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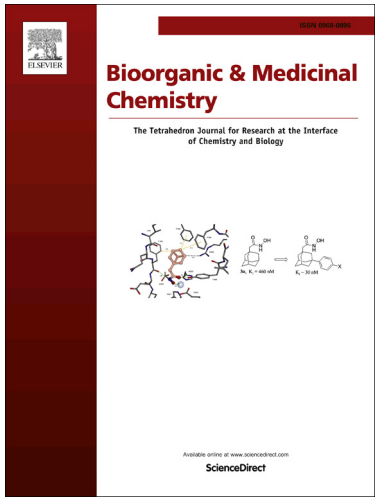

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Design and synthesis of new barbituric- and thiobarbituric acid derivatives as potent urease inhibitors: structure activity relationship and molecular modeling studies

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Abstract:

In this study 36 new compounds were synthesized by condensing barbituric acid or thiobarbituric acid and respective anilines (bearing different substituents) in the presence of triethyl orthoformate in good yields. *In vitro* urease inhibition studies against jack bean urease revealed that barbituric acid derived compounds **(1-9** and **19-27)** were found to exhibit low to moderate activity however thiobarbituric acid derived compounds **(10-18** and **28-36)** showed significant inhibition activity at low micro-molar concentrations. Among the synthesized compounds, compounds (**15**), (**12**), (**10**), (**36**), (**16**) and (**35**) showed excellent urease inhibition with IC_{50} values 8.53±0.027, 8.93±0.027, 12.96±0.13, 15±0.098, 18.9±0.027 and 19.7±0.63 μM, respectively, even better than the reference compound thiourea (IC_{50} = 21±0.011). The compound (**11**) exhibited comparable activity to the standard with IC_{50} value 21.83±0.19 μM. *In silico* molecular docking studies for

most active compounds (**10**), (**12**), (**15**), (**16**), (**35**) and (**36**) and two inactive compounds (**3**) and (**6**) were performed to predict the binding patterns.

Key words: Urease, barbituric, thiobarbituric, jack bean, in silico, triethyl orthoformate

1. Introduction:

Urease (urea amidohydrolase, EC 3.5.1.5) is a nickel-based enzyme that catalyzes the transformation of urea to carbon dioxide and ammonia. Urease is part of a range of living organisms including plants, algae, fungi and bacteria [1-3]. Urease plays a key function in nitrogen metabolism of plant during the germination process [4]. Even though having different origin of urease, all of them constitute highly conserved tertiary structures, common amino acid sequences in the active site and thus show same mechanism of action. Among numerous ureases, jack bean (*Canavalia ensiformis*) urease, the first enzyme crystallized [5] and best-characterized [6-8], has been widely employed in urease inhibition studies [9, 10]. The production of ammonia from urease is responsible for harmful complications in agriculture and health fields. During urea fertilization in agriculture, large quantities of ammonia are emitted into the atmosphere by high urease activity, it causes significant environmental and economic problems [11-13]. The enormous quantity of ammonia further stimulates plant damage by soil pH increase and ammonia toxicity [14]. Some bacterial ureases served as a critical factor involved in the progression of kidney stones, pyelonephritis, peptic ulcers, and other health complications [14, 15]. Urease in *Helicobacter pylori* is now accepted as a major cause of peptic ulcers [16-18], and its inhibition has been recognized as a potential therapeutic approach for peptic ulcers [19]. Therefore, urease inhibitors based anti-ulcer drugs have attracted great attention. Urease inhibitors have been broadly classified into two categories: (i) substrate-like inhibitors, such as hydroxyurea and hydroxamic acids, and (ii) mechanism-based inhibitors, such as phosphorodiamidates and imidazoles [20].

Recently, a variety of compounds including phosphoramidates [21], hydroxamic acids [21, 22], boric and boronic acids [23], heavy metal ions [24], quinones [25] and imidazoles [26] have been investigated for their urease inhibition activity. And more recently, barbiturates [27] and thiobarbiturates [28, 29] have been discovered as potent urease inhibitors.

Barbiturates and thiobarbiturates are medicinally imperative class of heterocyclic compounds, Barbiturates exhibites a wide range of biological activities including antibacterial, hypotensive, tranquilizing [30, 31], antioxidants [32], anticonvulsant and anesthetic [33], antiepileptic [34], sedatives and hypnotics [33-35], anticancer [36, 37], immuno-modulating [37], radio-sensitizing [38] and gelatinase inhibitors [39].

Similarly thiobarbiturates behave as HIV integrase inhibitors [40], anticonvulsant and anesthetic [33], antibacterial [41, 42], antifungal [43], antiviral [44] antitumor activities [45], tyrosinase inhibitors [46, 47] and anticancer with anti-inflammatory activities [48]. Literature review also revealed that barbiturates and thiobarbiturates show anti-tuberculosis [49], anti-diabetic and anti-bacterial properties [50].

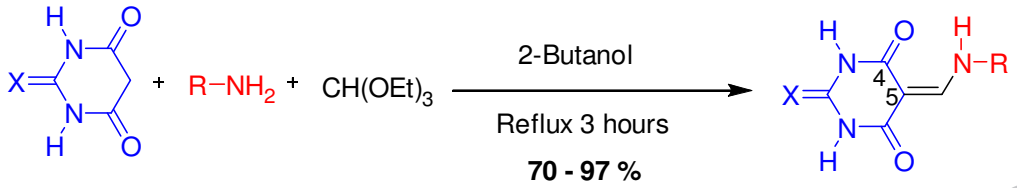
In view of these observations, it was thought worthwhile to synthesize a range of new and more potent derivatives of barbituric and thiobarbituric acids by incorporating substituted anilines at C-5 position of barbituric- or thiobarbituric acids. Research in the field of enzyme inhibition has enormous potential to introduce new drug candidates against different clinical conditions. Keeping in view the therapeutic importance of ureases, we have selected them as synthetic targets and to evaluate their urease inhibition potential to discover new lead molecules for the treatment of gastric ulcers, and other urease associated disorders. This method afforded the efficient synthesis of required compounds and also tolerated a range of functional groups including halo, -COOH, $-CF_3$, -SH, -OH, and NO_2.

2. Results and discussion

2.1. Chemistry

The desired substituted anilines based barbiturates and thiobarbiturates **(1-36)** were prepared in good to excellent yields (70–97%) according to the procedure given in the Scheme 1. The preparation of (**1-36)** was achieved from condensation of barbituric or thiobarbituric acid and the corresponding susbstituted anilines **(Table 1)**, in the presence of triethyl orthoformate and 2-butanol. Thus, a variety of functional groups such as carboxylic acid, hydroxyl, nitro, chloro, iodo, trifluoromethyl and thiol were tolerated under our reaction protocol. The chemical structures of all the synthesized compounds were establishe

d with the aid of spectroscopic and physical methods.



Scheme 1: Synthetic protocol for substituted anilines based barbiturates and thio-barbiturates; X = O or S and R= substituted aryls

2.2. Urease inhibition assay

Ureases are among the few enzymes that require nickel for activity. It is known that binding of nickel to urease is very specific and tight and the removal of metal ions can be achieved only by harsh treatment with denaturants or acids [51-53]. Literature studies revealed that biological activities of barbiturates and thiobarbiturates are different from each other [54]. The mechanism involved for the biological activities of barbiturates and thiobarbiturates is still not completely known, but recently, quantitative–structure–activity–relationship (QSAR) study has proposed that electronic factors play an important role in imparting biological activities to barbiturates and thiobarbiturates [55]. The thiobarbiturates were proved to possess better biological activities than barbiturates due to their better hydrogen bond acceptance ability which helps binding to the enzyme receptor. The reason for better hydrogen bond acceptance of sulfur atom was due to lower ionization energies and subsequently better electron donating abilities [54]. It has also been experimentally determined that sulfur atom in C=S group forms hydrogen bonds with enzymes (e.g. uridine phosphorylase) and biologically important thioureas [56, 57].

Recently, synthesis and urease inhibition activities of various thiobarbiturates have been reported and they showed excellent urease inhibition activity. The reason explained for the better activity was the possible involvement of two hydroxyl groups in the formation of hydrogen bonds with nickel atoms present in the enzyme. It was further reported that the

presence of one extra sulfur atom on the phenyl ring might also helpful in making coordination bonds with nickel [58].

Khan *et. al.,* synthesized and evaluated the *in vitro* urease inhibition activities of various arylidene barbiturates. It was suggested that the presence of highly electronegative fluoro group at *ortho*, *meta* or *para* positions enhanced the activity but the most activity was found when the fluoro group was at *para* position. Furthermore, molecular docking analysis showed that in most of the molecules, one of the carbonyl groups coordinates with both Ni atoms, while the other one is involved in the formation of hydrogen bonds with important active site residues, e.g., Ala170, Gly280 and Arg339 [27]. Nickel coordination might be one of the important factors for the activities of these compounds, as mostly known urease inhibitors also interact with nickel ions [59-62].

Previously we reported novel sulphonamides based barbiturates and thiobarbiturates and determined their urease inhibition activities. The thiobarbiturates (% inhibition 88.3-99.9 %) were found to be more potent than barbiturates. It was suggested that some coordinating sites such as, C=O group of thiobarbiturates ring and N-H groups, have been involved in chelating Ni (II) atoms of urease enzyme [29].

In current paper, we report the synthesis of structurally more diverse substituted aniline based barbiturate and thiobarbiturate derivatives. Khan et al reported arylidene barbiturates the present research include aniline derived compounds which provide an amino derivatives (=C-NHR). All the synthesized substituted anilines based barbiturates and thiobarbiturates (**1–36**) were evaluated for urease inhibitory activity. The compounds (**15**), (**12**), (**10**), (**36**), (**16**) and (**35**) showed excellent urease inhibition with IC_{50} values of 8.53±0.027, 8.93±0.027, 12.96±0.13, 15±0.098, 18.9±0.027 and 19.7±0.63 μM, respectively, much better than the standard urease inhibitor thiourea (IC_{50} = 21±0.011) (**Table 2**). The compound (**11**) also exhibited comparable activities to standard with IC_{50} value of 21.83±0.19 μM respectively. The compounds which showed less than 50% inhibition, their IC_{50} values were not calculated.

2.3. Structure activity relationship

In order to explain the structure–activity relationship and to get optimized urease inhibitors, both barbituric and thiobarbituric acids and substituted anilines were used to furnish the synthesis of substituted anilines based barbiturates and thiobarbiturates (**1-36**). These substituents include electron donating group such as hydroxyl, thiol and electron withdrawing groups such as carboxylic, nitro, trifluoromethyl, chloro, dichloro, trichloro and iodo. Furthermore, the positions of these groups were also changed in the phenyl ring to elaborate more detailed inhibition potential. The present investigation shows that thiobarbiturates (**10-18** and **28-36**) are more active than barbiturates (**1-9** and **19-27**). This is attributed to the presence of sulfur atom in thiobarbituric acid instead of oxygen atom in the barbituric acid, which plays an important role in imparting urease inhibition to these compounds. It is proposed that sulphur is less electronegative and bigger in size as compared to oxygen and make more feasible transfer or shifting of electrons from tetrahydropyrimidine ring to chelate the nickel ion present in the enzyme of bacteria, which is responsible for the activity of bacteria. Apart from this, other factors such as hydrophobic, steric and electronic properties may also amongst the possible reasons causing enhancement of the urease inhibition of these compounds.

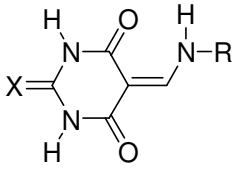
Amongst thiobarbiturates, **(10-18)** containing carboxyl group (-COOH) in their respective substituted anilines, inhibition potential varies due to changing position of carboxyl group (-COOH) and absence or presence of different other substituents in the phenyl ring. Compound **(15)**, bearing carboxyl group (–COOH) at C-2 position and nitro group ($-NO_2$) at C-5 position in the phenyl ring, was proved to be the most active with an IC_{50} value of 8.53±0.027 μM. Removing the nitro group ($-NO_2$) from the C-5 position of **(15)**, producing **(10)** (IC_{50} = 12.96±0.13) slightly diminished the activity, but it is still more active than standard thiourea. Changing the position of carboxyl group (-COOH) in the phenyl ring from C-2 to C-4, producing **(12)** (IC_{50} = 8.93±0.027 μM) again enhanced the activity. Thus, from the activity pattern it can be ascertained that the presence of carboxyl group (-COOH) at C-2 and C-4 positions in the phenyl ring plays a significant role in the high activities of these compounds and the activity is highest when nitro group ($-NO_2$) is also present at C-5 position along with carboxyl group (-COOH) at C-2 (**Table 1**). The possible

reason might be the involvement of carboxyl group (-COOH) to form hydrogen bonds with nickel atom present in the urease enzyme.

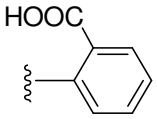
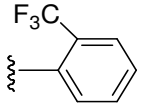
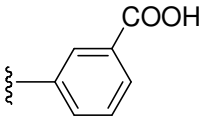
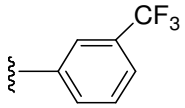
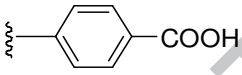
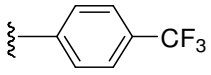
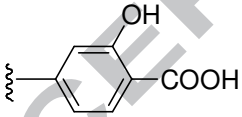
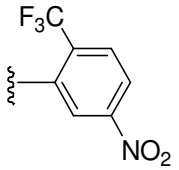
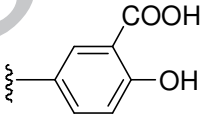
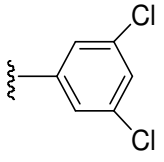
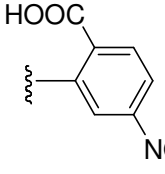
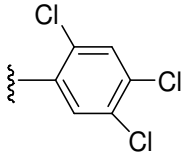
The presence of hydroxyl groups (-OH) at C-3 or C-4 positions in the phenyl ring slightly decreased the activities as compounds **(13)** and **(14)**. Similarly, the presence of nitro (-NO_2) at C-4 and chloro (-Cl) at C-6 positions in the phenyl ring also decreased the activities as in the compounds **(17)** and **(18)** respectively.

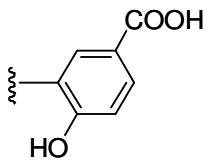
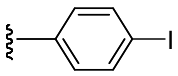
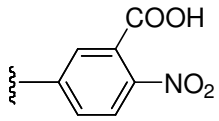
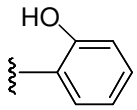
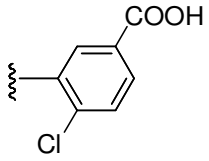
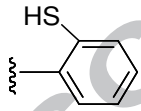
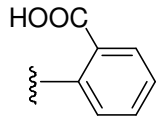
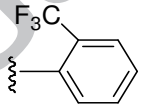
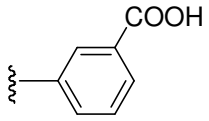
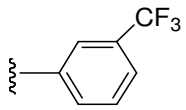
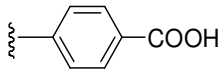
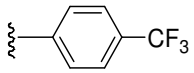
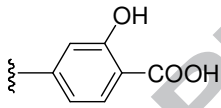
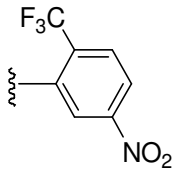
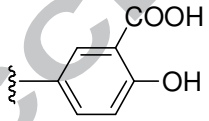
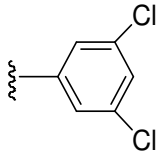
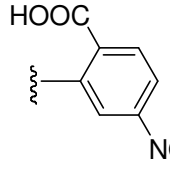
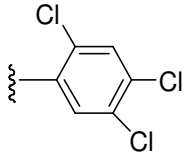
Amongst thiobarbiturates, **(28**-**36)** which are without carboxyl group (-COOH) in their respective substituted anilines, the compound **(36)** showed better urease inhibition activity with an IC_{50} = 15±0.098. It was due to the presence of thiol (-SH) group at C-2 position. Replacing the thiol (-SH) group from C-2 position with hydroxyl group (-OH) slightly decreased the activity, producing **(35)** with an IC_{50} value of 19.7±0.63 µM. From the activity pattern it can be ascertained that the presence of the thiol (-SH) or hydroxyl group (-OH) at C-2 position in the phenyl ring plays a significant role in the high activities of these compounds and the activity is higher when thiol group (-SH) is present at C-2 position instead of hydroxyl group (compounds **36** vs. **35**). The possible reason might be the involvement of thiol and hydroxyl groups to form hydrogen bonds with nickel atom present in the urease enzyme. However, the presence of trifluoromethyl group (-CF_3), dichloro, trichloro and iodo groups in the phenyl ring decreased the activities as in the compounds **(28-34)**.

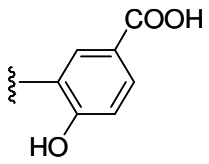
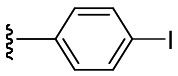
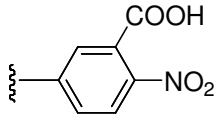
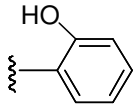
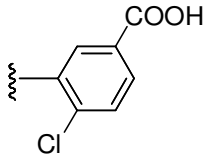
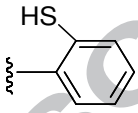
Table 1. Chemical structures, urease inhibition activity and IC_{50} values (mean± SEM, n=3) of substituted anilines based barbiturates and thiobarbiturates (ND, not determined).



(Compounds 1-36)

No	X	R	IC_{50}	No	X	R	IC_{50}
1	O		ND	**19**	O		ND
2	O		ND	**20**	O		ND
3	O		ND	**21**	O		ND
4	O		88.3±1.22	**22**	O		ND
5	O		ND	**23**	O		ND
6	O		ND	**24**	O		ND

7	O		ND	**25**	O		ND
8	O		25.8±0.47	**26**	O		ND
9	O		ND	**27**	O		52.6±0.29
10	S		12.96±0.13	**28**	S		65.3±0.57
11	S		21.83±0.19	**29**	S		24.56±0.098
12	S		**8.93±0.027**	**30**	S		136.4±0.50
13	S		32.3±0.16	**31**	S		28.7±0.32
14	S		ND	**32**	S		89.16±1.00
15	S		**8.53±0.027**	**33**	S		ND

16	S		18.9±0.027	**34**	S		41.33±0.29
17	S		39.76±0.072	**35**	S		19.7±0.63
18	S		26.66±0.95	**36**	S		15±0.098
		Thio-urea[a]	**21±0.011**				

[a] Thiourea standard inhibitor for antiurease activity

2.4. Molecular Modelling

We performed molecular modelling studies for all compounds which were more active than reference - thiourea (**10**, **12**, **15**, **16**, **35** and **36**) as well as for two inactive compounds (**3** and **6**) which were the analogues of the two most potent inhibitors (**12**) and (**15**). The urease from Jack bean was selected for the analysis among a group of ureases from a few different sources because our biological assay was based on such enzyme. Selection of the most active and inactive compounds for docking enabled us to reveal their binding mode and to show the important structural features responsible for the activity.

All analyzed derivatives of barbituric and thiobarbituric acid displayed similar, converged binding mode. Even the general orientation of each ligand in the active site was almost the same, the small differences appeared and were responsible for the large variations in the activity. The (thio)urea fragment of (thio)barbituric acid was engaged in complexation of nickel ions (Figure 1). However, thiobarbiturates were better complexing agents due to the easier thiolactam – thiolactim tautomerization, stronger acidic properties and easier subsequent ionization in comparison with barbiturates. Moreover, the scoring function obtained higher values for thiobarbiturates and therefore, derivatives containing sulfur

atom were potent while oxygen analogues were inactive. The NH group from (thio)barbituric moiety created hydrogen bond with C=O from backbone of Gly550 while the neighbouring carbonyl group formed H-bond with guanidine fragment of side chain of Arg609. In case of the most active compound (**15**) (Figure 1) carboxyl group from phenyl ring created salt bridge with the same arginine, and nitro group was orientated toward His594 and was able to form weak hydrogen bond. The phenyl ring could interact with imidazole ring of His593 by π- π stacking. In case of inactive compound (**6**) which was the analogue of inhibitor (**15**) all interactions were preserved (Figure 2). However, barbiturate – nickel complex was less stable than thiobarbiturate – nickel one and this led to inactivity. Inhibitor (**10**) which was devoid of nitro group was a little bit less active than compound (**15**) because of no interactions with His594. Compound (**12**) (Figure 3) contained only carboxyl substituent in the *para* position of the phenyl ring but it was sufficient to produce almost the same activity as in case of derivative (**15**) due to the creation of the hydrogen bond with His594. The difference between compounds (**3**) and (**12**) was exactly the same as between (**6**) and (**15**), and derivative (**3**) was also inactive. Inhibitor (**16**) due to the presence of hydroxyl substituent in position 2 of the phenyl ring and carboxyl in position 5 gave hydrogen bonds with Asp494 and His594, respectively. The second H-bond was weak and this might lead to decreased potency in comparison with compound (**15**) or (**12**). The change of carboxyl position from 5 to 4 could improve the potency because in position 4 carboxylate could form stronger hydrogen bond as in case of compound (**12**). Derivatives (**35**) and (**36**) possessed only one substituent in the phenyl ring in position 2 – hydroxyl and thiol group, respectively. In case of inhibitor (**35**) the phenyl ring was a little bit rotated and this hydroxyl group formed hydrogen bond with Asp494. Thiol group, present in compound (**36**), could ionize and form ionic interaction with Arg609. Such interaction was more profitable than hydrogen bond for derivative (**35**), and inhibitor (**36**) was a little bit more potent, however both compounds were still less active than (**15**) and (**12**).

The reference compound – thiourea was also able to interact with nickel cations and it mimicked the substrate of the enzyme. It was located a little bit farther from metal ions than our compounds but the distance was still good to obtain the inhibitory effect on the urease.

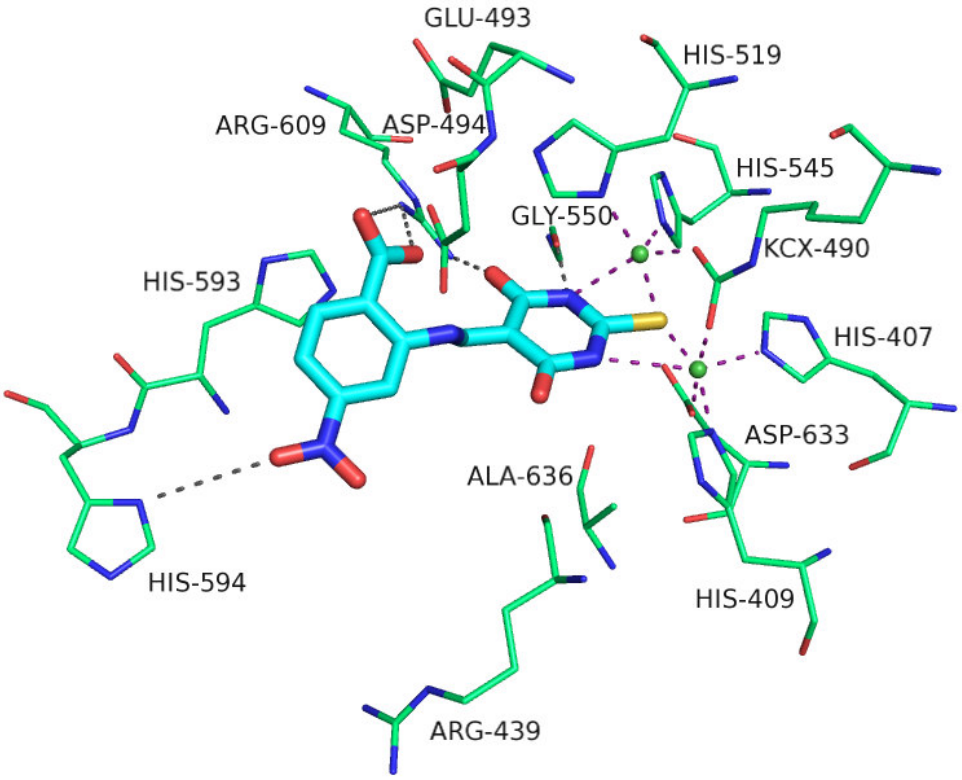


Figure **1**. The binding mode of the most active compound (**15**) within the active site of Jack bean urease. The most important feature of the bonding mode is formation of the complex with nickel ions. The violet dashed lines represent interactions with nickel cations while the grey ones ionic interactions and hydrogen bonds.

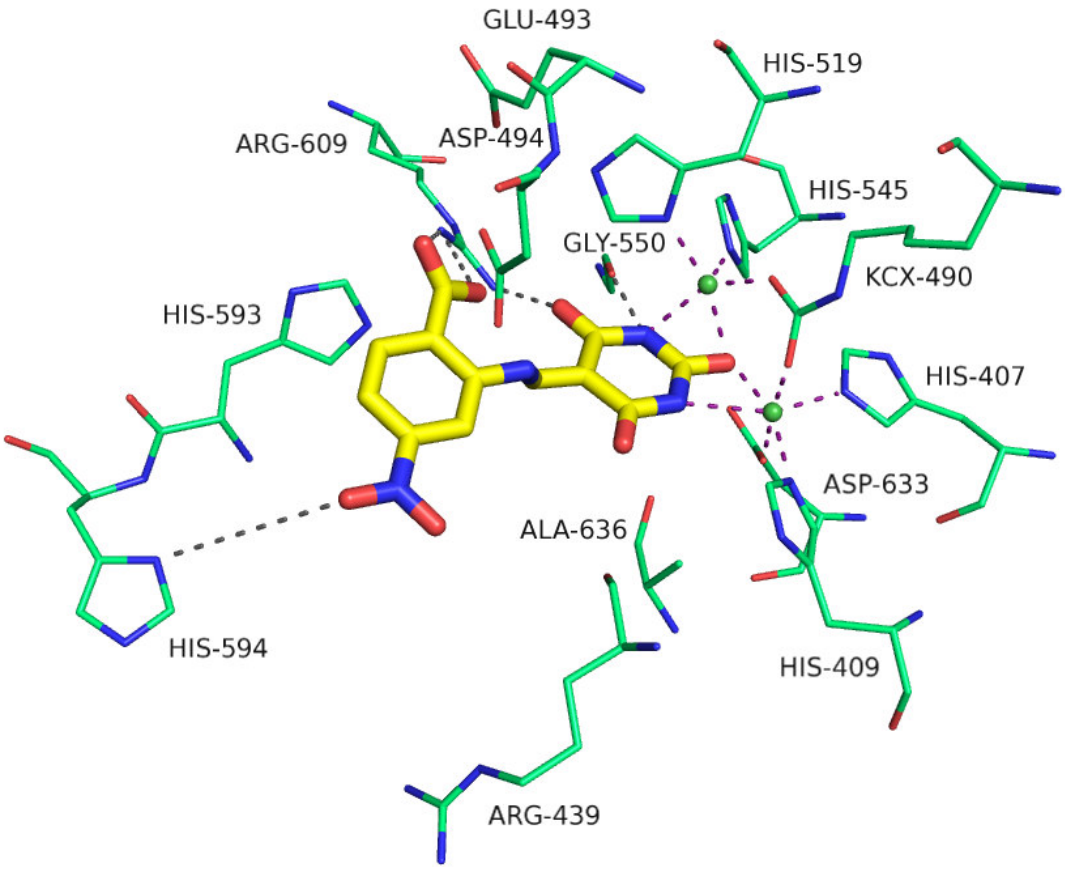


Figure **2**. The binding mode of the inactive compound (**6**) within the active site of Jack bean urease. The interactions scheme is quite the same as for the potent compound (**15**) but the complexing ability and stability of complex are lower. The violet dashed lines represent interactions with nickel cations while the grey ones ionic interactions and hydrogen bonds.

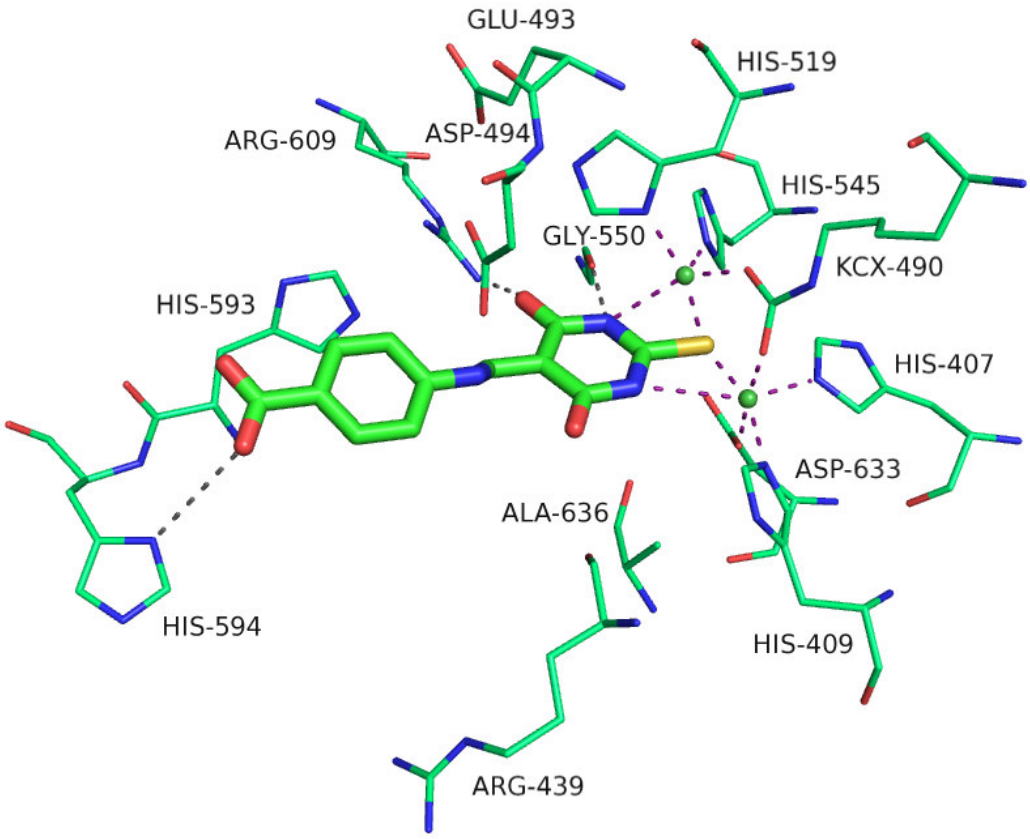


Figure **3**. The binding mode of compound (**12**) within the active site of Jack bean urease. The violet dashed lines represent interactions with nickel cations while the grey ones ionic interactions and hydrogen bonds.

3. Conclusion

In conclusion, a facile and high yield synthesis of novel substituted anilines based barbiturates and thiobarbiturates derivatives has been achieved. The *in-vitro* urease inhibition activities of these compounds revealed that thiobarbiturate derivatives were more active than the barbiturates, thus the thio-compounds (**10**), (**12**), (**15**), (**16**), (**35**) and (**36**) were found to be significant active among this tested series. The docking studies were also performed for the compounds (**10**, **12**, **15**, **16**, **35** and **36**) which were more active than reference - thiourea as well as for two inactive compounds (**3** and **6**) to investigate the binding patterns of these compounds with target enzyme. Our current findings may lead to thiobarbiturates based future urease inhibitors to treat ulcers and other urease related problems.

4. Experimental

4.1. General methods

^{1}H-NMR spectra were recorded in d_6-DMSO with Bruker AM 300 and AM 400 spectrometers (Rheinstetten – Forchheim, Germany) operating at 300MHz for compounds (**2, 3, 9, 15, 18, 21, 22, 23, 28, 32**) and at 400 MHz for compounds (**1, 4-8, 10-14, 16-17, 19-20, 24-27, 29-31, 33-36**). ^{1}H chemical shifts are reported in δ (ppm) and coupling constants in Hz. Splitting patterns were as follows s (singlet), d (doublet), dt (doublet of triplet), dd (double doublets), t (triplet), and m (multiplet). All *J* values are given in Hz and chemical shifts in δ-units. The ^{13}CNMR spectra were recorded in d_6-DMSO with Bruker AM 100 spectrometer (Rheinstetten – Forchheim, Germany) operating at 75 and 100 MHz. Tetramethylsilane (TMS) was taken as internal standard. Mass spectra were recorded with JEOL JMS600 and MAT312 operated with Electron Ionization mode. The progress of all reactions was monitored by TLC, which was performed on 2.0 × 5.0 cm aluminum sheets precoated with silica gel $60F_{254}$ to a thickness of 0.25 mm (Merck). The chromatograms were visualized under ultraviolet light (254–366 nm). The melting points were taken on Gallen Kamp apparatus and are uncorrected. **C**hemical reagents were purchased from the Merck Chemical Company in high purity. All the reagents were of commercial grade and used as obtained from supplier or redistilled as necessary.

4.2. General procedure for the synthesis of compounds (1-36)

0.5g (0.0039mol) of barbituric acid or thiobarbituric acid was added in 2-butanol (8-10 ml) and warmed till the solution become clear then added suitable substituted anilines (0.0039mol), *viz*. 2-amino benzoic acid, 3-amino benzoic acid, 4-amino benzoic acid, 4-amino salicylic acid, 5-amino salicylic acid, 2-amino-4-nitro benzoic acid, 3-amino-4-hydroxy benzoic acid, 5-amino-2-nitro benzoic acid, 3-amino-4-chloro benzoic acid, in the presence of slight excess of triethyl orthoformate (1ml). The resultant reaction mixture was heated under reflux for 3-4 hours. The solid product was collected by suction filtration in hot state. Then the product was washed with hot ethanol and dried. The products were obtained in good to excellent yields. The purity of the synthesized compounds was

checked by thin layer chromatography using appropriate solvent systems (Methanol:Chloroform; 1:1).

2-{[(2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (1)

Yield 72%, off-white solid, m.p. 310-314°C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 13.78 (1H, s, COO***H***), 13.36 (1H, d, *J* 13.8, =CHN***H***-), 10.95 (1H, s, CON***H***CO), 10.87 (1H, s, CON***H***CO), 8.61 (1H, d, *J* 13.8, =C***H***-N), 8.01 (1H, d, *J*7.6, Ar***H***), 7.8 (1H, d, *J* 8.4, Ar***H***), 7.69 (1H, t, *J* 7.6 Ar***H***), 7.31 (1H, t, *J* 7.6Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 167.6(C), 165(C), 163.7(C), 150.6(C), 149.8(CH), 139.9(C), 134.5(CH), 131.6(CH), 124.9(CH), 118.5(C), 116.8(CH), 94.2(C); MS (EI) *m/z* 275(M^+ 88%), 257(67), 229(80), 170(18), 143(100), 119(31).

3-{[(2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (2)

Yield 76%, off-white solid, m.p. 345-348°C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 13.19 (1H, s, COO***H***), 11.86 (1H, d, *J* 13.8, =CHN***H***-), 11.00 (1H, s, CON***H***CO), 10.87 (1H, s, CON***H***CO), 8.53 (1H, d, *J* 14.1, =C***H***-N), 7.94 (1H, s, Ar***H***), 7.79 (1H, d, *J* 7.8, Ar***H***), 7.77 (1H, d, *J* 7.8, Ar***H***), 7.54 (1H, t, *J* 7.8,Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166.6(C), 165.9(C), 163.5(C), 151.5(CH), 150.7(C), 139(C), 132.3(C), 130(CH), 126.4(CH), 122.9(CH), 119.1(CH), 93(C); MS (EI) *m/z* 275(M^+ 100%), 258(9), 230(20), 188(99), 144(29), 65(18).

4-{[(2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (3)

Yield 91%, off-white solid, m.p. 350-360 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 12.92 (1H, s, COO***H***), 11.9 (1H, d, *J* 13.5, =CHN***H***-), 11.06 (1H, s, CON***H***CO), 10.91 (1H, s, CON***H***CO), 8.59 (1H, d, *J* 13.5, =C***H***-N), 7.95 (2H, d, *J* 8.7,Ar***H***), 7.61 (2H, d, *J* 8.4, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166.5(C), 166(C), 163.4(C), 150.9(CH), 150.6(C), 142.1(C), 131(CH), 127.6(C), 118.1(CH), 93.7(C); MS (EI) *m/z* 275(M^+ 83%), 188(63), 144(29), 89(30), 65(100), 53(76).

2-hydroxy-4-{[(2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (4)

Yield 75%, brown solid, m.p. 324-330 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm);13.13 (1H, s, COO***H***), 11.77 (1H, d, *J* 13.6, =CHN***H***-),11.05 (1H, s, CON***H***CO), 10.90(1H, s, CON***H***CO), 9.76 (1H, s, O***H***), 8.53 (1H, d, *J* 13.6, =C***H***-N), 7.79 (1H, d, *J*8.4, Ar***H***), 7.08 (1H, s, Ar***H***), 7.03 (1H, d, *J* 8.8, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 171.3(C), 166(C), 163.3(C), 162.4(C), 150.7(CH), 150.6(C), 144.3(C), 132(CH), 110.1(C), 109.1(CH), 105.8(CH), 93.9(C); MS (EI) *m/z* 291(M^+ 54%), 273(21), 247(50), 159(36), 119(17), 103(20), 78(29), 53(100).

2-hydroxy-5-{[(2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (5)

Yield 74%, off-white solid, m.p. 252-260 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 13.41(1H, s, COO***H***),11.79 (1H, d, *J* 13.6, =CHN***H***-), 10.89 (1H, s, CON***H***CO), 10.75 (1H, s, CON***H***CO), 9.86 (1H, s, O***H***), 8.39 (1H, d, *J* 12.4, =C***H***-N),7.77 (1H, s, Ar***H***), 7.58 (1H, dd, *J* 8.8, Ar***H***), 6.96 (1H, d, *J* 8.8, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 171.5(C), 170.9(C), 165.9(C), 163.5(C), 159.6(C), 151.4(CH), 129.8(C), 124.2(CH), 120(CH), 117.2(CH), 113.8(C), 91.7(C); MS (EI) *m/z* 291(M^+ 48%), 273(94), 174(20), 146(43), 103(29), 79(41), 53(100).

4-nitro-2-{[(2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (6)

Yield 90%, light yellow solid, m.p. 338-346 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 14.44 (1H, s, COO***H***), 13.36 (1H, d, *J* 13.6, =CHN***H***-), 11.04 (1H, s, CON***H***CO), 10.95 (1H, s, CON***H***CO), 8.66 (1H, d, *J* 13.2, =C***H***-N), 8.51 (1H, s, Ar***H***), 8.2 (1H, d, *J* 8.4, Ar***H***), 8.03 (1H, dd, *J* 8.4 Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166.4(C), 165.1(C), 163.5(C), 150.6(C), 150.6(C), 150.4(CH), 141(C), 133.2(CH), 123.6(C), 118.5(CH), 112.4(CH), 95.3(C); MS (EI) *m/z* 320(M^+ 29%), 302(15), 274(27), 188(27), 91(13), 53(100).

4-hydroxy-3-{[(2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (7)

Yield 74%, off-white solid, m.p. 326-334 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 12.81 (1H, s, COO***H***), 12.01 (1H, d, *J* 14, =CHN***H***-),11.40 (1H, s, O***H***), 10.97 (1H, s, CON***H***CO), 10.84 (1H, s, CON***H***CO), 8.59 (1H, d, *J* 14, =C***H***-N), 7.99 (1H, s, Ar***H***), 7.67 (1H, dd, *J* 8.4, Ar***H***), 7.03 (1H, d, *J* 8.4 Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166.5(C), 166.2(C), 163.3(C), 151C), 150.6(C), 149.9(CH), 127.8(CH), 126(C), 122.4(C), 117(CH), 115.4(CH), 92.7(C); MS (EI) *m/z* 291(M^+ 87%), 181(21), 163(86), 153(58), 146(100), 118(22), 63(37).

2-nitro-5-{[(2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (8)

Yield 76%, yellow solid, m.p. 198-200 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 13.95 (1H, s, COO***H***), 11.89 (1H, d, *J* 13.6, =CHN***H***-), 11.08 (1H, s, CON***H***CO), 10.95 (1H, s, CON***H***CO), 8.6 (1H, d, *J* 13.6, =C***H***-N), 8.04 (1H, d, *J* 8.8 Ar***H***), 7.91 (1H, s, Ar***H***), 7.86 (1H, d, *J* 8.8 Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 168(C), 167.6(C), 165.3(C), 154.7(C), 151.5(C), 151(C), 148.4(CH), 133.8(C), 127.1(CH), 112.7(CH), 111.2(CH), 100.2(C); MS (EI) *m/z* 320(M^+ 70%), 128(100), 100(7), 85(30), 69(20).

4-chloro-3-{[(2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (9)

Yield 88%, off-white solid, m.p. 330-335 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 13.42 (1H, s, COO***H***), 12.33 (1H, d, *J* 13.2, =CHN***H***-), 11.16 (1H, s, CON***H***CO), 10.99 (1H, s, CON***H***CO), 8.65 (1H, d, *J* 13.2, =C***H***-N), 8.18 (1H, s, Ar***H***), 7.75 (1H, d, *J* 8.4, Ar***H***), 7.71 (1H, d, *J* 8.4 Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166.6(C), 166(C), 163.1(C), 151.1(CH), 150.5(C), 135.4(C), 131.2(C), 130.3(CH), 127.2(C), 126.8(CH), 118.3(CH), 94.2(C);MS (EI) *m/z* 309(M^+ 55%), 274(100), 231(70), 99(68), 53(78).

2-{[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (10)

Yield 89%, dark yellow solid, m.p. 340-344 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 13.84 (1H, s, COO***H***), 13.5 (1H, d, *J* 14, =CHN***H***-), 12.11 (1H, s, CON***H***CS), 12.02 (1H, s, CSN***H***CO), 8.65 (1H, d, *J* 14, =C***H***-N), 8.02 (1H, d, *J*8, Ar***H***), 7.84 (1H, d, *J* 8, Ar***H***), 7.69 (1H, t, *J* 8 Ar***H***), 7.34 (1H, t, *J* 8Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 178(C), 167.5(C), 162.9(C), 161.7(C), 150.9(CH), 139.5(C), 134.5(CH), 131.6(CH), 125.4(CH), 118.9(C), 117.2(CH), 95.4(C); MS (EI) *m/z* 291(M^+ 73%), 170(32), 143(43), 115(43), 77(64), 53(100).

3-{[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (11)

Yield 91%, light yellow solid, m.p. 332-338 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 13.2 (1H, s, COO***H***), 12.16 (1H, d, *J* 14, =CHN***H***-), 12.04 (1H, s, CON***H***CS), 12 (1H, s, CSN***H***CO), 8.58 (1H, d, *J* 14, =C***H***-N), 7.99 (1H, s, Ar***H***), 7.82 (1H, d, *J* 8, Ar***H***), 7.8 (1H, d, *J* 8, Ar***H***), 7.55 (1H, t, *J* 8,Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 177.9(C), 166.5(C), 163.7(C), 161.6(C), 152.5(CH), 138.8(C), 132.2(C), 130(CH), 126.9(CH), 123.3(CH), 119.6(CH), 94.3(C);MS (EI) *m/z* 291(M^+ 100%), 188(57), 137(39), 116(38), 89(26), 65(83), 53(65).

4-{[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}benzoic acid (12)

Yield 86%, yellow solid, m.p. 335-340 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 12.95 (1H, s, COO***H***), 12.21 (1H, d, *J* 14, =CHN***H***-), 12.07 (1H, s, CON***H***CS), 12.03 (1H, s, CSN***H***CO), 8.64 (1H, d, *J* 14, =C***H***-N), 7.96 (2H, d, *J* 8.8,Ar***H***), 7.62 (2H, d, *J* 8.8, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 177.9(C), 166.5(C), 163.7(C), 161.4(C), 151.9(CH), 141.8(C), 130.9(CH), 128(C), 118.5(CH), 95.00(C); MS (EI) *m/z* 291(M^+ 100%), 188(49), 144(46), 116(39), 89(29), 65(87), 53(61).

4-{[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}-2-hydroxy-benzoic acid (13)

Yield 76%, green solid, m.p. 300-306 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 13.17 (1H, s, COO***H***), 12.21 (1H, s, CON***H***CS),12.08 (1H, s, CSN***H***CO), 11.92 (1H, d, *J* 14, =CHN***H***-), 9.79 (1H, s, O***H***), 8.59 (1H, d, *J* 13.6, =C***H***-N), 7.81 (1H, d, *J*8.8, Ar***H***), 7.15 (1H, s, Ar***H***), 7.09 (1H, dd, *J* 8.8, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 177.9(C), 171.1(C), 163.6(C), 162.2(C), 161.3(C), 151.7(CH), 144(C), 131.9(CH), 110.5(C), 109.4(CH), 106.3(CH), 95.1(C); MS (EI) *m/z* 307(M^+ 53%), 263(27), 188(23), 103(32), 65(62), 53(100).

5-{[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}-2-hydroxy-benzoic acid (14)

Yield 77%, green solid, m.p. 300-305 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 13.46 (1H, s, COO***H***),12.08 (1H, s, CON***H***CS), 11.95 (1H, s, CSN***H***CO),11.95 (1H, d, *J* 14, =CHN***H***-), 9.90 (1H, s, O***H***), 8.44 (1H, d, *J* 14, =C***H***-N),7.86 (1H, s, Ar***H***), 7.72 (1H, dd, *J* 9.2, Ar***H***), 7.00 (1H, d, *J* 9.2, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 177.7(C), 170.8(C), 163.5(C), 161.5(C), 159.3(C), 152.4(CH), 130.2(C), 126.6(CH), 120.6(CH), 118.2(CH), 113.8(C), 93.5(C); MS (EI) *m/z* 307(M^+ 55%), 263(26), 188(25), 103(34), 65(63), 53(100).

2-{[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}-4-nitrobenzoic acid (15)

Yield 90%, light yellow solid, m.p. 320-326 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 14.5 (1H, s, COO***H***), 13.5 (1H, d, *J* 13.8, =CHN***H***-), 12.22 (1H, s, CON***H***CS), 12.13 (1H, s, CSN***H***CO), 8.74 (1H, d, *J* 13.5, =C***H***-N), 8.59 (1H, s, Ar***H***), 8.23 (1H, d, *J* 8.7, Ar***H***), 8.08 (1H, dd, *J* 8.7 Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 178.1(C), 166.3(C), 163(C), 161.6(C), 151.8(CH), 150.6(C), 140.7(C), 133.2(CH), 124(C), 119.1(CH), 113(CH), 96.3(C); MS (EI) *m/z* 336(M^+ 43%), 290(3), 216(17), 188(5), 114(19), 90(14), 69(37), 53(100).

3-{[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}-4-hydroxybenzoic acid (16)

Yield 76%, yellow solid, m.p. 323-330 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 12.77 (1H, s, COO***H***), 12.2 (1H, d, *J* 14.4, =CHN***H***-), 12.16 (1H, s, CON***H***CS), 12.03 (1H, s, CSN***H***CO), 11.5 (1H, s, O***H***), 8.66 (1H, d, *J* 14.4, =C***H***-N), 8.05 (1H, s, Ar***H***), 7.7 (1H, dd, *J* 8.4, Ar***H***), 7.03 (1H, d, *J* 8.4 Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 177.8(C), 166.6(C), 164.1(C), 161.5(C), 151.3(C), 150.9(CH), 128.4(CH), 125.7(C), 122.4(C), 117.6(CH), 115.5(CH), 94.1(C); MS (EI) *m/z* 307(M^+ 11%), 263(100), 188(23), 144(21), 120(77), 77(23), 53(53).

5-{[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}-2-nitrobenzoic acid (17)

Yield 72%, yellow solid, m.p.247- 250 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 14 (1H, s, COO***H***), 12.04 (1H, d, *J* 13.6, =CHN***H***-), 12.25 (1H, s, CON***H***CS), 12.12 (1H, s, CSN***H***CO), 8.66 (1H, d, *J* 13.6, =C***H***-N), 8.08 (1H, d, *J* 8.8 Ar***H***), 8 (1H, s, Ar***H***), 7.89 (1H, dd, *J* 9.2 Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 178(C), 165.7(C), 163.5(C), 161.3(C), 154.8(C), 152.3(CH), 142.6(C), 130.3(C), 125.8(CH), 119.3(CH), 111.2(CH), 95.9(C); MS (EI) *m/z* 336(M^+ 100%), 292(32), 155(49), 116(42), 103(40), 63(48), 53(81).

4-chloro-3-{[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl]amino}-benzoic acid (18)

Yield 86%, light yellow solid, m.p. 316-320 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 13.44 (1H, s, COO***H***), 12.45 (1H, d, *J* 13.2, =CHN***H***-), 12.31 (1H, s, CON***H***CS), 12.15 (1H, s, CSN***H***CO), 8.72 (1H, d, *J* 13.2, =C***H***-N), 8.23 (1H, s, Ar***H***), 7.77 (1H, dd, *J* 8.4, Ar***H***), 7.72 (1H, dd, *J* 8.4 Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 178(C), 165.9(C), 164.4(C), 161.2(C), 152.3(CH), 135.2(C), 131.3(C), 130.3(CH), 127.6(C), 127.3(CH), 118.8(CH), 95.5(C); MS (EI) *m/z* 325(M^+ 67%), 290(46), 222(44), 182(27), 171(26), 98.9(36), 53(100).

5-({[2-(trifluoromethyl)phenyl]amino}methylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (19)

Yield 93%, white solid, m.p. 300-307 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm); 12.47 (1H, d, *J* 12.8, =CHN***H***-), 11.15 (1H, s, CON***H***CO), 10.96 (1H, s, CON***H***CO), 8.61 (1H, d, *J* 12.8, =C***H***-N), 7.90 (1H, d, *J* 8 Ar***H***), 7.78 (1H, d, *J* 8.4, Ar***H***), 7.74 (1H, t, *J* 8, Ar***H***), 7.44 (1H, t, *J* 7.6, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166.7 (C), 163.1 (C), 152.3 (CH), 150.5 (C), 136.1 (C), 134.5(CH), 126.7 (CH), 126 (CH), 125 (C), 122.3(C), 119.8 (CH), 94.4 (C); MS (EI) *m/z* 299.1(M^+ 100%), 254(5.1), 212(45.9), 154(7.6), 126(6.4), 68(6.6).

5-({[3-(trifluoromethyl)phenyl]amino}methylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (20)

Yield 80%, White solid, m.p. 301-306 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 11.89 (1H, d, *J* 13.6, =CHN***H***-), 11.02 (1H, s, CON***H***CO), 10.88 (1H, s, CON***H***CO), 8.6 (1H, d, *J* 13.6, =C***H***-N), 7.98 (1H, s, Ar***H***), 7.82 (1H, d, *J* 8, Ar***H***), 7.61 (1H, t, *J* 8, ArH), 7.56 (1H, d, *J* 7.6, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 165.9 (C), 163.4 (C), 151.8 (CH), 150.6 (C), 139.6 (C), 130.7 (CH), 130.3 (C), 125.1 (C), 122.5 (CH), 122 (CH), 115.7 (CH), 93.3 (C); MS (EI) *m/z* 299.0(M^+ 100%), 212(75.7), 172(7), 144(3.3), 95(7.5).69(3.5).

5-({[4-(trifluoromethyl)phenyl]amino}methylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (21)

Yield 79%, white solid, m.p. 270-274 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 11.96 (1H, d, *J* 13.8, =CHN***H***-), 11.10 (1H, s, CON***H***CO), 10.95 (1H, s, CON***H***CO), 8.65 (1H, d, *J* 14.1, =C***H***-N), 8.06 (2H, d, *J* 8.7, Ar***H***), 7.74 (2H, d, *J* 9, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166 (C), 163.4 (C), 151.2 (CH), 150.8(C), 131 (C), 129.5 (2CH), 125.8(C), 123.7 (C), 120.1 (2CH), 93.5 (C); MS (EI) *m/z* 401.2(MH^+ 100%), 299.1(10.7), 212.1(10.7), 172.1(6.6), 144.1 (3.9), 95(6.7), 69(5).

5-({[5-nitro-2-(trifluoromethyl)phenyl]amino}methylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (22)

Yield 78%, yellow solid, m.p. 333-338 °C (decompose) ; ^{1}H NMR (d_6-DMSO, δ, ppm); 12.62 (1H, d, *J* 12, =CHN***H***-), 11.29 (1H, s, CON***H***CO), 11.11 (1H, s, CON***H***CO), 8.76 (1H, d, *J* 12, =C***H***-N), 8.51 (1H, d, *J* 9.2 Ar***H***), 8.42 (1H, s, Ar***H***), 8.20 (1H, d, *J* 9.2, Ar***H***),

^{13}C NMR (d_6-DMSO, δ, ppm): 166.8 (C), 162.7 (C), 151.3 (CH), 150.9 (C), 150.4(C), 143.38(C), 141.4 (C), 129.3 (CH), 122.8(CH), 120.7 (C), 120 (CH), 96.7 (C); MS (EI) *m/z* 334.2(M^+ 100%), 314.2(6.8), 273.2(9.7), 171.1(4.6), 154.1(17.3), 144.1(13.2), 75.1(8.2), 69.1(10.7).

5-{[(3,5-dichlorophenyl)amino]methylidene}pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (23)

Yield 94%, off white solid, m.p. 315-320 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 11.76 (1H, d, *J* 13.5, =CHN***H***-), 11.05 (1H, s, CON***H***CO), 10.90 (1H, s, CON***H***CO), 8.52 (1H, d *J* 13.5, =C***H***-N), 7.71 (2H, s, Ar***H***), 7.40 (1H, s, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 165.8 (C), 163.3(C), 151.7 (CH), 150.6 (C), 141.2 (C), 134.9 (C), 124.8 (CH), 117.6 (CH), 93.9(C); MS (EI) *m/z* 298.9(M^+ 100%), 171.9(6.1), 154(11.7), 144.9(18.8), 69(3.8).

5-{[(2,4,5-trichlorophenyl)amino]methylidene}pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (24)

Yield 73%, light yellow solid, m.p. 312-318 °C (decompose);); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.38 (1H, d, *J* 13.2, =CHN***H***-), 11.20 (1H, s, CON***H***CO), 11.06 (1H, s, CON***H***CO), 8.76 (1H, d, *J* 13.2, =C***H***-N), 8.34 (1H, s, Ar***H***), 8.00 (1H, s, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166.7(C), 162.9 (C), 151.3 (CH), 150.5 (C), 135.4 (C), 131.5 (C), 130.7 (CH), 127.6 (C), 122.1 (C), 119.4 (CH), 94.8 (C); MS (EI) *m/z* 346(M^+ 2%), 298(100), 144.9(4.8), 69(3.1).

5-{[(4-iodophenyl)amino]methylidene}pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (25)

Yield 90%, white solid, m.p. 306-310 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 11.78 (1H, d, *J* 13.6, =CHN***H***-), 11.01 (1H, s, CON***H***CO), 10.87 (1H, s, CON***H***CO), 8.49 (1H, d, *J* 13.6, =C***H***-N), 7.74 (2H, d, *J* 8.8, Ar***H***), 7.35 (2H, d, *J* 8.8, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166 (C), 163.4 (C), 151 (CH), 150.6 (C), 138.4 (C), 138.2(2CH), 129.7 (C), 120.7 (2CH), 92.9 (C).

5-{[(2-hydroxyphenyl)amino]methylidene}pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (26)

Yield 97%, yellow solid, m.p. 323-330 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.11 (1H, d, *J* 14.4, =CHN***H***-), 10.94 (1H, s, CON***H***CO), 10.81 (1H, s, CON***H***CO), 10.53

(1H, s, O***H***), 8.60 (1H, d, *J* 14.4, =C***H***-N), 7.58 (1H, d, *J* 8, Ar***H***), 7.05 (1H, t, *J* 7.6, Ar***H***), 6.95 (1H, d, *J* 7.6, Ar***H***), 6.87 (1**H** , t, *J* 7.6, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166.3 (C), 163.5 (C), 150.7 (C), 149.6(CH), 147 (C), 126.4 (CH), 126 (C), 120 (CH), 115.9 (CH), 115.7 (CH), 92.3 (C); MS (EI) *m/z* 231(M^+ 1.5%), 159(22.6), 120(100%), 69(3.6).

5-{[(2-sulfanylphenyl)amino]methylidene}pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (27)

Yield 70%, light yellow solid, m.p. 239-243 ^{o}C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.53 (1H, d, *J* 8.8, =CHN***H***-), 11.10 (1H, s, CON***H***CO), 11.03 (1H, s, CON***H***CO), 10.90 (1H, s, S***H***), 8.52 (1H, d, *J* 13.6, =C***H***-N), 7.73 (1H, d, *J* 8, Ar***H***), 7.32 (1H, d, *J* 7.2, Ar***H***), 7.11 (1H, m, Ar***H***), 7.08 (1H, m, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 166.2 (C), 163.2 (C), 150.6 (C), 150.3 (CH), 140.2 (C), 126.1 (CH), 124.2(C), 117.2 (CH), 115.8 (CH), 114.9 (CH), 93.8 (C); MS (EI) *m/z* 125(M^+ 100%), 95.0(3.2),69.0(12.0).

2-thioxo-5-({[2-(trifluoromethyl)phenyl]amino}methylidene)dihydropyrimidine-4,6 (1*H*,5*H*)-dione (28)

Yield 73%, yellow solid, m.p. 316-321 $^{\circ}$C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.59 (1H, d, *J* 12.6, =CHN***H***-), 12.31 (1H, s, CON***H***CS), 12.15 (1H, s, CSN***HCO***), 8.68(1H, d, *J* 12.6, =C***H***-N), 7.94 (1H, d, *J* 7.5, Ar***H***), 7.80 (1H, d, *J* 6.9, Ar***H***), 7.78 (1H, t, *J* 6.9, Ar***H***), 7.48 (1H, t, *J* 6.9, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 178 (C), 164.6 (C), 161.1 (C), 153.6 (CH), 135.8 (C), 134.5 (CH), 126.8 (CH), 126.6 (CH), 125.4 (C), 121.8 (C), 120.3 (CH), 95.6 (C).

2-thioxo-5-({[3-(trifluoromethyl)phenyl]amino}methylidene)dihydropyrimidine-4,6(1*H*,5*H*)-dione (29)

Yield 83%, light greenish, m.p. 312-318 $^{\circ}$C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.19 (1H, s, CON***H***CS), 12.06 (1H, d, *J* 13.6, =CHN***H***-), 12.05 (1H, s, CSN***HCO***), 8.66 (1H, d, *J* 14, =C***H***-N), 8.04 (1H, s, Ar***H***), 7.86 (1H, d, *J* 8, Ar***H***), 7.66 (1H, t, *J* 7.6, Ar***H***), 7.59 (1H, d, *J* 7.6, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm); 177.9 (C), 163.6 (C), 161.5 (C), 152.9 (CH), 139.3 (C), 130.7 (CH), 130.3 (C), 125.1 (C), 122.9 (CH), 122.5(CH), 116.2 (CH), 94.7 (C); MS (EI) *m/z* 315(M^+ 100%), 172.1(21.4), 155.0(9.3), 144(5), 75.1(3.5), 69.0(4.9).

2-thioxo-5-({[4-(trifluoromethyl)phenyl]amino}methylidene)dihydropyrimidine-4,6 (1*H*,5*H*)-dione (30)

Yield 76%, light yellow, m.p. 279-281 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.22 (1H, s, CON***H***CS), 12.09 (1H, s, CSN***H***CO), 12.00 (1H, d, *J* 14.4, =CHN***H***-), 8.69 (1H, d *J* 13.6, =C***H***-N), 8.00 (2H, m, Ar***H***), 7.74 (2H, m, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 178.2(C), 164.1(C), 161.8 (C), 152.2 (CH), 141.4 (C), 129.8 (2CH), 126.2 (C), 120.4 (2CH), 118.7 (C), 96.2 (C); MS (EI) *m/z* 315(M^+ 33.1%), 172(7.4), 144(4.0).

5-({[5-nitro-2-(trifluoromethyl)phenyl]amino}methylidene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (31)

Yield 97%, light yellow solid, m.p. 308-312°C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.77 (1H, d, *J* 12, =CHN***H***-), 12.45 (1H, s, CON***H***CS), 12.28 (1H, s, CSN***H***CO), 8.81 (1H, d, *J* 12, =C***H***-N), 8.54 (1H, d, *J* 9.2, Ar***H***), 8.48 (1H, s, Ar***H***), 8.25 (IH, d, *J* 9.2, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 178.2 (C), 164.7 (C), 160.8 (C), 152.6 (CH), 143.7(C), 141 (C), 129.2 (CH), 123.9(C), 122.8 (CH), 121.2 (C), 120.6 (CH), 97.7 (C); MS (EI) *m/z* 360.1(M^+ 100%), 172.1(5), 155(30.5), 144.1(19.5), 116(11.1), 76.1(5.5), 69.1(9.8).

5-{[(3,5-dichlorophenyl)amino]methylidene}-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (32)

Yield 79%, light yellow solid, m.p. 335-341°C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.20 (1H, s, CON***H***CS), 12.07 (1H, s, CSN***H***CO), 11.90 (1H, d, *J* 13.8, =CHN***H***-), 8.58 (1H, d, *J* 13.8, =C***H***-N), 7.75 (2H, s, Ar***H***), 7.43 (1H, s, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 178 (C), 163.6 (C), 161.4 (C), 152.7 (CH), 140.9 (C), 134.8 (C), 125.3 (CH), 118(CH), 95.1 (C); MS (EI) *m/z* 315(M^+ 100%), 172(23.4), 155(21.1), 145(18.2), 76.1(3.3), 69.1(6.6).

2-thioxo-5-{[(2,4,5-trichlorophenyl)amino]methylidene}dihydropyrimidine-4,6(1*H*,5*H*)-dione (33)

Yield 78%, white solid, m.p. 350-360 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.51 (1H, d, *J* 13.2, =CHN***H***-), 12.35 (1H, s, CON***H***CS), 12.17 (1H, s, CSN***H***CO), 8.82 (1H, d, *J* 13.2, =C***H***-N), 8.39 (1H, s, Ar***H***), 8.03 (1H, s, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 178 (C), 164.6 (C), 161 (C), 152.5 (CH), 135.1 (C), 131.5 (C), 130.8(CH), 128.2 (C), 122.4 (C), 119.8 (CH), 96 (C); MS (EI) *m/z* 351 (M^+ 100%), 315(16.2), 172(3.4), 155(13.2), 69(11.3).

5-{[(4-iodophenyl)amino]methylidene}-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (34)

Yield 90%, white solid, m.p. 329-335 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.17 (1H, s, CON***H***CS), 12.04 (1H, s, CSN***H***CO), 11.94 (1H, d, *J* 14, =CHN***H***-), 8.55 (1H, d, *J* 14, =C***H***-N), 7.76 (2H, d, *J* 8.8, Ar***H***), 7.39 (2H, d, *J* 8.8, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 177.8 (C), 163.7 (C), 161.5 (C), 152 (CH), 138.2 (2CH), 138.1 (C), 129.7 (C), 121.1 (2CH), 94.3 (C); MS (EI) *m/z* 373.1(M^+ 100%), 315.1(6.0), 155.0(3.8), 141.1(8.9), 76.0(14.2), 69.0(3.2).

5-{[(2-hydroxyphenyl)amino]methylidene}-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (35)

Yield 82%, light yellow solid, m.p. 323-330 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.30 (1H, d, *J* 14, =CHN***H***-), 12.13 (1H, s, CON***H***CS), 12.00 (1H, s, CSN***H***CO), 10.63 (1H, s, O***H***), 8.66 (1H, d, *J* 14.4, =C***H***-N), 7.62 (1H, d, *J* 8, Ar***H***), 7.08 (1H, t, *J* 7.6), 6.95 (1H, d, *J* 8, Ar***H***), 6.87 (1H, t, *J* 7.6, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 177.6 (C), 164.1 (C), 161.5 (C), 150.3 (CH), 147.3 (C), 126.9 (CH), 125.7 (C), 120(CH), 116.3(CH), 115.8 (CH), 93.8 (C); MS (EI) *m/z* 263(M^+ 100%), 188(19.1), 159(13.8), 65(5.3).

5-{[(2-sulfanylphenyl)amino]methylidene}-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (36)

Yield 80%, white solid, m.p. 225-228 °C (decompose); ^{1}H NMR (d_6-DMSO, δ, ppm): 12.66 (1H, d, *J* 14, =CHN***H***-), 12.17 (1H, s, CON***H***CS), 12.10 (1H, s, CSN***H***CO), 10.90 (1H, s, S***H***), 8.59 (1H, d, *J* 14, =C***H***-N), 7.79 (1H, d, *J* 8, Ar***H***), 7.33 (1H, d, *J* 7.6, Ar***H***),

7.14 (1H, m, Ar***H***), 7.12 (1H, m, Ar***H***); ^{13}C NMR (d_6-DMSO, δ, ppm): 177.8 (C), 164 (C), 161.5 (C), 152.2(CH), 140.1 (C), 126.1 (CH), 125.4 (C), 123(CH), 122.5 (CH), 117.7 (CH), 81.9 (C); MS (CI) *m/z* 264(MH^+ 1%), 175(11.8), 156(10.4), 144(34.8), 76(4.4), 69(36.2).

4.3. Urease inhibition assay

Reaction mixtures comprising 25µL of enzyme (jack bean urease) solution and 55 µL of buffers containing 100 mM urea were incubated with 5 µL of test compounds (0.5 mM concentration) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn. Briefly, 45 µL each phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprussside) and 70 µL of alkali reagent(0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50min, m using a micro-plate reader (Molecular Device, 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 soft Max Pro software (molecular Device, USA). The entire assays were performed at pH 6.8. Percentage inhibitions were calculated from the formula $100-(OD_{testwell}/OD_{control})\times100$. Thiourea was used as standard inhibitor of urease.

4.4 Molecular modelling studies

The three-dimensional structures of the compounds were built with Corina on-line tool [63]. Protonation states were predicted at pH of reaction mixture by Marvin online plugin [64]. The atom and bond types were checked and Gasteiger-Marsili charges were assigned by Sybyl-X 1.1 [65]. Finally, ligand structures were saved in the mol2 format.

The structure of Jack bean (*Canavalia ensiformis*) urease of resolution 1.49 Å was downloaded from Protein Data Bank (PDB code: 4GY7) [66]. Then, all histidine residues except His407, His409 and His545 (Nδ protonation) were protonated at Nε atom, ligand and water molecules were removed, and missing hydrogens were added using Hermes 1.5 [67]. The default coordination states were assigned to both nickel cations, and the unusual amino acids were checked. The binding site was defined as all amino acid residues within 12 Å from nickel ions. Dockings were performed with Gold 5.1 software [68] using genetic algorithm with default settings. GoldScore function and

analysis of binding mode were applied to find final ligand poses. Results were visualized by PyMOL 0.99rc2 [69]. Thiourea was used as a reference compound in the docking studies.

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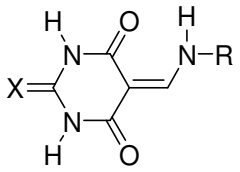
Figure captions

Figure **1**. The binding mode of the most active compound (**15**) within the active site of Jack bean urease. The most important feature of the bonding mode is formation of the complex with nickel ions. The violet dashed lines represent interactions with nickel cations while the grey ones ionic interactions and hydrogen bonds.

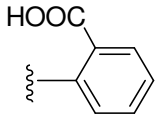
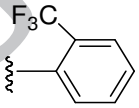
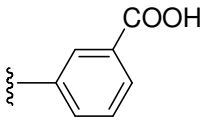
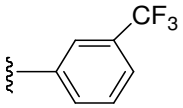
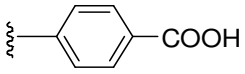
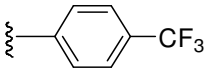
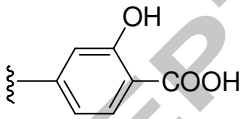
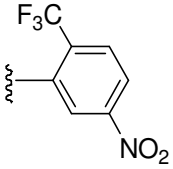
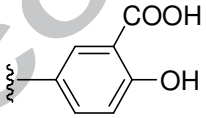
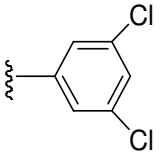
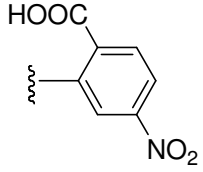
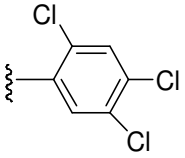
Figure **2**. The binding mode of the inactive compound (**6**) within the active site of Jack bean urease. The interactions scheme is quite the same as for the potent compound (**15**) but the complexing ability and stability of complex are lower. The violet dashed lines represent interactions with nickel cations while the grey ones ionic interactions and hydrogen bonds.

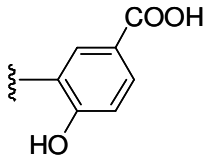
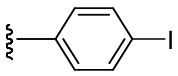
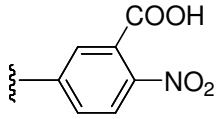
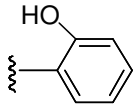
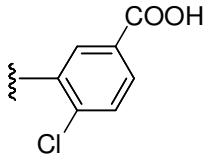
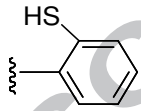
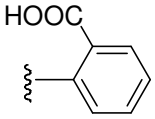
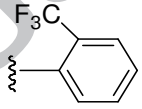
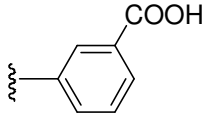
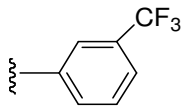
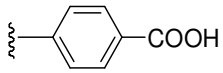
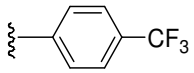
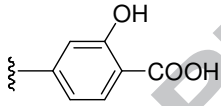
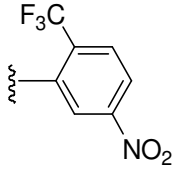
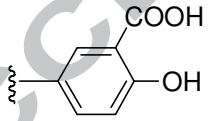
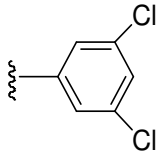
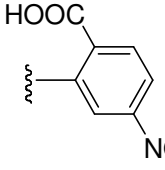
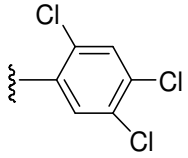
Figure **3**. The binding mode of compound (**12**) within the active site of Jack bean urease. The violet dashed lines represent interactions with nickel cations while the grey ones ionic interactions and hydrogen bonds.

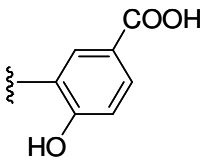
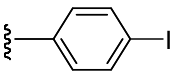
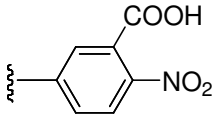
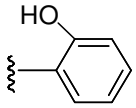
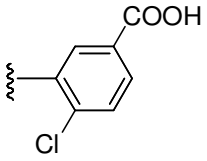
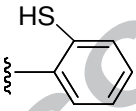
Table 1. Chemical structures, urease inhibition activity and IC_{50} values (mean± SEM, n=3) of substituted anilines based barbiturates and thiobarbiturates (ND, not determined).



(Compounds 1-36)

No	X	R	IC_{50}	No	X	R	IC_{50}
1	O		ND	**19**	O		ND
2	O		ND	**20**	O		ND
3	O		ND	**21**	O		ND
4	O		88.3±1.22	**22**	O		ND
5	O		ND	**23**	O		ND
6	O		ND	**24**	O		ND

7	O		ND	**25**	O		ND
8	O		25.8±0.47	**26**	O		ND
9	O		ND	**27**	O		52.6±0.29
10	S		12.96±0.13	**28**	S		65.3±0.57
11	S		21.83±0.19	**29**	S		24.56±0.098
12	S		**8.93±0.027**	**30**	S		136.4±0.50
13	S		32.3±0.16	**31**	S		28.7±0.32
14	S		ND	**32**	S		89.16±1.00
15	S		**8.53±0.027**	**33**	S		ND

16	S		18.9±0.027	**34**	S		41.33±0.29
17	S		39.76±0.072	**35**	S		19.7±0.63
18	S		26.66±0.95	**36**	S		15±0.098
		Thio-urea[a]	**21±0.011**				

[a] Thiourea standard inhibitor for antiurease activity

Fig 1

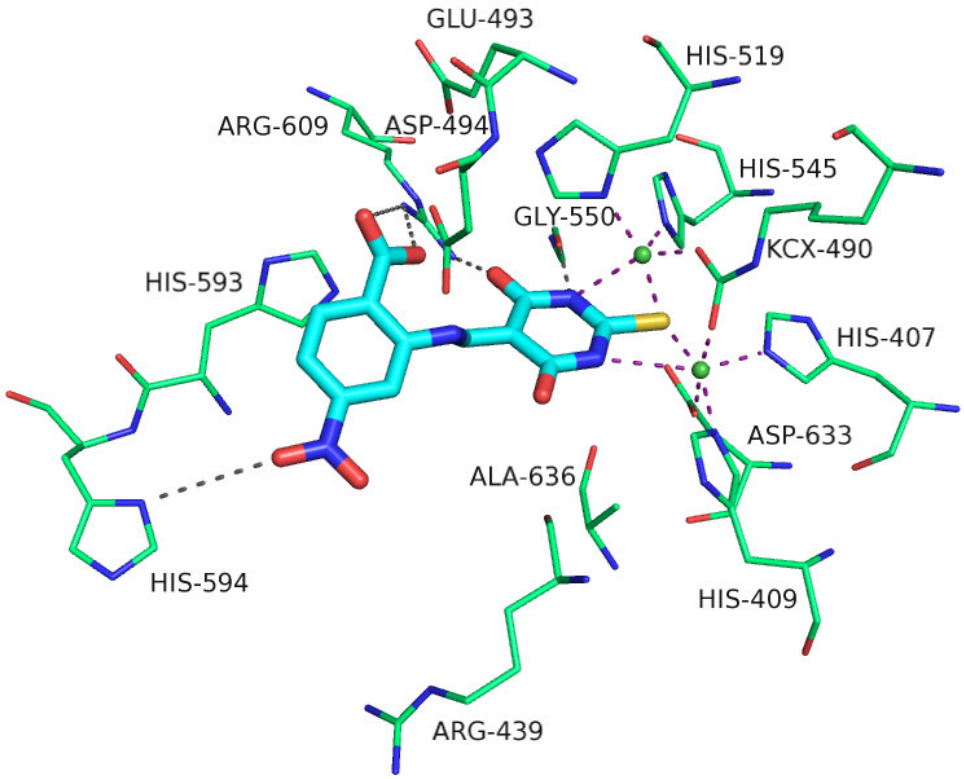


Fig 2

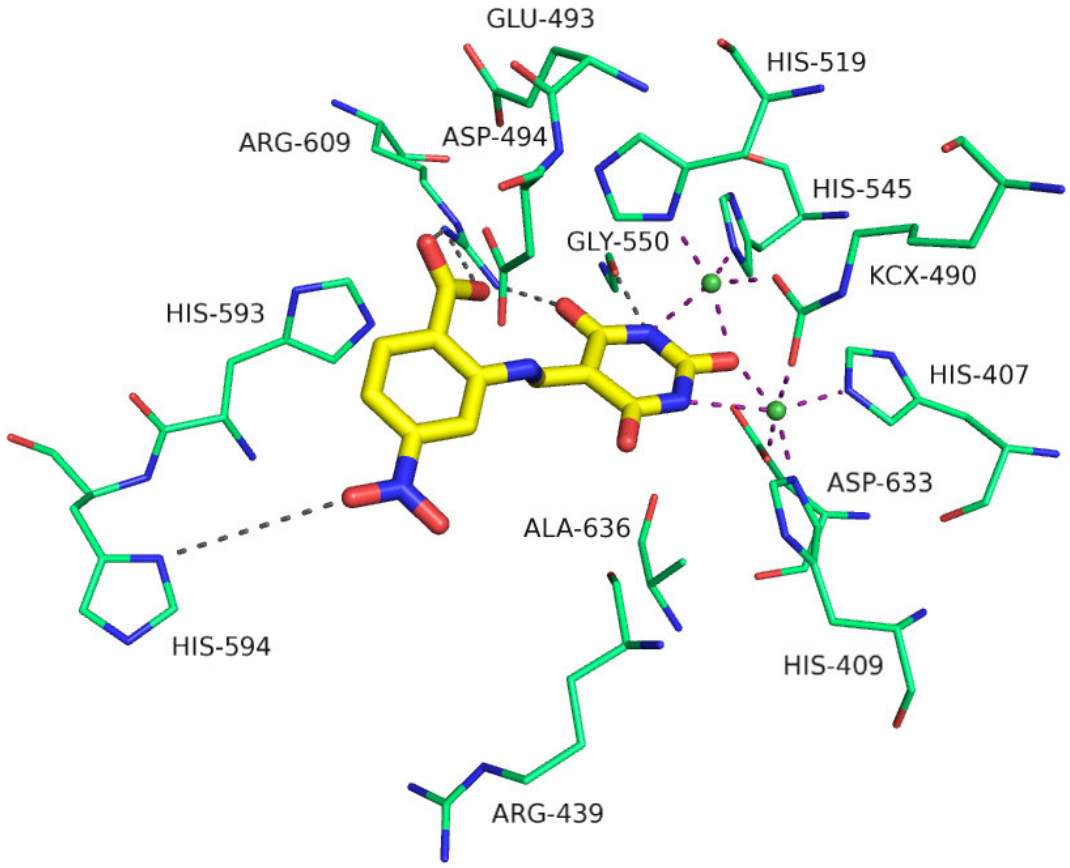
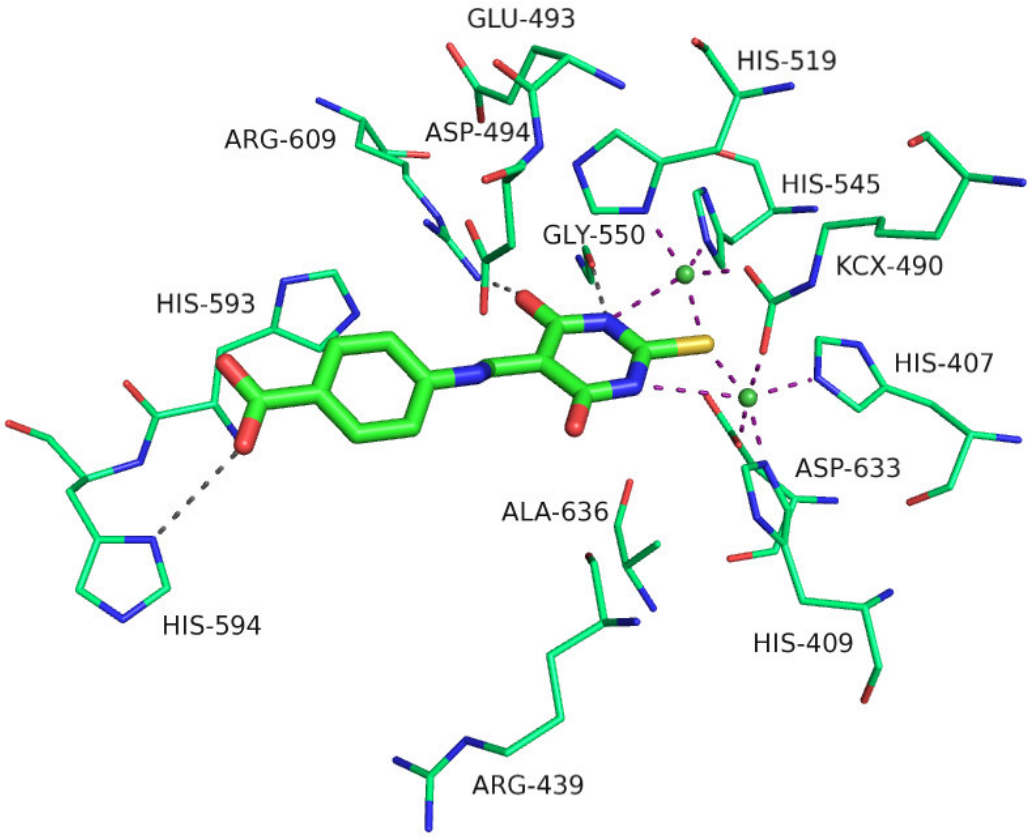
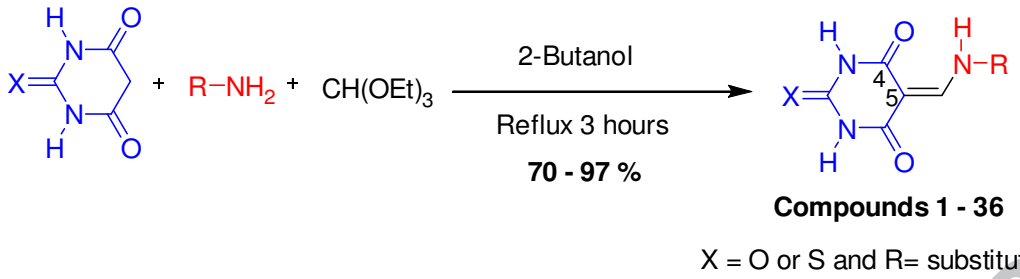


Fig 3

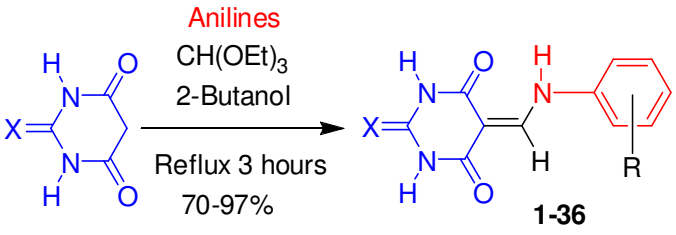


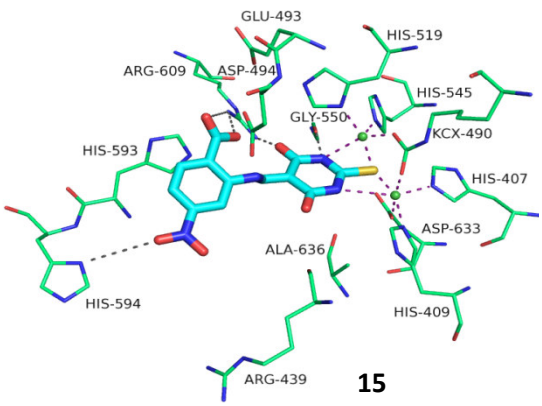
Scheme



Scheme 1: Synthetic protocol for substituted anilines based barbiturates and thio-barbiturates X = O or S and R= substituted aryls

Graphical Abstract





Binding mode of the most active compound (**15**) within the active site of Jack bean urease