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ARTICLE

Synthesis of *N*-Benzylidene-4-(5-methyl-1H-tetrazol-1-yl)benzenamines as Potent Antibacterial and Antifungal Agents

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ABSTRACT

In search of new antibacterial and antifungal agents with improved and broad spectrum potency, we designed and synthesized a series of novel *N*-benzylidene-4-(5-methyl-1*H*-tetrazol-1-yl)benzenamines (**6a-j**). All the synthesized compounds were evaluated for their *in vitro* antibacterial against Gram-positive and Gram-negative bacteria. The antifungal activities of the synthesized compounds were also evaluated. Some of the compounds (**6e**, **6i**, **6j**) showed better activities towards bacterial pathogens. Among the synthesized compounds, compound **6f** exhibited potent antibacterial activity against Gram-negative *Salmonella abony*, *Salmonella typhi*, *Escherichia coli* and Gram-positive *Bacillus subtilis* bacteria. Compound **6f** also shows potent antifungal activities against all the fungal pathogens.

KEYWORDS

Benzenamines, Microbial activities, Schiff base, Tetrazole.

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INTRODUCTION

The increasing number of pathogenic microbes frequently posed serious threats to the human health and other animals. The treatment of infectious diseases becomes important and remains a worldwide challenging problem with the emergence of resistance to the existing antimicrobial drugs [1-4]. Furthermore, systemic and cuticular fungal infections have drastically increased, specifically in individuals with suppressed immune systems. In particular, ESKAPE pathogens [5] (*i.e.*, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) with rapidly growing multi-drug resistant properties causes over 2 million hospital acquired infections and approximately 23,000 deaths per year. Pathogenic microbes like *Streptococcus* and *Pseudomonas* causes pneumonia and other bacteria such as *Salmonella*, *Shigella* and *Campylobacter* are responsible for foodborne illnesses. *Escherichia coli* cause skin infections, septicemia and respiratory infections. Eventually, there is an instant demand for the development of new broad spectrum antibacterial and antifungal agents with divergent and unique structure and with a mechanism of action possibly different from that of existing antimicrobial agents [6].

Schiff bases bearing different types of heterocycles were shown to exhibit an interesting and a wide range of pharmacological properties like antimicrobial [7], antibacterial [8], anti-

fungal [9], anti-inflammatory [10], analgesic [11], antitubercular [12], anticancer [13], anti-HIV [14], antioxidant [15], diuretics [16], *etc*. Studies have also shown that Schiff bases comprising bi or tridentate ligands are capable of forming stable complexes with transition metals [17,18].

Tetrazoles considered unusual due to the presence of four nitrogen atoms in a five-membered aromatic ring has attracted considerable attention as a non-classical bioisostere of carboxylic acid group in biologically active molecules [19]. Retained pharmacological effect and more favourable pharmacokinetic profile are often achieved by the replacement of carboxylate group with a metabolically stable tetrazolate group. The notable examples include PTB1B inhibitors [20], mGlu 1 receptor agonists [21], growth hormone secretagogues [22], *etc*. Tetrazole containing scaffolds namely vofopitant (I) acts as NK1 receptor antagonist [23], cilostazole (II) as a phosphodiesterase inhibitor [24], cefazaflur (III) as cephalosporin antibiotic [25], *etc*. In view of these facts, it was of interest to synthesize a novel series of Schiff bases bearing tetrazole nucleus and preliminary screening for the antibacterial and antifungal properties of the synthesized compounds.

EXPERIMENTAL

All the chemicals used were of AR grade and purchased from Sd-Fine chemicals, India. The progress of reaction and purity of the product were monitored by thin layer chromatography using precoated Silica 60/UV254 (SDFCL). IR spectra were recorded (KBr) on a Perkin Elmer 1650 spectrophotometer and reported in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on 400 MHz Varian Mercury plus 400 MHz FT NMR spectrometer using $CDCl_3$ as solvents and TMS as an internal standard. Chemical shifts are expressed as δ ppm scale relative to TMS (δ = 0.00 ppm). Melting points were obtained using melting points apparatus (Model MP-96) and are uncorrected.

Synthesis of 4-methylbenzylidene-(4-(5-methyl-1*H*-tetrazol-1-yl)phenyl)amine (6a): To a solution of 4-(5-methyl-1*H*-tetrazol-1-yl)benzenamine (**4**) (1.2 g, 0.5 mmol) in 20 mL ethanol were added 4-methylbenzaldehyde (**5a**) (0.52 g, 0.5 mmol). The mixture was stirred at room temperature for 30 min. poured into water, filtered and dried. Column chromatography (4:1::hexane-EtOAc) gave compound **6** as a pale yellow solid (1.19 g, 95 %); m.p. 139-141 ºC. IR (KBr, ν_{max}, cm^{-1}): 3106, 3071 (Ar-H), 2965, 2858 (CH_3), 1627 (C=N azomethine), 1581 (C=N tetrazole ring), 1499 (N=N tetrazole ring), 1453, 1410 and 1271 (N-N=N), 1092 and 1000 (tetrazole ring); ^1H NMR: δ ppm: 2.10 (s, 3H, CH_3); 2.49 (s, 3H, CH_3); 7.18 (d, 2H, Ar-H); 7.33 (d, 2H, Ar-H); 7.43 (d, 2H, Ar-H); 7.76 (d, 2H, Ar-H); 8.43 (s, 1H, N=CH); ^{13}C NMR: δ ppm: 8.39 (CH_3-tetrazole), 21.6 (CH_3-Ar), 119.9 (C, tetrazole), 125.7 (2C), 128.9 (2C), 129.2 (2C), 131.8 (2C), 133.7 (C-tetrazole), 141.8 (C-N), 152.2 (C, Ar), 152.8 (C, Ar), 160.3 (N=CH); MS (ESI) *m/z*: 278 $(M+1)^+$. Anal. calcd. (%) for $C_{16}H_{15}N_5$: C, 69.29; H, 5.45; N, 25.25; Found: C, 69.21; H, 5.46; N, 25.28.

4-Methoxybenzylidene-(4-(5-methyl-1*H*-tetrazol-1-yl)phenyl)amine (6b): 4-Methoxybenzaldehyde was used (0.63 g, 0.5 mmol) and the reaction proceeded for 30 min at room temperature afforded **6b**, 3063 (Ar-H), 2978, 2863 (CH_3), 1619 (C=N azomethine), 1587 (C=N tetrazole ring), 1490 (N=N tetrazole ring), 1441, 1403 and 1252 (N-N=N), 1096 and 1005 (tetrazole ring); 1H-NMR: δ ppm: 2.08 (s, 3H, CH3); 3.92 (s, 3H, OCH_3); 6.98 (d, 2H, Ar-H); 7.21 (d, 2H, Ar-H); 7.36 (d, 2H, Ar-H); 7.83 (d, 2H, Ar-H); 8.40 (s, 1H, N=CH); 13C-NMR: δ ppm: 9.0 (CH_3-tetrazole), 55.4 (CH_3-O), 119.4 (C, tetrazole), 123.7 (2C), 128.5 (2C), 130.2 (2C), 131.1 (2C), 132.9 (C-tetrazole), 133.8 (C-N), 151.1 (C, Ar), 152.4 (C, Ar), 162.2 (N=CH); MS (ESI) *m/z*: 294 $(M+1)^+$. Anal. calcd. (%) for $C_{16}H_{15}N_5O$: C, 65.52; H, 5.15; N, 23.88; O, 5.45; Found: C, 65.43; H, 5.18; N, 23.91; O, 5.46.

2-Chlorobenzylidene-(4-(5-methyl-1*H*-tetrazol-1-yl)-phenyl)amine (6c): 2-Chlorobenzaldehyde was used (0.61 g, 0.5 mmol) and the reaction proceeded for 30 min at room temperature afforded **6c** (1.58 g, 91 %); m.p. 128-130 °C; IR (KBr, ν_{max}, cm^{-1}): 3116, 3077 (Ar-H), 2981, 2876 (CH_3), 1604 (C=N azomethine), 1591 (C=N tetrazole ring), 1492 (N=N tetrazole ring), 1406 and 1277 (N-N=N), 1090 and 998 (tetrazole ring), 758 (C-Cl); ^1H NMR: δ ppm: 2.12 (s, 3H, CH_3); 7.13 (d, 2H, Ar-H); 7.39-7.46 (m, 3H, Ar-H); 7.55 (d, 2H, Ar-H); 7.73 (m, 1H, Ar-H); 8.95 (s, 1H, N=CH); ^{13}C NMR: δ ppm: 9.1 (CH_3-tetrazole), 120.1 (C, tetrazole), 121.08 (C, Ar), 123.3 (2C, Ar), 130.01 (C, Ar), 132.56 (2C, Ar), 133.32 (C-tetrazole), 133.96 (C-N), 137.31 (C, Ar), 150.01 (C, Ar) 151.01 (C, Ar), 151.99 (C, Ar), 160.88 (N=CH); MS (ESI) *m/z*: 299 $(M+1)^+$. Anal. calcd. (%) for $C_{16}H_{12}N_5Cl$: C, 60.51; H, 4.06; Cl, 11.91; N, 23.52; Found: C, 60.48; H, 4.10; Cl, 11.93; N, 23.56.

3-Chlorobenzylidene-(4-(5-methyl-1*H*-tetrazol-1-yl)phenyl)amine (6d): 3-Chlorobenzaldehyde was used (0.61 g, 0.5 mmol) and the reaction proceeded for 30 min at room temperature afforded **6d** (1.64 g, 95%); m.p. 136-138 ºC; IR (KBr, ν_{max}, cm^{-1}): 3123, 3089 (Ar-H), 2992, 2869 (CH_3), 1633 (C=N azomethine), 1597 (C=N tetrazole ring), 1503 (N=N tetrazole ring), 1405 and 1273 (N-N=N), 1097, 1068 and 995 (tetrazole ring), 717 (C-Cl); ^1H NMR: δ ppm: 2.11 (s, 3H, CH_3); 7.11 (d, 2H, Ar-H); 7.46 (d, 2H, Ar-H); 7.53-7.58 (m, 3H, Ar-H); 7.82 (m, 1H, Ar-H); 8.51 (s, 1H, N=CH); ^{13}C NMR: δ ppm: 9.08 (CH_3-tetrazole), 119.4 (C, tetrazole), 119.69 (C, Ar), 123.41 (2C, Ar), 128.47 (C, Ar), 130.0 (C, Ar), 132.89 (2C, Ar), 133.43 (C-tetrazole), 133.97 (C-N), 137.37 (C, Ar), 150.09 (C, Ar) 151.89 (C, Ar), 160.8 (N=CH); MS (ESI) *m/z*: 299 $(M+1)^+$. Anal. calcd. (%) for $C_{16}H_{12}N_5Cl$: C, 60.51; H, 4.06; Cl, 11.91; N, 23.52; Found: C, 60.49; H, 4.11; Cl, 11.92; N, 23.54.

4-Chlorobenzylidene-(4-(5-methyl-1*H*-tetrazol-1-yl)phenyl)amine (6e): 4-Chlorobenzaldehyde was used (0.61 g, 0.5 mmol) and the reaction proceeded for 30 min at room temperature afforded **6e** (1.67 g, 97 %); m.p. 142-144 ºC; IR (KBr, ν_{max}, cm^{-1}): 3101, 3062 (Ar-H), 2962, 2850 (CH_3), 1629 (C=N azomethine), 1598 (C=N tetrazole ring), 1500 (N=N tetrazole ring), 1407 and 1279 (N-N=N), 1130, 1099 and 1030 (tetrazole ring), 751 (C-Cl); ^1H NMR: δ ppm: 2.10 (s, 3H, CH_3); 7.13 (d, 2H, Ar-H); 7.28 (d, 2H, Ar-H); 7.48 (d, 2H, Ar-H); 7.82 (d, 2H, Ar-H); 8.49 (s, 1H, N=CH); ^{13}C NMR: δ ppm: 9.07 (CH_3-tetrazole), 119.46 (C, tetrazole), 123.34 (2C, Ar), 129.35 (2C, Ar), 130.1 (2C, Ar), 132.38 (2C, Ar), 138.24 (C-tetrazole), 142.41 (C-N), 150.82 (C, Ar), 152.48 (C, Ar), 160.17 (N=CH); MS (ESI) *m/z*: 299 $(M+1)^+$. Anal. calcd. (%) for $C_{16}H_{12}N_5Cl$: C, 60.51; H, 4.06; Cl, 11.91; N, 23.52; Found: C, 60.48; H, 4.05; Cl, 11.96; N, 23.57.

2-Nitrobenzylidene(4-(5-methyl-1*H*-tetrazol-1-yl)-phenyl)amine (6f): 2-Nitrobenzaldehyde was used (0.66 g, 0.5 mmol) and the reaction proceeded for 30 min at room temperature afforded **6f** (1.66 g, 93 %); mp 128-130 ºC; IR (KBr, ν_{max}, cm^{-1}): 3105, 3076 (Ar-H), 2989, 2887 (CH3), 1629 (C=N azomethine), 1607 (C=N tetrazole ring), 1494 (N=N tetrazole ring), 1403 and 1299 (N-N=N), 1105 and 1009 (tetrazole ring), 854 (C-NO_2); ^{1}H NMR: δ ppm: 2.10 (s, 3H, CH_3); 7.24 (d, 2H, Ar-H); 7.44 (d, 2H, Ar-H); 7.51-7.63 (m, 3H, Ar-H) 8.26 (m, 1H, Ar-H); 8.94 (s, 1H, N=CH); 13C-NMR: δ ppm: 9.11 (CH_3-tetrazole), 119.4 (C, tetrazole), 123.3 (C, Ar), 125.09 (2C, Ar), 129.04 (C, Ar), 130.66 (C, Ar), 131.06 (2C, Ar), 133.2 (C-tetrazole), 133.65 (2C, Ar), 137.42 (C-N), 149.27 (C, Ar), 151.57 (C, Ar) 159.68 (N=CH); MS (ESI) *m/z*: 309 $(M+1)^+$. Anal. calcd. (%) for $C_{16}H_{12}N_5O_2$: C, 58.44; H, 3.92; N, 27.26; O, 10.38; Found: C, 58.40; H, 3.93; N, 27.29; O, 10.41.

3-Nitrobenzylidene(4-(5-methyl-1*H*-tetrazol-1-yl)-phenyl)amine (6g): 3-Nitrobenzaldehyde was used (0.66 g, 0.5 mmol) and the reaction proceeded for 30 min at room temperature afforded **6g** (1.72 g, 96 %); m.p. 136-138 ºC; IR (KBr, ν_{max}, cm^{-1}): 3130, 3106 (Ar-H), 2982, 2859 (CH_3), 1624 (C=N azomethine), 1597 (C=N tetrazole ring), 1485 (N=N tetrazole ring), 1400 and 1293 (N-N=N), 1097 and 1003 (tetrazole ring), 845 (C- NO_2); ^{1}H NMR: δ ppm: 2.10 (s, 3H, CH_3); 7.28 (d, 2H, Ar-H); 7.38 (d, 2H, Ar-H); 7.48-7.51 (m, 3H, Ar-H); 8.51 (s, 1H, N=CH); 8.79 (m, 1H, Ar-H); ^{13}C NMR: δ ppm: 9.13 (CH_3-tetrazole), 119.5 (C, tetrazole), 123.5 (C, Ar), 124.22 (C, Ar), 128.01 (2C, Ar), 130.0 (C, Ar), 131.56 (2C, Ar), 133.25 (C-tetrazole), 133.88 (C-N), 136.02 (C, Ar), 148.72 (C, Ar) 152.51 (C, Ar), 158.64 (N=CH); MS (ESI) *m/z*: 309 $(M+1)^+$. Anal. calcd. (%) for $C_{16}H_{12}N_5O_2$: C, 58.44; H, 3.92; N, 27.26; O, 10.38; Found: C, 58.43; H, 3.94; N, 27.28; O, 10.44.

4-Nitrobenzylidene(4-(5-methyl-1*H*-tetrazol-1-yl)-phenyl)amine (6h): 4-Nitrobenzaldehyde was used (0.66 g, 0.5 mmol) and the reaction proceeded for 30 min at room temperature afforded **6h** (1.75 g, 98 %); m.p. 190-192 ºC; IR (KBr, ν_{max}, cm^{-1}): 3100, 3052 (Ar-H), 2971, 2856 (CH3), 1631 (C=N azomethine), 1596 (C=N tetrazole ring), 1506 (N=N tetrazole ring), 1409 and 1279 (N-N=N), 1162 and 1096 (tetrazole ring), 841 (C-NO_2); ^{1}H NMR: δ ppm: 2.11 (s, 3H, CH_3); 7.44 (dd, 2H, Ar-H); 7.52 (dd, 2H, Ar-H); 8.11 (d, 2H, Ar-H); 8.32 (d, 2H, Ar-H); 8.63 (s, 1H, N=CH); ^{13}C NMR: δ ppm: 9.12 (CH_3-tetrazole), 119.67 (C, tetrazole), 123.1 (2C, Ar), 129.16 (2C, Ar), 131.36 (2C, Ar), 132.38 (2C, Ar), 133.49 (C, Ar), 140.89 (C-tetrazole), 142.02 (C-N), 150.09 (C, Ar), 152.22 (C, Ar), 160.12 (N=CH); MS (ESI) *m/z*: 309 $(M+1)^+$. Anal. calcd. (%) for $C_{16}H_{12}N_5O_2$: C, 58.44; H, 3.92; N, 27.26; O, 10.38; Found: C, 58.41; H, 3.93; N, 27.23; O, 10.42.

***N*-((2-Chloroquinolin-3-yl)methylene)-4-(5-methyl-1*H*-tetrazol-1-yl)benzenamine (6i):** 2-Chloro-3-formylquinoline was used (0.82 g, 0.5 mmol) and the reaction proceeded for 30 min at room temperature afforded **6i** (1.54 g, 78 %); m.p. 201-202 ºC; IR (KBr, ν_{max}, cm^{-1}): 3114, 3062 (Ar-H), 2963, 2891 (CH3), 1623 (C=N azomethine), 1590 (C=N tetrazole ring), 1503 (N=N tetrazole ring), 1400 and 1287 (N-N=N), 1151 and 1088 (tetrazole ring); ^{1}H NMR: δ ppm: 2.52 (s, 3H, CH_3); 7.47-7.52 (m, 5H, Ar-H); 7.56-7.57 (d, 2H, Ar-H); 8.61 (s, 1H, Ar-H); 8.92 (s, 1H, Ar-H); 9.02 (s, 1H, N=CH); 13C-NMR: δ ppm: 9.86 (CH_3-tetrazole), 122.46 (C, tetrazole), 125.61 (C, Ar), 126.21 (2C, Ar), 127.1 (C, Ar), 131.62 (C, Ar), 134.23 (2C, Ar), 136.51 (C, Ar), 137.21 (C, Ar), 139.05 (C-tetrazole), 146.0 (C-N), 147.2 (C, Ar), 148.7 (C, Ar), 151.9 (C, Ar), 153.4 (C, Ar), 158.26 (N=CH); MS (ESI) *m/z*: 350 $(M+1)^+$. Anal. calcd. (%) for $C_{18}H_{13}N_6Cl$: C, 61.98; H, 3.76; Cl, 10.16; N, 24.09; Found: C, 61.89; H, 3.81; Cl, 10.23; N, 24.41.

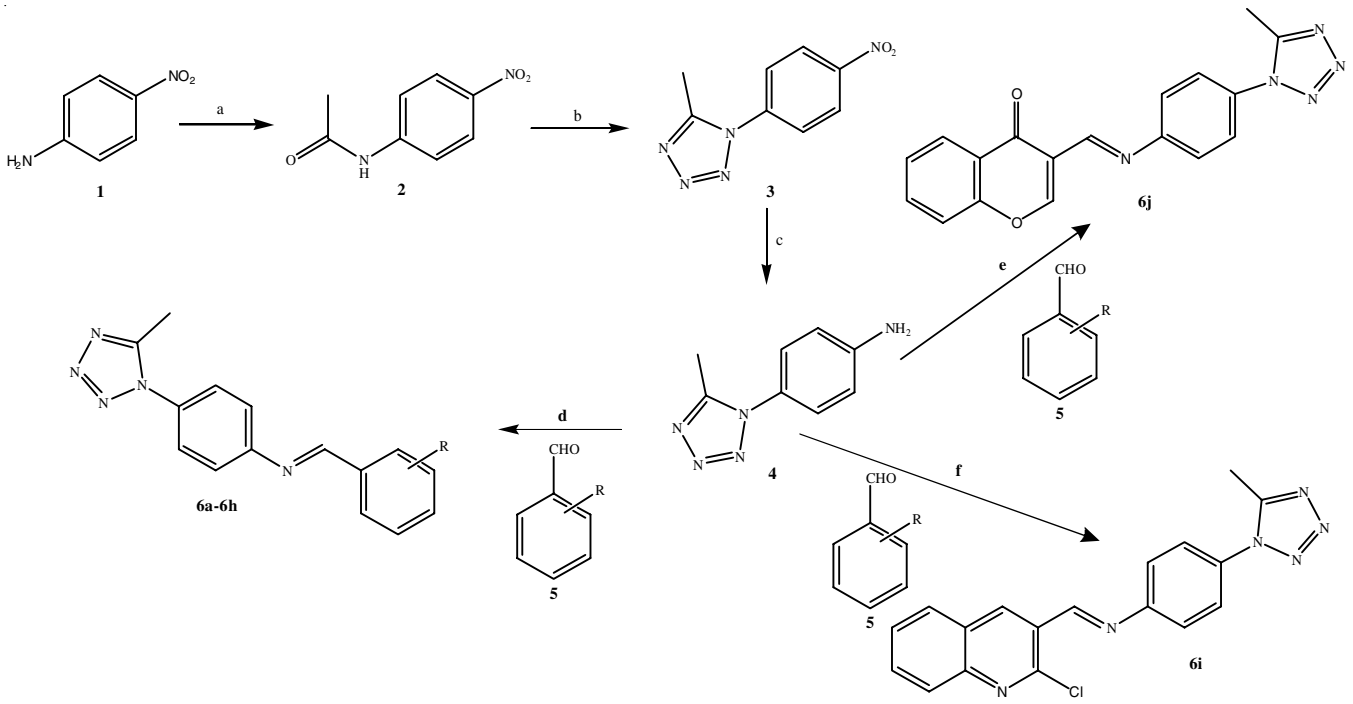
3-((4-(5-methyl-1*H*-tetrazol-1-yl)phenylimino)methyl)-4*H*-chromen-4-one (6j): 3-Formylchromone was used (0.75 g, 0.5 mmol) and the reaction proceeded for 30 min at room temperature afforded **6j** (1.64 g, 87%); m.p. 162-164 ºC; IR (KBr, ν_{max}, cm^{-1}): 3101, 3064 (Ar-H), 2977, 2865 (CH_3), 1618 (C=N azomethine), 1591 (C=N tetrazole ring), 1500 (N=N tetrazole ring), 1421 and 1267 (N-N=N), 1168 and 1110 (tetrazole ring); ^{1}H NMR: δ ppm: 2.62 (s, 3H, CH_3); 5.72 (s, 1H, arom.), 7.08 (m, 1H, Ar-H); 7.14 (m, 1H, Ar-H); 7.28-7.31 (m, 2H, Ar-H); 7.45-7.57 (m, 4H, Ar-H); 7.99 (s, 1H, N=CH); ^{13}C NMR: δ ppm: 9.78 (CH_3-tetrazole), 101.44 (C, arom.), 105.24 (C, arom.), 117.26 (C, tetrazole), 118.05 (C, arom.), 122.35 (C, arom.), 122.58 (2C, arom.), 126.18 (C, arom.), 126.39 (C, arom.), 129.18 (2C, Ar), 134.91 (C, arom.), 141.46 (C-tetrazole), 142.69 (C-N), 151.57 (C, Ar), 155.91 (N=CH), 182.06 (C=O), MS (ESI) *m/z*: 350 $(M+1)^+$. Anal. calcd. (%) for $C_{18}H_{13}N_5O_2$: C, 65.25; H, 3.95; N, 21.14; O, 9.66; Found: C, 65.21; H, 3.97; N, 21.17; O, 9.65.

RESULTS AND DISCUSSION

4-(5-Methyl-1*H*-tetrazol-1-yl)benzenamine (**4**) represents an adaptable starting material for the synthesis of Schiff bases. The introduction of heterocyclic moieties into the Schiff bases lead to enhance its biological activities. The synthesis of 4-(5-methyl-1*H*-tetrazol-1-yl)benzenamine (**4**) was carried using by reported method [26,27]. For the synthesis of 4-(5-methyl-1*H*-tetrazol-1-yl)benzenamine (**4**), initially, 4-nitroaniline (**1**) was acylated using acetic anhydride in the presence of pyridine to give 4-nitroacetanilide (**2**). Compound **2** by cyclization with NaN_3 in presence of $TiCl_4$ in acetonitrile, was converted to 5-methyl-1-(4-nitrophenyl)-1*H*-tetrazole (**3**). The catalytic reduction of compound **3** using $NaBH_4$ in the presence of $Ni(OAc)_2$ in water gives 4- (5-methyl-1*H*-tetrazol-1-yl)benzenamine (**4**). The target compounds were generated, in good yields, by stirring compound **4** with a range of selected substituted aldehydes in alcohol over a period of 10 min-5.5 h. The synthesis of tetrazole bearing Schiff bases are given in **Scheme-I**.

All the newly synthesized compounds gave moderate to high yields. Products were purified and characterized by various spectroscopic techniques. The IR spectra of compounds (**6a-j**) showed characteristic absorption bands at 1650-1620 cm^{-1} of C=N (azomethine) stretching [28], 1610-1580 cm^{-1} indicate the presence of C=N functional group of tetrazole ring [29], 1520-1480 cm^{-1} confirms the presence of N=N group of tetrazole ring [30] and 1300-1250 cm^{-1} revealed the presence of N-N=N linkage in the molecule [31,32]. IR, ^{1}H NMR, ^{13}C NMR and elemental analysis confirmed the structure of various novel (benzylidene)(4-(5-methyl-1*H*-tetrazol-1-yl)phenyl)amine derivatives (**6a-j**).

The antibacterial activity of the synthesized compounds was carried out by standard literature procedure using agar



5, 6; R. a, 4-CH_3, b, 4-OCH_3, c, 2-Cl, d, 3-Cl, e, 4-Cl, f, 2-NO_2, g, 3-NO_2, h, 4-NO_2

Scheme-I: Reaction conditions: a) Ac_2O, pyridine/DCM, b) NaN_3, $TiCl_4$/Acetonitrile, c) $NaBH_4$, $NiCl_2$/Water, d) Substituted aldehyde **5**/ EtOH, e) 3-formyl chromone/EtOH, f) 2-chloro, 3-formylquinoline/EtOH

diffusion method by finding the zone of inhibition of the drug sample against the standard drugs tetracycline for bacterial pathogens and nystatin for fungal pathogens [33]. Each test compound (10 mg) was dissolved in 1 mL of DMSO for preparing a stock solution of standard drugs. All the compounds were screened for their *in vitro* antibacterial activity against Gram-positive pathogens of *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus cereus* and *Staphylococcus aureus* and Gram-negative pathogens *viz*., *Salmonella typhi*, *Salmonella abony*, (c) *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aerogenosa* and *Shigella boydii*. The antifungal activity of all compounds was carried against *Candida albicans*, *Aspergillus niger* and *Saccharomyces cerevisiae*. The tests were carried out in duplicate. Apart from putting the controls of standard drug, controls with dimethyl sulphoxide (positive control) and without dimethyl sulphoxide (negative control) were also included in the test. The results were recorded as the average diameter of inhibition zone (IZ) of bacterial and fungal growth around the disk in mm by two-fold serial dilution method to determine the minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) in 1 mg/mL values are recorded in Table-1.

From Table-1, the results revealed that most of the compounds displayed capricious inhibitory effects on the growth of bacterial strains, as well as against the fungal strains. Among the tested compounds, the compounds **6a**, **6c**, **6e**, **6f**, **6i** and **6j** exhibited relatively good to moderate inhibitory profiles against Gram-positive and Gram-negative bacteria whereas the compound **6b**, **6d**, **6g** and **6h** showed no inhibitory effects on the growth of bacterial strains. The results revealed that compound **6a** exhibited divergent antibacterial activity against Gram-

TABLE-1
in vitro ANTIBACTERIAL ACTIVITY OF COMPOUNDS (**6a-j**)

Compound	Gram-negative bacteria						Gram-positive bacteria			
	Salmonella typhi	*Enterobacter aerogenes*	*Escherichia coli*	*Pseudomonas aerogenosa*	*Salmonella typhi*	*Shigella boydii*	*Bacillus subtilis*	*Bacillus Megaterium*	*Staphylococcus aureus*	*Bacillus cereus*
6a	–	–	10	13	06	12	14	13	08	15
6b	–	–	–	–	–	–	–	–	–	–
6c	13	–	15	06	07	14	–	11	12	10
6d	–	–	–	–	–	–	–	–	–	–
6e	07	–	09	10	-	08	11	07	–	12
6f	13	12	13	15	17	09	11	09	11	10
6g	–	–	–	–	–	–	–	–	–	–
6h	–	–	–	–	–	–	–	–	–	–
6i	–	–	11	12	–	–	–	15	08	11
6j	09	11	13	11	13	14	12	09	13	12
Tetracycline	22	20	20	33	21	26	25	20	30	25

negative bacteria while compound **6f** showed good activity against Gram-negative bacteria. Herein, compound **6i** was 75 % potent to tetracycline in inhibiting the growth of *B. megaterium* (MIC 15 mm), while compound **6f** was 80 % potent than tetracyclin against *S. abony* (MIC 17 mm). Among the series of compounds, **6f** and **6j** showed good to moderate growth inhibitory effects on the growth of all the bacterial strains while compounds **6a**, **6c**, **6e** and **6i** showed moderate to weak growth inhibitory activity as compared to tetracyclin. Besides, distinctive antibacterial profile was showed by compounds **6c** against both *E. coli* (MIC 15 mm) and *S. boydii* (MIC 14 mm).

Regarding the activity of compounds **6a-j** against fungal strains (Table-2), the results showed that compound **6f** was 50 % lesser than nystatin in inhibitory growth of *C. albicans* (MIC 12 mm), while the reactivity of other compounds **6a** and **6i** becomes less active as compared to standard drug nystatin (MIC 8 mm and 11 mm, respectively) while other tested compounds not showed growth inhibition of *C. albicans*. Furthermore, compound **6j** showed 50 % potent of nystatin in growth inhibition of *A. niger* (MIC 13 mm) and compound **6c** exhibited 60 % potent in growth inhibition of *S. cerevisiae* as compared to nystatin (MIC 12 mm).

TABLE-2
in vitro ANTIFUNGAL ACTIVITY OF COMPOUNDS (**6a-j**)

Compounds	*Candida albicans*	*Saccharomyces cerevisiae*	*Aspergillus niger*
6a	14	13	08
6b	–	–	–
6c	–	11	12
6d	–	–	–
6e	11	07	–
6f	11	09	11
6g	–	–	–
6h	–	–	–
6i	–	15	08
6j	12	09	13
Nystatin	25	20	30

The structure activity relationships (SAR) analysis in compounds **6a-j** bearing tetrazole moiety also revealed that compounds having methyl group at *para*-position (**6a**), nitro group at *ortho* (**6f**) and chromone containing compound (**6j**) exhibited potent antimicrobial activities against Gram-positive and Gram-negative bacteria as well as fungi, which indicated that *p*-methyl, *o*-nitro and chromone may play an important role on the antimicrobial activities. However, compounds having a nitro group at *meta* (**6g**) and *para* (**6h**) do not exhibit inhibitory activities which suggested that the position of group play a vital role in enhancing antimicrobial activities.

Conclusion

We have designed and synthesized a novel tetrazole bearing Schiff bases namely *N*-benzylidene-4-(5-methyl-1*H*-tetrazol-1-yl)benzenamine (**6a-h**), 3-((4-(5-methyl-1*H*-tetrazol-1-yl)-phenylimino)methyl)-4*H*-chromen-4-one (**6i**) and N-((2-chloroquinolin-3-yl)methylene)-4-(5-methyl-1*H*-tetrazol-1-yl)benzenamine (**6j**) from 4-(5-methyl-1*H*-tetrazol-1-yl)benzenamine (**4**) and screened for their antibacterial and antifungal activities. Generally, the results showed that compounds having 4-methyl (**6a**), 2-chloro (**6c**), 4-chloro (**6e**), 2-nitro (**6f**) group on aldehyde moeity displayed good to moderate growth of inhibition towards the tested pathogens. Compounds having quinoline (**6i**) and chromone (**6j**) ring exhibited potent antimicrobial activities against Gram-positive and Gram-negative bacteria as well as fungi. Among the tested compounds, compound **6f** shows most potent activity towards *S. abony*. The results are favourable for further studies in emerging new trends of tetrazole bearing schffolds as potential antimicrobials.

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