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Unsymmetrical urea and thiourea derivatives: An efficient nano BF_3-SiO_2 catalyzed PEG-400 mediated sonochemical synthesis and biological evaluation

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Abstract: An efficient and green approach has been developed for the synthesis of (substituted phenyl)-3-(4-(4-nitrophenylthio)phenyl)urea/thiourea derivatives **6(a-j)** using non-hazardous green solvent, PEG-400 under ultrasound irradiation conditions in the presence of a reusable silica-supported Lewis acid catalyst, nano-BF_3-SiO_2 *via* simple addition reaction of 4-(4-nitrophenylthio)aniline **(4)** with substituted phenyl isocyanates/isothiocyanates **5 (a-j)**. The advantages of developed method are convenient, offered higher yield of products with purity, less reaction time, easy work-up and reusability of the catalyst. Structures of the title products were established by IR, NMR (^{1}H, ^{13}C), mass spectral data and elemental analysis. Antimicrobial activity of the newly synthesized compounds was tested and the bio-screening data disclosed that urea derivatives, **6a** and **6d**, and thiourea derivatives, **6f**, **6i** and **6j** showed potential antimicrobial activity against the growth of selected microorganisms.

Keywords: 4-(4-Nitrophenylthio)aniline; nano-BF_3.SiO_2; PEG-400; ultrasonication; urea and thiourea derivatives; antimicrobial activity.

1. Introduction

Unsymmetrical urea and thiourea compounds have been gained considerable interest since their broad spectrum of biological applications found in medicine, agriculture, industry and petrochemicals[1] as well as used as intermediates and catalysts in organic synthesis. For example,

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diuron (DCMU) **(1)** is a commercially accessible herbicide,[2] morpholine urea derivative **(2)** is used in the treatment of chronic myelogenous leukemia[3] and urea scaffold **3** is used as a receptor tyrosine kinase (RTK) inhibitor[4] (Figure 1).

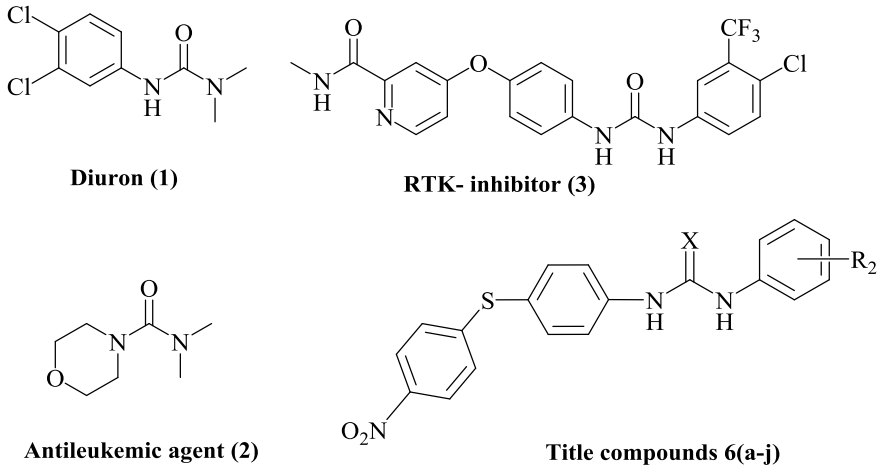


Figure 1.Some biologically active urea and thiourea derivatives.

The biological and synthetic applications of urea and thiourea derivatives are stimulated the researchers to make central attention from the last few decades for the development of new synthetic methodology and the synthesis of effective biologically active compounds with potential applications. However, numerous reports were found in the literature associated to the synthesis of symmetrical/unsymmetrical urea and thiourea derivatives individually using different surrogates like phosgene/thiophosgene,[5] isocyanates/isothiocyanates,[6] CS_2/CO_2,[7] dithiocarbamates,[8] and carbonyl-imidazoles.[9] The usage of phosgene/thiophosgene is most traditional path for the synthesis of urea and thioureas. However, some downsides are challenged to the chemists in the use of these surrogates such as some of them are too expensive to use on large scale, phosgene/thiophosgene emancipations many environmental and toxicology problems, and only symmetrical targets can be attained by this approach. Harsh conditions such as pressure, tedious work-up, strong basic reagents and long reaction time are also employed in the usage of CO_2/CS_2. Hence, the industries and academic researcher have been focused on the development of new alternative methods for the synthesis of urea and thiourea derivatives by using less toxic and hazardous reagents.[10]

In addition, various methods by alteration of catalysts and solvents for the synthesis of urea and thiourea derivatives are well documented in the literature.[11-12] Recently, commercially available isocyanates have used for the synthesis of disubstituted urea derivatives effectively.[13] Nowadays, the improvement in the environmental impact of industrial chemical processes has been attained more importance to reduce or eliminate the usage or generation of hazardous substances, solvents and catalysts in the synthetic processes. In order to usage of heterogeneous catalyst has become a major area of research in chemical synthesis. However, a heterogeneous catalyst, silica supported boron trifluoride (BF_3-SiO_2) is a bench-top catalyst which is associated more advantages like easy to handle, cheap, readily availability, eco-friendly, versatile, reusability, enable better accessibility of the reactants to the active sites and accomplished many acid catalyzed reactions.[14-15] Further, ultrasound is an alternative energy source to accelerate organic transformations ordinarily through heating instead of harsh conventional heating.[16] In the literature many homogeneous and heterogeneous catalyzed organic reactions[17] have established competently proceed *via* the formation and adiabatic collapse of transient cavitation bubbles in ultrasonication[18] by overcoming some of the demerits exposed in conventional methods. The remarkable advantages in the ultrasound irradiations are decrease the reaction time, increase the rate of the reaction, the yield enhancement of products with high purity, high efficiency and waste minimization. Hence, ultrasonic irradiations have been considered as important technique in organic synthesis.

In our continuing programme on synthetic methodologies,[19-20] herein, an efficient and nano silica supported boron trifluoride (Nano-$BF_3.SiO_2$) catalyzed high yielding procedure was accomplished for the synthesis of unsymmetrical urea and thiourea derivatives using PEG-400 as a green solvent under ultrasound irradiation and conventional conditions.

2. Experimental

All chemicals were procured from Aldrich, Merck and Sd Fine-chem. (India) were used without further purification. Solvents were distilled from the appropriate drying agents and degassed before use. The progress of the reaction and purity of the compounds were checked by TLC on Merck pre-coated silica GF254 plat using ethyl acetate:*n*-hexane as an eluent. The stationary phase, 100-120 silical gel and mobile phase, 5-50% ethyl acetate:*n*-hexane were used in column chromatography to the purification of title products. Melting points were determined in open capillaries on Guna digital Melting point apparatus and are uncorrected. IR spectra were recorded on JASCO FT-IR 5300 using KBr discs. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 using TMS as internal standard on Bruker 300 MHz spectrometer. Mass spectra were recorded on ESQUIRE 3000 mass spectrometer (positive mode). Results are presented as, IR bands in cm-1, chemical shift δ in ppm and J values in Hertz (Hz). Multiplicities are shown as the abbreviations: s (singlet), brs (broad singlet), d (doublet), dd (doublet of doublet), t (triplet) and m (multiplet). The numbering was given to the title compound for assigning the proper spectral characterization (Figure 1).

Sonication was performed using BANDELIN SONOREX® (Germany make) with a frequency of 35 kHz and a nominal power 200 W ultrasonic bath for ultrasonic irradiation with inbuilt heating 30-80 (°C), which is thermostatically adjustable. The reaction vessel was placed inside the ultrasonic bath containing water.

2.1. General Procedure for Synthesis of Compound 6b

2.1.1. Conventional Method

The mixture of 4-(4-nitrophenylthio)aniline **(4)** (1.0 mmol, 246 mg), 1-bromo-4-isocyanatobenzene (**5b**) (1.1 mmol, 216 mg) and the catalyst, 37% nano-$BF_3.SiO_2$ (0.30 g) were taken into a 50 mL round bottomed flask containing 10 mL of PEG-400. The reaction mixture was stirred about 2.0 hours at 60 ^oC and confirmed the reaction completion by TLC. The reaction mixture was dissolved in 15 mL of DCM at ambient temperature and filtered-off the contents to isolate the catalyst as residue followed by the residue was washed with DCM (5 mL x 3) to remove the stains on the catalyst. Water (20 mL) was added to the combined organic solution and the layers were separated. Then organic solution was concentrated to dryness under vacuum to get the crude product. The column chromatography was adopted to get the pure compound, 1-(4-bromophenyl)-3-(4-(4-nitrophenylthio)phenyl)urea (**6b**). The same procedure was adopted for the synthesis of remaining title compounds (**6a, 6c-j**) (Table 3).

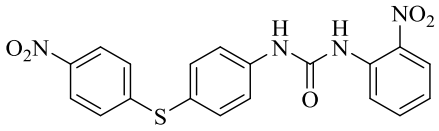
2.1.2. Ultrasonication Method

The mixture of 4-(4-nitrophenylthio)aniline **(4)** (1.0 mmol, 246 mg), 1-bromo-4-isocyanatobenzene (**5b**) (1.1 mmol, 216 mg) and the catalyst, 37% nano-$BF_3.SiO_2$ (0.30 g) were taken into a 100 mL vessel containing 10 mL of PEG-400. The reaction mixture was stirred at 50 ^oC for 25 min. under ultra sonicator and the reaction completion was judged by TLC. The reaction mixture was dissolved in 15 mL of DCM at room temperature and the reaction mixture was filtered-off to isolate the catalyst as residue followed by it was washed with DCM (5 mL x 3) to remove the stains on the catalyst. Water (20 mL) was added to the combined organic solution and then the layers were separated. Then organic solution was concentrated to dryness under vacuum to get the crude product. The column chromatography was adopted to get the pure compound, 1-(4-bromophenyl)-3-(4-(4-nitrophenylthio)phenyl)urea (**6b**). The same procedure was adopted for the synthesis of remaining title compounds (Table 3).

2.1.3. Recycling of the Catalyst, Nano-BF_3.SiO_2

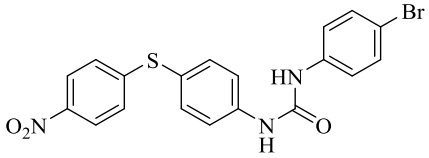
After completion of the reaction, the catalyst was removed from the reaction mixture by filtration as residue. The residue, nano-BF_3.SiO_2 was washed three to four times with 5 mL of DCM to remove the stains on the catalyst and then dried in oven at 80-90 ^oC for 2 h. The catalyst was reused up to three cycles with no significant decrease of catalytic activity.

*1-(2-Nitrophenyl)-3-(4-(4-nitrophenylthio)phenyl)urea (**6a**)*



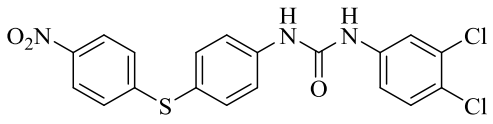
Yellow solid; Yield: 93%; M.p. 183-185; ^{1}H-NMR (DMSO-d_6) (δ/ppm): 8.27 (1H, s, -NH), 8.24 (1H, s, -NH), 8.05 (2H, d, J = 9.1 Hz, Ar-H), 8.01 (1H, d, J = 8.7 Hz, Ar-H), 7.58-7.66 (1H, m, Ar-H), 7.53 (2H, d, J = 9.0 Hz, Ar-H), 7.15-7.32 (4H, m, Ar-H), 7.07 (2H, d, J = 8.7 Hz, Ar-H); ^{13}C-NMR (DMSO-d_6) (δ/ppm): 152.7, 149.3, 145.1, 141.5, 139.3, 136.4, 132.4, 130.5, 127.3, 127.0, 124.6, 124.2, 123.7, 120.4, 117.4; IR (υ/cm^{-1}): 3352, 3268 (-N-H), 2924 (C-H), 1682 (-C=O), 1579, 1336 (-NO_2); MS (APCI, positive mode) (*m/z*) (%): 411 $(M+H)^+$ (100), 273 $(M-138+H)^+$ (50), 247 $(M-164+H)^+$ (30); Analysis (% Calculated/found) for $C_{19}H_{14}N_4O_5S$ (Mw 410.07) C: 55.60/55.67, H: 3.44/3.40, N: 13.65/13.59.

*1-(4-Bromophenyl)-3-(4-(4-nitrophenylthio)phenyl)urea (**6b**)*



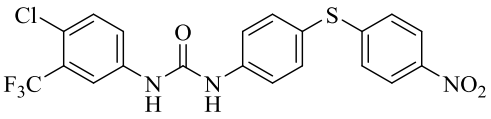
Light green solid; Yield: 92%; M.p. 236-238; ^{1}H-NMR (DMSO-d_6) (δ/ppm): 8.26 (1H, s, -NH), 8.23 (1H, s, -NH), 7.97 (2H, d, J = 9.3 Hz, Ar-H), 7.71 (2H, d, J = 8.7 Hz, Ar-H), 7.49 (2H, d, J = 9.0, Ar-H), 7.39 (2H, d, J = 8.7 Hz, Ar-H), 7.22 (2H, d, J = 8.4 Hz, Ar-H), 7.05 (2H, d, J = 8.4 Hz, Ar-H); ^{13}C-NMR (DMSO-d_6) (δ/ppm): 152.7, 149.4, 145.1, 141.8, 139.1, 136.5, 132.0, 131.9, 126.3, 124.6, 121.1, 120.9, 120.1; IR (υ/cm^{-1}): 3312, 3177 (-NH), 3084 (C-H), 1655 (-C=O), 1539, 1336 (NO_2), 1005 (C-Br); MS (APCI, positive mode) (*m/z*) (%): 446 $(M+2+H)^+$ (85), 444 $(M+H)^+$ (100), 384 $(M-60+H)^+$ (66), 276 $(M-168+H)^+$ (41), 247 $(M-197+H)^+$ (98), 124 $(M-320+H)^+$ (61).

*1-(3,4-Dichlorophenyl)-3-(4-(4-nitrophenylthio)phenyl)urea (**6c**)*



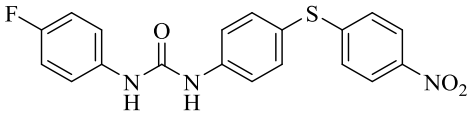
Light yellow solid; Yield: 90%; M.p. 235-236 (Lit. 238-240)[23]; ^{1}H-NMR (DMSO-d_6) (δ/ppm): 8.32 (1H, s, -NH), 8.29 (1H, s, -NH), 8.07 (2H, d, J = 9.0 Hz, Ar-H), 7.84 (1H, s, Ar-H),7.69 (2H, d, J = 8.7 Hz, Ar-H), 7.53 (2H, d, J = 9.0 Hz, Ar-H), 7.44 (1H, d, J = 6.3 Hz, Ar-H), 7.31 (2H, d, J = 8.7 Hz, Ar-H),7.18 (1H, d, J = 6.6 Hz, Ar-H); ^{13}C-NMR (DMSO-d_6) (δ/ppm): 153.1, 147.8, 141.2, 138.7, 136.3, 132.6, 132.1, 131.2, 130.8, 129.8, 128.3, 125.6, 124.0, 121.6, 120.5; IR (υ/cm^{-1}): 3372, 3259 (-N-H), 3038 (C-H), 1709 (-C=O), 1517, 1326 (NO_2), 1084 (Ar-Cl); MS (APCI, positive mode): (*m/z*) (%): 438 $(M+4+H)^+$ (43), 436 $(M+2+H)^+$ (17), 434 $(M+H)^+$ (100), 273 $(M-161+H)^+$ (27), 247 $(M-187+H)^+$ (84), 124 $(M-310+H)^+$ (41).

*1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-(4nitrophenylthio)phenyl)urea (**6d**)*



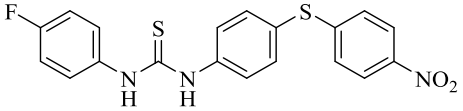
Light yellow solid; Yield: 92%; M.p. 224-227; ^{1}H-NMR (DMSO-d_6) (δ/ppm): 8.28 (1H, s, -NH), 8.22 (1H, s, -NH), 8.04 (2H, d, J = 8.7 Hz, Ar-H), 8.01 (1H, s, Ar-H), 7.65 (1H, d, J = 9.0 Hz, Ar-H), 7.41 (2H, d, J = 8.4 Hz, Ar-H), 7.32 (2H, d, J = 8.4 Hz, Ar-H), 7.13 (2H, d, J = 8.7 Hz, Ar-H), 7.07 (1H, d, J = 9.0 Hz, Ar-H); ^{13}C-NMR (DMSO-d_6) (δ/ppm): 152.7, 149.3, 145.1, 141.5, 139.3, 136.4, 132.4, 127.3, 127.0, 126.3, 124.6, 124.2, 123.7, 123.2, 120.4, 117.5; IR (υ/cm^{-1}): 3362, 3242 (-N-H), 3027 (C-H), 1687 (-C=O), 1537, 1334 (NO_2), 1396 (-C-F), 1030 (Ar-Cl); MS (APCI, positive mode) (*m/z*) (%): 470 $(M+2+H)^+$ (33), 468 $(M+H)^+$ (100), 273 $(M-195+H)^+$ (25), 247 $(M-221+H)^+$ (87), 124 $(M-344+H)^+$ (37); Analysis (% Calculated/found) for $C_{20}H_{13}ClF_3N_3O_3S$ (Mw: 467.03) C: 51.34/51.32, H: 2.80/2.76, N: 8.98/8.95.

*1-(4-Fluorophenyl)-3-(4-(4-nitrophenylthio)phenyl)urea (**6e**)*



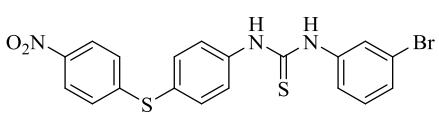
Off-white solid; Yield: 93%; M.p. 289-292 (Lit. 287-290)[23]; ^{1}H-NMR (DMSO-d_6) (δ/ppm): 8.24 (1H, s, -NH), 8.18 (1H, s, -NH), 8.11 (2H, d, J = 8.4 Hz, Ar-H), 7.91 (2H, d, J = 8.7 Hz, Ar-H), 7.65 (2H, d, J = 8.4 Hz, Ar-H), 7.54 (2H, d, J = 8.7 Hz, Ar-H), 7.25-7.38 (m, 4H, Ar-H); ^{13}C-NMR (DMSO-d_6) (δ/ppm): 168.5, 158.6, 148.2, 145.0, 140.2, 137.8, 134.0, 132.8, 129.3, 124.5, 121.3, 120.3, 116.1; IR (υ/cm^{-1}): 3352, 3240 (-N-H), 3018 (C-H), 1682 (-C=O), 1541, 1330 (NO_2), 1098 (-C-F); MS (APCI, positive mode) (*m/z*) (%): 384 $(M+H)^+$ (100), 246 $(M-138+H)^+$ (85).

*1-(4-Fluorophenyl)-3-(4-(4-nitrophenylthio)phenyl)thiourea (**6f**)*



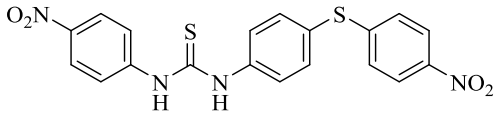
Brown solid; Yield: 92%; M.p. 108-110; ^{1}H-NMR (DMSO-d_6) (δ/ppm): 8.40 (2H, brs, -NH), 8.06 (2H, d, J = 9.3 Hz, Ar-H), 8.00 (2H, d, J = 7.2 Hz, Ar-H), 7.33 (2H, d, J = 7.2 Hz, Ar-H), 7.26 (2H, d, J = 7.8 Hz, Ar-H), 7.20 (2H, d, J = 9.0 Hz, Ar-H), 7.08 (2H, d, J = 7.5 Hz, Ar-H); ^{13}C-NMR (DMSO-d_6) (δ/ppm): 179.3, 160.3, 148.8, 143.7, 141.5, 137.5, 135.3, 133.5, 130.1, 128.7, 126.2, 123.7, 114.6; IR (υ/cm^{-1}): 3309, 3265 (-N-H), 2985 (C-H), 1216 (-C=S), 1578, 1331 (NO_2), 1276 (Ar-F); MS (APCI, positive mode) (*m/z*) (%): 384 $(M+H)^+$ (100), 273 $(M-111+H)^+$ (33), 247 $(M-137+H)^+$ (67), 202 $(M-183+H)^+$ (85), 124 $(M-260+H)^+$ (48).

1-*(3-Bromophenyl)-3-(4-(4-nitrophenylthio)phenyl)thiourea (**6g**)*



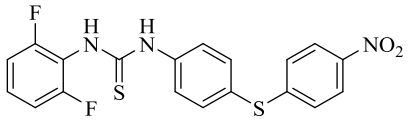
Pale Yellow solid; Yield: 91%; M.p. 114-116; ^{1}H-NMR (DMSO-d_6) (δ/ppm): 8.52 (1H, s, -NH), 8.29 (1H, s, -NH), 8.01 (2H, d, *J* = 8.7 Hz, Ar-H), 7.58 (2H, d, *J* = 8.7 Hz, Ar-H), 7.23 (2H, d, *J* = 8.1Hz, Ar-H), 6.74-6.98 (3H, m, Ar-H), 6.38-6.51 (3H, m, Ar-H); ^{13}C-NMR (DMSO-d_6) (δ/ppm): 179.1, 147.4, 142.4, 140.0, 138.1, 132.8, 131.2, 130.3, 128.9, 128.0, 126.4, 125.9, 124.7, 123.5, 122.6; IR (υ/cm^{-1}): 3261, 3177 (-N-H), 3028 (C-H), 1197 (-C=S), 1531, 1358 (NO_2), 1064 (Ar-Br); MS (APCI, positive mode) (*m/z*) (%): 462 $(M+2+H)^+$ (92), 460 $(M+H)^+$ (100), 273 $(M-187+H)^+$ (35), 247 $(M-213+H)^+$ (75); Analysis (% Calculated/found) for $C_{19}H_{14}BrN_3O_2S_2$ (Mw: 458.97) C: 49.57/49.53, H: 3.07/3.04, N: 9.13/9.07.

*1-(4-Nitrophenyl)-3-(4-(4-nitrophenylthio)phenyl)thiourea (**6h**)*



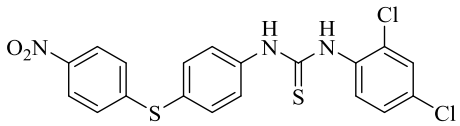
Pale Yellow solid; Yield: 91%; M.p. 106-109; ^{1}H-NMR (DMSO-d_6) (δ/ppm): 8.55 (1H, s, -NH), 8.27 (1H, s, -NH), 8.11 (2H, d, *J* = 8.7 Hz, Ar-H), 8.02 (2H, d, *J* = 8.1 Hz, Ar-H), 7.75 (2H, d, *J* = 8.7 Hz, Ar-H), 7.21 (2H, d, *J* = 8.4 Hz, Ar-H), 6.85 (2H, d, *J* = 8.4 Hz, Ar-H), 6.71 (2H, d, *J* = 8.7 Hz, Ar-H); ^{13}C-NMR (DMSO-d_6) (δ/ppm): 181.0, 147.6, 146.9, 144.5, 143.9, 140.6, 133.3, 132.9, 128.4, 126.8, 125.1, 124.0, 123.5; IR (υ/cm^{-1}): 3314, 3195 (-N-H), 3009 (C-H), 1210 (-C=S), 1542, 1355 (NO_2); MS (APCI, positive mode) (*m/z*) (%): 427 $(M+H)^+$ (100), 182 $(M-245+H)^+$ (82), 138 $(M-289+H)^+$ (52).

*1-(2,6-Difluorophenyl)-3-(4-(4-nitrophenylthio)phenyl)thiourea (**6i**)*



Pale brown solid; Yield: 93%; M.p. 134-136; ^{1}H-NMR (DMSO-d_6) (δ/ppm): 9.10 (1H, s, -NH), 8.92 (1H, s, -NH), 8.07 (2H, d, *J* = 8.4 Hz, Ar-H), 7.80 (2H, d, *J* = 8.7 Hz, Ar-H), 7.29 (2H, d, *J* = 8.4 Hz, Ar-H), 6.68-6.87 (5H, m, Ar-H); ^{13}C-NMR (DMSO-d_6) (δ/ppm): 179.3, 168.3 (d, J = 204.3 Hz), 147.3, 141.9, 138.6, 132.5, 131.2, 129.4, 126.6, 125.8, 124.8, 114.1, 112.6s; IR (υ/cm^{-1}): 3338, 3198 (-N-H), 3052 (C-H), 1205 (-C=S), 1528, 1350 (NO_2), 1096 (Ar-F); MS (APCI, positive mode) (*m/z*) (%): 418 $(M+H)^+$ (100), 384 $(M-63+H)^+$ (44), 247 $(M-171+H)^+$ (100), 172 $(M-246+H)^+$ (74).

*1-(2,4-Dichlorophenyl)-3-(4-(4-nitrophenylthio)phenyl)thiourea (**6j**)*



Light yellow solid; Yield: 90%; M.p. 105-107; ^{1}H-NMR (DMSO-d_6) (δ/ppm): 8.44 (1H, s, -NH), 8.36 (1H, s, -NH), 8.03 (2H, d, *J* = 9.0 Hz, Ar-H), 7.96 (1H, s, Ar-H), 7.62 (2H, d, *J* = 9.0 Hz, Ar-H), 7.54 (1H, d, *J* = 8.4 Hz, Ar-H), 7.18-7.28 (3H, m, Ar-H), 6.79 (2H, d, *J* = 8.7 Hz, Ar-H); ^{13}C-NMR (DMSO-d_6) (δ/ppm): 182.6, 145.9, 142.2, 138.1, 136.1, 133.9, 132.8, 131.9, 131.0, 129.9, 129.0, 128.5, 126.8, 125.4, 122.5; IR (υ/cm^{-1}): 3349, 3172 (-N-H), 3028 (C-H), 1208 (-C=S), 1539, 1324 (NO_2), 1012 (Ar-Cl); MS (APCI, positive mode) (*m/z*) (%): 454 $(M+4+H)^+$ (95), 452 $(M+2+H)^+$ (65), 450 $(M+H)^+$ (100), 273 $(M-177+H)^+$ (30), 247 $(M-203+H)^+$ (65).

2.2. Antimicrobial Activity

2.2.1. Antibacterial Activity

The bacteria such as *Escherichia coli*, *Bacillus subtilis* and *Streptococcus aureus* were chosen to screen the antibacterial activity of the newly synthesized urea and thiourea derivatives **6(a-j)** using disc diffusion method[21] and Streptomycin drug was used as a standard. Test samples in two different concentrations such as 50 and 100 µg/mL were prepared in DMSO. A standard inoculum (1-2 x 10^7c.f.u/mL 0.5 McFarland standards) was introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums. The discs measuring 6 mm in diameter were prepared from Whatmann No.1 filter paper and sterilized by dry heat at 120 ^oC for an hour. The dry sterilized discs previously soaked in known concentrations (50 and 100 µg/mL) of the test compounds were placed in nutrient agar medium. The culture palates were inverted and incubated for 24 h at 37 ^oC. The zone of inhibition around the disc was calculated and measured in millimeters. Blank test showed that DMSO used in the preparation of the test solutions does not affect the test organisms. All tests were repeated three times and average data has taken as final result. The inhibition zones of the tested compounds were compared with positive controls (Table 4).

2.2.2. Antifungal Activity

The antifungal activity of the synthesized urea and thiourea derivatives **6(a-j)** were screened against fungal strains such as *Fusarium oxysporum*, *Aspergillus flavus* and *Aspergillus niger* using agar disc-diffusion method[22] and Bovastin was used as a standard. The fungal strains were maintained on Potato Dextrose Agar (PDA) medium (Hi-Media). The culture from the slant was inoculated into the Potato Dextrose broth and incubated at 37 ˚C for 72 h. This culture (0.1 mL) was spread on the potato dextrose agar plate and a sterile glass spreader was used for even distribution of the inoculums. Sterile discs of Whatmann No.1 filter paper of about 6 mm diameter were impregnated on the surface of the media. Known concentrations (50 and 100 µg/mL) of test samples and standard in DMSO were prepared and applied on the discs and incubated for 72 h at 37 ˚C. The zone of inhibition around the disc was measured in millimeters. All tests were repeated three times and average data has taken as final result. The inhibition zones of the tested compounds were compared with positive controls (Table 5).

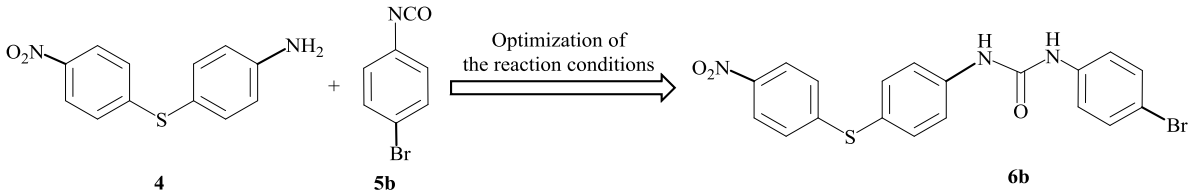
2.2.3. Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was determined by micro-broth-dilution method[22a]. To examine MICs of the test solutions, various serial concentrations by decreasing 2.5 µg/mL concentration for each time like 50, 47.5, 45, 42.5, 40, 37.5, 35 ... 5.0 and 2.5 µg/mL were prepared from the stock solution. Specifically, 0.1 mL of standardized inoculum (1-2 x 10^7 CFU/mL) was added to each test tube. The bacterial tubes were incubated aerobically for 24 h at 37 ^oC and fungal tubes were incubated for 72 h at 25 ^oC. Control was maintained for each test sample. The lowest concentration (highest dilution) of test compound that produced no visible signs of bacterial/fungal growth (no turbidity) when compared with the control tubes were regarded as MIC. The experimental results were tabulated in Table 6.

3. Results and Discussion

3.1. Chemistry

For the preparation of urea and thiourea derivatives, initially, 4-(4-nitrophenylthio)aniline (**4**) and 1-bromo-4-isocyanatobenzene (**5b**) were selected as models (Scheme 1).



4 5b 6b

Scheme 1. Model reaction to the optimization of reaction conditions.

Table 1. Optimization of catalyst, solvent and reaction temperature for the synthesis of urea derivative, **6b**[a].

Entry	Catalyst (quantity)	Solvent	Time	Yield (%)
1	No catalyst	THF (50 ^oC)	12.0 h	48
2	Et_3N	THF (50 ^oC)	6.5 h	72
3	DMPipz	THF (50 ^oC)	6.5 h	76
4	$CuCl_2$ (10 mol%)	THF (50 ^oC)	5.0 h	61
5	$FeCl_3$ (10 mol%)	THF (50 ^oC)	6.0 h	64
6	$AlCl_3$ (10 mol%)	THF (50 ^oC)	6.0 h	60
7	$ZnSO_4$ (10 mol%)	THF (50 ^oC)	6.0 h	58
8	$BF_3.OEt_2$ (15 mol%)	THF (50 ^oC)	4.0 h	80
9	37% Nano-$BF_3.SiO_2$ (0.2 g)	THF (50 ^oC)	2.5 h	84
10	37% Nano-$BF_3.SiO_2$ (0.2 g)	Toluene (80 ^oC)	4.0 h	72
11	37% Nano-$BF_3.SiO_2$ (0.2 g)	CH_3CN (70 ^oC)	4.0 h	77
12	37% Nano-$BF_3.SiO_2$ (0.2 g)	$CHCl_3$ (50 ^oC)	4.0 h	73
13	37% Nano-$BF_3.SiO_2$ (0.2 g)	DCM (50 ^oC)	4.0 h	75
14	37% Nano-$BF_3.SiO_2$ (0.2 g)	EtOH (60 ^oC)	3.0 h	84
15	37% Nano-$BF_3.SiO_2$ (0.2 g)	MeOH (60 ^oC)	3.0 h	78
16	37% Nano-$BF_3.SiO_2$ (0.2 g)	PEG-400 (60 ^oC)	2.0 h	85
17	37% Nano-$BF_3.SiO_2$ (0.2 g)	PEG-400 (60 ^oC)[b]	25 min	89
18	37% Nano-$BF_3.SiO_2$ (0.2 g)	PEG-400 (50 ^oC)[b]	25 min	88
19	37% Nano-$BF_3.SiO_2$ (0.2 g)	PEG-400 (40 ^oC) [b]	25 min	87
20	37% Nano-$BF_3.SiO_2$ (0.2 g)	PEG-400 (RT) [b]	25 min	81

[a]Model reaction was carried out using 4-(4-nitrophenylthio)aniline **(4)** (1 mmol) and 1-bromo-4-isocyanatobenzene **(5b)** (1.1 mmol); [b]Model reaction was carried out in ultrasonication.

At first to know the ability of reaction, the model reaction was carried out in THF without using any catalyst led to low yield of the product (48%) after a long reaction time (12 h) (Table 1 entry 1). Our considerable attention has focused to increase the reaction efficacy towards high yields, hence, the model reaction was examined in the presence of various bases like Et_3N, DMPipz and Lewis acid catalysts, $CuCl_2$, $FeCl_3$, $AlCl_3$, $ZnSO_4$, $BF_3.SiO_2$ and 37% nano-$BF_3.SiO_2$. Surprisingly, high yield of the product, 1-(4-bromophenyl)-3-(4-(4-nitrophenylthio) phenyl) urea (**6b**) was obtained in the presence of catalytic system, nano-$BF_3.SiO_2$ (84%) (Table 1 entry 9) and the rest of examined catalysts afforded moderate yields (Table 1 entry 2-8). Further, to distinguish the solvent effect and choose easily driven solvent for this reaction, the model reaction was carried out in different solvents such as toluene, $CHCl_3$, CH_3CN, DCM, EtOH, MeOH and PEG-400 (Table 1 entry 10-16). It was clear that in all the tested solvents moderate to high yields were obtained. However, EtOH (84%) and PEG-400 (85%) were afforded better yields of the product **6b** as compared with other solvents. Considering the environmental impacts and the reaction time, PEG-400 was selected as the solvent. Further, our attention was focused to reduce the reaction time; hence, the optimized reaction was carried out in ultrasonication at 40 ^oC (Table 1 entry 19) and observed remarkably high yield of the product **6b** (87%) in short reaction time (25 min.). Yet again to distinguish the temperature effect on the yield of the product, the model reaction was investigated at ambient temperature and different temperatures (Table 1 entry 17-20). As seen in the table, it was found that the reaction is proceed at 40 ºC and afforded high yield (81%) of the product in less time (25 min) and significant yield enhancement was observed upon increasing the temperature until 50 ^oC, and no noteworthy enhancement at 60 ^oC.

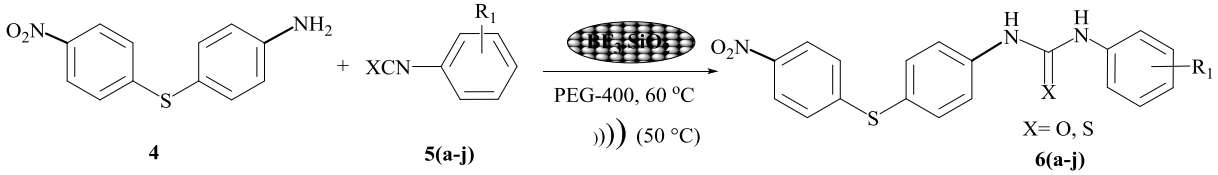
It is well known that the amount of catalyst also play an important role in the reaction, so, the model reaction was tested in PEG-400 by loading different amounts of the catalyst, 37% nano-BF_3-SiO_2 under conventional and ultrasonication conditions and the results are presented in Table 2, entry 1-5. As can be seen in Table 2, the optimum yield was observed at the usage of 0.30 g of 37% nano-BF_3-SiO_2 catalyst and no significant yield enhancement was observed even though when use more amount of catalyst. The reusability of the catalyst was also scrutinized up to five runs (Table 2, entry 4, 6-9) and it was observed that the usage of catalyst until 3rd run no significant yield variation was observed. The catalytic activity was reduced significantly while using the catalyst more than to three cycles.

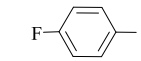
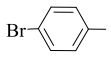
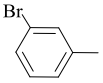
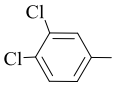
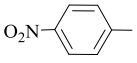
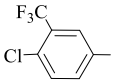
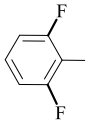
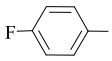
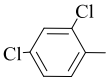
Table 2. Effect of loading of the catalyst for the synthesis of urea derivative, **6b**.[a]

Entry	Catalyst (g)	Time (h^b/min^c)	Yield (%)[b/c]
1	37% Nano-$BF_3.SiO_2$ (0.15 g)	2.0/25	78/83
2	37% Nano-$BF_3.SiO_2$ (0.20 g)	2.0/25	85/88
3	37% Nano-$BF_3.SiO_2$ (0.25 g)	2.0/25	8692
4	37% Nano-$BF_3.SiO_2$ (0.30 g)	2.0/25	88/92
5	37% Nano-$BF_3.SiO_2$ (0.35 g)	2.0/25	88/93
6	37% Nano-$BF_3.SiO_2$ (0.30 g) (2^{nd} run)	2.0/25	86/92
7	37% Nano-$BF_3.SiO_2$ (0.30 g) (3^{rd} run)	2.0/25	83/90
8	37% Nano-$BF_3.SiO_2$ (0.30 g) (4^{th} run)	2.0/25	82/87
9	37% Nano-$BF_3.SiO_2$ (0.30 g) (5^{th} run)	2.0/25	80/84

[a]Model reaction was carried out using 4-(4-nitrophenylthio)aniline **(4)**(1 mmol) and 1-bromo-4-isocyanatobenzene **(5b)** (1.1 mmol) in PEG-400 solvent; [b]Time and yield of the product **6b** in conventional condition; [c]Time and yield of the product **6b** in ultrasonication condition.

After we came to conclusion in the optimization of reaction conditions, the generality were patterned with various structurally diversified isocyanates and isothiocyanates to authenticate the scope of optimized reaction conditions (Scheme 2). All the desired urea and thiourea derivatives **6(a-j)** were obtained in excellent yields (Table 3), whereas isothiocyanates furnished good yields as compared with isocyanates. The reason might be isothiocyanates can competently co-ordinates with Lewis acid catalyst that lead to favorable addition to amine.



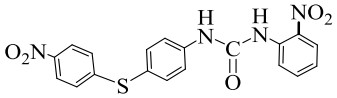
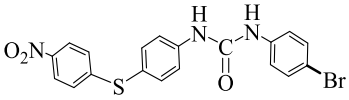
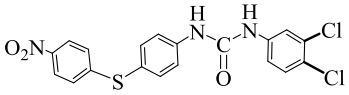
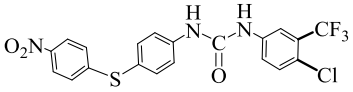
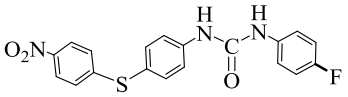
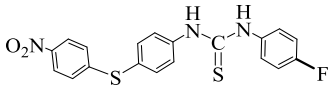
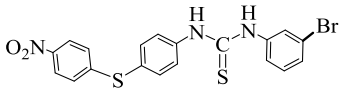
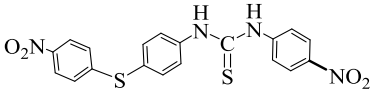
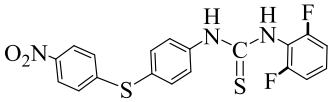
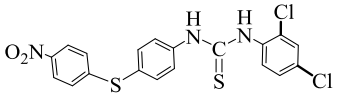
X	R_1-Ph-	Product	X	R_2-Ph-	Product
O		**6a**	S		**6f**
O		**6b**	S		**6g**
O		**6c**	S		**6h**
O		**6d**	S		**6i**
O		**6e**	S		**6j**

Scheme 2. Synthetic representation to the preparation of compounds **6(a-j)**.

3.2. Spectroscopic Data Analysis

The structures of the newly synthesized compounds were established by IR, NMR (^{1}H, ^{13}C), mass spectra and CHN analysis. In IR spectra, the absorption bands in the regions of 3378-3220 cm^{-1} and 3110-3218 cm^{-1} were corresponding to unsymmetrical -N-H stretching. The bands at 1630-1689 cm^{-1} and 1180-1230 cm^{-1} confirmed the functionalities -C=O in urea derivatives and C=S in thiourea derivatives, respectively. In ^{1}H NMR spectra, the chemical shift values in the region of 8.10-8.59 ppm as singlets/broad singlet corresponding to -NH proton and 6.40-7.90 ppm are due to the aromatic protons. In ^{13}C spectra, appearance of the carbon chemical shifts in the region of 151.6-153.8 ppm and 178.6-184.5 ppm confirmed the carbon (-C=O) in urea and thiourea (-C=S), respectively. In EI (+) mass spectra, appearance of the corresponding molecular ion peaks of the title compounds and their daughter ions with various relative intensities were given further evidence to the structures of title compounds. The observed composition of C, H, N in elemental analysis of title products was approximately coincided with theoretical C, H, N composition and provided a conclusive evidence for the proposed structures.

Table 3. Time and yield of the synthesized urea and thiourea derivatives **6(a-j)**.

Compd.	Product	Conventional		Ultrasonication		M.P (°C)
		Time (h)	Yield (%)	Time (min)	Yield (%)	
6a		2.5	89.0	35	93	183-185
6b		2.0	88.5	25	92	236-238
6c		3.0	85.5	30	90	235-236 (Lit. 238-240)[23]
6d		2.0	89.0	25	92	224-227
6e		2.0	88.0	25	93	289-292 (Lit. 287-290)[23]
6f		2.5	88.5	30	92	108-110
6g		2.5	89.0	35	91	114-116
6h		2.5	87.5	25	91	106-109
6i		2.5	90.0	25	93	134-136
6h		2.5	88.0	35	93	105-107

3.3. Pharmacology

The *in vitro* antibacterial activity against strains such as *Escherichia coli*, *Bacillus subtilis* and *Streptococcus aureus*, and antifungal activity against *Fusarium oxysporum*, *Aspergillus flavus* and *Aspergillus niger* were investigated at 50 and 100 μg/mL concentrations to the synthesized urea and thiourea derivatives **6(a-j)**. Disc-diffusion method[21] for antibacterial activity and agar disc-diffusion method[22] for antifungal activity were used to screen the activity, and the observed experimental results are tabulated in Table 4 and Table-5 respectively.

Table 4. Antibacterial activity of the title compounds **6(a-j).**

Product	Bacterial growth of zone of inhibition (mm)					
	Escherichia coli		***Bacillus subtillis***		***Streptococcus aureus***	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
6a	8.1	14.3	8.6	15.0	7.8	15.3
6b	3.2	9.2	---	6.2	4.2	10.5
6c	---	13.0	7.3	16.2	3.5	9.8
6d	10.8	15.4	8.2	16.3	10.8	15.3
6e	7.5	14.9	6.2	16.2	3.5	9.2
6f	9.0	15.4	9.1	14.8	10.1	16.0
6g	2.6	10.9	---	6.2	---	7.3
6h	---	8.7	4.2	10.2	7.0	14.2
6i	9.0	15.2	7.9	16.0	8.5	14.8
6j	8.2	13.6	7.4	15.1	7.9	15.5
Std.	12.0	16.5	10.0	18.0	12.5	17.0

Std. Standard- Streptomycin used as a standard for comparison of the antibacterial activity.

Table 5. Antifungal activity of the title compounds **6(a-j).**

Product	Fungal growth of zone of inhibition (mm)					
	Aspergillus flavus		***Aspergillus niger***		***Fusarium oxysporum***	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
6a	8.2	15.5	6.4	14.8	8.0	14.6
6b	---	7.1	---	8.0	---	4.9
6c	---	6.3	5.9	15.2	---	6.0
6d	9.0	16.4	9.4	15.1	7.9	15.6
6e	---	7.2	2.9	8.2	5.2	14.6
6f	5.7	13.7	8.8	14.3	8.0	16.5
6g	---	6.0	---	8.4	2.3	11.0
6h	6.1	14.8	3.2	9.7	---	5.9
6i	9.0	16.2	8.6	15.0	8.9	15.6
6j	8.1	14.7	8.0	14.4	7.2	14.3
Std.	11.0	18.0	12.0	17.0	12.5	18.0

Std. Standard – Bovastin used as a standard for comparison of the antifungal activity.

The standards, Streptomycin and Bovastin were used in antibacterial and antifungal activities, respectively for comparing the biological potency of the title compounds. The biological data displayed that some of the compounds did not exhibit any antimicrobial activity below the concentration of 50 µg/mL, but all the compounds exhibited antimicrobial activity at the concentration of 100 µg/mL. Whereas, urea compounds **6a** bonded with 2-nitro phenyl ring and **6d** connected with

3-trifluoromethyl-4-chloro phenyl ring, and thiourea derivatives **6f** having 4-fluoro phenyl entity, **6i** attached with 2,4-difluoro phenyl ring and **6j** associated with 2,4-dichloro phenyl ring exhibited potent activity on both tested bacteria and fungi. In particular, the compounds **6c** against *B. subtilis* and *A. niger*, **6e** against *B. subtilis*, *E. coli* and *F. oxysporum*, and **6h** against *S. aureus* and *A. flavus* showed promising growth of inhibition. In addition, minimum inhibitory concentrations were screened for these active compounds to know their potentiality using micro-broth dilution technique[23] and the results are tabulated in Table 6. The bio-screening results disclosed that all these tested potential compounds showed minimum inhibitory concentrations (MIC) in the range of 17.5-30.0 μg/mL. In whole bio-screening data observations, it was observed that thiourea derivatives showed potential antimicrobial activity as compared with urea derivatives.

Table 6. Minimum inhibitory concentration (MIC) of the active compounds.[a]

Compd.	*E. coli*	*B. subtillis*	*S. aureus*	*A. flavus*	*A. niger*	*F. oxysporum*
6a	20.0	25.0	27.5	22.5	30.0	20.0
6c	NT	27.5	45.0	NT	30.0	NT
6d	17.5	22.5	17.5	22.5	17.5	20.0
6e	25.0	27.5	50.0	NT	45.0	35.0
6f	25.0	20.0	20.0	30.0	20.0	22.5
6h	ND	40.0	35.0	27.5	40.0	NT
6i	17.5	25.0	22.5	25.0	22.5	22.5
6j	20.0	25.0	22.5	22.5	25.0	27.5
Std.[b]	7.5	5.0	7.5	---	---	---
Std.[c]	---	---	---	7.5	5.0	5.0

[a] - MIC values of the screened compounds were represented as μg/mL; Std.[b] -Streptomycin used as a standard for comparison of antibacterial activity; Std.[c] -Bovastin used as a standard for comparison of antifungal activity; *E. coli - Escherichia coli; B. subtillis - Bacillus subtillis; S. aureus - Streptococcus aureus; A. flavus - Aspergillus flavus; A. niger - Aspergillus niger; F. oxysporum - Fusarium oxysporum.*

4. Conclusion

In conclusion, we reported a green and effective synthetic protocol to the synthesis of urea and thiourea derivatives by the addition of amine to isocyanates/isothiocyanates in the presence of nano-$BF_3.SiO_2$ under ultrasonication and conventional conditions using a green solvent PEG-400. This protocol has several advantages, avoiding harmful organic solvents and harsh reaction conditions, high yield of the products with purity, less reaction time, easy work-up and reusability of the catalyst. However, one advantage in ultrasonication condition is less reaction time and low temperature (50 ºC) than that of conventional conditions. Structures of the newly synthesized title products were elucidated by spectral data such as IR, NMR (^{1}H, ^{13}C), mass and elemental analysis. The antimicrobial activity of the newly synthesized urea and thiourea derivatives were screened. The urea compounds **6a** and **6d**, and thiourea derivatives such as **6f**, **6i** and **6j** showed potent growth inhibition of both tested bacterial and fungal strains. In over all, thiourea derivatives showed potential antibacterial and antifungal activities when compared with urea derivatives. Particularly, fluorine substituted derivatives exhibited promising antimicrobial activity.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/OC

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