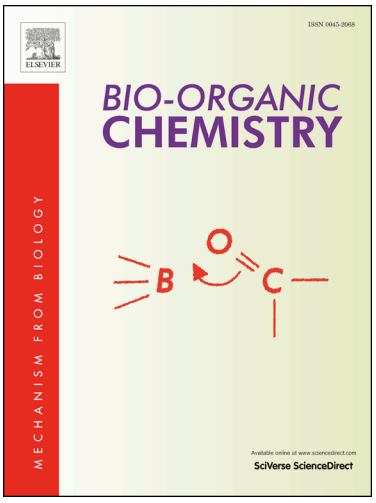

Synthesis, in vitro urease inhibitory activity, and molecular docking studies of thiourea and urea derivatives

Bilquees Bano, Kanwal, Khalid Mohammed Khan, Arif Lodhi, Uzma Salar, Farida Begum, Muhammad Ali, Muhammad Taha, Shahnaz Perveen

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Synthesis, *in vitro* urease inhibitory activity, and molecular docking studies of thiourea and urea derivatives

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Abstract: The current study deals with the synthesis of urea and thiourea derivatives **1-37** which were characterized by various spectroscopic techniques including FAB-MS, ^{1}H-, and ^{13}C-NMR. The synthetic compounds were subjected to urease inhibitory activity and compounds exhibited good to moderate urease inhibitory activity having IC_{50} values in range of 10.11-69.80 μM. Compound **1** (IC_{50} = 10.11 ± 0.11 μM) was found to be most active and even better as compared to the standard acetohydroxamic acid (IC_{50} = 27.0 ± 0.5 μM). A limited structure-activity relationship (SAR) was established and the compounds were also subjected to docking studies to confirm the binding interactions of ligands (compounds) with the active site of enzyme.

Keywords: Synthesis; urea; thiourea; *in vitro* urease activity; structure-activity relationship; molecular docking studies

Introduction

Urease (urea amidohydrolase E C 3.5.1.5) is an enzyme having two nickel atoms in its core structure and is responsible for the hydrolysis of urea into ammonia and CO_2 or carbamate [1-3]. The carbamate decomposes readily into another molecule of ammonia. These reactions causing the increase in the alkalinity of physiological system which is accountable for numerous adverse

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effects to animal and human health [4]. In addition, excess ammonia production is also harmful for agriculture and causes the death of cash crops. Ureases are found in fungi, bacteria, higher plants, and in soil as a soil enzyme. Bacterial ureases are large heteropolymeric metalloproteins with nickel (II) ions present in their active sites [5-7]. Many microbes use this enzyme in a reaction, which provides nitrogen for growth [8], and it is also involved in plant nitrogen metabolism during the germination process [9]. Elevated urease activity causes significant economic and environmental problems by releasing large amounts of ammonia into the atmosphere during urea fertilization in agriculture sector [10] which leads to plant damage primarily by depriving plants from their essential nutrients and secondarily by ammonia toxicity, thus resulting in the increased soil pH [11]. Urease plays a vital role in many pathogenic processes in animals as well as in humans. It played a major role in urinary catheter incrustation, peptic ulceration, kidney stone, pyelonephritis, urolithiasis, hepatic encephalopathy, and reactive arthritis [12-16]. Urease has also been reported as an immunogenic modulator in a number of pathogen-induced inflammatory reactions [17].

Urea and thiourea are versatile reagents in synthetic organic chemistry. They are important basic building blocks in the synthesis of heterocycles [18]. Thioureas and ureas (unsymmetrical or symmetrical) have drawn considerable attention due to their broad spectrum biological as well as pharmacological activities, and their application as pesticides [19]. A variety of thiourea and urea derivatives exhibited antimicrobial [20-21], antimalarial [22], analgesic, antiinflammatory [23], anticancer, antitumor [24], hypoglycemic [25], antituberculosis [26], and anti-HCV [27] activities. Despite having a large number of urease inhibitors being reported and marketed, there is still need of more potent inhibitors having less side effects and more efficacy. Since, our research group has been engaged in search of lead compounds as urease inhibitors. We have reported different classes of compounds as urease inhibitors including 5-nitroisatin-3-thiosemicarbazones, 1,3,4-oxazole-2(3*H*)-thiones, urea, and thiourea [28-29]. Therefore, in continuation of our research work and keeping in mind the reported urease inhibitory potential of urea and thiourea derivatives (**Figure-1**), we synthesized compounds **1**-**37** and screened them against urease enzyme. To the best of our knowledge, six compounds **1**, **2**, **25**, **28**, **32**, and **37** are known [30] while rest of the compounds are new.

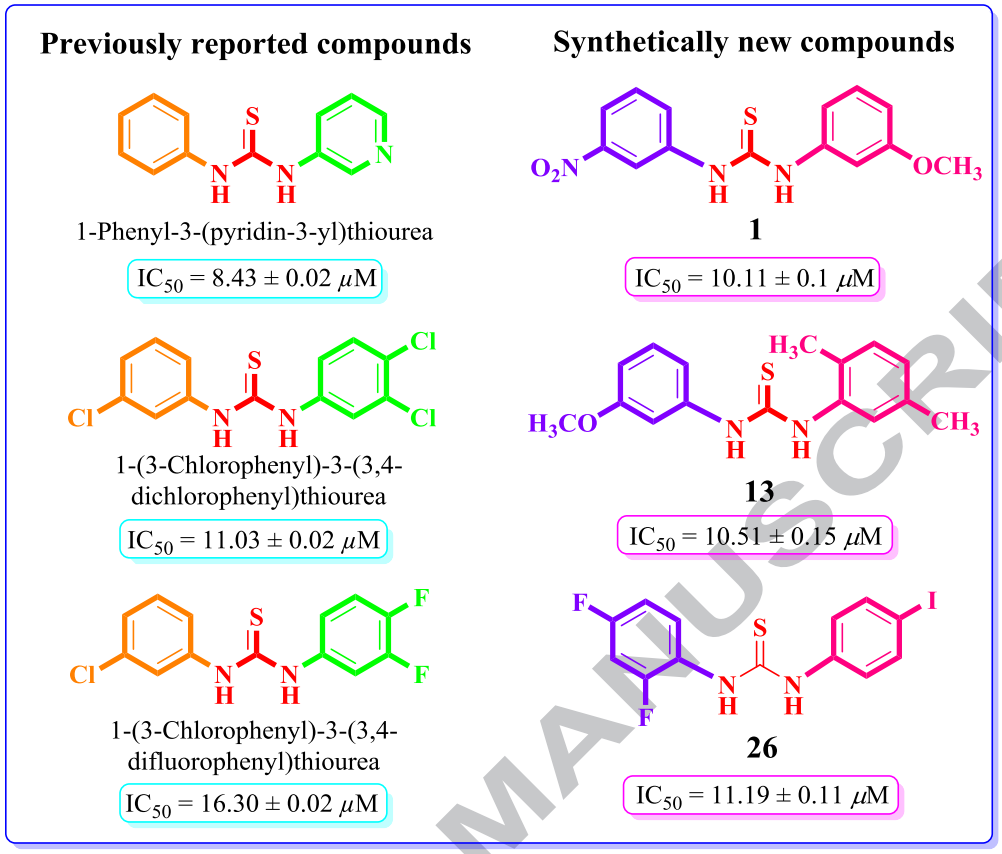
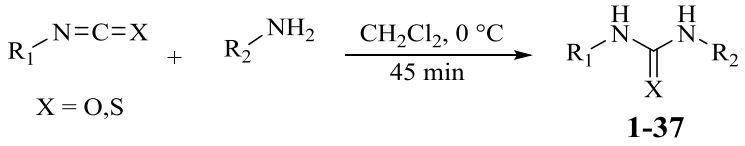


Figure-1: Rationale of the present study

Results and discussion

Chemistry

Urea and thiourea derivatives **1-37** were synthesized by the reaction of commercially available substituted phenyl isothiocyanate and isocyanate with a variety of substituted anilines in dichloromethane at 0 ˚C for 45 min according to literature procedure [31] as shown in **Scheme-1** (**Table-1**). In all experiments, precipitates were formed which were filtered and triturated with 10 mL of dichloromethane and excess of hexane. The product thus obtained was dried in vacuum and crystallized from methanol. The structures were determined using different spectroscopic methods such as FAB-MS, ^{1}H-, and ^{13}C-NMR.



1-37

Scheme-1: Synthesis of aryl urea and thiourea derivatives **1-37**

Spectral analysis of most active compound 1

NMR Spectroscopy

The ^{1}H-NMR was recorded in deuterated DMSO-d_6 on a 400 MHz instrument. A sharp singlet for NH appeared as the most downfield signal at δ_H 10.09. The molecule possess eight aromatic protons in which H-2′ resonated as singlet at δ_H 8.52. H-4′ resonated at δ_H 7.95 as doublet with coupling constant $J_{4',3'}$ = 8.0 Hz. H-6′ also appeared as doublet at δ_H 7.89 with coupling constant $J_{6',5'}$= 7.6 Hz showing *ortho* coupling with H-5′. A triplet of H-5′ appeared at δ_H 7.61 having coupling constant $J_{5'(4',6')}$ = 8.2 Hz, showing coupling with H-4′ and H-6′, respectively. Another triplet of H-5 was observed at δ_H 7.27 having coupling constant $J_{5(4,6)}$ = 8.0 Hz. A sharp singlet of H-2 appeared at δ_H 7.13. The doublet of H-6 was resonated at δ_H 7.01 showing *ortho* coupling with H-5 having coupling constant $J_{6,5}$ = 8.0 Hz. H-4 appeared at δ_H 6.74 as doublet showed coupling with H-5 with coupling constant $J_{4,5}$ = 7.6 Hz. The OCH_3 protons resonated at δ_H 3.73 as singlet as shown in **Figure-2A**.

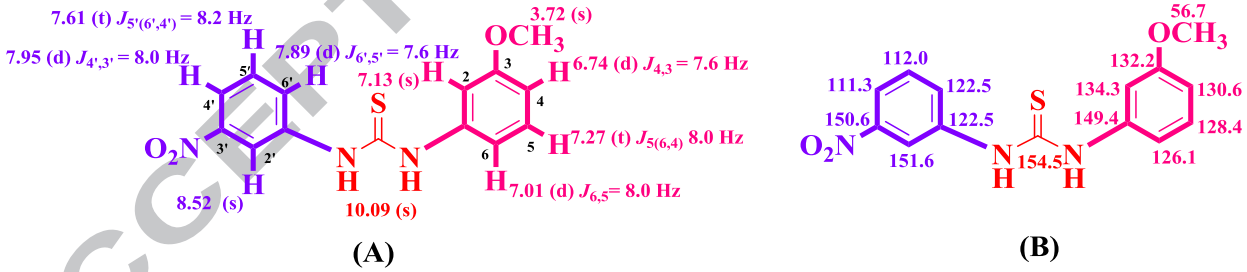


Figure-2: Representative ^{1}H- **(A)** and ^{13}C-NMR **(B)** chemical shift of compound **1**

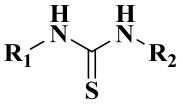
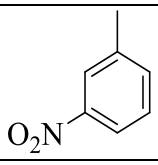
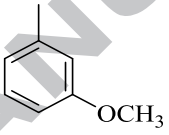
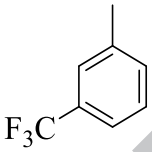
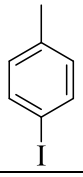
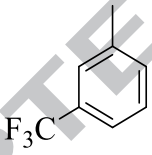
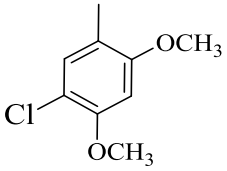
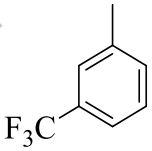
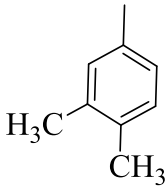
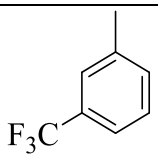
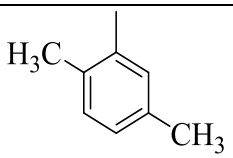
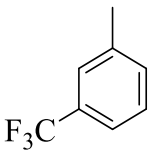
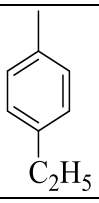
^{13}C-NMR broad-band decoupled spectrum (CD_3OD-d_4) showed total 14 carbon signals including eight methine, five quarternary, and one methoxy carbon. The most downfield signal was of thiocarbonyl group appeared at δ_C 154.5. Quarternary C-1, -3, -1′, and -3′ were resonated at δ_C 150.6, 149.4, 132.2, and 122.5, respectively. However, methine carbon C-2′ and C-2 appeared at δ_C 151.6 and δ_C 134.3, respectively. Signals of all remaining aromatic carbons appeared in the

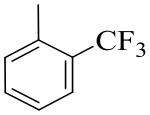
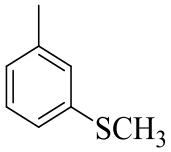
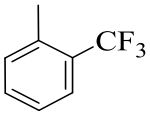
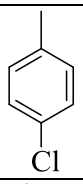
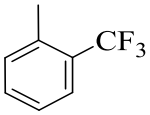
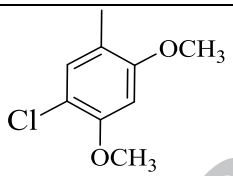
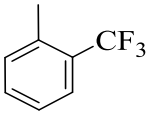
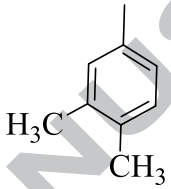
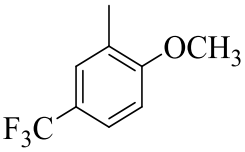
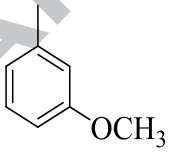
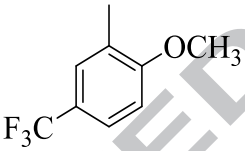
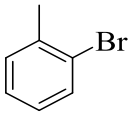
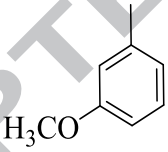
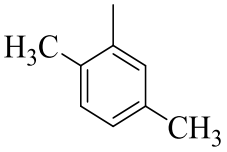
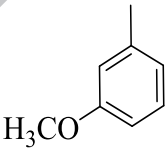
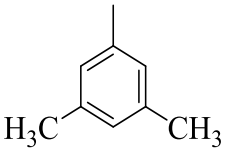
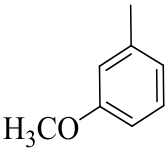
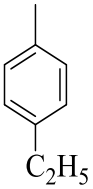
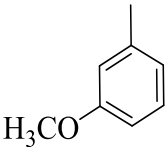
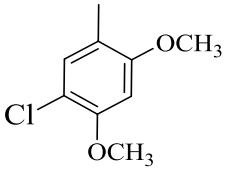
usual aromatic range δ_C 130.6-111.3. The most upfield signal belongs to methoxy carbon that appeared at δ_C 56.7 **Figure-2B**.

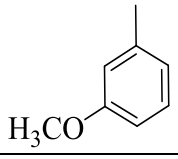
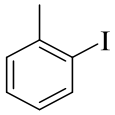
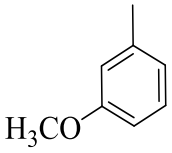
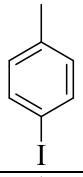
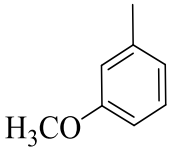
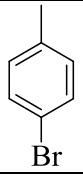
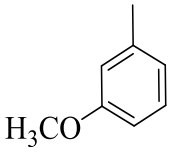
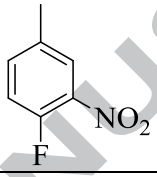
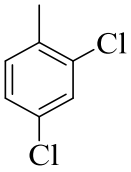
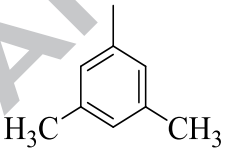
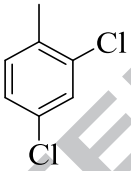
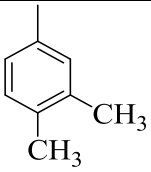
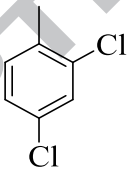
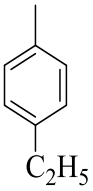
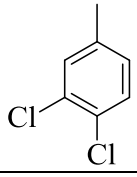
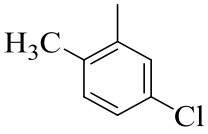
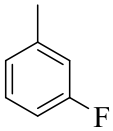
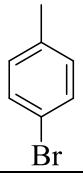
Mass Spectrometry

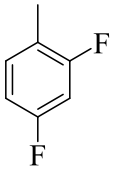
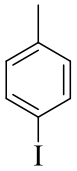
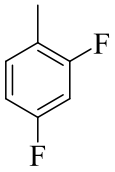
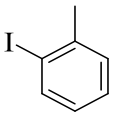
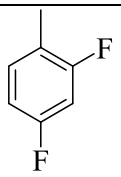
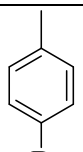
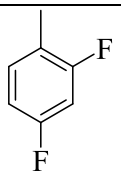
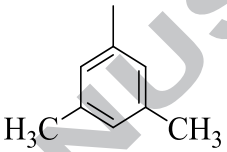
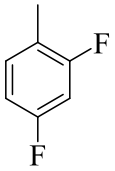
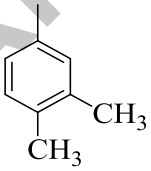
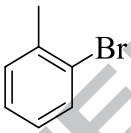
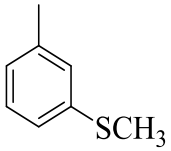
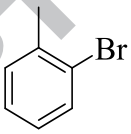
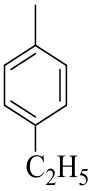
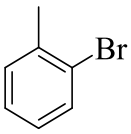
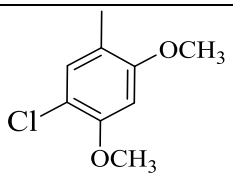
The compound **1** was unstable in EI-MS, thus was subjected to FAB-MS and showed the M+1 at *m/z* 304.

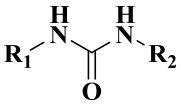
Table-1: Thiourea and urea derivatives **(1-37)**

Compounds	R_1	R_2	IC_{50} ± SEM[a] (μM)
 **Thiourea Derivatives**			
1			10.11 ± 0.11
2			11.24 ± 0.31
3			13.31 ± 0.15
4			19.48 ± 0.71
5			39.22 ± 0.61
6			61.86 ± 2.11

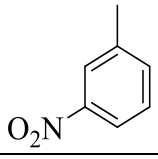
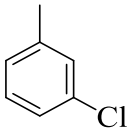
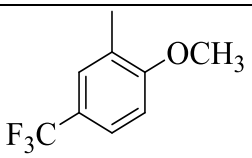
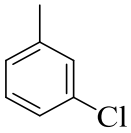
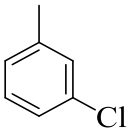
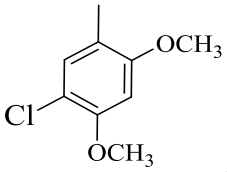
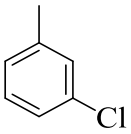
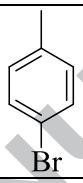
7			15.13 ± 0.71
8			69.80 ± 0.71
9			35.10 ± 2.11
10			67.12 ± 2.19
11			19.28 ± 0.31
12			22.23 ± 0.16
13			10.51 ± 0.15
14			17.32 ± 0.29
15			72.99 ± 3.71
16			17.11 ± 0.25

17			18.44 ± 0.61
18			60.33 ± 2.71
19			51.21 ± 0.11
20			67.42 ± 1.01
21			14.23 ± 0.31
22			17.00 ± 0.21
23			15.43 ± 0.36
24			19.16 ± 0.11
25			15.41 ± 0.15

26			11.19 ± 0.11
27			19.25 ± 0.21
28			17.29 ± 0.31
29			13.30 ± 0.11
30			18.19 ± 0.16
31			19.22 ± 0.31
32			23.01 ± 0.61
33			39.70 ± 0.71
Compounds	**R_1**	**R_2**	**IC_{50} ± SEM[a] (μM)**



Urea Derivatives

34			12.19 ± 0.15
35			13.34 ± 0.01
36			12.78 ± 0.18
37			17.12 ± 0.31
Acetohydroxamic acid[b]		27.0 ± 0.5	

SEM[a] is the standard error of the mean, Acetohydroxamic Acid[b] is the standard inhibitor for urease enzyme

Structure-activity relationship (SAR)

Synthetic compounds **1-37** were divide into two classes such as thiourea and urea derivatives. In general, differences in the activity of compounds were observed due to varying substitutions and positions at rings **A** and **B**. Mostly, the substituents at ring **B** are varied. To understand the better structure-activity relationship (SAR) of synthetic molecules with the enzyme binding site, the synthetic compounds were divided in three different categories based on substitutions at ring **A**, *i.e.* category (**i**) comprises of electron withdrawing substituents (nitro and trifluoromethyl), category (**ii**) with electron donating groups (methoxy), and category (**iii**) having halogen substituents (F, Cl, and Br), directly attached on the benzene ring).

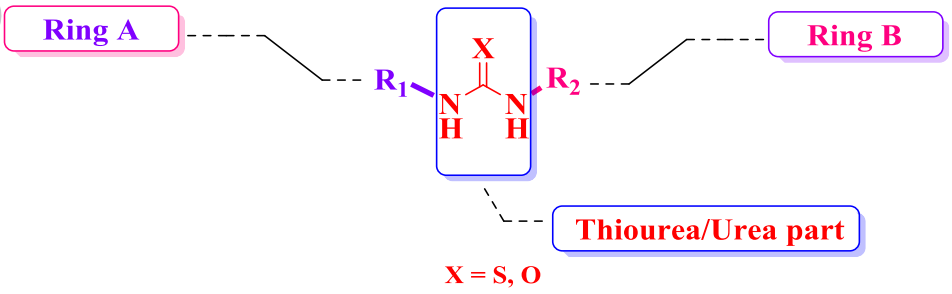
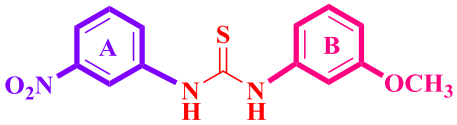


Figure-3: General structure of compounds

Nitro and trifluoromethyl substituted groups (Category i):

The highly active compound **1** (IC_{50} = 10.11 ± 0.11 μM) with nitro and methoxy groups at *meta* position of ring **A** and **B**, respectively, was found to be almost three-folds better active than the standard acetohydroxamic acid (IC_{50} = 27.0 ± 0.5 μM). The enhanced activity of this compound might be due to the interaction of nitro group with the active site of enzyme (**Figure-4**).

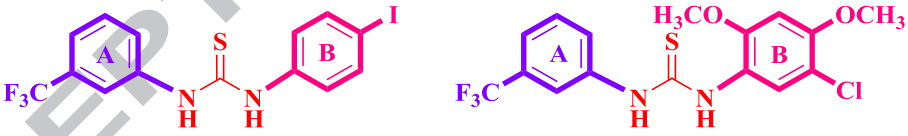


1

IC_{50} = 10.11 ± 0.1 μM

Figure-4: Structure-activity relationship of compound **1**

Compound **2** (IC_{50} = 11.24 ± 0.31 μM) having trifluoromethyl substituent at *meta* position of ring **A** and iodo group at *para* position of ring **B** also showed potent activity. The variation at ring **B** in compound **3** (IC_{50} = 13.31 ± 0.15 μM) bearing one chloro and two methoxy groups at *ortho*, *para*, and *meta* positions, respectively, resulted in the decreased activity as compared to compound **2**, but better than the standard acetohydroxamic acid (IC_{50} = 27.0 ± 0.5 μM). It indicates that the decline in activity is due to the addition of electron donating groups along with halogen (**Figure-5**).



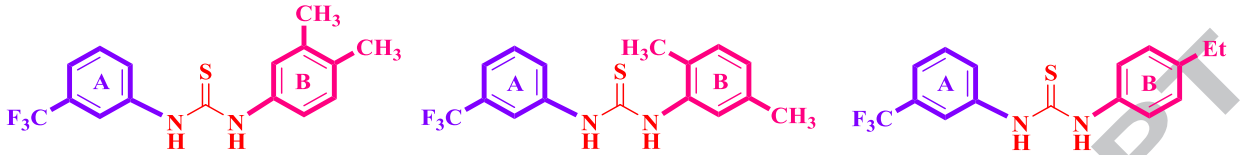
2 IC_{50} = 11.24 ± 0.31 μM

3 IC_{50} = 13.31 ± 0.15 μM

Figure-5: Structure-activity relationship of compounds **2** and **3**

Compounds **4** (IC_{50} = 19.48 ± 0.71 μM) and **5** (IC_{50} = 39.22 ± 0.61 μM) having CF_3 at *meta* position of ring **A** and two methyl groups at different positions of ring **B**. Amongst them, compound **4** having methyl groups at *meta* and *para* positions, respectively, showed good activity, however, the change in position of the methyl groups in compound **5** resulted in two folds decreased activity and it was further lost when two methyl groups were replaced with an ethyl group in compound **6** (IC_{50} = 61.86 ± 2.11 μM). Hence, the above activity pattern revealed

that the positions and nature of electron donating substituents at ring **B** matters a lot for good activity (**Figure-6**).



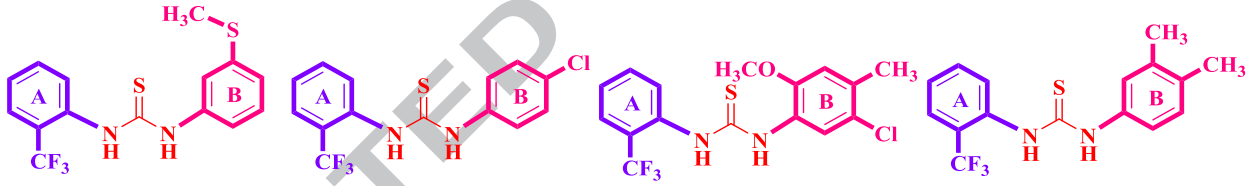
4
IC_{50} = 19.48 ± 0.71 μM

5
IC_{50} = 39.22 ± 0.61 μM

6
IC_{50} = 61.86 ± 2.11 μM

Figure-6: Structure-activity relationship of compounds **4**, **5**, and **6**

Compounds **7**, **8**, **9**, and **10** with trifluoromethyl group at *ortho* position of ring **A** also displayed good to moderate activity. Compound **7** (IC_{50} = 15.13 ± 0.71 μM) bearing thiomethyl group at *meta* position of ring **B** exhibited better potential as compared to the standard acetohydroxamic acid (IC_{50} = 27.0 ± 0.5 μM). The *para* chloro substituted compound **8** (IC_{50} = 69.80 ± 0.71 μM) showed lesser activity, however, mixed substitution of chloro with methoxy in compound **9** (IC_{50} = 35.10 ± 2.11 μM) resulted in good activity as compared to compound **8**. Compound **10** (IC_{50} = 67.12 ± 2.19 μM) having dimethyl aryl part was also weakly active (**Figure-7**).



7
IC_{50} = 15.13 ± 0.71 μM

8
IC_{50} = 69.80 ± 0.71 μM

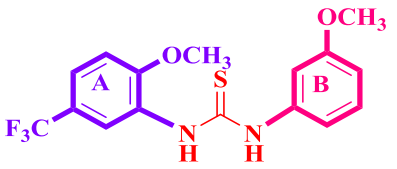
9
IC_{50} = 35.10 ± 2.11 μM

10
IC_{50} = 67.12 ± 2.19 μM

Figure-7: Structure-activity relationship of compounds **7**, **8**, **9**, and **10**

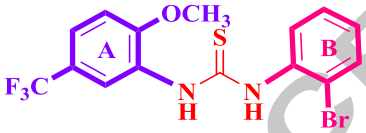
Among synthetic derivatives with 2-methoxy and 5-trifluoromethyl substitutions at ring **A**. Compound **11** (IC_{50} = 19.28 ± 0.31 μM) with methoxy group at *meta* position showed good activity. The replacement of methoxy with bromo at *ortho* position as in compound **12** (IC_{50} = 22.23 ± 0.16 μM), a decreased activity was observed in contrast to compound **11**, however, it remains more active than the standard acetohydroxamic acid (IC_{50} = 27.0 ± 0.5 μM) (**Figure-8**). Activities of compounds **11** and **12** can also be compared with compounds **3** (IC_{50} = 13.31 ± 0.15 μM) which lacks electron donating methoxy group at ring **A** but have a combination of halogen with methoxy groups on ring **B** was found to be more active. Which suggest that combination of electron donating groups with positive mesomeric effect on ring B such as halogen and methoxy

favors the good activity. Similarly, it further confirms by comparing the activities of **11** and **12** with compound **16** which lacks electron withdrawing trifluoromethyl at ring **A** but having combination of halogen and methoxy groups, was again found to be more active. Which further confirms the active participation of electron donating groups with positive mesomeric effect on ring **B**.



11

IC_{50} = 19.28 ± 0.31 μM



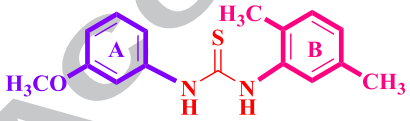
12

IC_{50} = 22.23 ± 0.16 μM

Figure-8: Structure-activity relationship of compounds **11** and **12**

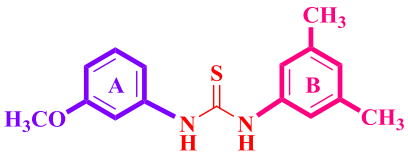
Methoxy substituted compounds (Category ii)

This category of compounds have methoxy group at *meta* position of ring **A**, among them compound **13** (IC_{50} = 10.51± 0.15 μM) with methyl substitutions at *ortho* and *meta* positions of ring **B** was the second most active compound of the series, this may be due to the interaction of methoxy and methyl groups with the active site of enzyme. However, positional difference of methyl substituents at ring **B** in compound **14** (IC_{50} = 17.32 ± 0.29 μM) resulted in decreased activity. The replacement of both methyl groups with a ethyl at *para* position of ring **B** leads to decreased activity as in compound **15** (IC_{50} = 72.99 ± 3.71 μM). It showed that methyl groups at particular positions are positively contributing in the activity (**Figure-9**).



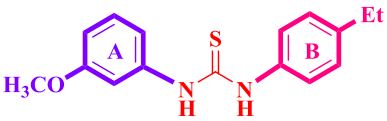
13

IC_{50} = 10.51 ± 0.15 μM



14

IC_{50} = 17.32 ± 0.29 μM



15

IC_{50} = 72.99 ± 3.71 μM

Figure-9: Structure-activity relationship of compounds **13**, **14**, and **15**

Among halogen substituted compounds at ring **B**, compound **16** (IC_{50} = 17.11 ± 0.25 μM) was found to have potent activity, this may be due to the presence of methoxy substituent along with

chloro group which enhanced the activity of compound as compared to its other halogen substituted analogues. The activity was slightly decreased in compound **17** (IC_{50} = 18.44 ± 0.61 μM) bearing iodo group, however, a sharp decline in the activity was observed when the position of iodo was changed from *ortho* to *para* as in compound **18** (IC_{50} = 60.33 ± 2.71 μM). Compounds **19** (IC_{50} = 51.21 ± 0.11 μM) and **20** (IC_{50} = 67.42 ± 1.01 μM) with bromo, fluoro, and nitro substitutions also showed weak activity as compared to the standard (**Figure-10**).

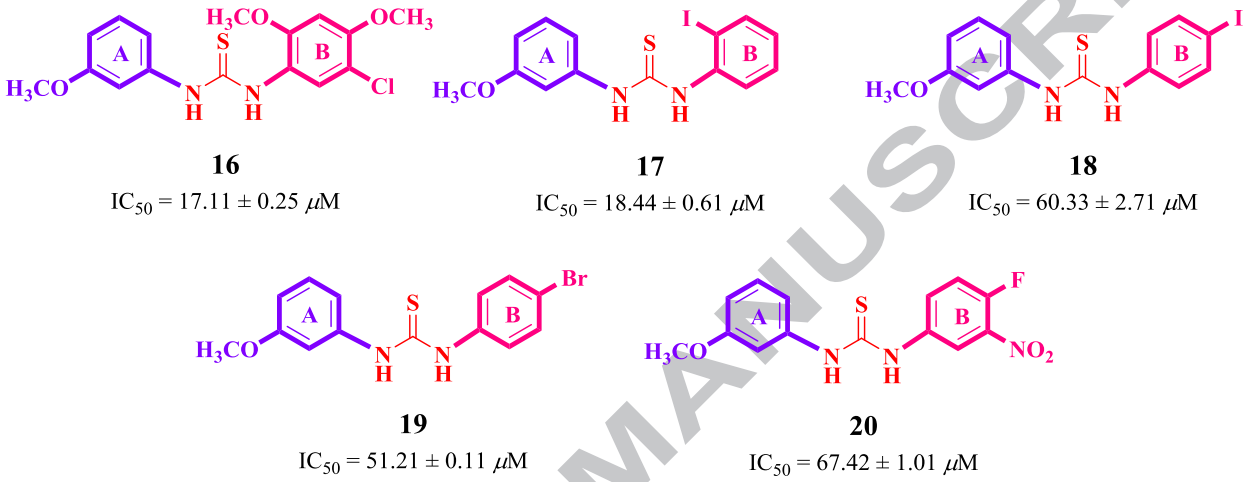
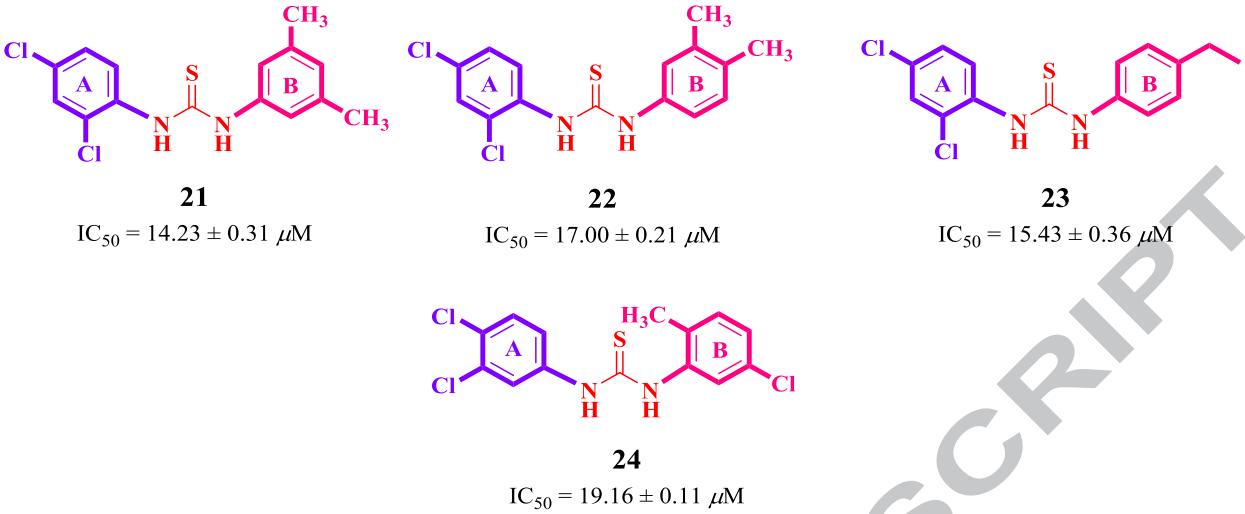


Figure-10: Structure-activity relationship of compounds **16**, **17**, **18**, **19**, and **20**

Halogen substituted compounds (iii)

The synthetic compounds with halogen substitution at ring **A** showed potential inhibitory activity and all compounds **21-33** were more active than the standard acetohydroxamic acid (IC_{50} = 27.00 ± 0.5 μM) except compound **33**.

The dichloro groups at ring **A** also enhanced the activity of compounds, among them compound **21** (IC_{50} = 14.23 ± 0.31 μM) with dimethyl substitutions at ring **B** was found to have superior activity. The change in positions of these methyl groups in compound **22** (IC_{50} = 17.00 ± 0.21 μM) resulted in lesser activity. Interestingly, compound **23** (IC_{50} = 15.43 ± 0.36 μM) having ethyl group at *para* position showed better activity, however, in the previous categories the substitution of ethyl group at ring **B** resulted in decreased activity. Compound **24** (IC_{50} = 19.16 ± 0.11 μM) with two chloro groups at *meta* and *para* positions of ring **A** and methyl and chloro groups at *ortho* and *meta* positions of ring **B**, respectively, showed better potential as compared to the standard acetohydroxamic acid (IC_{50} = 27.00 ± 0.5 μM) (**Figure-11**).



21
IC_{50} = 14.23 ± 0.31 μM

22
IC_{50} = 17.00 ± 0.21 μM

23
IC_{50} = 15.43 ± 0.36 μM

24
IC_{50} = 19.16 ± 0.11 μM

Figure-11: Structure-activity relationship of compounds **21**, **22**, **23**, and **24**

Mono fluoro compound **25** (IC_{50} = 15.41 ± 0.15 μM) with bromo group at the *para* position of ring **B** was found to have good activity. Addition of another fluoro group at ring **A** and iodo at *para* position of ring **B** in compound **26** (IC_{50} = 11.19 ± 0.11 μM) resulted in better activity which may be due to the addition of fluoro and iodo groups. The activity of compound **27** (IC_{50} = 19.25 ± 0.21 μM) was decreased by changing the position of iodo from *para* to *ortho*. The bromo substituted derivative **28** (IC_{50} = 17.29 ± 0.31 μM) showed good activity but less active than compound **26**. Compounds **29** (IC_{50} = 13.30 ± 0.11 μM) and **30** (IC_{50} = 18.19 ± 0.11 μM) exhibited good inhibitory activity than standard. Both of these compounds were positional isomers with respect to the positions of methyl group at ring **B**, in compound **29** the methyl groups were at both *meta* positions whereas in compound **30** with methyl groups at *meta* and *para* positions. This showed that particular positions of substituents at ring **B** are actively contributing in the activity (**Figure-12**).

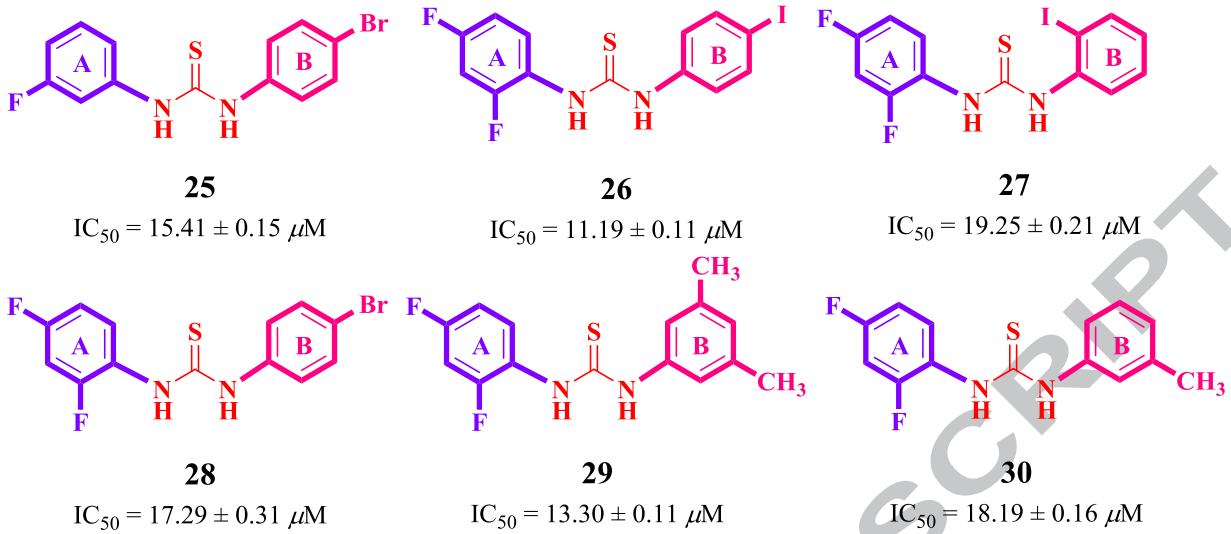


Figure-12: Structure-activity relationship of compounds **25**, **26**, **27**, **28**, **29**, and **30**

Among bromo substituted compounds, bearing bromo at *meta* position of ring **A**, compound **31** (IC_{50} = 19.22 ± 0.31 μM) with thiomethyl group at *meta* position of ring **B** was most active might be due to the interaction of the thiomethyl group with the active site. However, compound **32** (IC_{50} = 23.01 ± 0.61 μM) with ethyl group at *para* position exhibited less activity as compared to compound **31** even though it was more active than the standard acetohydroxamic acid (IC_{50} = 27.00 ± 0.5 μM). Compound **33** (IC_{50} = 39.70 ± 0.71 μM) bearing two methoxy and one chloro group at *ortho*, *para*, and *meta* positions of ring **B**, respectively, was found to be less active than the standard **Figure-13**.

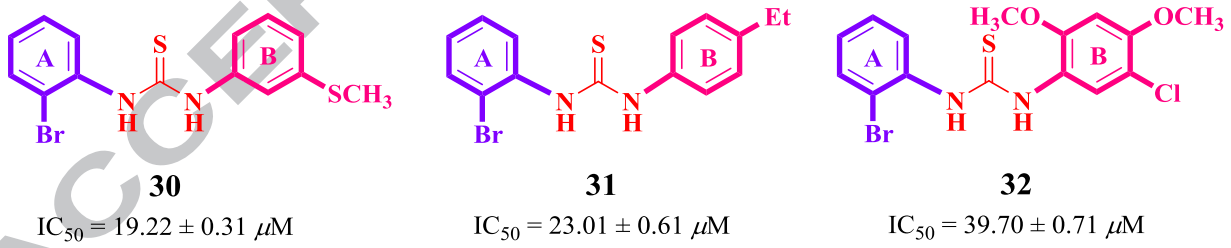


Figure-13: Structure-activity relationship of compounds **30**, **31**, and **32**

Urea Derivatives:

Urea derivatives also showed good inhibitory potential than standard acetohydroxamic acid (IC_{50} = 27.00 ± 0.5 μM), among them compound **34** (IC_{50} = 12.19 ± 0.15 μM) with chloro at *meta*

position of ring **B** and nitro at *meta* position of ring **A** was most active. However, compound **35** (IC_{50} = 13.34 ± 0.01 μM) having triflouro methyl and chloro group at ring **A** and *meta* chloro at ring **B** was found to be less active. Compound **36** (IC_{50} = 12.78 ± 0.18 μM) containing a *meta* chloro at ring **A** as well as *meta* chloro and two dimethoxy groups at *ortho* and *para* positions of ring **B** was found to be second most active among urea derivatives probably due to substitutions at ring **B**. The replacement of chloro with bromo and ejection of dimethoxy groups from ring **B** resulted in decreased activity in compound **37** (IC_{50} = 17.12 ± 0.31 μM) but it exhibited better activity than the standard (**Figure-14**).

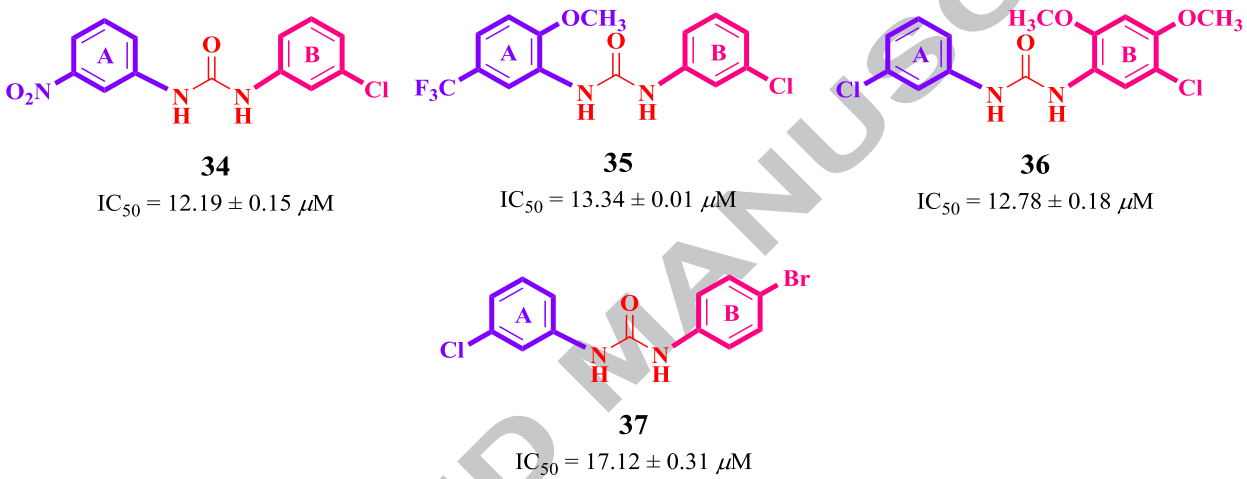


Figure-14: Structure-activity relationship of compounds **34**, **35**, **36**, and **37**

Molecular Docking Studies

Molecular docking studies were carried out to support the *in vitro* studies of the synthetic compounds. Enzyme selected for docking studies was downloaded from RCSB Protein Data Bank (PDB ID; 4UBP). The size of the grid was selected keeping in view the size of the ligands and the important residues of the enzyme *i.e.* H137, H139, A170, K220, H249, H275, G280, C322, H323, H324, R339, D363, A364 Ni798 and Ni799. The urease enzyme and the conformation adopted by the ligands in the active site is shown in **Figure-15**.

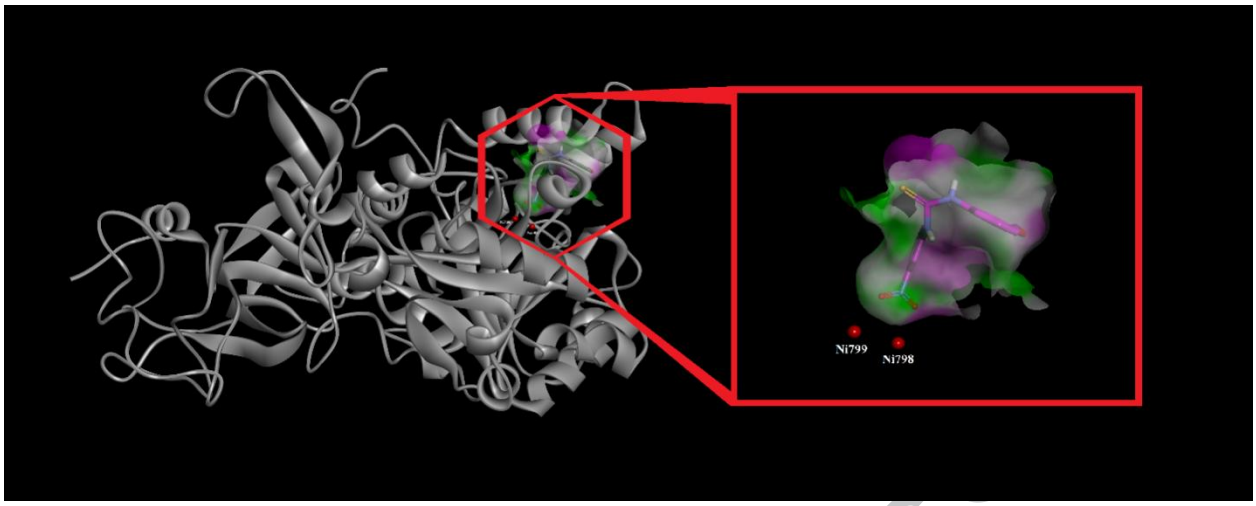


Figure-15: Urease from *Bacillus pasteurii* (Ribbon form in grey color), actives site of urease (enclosed in red color) and zoomed in docked conformation of compound **1** (Lilac colored sticks along with Nickle atoms colored in red)

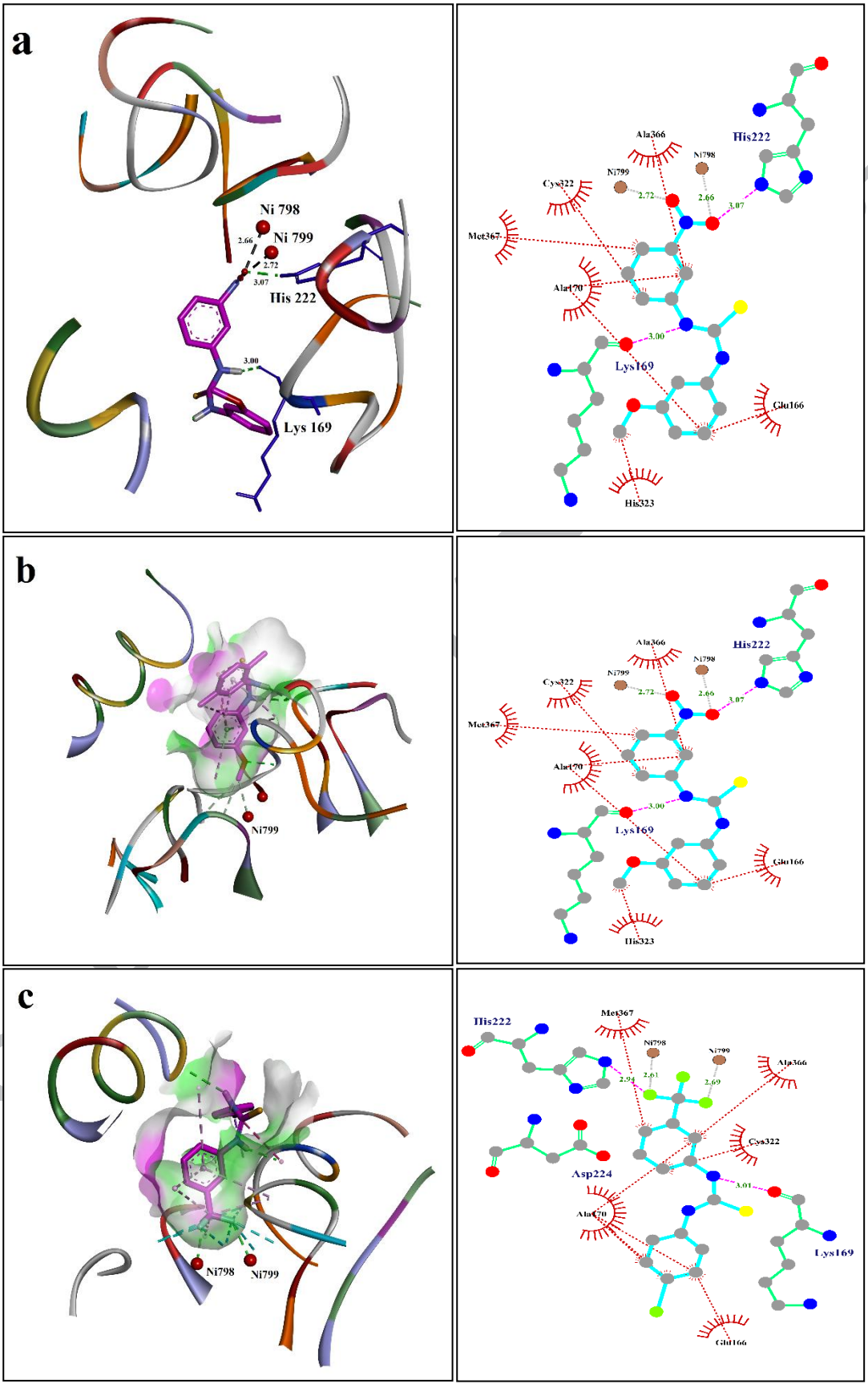
The top five most active compounds *i.e.* **1**, **13**, **2**, **29**, and **3** among the series were analyzed for their interactions with the active site of urease enzyme. Compound **1** with IC_{50} = 10.11 ± 0.11 μM was found forming different hydrogen bond and hydrophobic interactions with the side chain residues of active site. Two hydrogen bond interactions with a distance of 3.07 Å and 3.0 Å were formed by oxygen of nitro group as a hydrogen bond acceptor (HBA) and NH group of thiourea as a hydrogen bond donor (HBD) with the NH of imidazole and carbonyl oxygen of the side chain His222 and Lys169, respectively. In addition, the oxygens of nitro group at variable phenyl ring mediated metal bond interactions with both nickle atoms *i.e.* Ni 798 (2.66 Å) and Ni 799 (2.72 Å), respectively. Other interactions found in complex are shown in **Figure-16a**.

Compound **13** (IC_{50} = 10.51 ± 0.15 μM was found mediating three hydrogen bond interactions with the side chain amino acids of active site. A hydrogen bond interaction (3.29 Å) was formed between oxygen of methoxy and NH group of side chain His222 and two hydrogen bond interactions between both NH groups of thiourea and carboxyl oxygens of side chain Asp224 with a distance of 3.01 Å and 3.32 Å, respectively. The carbon of methoxy group at phenyl ring mediated metal bond interaction with Ni799 (3.06 Å). In addition, same carbon atom of methoxy group was found forming carbon bonding with the carboxy oxygens of KCX220 (3.76 Å) and Asp363 (mean = 3.69 Å). Other interactions found in stabilizing the complex were π-π stacked interaction between methoxybenzene ring and side chain His323, π-sulfur interaction between

sulfur of thiourea and side chain His323, π-cation interaction between methoxybenzene ring and side chain Arg339 and π-alkyl and alkyl interactions as shown in **Figure-16b.**

Third most active compound **2** among the series (IC_{50} = 11.24 ± 0.31 μM) was found forming two hydrogen bond interactions, first between NH group of thiourea as HBD and carbonyl oxygen of side chain Lys169 (3.01 Å), and second between fluorine atom of trifluoromethyl group and NH of imidazole ring as HBD of side chain His222 (2.94 Å) as shown in **Figure-16c**. Fluorine atoms of trifluoromethyl group also mediated metal acceptor interactions with Ni798 and Ni799 with a bond distance of 2.61 Å and 2.69 Å, respectively. Other interactions spotted between compound **2** and side chain residues of the active site are shown in **Figure-16c**.

Analysis of least energy conformations adopted by fourth most active compound **29** (IC_{50} = 13.30 ± 0.11 μM) and fifth most active compound **3** (IC_{50} = 13.31 ± 0.15 μM) in the binding site of urease showed that both were forming two hydrogen bond interactions (each) with the side chain residues. Also, both compounds are forming metal interactions with the nickle atoms of the binding site as shown in **Figure-16d** and **Figure-16e**. Summarizing the interactions of five most active compounds we can conclude that their high activities could be due to formation of interactions with the nickle atoms which are considered as the catalytic residues as they increase electrophilicity of carbonyl carbon of urea and nucleophilicity of water molecule during the hydrolysis of urea [32].



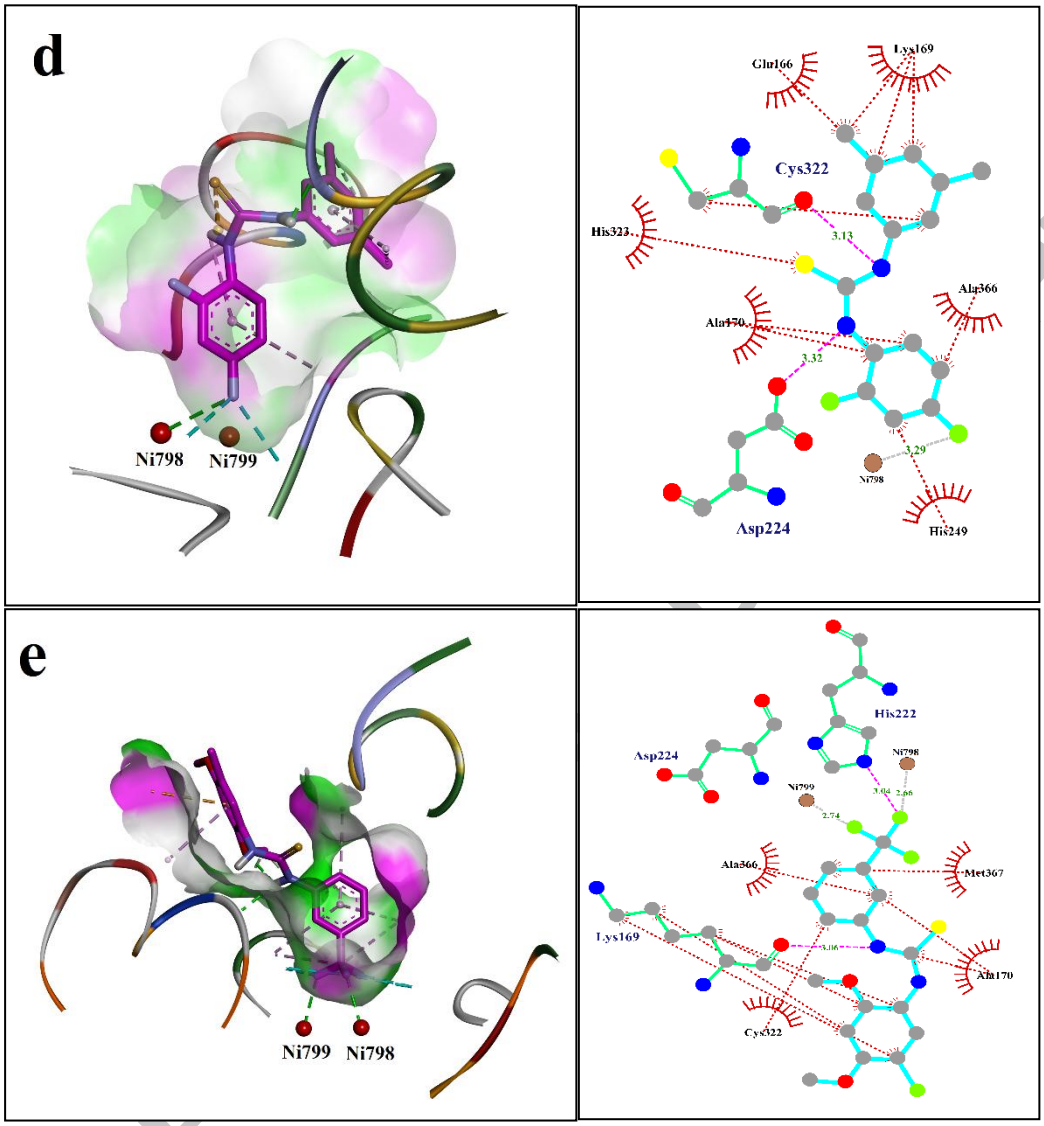


Figure-16: The ligand-protein interactions of most active compounds **(a) 1**, **(b) 13**, **(c) 2**, **(d) 29**, and **(e) 3** with the active site of urease from *Bacillus pasteurii* (4UBP) created by using Discovery Studio 17.2 and LigPlot^{+}. The left side displays 3D interactions of the compounds in the binding site. The right side shows the 2D interaction patterns. Dashed lines show the interactions among the ligand and the amino acids of the protein. Distances are in Angstrom.

Analyzing poses adopted by average active compound **9** (IC_{50} = 35.10 ± 2.11 μM) and least active compounds **18** (IC_{50} = 60.33 ± 2.71 μM) and **8** (IC_{50} = 69.80 ± 2.71 μM), it was found that these compounds were also involved in forming hydrogen bond interactions and other hydrophobic interactions which were observed in case of most active derivatives. The interactions which were not detected in least active compounds were the interactions of these compounds with the metal atoms in the active site of urease enzyme as shown in **Figure-17**.

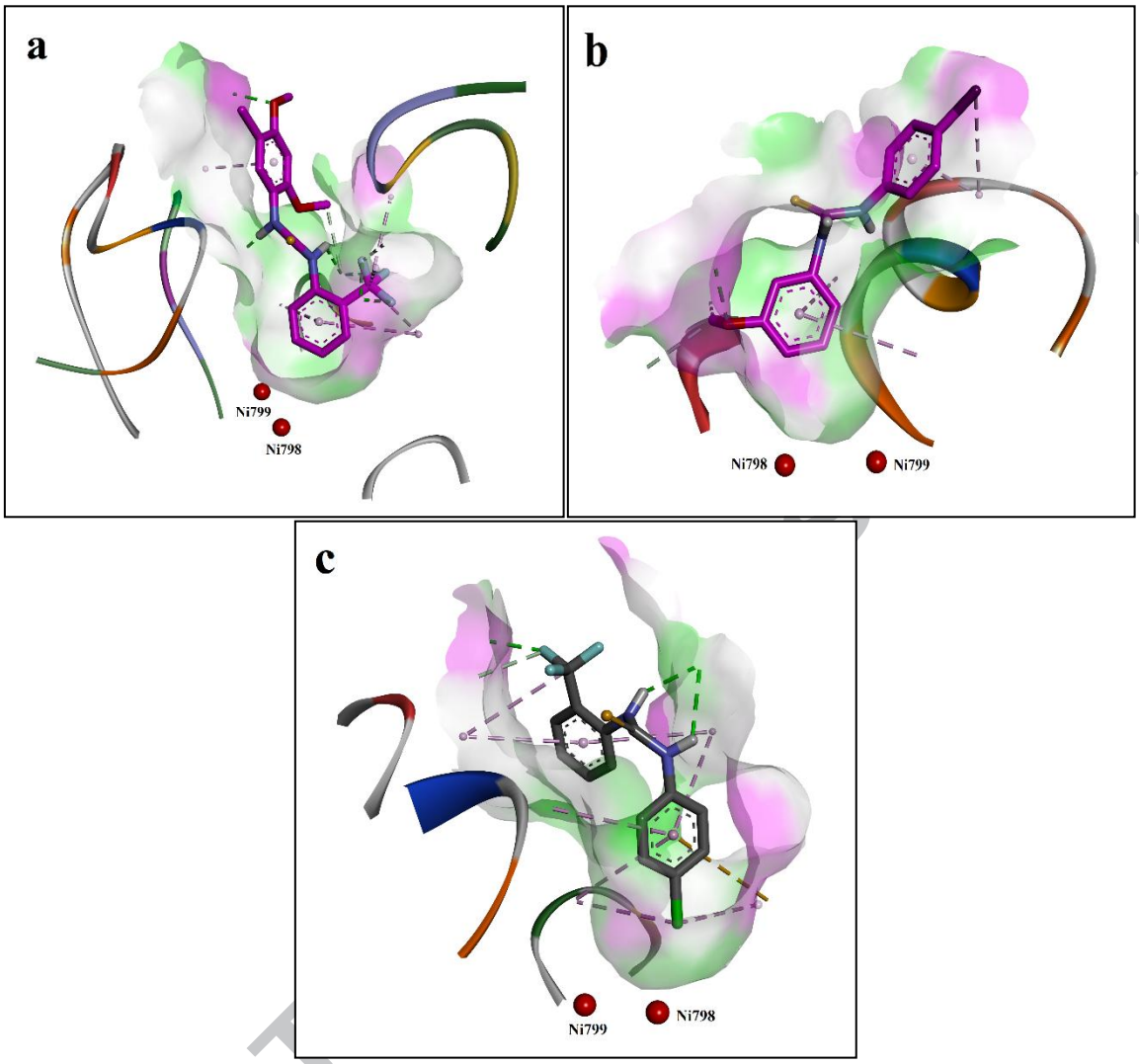


Figure-17: Three-Dimensional ligand-protein interactions of average active **(a) 9** and least active *i.e.* **(b) 18** and **(c) 8** compounds with the active site of urease from *Bacillus pasteurii* (4UBP) created by using Discovery Studio 17.2.

Concluding computational studies, it can be summarized that the potential of inhibition exhibited by synthetic compounds depends on the nature and position of substituents on both variable phenyl ring. Derivatives having substituents in direct interaction with the catalytic nickle atoms of enzyme's active site showed the highest inhibition among the series. In our study nitro groups, and halogens mainly made direct interactions with nickle atoms and it was also found that substituents at *meta* and *para* positions mainly resulted in minimizing the complex energy and this could be due to the fact that these two positions get closer to the nickle atoms as compared to substituent at *ortho* position. The substituents on the second variable ring also influence the activity and receptor ligand interactions *i.e.* substituents involved in forming hydrophobic

interactions and halogen interactions (particularly large size halogens) were found more active. Thiourea moiety also played a major role in stabilizing the complex as its NH groups acted as hydrogen bond donors. In some results, sulfur of thiourea group was also spotted forming π-sulfur interactions with the side chain amino acids particularly histamines, a well-known interaction playing important role in protein folding and stabilization.

Conclusion:

Urea and thiourea derivatives **1**-**37** were synthesized and evaluated for their urease inhibitory activity. Amongst synthetic compounds twenty-five compounds exhibited good inhibitory potential. Compounds **1** (IC_{50} = 1.13 ± 0.01 μM), **2** (IC_{50} = 11.24 ± 0.31 μM), **3** (IC_{50} = 13.31 ± 0.15 μM), **13** (IC_{50} = 10.51 ± 0.15 μM), and **29** (IC_{50} = 13.30 ± 0.11 μM) were found to be most active than the standard acetohydroxamic acid (IC_{50} = 27.0 ± 0.5 μM). Structure-activity relationship revealed that the different position of substituents mainly the *meta* and *para* substitutions at rings **A** and **B**, respectively, resulted in variable urease inhibitory activity. The docking studies further validated these results and showed efficient binding interaction between the active compound and urease enzyme proteins. Conclusively, a number of new lead compounds are identified as urease inhibitors which may be proceeded for further studies in search for better inhibitors.

Material and Methods:

^{1}H- and ^{13}C-NMR spectra were recorded on Bruker 300, 400, 75, and 100 MHz spectrometers, respectively. Mass experiments were carried out on a Finnigan MAT-311A (Germany) mass spectrometer. Thin-layer chromatography (TLC) was monitored on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Visualization of TLC chromatograms was performed by UV light at wavelengths of 254 and 365 nm. Dichloromethane of analytical grade was used as received from supplier RCI Labscan Limited, Thailand. Aniline and all isocyanates and isothiocyanates derivatives of analytical grades were used as received from the suppliers.

General procedure for the synthesis of compounds 1-37

Different substituted anilines (1 mmol) were dissolved in dichloromethane at 0 ˚C, reaction mixture was stirred for 10-15 min then isocyanates or isothiocayanates (1 mmol) were added, progress of reaction was monitored with TLC. After 45 min, a solid mass was appeared which was filtered, washed with hexane, triturated with 10 mL of dichloromethane and 50 mL of hexane and dried under vacuum. Crystallization from ethanol get the desired solid thiourea/urea. The structural determination was carried out by ^{1}H-, ^{13}C-NMR, and mass spectrometry.

Spectral Data for the Synthetic Compounds 1-37

1-(3-Methoxyphenyl)-3-(3-nitrophenyl)thiourea (1)

Yield: 75%, M.p.: 170-174°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.09 (s, 2H, NH), 8.52 (s, 1H, H-2'), 7.95 (d, $J_{4',5'}$ = 8.0 Hz, 1H, H-4') ,7.89 (d, $J_{6',5'}$ = 7.6 Hz, 1H, H-6'), 7.61 (t, $J_{5'(4',6')}$ = 8.0 Hz , 1H, H-5'), 7.27 (t, $J_{5(4,3)}$ = 8.0 Hz, 1H, H-5), 7.13 (s, 1H, H-2) ,7.01 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), 6.74 (d, $J_{4,5}$ = 7.6 Hz, 1H, H-4), 3.73 (s, 3H, OCH_3), ^{13}C-NMR (100 MHz, DMSO-d_6): δ154.5, 151.6, 150.6, 149.4, 134.3, 132.2, 130.6, 128.4, 126.1, 122.5, 122.5, 112.0, 111.3, 56.7. FAB^{+}: 304 (M+1)

1-(4-Iodophenyl)-3-(3-(trifluoromethyl)phenyl)thiourea (2)

Yield: 65%, M.p.: 231-235 $^{\circ}$C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.04 (s, 2H, NH), 7.92 (s, 1H, H-2), 7.74 (d, $J_{4,3}$ = 8.0 Hz, 1H, H-4), 7.68(d, $J_{5',6'}$ = $J_{2',3'}$ = 8.4 Hz, 2H, H-3'/H-5'), 7.57 (t, $J_{6(4,5)}$ = 7.6 Hz, 1H, H-6), 7.31 (d, $J_{6',5'}$ = $J_{2',3'}$ = 8.4 Hz, 2H, H-2'/H-6'), ^{13}C-NMR (100 MHz, DMSO-d_6): δ 163.2, 158.6, 146.4, 146.6, 141.6, 133.1, 130.5, 129.2, 128.7, 128.2, 127.8, 127.3, 125.1, 122.7, 120.9, 118.7, FAB^{+}: 423 (M+1).

1-(5-Chloro-2,4-dimethoxyphenyl)-3-(3-(trifluoromethyl) phenyl)thiourea (3)

Yield: 55%, M.p.: 172-176 $^{\circ}$C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.95 (s, 1H, NH), 9.31(s, 1H, NH), 7.99 (s, 1H, H-2), 7.75 (d, $J_{4,5}$ = 8.0 Hz, 1H, H-4), 7.64 (s, 1H, H-6'), 7.55 (t, $J_{5(4,6)}$ = 7.8 Hz, 1H, H-5), 7.44 (d, $J_{6,5}$ = 7.6 Hz, 1H, H-6), 6.85 (s, 1H, H-3'), FAB^{+}: 391 (M+1).

1-(3,4-Dimethylphenyl)-3-(3-(trifluoromethyl) phenyl)thiourea (4)

Yield: 85%, M.p.: 231-234 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.82 (s, 2H, NH), 7.93 (s, 1H, H-2), 7.73 (d, $J_{4,5}$ = 8.4 Hz, 1H, H-4), 7.52 (t, $J_{5(4,3)}$ = 7.8 Hz, 1H, H-5), 7.43 (d, $J_{6,5}$ = 7.6 Hz, 1H, H-6), 7.17 (ovp, 3H, H-5/H-6/H-2), 2.19 (s, 6H,CH_3), FAB$^+$: 325 (M+1).

1-(2,5-Dimethylphenyl)-3-(3-(trifluoromethyl) phenyl)thiourea (5)

Yield: 64%, [1] M.p.: 166-170 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.95(s, 1H, NH), 9.85 (s, 1H, NH), 7.94 (s, 1H, H-2), 7.73 (d, $J_{4,5}$ = 8.5 Hz, 1H, H-4), 7.54 (t, $J_{5(4,6)}$ = 8.0 Hz, 1H, H-5), 7.44 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), 7.10 (ovp, 1H, H-3'), 7.07 (s, 1H, H-6'), 6.89 (dd, $J_{4',3'}$ = 8.0 Hz, $J_{4',6'}$ = 2.0 Hz, 1H, H-4'), 3.74 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), FAB$^+$: 323 (M+1).

1-(4-Ethylphenyl)-3-(3-(trifluoromethyl) phenyl)thiourea (6)

Yield: 65%, M.p.: 249-251 °C; ^{1}H-NMR (400 MHz, MeOD-d_4): δ 7.85 (s, 1H, H-2), 7.68 (d, $J_{4,5}$ = 8.0 Hz, 1H, H-4), 7.51 (t, $J_{5(4,6)}$ = 7.8 Hz, 1H, H-5), 7.43 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), 7.31 (d, $J_{5,6}$ = $J_{6,5}$ = 8.4 Hz, 2H, H-5/H-6), 7.22 (d, $J_{2,3}$ = $J_{3,2}$ = 8.4 Hz, 2H, H-2/H-3). EI-MS: *m/z* (rel. abund. %) 324 (M$^+$, 67), 275 (49), 204 (15), 203 (37), 161 (61), 121 (68), 106 (100), 75 (24).

1-(3-(Methylthio)phenyl)-3-(2-(trifluoromethyl) phenyl)thiourea (7)

Yield: 72%, M.p.: 245-249 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.00 (s, 1H, NH), 9.35 (s, 1H, NH) ,7.73 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-3), 7.69 (t, $J_{4(5,6)}$ = 7.4 Hz, 1H, H-4), 7.56 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6),7.50 (ovp, 2H, H-5/H-2'), 7.27 (ovp, 2H, H-6'/H-5'),7.04 (d, $J_{4',3'}$ = 6.8 Hz, 1H, H-4'), 2.49 (s, 3H, CH_3), FAB$^+$: 343 (M+1).

1-(4-Chlorophenyl)-3-(2-(trifluoromethyl) phenyl)thiourea (8)

Yield: 82%, M.p.: 220-223 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.01 (s, 1H, NH), 9.39 (s, 1H, NH), 7.74 (d, $J_{3,4}$ = 7.6 Hz, 1H, H-3), 7.69 (t, $J_{4(5,6)}$ =7.6 Hz, 1H, H-4), 7.56 (ovp, 3H, H-6/H-3'/H-5')7.50 (t, $J_{5(4,6)}$ = 7.6 Hz, 1H, H-5), 7.39 (d, J = 8.0 Hz, 2H, H-6'/H-2'), FAB$^+$: 331 (M+1).

1-(5-Chloro-2,4-dimethoxyphenyl)-3-(2-(trifluoro methyl)phenyl)thiourea (9)

Yield: 85%, M.p.: 248-252 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.41 (s, 2H, NH) 7.85 (s, 1H, H-2'), 7.72 (d, $J_{3,4}$ = 7.6 Hz, 1H, H-43), 7.67 (t, $J_{5(4,6)}$ =7.6 Hz, 1H, H-5), 7.58 (d, $J_{6,5}$ = 8.0 Hz,

1H, H-6), 7.47 (t, $J_{4(3,2)}$ = 7.4 Hz, 1H, H-4), 6.84 (s, 1H, H-5'), 3.89 (s, 6H, OCH_3), FAB^+: 391 (M+1).

1-(3,4-Dimethylphenyl)-3-(2-(trifluoromethyl) phenyl)thiourea (10)

Yield: 75%, M.p.: 201-205 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.85 (s, 1H, NH), 9.13 (s, 1H, NH), 7.72 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-3), 7.65 (d, $J_{4,3}$ = 8.0 Hz, 1H, H-4), 7.57 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), 7.47 (t, $J_{5(4,6)}$ = 7.2 Hz, 1H, H-5), 7.22 (s, 1H, H-2'), 7.20 (d, $J_{6',5'}$ = 8.0 Hz, 1H, H-6'), 7.10 (d, $J_{5',6'}$ = 8.4 Hz, 1H, H-5'), 2.19 (s, 6H, 2-CH_3), FAB^+: 325 (M+1).

1-(2-Methoxy-5-(trifluoromethyl)phenyl)-3-(3-methoxyphenyl)thiourea (11)

Yield: 65%, M.p.: 244-248 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.18 (s, 1H, NH), 9.28 (s, 1H, NH), 8.45 (s, 1H, H-6'), 7.50 (d, $J_{4',5'}$ = 8.4 Hz, 1H, H-4'), 7.24 (ovp, 3H, H-3'/H-6/H-2), 7.05 (d, $J_{5,6}$ = 7.6 Hz, 1H, H-5), 6.74 (d, $J_{4,5}$ = 7.2 Hz, 1H, H-4), 3.91 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), ^{13}C-NMR (100 MHz, DMSO-d_6): δ 151.6, 150.5, 149.3, 134.2, 132.2, 130.5, 128.3, 127.3, 126.0, 122.4, 122.4, 112.0, 111.2, 56.6, 56.5. FAB^+, 357 (M+1).

1-(2-Bromophenyl)-3-(2-methoxy-5(trifluoro methyl) phenyl)thiourea (12)

Yield: 75%, M.p.: 214-218 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.86 (s, 1H, NH), 9.58 (s, 1H, NH), 8.59 (s, 1H, H-6'), 7.68 (d, $J_{4',5'}$ = 7.8 Hz, 1H, H-4'), 7.59 (d, $J_{3,4}$ = 7.5 Hz, 1H, H-3),7.50 (d, $J_{5,4}$ = 7.8 Hz, 1H, H-5), 7.41 (t, $J_{4(3,2)}$ = 7.5 Hz, 1H, H-4), 7.26 (ovp, 2H, H-3'/H-6), 3.93 (s, 3H, OCH_3), FAB^+: 405(M+1).

1-(2,5-Dimethylphenyl)-3-(3-methoxyphenyl)thiourea (13)

Yield: 55%; M.p.: 200-204°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.59 (s, 1H, NH), 9.26 (s, 1H, NH), 7.22 (ovp, 2H, /H-2/H-4), 7.10 (d, $J_{6,5}$ = 7.6 Hz, 1H, H-6),7.03 (d, $J_{5,6}$ = 5.6 Hz, 1H, H-5), 7.00 (s, 1H, H-2'), 6.97 (d, $J_{4',5'}$ = 7.6 Hz, 1H, H-4'), 6.69 (d, $J_{5',6'}$ = 8.0 Hz, 1H, H-5'), 3.72 (s, 3H, OCH_3), 2.24 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), FAB^+: 287(M+1).

1-(3,5-Dimethylphenyl)-3-(3-methoxyphenyl)thiourea (14)

Yield: 75%;[1] M.p.: 211-215 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.65 (s, 1H, NH), 9.62 (s, 1H, NH), 7.22 (t, $J_{5(4,3)}$ = 8.2 Hz, 1H, H-5), 7.15 (s, 1H, H-6'), 7.04 (s, 2H, H-2'/H-4') ,7.00 (d, $J_{6,5}$ =

7.6 Hz, 1H, H-6), 6.76 (s, 1H, H-2), 6.69 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,2}$ = 1.6 Hz, 1H, H-4), 3.72 (s, 3H, OCH_3), 2.23 (s, 6H, CH_3); FAB^+: 287(M+1).

1-(4-Ethylphenyl)-3-(3-methoxyphenyl)thiourea (15)

Yield: 85%, M.p.: 241-245 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.67 (s, 1H, NH), 9.66 (s, 1H, NH), 7.35(d, $J_{2',3'}$ = $J_{6',5'}$ = 8.0 Hz, 2H, H-2'/H-6'), 7.23 (d, $J_{4,5}$ = 8.4 Hz ,1H, H-4), 7.19 (ovp, 3H, H-3'/H-5'/H-2), 7.02 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), 6.69 (ovp, 1H, H-5), 3.72 (s, 3H, OCH_3), 2.59 (q, 2H, CH_2), 1.18 (t, 3H, CH_3), FAB$^+$: 287(M+1)

1-(5-Chloro-2,4-dimethoxyphenyl)-3-(3-methoxy phenyl)thiourea (16)

Yield: 65%, M.p.: 188-193 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.83 (s, 1H, NH), 9.05 (s, 1H, NH), 7.74 (s, 1H, H-2'), 7.23 (ovp, 2H, H-6/H-2), 7.02 (ovp, 1H, H-5) ,6.83 (s ,1H, H-5'), 6.70 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,2}$ = 2.0 Hz, 1H, H-4), 3.88 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), FAB$^+$: 353(M+1).

1-(2-Iodophenyl)-3-(3-methoxyphenyl)thiourea (17)

Yield: 65%, M.p.: 127-131 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.92 (s, 1H, NH), 9.31 (s, 1H, NH), 7.87 (d, $J_{3',4'}$ = 7.6 Hz, 1H, H-3'), 7.39 (ovp, 2H, H-4'/H-5'), 7.27(s, 1H, H-2), 7.24 (d, $J_{6',5'}$ = 8.4 Hz, 1H, H-6'), 7.03 (d, $J_{4,3}$ = 8.0 Hz, 1H, H-4), 6.99 (ovp, 1H, H-5), 6.73 (ovp, 1H, H-6), 3.74 (s, 3H, OCH_3), ^{13}C-NMR (75 MHz, DMSO-d_6): δ 160.3, 150.8, 148.6, 148.3, 132.0, 129.7, 128.3, 126.9, 126.2, 123.6, 121.1, 113.3, 109.8, 56.3. FAB$^+$: 385(M+1).

1-(4-Iodophenyl)-3-(3-methoxyphenyl)thiourea (18)

Yield: 75%, M.p.: 225-229 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.81(s, 2H, NH) ,7.64 (d, $J_{3',4'}$ = $J_{5',6'}$ = 8.4 Hz, 2H, H-3'/H-5'), 7.31 (d, $J_{2',3'}$ = $J_{6',5'}$ = 8.0 Hz, 2H, H-2'/H-6'), 7.21 (t, $J_{5(4,3)}$ = 8.0 Hz, 1H, H-5), 7.14 (s, 1H, H-2), 7.00 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), 6.70 (br.s, 1H, H-4), 3.72 (s, 3H, OCH_3), FAB$^+$: 285(M+1).

1-(4-Bromophenyl)-3-(3-methoxyphenyl)thiourea (19)

Yield: 85%, M.p.: 194-198 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.81 (s, 2H, NH), 7.50 (d, $J_{2',3'}$ = $J_{3',2'}$ = 8.0 Hz, 2H, H-2'/H-3') ,7.45 (d, $J_{5',6'}$ = $J_{6',5'}$ = 8.8 Hz, 2H, H-5'/H-6'), 7.22 (t, $J_{5(4,3)}$ = 8.2 Hz, 1H, H-5), 7.14 (s, 1H, H-2), 7.00 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), 6.71 (d, $J_{4,3}$ = 8.4 Hz, 1H, H-4), 3.72 (s, 3H, OCH_3), FAB$^+$: 337(M+1).

1-(4-Fluoro-3-nitrophenyl)-3-(3-methoxy phenyl) thiourea (20)

Yield: 55%, M.p.: 249-253 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.07 (s, 1H, NH), 9.99 (s, 1H, NH), 8.37 (d, $J_{6',2'}$ = 4.4 Hz, 1H, H-6'),7.87 (ovp, 1H, H-2'), 7.56 (t, $J_{5'\,(4',6')}$ =10.0 Hz, 1H, H-5'), 7.27 (t, $J_{5(6,4)}$ = 8.2 Hz, 1H, H-5), 7.11 (s, 1H, H-2), 7.00 (d, $J_{4,5}$ = 8.0 Hz, 1H, H-4), 6.75 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), FAB$^+$: 322 (M+1).

1-(2,4-Dichlorophenyl)-3-(3,5-dimethylphenyl)thiourea (21)

Yield: 85%, M.p.: 158-162 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.91 (s, 1H, NH), 9.32 (s, 1H, NH), 7.64 (s, 1H, H-3), 7.60 (d, $J_{5,6}$ = 8.8 Hz, 1H, H-5), 7.40 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), 7.08 (s, 2H, H-2'/H-4'), 6.79 (s, 1H, H-6'), 2.24 (s, 6H, CH_3), FAB$^+$, 325 (M+1).

1-(2,4-Dichlorophenyl)-3-(3,4-dimethylphenyl)thiourea (22)

Yield: 75%, M.p.: 218-222 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.90 (s, 1H, NH), 9.28 (s, 1H, NH), 7.65 (ovp, 1H, H-5), 7.60 (s, 1H, H-3), 7.41 (ovp, 1H, H-6), 7.21 (ovp, 3H, H-6'/ H-2'/H-5'), 2.20 (s, 6H, CH_3), FAB$^+$: 325 (M+1).

1-(2,4-Dichlorophenyl)-3-(4-ethylphenyl)thiourea (23)

Yield: 55%, M.p.: 162-166 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.96 (s, 1H, NH), 9.35 (s, 1H, NH), 7.66 (ovp, 1H, H-5), 7.60 (s, 1H, H-3), 7.42 (d, $J_{6,5}$ = 3.0 Hz, 1H, H-6), 7.39 (d, $J_{6',5'} = J_{2',3'}$ = 9.0 Hz, 2H, H-2'/H-6'), 7.20 (d, $J_{5',6'} = J_{3',2'}$ = 9.0 Hz, 2H, H-3'/H-5'), 2.62 (q, 2H, CH_2), 1.19 (t, 3H, CH_3), FAB$^+$: 325 (M+1).

1-(5-Chloro-2-methylphenyl)-3-(3,4-dichlorophenyl)thiourea (24)

Yield: 75%, M.p.: 180-184 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.96 (s, 1H, NH), 9.59 (s, 1H, NH), 7.89 (d, $J_{6,2}$ = 2.0 Hz, 1H, H-6), 7.57 (d $J_{5,6}$ = 8.4 Hz, 1H, H-5), 7.46 (dd, $J_{6,5}$ = 8.8 Hz, $J_{6,2}$ = 2.4 Hz, 1H, H-6), 7.35 (d, $J_{6',4'}$ = 1.6 Hz, 1H, H-6'), 7.28 (ovp, 2H, H-4'/H-3'), 2.19 (s, 3H, CH_3), FAB$^+$: 345 (M+1).

1-(4-Bromophenyl)-3-(3-fluorophenyl)thiourea (25)

Yield: 75%, M.p.: 168-172 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 2H, NH), 7.51 (d, $J_{3',2'} = J_{5',6'} = J_{4,5}$ = 8.0 Hz, 3H, H-3'/H-5'/H-4),7.45 (d, $J_{6',5'} = J_{2',3'}$ = 8.8 Hz, 2H, H-6'/H-2'), 7.37 (ovp, 1H, H-2), 7.24 (d, $J_{6,5}$ = 8.4 Hz, 1H, H-6), 6.95 (ovp, 1H, H-5), ^{13}C-NMR (100 MHz, DMSO-d_6): δ 154.2, 153.6, 152.0, 137.7, 136.9, 135.0, 133.3, 133.2, 129.0, 127.4, 126.0, 124.1, 123.5. FAB^{+}: 325 (M+1).

1-(2,4-Difluorophenyl)-3-(4-iodo phenyl)thiourea (26)

Yield: 65%, M.p.: 238-242 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.63 (s, 1H, NH), 9.60 (s, 1H, NH), 7.89(d, $J_{5,6}$ = 8.0 Hz, 1H, H-5), 7.66 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), 7.52 (ovp, 4H, H-2'/H-3'/H-5'/H-6'), 7.05 (ovp, 1H, H-3), FAB^{+}: 391 (M+1).

1-(2,4-Difluorophenyl)-3-(2-iodophenyl)thiourea (27)

Yield: 55%, M.p.: 164-170 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.52 (s, 1H, NH), 9.45 (s, 1H, NH), 7.89 (d , $J_{5,6}$ = 7.6 Hz, 1H, H-5), 7.66 (ovp, 1H, H-3), 7.42 (ovp, 2H, H-3'/H-6), 7.33 (ovp, 1H, H-5'), 7.09 (ovp, 2H, H-6'/H-4'), FAB^{+}: 389 (M+1).

1-(4-Bromophenyl)-3-(2,4-difluorophenyl)thiourea (28)

Yield: 65%, M.p.: 218-220 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 1H, NH) 9.45 (s, 1H, NH), 7.53 (ovp, 5H, H-2'/H-3'/H-5'/H-6'/H-3), 7.32 (ovp, 1H, H-5), 7.09 (t, $J_{6(5,4)}$ = 8.6 Hz, 1H, H-6), FAB^{+}: 345 (M+1).

1-(2,4-Difluorophenyl)-3-(3,5-dimethylphenyl)thiourea (29)

Yield: 65%, M.p.: 156-160 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.82 (s, 1H, NH), 9.26 (s, 1H, NH), 7.53 (ovp, 1H, H-3), 7.30 (ovp, 1H, H-5), 7.07 (ovp, 3H, H-6/H-2'/H-4'), 6.78 (s, 1H, H-6'), 2.49 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), ^{13}C-NMR (100 MHz, DMSO-d_6): δ 160.2, 153.7, 151.0, 148.0, 133.2, 132.0, 129.7, 126.7, 125.7, 123.6, 121.3, 117.0, 113.5, 60.3, 56.2. FAB^{+}: 293 (M+1).

1-(2,4-Difluorophenyl)-3-(3,4-dimethylphenyl)thiourea (30)

Yield: 75%, M.p.: 145-149 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.78 (s, 1H, NH), 9.20 (s, 1H, NH), 7.54 (ovp, 1H, H-3),7.30 (ovp, 1H, H-5), 7.18 (ovp, 2H, H-2'/H-6), 7.09 (ovp, 2H, H-5'/H-6'), 2.19 (s, 3H, CH_3), 2.18 (s, 3H,CH_3), FAB^{+}: 293 (M+1).

1-(2-bromophenyl)-3-(3-(methylthio)phenyl)thiourea (31)

Yield: 72%, M.p.: 200-204 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.99 (s, 1H, NH), 9.43 (s, 1H, NH), 7.66 (d, $J_{3,4}$ = 8.1 Hz, 1H, H-3), 7.52 (ovp, 2H, H-4'/H-6), 7.39 (t, $J_{5'(4',6')}$ = 7.5 Hz, 1H, H-5'), 7.25 (ovp, 3H, H-5/H-6'/H-4), 7.02 (s, 1H, H-2'), 2.49 (s, 3H, CH_3), FAB$^+$: 355 (M+1).

1-(2-bromophenyl)-3-(4-ethylphenyl)thiourea (32)

Yield: 82%, M.p.: 233-237 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.88 (s, 1H, NH), 9.25 (s, 1H, NH), 7.65 (d, $J_{3,4}$ = 7.6 Hz, 1H, H-3), 7.56 (d, J = 7.6 Hz, 1H, H-6), 7.41 (ovp, 3H, H-6'/H-5/H-4), 7.18 (ovp, 3H, H-5'/H-2'/H-3'), 2.58 (q, 2H, CH_2), 1.18 (s, 3H, CH_3), FAB$^+$: 335 (M+1).

1-(2-Bromophenyl)-3-(5-chloro-2,4-dimethoxyphenyl)thiourea (33)

Yield: 58%, M.p 214-218 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.43 (s, 1H, NH), 9.36 (s, 1H, NH), 7.84 (s, 1H, H-6'), 7.65 (ovp, 2H, H-4'/H-3'), 7.38 (s, 1H, H-2'), 7.18 (s, 1H, H-6'), 7.18 (s, 1H, H-6'), 6.84 (s, 1H, H-3'), 3.86 (s, 3H, OCH_3), FAB$^+$: 351(M+1).

1-(3-Chlorophenyl)-3-(3-nitrophenyl)urea (34)

Yield: 55%, M.p.: 168-172 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.30 (s, 1H, NH), 9.06 (s, 1H, NH), 8.53 (s, 1H, H-2'), 7.84 (d, $J_{4',5'}$ = 8.0 Hz, $J_{4',6'}$ = 1.2 Hz, 1H, H-4') ,7.72 (ovp, 2H, H-2/H-6'), 7.58 (t, $J_{5(4,3)}$ = 8.2 Hz, 1H, H-5), 7.31 (ovp, 2H, H-5'/H-6), 7.05 (ovp, 1H, H-4), ^{13}C-NMR (100 MHz, DMSO-d_6): δ 180.1, 160.9, 157.7, 140.31, 129.4, 127.3, 126.3, 126.2, 122.2, 120.5, 119.8, 119.7, 115.3, FAB$^+$: 292 (M+1).

1-(3-Chlorophenyl)-3-(2-methoxy-5-(trifluoromethyl)phenyl)urea (35)

Yield: 55%, M.p.: 188-193 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.86 (s, 1H, H-6'), 7.70 (d, $J_{4,5}$ = 8.0 Hz, 1H, H-4), 7.53 (t, $J_{5(3,4)}$ = 7.7 Hz, 1H, H-5), 7.45 (d, $J_{6,5}$ = 8.0 Hz, 1H, H-6), 7.33 (d, $J_{2',4'}$ = 1.5 Hz, 1H, H-2), 7.25 (d, $J_{5',6'}$= 8.0 Hz, 1H, H-3'), 7.21 (ovp, 1H, H-4') FAB$^+$, 345 (M+1).

1-(5-Chloro-2,4-dimethoxyphenyl)-3-(3-chlorophenyl)urea (36)

Yield: 60%, M.p.: 232-236 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.39 (s, 1H, NH), 8.18 (s, 1H, NH), 8.10 (s, 1H, H-2), 7.70 (s, 1H, H-2'), 7.28 (d, $J_{5,6}$ = 8.0 Hz, 1H, H-5), 7.19 (d, $J_{4,5}$ = 8.4 Hz,

1H, H-4), 7.00 (d, $J_{6,5}$ = 7.6 Hz, 1H, H-6), 6.85 (s, 1H, H-5'), 3.92 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), FAB^+: 341 (M+1).

1-(4-Bromophenyl)-3-(3-chlorophenyl)urea (37)

Yield: 70%, M.p.: 194-198 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.93 (s, 2H, NH), 7.67 (s, 1H, H-2), 7.45 (ovp, 4H, H-5'/H-6'/H-2'/H-3'), 7.29 (t, J = 9.0 Hz, 2H, H-4/H-5), 7.02 (d, $J_{6,5}$ = 7.2 Hz, 1H, H-6), FAB^+: 327 (M+1).

Bioassays

Urease inhibition assay

Reaction mixtures comprising 25 μL of enzyme (*Bacillus pasteurii* urease) solution and 55 μL of buffers containing urea (2-24 mM for *Bacillus pasteurii* urease) were incubated with 5 μL of test compounds at 30 °C for 4.15 min in 96-well plates. The increasing absorbance at 560 nm was measured after 10 min, using a microplate reader (Molecular Devices, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min.) were processed by using SoftMax Pro software (Molecular Devices, USA). All the assays were performed at pH 6.8 (3 mM sodium phosphate buffer) and 7 μg of phenol red per mL as indicator [33]. Acetohydroxamic acid was used as the standard inhibitor of urease. Percentage inhibitions were calculated from the formula.

$$100 - (OD_{testwell}/OD_{control}) \times 100$$

Molecular Docking Studies

To get insight of the interactions between the active site of urease enzyme and synthetic compounds, the molecular docking studies were carried out using AutodockVina [34]. The three-dimensional structure of urease from *Bacillus pasteurri* complexed with acetohydroxamic acid (HAE) at 1.55 Å (PDB ID: 4UBP) was retrieved from Protein Data Bank [www.rcsb.org/pdb]. Prior to conversion of pdb format of receptor file to pdbqt format, water molecules, Chain-A, Chain-B, and HAE complexed with Ni798 and Ni799 were removed using Discovery Studio Visualizer [35]. Atom type 't' and gasteiger charges 'q' were added to receptor file and was saved as pdbqt file using Autodock Tools [36]. Chembio3D Ultra (Version; 14.0.0.117) was used for sketching and minimizing the energies of synthesized compounds. Also, atom types along

with charges were added to ligands employing Openbabel (ver. 2.4.1) [37]. Other parameters for docking simulations were set as follow: Grid box size of 35 × 35 × 35 Å centered at X = 29.20, Y = 73.00, and Z = 72.75, number of maximum binding modes were set 50, energy difference of 4 KCal/mol was set between best and worst binding modes and exhaustiveness at 100, respectively. LigPlot^{+} [38] and Discovery Studio Visualizer were utilized to analyze the two-dimensional and three-dimensional structures of ligand protein complexes.

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Graphical abstract

Synthesis and *in vitro* urease inhibitory activity of thiourea and urea derivatives and their docking studies

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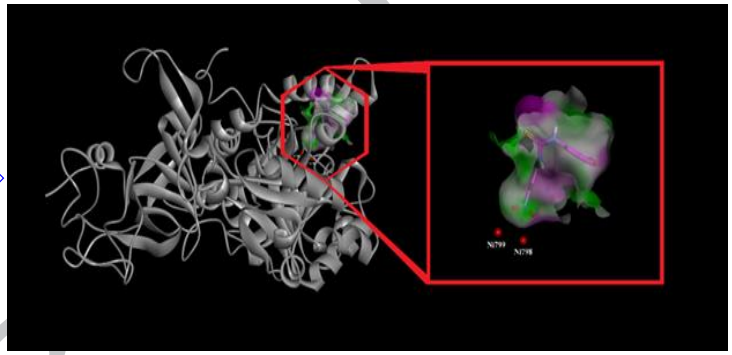
1

IC_{50} = 10.11 ± 0.1 μM

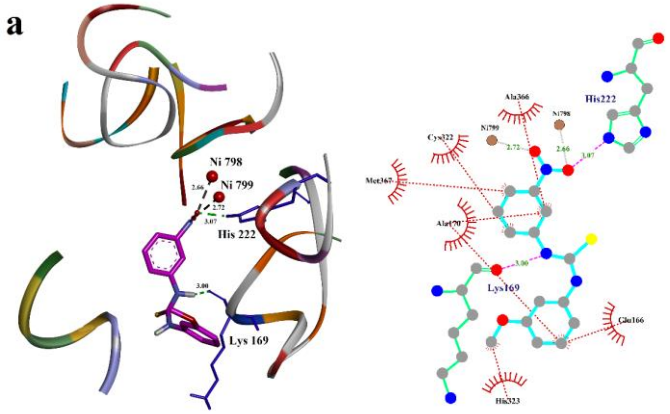
Most active compound **1**

Standard Acetohydroxamic acid

IC_{50} = 27.00 ± 0.5 μM



Urease from *Bacillus pasteurii* (Ribbon form in grey color), actives site of urease (enclosed in red color) and zoomed in docked conformation of compound 1 (Lilac colored sticks along with Nickle atoms colored in red)



The ligand-protein interactions of most active compounds (a) **1,**with the active site of urease from *Bacillus pasteurii* (4UBP) created by using Discovery Studio 17.2 and LigPlot+. The left side displays 3D interactions of the compounds in the binding site. The right side shows the 2D interaction patterns. Dashed lines show the interactions among the ligand and the amino acids of the protein. Distances are in Angstrom.

Highlights:

- Urea and thiourea derivatives were synthesized.
- Urease inhibitory activity of synthetic derivatives have been evaluated.
- Structure-activity relationship was established.
- *In silico* study of the most active compound was also done.