Research Article

Synthesis, Antioxidant, Antimicrobial, Antimycobacterial, and Cytotoxic Activities of Azetidinone and Thiazolidinone Moieties Linked to Indole Nucleus

A. R. Saundane and Prabhaker Walmik

Department of Postgraduate Studies and Research in Chemistry, Gulbarga University, Gulbarga 585 106, India

Correspondence should be addressed to A. R. Saundane; arsaundane@rediff.com

Received 29 June 2012; Revised 20 August 2012; Accepted 3 October 2012

Academic Editor: Marco Radi

Copyright © 2013 A. R. Saundane and P. Walmik. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

2-N-(2-Phenyl-1H-indol-3-yl)imino-4-arylthiazoles (3a−c) were used as key synthons for the preparation of (4-arylthiazol-2-yl)-4-(2-phenyl-1H-indol-3-yl)azetidin-2-ones (4a−c) and 3-(4-arylthiazol-2-yl)-2-(2-phenyl-1H-indol-3-yl)thiazolidin-4-ones (5a−c). These newly synthesized compounds have been characterized with the help of IR, 1H NMR, 13C NMR, and mass spectral studies. All compounds were screened for their antioxidant, antimicrobial, antimycobacterial, and cytotoxic activities. Some of the compounds displayed excellent activity.

1. Introduction

Free radicals and reactive oxygen species (ROS) including superoxide anion, hydrogen peroxide, and hydroxyl radical are being generated during normal cellular metabolism and bioorganic redox process. Furthermore, radical reactions play a significant role in the development of life-limiting chronic diseases such as cancer, hypertension, cardiac infarction, stroke, arteriosclerosis, rheumatoid arthritis, Alzheimer’s and Parkinson diseases, cataracts, and others [1–4]. Exposure of a normal cell to free radicals is known to damage structures and consequently interfere, with functions of enzymes and critical macromolecules (e.g., lipids, proteins and nucleic acids).

The human body possesses innate defense mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The unbalance between formation and detoxification of free radical species results in the progression of oxidative stress and leads to the development of chronic and degenerative diseases. Therefore, inhibition of oxidative damage by supplementation with an antioxidant and/or free radical scavengers might reduce the risk of these diseases [5, 6]. In the past decade, medicinal chemists, food chemists, and biologists have increasingly focused their attention on researching and testing for new and efficient natural or synthetic antioxidants as a protective strategy against these diseases by reducing and/or inhibiting free radical reactions.

Indole derivatives are biologically active chemicals present in microorganisms, plants, and animals representing an important class of therapeutic agent in medicinal chemistry [7–9]. Some of the indole derivatives are found to exhibit antibacterial [10–12], antifungal [13, 14], antiviral [15–17], antimalarial [18, 19], and anti-HIV [20] activities. Indole compounds are very efficient antioxidants, protecting both lipids and proteins from peroxidation and influences the antioxidant efficacy in biological systems [21, 22]. In recent years, many physiological properties of melatonin have been described resulting in much attention in the development of synthetic analogues of indole [23]. These compounds have structural similarity to melatonin.

2-Azetidinones, commonly known as β-lactams, are well-known heterocyclic compounds among organic and medicinal chemists [24]. The activities of famous antibiotics penicillins, aztreonam, and carbapenems are attributed to the presence of 2-azetidinone ring in their structure. Azetidinones are a very important class of compounds possessing a wide range of biological activities such as antimicrobial [25], antiviral [26], and others. Thiazolidin-4-one ring system is
the core structure in a variety of synthetic pharmaceuticals with broad spectrum of biological activities, for example, antifungal [27], antitubercular [28], and others.

Thiazole derivatives display a wide range of biological activities such as antimicrobial [29], anticancer [30], antitubercular [31], antihelminthic [32], and diuretic [33]. Antimicrobial activities of some substituted thiazoles are well established because they possess (S–C=N) toxophoric unit. Thiazoles have enhanced lipid solubility with hydrophilicity, easily metabolized by routine biochemical reactions and noncarcinogenic in nature [34].

Emerging infectious diseases and increasing number of multidrug-resistant microbial pathogens still make the treatment of infectious diseases an important and pressing global problem. Therefore, a substantial research for the discovery and synthesis of new classes of antimicrobial agents is needed [35, 36].

Tuberculosis (TB) remains among the world’s great public health challenges. Worldwide resurgence of TB is due to the two major problems: the acquired immunodeficiency syndrome (AIDS) epidemic, which started in the mid-1980s, and outbreak of multidrug-resistant tuberculosis (MDR-TB). Thus, there is an urgent need for anti-TB drugs with improved properties such as enhanced activity against MDR strain, reduced toxicity, shortened duration of therapy, rapid mycobacterical mechanism of action, and the ability to penetrate host cells and exert antimycobacterial effects in the intracellular environment. As a result, there is a pressing need for new antitubercular agents acting with greater potency and efficacy than the existing drugs [37].

Moreover, indole, thiazole, azetidinone, and thiazolidinone are well famed for their broad spectrum of biological activities. In the light of the above reports and in continuation of our research on the synthesis of bioactive indole derivatives [38–41], a drug strategy has been planned to synthesize indole derivatives containing thiazole, azetidinone, and thiazolidinone moieties with the hope to get improved biological activities.

2. Results and Discussion

A typical synthetic strategy employed to synthesize the indole derivatives (3–5) in excellent yields is depicted in Scheme 1. In the present paper, 4-aryltiazol-2-amin (1a–c) [42] on condensation with 2-phenyl-1H-indol-3-carboxaldehyde (2a) [43] in methanol using catalytic amount of acetic acid under reflux conditions afforded 2-N-(2-phenyl-1H-indol-3-yl)imino-4-arylthiazoles (3a–c) in excellent yields.

Compounds (3a–c) on cyclocondensation with chloroacetal chloride in presence of triethyl amine as a catalyst in dry benzene under reflux conditions yielded 3-chloro-1-(4 aryltiazole-2-yl)-4-(2-phenyl-1H-indol-3-yl)azetidin-2-ones (4a–c). Also 3a–c when subjected to cyclocondensation with mercaptoacetic acid in 1,4-dioxane under reflux temperature afforded 3-(4 aryltiazole-2-yl)-2-(2-phenyl-1H-indol-3-yl)thiazolidin-4-ones (5a–c). All the newly synthesized compounds were fully characterized on the basis of their elemental analysis and FT-IR, 1H NMR, 13C NMR, and mass spectral studies. Analytical and spectral data of the synthesized compounds are included in the experimental section.

2.1. Antioxidant Activities

2.1.1. 1,1-Diphenyl-2-pircyldrazyl (DPPH) Radical Scavenging Activity (RSA). DPPH is a stable free radical that can accept hydrogen radical or an electron and must thus be converted to a stable diamagnetic molecule. DPPH has an odd electron and so has a strong absorption band at 517 nm. When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons or hydrogen atoms taken up. Such a change in absorbance by this reaction has been extensively adopted to test the capacity of several molecules to act as free radical scavengers.

The scavenging effects of synthesized compounds (3–5) on the DPPH radical were evaluated. The results were compared with the standards 2-tert-butyl-4-methoxyphenol (butylated hydroxyanisole, BHA), 2-(1,1-dimethylethyl)-1,4-benzenediol (2-tert-butyl hydroquinone, TBHQ) and ascorbic acid (AA). The results suggested that the compounds 3a–c and 5a-c (68.14, 61.06, 64.89, 61.19, 68.14, and 72.27%) exhibited good radical scavenging activity at concentrations 100 µg/mL (Figure 1).

2.1.2. Ferric Ions (Fe3+) Reducing Antioxidant Power (FRAP). The reductive ability of synthesized compounds (3–5) was assessed by the extent of conversion of Fe3+/ferricyanide complex to the Fe2+/ferrous form. The reductive power of the synthesized compounds was observed at different concentrations and results were compared with standards BHA, TBHQ, and AA. The reducing ability of the synthesized compounds indicated that increase in the concentration of samples increases the reductive ability.

Reductive ability results suggested that compounds 3b, 4b, 4c, 5b, and 5c reduced metal ion complex to its lower oxidation state or take part in electron transfer reaction. In other words, these compounds showed the ability of electron donor to scavenge free radicals. The rest of the test compounds showed lower absorbance as compared to the standards. The higher the absorbance of the compounds, greater the reducing power (Figure 2).

2.1.3. Ferrous (Fe2+) Metal Ion Chelating Activity. Amongst the transition metals, iron is known to be the most important lipid oxidation prooxidant due to its high reactivity. The effective ferrous ion chelators may also afford protection against oxidative damage by removing ferrous ion (Fe2+) that may otherwise participate in hydroxyl radical generating Fenton type reactions [44]:

\[
{\text{Fe}^2+ + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-} \quad (1)
\]

Ferric (Fe3+) ion also produces radical from peroxides although the rate is tenfold less than that of ferrous (Fe2+) ion. Ferrous ion is the prooxidant among the various species...
of metal ions. Minimizing ferrous (Fe$^{2+}$) ion may afford protection against oxidative damage by inhibiting production of reactive oxygen species (ROS) and lipid production. The chelating effect of ferrous ion (Fe$^{2+}$) with test compounds was determined and results were compared with the standards BHA, TBHQ, and AA. Ferrozine can quantitatively form complex with ferrous ion in this method. In the presence of chelating agents, the complex formation is disrupted resulting in a decrease in red color of the complex. Measurement of color reduction, therefore, allows estimating the metal chelating activity of the coexisting chelators. Lower absorbance indicates higher metal chelating activity.

In this assay, synthesized compounds interfere with the formation of ferrous and ferrozine complex. Results suggested that the compounds 4a, 4b, and 5a exhibited good chelating activity. These compounds have ability to capture ferrous ion before ferrozine (Figure 3).

### 2.2 Antimicrobial Activity

Antibacterial results of the test compounds (Table 1) revealed that compound 4a showed the highest growth inhibitory effect against *E. coli*, 4a and 4c displayed highest growth inhibitory effect against *S. aureus*, 4a and 5a showed highest growth inhibitory effect against *K. pneumonia*, whereas 4a displayed the highest growth inhibitory effect against *P. aeruginosa*.

Further, the antifungal results indicated that compounds 4a and 5a showed the highest growth inhibitory effect against *A. oryzae*, 5a displayed the highest growth inhibitory effect against *A. niger*, 4c and 5a showed the highest growth inhibitory effect against *A. niger*. 4c and 5a showed the highest growth inhibitory effect against *A. niger*.
inhibitory effect against A. flavus, whereas 4c and 5a displayed the highest growth inhibitory effect against A. terreus.

The highest growth inhibitory effect against all bacteria by 4a and all fungi by 5a may be attributed to the presence of highly electronegative chlorine atom present in phenyl ring, azetidinone, and thiazolidinone moiety. These groups may be responsible for potent antibacterial and antifungal activities.

2.3. Antimycobacterial Activity. Compounds 4a, 4b, 5a, and 5c were assayed for inhibitory activity towards Mycobacterium tuberculosis H37Rv (ATCC-2794). The minimum inhibitory concentration (MIC expressed as μg/mL) was determined for each compound. Compound 4b exhibited the highest growth inhibitory effect against M. tuberculosis H37Rv (MIC= 12.5 μg/mL). Compound 4a exhibited the highest growth inhibitory effect against M. tuberculosis H37Rv (MIC= 25 μg/mL). Rest of the test compounds exhibited moderate to less activity.

2.4. Cytotoxic Assay. Compounds 4a, 4b, and 5a were evaluated for anticancer activity against A549 (Human Lung Adenocarcinoma) cell lines using standard drug. In vitro cytotoxicity was determined using standard MTT assay with protocol appropriate for the individual test system. MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] measures the metabolic activity of the viable cells. Compound 5a exhibited 100% cell lysis at concentration 10 μg/mL. Compound 5a exhibited good cytotoxicity due to the presence of chlorosubstitution at phenyl ring.

3. Conclusion

The present study revealed that the newly synthesized compounds having chlorosubstitution enhances the antioxidant, antimicrobial, antymycobacterial, and cytotoxic activity. The indole derivatives synthesized and tested in the present study were shown to be of reassuring importance for the development of new drugs. Therefore, our findings will provide great impact on chemist and biochemist for further investigations in the indole field.

4. Experimental Protocols

All the reagents were obtained commercially and used by further purification. Melting points were determined by an open capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography using silica-gel G-coated Al plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors. The IR (KBr pellet) spectra were recorded on a Perkin-Elmer (Spectrum One) FT-IR spectrometer. The 1H NMR and 13C NMR (DMSO-d6) spectra were recorded with a BRUKER NMR 500 and 125 MHz spectrometer respectively, the chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by electron impact method on JEOL GC mate spectrometer at 70 eV. Elemental analyses were performed on flash EA 1112 series elemental analyzer. All the compounds gave C, H, and N analysis within ± 0.4% of the theoretical values.

4-Arylthiazol-2-amines (1a-c) [42] and 2-phenyl-1H-indol-3-carboxaldehyde (2a) [43] were prepared by literature method.

General procedure for the synthesis of 2-N-(2-phenyl-1H-indol-3-yl)imino-4-arylthiazoles (3a–c).

A mixture of compounds (1a–c) (0.01 mol) and 2-phenyl-1H-indol-3-carboxaldehyde (2a) (0.01 mol) containing 4-5
drops of glacial acetic acid was refluxed in methanol (35 mL) on a water bath for 6 h. The reaction contents were cooled and poured into ice-cold water. The resulting solid was filtered, washed with sodium bisulphate solution followed by cold water, dried, and recrystallized from ethanol to get pure (3a-c).

2-N-(2-Phenyl-1H-indol-3-yl)imino-4-(4-chlorophenyl)thiazole (3a), Pale yellow crystals, Yield 67%, mp 127–28°C, Rf, 0.59 (ethylacetate: benzene, 1:1); IR (KBr): ν/cm⁻¹ 3251 (NH), 1655 (N=CH), 1601 (C=O), 749 (C=S–C); ¹H NMR (DMSO-d⁶): δ 12.48 (s, 1H, indole NH), 8.25 (s, 1H, N=CH), 7.32-7.86 (m, 13H, Ar-H), 6.10 (s, 1H, thiazole-CH); ¹³C NMR (DMSO-d⁶): δ 160.59, 143.63, 137.43, 137.16, 136.77, 135.93, 135.63, 134.39, 131.08, 130.82, 130.69, 124.72, 123.43, 120.20, 119.49, 117.99, 114.35, 114.27, 112.77, 112.58; Mass m/z: 413 (M⁺, 19%), 415 (M⁺+2, 6%); Anal. Calcd. for C₁₇H₁₁NO₅S: C, 75.96; H, 4.52; N, 11.07. Found: C, 76.02; H, 4.61; N, 11.12.

2-N-(2-Phenyl-1H-indol-3-yl)imino-4-(4-tolyl)thiazole (3b), Pale yellow crystals, Yield 64%, mp 152–53°C, Rf, 0.63 (ethylacetate: benzene, 1:1); IR (KBr): ν/cm⁻¹ 3244 (NH), 1649 (N=CH), 1608 (C=O), 746 (C=S–C); ¹H NMR (DMSO-d⁶): δ 12.43 (s, 1H, indole NH), 8.14 (s, 1H, N=CH), 7.28-7.89 (m, 13H, Ar-H), 6.07 (s, 1H, thiazole-CH), 2.39 (s, 3H, CH₃); Mass m/z: 393 (M⁺, 15%); Anal. Calcd. for C₂₅H₁₉NO₅S: C, 76.31; H, 4.87; N, 10.68. Found: C, 76.44; H, 4.90; N, 10.69.

2-N-(2-Phenyl-1H-indol-3-yl)imino-4-(4-toly]thiazole (3c), Pale yellow crystals, Yield 72%, mp 246–47°C, Rf, 0.66 (ethylacetate: benzene, 1:1); IR (KBr): ν/cm⁻¹ 3240 (NH), 1638 (N=CH), 1608 (C=O), 741 (C=S–C); ¹H NMR (DMSO-d⁶): δ 12.45 (s, 1H, indole NH), 8.27 (s, 1H, N=CH), 7.23-7.88 (m, 14H, Ar-H), 6.03 (s, 1H, thiazole-CH); Anal. Calcd. for C₂₅H₁₇NO₅S: C, 75.96; H, 4.52; N, 11.07. Found: C, 76.02; H, 4.61; N, 11.12.

General procedure for the synthesis of 3-chloro-1-(4-arylthiazol-2-yl)-4-(2-phenyl-1H-indol-3-yl)azetidin-2-ones (4a-c).

To Schiff’s base (3a-c) (0.02 mol) in dry benzene (30 mL) few drops of triethylamine and chloroacetyl chloride (0.02 mol) was added with stirring at room temperature during 15 min. The mixture was then refluxed for 1 h. Triethylamine hydrochloride formed was filtered off and washed several times with benzene. The filtrate and washing were combined and concentrated under reduced pressure and the residue obtained was washed with petroleum ether (40:60) to remove unreacted Schiff’s base. The product was dried and crystallized from 1,4-dioxane to get pure (4a-c).

### Table 1: Antimicrobial activity of synthesized compounds (3–5).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Antibacterial activity (zone of inhibition in mm)</th>
<th>Antifungal activity (zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC</td>
<td>SA</td>
</tr>
<tr>
<td>3a</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>3b</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>3c</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>4a</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>4b</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>4c</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>5a</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>5b</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>5c</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Data represented is the mean of three replicates.

EC: Escherichia coli (MTCC-723); SA: Staphylococcus aureus (ATCC-29513); KP: Klebsiella pneumonia (NCTC-13368); PA: Pseudomonas aeruginosa (MTCC-1688); AO: Aspergillus oryzae (MTCC-3567); AN: Aspergillus niger (MTCC-281); AF: Aspergillus flavus (MTCC-1973); AT: Aspergillus terreus (MTCC-1782).
Table 2: Antituberculosis activity of compounds (3–5).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MIC values (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>25</td>
</tr>
<tr>
<td>4b</td>
<td>12.5</td>
</tr>
<tr>
<td>5a</td>
<td>50</td>
</tr>
<tr>
<td>5c</td>
<td>50</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>3.125</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>6.25</td>
</tr>
</tbody>
</table>

MIC: minimum inhibitory concentrations.

Calcd. for C$_{27}$H$_{20}$N$_{5}$O$_{2}$Cl: C, 69.90; H, 4.29; N, 8.94. Found: C, 69.95; H, 4.32; N, 9.02%.

3-chloro-1-[(4-Phenylthiazol-2-yl)-4-(2-phenyl-1H-indol-3-yl)azetidin-2-one (4c), Brown crystals, Yield 63%, mp 140–41°C, Rf, 0.77 (ethylacetate: benzene, 1:1); IR (KBr): ν/cm$^{-1}$ 3131 (NH), 1712 (CO), 1614 (C=N), 771 (C–Cl), 740 (C–S–C); $^1$H NMR (DMSO-d$_6$): δ 12.43 (s, 1H, indole NH), 6.75–7.84 (m, 14H, Ar-H), 6.16 (s, 1H, thiazole-CH), 5.01 (s, 1H, NCH), 4.12 (s, 2H, SCH$_2$), 2.86 (s, 3H, CH$_3$); Anal. Calcd. for C$_{26}$H$_{26}$N$_{5}$O$_{2}$: C, 69.42; H, 4.60; N, 9.33%.

5. Biological Activities

5.1. Antioxidant Activity Assay

5.1.1. 1, 1-Diphenyl-2-picryl Hydrazyl (DPPH) Radical Scavenging Activity (RSA). The free radical scavenging activity (RSA) of compounds (3–5) at concentration (25, 50, 75, and 100 μg/mL) was carried out in the presence of freshly prepared solution of stable free radical DPPH (0.04% w/v) following Hatano's method [45], using 2-tert-butyl-4-methoxyphenol (butylated hydroxyanisole, BHA), 2-(1,1-dimethylethyl)-1,4-benzenediol (2-tert-butyl hydroquinone, TBHQ) and Ascorbic acid (AA) as standards. All the test analyses were performed on three replicates and results are averaged. The results in percentage are expressed as the ratio of absorption decrease of DPPH in the presence test compounds and absorption of DPPH in the absence of test compounds at 517 nm on ELICO SL 171 Mini Spec spectrophotometer. The percentage scavenging activity of the DPPH free radical was measured using the following equation (the results are shown in the Figure 1):

\[
\text{% DPPH Radical Scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100. \tag{2}
\]

5.1.2. Reducing Power Assay. The reducing power of the synthesized compounds (3-5) was determined according to the literature method [46]. Different concentrations of samples (25, 50, 75, and 100 μg/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH=6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. After which a portion of trichloroacetic acid (2.5 mL, 10%) was added to the mixture and centrifuged for 10 min, at 1000 Xg. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%). Then absorbance at λ 700 nm was measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The results are shown in the Figure 2.

5.1.3. Ferrous (Fe$^{2+}$) Metal Ion Chelating Activity. The chelating activity of ferrous ion by synthesized compounds (3-5) was estimated by following reported method [47]. The test samples (25, 50, 75, and 100 μg/mL) in ethanolic solution...
(0.4 mL) were added to a solution of FeCl$_2$ (0.05 mL, 2 mM). The reaction was initiated by the addition of ferrozine (0.2 mL, 5 mM) and the total volume was adjusted to 4 mL with ethanol. Ferrozine reacted with the divalent iron form stable magenta complex species that were very soluble in water. The mixture was shaken vigorously and kept at room temperature for 10 min. Then the absorbance of the solution was measured spectrophotometrically at λ 562 nm. All test analyses were run in triplicate and averaged. The percentage of inhibition of the ferrozine Fe$^{2+}$ complex formations was calculated using the following formula:

\[
\text{Ferrous ion chelating effect (\%) = } \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100.
\]

5.2. Antimicrobial Activity. The in vitro antimicrobial activity of the synthesized compounds was carried out against bacterial strains *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513), *Klebsiella pneumonia* (NCTC-13368) and *Pseudomonas aeruginosa* (MTCC-1688) and fungal species, *Aspergillus oryzae* (MTCC-3567), *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973), and *Aspergillus terreus* (MTCC-1782) by cup-plate method [48] using nutrient agar and PDA as medium, respectively. The holes of 6 mm diameter were punched carefully using a sterile cork borer and these were filled with test solution (1000 μg/mL in DMF) and DMF used as control. The plates were incubated at 37°C for 24 h and 72 h in case antibacterial and antifungal activity, respectively. The zones of inhibition around the wells were determined and the average based on triplicate measurements were recorded. The results are tabulated in the (Table 1).

5.3. Anti-TB Activity Using Alamar Blue Dye. The antimonycobacterial activities of compounds (3-5) was assessed against *M. tuberculosis* H37RV strain using micro plate Alamar blue dye assay (MABA) [49]. This methodology is nontoxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric methods. Briefly, 200 μL of sterile deionized water was added to all outer perimeter wells of sterile 96-well plate to minimize evaporation of medium in the test wells during incubation. The 96-wells plate received 100 μL of the middle brook 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 100 to 0.2 μg/mL and compared with standards pyrazinamide 3.125 μg/mL and streptomycin 6.25 μg/mL. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 μL freshly prepared 1:1 mixture of alamar blue reagent and 10% tween-80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC (minimal inhibition concentration) was defined as the lowest drug concentration which prevented the color change from blue to pink. The results are shown in the (Table 2).

The control contains FeCl$_3$ and ferrozine, complex formation molecule. The results are shown in the Figure 3.

5.4. MTT Assay

(1) MTT solution preparation: 10 mg MTT in 10 mL of Hanks balanced solution.

(2) Cell culture: the cell line were maintained in 96-well microtiter plate containing MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of gentamycin, Penicillin (100 Units/mL) and streptomycin (100 μg/mL) in the presence of 5% CO$_2$ at 37°C for 3–4 days. After 3–4 days the supernatant was removed, MEM media was replaced with Hanks balanced solution supplemented with Gentamycin, Penicillin, and Streptomycin and incubated overnight.

(3) Cytotoxicity assay: in vitro growth effect of test compound was assessed by calorimetric method [50]. Determination of conversion of MTT into “Formazan blue” by living cells. The supernatant was removed from the plate, then fresh Hanks balanced salt solution was added and treated with different concentrations of compounds diluted with DMSO. Control group contain only DMSO. After 24 h incubation at 37°C in a humidified atmosphere of 5% CO$_2$, the medium was replaced with MTT solution (100 μg/mL, 1 mg/mL in sterile Hanks balanced solution) and kept 4 h for incubation. The supernatant carefully aspirated, the precipitated crystals of “Formazan blue” were solubilized by adding DMSO (200 μg/mL) and absorbance was measured at λ 570 nm.

The results represent the mean of three readings. The concentration at which the absorbance of treated cells was reduced by 50 % with respect to the untreated control was calculated using the following formula:

\[
\text{Surviving cells (\%) } = \frac{\text{Mean absorbance of test compounds}}{\text{Mean absorbance at control}} \times 100.
\]

**Acknowledgments**

The authors are thankful to the Chairman, Department of Chemistry, Gulbarga University, Gulbarga, for providing laboratory facilities, Chairman, Department of Microbiology,
Gulbarga University, Gulbarga, for providing facilities to carry out antimicrobial activity, to the Principal, Maratha Mandal’s N. G. H. Institute of Dental Science and Research Centre, Belgaum-10, Karnataka, for carrying out antimycobacterial activity, and to the Director of Indian Institute of Technology, Chennai for providing 1H NMR, 13C NMR, and mass spectra. P. Walmik is thankful to Council of Scientific and Industrial Research New Delhi for providing the financial support as a (CSIR-SRF).

References


