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ORIGINAL ARTICLE

Synthesis and antimicrobial screening of tetra Schiff bases of 1,2,4,5-tetra (5-amino-1,3,4-thiadiazole-2-yl)benzene

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KEYWORDS

Tetra Schiff base;
1,3,4-Thiadiazole;
Antibacterial;
Antifungal

Abstract In the present study, novel tetra Schiff bases were synthesized by condensation of 1,2,4,5-tetra (5-amino-1,3,4-thiadiazole-2-yl)benzene with different aromatic aldehydes. The chemical structures were confirmed by means of IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. All compounds were screened for antibacterial (*Staphylococcus aureus* ATCC-9144, *Staphylococcus epidermidis* ATCC-155, *Micrococcus luteus* ATCC-4698, *Bacillus cereus* ATCC-11778, *Escherichia coli* ATCC-25922, and *Pseudomonas aeruginosa* ATCC-2853) and antifungal (*Aspergillus niger* ATCC-9029 and *Aspergillus fumigatus* ATCC-46645) activities by paper disc diffusion technique. The minimum inhibitory concentrations (MICs) of the compounds were also determined by agar streak dilution method. Among the synthesized compounds 1,2,4,5-tetra[5-(4-nitrobenzylideneamino)-1,3,4-thiadiazole-2-yl]benzene **7** was found to be the most potent antimicrobial activity with MICs of 3.4, 2.1, 1.2, 2.0, 3.1, 2.4, 1.1, and 1.7 μg/mL against the above mentioned respective strains.

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1. Introduction

Schiff bases represent an important class of organic compounds, especially in the medicinal and pharmaceutical fields. Thus, development and synthesis of novel Schiff base derivatives as potential chemotherapeutics still attract the attention of organic and medicinal chemist (Bharti et al., 2010; da Silva et al., 2011; Rosu et al., 2011). Many studies reported the biological activities of Schiff bases, including their anticancer, antibacterial, antifungal, and herbicidal activities (Gudipati

et al., 2011). 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, anticancer, cytotoxic, antimicrobial, antibacterial, anticonvulsant, etc. (Hranjec et al., 2011). A number of Schiff bases have been tested for antibacterial, antifungal, anticancer and herbicidal activities (Prasad et al., 2011; Pulate et al., 2011).

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and increasing number of multi-drug resistant microbial pathogens with particular relevance for Gram-positive bacteria. In spite of the large number of antibiotics and chemotherapeutics available for medical use, 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-thiadiaziles and their analogs have been known from the beginning of the 20th century (Bhat et al., 2011; Gilani et al., 2010). Literature survey revealed that slight modification in the structure can result in qualitative as well as quantitative changes in the activity (Alagawadi and Alegaon, 2010; Dong et al., 2010; Bhat et al., 2011). This prompted us to undertake the synthesis of various novel tetra Schiff bases derived from 1,2,4,5-tetra (5-amino-1,3,4-thiadiazole-2-yl)benzene and characterized using IR, ^{1}H NMR, ^{13}C NMR, and Elemental analysis with the aim of having improved activity. The synthesized compounds were screened for their antibacterial activity against four Gram-positive bacteria (*Staphylococcus aureus* ATCC-9144, *Staphylococcus epidermidis* ATCC-155, *Micrococcus luteus* ATCC-4698 and *Bacillus cereus* ATCC-11778), three Gram-negative bacteria (*Escherichia coli* ATCC-25922, *Pseudomonas aeruginosa* ATCC-2853 and antifungal (*Aspergillus niger* ATCC-9029 and *Aspergillus fumigatus* ATCC 46645) activities by paper disc diffusion technique. The minimum inhibitory concentrations (MICs) of the compounds were also determined by agar streak dilution method.

2. Experimental

2.1. Measurements

The melting points were taken in an open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FT-IR spectrometer MB 104 with KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker 300 NMR spectrometer operating at 400.13 and 100.77 MHz, respectively. Microanalyses were obtained with an Elemental analyses system GmbH VarioEL V300 element analyzer. The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF254, 200 mesh) aluminium plates (E Merk) and visualized in UV chamber. IR, ^{1}H NMR, ^{13}C NMR and elemental analysis were consistent with the assigned structures.

2.2. Synthesis

2.2.1. Synthesis of 1,2,4,5-tetra (5-amino-1,3,4-thiadiazole-2-yl)benzene **2**

A mixture of pyromellitic acid **1** (0.01 mol, 2.54 g) and (0.04 mol, 3.64 g) of thiosemicarbazide with (15 mL) of phosphorusoxy chloride was refluxed gently for 15 h, the completion of the reaction mixture monitored by TLC. After cooling, 125 mL of water was added, the mixture was then refluxed for 10 h, filtrated, and neutralized with potassium hydroxide. The precipitate was washed with water and recrystallized from (ethanol-water) to give the desired product. (Yield 80%). mp > 360 °C; IR (KBr) cm^{-1}: 3325–3298 (ν_{NH2}), 3081 (ν_{C-H}), 1610 ($\nu_{C=N}$), 1534 ($\nu_{C=C}$). ^{1}H NMR (DMF-d_6) δ ppm 8.87 (s, 2H, NH_2) (D_2O exchange, disappeared), 8.76 (s, 2H, NH_2) (D_2O exchange, disappeared), 8.70 (s, 2H, NH_2) (D_2O exchange, disappeared), 8.62 (s, 2H, NH_2) (D_2O exchange, disappeared), 7.50 (s, 1H, ar-H), 7.44 (s, 1H, ar-H). ^{13}C NMR (DMF-d_6) δ ppm 143.22–148.31 (6C, ar-C), 137.11–140.40 (8C, thiadiazole carbons). Anal. Found (calc.) for $C_{14}H_{10}N_{12}S_4$ (%): C, 35.44 (35.43); H, 2.13 (2.12); N, 35.41 (35.42), S, 27.04 (27.03).

2.2.2. General method of synthesis **3**–**10**

A mixture of 1,2,4,5-tetra (5-amino-1,3,4-thiadiazole-2-yl)benzene (0.01 mol) and appropriate aromatic aldehyde (0.04 mol) was dissolved in 35 mL of absolute ethanol. The mixture was then refluxed for 20–24 h with stirring, the completion of the reaction mixture monitored by TLC. After cooling to room temperature, a solid was obtained. The crude product was filtered, dried and recrystallized from ethanol.

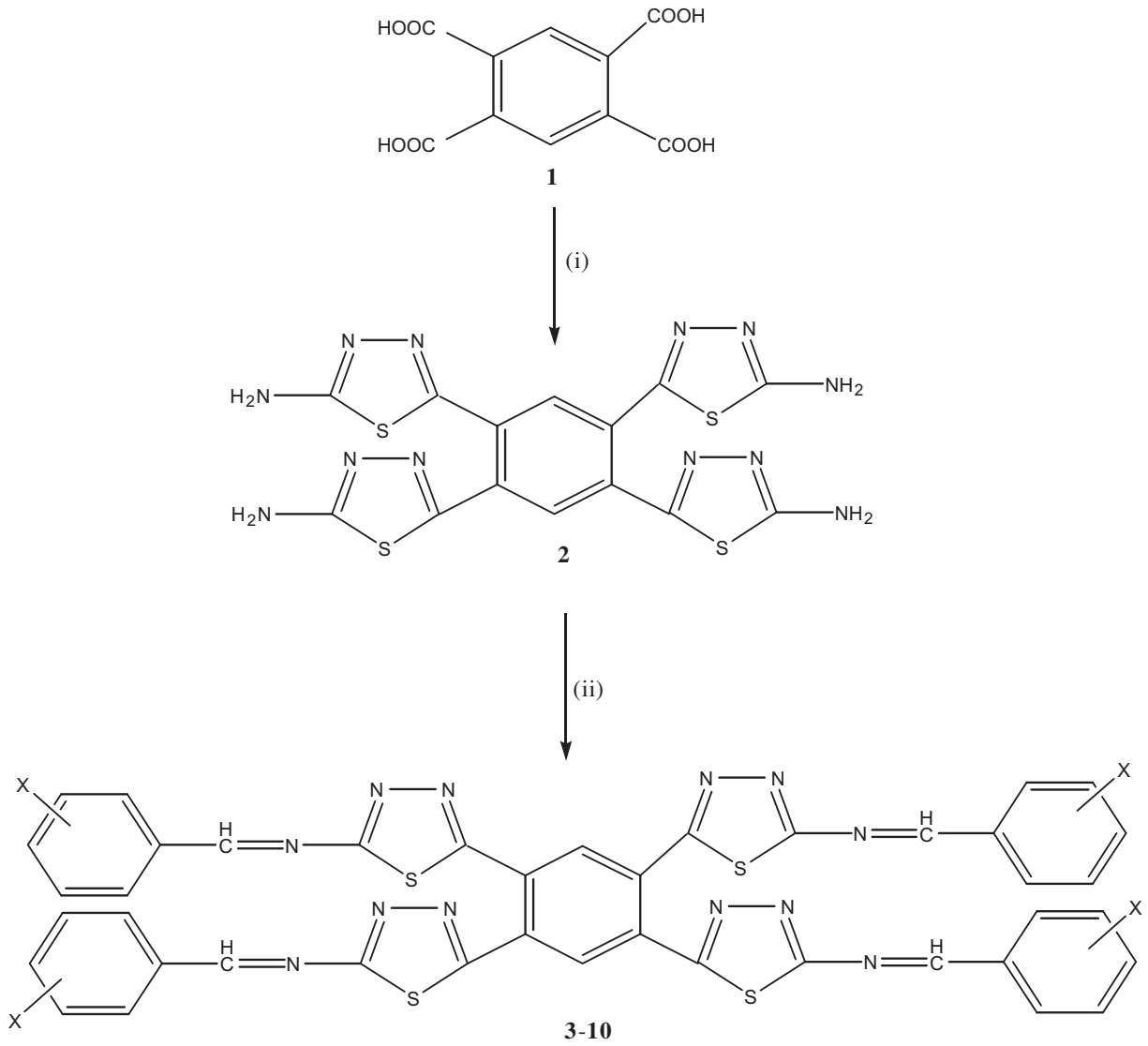
2.2.2.1. 1,2,4,5-Tetra[5-benzylideneamino-1,3,4-thiadiazole-2-yl]benzene ***3***. (Yield: 87%). mp 220–221 °C; IR (KBr) cm^{-1}: 3100 (ν_{C-H}), 1612 ($\nu_{C=N}$), 1560 ($\nu_{C=C}$). ^{1}H NMR (DMF-d_6) δ ppm 8.02–8.05 (d, 1H, ar-H), 7.95–8.01 (d, 1H, ar–H), 7.63–7.67 (d, 1H, ar–H), 7.57–7.60 (d, 1H, ar–H), 7.51–7.54 (d, 1H, ar–H), 7.50 (s, 1H, ar–H), 7.44–7.47 (d, 1H, ar–H), 7.40 (s, 1H, ar–H), 7.29–7.33 (d, 1H, ar–H), 7.19–7.22 (m, 3H, ar–H), 7.11–7.14 (m, 3H, ar–H), 7.00–7.03 (m, 3H, ar–H), 6.83–6.86 (m, 4H, ar–H), 5.74 (s, 1H, –CH = N–), 5.70 (s, 1H, –CH = N–), 5.63 (s, 1H, –CH = N–), 5.58 (s, 1H, –CH = N–). ^{13}C NMR (DMF-d_6) δ ppm 138.15–147.13 (12C, ar–C), 132.43–137.34 (12C, ar–C), 130.56–134.78 (6C, ar–C), 125.02–129.15 (8C, thiadiazole carbons), 115.31–119.40 (4C, –C = N–). Anal. Found (calc.) for $C_{42}H_{26}N_{12}S_4$ (%): C, 61.01 (61.00); H, 3.18 (3.17); N, 20.33 (20.32), S, 15.50 (15.51).

2.2.2.2. 1,2,4,5-Tetra[5-(4-methylbenzylideneamino)-1,3,4-thiadiazole-2-yl]benzene ***4***. (Yield: 73%). mp 200–201 °C; IR (KBr) cm^{-1} 3090 (ν_{C-H}), 1615 ($\nu_{C=N}$), 1560 ($\nu_{C=C}$). ^{1}H NMR (DMF–d_6) δ ppm 8.08–8.10 (d, 1H, ar–H), 7.97–8.00 (d, 1H, ar–H), 7.84–7.87 (d, 1H, ar–H), 7.61–7.65 (d, 1H, ar–H), 7.51 (s, 1H, ar–H), 7.53–7.55 (d, 1H, ar–H), 7.45–7.47 (d, 1H, ar–H), 7.43 (s, 1H, ar–H), 7.28–7.31 (m, 2H, ar–H), 7.21–7.25 (m, 2H, ar–H), 7.18–7.20 (m, 2H, ar–H), 6.02–7.07 (m, 2H, ar–H), 6.77–6.80 (m, 2H, ar–H), 5.79 (s, 1H, –CH = N–), 5.73 (s, 1H, –CH = N–), 5.66 (s, 1H, –CH = N–), 5.60 (s, 1H, –CH = N–). ^{13}C NMR (DMF–d_6) δ ppm 140.12–145.78 (12C, ar–C), 134.12–136.46 (12C, ar–C), 130.20–134.13 (6C, ar–C), 125.26–130.24 (8C, thiadiazole carbons). Anal. Found (calc.) for $C_{46}H_{34}N_{12}S_4$ (%): C, 62.57 (62.56); H, 3.87 (3.88); N, 19.04 (19.03), S, 14.51 (14.52).

2.2.2.3. 1,2,4,5-Tetra[5-(4-hydroxybenzylideneamino)-1,3,4-thiadiazole-2-yl]benzene ***5***. (Yield: 80%). mp > 360 °C; IR (KBr) cm^{-1} 3435 (ν_{O-H}), 3095 (ν_{C-H}), 1613 ($\nu_{C=N}$), 1560

Table 1 Effect of synthesized compounds on human pathogenic tested microorganisms.

Compounds	In vitro activity-zone of inhibition in mm (MIC in μg/mL)							
	S. aureus, ATCC-9144	*S. epidermidis*, ATCC-155	*M. luteus*, ATCC-4698	*B. cereus*, ATCC-11778	*E. coli*, ATCC-25922	*P. aeruginosa*, ATCC-2853	*A. niger*, ATCC-9092	*A. fumigatus*, ATCC-46645
1	5 (1.4)	3 (2.6)	4 (5.1)	1 (2.3)	2 (1.5)	3 (2.4)	6 (3.9)	4 (2.3)
2	19 (27.9)	20 (22.2)	18 (10.1)	19 (31.9)	19 (29.9)	16 (36.2)	20 (33.4)	23 (10.1)
3	24 (17.3)	22 (20.9)	26 (17.2)	21 (19.3)	23 (21.8)	25 (22.6)	27 (19.0)	24 (9.1)
4	21 (10.7)	19 (28.4)	20 (25.6)	23 (21.6)	19 (24.2)	27 (20.2)	26 (22.8)	25 (21.8)
5	25 (10.2)	22 (12.6)	22 (22.4)	23 (27.4)	20 (32.8)	23 (30.2)	21 (34.2)	20 (31.2)
6	24 (14.4)	25 (10.9)	26 (18.9)	25 (19.1)	24 (18.8)	23 (21.7)	27 (18.2)	24 (19.3)
7	28 (3.4)	26 (2.1)	24 (1.2)	23 (2.0)	23 (3.1)	24 (2.4)	17 (1.1)	24 (1.7)
8	19 (16.7)	23 (28.2)	24 (12.7)	23 (15.2)	20 (23.8)	24 (21.4)	21 (22.8)	23 (21.9)
9	25 (20.1)	19 (25.1)	25 (19.8)	24 (11.8)	22 (14.6)	17 (28.1)	18 (33.6)	19 (31.8)
10	20 (29.4)	17 (8.6)	19 (28.7)	21 (27.7)	24 (9.7)	22 (11.1)	19 (32.1)	20 (22.2)
Ciprofloxacin (100 μg/disc)	29 (0.2)	31 (0.39)	30 (0.1)	29 (0.3)	32 (0.2)	3 (0.25)	33 (0.1)	–
Ketoconazole (100 μg/disc)	–	–	–	–	–	–	30 (6.1)	29 (0.23)
Dimethylformamide	–	–	–	–	–	–	–	–



Comp. No.	X
3	H
4	*p*-CH_3
5	*p*-OH
6	*o*-OH
7	*p*-NO_2
8	*p*-Br
9	*p*-OCH_3
10	*p*-Cl

Scheme 1 Reagents and conditions: (i) thiosemicarbazide, $POCl_3$, reflux 15 h; (ii) appropriate aromatic aldehyde, absolute ethanol, reflux 10–24 h.

($\nu_{C=C}$). ^{1}H NMR (DMF–d_6) δ ppm 9.14 (s, 2H, 2OH) (D_2O exchange, disappeared), 9.23 (s, 2H, 2OH) (D_2O exchange, disappeared), 8.09–8.13 (d, 1H, ar–H), 7.93–8.03 (d, 1H, ar–H), 7.64–7.70 (d, 1H, ar–H), 7.56–7.58 (d, 1H, ar–H), 7.53 (1H, s, ar–H), 7.46–7.48 (d, 1H, ar–H), 7.43 (1H, s, ar–H), 7.35–7.38 (d, 1H, ar–H), 7.31–7.33 (d, 1H, ar–H), 7.25–7.27 (m, 3H, ar–H), 7.17–7.20 (m, 3H, ar–H), 7.13–7.14 (m, 3H, ar–H), 5.76 (s, 1H, –CH = N–), 5.70 (s, 1H, –CH = N–), 5.63 (s, 1H, –CH = N–), 5.58 (s, 1H, –CH = N–). ^{13}C NMR (DMF–d_6) δ ppm 143.05–147.14 (12C, ar–C), 138.10–141.72 (12C, ar–C), 133.89–136.67 (6C, ar–C), 126.90–129.65 (8C, thiadiazole carbons). Anal. Found (calc.) for $C_{42}H_{26}N_{12}O_4S_4$ (%): C, 56.61 (56.62); H, 2.95 (2.94); N, 18.87 (18.86), S, 14.41 (14.40).

2.2.2.4. 1,2,4,5-Tetra[5-(2-hydroxybenzylideneamino)-1,3,4-thiadiazole-2-yl]benzene ***6***. (Yield: 59%). mp > 360 °C; IR (KBr) cm^{-1} 3423 (ν_{O-H}), 3010 (ν_{C-H}), 1617 ($\nu_{C=N}$), 1543 ($\nu_{C=C}$). ^1H NMR (DMF–d_6) δ ppm 9.20 (s, 2H, 2OH) (D_2O exchange, disappeared), 9.27 (s, 2H, 2OH) (D_2O exchange, disappeared), 8.13–8.16 (d, 1H, ar–H), 8.06–8.09 (d, 1H, ar–H), 7.91–7.94 (d, 1H, ar–H), 7.81–7.83 (d, 1H, ar–H), 7.75–7.78 (d, 1H, ar–H), 7.69–7.71 (d, 1H, ar–H), 7.61–7.63 (d, 1H, ar–H), 7.54–7.57 (m, 3H, ar–H), 7.51 (1H, s, ar–H), 7.47–7.50 (m, 3H, ar–H), 7.45 (1H, s, ar–H), 6.40–7.43 (m, 3H, ar–H), 5.75 (s, 1H, –CH = N–), 5.72 (s, 1H, –CH = N–), 5.61 (s, 1H, –CH = N–), 5.56 (s, 1H, —CH = N–). ^{13}C NMR (DMF–d_6) δ ppm 145.15–147.04 (12C, ar–C), 140.12–143.92 (12C, ar–C), 134.56–138.17 (6C, ar–C), 127.80–130.19 (8C, thiadiazole carbons). Anal. Found (calc.) for $C_{42}H_{26}N_{12}O_4S_4$ (%): C, 56.61 (56.62); H, 2.95 (2.94); N, 18.87 (18.86), S, 14.39 (14.40).

2.2.2.5. 1,2,4,5-Tetra[5-(4-nitrobenzylidene)-1,3,4-thiadiazole-2-yl]benzene 7. (Yield: 78%). mp 235–236 °C; IR (KBr) cm^{-1} 3076 (ν_{C-H}), 1614 ($\nu_{C=N}$), 1543 ($\nu_{C=C}$), 1512, 1356 (ν_{N-O}). ^1H NMR (DMF–d_6) δ ppm 8.16–8.19 (d, 1H, ar–H), 8.09–8.11 (d, 1H, ar–H), 8.03–8.05 (d, 1H, ar–H), 7.92–7.95 (d, 1H, ar–H), 7.85–7.87 (d, 1H, ar–H), 7.81–7.83 (d, 1H, ar–H), 7.74–7.76 (d, 1H, ar–H), 7.68–7.69 (m, 3H, ar–H), 7.60–7.63 (m, 3H, ar–H), 7.55–7.57 (m, 3H, ar–H), 7.53 (1H, s, ar–H), 7.47 (1H, s, ar–H), 5.71 (s, 1H, –CH = N), 5.68 (s, 1H, –CH = N–), 5.63 (s, 1H, –CH = N–), 5.59 (s, 1H, –CH = N–). ^{13}C NMR (DMF–d_6) δ ppm 143.05–149.11 (12C, ar–C), 138.78–141.12 (12C, ar–C), 132.06–136.27 (6C, ar–C), 127.99–130.67 (8C, thiadiazole carbons). Anal. Found (calc.) for $C_{42}H_{22}N_{16}O_8S_4$ (%): C, 50.08 (50.09); H, 2.21 (2.20); N, 22.27 (22.26), S, 12.73 (12.74).

2.2.2.6. 1,2,4,5-Tetra[5-(4-bromobenzylideneamino)-1,3,4-thiadiazole-2-yl]benzene ***8***. (Yield: 85%). mp 287–288 °C; IR (KBr) cm^{-1} 3065 (ν_{C-H}), 1613 ($\nu_{C=N}$), 1564 ($\nu_{C=C}$). ^1H NMR (DMF–d_6) δ ppm 8.11–8.14 (d, 1H, ar–H), 8.04–8.07 (d, 1H, ar–H), 7.89–7.92 (d, 1H, ar–H), 7.83–7.85 (d, 1H, ar–H), 7.78–7.80 (d, 1H, a–H), 7.72–7.74 (d, 1H, ar–H), 7.66–7.69 (d, 1H, ar–H), 7.60–7.63 (m, 3H, ar–H), 7.56–7.58 (m, 3H, ar–H), 6.52–7.54 (m, 3H, ar–H), 7.50 (1H, s, ar–H), 7.46 (1H, s, ar–H), 5.74 (s, 1H, –CH = N), 5.66 (s, 1H, –CH = N–), 5.63 (s, 1H, –CH = N–), 5.54 (s, 1H, –CH = N–). ^{13}C NMR (DMF–d_6) δ ppm 142.21–147.34 (12C, ar–C), 137.08–140.24 (12C, ar–C), 131.56–134.74 (6C, ar–C), 124.91–128.57 (8C, thiadiazole carbons). Anal. Found (calc.) for $C_{42}H_{22}Br_4N_{12}S_4$ (%): C, 44.14 (44.15); H, 1.95 (1.94); N, 14.70 (14.71), S, 11.22 (11.23).

2.2.2.7. 1,2,4,5-Tetra[5-(4-methoxybenzylideneamino)-1,3,4-thiadiazole-2-yl]benzene ***9***. (Yield: 68%). mp > 360 °C; IR (KBr) cm^{-1} 3045 (ν_{C-H}), 2967 ($\nu_{C-H\ aliphatic}$), 1615 ($\nu_{C=N}$), 1536 ($\nu_{C=C}$). ^1H NMR (DMF–d_6) δ ppm 8.09–8.11 (d, 1H, ar–H), 8.02–8.04 (d, 1H, ar–H), 7.94–7.96 (d, 1H, ar–H), 7.85–7.87 (d, 1H, ar–H), 7.81–7.83 (d, 1H, ar–H), 7.77–7.79 (d, 1H, ar–H), 7.70–7.72 (d, 1H, ar–H), 7.62–7.64 (m, 3H, ar–H), 7.58–7.61 (m, 3H, ar–H), 7.53 (1H, s, ar–H), 7.48 (1H, s, ar–H), 6.56–6.59 (m, 3H, ar–H), 5.71 (s, 1H, –CH═N), 5.69 (s, 1H, –CH═N–), 5.63 (s, 1H, –CH═N–), 5.59 (s, 1H, –CH═N–). ^{13}C NMR (DMF–d_6) δ ppm 144.35–149.67 (12C, ar–C), 136.13–140.97 (12C, ar–C), 130.79–134.69 (6C, ar–C), 125.57–129.86 (8C, thiadiazole carbons). Anal. Found (calc.) for $C_{46}H_{34}N_{12}O_4S_4$ (%): C, 58.33 (58.34); H, 3.61 (3.62); N, 17.74 (17.75), S, 13.55 (13.54).

2.2.2.8. 1,2,4,5-Tetra[5-(4-chlorobenzylideneamino)-1,3,4-thiadiazole-2-yl]benzene ***10***. (Yield: 75%). mp 243–244 °C; IR (KBr) cm^{-1} 3063 (ν C–H), 1613 (ν C = N), 1542 (ν C = C). ^1H NMR (DMF–d6) δ ppm 8.10–8.14 (d, 1H, ar–H), 8.07–8.11 (d, 1H, ar–H), 7.93–7.96 (d, 1H, ar–H), 7.88–7.90 (d, 1H, ar–H), 7.83–7.85 (d, 1H, ar–H), 7.75–7.77 (d, 1H, ar–H), 7.70–7.72 (d, 1H, ar–H), 7.63–7.65 (m, 3H, ar–H), 7.56–7.58 (m, 3H, ar–H), 6.48–7.51 (m, 3H, ar–H), 7.49 (1H, s, ar–H), 7.46 (1H, s, ar–H), 5.72 (s, 1H, –CH═N), 5.70 (s, 1H, –CH═N–), 5.62 (s, 1H, –CH═N–), 5.60 (s, 1H, –CH═N–). ^{13}C NMR (DMF–d_6) δ ppm 145.12–150.03 (12C, ar–C), 135.01–139.94 (12C, ar–C), 129.78–132.48 (6C, ar–C), 126.47–130.11 (8C, thiadiazole carbons). Anal. Found (calc.) for $C_{42}H_{22}Cl_4N_{12}S_4$ (%): C, 52.30 (52.29); H, 2.31 (2.30); N, 17.41 (17.42), S, 13.30 (13.29).

2.3. Biology investigation

2.3.1. Antimicrobial screening

The antibacterial activity of the synthesized compounds was tested against four Gram-positive bacteria (*S. aureus* ATCC-9144, *S. epidermidis* ATCC-155, *M. luteus* ATCC-4698 and *B. cereus* ATCC-11778) and three Gram-negative bacteria (*E. coli* ATCC-25922 and *P. aeruginosa* ATCC-2853) using nutrient agar medium. The antifungal activities of the compounds were tested against fungi and molds namely *A. niger* ATCC-9029 and *A. fumigatus* ATCC-46645 using sabouraud dextrose agar medium.

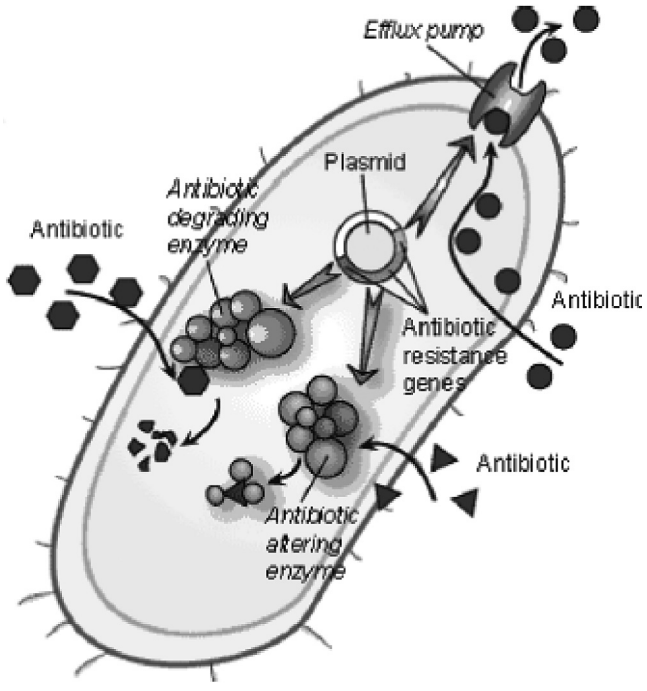


Figure 1 Illustration of how some antimicrobial agents are rendered ineffective (Fluit et al., 2001).

2.3.2. Paper disc diffusion technique

The sterilized (Gillespie, 1994) (autoclaved at 120 °C for 30 min) medium (40–50 °C) was inoculated (1 mL/100 mL of medium) with the suspension (10^5 cfu mL^{-1}) of the microorganism (matched to McFarland barium sulfate standard) and poured into a petridish to give a depth of 3–4 mm. The paper was impregnated with the test compounds. The paper impregnated with the test compounds (μg mL^{-1} in dimethyl formamide) was placed on the solidified medium. The plates were preincubated for 1 h at room temperature and incubated at 37 °C for 24 and 48 h for antibacterial and antifungal activities, respectively. Ciprofloxacin (100 μg/disc) and Ketoconazole (100 μg/disc) were used as standard for antibacterial and antifungal activities, respectively. The observed zone of inhibition is presented in Table 1.

2.3.3. Minimum inhibitory concentration (MIC)

MIC (Hawkey and Lewis, 1994) of the synthesized compounds was determined by agar streak dilution method. A stock solution of the synthesized compound (100 μg mL^{-1}) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar (nutrient agar for antibacterial activity and sabouraud dextrose agar medium for antifungal activity). A specified quantity of the medium (40–50 °C) containing the compound was poured into a petridish to give a depth of 3–4 mm and allowed to solidify. Suspension of the microorganism was prepared to contain approximately 10^5 cfu mL^{-1} and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37 °C for 24 and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in Table 1.

3. Results and discussion

3.1. Synthesis

The synthesis involves reaction of pyromellitic acid **1** with thiosemicarbazide, using phosphorusoxy chloride for dehydrative cyclization to produce 1,2,4,5-tetra (5-amino-1,3,4-thiadiazole-2-yl)benzene **2**. The Schiff bases under investigation were synthesized in the usual way for the preparation of anils (Gaber et al., 2001) by condensation of 1,2,4,5-tetra (5-amino-1,3,4-thiadiazole-2-yl)benzene **2** with different aromatic aldehydes in 1:4 molar proportion using absolute ethanol as solvent. The reaction mixture was heated under reflux for about 24 h, then filtered off and washed with ethanol. The compounds were purified by repeated recrystallization from ethanol and then dried under vacuum. The synthetic scheme illustrates the way used for the synthesis of target compounds (Scheme 1). The structures of the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and elemental analysis.

3.2. Antimicrobial evaluation

Microorganisms have existed on the earth for more than 3.8 billion years and exhibit the greatest genetic and metabolic diversities. They are an essential component of the biosphere and serve an important role in the maintenance and sustainability of ecosystems. It is believed that they compose about 50% of the living biomass. In order to survive, they have evolved mechanisms that enable them to respond to selective pressure exerted by various environments and competitive challenges. The disease-causing microorganisms have particularly been vulnerable to man's selfishness for survival who has sought to deprive them of their habitat using antimicrobial agents. These microorganisms have responded by developing resistance mechanisms to fight off this offensive. Currently antimicrobial resistance among bacteria, viruses, parasites, and other disease-causing organisms is a serious threat to infectious disease management globally (Dun, 1999).

In order to appreciate the mechanisms of resistance, it is important to understand how antimicrobial agents act. Antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. The understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways how resistance against them develops. However, the mechanism of action of antimicrobial agents can be categorized further based on the structure of the bacteria or the function that is affected by the agents. These include generally the following:

- Inhibition of the cell wall synthesis
- Inhibition of ribosome function
- Inhibition of nucleic acid synthesis
- Inhibition of folate metabolism
- Inhibition of cell membrane function

Microorganisms were increasingly becoming resistant to ensure their survival against the arsenal of antimicrobial agents to which they were being bombarded. They achieved this through different means but primarily based on the chemical structure of the antimicrobial agent and the mechanisms through which the agents acted. The resistance mechanisms, therefore, depend on which specific pathways are inhibited by the drugs and the alternative ways available for those pathways that the organisms can modify to get a way around in order to survive (Yeaman and Yount, 2003; Tenover, 2006). Resistance can be described in two ways:

a) intrinsic or natural whereby microorganisms naturally do not posses target sites for the drugs and, therefore, the drug does not affect them or they naturally have low permeability to those agents because of the differences in the chemical nature of the drug and the microbial membrane structures especially for those that require entry into the microbial cell in order to effect their action or
b) acquired resistance whereby a naturally susceptible microorganism acquires ways of not being affected by the drug.

Acquired resistance mechanisms can occur through various ways, Fig. 1.

For this reason all the synthesized compounds were evaluated for *in vitro* antimicrobial activities against *S. aureus* (ATCC-9144), *S. epidermidis* (ATCC-155), *M. luteus* (ATCC-4698), *B. cereus* (ATCC-11778), *E. coli* (ATCC-25922),

P. aeruginosa (ATCC-2853) as examples of Gram positive and negative bacteria. They were also evaluated for their in vitro antifungal potential against *A. niger* (ATCC-9029) as example of fungi and *A. fumigatus* (ATCC-46645) as example of molds. All the fungal and mold strains were clinical isolates, identified with conventional morphological and biochemical methods.

From the results we can see that the synthesized compounds were moderately active against tested microorganisms with the range of MIC values for *S. aureus* (3.4–29.4 μg/mL), *S. epidermidis* (2.1–28.2 μg/mL), *M. luteus* (1.2–28.7 μg/mL), *B. cereus* (2.0–27.7 μg/mL), *E. coli* (3.1–32.8 μg/mL), *P. aeruginosa* (2.4–36.2 μg/mL), *A. niger* (1.1–34.2 μg/mL) and *A. fumigatus* (1.7–31.8 μg/mL) and the compound 1,2,4,5-tetra[5-(4-nitrobenzylideneamino)-1,3,4-thiadiazole-2-yl]benzene **7** was found to exhibit the most potent antimicrobial activity with the MICs of 3.4, 2.1, 1.2, 2.0, 3.1, 2.4, 1.1and 1.7 μg/mL against *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus*, *E. coli*, *P. aeruginosa*, *A. niger* and *A. fumigatus*, respectively. From SAR studies it is clear that compound **7** exhibited significant antimicrobial activity when compared to standard drugs Ciprofloxin and Ketoconazole. Other compounds **1**–**6**, **8**–**10** showed good antibacterial and antifungal activities. The most potent antibacterial and antifungal activities exhibited by compound **7** might be due to the presence of strong electron withdrawing substituent two nitro groups on the benzylideneamino moiety of the 1,3,4-thiadiazole. While other compounds, though they contain weak electron withdrawing groups like bromo, chloro and electron donating groups like methoxy, methyl, hydroxy groups unfortunately produced weak antimicrobial activity (Table 1).

4. Conclusion

The antimicrobial activity of the synthesized compounds may be due to the presence of the versatile pharmacophore which might increase the lipophilic character of the molecules, which facilitate the crossing through the biological membrane of the microorganism and thereby inhibit their growth. For this reason, we can see that compound 1,2,4,5-tetra[5-(4-nitrobenzylideneamino)-1,3,4-thiadiazole-2-yl]benzene **7** was found to exhibit the most potent antimicrobial activity against *S. aureus* ATCC-9144, *S. epidermidis* ATCC-155, *M. luteus* ATCC-4698, *B. cereus* ATCC-11778, *E. coli* ATCC-25922, *P. aeruginosa* ATCC-2853, *A. niger* ATCC-9029 and *A. fumigatus* ATCC-46645 when compared with Ciprofloxin and Ketoconazole. In conclusion, we reported here a simple and convenient route for the synthesis of some new derivatives based on 1,3,4-thiadiazole for antimicrobial evaluation.

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