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Solution-phase microwave assisted parallel synthesis, biological evaluation and in silico docking studies of *N*,*N*′-disubstituted thioureas derived from 3-chlorobenzoic acid

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ABSTRACT

A facile and robust microwave-assisted solution phase parallel synthesis protocol was exercised for the development of a 38-member library of *N*,*N*′-disubstituted thiourea analogues (**1**–**38**) by using an identical set of conditions. The reaction time for synthesis of *N*,*N*′-disubstituted thiourea analogues was drastically reduced from a reported duration of 8–12 h for conventional methods to only 1.5–2.0 min. All the derivatives (**1**–**38**) were characterized by physico-analytical techniques such as elemental analysis in combination with FT-IR, ^{1}H, ^{13}C NMR and by single crystal XRD analysis have also been performed. These compounds were screened for their in vitro urease inhibition activities. Majority of compounds exhibited potent urease inhibition activities, however, the most significant activity was found for **16**, with an IC_{50} value of 1.23 ± 0.1 μM. Furthermore, the synthesized compounds were screened for their cytotoxic potential against lungs cancer cell lines. Cell culture studies demonstrated significant toxicity of the compounds on the cell lines, and the levels of toxicity were altered in the presence of various side groups. The molecular docking studies of the most potent inhibitors were performed to identify the probable binding modes in the active site of the urease enzymes. These compounds have a great potential and significance for further investigations.

1. Introduction

Urease (urea amidohydrolase; E.C.3.5.1.5), a nickel-containing metalloenzyme with an ability to catalyze the hydrolysis of urea to ammonia and carbamates, is considered as an important virulence factor in the pathogenesis of several diseases.[1,2] It is predominantly found in numerous living organisms, such as bacteria, fungi, higher plants, and some invertebrates.[3,4] Urease is important for human and animal health due to its involvement in the development of several infections associated with stones and plays a vibrant role in pathogenesis of urolithiasis, pyelonephritis, and hepatic encephalopathy.[4] In addition, high concentration of ammonia from the hydrolysis reaction as well as elevated pH level also imparts several negative effects in the fields of medicine and agriculture.[5–8] To counteract these negative effects, inhibition of urease is an attractive strategy and a diverse variety of urease inhibitors have been reported[1,9–12], however, due to their toxicity or instability, the development of potent urease inhibitors with fewer toxic effects is highly needed.

Similarly, cancer still remains a potentially life-threatening disease and is the second cause of death in the US, with an expected 1,665,540 cancer cases in 2014. The high volume of data related to cancer deaths indicates that 90% of the patients die due to chronic tumor metastases.[13] Cancer is chronic disease, initiated by complex processes, which are directly associated to a fundamental cycle of life, i.e., cell division and may be induced by exogenous agents and is characterized by uncontrolled cell proliferation leading to tumor formation.[13,14] Several external (chemicals, radiation, infectious microorganisms, and tobacco) and internal (hormones, mutations and immune conditions) factors contribute enormously to the eruption of cancers.[14,15] Despite the development of several anticancer drugs, the continued efforts to identify new anticancer

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agents with less side effects and toxicity is the subject of current medicinal chemistry research.[16,17]

Aryl/aroyl substituted thioureas have found potential applications for the structural developments in heterocyclic chemistry and modifications with remarkable pharmacological and biological implications. In the past few decades, in vitro studies have shown that polyfunctional thioureas are potential herbicides,[18] insecticides,[19] plant-growth regulators[20] and antihypertensive agents,[21] antitubercular, antimalarial, anticancer,[22] antithyroid, antihelmintic and TRPV1 receptor antagonists.[23] Thioureas may be used as precursors to yield active compounds of pharmacological importance by treating with other intermediates bearing different functionalities. For example, thioureas condense with α-halocarbonyl compounds to produce 2-amino-1,3-thiazoles[24] and arylthioureas can be converted into benzothiazoles in the presence of bromine as an oxidizing agent.[25] The synthesis of iminothiazolines,[26] thiohydantoins,[27] 1,3,5-triazines[28] and 2-amino-oxazolidines[29] from thioureas has been well explored.

Mostly, *N*,*N*′-disubstituted thioureas are synthesized by treating primary and secondary amines with $Na^+/K^+/NH_4^+$ thiocyanates,[21] aroyl isothiocyanates with primary and secondary amines followed by acid hydrolysis[30] and isothiocyanates with ammonia or amines.[31] The de-benzoylation of *N*-substituted and *N*,*N*′-disubstituted thioureas with hydrazine hydrate under solvent-free conditions yields mono- and *N*,*N*′-disubstituted thioureas[32] and a synthesis of unsubstituted thioureas with primary alkyl amines at high temperature have also been reported.[33] In the recent decades, numerous new methodologies have been exercised for the design and synthesis of *N*,*N*′-substituted thioureas.[34] However, many of these methods are not very popular due to some disadvantages, specifically, long reaction time under high thermal conditions and the use of large quantity of solvents. The development of efficient, mild and environment friendly protocols are still need of the day. The microwave supported organic syntheses have received great attention in the recent years that greatly facilitated the organic chemists to increase the number and purity of the products. It has also inherent advantages of high chemical yield and shorter reaction time. Recently, solvent free microwave assisted synthesis of substituted thioureas have been reported.[35–37] These solvent free syntheses have many objections regarding purity of the products due to the presence of inorganic byproducts, non-uniform heating and restricted molecular activities in the viscous phase. Keeping in view these issues and in perpetuation to our previous work on different types of thioureas, their coordination and medicinal chemistry,[38,39] we report here the microwave assisted solution-phase parallel synthesis of *N*,*N*′-disubstituted thioureas and their biological activities such as urease inhibition both in vitro & *in virtual* and anticancer activities because of their inherent drug like significance. We speculated that our designed thiourea compounds could effectively inhibit the urease due to structural similarity with the natural substrate of urease, i.e., urea and the diverse structural features introduced on both diversity points of thiourea would probably exert positive effects on activity through the binding of molecules to active site of the enzyme. The results of this study are presented in the subsequent discussion.

2. Experimental

2.1. Materials and methods

All experiments were carried out under the specified conditions of temperature and normal pressure. Solvents were purified and distilled from the drying agents, stored over molecular sieves 4 Å and degassed before use. Microwave syntheses were carried out using a CEM MARS 6 microwave synthesizer with low absorption power settings. The experiments were carried out in standard Pyrex capable glass vials (20 mL).

NMR spectra were recorded on a Bruker ARX, 300 MHz spectrometer. ^{1}H NMR (300.13 MHz): internal standard solvent $CDCl_3$ (7.28 ppm from TMS): internal standard TMS; ^{13}C NMR (75.47 MHz): internal standard solvent $CDCl_3$ (77.0 ppm from TMS): internal standard TMS; the splitting of proton resonances in the reported ^{1}H NMR spectra are defined as s = singlet, d = doublet, t = triplet, q = quartet and m = complex pattern; coupling constants are reported in Hz. FT-IR spectra were recorded as KBr pellets on a Bio-Rad Excalibur FT-IR Model FTS 3000 MX (400–4000 cm^{-1}) and the elemental analyses were performed using a LECO-932 CHNS analyzer. The melting points were determined on a Bio Cote SMP10-UK and are uncorrected. The chemicals like 3-chlorobenzoic acid, ammonium thiocyanate, thionyl chloride and primary/secondary amines, were purchased from Sigma–Aldrich and used as received.

2.2. Microwave assisted solution-phase parallel synthesis of *N*, *N*′-disubstituted thioureas (1–38)

Freshly prepared 3-chlorobenzoyl isothiocyanate was mixed in a 1:1 molar ratio with the desired primary amines in dry THF, in half capped cylindrical glass vessels. The reaction mixtures were subjected to microwave heating using the low absorption mode at 60–65 °C for about 1.5–2.0 min. On cooling, the reaction mixtures were slowly poured into HCl acidified (pH 4–5) chilled water in to ditto labeled beakers (100 mL) and stirred well with a glass rod. The solids were separated by filtration and dried at room temperature (Scheme 1). The yields were above 85% for each of the substituted aroylthioureas (**1**–**38**).

2.3. Spectroscopic data (1–38)

The spectroscopic data for the compounds (**1**–**38**) is given below.

2.3.1. 1-(3-Chlorobenzoyl)-3-phenylthiourea (1)

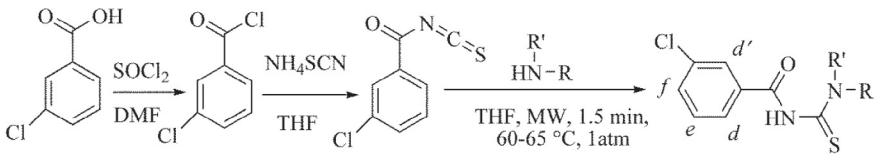
Yield 88%; white solid; mp 135–136 °C; FT-IR (KBr, cm^{-1}) 3217, 3134, 3014, 1972, 1590, 1530, 1329, 1250, 1157, 1016, 869, 716, 692, 542, 490; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (t, 1H, J = 7.2 Hz, ArH_a), 7.45 (t, 2H, J = 8.1 Hz, Ar$H_{b=b'}$), 7.51 (t, 1H, J = 7.8 Hz, ArH_e), 7.64 (d, 2H, J = 8.1 Hz, ArH_f), 7.74 (d, 2H, J = 8.1 Hz, Ar$H_{c=c'}$), 7.77 (d, 1H, J = 7.5 Hz, ArH_d), 7.91 (s, 1H, Ar$H_{d'}$), 9.06 (s, CONH), 12.47 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 124.16 (2C), 125.33, 127.03, 127.98, 128.99 (2C), 130.53, 133.42, 133.80,135.66, 137.47 (Ar-C), 165.61 (C=O), 178.03 (C=S); Anal. Calcd for $C_{14}H_{11}N_2OSCl$; C, 57.83; H, 3.81; N, 9.63; S, 11.03 Found: C, 57.81; H, 3.80; N, 9.65; S, 11.00.

2.3.2. 1-(3-Chlorobenzoyl)-3-(2-chlorophenyl)thiourea (2)

Yield 92%; white solid; mp 131–132 °C; FT-IR (KBr, cm^{-1}) 3285, 3236, 3022, 1676, 1658, 1591, 1531, 1439, 1347, 1256, 1158, 1079, 876, 769, 746, 692, 563, 493; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (d, 1H, J = 7.5 Hz, ArH_c), 7.37 (t, 1H, J = 7.8 Hz, ArH_a), 7.48–7.54 (m, 2H, Ar$H_{e,\ b}$), 7.64 (d, 1H, J = 8.1 Hz, Ar$H_{b'}$), 7.79 (d, 1H, J = 7.8 Hz, ArH_f),7.94 (s, 1H, Ar$H_{d'}$), 8.41 (d, 1H, J = 7.8 Hz, ArH_d) 9.15 (s, CONH), 12.64 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 125.43, 126.13, 126.98, 127.86, 127.98, 128.10, 129.73, 130.52, 133.25, 133.86, 135.89, 135.67 (Ar-C), 165.42 (C=O), 178.32 (C=S); Anal. Calcd for $C_{14}H_{10}N_2OSCl_2$; C, 57.70; H, 3.10; N, 8.61; S, 9.86 Found: C, 57.70; H, 3.10; N, 8.63; S, 9.85.

2.3.3. 1-(3-Chlorobenzoyl)-3-(3-chlorophenyl)thiourea (3)

Yield 95%; white solid; mp 132–133 °C; FT-IR (KBr, cm^{-1}) 3310, 3232, 3048, 1657, 1593, 1530, 1423, 1252, 1159, 1078, 893, 779,



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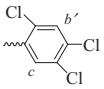
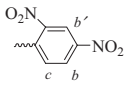
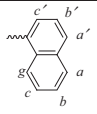
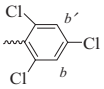
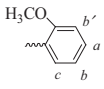
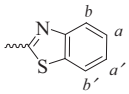
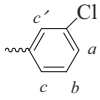
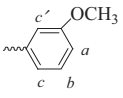
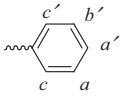
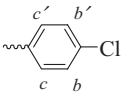
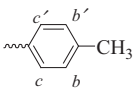
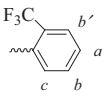
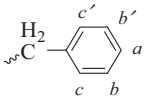
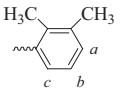
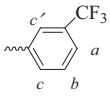
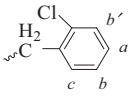
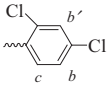
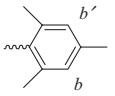
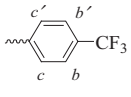
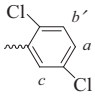
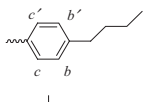
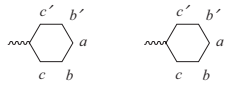
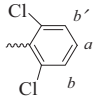
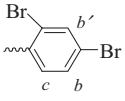
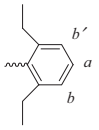
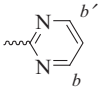
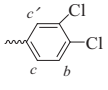
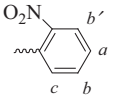
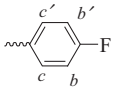
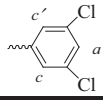
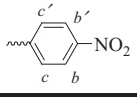
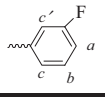
1		H	11		H	21		H	31		H
2		H	12		H	22		H	32		H
3		H	13		H	23		H	33		CH_3
4		H	14		H	24		H	34		H
5		H	15		H	25		H	35		H
6		H	16		H	26		H	36		H
7		H	17		H	27		H	37		
8		H	18		H	28		H	38		H
9		H	19		H	29		H			
10		H	20		H	30		H			

Scheme 1. Synthesis of *N*,*N*′-disubstituted thioureas (**1**–**38**).

757, 676565, 484; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (d, 1H, J = 6.9 Hz, ArH_c), 7.36 (t, 1H, J = 8.1 Hz, ArH_b), 7.51 (t, 1H, J = 8.1 Hz, ArH_e), 7.59 (d, 1H, J = 8.1 Hz, ArH_a), 7.65 (d, 1H, J = 8.1 Hz, ArH_f), 7.76 (d, 1H, J = 8.1 Hz, ArH_d), 7.87 (s, 1H, J = 8.1 Hz, Ar$H_{d'}$), 9.09 (s, CON*H*), 12.53 (s, CSN*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 122.19, 124.10, 125.38, 127.09, 128, 129.93, 130.56, 133.21, 133.92, 134.50, 135.69, 138.55 (Ar-*C*), 165.71 (C=O), 178.10 (*C*=S); Anal. Calcd for $C_{14}H_{10}N_2OSCl_2$; C, 57.70; H, 3.10; N, 8.61; S, 9.86 Found: C, 57.69; H, 3.09; N, 8.62; S, 9.86.

2.3.4. 1-(3-Chlorobenzoyl)-3-(4-chlorophenyl)thiourea (4)

Yield 96%; white solid; mp 166–167 °C; FT-IR (KBr, cm^{-1}) 3214, 3046, 1667, 1605, 1532, 1487, 1358, 1260, 1161, 1090, 1005, 874, 826, 697, 602, 503; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, 2H, J = 9.0 Hz, Ar$H_{c=c'}$), 7.50 (t, 1H, J = 7.8 Hz, ArH_e), 7.63–7.69 (m, 3H, Ar$H_{f,\ b,\ b'}$), 7.77 (d, 1H, J = 7.8 Hz, ArH_d), 7.91 (s, 1H, Ar$H_{d'}$), 9.18 (s, CON*H*), 12.50 (s, CSN*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 125.41 (2C), 128.02, 129.09 (2C), 130.53, 132.35, 133.24, 133.28, 135.65, 135.97 (Ar-*C*), 165.78 (*C*=O), 178.21 (*C*=S); MS (EI) m/z 323.6 (M^+), 138.8 (base peak); Anal. Calcd for $C_{14}H_{10}N_2OSCl_2$; C, 57.70; H, 3.10; N, 8.61; S, 9.86 Found: C, 57.69; H, 3.09; N, 8.65; S, 9.87.

2.3.5. 1-(3-Chlorobenzoyl)-3-(2,3-dichlorophenyl)thiourea (5)

Yield 85%; white solid; mp 183–184 °C; FT-IR (KBr, cm^{-1}) 3229, 3152, 3022, 1677, 1586, 1518, 1344 1251, 1160, 1160, 875, 787, 696, 459; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.43 (t, 1H, J = 8.0 Hz, ArH_b), 7.55–7.60 (m, 2H, Ar$H_{e,\ c}$), 7.73 (d, 1H, J = 8.0 Hz, ArH_a), 7.89 (d, 1H, J = 8.1 Hz, ArH_f), 8.0 (d, 1H, J = 8.0 Hz, ArH_d), 8.04 (s, 1H, Ar$H_{d'}$), 12.01 (s, CON*H*), 12.51 (s, CSN*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 127.7, 128.0, 128.2, 129.0, 129.2, 130.8, 132.4, 133.4,

133.6, 134.3, 138.0 (Ar-*C*), 167.6 (*C*=O), 180.9 (*C*=S); Anal. Calcd for $C_{14}H_9N_2OSCl_3$; C, 46.75; H, 2.52; N, 7.79; S, 8.92 Found: C, 46.75; H, 2.51; N, 7.80; S, 8.92.

2.3.6. 1-(3-Chlorobenzoyl)-3-(2,4-dichlorophenyl)thiourea (6)

Yield 95%; white solid; mp 189–190 °C; FT-IR (KBr, cm^{-1}) 3258, 3092, 3001, 1686, 1578, 1527, 1469, 1323, 1248, 1161, 1094, 866, 812, 723, 693, 601553, 443; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.49 (d, 1H, J = 8.6 Hz, ArH_c), 7.57 (t, 1H, J = 8.0 Hz, ArH_e), 7.73 (d, 1H, J = 8.0 Hz, ArH_b), 7.75 (s, 1H, Ar$H_{b'}$), 7.92 (d, 1H, J = 8.0 Hz, ArH_f), 8.02 (d, 1H, J = 8.6 Hz, ArH_d), 8.04 (s, 1H, Ar$H_{d'}$), 12.01 (s, CONH), 12.49 (s, CSNH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 127.9, 128.0, 129.0, 129.5, 129.8, 130.2, 130.8, 132.0, 133.4, 133.6, 134.4, 135.2 (Ar-*C*), 167.6 (*C*=O), 180.7 (*C*=S); MS (EI) m/z 359.5 (M^+), 138.8 (base peak); Anal. Calcd for $C_{14}H_9N_2OSCl_3$; C, 46.75; H, 2.52; N, 7.79; S, 8.92 Found: C, 46.74; H, 2.50; N, 7.81; S, 8.91.

2.3.7. 1-(3-Chlorobenzoyl)-3-(2,5-dichlorophenyl)thiourea (7)

Yield 86%; white solid; mp 201–202 °C; FT-IR (KBr, cm^{-1}) 3244, 3095, 2988, 1680, 1590, 1545, 1528, 1408, 1344, 1256, 1165, 1088, 898, 812, 704, 545; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.42 (d, 1H, J = 8.6 Hz, ArH_a), 7.57 (t, 1H, J = 8.0 Hz, ArH_e), 7.63 (d, 1H, J = 8.5 Hz, Ar$H_{b'}$), 7.73 (d, 1H, J = 8.6 Hz, ArH_f), 7.93 (d, 1H, J = 8.0 Hz, ArH_d), 8.05 (s, 1H, ArH_c), 8.22 (s, 1H, Ar$H_{d'}$), 12.05 (s, CONH), 12.50 (s, CSNH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 127.6, 127.7, 128.0, 128.4, 129.0, 130.9, 131.3, 131.7, 133.4, 133.6, 134.4, 137.1 (Ar-*C*), 167.7 (*C*=O), 180.6 (*C*=S); Anal. Calcd for $C_{14}H_9N_2OSCl_3$; C, 46.75; H, 2.52; N, 7.79; S, 8.92 Found: C, 46.75; H, 2.51; N, 7.83; S, 8.93.

2.3.8. 1-(3-Chlorobenzoyl)-3-(2,6-dichlorophenyl)thiourea (8)

Yield 87%; white solid; mp 200–201 °C; FT-IR (KBr, cm^{-1}) 3152, 3068, 1677,1564, 1514, 1250, 1159, 875, 789, 720, 664, 462; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (t, 1H, J = 7.5 Hz, ArH_a), 7.46 (d, 2H, J = 8.1 Hz, Ar$H_{b=b'}$), 7.51 (t, 1H, J = 8.1 Hz, ArH_e), 7.65 (d, 1H, J = 7.8 Hz, ArH_f), 7.80 (d, 1H, J = 7.8 Hz, ArH_d), 9.28 (s, CONH), 11.90 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 125.49 (2C), 128.19, 128.64 (2C), 129.80, 130.51, 133.18, 133.26, 133.91, 134.40, 135.67 (Ar-*C*), 165.69 (*C*=O), 180.39 (*C*=S); Anal. Calcd for $C_{14}H_9N_2OSCl_3$; C, 46.75; H, 2.52; N, 7.79; S, 8.92 Found: C, 46.74; H, 2.50; N, 7.81; S, 8.91.

2.3.9. 1-(3-Chlorobenzoyl)-3-(3,4-dichlorophenyl)thiourea (9)

Yield 88%; white solid; mp 176–177 °C; FT-IR (KBr, cm^{-1}) 3242, 3096, 3028, 1666, 1607, 1531, 1474, 1356, 1300, 1260, 1162, 1132, 897, 874, 810, 683, 436; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48–7.51 (m, 2H, Ar$H_{c,\ b}$), 7.57 (t, 1H, J = 8.7 Hz, ArH_e), 7.67 (d, 1H, J = 7.8 Hz, ArH_f), 7.77 (d, 1H, J = 7.8 Hz, ArH_d), 7.90 (s, 1H, Ar$H_{c'}$), 7.99 (s, 1H, Ar$H_{d'}$) 9.07 (s, CONH), 12.55 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 123.30, 125.37, 125.65, 127.99, 130.50, 130.59, 132.79, 133.08, 134.01, 135.74, 136.79 (Ar-*C*), 165.76 (*C*=O), 178.13 (*C*=S); Anal. Calcd for $C_{14}H_9N_2OSCl_3$; C, 46.75; H, 2.52; N, 7.79; S, 8.92 Found: C, 46.75; H, 2.51; N, 7.82; S, 8.92.

2.3.10. 1-(3-Chlorobenzoyl)-3-(3,5-dichlorophenyl)thiourea (10)

Yield 86%; white solid; mp 185–186 °C; FT-IR (KBr, cm^{-1}) 3241, 3089, 3050, 1673, 1588, 1565, 1531, 1445, 1351, 1267, 1160, 1119, 852, 797, 732, 689, 559, 431; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.50 (s, 1H, ArH_a), 7.57 (t, 1H, J = 8.0 Hz, ArH_e), 7.73 (d, 1H, J = 8.0 Hz, ArH_f), 7.81 (s, 2H, Ar$H_{c,\ c'}$), 7.91 (d, 1H, J = 8.0 Hz, ArH_d), 8.02 (s, 1H, Ar$H_{d'}$), 11.87 (s, CONH), 12.44 (s, CSNH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 123.7 (2C), 126.2, 127.9, 128.9, 130.9, 133.4, 133.6, 134.5, 140.9 (Ar-*C*), 167.0 (*C*=O), 180.0 (*C*=S); Anal. Calcd for $C_{14}H_9N_2OSCl_3$; C, 46.75; H, 2.52; N, 7.79; S, 8.92 Found: C, 46.73; H, 2.49; N, 7.81; S, 8.92.

2.3.11. 1-(3-Chlorobenzoyl)-3-(2,4,5-trichlorophenyl)thiourea (11)

Yield 88%; white solid; mp 213–214 °C; FT-IR (KBr, cm^{-1}) 3295, 3086, 3068, 2990, 1982, 1564, 1522, 1470, 1368, 1308,1252, 1155, 1130, 1079, 873, 737, 683, 606, 559, 478; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.56 (t, 1H, J = 8.0 Hz, ArH_e), 7.71 (d, 1H, J = 7.5 Hz, ArH_f), 7.92 (d, 1H, J = 7.5 Hz, ArH_d), 7.98 (s, 1H, ArH_c), 8.03 (s, 1H, Ar$H_{b'}$), 8.41 (s, 1H, Ar$H_{d'}$), 12.00(s, CONH), 12.52 (s, CSNH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 127.9, 128.0, 128.6, 128.8, 129.0, 130.0. 130.8, 131.0, 133.5, 133.6, 134.2, 136.1 (Ar-*C*), 167.7 (*C*=O), 180.7 (*C*=S); MS (EI) m/z 356.6 (M^+ = M-Cl), 138.8 (base peak); Anal. Calcd for $C_{14}H_8N_2OSCl_4$; C, 42.67; H, 2.05; N, 7.11; S, 8.14 Found: C, 42.66; H, 2.00; N, 7.13; S, 8.13.

2.3.12. 1-(3-Chlorobenzoyl)-3-(2,4,6-trichlorophenyl)thiourea (12)

Yield 95%; white solid; mp 179–180 °C; FT-IR (KBr, cm^{-1}) 3412, 3147, 3040, 1677, 1571, 1482, 1341, 1234, 1159, 1079, 880, 818, 728, 652, 565, 490; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (s, 2H, Ar$H_{b=b'}$), 7.51 (t, 1H, J = 8.1 Hz, ArH_e), 7.66 (d, 1H, J = 7.8 Hz, ArH_f), 7.77 (d, 1H, J = 7.8 Hz, ArH_d), 7.93 (s, 1H, Ar$H_{d'}$), 9.29 (s, CONH), 11.86 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 125.49 (2C), 128.19, 128.71 (2C), 130.53, 132.16, 133.02, 134.01, 134.89, 135.01, 135.70 (Ar-*C*), 165.76 (*C*=O), 180.42 (*C*=S); Anal. Calcd for $C_{14}H_8N_2OSCl_4$; C, 42.67; H, 2.05; N, 7.11; S, 8.14 Found: C, 42.64; H, 2.01; N, 7.12; S, 8.14.

2.3.13. 1-(3-Chlorobenzoyl)-3-(2,3,5,6-tetrachlorophenyl) thiourea (13)

Yield 89%; white solid; mp 201–202 °C; FT-IR (KBr, cm^{-1}) 3487, 3416, 3119, 3046, 1677, 1568, 1512, 1470, 1341, 1242, 1157, 883, 740, 715, 589, 415; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (t, 1H, J = 7.8 Hz, ArH_e), 7.66–7.68 (m, 2H, Ar$H_{a,\ f}$), 7.8 (d, 1H, J = 7.8 Hz, ArH_d), 7.95 (s, 1H, Ar$H_{d'}$), 9.26 (s, CONH), 12.02 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 125.5 (2C), 128.19 (2C), 130.58, 130.68, 132.06, 132.27, 134.10, 135.76 (Ar-*C*), 165.82 (*C*=O), 180.01 (*C*=S); Anal. Calcd for $C_{14}H_7N_2OSCl_5$; C, 39.24; H, 1.65; N, 6.54; S, 7.48 Found: C, 39.28; H, 1.65; N, 6.55; S, 7.48.

2.3.14. 1-(3-Chlorobenzoyl)-3-(4-methylphenyl)thiourea (14)

Yield 95%; white solid; mp 153–154 °C; FT-IR (KBr, cm^{-1}) 3307, 3046, 1672, 1557, 1467, 1427, 1358, 1276, 1250, 1148, 1078, 815, 722, 680, 604, 508, 418; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (s, 3H, *p*-CH_3), 7.25 (d, 2H, J = 8.4 Hz, Ar$H_{c=c'}$), 7.50 (t, 1H, J = 8.1 Hz, ArH_e), 7.57 (d, 2H, J = 8.4 Hz, Ar$H_{b=b'}$), 7.63 (d, 1H, J = 7.8 Hz, ArH_f), 7.77 (d, 1H, J = 7.8 Hz, ArH_d), 7.91 (s, 1H, Ar$H_{d'}$), 9.13 (s, CONH), 12.37 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.51 (*o*-CH_3), 124.23 (2C), 125.36, 128.00, 129.58 (2C), 130.50, 133.46, 133.74, 134.89, 135.61, 137.1 (Ar-*C*), 165.62(*C*=O), 178.18 (*C*=S); Anal. Calcd for $C_{15}H_{13}N_2OSCl$; C, 59.11; H, 4.30; N, 9.19; S, 10.52 Found: C, 59.10; H, 4.29; N, 9.21; S, 10.51.

2.3.15. 1-(3-Chlorobenzoyl)-3-(2,3-dimethylphenyl)thiourea (15)

Yield 96%; white solid; mp 161–162 °C; FT-IR (KBr, cm^{-1}) 3217, 3065, 2935, 1674, 1566, 1514, 1381, 1330, 1247, 1169, 1064, 832, 723, 602, 550, 444; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.25 (s, 3H, *m*-CH_3), 2.36 (s, 3H, *o*-CH_3) 7.17–7.23 (m, 2H, Ar$H_{a,\ c}$), 7.42 (t, 1H, J = 7.1 Hz, ArH_b), 7.51 (t, 1H, J = 8.1 Hz, ArH_e), 7.65 (d, 1H, J = 8.1 Hz, ArH_f), 7.80 (d, 1H, J = 8.1 Hz, ArH_d), 9.21 (s, CONH), 12.10 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.24 (*m*-CH_3), 20.51 (*o*-CH_3), 124.44, 125.41,125.91, 128.09, 129.59, 130.49, 132.58, 133.44, 133.75, 135.65, 138.10 (Ar-*C*), 165.73 (*C*=O), 179.63 (*C*=S); MS (EI) m/z 317.8 (M^+), 110.9 (base peak); Anal. Calcd for $C_{16}H_{15}N_2OSCl$; C, 60.28; H, 4.74; N, 8.79; S, 10.06 Found: C, 60.27; H, 4.74; N, 8.80; S, 10.01.

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2.3.16. 1-(3-Chlorobenzoyl)-3-(2,4,6-trimethylphenyl)thiourea (16)

Yield 90%; white solid; mp 160–161 °C; FT-IR (KBr, cm^{-1}) 3165, 3026, 2917, 1675, 1531, 1514, 1341, 1250, 1171, 1147, 1070, 860, 748, 721, 701, 655, 615, 436; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.27 (s, 6H, 2[*o*-CH_3]), 2.32 (s, 3H, *p*-CH_3), 6.96 (s, 2H, Ar$H_{b=b'}$), 7.48 (t, 1H, J = 8.1 Hz, ArH_e), 7.62 (d, 1H, J = 6.9 Hz, ArH_f), 7.78 (d, 1H, J = 6.9 Hz, ArH_d), 7.93 (s, 1H, Ar$H_{d'}$), 9.35 (s, CONH), 11.69 (s, CSNH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.0 (2C, *o*-CH_3), 21.01 (*p*-CH_3), 125.4, 128.0 (2C), 129.0 (2C), 130.3, 132.6, 133.3, 133.6, 134.8, 135.4, 138.0 (Ar-*C*), 165.7 (*C*=O), 179.8 (*C*=S); Anal. Calcd for $C_{17}H_{17}N_2$-OSCl; C, 61.34; H, 5.15; N, 8.42; S, 9.63 Found: C, 61.34; H, 5.14; N, 8.44; S, 9.64.

2.3.17. 1-(3-Chlorobenzoyl)-3-(3-bromophenyl)thiourea (17)

Yield 89%; white solid; mp 165–166 °C; FT-IR (KBr, cm^{-1}) 3250, 3161, 1679, 1578, 1530, 1419, 1322, 1252, 1158, 1078, 800, 712, 684, 600, 478, 448; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.36 (t, 1H, J = 8.2 Hz, ArH_b), 7.45 (d, 1H, J = 7.5 Hz, ArH_c), 7.54 (t, 1H, J = 8.1 Hz, ArH_e), 7.61 (d, 1H, J = 8.1 Hz, ArH_a), 7.70 (d, 1H, J = 7.5 Hz, ArH_f), 7.91 (d, 1H, J = 7.5ArH_d), 8.01 (s, 1H, Ar$H_{c'}$), 8.05 (s, 1H, Ar$H_{d'}$), 11.80 (s, CONH), 12.49 (s, CSNH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 121.5, 123.9, 127.3, 127.9, 128.7, 129.5, 130.8, 131.0, 133.3, 133.6, 134.5, 139.9 (Ar-*C*), 167.2 (*C*=O), 179.7 (*C*=S); MS (EI) *m*/*z* 369.6 (M^+), 138.8 (base peak); Anal. Calcd for $C_{14}H_{10}N_2$OSBrCl; C, 45.49; H, 2.73; N, 7.58; S, 8.67 Found: C, 45.47; H, 2.72; N, 7.60; S, 8.65.

2.3.18. 1-(3-Chlorobenzoyl)-3-(2,4-dibromophenyl)thiourea (18)

Yield 90%; white solid; mp 160–161 °C; FT-IR (KBr, cm^{-1}) 3250, 3040, 3020, 3013, 1682, 1568, 1527, 1466, 1323, 1252, 1160, 1075, 1046, 868, 764, 698, 590, 453; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.57 (t, 1H, J = 8.2 Hz, ArH_e), 7.66 (d, 1H, J = 8.1 Hz, ArH_c), 7.72 (d, 1H, J = 8.1 Hz, ArH_b), 7.82 (d, 1H, J = 8.1 Hz, ArH_f), 7.92 (d, 1H, J = 8.1 Hz, ArH_d),7.99 (s, 1H, J = 8.1Ar$H_{b'}$), 8.04 (s, 1H, J = 8.1 Hz, Ar$H_{d'}$) 12.07 (s, CONH), 12.41 (s, CSNH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 120.5, 121.2, 128.0, 129.0, 130.7, 130.9, 131.4, 133.4, 133.6, 134.4, 135.0, 137.0 (Ar-*C*), 167.6 (*C*=O), 180.8 (*C*=S); Anal. Calcd for $C_{14}H_9N_2OSBr_2Cl$; C, 37.49; H, 2.02; N, 6.25; S, 7.15 Found: C, 37.45; H, 2.00; N, 6.27; S, 7.10.

2.3.19. 1-(3-Chlorobenzoyl)-3-(2-nitrophenyl)thiourea (19)

Yield 84%; white solid; mp 155–156 °C; FT-IR (KBr, cm^{-1}) 3283, 3232, 3020, 1666, 1660, 1590, 1530, 1438, 1345, 1252, 1155, 1076, 873, 766, 746, 690, 562, 483; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (d, 1H, J = 7.5 Hz, ArH_c), 7.36 (t, 1H, J = 7.8 Hz, ArH_a), 7.47–7.53 (m, 2H, Ar$H_{e,\ b}$), 7.63 (d, 1H, J = 8.1 Hz, Ar$H_{b'}$), 7.76 (d, 1H, J = 7.8 Hz, ArH_f), 7.92 (s, 1H, Ar$H_{d'}$), 8.43 (d, 1H, J = 7.8 Hz, ArH_d) 9.18 (s, CONH), 12.62 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 124.3, 125.1, 126.2, 126.8, 127.5, 128.3, 129.6, 130.8, 133.1, 133.7, 135.6, 135.8 (Ar-*C*), 165.3 (*C*=O), 178.5 (*C*=S).

2.3.20. 1-(3-Chlorobenzoyl)-3-(4-nitrophenyl)thiourea (20)

Yield 83%; yellow solid; mp 169–170 °C; FT-IR (KBr, cm^{-1}) 3287, 3045, 3032, 1691, 1594, 1557, 1510, 1324, 1258, 1161, 1107, 881, 853, 752, 732, 676, 608, 496, 462; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (t, 1H, J = 7.8 Hz, Ar$H_{e'}$), 7.67 (d, 1H, J = 8.1 Hz, ArH_f), 7.79 (d, 1H, J = 8.1 Hz, ArH_d),7.92 (s, 1H, Ar$H_{d'}$), 8.06 (d, 2H, J = 9.3 Hz, Ar$H_{c=c'}$), 8.31 (d, 2H, J = 9.3 Hz, Ar$H_{b=b'}$), 9.19 (s, CONH), 12.96 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 123.23(2C), 124.65 (2C), 125.45, 128.04, 130.64, 132.94, 134.15, 135.77, 143.09, 145.25 (Ar-*C*), 165.92 (*C*=O), 177.89 (*C*=S); MS (EI) *m*/*z* 334.7 (M^+), 138.8 (base peak); Anal. Calcd for $C_{14}H_{10}N_3$-O_3SCl; C, 50.08; H, 3.00; N, 12.51; S, 9.55 Found: C, 50.02; H, 3.01; N, 12.53; S, 9.54.

2.3.21. 1-(3-Chlorobenzoyl)-3-(2, 4-dinitrophenyl)thiourea (21)

Yield 83%; yellow solid; mp 161–162 °C; FT-IR (KBr, cm^{-1}) 3450, 3335, 3203, 3107, 1691, 1594, 1557, 1510, 1324, 1258, 1161, 1107, 881, 853, 752, 732, 676, 608, 496, 462; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (d, 1H, J = 7.8 Hz, ArH_c), 7.45 (t, 1H, J = 8.1 Hz, ArH_e), 7.760 (d, 1H, J = 8.1 Hz, ArH_f), 7.75 (d, 1H, J = 7.8 Hz, ArH_d), 7.87 (s, 1H, Ar$H_{d'}$), 8.21 (d, 2H, J = 9.1 Hz, ArH_b), 9.11 (s, 1H, Ar$H_{b'}$), 9.25 (s, CONH), 13.3 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 122.3, 125.5, 128.3, 128.9, 130.8, 131.6, 133.2, 134.3, 135.6, 143.2, 145.3, 147.2 (Ar-*C*), 166.3 (*C*=O), 179.3 (*C*=S); Anal. Calcd for $C_{14}H_9N_4O_5$SCl; C, 44.16; H, 2.38; N, 14.71; S, 8.42 Found: C, 44.14; H, 2.35; N, 14.73; S, 8.40.

2.3.22. 1-(3-Chlorobenzoyl)-3-(2-methoxyphenyl)thiourea (22)

Yield 89%; white solid; mp 131–132 °C; FT-IR (KBr, cm^{-1}) 3256, 3013, 2941, 1678, 1904, 1558, 1535, 1487, 1464, 1364, 1316, 1246, 1180, 1153, 1111, 1022, 739, 708, 683, 488; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97 (s, 3H, *o*-OCH_3), 6.98 (d, 1H, J = 8.1 Hz, ArH_c), 7.04 (t, 1H, J = 8.1 Hz, ArH_a), 7.24 (t, 1H, J = 8.1 Hz, ArH_b), 7.49 (t, 1H, J = 8.1 Hz, ArH_e), 7.63 (d, 1H, J = 7.5 Hz, ArH_f), 7.78 (d, 1H, J = 7.5 Hz, ArH_d), 7.94 (s, 1H, Ar$H_{d'}$), 8.77 (d, 1H, J = 8.1 Hz, Ar$H_{b'}$), 9.05 (s, CONH), 12.78 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0 (*o*-OCH_3), 110.62, 120.26, 122.89, 125.34, 126.95, 127.01,128.04,130.44, 132.59, 133.63, 135.57, 150.69 (Ar-*C*), 165.16 (*C*=O), 176.27 (*C*=S); Anal. Calcd for $C_{15}H_{13}N_2O_2$SCl; C, 56.16; H, 4.08; N, 8.73; S, 10.00 Found: C, 56.15; H, 4.00; N, 8.75; S, 9.98.

2.3.23. 1-(3-Chlorobenzoyl)-3-(3-methoxyphenyl)thiourea (23)

Yield 85%; white solid; mp 110–111 °C; FT-IR (KBr, cm^{-1}) 3275, 3045, 2961, 1677, 1597, 1561, 1524, 1466, 1352, 1279, 1246, 1155, 1034, 908, 827, 802, 676, 554, 450; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 (s, 3H, *p*-OCH_3), 6.85 (d, 1H, J = 8.1 Hz, ArH_c), 7.22 (d, 1H, J = 8.1 Hz, ArH_a), 7.33 (t, 1H, J = 8.1 Hz, ArH_b), 7.48–7.53 (m, 2H, Ar$H_{e,\ c'}$), 7.64 (d, 1H, J = 7.8 Hz, ArH_f), 7.75 (d, 1H, J = 7.8 Hz, ArH_d), 7.91 (s, 1H, Ar$H_{d'}$), 9.10 (s, CONH), 12.54 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.47 (*p*-OCH_3), 109.47, 112.71, 116.16, 127.98, 129.36, 129.68, 130.52, 133.39, 133.79, 135.64, 138.53, 159.92 (Ar-*C*), 165.62 (*C*=O), 177.73 (*C*=S); Anal. Calcd for $C_{15}H_{13}$-N_2O_2SCl; C, 56.16; H, 4.08; N, 8.73; S, 10.00 Found: C, 56.14; H, 4.02; N, 8.76; S, 9.99.

2.3.24. 1-(3-Chlorobenzoyl)-3-(2-trifluoromethylphenyl) thiourea (24)

Yield 86%; white solid; mp 150–151 °C; FT-IR (KBr, cm^{-1}) 3251, 3119, 3057, 1675, 1568, 1523, 1320, 1245, 1159, 1115, 1032, 874, 760, 744, 710, 603, 548; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.49 (m, 2H, Ar$H_{\ b,\ e}$), 7.60–7.65 (m, 2H, Ar$H_{a,\ c}$), 7.73 (d, 1H, J = 7.5 Hz, ArH_f), 7.77 (d, 1H, J = 7.5 Hz ArH_d), 7.92 (d, 1H, J = 7.6 Hz, Ar$H_{b'}$), 7.95 (s, 1H, Ar$H_{d'}$), 9.38 (s, CONH), 12.44 (s, CSNH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 123.93, 124.60 & 124.81 (^{13}C-^{19}F, ^1J = 26.5 Hz, *C*F_3), 125.5, 126.30 & 126.33 (^{13}C-^{19}F, ^2J = 3.75 Hz), 127.5, 128.0, 129.6, 130.3, 132.1, 133.0, 133.7, 135.3, 135.6 (Ar-*C*), 165.7 (*C*=S), 180.3 (*C*=O); Anal. Calcd For $C_{15}H_{10}N_2OSClF_3$; C, 50.22; H, 2.81; N, 7.81; S, 8.90 Found: C, 50.19; H, 2.79; N, 7.83; S, 8.89.

2.3.25. 1-(3-Chlorobenzoyl)-3-(3- trifluoromethylphenyl) thiourea (25)

Yield 85%; white solid; mp 121–122 °C; FT-IR (KBr, cm^{-1}) 3334, 3219, 3033, 1676, 1601, 1574, 1527, 1454, 1329, 1252, 1158, 1111, 890, 799, 742, 718, 691, 608, 486; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49–7.57 (m, 3H, Ar$H_{e,\ c,\ b}$), 7.66 (d, 1H, J = 7.8 Hz, ArH_f), 7.78 (d, 1H, J = 7.8 Hz, ArH_d), 7.91 (s, 1H, Ar$H_{d'}$), 7.96 (d, 1H, J = 7.8 Hz, ArH_a), 8.05 (s, 1H, Ar$H_{c'}$) 9.14 (s, CONH), 12.64 (s, CSNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 120.93, 123.6, 125.42& 126.62 (^{13}C-^{19}F, ^1J = 25.1 Hz), 127.30,128.01, 129.51, 130.58, 131.20, 131.63,

133.15, 133.98, 135.71, 137.99 (Ar-*C*), 165.81 (*C*═O), 178.37 (*C*═S); MS (EI) *m/z* 357.7 (M^+), 138.9 (base peak); Anal. Calcd For $C_{15}H_{10}N_2OSClF_3$; C, 50.22; H, 2.81; N, 7.81; S, 8.90 Found: C, 50.17; H, 2.78; N, 7.81; S, 8.90.

2.3.26. 1-(3-Chlorobenzoyl)-3-(4-trifluoromethylphenyl) thiourea (26)

Yield 88%; white solid; mp 112–113 °C; FT-IR (KBr, cm^{-1}) 3225, 3116, 3042, 1669, 1605, 1568, 1531, 1414, 1356, 1327, 1261, 1165, 1113, 1067, 876, 837, 789, 730, 700, 608, 589, 459, 422; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (t, 1H, *J* = 6.2 Hz, Ar*H* $_e$), 7.63 (d, 1H, *J* = 6.2 Hz, ArH_f), 7.67 (d, 2H, *J* = 6.9 Hz, Ar$H_{c=c'}$), 7.76 (d, 1H, *J* = 6.5 Hz, ArH_d), 7.90 (m, 3H, Ar$H_{b,\ b',\ d'}$), 9.24 (s, CON*H*), 12.71 (s, CSN*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 123.7 (2C), 125.71 & 125.91 (^{13}C-^{19}F, ^1J = 25 Hz, *C*F_3), 125.4 (2C), 126.0& 126.09 (2C,^{13}C-^{19}F, ^2J = 3.75), 130.0, 130.5, 133.1, 133.8, 135.6, 140.5 (Ar-*C*), 165.8 (*C*═O), 178.0 (*C*═S); Anal. Calcd For $C_{15}H_{10}N_2OSClF_3$; C, 50.22; H, 2.81; N, 7.81; S, 8.90 Found: C, 50.20; H, 2.80; N, 7.82; S, 8.90.

2.3.27. 1-(3-Chlorobenzoyl)-3-(*n*-butylphenyl)thiourea (27)

Yield 85%;, white solid; mp 142.5–142.7 °C; FT-IR (KBr, cm^{-1}) 3316, 3226, 3025, 2954, 2928, 2854, 1668, 1597, 1553, 1510, 1446, 1423, 1350, 1278, 1248, 1146, 1078, 832, 810, 719, 679, 526, 493; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, 3H, J = 7.5 Hz, -$C_\alpha H_2$-$C_\beta H_2$-$C_\gamma H_2$-$C_\delta H_3$), 1.37 (sex, 2H, J = 6.9 Hz, -$C_\gamma H_2$-), 1.61(p, 2H, J = 7.6 Hz, -$C_\beta H_2$-), 2.62(t, 2H, J = 7.5 Hz, -$C_\alpha H_2$-), 7.22 (d, 2H, J = 8.1 Hz, Ar$H_{c=c'}$), 7.45 (t, 1H, J = 8.1 Hz, ArH_e), 7.59(d, 2H, J = 8.2 Hz, Ar$H_{b=b'}$), 7.71–7.83 (m, 2H, Ar$H_{d,\ f}$), 7.89 (s, 1H, Ar$H_{d'}$), 9.28 (s, CON*H*), 12.42 (s, CSN*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.8, 22.23, 33.33, 35.11(*n*-butyl), 123.7(2C), 125.4, 127.9, 128.72 (2C), 130.3, 133.4, 133.5, 134.9, 135.3, 141.8(Ar-*C*), 165.7(C-7), 177.9 (C-8); MS (EI) *m/z* (M^+); Anal. Calcd for $C_{18}H_{19}ClN_2OS$; C, 62.33, H 5.52, N 8.08, S 9.24 Found: C 62.28, H 5.51, N 8.01, S 9.22.

2.3.28. 1-(3-Chlorobenzoyl)-3-(2, 6-diethylphenyl)thiourea (28)

Yield 89%; white solid; mp 156–157 °C; FT-IR (KBr, cm^{-1}) 3192, 3158, 3066, 2968, 2934, 2873, 1672, 1530, 1509, 1420, 1337, 12471202, 1159, 1111, 1065, 875, 812, 714, 617, 478, 444; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.26 (t, 6H, *J* = 4.5 Hz, 2 [*o*-CH_2–CH_3]), 2.67 (q, 4H, *J* = 4.5 Hz, 2 [*o*-CH_2–CH_3]), 7.21 (d, 2H, *J* = 7.6 Hz, Ar$H_{b=b'}$), 7.33 (t, 1H, *J* = 7.5 Hz, ArH_a), 7.50 (t, 1H, *J* = 7.5 Hz, ArH_e), 7.64 (d, 1H, *J* = 8.2 Hz, ArH_f), 7.80 (d, 1H, *J* = 8.2 Hz, ArH_d), 7.95 (s, 1H, Ar$H_{d'}$), 9.28 (s, CON*H*), 11.80 (s, CSN*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.5 (2C,-*C*H_3), 24.7 (2C, –*C*H_2–), 125.4, 126.5 (2C), 128.0, 128.8, 130.4 (2C), 133.3, 133.7, 134.0, 135.5, 140.9 (Ar-*C*), 165.7 (*C*═O), 180.18 (*C*═S); MS (EI) *m/z* 346.1 (M^+), 139.0 (base peak); Anal. Calcd for $C_{18}H_{19}N_2OSCl$; C, 62.33; H, 5.52; N, 8.08; S, 9.24 Found: C, 62.31; H, 5.51; N, 8.11; S, 9.23.

2.3.29. 1-(3-Chlorobenzoyl)-3-(3-fluorophenyl)thiourea (29)

Yield 89%; white solid; mp 141–142 °C; FT-IR (KBr, cm^{-1}) 3298, 3228, 3061, 1668, 1541, 1528, 1470, 1246, 1163, 1051, 897, 801, 746, 683, 609, 538, 455; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.0 (d, 1H, *J* = 7.5 Hz, ArH_c), 7.36–7.39 (m, 2H, Ar$H_{a,\ c'}$), 7.48 (t, 1H, *J* = 8.1 Hz, ArH_e), 7.62 (d, 1H, *J* = 7.5 Hz, ArH_f), 7.71–7.75 (m, 2H, Ar$H_{d,\ b}$), 7.89 (s, 1H, Ar$H_{d'}$) 9.14 (s, CON*H*), 12.58 (s, CSN*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 111.3 (d, ^{13}C-^{19}F, ^2J = 25 Hz), 113.9 (d, ^{13}C-^{19}F, ^2J = 25 Hz), 119.5, 125.5, 128.0, 130.1, 130.3, 130.6, 133.3, 133.5, 135.7, 163.0 (d, ^{13}C-^{19}F, ^1J = 250 Hz) (Ar-*C*), 165.9 (*C* = O), 177.9 (*C*═S); Anal. Calcd for $C_{14}H_{10}N_2OSClF$; C, 54.46; H, 3.26; N, 9.07; S, 10.39 Found: C, 54.47; H, 3.25; N, 9.11; S, 10.39.

2.3.30. 1-(3-Chlorobenzoyl)-3-(4-fluorophenyl)thiourea (30)

Yield 90%; white solid; mp 151–152 °C; FT-IR (KBr, cm^{-1}) 3216, 3044, 1667, 1615, 1569, 1537, 1504, 1360, 1261, 1227, 1159, 1115, 1082, 878, 820, 730, 697, 606, 539, 511, 475, 434; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (m, 2H, Ar$H_{c=c'}$), 7.49 (t, 1H, *J* = 7.6 Hz, ArH_e), 7.62–7.66 (m, 3H, Ar$H_{b,\ b',\ f}$), 7.75 (d, 1H, *J* = 8.9 Hz, ArH_d), 7.88 (s, 1H, Ar$H_{d'}$) 9.16 (s, CON*H*), 12.38 (s, CSN*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 115.8 (d, 2C, ^2J = 25 Hz, ^{13}C-^{19}F), 125.3, 126.2 (d, 2C, ^3J = 6.0 Hz, ^{13}C-^{19}F), 127.9, 120.4, 133.2, 133.4, 133.7, 135.6, 159.0 (d, ^1J = 250 Hz, ^{13}C-^{19}F) (Ar-*C*), 165.7 (*C*═O), 178.6 (*C*═S); Anal. Calcd for $C_{14}H_{10}N_2OSClF$; C, 54.46; H, 3.26; N, 9.07; S, 10.39 Found: C, 54.45; H, 3.25; N, 9.10; S, 10.38.

2.3.31. 1-(3-Chlorobenzoyl)-3-(1-naphthyl)thiourea (31)

Yield 90%; intense violet; mp 164–165 °C; FT-IR (KBr, cm^{-1}) 3231, 3165, 2919, 1670, 1568, 1509, 1328, 1246, 1173, 1156, 1066, 883, 773, 746, 713, 475, 444; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (t, 1H, *J* = 8.2 Hz, Ar$H_{b'}$), 7.47 (t, 1H, *J* = 8.2 Hz, ArH_b), 7.53–7.65 (m, 3H, Ar$H_{e,\ a,\ a}$), 7.70 (d, 1H, *J* = 7.5 Hz, Ar$H_{c'}$), 7.86 (d, 1H, *J* = 8.1 Hz, ArH_f), 7.92 (d, 1H, *J* = 7.5 Hz, ArH_d), 7.98 (s, 1H, Ar$H_{d'}$), 8.02 (d, 2H, *J* = 7.5 Hz, ArH_g), 9.5 (s, CON*H*), 12.66 (s, CSN*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 121.5, 123.8, 125.2, 125.5, 126.1, 126.4, 126.9, 128.0, 128.2, 128.4, 128.6, 129.9, 130.4, 133.0, 133.7,134.1 (Ar-*C*), 165.0 (*C*═O), 180.2 (*C*═S); Anal. Calcd for $C_{18}H_{13}N_2OSCl$; C, 63.43; H, 3.84; N, 8.22; S, 9.41 Found: C, 63.42; H, 3.83; N, 8.23; S, 9.40.

2.3.32. 1-(3-Chlorobenzoyl)-3-(2-benzothiazol)thiourea (32)

Yield 86%; white solid; mp 160–161 °C; FT-IR (KBr, cm^{-1}) 3285, 3042, 3033, 1698, 1592, 1552, 1511, 1329, 1253, 1164, 1109, 885, 853, 752, 735, 674, 608, 492, 463; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (t, 1H, *J* = 7.8 Hz, Ar$H_{e'}$), 7.60 (d, 2H, *J* = 9.1 Hz, Ar$H_{a=a'}$), 7.66 (d, 1H, *J* = 8.1 Hz, ArH_f), 7.75 (d, 1H, *J* = 8.1 Hz, ArH_d), 7.95 (s, 1H, Ar$H_{d'}$), 8.31 (d, 2H, *J* = 9.3 Hz, Ar$H_{b=b'}$), 9.18 (s, CON*H*), 12.94 (s, CSN*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 123.1, 124.65, 125.4, 128.0, 130.6, 132.9, 134.1, 135.7, 136.6, 143.0, 145.2, 148.9, 165.8 (*C*═O), 174.5 (*C*NNS), 177.9 (*C*═S); MS (EI) *m/z* 334.7 (M^+), 138.8 (base peak); Anal. Calcd for $C_{14}H_{10}N_3O_3SCl$; C, 50.08; H, 3.00; N, 12.51; S, 9.55 Found: C, 50.02; H, 3.01; N, 12.53; S, 9.54.

2.3.33. 1-(3-Chlorobenzoyl-3-(*N*-methylphenyl)thiourea (33)

Yield 88%; white solid; mp 130–131 °C; FT-IR (KBr, cm^{-1}) 3240, 3200, 3027, 2933, 1697, 1570, 1515, 1437, 1387, 1277, 1248, 1177, 1118, 1059, 916, 763, 735, 638, 550, 465; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.78 (s, 3H, N-*C*H_3), 7.26–8.40 (m, 9H, Ar-*H*), 8.5 (s, CON*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 45.7 (N-*C*H_3), 125.4, 125.5, 127.8 (2C), 127.9 (2C),128.0 (2C), 129.5 (2C), 130.0, 132.7, 133.4, 134.5, 134.9 (Ar-*C*), 161.59 (*C*═O), 175.76 (*C*═S); Anal. Calcd for $C_{15}H_{13}N_2OSCl$; C, 59.11; H, 4.30; N, 9.19; S, 10.52 Found: C, 59.11; H, 4.29; N, 9.22; S, 10.52.

2.3.34. 1-(3-Chlorobenzoyl)-3-benzylthiourea (34)

Yield 88%; white solid; mp 113–114 °C; FT-IR (KBr, cm^{-1}) 3310, 3236, 3209, 3020, 2960, 1674, 1641, 1541, 1322, 1252, 1170, 1070, 804, 748, 697, 617, 478; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.93 (d, 2H, *J* = 5.4 Hz, –*C*H_2–), 7.34–7.85 (m, 9H, Ar*H*), 9.12 (s, CON*H*), 10.94 (s, CSN*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 49.89 (–*C*H_2–), 125.33, 127.94 (2C), 128.94 (2C), 129.96, 130.44, 131.63, 133.62, 135.53, 135.99, 137.81 (Ar-*C*), 165.54 (*C*═O), 179.76 (*C*═S); MS (EI) *m/z* 269.9 (M^+ = M-Cl), 104.9 (base peak); Anal. Calcd for $C_{15}H_{13}N_2OSCl$; C, 59.11; H, 4.30; N, 9.19; S, 10.52 Found: C, 59.12; H, 4.28; N, 9.20; S, 10.50.

2.3.35. 1-(3-Chlorobenzoyl)-3-(2-chlorobenzyl)thiourea (35)

Yield 87%; white solid; mp 124–125 °C; FT-IR (KBr, cm^{-1}) 3294, 3040, 2981, 1680, 1605, 1561, 1522, 1346, 1244, 1149, 1110, 914, 829, 735, 673, 604, 453; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.98 (d, 2H, *J* = 5.4 Hz, –*C*H_2–), 7.23–7.56 (m, 6H, Ar*H*), 7.67 (d, 1H, *J* = 7.5 Hz, ArH_d), 7.82 (s, 1H, Ar$H_{d'}$), 9.21 (s, CON*H*), 10.06 (s, CSN*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 47.3 (–*C*H_2–),

125.4, 126.2, 126.9, 127.9, 128.4, 129.4,129.6, 130.3, 130.5, 133.0, 133.4, 135.3 (Ar-*C*), 165.6 (*C*=O), 179.8 (*C*=S); Anal. Calcd for $C_{15}H_{12}N_2OSCl_2$; C, 53.11; H, 3.57; N, 8.26; S, 9.45 Found: C, 53.12; H, 3.57; N, 8.28; S, 9.45.

2.3.36. 1-(3-Chlorobenzoyl)-3-cyclohexylthiourea (36)

Yield 89%; white solid; mp 90–91 °C; FT-IR (KBr, cm^{-1}) 3323, 3227, 3170, 3048, 2928, 2854, 1672, 1627, 1542, 1341, 1262, 1169, 1022, 778, 707, 664, 623; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23–2.17 (m, 10H, Cyclohexane –CH_2–), 4.26–4.35 (m, 1H, Cyclohexane –C*H*–), 7.51 (t, 2H, *J* = 7.6 Hz, Ar$H_{e=e'}$), 7.63 (t, 1H, *J* = 7.6 Hz, ArH_f), 7.84 (d, 2H, *J* = 7.6 Hz, Ar$H_{d=d'}$), 8.94 (s, CON*H*), 10.72 (s, CSN*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3 (2C), 25.4, 33.26 (2C), 57.62 (Cyclohexane-*C*), 127.3 (2C), 129.1 (2C), 131.8, 133.5 (Ar-*C*), 166.8 (*C*=O), 178.22 (*C*=S); Anal. Calcd for $C_{14}H_{17}N_2$-OSCl; C, 56.65; H, 5.77; N, 9.44; S, 10.80 Found: C, 56.64; H, 5.77; N, 9.46; S, 10.79.

2.3.37. 1-(3-Chlorobenzoyl)-3-dicyclohexylthiourea (37)

Yield 80%; white solid; mp 120–121 °C; FT-IR (KBr, cm^{-1}) 3328, 3177, 3055, 2946, 2864, 1662, 1617, 1526, 1335, 1264, 1168, 1020, 774, 709, 668, 626; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24–2.20 (m, 20H, Cyclohexane –CH_2-), 4.22–4.34 (m, 2H, Cyclohexane –C*H*-), 7.53 (t, 2H, *J* = 7.6 Hz, Ar$H_{e=e'}$), 7.64 (t, 1H, *J* = 7.6 Hz, ArH_f), 7.82 (d, 2H, *J* = 7.6 Hz, Ar$H_{d=d'}$), 8.98 (s, CON*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3 (4C), 25.4, 33.26 (4C), 57.62 (Cyclohexane-*C*), 127.3 (4C), 129.1 (4C), 131.8, 133.5 (Ar-*C*), 167.0 (*C*=O), 178.2 (*C*=S); Anal. Calcd for $C_{20}H_{27}N_2OSCl$; C, 63.39; H, 7.18; N, 7.39; S, 8.46 Found: C, 63.37; H, 7.17; N, 7.40; S, 8.78.

2.3.38. 1-(3-Chlorobenzoyl)-3-(pyrimidin-2-yl)thiourea (38)

Yield 88%; yellow solid; mp 180–181 °C; FT-IR (KBr, cm^{-1}) 3158, 3048, 2991, 2939, 1722, 1656, 1587, 1561, 1515, 1439, 1412, 1331, 1244, 1206, 1175, 1126, 916, 808, 729, 677, 567, 503, 456; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.30 (t, 1H, *J* = 6.3 Hz, pyrimidin–H_a), 7.61 (t, 1H, *J* = 6.3 Hz, ArH_e), 7.73 (d, 1H, *J* = 6.8 Hz, ArH_f), 7.91 (d, 1H, *J* = 6.8 Hz, ArH_d), 7.97 (s, 1H, Ar$H_{d'}$), 8.76 (d, 2H, *J* = 4.2 Hz, pyrimidin-$H_{b=b'}$), 12.2 (s, 1H, CON*H*), 13.5 ($s_{(broad)}$, 1H, CSN*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 117.6,157.5,158.9 (2C) (pyrimidin), 127.4, 128.5, 131.4, 133.2, 134.0, 135.7 (Ar-*C*), 166.3 (*C*=O), 178.4 (*C*=S); MS (EI) *m*/*z* 291.7 (M^+), 138.8 (base peak); Anal. Calcd for $C_{12}H_9N_4OSCl$; C, 49.23; H, 3.10; N, 19.14; S, 10.95 Found: C, 49.23; H, 3.08; N, 19.19; S, 10.94.

2.4. Data collection and structural refinement of 13, 16, 24, 28 and 30

Crystals of the compounds **13**, **16**, **24**, **28** and **30** were grown by the slow evaporation in ethanol/dichloromethane (1:1). The colorless crystals were mounted in a random orientation on a glass fiber and the diffraction data collections were performed with a Stoe IPDS-II two circle (**13**) and Rigaku AFC7R Mercury CCD diffractometer (**16**, **24**, **28** and **30**) with graphite monochromatic MoKα radiation (λ = 0.71073 Å).[40] The structures were solved by direct methods using SHELXS-97 and refined with full-matrix least-squares on F^2 with SHELXL-97 and absorption correction with MULABS option in PLATON and REQABS.[41] Structures were solved by direct and Fourier methods and refined by full-matrix least-squares based on F^2.[42] All non-hydrogen atoms were refined anisotropically, hydrogen atoms were determined from difference Fourier maps and refined at idealized positions riding on their attached C or N atoms. For **16** there are three crystallographically independent but chemically equal molecules along with a solvate per asymmetric unit.

2.5. Biological assays

2.5.1. Measurement of urease activity

Newly synthesized series of *N*,*N*′-disubstituted thioureas (**1**–**38**) were screened for inhibitory activity against urease. The activity was measured by determining the amount of ammonia liberated during the indophenol by using standard protocol.[39,43] The compounds were initially screened at 1 mM concentration. Thioureas exhibiting ⩾50% inhibition were selected for further determination of dose–response curves against all the compounds. For this purpose 7–9 serial dilutions of each compound were prepared in assay buffer and their dose response curves were obtained by adopting the same methods used for initial screening. All experiments were performed in triplicate. Results reported are mean of three independent experiments (±SEM) and expressed as percent inhibitions calculated by the formula.

$$\text{Inhibition}(\%) = [100 - (\text{Abs of test compound}/\text{Abs of control}) \times 100]$$

IC_{50} values of potential inhibitors (⩾50%) were obtained with the help of non-linear regression analysis program of Graph Pad prism 5.0 Software Inc., San Diego, California, USA.

2.5.2. Cytotoxicity assay by sulforhodamine B (SRB)

Lung carcinoma (H-157), (ATCC CRL-5802) cell lines were kept in RPMI-1640 having heat-inactivated fetal bovine serum (10%) glutamine (2 mM), pyruvate (1 mM), 100 U/mL penicillin and 100 μg/mL streptomycin in T-75 cm^2 sterile tissue culture flasks in a 5% CO_2 incubator at 37 °C.[44] 96 well plates were used for growing H-157 cells by inoculating 10^4 cells per 100 μL per well and plates were incubated in CO_2 incubator at 37 °C. Within 24 h, a uniform monolayer was formed which was used for experiments. The compounds (100 μM) were inoculated in test wells while control and blank wells were also prepared containing standard drug (vincristine) and culture media with cells, respectively.[44,45] The plates were then incubated for 48 h. After that cells were fixed with 50 μL of 50% ice cold trichloroacetic acid solution (TCA) at 4 °C for 1 h. The plates were washed 5 times with phosphate-buffered saline (PBS) and air dried. Fixed cells were further treated with 0.4% w/v sulforhodamine B dye prepared in 1% acetic acid solution and left at room temperature for 30 min. After that the plates were rinsed with 1% acetic acid solution and allowed to dry. In order to solubilize the dye, the dried plates were treated with 10 mM Tris base solution for 10 min at room temperature. Absorbance was measured at 490 nm subtracting the background measurement at 630 nm. All experiments were performed in triplicate. Results reported are mean of three independent experiments (±SEM) and expressed as percent inhibitions calculated by the formula.

$$\text{Inhibition}\ (\%) = [100 - (\text{Abs of test compound}/\text{Abs of control}) \times 100]$$

2.5.3. Docking protocols

2.5.3.1. Structure selection and preparation. Molecular docking studies were conducted to investigate putative interactions of the compounds in complex with the urease enzyme. In order to perform efficient docking studies, the crystallographic structure of Jack bean urease (PDB ID: 3LA4) was obtained from the RCSB PDB database[46] and prepared. Prior to docking experiments, the structures of the enzyme and the compounds were prepared as follows. The enzyme structure was protonated with the Protonate3D[47] algorithm implemented in the molecular modeling tool MOE.[48] The structure was energy minimized using Amber99 force field including all crystallographic solvent molecules. The

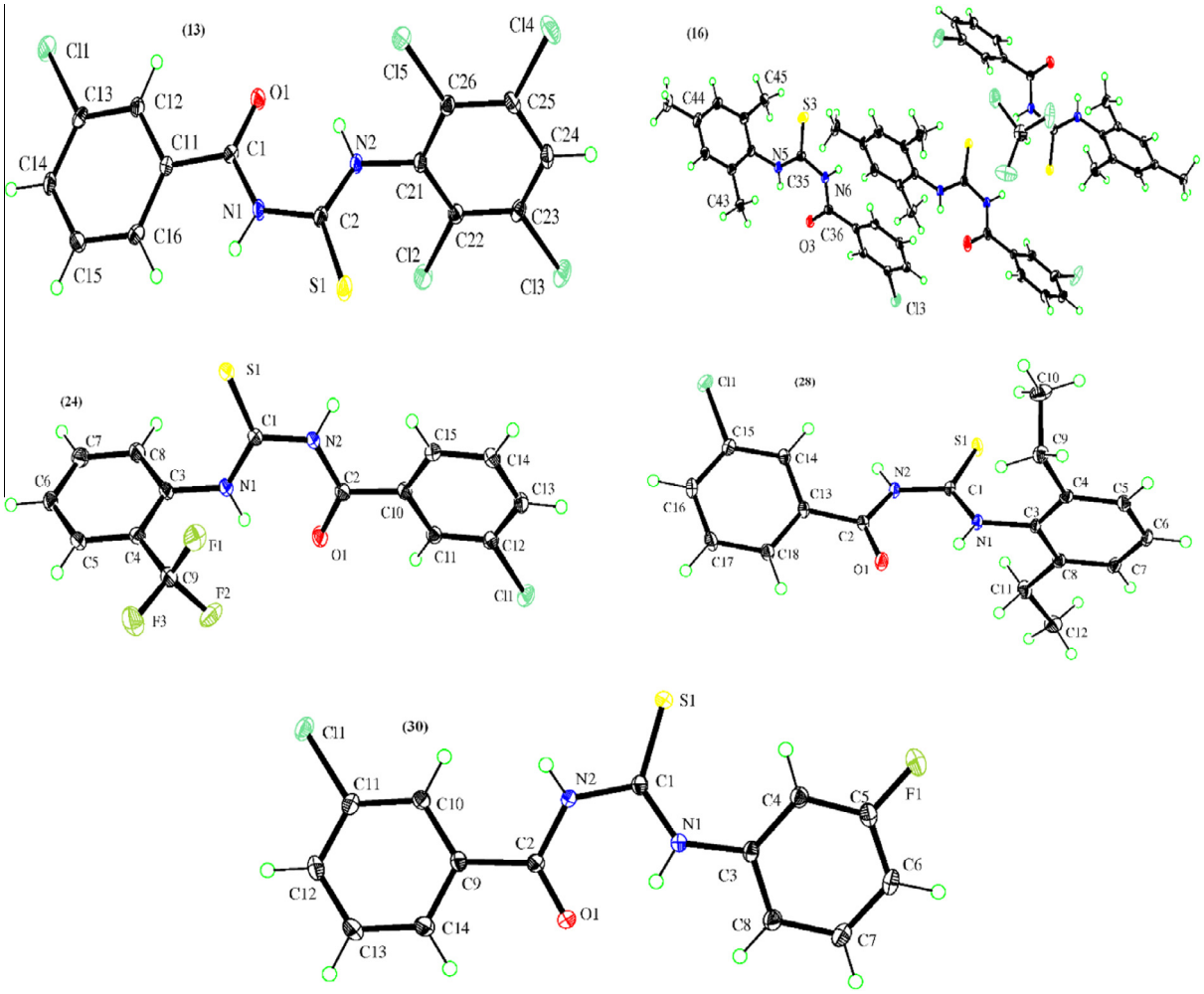

Figure 1. ORTEP structures of **13**, **16**, **24**, **28** and **30** showing anisotropic displacement ellipsoids drawn at the 35% probability level. Structure of **16** consists of three independent conformers with chloroform as a solvate.

backbone atoms were restrained with a small force in order to avoid collapse of the binding pockets during energy minimization calculations. After minimization, the water molecules and co-crystallized bound compounds were removed.

2.5.3.2. Compounds preparation. The 3D structural coordinates of the compounds were generated for all the compounds using MOE followed by assignment of protonation and ionization states in physiological pH range by using the 'wash' module. Afterwards, the compounds' structures were energy minimized with the MMFF94x force field for docking studies.[48]

2.5.3.3. Docking studies. Docking studies of all the compounds were performed using LeadIT from BioSolveIT, GmbH Germany.[49] Receptor was loaded by Load or Prepare Receptor utility of the LeadIT software, while selecting the metal ions as part of the receptor. The binding site for Jack bean urease was defined in 7.5 Å spacing of the amino acid residues surrounding the bound phosphate. By FlexX utility of LeadIT, docking of compounds was performed. Default docking parameters were not changed and top 30 highest scoring docked positions were kept for further analysis. The Discovery studio visualizer v4, was used for visualizing the results.[50] Using HYDE visual affinity[51] program of LeadIT, the binding mode analysis of the docked poses were evaluated. Binding free energy, i.e., ΔG was determined for each pose. Poses with lowest ΔG values were considered as the most stable pose with highest affinity for interaction with the receptor.

2.6. Supplementary data

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited to the Cambridge Crystallographic Data Centre as supplementary publication CCDC no. 605433, 1453877–1453880 for **13**, **16**, **24**, **28** and **30**, respectively. Copies of available materials can be obtained, free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB21EZ, UK, (Fax:+44-(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

3. Results and discussion

3.1. Chemistry

Solution-phase microwave assisted parallel synthesis of *N*,*N*′-disubstituted thioureas was performed using CEM MARS 6 microwave reactor, specifically for organic synthesis. A small library of thirty eight members (**1**–**38**) was successfully developed and fully characterized by modern analytical techniques. The purity level of the compounds was established by TLC with Merck Kieselgel GF 254 plates and elemental analysis. Their structures were

Table 1
Crystallographic data and structure refinement for compounds **13**, **16**, **24**, **28** and **30**

Compound	**13**	**16**	**24**	**28**	**30**
Formula	$C_{14}H_7Cl_5N_2OS$	$C_{17}H_{17}ClN_2OS$, $C_{0.33}$ $H_{0.33}$ Cl	$C_{15}H_{10}ClF_3N_2OS$	$C_{18}H_{19}ClN_2OS$	$C_{14}H_{10}ClFN_2OS$
FW	428.53	372.62	358.76	346.86	308.75
Crystal system	Triclinic	Triclinic	Triclinic	Triclinic	Triclinic
Space group	P-1	P-1	P-1	P-1	P-1
a (Å)	7.8062(11)	10.860(2)	7.636(3)	8.1763(17)	8.6742(15)
b (Å)	8.4597(12)	15.783(4)	8.275(3)	9.071(2)	8.7810(19)
c (Å)	12.7760(19)	16.253(4)	12.148(4)	12.155(3)	9.1296(18)
α, β, γ (°)	93.374(11), 99.840(11), 97.379(11)	101.677(3), 101.111(3), 90.700(3)	88.564(11), 79.230(10), 83.530(9)	85.069(7), 77.163(7), 78.649(7)	86.151(6), 73.428(7), 79.084(6)
V (Å^3)	821.583	2673.22	749.3(5)	861.0(3)	654.4(2)
Z	2	6	2	2	2
T (K)	173(2)	123(2)	123(2)	123(2)	123(2)
D_C Mg/m^3	1.732	1.389	1.590	1.338	1.567
μ (mm^{-1})	1.012	0.487	0.431	0.349	0.459
F(000)	428	1160	364	364	316
Crystal size (mm^3)	0.37 × 0.32 × 0.22	0.40 × 0.32 × 0.25	0.30 × 0.24 × 0.15	0.38 × 0.28 × 0.12	0.50 × 0.45 × 0.35
θ range (°)	3.8 to 25.7	3.01 to 27.48°	3.00 to 27.47°	3.13 to 27.46°	3.11 to 27.46°
Index ranges	−9<=h ⩽ 9, −10 ⩽ k ⩽ 9, −15 ⩽ l ⩽ 15	−14 ⩽ h ⩽ 12, −15 ⩽ k ⩽ 20, −21 ⩽ l ⩽ 13	−9 ⩽ h ⩽ 9, −7 ⩽ k ⩽ 10, −15 ⩽ l ⩽ 15	−8 ⩽ h ⩽ 10, −11 ⩽ k ⩽ 10, −15 ⩽ l ⩽ 14	−8 ⩽ h ⩽ 10, −11 ⩽ k ⩽ 10, −15 ⩽ l ⩽ 14
Reflections collected	5914	21729	5870	6831	5034
Indept. Reflect. [*R*(int)]	3025 [*R*(int) = 0.0397]	12097 [*R*(int) = 0.0249]	3355 [*R*(int) = 0.0216]	3865 [*R*(int) = 0.0171]	2897 [*R*(int) = 0.0196]
Completeness to *θ* = 28°	98.5%	98.8%	97.5%	97.9%	% 96.5
Max. & min. transmission	0.7057, 0.8080	0.8879, 0.8290	0.8816, 0.9382	0.9594, 0.8789	0.8560, 0.8031
Data/restraints/parameters	3025/0/217	12097/0/664	3355/0/208	3865/0/210	2897/0/189
Goodness-of-fit on F^2	1.021	1.088	1.038	1.088	1.051
R_1 [$I > 2\sigma(I)$]	0.0336	0.0430	0.0373	0.0350	0.0360
wR_2 [all data]	0.0908	0.1059	0.0960	0.0831	0.0981
Largest peak and hole (e Å^{-3})	0.294 and −0.434	0.423 and −0.515	0.368 and −0.319	0.319 and −0.231	0.963 and −0.271

investigated by FT-IR, ^{1}H- and ^{13}C-NMR (Bruker ARX, 300 MHz spectrometer) and single crystal XRD. The reaction intermediate, 3-cholorobenzoyl isothiocyanate was synthesized by conventional method and purified by steam distillation technique. Stoichiometric quantities of 3-cholorobenzoyl and respective primary/secondary amines were subjected to cylindrical glass vials with THF as solvent. Irradiated the glass vials under identical set of conditions of power and exposure time. The nature of substrates primary/secondary amines (H_2NR/ HNRŔ), strongly influence the yield of the final products (Scheme 1). The substituents like F, Cl, Br, CF_3, OR, R and $-NO_3$ attached at the *ortho* and *para* positions on the aromatic ring in H_2NR/HNRR′ moiety show strong mesomeric effect (+R). This has been reflected in the increase of product yield in the following order; Br/Cl > R > OR > F > CF_3 > cyclohexyl > $-NO_3$. The cyclohexyl and $-NO_3$ containing substrate has shown lowest yield due to conformational isomerism and high electron with drawing effect, respectively.[39] The complete description of the substituents for derivatives (**1**–**38**) is illustrated in Scheme 1.

3.2. Spectroscopic studies

The major absorption bands recorded in the FT-IR spectra of the synthesized *N*,*N*′-disubstituted thioureas (**1**–**38**) are delineated in spectroscopic data separately. The assignments are in good agreement with the literature reported values.[52] The absence of ν (S-H) absorption band at 2530–2590 cm^{-1} confirmed the change of isothiocyanate moiety into thiourea (–NHCSNH–) group. The characteristic IR bands of *N*,*N*′-disubstituted thioureas were between/near to; 3120–3382 (NH), 2950–3100 Ph(CH), 1650–1730 (C═O), 1550–1630 (CN), 1250–1275 (C═S) and 1150–1190 (C—S). A medium strong band at 1670–1680 cm^{-1} suggested a possible hydrogen bond interactions between the NH-group and the carbonyl O-atom, instead of the expected normal carbonyl (C═O) absorption around 1710 cm^{-1}. The band around 22530–2590 cm^{-1} associated to SH was not appeared which indicated the absence of the N═CSH tautomerism in all the compounds (**1**–**38**).[52–54]

The ^{1}H NMR data for the compounds (**1**–**38**) showed that the NH proton resonates considerably downfield and chemical shifts were found around 9.00–9.55 and 10.80–12.78 ppm for free and hydrogen bonded NH, respectively, and aromatic protons appeared downfield 7.25 to 8.55 ppm. The coordinating or highly polar solvents like DMSO-d_6 had prominent effect on the free NH protons chemical shift and appeared more downfield as compared with the solvents like C_6D_6, $CDCl_3$ and CD_2Cl_2. Such shifts could be ascribed to the probable hydrogen bonding between the NH and sulfoxide (S═O) moiety.[52]

The ^{13}C NMR data depicted all the signals due to magnetically distinctive carbons present in the compounds (**1**–**38**). The carbon resonances associated with the aromatic moieties of thiourea ligands were consigned on the basis of signal intensities and were compared with the literature values.[55] The chemical shifts related to the carbons in CONH and CSNH groups of the thioureas resonate around 165–170 and 175–185 ppm, respectively.

3.3. Structural studies

The five molecular structures **13**, **16**, **24**, **28** and **30** (Fig. 1) consist of similar thiourea cores (–CONHCSNH–) with different substitution patterns. The cores are mostly planar with O–C–N–C and C–N–C–S torsion angles of −4.5(4)° and 174.7(2)° for **13**, −6.0(3)° (molecule I), 3.1(3)° (molecule II), 7.5(3)° (molecule III) and 175.87(16)° (I), −178.19(15)° (II), 178.26(14)° (III) for **16**, 12.2(2)° and 172.65(12)° for **24**, −9.4(2)° and −179.38(11)° for **28**, 2.5(2)° and 175.07(12)° for **30** as well. The aromatic ring planes make dihedral angles of 59.74(1)°, 54.82(5)°, 88.27(3)°, 71.32(1)° and 21.31(5)° for (**13**), (**16**I), (**24**), (**28**) and (**30**), respectively. In general, there are no unexpected variations in geometric parameters. All

Table 2
Selected bond lengths (Å) and angles (°) for compounds **13**, **16**, **24**, **28** and **30**

13							
C(2)–S(1)	1.650(2)	O(1)–C(1)–N(1)	121.9(2)	C(1)–N(1)	1.386(3)	N(2)–C(2)–N(1)	114.9(2)
C(1)–O(1)	1.229(3)	C(1)–N(1)–C(2)	128.7(2)	C(2)–N(1)	1.402(3)	N(2)–C(2)–S(1)	125.73(17)
16				**28**			
C(1)–S(1)	1.6690(16)	C(1)–N(1)–C(3)	124.61(14)	C(1)–S(1)	1.6734(14)	C(1)–N(1)–C(3)	123.99(11)
C(2)–O(1)	1.222(2)	N(1)–C(1)–N(2)	116.56(14)	C(2)–O(1)	1.2239(17)	N(1)–C(1)–N(2)	116.85(11)
C(1)–N(1)	1.325(2)	N(1)–C(1)–S(1)	124.15(13)	C(1)–N(1)	1.3286(17)	N(1)–C(1)–S(1)	124.14(10)
C(1)–N(2)	1.399(2)	O(1)–C(2)–N(2)	122.43(17)	C(1)–N(2)	1.3929(16)	O(1)–C(2)–N(2)	122.78(12)
C(2)–N(2)	1.385(2)	N(2)–C(1)–S(1)	119.29(13)	C(2)–N(2)	1.3799(17)	N(2)–C(1)–S(1)	119.01(10)
24				**30**			
C(1)–S(1)	1.6669(17)	C(1)–N(1)–C(3)	123.41(13)	C(1)–S(1)	1.6713(15)	C(1)–N(1)–C(3)	131.83(13)
C(2)–O(1)	1.225(2)	N(1)–C(1)–N(2)	116.13(14)	C(2)–O(1)	1.2244(19)	N(1)–C(1)–N(2)	114.66(13)
C(1)–N(1)	1.337(2)	N(1)–C(1)–S(1)	124.24(12)	C(1)–N(1)	1.333(2)	N(1)–C(1)–S(1)	128.38(12)
C(1)–N(2)	1.3920(19)	O(1)–C(2)–N(2)	122.39(14)	C(1)–N(2)	1.4032(19)	O(1)–C(2)–N(2)	122.65(14)
C(2)–N(2)	1.383(2)	N(2)–C(1)–S(1)	119.59(12)	C(2)–N(2)	1.3780(19)	N(2)–C(1)–S(1)	116.95(11)

Table 3
The intermolecular and intramolecular hydrogen bonds for compounds **13**, **16**, **24**, **28** and **30**. Hydrogen-bond geometry: Distance, Å; angle,°

Compound	*D*–H···*A*	*D*–H(Å)	H···*A*(Å)	*D*···*A*(Å)	*D*–H···*A*(°)
13	N2–H2···O1	0.85(3)	1.96(3)	2.644(2)	136(2)
16	N1–H1···O1	0.83(2)	1.97(2)	2.6509(19)	139(2)
	N2–H2···S2	0.85(2)	2.53(2)	3.3730(18)	167.9(18)
	N3–H3···O2	0.818(18)	2.015(19)	2.6598(19)	135.4(18)
	N3–H3···O3^{i}	0.818(18)	2.39(2)	3.0280(19)	135.7(17)
	N4–H4···S1	0.85(2)	2.55(2)	3.3878(18)	170.9(18)
	N5–H5···O3	0.84(2)	2.00(2)	2.674(2)	136(2)
	N5–H5···O2^{ii}	0.84(2)	2.58(2)	3.285(2)	142(2)
24	N1–H1···O1	0.88	1.92	2.6227(18)	135.4
	N2–H2···S1^{i}	0.88	2.57	3.4251(17)	163.5
28	N1–H1···O1	0.88	1.95	2.6425(15)	134.9
	N2–H2···S1^{i}	0.88	2.56	3.3951(13)	159.0
30	N1–H1···O1	0.86(2)	1.88(2)	2.6227(18)	144(2)
	N2–H2···S1^{i}	0.79(2)	2.77(2)	3.4787(15)	151(2)

Symmetry codes: (i) *x*, *y*, *z* − 1; (ii) *x*, *y*, *z* + 1(**16**); (i) −*x* + 1, −*y* + 1, −*z* + 1(**24**); (i) −*x* + 1, −*y* + 1, −*z* + 1(**28**); (i) −*x* + 1, −*y* + 1, −*z* + 1(**30**).

the molecular structures show an intramolecular N—H···O hydrogen bond stabilizing the (–CONHCSNH–) geometry, forming a pseudo six membered ring (Table 3). Crystallographic and refinement details are shown in Table 1. Selected bond lengths and angles are given in Table 2.

3.4. Pharmacology

3.4.1. Urease inhibition assay

The synthesized *N*,*N*′-disubstituted thioureas (**1**–**38**) were screened for inhibitory activity against Jack bean urease and the result are given in Table 4. The results revealed that most of the compounds in the series possess lower IC_{50} values than the standard thiourea (IC_{50} = 22.3 ± 1.2 μM). Compound **16** with 2,4,6-trimethylphenyl substitution was found to be most potent compound among the series having an IC_{50} value of 1.23 ± 0.1 μM. The compound was found to be ~18-fold more active than the standard inhibitor, thiourea having IC_{50} value of 22.3 ± 1.2 μM. Whereas, the compound **23** having 2,4-dinitrophenyl substitution was the least potent with 21.7% inhibition. Compound **5** having 2,3-dichlorophenyl and compound **38** with 2-pyrimidinyl substitution, presented IC_{50} value of 1.92 ± 0.1 and 2.11 ± 0.1 μM, respectively. The substitution of different groups including electron-donating and electron-withdrawing groups at the phenyl ring resulted in varying inhibition patterns depending upon type and position of substituent. Some of the substituents on phenyl ring, improved inhibitory activities of compounds and some resulted in reduction in inhibition capacity.

It was noted that dichlorophenyl and trichlorophenyl thiourea derivatives were more active against urease as compared to tetrachlorophenyl derivatives. Similarly, all the trifluoromethylphenyl derivatives were potent inhibitors of urease in the series.

3.4.2. Anticancer activity of *N*,*N*′-disubstituted thioureas

The anticancer drugs used in chemotherapy are systemic antiproliferative agents, which preferentially kill those cells that are dividing. In the present study, antiproliferative activity of the newly synthesized thiourea derivatives was measured by the cell growth inhibition against lung carcinoma (H157) through SRB assay and the % inhibition was presented in Table 5. Vincristine, a standard anticancer drug, was used as reference for test compounds. The results showed that all the compounds exhibit mild to moderate anticancer activity against H157 cell lines at 100 μM.

Compound **12** having 2,4,6-trichlorophenyl substitution was the potent compound against H157 with 60.9% inhibition. The nature and type of the side groups present in compounds **1**–**38** is one of the most important factors in the appearance of anticancer effect. The substitution at phenyl groups effects the inhibitory potential of compounds. Halogenated substitution at phenyl ring has greater influence on anticancer activity.

3.4.3. Molecular docking

3.4.3.1. Molecular docking studies. The structures of di-substituted thioureas were docked to the crystallographic structure of Jack bean urease PDB ID: 3LA4. Active site of the receptor contain bi-nickel center. The binding pattern of synthesized inhibitors were established by molecular docking studies. As evident from in vitro results compound **16** was the most suitable for docking studies. After reproducing the co-crystallized reference ligand into the active site of receptor, all the derivatives were docked in active

Table 4
In vitro urease inhibitory activity of *N*,*N*′-disubstituted thioureas (**1**–**38**)

No	IC_{50} ± SEM (μM)/%inhibition	No	IC_{50} ± SEM (μM)/%inhibition
1	3.24 ± 0.3	**20**	28.1%
2	25.7%	**21**	23.1%
3	29.1%	**22**	3.79 ± 0.8
4	11.7 ± 0.2	**23**	21.7%
5	1.92 ± 0.1	**24**	5.58 ± 0.2
6	1.34 ± 0.1	**25**	2.86 ± 0.1
7	9.72 ± 0.7	**26**	7.04 ± 0.3
8	5.38 ± 0.3	**27**	36.9%
9	36.7%	**28**	12.6 ± 0.5
10	6.53 ± 0.4	**29**	2.91 ± 0.4
11	7.41 ± 0.5	**30**	29.9%
12	3.64 ± 0.2	**31**	37.1%
13	32.7%	**32**	27.9%
14	12.4 ± 0.4	**33**	17.5 ± 0.4
15	4.45 ± 0.3	**34**	16.4 ± 0.6
16	1.23 ± 0.1	**35**	27.4%
17	20.1 ± 0.9	**36**	14.3 ± 0.2
18	6.32 ± 0.3	**37**	29.9%
19	8.59 ± 0.7	**38**	2.11 ± 0.1
Thiourea	22.3 ± 1.2	—	—

Table 5
Anticancer activity of *N*,*N*′-disubstituted thioureas (**1**–**38**) against lung carcinoma (H-157) at 100 μM

Codes	%Inhibition	Codes	%Inhibition
1	45.2	**20**	32.7
2	45.4	**21**	36.1
3	52.9	**22**	42.8
4	52.6	**23**	42.2
5	50.7	**24**	36.9
6	38.9	**25**	48.9
7	46.6	**26**	44.6
8	42.2	**27**	47.7
9	39.4	**28**	41.2
10	48.5	**29**	42.7
11	43.3	**30**	36.3
12	60.9	**31**	42.2
13	45.5	**32**	48.8
14	39.4	**33**	45.1
15	41.7	**34**	40.5
16	43.7	**35**	27.6
17	32.4	**36**	48.5
18	38.2	**37**	47.8
19	36.4	**38**	57.2
Vincristine	74.6	—	—

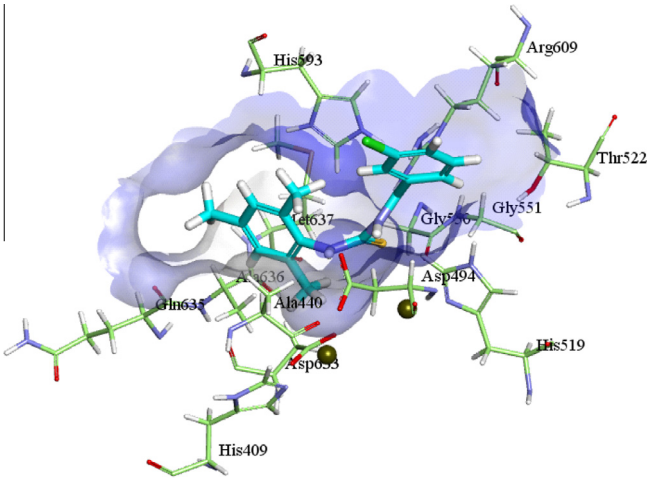

Figure 2. Putative binding mode of **16** bound to the active site of Jack bean Urease. Carbon atoms of **16** are colored blue and that of protein are light green. The two nickel cations in the active site are represented as small dark green spheres.

Table 6
Docking and Hyde scores and their corresponding ranks by Hyde affinity assessment

Codes	FlexX score of the top ranking pose	Poser rank	Binding free energy ΔG (kJ mol^{-1})
1	−23.34	2	−17
2	−26.51	5	−14
3	−22.14	1	−18
4	−22.06	2	−17
5	−25.73	1	−13
6	−25.10	5	−7
7	−24.63	2	−13
8	−21.85	6	−16
9	−21.82	3	−11
10	−24.47	1	−16
11	−28.54	3	−11
12	−19.90	1	−16
13	−18.17	3	−10
14	−36.54	3	−12
15	−20.16	17	−10
16	−20.98	18	−15
17	−21.65	4	−18
18	−18.79	2	−10
19	−22.08	6	−14
20	−24.14	22	−11
21	−18.64	8	−16
22	−20.58	10	−10
23	−19.75	10	−1
24	−24.84	10	−19
25	−26.04	8	−25
26	−16.69	8	−20
27	−28.48	19	−7
28	−20.40	10	−16
29	−29.08	2	−13
30	−28.82	13	−1
31	−20.66	1	−11
32	−16.58	4	−8
33	−29.34	14	−5
34	−22.33	4	−7
35	−25.06	1	−14
36	−26.38	5	−13
37	−26.22	4	−11
38	−22.13	17	−2

site. There was a common inclination for most of residues in the active site to which all the compounds were interacting. It was noticed that all the compounds show interactions with amino acids Ala440, Arg609, Asp494, His409, His492, His519, Ala636, Met637 and His593. The hydrogen bonding interactions were identified between the oxygen and hydrogen of the inhibitors with residues Arg609, Asp494, Ala440 and His593 as described previously.[39,56] Additionally, hydrophobic interactions of the inhibitors were recognized within the active site residues. Figure 2 reports a prioritized binding mode model of the potent inhibitor **16**.

3.4.3.2. Binding mode analysis. The putative binding mode of compound **16**, the most potent urease inhibitor was shown in Figure 2. Trimethylphenyl substituent was located towards the bottom of the binding pocket, where aromatic interactions with the surrounding residues and the nickel ions in the deep pocket were seen. While the 3-chlorobenzoyl side of the compound was oriented towards residue Arg609 and Thr522. All the compounds do not interact with any Ni atom in the active site, however, weak interactions were found by most of the inhibitors with ni842 in the catalytic site of urease enzyme. Figure 2 represents the alignment of potent inhibitor inside the active pocket of 3LA4 by forming hydrogen bond interactions with His593 and Arg609. Moreover, π–π interactions were noticed by trimethyl phenyl ring of the derivative with active site residues.

3.4.3.3. HYDE assessment. The HYDE affinity assessment was performed for first 30 top ranking docked conformations. The

binding free energy ΔG, FlexX docking score and their most favorable poses are given in Table 6 for all the derivatives. Most of the compounds binds to the receptor with a very high binding affinity.

4. Conclusion

A series of *N*,*N*′-disubsituted thiourea analogues were prepared using facile microwave-assisted solution phase parallel synthesis. The synthesized compounds were evaluated for in vitro urease inhibitory activity. Most of the compounds exhibited excellent urease inhibition, among which, compound **16** shows potent urease inhibitory activity with an IC_{50} value 1.23 ± 0.1 μM. All the synthesized derivatives were screened for their anticancer activities. Compound **12** was the potent compound against H157 with 60.91% inhibition. The docking studies further unlocked the binding site interactions of the potent inhibitors. Furthermore, the results suggest that these derivatives may be used as preventive and chemotherapeutic agents for cancer. Hence it is recommended to find the correlation of urease inhibitors and their role in the therapy of cancer.

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References and notes

1. Ibrar, A.; Khan, I.; Abbas, N. *Arch. Pharm. Chem. Life Sci.* **2013**, *346*, 423.
2. Dixon, N. E.; Gazzola, T. C.; Blakeley, R. L.; Zermer, B. *J. Am. Chem. Soc.* **1975**, *97*, 4131.
3. Zaborska, W.; Kot, M.; Superata, K. *J. Enzym. Inhib. Med. Chem.* **2002**, *17*, 247.
4. Lodhi, M. A.; Hussain, J.; Abbasi, M. A.; Jassbi, A. R.; Choudhary, M. I.; Ahmad, V. U. *J. Enzym. Inhib. Med. Chem.* **2006**, *21*, 531.
5. Zonia, L. E.; Stebbins, N. E.; Polacco, J. C. *Plant Physiol.* **1995**, *107*, 1097.
6. Collins, C. M.; DÓrazio, S. E. F. *Mol. Microbiol.* **1993**, *9*, 907.
7. Montecucco, C.; Rappuoli, R. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 457.
8. Zhengping, W.; Van Cleemput, O.; Demeyer, P.; Baert, L. *Biol. Fertil. Soils* **1991**, *11*, 41.
9. Upadhyay, L. S. B. *Indian J. Biotechnol.* **2012**, *11*, 381.
10. Kosikowska, P.; Berlicki, L. *Expert. Opin. Ther. Pat.* **2011**, *21*, 945.
11. Amtul, Z.; Rahman, A. U.; Siddiqui, R. A.; Choudhary, M. I. *Curr. Med. Chem.* **2002**, *9*, 1323.
12. Follmer, C. *J. Clin. Pathol.* **2010**, *63*, 424.
13. Kumar, R.; Shin, W. S.; Sunwoo, K.; Kim, W. Y.; Koo, S.; Bhuniya, S.; Kim, J. S. *Chem. Soc. Rev.* **2015**, *44*, 6670.
14. Ahmed, M. F.; Belal, A. *Res. Chem. Intermed.* **2015**. http://dx.doi.org/10.1007/s11164-015-2048-8.
15. Solomon, V. R.; Hua, C.; Lee, H. *Bioorg. Med. Chem.* **2009**, *17*, 7585.
16. Kattimani, P. P.; Kamble, R. R.; Kariduraganavar, M. Y.; Dorababu, A.; Hunnur, R. K. *Eur. J. Med. Chem.* **2013**, *62*, 232.
17. Nasr, T.; Bondock, S.; Youns, M. *Eur. J. Med. Chem.* **2014**, *76*, 539.
18. Li, Z. H.; Zhang, Y.; Peng, Z. H.; Wang, Y. G. *Huaxueshiji* **2002**, *24*, 214.
19. Madan, V. K.; Taneja, A. D.; Kudesia, V. P. *J. Indian Chem. Soc.* **1991**, *68*, 162.
20. (a) Yonova, P. A.; Stoilkova, G. M. *J. Plant Growth Regul.* **2004**, *23*, 280; (b) Brown, B. T.; Harris, R. L. N. *Pestic. Sci.* **1973**, *4*, 215.
21. Loev, B.; Bender, P. E.; Bowman, H.; Helt, A.; McLean, R.; Jen, T. *J. Med. Chem.* **1972**, *15*, 1024.
22. (a) Hearn, M. J.; Chen, M. F.; Cynamon, M. H.; Wang'ondu, R.; Webster, E. R. *J. Sulfur Chem.* **2006**, *27*, 149; (b) Mahajan, A.; Yeh, S.; Nell, M.; Van Rensburg, C. E. J.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5683.
23. (a) Nakamura, H.; Noh, J. Y.; Itoh, K.; Fukata, S.; Miyauchi, A.; Hamada, N. *J. Clin. Endocrinol. MeTable* **2007**, *92*, 2157; (b) Duan, L.-P.; Xue, J.; Xu, L.-L.; Zhang, H.-B. *Molecules* **2010**, *15*, 6941; (c) Chung, J. U.; Kim, S. Y.; Lim, J. O.; Choi, H. K.; Kang, S. U.; Yoon, H. S.; Ryu, H. C.; Kang, D. W.; Lee, J.; Kang, B.; Choi, S.; Toth, A.; Pearce, L. V.; Pavlyukovets, V. A.; Lundberg, D. J.; Blumberg, P. M. *Bioorg. Med. Chem.* **2007**, *15*, 6043.
24. (a) Kearney, P. C.; Frenandez, M.; Flygare, J. A. *J. Org. Chem.* **1998**, *63*, 196; (b) Boga, C.; Forlani, L.; Silvestroni, C.; Corradi, A. B.; Sagarabotto, P. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1363.
25. Patil, D. G.; Chedekel, M. R. *J. Org. Chem.* **1984**, *49*, 997.
26. Kasmi, S.; Hamelin, J.; Benhaoua, H. *Tetrahedron Lett.* **1998**, *39*, 8093.
27. (a) Kidwai, M.; Venkataramanan, R.; Dave, B. *Green Chem.* **2001**, *3*, 278; (b) Paul, S.; Gupta, M.; Gupta, R.; Loupy, A. *Synthesis* **2002**, 75.
28. Anshu, D.; Kapil, A.; Meha, S. *Synth. Commun.* **2004**, *34*, 1141.
29. Heinelt, U.; Schultheis, D.; Jager, S.; Iindenmaire, M.; Pollex, A.; Beckmann, H. S. G. *Tetrahedron* **2004**, *60*, 9883.
30. (a) Frank, R. L.; Smith, P. V. *Org. Synth. Coll. III* **1955**, 735; (b) Rasmussen, C. R.; Villani, F. J. J.; Weaner, L. E.; Reynolds, B. E.; Hood, A. R.; Hecker, L. R.; Nortey, S. O.; Hanslin, A.; Costanzo, M. J.; Powell, E. T.; Molinari, A. J. *Synthesis* **1988**, 456.
31. Moor, M. L.; Crossely, F. S. *Org. Synth. Coll. III* **1955**, 617.
32. Kodomari, M.; Suzuki, M.; Tanigawa, K.; Aoyama, T. *Tetrahedron Lett.* **2005**, *46*, 5841.
33. Erickson, J. G. *J. Org. Chem.* **1956**, *21*, 483.
34. (a) Vázquez, J.; Bernès, S.; Reyes, Y.; Moya, M.; Sharma, P.; Alvarez, C.; Gutiérrez, R. *Synthesis* **2004**, 1955; (b) Katritzky, A. R.; Kirichenko, N.; Rogovoy, B. V.; Kister, J.; Tao, H. *Synthesis* **2004**, 1799; (c) Ciszewski, L.; Xu, D.; Repic, O.; Blacklock, T. J. *Tetrahedron Lett.* **2004**, *45*, 8091.
35. Zeng, R. S.; Zou, J. P.; Mu, X. J.; Shen, Q. *Chinese J. Chem.* **2003**, *21*, 1652.
36. Wei, T. B.; Zhang, Y. M.; Wei, W. *J. Chem. Res. Synop.* **2003**, *10*, 666.
37. Wei, T. B.; Lin, Q.; Zhang, Y. M.; Wei, W. *Synth. Commun.* **2004**, *34*, 181.
38. (a) Rauf, M. K.; Imtiaz-ud-Din; Badshah, A.; Gielen, M.; Ebihara, M.; de Vos, D.; Ahmed, S. *J. Inorg. Biochem.* **2009**, *103*, 1135; (b) Rauf, M. K.; Yaseen, S.; Badshah, A.; Zaib, S.; Arshad, R.; Imtiaz-ud-Din; Tahir, M. N.; Iqbal, J. *J. Biol. Inorg. Chem.* **2015**, *20*, 541; (c) Yaseen, S.; Rauf, M. K.; Zaib, S.; Badshah, A.; Tahir, M. N.; Ali, M. I.; Imtiaz-ud-Din; Shahid, M.; Iqbal, J. *Inorg. Chim. Acta* **2016**, *443*, 69.
39. Rauf, M. K.; Talib, A.; Badshah, A.; Zaib, S.; Shoaib, K.; Shahid, M.; Flörke, U.; Imtiaz-ud-Din; Iqbal, J. *Eur. J. Med. Chem.* **2013**, *70*, 487.
40. (a) Stoe & Cie. X-AREA. Stoe & Cie, Darmstadt, Germany, 2001, G. M. Sheldrick, SADABS. 2004, University of Göttingen, Germany; (b) Molecular Structure Corporation & Rigaku 2001, CrystalClear. MSC, The Woodlands, Texas, USA, and Rigaku Corporation, Tokyo, Japan; (c) Molecular Structure Corporation & Rigaku 2004, TEXSAN. MSC, The Woodlands, Texas, USA, and Rigaku Corporation, Tokyo, Japan
41. (a) Spek, A. L. *J. Appl. Crystallogr.* **2003**, *36*, 7; (b) Rigaku *REQAB*; Rigaku Corporation: Tokyo, Japan, 1998.
42. Sheldrick, G. M. *Acta Cryst. A* **2008**, *64*, 112.
43. Weatherburn, M. W. *Anal. Chem.* **1967**, *39*, 971.
44. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
45. Khan, I.; Zaib, S.; Ibrar, A.; Rama, N. H.; Simpson, J.; Iqbal, J. *Eur. J. Med. Chem.* **2014**, *78*, 167.
46. Balasubramanian, A.; Ponnuraj, K. *J. Mol. Biol.* **2010**, *400*, 274.
47. Labute, P. *Protonate 3D*; Chemical Computing Group, 2007. http://www.chemcomp.com/journal/proton.htm.
48. MOE (The Molecular Operating Environment) Version 2014.0901. Chemical Computing Group, (CCG) http://www.chemcomp.com/MOEMolecular_Operating_ Environment.htm.
49. LeadIT, 2014, at http://www.biosolveit.de/LeadIT/.
50. Accelrys Software Inc. *Discovery Studio Modeling Environment, Release 4.0*; Accelrys Software: San Diego, CA, 2013.
51. Schneider, N.; Lange, G.; Hindle, S.; Klein, R.; Rarey, M. *J. Comput. Aided Mol. Des.* **2013**, *27*, 15.
52. Fregona, D.; Giovagnini, L.; Ronconi, L.; Marzano, C.; Treeevisan, A.; Sitran, S.; Bordin, B. *J. Inorg. Biochem.* **2003**, *93*, 181.
53. Wiles, D. M.; Gingkas, B. A.; Suprlnchuk, T. *Can. J. Chem.* **1967**, *45*, 469.
54. Cesaro, S. N. *Vib. Spectrosc.* **1998**, *16*, 55.
55. Kolinowski, H. O.; Berger, S.; Brown, S. ^{13}C *NMR Spectroscopy*; Thieme-Verlag: Stuttgart, Germany, 1984.
56. Ahmad, M.; Pervez, H.; Zaib, S.; Yaqub, M.; Naseer, M. M.; Khan, S. U.; Iqbal, J. *RSC Adv.* **2016**, *6*, 60826.