

SYNTHESIS AND *IN-VITRO* ANTIBACTERIAL ACTIVITY OF SOME NEW UREA, THIOUREA AND THIOSEMICARBAZIDE DERIVATIVES

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ABSTRACT

Five title compounds: (1-(4-chlorophenyl)(2-hydroxy phenyl) methyl urea), (1-(2-hydroxyphenyl)(4-hydroxyphenyl) methyl urea), (1-(4-chlorophenyl)(2-hydroxyphenyl) methyl thiourea), (2-(4-chlorophenyl)(2-hydroxyphenyl) methyl hydrazine carbo thioamide), (2-(2-hydroxyphenyl)(4-hydroxyphenyl) methyl hydrazine carbo thioamide) have been synthesized by condensing urea, thiourea or thiosemicarbazide with phenol and substituted aromatic aldehydes in one pot. The newly synthesized compounds have been characterized by spectral data. All the test compounds were screened for *in vitro* antibacterial activity against *B.Subtilis*, *S.Aureus*, *S.Typhi* and *S.Dysentery* and showed significant antibacterial activity against the bacterial strains used. The values compare well with the potency of Ampicillin in respective assay.

Keywords: Phenol, Benzaldehyde, Thiosemicarbazide, Antibacterial activity.

INTRODUCTION

Although a large number of antibiotics and chemotherapeutics are available for medical use, the antimicrobial resistance created a substantial need of new class of antibacterial agents in the last decade's¹⁻⁴. Derivatives of urea, thiourea and thiosemicarbazide play a vital role in the field of medicinal chemistry by regulating various pharmacological activities. Literature survey reveals that urea and thiourea derivatives showed a broad spectrum of biological Activities as anti-HIV, antiviral, HDL- elevating antibacterial, analgesic properties⁵⁻⁸. 1, 3- disubstituted urea derivatives by condensation with benzaldehyde were evaluated for their antiproliferative activity against a panel of human tumor cell lines⁹. The survey also reveals that the thiosemicarbazide derivatives were found to posse's antibacterial activity against *S.aereus* and *E.coli*. Encouraged by these observations, it was thought worthwhile to synthesize some new title compounds and screen them for their possible antibacterial activity. In the present work, the synthesis and antibacterial activity of some new urea, thiourea and thiosemicarbazide derivatives (Compounds 1-5) were reported.

MATERIAL AND METHODS

All reagents and solvents were purchased from Merck and used without further purification. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Crude products were purified by column chromatography on silica gel of 60-120 mesh. IR spectral data were recorded on Thermo Nicolet FT-IR spectrophotometer in KBr medium, ¹H-NMR data recorded as solutions in DMSO-*d*₆ on a Bruker AC 300 MHz spectrometer and LC-MS spectra were recorded on a Jeol-8X 102 (FAB) Mass Spectrometer. The test compounds were synthesized by the following procedures.

Synthesis of compound-1: (1-(4-chlorophenyl) (2-hydroxyphenyl)methyl) thiourea); (Scheme-1)

To the ethanol solution of phenol (0.01 moles; 0.941g) and 4-chloro benzaldehyde (0.01moles; 1.4g), a hot solution of 0.01 moles of thiourea (0.76g) in ethanol was added drop wise with stirring and the reaction mixture was heated at 80°C for 6hrs with constant stirring. The mixture was then left overnight in a freezer. The formed solid was filtered off, dried and recrystallized from ethanol to afford 2.05g (70%) of **1**; M.P: 202-218° C.

IR (KBr/ max cm⁻¹): 3507 (AR-OH; stretching), 3236 (NH₂ stretching), 1538, (C=S), 1232 (CN), 770 (C-Cl, STRETCHING). ¹H NMR (DMSO-*d*₆): δ 7.38- 6.9 (m, 8H, Ar-H), δ 9.68 (s, aromatic C-OH), δ 9.53 (s, NH₂, thiourea), δ 2.7 (s, methine) LC-MS m/z: 292. Elemental analysis calculated For C₁₄H₁₃ClN₂O₂S: C, 57.43; H, 4.48; Cl, 12.11; N, 9.57; O, 5.46; S, 10.95

Synthesis of Compound-2: (1-(4-chlorophenyl) (2 hydroxyphenyl) methyl urea); (Scheme-2)

To a mixture of phenol (0.01 moles: 0.941g) and 4- chloro benzaldehyde (0.01moles; 1.4g), a solution of 0.01 moles of urea (0.60g) in hot ethanol was added drop wise with stirring and the reaction mixture was heated at 80-90°C for 6hrs with constant stirring. After the mixture was cooled the formed solid separated was filtered off, dried and recrystallized from ethanol to afford 1.88g (68%) of **2**; M.P: 176-188° C.

IR (KBr) (ν, cm⁻¹): 3504 (Ar-OH; stretching), 3247 (NH₂; stretching), 1635 (C=O), 1235 (CN) 780 (C-Cl, Stretching). ¹H NMR (DMSO-*d*₆): δ 7.4-6.8 (m, 8H, Ar-H), δ 11.8 (s, H, OH), δ 11.5 (s, 2H, NH₂), δ 8.27 (d, H, NH), δ 3.8 (q, H, NH), δ 2.9 (s, H, CH). LC-MS m/z: 276. Elemental analysis calculated For C₁₄H₁₃ClN₂O₂: C, 60.77; H, 4.74; Cl, 12.81; N, 10.12; O, 11.56.

Synthesis of compound-3: (2-(4-chlorophenyl) (2-hydroxyphenyl) methyl) hydrazine carbothioamide); (Scheme-3)

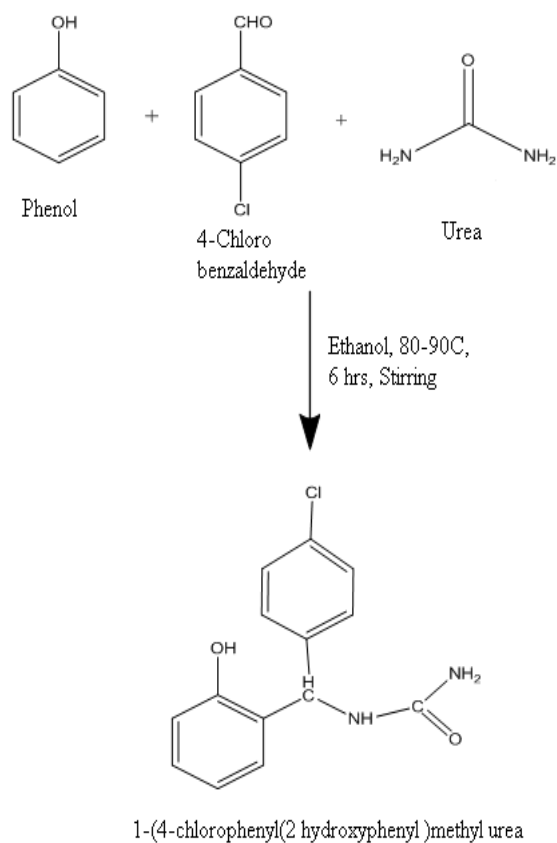
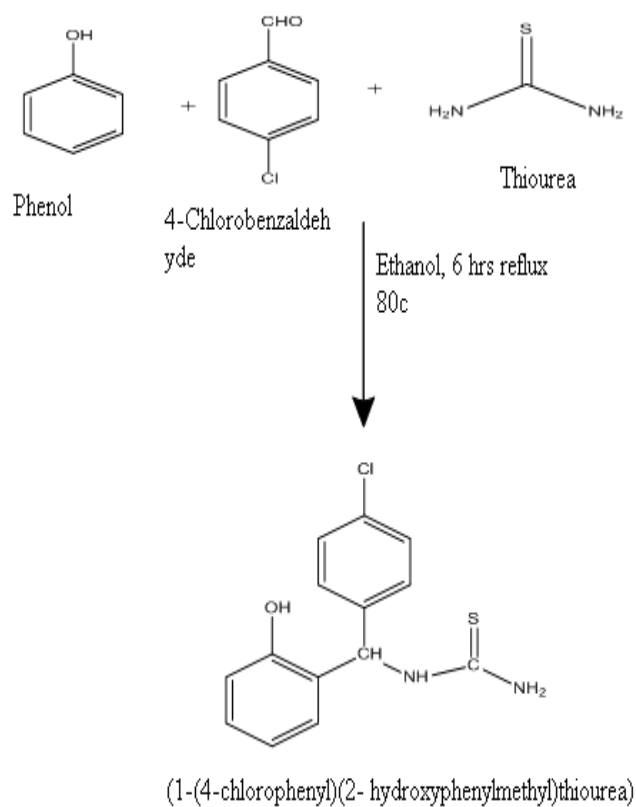
To a mixture of phenol (0.01 moles; 0.941g) 4- chloro benzaldehyde (0.01moles; 1.4g), a solution of 0.01 moles of thiosemicarbazide (0.91g) in hot ethanol was added drop wise with stirring and the reaction mixture was stirred at room temperature for 5hrs. After the mixture was cooled for 24 hrs, the formed solid was filtered off, washed with hexane, dried and recrystallized from ethanol to afford 2.18g (71%) of **3**; M.P: 176-190° C;

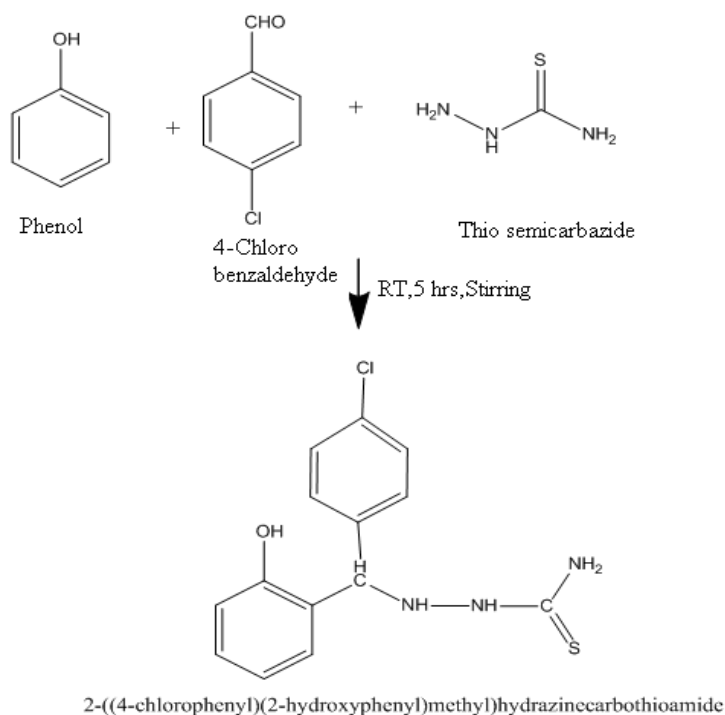
IR (KBr) (ν, cm⁻¹): 3500 (Ar-OH; stretching), 3242 (NH₂; stretching), 1534 (CS), 1294 (CN), 760 (C-Cl, Stretching). ¹H NMR (DMSO-*d*₆): δ 7.52- 6.9 (m, 8H, Ar-H), δ 11.4 (s, H, OH), δ 11.2 (s, 2H, NH₂), δ 8.04 (d, H, NH), δ 3.4 (q, H, NH), δ 2.5 (s, H, CH). LC-MS m/z: 307. Elemental analysis calculated For C₁₄H₁₄ClN₃O₂S: C, 54.63; H, 4.58; Cl, 11.52; N, 13.65; O, 5.20; S, 10.42.

Synthesis of Compound-4: (2-(2-hydroxyphenyl) (4-hydroxyphenyl) (methyl) hydrazinecarbothioamide); (Scheme-4)

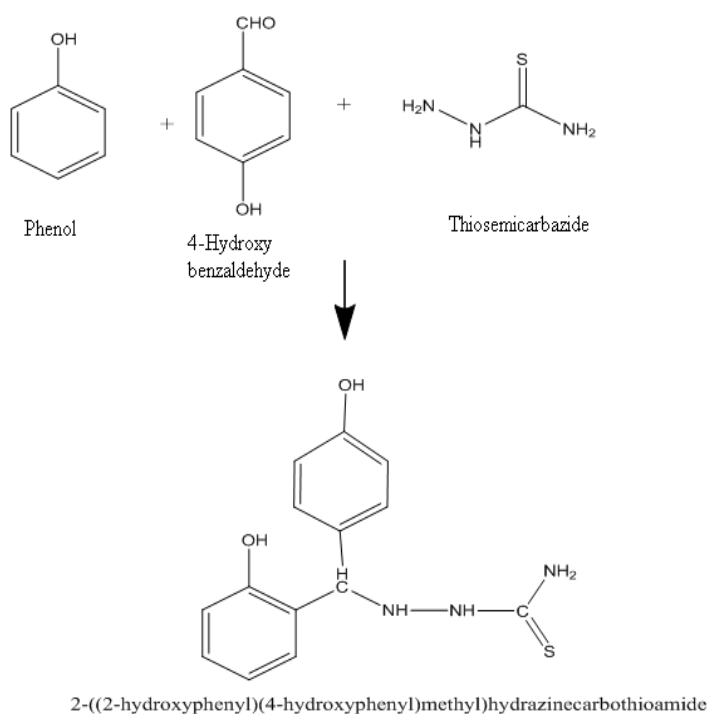
To a mixture of phenol (0.01moles; 0.94g) and 4-hydroxybenzaldehyde (0.01moles; 1.22g), a small amount of 0.01 moles; (0.91g) of thiosemicarbazide was added dropwise in an icebath and the reaction mixture was stirred continuously for 6hours. The separated solid was filtered off, washed with hexane, dried and recrystallized from ethanol to afford 1.87g (67%) of **4**; M.P: 190-202° C;

IR (KBr) (ν, cm⁻¹): 3510 (Ar-OH; stretching), 3041 (C-H stretching), 1534 (CS). ¹H NMR (DMSO-*d*₆): δ 7.43-7.14 (8H, m, Ar-H), δ 11.4 (s, H, OH), δ 11.2 (s, 2H, NH₂), δ 8.04 (d, H, NH), δ 3.4 (q, H, NH), δ 2.5 (s, H, CH). LC-MS m/z: 289. Elemental analysis calculated For C₁₄H₁₅N₃O₂S: C, 58.11; H, 5.23; N, 14.52; O, 11.06; S, 11.08





Scheme-3



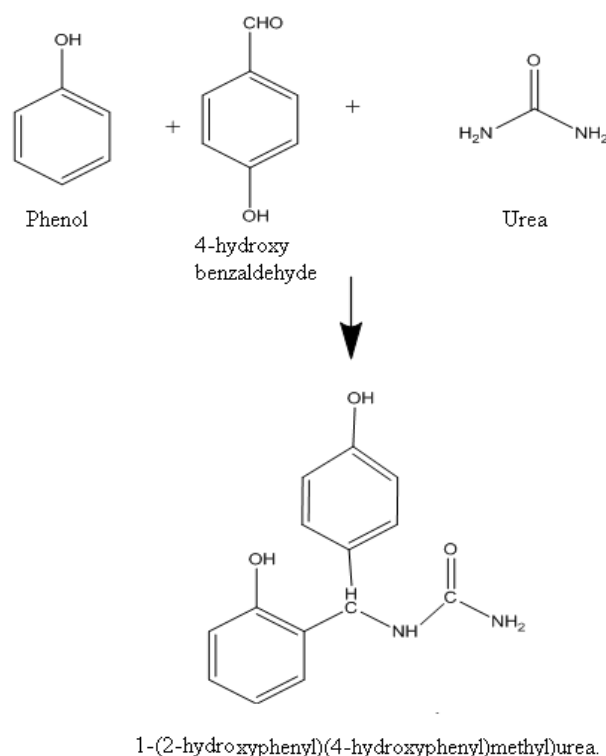
Scheme-4

Synthesis of Compound-5: (1-(2-hydroxyphenyl) (4-hydroxyphenyl) methyl) urea; (Scheme-5)

To a mixture of phenol (0.01moles; 0.94g) and 4-hydroxybenzaldehyde (0.01moles; 1.22g), a small amount of 0.01 moles; 0.60g of urea was added drop wise at 80°C in a water bath and the reaction mixture was stirred continuously for 6 hours. And the reaction mixture was washed with hexane. The formed solid was filtered off,

dried and recrystallized from ethanol to afford 1.76g (68%) of 5; M.P: 178-180° C;

IR (KBr) (ν , cm⁻¹): 3504 (Ar-OH; stretching), 3247 (NH₂; stretching), 1635 (C=O), 1235 (CN). ¹H NMR (DMSO-*d*₆): δ 7.2- 6.9 (m, 8H, Ar-H), δ 11.8 (s, H, OH), δ 11.5 (s, 2H, NH₂), δ 8.27 (d, H, NH), δ 3.8 (q, H, NH), δ 2.9 (s, H, CH). LC-MS m/z: 258. Elemental analysis calculated For C₁₄H₁₄N₂O₃: C, 65.11; H, 5.46; N, 10.85; O, 18.58.



Scheme-5

Antibacterial assay

The antibacterial assay includes two Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and gram negative bacteria: *Salmonella typhi*, *Shigella dysentery* which were obtained from the Department of Biotechnology, GITAM University, Visakhapatnam. Ampicillin was used as a positive control while DMSO served as a negative control. Agar well diffusion method¹⁰⁻¹² was used to determine the antibacterial efficacy of the synthesized compounds. Bacterial inoculum (105CFU/ml) was spread on Muller Hilton agar. After the inoculum dried, 6mm diameter wells were made in the agar plate with a sterile cork borer. The synthesized compounds dissolved in DMSO were added to the wells at a concentration of 10mg/ml. The petriplates were incubated at 37°C for 24 hrs. The zones of inhibition were measured in mm to estimate the potency of the test compounds. The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The compounds **4**, **1**, **5**

showed very good activity against all the bacterial strains. The observed zone of inhibition is presented in **table-1**.

RESULTS AND DISCUSSION

Phenol and 4-chlorobenzaldehyde were condensed with urea, thiourea and thiosemicarbazide to give compounds 1-3. Similarly phenol and 4-hydroxybenzaldehyde were condensed with urea and thiosemicarbazide to give compounds 4-5. The title compounds 1-5, exhibited characteristic bands in their IR spectra, in the regions 3507(Ar-OH; stretching), 3236 (NH₂ stretching), 1538 (C=S), 1232(CN), 770(C-Cl, Stretching). The proton NMR spectral data of the title compounds 1-5 the methine proton appeared as singlet at δ 2.7, the entire aromatic protons resonated as multiplet at 7.43-6.90 and M⁺ ion peaks in their LC-MS at m/z ranged from 292, 276, 307, 289, 258 respectively. The purity and homogeneity of the compounds were confirmed by melting point and thin layer chromatography. The antimicrobial efficacies for the synthesized compounds (1-5) are reported in **table 1**.

Table 1: Effect Of Synthesised Compounds On The Selected Bacterial Strains Diameter of the zone of inhibition (in mm)

S. No.	Name Of The Organism	Compound-1	Compound-2	Compound-3	Compound-4	Compound-5	Ampicillin
1	Staphylococcus aureus	12	11	10	13	15	16
2	Bacillus subtilis	16	11	10	13	14	16
3	Salmonella typhi	12	13	14	16	12	NA
4	Shigella dysentery	14	15	12	16	12	NA

Ampicillin was used as a standard for the antibacterial activity. The hydroxyl substituted compounds showed pronounced antibacterial activity. Literature survey reveals that electron withdrawing or donating groups amend the lipophilicity of the test compounds, which in turn alters permeability across the bacterial cell membrane. Among the tested compounds for the anti bacterial efficacy compound-1 found to be more efficient than the other synthesized compounds against *Bacillus subtilis*. Compound -5 is more efficient than the other compounds against *S.aureus*. Ampicillin has no effect on gram negative bacteria (*S.typhi*,

S.dysentery) whereas all the synthesized compounds are effective against the gram negative bacteria.

CONCLUSION

All the newly synthesized compounds showed moderate to good potency against different bacterial strains. Out of which compound-1, 4 & 5 showed prominent antibacterial activity which develops a productive environment for the development of new class of antibacterial agents.

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