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Research Article

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Synthesis, Characterization and Biological screening of novel 2,5-disubstituted-1,3,4-thiadiazole derivatives

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ABSTRACT

In the present study, novel Schiff bases of 2,5-disubstituted-1,3,4-thiadiazole have been synthesized. The chemical structures were confirmed by IR, ^{1}H NMR and elemental analysis. Acute toxicity study of synthesized compounds was performed. All compounds were screened for antibacterial (Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Bacillus cereus, Escherichia coli, and Pseudomonas aeruginosa)activities and antifungal (Aspergillus niger) activities. The antioxidant activity of a series of novel 2,5-Disubstituted-1,3,4-thiadiazole was determined by DPPH radical scavenging , ferrous reducing power, Fe^{2+} chelating activity assay. . The minimum inhibitory concentrations (MICs) of the compounds were determined by agar streak dilution method. Among the synthesized compounds, 5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxy benzylidene)-4-aminophenyl}-1,3,4-thiadiaz-ole ***8f*** *was found to be the most potent antimicrobial activity with MICs of 0.12, 3.1, 2.9, 0.25, 0.16, 0.27 and 2.4 µg/mL against the above mentioned respective strains. The derivatives showed good antioxidant capacity in DPPH radical scavenging assay, when compared to other in vitro models and the IC_{50} value of most active derivative* ***8f*** *was found to be 245.67µg/mL. The total phenolic content using Folin's-Ciocalteu reagent indicated that 1mg of most active derivative* ***8f*** *contains 624.5 µg with gallic acid equivalent.*

Key words: Acute Toxicity Study, Antibacterial activity, Antifungal activity, Schiff bases, Minimum inhibitory concentration, Gallic acid, L-ascorbic acid, Antioxidant, Thiadiazole.

INTRODUCTION

Schiff bases represent an important class of organic compounds, especially in the therapeutic and pharmaceutical fields. Thus, development and synthesis of novel Schiff base derivatives as potential chemotherapeutics still attracts the attention of organic and medicinal chemists [1, 2, 3]. Many studies reported the biological activities of Schiff bases, including their antineoplastic, antibacterial, antifungal and herbicidal activities [4]. Schiff bases, derived mostly from variety of heterocyclic rings, were reported to possess a broad spectrum and a wide variety of biological activities including antiviral, antineoplastic, cytotoxic, antimicrobial, antibacterial, anticonvulsant, etc [5]. A number of Schiff bases have been tested for antibacterial, antifungal, anticancer and herbicidal activities [6, 7].

The emergence of old and new antibiotic-resistant bacterial strains in the last decades constitutes a substantial need for the new class of antibacterial agents.

The varied biological activities of 1,3,4-thiadiaoles and their analogs have been known from the beginning of the 20th century [8, 9]. Literature survey revealed that slight modification in the structure can result in qualitative as well as quantitative changes in the activity [8, 10, 11]. This prompted us to undertake the synthesis of various novel Schiff bases derived from 2,5-Disubstituted-1,3,4-thiadiazole and characterized using IR, ^{1}H NMR and Elemental analysis with the aim of having improved activity.

Antioxidant plays a major role in the living system and it prevent oxidative damage, when the oxidative damage occur in living system results in cancer, cardiovascular disease and diabetes [12]. The reactive oxygen species such as superoxide, hydrogen peroxide, hydroxyl, nitric oxide radical are various forms of activated oxygen generated from biological reaction as oxidation product [13]. ROS are continuously produced during regular physiological process and it may cause cellular injuries, leading to the accumulation of lipid peroxides in biological membranes, damaging crucial biomolecules such as nucleic acids, lipids, proteins, polyunsaturated fatty acids and carbohydrates. The DNA damage can cause the mutation in living system. The oxidative stress leads to the pathogenesis of various lung disorders like asthma, chronic obstructive, pulmonary disorders, acute lung injury and lung cancer [14]. The ROS directly stimulate histamine release from mast cells and mucus secretion from airway epithelial cells resulting in asthma.

Free radicals and reactive oxygen species have been involved in the etiology and pathophysiology of gastric ulcers. The collapse of mucosal defense mechanism ultimately leads to gastric inflammation and ulceration [15]. Antioxidants have a significant role in the protection against the ulceration of gastric mucosa. It has been demonstrated that many synthetic agents possess potent antioxidant actions are effective choice in healing experimentally induced gastric ulcers [16, 17].

The synthesized compounds were screened for their antibacterial activity against four Gram-positive bacteria (Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus and Bacillus cereus) and two Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and antifungal activity against fungi (*Aspergilus niger*). The minimum inhibitory concentrations (MICs) of the compounds were determined by agar streak dilution method.

The antioxidant activity of a series of novel 2,5-Disubstituted-1,3,4-thiadiazole were determined by DPPH radical scavenging , ferrous reducing power, Fe^{2+} chelating activity assay, nitric oxide radical scavenging activity, $ABTS^+$ radical cation decolourisation assay, superoxide anion and hydrogen peroxide radical scavenging activities.

MATERIALS AND METHODS

Measurements

The melting points were taken in an open capillary tube and uncorrected. The IR spectra of the compounds were recorded on FT-IR spectrometer with KBr pellets. ^{1}H spectra was recorded using NMR spectrometer operating at 400.13 MHz. Microanalyses were obtained with an Elemental analysis. The purity of compounds was checked by TLC on pre-coated SiO_2 gel (HF254, 200mesh) aluminium plates (E Merk) and visualized in UV chamber. IR, ^{1}H NMR and elemental analysis were consistent with the assigned structures.

Synthesis

General method of synthesis of Substituted diacyl hydrazine (3a-f)

To a stirred suspension of substituted salicylic hydrazine (1 mol) in 15 mL toluene, 0.96 g of methyl sulfonic acid was added at the right time. The mixture was stirred for 10 min, afterwards 1 mol of substituted benzoyl chloride was added. After that stirring continued for 3 h. The mixture was cooled, purified in crushed ice. The mixture was filtered, washed and dried.

N1-(2-Aminobenzoyl)-N2-(2-hydroxybenzoyl)hydrazine **3a:**
(Yield: 89%). mp 125-126^{o}C; IR (KBr) cm^{-1}:3630($\nu_{O-H)}$,3200(ν_{N-H}), 3100(ν_{C-H}), 1710($\nu_{C=O}$), 1570($\nu_{C=C}$), 1310(ν_{C-N}), 755(o-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm 10.87 (s, 1H, ar-OH), 7.85 (d, 1H, ar-H), 7.58 (t, 1H, ar-H), 7.49 (d, 1H, ar-H), 7.46 (t, 1H, ar-H), 7.29 (t, 1H, ar-H), 7.28 (d, 1H, ar-H), 7.03 (t, 1H, ar-H), 6.88 (d, 1H, ar-H),

4.85 (s, 2H, ar-NH_2), 4.58 (d, 1H, NH), 4.58 (d, 1H, NH). ^{13}C NMR (DMF-d6) δ ppm: 163.01, 163.01, 158.74, 146.49, 131.85, 130.81, 128.15, 128.09, 125.07, 121.98, 120.36, 118.92, 117.26, 115.66. Anal. Found (calc.) for $C_{14}H_{13}N_3O_3$ (%): C, 61.89, H, 4.86, N, 15.43, O, 17.61.

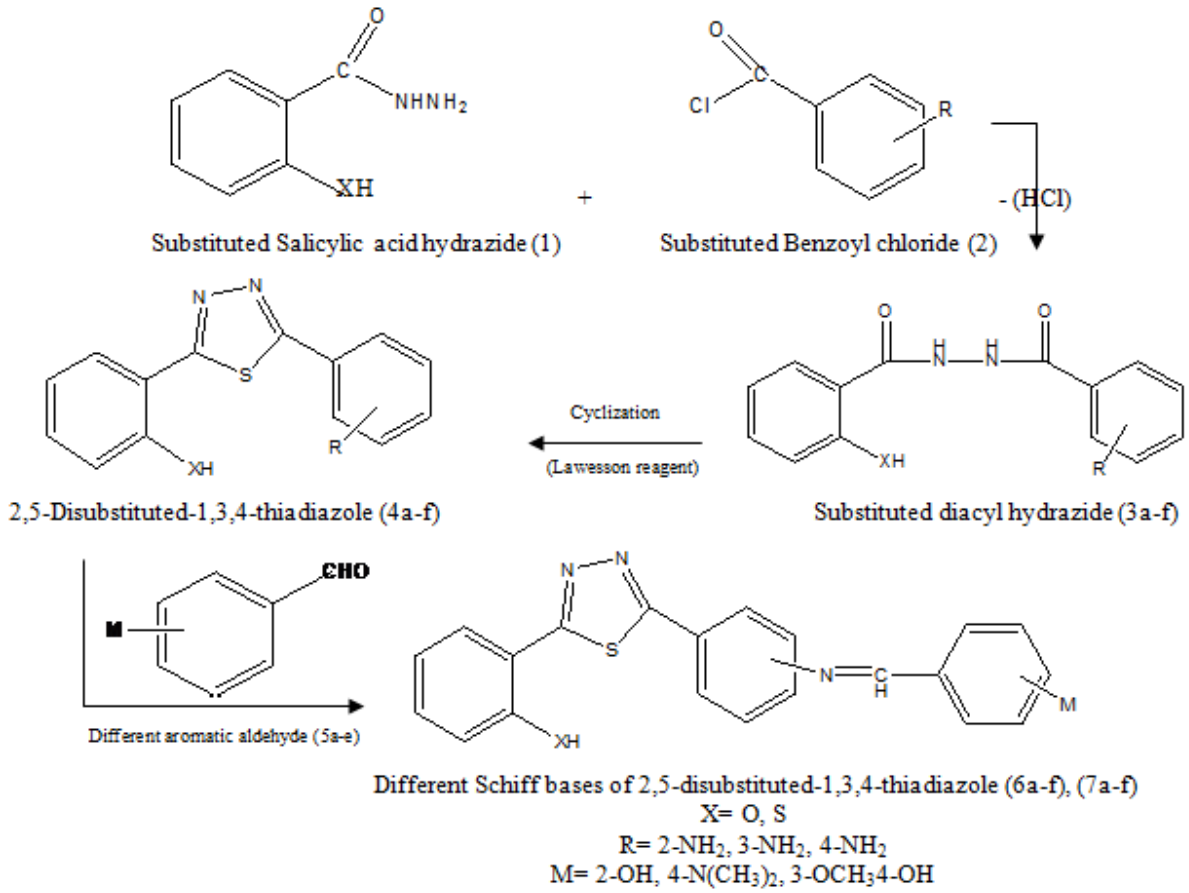

Figure 1: Scheme

N1-(2-Aminobenzoyl)-N2-(2-mercaptobenzoyl)hydrazine **3b:**
(Yield: 76%). mp 129-130^{o}C; IR (KBr) cm^{-1}: 3190(ν_{N-H}), 3095(ν_{C-H}), 1712($\nu_{C=O}$), 1560($\nu_{C=C}$), 1350(ν_{C-N}), 760(o-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 7.87 (d, 1H, ar-H), 7.67 (t, 1H, ar-H), 7.59 (t, 1H, ar-H), 7.58 (t, 1H, ar-H), 7.48 (d, 1H, ar-H), 7.40 (d, 1H, ar-H), 7.28 (t, 1H, ar-H), 6.87 (d, 1H, ar-H), 4.75 (s, 2H, ar-NH_2), 4.58 (d, 1H, NH), 4.58 (d, 1H, NH), 2.76 (s, 1H, ar-SH). ^{13}C NMR (DMF-d6) δ ppm: 163.011, 163.011, 146.496, 138.7, 132.39, 131.21, 130.81, 130.55, 130.3, 128.09, 128.0, 125.07, 120.36, 115.66. Anal. Found (calc.) for $C_{14}H_{13}N_3O_2S$ (%): C, 58.46, H, 4.63, N, 14.51, O, 11.19, S, 11.21.

N1-(3-Aminobenzoyl)-N2-(2-hydroxybenzoyl)hydrazine **3c:**
(Yield: 87%). mp 161-162^{o}C; IR (KBr) cm^{-1}: 3620($\nu_{O-H)}$, 3195(ν_{N-H}), 3065(ν_{C-H}), 1723($\nu_{C=O}$), 1530($\nu_{C=C}$), 1190(ν_{C-N}), 760(o-disubstituted benzene), 705,795(m-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.78 (s, 1H, ar-OH), 7.98 (s, 1H, ar-H), 7.84 (d, 1H, ar-H), 7.46 (t, 1H, ar-H), 7.45 (d, 1H, ar-H), 7.42 (t, 1H, ar-H), 7.29 (t, 1H, ar-H), 7.26 (d, 1H, ar-H), 7.03 (d, 1H, ar-H), 4.72 (s, 2H, ar-NH_2), 4.58 (d, 1H, NH), 4.58 (d, 1H, NH). ^{13}C NMR (DMF-d6) δ ppm: 164.35, 163.011, 158.73, 146.73, 134.57, 131.85, 128.15, 127.52, 127.14, 121.98, 118.917, 117.26, 116.35, 115.48. Anal. Found (calc.) for $C_{14}H_{13}N_3O_3$ (%): C, 61.85, H, 4.72, N, 15.46, O, 17.48.

N1-(3-Aminobenzoyl)-N2-(2-mercaptobenzoyl)hydrazine **3d:**
(Yield: 75%). mp 168-169^{o}C; IR (KBr) cm^{-1}: 3192(ν_{N-H}), 3026(ν_{C-H}), 1718($\nu_{C=O}$), 1567($\nu_{C=C}$), 1250(ν_{C-N}), 763(o-disubstituted benzene), 702,798(m-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 7.98 (s, 1H, ar-H), 7.87 (d,

1H, ar-H), 7.67 (d, 1H, ar-H), 7.62 (d, 1H, ar-H), 7.59 (t, 1H, ar-H), 7.42 (t, 1H, ar-H), 7.40 (t, 1H, ar-H), 7.26 (d, 1H, ar-H), 4.82 (s, 2H, ar-NH_2), 4.57 (d, 1H, NH), 4.57 (d, 1H, NH), 2.18 (s, 1H, ar-SH). ^{13}C NMR (DMF-d6) δ ppm: 164.35, 163.01, 146.73, 138.7, 134.57, 132.39, 131.21, 130.55, 130.3, 128.0, 127.52, 127.14, 116.35, 115.48. Anal. Found (calc.) for $C_{14}H_{13}N_3O_2S$ (%): C, 58.48, H, 4.59, N, 14.75, O, 11.18, S, 11.19.

N1-(4-Aminobenzoyl)-N2-(2-hydroxybenzoyl)hydrazine **3e:**
(Yield: 91%). mp 192-193^{o}C; IR (KBr) cm^{-1}: 3642($\nu_{O-H)}$, 3200(ν_{N-H}), 3029(ν_{C-H}), 1698($\nu_{C=O}$), 1577($\nu_{C=C}$), 1286(ν_{C-N}), 753(o-disubstituted benzene), 821(p-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.68 (s, 1H, ar-OH), 7.78 (d, 1H, ar-H), 7.66 (d, 1H, ar-H), 7.66 (d, 1H, ar-H), 7.46 (t, 1H, ar-H), 7.29 (t, 1H, ar-H), 7.03 (d, 1H, ar-H), 6.87 (d, 1H, ar-H), 6.87 (d, 1H, ar-H), 4.87 (s, 2H, ar-NH_2), 4.57 (d, 1H, NH), 4.57 (d, 1H, NH). ^{13}C NMR (DMF-d6) δ ppm: 164.35, 163.01, 158.73, 149.06, 133.82, 131.85, 128.69, 128.15, 121.98, 118.91, 117.26, 113.23, 113.23. Anal. Found (calc.) for $C_{14}H_{13}N_3O_3$ (%): C, 61.91, H, 4.79, N, 15.42, O, 17.52.

N1-(4-Aminobenzoyl)-N2-(2-mercaptobenzoyl)hydrazine **3f:**
(Yield: 72%). mp 198-199^{o}C; IR (KBr) cm^{-1}: 3187(ν_{N-H}), 3056(ν_{C-H}), 1727($\nu_{C=O}$), 1582($\nu_{C=C}$), 1328(ν_{C-N}), 742(o-disubstituted benzene), 810(p-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 7.87 (d, 1H, ar-H), 7.67 (d, 1H, ar-H), 7.66 (d, 1H, ar-H), 7.66 (d, 1H, ar-H), 7.58 (t, 1H, ar-H), 7.40 (t, 1H, ar-H), 6.87 (d, 1H, ar-H), 6.57 (d, 1H, ar-H), 4.87 (s, 2H, ar-NH_2), 4.57 (d, 1H, NH), 4.57 (d, 1H, NH), 2.39 (s, 1H, ar-SH). ^{13}C NMR (DMF-d6) δ ppm: 164.35, 163.01, 149.06, 138.7, 133.82, 132.39, 131.21, 130.55, 130.3, 128.69, 128.69, 128.0, 113.23, 113.23. Anal. Found (calc.) for $C_{14}H_{13}N_3O_2S$ (%): C, 58.46, H, 4.52, N, 14.79, O, 11.12, S, 11.19.

General procedure for synthesis of 2,5-Disubstituted-1,3,4-thiadiazole derivatives (4a-f):
2,5-disubstituted-1,3,4-thiadiazoles were prepared from the reaction of diacylhydrazines (0.1mol) with sulphur source (Lawesson's reagent). The reaction involves thionation of the carbonyl group followed by cyclization with loss of H_2S.The use of Lawesson's reagent gave higher yield and cleaner reaction. The mixture was refluxed for 3 h. The mixture was cooled, purified by crushed ice, filtered, washed and dried.

2-(2-Aminophenyl)-5-(2-hydroxyphenyl)-1,3,4-thiadiazole **4a:**
(Yield: 86%). mp 121-122^{o}C; IR (KBr) cm^{-1}: 3631($\nu_{O-H)}$,3300(ν_{N-H}), 3012(ν_{C-H}), 1572($\nu_{C=C}$), 1610($\nu_{C=N}$), 757(o-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.95 (s, 1H, ar-OH), 7.89 (d, 1H, ar-H), 7.84 (d, 1H, ar-H), 7.58 (t, 1H, ar-H), 7.54 (t, 1H, ar-H), 7.49 (t, 1H, ar-H), 7.39 (t, 1H, ar-H), 7.18 (d, 1H, ar-H), 6.75 (d, 1H, ar-H), 4.87 (s, 2H, ar-NH_2). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 157.89, 147.38, 131.85, 130.81, 130.66, 128.3, 127.82, 127.58, 119.4, 117.8, 116.7, 115.08. Anal. Found (calc.) for $C_{14}H_{11}N_3OS$ (%): C, 62.35, H, 4.16, N, 15.54, O, 5.85, S, 11.92.

2-(2-Aminophenyl)-5-(2-mercaptophenyl)-1,3,4-thiadiazole **4b:**
(Yield: 74%). mp 128-129^{o}C; IR (KBr) cm^{-1}: 3190(ν_{N-H}), 3095(ν_{C-H}), 1560($\nu_{C=C}$), 1650($\nu_{C=N}$), 762(o-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 7.87 (d, 1H, ar-H), 7.82 (d, 1H, ar-H), 7.78 (d, 1H, ar-H), 7.58 (t, 1H, ar-H), 7.57 (t, 1H, ar-H), 7.53 (t, 1H, ar-H), 7.40 (t, 1H, ar-H), 6.75 (d, 1H, ar-H), 4.76 (s, 2H, ar-NH_2), 2.15 (s, 1H, ar-SH). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 147.38, 131.32, 131.21, 130.80, 130.66, 130.66, 130.55, 127.82, 127.82, 127.58,127.58, 115.08. Anal. Found (calc.) for $C_{14}H_{11}N_3S_2$ (%): C, 58.85, H, 3.82, N, 14.76, S, 22.32.

2-(3-Aminophenyl)-5-(2-hydroxyphenyl)-1,3,4-thiadiazole **4c:**
(Yield: 89%). mp 149-150^{o}C; IR (KBr) cm^{-1}: 3623($\nu_{O-H)}$, 3395(ν_{N-H}), 3069(ν_{C-H}), 1537($\nu_{C=C}$), 1632($\nu_{C=N}$), 763(o-disubstituted benzene), 707,798(m-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.88 (s, 1H, ar-OH), 8.05 (d, 1H, ar-H), 7.58 (t, 1H, ar-H), 7.49 (d, 1H, ar-H), 7.46 (s, 1H, ar-H), 7.39 (t, 1H, ar-H), 7.38 (t, 1H, ar-H), 7.19 (d, 1H, ar-H), 6.61 (d, 1H, ar-H), 4.82 (s, 2H, ar-NH_2). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 157.89, 146.73, 131.85, 131.01, 128.3, 127.14, 126.92, 119.21, 117.8, 116.7, 116.35, 115.48. Anal. Found (calc.) for $C_{14}H_{11}N_3OS$ (%): C, 62.39, H, 4.16, N, 15.54, O, 5.84, S, 11.95.

2-(3-Aminophenyl)-5-(2-mercaptophenyl)-1,3,4-thiadiazole **4d:**
(Yield: 78%). mp 152-153^{o}C; IR (KBr) cm^{-1}: 3390(ν_{N-H}), 3073(ν_{C-H}), 1585($\nu_{C=C}$), 1646($\nu_{C=N}$), 738(o-disubstituted benzene), 697,758(m-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 8.03 (d, 1H, ar-H), 7.84 (d, 1H, ar-H), 7.67 (s, 1H, ar-H), 7.55 (t, 1H, ar-H), 7.52 (d, 1H, ar-H), 7.48 (t, 1H, ar-H), 7.27 (t, 1H, ar-H), 6.64 (d, 1H, ar-H), 4.86 (s, 2H, ar-NH_2), 2.28 (s, 1H, ar-SH). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 146.73, 131.32, 131.21, 131.01,

130.66, 130.55, 127.82, 127.58, 127.14, 126.92, 116.35, 115.48. Anal. Found (calc.) for $C_{14}H_{11}N_3S_2$ (%): C, 58.85, H, 3.74, N, 14.76, S, 22.42.

2-(4-Aminophenyl)-5-(2-hydroxyphenyl)-1,3,4-thiadiazole **4e:**
(Yield: 83%). mp 192-193^{o}C; IR (KBr) cm^{-1}: 3628($\nu_{O-H)}$, 3345(ν_{N-H}), 3054(ν_{C-H}), 1523($\nu_{C=C}$), 1649($\nu_{C=N}$), 742(o-disubstituted benzene), 832(p-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.76 (s, 1H, ar-OH), 7.88 (d, 1H, ar-H), 7.63 (d, 1H, ar-H), 7.63 (d, 1H, ar-H), 7.58 (t, 1H, ar-H), 7.49 (t, 1H, ar-H), 7.18 (d, 1H, ar-H), 6.72 (d, 1H, ar-H), 6.72 (d, 1H, ar-H), 4.85 (s, 2H, ar-NH_2). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 157.89, 149.06, 131.85, 131.01, 128.55, 128.55, 128.3, 119.4, 117.8, 116.7, 113.7, 113.7. Anal. Found (calc.) for $C_{14}H_{11}N_3OS$ (%): C, 62.39, H, 4.19, N, 15.56, O, 5.86, S, 11.95.

2-(4-Aminophenyl)-5-(2-mercaptophenyl)-1,3,4-thiadiazole **4f:**
(Yield: 81%). mp 201-202^{o}C; IR (KBr) cm^{-1}: 3445(ν_{N-H}), 3053(ν_{C-H}), 1526($\nu_{C=C}$), 1619($\nu_{C=N}$), 754(o-disubstituted benzene), 826(p-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 7.82 (d, 1H, ar-H), 7.77 (d, 1H, ar-H), 7.66 (d, 1H, ar-H), 7.66 (d, 1H, ar-H), 7.58 (t, 1H, ar-H), 7.53 (t, 1H, ar-H), 6.73 (d, 1H, ar-H), 6.73 (d, 1H, ar-H), 4.83 (s, 2H, ar-NH_2), 2.18 (s, 1H, ar-SH). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 149.06, 131.32, 131.27, 131.01, 130.66, 130.55, 128.55, 128.55, 127.82, 127.58, 113.7, 113.7. Anal. Found (calc.) for $C_{14}H_{11}N_3S_2$ (%): C, 58.84, H, 3.76, N, 14.65, S, 22.42.

General procedure for synthesis of different Schiff base of 1,3,4-thiadiazole derivatives (6-8a-f):
0.01mol was dissolved in 30ml of ethanol containing few drops of glacial acetic acid. The appropriate aromatic aldehyde was added, and reaction mixture was refluxed for 5h at 70^{o}C. The reaction mixture was cooled, filtered, washed dried and recrystalized with ethanol.

5-(2-Hydroxyphenyl)-2-{N-(2-hydroxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole **6a**: (Yield: 75%). mp 170-171^{o}C; IR (KBr) cm^{-1}: 3608($\nu_{O-H)}$, 3012(ν_{C-H}), 1564($\nu_{C=C}$), 1618($\nu_{C=N}$), 748(o-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 10.625 (s, 1H, ar-OH), 8.654 (s, 1H, ar-CH), 8.167 (d, 1H, ar-H), 8.025 (d, 1H, ar-H),7.762 (d, 1H, ar-H), 7.666 (t, 1H, ar-H), 7.617 (d, 1H, ar-H), 7.552 (t, 1H, ar-H), 7.488 (t, 1H, ar-H), 7.424 (t, 1H, ar-H), 7.357 (t, 1H, ar-H), 7.206 (d, 1H, ar-H), 7.047 (d, 1H, ar-H). 13C NMR (DMF-d6) δ ppm: 163.9, 163.87, 163.87, 161.22, 157.89, 144.54, 133.05, 132.39, 131.85, 131.85, 130.66, 128.3, 127.82, 127.58, 125.08, 119.4, 119.16, 119.11, 117.8, 117.21, 116.7. Anal. Found (calc.) for $C_{21}H_{15}N_3O_2S$ (%): C, 67.50, H, 4.11, N, 11.21, O, 8.61, S, 8.54.

5-(2-Mercaptophenyl)-2-{N-(2-hydroxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole **6b** (Yield: 71%). mp 182-183^{o}C; IR (KBr) cm^{-1}: 3632($\nu_{O-H)}$, 3065(ν_{C-H}), 1524($\nu_{C=C}$), 1657($\nu_{C=N}$), 749(o-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 10.519 (s, 1H, ar-OH), 8.663 (s, 1H, ar-CH), 8.15 (d, 1H, ar-H), 7.918 (d, 1H, ar-H), 7.781 (d, 1H, ar-H), 7.767 (d, 1H, ar-H), 7.73 (t, 1H, ar-H), 7.61(d, 1H, ar-H), 7.605 (t, 1H, ar-H), 7.588 (t, 1H, ar-H), 7.501 (t, 1H, ar-H), 7.438 (t, 1H, ar-H), 7.358 (t, 1H, ar-H), 7.052 (d, 1H, ar-H), 2.321 (s, 1H, ar-SH). 13C NMR (DMF-d6) δ ppm: 163.9, 163.87, 163.87, 161.22, 144.54, 133.05, 132.39, 131.85, 131.32, 131.21, 130.66, 130.66, 130.55, 127.82, 127.82, 127.58, 127.58, 125.08, 119.16, 119.11, 117.21. Anal. Found (calc.) for $C_{21}H_{15}N_3OS_2$ (%): C, 64.69, H, 3.81, N, 10.71, O, 4.10, S, 16.37.

5-(2-Hydroxyphenyl)-2-{N-(2-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole **6c** (Yield: 68%). mp 189-190^{o}C; IR (KBr) cm^{-1}: 3623($\nu_{O-H)}$, 3042(ν_{C-H}), 1582($\nu_{C=C}$), 1637($\nu_{C=N}$), 739(o-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 10.654 (s, 1H, ar-OH), 8.788 (s, 1H, ar-CH), 7.932 (s, 1H, ar-H), 7.859 (d, 1H, ar-H), 7.729 (d, 1H, ar-H), 7.71 (d, 1H, ar-H), 7.654 (t, 1H, ar-H), 7.535 (t, 1H, ar-H), 7.514 (t, 1H, ar-H), 7.514 (t, 1H, ar-H), 7.451 (t, 1H, ar-H), 7.32 (t, 1H, ar-H), 7.118 (d, 1H, ar-H), 7.02 (d, 1H, ar-H). 13C NMR (DMF-d6) δ ppm: 163.87, 163.87, 162.45, 161.22, 157.89, 143.70, 132.39, 131.85, 131.85, 131.01, 128.3, 127.14, 126.92, 121.25, 119.4, 119.16, 119.11, 117.8, 117.21, 116.7, 116.35. Anal. Found (calc.) for $C_{21}H_{15}N_3O_2S$ (%): C, 67.51, H, 4.11, N, 11.21, O, 8.49, S, 8.51.

5-(2-Mercaptophenyl)-2-{N-(2-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole **6d** (Yield: 65%). mp 176-177^{o}C; IR (KBr) cm^{-1}: 3641($\nu_{O-H)}$, 3029(ν_{C-H}), 1574($\nu_{C=C}$), 1643($\nu_{C=N}$), 757(o-disubstituted benzene), 694, 771 (m-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 10.512 (s, 1H, ar-OH), 8.765 (s, 1H, ar-CH), 7.964 (s, 1H, ar-H), 7.902 (d, 1H, ar-H), 7.758 (d, 1H, ar-H), 7.754 (d, 1H, ar-H), 7.739 (d, 1H, ar-H), 7.609 (t, 1H, ar-H), 7.574 (t, 1H, ar-H), 7.55 (d, 1H, ar-H), 7.455 (t, 1H, ar-H), 7.425 (t, 1H, ar-H), 7.321 (t, 1H, ar-H), 7.025 (d, 1H, ar-H), 2.311 (s,

1H, ar-H). 13C NMR (DMF-d6) δ ppm: 163.87, 163.87, 162.45, 161.22, 143.70, 132.39, 131.85, 131.32, 131.21, 131.01, 130.66, 130.55, 127.82, 127.82, 127.58, 127.14, 126.92, 121.25, 119.16, 119.11, 117.21, 116.35. Anal. Found (calc.) for $C_{21}H_{15}N_3OS_2$ (%): C, 64.71, H, 3.81, N, 10.72, O, 4.10, S, 16.39.

5-(2-Hydroxyphenyl)-2-{N-(2-hydroxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole **6e** (Yield: 69%). mp 203-204°C; IR (KBr) cm^{-1}: 3637(ν_{O-H}), 3081(ν_{C-H}), 1541($\nu_{C=C}$), 1638($\nu_{C=N}$), 746(o-disubstituted benzene), 816(p-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 10.608 (s, 1H, ar-OH), 8.9 (s, 1H, ar-CH), 8.026 (d, 1H, ar-H), 7.953 (d, 1H, ar-H), 7.953 (d, 1H, ar-H), 7.761 (d, 1H, ar-H), 7.667 (t, 1H, ar-H), 7.615 (d, 1H, ar-H), 7.615 (d, 1H, ar-H), 7.552 (t, 1H, ar-H), 7.487 (t, 1H, ar-H), 7.356 (t, 1H, ar-H), 7.206 (d, 1H, ar-H), 7.045 (d, 1H, ar-H). 13C NMR (DMF-d6) δ ppm: 163.87, 163.87, 162.45, 161.22, 157.89, 149.62, 132.39, 131.85, 131.85, 131.01, 128.55, 128.55, 128.3, 119.4, 119.16, 119.11, 117.8, 116.7. Anal. Found (calc.) for $C_{21}H_{15}N_3O_2S$ (%): C, 67.52, H, 4.09, N, 11.29, O, 8.51, S, 8.51.

5-(2-Mercaptophenyl)-2-{N-(2-hydroxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole **6f** (Yield: 68%). mp 209-210°C; IR (KBr) cm^{-1}: 3638(ν_{O-H}), 3081(ν_{C-H}), 1542($\nu_{C=C}$), 1628($\nu_{C=N}$), 747(o-disubstituted benzene), 824 (p-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 10.489 (s, 1H, ar-OH), 8.908 (s, 1H, ar-CH), 7.971 (d, 1H, ar-H), 7.971 (d, 1H, ar-H), 7.918 (d, 1H, ar-H), 7.779 (d, 1H, ar-H), 7.768 (s, 1H, ar-H), 7.605 (t, 1H, ar-H), 7.602 (d, 1H, ar-H), 7.602 (d, 1H, ar-H), 7.588 (t, 1H, ar-H), 7.5 (t, 1H, ar-H), 7.348 (t, 1H, ar-H), 7.051 (d, 1H, ar-H), 2.322 (s, 1H, ar-SH). 13C NMR (DMF-d6) δ ppm: 163.87, 163.87, 162.45, 161.22, 149.62, 132.39, 131.85, 131.32, 131.21, 131.01, 130.66, 130.55, 128.55, 128.55, 127.82, 127.58, 123.52, 123.52, 119.16, 119.11, 117.21. Anal. Found (calc.) for $C_{21}H_{15}N_3OS_2$ (%): C, 64.71, H, 3.81, N, 10.71, O, 4.10, S, 16.38.

5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-2-aminophenyl}-1,3,4-thiadiazole **7a**
(Yield: 71%). mp 176-177°C; IR (KBr) cm^{-1}: 3634(ν_{O-H}), 3058(ν_{C-H}), 1534($\nu_{C=C}$), 1659($\nu_{C=N}$), 739(o-disubstituted benzene), 828(p-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 10.572 (s, 1H, ar-H), 8.615 (s, 1H, ar-CH), 8.034 (d, 1H, ar-H), 7.731 (d, 1H, ar-H), 7.678 (t, 1H, ar-H), 7.634 (d, 1H, ar-H), 7.634 (d, 1H, ar-H), 7.621 (t, 1H, ar-H), 7.535 (t, 1H, ar-H), 7.521 (t, 1H, ar-H), 7.246 (d, 1H, ar-H), 7.185 (d, 1H, ar-H), 6.746 (d, 1H, ar-H), 6.746 (d, 1H, ar-H), 2.917 (s, 6H, $2CH_3$). 13C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.39, 157.89, 151.43, 144.54, 133.05, 131.85, 130.66, 130.3, 130.3, 128.3, 127.82, 127.58, 125.08, 124.6, 119.4, 117.8, 116.7, 111.54, 111.54, 40.30, 40.30. Anal. Found (calc.) for $C_{23}H_{20}N_4OS$ (%): C, 68.86, H, 5.09, N, 13.86, O, 3.86, S, 8.05.

5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-2-aminophenyl}-1,3,4-thiadiazole **7b**
(Yield: 61%). mp 171-172°C; IR (KBr) cm^{-1}: 3614(ν_{O-H}), 3046(ν_{C-H}), 1527($\nu_{C=C}$), 1628($\nu_{C=N}$), 763(o-disubstituted benzene), 806(p-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 8.623 (s, 1H, ar-CH), 8.069 (d, 1H, ar-H), 7.885 (d, 1H, ar-H), 7.75 (d, 1H, ar-H), 7.76 (t, 1H, ar-H), 7.646 (t, 1H, ar-H), 7.645 (d, 1H, ar-H), 7.645 (d, 1H, ar-H), 7.547 (t, 1H, ar-H), 7.518 (t, 1H, ar-H), 7.237 (d, 1H, ar-H), 6.666 (d, 1H, ar-H), 6.666 (d, 1H, ar-H), 2.917 (s, 6H, $2CH_3$), 2.376 (s, 1H, ar-SH). 13C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.39, 151.43, 144.54, 133.05, 131.32, 131.21, 130.66, 130.66, 130.55, 130.3, 130.3, 127.82, 127.82, 127.58, 127.58, 125.08, 124.6, 111.54, 111.54, 40.30, 40.30. Anal. Found (calc.) for $C_{23}H_{20}N_4S_2$ (%): C, 66.26, H, 4.76, N, 13.41, S, 15.48.

5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole **7c** (Yield: 68%). mp 195-196°C; IR (KBr) cm^{-1}: 3648(ν_{O-H}), 3071(ν_{C-H}), 1547($\nu_{C=C}$), 1618($\nu_{C=N}$), 764(o-disubstituted benzene), 692, 762(m-disubstituted benzene), 825(p-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 10.618 (s, 1H, ar-OH), 8.673 (s, 1H, ar-CH), 7.773 (s, 1H, ar-H), 7.724 (d, 1H, ar-H), 7.624 (t, 1H, ar-H), 7.51(t, 1H, ar-H), 7.506 (d, 1H, ar-H), 7.506 (d, 1H, ar-H),7.49 (t, 1H, ar-H), 7.454 (d, 1H, ar-H), 7.377 (d, 1H, ar-H), 7.094 (d, 1H, ar-H), 6.614 (d, 1H, ar-H), 6.614 (d, 1H, ar-H), 2.891 (s, 6H, $2CH_3$). 13C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.55, 157.89, 151.43, 143.70, 131.85, 131.01, 130.3, 130.3, 128.3, 127.14, 126.92, 124.6, 121.25, 119.4, 117.8, 116.7, 116.35, 111.54, 111.54, 40.3, 40.3. Anal. Found (calc.) for $C_{23}H_{20}N_4OS$ (%): C, 68.91, H, 5.09, N, 13.91, O, 3.86, S, 8.05.

5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole **7d**
(Yield: 62%). mp 185-186°C; IR (KBr) cm^{-1}: 3627(ν_{O-H}), 3082(ν_{C-H}), 1562($\nu_{C=C}$), 1673($\nu_{C=N}$), 761(o-disubstituted benzene), 706, 782 (m-disubstituted benzene), 834(p-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 8.673 (s, 1H, ar-CH), 7.801 (s, 1H, ar-H), 7.747 (d, 1H, ar-H), 7.737 (d, 1H, ar-H), 7.553 (t, 1H, ar-H), 7.523 (t, 1H, ar-H), 7.517 (d, 1H, ar-H), 7.517 (d, 1H, ar-H), 7.491 (t, 1H, ar-H), 7.471 (d, 1H, ar-H), 7.414 (d, 1H, ar-H), 6.615 (d, 1H, ar-H), 2.892 (s, 6H, $2CH_3$), 2.324 (s, 1H, ar-SH). 13C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.55, 151.43,

143.70, 131.32, 131.21, 131.01, 130.66, 130.55, 130.3, 130.3, 127.82, 127.58, 127.14, 126.92, 124.6, 121.25, 116.35, 111.54, 111.54, 40.3, 40.3. Anal. Found (calc.) for $C_{23}H_{20}N_4S_2$ (%): C, 66.26, H, 4.79, N, 13.36, S, 15.37.

5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole **7e** (Yield: 58%). mp 209-210^{o}C; IR (KBr) cm^{-1}: 3648(ν_{O-H}), 3047(ν_{C-H}), 1568($\nu_{C=C}$), 1654($\nu_{C=N}$), 765(o-disubstituted benzene), 826(p-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 10.592 (s, 1H, ar-OH), 8.805 (s, 1H, ar-CH), 7.785 (d, 1H, ar-H), 7.785 (d, 1H, ar-H), 7.731 (d, 1H, ar-H), 7.621 (t, 1H, ar-H), 7.544 (d, 1H, ar-H), 7.544 (d, 1H, ar-H), 7.521 (t, 1H, ar-H), 7.406 (d, 1H, ar-H), 7.406 (d, 1H, ar-H), 7.185 (d, 1H, ar-H), 6.745 (d, 1H, ar-H), 2.917 (s, 6H, 2CH_3). 13C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.55, 157.89, 151.43, 149.62, 131.85, 131.01, 130.3, 130.3, 128.55, 128.55, 128.3, 124.6, 123.52, 123.52, 119.4, 117.8, 116.7, 111.54, 111.54, 40.3, 40.3. Anal. Found (calc.) for $C_{23}H_{20}N_4OS$ (%): C, 68.91, H, 5.11, N, 13.87, O, 3.92, S, 8.14.

5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole **7f** (Yield: 61%). mp 212-213^{o}C; IR (KBr) cm^{-1}: 3647(ν_{O-H}), 3046(ν_{C-H}), 1575($\nu_{C=C}$), 1639($\nu_{C=N}$), 759(o-disubstituted benzene), 834(p-disubstituted benzene). $_1$H NMR (DMF-d_6) δ ppm: 8.813 (s, 1H, ar-CH), 7.884 (d,1H, ar-H), 7.814 (d,1H, ar-H), 7.814 (d,1H, ar-H), 7.75 (d,1H, ar-H), 7.630 (d,1H, ar-H), 7.630 (d,1H, ar-H), 7.55 (t, 1H, ar-H), 7.518 (t, 1H, ar-H), 7.350 (d,1H, ar-H), 7.350 (d,1H, ar-H), 6.664 (d,1H, ar-H), 6.664 (d,1H, ar-H), 2.916 (s, 6H, 2CH_3), 2.316 (s, 1H, ar-SH). 13C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.55, 151.43, 149.62, 131.32, 131.21, 131.01, 130.66, 130.55, 130.3, 128.55, 128.55, 127.82, 127.58, 124.6, 123.52, 123.52, 111.54, 111.54, 40.3, 40.3. Anal. Found (calc.) for $C_{23}H_{20}N_4S_2$ (%): C, 66.39, H, 4.79, N, 13.38, S, 15.37.

5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole **8a:**
(Yield: 57%). mp 212-213^{o}C; IR (KBr) cm^{-1}: 3647(ν_{O-H}), 3069(ν_{C-H}), 1572($\nu_{C=C}$), 1674($\nu_{C=N}$), 746(o-disubstituted benzene), 698, 778(m-disubstituted benzene), 834(p-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.56 (s, 1H, ar-OH), 8.61 (s, 1H, ar-CH), 7.92 (d,1H, ar-H), 7.74 (d,1H, ar-H), 7.68 (d, 1H, ar-H), 7.67 (t, 1H, ar-H), 7.64 (t, 1H, ar-H), 7.55 (t, 1H, ar-H), 7.53 (t, 1H, ar-H), 7.41 (d,1H, ar-H), 7.27 (s, 1H, ar-H), 7.11 (d,1H, ar-H), 6.87 (d,1H, ar-H), 3.80 (s, 3H, CH_3). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.51, 157.89, 149.81, 147.83, 144.54, 133.05, 131.85, 130.66, 128.74, 128.3, 127.95, 127.82, 127.58, 125.08, 119.4, 117.8, 116.7, 115.53, 107.34, 56.15. Anal. Found (calc.) for $C_{22}H_{17}N_3O_3S$ (%): C, 65.42, H, 4.31, N, 10.49, O, 11.95, S, 7.87.

5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4 -thiadiazole **8b:**
(Yield: 64%). mp 201-202^{o}C; IR (KBr) cm^{-1}: 3618(ν_{O-H}), 3056(ν_{C-H}), 1558($\nu_{C=C}$), 1619($\nu_{C=N}$), 742(o-disubstituted benzene), 706, 785(m-disubstituted benzene), 831(p-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.62 (s, 1H, ar-OH), 8.62 (s, 1H, ar-CH), 8.07 (d,1H, ar-H), 7.75 (d,1H, ar-H), 7.75 (d,1H, ar-H), 7.69 (d,1H, ar-H), 7.67 (t, 1H, ar-H), 7.59 (t, 1H, ar-H), 7.57 (t, 1H, ar-H), 7.56 (t, 1H, ar-H), 7.42 (d, 1H, ar-H), 6.81 (d, 1H, ar-H), 3.80 (s, 3H, CH_3), 2.32 (s, 1H, ar-SH). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.51, 149.81, 147.83, 144.54, 133.05, 131.32, 131.21, 130.66, 130.66, 130.55, 128.74, 127.95, 127.82, 127.82, 127.58, 127.58, 125.08, 115.53, 107.34, 56.15. Anal. Found (calc.) for $C_{22}H_{17}N_3O_2S_2$ (%): C, 62.95, H, 4.15, N, 10.13, O, 7.69, S, 15.34.

5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole **8c:**
(Yield: 59%). mp 215-216^{o}C; IR (KBr) cm^{-1}: 3624(ν_{O-H}), 3068(ν_{C-H}), 1557($\nu_{C=C}$), 1653($\nu_{C=N}$), 736(o-disubstituted benzene), 698, 774(m-disubstituted benzene), 831(p-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.62 (s, 1H, ar-OH), 8.71 (s, 1H, ar-CH), 7.89 (s, 1H, ar-H), 7.72 (d,1H, ar-H), 7.64 (d,1H, ar-H), 7.62 (t, 1H, ar-H), 7.61 (t, 1H, ar-H), 7.52 (d, 1H, ar-H), 7.51 (t, 1H, ar-H), 7.28 (d,1H, ar-H), 7.23 (s,1H, ar-H), 7.09 (d,1H, ar-H), 6.85 (d,1H, ar-H), 3.73 (s, 3H, CH_3). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.51, 157.89, 149.81, 147.83, 143.70, 131.85, 131.01, 128.74, 128.3, 127.95, 127.14, 126.92, 121.25, 119.4, 117.8, 116.7, 116.35, 115.53, 107.34, 56.15. Anal. Found (calc.) for $C_{22}H_{17}N_3O_3S$ (%): C, 65.53, H, 4.28, N, 10.48, O, 11.82, S, 7.83.

5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4 -thiadiazole **8d:**
(Yield: 66%). mp 212-213^{o}C; IR (KBr) cm^{-1}: 3637(ν_{O-H}), 3054(ν_{C-H}), 1585($\nu_{C=C}$), 1673($\nu_{C=N}$), 745(o-disubstituted benzene), 708, 792 (m-disubstituted benzene), 837(p-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.59 (s, 1H, ar-OH), 8.71 (s, 1H, ar-CH), 7.92 (s, 1H, ar-H), 7.74 (d, 1H, ar-H), 7.73 (d, 1H, ar-H), 7.71 (d, 1H, ar-H), 7.55 (d, 1H, ar-H), 7.54 (t, 1H, ar-H), 7.52 (t, 1H, ar-H), 7.52 (t, 1H, ar-H), 7.28 (d, 1H, ar-H), 3.74 (s, 3H, CH_3), 2.34 (s, 1H, ar-SH). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.51, 149.81, 147.83, 143.70, 131.32, 131.21, 131.01, 130.66, 130.55, 128.74, 127.95, 127.82, 127.58, 127.14, 126.92, 121.25, 116.35, 115.53, 107.34, 56.15. Anal. Found (calc.) for $C_{22}H_{17}N_3O_2S_2$ (%): C, 62.91, H, 4.12, N, 10.06, O, 7.58, S, 15.34.

5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole **8e:** (Yield: 55%). mp 224-225^{o}C; IR (KBr) cm^{-1}: 3623(ν_{O-H}), 3074(ν_{C-H}), 1589($\nu_{C=C}$), 1671($\nu_{C=N}$), 762(o-disubstituted benzene), 695, 798 (m-disubstituted benzene), 834(p-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.61 (s, 1H, ar-OH), 8.86 (s, 1H, ar-CH), 7.74 (d, 1H, ar-H), 7.72 (d, 1H, ar-H), 7.72 (d, 1H, ar-H), 7.65 (d, 1H, ar-H), 7.65 (d, 1H, ar-H), 7.64 (t, 1H, ar-H), 7.53 (t, 1H, ar-H), 7.40 (d, 1H, ar-H), 7.27 (d, 1H, ar-H), 7.10 (d, 1H, ar-H), 6.87 (d, 1H, ar-H), 3.80 (s, 3H, CH_3). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.51, 157.89, 149.81, 149.62, 147.83, 131.85, 131.01, 128.74, 128.55, 128.55, 128.3, 127.95, 123.52, 123.52, 119.4, 117.8, 116.7, 115.53, 107.34, 56.15. Anal. Found (calc.) for $C_{22}H_{17}N_3O_3S$ (%): C, 65.52, H, 4.29, N, 10.34, O, 11.86, S, 7.92.

5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4 -thiadiazole **8f:** (Yield: 63%). mp 219-220^{o}C; IR (KBr) cm^{-1}: 3611(ν_{O-H}), 3075(ν_{C-H}), 1563($\nu_{C=C}$), 1645($\nu_{C=N}$), 739(o-disubstituted benzene), 699, 758 (m-disubstituted benzene), 831(p-disubstituted benzene). ^{1}H NMR (DMF-d_6) δ ppm: 10.63 (s, 1H, ar-OH), 8.87 (s, 1H, ar-CH), 7.75 (d, 1H, ar-H), 7.75 (d, 1H, ar-H), 7.74 (d, 1H, ar-H), 7.74 (d, 1H, ar-H), 7.66 (d, 1H, ar-H), 7.66 (d, 1H, ar-H), 7.59 (t, 1H, ar-H), 7.57 (t, 1H, ar-H), 7.42 (d, 1H, ar-H), 7.28 (s, 1H, ar-H), 6.81 (d, 1H, ar-H), 3.80 (s, 3H, CH_3), 2.31 (s, 1H, ar-SH). ^{13}C NMR (DMF-d6) δ ppm: 163.87, 163.87, 159.51, 149.81, 149.62, 147.83, 131.32, 131.21, 131.96, 130.66, 130.55, 128.74, 128.55, 128.55, 127.95, 127.82, 127.58, 123.52, 123.52, 115.53, 107.34, 56.15. Anal. Found (calc.) for $C_{22}H_{17}N_3O_2S_2$ (%): C, 62.91, H, 4.11, N, 10.09, O, 7.56, S, 15.33.

Acute Toxicity Studies

The Acute toxicity of the synthesized compounds was determined by using Wistar rat of either sex (150 -250 g) maintained under standard husbandry conditions. The animals were fasted overnight prior to the experiment and fixed dose (OECD) guideline no. 425 method of (CPCSEA) was adopted for toxicity studies. Effective dose (ED_{50} – therapeutic dose) was taken as 1/10th of lethal dose [18].

Biological Investigation

Antimicrobial screening

The antibacterial activity of the synthesized compounds was tested against four Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus* and *Bacillus cereus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using the nutrient agar medium and antifungal activity against fungi (*A. niger*) using sabouraud dextrose agar medium.

Minimum inhibitory concentration (MIC)

MIC [19] of the synthesized compounds was determined by agar streak dilution method. A stock solution of synthesized compound (100µg/mL) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in the specified quantity of sterile agar (nutrient agar for antibacterial activity and sabouraud dextrose agar medium for antifungal activity). A specified quantity of the medium (40-50^{o}C) containing the compound was poured into a petridish to give a depth of 4mm and allowed to solidify. Suspension of the microorganism was prepared and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37^{o}C for 24 and 48 h for bacteria and fungi, respectively. MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate.

Total Phenolic content

The total Phenolic content of the synthesized compounds was determined using Gallic acid equivalence (GAE) [20]. The compounds were diluted in methanol in the concentration of 100 µg/mL and 0.5 mL of the sample solution was transferred to a 10 mL volumetric flask, to which 0.5 mL undiluted Folin's-Ciocalteu reagent was added. After one minute, 1.5 mL of 20% (w/v) Na_2CO_3 was added and the volume was made up to 10mL with distilled water. The reaction mixture incubated at 25^{o}C for one hour and the absorbance was measured at 760 nm and compared with a pre-prepared gallic acid calibration curve. The blue color formation is the end point of a reaction mixture.

DPPH radical scavenging activity

The ability of synthesized derivatives to scavenge DPPH radical was assessed using Mondal et al [21] method with modification. Briefly, dilutions of different concentrations (200 – 1000 µg/mL) were mixed with 3.0 mL DPPH (0.5 mmol/L in methanol), the resultant absorbance was recorded at 517 nm after 30 min. incubation at 37^{o}C. The percentage of scavenging activity was derived using the following formula, Percentage of inhibition (%) = [($A_{control}$ – A_{sample}) / $A_{control}$] X 100, where $A_{control}$ – absorbance of DPPH, A_{sample} – absorbance of reaction mixture (DPPH with Sample).

Ferrous reducing power

The reducing ability of synthesized compounds was measured according to the method of Oyaizu [22]. Dilutions of various concentrations (200 – 1000 µg/mL) in methanol were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50^{o}C for 20 min. with TCA (10%: 2.5 mL). Then mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (1%) and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power. The reducing power of synthesized compounds was compared with that of standard antioxidant L-ascorbic acid.

Fe^{2+} chelating activity assay

The chelating activity of the synthesized compounds was evaluated by measuring the Fe^{2+} chelating activity according to the method of Dinis, et al [23]. Dilutions of various concentrations (200 – 1000 µg/mL) in methanol, 1.6 mL of distilled water and 0.05 mL of $FeCl_2$ (2mM) were added and after 30 s, 0.1 mL of ferrozine (5mM) added. The reaction mixture was incubated for 10min at 30^{o}C and the absorbance of Fe^{2+} ferrozine complex was measured at 562 nm. A lower absorbance indicates a higher chelating power. The chelating activity of the compounds on Fe^{2+} was compared with that of EDTA (0.01 mM) and citric acid (0.025 M). The percentage of chelating activity calculated using the formula: % of chelating activity = (A_1 – A_2) X 100, where A_1 – absorbance of the reaction mixture without compounds and A_2 – absorbance of the reaction mixture with compounds.

RESULTS AND DISCUSSION

Synthesis

The synthesis involves reaction of substituted salicylic acid hydrazide **1** with substituted benzoyl chloride **2** which resulted in the formation of substituted diacyl hydrazines **(3a-f)**. Cyclization of substituted diacyl hydrazines was carried out using Lawesson reagent, which resulted in formation of 2,5-Disubstituted-1,3,4-thiadiazoles **(4a-f)**. 2,5-Disubstituted-1,3,4-thiadiazoles undergo reaction with different aromatic aldehydes to form different Schiff bases. The compounds were purified by repeated recrystallization from ethanol and then dried under vacuum [24, 25]. The synthetic scheme illustrates the way used for the synthesis of target compounds (Figure 1). The structures of compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and elemental analysis.

Acute Toxicity Studies

The synthesized compounds were investigated for their toxicity (Table 1), (Figure 2). It was ascertained that all tested compounds have low toxicity, the lowest toxicity was showed by **6f, 7f, 8e** and **8f.**

Antimicrobial evaluation

All the synthesized Schiff bases were evaluated for in vitro antibacterial activity against four Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus and Bacillus cereus*) and two Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa*) and antifungal activity against fungi (*Aspergillus niger*) [26, 27, 28].

From the results, we can see that the synthesized Schiff bases were moderately active against tested microorganisms with the range of MIC values for S. aureus (0.12-21.5 µg/mL), S. epidermidis (0.35-21.6 µg/mL), M. luteus (0.1-22.6 µg/mL), B. cereus (0.25-21.4 µg/mL), E. coli (0.16-19.8 µg/mL), P. aeruginosa (0.21-21.2 µg/mL), A. niger (2.4-21.6 µg/mL). The compound 5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiaz -ole **8f** was found to exhibit the most potent antimicrobial activity with the MICs of 0.12, 3.1, 2.9, 0.25, 0.16, 0.27 and 2.4 µg/mL against S. aureus, S. epidermidis, M. luteus, B. cereus, E. coli, P. aeruginosa and A. niger respectively and also more active than Ciprofloxacin and Ketoconazole against *Staphylococcus aureus, Escherichia coli, Aspergilus niger*. From the SAR studies, clearly compound **8f** exhibited significant antimicrobial activity when compared to standard drugs Ciprofloxacin and Ketoconazole. Other compounds **6a-f, 7a-f, 8a-e** also showed good antibacterial and antifungal activities. The compound **8f** showed maximum activity might be due to para substitution, electron withdrawing group, ortho substituted sulfahydril group in disubstituted 1,3,4-thiadiazole. While the other compounds, though they contain electron donating group, ortho substituted hydroxyl group unfortunately produced weak antimicrobial activity (Table 2), (Figure 3).

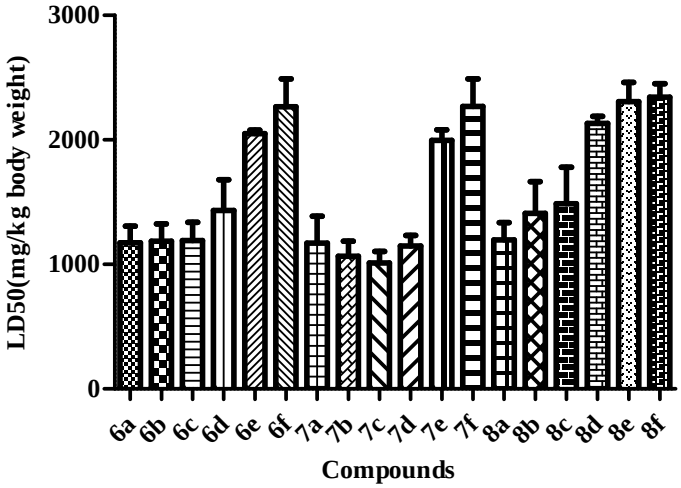

Figure 2: LD_{50} of Synthesized compounds

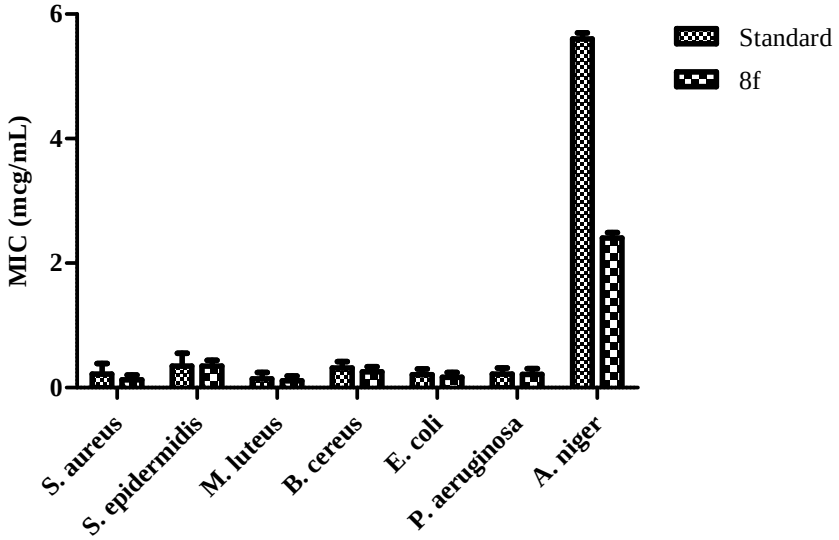

Figure 3: Antimicrobial studies of most active synthesized compound

Antioxidant activity

Total Phenolic content

The Total Phenolic content in synthesized derivatives was obtained (Table 3). The Gallic acid linear curve obtained using the y = 0.002 x + 0.063, R^2 = 0.997 (Figure 4). The Total Phenolic of the synthesized compounds is given in Table 3.

DPPH radical scavenging activity

The photometric evaluation of the antioxidant capacity of the synthesized compounds showed good antioxidant capacity (Figure 5). The IC_{50} value of the most active synthesized compounds (8e, 8f) and standard antioxidant (ascorbic acid) such as 273.28 µg/mL, 245.67 µg/mL, 169.48 µg/mL respectively. A lower IC_{50} value indicates a higher free radical scavenging activity[29].

Ferrous reducing power

The reducing ability of synthesized compounds increased with increasing concentration of the sample. The higher absorbance value indicated that high antioxidant capacity [30]. The result showed significant value in 8e and 8f

compared to other compounds. The result showed that the synthesized compounds posses ferric ions (Fe^{3+}) reducing ability (Figure 6).

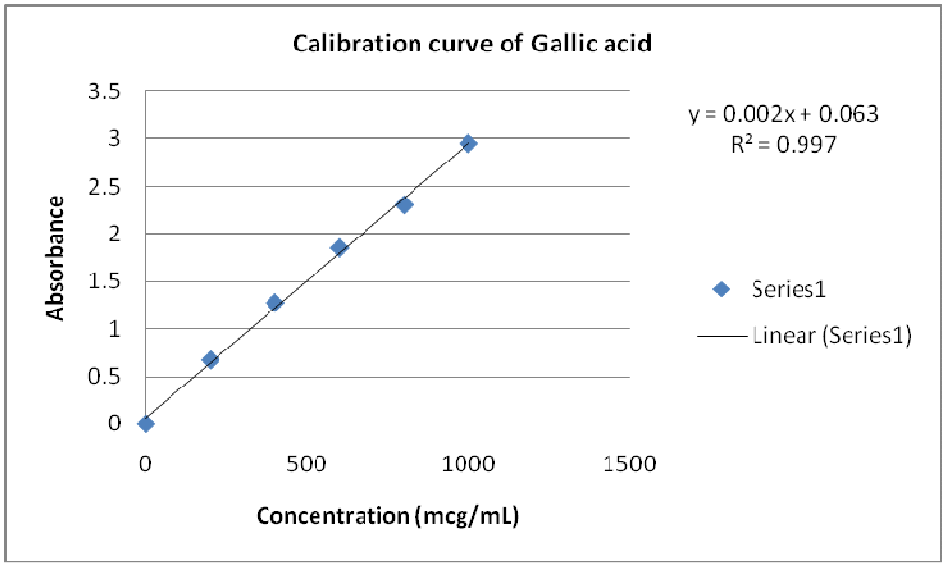

Figure 4: Calibration curve of Gallic acid

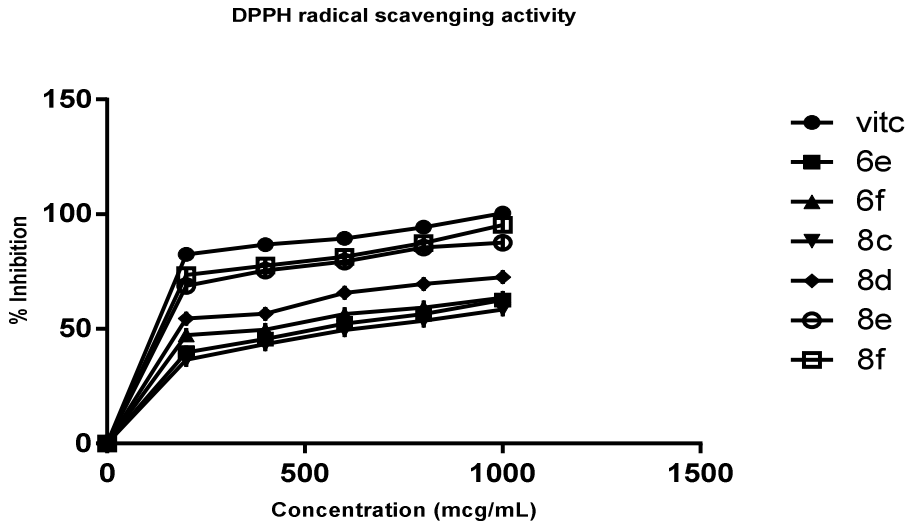

Figure 5: DPPH radical scavenging activity
Note: 6a-d, 7a-f, 8a-b not done due to less total Phenolic content

Fe^{2+} chelating activity assay

The Fe^{2+} chelating activity of synthesized compounds showed potential chelating power and the IC_{50} value for 8e, 8f and standard was found to be 633.26 µg/mL, 448.92 µg/mL, 318.05 µg/mL respectively (Figure 7). The iron generates free radicals through the fenton and Haber – Weiss reactions that prevent the oxidation damage.

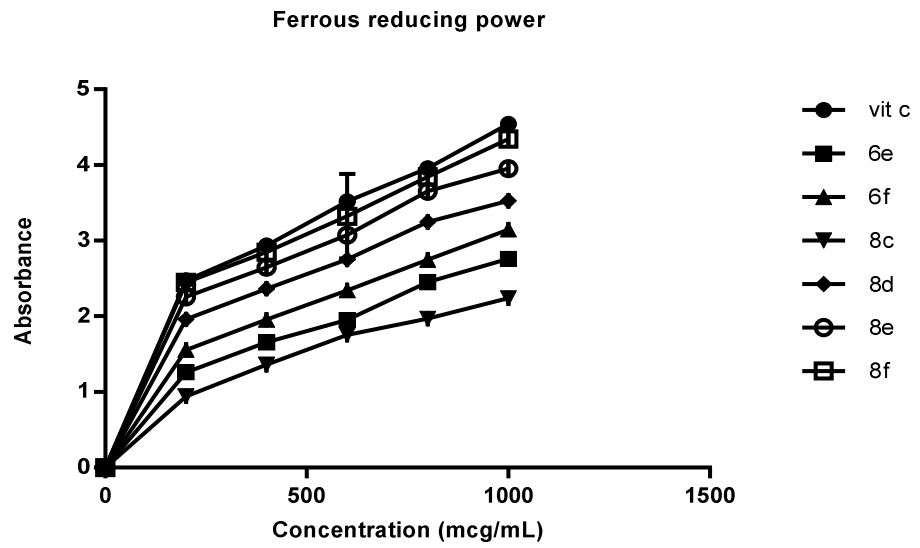

Figure 6: Ferrous reducing power
Note: 6a-d, 7a-f, 8a-b not done due to less total Phenolic content

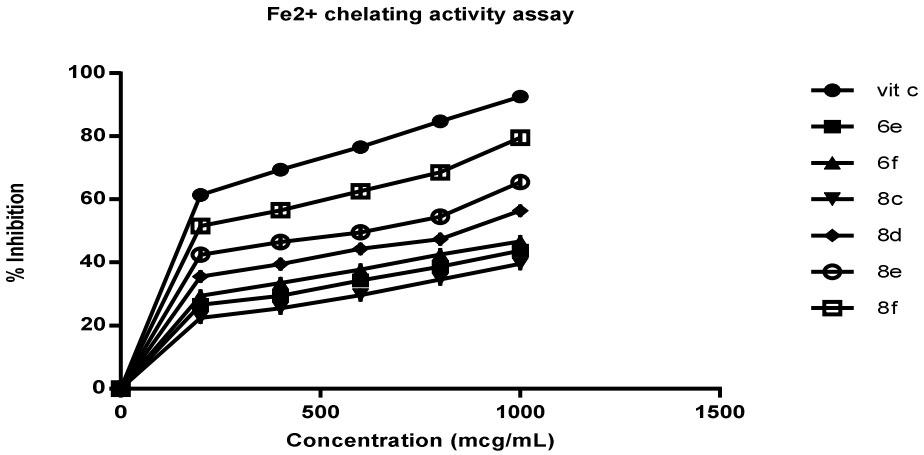

Figure 7: Fe^{2+} chelating activity assay
Note: 6a-d, 7a-f, 8a-b not done due to less total Phenolic content

Table 1: LD_{50} value of synthesized compounds

	LD_{50} (mg/kg body weight)			
Comp.	**24hours**	**48hours**	**7 days**	**Average**
6a	1250	1250	1020	1173.3
6b	1265	1265	1025	1185
6c	1275	1275	1075	1208.3
6d	1575	1575	1145	1431.6
6e	2065	2065	2015	2048.3
6f	2395	2395	2010	2266.6
7a	1295	1295	925	1171.6
7b	1135	1135	920	1063.3
7c	1065	1065	905	1011.6
7d	1195	1195	1045	1145
7e	2045	2045	1895	1995
7f	2395	2395	2015	2268.3
8a	1275	1275	1035	1195
8b	1425	1425	935	1261.6
8c	1655	1655	1145	1485
8d	2165	2165	2065	2131.6
8e	2395	2395	2125	2305
8f	2405	2405	2215	2341.6

Table 2: Effect of synthesized compounds on human pathogenic tested microorganisms

	In vitro antimicrobial activity of Synthesized compounds expressed as the MIC (µg/mL)						
Compounds	S. aureus	S. epidermidis	M. luteus	B. cereus	E. coli	P. aeruginosa	A. niger
6a	19.35±.12	16.81±.07	22.5±.08	21.35±.05	19.7±.08	17.31±.07	21.2±.08
6b	18.35±.12	21.58±.07	14.55±.05	17.16±.10	18.3±.08	21.21±.11	19.41±.09
6c	14.56±.15	15.16±.08	13.71±.07	15.25±.05	19.61±.07	18.25±.10	20.3±.08
6d	13.53±.12	14.55±.05	12.23±.08	17.71±.09	14.5±.08	19.11±.04	21.51±.09
6e	3.7±.08	12.31±.07	14.18±.07	4.58±.04	0.96±.01	3.61±.09	5.13±.05
6f	0.18±.01	5.28±.07	2.73±.08	0.27±.01	0.16±.01	0.37±.01	4.41±.07
7a	20.48±.07	21.3±.06	17.51±.09	17.41±.07	16.38±.09	18.5±.08	14.7±.08
7b	21.5±.08	18.38±.07	14.5±.08	16.36±.05	12.7±.06	19.3±.08	15.3±.08
7c	18.53±.10	20.51±.07	16.71±.09	17.4±.08	14.48±.07	14.41±.09	17.48±.11
7d	17.26±.05	16.41±.07	12.8±.08	14.53±.08	17.41±.07	13.83±.10	18.78±.11
7e	14.41±.14	13.73±.08	17.51±.07	18.43±.05	14.2±.08	17.41±.07	17.11±.07
7f	0.22±.01	4.8±.08	6.2±.08	14.75±.05	0.64±.01	5.7±.08	4.16±.05
8a	12.56±.05	19.33±.08	18.61±.09	12.21±.09	15.71±.13	18.5±.08	14.2±.08
8b	14.71±.09	17.75±.08	17.71±.09	12.18±.09	14.15±.05	11.21±.07	18.21±.09
8c	15.61±.07	16.86±.05	14.8±.08	9.63±.08	17.68±.09	10.4±.08	5.38±.11
8d	3.14±.01	5.51±.07	2.81±.09	4.15±.05	0.75±.01	4.23±.08	3.7±.08
8e	0.21±.01	0.38±.01	0.12±.01	0.28±.01	0.16±.01	0.22±.01	3.55±.05
8f	0.12±.01	0.35±.01	0.11±.01	0.24±.01	0.14±.01	0.20±.01	2.3±.08
Ciprofloxacin (100 µg/disc)	0.21±.01	0.37±.01	0.15±.01	0.32±.01	0.21±.01	0.23±.01	
Ketoconazole (100 µg/disc)							5.6±.08

Table 3: Total Phenolic content of 6a-8f

S no.	Compounds	Absorbance	Total Phenolic content
1	6a	1.026	481.5
2	6b	1.104	520.5
3	6c	1.056	496.5
4	6d	1.149	543
5	6e	1.193	565
6	6f	1.204	570.5
7	7a	0.963	450
8	7b	0.979	458
9	7c	1.012	474.5
10	7d	1.027	482
11	7e	1.019	478
12	7f	1.125	531
13	8a	1.011	474
14	8b	1.026	481.5
15	8c	1.164	550.5
16	8d	1.267	602
17	8e	1.298	617.5
18	8f	1.312	624.5

CONCLUSION

The antimicrobial activity of the synthesized compounds may be due to the presence of various pharmacophore with might increase the lipophilic character of the molecules, which might facilitate crossing through biological membrane of the microorganism and thereby inhibit their growth.

The antioxidant activity of the synthesized compounds may be due to the presence of SH and OH groups that can donate hydrogen atom and exist in radical form. This can scavenge the free radicals which involved the destructive biochemical reaction. In conclusion, we reported here a simple and convenient route for synthesis of some new derivatives of 1,3,4-thiadiazole for antioxidant and antimicrobial evaluation.

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