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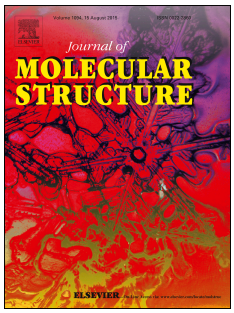


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# Synthesis of Novel Azol-β-Lactam Derivatives starting from phenyl piperazine and Investigation of their Antiurease activity and Antioxidant Capacity Comparing with their Molecular Docking Studies

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**Abstract:** This study reports the synthesis, biological investigation and molecular docking of novel β-lactam derivatives bearing 1,3,4-thiadiazole and 1,3,4-oxadiazole ring system. The synthesized compounds were evaluated for *in vitro* antiurease activity and antioxidant capacity. Almost all compounds showed excellent antiurease activity compared to thiourea, standard drug. Also, in silico ADME (absorption, distribution, metabolism, elimination) prediction and molecular docking studies were performed.

**Keywords:** Azole, phenyl piperazine, β-Lactam, Antioxidant Capacity, Antiurease activity, Molecular Docking

## 1. Introduction

β-Lactams are a major class of antibiotics defined by the presence of an azetidine-2-one ring, which is the nucleus of biological activity [1]. The azetidine-2-one ring system is a widespread structural character of a number of broad spectrum β-lactam antibiotics, such as penicillins, cephalosporins, carbapenems, nocardicins and monobactams that have been widely used as chemotherapeutic agents for treating microbial diseases [2]. This ring system also possess many biological activities such as cholesterol absorption inhibitors [3], human cytomegalovirus protease inhibitors [4], thrombin inhibitors [5], antihyperglycemic [6], anti-tumour [7], anti-HIV [8], antiinflammatory, analgesic activities [9] and serine-dependent enzyme inhibitors [10]. However, microorganisms have developed resistance to compounds containing beta-lactam ring as in any drug class. Therefore, the synthesis of new compounds with more effective and broad spectrum than the existing ones has gained importance. In this context, a kind of varied techniques have been reported for the synthesis of β-lactam

derivatives, including cyclization reactions, carbene insertion reactions, rearrangement of heterocyclic compounds, Reformatsky reaction, Ugi 4CR/cyclization and [2 + 2] ketene-imine cycloaddition [11-17].

Azoles are one of the crucial classes of nitrogen containing heterocycles that displayed diverse biological activities such as anti-bacterial, anti-malarial, anti-fungal, anti-HIV, antiinflammatory and anti-TB properties [18-20]. Among these class, thiadiazole and oxadiazole moieties have an important role in the fields of heterocyclic and medicinal chemistry because of their nitrogen, sulfur and oxygen atoms. Thiadiazole has chamred a great deal of concern as a favored scaffold due to its considerable therapeutic potential. The sulfur atom of thiadiazole ring gives improved liposolubility and the mesoionic nature of 1,3,4-thiadiazoles also allows these compounds to cross cellular membranes and interact with biological targets with distinct affinities [21-24]. Thiadiazoles show a wide spectrum of biological activities such as the inhibition of CAs, cyclooxygenases (COXs), neutral endopeptidase (NEP), aminopeptidase N (APN), matrix metalloproteinases (MMPs), phosphodiesterases (PDEs) and c-Src/Abl tyrosine kinase [25]. 1,3,4-Oxadiazole structure is a crucial group in heterocyclic chemistry due to their significant bioactivities and they have a broad application in organic synthesis [26, 27]. These compounds are affectingly being used as antibacterial agents, anticancer, anti-Parkinson, anti-HIV, and anti-proliferative agents [28-33].

Urease play a vital role in pathogenesis of peptide ulcers which cause cancer as helping the helicobacter pylori baceria to standat low pH of the stomach during colonizaition [34. 35]. Antioxidants are generally hydrogen donors or electron donorsto the reactive site in neutralizing free radicals. Antioxidants areextensively studied for their capacity for protect organism and cellfrom damage that is induced by oxidative stress. Scientists in manydifferent disciplines become more interested in new compounds,either synthesized or obtained from natural sources that couldprovide active components to prevent or reduce the impact ofoxidative stress on cell [36-38].

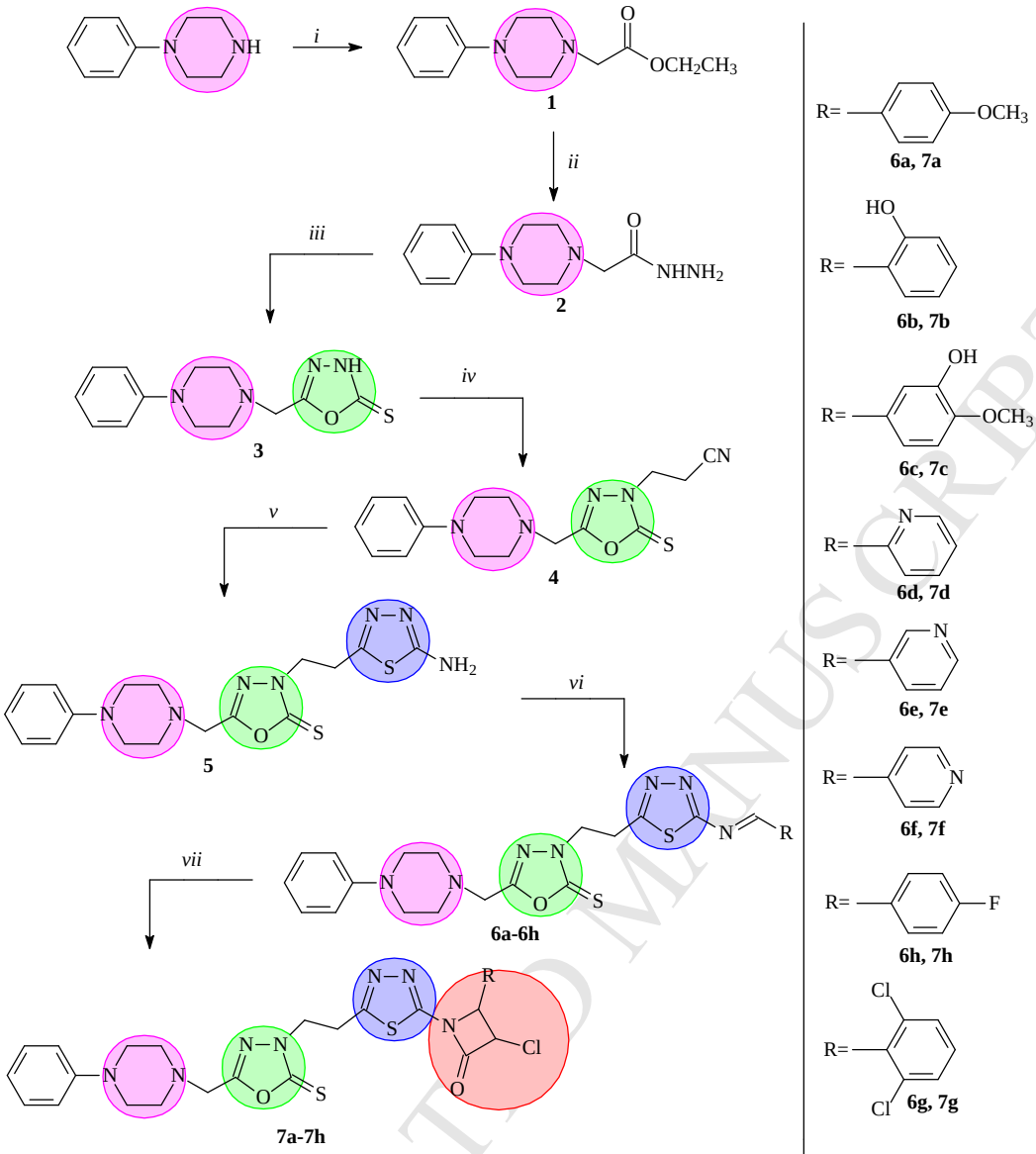
Our group has already reported oxadiazole and thiadiazole scaffold as potent antiurease, α-glucosidase inhibitor and antimicrobial activity [39, 40]. Therefore, we considered the synthesis of new hybrid compounds carrying oxadiazole, thiadiazole and β-lactam rings in the same structure, and these new compounds were thought to be more potent inhibitors. In the present research, the designed hybrid molecules were screened for their antioxidant capacity and potential of enzyme inhibition against urease enzyme. *In silico* studies were also

performed to figure out different kinds of interactions with active pocket of urease enzyme. In addition, one of the computational studies, ADME prediction were applied for all compounds.

## 2. Experimental

### 2.1. Chemistry

All the chemicals were purchased from *FlukaChemie AG Buchs* (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. The mobile phase was ethyl acetate:diethyl ether (1:2), and detection was made using UV light. FT-IR spectra were recorded using a *Perkin Elmer* 1600 series FTIR spectrometer. $^{1}$H NMR and $^{13}$C NMR spectra were registered in DMSO-$d_6$ on a *BRUKER AVENE II* 400 MHz NMR Spectrometer (400.13 MHz for $^{1}$H and 100.62 MHz for $^{13}$C). The chemical shifts are given in ppm, *J* values are given in Hz. The Mass spectra were obtained on a *Quattro LC-MS* (70 eV) Instrument. The yields, melting points and crystallization solvent of all compounds were given in Table 1.




**Scheme 1.** Synthetic pathway for the compounds. *i*: $BrCH_2COOEt$, $NEt_3$, THF, *ii*: $NH_2NH_2.H_2O$, EtOH, *iii*: $CS_2$, KOH, EtOH, *iv*: $CH_2CHCN$, $NEt_3$, EtOH, *v*: $NH_2CSNHNH_2$, $CF_3COOH$, *vi*: RHCO, *vii*: $ClCH_2COCl$, 1,4-dioxane, $NEt_3$.

### 2.2. 5-((4-phenylpiperazin-1-yl)methyl)-1,3,4-oxadiazole-2(3H)-thione (3)

Compound **2** (10 mmol) and $CS_2$ (20 mmol) were added to a solution of KOH (10 mmol) in 20 mL $H_2O$ and 20 mL ethanol and the reaction mixture was refluxed for 13 h. Then, the reaction content was acidified with conc. HCl to pH 6. The precipitate formed was filtered off, washed with $H_2O$ and recrystallized from ethanol to afford the desired compound.

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3011 (aromatic CH), 2841 (SH), 1442 (C=N). $^1H$ NMR (DMSO-$d_6$, δ ppm): 2.51 (4H, d, *J*= 1.6 Hz, $2CH_2$), 3.15 (4H, s, $2CH_2$), 3.44 (2H, d, *J*= 2.0 Hz, $CH_2$), 6.79 (1H, t, *J*= 12.0 Hz, arH), 6.93 (2H, d, *J*= 8.0 Hz, arH), 7.21 (2H, t, *J*= 12.0 Hz, arH), 14.42

(1H, brs, SH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 48.52 ($2CH_2$), 51.56 ($CH_2$), 52.41 ($2CH_2$), arC: [116.00 (2CH), 119.45 (CH), 129.36 (2CH), 151.29 (C)], 161.09 (oxadiazole C-5), 178.48 (oxadiazole C-2). EI MS *m/z* (%): 315.22 ($[M+K]^+$, 40, 277.23 ($[M + 1]^+$, 100), 175.16 (47), 106.04 (45).

### 2.3. 3-(5-((4-phenylpiperazin-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)propane nitrile (4)

To a solution of the compound **3** (10 mmol) in ethanol was added acrylonitrile (50 mmol) in the presence of triethylamine (50 mmol) and reaction mixture was refluxed for 6h. After evaporating the solvent under reduced pressure, a solid obtained. The crude product was recrystallized from ethanol to afford the desired product.

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3073 (aromatic CH), 2253 (CN), 1461 (C=N), 1220 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.48 (2H, s, $CH_2$), 2.64 (4H, s, $2CH_2$), 3.12 (2H, s, $CH_2$), 3.30 (2H, s, $CH_2$), 3.77 (2H, s, $CH_2$), 4.28 (2H, t, *J*= 12.4 Hz, $CH_2$), 6.75 (1H, t, *J*= 13.6 Hz, arH), 6.91 (2H, d, *J*= 8.0 Hz, arH), 7.18 (2H, t, *J*= 12.0 Hz, arH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 16.13 ($CH_2$), 44.62 ($CH_2$), 48.57 ($2CH_2$), 51.54 ($CH_2$), 52.40 ($2CH_2$), arC: [115.99 (2CH), 118.37 (CH), 129.36 (2CH), 151.36 (C)], 119.43 (CN), 159.91 (oxadiazole C-5), 176.77 (oxadiazole C-2). EI MS *m/z* (%): 368.36 ($[M + K]^+$, 100), 175.28 (25), 113.02 (60).

### 2.4. 3-(2-(5-amino-1,3,4-thiadiazol-2-yl)ethyl)-5-((4-phenylpiperazin-1-yl)methyl)-1,3,4-oxadiazole-2(3H)-thione (5)

A mixture of the compound **4** (10 mmol) and thiosemicarbazide (10 mmol) were combined in ethanol in the presence of trifluoroacetic acid (5 mL) in an oil bath and then was refluxed for 9 h. Then the reaction content was neutralized with diluted $NH_3$ to pH 6. Water was decanted from the mixture and the remaining fatty fraction was treated with ether to solidify the crude product. The resulting solid was filtered and purified by crystallization from acetone.

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3279 ($NH_2$), 3084 (aromatic CH),1527 (C=N), 1299 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.48 (2H, s, $CH_2$), 2.74 (4H, s, $2CH_2$), 3.17 (4H, s, $2CH_2$), 3.88 (2H, s, $CH_2$), 4.32 (2H, s, $CH_2$), 6.78 (1H, s, arH), 6.93 (1H, d, *J*= 6.0 Hz, arH), 7.02 (1H, s, arH), 7.17 (2H, d, *J*= 19.2 Hz, arH), 7.27 (2H, s, $NH_2$). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 27.59 ($CH_2$), 47.95 ($2CH_2$), 48.47 ($CH_2$), 51.38 ($CH_2$), 52.39 ($2CH_2$), arC: [116.02 (2CH), 119.46 (CH), 129.37 (2CH), 151.27 (C)], 153.89 (oxadiazole C-5), 161.94 (thiadiazole C-2), 169.28 (thiadiazole C-5), 178.60 (oxadiazole C-2). EI MS *m/z* (%): 405.40 ($[M + 2]^+$, 22), 404.40 ($[M + 1]^+$, 100), 305.54 (21), 304.47 (88), 175.21 (25).

### 2.5. General method for the synthesis of compounds 6a-h

To a solution of compound **5** (10 mmol) and corresponding aromatic aldehyde (20 mmol) was refluxed for 2 h at 125 ºC in an oil bath. After completion of the reaction, pure water was added to the flask and exracted with dichloromethane (1:3). Organic phase was dried with $Na_2SO_4$. After filtration off the solvent was evaporated under reduced pressure, a solid obtained. The crude product was recrystallized from an appropriate solvent (Table 1) to afford the desired product.

#### 2.5.1. 3-(2-(5-((4-methoxybenzylidene)amino)-1,3,4-thiadiazol-2-yl)ethyl)-5-((4-phenyl piperazin-1-yl)methyl)-1,3,4-oxadiazole-2(3H)-thione (6a)

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3044 (aromatic CH), 1576 (C=N), 1256 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.67 (4H, s, $2CH_2$), 3.04 (2H, s, $CH_2$), 3.65 (4H, s, $2CH_2$), 3.80 (3H, s, $OCH_3$), 3.97 (2H, s, $CH_2$), 5.12 (2H, s, $CH_2$), 6.61 (3H, t, *J*= 4.0 Hz, arH), 6.92 (2H, d, *J*= 8.0 Hz, arH), 7.06 (2H, d, *J*= 4.0 Hz, arH), 7.17 (2H, d, *J*= 4.0 Hz, arH), 8.36 (1H, s, CH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 29.56 ($CH_2$), 46.65 ($CH_2$), 50.47 ($2CH_2$), 52.63 ($2CH_2$), 55.89 ($CH_2$), 59.92 ($OCH_3$), ar: [113.02 (2CH), 118.86 (CH), 122.40 (CH), 126.18 (2CH), 129.09 (CH), 129.15 (2CH), 137.27 (C), 151.14 (C), 164.34 (C)], 155.11 (thiadiazole C-2), 161.86 (oxadiazole C-2), 166.70 (thiadiazole C-5), 169.66 (CH), 178.11 (oxadiazole C-5). EI MS *m/z* (%): 128 (50), 175 (100), 404 (69), 522 ($[M+1]^+$, 59).

#### 2.5.2. 3-(2-(5-((2-hydroxybenzylidene)amino)-1,3,4-thiadiazol-2-yl)ethyl)-5-((4-phenyl piperazin-1-yl)methyl)-1,3,4-oxadiazole-2(3H)-thione (6b)

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3044 (aromatic CH),1573 (C=N), 1271 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.57 (4H, s, $2CH_2$), 3.12 (2H, s, $CH_2$), 3.59 (4H, s, $2CH_2$), 3.78 (2H, s, $CH_2$), 4.52 (2H, s, $CH_2$), 6.71 (2H, d, *J*= 4.0 Hz, arH), 6.74 (1H, s, arH), 6.82 (1H, d, *J*= 4.0 Hz, arH), 6.92-7.01 (2H, m, arH), 7.11-7.18 (2H, m, arH), 7.47-7.51 (1H, m, arH), 9.17 (1H, s, CH), 11.27 (1H, s, OH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 29.81 ($CH_2$), 43.14 ($CH_2$), 46.68 ($CH_2$), 52.64 ($2CH_2$), 59.89 ($2CH_2$), ar: [113.05 (CH), 116.71 (2CH), 120.83 (C), 126.18 (CH), 127.02 (CH), 127.41 (CH), 128.59 (2CH), 133.48 (CH), 151.04 (C), 158.65 (C)], 155.12 (thiadiazole C-2), 162.13 (oxadiazole C-2), 166.25 (thiadiazole C-5), 169.53 (CH), 178.69 (oxadiazole C-5). EI MS *m/z* (%):102.27 (53), 323.32 (76), 327.35 (35), 447.60 (100), 448.48 (29).

**2.5.3. 3-(2-(5-((3-hydroxy-4-methoxybenzylidene)amino)-1,3,4-thiadiazol-2-yl)ethyl)-5-((4-phenylpiperazin-1-yl)methyl)-1,3,4-oxadiazole-2(3H)-thione (6c)**

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3125 (OH), 3024 (aromatic CH), 1587 (C=N), 1236 (C=S). $^{1}$H NMR (DMSO-$d_6$, δ ppm): 2.79 (4H, t, *J*= 8.0 Hz, $2CH_2$), 2.97 (2H, q, *J*= 12.0 Hz, $CH_2$), 3.23 (4H, t, *J*= 16.0 Hz, $2CH_2$), 3.49 (2H, s, $CH_2$), 3.98 (3H, s, $OCH_3$), 4.36 (2H, q, *J*= 16.0 Hz, $CH_2$), 6.84 (1H, d, *J*= 8.0 Hz, arH), 6.89 (1H, d, *J*= 8.0 Hz, arH), 6.90-6.99 (3H, m, arH), 7.28 (1H, t, *J*= 16.0 Hz, arH), 7.43 (2H, d, *J*= 8.0 Hz, arH), 8.74 (1H, s, CH), 9.84 (1H, s, OH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 27.29 ($CH_2$), 46.51 ($CH_2$), 47.70 ($CH_2$), 51.51 ($2CH_2$), 52.29 ($2CH_2$), 56.98 ($OCH_3$), ar: [109.95 (CH), 113.10 (CH), 126.17 (2CH), 127.87 (CH), 128.25 (CH), 129.13 (2CH), 132.53 (C), 150.72 (C), 152.44 (C), 155.10 (C)], 159.98 (thiadiazole C-2), 162.83 (oxadiazole C-2), 163.77 (CH), 169.79 (thiadiazole C-5), 180.51 (oxadiazole C-5). EI MS *m/z* (%): 560.39 ($M^+$, 100).

**2.5.4. 5-((4-phenylpiperazin-1-yl)methyl)-3-(2-(5-((pyridin-2-ylmethylene)amino)-1,3,4-thiadiazol-2-yl)ethyl)-1,3,4-oxadiazole-2(3H)-thione (6d)**

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3042 (aromatic CH), 1598 (C=N), 1218 (C=S). $^{1}$H NMR (DMSO-$d_6$, δ ppm): 2.61 (4H, s, $2CH_2$), 3.12 (4H, d, *J*= 8.0 Hz, $2CH_2$), 3.32 (2H, s, $CH_2$), 3.73 (2H, s, $CH_2$), 4.33 (2H, d, *J*= 8.0 Hz, $CH_2$), 6.79 (1H, d, *J*= 8.0 Hz, arH), 6.92 (2H, t, *J*= 16.0 Hz, arH), 7.13 (1H, s, arH), 7.21 (1H, s, arH), 7.70-7.84 (1H, m, arH), 7.93-8.05 (1H, m, arH), 8.32 (1H, s, CH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 24.38 ($CH_2$), 46.70 ($CH_2$), 51.88 ($2CH_2$), 52.62 ($2CH_2$), 59.89 ($CH_2$), ar: [118.73 (CH), 122.29 (CH), 126.21 (2CH), 127.63 (CH), 129.13 (2CH), 136.31 (CH), 148.81 (CH), 151.49 (C), 155.74 (C)], 157.69 (thiadiazole C-2), 161.95 (oxadiazole C-2), 167.38 (thiadiazole C-5), 169.59 (CH), 179.66 (oxadiazole C-5). EI MS *m/z* (%): 106 (63), 175 (100), 285 (63), 301 (62), 315 (60), 369 (50), 493 ($[M+1]^+$, 20).

**2.5.5. 5-((4-phenylpiperazin-1-yl)methyl)-3-(2-(5-((pyridin-3-ylmethylene)amino)-1,3,4-thiadiazol-2-yl)ethyl)-1,3,4-oxadiazole-2(3H)-thione (6e)**

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3065 (aromatic CH), 1599 (C=N), 1220 (C=S). $^{1}$H NMR (DMSO-$d_6$, δ ppm): 2.51 (4H, s, $2CH_2$), 3.18 (4H, s, $2CH_2$), 3.39 (2H, s, $CH_2$), 3.66 (2H, s, $CH_2$), 4.38 (2H, t, *J*= 16.0 Hz, $CH_2$), 6.85 (1H, t, *J*= 16.0 Hz, arH), 7.0 (2H, d, *J*= 8.0 Hz, arH), 7.25 (2H, t, *J*= 12.0 Hz, arH), 7.67 (1H, q, *J*= 12.0 Hz, arH), 7.77 (1H, q, *J*= 16.0 Hz, arH), 8.17 (1H, d, *J*= 8.0 Hz, arH), 8.28 (1H, d, *J*= 8.0 Hz, arH), 8.74 (1H, s, CH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 26.08 ($CH_2$), 46.49 ($CH_2$), 51.82 ($CH_2$), 52.45 ($2CH_2$), 52.64 ($2CH_2$), ar: [118.82 (2CH), 122.32 (CH), 126.13 (2CH), 129.59 (CH), 135.48 (CH), 137.45 (C), 150.76 (C), 151.37 (CH),

154.45 (C)], 157.44 (thiadiazole C-2), 162.36 (oxadiazole C-2), 165.97 (CH), 169.52 (thiadiazole C-5), 180.30 (oxadiazole C-5). EI MS *m/z* (%): 478 (100), 482 (53), 493 ($[M+2]^+$, 90), 494 (43), 512 (53).

**2.5.6. 5-((4-phenylpiperazin-1-yl)methyl)-3-(2-(5-((pyridin-4-ylmethylene)amino)-1,3,4-thiadiazol-2-yl)ethyl)-1,3,4-oxadiazole-2(3H)-thione (6f)**

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3097 (aromatic CH), 1599 (C=N), 1215 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.51 (4H, s, $2CH_2$), 3.23 (4H, s, $2CH_2$), 3.29 (2H, s, $CH_2$), 4.0 (2H, s, $CH_2$), 4.35 (2H, t, *J*= 16.0 Hz, $CH_2$), 6.82 (1H, t, *J*= 16.0 Hz, arH), 6.97 (2H, d, *J*= 8.0 Hz, arH), 7.24 (2H, d, *J*= 8.0 Hz, arH), 7.57 (1H, d, *J*= 8.0 Hz, arH), 7.83 (1H, d, *J*= 8.0 Hz, arH), 8.71-8.75 (2H, m, arH), 8.90 (1H, s, CH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 27.97 ($CH_2$), 44.25 ($CH_2$), 46.41 ($CH_2$), 52.13 ($2CH_2$), 53.28 ($2CH_2$), ar: [113.00 (2CH), 126.13 (CH), 127.48 (CH), 128.87 (2CH), 136.85 (C), 144.65 (CH), 150.36 (C), 154.96 (CH), 156.06 (CH)], 154.96 (thiadiazole C-2), 160.51 (oxadiazole C-2), 164.83 (CH), 168.10 (thiadiazole C-5), 174.88 (oxadiazole C-5). EI MS *m/z* (%): 108 (100), 140 (43), 404 (32), 493 ($[M+2]^+$, 25).

**2.5.7. 3-(2-(5-((2,6-dichlorobenzylidene)amino)-1,3,4-thiadiazol-2-yl)ethyl)-5-((4-phenyl piperazin-1-yl)methyl)-1,3,4-oxadiazole-2(3H)-thione (6g)**

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3054 (aromatic CH), 1586 (C=N), 1225 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.80 (4H, s, $2CH_2$), 2.96 (4H, s, $2CH_2$), 3.25 (2H, s, $CH_2$), 3.73 (2H, d, *J*= 16.0 Hz, $CH_2$), 4.36 (2H, q, *J*= 20.0 Hz, $CH_2$), 6.89-6.98 (3H, m, arH), 7.13 (1H, s, arH), 7.25-7.30 (4H, m, arH), 8.75 (1H, s, CH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 26.45 ($CH_2$), 46.67 ($CH_2$), 51.49 ($CH_2$), 51.98 ($2CH_2$), 54.13 ($2CH_2$), ar: [126.11 (2CH), 127.64 (CH), 128.31 (2CH), 128.48 (CH), 129.93 (2CH), 132.30 (C), 133.73 (C), 151.91 (C)], 152.88 (thiadiazole C-2), 155.00 (oxadiazole C-2), 164.15 (CH), 169.33 (thiadiazole C-5), 176.57 (oxadiazole C-5). EI MS *m/z* (%): 153.17 (100), 188.91 (82), 205.28 (77), 307.22 (75), 582.39 ($[M+Na]^+$, 37).

**2.5.8. 3-(2-(5-((4-fluorobenzylidene)amino)-1,3,4-thiadiazol-2-yl)ethyl)-5-((4-phenyl piperazin-1-yl)methyl)-1,3,4-oxadiazole-2(3H)-thione (6h)**

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3063 (aromatic CH), 1599 (C=N), 1222 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.51 (4H, s, $2CH_2$), 3.17 (4H, s, $2CH_2$), 3.39 (2H, s, $CH_2$), 4.41 (2H, s, $CH_2$), 4.62 (2H, s, $CH_2$), 6.90 (1H, t, *J*= 16.0 Hz, arH), 7.05 (3H, d, *J*= 8.0 Hz, arH), 7.28 (3H, t, *J*= 16.0 Hz, arH), 7.45 (1H, t, *J*= 16.0 Hz, arH), 8.01 (1H, t, *J*= 16.0 Hz, arH), 9.99 (1H, s, CH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 28.33 ($CH_2$), 42.30 ($CH_2$), 46.39 ($CH_2$), 52.10 ($2CH_2$), 53.16 ($2CH_2$), ar:

[113.08 (2CH), 126.20 (2CH), 127.43 (2CH), 127.75 (CH), 128.91 (2CH), 137.64 (C), 144.81 (C), 161.75-164.18 ($d_{C-F}$, *J*=243.0 Hz, C)], 155.02 (thiadiazole C-2), 160.63 (oxadiazole C-2), 162.80 (thiadiazole C-5), 169.19 (CH), 177.75 (oxadiazole C-5). EI MS *m/z* (%): 205.18 (100), 486.25 (18), 509.21 ($[M]^+$, 22).

### 2.6. General method for the synthesis of compounds 7a-h

A mixture of compound **6** (10 mmol), triethylamine (5 mmol) and chloroacetyl chloride (5 mmol) were combined in dioxane and stirred in an ice bath (0-5 °C) for 10 h. The reaction mixture was poured into ice-water, the resulting solid was filtered, dried and crystallized from an appropriate solvent.

#### 2.6.1. 3-Chloro-4-(4-methoxyphenyl)-1-(5-(2-(5-((4-phenylpiperazin-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethyl)-1,3,4-thiadiazol-2-yl)azetidin-2-one (7a)

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3032 (aromatic CH), 1698 (C=O), 1600 (C=N), 1496 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.37 (4H, t, *J*=12.0 Hz, $2CH_2$), 2.93 (2H, s, $CH_2$), 3.28 (4H, t, *J*=8.0, $2CH_2$), 3.42 (2H, s, $CH_2$), 3.74 (3H, s, $OCH_3$), 4.24 (2H, s, $CH_2$), 4.74 (1H, s, CH), 5.04 (1H, s, CH), 6.97 (2H, d, *J*=8.0 Hz, arH), 7.36 (1H, s, arH), 7.40 (2H, d, *J*= 8.0 Hz, arH), 7.53-7.57 (m, 2H, arH), 8.03 (2H, d, *J*= 8.0 Hz, arH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 24.39 ($CH_2$), 46.66 ($CH_2$), 50.29 ($CH_2$), 51.89 ($2CH_2$), 53.83 ($2CH_2$), 56.27 ($OCH_3$), 60.13 (CH), 67.57 (CH), ar: [113.13 (2CH), 116.39 (2CH), 121.35 (CH), 126.13 (2CH), 129.13 (2CH), 137.41 (C), 149.59 (C), 155.05 (C)], 145.07 (thiadiazole C-5), 161.29 (oxadiazole C-2), 166.48 (thiadiazole C-2), 168.90 (C=O), 180.51 (oxadiazole C-5). EI MS m/z (%): 313.25 (30), 462.27 (100), 637.23 ($[M+K]^+$, 57).

#### 2.6.2. 3-Chloro-4-(2-hydroxyphenyl)-1-(5-(2-(5-((4-phenylpiperazin-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethyl)-1,3,4-thiadiazol-2-yl)azetidin-2-one (7b)

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3026 (aromatic CH), 1703 (C=O), 1578 (C=N), 1458 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.41 (4H, s, $2CH_2$), 2.64 (2H, s, $CH_2$), 3.29 (4H, s, $2CH_2$), 3.42 (2H, s, $CH_2$), 4.29 (2H, s, $CH_2$), 4.98 (1H, s, CH), 5.45 (1H, s, CH), 6.98 (2H, d, *J*=8.0 Hz, arH), 7.04 (1H, d, *J*= 8.0 Hz, arH), 7.25 (1H, t, *J*= 12.0 Hz, arH), 7.44-7.48 (m, 2H, arH), 7.54 (2H, d, *J*= 7.0 Hz, arH), 7.67 (1H, d, *J*= 8.0 Hz, arH), 9.62 (1H, s, OH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 21.26 ($CH_2$), 50.82 ($CH_2$), 53.24 ($2CH_2$), 55.49 ($2CH_2$), 59.894(CH), 62.11 (CH), ar: [119.17 (2CH), 120.58 (CH), 123.89 (2CH), 125.42 (CH), 127.67 (C), 130.50 (2CH), 131.67 (CH), 153.23 (C), 157.12 (C)], 144.84 (thiadiazole C-5), 163.21 (oxadiazole C-2), 167.43

(thiadiazole C-2), 171.47 (C=O), 180.12 (oxadiazole C-5). EI MS m/z (%): 606.24 (100), 608.25 ($[M+1]^+$, 83), 609.32 (24).

**2.6.3. 3-Chloro-4-(3-hydroxy-4-methoxyphenyl)-1-(5-(2-(5-((4-phenylpiperazin-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethyl)-1,3,4-thiadiazol-2-yl)azetidin-2-one (7c)**

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3319 (-OH), 3063 (aromatic CH), 1678 (C=O), 1595 (C=N), 1245 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.41 (4H, s, $2CH_2$), 2.87 (2H, s, $CH_2$), 3.16 (4H, s, $2CH_2$), 3.54 (2H, s, $CH_2$), 3.81 (3H, s, $OCH_3$), 4.31 (2H, s, $CH_2$), 5.19 (1H, s, CH), 5.41 (1H, s, CH), 6.30 (1H, bs, OH), 6.78 (1H, t, *J*=12.0 Hz, arH), 6.92-6.95 (3H, m, arH), 7.20-7.25 (3H, m, arH), 7.27-7.35 (1H, m, arH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 26.86 ($CH_2$), 46.71 ($CH_2$), 51.51 ($CH_2$), 52.71 ($2CH_2$), 55.21 ($2CH_2$), 57.67 ($OCH_3$), 59.95 (CH), 66.08 (CH), ar: [113.11 (CH), 117.61 (CH), 119.60 (2CH), 122.22 (2CH), 126.19 (2CH), 137.31 (C), 143.04 (C), 150.90 (C)], 145.19 (thiadiazole C-5), 160.05 (oxadiazole C-2), 166.18 (thiadiazole C-2), 169.63 (C=O), 178.40 (oxadiazole C-5). EI MS m/z (%): 556.21 (100), 556.47 (91), 600.33 (22), 614.42 ($M^+$, 26).

**2.6.4. 3-Chloro-1-(5-(2-(5-((4-phenylpiperazin-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethyl)-1,3,4-thiadiazol-2-yl)-4-(pyridin-2-yl)azetidin-2-one (7d)**

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3061 (aromatic CH), 1681 (C=O), 1587 (C=N), 1239 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.59 (4H, s, $2CH_2$), 2.82 (2H, s, $CH_2$), 3.13 (4H, s, $2CH_2$), 3.46 (2H, s, $CH_2$), 4.12 (2H, s, $CH_2$), 5.09 (1H, s, CH), 5.38 (1H, s, CH), 6.77 (1H, t, *J*=16.0 Hz, arH), 6.91 (2H, d, *J*=8.0 Hz, arH), 7.02 (2H, d, *J*= 12.0 Hz, arH), 7.20 (2H, t, *J*=12.0 Hz, arH), 8.05 (2H, d, *J*= 12.0 Hz, arH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 26.71 ($CH_2$), 46.38 ($CH_2$), 49.78 ($CH_2$), 52.12 ($2CH_2$), 52.97 ($2CH_2$), 58.92 (CH), 66.05 (CH), ar: [116.13 (2CH), 119.86 (CH), 121.35 (CH), 126.13 (CH), 128.92 (2CH), 138.45 (CH), 151.22 (C), 153.17 (CH), 165.14 (C)], 147.95 (thiadiazole C-5), 161.64 (oxadiazole C-2), 166.59 (thiadiazole C-2), 169.96 (C=O), 176.58 (oxadiazole C-5). EI MS m/z (%): 101.13 (36), 554.32 (100), 570.28 ($[M+1]^+$, 48).

**2.6.5. 3-Chloro-1-(5-(2-(5-((4-phenylpiperazin-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethyl)-1,3,4-thiadiazol-2-yl)-4-(pyridin-3-yl)azetidin-2-one (7e)**

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3019 (aromatic CH), 1702 (C=O), 1579 (C=N), 1460 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.44 (4H, s, $2CH_2$), 2.68 (2H, s, $CH_2$), 3.22 (4H, s, $2CH_2$), 3.52 (2H, s,

$CH_2$), 4.23 (2H, s, $CH_2$), 5.06 (1H, s, CH), 5.41 (1H, s, CH), 6.97 (2H, d, *J*=12.0 Hz, arH), 7.27 (3H, d, *J*=8.0 Hz, arH), 7.34 (2H, t, *J*= 12.0 Hz, arH), 8.04 (2H, d, *J*= 12.0 Hz, arH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 25.60 ($CH_2$), 46.66 ($CH_2$), 51.89 ($CH_2$), 52.46 ($2CH_2$), 53.35 ($2CH_2$), 58.72 (CH), 67.64 (CH), ar: [116.73 (2CH), 119.09 (CH), 126.09 (CH), 127.65 (CH), 129.49 (CH), 134.03 (CH), 141.60 (C), 147.89 (CH), 148.94 (CH), 152.54 (C)], 145.29 (thiadiazole C-5), 161.93 (oxadiazole C-2), 166.57 (thiadiazole C-2), 169.18 (C=O), 176.61 (oxadiazole C-5). EI MS m/z (%): 168.84 (100), 205.15 (96), 360.51 (46), 570.31 ($[M+1]^+$, 68).

### 2.6.6. 3-Chloro-1-(5-(2-(5-((4-phenylpiperazin-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethyl)-1,3,4-thiadiazol-2-yl)-4-(pyridin-4-yl)azetidin-2-one (7f)

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3030 (aromatic CH), 1701 (C=O), 1579 (C=N), 1496 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.39 (4H, s, $2CH_2$), 2.84 (2H, s, $CH_2$), 3.26 (4H, s, $2CH_2$), 3.54 (2H, s, $CH_2$), 4.20 (2H, s, $CH_2$), 4.93 (1H, s, CH), 5.95 (1H, s, CH), 6.92 (1H, d, *J*=12.0 Hz, arH), 7.37-7.40 (7H, m, arH), 8.01 (1H, d, *J*= 8.0 Hz, arH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 25.93 ($CH_2$), 47.78 ($CH_2$), 51.74 ($CH_2$), 52.03 ($2CH_2$), 54.05 ($2CH_2$), 60.49 (CH), 70.14 (CH), ar: [117.77 (2CH), 121.64 (CH), 124.58 (2CH), 128.89 (2CH), 148.22 (C), 151.31 (2CH), 154.94 (C)], 145.48 (thiadiazole C-5), 161.70 (oxadiazole C-2), 166.65 (thiadiazole C-2), 169.62 (C=O), 178.69 (oxadiazole C-5). EI MS m/z (%): 205.21 (42), 447.54 (38), 606.24 (100), 608.38 ($[M+K]^+$, 69).

### 2.6.7. 3-Chloro-4-(2,6-dichlorophenyl)-1-(5-(2-(5-((4-phenylpiperazin-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethyl)-1,3,4-thiadiazol-2-yl)azetidin-2-one (7g)

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3058 (aromatic CH), 1681 (C=O), 1588 (C=N), 1239 (C=S). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.52 (4H, s, $2CH_2$), 2.87 (2H, s, $CH_2$), 3.41 (4H, s, $2CH_2$), 3.69 (2H, s, $CH_2$), 4.93 (2H, s, $CH_2$), 5.71 (1H, s, CH), 6.02 (1H, s, CH), 6.98 (2H, d, *J*=8.0 Hz, arH), 7.39 (4H, t, *J*=20.0 Hz, arH), 8.03 (2H, d, *J*= 8.0 Hz, arH). $^{13}$C NMR (DMSO-$d_6$, δ ppm): 24.56 ($CH_2$), 46.72 ($CH_2$), 48.65 ($CH_2$), 50.29 ($2CH_2$), 52.35 ($2CH_2$), 59.98 (CH), 62.18 (CH), ar: [115.99 (2CH), 121.71 (CH), 126.17 (2CH), 129.38 (2CH), 132.59 (CH), 134.46 (2C), 135.90 (C), 151.36 (C)], 145.18 (thiadiazole C-5), 160.50 (oxadiazole C-2), 165.27 (thiadiazole C-2), 168.34 (C=O), 179.61 (oxadiazole C-5). EI MS m/z (%): 191.93 (40), 239.95 (55), 242.02 (60), 443.26 (73), 613.35 (52), 637.27 ($M^+$, 100).

**2.6.8. 3-Chloro-4-(4fluorophenyl)-1-(5-(2-(5-((4-phenylpiperazin-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethyl)-1,3,4-thiadiazol-2-yl)azetidin-2-one (7h)**

FT-IR ($\upsilon_{max}$, $cm^{-1}$): 3065 (aromatic CH), 1696 (C=O), 1582 (C=N), 1238 (C=S). $^{1}H$ NMR (DMSO-$d_6$, δ ppm): 2.65 (4H, s, $2CH_2$), 2.96 (2H, s, $CH_2$), 3.16 (4H, s, $2CH_2$), 3.52 (2H, s, $CH_2$), 4.31 (2H, s, $CH_2$), 4.92 (1H, s, CH), 5.26 (1H, s, CH), 7.05 (2H, d, *J*=8.0 Hz, arH), 7.16 (1H, s, arH), 7.19 (2H, d, *J*= 8.0 Hz, arH), 7.42 (2H, s, arH), 7.78 (2H, d, *J*= 8.0 Hz, arH). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 25.81 ($CH_2$), 46.29 ($CH_2$), 48.65 ($CH_2$), 50.28 ($2CH_2$), 52.03 ($2CH_2$), 60.61 (CH), 67.41 (CH), ar: [115.98 (2CH), 118.59 (CH), 121.60 (CH), 126.17 (CH), 127.13 (CH), 128.89 (CH), 129.39 (2CH), 137.36 (C), 151.31 (C), 159.47-161.72 ($d_{C-F}$, *J*=245.0 Hz, C)], 144.15 (thiadiazole C-5), 164.30 (oxadiazole C-2), 167.64 (thiadiazole C-2), 169.81 (C=O), 179.62 (oxadiazole C-5). EI MS m/z (%): 120 (67), 213 (55), 314 (40), 452 (31), 587.42 ($[M+1]^+$, 100), 588 ($[M+2]^+$, 52).

## 3. Results and Discussion

### 3.1. Chemistry

1,3,4-thiadiazole-β-lactam hybrid compounds **7a-h** have been prepared according to the synthetic procedure introduced in Scheme 1. Compounds **1** and **2** were synthesized with the known substitution reactions, separately with ethyl bromoacetate and hydrazine hydrate. Compound **3**, containing 1,3,4-oxadiazole ring, was obtained by cyclization of compound **2** with $CS_2$ in basic media. 3-(5-((4-phenylpiperazin-1-yl)methyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)propan nitrile (**4**) was synthesized by the reaction of compound **3** and acrylonitrile. The $CH_2$ signals in the structure formed by the addition of the acrylonitrile group to 1,3,4-oxadiazole were observed between 3.12 and 4.28 ppm in $^{1}H$ NMR spectrum. The quaternary carbon atom of –CN group was resonated in 119.43 ppm in $^{13}C$ NMR spectrum. These findings approved the formation of the compound **4**. Compound **5**, comprising a 1,3,4-thiadiazol-5-amino ring, was achieved by reacting compound **4** with thiosemicarbazide in the presence of trifluoroacetic acid, as a catalyst. The appearance of $-NH_2$ group was shown in 7.27 ppm in $^{1}H$ NMR spectrum and this supported the formation of the thiadiazole ring of compound 4 with thiosemicarbazide. In addition, the absence of nitrile carbon in the $^{13}C$ NMR spectrum and the resonance of the 1,3,4-thiadiazole C-2 and C-2 carbons at the relevant sites promoted the compound **5**.

The free amino group of compound **5** in 1,3,4-thiadiazole ring at C-2 position, were reacted with various aldehydes, and corresponding Schiff base derivatives (**6a-h**) were obtained. The

disappearance of amino group in the FT-IR spectrum for compounds **6a-h** and the resonance of the CH protons in the imine group (-N=CH) between 8.32 and 9.17 ppm showed that the structure of Schiff bases was formed. It is interesting to note that the compounds having imine group may exist as *E*/*Z* geometrical isomers due to the C=N double bond and as cis/trans amide conformers [41-43]. According to the previous studies [42], in dimethyl-$d_6$ sulfoxide solution, this kind of compounds are present in higher percentage in the form of geometrical *E* isomer about C=N double bond. Another evidence for supporting the formation of compounds **6a-h**, carbon atom signals of HC=N were monitored between 163.87 and 169.66 ppm in $^{13}$C NMR spectrum. Schiff base derivatives of compounds **6a-h** were reacted by dropwise addition of chloroacetyl chloride in an ice bath in the presence of triethylamine in dioxane solvent to afford the target compounds of β-lactam derivatives of compounds **7a-h** (Scheme 1). The protons in the imine group disappeared and the protons on the carbon atom to which the Cl atom bound in the beta-lactam were resonated between 5.04 and 6.02 ppm in $^{1}$H NMR spectrum. Meanwhile, this carbon atom and carbonyl carbon atom appeared in 62.11-70.14 and 168.34-171.47 ppm in $^{13}$C NMR, respectively.

**Table 1.** Reaction yields, melting points and crystallization solvents of all compounds.

| Compound | Yield (%) | Melting Point ($^{o}$C) | Crystallization Solvent |
|---|---|---|---|
| **3** | 86 | 178-179 | EtOH |
| **4** | 82 | 145-146 | EtOH |
| **5** | 56 | 155-156 | Acetone |
| **6a** | 79 | 178-179 | EtOH |
| **6b** | 89 | 181-182 | EtOH |
| **6c** | 81 | 188-189 | EtOH |
| **6d** | 83 | 200-201 | MeOH |
| **6e** | 78 | 175-177 | MeOH:$H_2O$ (1:3) |
| **6f** | 82 | 189-190 | MeOH:$H_2O$ (1:3) |
| **6g** | 76 | 205-206 | EtOH |
| **6h** | 69 | 165-166 | MeOH |
| **7a** | 64 | 202-203 | Acetone |
| **7b** | 68 | 205-206 | EtOH |
| **7c** | 72 | 166-167 | EtOH |
| **7d** | 58 | 175-176 | MeOH |
| **7e** | 65 | 199-200 | MeOH |
| **7f** | 70 | 160-161 | Acetone |
| **7g** | 73 | 181-182 | EtOH |
| **7h** | 66 | 192-193 | EtOH |

### 3.2. Biological Activity

#### 3.2.1. Antioxidant Capacity

DPPH (2.2-diphenyl-1-picrylhydrazyl) radical scavenging activity was performed the synthesized compounds with different chemicals was determined using the free radical DPPH (2.2-diphenyl-1-picrylhydrazyl), as described by Blois [50]. A 100-µL: chemical solution was mixed with 1 mL of freshly prepared methanolic DPPH solution. The reaction mixture was incubated for 30 min at room temperature in the dark and was then measured at 520 nm. The activity was expressed as µmol Trolox equivalent. FRAP (the ferric reducing ability of plasma) was measured using the method described by Benzie & Strain [51] with some modification. To 100 µL of each sample was added 2900 µL freshly prepared FRAP reagent containing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ (2,4,6-tripyridyle-s-triazine) and 20 mmol/L $FeCl.6H_2O$ in proportions of 10:1:1 (v/v/v). The mixture was incubated for 30 min at 37 ºC and measured at 593 nm. The values were given as µmol of Trolox/g. CUPRAC (cupric ion reducing antioxidant capacity) was measured following the procedure described by Apak *et al.* [52] with some modification. Briefly, 100 µL of each chemical solution was mixed with 900 µL bidistilled water, 1 mL acetate buffer solution (1 mmol/L, pH: 7.0), 1 mL $CuCl_2$ (10 mmol/L) and 1 mL 7.5 mmol/L neocuproine to a final volume of 4 mL. The reaction mixture was then incubated in the dark for 30 min at room temperature and the absorbance of the reaction mixture was measured at 450 nm against a water blank. Trolox was used as the standard calibration curves and the results were expressed as µmol Trolox equivalent per g.

DPPH, FRAP and CUPRAC assays were performed to determine the antioxidant capacity (AC, µmol TE/g) of the synthesized compounds. All compounds displayed good-moderate antioxidant activity (Table 2). Trolox was used as standard for DPPH radical scavenging method and the results are given $SC_{50}$ value. According to DPPH method, **6a**, **6e** and **6h** exhibited good activity with 0,12 ± 0,01, 0,26 ± 0,01 and 0,38± 0,01 $SC_{50}$ values, respectively. On the other hand, **7b** (7529.53 ± 2.57 µmol TE/g) and **6e** (7175.01 ± 20.00 µmol TE/g) for CUPRAC, **7b** (4,453 ± 0,006) for FRAP showed the highest AC values among the synthesized compounds (Table 2).

**Table 2.** Antioxidant capacity of newly synthesized compounds

| Compound No | CUPRAC (µmol TE/g) | DPPH (mg/mL) $SC_{50}$ | FRAP (µmol TE/g) |
|---|---|---|---|
| **3** | 1986.28±18.56 | 3,24±0,01 | 1,269±0,002 |
| **4** | 29.54±2.57 | 7,85±0,02 | 0,434±0,006 |
| **5** | 2431.99±16.10 | 3,18±0,04 | 0,459±0,004 |
| **6a** | 3950.34±16.25 | 0,12±0,01 | 4,557±0,021 |
| **6b** | 365.14±2.36 | 3,28±0,02 | 0,136±0,003 |

| | | | |
|---|---|---|---|
| **6c** | 348.64±26.00 | 5,06±0,01 | 0,947±0,016 |
| **6d** | 875.41±10.00 | 2,61±0,02 | 1,912±0,032 |
| **6e** | 7175.01±20.00 | 0,26±0,01 | 1,710±0,056 |
| **6f** | 123.97±4.61 | 1,18±0,01 | 0,421±0,009 |
| **6g** | 431.98± 16.10 | 3,18±0,04 | 0,459±0,004 |
| **6h** | 1843.56±3.00 | 0,38±0,01 | 0,564±0,004 |
| **7a** | 1986.11±23.42 | 4,29±0,03 | 1,119±0,030 |
| **7b** | 7529.53±2.57 | 0,81±0,00 | 4,453±0,006 |
| **7c** | 756.14±10.00 | 1,18±0,01 | 0,828±0,018 |
| **7d** | 1745.12± 1.00 | 2,13±0,02 | 0,486±0,020 |
| **7e** | 1954.34± 4.58 | 3,23±0,06 | 0,592±0,000 |
| **7f** | 1265.29±2.00 | 1,79±0,05 | 0,673±0,004 |
| **7g** | 1048.11±6.00 | 2,13±0,03 | 0,218±0,001 |
| **7h** | 1278.35±14.00 | 4,29±0,01 | 1,229±0,016 |
| **Trolox** | | 0,04±0,00 | |

**3.2.2. Urease Inhibitory Activity** [53]

Urease activity was defined by using Weatherburn process. Reaction mixtures including 25 µL of Jack Bean urease, 55 µL of buffer (0.01 mol/L $K_2HPO_4$, 1 mmol/L EDTA and 0.01 mol/L LiCl, pH 8.2) and 10 mmol/L urea were incubated with 5 µL of the test compounds at room temperature for 15 min in microtiter plates. The production of ammonia was measured following the indophenol method and was used to determine the urease inhibitory activity. The phenol reagent (45 µL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 µL, 0.5% w/v sodium hydroxide and 0.1% v/v NaOCl) were added to each well. This mixture was incubated for 15 minutes more at 35 °C and optical density was measured at 625 nm against a blank solution including distilled water instead of enzyme. For the determination of the $IC_{50}$ value of the extracts, activity assays were conducted at five different extract concentration and dose response curve was generated. Thiourea was used as standard inhibitor.

The obtained compounds were examined for their *in vitro* urease inhibitory activity against Jack bean urease. All the compounds displayed range from good to excellent activity compared to thiourea used as standard drug. Especially, among the compounds **6a**, **7b** and **7f** displayed excellent inhibition with 0.98 ± 0.03; 1.15 ± 0.00 and 1.54 ± 0.01 $IC_{50}$ values, respectively (Table 3).

**Table 3.** Inhibitory activities of the synthesized compounds against Jack bean urease

| **Compound No** | **Antiurease $IC_{50}$ (mg/mL)** | **Compound No** | **Antiurease $IC_{50}$ (mg/mL)** |
|---|---|---|---|
| **3** | 6.87 ± 0.01 | **6h** | 3.18 ± 0.02 |

| | | | |
|---|---|---|---|
| **4** | 8.54 ± 0.02 | **7a** | 2.55 ± 0.03 |
| **5** | 3.45 ± 0.04 | **7b** | 1.15 ± 0.00 |
| **6a** | 0.98 ± 0.03 | **7c** | 4.75 ± 0.01 |
| **6b** | 2.54 ± 0.02 | **7d** | 5.36 ± 0.02 |
| **6c** | 3.72 ± 0.01 | **7e** | 2.85 ± 0.02 |
| **6d** | 6.43 ± 0.02 | **7f** | 1.54 ± 0.01 |
| **6e** | 5.62 ± 0.04 | **7g** | 5.84 ± 0.03 |
| **6f** | 3.27 ± 0.02 | **7h** | 6.54 ± 0.01 |
| **6g** | 3.45 ± 0.04 | **Thiourea** | 12,02 ± 0,06 |

### 3.3. ADME Prediction

*In silico* ADME study, which is one of the computational calculations, was applied for the synthesized compounds. In this study, we calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLogP), number of hydrogen bond acceptors (*n*-ON), number of hydrogen bonds donors (*n*-OHNH), topological polar surface area (TPSA), number of rotatable bonds (*n*-ROTB) and Lipinski's rule of five [45] using Molinspiration online property calculation toolkit [45]. Absorption (% ABS) was calculated by: % ABS =109 x(0.345xTPSA) [46]. From all these parameters (Table 4), all synthesized compounds exhibited a good % ABS (75.73–92.33%). It was monitored that none of the compound violated Lipinski's rule of five except for **7c** (Table 4). It violated due the number of oxygen and nitrogen atoms in the structure and molecular weight. It can be suggested that other compounds may be developed as good drug candidates. A molecule probably to be evolved as an orally active drug candidate should exhibit no more than one violation of the following four criteria: logP (octanol–water partition coefficient) ≤5, molecular weight ≤500, number of hydrogen bond acceptors ≤10 and number of hydrogen bond donors ≤5 [47]. Since the obtained compounds (except for **7c**) followed this rule for orally active drug and therefore can be promoted as oral drug candidates.

**Table 4.** Pharmacokinetic parameters for good oral bioavailability of synthesized compound **3-7**.

| **Entry** | **% ABS** | **TPSA ($A^2$)** | **n-ROTB** | **MV** | **MW** | **miLog *P*** | **n-ON acceptors** | **n-OHNH donors** | **Nviolations** |
|---|---|---|---|---|---|---|---|---|---|
| | - | - | - | - | ≤500 | ≤5 | ≤10 | ≤5 | ≤1 |
| **3** | 92.33 | 48.30 | 3 | 244.45 | 276.37 | 0.62 | 5 | 1 | 0 |
| **4** | 87.88 | 61.23 | 5 | 295.29 | 329.43 | 0.83 | 6 | 0 | 0 |
| **5** | 78.21 | 89.25 | 6 | 343.53 | 403.54 | 1.11 | 8 | 2 | 0 |
| **6a** | 79.74 | 84.82 | 9 | 452.45 | 521.67 | 3.22 | 9 | 0 | 1 |
| **6b** | 76.21 | 95.02 | 8 | 434.93 | 507.64 | 3.10 | 9 | 1 | 1 |
| **6c** | 69.90 | 113.34 | 12 | 453.89 | 525.66 | 2.40 | 10 | 2 | 1 |
| **6d** | 77.16 | 97.27 | 11 | 416.17 | 480.62 | 1.89 | 9 | 1 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **6e** | 77.16 | 97.27 | 11 | 416.17 | 480.62 | 1.82 | 9 | 1 | 0 |
| **6f** | 77.16 | 97.27 | 11 | 416.17 | 480.62 | 1.77 | 9 | 1 | 0 |
| **6g** | 79.89 | 84.38 | 11 | 447.40 | 548.52 | 4.32 | 8 | 1 | 1 |
| **6h** | 79.89 | 84.38 | 11 | 425.26 | 497.62 | 3.22 | 8 | 1 | 0 |
| **7a** | 76.99 | 92.77 | 9 | 497.31 | 598.15 | 3.15 | 10 | 0 | 1 |
| **7b** | 73.20 | 103.76 | 8 | 479.78 | 584.13 | 3.03 | 10 | 1 | 1 |
| **7c** | 70.02 | 113.00 | 9 | 505.33 | 614.15 | 2.43 | 11 | 1 | 2 |
| **7d** | 75.73 | 96.43 | 8 | 467.61 | 569.12 | 1.92 | 10 | 0 | 1 |
| **7e** | 75.73 | 96.43 | 8 | 467.61 | 569.12 | 1.85 | 10 | 0 | 1 |
| **7f** | 75.73 | 96.43 | 8 | 467.61 | 569.12 | 1.80 | 10 | 0 | 1 |
| **7g** | 80.18 | 83.53 | 8 | 498.83 | 637.02 | 4.35 | 9 | 0 | 1 |
| **7h** | 80.18 | 83.53 | 8 | 476.69 | 586.12 | 3.25 | 9 | 0 | 1 |

### 3.4. Molecular Docking Studies

To investigate possible binding interactions as well as to support the in vitro activity of the studied compounds, molecular docking studies were performed using Maestro Molecular Modeling platform (version10.5) by Schrödinger [48] for all newly synthesized β-lactam derivatives. The protocol of docking study was given in Supplementary File.

The most active compounds (**6a**, **7b** and **7f**) were further investigated to clarify the probable binding modes and provide straightforward knowledge for binding interactions of the protein-ligand complexes. The active site residues of the Jack bean urease (JBU) consist of His407, His409, Arg439, Ala440, Lys490, His492, Asp494, His519, His545, Cys592, His593, His594, Arg609, Asp633, Ala636, and Met637 [49]. All compounds showed better docking scores ranging from -5.33 kcal/mol to -9.21 kcal/mol than the reference inhibitor thiourea in Table 5.

**Table 5.** Docking scores, Lipophilic contact plus phobic attractive term, Hydrogen-bonding term and polar interactions term in the active site

| Comp. No | Docking Score | Glide Lipo | Glide Hbond | Glide Esite | Key Interacting Residues | |
|---|---|---|---|---|---|---|
| | | | | | H-Bond | Pi-Pi Stacking |
| 3 | -5.329 | -0.667 | -0.32 | -0.1 | Ala636 | Arg609 |
| 4 | -5.943 | -2.419 | 0 | -0.103 | - | Arg439 |
| 5 | -5.984 | -2.355 | -0.14 | -0.307 | Asp633, Arg439 | His593 |
| **6a** | **-8.856** | **-3.673** | **-0.325** | -0.101 | Cys592, His593 | His594, His593 |
| 6b | -7.192 | -2.441 | -0.456 | 0 | Gly638, Gln635 | - |
| 6c | -7.6 | -2.922 | -0.303 | -0.299 | Thr571, Asp633 | - |
| 6d | -5.862 | -1.534 | -0.32 | -0.331 | Arg609, Cys592 | His594, Arg609 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6e | -7.81 | -3.249 | 0 | -0.173 | - | His585 |
| 6f | -7.342 | -2.531 | -0.379 | -0.095 | Cys592, Gly638 | Arg439 |
| 6g | -7.332 | -3.695 | -0.176 | 0 | His593 | Arg639 |
| 6h | -6.595 | -1.222 | -0.372 | 0 | Arg639, Met637 | Arg439 |
| 7a | -9.199 | -4.479 | 0 | -0.104 | - | His585 |
| **7b** | **-9.208** | **-0.467** | **-0.292** | -0.237 | Ala440, Arg439 | - |
| 7c | -8.569 | -1.003 | -0.247 | -0.103 | Cys592, Ala636 | Arg439 |
| 7d | -7.279 | -2.705 | -0.429 | -0.138 | Cys592, Arg439, | Arg609, His594 |
| 7e | -7.85 | -3.443 | 0 | -0.179 | - | His593 |
| **7f** | **-8.111** | **-4.136** | **-0.589** | -0.023 | Arg609, Thr571 | His593 |
| 7g | -7.945 | -3.971 | 0 | -0.021 | - | His545, His585 |
| 7h | -7.541 | -3.884 | 0 | -0.037 | - | His593, His585 |
| Thiourea | -4.981 | 0 | -0.49 | -0.377 | Gly550, His409 | - |

According to the docking studies, the top scoring poses and ligand interaction diagrams of most active compounds were shown in Figure 1. The compound **6a** forms a strong hydrogen bond between the His593 residue and the nitrogen of the thiadiazole ring (NH–N) at distance of 2.31 Å. The Cys592 (marked as CME592), which located at the mobile flap covering the active site is forming hydrogen bond with the oxygen of phenoxy moiety (OH–O) at distance of 2.10 Å. The pi-pi stacking interactions with the amino acid residues of His592 and His593 through the phenoxy ring and thiadiazole of **6a** was also observed in protein-ligand complex in Figure 1. The phenoxy ring and thiadiazole moiety of **6a** were embedded in an exposed solvent area as well. The best orientation of compound **7b** was located the active site through two hydrogen bonds with amino acid residues Arg439 and Ala440. The phenol ring of compound **7b** was embedded towards the $Ni^{+2}$ cations of JBU. It was clearly observed that compound **7f** formed two hydrogen bond interactions with Arg609 and Thr571 residues and formed pi-pi stacking interaction with His593 residue of JBU. The **7f**-enzyme complex showed that Z-shape form of ligand penetrated toward the catalytic site of enzyme and located within binding pocket of urease. The benzyl group of compound **7f** was extended with a displacement towards the exposed solvent region. Similar interactions could be seen for the other newly synthesized β-lactam derivatives with docking scores, lipophilic contact plus

phobic attractive term, Hydrogen-bonding term and polar interactions term of Glide Scores in Table 5.

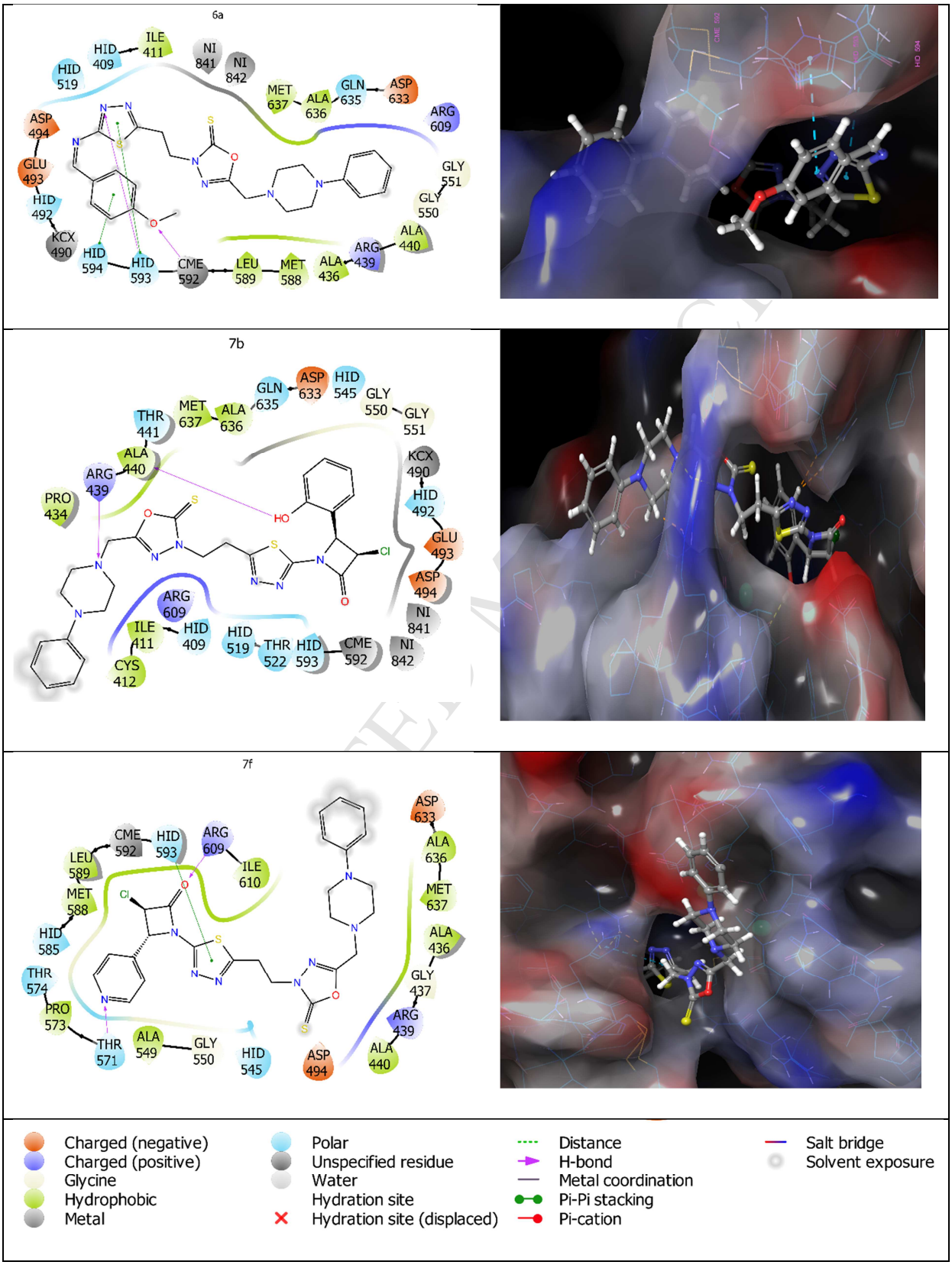



**Figure 1.** Schematic view and 3D top-docking poses of compound 6a, 7b and 7f in the active sites of JBU

According to in silico results, the compounds containing β-lactam ring had better docking score than their analog compounds without β-lactam ring. It is mostly due to fact that β-lactam ring was gained to molecules two more rotatable bonds and one more hydrogen bond acceptor so that the compounds containing β-lactam ring had more strong binding interaction.

## 3. Conclusion

In summary we report here the synthesis of some new kind of β-lactam derivatives containing azole rings such as oxadiazole and thiadiazole with phenyl piperazine ring in one molecule. And investigate their antiurease activity and antioxidant capacity comparing with their molecular docking studies. And beside this compound **6a**, which is a Schiff base containing a 4-methoxyphenyl group, compound **7b** which is a β-lactam derivative containing 2-hydoxyphenyl group and compound **7f** contains a 4-pyridine ring showed excellent antiureas activity and have excellent antioxidant capacity.

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### Conflicts of interest

There are no conflicts to declare.

## References

[1] F. Broccolo, G. Cainelli, G. Caltabiano, C.E.A. Cocuzza, C.G. Fortuna, P. Galletti, D. Giacomini, G. Musumarra, R. Musumeci, A. Quintavalla, J. Med. Chem. 49 (2006), 2804.

[2] A.K. Halve, D. Bhadauria, R. Dubey, Bioorg. Med. Chem. Lett. 17 (2007) 341.

[3] S.B. Rosenblum, T. Huynh, A. Afonso, HR. Jr. Davis, N. Yumibe, J.W. Clader, D.A. Burnett, J. Med. Chem. 41 (1998) 973.

[4] A.D. Borthwick, G. Weingarte, T.M. Haley, M. Tomaszewski, W. Wang, Z. Hu, J. Bedard, H. Jin, L. Yuen, T.S. Mansour, Bioorg. Med. Chem. Lett. 8 (1998) 365.

[5] W.T. Han, A.K. Trehan, J.J.K. Wright, M.E. Federici, S.M. Seiler, N.A. Meanwell, Bioorg. Med. Chem. 3 (1995) 1123.

[6] R.K. Goel, M.P. Mahajan, S.K.J. Kulkarni, Pharm. Pharm. Sci. 7 (2004) 80.

[7] G. Veinberg, I. Shestakova, M. Verma, I. Kenepe, E. Lukevics, Bioorg. Med. Chem. Lett. 14 (2004) 147.

[8] T. Sperka, J. Pitlik, P. Bagossi, J. Tozser, Bioorg. Med. Chem. Lett. 15 (2005) 3086.

[9] C. Saturnino, B. Fusco, P. Saturnino, G. De Martino, F. Rocco, J.C. Lanceolat, Biol. Pharm. Bull. 23 (2000) 654.

[10] (a) M.I. Konaklieva, Curr. Med. Chem.-Anti-Infect. Agents 1 (2002) 215; (b) A. Clemente, A. Domingos, A.P. Grancho, J. Iley, R. Moreira, J. Neres, N. Palma, A.B. Santana, E. Valente, Bioorg. Med. Chem. Lett. 11 (2001) 1065.

[11] G.S. Singh, M. D'hooghe, N. De Kimpe, Azetidines, azetines and azetes: monocyclic. In: Stevens CV, ed. Comprehensive Heterocyclic Chemistry III. Oxford, UK: Elsevier, 1 (2008) vol. 2.

[12] J.C. Walton. Top Curr. Chem. 264 (2006) 163.

[13] P.A. Magriotis. Angew. Chem. Int. Ed. 40 (2001) 4377.

[14] M. Gomez-Gallego, M.J. Mancheno, A.M. Sierra. Tetrahedron. 56 (2000) 5743.

[15] R.G. Soengas, Y. Segade, C. Jimenez, J. Rodríguez. Tetrahedron. 67 (2011) 2617.

[16] E. Ghabraie, S. Balalaie, S. Mehrparvar, F. Rominger. J Org. Chem. 17 (2014) 7926.

[17] R. Ocampo, J.W.R. Dolbier. Tetrahedron. 60 (2004) 9325.

[18] G.B. Bagihalli, P.G. Avaji, S.A. Patil, P.S. Badami. Eur. J Med. Chem. 43 (2008) 2639–2649.

[19] T. Akhtar, S. Hameed, N.A. Al-Masoudi, K.M. Khan Heteroat. Chem. 18 (2007) 316–322.

[20] M. Wujec, A. Siwek, M. Swatko-Ossor, L. Mazur, Z. Rzaczynska. J Heterocycl. Chem. 45 (2008) 1893–1896.

[21] Y. Li, J. Geng, Y. Liu, S. Yu, G. Zhao. ChemMedChem. 8 (2013) 27.

[22] S. Haider, M.S. Alam, H. Hamid. Eur. J Med. Chem. 92 (2015) 156.

[23] A.K. Jain, S. Sharma, A. Vaidya, V. Ravichandran, R.K. Agrawal. Chem. Biol. Drug. Des. 81 (2013) 557.

[24] Y. Hu, C-Y. Li, X-M. Wang, Y-H. Yang, H-L. Zhu. Chem. Rev. 114 (2014) 5572.

[25] J. Matysiak. Mini-Rev. Med. Chem. 15 (2015) 762.

[26] M.S. Chande, A.A. Godbole, E. Coutinho, P. Desai, Ind. J. Chem. 42B (2004) 397.

[27] M. Yar, M. Bajda, R.A. Mehmood, L.R. Sidra, N. Ullah, L. Shahzadi, Lett. Drug. Des. Discov. 11 (2014) 331–338.

[28] M. Amir, S. Shahani, Ind. J. Heterocycl. Chem. 8 (1998) 107–110.

[29] J. Hazarika, J.C.S. Kataky, Ind. J. Heterocycl. Chem. 7 (1998) 83–92.

[30] B.S. Holla, C.S. Prasanna, B. Poojary, K.S. Rao, K. Shridhara, U.G. Bhat, Ind. J. Chem. 43B (2004) 864–868.

[31] M.A. Abbasi, B. Shahzad, Aziz-ur-Rehman, S. Rasool, K. Nafeesa, M.A. Ashraf, World J. Pharm. Sci. 2 (2014) 32–40.

[32] K. Rubab, M.A. Abbasi, S.Z. Siddiqui Aziz-ur-Rehman, M.N. Akhtar, Trop. J. Pharm. Res. 15 (2016) 1515–1524.

[33] K. Rubab, M.A. Abbasi, Aziz-ur-Rehman, M. Ashraf, S.Z. Siddiqui, A. Shaukat, Trop. J. Pharm. Res. 15 (2016) 1525–1533.

[34] S.S. Devesa, W.J. Blot, J.F. Fraumeni, Changing patterns in the incidence ofesophageal and gastriccarcinoma in the United States, J. Canc. 83 (1998)2049e2053.

[35] C.P. Howson, T. Hiyama, E.L. Wynder, The decline of gastric cancer: epide-miology of an unplanned triumph, J. Epidemiol. Rev. 8 (1986) 1e27.

[36]A.C. Bustamante, J.V. Paredes, A.F. Parra, A.P. Peraza, J.M. Martínez,M.S. Martínez, A.R. Organillo, Antioxidant activity of butyl- and phenyl-stannoxanes derived from 2-, 3- and 4-pyridinecarboxylic acids, Molecules 15(2010) 5445e5459.

[37] N. Dharmaraj, P. Viswanathamurthi, K. Natarajan, Ru(II) complexes containingbidendate Schiff bases and their antifungal activity, Transit. Met. Chem. 26(2001) 105e110.

[38] C. Basu, S. Chowdhury, R. Banerjee, H.S. Evans, S. Mukherjee, Polyhedron 26(2007) 3617e3624.[

[39] A. Demirbas, D. Sahin, N. Demirbas, S.A. Karaoglu. Eur. J Med. Chem. 44 (2009) 2896–2903.

[40] H. Bayrak, A. Demirbas, N. Demirbas, S.A. Karaoglu. Eur. J Med. Chem. 45 (2010) 4726-4732.

[41] N. Galic, B. Peric, B. Kojic-Prodic, Z. Cimerman, J. Mol. Struct. 559 (2001) 187-194.

[42] E. Wyrzykiewicz, D. Prukah. J. Heterocycl. Chem. 35 (1998) 381-387.

[43] H. Bayrak, A. Demirbas, S.A. Karaoglu, N. Demirbas, Eur. J Med. Chem. 44 (2009) 1057-1066.

[44] Lipinski, C. A.; Lombardo, L.; Dominy, B. W.; Feeney, P. J. Adv. Drug Delivery Rev. 46 (2001) 3.

[45] Molinspiration Chemoinformatics, Brastislava, Slovak Republic, Available from: http://www.molinspiration.com/cgi-bin/properties, 2015.

[46] Y. Zhao, M.H. Abraham, J. Lee, A. Hersey, N.C. Luscombe, G. Beck, B. Sherborne, I. Cooper. Pharm. Res. 19 (2002) 1446.

[47] P. Ertl, B. Rohde, P.J. Selzer. Med. Chem. 43 (2000) 3714.

[48] Schrödinger Suite 2009 Induced Fit Docking protocol; Glide version 5.5, S., LLC, New York, NY, 2009; Prime version 2.1, Schrödinger, LLC, New York, NY, 2009.

[49] G. Akyüz, E. Menteşe, M. Emirik, N. Baltaş. Bioorganic Chemistry. 80 (2018) 121-128.

[50] M.S. Blois. Nature 181 (1958) 1199-1200.

[51] I.F.F. Benzie, J.J. Strain, Methods Enzymol. 299 (1999) 15-27.

[52] R. Apak, K. Guclu, M. Ozyurek, S.E. Karademir, J. Agric. Food Chem. 52 (2004) 7970-7981.

[53] M.W. Weatherburn, Anal. Chem. 39 (1967) 971-974.

[54] S.W.I. Siu, K. Pluhackova, R.A. Böckmann, J. Chem. Theory Comput., 8 (2012), 1459-1470

[55] A. Balasubramanian, K. Ponnuraj. J. Mol. Biol. 400 (2010) 274-283.

[56] R.A. Friesner, R.B. Murphy, M.P. Repasky, L.L. Frye, J.R. Greenwood, T.A. Halgren, P.C. Sanschagrin, D.T. Mainz. J. Med. Chem. 49 (2006) 6177-6196.