Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(17):33-39
(http://www.derpharmachemica.com/archive.html)

Design, Synthesis and Bioevaluation of New Urea/Thiourea Derivatives of 3,5-dichloro-4-hydroxy-aniline

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ABSTRACT

Synthesis of a series of new urea and thiourea derivatives of 3,5-dichloro-4-hydroxyaniline was accomplished by reacting various isocyanates and isothiocyanates in the presence of triethylamine at 60°C. All the title compounds obtained in high yields and they were characterized by IR, ^{1}H, ^{13}C-NMR, mass spectral and elemental analysis. All the title compounds exhibited potent antioxidant and antimicrobial activities.

Keywords: 3,5-dichloro-4-hydroxyaniline, Isocyanates, Isothiocyanates, Urea/Thiourea derivatives, Antioxidant, Antimicrobial activity

INTRODUCTION

Urea and thiourea derivatives possess potent anticancer properties [1-3]. Thiourea derivatives display a wide range of biological activity including antibacterial, anti-fungal, antitubercular, anthelmintic, rodenticide, insecticidal and plant growth regulatory properties [4-6]. Urea and thiourea are multifaceted functional groups that are commonly found in natural products and often display a wide range of biological activities [7], such as antioxidant [8], antiviral [9], anti-inflammatory [10], antimalarial [11,12], anti-HIV [13] inhibitors in the corrosion field [14], catalysts in chemical reactions [15], as pesticides in agriculture [16], herbicides [17] and in pharmaceutical industry [18]. Their bio-activity lies in the structure of the thiourea derivatives which are more efficacious as antimicrobial and anticancer agents. Some thiourea compounds were reported to be Non-nucleoside Inhibitors (NNIs) of the Reverse Transcriptase (RT) enzyme of the Human Immunodeficiency Virus (HIV) [19]. Diaryl substituted heterocyclic urea derivatives were found to inhibit cholesterol O-acy1 Transferase (ACAT) as hypocholesterolemic agents *in vitro* and *in vivo* studies [20], but N, N-disubstituted cyclic urea-3- benzamide was found to be HIV protease inhibitor; which is useful in the treatment of AIDS [21].

In addition to the nitrogen functionalities such as urea and thiourea which have been found in broad spectrum of biological activities like antidiabetic [22] especially P-heterocyclic pyridine urea and thiourea derivatives showed significant anti-inflammatory activities both *in vivo* and *vitro* [23] and also acts as antiviral especially piperazine doped with febuxostat urea and thiourea derivatives found to be promising antiviral agents against Tobacco Mosaic Virus [24]. The compounds penfluzuron [25] 1 and buprofezin [26] 2 are one class of the Insect Growth Regulators (IGR), which inhibit chitin synthesis and are responsible for the formation of insect cuticle, regorafinib 3 is a well-known anticancer drug and compound 4 (Figure 1) is found to be non-nucleoside inhibitor of the reverse transcriptase enzyme of HIV [27].

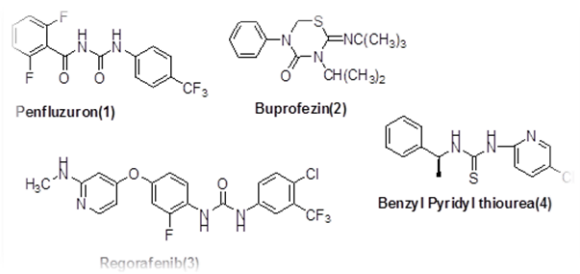


Figure 1: Some important drug molecules of urea and thiourea

A thorough investigation relating to the structure and the activity of the thiourea derivatives as well as their stability under biological conditions is required. These detailed investigations could be helpful in designing more potent antimicrobial agents for the therapeutic use since varying substituents on a basic structural frame work is a common method for drug design in medicinal chemistry.

In view of their broad spectrum of bioactivities the authors have designed and accomplished the synthesis of a series of new 1-(3,5-dichloro-4-hydroxy phenyl) urea/thiourea derivatives (3a-3j) and evaluated their antimicrobial and antioxidant activities.

MATERIALS AND METHODS

All the solvents and reagents were dried and purified before use by adopting the standard procedures and techniques. Progress of the reaction and purity of the compounds were monitored by Thin Layer Chromatography (TLC) on aluminium sheet of silica gel 60F 254, E-Merk, Germany using iodine as visualizing agent. Melting points (MP) were determined in open capillary tubes using a calibrated thermometer by GUNA digital melting point apparatus, expressed in degrees centigrade (°C) and are uncorrected. Infrared spectra (v in cm^{-1}) were recorded as KBr pellets on a Perkin-Elmer, FTIR 100 spectrophotometer and calibrated by using a standard polystyrene film. ^1H and ^{13}C-NMR spectra were recorded as solutions in Deuterated Dimethyl Sulfoxide (DMSO-d_6) on a Bruker AMX 400 MHz spectrometer operating at 400 MHz for ^1H, 100 MHz for ^{13}C-NMR. The ^1H and ^{13}C chemical shifts were expressed in Parts Per Million (ppm) with reference to Tetramethylsilane (TMS). A few of the compounds were recorded by Liquid Chromatography-Mass Spectra (LC-MS) on a LC-MS 2010, Shimadzu, Japan Mass spectrometer. Elemental analysis was performed on Thermo Finnigan instrument at University of Hyderabad. The reactions were carried out in a round bottomed 50 ml flask fitted with a reflux condenser, a magnetic stirrer-cum hot plate was used for stirring and heating the reaction mixtures. Rotary evaporator was used for removing the solvent from the reaction mixture.

General procedure for the synthesis of compounds (3a-j)

To a stirred solution of 4-hydroxy, 3,5-dichloro aniline (1) in dry Tetrahydrofuran (THF) (10 ml), various isocynates and isothiocynates (2) were added at 10-15°C in the presence of Triethylamine (TEA). After completion of the addition, the reaction mixture was stirred for 2-3 h at 60°C. The reaction progress was monitored by TLC. After completion of the reaction, $Et_3N.HCl$ was removed by filtration and the solvent in a Rota-evaporator to obtain crude product. It was purified by silica gel column chromatography eluting with hexane: ethylacetate (10-30%) to afford the title compounds 3(a-j) (Table 1).

1-(3,5-Dichloro-4-hydroxy phenyl)-3-(4-nitrophenyl) urea (3a): Yield-75%, light green solid, m.p. 180-182°C; IR (KBr) (V_{max} cm^{-1}): 1640 (C=O), 3214 (NH), 3354 (OH); ^1H-NMR (DMSO-d_6) δ ppm: 9.89 (s, 1H, OH), 9.59 (s, 1H, NH), 8.94 (s, 1H, NH), 7.67-7.61 (m, 4H, Ar-H), 7.59-7.56 (m, 2H, Ar-H); Molecular Formulae: $C_{13}H_9Cl_2N_2O_4$.

1-(3,5-Dichloro-4-hydroxyphenyl)-3-(2,4-difluorophenyl) urea (3b): Yield-80%, yash coloured solid, m.p. 185-187°C; IR (KBr) (V_{max} cm^{-1}): 1633 (C=O), 3290 (NH), 3414 (OH); ^1H-NMR (DMSO-d_6) δ ppm: 9.94 (s, 1H, OH), 8.82 (s, 1H, NH), 8.76 (s, 1H, NH),7.99-7.97 (m, 2H, Ar-H), 7.60 (s, 1H, Ar-H), 7.58 (s, 1H, Ar-H), 7.32 (s, 1H, Ar-H); Molecular Formulae: $C_{13}H_8Cl_2F_2N_2O_2$.

1-(3,5-Dichloro-4-hydroxyphenyl)-3-(4-bromophenyl) urea (3c): Yield-82%, yash colour solid, m.p. 167-169°C; IR (KBr) (V_{max} cm^{-1}): 1698 (C=O), 3229 (NH), 3565 (OH); ^1H-NMR (DMSO-d_6) δ ppm: 9.03 (s, 1H, NH), 8.85 (s, 1H, NH), 7.43-7.34 (m, 6H, Ar-H); ^{13}C-NMR (DMSO) δ ppm: 153.0 (C=O), 146.2, 139.6, 131.9, 131.8, 122.9, 120.7, 119.4, 113.7; Anal. Calcd. For $C_{13}H_9BrCl_2N_2O_2$: C-41.52, H-2.41, N-7.45; found: C-41.62, H-2.38, N-7.51. LC-MS m/z 374 (100, $M^{+\bullet}$), 376 (72, $(M+2)^{+\bullet}$), 372(45).

1-(3,5-Dichloro-4-hydroxyphenyl)-3-(3,4-dichlorophenyl) urea (3d): Yield-75%, yash colour solid, m.p. 190-192°C; IR (KBr) (V_{max} cm^{-1}): 1640 (C=O), 3317 (NH), 3549 (OH); ^1H-NMR (DMSO-d_6) δ ppm: 9.40 (s, 1H, OH), 9.28 (s, 1H, NH), 8.89 (s, 1H, NH), 7.75 (s, 1H, Ar-H), 7.40-7.21 (m, 4H, Ar-H); Molecular formulae: $C_{13}H_8Cl_4N_2O_2$.

1-(3,5-Dichloro-4-hydroxyphenyl)-3-(4-fluorophenyl) urea (3e): Yield-80%, dark yash colour solid, m.p 162-164°C; IR (KBr) (V_{max} cm^{-1}): 1649 (C=O), 3315 (NH), 3496 (OH); ^1H-NMR (DMSO-d_6) δ ppm: 8.76 (S, 1H, NH), 8.50 (S, 1H, NH), 7.36-7.31 (m, 6H, Ar-H); Molecular formulae: $C_{13}H_9FCl_2N_2O_2$.

1-(3, 5-Dichloro-4-hydroxyphenyl)-3-(1-allyl) thiourea (3f): Yield-68%, dark cement colour solid, m.p 175-177°C; IR (KBr) (V_{max} cm^{-1}): 1347 (C=S), 3428 (NH), 3547 (OH);^1H-NMR (DMSO-d_6) δ ppm: 9.74 (s, 1H, NH), 9.13 (s, 1H, NH), 7.42-7.21 (m, 2H, Ar-H), 5.76-5.71 (m, 4H, CH_2=CH), 4.99-4.96 (m, 2H, Ali-H); Molecular formulae: $C_{10}H_{10}Cl_2N_2OS$.

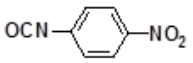
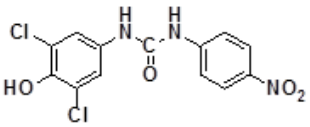
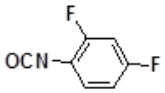
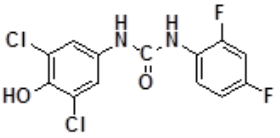
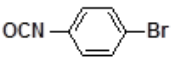
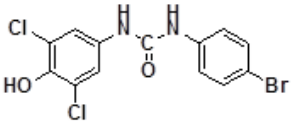
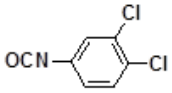
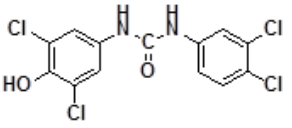
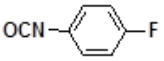
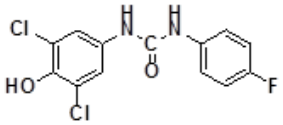
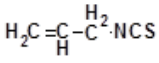
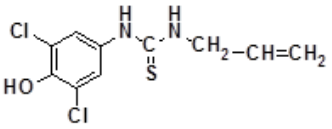
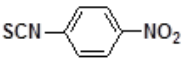
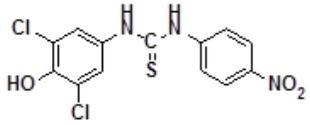
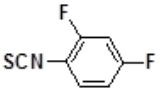
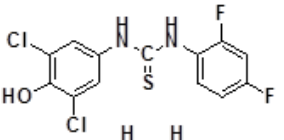
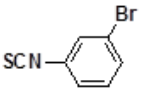
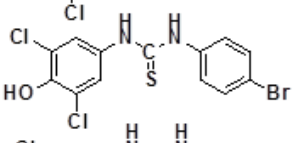
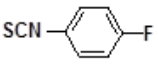
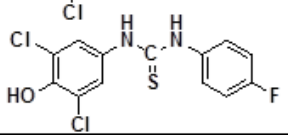
1-(3,5-Dichloro-4-hydroxyphenyl)-3-(4-nitrophenyl) thiourea (3g): Yield-75%, yellow colour solid, m.p. 205-207°C; IR (KBr) (V_{max} cm^{-1}): 1318 (C=S), 3270 (NH), 3563 (OH); ^1H-NMR (DMSO-d_6) δ ppm: 10.42 (s, 1H, NH), 10.14 (s, 1H, NH), 8.30- 8.18 (m, 2H, Ar-H), 7.86-7.77 (m, 2H, Ar-H), 7.49-7.41 (m, 2H, Ar-H); ^{13}C-NMR (DMSO) δ ppm: 180.2 (C=S), 147.2, 146.4, 143.0, 132.0, 125.6, 125.3, 124.9, 124.8, 122.6, 122.4, 122.2, 122.1; Anal. Calcd. For $C_{13}H_9Cl_2N_3O_3S$: C-43.59, H-2.53, N-11.73; found: C-4.52, H-2.58, N-11.65. LC-MS m/z 355 (100, $M^{+\bullet}$), 357 (66, $M+2)^{+\bullet}$.

1-(3, 5-Dichloro-4-hydroxyphenyl)-3-(2, 4-difluorophenyl) thiourea (3h): Yield-70%, cement colour solid, m.p. 185-187°C; IR (KBr) (V_{max} cm^{-1}): 1349 (C=S), 3237 (NH), 3478 (OH); ^1H-NMR (DMSO-d_6) δ ppm: 9.82 (s, 1H, OH), 9.48 (s, 1H, NH), 9.08 (s, 1H, NH), 7.56-7.30 (m, 4H, Ar-H), 7.10 (s, 1H, Ar-H). Molecular formulae: $C_{13}H_8Cl_2F_2N_2OS$.

1-(3, 5-Dichloro-4-hydroxyphenyl)-3-(3-bromophenyl) thiourea (3i): Yield-68%, dark cement colour solid, m.p. 171-173°C; IR (KBr) (V_{max} cm^{-1}): 1356 (C=S), 3330 (NH), 3584 (OH); ^1H-NMR (DMSO- d_6) δ Ppm: 8.68 (s, 1H, NH), 8.46 (s, 1H, NH), 7.43-7.11 (m, 6H, Ar-H); Molecular Formulae: $C_{13}H_9Cl_2BrN_2OS$.

1-(3, 5-Dichloro-4-hydroxyphenyl)-3-(4-fluorophenyl) thiourea (3j): Yield-80%, yash colour solid, m.p. 185-187°C; IR (KBr) (V_{max} cm^{-1}): 1347 (C=S), 3400 (NH), 3654 (OH); ^1H-NMR (DMSO-d_6) δ ppm: 9.89 (s, 1H, OH), 9.59 (s, 1H, NH), 8.94 (s, 1H, NH) 7.56-7.10 (m, 6H, Ar-H); ^{13}C-NMR (DMSO) δ ppm: 180.2 (C=S), 162.7, 142.6, 134.1, 132.5, 131.O, 126.4, 125.7, 122.6 ; Molecular formulae: $C_{13}H_9Cl_2N_2O_4$.

Table 1: Physical data of the new urea/thiourea compounds (3a-j)

S.No	R-X	Product	Yield(%)	m.p (°C)
3a			75	180-182
3b			80	185-187
3c			82	167-169
3d			75	190-192
3e			70	162-164
3f			68	175-177
3g			75	205-207
3h			70	185-187
3i			68	171-173
3j			72	190-192

BIOLOGICAL ACTIVITY

Antioxidant activity

All the newly synthesized urea/thiourea derivatives 3(a-j) were screened for their antioxidant activity by three radical scavenging methods of 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Hydrogen Peroxide (H_2O_2), nitric oxide.

DPPH radical scavenging activity

The DPPH [28], has been widely used to evaluate the free radical scavenging capacity of the synthesized antioxidants. The strong absorption maximum at 517 nm was observed in the DPPH due to the presence of radical when the radical of the DPPH becomes paired with an electron or acceptance of the hydrogen radical from the antioxidant then the absorbance of DPPH reduces. All the newly synthesized urea/thiourea derivatives 3(a-j) and natural antioxidant, ascorbic acid were prepared in different concentrations (50 and 100 µg/ml) in DMSO and homogeneity of the test samples were attained using magnetic stirrer. DPPH solution was prepared in methanol and adjusted the concentration to 0.004% w/v by adding MeOH. The DPPH solution (4 ml, 0.004% w/v) was added to aliquot of standard solution and tested samples solution (1 ml of each) of various concentrations in a set of test tubes and shaken vigorously. Recorded the room temperature and kept for 30 min. in the dark to complete the reaction the absorbance values of the tested samples were recorded against blank at 517 nm. The natural antioxidant, ascorbic acid was used as a positive control. The scavenging capacity of DPPH radicals was calculated using the following equation. The experiment was repeated in triplicate and the average values are tabulated in Table 2.

% of scavenging = [Abs DPPH-Abs Sample/Abs DPPH] × 100

Where, DPPH is the absorbance of the control (DPPH solution without the test compound solution) and Asample is the absorbance of the test sample DPPH solution with the test compound solution).

Table 2: Antioxidant activity of urea/thiourea derivatives 3(a-j) by DPPH method

Compound	% of activity in different concentrations in µg/ml	
	50 µg/ml	100 µg/ml
3a	60.74	66.15
3b	58.54	65.41
3c	78.23	83.53
3d	68.28	74.13
3e	72.51	76.59
3f	82.66	89.39
3g	59.05	66.45
3h	75.95	81.14
3i	70.06	74.89
3j	54.23	59.86
Std	79.61	86.15

Std-Ascorbic acid was used as a standard

H_2O_2 scavenging activity

The H_2O_2 radical scavenging activity of the tested samples was determined according to the protocol of Navabi et al [29]. H_2O_2 40 mm solution was prepared in phosphate buffer (pH 7.4) and the concentration of H_2O_2 was determined by absorption at 230 nm using a spectrophotometer. Two concentrations (50 and 100 µg/ml) of standard antioxidant, Butylated Hydroxy Toluene (BHT) and synthesized compounds in a set of test tubes were prepared. The absorbance of tested samples at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2. The experiment was carried out in triplicate and the results are presented in Table 3.

Table 3: Antioxidant activity of urea/thiourea derivatives 3(a-j) by H_2O_2 method

Compound	% of activity in different concentrations in µg/ml	
	50 µg/ml	100 µg/ml
3a	74.53	79.13
3b	65.42	70.15
3c	70.33	74.58
3d	79.54	89.32
3e	70.86	76.27
3f	69.50	73.26
3g	76.45	83.83
3h	66.24	70.26
3i	77.79	85.57
3j	71.25	75.62
Std	76.98	85.41

Std-Butylated hydroxy toluene was used as a standard

Nitric oxide (NO) radical scavenging method

Green and Marcocci [31-33], modified method was employed for investigation of the nitric oxide radical scavenging activity of the title compounds. All the newly synthesized compounds and natural antioxidant, ascorbic acid in two concentrations (50 and 100 µg/ml) were prepared in DMSO and the homogeneous solutions were achieved by stirring on magnetic stirrer. Nitric oxide radicals were generated from 1 ml of sodium nitroprusside (10 mM) and 1.5 ml of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations of the test compounds in a set of test tubes and incubated for 150 min at 25°C. After incubation, 1 ml of the reaction mixture was treated with 1 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dichloride) and absorbance of the chromophore was measured at 546 nm. The standard used in the present method was BHT. Nitric oxide scavenging activity was calculated by the following equation.

$$\% \text{ of scavenging} = [(A_{control}-A_{sample})/A_{blank}] \times 100$$

Where, Acontrol is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Tests were carried out in triplicate, and the average value is taken and the results are presented in Table 4.

Table 4: Antioxidant activity of urea/thiourea derivatives 3(a-j) by nitric oxide method

Compounds	% of activity in different concentrations in µg/ml	
	50 µg/ml	100 µg/ml
3a	52.07	60.35
3b	55.72	64.49
3c	54.02	62.23
3d	65.18	72.31
3e	68.25	76.25
3f	70.32	75.16
3g	77.48	81.41
3h	68.50	73.24
3i	61.24	66.85
3j	68.73	72.30
Std	78.42	85.59

Std-Butylated hydroxy toluene was used as a standard

Antibacterial activity

The synthesized urea and thiourea derivatives 3(a-j) of 4-hydroxy-3,5-dichloro aniline were tested for their antibacterial potency against four bacterial strains such as *Bacillus subtilis* and *Staphylococcus aureus* and *Pseudomonas aeruginosa* and *Escherichia coli* at 100 µg/ml concentration. Agar well diffusion method [32,34] was followed to examine the activity and norfloxacin was used as a standard. Each title compound (2 mg), standard drug (norfloxacin) were dissolved in 2 ml of DMSO and further diluted to prepare 100 µg/ml concentration of test solution and accurately 1 ml of these prepared samples were used for test. Centrifuged pellets of bacteria from a 24 h old culture containing approximately 10^5-10^6 Colony Forming Unit (CFU) per ml was spread on the surface of Muller Hington Agar (MHA) plates. Nutrient agar medium was prepared by suspending nutrient agar 20 g in one liter of distilled water (pH 7.0), autoclaved and cooled to 45°C. Then it was seeded with 10 ml of prepared inoculum to have 10^6 CFU/ml. Petri dishes were prepared by pouring 75 ml of seeded nutrient agar. Wells were created in medium with the help of a sterile metallic borer and test solutions were added. Experimental plates were incubated for 24 h and antibacterial activity was assayed by measuring zones of inhibition in diameter around the well. The zone of inhibition of the tested solution was compared with standard. The bacterial assays were performed in triplicate and average results are presented in Table 5. It is established that DMSO does not inhibit the growth of bacterial and fungal strains.

Table 5: Antibacterial activity of the title urea and thioureas 3(a-j)[a]

Compounds*	Zone of inhibition (mm)			
	Staphylococcus aureus	***Bacillus subtilis***	***Escheriea coli***	***Pseudomonas aeruginosa***
3a	14.7	18.5	15.0	20,2
3b	9.5	18.0	13.6	19.5
3c	12.2	15.8	8.5	17.3
3d	6.9	16.2	8.0	15.5
3e	10.6	15.7	10.3	16.4
3f	9.0	16.5	7.4	14.5
3g	12.5	19.5	11.9	19.0
3h	8.2	15.6	11.0	14.8
3i	10.2	20.2	12.5	16.0
3j	13.1	19.5	14.0	20.0
Std	32.1	28.5	35.0	28.6

*Concentration 100 µg/ml; a-The zone of inhibition of the title compounds was measured in mm; Std-Norfloxacin was used as a standard

Antifungal activity

The antifungal strains, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* were used to investigate antifungal potency of the newly synthesized urea and thiourea derivatives 3(a-j) using the agar disc-diffusion method [35-38]. The fungal strains were maintained in Potato Dextrose Agar (PDA) medium (Hi-Media), a loopful of culture from the slant was inoculated in to the potato dextrose broth and incubated at 37°C for 48-72 h. Then 0.1 ml of the culture was spread on the potato dextrose agar plate and a sterile glass spreader was used for even distribution of the inoculum. All the compounds were dissolved in DMSO. Sterile discs of Whatman No.1 filter paper of about 6 mm diameter were impregnated on the surface of the media. Blank test showed that DMSO does not affect the growth of the test organisms. 100 µg/ml concentration of the test compounds were prepared and applied on the discs and incubated at 37°C for 48-72 h. The zone of inhibition around the disc was calculated as the edge to edge zone of the confluent growth in millimeters. All tests were repeated three times and the average data was taken as the final result. Nystatin was used as a reference drug and the inhibition zones of the test compounds were compared with controls **(**Table 6).

Table 6: Antifungal activity of the title urea and thioureas 3(a-j)[a]

Compound*	Zone of inhibition (mm)		
	Aspergillus niger	***Bacillus albicans***	***Fusarium oxysporum***
3a	13.4	14.2	13.9
3b	20.5	19.1	22.7
3c	9.2	13.2	18.1
3d	22.6	19.3	22.5
3e	10.5	14.9	14.2
3f	20.1	19.5	18.9
3g	22.5	19.6	24.7
3h	15.3	20.1	18.6
3i	9.5	20.4	23.7
3j	10.3	15.2	14.3
Std	26.8	23.0	28.5

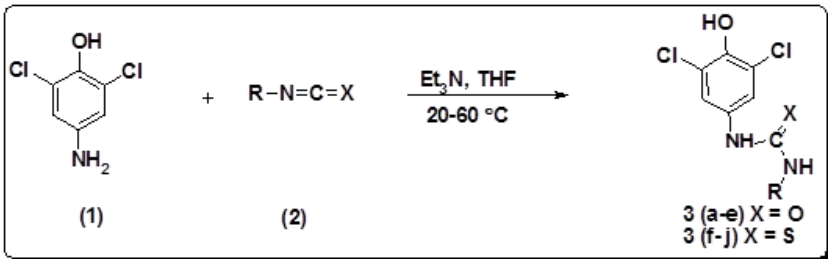
a-The zone of inhibition of the title compounds was measured in mm; *concentration 100 µg/ml; **Std**-Nystatin was used as a standard

RESULTS AND DISCUSSION

Chemistry

3,5-Dichloro-4-hydroxyaniline (1) was reacted with bioactive phenylisocyantes 2a-e and phenyisothiocyanates 2f-j in THF using triethylamine as a base at 60°C to afford 1- (3, 5-dichloro-4-hydroxy phenyl) substituted urea derivatives 3(a-e) and the corresponding thiourea derivatives 3(f-j) in high yields. The progress of the reaction was monitored by TLC. After completion of the reaction; the reaction mixture was concentrated under vacuum and then subjected to silica gel column chromatography using Ethyl acetate: Hexane (1:2) as an eluent and obtained pure products.

The IR spectra of 3a-j showed stretching absorptions in the regions 3558-3635, 3228-3428, 1630-1698, 1318-1350 cm^{-1} for O-H, N-H, C=O and C=S respectively. The proton NMR chemical shifts for compounds 3(a-j) are given in experimental part. The NH protons attached to –C=O/-C=S appeared as two distinct singlets in the region 8.50-9.80 ppm and the OH protons resonated as singlets in the region 9.25-10.38 ppm. The aromatic protons are resonated in their corresponding regions as singlet/doublet/triplet/multiplets based on their structure orientation. The ^{13}C-NMR chemical shifts for three of the title compounds (3c, 3g, 3j) are given in the experimental part. Aromatic carbons of the title compounds gave signals in the expected region. The C=O carbon of the compound 3c resonated at 153.0 ppm whereas the –C=S carbon of the compounds 3g and 3j gave a singlets at δ=180.2. Elemental analysis was obtained for two of the title compounds. They gave satisfactory C, H, N elemental analytical data. The molecular ions and isotopic ions of obtained mass spectra (3c & 3g) are in good agreement with the proposed structures. All the newly synthesized compounds were screened for antioxidant, antibacterial and antifungal activity (Scheme 1).



Scheme 1: Synthesis of urea and thiourea derivatives 3(a-j) from 3,5-dichloro-4-hydroxy-aniline

Antioxidant activity

The antioxidant activity of the titled compounds was evaluated by DPPH method, H_2O_2 scavenging method and nitric oxide scavenging method at two different concentrations 50 and 100 µg/ml. Antioxidant data results revealed that compounds 3f and 3c in DPPH method, 3d and 3i in H_2O_2 method and 3f and 3g in nitric oxide method exhibited good scavenging activities. Respectively the reason might be the presence of nitro, bromine and chloro groups in the urea/thioureas which are involved in rapid abstraction of free radicals. The rest of the compounds showed moderate antioxidant activity and the results are summarized in Table 2 (DPPH method), Table 3 (H_2O_2 method) and Table 4 (Nitric oxide method) respectively.

Antimicrobial activity

All the newly synthesized compounds were screened for their antibacterial activity against two Gram-positive bacterial strains *B. subtilis* and *S. aureus* and two Gram-negative bacterial strains *P. aeruginosa* and *E. coli* using agar well diffusion method. Norfloxacin was used as an antibacterial standard. The results revealed that majority of the tested compounds 3a-j showed moderate to good inhibitory activity against all the strains. Compounds 3a > 3j > 3c > 3g showed high activity. The reason might be presence of nitro group, bromo and fluoro groups attached to the aryl ring and the antibacterial zones of inhibition (mm) of the title compounds are given in Table 5.

The antifungal activity of the newly synthesized compounds 3a-j were screened against three fungal pathogens *A. niger*, *C. albicans* and *F. oxysporum* using disc diffusion method and the results were compared with the standard drug, Nystatin. Among the compounds 3d > 3g > 3b > 3f exhibited significant inhibition activity at 100 µg/ml against fungal pathogens. The compounds 3h, 3a and 3j showed moderate activity. The diameter of zones of inhibition values are given in Table 6.

CONCULSION

In the present study, a series of new 1- (3, 5-Dichloro-4-hydroxy phenyl) substituted urea/thiourea derivatives were synthesized in high yields adopting simple procedure. The new title compounds were assessed for antimicrobial and anti-oxidant activity. All the title compounds 3(a-j) screened for their antioxidant activity by DPPH radical-scavenging, H_2O_2 radical scavenging and nitric oxide radical scavenging methods. Antimicrobial screening data of the title urea and thiourea derivatives 3(a-j) revealed that majority of the compounds showed potent antifungal activity. A few of the title compounds exhibited good anti-oxidant activity in all the three methods.

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