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Synthesis of novel *N*-(1,3-thiazol-2-yl)benzamide clubbed oxadiazole scaffolds: Urease inhibition, Lipinski rule and molecular docking analyses

Muhammad Athar Abbasi[a,b,*], Hussain Raza[a], Aziz-ur-Rehman[b], Sabahat Zahra Siddiqui[b], Syed Adnan Ali Shah[c], Mubashir Hassan[a], Sung-Yum Seo[a,*]

[a] College of Natural Science, Department of Biological Sciences, Kongju National University, Gongju 32588, South Korea
[b] Department of Chemistry, Government College University, Lahore 54000, Pakistan
[c] Faculty of Pharmacy and Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Level 9, FF3, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia

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ABSTRACT

Present work aimed to synthesize some unique bi-heterocyclic benzamides as lead compounds for the *in vitro* inhibition of urease enzyme, followed by *in silico* studies. These targeted benzamides were synthesized in good yields through a multi-step protocol and their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, EI-MS and elemental analysis. The *in vitro* screening results showed that most of the ligands exhibited good inhibitory potentials against the urease. Chemo-informatics analysis envisaged that all these compounds obeyed the Lipinski's rule. Molecular docking results showed that **7h** exhibited good binding energy value (−8.40 kcal/mol) and was bound within the active region of urease enzyme. From the present investigation, it was inferred that some of these potent urease inhibitors might serve as novel templates in drug designing.

1. Introduction

The bioactivity potential of heterocyclic compounds has been greatly employed in the pharmaceutical industries for the discovery of new drug candidates. Different classes of heterocyclic compounds possess a broad range of pharmacological activities [1]. One important class of heterocyclic compound that contains one sulfur atom is known as thiazole. This class is present in many natural and synthetic products with a wide range of pharmacological activities, such as antiviral, anticancer, antibacterial, antifungal, anticonvulsant, anti-parkinsonian and anti-inflammatory activities that can be well illustrated by the large number of drugs in the market containing this functional group. As examples, the anticonvulsant riluzole, the anti-parkinsonian talipexole, the antischistosomal miridazole, the anthelmintic tiabendazole, the anti-ulcer alizatidine, the vitamin B, the antibacterial sulfathiazole, and the antiviral ritonavir can be cited. Thiazole ring also finds applications in other fields, such as polymers, liquid crystals, photonucleases, fluorescent dyes, insecticides, and antioxidants [2,3].

1,3,4-Oxadiazoles form an important class of five-member heterocyclic compounds with a wide range of biological activities. The importance of the oxadiazoles' nucleus is well established in agricultural and pharmaceutical chemistry as far as its corresponding derivatives are used as antipyretic, analgesic, antidepressant, antimicrobial, antiviral, fungicidal, antineoplasic, anti-inflammatory agents, central nervous system stimulants, anticonvulsive, anticancer, and antihypertensive agents [4]. 2,5-Disubstituted-1,3,4-oxadiazole is a versatile pharmacophore which also exhibits biological activities like hypnotic, sedative, anti-HIV, hypoglycaemic, antioxidant, genotoxic, and insecticidal activities [5].

Urease (EC 3.5.1.5; urea amidohydrolase) is a metal containing enzyme that catalyzes the hydrolysis of urea into ammonia and carbamate. Ureases are widespread in nature among plants, bacteria, fungi, algae and invertebrates. Urease producing bacteria have a harmful effect on human health. *Helicobacter pylori* (*H. pylori*) are one of the most successful human bacterial parasites, which colonize more than half of the human population. It can survive at the low pH of stomach during colonization. Most infected people would be asymptomatic in their life time. Unfortunately, about 15–20% of them would develop into severe gastroduodenal pathologies, such as stomach and duodenal ulcers, adenocarcinomas and stomach lymphomas. Other urease associated diseases include hepatic encephalopathy, urolithiasis, urinary catheter encrustation, pyelonephritis and hepatic coma. Recently, some 3-arylpropionylhydroxamic acid derivatives [6], reductive derivatives of flavonoids [7], and bisindolylmethane

* Corresponding authors at: Department of Chemistry, Government College University, Lahore 54000, Pakistan (M. Athar Abbasi); College of Natural Science, Department of Biological Sciences, Kongju National University, Gongju 32588, South Korea (S.-Y. Seo).
E-mail addresses: abbasi@gcu.edu.pk (M. Athar Abbasi), dnalove@kongju.ac.kr (S.-Y. Seo).

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thiosemicarbazides [8] were reported as potential urease inhibitors, but still there is considerable need for the alternative or novel treatment of *H. pylori* associated diseases.

Therefore, in continuation of our previous effort on urease inhibition by the related bi-heterocyclic molecules [9], this work was aimed to provide some novel thiazole-oxadiazole hybrid scaffolds as potential inhibitors of jack bean urease. Moreover, the molecular docking analysis was carried out to ascertain their interactions with the enzyme.

2. Materials and methods

2.1. General

All the chemicals, along with analytical grade solvents, were purchased from Sigma Aldrich, Alfa Aesar (Germany), or Merck through local suppliers. Pre-coated silica gel Al-plates were used for TLC with ethyl acetate and *n*-hexane as solvent system (25:75). Spots were detected by UV_{254}. Gallonkamp apparatus was used to detect melting points in capillary tubes. IR spectra (ν, cm^{-1}) were recorded by KBr pellet method in the Jasco-320-A spectrophotometer. ^1H NMR spectra (δ, ppm) were recorded at 600 MHz (^{13}C NMR spectra, at 150 MHz) in DMSO-d_6 using the Bruker Advance III 600 As- cend spectrometer using BBO probe. EI-MS spectra were measured on a JEOL JMS-600H instrument with data processing system. The coupling constant (J) is given in Hz and chemical shift (δ) in ppm. The abbreviations used in interpretation of ^1H NMR spectra are as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br.t, broad triplet; q, quartet; quint, quintet; sex, sextet; sep, septet; m, multiplet, dist. distorted.

2.2. Procedure for the synthesis of ethyl 2-[2-(benzoylamino)-1,3-thiazol-4-yl]acetate (3)

Ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate (**2**; 0.05 mol) was taken in a 2% aqueous Na_2CO_3 solution (200 mL) in a 500 mL RB flask and benzoyl chloride (**1**; 0.05 mol) was added drop wise and mixture was stirred for 30 min. The reaction mixture became a solid adhering paste. Crushed ice (ca. 10 g) is then added to the contents of the flask and stirred well with a glass rod. The thick reaction mixture gradually became soft by the gradual dispersion of traces of benzoyl chloride in the aqueous phase during stirring and finally the supernatant aqueous layer becomes clear. The reaction progress was observed by TLC using *n*-hexane and ethyl acetate solvent system (6:4). After 5 h stirring and gradual heating, the product was precipitated, filtered and washed with water to get it freed from any adhering unreacted material. After drying, ethyl 2-[2-(benzoylamino)-1,3-thiazol-4-yl]acetate (**3**) was obtained as a crystalline solid.

2.3. Synthesis of N-[4-(2-hydrazino-2-oxoethyl)-1,3-thiazol-2-yl] benzamide (4)

Ethyl 2-[2-(benzoylamino)-1,3-thiazol-4-yl]acetate (**3**; 0.05 mol) was taken in methanol (200 mL) in a 500 mL RB flask, hydrazine hydrate (0.05 mol) was added drop wise and mixture was refluxed for 02 h. The reaction progress was observed by TLC using *n*-hexane and ethyl acetate solvent system (4:6). After completion of reaction, the mixture was allowed to cool at room temperature to get white colored precipitates of the product, *N*-[4-(2-hydrazino-2-oxoethyl)-1,3-thiazol-2-yl]benzamide (**4**). It was filtered and washed with methanol.

2.4. Synthesis of N-{4-[(5-sulfanyl-1,3,4-oxadiazol-2-yl)methyl]-1,3-thiazol-2-yl}benzamide (5)

The hydrazide (**4**; 0.025 mol) was taken in C_2H_5OH (20 mL) in a 250 mL RB flask at 28 °C and then solid KOH (0.024 mol) was added. The contents were dissolved on reflux. Carbon disulphide (0.05 mol) was poured into the reaction mixture drop wise at 28 °C and the solution was refluxed again for 10 h. The reaction progress was noted with TLC using *n*-hexane and ethyl acetate solvent system (7:3). After completion of reaction, excess of ethanol was evaporated and sufficient ice cold distilled water was added followed by addition of dilute HCl till constant pH of 4–5. Light peach colored precipitates of *N*-{4-[(5-sulfanyl-1,3,4-oxadiazol-2-yl)methyl]-1,3-thiazol-2-yl}benzamide (**5**) were filtered and washed with distilled water.

^1H NMR: 12.72 (s, 1H, SH), 8.09 (dist.dd, J = 2.1, 6.1 Hz, 2H, H-2 & H-6), 7.63 (br.t, J = 7.3 Hz, 1H, H-4), 7.54 (br.t, J = 6.8 Hz, 2H, H-3 & H-5), 7.22 (s, 1H, H-5′), 4.23 (s, 2H, CH_2-6′); ^{13}C NMR: 177.86 (C-2″), 165.08 (C-7), 161.78 (C-2′), 158.89 (C-5″), 142.73 (C-4′), 132.59 (C-4), 131.83 (C-1), 128.53 (C-2 & C-6), 128.05 (C-3 & C-5), 111.50 (C-5′), 27.70 (C-6′).

2.5. General procedure for the synthesis of N-(4-{[5-(substituted-sulfanyl)-1,3,4-oxadiazol-2-yl]methyl}-1,3-thiazol-2-yl)benzamides (7a-n)

The nucleophilic compound **5** (0.47 mmol) was dissolved in *N*,*N*-dimethyl formamide (DMF, 5–10 mL) in a 100 mL RB flask. Solid LiH (0.005 g) was added and mixture was stirred for half an hour for activation of **5.** Then, from different electrophiles (**6a-n**, aralkyl/alkyl halides), one in each respective reaction was added in equimolar ratio and the mixture was further stirred for 3–5 h. The reaction was monitored by TLC using *n*-hexane and ethyl acetate solvent system (8:2). After completion, ice cold distilled water was added to the reaction flask. The respective products, **7a-n,** were collected by filtration or solvent extraction.

2.5.1. N-(4-{[5-(Benzylsulfanyl)-1,3,4-oxadiazol-2-yl]methyl}-1,3-thiazol-2-yl)benzamide (7a)

Light brown solid; yield: 83%; m.p.: 249–250 °C; Mol. Formula: $C_{20}H_{16}N_4O_2S_2$; Mol. Mass: 408 g mol^{-1}; IR: 3357 (N—H str.), 3052 (C—H str. of aromatic ring), 2928 (–CH_2– str.), 1665 (C═O str.), 1648 (C═N str.), 1570 (C═C str. of aromatic ring); ^1H NMR: 12.68 (s, 1H, –NH-CO-7), 8.08 (br.d, J = 7.8 Hz, 2H, H-2 & H-6), 7.63 (br.t, J = 7.5 Hz, 1H, H-4), 7.53 (br.t, J = 7.4 Hz, 2H, H-3 & H-5), 7.46 (br.d, J = 7.6 Hz, 2H, H-2‴ & H-6‴), 7.39 (dt, J = 7.7, 1.6 Hz, 2H, H-3‴ & H-5‴), 7.25 (br.t, J = 7.4, 1H, H-4‴), 7.17 (s, 1H, H-5′), 4.47 (s, 2H, CH_2-7‴), 4.34 (s, 2H, CH_2-6′); ^{13}C NMR: 165.61 (C-7), 164.97 (C-2″), 162.88 (C-2′), 158.84 (C-5″), 143.26 (C-4′), 139.55 (C-1‴), 132.51 (C-4), 131.75 (C-1), 130.48 (C-2‴ & C-6‴), 131.66 (C-3‴ & C-5‴), 128.47 (C-2 & C-6), 128.02 (C-3 & C-5), 127.99 (C-4‴), 111.10 (C-5′), 34.86 (C-7‴), 27.45(C-6′); Anal. Calc. for $C_{20}H_{16}N_4O_2S_2$ (408.07): C, 58.80; H, 3.95; N, 13.72. Found: C, 58.74; H, 3.88; N, 13.77; EI-MS: m/z 408 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 105 $[C_8H_5O]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$.

2.5.2. N-[4-({5-[(2-Chlorobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl} methyl)-1,3-thiazol-2-yl]benzamide (7b)

Off white solid; yield: 86%; 281–280 °C; Mol. Formula: $C_{20}H_{15}ClN_4O_2S_2$; Mol. Mass: 442 g mol^{-1}; IR: 3360 (N—H str.), 3050 (C—H str. of aromatic ring), 2930 (-CH_2- str.), 1662 (C═O str.), 1644 (C═N str.), 1580 (C═C str. of aromatic ring), 685 (C—Cl str.); ^1H NMR: 12.68 (s, 1H, –NH-CO-7), 8.08 (dist. dd, J = 1.3, 8.4 Hz, 2H, H-2 & H-6), 7.62 (dist. dt, J = 1.2, 7.4 Hz, 1H, H-4), 7.53 (dist.t, J = 7.9 Hz, 2H, H-3 & H-5), 7.50 (br.d, J = 8.0 Hz, 2H, H-2‴ & H-6‴), 7.36–7.30 (m, 3H, H-3‴, H-4‴ & H-5‴), 7.17 (s, 1H, H-5′), 4.46 (s, 2H, CH_2-7‴), 4.33 (s, 2H, CH_2-6′); ^{13}C NMR: 165.62 (C-7), 164.97 (C-2″), 162.88 (C-2′), 158.75 (C-5″), 143.25 (C-4′), 139.24 (C-1‴), 132.84 (C-2‴), 132.50 (C-4), 131.76 (C-1), 130.18 (C-4‴), 128.71 (C-5‴), 128.45 (C-2 & C-6), 128.01 (C-3 & C-5), 127.55 (C-3‴), 127.53 (C-6‴), 111.10 (C-5′), 34.81 (C-7‴), 27.36 (C-6′); Anal. Calc. for $C_{20}H_{15}ClN_4O_2S_2$ (442.03): C, 54.23; H, 3.41; N, 12.65. Found: C, 54.18; H, 3.49; N, 12.57; EI-MS: m/z 444 $[M+2]^+$, 442 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 125 $[C_7H_6Cl]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$.

*2.5.3. N-[4-({5-[(3-Chlorobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl} methyl)-1,3-thiazol-2-yl]benzamide (**7c**)*

Brown solid; yield: 81%; 288–289 °C; Mol. Formula: $C_{20}H_{15}ClN_4O_2S_2$; Mol. Mass: 442 g mol^{-1}; IR: 3350 (N—H str.), 3026 (C—H str. of aromatic ring), 2923 (-CH_2- str.), 1663 (C═O str.), 1647 (C═N str.), 1584 (C═C str. of aromatic ring), 686 (C—Cl str.); ^{1}H NMR: 12.68 (s, 1H, -NH-CO-7), 8.08 (br.d, *J* = 8.4 Hz, 2H, H-2 & H-6), 7.66 (br.s, 1H, H-2‴), 7.63 (br.t, *J* = 7.4 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.6 Hz, 2H, H-3 & H-5), 7.45 (br.d, *J* = 7.9 Hz, 1H, H-4‴), 7.40 (br.d, *J* = 7.7 Hz, 1H, H-6‴), 7.25 (br.t, *J* = 7.7 Hz, 1H, H-5‴), 7.17 (s, 1H, H-5′), 4.47 (s, 2H, CH_2-6′), 4.34 (br.s, 2H, CH_2-7‴); ^{13}C NMR: 165.68 (C-7), 165.06 (C-2″), 162.97 (C-2′), 158.84 (C-5″), 143.31 (C-4′), 139.55 (C-1‴), 132.55 (C-4), 131.85 (C-1), 131.66 (C-3‴), 130.51 (C-4‴), 130.48 (C-5‴), 128.50 (C-2 & C-6), 128.08 (C-3 & C-5), 128.01 (C-2‴), 121.49 (C-6‴), 111.16 (C-5′), 34.86 (C-7‴), 27.45 (C-6′); Anal. Calc. for $C_{20}H_{15}ClN_4O_2S_2$ (442.03): C, 54.23; H, 3.41; N, 12.65. Found: C, 54.15; H, 3.44; N, 12.55; EI-MS: *m/z* 444 $[M+2]^+$, 442 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 125 $[C_7H_6Cl]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$.

*2.5.4. N-[4-({5-[(4-Chlorobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl} methyl)-1,3-thiazol-2-yl]benzamide (**7d**)*

Brown amorphous liquid ; yield: 84%; 269–270 °C; Mol. Formula: $C_{20}H_{15}ClN_4O_2S_2$; Mol. Mass: 442 g mol^{-1}; IR: 3350 (N—H str.), 3033 (C—H str. of aromatic ring), 2921 (-CH_2- str.), 1666 (C═O str.), 1649 (C═N str.), 1585 (C═C str. of aromatic ring), 688 (C—Cl str.); ^{1}H NMR: 12.67 (s, 1H, -NH-CO-7), 8.08 (br.d, *J* = 7.8 Hz, 2H, H-2 & H-6),7.63 (br.t, *J* = 7.5 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.4 Hz, 2H, H-3 & H-5), 7.50 (br.d, *J* = 8.2 Hz, 2H, H-2‴ & H-6‴), 7.35 (br.d, *J* = 8.2 Hz, 2H, H-3‴ & H-5‴), 7.17 (s, 1H, H-5′), 4.46 (s, 2H, CH_2-6′), 4.31 (s, 2H, CH_2-7‴); ^{13}C NMR: 165.67 (C-7), 165.05 (C-2″), 162.98 (C-2′), 158.82 (C-5″), 143.35 (C-4′), 136.71 (C-1‴), 135.74 (C-4‴), 132.57 (C-4), 131.86 (C-1), 130.42 (C-3‴ & C-5‴), 128.96 (C-2‴ & C-6‴), 128.50 (C-2 & C-6), 128.09 (C-3 & C-5), 111.09 (C-5′), 35.57 (C-7‴), 27.52 (C-6′); Anal. Calc. for $C_{20}H_{15}ClN_4O_2S_2$ (442.03): C, 54.23; H, 3.41; N, 12.65. Found: C, 54.29; H, 3.48; N, 12.53; EI-MS: *m/z* 444 $[M+2]^+$, 442 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 125 $[C_7H_6Cl]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$.

*2.5.5. N-[4-({5-[(2-Bromobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl} methyl)-1,3-thiazol-2-yl]benzamide (**7e**)*

Light Brown solid; yield: 77%; 306–307 °C; Mol. Formula $C_{20}H_{15}BrN_4O_2S_2$; Mol. Mass: 486 g mol^{-1}; IR: 3354 (N—H str.), 3042 (C—H str. of aromatic ring), 2926 (-CH_2- str.), 1668 (C═O str.), 1653 (C═N str.), 1581 (C═C str. of aromatic ring), 575 (C—Br str.); ^{1}H NMR: 12.67 (s, 1H, -NH-CO-7), 8.07 (br.d, *J* = 7.7 Hz, 2H, H-2 & H-6), 7.62 (br.t, *J* = 7.4 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.3 Hz, 2H, H-3 & H-5), 7.58 (br.d, *J* = 7.6 Hz, 1H, H-3‴), 7.37–7.34 (m, 3H, H-4‴, H-5‴ & H-6‴), 7.17 (s, 1H, H-5′), 4.46 (s, 2H, CH_2-6′), 4.33 (s, 2H, CH_2-7‴); ^{13}C NMR: 165.64 (C-7), 165.02 (C-2″), 162.96 (C-2′), 158.35 (C-5″), 143.34 (C-4′), 136.34 (C-1‴), 132.33 (C-3‴), 132.50 (C-4), 131.84 (C-5‴), 131.60 (C-1), 129.68 (C-4‴), 128.45 (C-2 & C-6), 128.00 (C-3 & C-5), 127.68 (C-6‴), 123.98 (C-2‴), 111.10 (C-5′), 34.82 (C-7‴), 27.46 (C-6′); Anal. Calc. for $C_{20}H_{15}BrN_4O_2S_2$ (485.98): C, 59.29; H, 3.10; N, 11.50. Found: C, 59.18; H, 3.16; N, 11.46; EI-MS: *m/z* 488 $[M+2]^+$, 486 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 169 $[C_7H_6Br]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$.

*2.5.6. N-[4-({5-[(3-Bromobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl} methyl)-1,3-thiazol-2-yl]benzamide (**7f**)*

Creamy brown powder; yield: 89%; m.p.: 282–283 °C; Mol. Formula: $C_{20}H_{15}BrN_4O_2S_2$; Mol. Mass: 486 g mol^{-1}; IR: 3355 (N—H str.), 3041 (C—H str. of aromatic ring), 2923 (-CH_2- str.), 1666 (C═O str.), 1652 (C═N str.), 1583 (C═C str. of aromatic ring), 591 (C-Br str.); ^{1}H NMR: 12.67 (s, 1H, -NH-CO-7), 8.07 (br.d, *J* = 7.7 Hz, 2H, H-2 & H-6),7.62 (br.t, merged *J* = 7.4 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.3 Hz, 2H, H-3 & H-5), 7.65 (br.s, 1H, H-2‴), 7.46 (dist.d, *J* = 7.9 Hz, 1H, H-6‴), 7.38 (dist.d, *J* = 7.8 Hz, 1H, H-4‴), 7.25 (br.t, *J* = 7.8 Hz, 1H, H-5‴), 7.17 (s, 1H, H-5′), 4.46 (s, 2H, CH_2-6′), 4.33 (s, 2H, CH_2-7‴); ^{13}C NMR: 165.61 (C-7), 165.04 (C-2″), 162.89 (C-2′), 158.82 (C-5″), 143.24 (C-4′), 139.50 (C-1‴), 132.50 (C-4), 131.85 (C-1), 131.60 (C-2‴), 130.45 (C-4‴), 130.41 (C-5‴), 128.45 (C-2, C-6 & C-6‴, merged), 128.00 (C-3 & C-5), 127.95 (C-3‴), 111.10 (C-5′), 34.72 (C-7‴), 27.35 (C-6′); Anal. Calc. for $C_{20}H_{15}BrN_4O_2S_2$ (485.98): C, 59.29; H, 3.10; N, 11.50. Found: C, 59.36; H, 3.07; N, 11.44; EI-MS: *m/z* 488 $[M+2]^+$, 486 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 169 $[C_7H_6Br]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$.

*2.5.7. N-[4-({5-[(4-Bromobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl}methyl)-1,3-thiazol-2-yl]benzamide (**7g**)*

Brown solid; yield: 81%; m.p.: 294–295 °C; Mol. Formula: $C_{20}H_{15}BrN_4O_2S_2$; Mol. Mass: 486 g mol^{-1}; IR: 3350 (N—H str.), 3032 (C—H str. of aromatic ring), 2921 (-CH_2- str.), 1667 (C═O str.), 1650 (C═N str.), 1585 (C═C str. of aromatic ring), 588 (C—Br str.); ^{1}H NMR: 12.67 (s, 1H, -NH-CO-7), 8.08 (br.d, *J* = 7.8 Hz, 2H, H-2 & H-6), 7.63 (br.t, *J* = 7.4 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.8 Hz, 2H, H-3 & H-5), 7.44 (br.d, *J* = 7.9 Hz, 2H, H-3‴ & H-5‴), 7.39 (br.d, *J* = 7.8 Hz, 2H, H-2‴ & H-6‴), 7.17 (s, 1H, H-5′), 4.47 (s, 2H, CH_2-6′), 4.34 (s, 2H, CH_2-7‴); ^{13}C NMR: 165.60 (C-7), 165.05 (C-2″), 162.88 (C-2′), 158.83 (C-5″), 143.29 (C-4′), 132.96 (C-1‴), 132.51 (C-4), 131.88 (C-1), 131.39 (C-3‴ & C-5‴), 131.28 (C-2‴ & C-6‴), 128.50 (C-2 & C-6), 128.04 (C-3 & C-5), 121.88 (C-4‴), 111.11 (C-5′), 34.76 (C-7‴), 27.54 (C-6′); Anal. Calc. for $C_{20}H_{15}BrN_4O_2S_2$ (485.98): C, 59.29; H, 3.10; N, 11.50. Found: C, 59.34; H, 3.19; N, 11.41; EI-MS: *m/z* 488 $[M+2]^+$, 486 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 169 $[C_7H_6Br]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$.

*2.5.8. N-[4-({5-[(4-Fluorobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl}methyl)-1,3-thiazol-2-yl]benzamide (**7h**)*

Lemon yellow solid; yield: 84%; m.p.: 278–279 °C; Mol. Formula $C_{20}H_{15}FN_4O_2S_2$; Mol. Mass: 426 gmol^{-1}; IR: 3350 (N—H str.), 3017 (C—H str. of aromatic ring), 2915 (-CH_2- str.), 1663 (C═O str.), 1651 (C═N str.), 1584 (C═C str. of aromatic ring), 1162 (C—F str.); ^{1}H NMR: 12.67 (s, 1H, -NH-CO-7), 8.08 (br.d, *J* = 7.9 Hz, 2H, H-2 & H-6), 7.63 (br.t, *J* = 7.8 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.9 Hz, 2H, H-3 & H-5), 7.43 (dd, *J* = 5.5, 8.1 Hz, 2H, H-2‴ & H-6‴, due to coupling with F_{19}), 7.17 (s, 1H, H-5′), 7.14 (br.t, *J* = 8.8 Hz, 2H, H-3‴& H-5‴, due to coupling with F_{19}), (s, 1H, H-5′), 4.47 (s, 2H, CH_2-6′), 4.35 (s, 2H, CH_2-7‴); ^{13}C NMR: 165.61 (C-7), 165.04 (C-2″), 162.89 (C-2′), 162.36 & 160.74 (C-4‴, coupling with F_{19}), 158.85 (C-5″), 143.29 (C-4′), 132.96 & 132.94 (C-1‴, due to coupling of F_{19}), 132.52 (C-4), 131.89 (C-1), 131.10 & 131.05 (C-2″ & C-6″, due to coupling of F_{19}), 128.50 (C-2 & C-6), 128.09 (C-3 & C-5), 115.38 & 115.24 (C-3″ & C-5″, due to coupling of F_{19}), 111.10 (C-5′), 34.79 (C-7‴), 27.54 (C-6′); Anal. Calc. for $C_{20}H_{15}FN_4O_2S_2$ (426.06): C, 56.32; H, 3.55; N, 13.14. Found: C, 56.26; H, 3.45; N, 13.23; EI-MS: *m/z* 426 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 109 $[C_7H_6F]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$.

*2.5.9. N-[4-({5-[(2-Methylbenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl} methyl)-1,3-thiazol-2-yl]benzamide (**7i**)*

Yellowish amorphous solid; yield: 83%; m.p.: 196–197 °C; Mol. Formula: $C_{21}H_{18}N_4O_2S_2$; Mol. Mass: 422 gmol^{-1}; IR: 3347 (N—H str.), 3023 (C—H str. of aromatic ring), 2919 (-CH_2- str.), 1665 (C═O str.), 1649 (C═N str.), 1587 (C═C str. of aromatic ring); ^{1}H NMR: 12.67 (s, 1H, -NH-CO-7), 8.08 (br.d, *J* = 7.7 Hz, 2H, H-2 & H-6), 7.63 (br.t, *J* = 7.8 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.5 Hz, 2H, H-3 & H-5), 7.35 (br.d, *J* = 8.0 Hz, 1H, H-6‴), 7.21–7.19 (m, 2H, H-3‴ & H-4‴), 7.17 (s, 1H, H-5′), 7.13–7.12 (m, 1H, H-5‴), 4.47 (s, 2H, CH_2-6′), 4.34 (s, 2H, CH_2-7‴), 2.38 (s, 3H, CH_3-8‴); ^{13}C NMR: 165.66 (C-7), 165.04 (C-2″), 162.98 (C-2′), 158.85 (C-5″), 143.32 (C-4′), 136.30 (C-1‴), 132.95 (C-2‴), 132.52 (C-4), 131.86 (C-1), 130.41 (C-3‴), 129.86 (C-4‴), 128.50 (C-2 & C-6), 128.14 (C-6‴), 128.09 (C-3 & C-5), 126.02 (C-5‴), 111.15

(C-5′), 34.84 (C-7‴), 27.91(C-6′), 20.03 (C-8‴); Anal. Calc. for $C_{21}H_{18}N_4O_2S_2$ (422.09): C, 59.69; H, 4.29; N, 13.26. Found: C, 59.76; H, 4.18; N, 13.13; EI-MS: *m/z* 422 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 105 $[C_8H_5O]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$.

2.5.10. N-(4-{[5-(Ethylsulfanyl)-1,3,4-oxadiazol-2-yl]methyl}-1,3-thiazol-2-yl)benzamide (7j).

Dark brown gummy solid; yield: 82%; Mol. Formula: $C_{15}H_{14}N_4O_2S_2$; Mol. Mass: 346 $gmol^{-1}$; IR: 3361 (N—H str.), 3044 (C—H str. of aromatic ring), 2932 (-CH_2- str.), 1666 (C=O str.), 1647 (C=N str.), 1587 (C=C str. of aromatic ring); ^{1}H NMR: 12.69 (s, 1H, -NH-CO-7), 8.08 (br.d, *J* = 7.3 Hz, 2H, H-2 & H-6),7.63 (br.t, *J* = 7.4 Hz, 2H, H-1), 7.53 (br.t, *J* = 7.6 Hz, 2H, H-3 & H-5), 7.19 (s, 1H, H-5′), 4.34 (s, 2H, CH_2-6′), 3.22 (q, *J* = 7.3 Hz, 2H, CH_2-1′′), 1.44 (t, *J* = 7.3 Hz, 3H, CH_3-2′′); ^{13}C NMR: 165.30 (C-7), 165.03 (C-2″), 163.65 (C-2′), 158.82 (C-5″), 143.41 (C-4′), 132.59 (C-4), 131.83 (C-1), 128.45 (C-2 & C-6), 128.03 (C-3 & C-5), 111.03 (C-5′), 33.73 (C-1‴), 27.16 (C-6′), 14.82 (C-2‴); Anal. Calc. for $C_{15}H_{14}N_4O_2S_2$ (346.06): C, 52.00; H, 4.07; N, 16.17. Found: C, 51.93; H, 3.94; N, 16.28; EI-MS: *m/z* 346 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$, 29 $[C_2H_5]^+$.

2.5.11. N-(4-{[5-(1-Propylsulfanyl)-1,3,4-oxadiazol-2-yl]methyl}-1,3-thiazol-2-yl)benzamide (7k).

Light brown greasy solid; yield: 84%; m.p.: 184–185 °C; Mol. Formula: $C_{16}H_{16}N_4O_2S_2$; Mol. Mass: 360 $gmol^{-1}$; IR: 3363 (N—H str.), 3051 (C—H str. of aromatic ring), 2936 (-CH_2- str.), 1665 (C=O str.), 1644 (C=N str.), 1582 (C=C str. of aromatic ring); ^{1}H NMR: 12.70 (s, 1H, -NH-CO-7), 8.09 (br.d, *J* = 7.3 Hz, 2H, H-2 & H-6), 7.63 (br.t, *J* = 7.4 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.6 Hz, 2H, H-3 & H-5), 7.19 (s, 1H, H-5′), 4.35 (s, 2H, CH_2-6′), 3.19 (t, *J* = 7.2 Hz, 2H, CH_2-1‴), 1.73 (sext., *J* = 7.3 Hz, 2H, CH_2-2‴), 0.95 (t, *J* = 7.3 Hz, 3H, CH_3-3‴); ^{13}C NMR: 165.31 (C-7), 165.02 (C-2″), 163.65 (C-2′), 158.82 (C-5″), 143.40 (C-4′), 132.49 (C-4), 131.84 (C-1), 128.45 (C-2 & C-6), 128.03 (C-3 & C-5), 111.03 (C-5′), 33.74 (C-1‴), 27.39 (C-6′), 22.24 (C-2‴), 12.68 (C-3‴); Anal. Calc. for $C_{16}H_{16}N_4O_2S_2$ (360.07): C, 53.31; H, 4.47; N, 15.54. Found: C, 53.23; H, 4.41; N, 15.51; EI-MS: *m/z* 360 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$, 43 $[C_3H_7]^+$.

2.5.12. N-(4-{[5-(1-Pentylsulfanyl)-1,3,4-oxadiazol-2-yl]methyl}-1,3-thiazol-2-yl)benzamide (7l).

Brown solid; yield: 89%; m.p.: 207–208 °C; Mol. Formula: $C_{18}H_{20}N_4O_2S_2$; Mol. Mass: 388 g mol^{-1}; IR: 3361 (N—H str.), 3049 (C—H str. of aromatic ring), 2933 (-CH_2- str.), 1666 (C=O str.), 1651 (C=N str.), 1586 (C=C str. of aromatic ring); ^{1}H NMR: 12.69 (s, 1H, -NH-CO-7), 8.08 (br.d, *J* = 7.8 Hz, 2H, H-2 & H-6), 7.63 (br.t, *J* = 7.7 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.7 Hz, 2H, H-3 & H-5), 7.19 (s, 1H, H-5′), 4.35 (s, 2H, CH_2-6′), 3.19 (t, *J* = 7.3 Hz, 2H, CH_2-1‴), 1.70 (quint., *J* = 7.3 Hz, 2H, CH_2-2‴), 1.37–1.33 (m, 2H, CH_2-3‴), 1.32–1.26 (m, 2H, CH_2-4‴), 0.86 (t, *J* = 7.0, 3H, CH_3-5‴); ^{13}C NMR: 165.33 (C-7), 165.03 (C-2″), 163.66 (C-2′), 158.83 (C-5″), 143.39 (C-4′), 132.48 (C-4), 131.85 (C-1), 128.45 (C-2 & C-6), 128.03 (C-3 & C-5), 111.04 (C-5′), 37.74 (C-1‴), 29.95 (C-2‴), 28.55 (C-3‴), 27.53(C-6′), 21.48 (C-4‴), 13.73 (C-5‴); Anal. Calc. for $C_{18}H_{20}N_4O_2S_2$ (388.10): C, 55.65; H, 5.19; N, 14.42. Found: C, 55.58; H, 5.11; N, 14.36; EI-MS: *m/z* 388 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$, 71 $[C_5H_{11}]^+$.

2.5.13. N-(4-{[5-(2-Pentylsulfanyl)-1,3,4-oxadiazol-2-yl]methyl}-1,3-thiazol-2-yl)benzamide (7m).

Light green greasy gummy solid; yield: 81%; Mol. Formula $C_{18}H_{20}N_4O_2S_2$; Mol. Mass: 388 $gmol^{-1}$; IR: 3362 (N—H str.), 3048 (C—H str. of aromatic ring), 2931 (-CH_2- str.), 1664 (C=O str.), 1649 (C=N str.), 1584 (C=C str. of aromatic ring); ^{1}H NMR: 12.69 (s, 1H, -NH-CO-7), 8.08 (br.d, *J* = 7.5 Hz, 2H, H-2 & H-6), 7.63 (br.t, *J* = 7.4 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.5 Hz, 2H, H-3 & H-5), 7.19 (s, 1H, H-5′), 4.35 (s, 2H, CH_2-6′), 3.68–3.65 (m, 1H, H-2‴), 1.61–1.59 (m, 2H, CH_2-3‴), 1.41–1.36 (m, 5H, CH_2-4‴ & CH_3-1‴), 0.86 (t, 3H, CH_3-5‴); ^{13}C NMR: 165.46 (C-7), 164.96 (C-2″), 162.81 (C-2′), 158.73 (C-5″), 143.39 (C-4′), 132.50 (C-4), 131.76 (C-1), 128.45 (C-2 & C-6), 128.03 (C-3 & C-5), 111.07 (C-5), 43.44 (C-3‴), 37.97 (C-2‴), 27.38 (C-6′), 21.06 (C-1‴), 19.47 (C-4‴), 13.39 (C-5‴); Anal. Calc. for $C_{18}H_{20}N_4O_2S_2$ (388.10): C, 55.65; H, 5.19; N, 14.42. Found: C, 55.54; H, 5.14; N, 14.38; EI-MS: *m/z* 388 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 105 $[C_8H_5O]^+$, 77 $[C_6H_5]^+$, 71 $[C_5H_{11}]^+$.

2.5.14. N-(4-{[5-(Phenethylsulfanyl)-1,3,4-oxadiazol-2-yl]methyl}-1,3-thiazol-2-yl)benzamide (7n)

Lemon yellow solid; yield: 83%; m.p.: 255–256 °C; Mol. Formula: $C_{21}H_{18}N_4O_2S_2$; Mol. Mass: 422 $gmol^{-1}$; IR: 3357 (N—H str.), 3032 (C—H str. of aromatic ring), 2927 (-CH_2- str.), 1664 (C=O str.), 1653 (C=N str.), 1586 (C=C str. of aromatic ring); ^{1}H NMR: 12.72 (s, 1H, -NH-CO-7), 8.08 (br.d, *J* = 7.6 Hz, 2H, H-2 & H-6), 7.62 (br.t, *J* = 7.3 Hz, 1H, H-4), 7.53 (br.t, *J* = 7.3 Hz, 2H, H-3 & H-5), 7.28 (dis.t, *J* = 7.1 Hz, 2H, H-3‴ & H-5‴), 7.23–7.18 (m, 4H, H-5′, H-2‴, H-4‴& H-6‴), 4.35 (s, 2H, CH_2-6′), 3.46 (br.t, *J* = 7.3 Hz, 2H, CH_2-8‴), 3.01 (br.t, *J* = 7.5 Hz, 2H, CH_2-7‴); ^{13}C NMR: 165.42 (C-7), 165.06 (C-2″), 163.50 (C-2′), 158.84 (C-5″), 143.44 (C-4′), 139.09 (C-1‴), 132.56 (C-4), 131.83 (C-1), 128.58 (C-2 & C-6), 128.37 (C-3‴ & C-5‴), 128.51 (C-2‴ & C-6‴), 128.07 (C-3 & C-5), 126.50 (C-4‴), 111.15 (C-5′), 34.90 (C-7‴), 33.09 (C-8‴), 27.44 (C-6′); Anal. Calc. for $C_{21}H_{18}N_4O_2S_2$ (422.09): C, 59.69; H, 4.29; N, 13.26. Found: C, 59.63; H, 4.25; N, 13.21; EI-MS: *m/z* 422 $[M]^+$, 318 $[C_{13}H_{10}N_4O_2S_2]^+$, 243 $[C_{12}H_9N_3OS]^+$, 105 $[C_8H_5O/C_8H_9]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$.

2.6. Urease inhibition assay

This enzyme assay is the customized form of the commonly known Berthelot assay [10]. The assay mixture of 85 μL is prepared containing 10 μL of phosphate buffer of pH 7.0 (in each well in the 96-well plate), 10 μL of sample solution and 25 μL of enzyme solution (0.135 units). Contents were pre-incubated at 37 °C for 5 min. Then, 40 μL of urea stock solution (20 mM) was added to each well with incubation for 10 min at 37 °C. It is followed by the addition of 115 μL phenol hypochlorite reagents (freshly prepared by mixing 45 μL phenol with 70 μL of alkali) per well. For color development, incubation was carried out for further 10 min at 37 °C. Absorbance was measured at 625 nm. The percentage enzyme inhibition and IC_{50} values were calculated using the following formula:

$$\text{Inhibition}(\%) = \frac{\text{Control}-\text{Test}}{\text{Control}} \times 100$$

Where, Control is the total enzyme activity without inhibitor and Test, the activity in the presence of test compound. IC_{50} values were calculated using the EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, US).

2.6.1. Statistical analysis

Statistical analysis was performed by Microsoft Excel 2010 for all the thrice measured values and the results are presented as mean ± SEM.

2.7. Computational methodology

2.7.1. Repossession of Jack bean urease from protein data Bank

The three dimensional (3D) structure of Jack bean urease (*C. ensiformis*) having PDBID 4H9M (https://www.rcsb.org/structure/4h9m) was accessed from Protein Data Bank (PDB). The target protein (Urease) further energy minimized by Chiron online server [11]. The Ramachandran plot and values we accessed from PDB and receptor architecture along with α-helices, β-sheets, coils and turns percentage values were predicted from online server VADAR 1.8 [12].

2.7.2. Chemo-informatics properties of synthesized compounds

The synthesized ligands, **7a-n**, were evaluated on the basis of chemo-informatics properties and Lipinski rule of five (RO5). Multiple online servers such as Molinspiration (http://www.molinspiration.com/), and Molsoft (http://www.molsoft.com/) were employed to predict the molecular properties and bioactivity score of designed ligands, **7a-n**.

2.7.3. Molecular docking

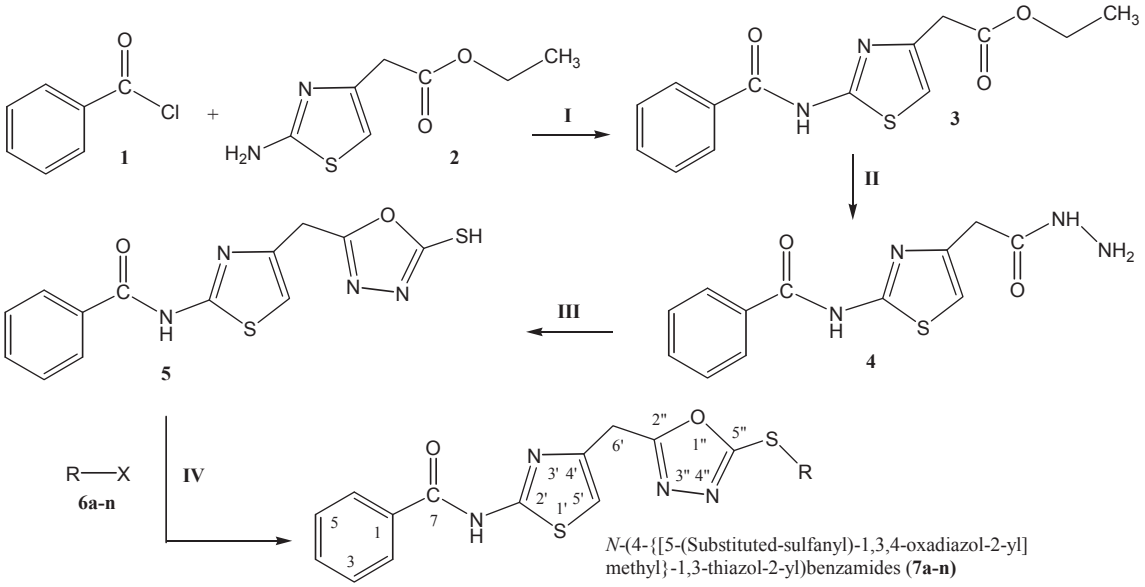
Before docking experiment all the synthesized chemical structures were sketched in ACD/ChemSketch tool and accessed in mol format. Furthermore, UCSF Chimera 1.10.1 tool was employed to energy minimization of each ligand separately having default parameters such as steepest descent steps 100 with step size 0.02 (Å), conjugate gradient steps 100 with step size 0.02 (Å) and update interval was fixed at 10. Finally, Gasteiger charges were added using Dock Prep in ligand structure to obtain the good structure conformation. Molecular docking experiment was employed on all the ligands, **7a-n**, against Jack bean urease by using virtual screening tool PyRx with VINA Wizard approach [13]. The grid box parameters values in VINA search space (X = 10.22, Y = 24.56 and Z = 46.18) were adjusted with default exhaustiveness value = 8 to maximize the binding conformational analysis. We have adjusted sufficient grid box size on biding pocket residues to allow the ligand to move freely in the search space. All the synthesized ligands were docked separately against target protein. In all docked complexes, the ligands conformational poses were keenly observed to obtain the best docking results. The generated docked complexes were evaluated on the basis of lowest binding energy (kcal/mol) values and binding interaction pattern between ligands and receptor. The graphical depictions of all the docked complexes were accomplished by UCSF Chimera 1.10.1 [14] and Discovery Studio (2.1.0), respectively [15].

3. Results and discussion

3.1. Chemistry

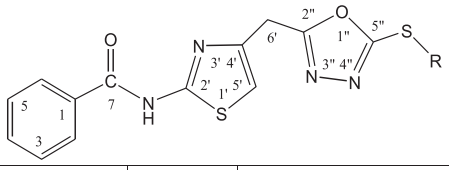
The synthetic pathway, used for the synthesis of novel bi-heterocyclic benzamides, is indicated in Scheme 1 and varying substituents are listed in Table 1. Initially, benzoylation of ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate (**2**) with benzoyl chloride **(1)** in aqueous weak basic medium was carried out to insert a benzamide functionality, whereby, ethyl 2-[2-(benzoylamino)-1,3-thiazol-4-yl]acetate (**3**) was obtained. This molecule **3** bearing an ester functionality, which is prone to a nucleophilic substitution reaction, was reacted with hydrazine hydrate in methanol to acquire the corresponding hydrazide, *N*-[4-(2-hydrazino-2-oxoethyl)-1,3-thiazol-2-yl] benzamide (**4**). This substitution reaction was completed by refluxing the mixture for two hours. The hydrazide, **4**, was made to react with carbon disulfide in the presence of KOH, an activator for cyclization, to yield a thiol containing nucleophile, *N*-{4-[(5-sulfanyl-1,3,4-oxadiazol-2-yl)methyl]-1,3-thiazol-2-yl}benzamide (**5**). Finally, *S*-substitution was carried out by reacting thiol **5** with different aralkyl/alkyl halides, **6a-n,** to obtain the targeted bi-heterocyclic amides, *N*-(4-{[5-(substituted-sulfanyl)-1,3,4-oxadiazol-2-yl]methyl}-1,3-thiazol-2-yl)benzamides (**7a-n**). The molecular structures of these synthesized derivatives were corroborated by their IR EI-MS, ^{1}H NMR and ^{13}C NMR spectral data along with elemental analysis.

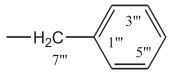
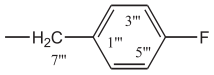
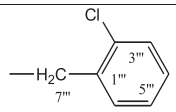
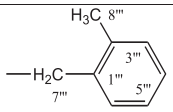
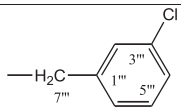
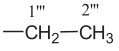
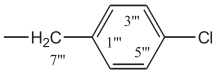
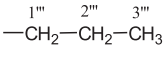
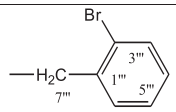
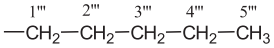
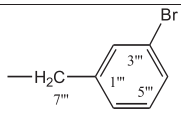
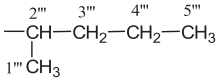
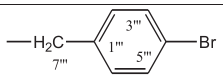
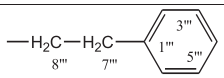
The structural assignment of one of the compounds is elaborated hereby for the benefit of the readers. The molecule **7c** was acquired as a brown solid in 81% yield having melting point 288–289 °C. Its molecular formula, $C_{20}H_{15}ClN_4O_2S_2$, was recognized by CHN analysis data and molecular mass was justified by the molecular ion peak $[M]^+$, and higher isotope peak $[M+2]^+$ of almost one-third intensity, in its EI-MS at *m/z* 442 and 444, respectively. The mass fragmentation pattern also supported to erect the said molecular formula. Moreover, counting the number of protons in its ^{1}H NMR spectrum and the carbon resonances in its ^{13}C NMR spectrum, also supplemented the assignment of molecular formula. The vibrational studies of the molecules were examined using FT-IR spectroscopy to affirm various functionalities. The significant absorption bands in IR spectrum appeared at ν 3350 (N—H str.), 3026 (C—H str. of aromatic ring), 2923 (-CH_2- str.), 1663 (C=O str.), 1647 (C=N str.), 1584 (C=C str. of aromatic ring), 686 (C—Cl str.) cm^{-1}. The highly deshielded signal at δ 12.68 (s, 1H, -NH-CO-7) in its ^{1}H NMR spectrum was rational for an amidic hetero-atom proton (Fig. 1a). A benzoyl group in the molecule was apparent by its discrete signals at δ 8.08 (br.d, J = 8.4 Hz, 2H, H-2 & H-6), 7.63 (br.t, J = 7.4 Hz, 1H, H-4), and 7.53 (br.t, J = 7.6 Hz, 2H, H-3 & H-5) [9]. The other signals, in the aromatic region, were an attribute of 3-chlorobenzyl moiety, resonating as δ 7.66 (br.s, 1H, H-2‴), 7.45 (br.d, J = 7.9 Hz, 1H, H-4‴), 7.40 (br.d, J = 7.7 Hz, 1H, H-6‴), and 7.25 (br.t, J = 7.7 Hz, 1H, H-5‴), (Fig. 1b). The benzylic methylene appeared as a broad-singlet at 4.34 (br.s, 2H, CH_2-7‴). A singlet at δ 7.17 (s, 1H, H-5′) was accounted for a 1,3-thiazol-2-yl moiety while a methylene, connecting the two heterocycles (2-amino-1,3-thiazol-4-yl and



Scheme 1. Outline for the synthesis of *N*-(4-{[5-(substituted-sulfanyl)-1,3,4-oxadiazol-2-yl]methyl}-1,3-thiazol-2-yl)benzamides. Reagents & Conditions: (I) Aq. 2% Na_2CO_3/stirring and heating for 5 hrs. (II) MeOH/N_2H_4·H_2O/refluxing for 2 hrs. (III) EtOH/CS_2/KOH/refluxing for 10 hrs. (IV) DMF/LiH/stirring for 3–5 hrs.

Table 1
Different –R (aralkyl/alkyl) groups in Scheme 1.



Compd.	-R_1	Compd.	-R_1
6a, 7a		**6h, 7h**	
6b, 7b		**6i, 7i**	
6c, 7c		**6j, 7j**	
6d, 7d		**6k, 7k**	
6e, 7e		**6l, 7l**	
6f, 7f		**6m, 7m**	
6g, 7g		**6n, 7n**	

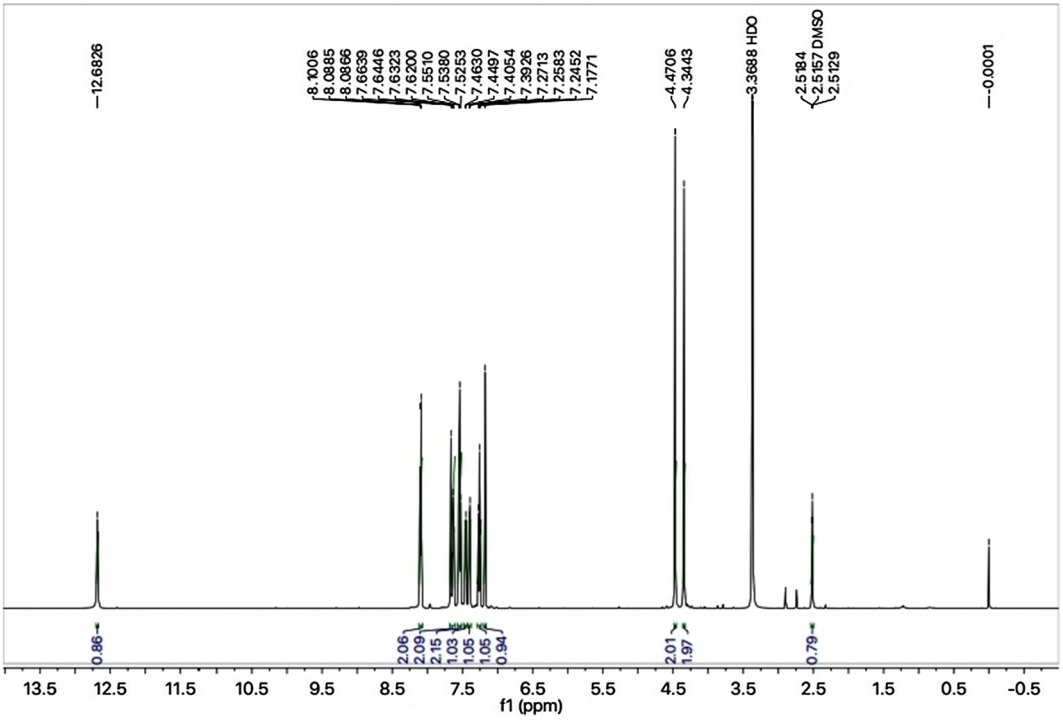


Fig. 1a. ^{1}H NMR spectrum of **7c**.

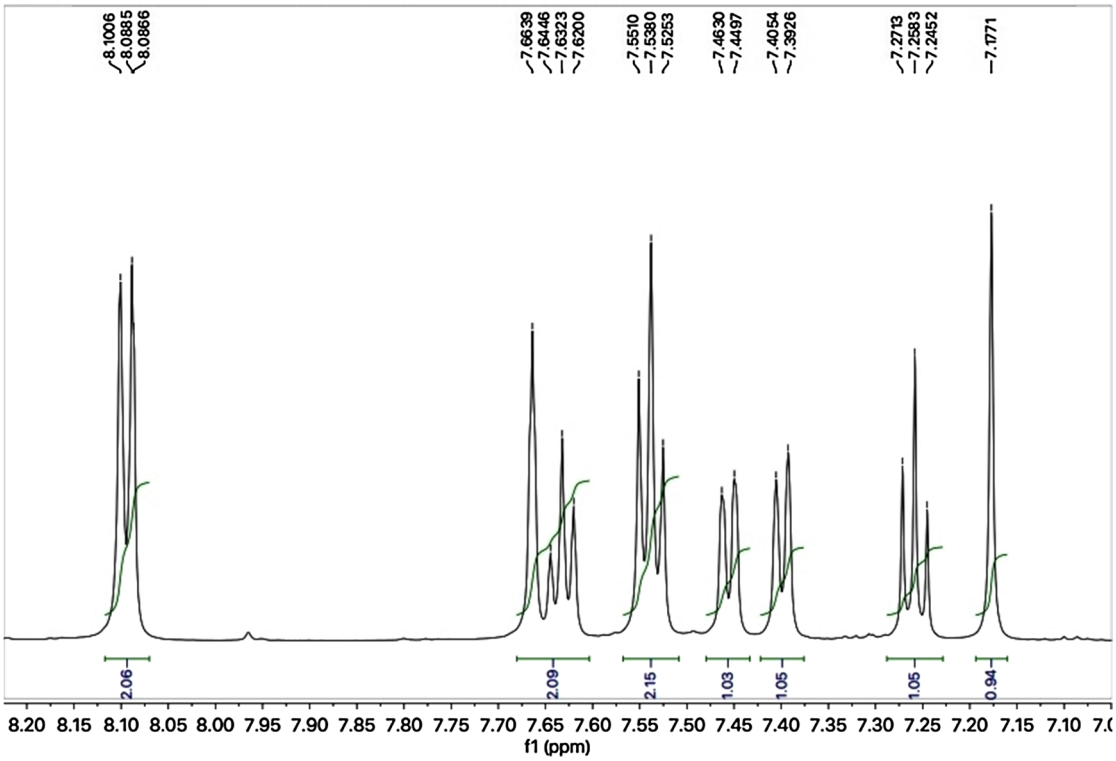


Fig. 1b. Expanded aromatic region of ^{1}H NMR spectrum of **7c**.

1,3,4-oxadiazol-2-yl), was characterized as a broad-singlet resonating at δ 4.47 (br.s, 2H, CH_2-6′).

The ^{13}C NMR spectrum (Fig. 2) thoroughly corroborated the carbon skeleton of the molecule. 5-Sulfanyl-1,3,4-oxadiazol-2yl heterocyclic core was identified through two quaternary signals at δ 165.06 (C-2″), and 158.84 (C-5″). The 1,3-thiazol-2-yl-4-(substituted-methyl) moiety was rational by two quaternary carbon resonances at δ 162.97 (C-2′), and 143.31 (C-4′), a methine signal at δ 111.16 (C-5′), while the

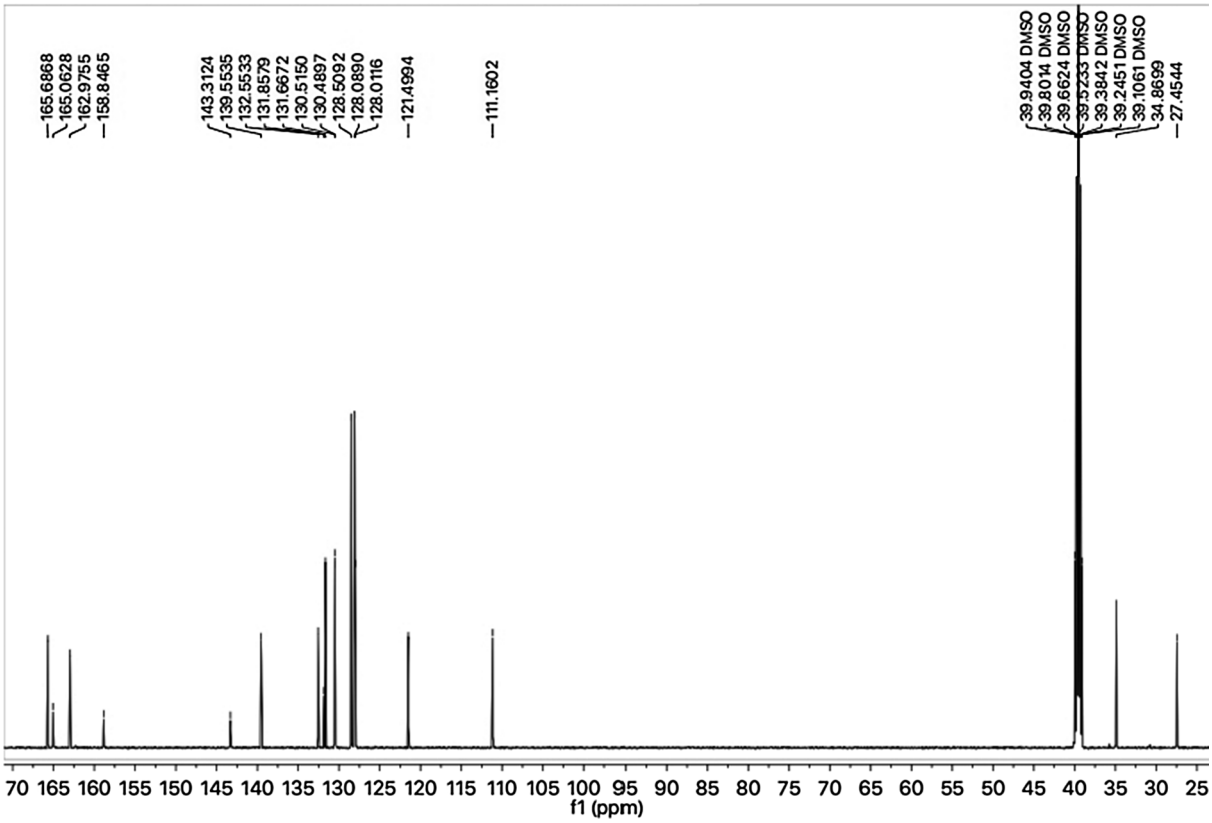
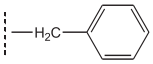
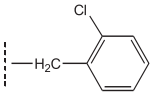
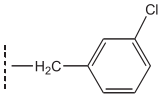
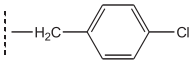
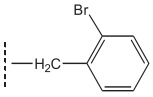
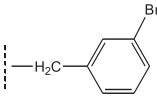
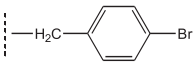
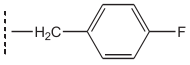
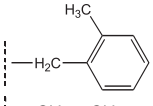
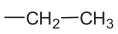
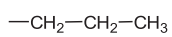
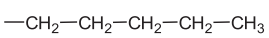
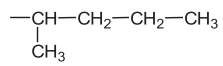
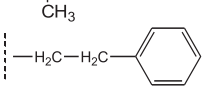


Fig. 2. ^{13}C NMR spectrum of **7c**.

Table 2
Percent inhibition at 0.5 mM and IC_{50} values for urease enzyme.

Compound	Aralkyl or Alkyl Part	Urease inhibition	
		Inhibition (%)	IC_{50} (μM)
7a		71.69 ± 0.12	51.26 ± 0.38
7b		76.52 ± 0.13	48.15 ± 0.21
7c		74.82 ± 0.13	14.24 ± 0.09
7d		76.34 ± 0.17	47.82 ± 0.43
7e		89.85 ± 0.14	3.58 ± 0.02
7f		77.93 ± 0.15	43.66 ± 0.49
7g		96.17 ± 0.07	3.79 ± 0.04
7h		98.24 ± 0.06	2.17 ± 0.41
7i		97.83 ± 0.11	3.19 ± 0.21
7j		98.29 ± 0.09	2.59 ± 0.09
7k		94.31 ± 0.07	5.58 ± 0.10
7l		95.13 ± 0.09	2.99 ± 0.45
7m		95.29 ± 0.06	3.77 ± 0.23
7n		94.24 ± 0.06	5.91 ± 0.45
Thiourea (Standard)		98.12 ± 0.18	21.11 ± 0.12

Note. IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated from the inhibition data obtained after doing assays at high dilutions of the compounds as given in assay method and data was computed using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA). Data is mean of three values (mean ± s.e.m., n = 3).

methylene group, linked to two heterocyles, appeared at δ 27.45 (C-6′). A benzoyl group was undoubtedly identified by its peculiar signals at δ 165.68 (C-7), 132.55 (C-4), 131.85 (C-1), 128.50 (C-2 & C-6), and 128.08 (C-3 & C-5). The 3-chlorobenzyl moiety was characterized by typical six signals for a 1,3-di-substituted phenyl ring appearing at δ 139.55 (C-1‴), 131.66 (C-3‴), 130.51 (C-4‴), 130.48 (C-5‴), 128.01 (C-2‴), and 121.49 (C-6‴), along with a benzylic methylene resonating at δ 34.86 (C-7‴). The mass fragmentation pattern of this compound was also fully justifying the deduced units and the data of prominent peaks is given in the experimental section. Thus, based on cumulative spectral evidences, the structure of **7c** was confirmed and it was named *N*-[4-({5-[(3-chlorobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl}methyl)-1,3-thiazol-2-yl]benzamide. A similar exercise was implemented for the structural analysis of other bi-heterocyclic amides.

3.2. Urease inhibition and structure-activity relationship

The newly synthesized bi-heterocyclic benzamides were screened against urease enzyme and a varying degree of inhibition was displayed by these molecules ranging from 2.17 ± 0.41 to 51.26 ± 0.38 μM, relative to standard inhibitor thiourea having value IC_{50} of 21.11 ± 0.12 μM (Table 2). Some of the compounds, for example, **7h**, **7j** and **7l**, displayed IC_{50} values of 2.17 ± 0.41, 2.59 ± 0.09, and 2.99 ± 0.45 μM, respectively, which are much lower than the standard, and it indicated that these molecules were exhibiting many folds better inhibitory potential than the standard thiourea.

Although, the observed activity is the resultant of a whole molecule, but a limited structure–activity relationship (SAR) was rationalized by analyzing the effect of different aralkyl or alkyl groups (-R) attached to

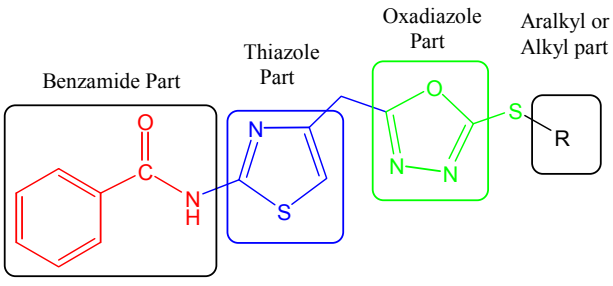


Fig. 3. General structural features of compounds, **7a-n**.

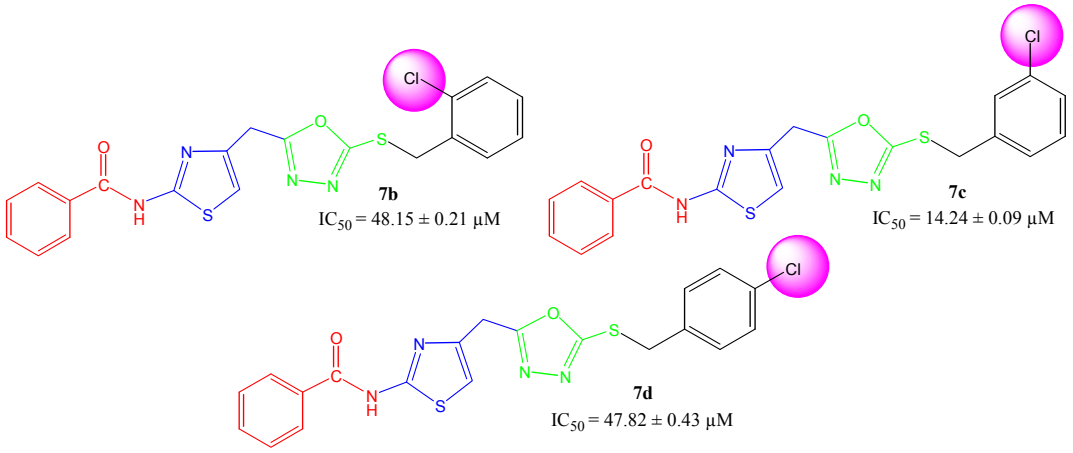


Fig. 4. Structure-activity relationship of compounds **7b**, **7c**, and **7d**.

sulfur atom on the inhibitory potential. Fig. 3 displayed the general structural features of the synthetic compounds.

Among the three chlorinated regio-isomers, **7b**, **7c** and **7d**, the molecule **7c** bearing a 2-chloro group exhibited better inhibitory potential (IC_{50} = 14.24 ± 0.09 μM), as compared to **7b** (IC_{50} = 48.15 ± 0.21 μM) and **7d** (IC_{50} = 47.82 ± 0.43 μM), in which chloro group was present at 2-position and 4-position, respectively. The compounds **7b** and **7d** having chloro group at *ortho* and *para* position respectively, resembled in their inhibitory activity. It was outcome from the results that probably more efficient interactions are made by the compound with enzyme, when a medium sized inductively withdrawing chloro group is present at *meta* position instead of *ortho* and *para* position (Fig. 4).

However, a reverse trend was observed when a relatively bulky bromo group was present in the molecules. Hereby, the molecule **7e** (IC_{50} = 3.58 ± 0.02 μM) with *ortho* and **7g** (IC_{50} = 3.79 ± 0.04 μM) with *para* bromo group exhibited mutually resembling and promising inhibitory potential as compared to *meta* isomer (**7f**; IC_{50} = 43.66 ± 0.49 μM). Similarly, when a small sized fluoro group was present at *para* position in **7h** (IC_{50} = 2.17 ± 0.41 μM), this molecule showed even a more promising inhibitory potential relative to bromo analogue. Moreover, this compound was found to be most active among all synthetic derivatives and possessed almost ten folds better inhibitory potential as compared to standard thiourea (IC_{50} = 21.11 ± 0.12 μM). It means that when a small sized group is present at 4-position of benzyl ring (Fig. 5), the molecule is more efficiently interacting with enzyme as compared to other analogues.

Compound **7a** (IC_{50} = 51.26 ± 0.38 μM) having an un-substituted benzyl group was the least active in the series. However, a considerably good inhibitory potential was rendered to the compound **7n** (IC_{50} = 5.91 ± 0.45 μM) in which the same un-substituted phenyl ring was separated through two methylenes (phenethyl group). Even a better inhibitory activity, relative to **7a**, was observed in case of **7i** (IC_{50} = 3.19 ± 0.21 μM) where a small sized methyl group was present at 2-position. It means that presence of a small substituent on benzyl group made the molecule as more suitable inhibitor relative to an un-substituted compound (Fig. 6).

The aliphatic *S*-substituted derivatives (Fig. 7) exhibited overall good inhibitory potentials against the target enzyme. On comparison of **7j** (IC_{50} = 2.59 ± 0.09 μM) and **7k** (IC_{50} = 5.58 ± 0.10 μM), it was obvious that former compound with smaller ethyl group was more active than the latter higher homologous derivative. Indeed, **7j** was identified as second most active compound in the presently studied synthetic series. The compound **7l** (IC_{50} = 2.99 ± 0.45 μM) with five-carbon straight aliphatic chain was more active than its branched chain isomer **7m** (IC_{50} = 3.77 ± 0.23 μM). However, an interesting feature was observed on comparison of inhibitory potential of **7k** and **7l**, whereby a molecule with relatively long aliphatic chain (**7l**) was more

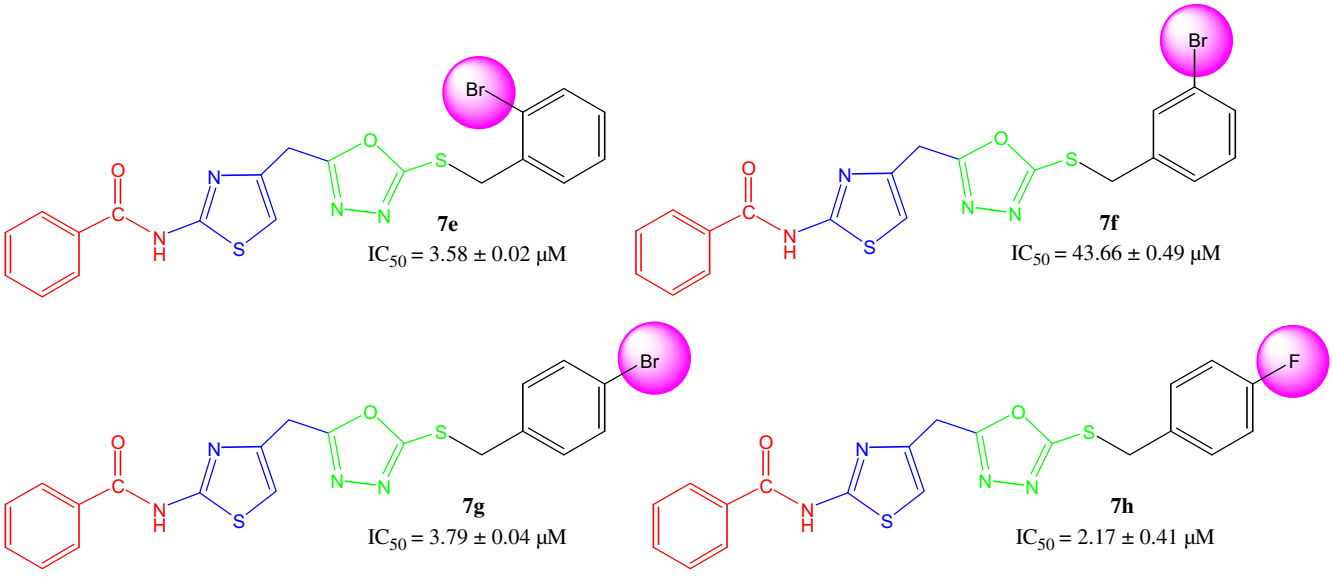


Fig. 5. Structure-activity relationship of compounds **7e**, **7f**, **7g** and **7h**.

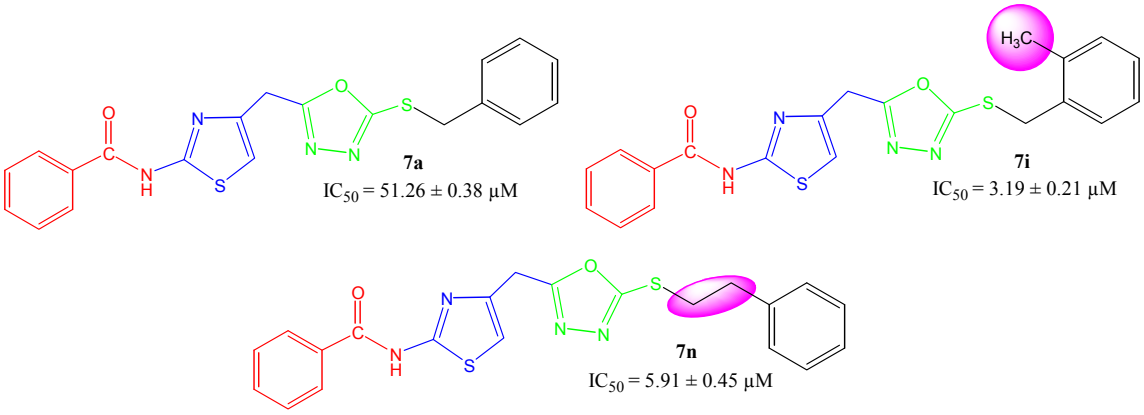


Fig. 6. Structure-activity relationship of compounds **7a**, **7i**, and **7n**.

active than a compound with smaller chain (**7k**). It might be related to an enhanced lipophilic character of **7l** by virtue of which it might make relatively more efficient interactions with the active site of the enzyme.

So, it was inferred from the structure-activity relationship that molecules usually with *ortho* or *para*-substituent on aromatic ring of benzyl group as well as compounds with *S*-substituted aliphatic groups were generally good inhibitors of the target enzyme. Synthesized compounds can be arranged in the following row according to their inhibitory activity: **7h** > **7j** > **7l** > **7i** > **7e** > **7m** > **7g** > **7k** > **7n** > **7c** > **7f** > **7b** > **7a** (see Table 2 for IC_{50} values).

3.3. In-silico analysis

3.3.1. Structural assessment of jack bean urease

Jack bean urease is a class of hydrolase protein having tetra domains (1–4) with different numbers of residues. The domain 4 is most important due presence of binding pocket and its catalytic behavior (Fig. 8). The VADAR analysis justified that Jack bean urease architecture consist of 27% α-helices, 31% β-sheets and 41% coils. Moreover, Ramachandran plots and values indicated that 97.5% of residues were present in favored regions which shows the good precision of phi (φ) and psi (ψ) angles among the coordinates of jack bean urease structure (Fig. S1).

3.3.2. Chemo-informatics properties and Lipinski's rule (RO5) of synthesized compounds

The predicted chemo-informatic properties were evaluated by computational tools. Results exposed that compounds **7a-n** have better predicted value of molecular weight (g/mol), hydrogen bond acceptor and donor, log*P*, polar surface area (A^2) and molar volume (A^3). All the predicted values of ligands, **7a-n**, showed good comparable results

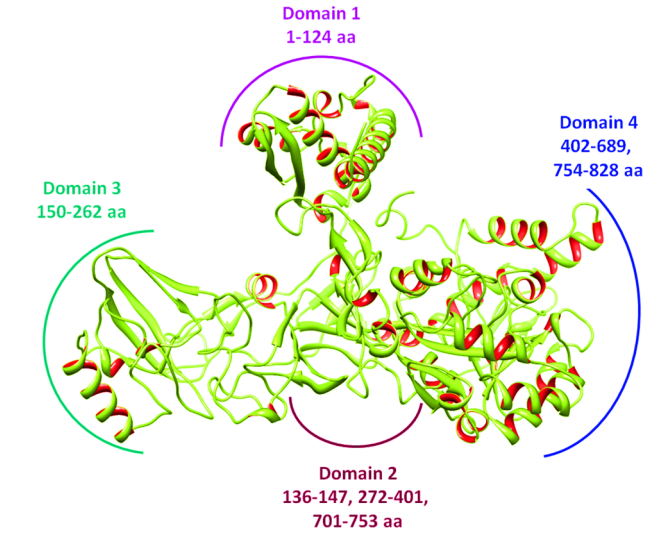


Fig. 8. The general structure of jack bean urease protein.

compared to standard values (Table 3). Moreover, the Lipinski's rule of five (RO5) analysis depicted that all the chemical structures, **7a-n**, obey they RO5 rule and possess good comparable values against standard molecular weight (< 5000 g/mol), HBA (< 10), HBD (< 5) and logP (< 5) values [16]. The RO5 deviation results in poor absorption of compounds. However there are plenty of examples are available for RO5 violation amongst the existing drugs [17,18]. Furthermore, the polar surface area (PSA) of a molecule is defined as the surface sum over all polar atoms, primarily oxygen and nitrogen, also including their attached hydrogen atoms. The PSA parameter is commonly used

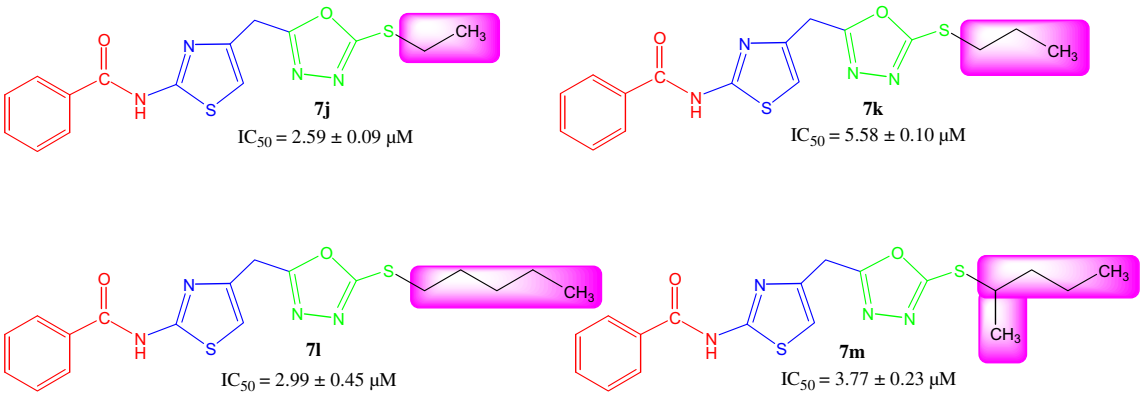


Fig. 7. Structure-activity relationship of compounds **7j**, **7k**, **7l** and **7m**.

Table 3
Chemo-informatic properties of ligands.

Properties	7a	7b	7c	7d	7e	7f	7g	7h	7i	7j	7k	7l	7m	7n
Mol.Wt	408	442	442	442	485	485	485	426	422	246	360	388	388	422
HBA	7	7	7	7	7	7	7	7	7	7	7	7	7	7
HBD	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Log*P*	4.05	4.64	4.76	4.76	4.78	4.90	4.90	4.31	4.33	2.97	3.45	4.42	4.42	4.42
PSA (A^2)	63	63	63	63	63	63	63	63	63	63	63	63	63	63
Mol vol.(A^3)	362	378	379	379	383	378	384	368	382	307	329	365	357	383
nrotb	7	7	7	7	7	7	7	7	7	6	6	9	8	8
Drug Score	0.29	0.56	0.53	0.64	0.24	0.29	0.51	0.67	0.42	0.34	0.79	0.49	1.13	0.61
GPCR ligand	−0.66	−0.66	−0.63	−0.66	−0.77	−0.73	−0.72	−0.63	−0.67	−0.83	−0.73	−0.65	−0.82	−0.56
Ion channel modulator	−1.25	−1.28	−1.21	−1.21	−1.30	−1.28	−1.27	−1.21	−1.32	−1.44	−1.39	−1.27	−1.39	−1.13
Kinase inhibitor	−0.20	−0.25	−0.23	−0.21	−0.31	−0.26	−0.24	−0.17	−0.31	−0.39	−0.40	−0.39	−0.50	−0.31
Nuclear receptor ligand	−1.12	−1.10	−1.11	−1.11	−1.23	−1.20	−1.18	−1.07	−1.11	−1.42	−1.33	−1.17	−1.25	−1.06
Protease inhibitor	−0.24	−0.29	−0.27	−0.27	−0.41	−0.35	−0.33	−0.25	−0.29	−0.39	−0.32	−0.23	−0.38	−0.20
Enzyme inhibitor	−0.41	−0.48	−0.42	−0.42	−0.52	−0.47	−0.46	−0.41	−0.45	−0.57	−0.50	−0.39	−0.51	−0.38

*Mol. Wt = Molecular Weight (g/mol), HBA = Hydrogen Bond Acceptor, HBD = Hydrogen Bond Donor, PSA = Polar Surface Area.

Table 4
The docking energy values.

Docking complexes	Energy values (kcal/mol)
7a	−8.40
7b	−8.50
7c	−8.30
7d	−8.40
7e	−8.60
7f	−8.50
7g	−8.40
7h	−8.40
7i	−8.60
7j	−7.50
7k	−7.80
7l	−7.40
7m	−7.80
7n	−8.40

for drug's optimization ability to permeate cells. Prior research data showed the standard value of PSA (< 89 A^2) [19]. Our predicted results showed that compounds, **7a-n**, possessed less than standard values PSA results. This simple topological parameter is a measure of molecular flexibility. The number of rotatable bonds is also considered as a very good descriptor of oral bioavailability of drugs [20]. Rotatable bond is defined as any single non-ring bond, bounded to nonterminal heavy (i.e., non-hydrogen) atom. Amide C—N bonds are not considered because of their high rotational energy barrier. Prior research data showed that the number of rotatable bonds in ligands must be in range < 10 which depicts high probability of good oral bioavailability in rat [20]. Our predicted results showed that all the ligands were fall in standard range which showed their good oral bioavailability behavior.

The drug score is a complex of basic molecular parameters are hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility. Our results showed that all the synthetic compounds, **7a-n**, showed good drug score values, which depicts its good drug like behavior and may be considered as drug candidate molecules against urease. The overall predicted results values of all compounds is mentioned in Table 3.

3.3.3. Molecular docking analysis

3.3.3.1. Docking energy evaluation of ligands. To predict the best conformational position within the active region of urease all the synthesized ligands, **7a-n**, were docked against receptor molecule. The generated docked complexes were examined on the basis of minimum energy values (kcal/mol) and bonding interaction pattern such as hydrogen and hydrophobic, respectively. Docking results justified that all compounds, **7a-n**, depict good energy values (kcal/mol) (Table 4). The standard error value for Autodock is reported as 2.5 kcal/mol (http://autodock.scripps.edu/). However, all the synthesized compounds have no big docking energy value difference more than standard error value. Although, the basic nucleus of all the synthesized compounds was same and most of ligands possess good efficient energy values with no big energy value fluctuations (Table 4).

The docking energy calculation is done by Eq. (1).

$$\Delta G\text{binding} = \Delta G\text{gauss} + \Delta G\text{repulsion} + \Delta G\text{hbond} + \Delta G\text{hydrophobic} + \Delta G\text{tors} \quad (1)$$

ΔG gauss Attractive term for dispersion, two gaussian functions, ΔGrepulsion Square of the distance if closer than a threshold value, ΔG

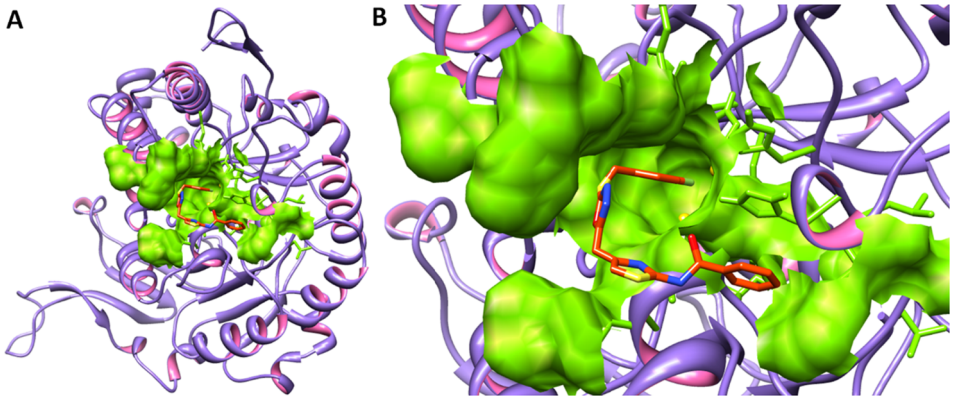


Fig. 9. Docking complex **7h** against urease. The urease structure is highlighted in ribbon format having purple color while the binding pocket is justified in green color in surface format. The ligand structure is depicted in brown color with functional moiety in different colors.

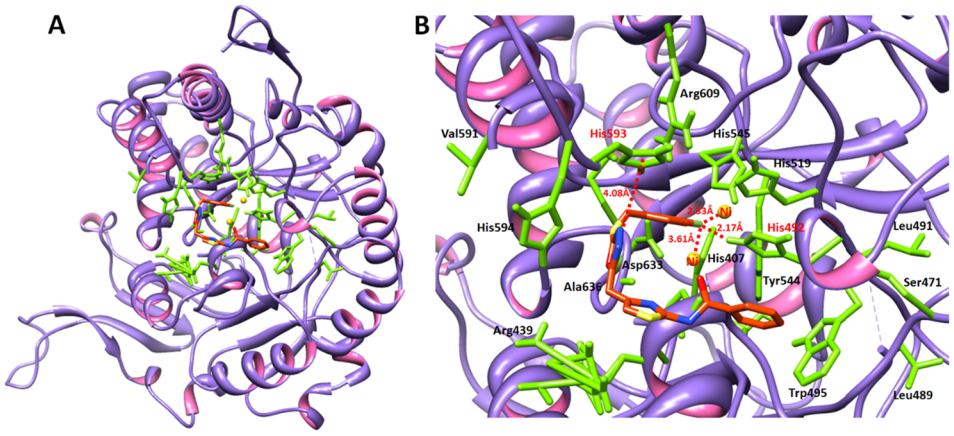


Fig. 10. Docking complex of **7h**. (A) The general overview of docking depiction. The protein structure is represented in purple color in ribbon format while ligand is highlighted brown color. (B) The closer view of binding pocket interaction with best conformation position of ligand **7h** against target protein. The ligand molecule is depicted in brown color while their functional groups such as amino, sulfur and fluorine are showed in blue, yellow and green colors, respectively. The binding pocket residues are highlighted in green color. The binding interaction shows in red dotted lines with distances mentioned in angstrom (Å). Two nickel atoms are represented in yellow circle.

hbond Ramp function - also used for interactions with metal ions, ΔG hydrophobic Ramp function, ΔG tors Proportional to the number of rotatable bonds

3.3.3.2. Binding pocket and binding interaction analyses. The binding pocket is present in domain 4 where nickel metals are present [21]. Based on *in vitro* analysis **7h** showed good enzyme inhibition potential therefore, ligand **7h** was selected to check the binding interaction pattern. The **7h**-docking complex showed that twisted form of ligand having fluoro group show penetration toward nickel metal and adjusted within binding pocket of urease. The substituted functional moiety showed good conformation position inside the active region of target protein (Fig. 9).

Fig. 10 showed the binding interaction pattern of **7h** and urease. In detail binding analysis it was observed that two halogen metal interactive bonds were between the fluoro group of **7h** and nickel metals of urease having 2.33 and 3.61 Å, respectively. Similarly, same bezyl fluorine form another hydrogen bond with His492 at a distance 2.17 Å. The oxadizole ring form hydrophobic interaction with His593 having distance 4.08 Å. The presence of 4-fluoro group at benzyl moiety showed good results both in *in vitro* and docking analysis. The docking complex binding pocket residues also showed good correlation with published data which strengthened our docking results [22–25]. All other compounds docking complexes are mentioned in supplementary data (Figs S2-S15).

4. Conclusion

We have described the successful synthesis of bi-heterocyclic benzamides molecules through a multi-step protocol under facile conditions and these novel compounds were obtained in good yields. Our present study proved fruitful as we succeeded to explore some potent inhibitors of urease enzyme, which can find their utility as promising therapeutic agents in drug discovery and designing program. Both *in vitro* and in silico results showed that compound **7h** has good inhibitory potential ($IC_{50:}$ 2.17 ± 0.41 μM) with a good binding energy value (−8.40 kcal/mol) and could serve a key compound for further studies as a potent urease inhibitor.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.018.

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