Synthesis and Antibacterial Screening of 1,3,4-Thiadiazoles, 1,2,4-Triazoles, and 1,3,4-Oxadiazoles Containing Piperazine Nucleus

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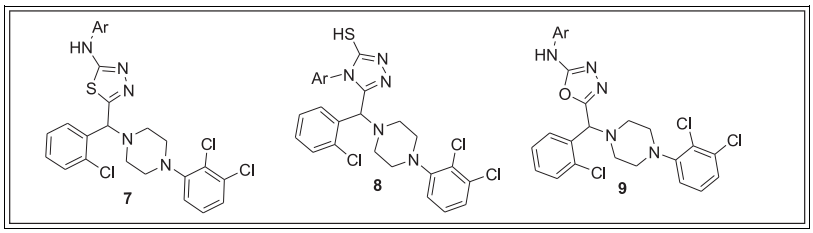

A series of novel 1,3,4-thiadiazoles, 1,2,4-triazoles, and 1,3,4-oxadiazoles were synthesized by cyclization of substituted 1-(2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetyl)thiosemicarbazide. The structures of all newly synthesized compounds were elucidated on the basis of spectral studies. Some of them were screened for their antibacterial activity. The compounds **6b**, **6c**, **8e**, **9a,** and **9b** have shown moderate activity towards *Bacillus Subtilis* and *Escherichia Coli*.

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INTRODUCTION

Piperazines are the saturated analogues of pyrazines having nitrogen atoms at opposite positions. Piperazines are an important class of compounds with biological activities like anthelmintic [1], α_1-adrenergic receptor blockers [2], and antibacterial [3]. Aryl piperazine derivatives show remarkable activity in neuropathic pain without sedative side effect [4]. Some important marketed drugs that contain piperazine nucleus are Cetirizine (antihistamine), Perphenazine (antipsychotic), Trazodone (antidepressant), and Ranolazine (antianginal).

Thiosemicarbazides are the valuable starting compounds used for the synthesis of azoles. Thiosemicarbazides are important compounds having a variety of applications [5]. They have shown activities like anticancer [6], anti-HIV [7], antimalarial [8], antifungal [9], and antibacterial [10].

Thiadiazoles are five-membered heterocyclic compounds containing two nitrogen atoms and one sulfur atom as part of the aromatic ring. Recently, intense investigation has been carried out on thiadiazoles having different substituents. They show various biological activities like antibacterial [11], antioxidant [12], antimicrobial [13] cyclooxygenase-2 inhibitors [14], antifungal [15], and antidepressant [16].

1,2,4-Triazoles are important group of heterocyclic compounds characterized by a five-membered ring having three nitrogen atoms. The biological activities of 1,2,4-triazoles have been investigated by various studies. They possess biological activities like antitubercular [17], antitumor [18], analgesic [19], diuretic [19], anti-inflammatory [20], anticonvulsant [20], and antimicrobial [21].

1,3,4-Oxadiazoles are five-membered heterocyclic compounds containing two nitrogen atoms and one oxygen atom. The heterocyclic compounds anchored with 1,3,4-oxadiazoles exhibit the pharmacological activities like antimicrobial [22], anthelmintic [23], anti-inflammatory [24], analgesic [24], antimitotic [25], and anti-HIV [26].

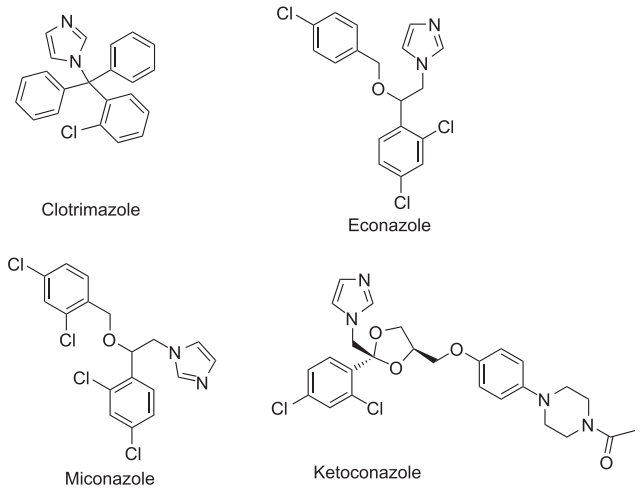
Literature survey showed that incorporation of halogen in heterocyclic compounds increases their activities. Several synthetic organic chemists have synthesized halogenated heterocycles and evaluated them for biological screening. Kumar and coworkers have synthesized series of 3-[4′(*p*-chlorophenyl)thiazol-2′-yl]-2-[(substituted azetidinone/thiazolidinone)-aminomethy]-6-bromoquinazolin-4-ones and reported their anti-inflammatory and analgesic activities [27]. Several commercially available drugs also contain chlorinated heterocyclic compounds such as clotrimazole, econazole, miconazole, and ketoconazole (Scheme 1).

Present research article describes the synthesis and antibacterial screening of thiadiazoles, triazoles, and oxadiazoles containing piperazine nucleus.

RESULTS AND DISCUSSION

Substituted 1-(2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl) piperazin-1-yl)acetyl)-4-phenyl thiosemicarbazide **6** was synthesized in five steps starting from 2-(2-chlorophenyl)acetic acid **1**. 2-(2-Chlorophenyl)acetic acid **1** was converted into 2-bromo-2-(2-chlorophenyl)acetic acid **2** by using *N*-bromosuccinimide [28]. Methyl ester **3** of compound **2** was treated with 1-(2,3-dichlorophenyl)piperizine hydrochloride in

Scheme 1



presence of base to afford methyl 2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetate **4** [29]. 2-(2-Chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl) acetohydrazide **5** was prepared by refluxing compound **4** with hydrazine hydrate. Condensation of compound **5** with different aryl isothiocyanates in alcohol furnished the corresponding thiosemicarbazides **6**. Thiosemicarbazides undergo cyclisation to give thiadiazoles in acidic condition, whereas to triazoles in basic medium. Oxadiazoles were prepared by treating thiosemicarbazides with iodine and potassium iodide in NaOH (Scheme 2). The spectral analysis supports these transformations.

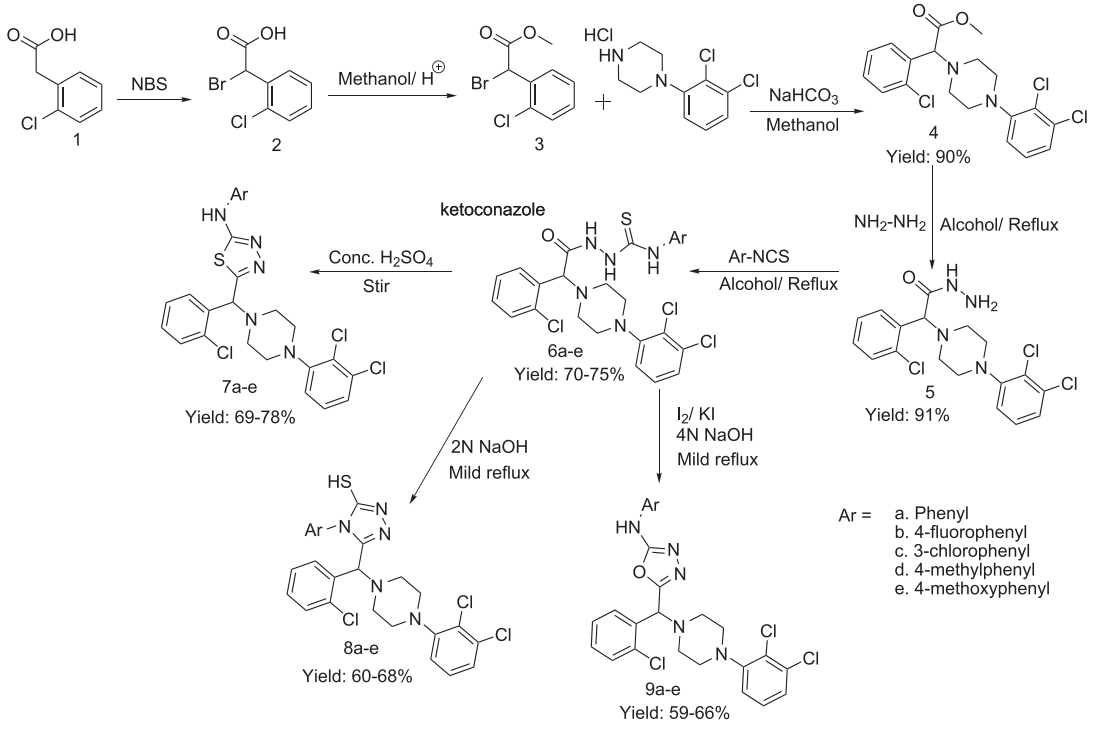
The antibacterial activity of some of the newly synthesized compounds was carried out by agar well diffusion method. Two bacterial species were chosen for the study: one was Gram Positive *Bacillus Subtilis* and another was Gram-negative *Escherichia Coli*. Ampicillin was used as a standard drug for this study.

EXPERIMENTAL

Melting points were determined in open capillary tubes in liquid paraffin bath and are uncorrected. IR spectra were recorded on Perkin Elmer Spectrophotometer using potassium bromide discs. NMR spectra were recorded on a Varian NMR 400 MHz spectrometer (Varian Inc., Switzerland), and chemical shifts are given in δ ppm relative to TMS using deuterated DMSO and deuterated chloroform as a solvents. Mass spectra were recorded on Water's Acquity Ultra Performance TQ Detector Mass Spectrometer. Elemental analysis was performed on the Elemental Instrument Model Vario Micro Cube.

Preparation of methyl 2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetate (4) [29]. To the solution of methyl-2-bromo-2-(2-chlorophenyl)acetate (0.1 mol) in methanol, 1-(2,3-dichlorophenyl)piperazine hydrochloride (0.12 mol) and sodium bicarbonate

Scheme 2



(0.28 mol) were added. The reaction mixture was refluxed for 6 h. After completion of reaction, the product was extracted with dichloromethane. After the evaporation of solvent, solid obtained was recrystallized with ethanol to acquire pure product as a white solid powder.

Methyl 2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl) piperazin-1-yl)acetate (4). Yield 90.0%; mp 98°C; ir (KBr, cm^{-1}): 1044 (Ar–Cl),1190 (C–O), 1476 & 1579 (C=C), 1742 (C=O); ^{1}H nmr (DMSO-d_6): δ 2.68 (m, 4H, –CH_2, piperazine), 3.08 (m, 4H, –CH_2, piperazine), 3.71 (s, 3H, –OCH_3), 4.77 (s, 1H, –CH), 6.94 (dd, 1H, Ar–H), 7.11–7.16 (m, 2H, Ar–H), 7.23–7.31 (m, 3H, Ar–H), 7.41 (d, 1H, Ar–H), 7.67 (m, 1H, Ar–H); ms: m/z 413 (M+H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{19}H_{19}Cl_3N_2O_2$: C, 55.16; H, 4.63; N, 6.77. Found: C, 55.14; H, 4.60; N, 6.74.

Preparation of 2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide (5). Methyl 2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetate (0.01 mol) and hydrazine hydrate (0.31 mol) were dissolved in 30 mL of ethanol and refluxed for 24 h. After completion of reaction, the contents were cooled, and the solid obtained was filtered and washed with ethanol. Then it was recrystallized from ethanol to obtain 2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide as a white powder.

2-(2-Chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl) acetohydrazide (5). Yield 91.0%; mp 148°C; ir (KBr, cm^{-1}): 1043 (Ar–Cl), 1450 & 1577 (C=C), 1678 (C=O), 3175 (N–H); ^{1}H nmr (DMSO-d_6): δ 2.54 (m, 4H, –CH_2, piperazine), 2.98 (m, 4H, –CH_2, piperazine), 4.35 (s, 2H, –NH_2), 4.39 (s, 1H, –CH), 7.14 (m, 1H, Ar–H), 7.29–7.43 (m, 5H, Ar–H), 7.83–7.85 (dd, 1H, Ar–H), 9.5 (s, 1H, NH). ms: m/z 413 (M+H) with (M +2), (M+4) & (M+6) peaks; *Anal.* Calcd. for $C_{18}H_{19}Cl_3N_4O$: C, 52.25; H, 4.63; N, 13.54. Found: C, 52.23; H, 4.60; N, 13.57.

Preparation of 1-(2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetyl)-4- phenylthiosemicarbazide (6). Equimolar amount (5 mmol) of compound **5** and aryl isothiocyanate was dissolved in 15 mL of ethanol. The reaction mixture was heated under reflux for 2 h. The progress of reaction was monitored by TLC (80% Pet ether + 20% ethyl acetate). After completion of reaction, contents were cooled, and the solid obtained was filtered and recrystallized from ethanol to acquire the pure product 1-(2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetyl)-4- phenylthiosemicarbazide **6**.

1-(2-(2-Chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetyl)-4-phenylthiosemicarbazide (6a). Yield 75%; mp 195°C; ir (KBr, cm^{-1}): 1040 (Ar–Cl), 1498 & 1598 (C=C), 1655 (C=O), 3206 (N–H), 3280 (N–H); ^{1}H nmr (DMSO-d_6): δ 2.68 (m, 4H, –CH_2, piperazine), 2.99 (m, 4H, –CH_2, piperazine), 4.79 (s, 1H, –CH), 7.10–7.14 (m, 2H, Ar–H), 7.25–7.38 (m, 7H, Ar–H), 7.42–7.49 (m, 3H, Ar–H), 9.42 (bs, 1H, –NH), 9.60 (bs, 1H, –NH), 10.25 (bs, 1H, –NH); ms: m/z 548 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{25}H_{24}Cl_3N_5OS$: C, 54.70; H, 4.41; N, 12.76. Found: C, 54.73; H, 4.45; N, 12.80.

1-(2-(2-Chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetyl)-4-(4-fluorophenyl) thiosemicarbazide (6b). Yield 73%; mp 189°C; ir (KBr, cm^{-1}): 1042 (Ar–Cl), 1132 (Ar–F), 1497 & 1598 (C=C), 1657 (C=O), 3207 (N–H), 3285 (N–H); ^{1}H nmr (DMSO-d_6): δ 2.66 (m, 4H, –CH_2, piperazine), 3.00 (m, 4H, –CH_2, piperazine), 4.80 (s, 1H, –CH), 7.09–7.17 (m, 3H, Ar–H), 7.25–7.49 (m, 7H, Ar–H), 7.60–7.63 (m, 1H, Ar–H), 9.45 (bs, 1H, –NH), 9.71 (bs, 1H, –NH), 10.35 (bs, 1H, –NH); ms: m/z 566 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{25}H_{23}Cl_3FN_5OS$: C, 52.97; H, 4.09; N, 12.35. Found: C, 53.00; H, 4.13; N, 12.40.

4-(3-Chlorophenyl)-1-(2-(2-chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetyl) thiosemicarbazide (6c). Yield 72%; mp 187°C; ir (KBr, cm^{-1}): 1044 (Ar–Cl), 1499 & 1597 (C=C), 1658 (C=O), 3208 (N–H), 3284 (N–H); ^{1}H nmr (DMSO-d_6): δ 2.68 (m, 4H, –CH_2, piperazine), 3.10 (m, 4H, –CH_2, piperazine), 4.85 (s, 1H, –CH), 7.09–7.12 (m, 3H, Ar–H), 7.26–7.39 (m, 7H, Ar–H), 7.47–7.51 (m, 1H, Ar–H), 9.52 (bs, 1H, –NH), 9.95 (bs, 1H, –NH), 10.26 (bs, 1H, –NH); ms: m/z 582 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{25}H_{23}Cl_4N_5OS$: C, 51.47; H, 3.97; N, 12.01. Found: C, 51.50; H, 4.03; N, 12.06.

1-(2-(2-Chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetyl)-4-p-tolylthiosemicarbazide (6d). Yield 70%; mp 185°C; ir (KBr, cm^{-1}): 1041 (Ar–Cl), 1497 & 1598 (C=C), 1655 (C=O), 3207 (N–H), 3284 (N–H); ^{1}H nmr (DMSO-d_6): δ 2.25 (s, 3H, –CH_3), 2.69 (m, 4H, –CH_2, piperazine), 3.00 (m, 4H, –CH_2, piperazine), 4.79 (s, 1H, –CH), 7.11–7.13 (m, 3H, Ar–H), 7.25–7.37 (m, 6H, Ar–H), 7.47–7.49 (m, 1H, Ar–H), 7.67–7.69 (m, 1H, Ar–H) 9.52 (bs, 1H, –NH), 9.63 (bs, 1H, –NH), 10.31 (bs, 1H, –NH); ms: m/z 562 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{26}H_{26}Cl_3N_5OS$: C, 55.47; H, 4.66; N, 12.44. Found: C, 55.50; H, 4.70; N, 12.47.

1-(2-(2-Chlorophenyl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetyl)-4-(4-methoxyphenyl) thiosemicarbazide (6e). Yield 74%; mp 192°C; ir (KBr, cm^{-1}): 1043 (Ar–Cl), 1495 & 1599 (C=C), 1653 (C=O), 3206 (N–H), 3285 (N–H); ^{1}H nmr (DMSO-d_6): δ 2.68 (m, 4H, –CH_2, piperazine), 3.00 (m, 4H, –CH_2, piperazine), 3.72 (s, 3H, –OCH_3), 4.79 (s, 1H, –CH), 6.88 (d, 2H, Ar–H), 7.09–7.12 (m, 1H, Ar–H), 7.25–7.37 (m, 5H, Ar–H), 7.46–7.48 (m, 1H, Ar–H) 7.65–7.68 (m, 2H, Ar–H), 9.42 (bs, 1H, –NH), 9.57 (bs, 1H, –NH), 10.27 (bs, 1H, –NH); ms: m/z 578 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{26}H_{26}Cl_3N_5O_2S$: C, 53.94; H, 4.53; N, 12.10. Found: C, 53.97; H, 4.58; N, 12.14.

Preparation of 5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl) piperazin-1-yl)methyl)-*n*-phenyl-1,3,4-thiadiazol-2-amine (7). Thiosemicarbazide **6** (1 mmol) was dissolved in 4 mL of conc. H_2SO_4 in a 100 mL Round Bottom Flask (RBF). The reaction mixture was stirred at room temperature for 3 h. After completion of reaction, 20 g of crushed ice was added in it. The solid obtained was separated by filtration and recrystallized from ethanol to afford thiadiazoles **7**.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-n-phenyl-1,3,4-thiadiazol-2-amine (7a). Yield 72%; mp 167°C; ir (KBr, cm^{-1}): 1041 (Ar–Cl), 1478 & 1597 (C=C), 3211(N–H); ^{1}H nmr (DMSO-d_6): δ 3.20 (m, 4H, $–CH_2$, piperazine), 4.09 (m, 4H, $–CH_2$, piperazine), 5.26 (s, 1H, –CH), 7.17–7.19 (m, 2H, Ar–H), 7.32–7.42 (m, 6H, Ar–H), 7.44–7.46 (m, 2H, Ar–H) 7.51–7.53 (m, 1H, Ar–H), 7.62–7.65 (m, 1H, Ar–H), 9.79 (s, 1H, –NH); ms: m/z 530 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{25}H_{22}Cl_3N_5S$: C, 56.56; H, 4.18; N, 13.19. Found: C, 56.59; H, 4.18; N, 13.22.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-n-(4-fluorophenyl)-1,3,4-thiadiazol-2-amine (7b). Yield 78%; mp 157°C; ir (KBr, cm^{-1}): 1052 (Ar–Cl), 1134 (Ar–F), 1478 & 1597 (C=C), 3225 (N–H); ^{1}H nmr (DMSO-d_6): δ 3.19 (m, 4H, $–CH_2$, piperazine), 4.10 (m, 4H, $–CH_2$, piperazine), 5.26 (s, 1H, –CH), 7.15–7.20 (m, 2H, Ar–H), 7.30-7.39 (m, 5H, Ar–H), 7.48–7.50 (m, 2H, Ar–H) 7.62–7.63 (m, 1H, Ar–H), 7.68–7.70 (m, 1H, Ar–H), 9.90 (s, 1H, –NH); ms: m/z 548 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd. for $C_{25}H_{21}Cl_3FN_5S$: C, 54.70; H, 3.86; N, 12.76. Found: C, 54.75; H, 3.90; N, 12.80.

N-(3-Chlorophenyl)-5-((2-chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-1,3,4-thiadiazol-2-amine (7c). Yield 70%; mp 159°C; ir (KBr, cm^{-1}): 1052 (Ar–Cl), 1477 & 1598 (C=C), 3222 (N–H); ^{1}H nmr (DMSO-d_6): δ 3.17 (m, 4H, $–CH_2$, piperazine), 4.08 (m, 4H, $–CH_2$, piperazine), 5.25 (s, 1H, –CH), 7.14–7.20 (m, 2H, Ar–H), 7.31–7.39 (m, 5H, Ar–H), 7.47–7.50 (m, 2H, Ar–H), 7.60–7.63 (m, 1H, Ar–H), 7.65–7.68 (m, 1H, Ar–H), 9.95 (s, 1H, –NH); ms: m/z 564 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{25}H_{21}Cl_4N_5S$: C, 53.11; H, 3.74; N, 12.39. Found: C, 53.15; H, 3.78; N, 12.43.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-n-p-tolyl-1,3,4-thiadiazol-2-amine (7d). Yield 69%; mp 158°C; ir (KBr, cm^{-1}): 1050 (Ar–Cl), 1477 & 1595 (C=C), 3220 (N–H); ^{1}H nmr (DMSO-d_6): δ 2.25 (s, 3H, $–CH_3$), 3.24 (m, 4H, $–CH_2$, piperazine), 3.59 (m, 4H, $–CH_2$, piperazine), 5.48 (s, 1H, –CH), 7.11–7.17 (m, 3H, Ar–H), 7.25–7.29 (m, 2H, Ar–H), 7.30–7.34 (m, 4H, Ar–H) 7.63–7.66 (m, 1H, Ar–H), 7.72–7.75 (m, 1H, Ar–H), 9.71 (s, 1H, –NH); ms: m/z 544 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{26}H_{24}Cl_3N_5S$: C, 57.31; H, 4.44; N, 12.85. Found: C, 57.34; H, 4.48; N, 12.88.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-n-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (7e). Yield 67%; mp 162°C; ir (KBr, cm^{-1}): 1051 (Ar–Cl), 1478 &1599 (C=C), 3223 (N–H); ^{1}H nmr (DMSO-d_6): δ 3.27 (m, 4H, $–CH_2$, piperazine), 3.72 (s, 3H, $–OCH_3$), 4.26 (m, 4H, $–CH_2$, piperazine), 5.57 (s, 1H, –CH), 6.74 (d, 8 Hz, 2H, Ar–H), 6.91–7.17 (m, 2H, Ar–H), 7.30–7.35 (m, 4H, Ar–H) 7.46–7.59 (m, 2H, Ar–H), 7.65–7.67 (m, 1H, Ar–H), 9.66 (s, 1H, –NH); ms: m/z 560 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd For $C_{26}H_{24}Cl_3N_5OS$: C, 55.67; H, 4.31; N, 12.49. Found: C, 55.71; H, 4.35; N, 12.52.

Preparation of 5-((2-chlorophenyl)(4-(2,3-dichlorophenyl) piperazin-1-yl)methyl)-4-phenyl-4*h*-1,2,4-triazole-3-thiol (8). Thiosemicarbazide **6** (1 mmol) was dissolved in 10 mL of 2N NaOH. The reaction mixture was heated under mild reflux. The progress of reaction was monitored by TLC (80% Pet ether + 20% Ethyl acetate). After completion of reaction, contents were cooled and poured into crushed ice. Then it was acidified with glacial acetic acid. The product was separated by filtration and recrystallized from ethanol to acquire corresponding triazoles **8**.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-4-phenyl-4h-1,2,4-triazole-3-thiol (8a). Yield 65%; mp 233°C; ir (KBr, cm^{-1}): 1044 (Ar–Cl), 1498 & 1592, (C=C), 3065 (NH); ^{1}H nmr ($CDCl_3$): δ 2.77 (m, 4H, $–CH_2$, piperazine), 3.00 (m, 4H, $–CH_2$, piperazine), 5.13 (s, 1H, –CH), 6.89–7.00 (m, 2H, Ar–H), 7.15–7.19 (m, 3H, Ar–H), 7.23–7.28 (m, 2H, Ar–H) 7.49–7.56 (m, 5H, Ar–H), 11.16 (s, 1H, –SH); ms: m/z 530 (M–H) with (M +2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{25}H_{22}Cl_3N_5S$: C, 56.56; H 4.18; N, 13.19. Found: C, 56.60; H, 4.22; N, 13.23.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-4-(4-fluorophenyl)-4h-1,2,4-triazole-3-thiol (8b). Yield 68%; mp 228°C; ir (KBr, cm^{-1}): 1048 (Ar–Cl), 1140 (Ar–F), 1497 & 1599 (C=C), 3071 (NH); ^{1}H nmr ($CDCl_3$): δ 2.74 (m, 4H, $–CH_2$, piperazine), 3.02 (m, 4H, $–CH_2$, piperazine), 5.14 (s, 1H, –CH), 6.90–6.92 (m, 2H, Ar–H), 7.10–7.16 (m, 3H, Ar–H), 7.26–7.31 (m, 2H, Ar–H) 7.48-7.56 (m, 4H, Ar–H), 11.05 (s, 1H, –SH); ms: m/z 548 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{25}H_{21}Cl_3FN_5S$: C, 54.70; H 3.86; N, 12.76. Found: C, 54.74; H, 3.91; N, 12.80.

4-(3-Chlorophenyl)-5-((2-chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-4h-1,2,4-triazole-3-thiol (8c). Yield 65%; mp 226°C; ir (KBr, cm^{-1}): 1050 (Ar–Cl), 1495 & 1598 (C=C), 3075(NH); ^{1}H nmr ($CDCl_3$): δ 2.74 (m, 4H, $–CH_2$, piperazine), 3.02 (m, 4H, $–CH_2$, piperazine), 5.14(s, 1H, –CH), 6.90–6.93 (m, 2H, Ar–H), 7.10–7.17(m, 2H, Ar–H), 7.26–7.33 (m, 4H, Ar–H) 7.48–7.50 (m, 3H, Ar–H), 11.08 (s, 1H, –SH); ms: m/z 564 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.*

Calcd for $C_{25}H_{21}Cl_4N_5S$: C, 53.11; H 3.74; N, 12.39. Found: C, 53.15; H, 3.78; N, 12.41.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-4-p-tolyl-4h-1,2,4-triazole-3-thiol (8d). Yield 66%; mp 228°C; ir (KBr, cm^{-1}): 1045 (Ar–Cl), 1495 & 1596 (C=C), 3072 (NH); ^1H nmr ($CDCl_3$): δ 2.43 (s, 3H, $–CH_3$), 2.67 (m, 4H, $–CH_2$, piperazine), 3.01 (m, 4H, $–CH_2$, piperazine), 5.08 (s, 1H, –CH), 7.06–7.14 (m, 3H, Ar–H), 7.25–7.52 (m, 5H, Ar–H), 7.53–7.55 (m, 2H, Ar–H), 7.78–7.80 (m, 1H, Ar–H) 11.50 (s, 1H, –SH); ms: m/z 544 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal* .Calcd. for $C_{26}H_{24}Cl_3N_5S$: C, 57.31; H 4.44; N, 12.85. Found: C, 57.35; H, 4.48; N, 12.88.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-4-(4-methoxyphenyl)-4h-1,2,4-triazole-3-thiol (8e). Yield 60%; mp 226°C; ir (KBr, cm^{-1}): 1047 (Ar–Cl), 1497 &1598, (C=C), 3074 (NH); ^1H nmr ($CDCl_3$): δ 2.63 (m, 4H, $–CH_2$, piperazine), 3.00 (m, 4H, $–CH_2$, piperazine), 3.89 (s, 3H, $–OCH_3$), 5.28 (s, 1H, –CH), 7.05–7.13 (m, 3H, Ar–H), 7.28–7.55 (m, 5H, Ar–H), 7.58–7.50 (m, 2H, Ar–H), 7.69–7.72 (m, 1H, Ar–H) 11.32 (s, 1H, –SH); ms: m/z 560 (M–H) with (M+2), (M +4) & (M+6) peaks; *Anal.* Calcd for $C_{26}H_{24}Cl_3N_5OS$: C, 55.67; H 4.31; N, 12.49. Found: C, 55.70; H, 4.35; N, 12.52.

Preparation of 5-((2-chlorophenyl)(4-(2,3-dichlorophenyl) piperazin-1-yl)methyl)-*n*-phenyl-1,3,4-oxadiazol-2-amine (9). The mixture thiosemicarbazide **6** (1 mmol), potassium iodide (2 mmol), iodine (2 mmol) was dissolved in 4N sodium hydroxide (10 mL). The reaction mixture was heated under mild reflux for 5 h. The progress of reaction was monitored by TLC (80% Pet ether + 20% Ethyl acetate). After completion of reaction, contents were poured over crushed ice and were extracted with ethyl acetate. The crude product isolated after evaporation of ethyl acetate was recrystallized from ethanol to acquire pure product **9**.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-n-phenyl-1,3,4-oxadiazol-2-amine (9a). Yield 62%; mp 199°C; ir (KBr, cm^{-1}): 1046 (Ar-Cl), 1498 & 1599 (C=C), 3249 (NH); ^1H nmr ($CDCl_3$): δ 2.78 (m, 4H, $–CH_2$, piperazine), 3.09 (m, 4H, $–CH_2$, piperazine), 5.43 (s, 1H, –CH), 6.95 (m,1H, Ar–H), 7.07–7.15 (m, 5H, Ar–H), 7.26–7.43 (m, 5H, Ar–H) 7.89–7.91 (m, 1H, Ar–H) 9.85 (s, 1H, –NH); ms: m/z 514 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{25}H_{22}Cl_3N_5O$: C, 58.32; H 4.31; N, 13.60. Found: C, 58.36; H, 4.35; N, 13.63.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-n-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (9b). Yield 64%; mp 202°C; ir (KBr,cm^{-1}): 1043 (Ar–Cl), 1138 (Ar–F), 1497 & 1596 (C=C), 3251 (NH); ^1H nmr ($CDCl_3$): δ 2.70 (m, 4H, $–CH_2$, piperazine), 3.18 (m, 4H, $–CH_2$, piperazine), 4.83 (s, 1H, –CH), 7.10–7.18 (m, 2H, Ar–H), 7.25–7.39 (m, 6H, Ar–H), 7.48–7.50 (m, 1H, Ar–H) 7.66–7.70 (m, 2H, Ar–H) 9.90 (s, 1H, –NH); ms: m/z 532 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{25}H_{21}Cl_3FN_5O$: C, 56.35; H 3.97; N, 13.14. Found: C, 56.38; H, 4.00; N, 13.19.

N-(3-Chlorophenyl)-5-((2-chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-1,3,4-oxadiazol-2-amine (9c). Yield 63%; mp 210°C; ir (KBr, cm^{-1}): 1042 (Ar–Cl), 1496 & 1599 (C=C), 3250 (NH); ^1H nmr ($CDCl_3$): δ 2.68 (m, 4H, $–CH_2$, piperazine), 3.15 (m, 4H, $–CH_2$, piperazine), 4.80 (s, 1H, –CH), 7.10–7.18 (m, 2H, Ar–H), 7.24–7.37 (m, 6H, Ar–H), 7.48–7.51 (m, 1H, Ar–H) 7.64–7.68 (m, 2H, Ar–H) 9.88 (s, 1H, –NH); ms: m/z 548 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{25}H_{21}Cl_4N_5O$: C, 54.67; H 3.85; N, 12.75. Found: C, 54.71; H, 3.89; N, 12.80.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-n-p-tolyl-1,3,4-oxadiazol-2-amine (9d). Yield 59%; mp 195°C; ir (KBr, cm^{-1}): 1045 (Ar–Cl), 1498 & 1598 (C=C), 3252 (NH); ^1H nmr ($CDCl_3$): δ 2.43 (s, 3H, $–CH_3$), 2.67 (m, 4H, $–CH_2$, piperazine), 3.01 (m, 4H, $–CH_2$, piperazine), 5.12 (s, 1H, –CH), 6.90–6.93 (m, 2H, Ar–H), 7.13–7.16 (m, 3H, Ar–H), 7.28–7.32 (m, 4H, Ar–H) 7.58–7.63 (m, 2H, Ar–H) 9.90 (s, 1H, –NH); ms: m/z 528 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{26}H_{24}Cl_3N_5O$: C, 59.05; H 4.57; N, 13.24. Found: C, 59.10; H, 4.60; N, 13.27.

5-((2-Chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl) methyl)-n-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (9e). Yield 66%; mp 197°C; ir (KBr, cm^{-1}): 1043 (Ar–Cl), 1497 & 1599 (C=C), 3255 (NH); ^1H nmr ($CDCl_3$): δ 2.68 (m, 4H, $–CH_2$, piperazine), 3.02 (m, 4H, $–CH_2$, piperazine), 3.80 (s, 3H, $–OCH_3$), 5.13 (s, 1H, –CH), 6.89–6.97 (m, 3H, Ar–H), 7.09–7.15(m, 2H, Ar–H), 7.25–7.29 (m, 5H, Ar–H) 7.57–7.60 (m, 1H, Ar–H) 9.91 (s, 1H, –NH); ms: m/z 544 (M–H) with (M+2), (M+4) & (M+6) peaks; *Anal.* Calcd for $C_{26}H_{24}Cl_3N_5O_2$: C, 57.31; H 4.44; N, 12.85. Found: C, 57.35; H, 4.47; N, 12.89.

Table 1

Antibacterial screening of synthesized compounds.

Compound	*E. Coli*	*B. Subtilis*	Compound	*E. Coli*	*B. Subtilis*
6a	11	12	**8a**	13	13
6b	**15**	**16**	**8b**	14	12
6c	**14**	**17**	**8c**	15	11
6d	13	12	**8d**	13	14
6e	12	16	**8e**	**13**	**15**
7a	9	14	**9a**	**15**	**15**
7b	8	14	**9b**	**14**	**16**
7c	9	15	**9c**	15	12
7d	9	13	**9d**	13	16
7e	11	15	**9e**	15	13
Standard drug: Ampicillin				16	17

ANTIBACTERIAL SCREENING

Bacillus Subtilis and *E. Coli* species were chosen for the study of antibacterial activity. The activity was tested by agar well diffusion method. The bacteria were cultured on nutrient agar. The concentration of the compounds taken was 10 mg/mL of which 0.1 mL was used in this assay. Ampicillin was used as a standard drug and its final concentration used was 1 mg. The zone inhibition of antibacterial activity was measured in mm, and the results were produced as an average of three repeated assays. The result of this assay is given in Table 1. It was found that the compounds **6b**, **6c**, **8e**, **9a,** and **9b** have shown moderate activity towards both bacterial species.

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