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Synthesis, antimicrobial, antioxidant, cytotoxic, antiurease and molecular docking studies of *N*-(3-trifluoromethyl)benzoyl-*N′*-aryl thiourea derivatives

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ABSTRACT

An irrefutable advancement has been noted for the infectious diseases caused due to ureolytic bacteria through the development of various drugs. Keeping in mind the extremely valuable synthetic utility and medicinal significance of thiourea derivatives, synthesis of new 3-trifluoromethyl benzoic acid thiourea derivatives **(3a–j)** were carried out. The biological potential of all compounds in terms of antimicrobial, antioxidant, cytotoxic and antiurease activities were studied. The compounds **3a, 3c** and **3i** with dichloro and methoxy groups substitution on the aryl group showed significant activity against all strain of bacteria while moderate to no activity was observed in remaining compounds. Whereas the antifungal evaluation showed that all compounds were active againts *C. Albican* and no activity was observed against *C. Prapsilosis*. The cytotoxic findings revealed the non-toxic nature of these compounds as IC_{50} values of majority of the compounds are above 100 µm except for compounds **3f** and **3g**. In addition, these compounds exhibited better antioxidant potential as 100 µm concentration inhibited > 50% reactive oxygen species (ROS) production except compounds **3e, 3f** and **3j**. The compound **3a** proved to be the most potent urease inhibitor showing the highest enzyme % inhibition (93.1%) with IC_{50} value of 8.17 ± 0.24 µM and found more active as compare to standard followed by compound **3e** (92.6%), **3h** (91.6%), **3d** (90.8%), **3b** (90.6%) and **3f** (90.0%) with their respective IC_{50} values. All the synthesized compounds were docked into the binding cavity of Urease (PDB ID: 4ubp). The most active compound 3a was also ranked as top on the docking score as it was found to show valuable interactions with the target protein along with good docking scores. Hence our results revealed that the synthesized compounds have potential to be used as potent urease inhibitors after further detailed mechanistic studies.

1. Introduction

Urease is an enzyme which contains nickel metal as a catalyst for the hydrolysis of urea into ammonia and carbondioxide. Ureases are widespread in nature among invertebrates, plants, algae, fungi and bacteria. Urease producing bacteria have severe effects on human health. In humans the urease producing bacteria *H. pylori* is the main reason of urinary tract and gastrointestinal infections which leads towards the peptic ulcer and stomach cancer. Other urease associated diseases include urolithiasis, hepatic encephalopathy, hepatic coma, urinary catheter encrustation, and pyelonephritis. During colonization, *H. pylori* exists at the low pH in stomach, which is the main reason for the occurrence of gastric and peptic ulcers and, sometimes it may also lead to cancer [1,2], a global problem of today. Since discovery of the

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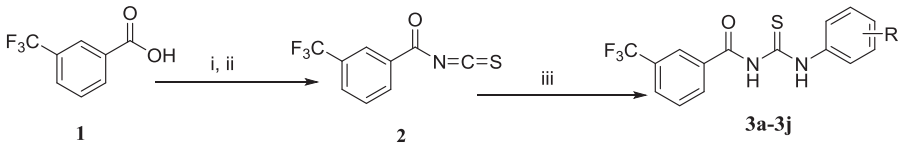


3a. R=2,6-Dichloro **3f.** R=4-Cyano
3b. R=3-Chloro **3g.** R=H
3c. R=2,5-Dichloro **3h.** R=2,4-Dinitro
3d. R=4-Nitro **3i.** R=3,4=Dimethoxy
3e. R=2-Fluoro **3j.** R=3-Bromo

Scheme 1. Synthesis of thiourea derivatives **(3a–j)**, i. $SOCl_2$, 2 h reflux, ii. KSCN, acetone, 35 min stirring, iii. substituted anilines, acetone, 1 h reflux.

plant urease inhibitors such as from *Canavalia ensiformis* (Fabaceae), urease has been comprehensively used as a model to develop new urease inhibitors for both agricultural and clinical use [2,3].

The discovery of new potent urease inhibitors purified from *C. ensiformis* with versatile applications is in part particularly owing to the amino acid sequencing similarities among ureases though isolated from multiple species. These similarities suggest the presence of common ancestor of urease [3]. They have gained significant attention owing to their influential role in medical health pertaining to their efficient antiurease potential in microorganisms [4]. With the advancement of knowledge in the field of biological systems and with improved technology, still there is a dire need for the discovery of new drugs which is though very time consuming, expensive and tedious task. Therefore it is indispensable to not ignore the findings of failure routs at an early stage of drug development process which will naturally be of immense utility [5]. Thioureas and its derivatives are playing their crucial role in the field of medicinal chemistry as well as drug development. Besides their harmful effects, they are not only used as effective phenoloxidase inhibitors but also used as potent herbicides, fungicides, and rodenticides. In the quest of synthesizing potent biologically active moieties, thiourea and it's various compounds have gained chemists' great devotion in terms of their antimicrobial, anticancer as well as anti-inflammatory potential evaluation [6]. Among numerous methods used for the synthesis of thioureas, condensation of primary and secondary amine with isothiocyanate constitutes one of the most widely accepted general methods [7,8].

Keeping in view the synthetic and biological significance of thioureas, we planned the synthesis of meta-triflurомethyl benzoyl thioureas and explored their antimicrobial, antioxidant, cytotoxic and antiurease activities as well as their molecular docking studies.

2. Materials and methods

We purchased 3-(trifluoromethyl)benzoic acid from Alfa Aesar, thionyl chloride from Riedeldehaen (RDH), Germany, potassium thiocyanate from MERCK, acetone from NORMAPUR, substituted anilines, dichloromethane, *n*-hexane and ethyl acetate were purchased from DAE JUNG. Progress of the reactions was monitored through TLC, Merck DC finished foils silica gel 60 F_{254} on aluminum foil and Macherey finished foils Alugram® Sil G/UV254. UV lamp (254 nm and/or 366 nm) was used for the detection of chromatograms. Melting points were recorded on SMP 20 digital Melting point apparatus. BRUKER FT-IR Spectrometer Model Tensor II in Quaid-i-Azam University Islamabad was used to record the fourier transform infrared spectra of the synthesized compounds by using ATR method. For NMR studies Bruker made in Switzerland, NMR spectrometer, AVANCE at 300 MHz in Quaid-i-Azam University Islamabad for their ^{1}H and ^{13}C NMR spectra. DMSO-d_6 was used as the solvent for recording of respective spectras and TMS was used as internal reference. JEOL MS 600H-1 Instrument was used to record the electron ionization mass spectra of the synthesized compounds. Finnigan MAT95 or Varian MAT 311; Bruker FT, CIR, AMD 402 (AMD Intectra). Synergy HT BioTek® USA micro plate reader, digital electronic weighing balance-Precisa Instruments, Switzerland. Sonicator-Elmasonic, Germany, Urease enzyme (Sigma Aldrich), urea and thiourea (Merck), phenol reagent (Pak Land scientific productions), sodium nitro prusside (BDH laboratory supplies Poole, BH 15 ITD, England).

2.1. Experimental

2.1.1. General procedure for the synthesis of thiourea derivatives (3a–j)

Meta-triflurомethyl benzoic acid (1.0 eq) was dissolved in thionylchloride (1.5 eq) with 2 h reflux. The completed reaction produced respective acid chloride which was cooled at room temperature. The freshly prepared acid chloride was poured into the potassium thiocyanate solution in acetone after stirring at room temperature for 35 min. The respective thiourea was obtained by introducing a specific aniline solution to the fresh isothiocyanate solution with subsequent 1 h reflux. The progress of the reaction was monitored through TLC. After the reaction completion and cooling to room temperature, the reaction mixture was poured into the ice-cold water. Precipitates of synthesized thiourea were filtered, dried and purified by recrystallization with dichloromethane [9–11] (see Scheme 1).

2.1.2. Characterization of thiourea derivatives (3a–j)

2.1.2.1. N-(3-triflouromethylphenyl)-N′-(2,6-diclorophenyl) thiourea (3a). Starting with 3-(trifluoromethyl)benzoic acid (500 mg, 2.6 mmol), $SOCl_2$ (460 mg, 3.94 mmol, 0.28 ml), KSCN (252 mg, 2.6 mmol), 2,6-dichloroaniline (421 mg, 2.6 mmol) and acetone (10 ml), **3a** was isolated as yellow crystals (542 mg, 53%). ^{1}H NMR (DMSO-d_6, δ = ppm): 12.24 (s, 1H, CONH), 12.00 (s, 1H, CSNH), 8.38 (s, 1H_{Ar}), 8.29 (d, J = 7.8H_{Ar}), 8.04 (d, J = 7.8H_{Ar}), 7.79 (t, J = 7.8 Hz, 1H_{Ar}), 7.57 (d, J = 7.8 Hz, 2H_{Ar}), 7.40 (t, J = 8.7 Hz, 1H_{Ar}). ^{13}C NMR (DMSO-d_6, δ = ppm): 181.4 (CS), 167.2 (CO), 134.8 (C), 134.1 (2C-Cl), 133.4 (C), 133.3 (C), 130.2 (q, J = 48.1 Hz, CF_3), 130.0 (C), 129.7 (C), 129.3 (C), 129.0 (2C), 126.0 (q, J = 48.1 Hz, CCF_3), 122.4. FT-IR (ATR, cm^{-1}): 3150 (N—H), 2968 (=C—H_{Ar}), 1673 (C=O), 1588 (C=C_{Ar}), 1377 (C—F), 1243 (C=S), 1123 (C—N), 730 (C—Cl). HRMS calculated for $C_{15}H_9Cl_2F_3N_2OS$ $[M+H]^+$: 392.9837; found 392.9837.

2.1.2.2. N-(3-triflouromethylphenyl)-N′-(3-clorophenyl) thiourea (3b). Starting with 3-(trifluoromethyl)benzoic acid (250 mg, 1.3 mmol), $SOCl_2$ (232 mg, 1.95 mmol, 0.141 ml), KSCN (126 mg, 1.3 mmol), 3-chloroaniline (204 mg, 1.3 mmol, 0.169 ml) and acetone (10 ml), **3b** was isolated as yellowish orange powder (324 mg, 69%).^{1}H NMR (DMSO-d_6, δ = ppm): 12.48 (s, 1H, CONH), 12.00 (s, 1H, CSNH), 8.34 (s, 1H_{Ar}), 8.25 (d, J = 7.8 Hz, 1H_{Ar}), 8.03 (d, J = 8.1 Hz, 1H_{Ar}), 7.94 (s, 1H_{Ar}), 7.79 (t, J = 7.8 Hz, 8.1 Hz, 1H_{Ar}), 7.58 (d, J = 8.4 Hz, 1H_{Ar}), 7.46 (t, J = 7.8 Hz, 8.1 Hz, 1H_{Ar}), 7.35 (d, J = 8.1 Hz, 1H_{Ar}). ^{13}C NMR (DMSO-d_6, δ = ppm): 179.8 (CS), 167.2 (CO), 139.9 (C), 133.6 (C), 133.3 (C), 133.2 (C), 130.8 (C), 130.2 (C), 130.0 (q, J = 48.1 Hz, CF_3), 129.7 (C), 129.2 (C), 126.7 (C), 125.9 (q, J = 48.1 Hz, CCF_3), 124.5, 123.6. FT-IR (ATR, cm^{-1}): 3338 (N—H), 2993 (=C—H_{Ar}), 1677 (C=O), 1585 (C=C_{Ar}), 1328 (C—F), 1245 (C=S), 1174 (C—N), 804 (C—Cl). MS (EI, 70 eV); m/z (%) = 358(M+•, 8), 305(2), 189(2), 173(M+, 100),

169(18), 145(48), 125(7), 99(6), 75(8). HRMS (ESI) calculated for $C_{15}H_{10}ClF_3N_2OS$ $[M+H]^+$: 359.0228; found 359.0227.

2.1.2.3. N-(3-triflouromethylphenyl)-N′-(2,5-dicloropheny) thiourea ***(3c)***. Starting with 3-(trifluoromethyl)benzoic acid (250 mg, 1.3 mmol), $SOCl_2$ (232 mg, 1.95 mmol, 0.141 ml), KSCN (126 mg, 1.3 mmol), 2,5-dichloroaniline(210 mg, 1.3 mmol) and acetone (10 ml), **3c** was isolated asa yellow powder (311 mg, 61%). ^{1}H NMR (DMSO-d_6, δ = ppm): 12.66 (s, 1H, CONH), 12.26 (s, 1H, CSNH), 8.35 (s, $1H_{Ar}$), 8.25 (d, J = 10.5 Hz, $1H_{Ar}$), 8.04 (d, J = 7.8 Hz, $1H_{Ar}$), 7.79 (t, J = 7.8 Hz, $1H_{Ar}$), 7.63 (s, $1H_{Ar}$), 7.44 (d, J = 2.4 Hz, $1H_{Ar}$), 7.41 (d, J = 2.4 Hz, $1H_{Ar}$). ^{13}C NMR (DMSO-d_6, δ = ppm): 180.6 (CS), 167.7 (CO), 137.1 (C), 133.4 (C), 133.3 (C), 131.6 (C), 130.3 (C), 130.2 (q, J = 48.1 Hz, CF_3), 129.6 (C), 129.2 (C), 128.4 (C), 127.7 (C), 127.6 (C), 126.1 (q, J = 48.1 Hz, CCF_3), 122.4. FT-IR (ATR, cm^{-1}): 3337 (N−H), 2920 (=C−H_{Ar}), 1682 (C=O), 1575 (C=C_{Ar}), 1322 (C−F), 1241 (C=S), 1172 (C−N), 810 (C−Cl). HRMS (ESI) calculated for $C_{15}H_9Cl_2F_3N_2OS$ $[M+H]^-$: 390.9701; found 390.9692.

2.1.2.4. N-(3-triflouromethylphenyl)-N′-(4-nitrophenyl) thiourea ***(3d)***. Starting with 3-(trifluoromethyl)benzoic acid (250 mg, 1.3 mmol), $SOCl_2$ (232 mg, 1.95 mmol, 0.141 ml), KSCN (126 mg, 1.3mmoles), 4-nitoaniline (176 mg, 1.3 mmol) and acetone (10 ml), **3d** was isolated as a light yellow powder (293 mg, 61%).^{1}H NMR (DMSO-d_6, δ = ppm): 12.72 (s, 1H, CONH), 12.10 (s, 1H, CSNH), 8.34 (s, $1H_{Ar}$), 8.29 (d, J = 9.0 Hz, $1H_{Ar}$), 8.25 (d, J = 7.8, $1H_{Ar}$), 8.06 (t, J = 9.3 Hz, $1H_{Ar}$), 8.04 (dd, J = 8.4 Hz, $2H_{Ar}$), 7.79 (t, J = 7.8 Hz, 8.1 Hz, $2H_{Ar}$). FT-IR (ATR, cm^{-1}): 3302 (N−H), 2946 (=C−H_{Ar}), 1681 (C=O), 1608 (C=C_{Ar}), 1518 (N−O), 1330 (C−F), 1251 (N−O). MS (EI, 70 eV); m/z (%) = 369(M+•, 27), 316(3), 189(6), 180(27), 173(M+, 100), 145(84), 138(11). HRMS (ESI) calculated for $C_{15}H_{10}Cl_2F_3N_3O_3S$ $[M+H]^+$ and $[M-H]^-$: 370.0467; found 370.0467 and 368.0329; found 368.0322.

2.1.2.5. N-(3-triflouromethylphenyl)-N′-(2-fluorophenyl) thiourea ***(3e)***. Starting with 3-(trifluoromethyl)benzoic acid (250 mg, 1.3 mmol), $SOCl_2$ (232 mg, 1.95 mmol, 0.141 ml), KSCN (126 mg, 1.3 mmol), 2-fluoroaniline (144 mg, 1.3 mmol, 0.125 ml) and acetone (10 ml), **3e** was isolated as yellow powder (275 mg, 62%).^{1}H NMR (DMSO-d_6, δ = ppm): 12.45 (s, 1H, CONH), 12.14 (s, 1H, CSNH), 8.32 (s, $1H_{Ar}$), 8.25 (d, J = 8.1 Hz, $1H_{Ar}$), 8.03 (d, J = 7.5, $1H_{Ar}$), 7.78 (t, J = 7.8 Hz, 8.1 Hz, $1H_{Ar}$), 7.38–7.34 (m, $2H_{Ar}$), 7.32–7.24 (m, $2H_{Ar}$). FT-IR (ATR, cm^{-1}): 3233 (N−H), 2930 (=C−H_{Ar}), 1653 (C=O), 1617 (C=C_{Ar}), 1336 (C−F), 1314 (C−F). MS (EI, 70 eV); m/z (%) = 342(M+•, 20), 323(12), 283(62), 189(4), 173(M+,100), 153(40), 145(96), 111(16), 95(17). HRMS (ESI) calculated for $C_{15}H_{10}F_4N_2OS$ $[M+H]^+$ and $[M-H]^-$:343.0526; found 343.0522 and 341.0379; found 341.0377.

2.1.2.6. N-(3-triflouromethylphenyl)-N′-(4-cyanophenyl) thiourea ***(3f)***. Starting with 3-(trifluoromethyl)benzoic acid (250 mg, 1.3 mmol), $SOCl_2$ (232 mg, 1.95 mmol, 0.141 ml), KSCN (126 mg, 1.3 mmol), 4-cyanoaniline (153 mg, 1.3 mmol) and acetone (10 ml), **3f** was isolated as yellow powder (270 mg, 60%).^{1}H NMR (DMSO-d_6, δ = ppm): 12.64 (s, 1H, CONH), 12.08 (s, 1H, CSNH), 8.34 (s, $1H_{Ar}$), 8.25 (d, J = 7.8 Hz, $1H_{Ar}$), 8.04 (d, J = 7.8 Hz, $1H_{Ar}$), 7.99 (d, J = 8.7 Hz, $2H_{Ar}$), 7.90 (d, J = 8.7 Hz, $1H_{Ar}$), 7.80 (t, J = 7.8 Hz, $1H_{Ar}$). ^{13}C NMR (DMSO-d_6, δ = ppm): 179.6 (CS), 167.1 (CO), 142.6 (2C), 133.6 (C), 133.4 (2C), 130.2 (C), 130.0 (q, J = 48.1 Hz, CF_3), 129.6 (C), 129.2 (C), 126.0 (q, J = 48.1 Hz, CCF_3), 124.8 (2C), 122.4 (C), 119.1 (C). FT-IR (ATR, cm^{-1}): 3209 (N−H), 3028 (=C−H_{Ar}), 2229 (C≡N), 1662 (C=O), 1319 (C−F). MS (EI, 70 eV); m/z (%) = 349(M+•, 74), 330(3), 290(4), 189(13), 173(M+, 100), 160(98), 145(92), 118(30), 102(16). HRMS (ESI) calculated for $C_{16}H_{10}F_3N_3OS$ $[M+H]^+$: 350.0569; found 350.0569.

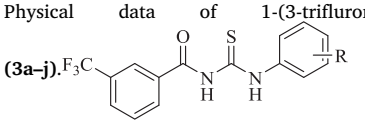
2.1.2.7. N-(3-triflouromethylphenyl)-N′-phenyl thiourea ***(3g)***. Starting with 3-(trifluoromethyl)benzoic acid 1(250 mg, 1.3 mmol), $SOCl_2$ (232 mg, 1.95 mmol, 0.141 ml), KSCN (126 mg, 1.3 mmol), aniline (121 mg, 1.3 mmol, 0.118 ml) and acetone (10 ml), **3 g** was isolated as yellow powder (290 mg, 69%). ^{1}H NMR (DMSO-d_6, δ = ppm): 12.49 (s, 1H, CONH), 11.95 (s, 1H, CSNH), 8.33 (s, $1H_{Ar}$), 8.24 (d, J = 7.8 Hz, $1H_{Ar}$), 8.02 (d, J = 7.5 Hz, $1H_{Ar}$), 7.85 (t, J = 7.8 Hz, $1H_{Ar}$), 7.69 (d, J = 7.5 Hz, $2H_{Ar}$), 7.44 (t, J = 7.5 Hz, 8.1 Hz, $2H_{Ar}$), 7.28 (t, J = 7.2 Hz, 7.5 Hz, $1H_{Ar}$). ^{13}C NMR (DMSO-d_6, δ = ppm): 179.4 (CS), 167.4 (CO), 138.4 (C), 133.7 (C), 133.3 (C), 130.1 (C), 129.9 (q, J = 48.1 Hz, CF_3), 129.6 (C), 129.1 (2C), 126.8 (C), 125.9 (q, J = 48.1 Hz, CCF_3), 124.8 (2C), 122.4 (C). FT-IR (ATR, cm-1): 3311 (N−H), 3015 (=C−H_{Ar}), 1681 (C=O), 1330 (C−F). HRMS (ESI) calculated for $C_{15}H_{11}F_3N_2OS$ $[M+H]^+$: 325.0616; found 325.0616.

2.1.2.8. N-(3-triflouromethylphenyl)-N′-(2,5-dinitrophenyl) thiourea ***(3h)***. Starting with 3-(trifluoromethyl)benzoic acid 1(250 mg, 1.3 mmol), $SOCl_2$ (232 mg, 1.95 mmol, 0.141 ml), KSCN (126 mg, 1.3 mmol), 2,4-dinitroaniline (238 mg, 1.3 mmol) and acetone (10 ml), **3 h** was isolated as light yellow powder (385 mg, 72%).^{1}H NMR (DMSO-d_6, δ = ppm): 12.66 (s, 1H, CONH), 12.27 (s, 1H, CSNH), 8.32 (s, $1H_{Ar}$), 8.28 (d, J = 7.2 Hz, $1H_{Ar}$), 8.01 (d, J = 8.4 Hz, $1H_{Ar}$), 7.81 (t, J = 6.9 Hz, 7.5 Hz, $1H_{Ar}$), 7.74 (d, J = 2.4 Hz, $2H_{Ar}$), 7.62 (d, J = 8.7 Hz, $1H_{Ar}$), 7.45–7.39 (m, $1H_{Ar}$). ^{13}C NMR (DMSO-d_6, δ = ppm): 180.6 (CS), 167.7 (CO), 137.1 (C), 136.5 (C), 135.0 (C), 130.1 (C), 129.9 (q, J = 48.1 Hz, CF_3), 129.6 (C), 129.1 (2C), 126.8 (C), 125.9 (q, J = 48.1 Hz, CCF_3), 124.8 (2C), 122.4 (C). FT-IR (ATR, cm^{-1}): 3278 (N−H), 2916 (=C−H_{Ar}), 1649 (C=O), 1577 (NO_2), 1335 (C−F), 1299 (NO_2). HRMS (ESI) calculated for $C_{15}H_9F_3N_4O_5S$ $[M]^+$ not possible.

2.1.2.9. N-(3-triflouromethylphenyl)-N′-(3,4-dimethoxyphenyl) thiourea ***(3i)***. Starting with 3-(trifluoromethyl)benzoic acid 1(200 mg, 1.05 mmol), $SOCl_2$ (187 mg, 1.57 mmol, 0.114 ml), KSCN (102 mg, 1.05 mmol), 3,4-dimethoxyaniline (80 mg, 1.3 mmol) and acetone (10 ml), **3i** was isolated as yellow powder (293 mg, 73%).^{1}H NMR (DMSO-d_6, δ = ppm): 12.41 (s, 1H, CONH), 11.90 (s, 1H, CSNH), 8.33 (s, $1H_{Ar}$), 8.23 (d, J = 7.8 Hz, $1H_{Ar}$), 8.02 (d, J = 7.8 Hz, $1H_{Ar}$), 7.78 (t, J = 7.8 Hz, $1H_{Ar}$), 7.38 (d, J = 2.4 Hz, $1H_{Ar}$), 7.20 (dd, J = 2.25 Hz, 8.55 Hz, $1H_{Ar}$), 6.99 (d, J = 8.7 Hz, $1H_{Ar}$), 3.78 (s, 3H, $OCH_{3'}$), 3.76 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, δ = ppm): 179.0 (CS), 167.3 (CO), 148.8 (C), 147.5 (C), 133.7 (C), 133.2 (C), 131.3 (C), 130.1 (C), 129.7 (q, J = 48.1 Hz, CF_3), 129.2 (C), 126.0 (q, J = 48.1 Hz, CCF_3), 116.9 (C), 113.2 (C), 111.8 (C), 109.2 (C), 56.1 ($OCH_{3'}$), 56.0 (OCH_3). FT-IR (ATR, cm^{-1}): 3182 (N−H), 2844 (C−H), 3013 (=C−H_{Ar}), 1674 (C=O), 1326 (C−F), 1150 (C−O). HRMS (ESI) calculated for $C_{17}H_{15}F_3N_2O_3S$ $[M+H]^+$ and $[M-H]^-$: 385.0826; found 385.0828 and 383.0689; found 383.0682.

2.1.2.10. N-(3-triflouromethylphenyl)-N′-(3-bromophenyl) thiourea ***(3j)***. Starting with 3-(trifluoromethyl)benzoic acid 1 (250 mg, 1.3 mmol), $SOCl_2$ (232 mg, 1.95 mmol, 0.0141 ml), KSCN (126 mg, 1.3 mmol), 3-bromoaniline (210 mg, 1.3 mmol) and acetone (10 ml), **3j** was isolated as light yellow powder (201 mg, 58%).^{1}H NMR (DMSO-d_6, δ = ppm): 12.49 (s, 1H, CONH), 12.16 (s, 1H, CSNH), 8.37 (s, $1H_{Ar}$), 8.27 (d, J = 8.1 Hz, $1H_{Ar}$), 8.04 (d, J = 7.8 Hz, $1H_{Ar}$), 7.91 (dd, J = 1.2 Hz, 8.1 Hz, $1H_{Ar}$), 7.79 (s, $1H_{Ar}$), 7.75 (dd, J = 0.9 Hz, 9.0 Hz, $1H_{Ar}$), 7.48 (dt, J = 1.2 Hz, 7.8 Hz, $1H_{Ar}$), 7.29 (dt, J = 1.5 Hz, 7.8 Hz, $1H_{Ar}$). ^{13}C NMR (DMSO-d_6, δ = ppm): 180.7 (CS), 167.6 (CO), 137.3 (C), 133.4 (C), 133.4 (C), 133.1 (C), 130.3 (C), 130.2 (C), 130.1 (q, J = 48.1 Hz, CF_3), 129.7 (C), 129.2 (C), 128.3 (C), 126.1 (q, J = 48.1 Hz, CFF_3), 122.4 (C), 119.8 (C). FT-IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3241 (N−H), 3051 (=C−H_{Ar}), 1688 (C=O), 1527, 606 (C−F). HRMS (ESI) calculated for $C_{15}H_{10}BrF_3N_2OS$ $[M-H]^-$: 400.9581; found 400.9576.

Table 1
Physical data of 1-(3-trifluromethyl benzoyl)-3-arylthioureas **(3a–j)**.



Sr. No.	R	Color	M.P °C	[a]R_f	Yield (%)
3a.	2,6-Dichloro	Yellow crystal	170–172	0.51	67
3b.	3-Chloro	Yellowish orange	120–122	0.53	75
3c.	2,5-Dichloro	Yellowish	141–143	0.57	61
3d.	4-Nitro	Light yellow	153–155	0.71	71
3e.	2-Fluoro	Yellow powder	107–108	0.63	65
3f.	4-Cyano	Yellow powder	171–173	0.70	67
3g.	H	Yellow powder	173–174	0.54	69
3h.	2,4-Dinitro	Light yellow	142–144	0.52	72
3i.	3,4-Dimethoxy	Yellow powder	101–102	0.46	83
3j.	3-Br	Light yellow powder	185–187	0.54	61

[a] Pet.ether:Ethylacetate, 7:3.

2.2. Antibacterial potential

Disc diffusion method was followed to assess the antibacterial activity of synthesized compounds **(3a–j)** by using test samples (4 mg/mL in DMSO), nutrient broth (Sigma Aldrich, USA), DMSO cefixime (4 mg/mL in DMSO) (Standard antibiotic) and four strains of bacteria *Pesudomonas aerouginosa* (ATTC# 9027), *E. Coli*, *S. Aureus* (ATTC# 6538) and MRSA 10 were used. The plates were incubated for 24 h at 37 °C. Antibacterial activity was measured as the zone of inhibition in mm. The experiment was carried out in triplicates [12].

2.3. Antifungal potential

The antifungal potential of each synthesized compound was determined by 'Disc Diffusion Method'. Test samples (4 mg/mL in DMSO), SDA (Sabouraud Dextrose Agar, DMSO, standard drug fluconazole (4 mg/mL in DMSO), laminar flow hood, incubator, petri plate, blank discs and two fungal strains *C. albicans* (ATCC No. 9002) and C. parapsilosis (ATCC #22019) were used. The plates were incubated for 48 h at 28 °C. Antifungal effect was measured as the zone of inhibition in mm. The experiment was carried out in triplicates [12].

2.4. Oxidative burst assay

Oxidative burst assay was determined of the synthetic compounds by using Luminol-enhanced chemiluminescence assay after few modifications [13]. Briefly 25 µL of diluted whole blood was added in Hanks balanced salt solution supplemented with calcium and magnesium chlorides [$HBSS^{++}$; Sigma, St. Louis, USA] then 25 µL of compounds was added to be tested (250 µg/mL) in a 96 well plates [Costar, NY, USA]. In the thermostat chamber of luminometer [Labsystems, Helsinki, Finland] plate was incubated at 37 °C for 15 min. Each well, after incubation was given 25 µL of serum opsonized zymosan (SOZ) [Fluka, Buchs, Switzerland] and luminol [Research Organics, Cleveland, OH, USA] respectively. In luminometer, the level of the ROS was recorded in term of relative light units (RLU).

2.5. Anticancer/cytotoxic evaluation

The cytotoxicity of tested compounds was evaluated by determining cell viability using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. NCI-H460 cells and NIH 3 T3 cells were seeded in a 96 well plate [Costar, NY, USA] at a density of 10,000 and 15,000 cells per well respectively. After 24 h of incubation, cells were treated with compounds at different concentrations (1.95–250 µM). After 48 h of incubation cell viability was determined as described previously [14]. Briefly, 10 µL MTT (Biobasic, Canda) dye was added in each well, followed by 4 h of incubation and DMSO was used to solubilize the formazan crystals and absorbance was measured at 570 nm. The IC_{50} of compounds at concentrations was calculated for both normal and cancer cell lines.

2.6. Antiurease potential

Anti-urease assay was executed according to previously reported protocol with few modifications. First of all 5 µL of sample compound and 25 µL (0.25 mg/mL) of enzyme were incubated at 37 °C for 15 min with the subsequent addition of 55 µL of substrate (urea) and re-incubation under similar conditions. Absorbance was measured at 630 nm after incubation and was taken as pre read. Then phenol (45 µL) and alkali reagent (70 µL) were added into the mixture to initiate the reaction followed by 50 min incubation. Absorbance at 630 nm taken as after read was measured after incubation. Thiourea served as positive control (urease inhibitor) with methanol as a blank control. The %inhibition of urease was calculated through following formula:

$$Inhibition\ \ (\%) = 100 - (A_c/A_f) \times 100$$

Serial dilutions were done for the calculation of IC_{50} values. The results were computed by using EZ-fit enzyme kinetics software [12].

3. Results and discussion

3.1. Chemistry

The characterization of the synthesized compounds was carried out by FT-IR, ^1H & ^{13}C NMR, EI-MS and HRMS spectroscopic techniques. The physical data of all compounds **(3a–j)** is listed in Table 1.

The FT-IR spectra of the respective thiourea derivative confirmed the synthesis by the appearance of absorption bands for NH (secondary amine) in the region of 3338–3150 cm^{-1}. The presence of a strong band ranging from 3028 to 2918 cm^{-1} confirm the presesnce of =C—H bond. Whereas the cabonyl strong absorption band appeared at a range of 1688–1649 cm^{-1} in all compounds. The other significant absorption bands for C=C_{Ar}, C—F, C=S and C—N appeared ranging from 1538 to 1508, 1333to 1243, 1262 to 1169 and 1154 to 1109 cm^{-1} respectively. The structure of the particular *N*-(3-triflourophenyl)-*N′*-arylthioureas (1–10) were further confirmed by ^1H NMR data by the appearance of characteristic signals a very down field one proton singlet in the range of 12.24–12.72 ppm for CONH and another characteristic down field singlet in the rage of 11.90–12.97 ppm for CSNH. While the signals for the rest of protons were accordingly. In the ^{13}C NMR characteristic signals for C=S group were found in the range of 179.0–181.4 while signal for C=O were found in the range of 167.1–167.9 confirmed the structure of *N*-aroyl-*N′*-aryl thiourea structure [15,16]. The structure of **3a–j** were confirmed by the HRMS and EIMS mass spectrometric data which were in accordance.

3.2. Antimicrobial activities

Various thiourea derivatives showed significant antimicrobial potential against resistant and ATCC strains [17]. Therefore, all the synthesized compounds were subjected to antimicrobial activities i.e antibacterial and antifungal activity to test their significance in the field of medicines. Antibacterial activities were carried out by using disc diffusion method where 4 mg of samples dissolved in DMSO were tested against *P. aerouginosa*, *E. coli*, *S. aureus* and MRSA-10 strains of bacteria while cefixime was used as a standard drug. Compounds **3a**, **3c** and **3i** with dichloro and methoxy groups substitution of the aryl group showed significant activity against all strain of bacteria (Table 2) while rest of the compounds showed moderate to no activity. These findings are correlated with previous reports that halogen and methoxy substituted thiourea derivatives showed significant activities against bacterial and fungal strains [18]. Kaswala and coworkers reported that

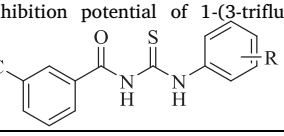
Table 2
Antimicrobial activities of 1-(3-triflurometyl benzoyl)-3-arylthioureas **(3a–j).**

Compounds	Bacterial strain (Zone of inhibition mm)				Antifungal activity ZOI (mm)	
	Pesudomonas aerouginosa	*E.coli*	*S. aureus*	*MRSA-10*	*C.albican*	*C.prapsilosis*
3a	18.1	9.1	19.1	9.1	17.1	–
3b	9.1	–	–	–	14.1	–
3c	14.1	–	13.1	11.1	19.1	–
3d	10.1	–	–	–	20.1	–
3e	13.1	–	–	10.1	16.1	
3f	–	9.2	–	–	14.1	
3g	–	–	–	–	20.2	–
3h	–	–	–	–	16	
3i	20.1	10.1	23.1	–	19.0	–
3j	9.2	–	–	11.1	16.1	–
Cefixime	23.2	40.2	30.0	15.6	42.1	–
DMSO	0.0	0.0	0.0	0.0	0.0	

Table 3
Anticancer/cytotoxicity activity & oxidative burst assay.

Compounds	NIH-3T3 IC_{50} ± S.D	NCI-H460 IC_{50} ± S.D	Oxidation (% Inhibition)
3a	> 250	> 250	81.7 ± 10.4
3b	> 250	> 250	87.3 ± 11.2
3c	> 250	> 250	90.5 ± 9.5
3d	> 250	> 250	85.7 ± 12.5
3e	> 250	> 250	Inactive
3f	54.83 ± 2.36	> 250	Inactive
3g	33.31 ± 2.22	> 250	67.5 ± 3.9
3h	93.01 ± 2.93	> 250	89.9 ± 9.1
3i	146.57 ± 0.77	> 250	82.7 ± 0.7
3j	168.26 ± 4.25	> 250	44.4 ± 3.6
Standard Drug Cisplatin	> 100	19.05 ± 0.45	–

Table 4
Urease inhibition potential of 1-(3-triflurometyl benzoyl)-3-arylthioureas **(3a–j)**.



Compounds	R	% Inhibition	IC_{50} ± SEM[a] [μM]
3a	2,6-dichloro	93.1	8.17 ± 0.24
3b	3-Chloro	90.6	10.90 ± 1.31
3c	2,5-dichloro	77.6	21.60 ± 0.27
3d	4-nitro	90.8	9.82 ± 0.22
3e	2-fluoro	92.6	9.19 ± 0.78
3f	4-cyano	90.0	13.90 ± 0.57
3g	H	91.6	17.90 ± 0.48
3h	2,4-dinitro	87.9	11.69 ± 0.38
3i	3,4-dimethoxy	72.8	13.28 ± 0.39
3j	3-Br	86.2	14.71 ± 0.58
STD	Thiourea	98.0	21 ± 0.28

[a] SEM; the standard error of the mean.

methoxy and chloro substituted thiourea showed potent effects against *S. aureus*. In current study dichloro substituted compounds **(3a, 3c)** showed promising activities against *S. aureus, P. aeruginosa* and *C. albicans*. These findings are correlated with another report by Stefenska and colleagues reported that antimicrobial screening of synthesized compounds showed substantial antibacterial activity. They observed that compounds having electron withdrawing groups especially chlorine revealed promising antibacterial potential [19]. It is also reported that methoxy group could be important for binding of molecules to the target but, has less significant role in activity as compared to halogens. Therefore, halogen substituted compounds as compared to methoxy groups found more active against various bacterial and fungal

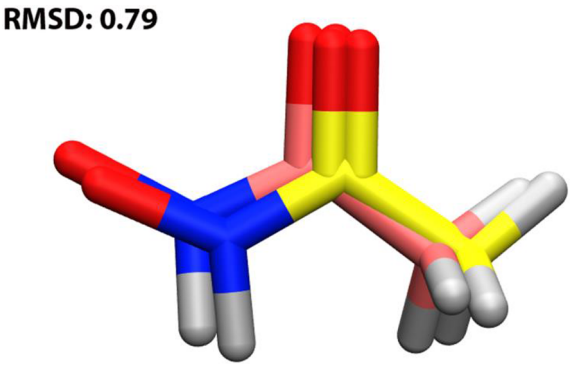


Fig. 1. Superposition of co-crystalized ligand and docked conformation, orange represent the co-crystalized ligand and the yellow the re-docked conformation of the ligand.

strains [20]. Soni et al., reported that chloro substituted compounds showed more than 80% bacterial inhibition as compared to methoxy substituted aryl thiourea derivatives [21].

Antifungal activities were performed using disc diffusion method against two fungal strain *C. albican* and *C. prapsilosis*. All the compounds showed activity against *C. albican* while no activity was observed against *C. prapsilosis* strain of fungi (Table 2).

3.3. Anticancer/cytotoxic and oxidative burst assay potential

All synthesized compounds were evaluated for their cytotoxic and antioxidant activity in cellular assays. Here Table 3 contains the summary of anticancer/cytotoxic results.

The synthesized compounds are found non-toxic as IC_{50} values of majority of the compounds are above 100 μm except for compounds 3f and 3g which contain electron withdrawing CN **(3f)** and H **(3g)** substitution at C-4 on one of the phenyl ring of thiourea bridge. The compound 3f with IC_{50} value 54.83 ± 2.36 and 3g with IC_{50} value 33.31 ± 2.22 were found significantly toxic on NIH-3T3 cells. While none of the compounds was found toxic against NCI-H460 cells. In addition these compounds possess **(3a–j)** better antioxidant potential as 100 μM concentration inhibited > 50% reactive oxygen species (ROS) production except compounds **3e**, **3f**, and **3j**. It could be related to the presence of electron donating substituents 2-F **(3e)** and 3-Br **(3j)** and electron withdrawing group 4-CN **(3f)** on one of the phenyl ring of thiourea bridge. Thus the nature and position of substituents of synthesized compounds play a crucial role in determining the extent of their activity.

Table 5
Docking scores and interactions detail of 1-(3-trifluoromethyl benzoyl)-3-arylthioureas **(3a–j)**.

Compound	Docking Score	Interaction Report							
		Ligand		Receptor			Interaction	Distance	E (kcal/mol)
3a	−11.0572	N	10	O	CYS	322	H-donor	2.43	−0.5
		O	11	CA	ALA	166	H-acceptor	3.09	−0.7
		F	24	NH2	ARG	339	H-acceptor	3.07	−0.9
		C	3	5-ring HIS	323	H-pi	4.66	−0.3	
3b	−10.08797	S	12	SG	CYS	322	H-donor	3.79	−0.7
		O	11	CE1	HIS	324	H-acceptor	2.80	−0.5
		S	12	CB	ALA	366	H-acceptor	3.32	−0.8
3c	−8.6077	S	12	CE	LYS	169	H-acceptor	3.80	−1.7
		F	24	NH2	ARG	339	H-acceptor	2.87	−1.1
3d	−10.5906	C	17	OD2	ASP	363	H-donor	3.21	−2.1
		O	21	CA	GLY	280	H-acceptor	3.15	−0.8
		F	24	NZ	LYS	169	H-acceptor	2.29	0.1
3e	−10.8393	N	10	O	CYS	322	H-donor	2.65	−4.1
		S	12	CE	LYS	169	H-acceptor	3.65	−0.8
		F	23	NH2	ARG	339	H-acceptor	2.50	−0.9
3f	−9.2167	N	10	O	CYS	322	H-donor	2.76	−4.2
		S	12	CE	LYS	169	H-acceptor	3.11	−1.4
3g	−8.7167	S	12	NZ	LYS	169	H-acceptor	4.18	−1.3
3h	−10.2678	C	4	OD1	ASP	224	H-donor	2.81	−2.3
		O	28	CB	LYS	169	H-acceptor	2.94	−0.8
		O	28	CE	LYS	169	H-acceptor	3.22	−0.9
3i	−9.5419	S	12	CE	LYS	169	H-acceptor	3.66	−2.0
		O	23	CA	ALA	366	H-acceptor	2.87	−0.8
3j	−8.9071	N	8	SG	CYS	322	H-donor	3.20	−2.4
		S	12	CA	ALA	170	H-acceptor	3.36	−1.1

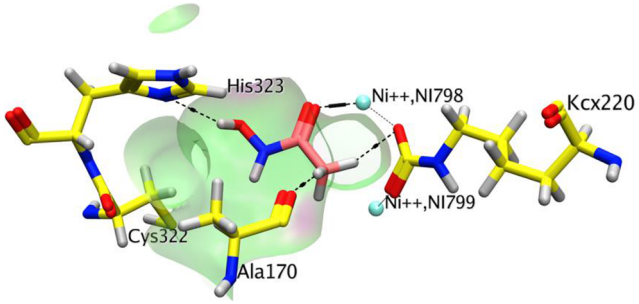


Fig. 2. Three dimensional Interaction of standard compound (thiourea) in the active site of urease.

3.4. Urease inhibition potential

Anti-urease assay evaluation was arried out following the previously reported method [22,23] with few modifications. All ten compounds **3a–j** were tested for their urease inhibition potential. The IC_{50} values obtained were found ranging from 8.17 ± 0.24 to 21.60 ± 0.27 μM. The results showed that all compounds exhibited significant urease inhibition (Table 4).

The compound 3a proved to be the most potent urease inhibitor showing the highest enzyme % inhibition (93.1%) with IC_{50} value of 8.17 ± 0.24 μM and found superior to the thiourea (standard inhibitor) followed by 3e (92.6%), 3 h (91.6%), 3d (90.8%), 3b (90.6%) and 3f (90.0%) with IC_{50} values of 9.19 ± 0.78, 17.90 ± 0.48, 10.90 ± 1.31, 9.82 ± 0.22 and 13.90 ± 0.57 μM respectively. In this experiment thiourea itself was used as a reference standard urease inhibitor with IC_{50} value of 21 ± 0.28 μM. While rest of the compounds also showed good urease inhibitory activity. Our findings were appraisable and significantly correlated with previous reports that thiourea showed urease inhibition activity [23,24].

An essential receptor moiety in thiourea is considered as a core pharmacophore group whereas the key sites for receptor ability are considered to be the two different N—H groups. *N*-(3-triflourophenyl)-*N′*-(2,6-diclorophenyl) thiourea **3a** bearing two chlorine substituents at 2 and 6 positions was found to posses significantly enhanced urease inhibition. The plausable explanation of this high activity is found to be related with the electron withdrawing nature of chlorine atom which influences the inhibition activity against urease enzyme.

N-(3-Triflourophenyl)-*N′*-(2-fluorophenyl) thiourea **3e** compared to *N*-(3-Triflourophenyl)-*N′*-phenyl thiourea 3h and *N*-(3-Triflourophenyl)-*N′*-(4-nitrophenyl)thiourea **3d** exhibited second highest urease inhibition activity among the all synthesized compounds probably due to the presence of highly electronegative atom, fluorine at otho position of the thiourea bridge which plays a crucial role in enhencement of UI potential compared to simple phenyl and the electron withdrawing nitro group at para position. The obtained results showed the overall inhibiting power decreases from 2,6-DiCl**(3a)** > 2-F **(3e)** > phenyl**(3 g)** > 4-NO_2**(3d)** > 3-Cl**(3b)** > 4-CN**(3f)** > 2,5-diNO_2**(3 h)** > 3-Br**(3j)** > 3,6-diCl**(3c)** > 3,4-diOCH_3**(3i)** analogs.

The current study demonstrate that the substitution pattern and functional group nature attached to the phenyl ring to the thiourea bridge plays a vital role on the urease inhibitory potential of thiourea class of compounds. All thiourea derivatives were found significantly active urease inhibitors ranging from 8.17 ± 0.24 to 21.60 ± 0.27 μM probably due to the presence of 3-CF_3 group at the core phenyl group of the thiourea.

4. Molecular docking studies

This study was expanded to obtain more information about the valuable interactions of the active compounds with the target protein of urease through the software package MOE (*Molecular Operating Environment*) [25].

4.1. Preparation of receptor protein

Protein molecule, Urease (PDB id code 4ubp) was obtained from Protein Data Bank. Solvent was removed followed by 3D protonation of the protein molecule. Energy minimization algorithm of MOE tool was used to minimize the energy of the protein molecule. This minimized structure was used in docking procedure as receptor molecule.

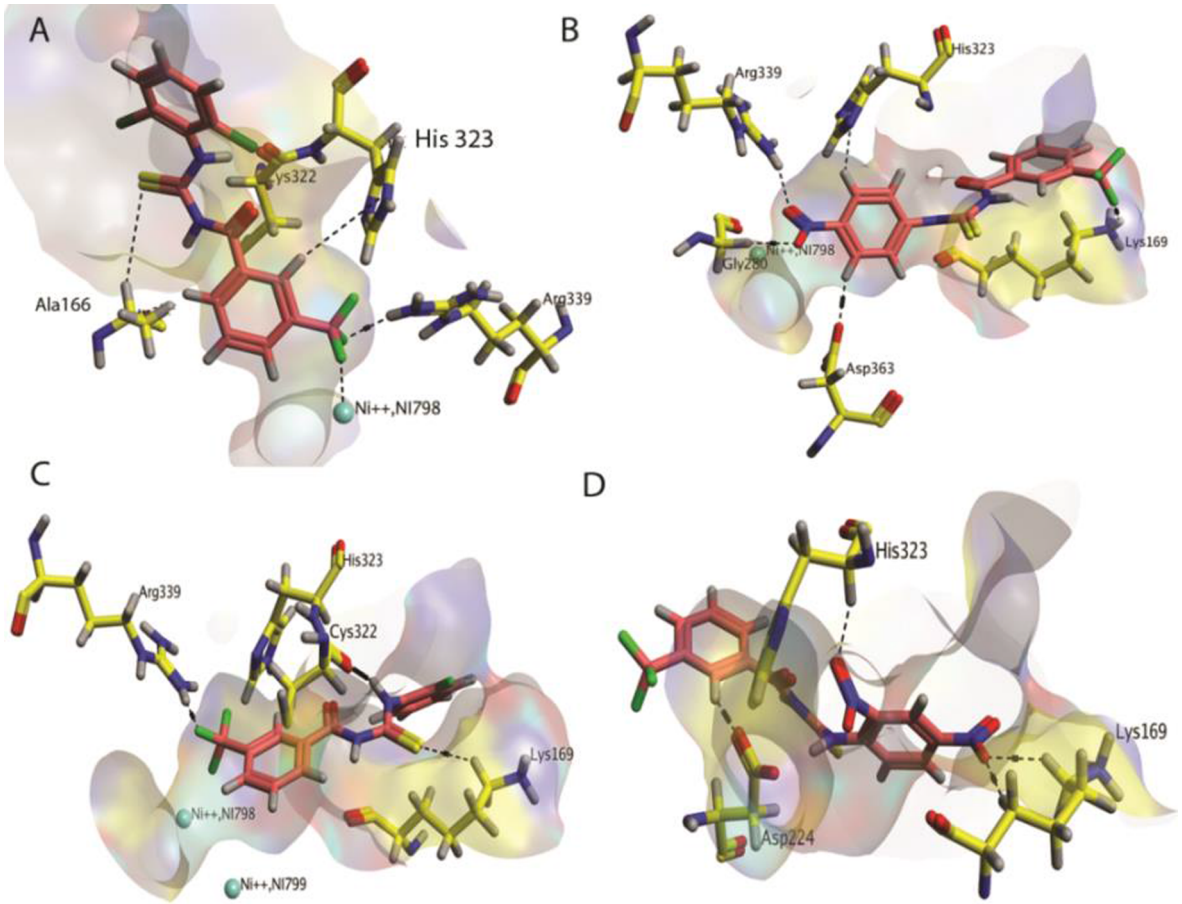


Fig. 3. Three dimensional Interaction of compounds **3a**, **3d**, **3e** and **3h** with active site residues of Urease. Ligands are shown in orange color (stick model), key residues of the active site are shown in yellow stick model, Nickle ions are shown as cyan dots, Hydrogen bonding and other interaction is shown in dark color dotted lines. (A) 3D interaction mode of compound 3a with the active site residues His323, Cys322, Arg339, Ala166 and Ni ion. (B) 3D interactions network of compound 3d with the key residues His323, Lys169, Asp 363, Gly280 and Arg339 (C) 3D bonding mode of compound 3e with Arg339, Cys322 and Lys169 (D) Hydrogen bonding and polar interaction of compound 3h with His323, Lys169 and Asp224.

4.2. Preparation of ligands

To study the binding modes of the synthesized compounds given in Table 1, all ligands were docked into the binding site of the target enzyme Urease. The generation of 3D structures of the synthesized compounds was carried out through the builder tool implemented in MOE followed by their 3D protonation. Whereas energy minimization was calculated using the default parameters of the MOE (gradient: 0.05, Force Field: MMFF94X).

4.3. Molecular docking method

For docking studies, the default parameters of MOE were used i.e., Placement: Triangle Matcher, Re-scoring 1: London dG, Refinement: Forcefield, Rescoring 2: GBVI/WSA. The top ranked conformations on the basis of docking score were selected for further analysis after allowing formation of 10 conformations for each ligand. In order to validate the docking protocol, the 3D structure of the urease enzyme in complex with acetohydroxamic acid was retrieved from the protein data bank (PDB ID 4UBP) and re-docked in the active site of the enzyme. The ligand binding site for docking was defined as a collection of amino acids enclosed within a sphere of 4.5 Å radius around the coordinates of the ligand, which is the inhibitor molecule present in the binding pocket of urease. The top ranked conformations of the ligands were saved in a separate database for further evaluation. The reliability of the docking protocol was confirmed by 0.79 Å RMSD from docked and co-crystallized ligand [26]. Fig. 1 showed the superposition of the docked and co-crystallized ligands.

The results of the correlation between the docking scores and the experimental values are given in Table 5.

4.4. Protein and ligands interaction details

The already validated docking protocol was used to dock these newly synthesized compounds. Furthermore, the standard thiourea was also docked in the active site of urease to check the fitting of the standard compound (Fig. 2).

All the synthesized thiourea derivatives **(3a–j)** were docked into the binding cavity of Urease (PDB ID: 4ubp) was carried out. Based on the IC_{50} values, the top active compounds along with the intermediate compounds were obtained to express ligands interactions with the target proteins along with good docking scores. On the basis of docking score the most active compound 3a was also ranked as top. The compound 3a was found deeply bound into the urease binding cavity making interactions valuable with both Nickel ion present in the binding cavity and the residues Arg339, His323, Cys322, Ala166 (Fig. 3A). Whereas H-pi interaction of the basic His323 and the trifluorophenyl group of the compounds was also noted. Arg339 and Ala166 were found in hydrogen acceptor interaction with the trifluorophenyl and thiourea groups respectively. A hydrogen donor interaction was also predicted in the generated ligand interaction report between Cys322 and the carbonyl oxygen of the compound. Fluorine of trifluorophenyl group also showed a metal-ion interaction with the Ni ion of the binding cavity. The important feature observed in the

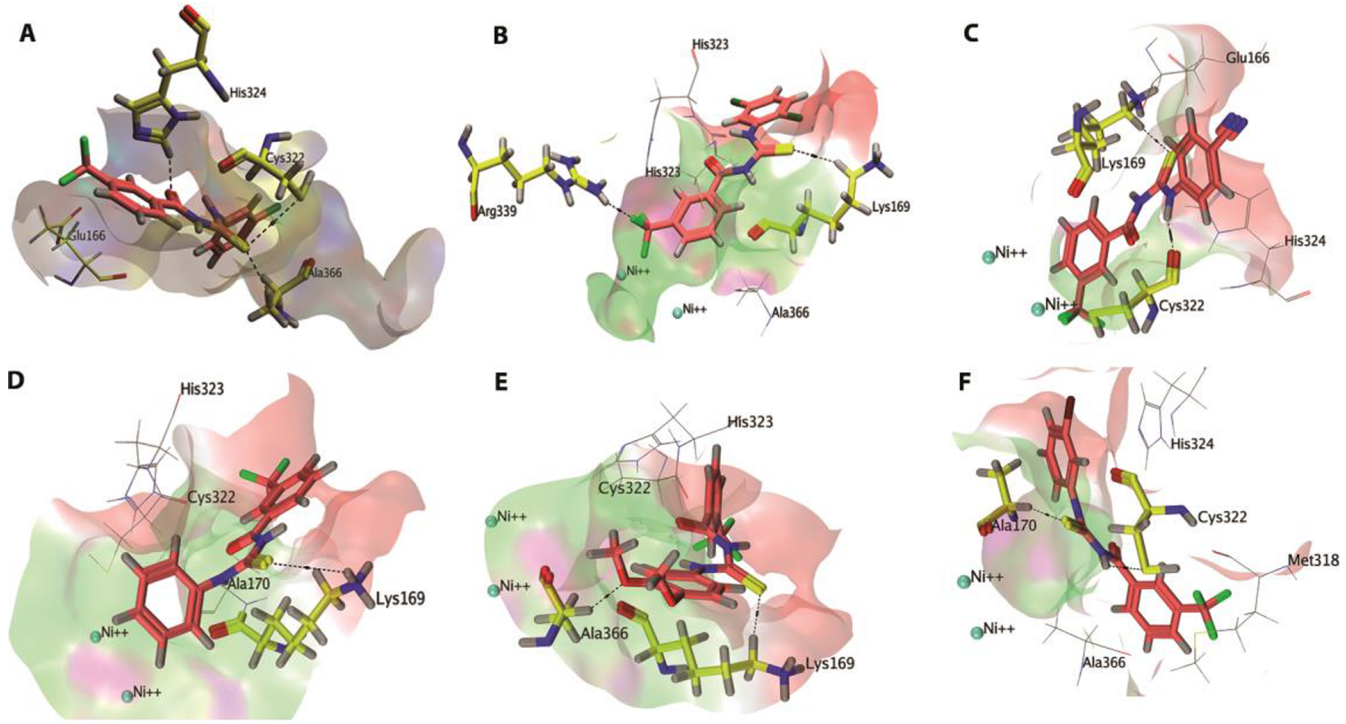


Fig. 4. Three dimensional Interaction of compounds (A) 3b, (B) 3c, (C) 3f, (D) 3g, (E) 3i and (F) 3j with active site residues of Urease.

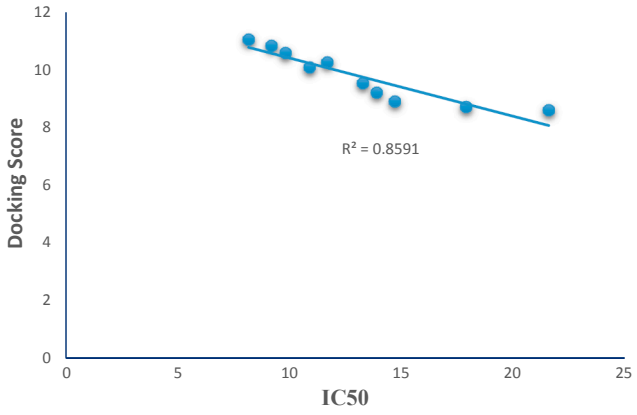


Fig. 5. The correlation graph between the docking scores along with experimental results (IC_{50}).

structure of this ligand regarding interaction was the trifluorophenyl group and thiourea (Fig. 3).

Among the other active compounds, compound 3d was found forming five interactions with the residues His323, Lys169, Gly280, Asp363 and Arg339 as shown in Fig. 3B. His323 was observed in pi-H interaction with the nitrobenzene part of the compound (in binding model only) whereas Lys169 showed the backbone hydrogen acceptor interaction with the fluorine of trifluorophenyl group. Similar interactions were observed for Gly280 and Arg339 (in binding mode only) with the oxygen atoms of the nitrophenyl group. A hydrogen donor interaction was also observed between Asp363 and the nitrophenyl moiety. The presence of nitro group instead of Cl compared with 3a in binding with the receptor protein showed the non-involvement of thiourea moiety.

Compound 3e is found as the second most active compound with three significant interactions with the residues Arg339, Cys322 and Lys169 (Fig. 3C). Two hydrogen acceptor interactions were found for the Arg339 and Lys169 with triflourophenyl and thiourea groups of the ligand respectively. The NH of the thiourea moiety of compound 3e made a hydrogen donor interaction with Cys322 of the binding cavity. The fluorophenyl group inserted here in compound **3e** presented inert behavior to be involved in the binding directly. Being the second most active compound, the lower number of interactions shown by compound **3e** as compared to compound **3d** were unexpected, however, the higher bonding energies and good docking score (Table 5) calculated for this compound may support the overall stability of the possible predicted complex and hence its activity.

Compound 3h with 2,5-dinitrophenyl substitution showed three interactions with the target protein (Fig. 3D). His323 (in binding mode only) and Lys169 showed hydrogen acceptor interactions with the oxygen of dinitrophenyl moiety whereas Asp224 formed hydrogen donor interaction with the triflourophenyl substituent group. Furthermore, the binding modes of compounds **3b**, **3c**, **3f**, **3g**, **3i** and **3j** are shown in Fig. 4.

The molecular docking study of these compounds showed the fact that the nucleophilic, light and polar substituents like CF_3, NO_2, NH, C═O and C═S in these synthesized ligands showed better interaction modes with target protein and therefore have good inhibitory activities. The docking scores which predicts the overall stability and fitness of the possible ligand-protein complex and the interaction detail for all these studied compounds is given in Table 5. The correlation graph shown in Fig. 5 revealed a good agreement between the experimental and docking results indicated from the correlation coefficient calculated ($R^2 = 0.8591$) for predicted docking score and IC_{50} values of the synthesized compounds **(3a–j)**.

Thus literature reveals that variously substituted thioureas and their cyclized heterocycles as well as their different metal complexes had been synthesized and biologically evaluated [10,27] but herein we report *N,N′*-disubstituted thiourea derivatives which were evaluated for the first time for their antiurease potential along with their respective antimicrobial, antioxidant and anticancer/ctotoxic activities.

5. Conclusion

In conclusion, we report new analogs **(3a–j)** of *N*-(3-trifluoromethyl)benzoyl-*N′*-aryl thiourea where compounds **3a**, **3c** and **3i**

with dichloro and methoxy groups substitution on the aryl group showed significant activity against all strain of bacteria. The antifungal evaluation showed that all compounds were active againts *C. Albican* and no activity was observed against *C. Prapsilosis*. In addition, their cytotoxic findings revealed non-toxic nature of these compounds except for compounds **3f** and **3g** while they exhibited better antioxidant potential. Furthermore the compound **3a** proved to be the most potent urease inhibitor showing the highest enzyme % inhibition (93.1%) with IC_{50} value of 8.17 ± 0.24 μM followed by compounds **3e**, **3d**, **3b** and **3f**. All the synthesized compounds were docked into the binding cavity of Urease. The most active compound **3a** was also ranked as top on the docking score as it was found to show valuable interactions with the target protein along with good docking scores. Herein we propose that the synthesized compounds have potential to be used as potent urease inhibitors after further detailed mechanistic investigations.

Acknowledgement

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.102946.

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