Schiff bases of 4-(methylthio)benzaldehydes: Synthesis, characterization, antibacterial, antioxidant and cytotoxicity Studies

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ABSTRACT

A series of new Schiff bases of 4-(methylthio)benzaldehyde derivatives 3(a-i) were synthesized by the reaction of 4-(methylthio)benzaldehyde with various amines 2(a-i). Newly synthesized compounds were characterized by elemental analyses, UV-visible, FT-IR, Mass and $^1$H NMR spectral studies. All compounds were evaluated for their in vitro antibacterial activity against clinically isolated strains i.e., E. Coli, P. Fluorescence, M. Luteus and B. Subtilis. These compounds were screened for their antioxidant activity by 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) and ferrous ion chelating assay (Fe$^{2+}$) methods. The cytotoxicity assay was performed by tryphan blue dye exclusion method. Compounds 3g, 3h and 3i exhibited good antibacterial activity when compared with other compounds in the series against tested pathogenic bacterial strains. All the compounds showed antioxidant activity, where compound 3b was the best radical scavenger and Fe$^{2+}$ ion scavenger. These findings showed that the Schiff bases of 4-(methylthio)benzaldehyde derivatives possess antioxidant activity with different mechanism of actions towards the different free radicals tested. Among these derivatives, 3b and 3h had the strongest activity against human peripheral lymphocytes.

1. Introduction

4-(Methylthio)benzaldehyde is a essential moiety for the synthesis of various pharmaceutical and biologically active compounds. It is the intermediate for the synthesis of a pyrrole derivatives showing anti-inflammatory activity.1, 2 Antimicrobial drug discovery research, accompanied by clinical development, has historically been conducted by large pharmaceutical companies. Infections caused by microbes are a serious menace to the health of human beings and often have connection to some other diseases, whenever the body system gets debilitated. The number of different classes of
antibacterial\textsuperscript{3, 4} and antifungal agents\textsuperscript{5} has been discovered. The extensive use of antibiotics has led to the appearance of multi-drug resistant microbial pathogens.\textsuperscript{6}

The compounds containing an imine groups are important in elucidating the mechanism of transamination and racemisation reactions in biological systems.\textsuperscript{7, 8} Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behaviors have been studied.\textsuperscript{9} They have been synthesized from a variety of compounds, such as amino thiazoles, 2-hydroxy-1-naphthalaniline, amino sugars, aromatic aldehydes, ketones, isatin, triazole ring, thiosemicarbazides, amino acids, pyrazolone, etc.\textsuperscript{10, 11} Antimicrobial and anticancer activities of Schiff bases have been reported\textsuperscript{12} and they are active against a wide range of organisms. Many Schiff bases are known to be medicinally important and are used to design medicinal compounds.\textsuperscript{13}

Free radical contains an odd number of electrons which makes it unstable, short lived and highly reactive. Therefore, it reacts quickly with other compounds in order to capture the needed electron to gain stability. Generally, free radical attacks the nearest stable molecule, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction cascade resulting in disruption of a living cell.\textsuperscript{14, 15} There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds, nitrogen compounds as well as ascorbic acid.\textsuperscript{16, 17} The primary antioxidants comprise essentially sterically hindered phenols and secondary aromatic amines.\textsuperscript{18} These antioxidants act usually both through chain transfer and chain termination. The first step of the reactive radical’s termination by this type of antioxidants is hydrogen atom transfer from the antioxidant molecule to the reactive radical intermediate.\textsuperscript{19} In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma while lipid-soluble antioxidants protect cell membranes from lipid peroxidation.\textsuperscript{20} These compounds may be synthesized in the body or obtained from the diet.\textsuperscript{21} A significant part of drug discovery in the past few years has been focused on agents to prevent or treat cancer. This is not surprising because, in most developed countries and, to an increasing extent, cancer is among the three most common causes of death and morbidity. Cancer treatments may involve surgery, radiotherapy, and chemotherapy and often a combination of two or all three is employed. Cytotoxicity studies involve the analysis of morphological damage or inhibition of zone of outgrowth induced by the chemicals tested.\textsuperscript{22} The methods used for the evaluation of Cytotoxicity i.e. blue dye exclusion assay in that the tryphan blue was a vital stain used to selectively color dead tissues or cells blue based on the principle that live (viable) cells actively pump out the dye by efflux mechanism where as dead (nonviable) cells do not and MTT [(3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide] which measures the metabolic activity of the viable cells.\textsuperscript{23} In this respect, the present paper reports the synthesis and biological activities of a new class of Schiff bases of 4-(methylthio)benzaldehyde derivatives 3(a-i).

2. Experimental

2.1. Chemistry

All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd. Melting range was determined by Veego Melting Point VMP III apparatus. The UV-Visible spectrum was recorded on UV-1800 SHIMADZU UV spectrometer with quartz cell of 1.0 cm path length. An elemental analysis was recorded on Costech ECS 4010 CHNS-O Elemental Analyzer. The FT-IR spectra was recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer and were quoted in cm\textsuperscript{-1}.\textsuperscript{1} H NMR spectra was recorded on Bruker DMX 300, 400MHz spectrometer using DMSO-d\textsubscript{6} as solvent and TMS as an internal standard. Mass spectral data were obtained by LC-MSD Trap XCT. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck-made TLC plates.
2.2. General procedure for the synthesis of Schiff bases of 4-(methylthio)benzaldehyde derivatives (3a-i)

Equimolar concentrations of 4-(methylthio)benzaldehyde (3.28 mmol, 1) and different amines (3.28 mmol, 2a-i) were refluxed for 7-8 hr using methanol (25 ml) and 2-3 drops of conc. sulfuric acid was added to the mixture. The progress of the reaction was followed by TLC until the reaction was complete. It was poured to ice cold water, the precipitate was filtered, washed with excess of distilled water and the residue was recrystallized from ethanol, DMF and ethyl acetate. Compounds 3(a-i) were prepared by the method summarized in Scheme 1.

![Scheme 1](image)

Scheme 1 Reagents and conditions: (i) Methanol, reflux, H^+, 7-8 hr

2.2.1. (E)-N-(4-(Methylthio)benzylidene)-3,5-dibromopyrazin-2-amine (3a)

The product was synthesized by the reaction of 1 and 3,5-dibromopyrazin-2-amine (2a). It was obtained as a yellow solid. FT-IR (KBr, cm\(^{-1}\)): 3078 (Ar-H), 1671 (HC=N), 1468 (C=C), 1298 (C-S), 527 (C-Br). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm: 2.39 (s, 3H, CH\(_3\)), 7.23 (d, 2H, Ar-H), 7.61 (d, 2H, Ar-H), 7.98 (s, 1H, CH=N), 8.81 (s, 1H, Pyrazine-H). MS (ESI) m/z: 387.80. Anal. Calcd. for C\(_{12}\)H\(_9\)Br\(_2\)N\(_3\)S (in %): C, 37.23; H, 2.34; N, 10.86. Found: C, 37.20; H, 2.10; N, 10.91.

2.2.2. (E)-2-(4-(Methylthio)benzylideneamino)-6-methylpyrimidin-4-ol (3b)

The product was synthesized by the reaction of 1 and 2-amino-6-methylpyrimidin-4-ol (2b). It was obtained as a white solid. FT-IR (KBr, cm\(^{-1}\)): 3514 (O-H), 3059 (Ar-H), 1660 (HC=N), 1454 (C=C), 1331 (C-S). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm: 2.35 (s, 3H, CH\(_3\)), 2.46 (s, 3H, CH\(_3\)), 5.20 (s, 1H, OH), 6.71 (s, 1H, Ar-H), 7.29 (d, 2H, Ar-H), 7.69 (d, 2H, Ar-H), 8.01 (s, 1H, CH=N). MS (ESI) m/z: 260.11. Anal. Calcd. for C\(_{13}\)H\(_{13}\)N\(_3\)OS (in %): C, 60.21; H, 5.05; N, 16.20. Found: C, 60.18; H, 5.28; N, 16.12.

2.2.3. (E)-N-(4-(Methylthio)benzylidene)-4-bromo-2-chloro-6-methylbenzenamine (3c)

The product was synthesized by the reaction of 1 and 4-bromo-2-chloro-6-methylbenzenamine (2c). It was obtained as a white solid. FT-IR (KBr, cm\(^{-1}\)): 3061 (Ar-H), 1661 (HC=N), 1468 (C=C), 1291 (C-S), 728 (C-Cl), 521 (C-Br). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm: 2.34 (s, 3H, CH\(_3\)), 2.42 (s, 3H, CH\(_3\)), 7.20-7.61 (m, 6H, Ar-H), 8.29 (s, 1H, CH=N). MS (ESI) m/z: 355.41. Anal. Calcd. for C\(_{15}\)H\(_{13}\)BrClNS (in %): C, 50.79; H, 3.69; N, 3.95. Found: C, 50.83; H, 3.71; N, 3.72.

2.2.4. (E)-N-(4-(Methylthio)benzylidene)thiazol-2-amine (3d)

The product was synthesized by the reaction of 1 and thiazol-2-amine (2d). It was obtained as an off white solid. FT-IR (KBr, cm\(^{-1}\)): 3083 (Ar-H), 1665 (C=N), 1474 (C=C), 1310 (C-S). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm: 2.31 (s, 3H, CH\(_3\)), 7.11 (d, 1H, thiazole-H), 7.24 (d, 2H, Ar-H), 7.63 (d, 2H, Ar-H), 7.92 (d, 1H, thiazole-H), 8.78 (s, 1H, CH=N). MS (ESI) m/z: 235.40. Anal. Calcd. for C\(_{11}\)H\(_{10}\)N\(_2\)S\(_2\) (in %): C, 56.38; H, 4.30; N, 11.95. Found: C, 56.42; H, 4.12; N, 11.71.
2.2.5. (E)-5-(4-(Methylthio)benzylideneamino)-1,3,4-thiadiazole-2-thiol (3e)

The product was synthesized by the reaction of 1 and 5-amino-1,3,4-thiadiazole-2-thiol (2e). It was obtained as a white solid. FT-IR (KBr, cm⁻¹): 3014 (Ar-H), 2589 (S-H), 1659 (C=N), 1453 (C=C), 1328 (C=S). 1H NMR (DMSO-d₆) δ ppm: 2.40 (s, 3H, CH₃), 2.81 (s, 1H, S-H), 7.25 (d, 2H, Ar-H), 7.65 (d, 2H, Ar-H), 8.49 (s, 1H, CH=N). MS (ESI) m/z: 267.98. Anal. Calcd. for C₁₀H₉N₃S₃ (in %): C, 44.92; H, 3.39; N, 15.71. Found: C, 44.80; H, 3.10; N, 15.84.

2.2.6. (E)-N-(4-(Methylthio)benzylidene)-4-chloropyridin-2-amine (3f)

The product was synthesized by the reaction of 1 and 4-chloropyridin-2-amine (2f). It was obtained as a white solid. FT-IR (KBr, cm⁻¹): 3020 (Ar-H), 1670 (C=N), 1440 (C=C), 1328 (C=S), 714 (C-Cl). 1H NMR (DMSO-d₆) δ ppm: 2.31 (s, 3H, CH₃), 7.19 (d, 2H, Ar-H), 7.69 (d, 2H, Ar-H), 7.41-8.69 (m, 3H, Py-H), 8.55 (s, 1H, CH=N). MS (ESI) m/z: 263.53. Anal. Calcd. for C₁₃H₁₁ClN₂S (in %): C, 59.42; H, 4.22; N, 10.66. Found: C, 59.45; H, 4.26; N, 10.69.

2.2.7. (E)-N-(4-(Methylthio)benzylidene)-3-chloro-5-(trifluoromethyl)pyridin-2-amine (3g)

The product was synthesized by the reaction of 1 and 3-chloro-5-(trifluoromethyl)pyridin-2-amine (2g). It was obtained as a white solid. FT-IR (KBr, cm⁻¹): 3021 (Ar-H), 1676 (C=N), 1449 (C=C), 1309 (C-S), 1106 (C-F), 718 (C-Cl). 1H NMR (DMSO-d₆) δ ppm: 2.47 (s, 3H, CH₃), 7.28 (d, 2H, Ar-H), 7.81 (d, 2H, Ar-H), 8.12 (s, 1H, Py-H), 8.32 (s, 1H, CH=N), 8.85 (s, 1H, Py-H). MS (ESI) m/z: 331.36. Anal. Calcd. for C₁₄H₁₀ClF₃N₂S (in %): C, 50.84; H, 3.05; N, 8.47. Found: C, 50.68; H, 3.24; N, 8.51.

2.2.8. (E)-N₁-(4-(Methylthio)benzylidene)-4-methyl-N₃-(4-(pyridin-4-yl)pyrimidin-2-yl)benzene-1,3-diamine (3h)

The product was synthesized by the reaction of 1 and 6-methyl-N₁-(4-(pyridin-4-yl)pyrimidin-2-yl)benzene-1,3-diamine (2h). It was obtained as a brownish white solid. FT-IR (KBr, cm⁻¹): 3162 (N-H), 3074 (Ar-H), 1660 (C=N), 1456 (C=C), 1327 (C-S), 1150 (C-N). 1H NMR (DMSO-d₆) δ ppm: 2.13 (s, 3H, CH₃), 2.42 (s, 3H, CH₃), 6.58 (d, 1H, Ar-H), 6.98 (d, 1H, Ar-H), 7.11 (s, 1H, Ar-H), 7.23 (d, 2H, Ar-H), 7.41-7.31 (d, 2H, pyrimidine-H), 7.55 (t, 1H, pyridine-H), 7.80 (d, 2H, Ar-H), 8.11 (s, 1H, CH=N), 8.49 (d, 1H, pyridine-H), 8.70 (d, 1H, pyridine-H), 8.77 (s, 1H, pyridine-H), 9.23 (s, 1H, N-H). MS (ESI) m/z: 412.07. Anal. Calcd. for C₂₄H₂₁N₅S (in %): C, 70.05; H, 5.14; N, 17.02. Found: C, 70.14; H, 5.30; N, 17.26.

2.2.9. (E)-4-(4-(Methylthio)benzylidene)-3-(2-methyl-5-nitrophenyl)guanidine (3i)

The product was synthesized by the reaction of 1 and (2-methyl-5-nitrophenyl)guanidine (2i). It was obtained as a brownish white solid. FT-IR (KBr, cm⁻¹): 3170 (N-H), 3074 (Ar-H), 1660 (C=N), 1456 (C=C), 1327 (C-S), 1150 (C-N). 1H NMR (DMSO-d₆) δ ppm: 2.13 (s, 3H, CH₃), 2.42 (s, 3H, CH₃), 5.24 (s, 1H, NH), 7.12 (d, 1H, Ar-H), 7.21 (d, 2H, Ar-H), 7.34 (s, 1H, Ar-H), 7.61 (d, 1H, Ar-H), 7.71 (d, 2H, Ar-H), 7.95 (s, 1H, CH=N), 9.21 (s, 1H, NH). MS (ESI) m/z: 329.12. Anal. Calcd. for C₁₆H₁₆N₄O₂S (in %): C, 58.52; H, 4.91; N, 17.06. Found: C, 58.41; H, 4.80; N, 17.26.

2.3. Antibacterial activity

Broth dilution assay was carried out according to the method developed in a microtitre plate (96 well plate) with slight modifications. In brief overnight culture of the above mentioned pathogens were made every time in Muller Hinton’s broth and were diluted with the fresh Muller Hinton’s broth till the A₆₀₀ reaches 0.05. 100 µl of the each diluted bacterial cultures (A₆₀₀= 0.05) were dispensed to their respective wells (96 well polypropylene micro titer plate) in triplicates. A blank is maintained
which contains only sterile Muller Hinton’s broth. The plates were covered with sterile aluminum foil to avoid contamination and were incubated at 37 °C for 18 hr in a refrigerated bacteriological incubator. The plate was read in UV-Visible microplate spectrophotometer at 600 nm (photometric) with 10 seconds of shaking; the values obtained for each pathogen and drug of different concentrations were averaged and are negative with the empty broth (Blank). The minimal inhibitory concentration (MIC) was determined by broth microdilution method. The MIC value was defined as the lowest concentration of compounds whose absorbance was comparable with the negative control wells (broth only, without inoculum). MIC values and comparison with standard antibiotic (Carbenicillin) were tabulated as the mean of three replicates.

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging assay

The free radical scavenging activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier.27-29 The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and stored at 20 °C until required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100 μl of the sample at various concentrations (20 - 100 μg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared as above without any sample. Ascorbic acid (Vit-C) was used as positive control. All the experiments were done in triplicates and the values are averaged. A dose responsive curve was plotted to determine the IC50 values. IC50 is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity.30 All the tests were run in triplicate and averaged.

Scavenging effect (%) = [(control absorbance - sample absorbance) / control absorbance] × 100

2.4.2. Ferrous ion chelating assay

The chelating activity of the Schiff base derivatives for ferrous ions (Fe2+) was measured according to the method.31 Briefly, 0.5 mL different concentration of synthesized compounds was added to a solution of 2 mM FeCl2 (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). The mixture was shaken vigorously and left at room temperature for 10 min. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. Absorbance of the solution was then measured spectrophotometrically at 562 nm. EDTA was used as a positive control. All the experiments were done in triplicates and the values are averaged. A dose responsive curve was plotted to determine the IC50 values. IC50 is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity.32 All the tests were run in triplicate and averaged. The percentage inhibition of ferrozine-Fe2+ complex formation by the compounds was calculated as:

Percentage of inhibition (%) = [(A0 - A1)/A0] × 100,

where A0 was the absorbance of the control, and A1 was the absorbance of the test sample.

2.5. Cytotoxicity assay

2.5.1. Isolation of human peripheral lymphocytes

The peripheral lymphocytes were isolated from 10 to 15 ml of the freshly drawn venous blood from healthy male donors aged between 22-26 years. Blood was collected in anticoagulant Acid Citrate Dextrose (ACD) (85 mM citric acid, 71 mM Trisodium Citrate, 165 mM D-Glucose) in the ratio of 5:1. To this four volumes of hemolysing buffer (0.85% NH4Cl in 10 mM Tris buffer, pH 7.4) was added,
mixed well and incubated at 4 °C for 30 min. Then the cells were centrifuged at 12000 rpm for 12 min, the supernatant was discarded, pellet was washed thrice with 10 ml of Hanks Balanced Salt Solution (HBSS) (137 mM NaCl, 5 mM KCl, 8.5 mM Phosphate buffer pH 7.4, 0.8 mm MgSO₄ and 5 mM D-Glucose) and suspend in the same buffer solution. Cells were suspended in HBSS and it is stored at 4 °C.

2.5.2. Cell viability test

The cell viability was determined by Tryphan blue dye exclusion method. To 10 µl of the cell suspension (Peripheral lymphocytes) an equal volume of 0.4% trypan blue was added. The cells were then charged to hemocytometer and the cell number was counted. The dead cells being permeable to tryphan blue appeared blue against white colour of the viable cells. The percent cell viability was calculated using the formula:

\[
\text{Percent of viability} = \left[\frac{\text{No. of viable cells}}{\text{Total no. of cells}}\right] \times 100
\]

3. Results and discussion

3.1. Chemistry

The Schiff bases of 4-(methylthio)benzaldehyde derivatives 3(a-i) were synthesized according to Scheme 1. Formation of Schiff bases of 4-(methylthio)benzaldehyde derivatives 3(a-i) was confirmed by recording their ¹H NMR, LC-MS, Elemental analysis FT-IR and UV-visible spectra. The synthesis employs readily available starting materials and simple procedures making this method very attractive and convenient for the synthesis of various Schiff bases compounds. The chemical structures and physical data of all the synthesized compounds are tabulated in Table 1 and Table 2, respectively.

| Table 1. Chemical structure of the synthesized compounds 3(a-i) |
|---------------------------------|---------------------------------|
| **Compound** | **R** | **Structure** |
| 3a | ![3a](image) | ![3a](image) |
| 3b | ![3b](image) | ![3b](image) |
| 3c | ![3c](image) | ![3c](image) |
| 3d | ![3d](image) | ![3d](image) |
| 3e | ![3e](image) | ![3e](image) |
The 4-(methylthio)benzaldehyde (1) was reacted with various amines (R-NH$_2$, 2a-i) in methanol to obtain Schiff bases of 4-(methylthio)benzaldehyde derivatives 3(a-i) in good yield (68-82%). The UV spectra of 3(a-i) were recorded using suitable solvents in the range of 200 - 800 nm. The electronic absorption spectra of compounds show new bands and appearance of wavelength absorption band in the UV region in UV-visible spectrum owing to confirms the formation of new compounds. The FT-IR spectra of 3(a-i) were recorded using KBr pellets in the range of 4000 - 400 cm$^{-1}$. The absence of NH$_2$ and C=O absorption bands in the IR spectra confirmed that the synthesized compounds. The absorption bands at 3060 - 3080 cm$^{-1}$ are assigned to the aromatic-H stretch. The absorption band at 1728 cm$^{-1}$ is due to the HC=O stretch in compound 1. The appearance of a medium to strong absorption bands at 1659-1676 cm$^{-1}$ due to a stretching vibration of the azomethine (HC=N) bond formation in the synthesized compound. The strong bands at 527 cm$^{-1}$ and 521 cm$^{-1}$ are assigned to the C-Br stretch in 3a and 3c, respectively. New bands appeared at 1106 cm$^{-1}$ (3g) corresponding to C-F stretching frequency. The strong bands at 728 cm$^{-1}$ and 714 cm$^{-1}$ are assigned to the C-Cl stretch in 3e and 3f, respectively. The characteristic resonance peaks in $^1$H NMR for the new compounds were reported using DMSO-d$_6$. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures. The proton spectral data of the starting material, 4-(methylthio)benzaldehyde (1) shows resonance at $\delta$ 10.08 ppm (s, 1H, CHO). In all the synthesized compounds 3(a-i) the above resonance disappeared and additional resonances assigned to the –CH=N ($\delta$ 7.95 – 8.80 ppm) were observed which confirmed the product. The mass spectra of 3a showed molecular ion peak at m/z 387.80 which is in agreement with the molecular formula C$_{12}$H$_9$Br$_2$N$_3$S.
elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within ± 0.4 %.

3.2. Biology

The investigation of antibacterial screening data revealed that all tested compounds showed antibacterial activity against four pathogenic bacterial strains. Among the series 3a-i, compounds 3g, 3h and 3i exhibited a significant antibacterial activity against Gram positive and Gram negative bacteria. Compounds 3c, 3e, 3g and 3d showed good zone of inhibition against tested bacterial strains in comparison to standard drug. Compounds 3a, 3f and 3b showed moderate inhibitory activity against tested bacterial strains. The Percentage of inhibition and MIC results were compared with standard drug Carbenicillin as depicted in Table 3 and Table 4 respectively. Compound 3h was found to be more potent against gram positive and gram negative bacterial strains with the 94-99 % (at 500 µg) zone of inhibition. The nature of the linkage (substituent on aromatic ring) influences the antibacterial activity. Compounds 3a-i showed antibacterial activity in the order: 3h > 3i > 3g > 3e > 3c > 3d > 3a > 3f > 3b against tested bacterial strains (Fig.1). However, the activities of the tested compounds are less than those of standard antibacterial agents used.

### Table 3. Percentage inhibition of the tested compounds

| Compound | E.Coli | P.Fluorescence | M.Luteus | B.Subtilis |
|----------|--------|----------------|----------|------------|
| 3a       | 51.84  | 42.86          | 44.55    | 46.91      |
| 3b       | 46.99  | 36.31          | 37.63    | 38.75      |
| 3c       | 65.10  | 60.12          | 57.74    | 55.24      |
| 3d       | 60.94  | 58.24          | 50.19    | 53.13      |
| 3e       | 69.96  | 65.05          | 61.87    | 60.16      |
| 3f       | 50.72  | 41.21          | 43.73    | 39.42      |
| 3g       | 90.44  | 87.90          | 94.87    | 93.82      |
| 3h       | 94.91  | 98.67          | 96.88    | 97.84      |
| 3i       | 93.40  | 93.38          | 96.18    | 96.92      |
| Standard | 96.29  | 98.49          | 97.58    | 98.60      |

**Fig. 1.** Anti-bacterial activity of the tested compounds
Table 4. MIC of the tested compounds

| Compound | E.Coli % | P.Fluorescence % | M.Luteus % | B.Subtilis % |
|----------|----------|------------------|------------|--------------|
| 3a       | 320      | 360              | 350        | 340          |
| 3b       | 340      | 410              | 400        | 390          |
| 3c       | 120      | 150              | 180        | 190          |
| 3d       | 150      | 175              | 300        | 275          |
| 3e       | 110      | 120              | 140        | 150          |
| 3f       | 335      | 370              | 361        | 425          |
| 3g       | 40       | 50               | 25         | 30           |
| 3h       | 20       | 10               | 15         | 15           |
| 3i       | 25       | 15               | 20         | 20           |
| Standard | 16       | 08               | 12         | 13           |

The compound 3b showed higher radical inhibition activity due to the presence of hydroxy group (electron donating group) in the aromatic ring.\(^{34}\) Percentage of DPPH radical scavenging activity and IC\(_{50}\) values were depicted in Table 5.

Table 5. DPPH radical scavenging activity of the tested compounds

| Compounds | % of Scavenging activity | IC\(_{50}\) (µg/ml) |
|-----------|--------------------------|-------------------|
|           | 20 | 40 | 60 | 80 | 100 |          |
| 3a        | 58 | 62 | 68 | 70 | 78  | 17.24    |
| 3b        | 12 | 15 | 45 | 48 | 50  | 100.00   |
| 3c        | 54 | 59 | 64 | 68 | 74  | 18.51    |
| 3d        | 48 | 52 | 56 | 58 | 60  | 38.46    |
| 3e        | 46 | 51 | 54 | 57 | 61  | 38.46    |
| 3f        | 56 | 59 | 63 | 67 | 75  | 17.85    |
| 3g        | 24 | 33 | 41 | 55 | 68  | 72.72    |
| 3h        | 24 | 30 | 38 | 41 | 48  | 104.16   |
| 3i        | 15 | 17 | 25 | 32 | 47  | 106.38   |
| Standard  | 59 | 61 | 64 | 69 | 74  | 16.94    |

Fig. 2. DPPH radical scavenging activity of the tested compounds

Fig. 3. Ferrous ion chelating activity of the tested compounds

The aromatic ring system with halogens like chlorine or fluorine in 3c, 3f and 3g were found to be more active than other compounds in the series (Fig.2). Thiazole group in 3d and thiadiazole group in 3e are found to be similar antioxidant activity. The nature of the functional groups is crucial for biological activity. All the investigated substances were capable of chelating Fe\(^{2+}\) ions. Fe\(^{2+}\) ions initiate free radicals through the Fenton and Haber-Weiss reaction. Fenton Weiss reaction is a reaction between ferrous ion and hydrogen peroxide which produces highly reactive hydroxyl radicals implicated in many diseases.\(^{35}\) The metal chelating
effects of the samples were dependent on concentration and linearly increased with the sample concentration increased. The affinity of 3a-i for ferrous ions was relatively low comparison to EDTA (Fig.3). However the activity of 3b was nearer to standard. Thiazole group in 3d and thiadiazole group in 3e are found to be similar antioxidant activity. The aromatic ring system with halogens like chlorine or fluorine in 3a, 3c, 3f and 3g were found to be less active than other compounds in the series. Percentage of ferrous ion chelating activity was depicted in Table 6.

**Table 6. Ferrous Ion Chelating activity of the tested compounds**

| Compounds | % of Ferrous ion chelating activity | IC50 (µg/ml) |
|-----------|------------------------------------|-------------|
| 3a        | 40       | 50 | 62 | 68 | 71 | 40.00 |
| 3b        | 66       | 65 | 80 | 85 | 95 | 15.15 |
| 3c        | 26       | 34 | 41 | 48 | 58 | 83.33 |
| 3d        | 61       | 68 | 74 | 90 | 91 | 16.12 |
| 3e        | 58       | 62 | 62 | 91 | 91 | 16.24 |
| 3f        | 34       | 42 | 49 | 52 | 66 | 61.22 |
| 3g        | 32       | 38 | 41 | 48 | 52 | 83.33 |
| 3h        | 32       | 77 | 80 | 84 | 84 | 25.97 |
| 3i        | 42       | 51 | 65 | 79 | 82 | 39.21 |
| Standard  | 72       | 79 | 81 | 83 | 88 | 13.88 |

Toxicity study is the most important aspect of the new drug development program as far as the safety evaluation is concerned. Therefore, an attempt was made to determine the cytotoxicity by Tryphan blue exclusion method, which was very simple & precise. Tryphan blue exclusion method is based on the ability of viable cells to be impermeable to Tryphan blue. The inhibition percentage with regard to cytotoxicity of compounds was found to be 50-90 %. Percentage of cell viability of peripheral lymphocytes was depicted in Table 7. From this study, it was observed that compounds 3h and 3b are shown to be more cytotoxicity activity, may be the presence of pyrimidine. Compounds 3f and 3g showed moderate to good cytotoxicity against normal peripheral lymphocytes. Compound 3i exhibited more active when compared to 3a, 3d and 3e.

**Table 7. Cytotoxicity activity of the tested compounds**

| Compound | % of viable cells |
|----------|------------------|
| 3a       | 54               |
| 3b       | 80               |
| 3c       | 59               |
| 3d       | 51               |
| 3e       | 50               |
| 3f       | 71               |
| 3g       | 69               |
| 3h       | 90               |
| 3i       | 66               |
| Control  | 95               |
| Mitomycin C | 43           |
| Toluene  | 37               |
| CuSO4    | 80               |

4. Conclusion

In conclusion, a series of Schiff bases of 4-(methylthio)benzaldehyde derivatives 3(a-i) were synthesized in good yield, characterized by different spectral studies and their biological activity has been evaluated. Compounds 3g, 3h and 3i exhibited a significant antibacterial activity against Gram positive and Gram negative bacteria. The compound 3b showed higher radical inhibition and Fe2+ chelating activity. Compounds 3h and 3b are shown to be more cytotoxicity activity. The in-vitro
cytotoxicity assay offer quick, simple and efficient way of testing the toxicity and forms an important tool for high throughput screening of synthesized compounds.

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