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Research Article

Piperazine and thiourea containing analogs of phenyl acetic acid: Synthesis and their antimicrobial activity

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Abstract : New classes of compounds have been synthesized from acidchloride of 2-[(2,6-dichlrophenyl) amino]phenyl acetic acid and 2-[(2,6-dichlrophenyl)amino]phenyl acetoxy acetic acid by treating with various substituted piperazine. Compounds **15a-o** have been prepared from arylthiourea. The structures of new compounds were supported by elemental analysis IR and ^{1}H NMR. All the compounds were evaluated for their antibacterial and antifungal activity.

INTRODUCTION

Heterocyclic compounds have enjoyed a prominent place in medicinal chemistry. Piperazine derivatives have been the subject of chemical and biological studies due to their various interesting pharmacological activities. Historically, most of them have been used as antihelminthics agent with very wide therapeutic index. Their therapeutic effects have been studied for number of pathological conditions including antibacterial [1,2], anticancer [3], antidepressant [4], anti-HIV [5], anti-inflammatory [6], antitumor [7], antimalarial [8] and antioxidative [9]. Thiourea derivatives have been suggested for potential therapeutic agents by several investigators. They have used as antibacterial [10], anticancer [11,12], antimalarial [13], antitubercular [14], antiviral [15], anti-HIV [16] and anti-nociceptive [17], Looking to the large amount of work has been carried out so far; the field is wide open and need extensive investigation to understand the biological activities. Attention has been devoted to the development of safe and effective heterocycles that display noticeable medicinal activities. New challenging problems are multi-drug resistant microorganisms, which pose to the medicinal chemist. Based on this

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background and continuation of our research program on the synthesis new compounds with phenyl acetic acid derivatives [18-20]; we have decided to explore new biologically active compounds. Ultimately, this approach leads us to focus on to study antibacterial and antifungal activity of piperazine and thiourea derivatives. The synthesis of new derivatives has been outlined in Scheme-**I**, **II** and **III**. The antibacterial and antifungal activities were compared with standard drug penicillin, chloramphenicol, ampicillin and griseofulvin respectively. The antibacterial and antifungal testing was carried out by ***cup-plate*** method [21].

The structures of compounds were established from their elemental analysis and spectral data. The absorption bands of –COOH group of lead molecules **(1** and **8)** observed at 1730 cm^{-1}, which sifted to 1675-1650 cm^{-1} after the formation of amides. The IR spectrum of piperazine containing phenyl acetic acid derivatives showed absorption bands at 1114-1090 cm^{-1} for C-N (piperazine), 1367-1345 cm^{-1} for C-N, 789-778 cm^{-1} for C-Cl, 3450-3433 cm^{-1} corresponds to –NH stretching; for $–CH_2$ showed asymmetric at 2929-2915 cm^{-1} and symmetric stretching at 2848-2834 cm^{-1}; The amides displayed a strong absorption band near 1675-1650 cm^{-1} indicate the presence of carbonyl of secondary amide I, 1565-1550 cm^{-1} amide II and 1245-1230 cm^{-1} of amide III. Thiourea derivatives showed absorption band at 1170-1150 due to C=S stretching vibration, one sharp band observed near 1750-1735 cm^{-1} indicate the C=O stretching for ester linkage and band observed near 1280-1000 cm^{-1} indicate the C-O stretching vibration. ^1H NMR for 8 protons of piperazine showed a multiplet at δ 2.76-3.40 and a singlet observed at δ 3.52-3.73 for $–CH_2$-CO- protons. Phenyl acetoxy acetic acid derivatives of piperazine showed singlet at δ 3.71-3.82 and δ 4.52-4.69 for $–CH_2COO$- and $–O-CH_2$-CO- respectively. Thiourea derivatives showed singlet at δ 8.38-8.52 and 8.05-8.15 for –C=S-NH- and –CONH- respectively.

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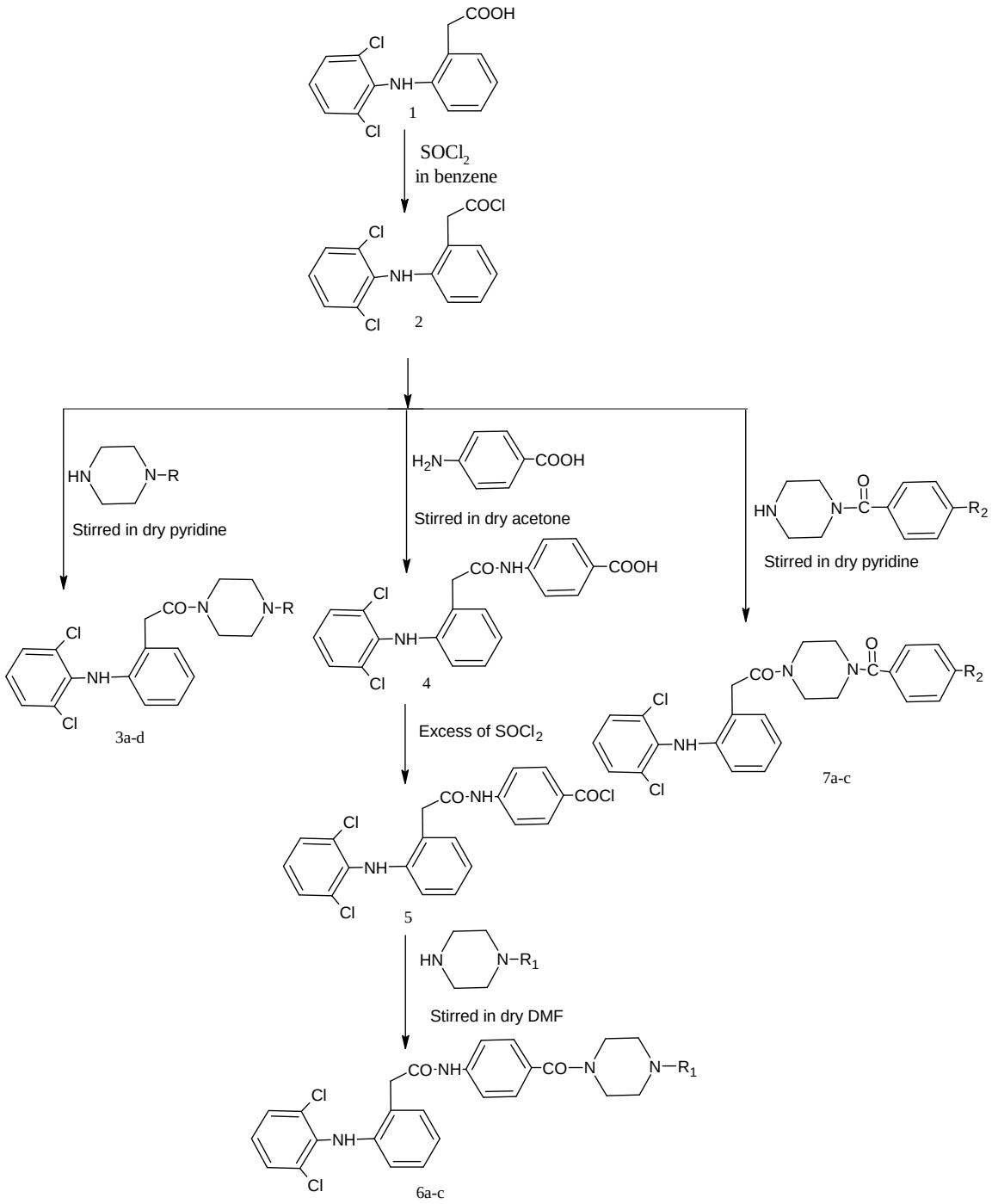

Scheme I

R = 3a. -H 3b. $-CH_3$ 3c. $-CH_2CH_2OH$ 3d. $4\text{-}NO_2\text{-}C_6H_5$

R_1 = 6a. -H 6b. $-CH_3$ 6c. $-CH_2CH_2OH$

R_2 = 7a. -Cl 7b. $-NO_2$ 7c. $-NHCOCH_3$

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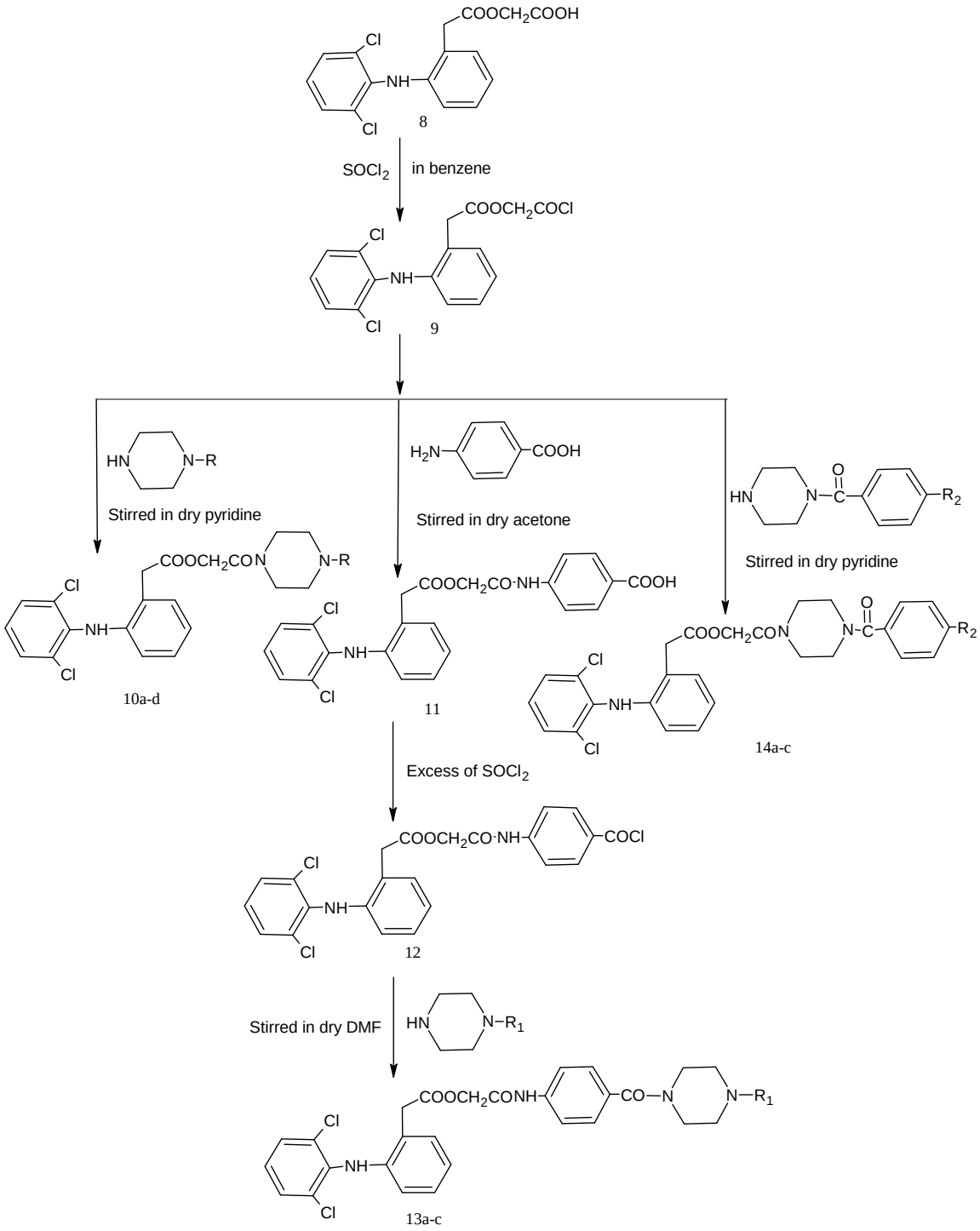

Scheme II

R = 10a. -H 10b. $-CH_3$ 10c. $-CH_2CH_2OH$ 10d. $4\text{-}NO_2\text{-}C_6H_5$

R_1 = 13a. -H 13b. $-CH_3$ 13c. $-CH_2CH_2OH$

R_2 = 14a. -Cl 14b. $-NO_2$ 14c. $-NHCOCH_3$

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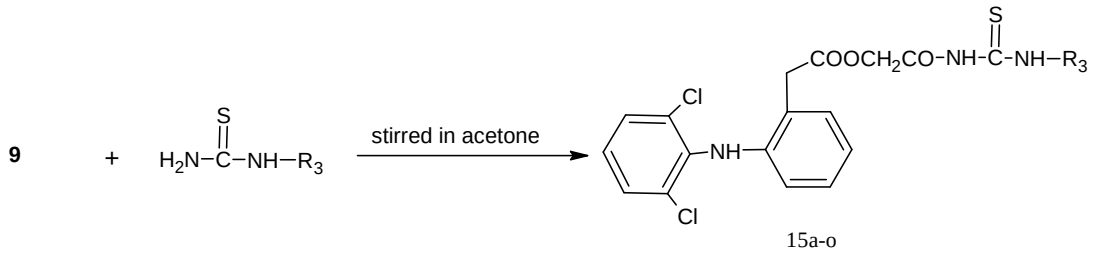

Scheme III

R_3 =

15a.	$-C_6H_5$	15b.	$2\text{-}NO_2\text{-}C_6H_4$	15c.	$3\text{-}NO_2\text{-}C_6H_4$	15d.	$4\text{-}NO_2\text{-}C_6H_4$	15e.	$2\text{-}CH_3\text{-}C_6H_4$
15f.	$3\text{-}CH_3\text{-}C_6H_4$	15g.	$4\text{-}CH_3\text{-}C_6H_4$	15h.	$2\text{-}OCH_3\text{-}C_6H_4$	15i.	$3\text{-}OCH_3\text{-}C_6H_4$	15j.	$4\text{-}OCH_3\text{-}C_6H_4$
15k.	$2\text{-}Cl\text{-}C_6H_4$	15l.	$3\text{-}Cl\text{-}C_6H_4$	15m.	$4\text{-}Cl\text{-}C_6H_4$	15n.	$4\text{-}COOH\text{-}C_6H_4$	15o.	$2,6\text{-}(Cl)_2\text{-}C_6H_3$

Material and Methods

Experimental

Melting points were measured by open capillary method and all were uncorrected. IR absorption spectra were recorded on Parkin Elmer-838 FT IR spectrometer using KBr pellet and ^{1}H-NMR spectra were recorded in DMSO-d_6 /D_2O on Bruker DRX-300 (300 MHz FT NMR) instrument (chemical shifts in δ ppm). The purity of products routinely checked by TLC using silica gel in benzene: ethyl acetate, benzene: ethanol, acetone: ethanol & ethyl acetate: ethanol solvents. The compound 2-[(2,6-dichlorophenyl)amino]phenyl acetoxy acetyl chloride **(2)** was prepared from 2-[(2,6-dichlorophenyl)amino] phenyl acetoxy acetic acid **(1)** by reported method [22].

2.1.1 General procedure for the preparation of 2-[(2, 6-dichlorophenyl)amino]-N^1-phenyl acetyl-N^4-(substituted)piperazine (3a-d)

An appropriate mixture of 2-[(2,6-dichlorophenyl)amino]phenyl acetyl chloride (5 mmol) in dry pyridine (15 ml) was taken and added portion wise to an ice-cold mixture of *N*- (substituted) piperazine (5 mmol) in dry pyridine (15 ml) with occasional stirring. After completion of addition, stirred for 2 h. and was refluxed for 3 h. The whole content was poured into acidic ice-cold water and the solid thus obtained was separated and washed thoroughly with water, dried and recrystallised from ethanol.

2-[(2, 6-Dichlorophenyl)amino]phenyl acetyl piperazine (3a)

Yield 69 %, mp 230-232°C, Anal. Calculated % $C_{18}H_{19}N_3OCl_2$: C-59.35, H-5.22, N- 11.54. Found C-59.28, H-5.20, N-11.48. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 2925, 2845 (C-H); 1670 (>CO-N<); 1360 (C-N); 1110 (C-Npip); 745(N-H wag); 785 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): spectrum, δ, ppm: 2.70-3.40 m (8H $_{pip}$), 3.20 s (1H, NH_{pip}) 3.68 s (2H, -CH_2-CO-), 6.50-7.82 m (7H_{arom}); 9.10 s (1 H, -NH).

2-[(2, 6-Dichlorophenyl)amino]-N^1-phenyl acetyl-N^4- methyl piperazine (3b)

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Yield 60 %, mp 130-134°C, Anal. Calculated % $C_{19}H_{21}N_3OCl_2$: C-60.33, H-5.55, N- 11.11. Found C-60.27, H-5.59, N-11.02. IR (KBr ν_{max} cm^{-1}): 3448 (-NH); 2920, 2843 (C-H); 1674 (>CO-N<); 1362 (C-N); 1105 (C-Npip); 741 (N-H wag); 787 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.91 s (3H, -N-CH_3); 2.61-3.43 m (8H_{pipe}); 3.73 s (2H, -CH_2-CO-), 6.58-7.82 m (7H_{arom}); 10.12 s (1 H, -NH).

2-[(2, 6-Dichlorophenyl)amino]-*N*^{1}-phenyl acetyl-*N*^{4}-2-hydroxyethyl piperazine (3c)

Yield 58 %, mp 112-115°C, Anal. Calculated % $C_{20}H_{23}N_3O_2Cl_2$: C-58.84, H-5.63, N- 10.29. Found C-58.80, H-5.57, N-11.19. IR (KBr ν_{max} cm^{-1}): 3434 (-NH); 3332 (-OH); 2925, 2848 (C-H); 1668 (>CO-N<); 1360 (C-N); 1109 (C-Npip); 744 (N-H wag); 781 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.70-3.44 m (8H_{pipe}); 3.55 m (4H, N-$(CH_2)_2$-O); 3.70 s (2H, -CH_2-CO-); 4.48 (-CH_2OH); 6.51-7.89 m (7H_{arom}); 9.14 s (1 H, -NH).

2-[(2, 6-Dichlorophenyl)amino]-*N*^{1}-phenyl acetyl-*N*^{4}- 4-nitro phenyl piperazine (3d)

Yield 56 %, mp 184-186°C, Anal. Calculated % $C_{24}H_{22}N_4O_3Cl_2$: C-59.39, H-4.53, N- 11.54. Found C-59.28, H-4.49, N-11.50. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 2920, 2845 (C-H); 1673 (>CO-N<); 1550, 1340 (-NO_2); 1365 (C-N); 1100 (C-Npip); 750 (N-H wag); 780 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.76-3.48 m (8H_{pipe}); 3.64 s (2H, -CH_2-CO-), 6.44-7.93 m (11H_{arom}); 9.04 s (1 H, -NH).

2.1.2 General procedure for the preparation of 2-[(2, 6-dichlorophenyl)amino]phenyl acetamido benzoic acid (4)

2-[(2, 6-Dichlorophenyl)amino]phenyl acetyl chloride (5 mmol) was dissolved in dry acetone (20 ml) and was added in well stirred ice-cold mixture of 4-amino benzoic acid (6.85 g, 50 mmol) in dry acetone (40 ml). During the addition neutral *p*H was maintained by the addition of 5N NaOH (5 ml) and stirring was continued at the room temperature for 4 h.; refluxed for 5 h. in water bath. The excess of solvent was evaporated in vacuum. The obtained jelly like solid mass was poured into crushed ice. The isolated product was washed with diluted HCl and then with water. It was recrystallised from absolute alcohol.

Yield 65 %, mp 195-198°C, Anal. Calculated % $C_{21}H_{16}N_2O_3Cl_2$: C-60.74, H-3.88, N- 6.75. Found C-60.70, H-3.83, N-6.71. IR (KBr ν_{max} cm^{-1}): 3443 (-NH); 2922, 2840 (C-H); 1710 (-COOH); 1668, 1562, 1239 (amide I, II, III); 1365 (C-N); 747 (N-H wag); 786 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.70 s (2H, -CH_2-CO-); 6.33-7.95 m (11H_{arom}); 9.09 s (1 H, -NH), 12.04 s (1H, -COOH).

2.1.3 General procedure for the preparation of 2-[(2, 6-dichlorophenyl)amino]phenyl acetamido benzoyl chloride (5)

To a mixture of 2-[(2,6-dichlorophenyl)amino]phenyl acetamido benzoic acid (2.07 g, 5 mmol) was refluxed with excess of thionyl chloride (15 ml) for 6 h. The excess of thionyl chloride was distilled off and its traces were removed by the azeotropic distillation with dry benzene. The obtained acid chloride was directly used for next step without further purification. mp 135-138°C

2.1.4 General procedure for the preparation of 2-[(2,6-Dichlorophenyl)amino]phenyl acetamido-*N*^{1}-benzoyl-*N*^{4}-(substituted) piperazine (6a-c)

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2-[(2,6-Dichlorophenyl)amino]phenyl acetamido benzoyl chloride (5 mmol) dissolved in dry pyridine (15 ml) and was added in to well stirred chilled solution of substituted piperazine (10 mmol) in a dry pyridine (10 ml). After completion of the addition, the reaction mixture was stirred further 1.5 h. at room temperature and refluxed for 3 h. in oil bath, whole contents were hot to pour in to acidic ice-cold water. The separated solid was filtered, washed thoroughly with water, dried and recrystallised from methanol.

2-[(2, 6-Dichloro phenyl)amino]phenyl acetamido-N^1- benzoyl piperazine (6a)

Yield 66 %, mp 170-174°C, Anal. Calculated % $C_{25}H_{24}N_4O_2Cl_2$: C-62.12, H-5.00, N- 11.59. Found C-62.08, H-4.90, N-11.52. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 2920, 2845 (C-H); 1670, 1640 (amide I); 1565, 1235 (amide II, III); 1360 (C-N); 1100 (C-N_{pip}); 745 (N-H wag); 780 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.80-3.40 m (8H_{pip}); 2.67 s (1H_{pip}); 3.68 s (2H, -CH_2-CO-); 6.31-7.98 m (11H_{arom}); 9.07 s (1 H, -NH).

2-[(2, 6-Dichlorophenyl)amino]phenyl acetamido-N^1- benzoyl- N^4- methyl piperazine (6b)

Yield 69 %, mp 132-135°C, Anal. Calculated % $C_{26}H_{26}N_4O_2Cl_2$: C-62.78, H-5.27, N- 11.26. Found C-62.69, H-5.16, N-11.19. IR (KBr ν_{max} cm^{-1}): 3445 (-NH); 2922, 2845 (C-H); 1675, 1650 (amide I); 1565, 1240 (amide II, III); 1360 (C-N); 1105 (C-N_{pip}); 750 (N-H wag); 785 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.17 s (3H –N-CH_3), 2.70-3.45 m (8H_{pipe}); 3.61 s (2H, -CH_2-CO-), 6.34-7.90 m (7H_{arom}); 9.10 s (1 H, -NH).

2-[(2, 6-Dichlorophenyl)amino]phenyl acetamido-N^1- benzoyl- N^4-2-hydroxy ethyl piperazine (6c)

Yield 61 %, mp 151-153°C, Anal. Calculated % $C_{27}H_{26}N_4O_3Cl_2$: C-61.48, H-5.35, N- 10.62. Found C-61.40, H-5.21, N-10.54. IR (KBr ν_{max} cm^{-1}): 3447 (-NH); 3320 (-OH); 2925, 2840 (C-H); 1672, 1645 (amide I); 1568, 1243 (amide II, III); 1367 (C-N); 1107 (C-N_{pip}); 754 (N-H wag); 782 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.68-3.45 m (8H_{pipe}); 3.52 m (N-$(CH_2)_2$-O); 3.69 s (2H, -CH_2-CO-); 4.41 (-CH_2OH); 6.38-7.89 m (11H_{arom}); 9.01 s (1 H, -NH).

2.1.5 General procedure for the preparation of 2-[(2, 6-Dichlorophenyl)amino]-N^1-phenyl acetyl-N^4-(substituted)benzoyl piperazine (7a-c)

Take a mixture of appropriate 2-[(2,6-dichlorophenyl)amino]phenyl acetyl chloride (5 mmol) in dry pyridine (15 ml) was added portion wise to an ice-cold mixture of 4-substituted benzoyl piperazine (5 mmol) in dry pyridine (15 ml) with occasional stirring, then the mixture was stirred further for 2 h. and refluxed for 3-4 h. The whole content was kept over night at room temperature and treated with acidic ice-cold water. The separated solid was filtered, washed with water and recrystallised from ethanol. **TLC:** ethyl acetate: methanol (1:1).

2-[(2, 6-Dichlorophenyl)amino]-N^1-phenyl acetyl- N^4-4- nitrobenzoyl piperazine (7a)

Yield 63 %, mp 228-231°C, Anal. Calculated % $C_{25}H_{22}N_4O_4Cl_2$: C-58.49, H-4.28, N- 10.92. Found C-58.41, H-4.19, N-10.84. IR (KBr ν_{max} cm^{-1}): 3441 (-NH); 2915, 2843 (C-H); 1678, 1650 (amide I); 1550, 1340 (-NO_2); 1365 (C-N); 1102 (C-N_{pip}); 755 (N-H wag); 788 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.61-3.42 m (8H_{pip}); 3.66 s (2H, -CH_2-CO-), 6.42-7.95 m (11H_{arom}); 9.08 s (1 H, -NH).

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2-[(2, 6-Dichlorophenyl)amino]-N^1-phenyl acetyl- N^4- 4-chlorobenzoyl piperazine (7b)

Yield 58 %, mp 214-216°C, Anal. Calculated % $C_{25}H_{22}N_3O_2Cl_3$: C-59.72, H-4.37, N- 8.35. Found C-59.66, H-4.31, N-8.31. IR (KBr ν_{max} cm^{-1}): 3445 (-NH); 2924, 2840 (C-H); 1670, 1650 (amide I); 1360 (C-N); 1090 (C-N_{pip}); 745 (N-H wag); 780 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.73-3.46 m (8H_{pip}); 3.58 s (2H, -CH_2-CO-), 6.35-7.89 m (11H_{arom}); 9.09 s (1 H, -NH).

2-[(2, 6-Dichlorophenyl)amino]-N^1-phenyl acetyl- N^4- acetamido benzoyl piperazine (7c)

Yield 69 %, mp 189-192°C, Anal. Calculated % $C_{27}H_{26}N_4O_3Cl_2$: C-61.72, H-4.94, N- 10.66. Found C-61.66, H-4.88, N-10.58. IR (KBr ν_{max} cm^{-1}): 3445 (-NH); 2920, 2845 (C-H); 1670, 1642 (amide I); 1565, 1245 (amide II, III); 1360 (C-N); 1105 (C-N_{pip}); 750 (N-H wag); 785 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.62-3.42 m (8H_{pip}); 2.5 s, (3H, -CH_3); 3.68 s (2H, -CH_2-CO-), 6.40-7.96 m (11H_{arom}); 9.01 s (1 H, -NH), 10.03 s (1H, -NHCO).

2.1.6 General procedure for the preparation of 2-[(2, 6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl-N^4-(substituted) piperazine (10a-d)

2-[(2, 6-Dichlorophenyl)amino]phenyl acetoxy acetyl chloride (5 mmol) was dissolved in dry DMF (25 ml) and was added portion wise into the ice-cold mixture of *N*-substituted piperazine (5 mmol) in dry DMF (15 ml) with constant stirring at 0-10 ºC for 6 h. and refluxed for 3-4 h. in oil bath & was kept overnight at room temperature. Next day the whole content was poured in to crushed ice, separated solid was filtered, washed successively with water and 10% $NaHCO_3$ solution. The isolated product was recrystallised from ethanol. **TLC:** ethyl acetate: benzene (1:1)

2-[(2, 6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl piperazine (10a)

Yield 69 %, mp 162-165°C, Anal. Calculated % $C_{20}H_{21}N_3O_3Cl_2$: C-56.88, H-4.97, N- 9.95. Found C-56.80, H-4.95, N-9.90. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 2923, 2840 (C-H); 1720 (-C=O); 1640 (amide I); 1355 (C-N); 1096 (C-N_{pip}); 743 (N-H wag); 778 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.80-3.48 m (8H_{pip}); 2.7 s (1H_{pip}); 3.78 s (2H, –CH_2COO-); 4.66 s (2H, -O-CH_2-CO-), 6.33-7.90 m (7H_{arom}); 9.15 s (1 H, -NH).

2-[(2, 6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl –N^4-methyl piperazine (10b)

Yield 62 %, mp 109-112°C, Anal. Calculated % $C_{21}H_{23}N_3O_3Cl_2$: C-57.81, H-5.27, N- 9.63. Found C-57.84, H-5.20, N-9.58. IR (KBr ν_{max} cm^{-1}): 3447 (-NH); 2922, 2845 (C-H); 1715 (-C=O); 1645 (amide I); 1359 (C-N); 1098 (C-N_{pip}); 745 (N-H wag); 787 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.85 s (3H –N-CH_3); 2.65-3.40 m (8H_{pip}); 3.78 s (2H, –CH_2COO-); 4.62 s (2H, -O-CH_2-CO-), 6.60-7.74 m (7H_{arom}); 9.52 s (1 H, -NH).

2-[(2, 6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl –N^4-2-hydroxy ethyl piperazine (10c)

Yield 65 %, mp 135-137°C, Anal. Calculated % $C_{22}H_{25}N_3O_4Cl_2$: C-56.66, H-5.36, N- 9.01. Found C-56.61, H-5.29, N-8.96. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 3320 (-OH); 2925, 2842 (C-H); 1722 (-C=O); 1680 (amide I); 1355 (C-N); 1100 (C-N_{pip}); 750 (N-H wag); 780 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.62-3.42 m (8H_{pip}); 3.58 m (N-$(CH_2)_2$-O); 3.72 s (2H, -CH_2-CO-); 4.40 (-CH_2OH); 4.68 s (2H, -O-CH_2-CO-), 6.68-7.70 m (7H_{arom}); 9.40 s (1 H, -NH).

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2-[(2, 6- Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl –N^4-4-nitro phenyl piperazine (10d)

Yield 60%, mp 84-86°C, Anal. Calculated % $C_{26}H_{24}N_4O_5Cl_2$: C-57.47, H-4.41, N-10.31. Found C-57.38, H-4.40, N-10.22. IR (KBr ν_{max} cm^{-1}): 3449 (-NH); 2928, 2840 (C-H); 1712 (-C=O); 1683 (amide I); 1543,1346 (-NO_2); 1352 (C-N); 1105 (C-Npip); 756 (N-H wag); 785 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.62-3.43 m (8H_{pip}); 3.80 s (2H, –CH_2COO-); 4.60 s (2H, -O-CH_2-CO-), 6.74-7.90 m (11H_{arom}); 9.30 s (1 H, -NH).

2.1.7 General procedure for the preparation of 2-[(2,6-Dichlorophenyl)amino]phenyl acetoxy acetamido N^1-benzoyl-N^4-(substituted) piperazine (13a-c).

The compound 2-[(2,6-dichlorophenyl)amino]phenyl acetamido benzoyl chloride (5 mmol) dissolved in dry DMF (20 ml) and was added in to well stirred ice-cold solution of *N*-substituted piperazine (5 mmol) dissolved in dry DMF (15 ml); stirred the reaction mixture at 0-10ºC for 8 h. and refluxed for 3-4 h. Then the content was poured in to cold water. The isolated product was filtered and washed successively with 5 % $NaHCO_3$ solution. The product was recrystallised from ethanol.

TLC: ethyl acetate: acetone (1:1).

2-[(2, 6-Dichlorophenyl)amino]phenyl acetoxy acetamido-N^1-benzoyl piperazine (13a)

Yield 66 %, mp 170-174 °C, Anal. Calculated % $C_{27}H_{26}N_4O_4Cl_2$: C-59.90, H-4.80, N- 10.35. Found C-59.79, H-4.71, N-10.30. IR (KBr ν_{max} cm^{-1}): 3443 (-NH); 2925, 2840 (C-H); 1722 (-C=O); 1670, 1640 (amide I); 1540, 1250 (amide II, III); 1355 (C-N); 1105 (C-N_{pip}); 745 (N-H wag); 780 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.60-3.47 m (9H_{pip}); 3.71 s (2H, –CH_2COO-); 4.60 s (2H, -O-CH_2-CO-), 6.60-7.79 m (11H_{arom}); 9.45 s (1 H, -NH).

2-[(2, 6-Dichlorophenyl)amino]phenyl acetoxy acetamido-N^1- benzoyl- N^4-methyl Piperazine (13b)

Yield 59 %, mp 195-197 °C, Anal. Calculated % $C_{28}H_{28}N_4O_4Cl_2$: C-60.55, H-5.04, N- 10.09. Found C-60.51, H-4.97, N-10.00. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 2929, 2841 (C-H); 1651, 1543, 1257 (amide I, II, III); 1352 (C-N); 1101 (C-N_{pip}); 747 (N-H wag); 788 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.82 s (3H –N-CH_3); 2.72-3.44 m (8H_{pip}); 3.72 s (2H, –CH_2COO-); 4.68 s (2H, -O-CH_2-CO-), 6.57-7.78 m (11H_{arom}); 9.48 s (1 H, -NH).

2-[(2, 6-Dichlorophenyl)amino]phenyl acetoxy acetamido-N^1- benzoyl- N^4-2-hydroxy ethyl Piperazine (13c)

Yield 61 %, mp 134-135 °C, Anal. Calculated % $C_{29}H_{30}N_4O_5Cl_2$: C-59.50, H-5.12, N- 9.57. Found C-59.41, H-5.09, N-9.48. IR (KBr ν_{max} cm^{-1}): 3448 (-NH); 3340 (-OH); 2925, 2840 (C-H); 1660, 1630 (amide I); 1540, 1240 (amide II, III); 1350 (C-N); 1110 (C-N_{pip}); 750 (N-H wag); 785 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.68-3.47 m (8H_{pip}); 3.54 m (N-$(CH_2)_2$-O); 3.79 s (2H, –CH_2COO-); 4.44 (-CH_2OH); 4.66 s (2H, -O-CH_2-CO-), 6.60-7.78 m (11H_{arom}); 9.43 s (1 H, -NH).

2.1.8 General procedure for the preparation of 2-[(2,6-Dichlorophenyl)amino]-N^1- phenyl acetoxy acetyl- N^4- 4- substituted benzoyl piperazine (14a-c)

To a suspension of 4-substituted benzoyl piperazine (5 mmol) in dry pyridine (25 ml), and cool in ice-bath. Add a cold solution of 2-[(2,6-dichlorophenyl)amino]phenyl acetamido benzoyl chloride (5 mmol) in pyridine

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portion wise with vigorous stirring. During the addition temperature of the reaction mixture was maintained between 0-10 ºC and refluxed it for 7-8 h., whole contents were kept over night at room temperature. Next day it was treated with acidic cold water thoroughly with 10% $NaHCO_3$ solution and then with water, dried and recrystallised from ethanol. **TLC:** ethyl acetate: acetone (1: 1).

2-[(2,6-Dichlorophenyl)amino]-*N*^{1}-phenyl acetoxy acetyl- *N*^{4}- 4-chloro benzoyl piperazine (14a)

Yield 56 %, mp 274-277 °C, Anal. Calculated % $C_{27}H_{24}N_3O_4Cl_3$: C-57.82, H-4.27, N- 7.49. Found C-57.71, H-4.19, N-7.41. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 2921, 2844 (C-H); 1640 (CO-N); 1352 (C-N); 1110 (C-N_{pip}); 753 (N-H wag); 787 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.70-3.45 m (8H_{pip}); 3.77 s (2H, –CH_2COO-); 4.66 s (2H, -O-CH_2-CO-), 6.75-7.92 m (11H_{arom}); 9.35 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-*N*^{1}-phenyl acetoxy acetyl- *N*^{4}- 4-nitro benzoyl piperazine (14b)

Yield 59 %, mp 244-247 °C, Anal. Calculated % $C_{27}H_{24}N_4O_6Cl_2$: C-56.75, H-4.20, N- 9.80. Found C-56.70, H-4.16, N-9.86. IR (KBr ν_{max} cm^{-1}): 3445 (-NH); 2929, 2847 (C-H); 1714 (-C=O); 1670, 1648 (amide I); 1544, 1347 (-NO_2); 1350 (C-N); 1107(C-N_{pip}); 755 (N-H wag); 784 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.73-3.49 m (8H_{pip}); 3.82 s (2H, –CH_2COO-); 4.66 s (2H, -O-CH_2-CO-), 6.68-7.88 m (11H_{arom}); 9.39 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-*N*^{1}-phenyl acetoxy acetyl- *N*^{4}- acetamido benzoyl piperazine (14c)

Yield 62 %, mp 258-261 °C, Anal. Calculated % $C_{29}H_{28}N_4O_5Cl_2$: C-59.70, H-4.80, N- 9.60. Found C-59.62, H-4.71, N-9.54. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 2920, 2845 (C-H); 1720 (-C=O); 1680, 1640 (amide I); 1565, 1240 (amide II, III); 1357 (C-N); 1092 (C-N pip); 745 (N-H wag); 785 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.73-3.42 m (8H_{pip}); 2.5 s (-CH_3) 3.76 s (2H, –CH_2COO-); 4.61 s (2H, -O-CH_2-CO-), 6.65-7.82 m (11H_{arom}); 9.32 s (1 H, -NH) 11.03 s (1H, -NHCO).

2.1.9 General procedure for the preparation of 2-[(2,6-Dichlorophenyl)amino]-*N*^{1}-phenyl acetoxy acetyl-*N*^{2}-aryl thiourea (15a-o)

An appropriate amount of 2-[(2,6-dichlorophenyl)amino]phenyl acetoxy acetyl chloride (5 mmol) portion wise was added in a mixture of aryl thiourea (5 mmol) in dry acetone in ice bath about 1.5 h. with constant stirring. The temperature of the reaction mixture was maintained to 0-10 ºC, meanwhile 5N NaOH (5 ml) was added in to it and maintained *p*H~7. The reaction mixture was refluxed in water bath for 6-7 h. The excess of acetone was distilled off and the solid was thoroughly washed with water and aqueous $NaHCO_3$ (10%) solution. Final compound was obtained in a radish brown powder form.

2-[(2,6-Dichlorophenyl)amino]-*N*^{1}-phenyl acetoxy acetyl-*N*^{2}-phenyl thiourea (15a)

Yield 55 %, mp 254-255 °C, Anal. Calculated % $C_{23}H_{19}N_3O_3SCl_2$: C-56.56, H-3.89, N- 8.61. Found C-6.50, H-3.81, N-8.51. IR (KBr ν_{max} cm^{-1}): 3443 (-NH); 2917, 2844 (C-H); 1745 (C=O);1672, 1522, 1264 (amide I, II, III); 1351 (C-N); 1173 (C=S), 747 (N-H wag); 782 (C-Cl).^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.80 s (2H, –CH_2COO-); 4.66 s (2H, -O-CH_2-CO-), 6.80-7.81 m (11H_{arom}); 8.15 s (1H, -CONH); 8.41 s (1H, -C=S-NH-); 9.21 s (1 H, -NH).

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2-[(2,6-Dichlorophenyl)amino]-*N*[1]-phenyl acetoxy acetyl-*N*[2]-2-nitrophenyl thiourea (15b)

Yield 65 %, mp 184-186 °C, Anal. Calculated % $C_{23}H_{18}N_4O_5SCl_2$: C-51.79, H-3.37, N- 10.51. Found C-51.71, H-3.27, N-10.45. IR (KBr ν_{max} cm^{-1}): 3437 (-NH); 2918, 2846 (C-H); 1737 (C=O); 1668, 1522, 1267 (amide I, II, III); 1562, 1357 (-NO_2); 1345 (C-N); 1167 (C=S), 747 (N-H wag); 785 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.82 s (2H, –CH_2COO-); 4.66 s (2H, -O-CH_2-CO-), 6.82-7.80 m (11H_{arom}); 8.15 s (1H, -CONH); 8.40 s (1H, -C=S-NH-); 9.16 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-*N*[1]-phenyl acetoxy acetyl-*N*[2]-3-nitrophenyl thiourea (15c)

Yield 70 %, mp 169-171 °C, Anal. Calculated % $C_{23}H_{18}N_4O_5SCl_2$: C-51.79, H-3.37, N- 10.51. Found C-51.70, H-3.30, N-10.42. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 2920, 2840 (C-H); 1740 (C=O);1670, 1525, 1260 (amide I, II, III); 1555, 1352 (-NO_2); 1347 (C-N); 1170 (C=S), 740 (N-H wag); 780 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.88 s (2H, –CH_2COO-); 4.60 s (2H, -O-CH_2-CO-), 6.78-7.81 m (11H_{arom}); 8.13 s (1H, -CONH); 8.42 s (1H, -C=S-NH-); 9.25 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-*N*[1]-phenyl acetoxy acetyl-*N*[2]-4-nitrophenyl thiourea (15d)

Yield 50 %, mp 133-136 °C, Anal. Calculated % $C_{23}H_{18}N_4O_5SCl_2$: C-51.79, H-3.37, N- 10.51. Found C-51.69, H-3.33, N-10.50. IR (KBr ν_{max} cm^{-1}): 3448 (-NH); 2915, 2836 (C-H); 1734 (C=O);1678, 1521, 1267 (amide I, II, III); 1547, 1350 (-NO_2); 1355 (C-N); 1165 (C=S), 743 (N-H wag); 788 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.80 s (2H, –CH_2COO-); 4.62 s (2H, -O-CH_2-CO-), 6.72-7.88 m (11H_{arom}); 8.10 s (1H, -CONH); 8.40 s (1H, -C=S-NH-); 9.20 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-*N*[1]-phenyl acetoxy acetyl-*N*[2]-2-methylphenyl thiourea (15e)

Yield 52 %, mp 179-182 °C, Anal. Calculated % $C_{24}H_{21}N_3O_3SCl_2$: C-57.38, H-4.18, N- 8.36. Found C-57.31, H-4.10, N- 8.29. IR (KBr ν_{max} cm^{-1}): 3435 (-NH); 2915, 2838 (C-H); 1748 (C=O);1670, 1534, 1255 (amide I, II, III); 1353 (C-N); 1166 (C=S), 746 (N-H wag); 788 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.32 s (3H, -CH_3); 3.81 s (2H, –CH_2COO-); 4.65 s (2H, -O-CH_2-CO-), 6.71-7.78 m (11H_{arom}); 8.11 s (1H, -CONH); 8.45 s (1H, -C=S-NH-); 9.13 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-*N*[1]-phenyl acetoxy acetyl-*N*[2]-3-methyphenyl thiourea (15f)

Yield 50 %, mp 229-230 °C, Anal. Calculated % $C_{24}H_{21}N_3O_3SCl_2$: C-57.38, H-4.18, N- 8.36. Found C-57.30, H-4.08, N-8.33. IR (KBr ν_{max} cm^{-1}): 3433 (-NH); 2915, 2844 (C-H); 1740 (C=O); 1674, 1522, 1261 (amide I, II, III); 1357 (C-N); 1165 (C=S), 745 (N-H wag); 784 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.24 s (3H, -CH_3); 3.88 s (2H, –CH_2COO-); 4.60 s (2H, -O-CH_2-CO-), 6.77-7.89 m (11H_{arom}); 8.05 s (1H, -CONH); 8.43 s (1H, -C=S-NH-); 9.17 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-*N*[1]-phenyl acetoxy acetyl-*N*[2]-4-methyphenyl thiourea (15g)

Yield 65 %, mp 244-246°C, Anal. Calculated % $C_{24}H_{21}N_3O_3SCl_2$: C-57.38, H-4.18, N- 8.36. Found C-57.34, H-4.11, N-8.27. IR (KBr ν_{max} cm^{-1}): 3450 (-NH); 2920, 2845 (C-H); 1740 (C=O);1680, 1535, 1270 (amide I, II, III); 1352 (C-N); 1170 (C=S), 740 (N-H wag); 785 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.29 s (3H, -CH_3); 3.83 s (2H, –

CH_2COO-); 4.63 s (2H, -O-CH_2-CO-), 6.83-7.86 m (11H_{arom}); 8.11 s (1H, -CONH); 8.45 s (1H, -C=S-NH-); 9.10 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl-N^2-2-methoxylphenyl thiourea (15h)

Yield 61 %, mp 224-225°C, Anal. Calculated % $C_{24}H_{21}N_3O_4SCl_2$: C-55.61, H-4.05, N- 8.11. Found C-55.56, H-4.00, N- 8.02. IR (KBr ν_{max} cm^{-1}): 3435 (-NH); 2917, 2843 (C-H); 1745 (C=O);1670, 1525, 1262 (amide I, II, III); 1363 (C-N); 1166 (C=S); 1025, 1212(C-O-C); 744 (N-H wag); 789 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.72 s (3H, -OCH_3); 3.83 s (2H, –CH_2COO-); 4.63 s (2H, -O-CH_2-CO-), 6.83-7.86 m (11H_{arom}); 8.11 s (1H, -CONH); 8.45 s (1H, -C=S-NH-); 9.10 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl-N^2-3-methoxyphenyl thiourea (15i)

Yield 55 %, mp 179-181°C, Anal. Calculated % $C_{24}H_{21}N_3O_4SCl_2$: C-55.61, H-4.05, N- 8.11. Found C-55.52, H-3.96, N-8.00. IR (KBr ν_{max} cm^{-1}): 3437 (-NH); 2918, 2841 (C-H); 1742 (C=O);1672, 1520, 1265 (amide I, II, III); 1359 (C-N); 1163 (C=S); 1025, 1212(C-O-C); 741 (N-H wag); 782 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.78 s (3H, -OCH_3); 3.85 s (2H, –CH_2COO-); 4.66 s (2H, -O-CH_2-CO-), 6.82-7.80 m (11H_{arom}); 8.10 s (1H, -CONH); 8.47 s (1H, -C=S-NH-); 9.15 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl-N^2-4-methoxyphenyl thiourea (15j)

Yield 50 %, mp 239-241°C, Anal. Calculated % $C_{24}H_{21}N_3O_4SCl_2$: C-55.61, H-4.05, N- 8.11. Found C-55.60, H-3.99, N-8.04. IR (KBr ν_{max} cm^{-1}): 3445 (-NH); 2925, 2840 (C-H); 1745 (C=O); 1680, 1540, 1250 (amide I, II, III); 1355 (C-N); 1160 (C=S); 1025, 1212(C-O-C); 750 (N-H wag); 785 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.75 s (3H, -OCH_3); 3.80 s (2H, –CH_2COO-); 4.67 s (2H, -O-CH_2-CO-), 6.88-7.81 m (11H_{arom}); 8.15 s (1H, -CONH); 8.40 s (1H, -C=S-NH-); 9.16 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl-N^2-2-chlorophenyl thiourea (15k)

Yield 51 %, mp 214-216°C, Anal. Calculated % $C_{23}H_{18}N_3O_3SCl_3$: C-2.82, H-3.44; N- 8.04. Found C-52.79, H-3.39, N-7.98. IR (KBr ν_{max} cm^{-1}): 3442 (-NH); 2923, 2847 (C-H); 1748 (C=O);1677, 1522, 1263 (amide I, II, III); 1352 (C-N); 1169 (C=S), 747 (N-H wag); 788 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.78 s (2H, –CH_2COO-); 4.65 s (2H, -O-CH_2-CO-), 6.80-7.87 m (11H_{arom}); 8.14 s (1H, -CONH); 8.43 s (1H, -C=S-NH-); 9.22 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl-N^2-3-chlorophenyl thiourea (15l)

Yield 60 %, mp 164-165°C, Anal. Calculated % $C_{23}H_{18}N_3O_3SCl_3$: C-52.82, H-3.44, N- 8.04. Found C-52.74, H-3.35, N-7.94. IR (KBr ν_{max} cm^{-1}): 3445 (-NH); 2920, 2845 (C-H); 1735 (C=O); 1665, 1540, 1265 (amide I, II, III); 1360 (C-N); 1165 (C=S), 750 (N-H wag); 780 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm):3.84 s (2H, –CH_2COO-); 4.69 s (2H, -O-CH_2-CO-), 6.82-7.86 m (11H_{arom}); 8.08 s (1H, -CONH); 8.52 s (1H, -C=S-NH-); 9.24 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl-N^2-4-chlorophenyl thiourea (15m)

Yield 65 %, mp 230-232°C, Anal. Calculated % $C_{23}H_{18}N_3O_3SCl_3$: C-52.82, H-3.44, N- 8.04. Found C-52.77, H-3.40, N-8.00. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 2920, 2845 (C-H); 1740 (C=O); 1680, 1540, 1250 (amide I, II, III); 1355 (C-

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N); 1173 (C=S), 745 (N-H wag); 785 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.75 s (2H, –CH_2COO-); 4.60 s (2H, -O-CH_2-CO-), 6.77-7.85 m (11H_{arom}); 8.14 s (1H, -CONH); 8.45 s (1H, -C=S-NH-); 9.20 s (1 H, -NH).

2-[(2,6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl-N^2-4-carboxyphenyl thiourea (15n)

Yield 55 %, mp 234-236°C, Anal. Calculated % $C_{24}H_{19}N_3O_5SCl_2$: C-54.16, H-3.56, N- 7.89. Found C-54.11, H-3.47, N-7.81. IR (KBr ν_{max} cm^{-1}): 3447 (-NH); 2923, 2834 (C-H); 1745 (C=O);1677, 1521, 1263 (amide I, II, III); 1357 (C-N); 1171 (C=S), 742 (N-H wag); 778 (C-Cl).^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.72 s (2H, –CH_2COO-); 4.67 s (2H, -O-CH_2-CO-), 6.71-7.83 m (11H_{arom}); 8.15 s (1H, -CONH); 8.38 s (1H, -C=S-NH-); 9.12 s (1 H, -NH); 12.40 s (1H, -COOH).

2-[(2,6-Dichlorophenyl)amino]-N^1-phenyl acetoxy acetyl-N^2-2,6-dichlorophenyl thiourea (15o)

Yield 50 %, mp 264-266°C, Anal. Calculated % $C_{23}H_{17}N_3O_3SCl_4$: C-49.56, H-3.05, N- 7.54. Found C-49.50, H-3.00, N-7.48. IR (KBr ν_{max} cm^{-1}): 3440 (-NH); 2920, 2840 (C-H); 1740 (C=O); 1670, 1540, 1245 (amide I, II, III); 1350 (C-N); 1150 (C=S), 755 (N-H wag); 780 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.89 s (2H, –CH_2COO-); 4.62 s (2H, -O-CH_2-CO-), 6.72-7.88 (m, 10H_{arom}); 8.10 (s, 1H, -CONH); 8.40 (s, 1H, -C=S-NH-); 9.25 s (1 H, -NH).

RESULTS AND DISCUSSION

Antibacterial activity

All the compounds reported in Table 1 were screened for antibacterial and antifungal *in vitro* activity against *S. aureus, B. subtilis, E. coli, P. vulgaris and C. albicans* respectively using cup plate method at 50 µg/ml concentration and compared standard drugs penicillin, chloramphenicol, ampicillin and greseofulvin.

From the experimental data it was observed that piperazine derivatives of compounds **3, 4** and **7** were moderately active against gram positive bacteria *S. aureus* & *B. subtilis;* However significant activity was observed for **7a** (R_2 = Cl) against *S. aureus*.

In case of gram-negative bacterial strains, the reference drugs data was compared with tested compounds data and it was clearly observed that the **3c (**R = -CH_2CH_2OH**), 4c (**R_1 = -CH_2CH_2OH**), 3d** (R= 4-NO_2-C_6H_5)**, 6b** (R_1 = -CH_3) , **7a** (R_2 = –Cl) and **7b** (R_2 =-NO_2) against *E. coli* showed good activity. Compounds **6c** (R_1 =-CH_2CH_2OH), **7a** (R_2 = –Cl) and **7c** (R_2=$NHCOCH_3$) showed promising activity against *P. vulgaris* bacterial species; while other compounds showed mild to moderate activity.

From the experimental data, it has been concluded the piperazine derivatives of **10, 13** and **14** were found to be mild to moderately active against *S .aureus* and *B. subtilis* bacteria. The maximum activity was observed for **14a** (R_2 = -Cl) against *S. aureus* and *B. subtilis* respectively.

In case of Gram-negative bacteria, most of the compounds showed promising activity. The compounds **10a (**R= H**)**, **Xc** (R = -CH_2CH_2OH), **13c** (R_1 = -CH_2CH_2OH**), 10d** (R = 4-NO_2-C_6H_5) and **14b** (R_2 = 4-NO_2) groups exhibited significant activity against *E. coli*.

The activity exhibited by the reference antibiotics penicillin, chloramphenicol and ampicillin were compared with freshly prepared derivatives, it was clearly observed that the **14a** (R_2 = -Cl)

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showed comparatively active with chloramphenicol against *p. vulgaris*.

From the results; the thiourea derivatives showed mild activity against gram positive bacterial strain. The significant activity was observed in **15c** (R_3 = 3-$NO_2C_6H_4$) and **15o** (R_3 = 2,6-$(Cl)_2$-C_6H_3) against *S. aureus* and *B. subtilis* respectively. In case of gram-negative bacterial strains, the maximum activity observed in **15m** (R_3= 4-Cl-C_6H_4), **15n** (R_3 = 4-COOH-C_6H_4) and **15o** (R_3 = 2,6-$(Cl)_2$-C_6H_3) against *E. coli*. Compounds **15k (**R_3 = 2-Cl-C_6H_4), **15n** (R_3 = 4-COOH-C_6H_4 and **15o** (R_3 = 2,6-$(Cl)_2$-C_6H_3) against *P. vulgaris* exhibited remarkable activity while, remaining all the compounds show mild to moderate activity.

Antifungal activity

A perusal of the antifungal experimental data, countless compounds showed mild to moderate antifungal activity against *C. albicans*. The highest activity was displayed by **7a** (R_2 = -Cl). However, compounds **14b** (R_2 = -NO_2), **15g** (R_3 = 4-CH_3), **15j** (R_3 **=** 4-OCH_3-C_6H_4) and **15n** (R_3 = 4-COOH-C_6H_4) showed significant activity against *C. albicans.*

Table I Antimicrobial activity of synthesized compounds

Compound	R	Zone of inhibition in mm				
		Gram-positive		Gram-negative		Antifungal
		S. aureus	*B. subtilis*	*E. coli*	*P. vulgaris*	*C. albicans*
3a	H	13	11	14	12	15
3b	4-CH_3	14	16	15	14	15
3c	4-CH_2CH_2OH	16	09	18	14	14
3d	4-NO_2-C_6H_5	14	10	19	11	16
6a	H	14	10	16	14	14
6b	4-CH_3	13	12	19	17	16
6c	4-CH_2CH_2OH	12	16	20	19	13
7a	4-Cl	19	12	18	19	18
7b	4-NO_2	16	14	19	16	13
7c	4-$NHCOCH_3$	14	16	16	19	15
10a	H	14	11	18	17	16
10b	4-CH_3	12	14	16	17	15
10c	4-CH_2CH_2OH	15	15	18	14	16
10d	4-NO_2-C_6H_5	12	14	19	16	14
13a	H	12	11	16	10	15
13b	4-CH_3	16	14	17	16	15
13c	4-CH_2CH_2OH	14	12	19	18	13
14a	4-Cl	18	17	17	20	14
14b	4-NO_2	14	11	19	17	18
14c	4-$NHCOCH_3$	11	10	16	14	14
15a	H	14	12	15	12	12
15b	2-NO_2	16	15	16	11	16
15c	3-NO_2	17	13	15	11	14

15d	4-2-NO_2	14	15	17	13	16
15e	2-CH_3	16	12	15	14	14
15f	3- CH_3	12	11	14	12	17
15g	4- CH_3	13	13	17	16	19
15h	2-OCH_3	14	14	19	13	16
15i	3- OCH_3	12	10	16	14	12
15j	4- OCH_3	14	13	17	16	18
15k	2-Cl	14	14	16	18	16
15l	3- Cl	12	11	18	16	14
15m	4- Cl	16	15	18	13	16
15n	4-COOH	16	16	19	18	18
15o	2,6-$(Cl)_2$	12	20	22	19	16
Penicillin		30	28	20	21	-
Chloramphenicol		28	25	21	20	-
Ampicillin		26	28	22	21	-
Greseofulvin		-	-	-	-	23

CONCLUSION

In the present investigation, 3**5** new aceclofenac containing piperazinyl and thiourea derivatives were synthesized and characterized by spectral analysis. They were screened for antibacterial and antifungal activity using cup plate method and compared with standard drugs. The activity was recognized to the presence of chloro, hydroxyl and methoxy groups on the condensed heterocyclic system containing aceclofenac. In the view of the above result and to identify new candidates that may value in designing new, selective, less toxic anti-microbial agents.

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