Synthesis, Spectral Studies and Antimicrobial Activity of imidazo[2,1-b][1,3,4]thiadiazole derivatives

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ABSTRACT

Thiadiazole is a heterocyclic compound containing both two nitrogen atom and one sulfur atom as a part of the aromatic five-membered ring. The imidazo [2,1-b]-1,3,4-thiadiazole ring system is the core skeleton of well known immunomodulator levamisole. The synthesis and antimicrobial activity of nine 6-Phenyl-2-substituted imidazo [2,1-b]-1,3,4-thiadiazole derivatives were reported against Gram +ve bacteria Bacillus subtilis (MTCC 121), Staphylococcus aureus (MTCC 87), Gram –ve bacteria Pseudomonas aeruginosa (MTCC 424), Escherichia coli (MTCC 40), and fungal strains Candida albicans (MTCC 183), Fusarium solani (MTCC 2935), Fusarium oxyporium (MTCC 2840). Ciprofloxacin and Fluconazole were used as standard drug for antibacterial and antifungal activity respectively. The synthesized compound (6) and (5b) had moderate antibacterial activity especially with Gram negative Escherichia coli (MTCC 40) where (5a) and (5f) had good antibacterial activity. The structures of the synthesized compounds were established by IR, NMR and Mass spectral studies.

Keywords: Thiadiazole, Antimicrobial, Imidazo [2,1-b]-1,3,4-thiadiazole, Thiosemicarbazide

INTRODUCTION

An anti-microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans [1]. The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Now, most of these infections can be cured easily with a short course of antimicrobials. However, with the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents. The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbe completely, allowing the microbe to survive, change, and become resistant to the poisons and or heavy metals.

Microorganisms 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 ecosystem [2]. Antimicrobials differ in their physical, chemical and pharmacological properties. They also differ in their antibacterial spectrum of
activity and mechanism of action. The major mechanism of action include inhibition of cell wall synthesis, e.g., penicillin, inhibition of ribosome function, e.g., erythromycin, inhibition of nucleic acid synthesis, e.g., hydroxyurea, inhibition of folate metabolism, e.g., methotrexate and inhibition of membrane function, e.g., polymixin [3]. Compounds containing imidazo [2,1-b][1,3,4] thiadiazole heterocyclic system are known to possess interesting pharmacological properties such as Anticancer [4], Antitubercular [5], Antibacterial [6], Antifungal [7], Anticonvulsant, Analgesic [8], Anti-secretory [9], Anti-inflammatory [10], Cardiotonic [11].

The imidazo [2,1-b]-1,3,4-thiadiazole ring system is the core skeleton of well known immunomodulator levamisole [12]. The use of antimicrobial agents is critical to successful treatment of infectious diseases. Although there are numerous classes of drugs that are routinely used to treat infections in humans, there are several reasons why the discovery and development of new antimicrobial agents are important. Over the past decade there has been an increased development of resistance in organisms that are typical pathogens in humans. These include methicillin/oxacillin-resistant Staphylococcus aureus, vancomycin resistant and intermediate Staphylococcus aureus, vancomycin-resistant Enterococcus, gram-negative bacilli that produce extended spectrum beta-lactamases, carbapenem-resistant Klebsiella pneumoniae, and Pseudomonas and Acinetobacter strains that are resistant to all antibiotics that are typically used for treatment. This increased resistance has limited the selection of antimicrobials that may be used to treat specific organisms. New antimicrobials are also needed for certain groups of organisms. Very limited numbers of antimicrobials are available to treat infections caused by fungi and mycobacteria. Infections with these organisms continue to be a major concern.

Although many infectious diseases have been known for thousands of years, over the past 30 years a number of new infectious diseases have been discovered. Some examples include Lyme disease caused by Borrelia burgdorferi, Legionnaires’ disease caused by Legionella pneumophila, peptic ulcers caused by Helicobacter pylori, antibiotic associated diarrhea caused by Clostridium difficile and AIDS caused by Human Immunodeficiency Virus [12].

Chemotherapy for cancer treatment, immunosuppressive drugs for treatment of autoimmune diseases and organ transplant recipients and infections (such as AIDS) that alter the effectiveness of the host immune system render individuals at high risk for fungal infections and certain mycobacterial infections [13]. Often these infections are caused by environmental organisms that would not typically cause disease in a normal host[14].

EXPERIMENTAL

The commercial chemicals employed for the present work were purchased from Sigma-Aldrich, Merck India and Loba Chem. All the solvents were used after distillation. Thin layer chromatography was performed on E Merck silica gel 60F254 precoated plates and the identification was done with UV light and iodine chambers. Various solvent systems used for developing chromatograms were chloroform: methanol (9:1), chloroform: methanol (9.5:0.5), benzene: acetone (9:1). Melting points were determined in open capillaries on a Buchi-apparatus and are uncorrected. The identification and characterization of the compounds were carried out by determining melting point, IR, 1H NMR, 13C NMR and Mass spectroscopy. All the infra red (IR) spectra (KBr) were recorded on the FT-IR Perkin-Elmer spectrometer (4000-400 cm⁻¹). 1H NMR and 13C NMR spectra were recorded on Bruker Avance II 400 spectrometer where TMS was used as internal standard and chemical shifts are expressed as δ ppm. The Mass spectra were run on micromas Q-T of micro spectrometer at SAIF Panjab University, Chandigarh.

Synthesis of 5-p-substituted-1,3,4-thiadiazol-2-amine 3(a-b)

Substituted benzoic acid (1a-b, 6.8 g, 0.05 mol) and thiosemicarbazide (4.5 g, 0.05 mol) in phosphorous oxychloride (30 ml) were refluxed gently for 1-2 h. The solution was allowed to cooled and then water (90 ml) was added carefully (Scheme.1). The separated solid was filtered and suspended in water and basified with aqueous potassium hydroxide. The solid was filtered, washed with water, recrystallized with ethanol.

5-Methyl-1,3,4-thiadiazol-2-amine (3a): This compound was obtained as crystalline solid/ light pink. Yield: 71%; Melting point: 220-223°C; Rf 0.56 (Benzene: Acetone 7:3).
5-Fluoro-1,3,4-thiadiazol-2-amine (3b): This compound was obtained as crystalline solid/light yellow. Yield: 65%; Melting point: 202-205°C; \( R_f \) 0.42 (Benzene: Acetone 7:3).

Synthesis of 6-phenyl-2-p-substituted imidazo[2,1-b][1,3,4]thiadiazole 5(a-f)
A mixture of equimolar quantities of 5-p-substituted thiadiazole amine (3a-b, 1.91 g, 0.01 mol) and substituted phenacyl bromide (1.99 g, 0.01 mol) was refluxed in dry ethanol for 18-24 h (Scheme 1). The excess solvent was evaporated through vacumm filtration. The obtained solid product was suspended in water and the solution was neutralized by sodium carbonate solution to get water free base. The crude product was filtered, washed with water, dried and recrystallized with ethanol.

6-Phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole 5(a): State: crystalline solid/ light brown; Yield: 41.43%; Melting Point: 170-173°C; \( R_f \) 0.62 (Benzene: Acetone 9:1); \(^1\)H NMR (400MHz, CDCl\(_3\), ppm): 8.01 (s, 1H, CH\(_2\)), 7.5- 7.8 (m, 9H, Ar-H), 2.15 (s, 3H, CH\(_3\))

6-(4-Methoxyphenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole 5(b): This compound was obtained as crystalline solid/ light brown. Yield: 45.02%; Melting point: 179-182°C; \( R_f \) 0.42 (Chloroform: Methanol 9:1); IR (KBr, \( \nu \), cm\(^{-1}\)): 3134 (Ar-C-H), 2936 (C-H); \(^1\)H NMR (400MHz, CDCl\(_3\), ppm): 3.8 (s, 3H, OCH\(_3\)), 7.9 (s, 1H, CH\(_3\)), 6.9-7.7 (m, 8-H, Ar-H)
6-(4-Nitrophenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (5c): This compound was obtained as crystalline solid/ light orange. Yield: 48.43%; Melting point: 224-247°C; $R_f$ 0.48 (Benzene: Acetone 9:1); IR (KBr, $\nu$, cm$^{-1}$): 1518, 1340 (N=O), 3153 (Ar-C-H stretch), 2924 (C-H); $^1$H NMR (400MHz, CDCl$_3$, ppm): 8.9 (s, 1H, CH), 2.5 (s, 3H, CH$_3$), 7.4-8.2 (m, 9H, Ar-H)

2-(4-Fluorophenyl)-6-(4-Methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole (5d): This compound was obtained as brown solid/ pale brown. Yield: 42.05%; Melting point: 217-220°C; $R_f$ 0.58 (Benzene: Acetone 7:1); IR (KBr, $\nu$, cm$^{-1}$): 1250 (C-F), 3089 (Ar-C-H stretch); $^1$H NMR (400MHz, DMSO-d$_6$, ppm): 3.8 (s, 3H, OCH$_3$), 8.3 (s, 1H, CH), 6.9-8.0 (m, 8H, Ar-H)

2-(4-Fluorophenyl)-6-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (5e): This compound was obtained as crystalline solid/ light yellow. Yield: 38%; Melting point: 231-235°C; $R_f$ 0.36 (Chloroform: Methanol 9:1); IR (KBr, $\nu$, cm$^{-1}$): 1226 (C-F), 3100 (Ar-C-H stretch), 2921(C-H); $^1$H NMR (400MHz, DMSO-d$_6$, ppm): 8.1(s, 1H, CH), 7.2-7.9 (m, 8H, Ar-H), 2.3 (s, 1H, CH$_3$)

2,6-Bis(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazole (5f): This compound was obtained as crystalline solid/ light brown. Yield: 42%; Melting point: 239-242°C; $R_f$ 0.55 (Benzene: Acetone 9:1); IR (KBr, $\nu$, cm$^{-1}$): 1240 (C-F), 3098 (Ar-C-H stretch); $^1$H NMR (400MHz, DMSO-d$_6$, ppm): 8.3 (s, 1H, CH), 7.1-7.9 (m, 9H, Ar-H)

6-Phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (6): Vilsmeir- Haack reagent was prepared by adding phosphoryl-chloride (3 ml) in dimethyl formamide (20 ml) at 0°C with stirring. Then 6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazoles (1.91 g 0.01 mol) was added to the reagent and stirred for 2 h at room temperature and at 60°C for additional 2 h (Scheme.1). The mixture was then poured in sodium carbonate solution and stirred at 90°C for 2 h. After cooling, the mixture was diluted with water and extracted with chloroform. The residue obtained was recrystallized from ethanol solvent to get crystalline solid.

6-Phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (6): This compound was obtained as crystalline solid/ light brown. Yield: 35%; Melting point: 174-178°C; $R_f$ 0.45 (Benzene: Acetone 9:1); IR (KBr, $\nu$, cm$^{-1}$): 1602 (C=O), 3116 (Ar-C-H), 2918 (C-H); $^1$H NMR (400MHz, DMSO-d$_6$, ppm): 10.62 (s, 1H, CHO), 6.7-8.04 (m, 9-H, Ar-H), 2.3 (s, 3H, CH$_3$)

5-Nitroso-6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (7): To a well stirred solution of (1.9 g, 0.01mol) 6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazoles (1.91 g 0.01 mol) was added to the reagent and stirred for 2 h at room temperature and at 60°C for additional 2 h (Scheme.1). The mixture was then poured in sodium carbonate solution and stirred at 90°C for 2 h. After cooling, the mixture was diluted with water and extracted with chloroform. The residue obtained was recrystallized from ethanol solvent to get crystalline solid.

5-Nitroso-6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (7): This compound was obtained as crystalline solid/ light brown. Yield: 40%; Melting point: 142-145°C; $R_f$ 0.42 (Benzene: Acetone 9:1); IR (KBr, $\nu$, cm$^{-1}$): 1511, 1344 (N=O), 3116 (Ar-C-H), 2918 (C-H); $^1$H NMR (400MHz, CDCl$_3$, ppm): 7.1-7.9 (m, 9H, Ar-H), 2.3 (s, 1H, CH$_3$)

5-Bromo-6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (8): To a well stirred solution of 6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazoles (0.01 mol) in glacial acetic acid (5 ml) and anhydrous sodium acetate (0.02 mol) was added bromine (0.1 ml) dropwise with stirring at room temperature (Scheme.1). After the addition, stirring was continued for 2 h. The reaction mixture was poured on to ice cold water and basified with ammonium solution. The separated solid was collected, washed with water and recrystallized with ethanol.
5-Bromo-6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (8): This compound was obtained as crystalline solid/ light brown. Yield: 45%; Melting point: 162-165°C; \( R_f \) 0.54 (Benzen: Acetone 9:1); IR (KBr, v cm\(^{-1}\)): 686 (C-Br), 3116 (Ar-C-H), 2917 (C-H); \(^1\)HNMR (400MHz, CDCl\(_3\), ppm): 7.2-8.0 (m, 9H, Ar-H), 2.15 (s, 3H, CH\(_3\))

**Antimicrobial screening by Agar well diffusion method**

Antimicrobial activity of new synthesized compounds 5(a-f), and 6-8 was carried out by the Cup-Plate Agar Diffusion method against Gram +ve bacteria strains like *Staphylococcus aureus* (MTCC 87), *Bacillus subtilis* (MTCC 121), and Gram –ve bacterial strains like *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 40) and fungal strains like *Candida albicans* (MTCC 183), *Fusarium solani* (MTCC 2935), *Fusarium oxyporum* (MTCC 2840). All microorganisms were obtained from Institution of Microbial Technology (IMTECH) Chandigarh. The standards Ciprofloxacin and Fluconazole were dissolved in DMSO to get a concentration 100μg/ml for testing antibacterial activity. Each test compound was dissolved in DMSO to get a concentration of 100μg/ml for testing antibacterial activity. The zone of inhibition were observed and measured in mm.

**RESULTS AND DISCUSSION**

As per the proposed protocol the synthesis of varied substituted imidazo[2,1-b][1,3,4]thiadiazoles is carried out. The 5-substituted 1,3,4-thiadiazoles were synthesized in good yields by reacting thiosemicarbazide with substituted benzoic acid in presence of phosphorous oxy-chloride. The reaction proceeds via the intermediate iminothiadiazole which under reflux temperature spontaneously undergoes dehydrocyclisation to form the desired fused heterocycle. The electronic and steric factors at 5\( \theta \) position of 2-amino-5-substituted-1,3,4-thiadiazole are crucial in determining the course of its reaction with substituted α-haloarylketones. The strongly electronegative groups imparts less nucleophilic character to the nitrogen at 4\( \theta \) position of the 1,3,4-thiadiazole. Thus the different imidazo [2,1-b][1,3,4]thiadiazole derivatives were undergo electrophillic substitution reaction. All reactions were monitored through TLC observation till the completion using suitable mobile phase each time. After completion the reaction, the products were purified by using suitable solvents e.g. ethanol. The structures of all synthesized derivatives were confirmed by various analytical and spectral data. The brief outcomes of the work are given in Table 1.

**Table 1: Physical characteristic of synthesized compounds**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Compound</th>
<th>Molecular Formula</th>
<th>Molecular Weight (g)</th>
<th>Melting point (°C)</th>
<th>Yield (%)</th>
<th>( R_f )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5a</td>
<td>C(_7)H(_6)N(_2)S</td>
<td>291.37</td>
<td>170-173</td>
<td>41</td>
<td>0.62</td>
</tr>
<tr>
<td>2</td>
<td>5b</td>
<td>C(_7)H(_6)N(_2)O(_2)S</td>
<td>321.40</td>
<td>179-183</td>
<td>45</td>
<td>0.42</td>
</tr>
<tr>
<td>3</td>
<td>5c</td>
<td>C(_7)H(_6)N(_2)O(_2)</td>
<td>336.34</td>
<td>244-247</td>
<td>48</td>
<td>0.48</td>
</tr>
<tr>
<td>4</td>
<td>5d</td>
<td>C(_7)H(_6)FN(_2)OS</td>
<td>325.26</td>
<td>217-220</td>
<td>42</td>
<td>0.59</td>
</tr>
<tr>
<td>5</td>
<td>5e</td>
<td>C(_7)H(_6)N(_2)S</td>
<td>309.36</td>
<td>231-235</td>
<td>38</td>
<td>0.63</td>
</tr>
<tr>
<td>6</td>
<td>5f</td>
<td>C(_7)H(_6)F(_2)N(_2)S</td>
<td>313.20</td>
<td>241-243</td>
<td>42</td>
<td>0.55</td>
</tr>
<tr>
<td>7</td>
<td>5g</td>
<td>C(_7)H(_6)N(_2)OS</td>
<td>319.38</td>
<td>174-178</td>
<td>35</td>
<td>0.53</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>C(_7)H(_6)N(_2)O</td>
<td>320.07</td>
<td>142-145</td>
<td>40</td>
<td>0.42</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>C(_7)H(_6)BrN(_2)S</td>
<td>370.27</td>
<td>162-165</td>
<td>45</td>
<td>0.54</td>
</tr>
</tbody>
</table>

The \( R_f \) value, melting point (m.p.), IR, \(^1\)HNMR, \(^13\)CNMR, and MS was studied for all the derivatives. The \( R_f \) value was observed between 0.4 - 0.63 (Table 1) using different solvent system and detecting agent. The \( R_f \) value for different imidazo-thiadiazole was found to be higher than the synthesized compounds. The melting point of all the compounds are in range between 145-247 °C (Table 1) and uncorrected. The melting point of all the aminothiadiazole was found to be in agreement with the literature. The yield of all synthesized derivatives was found in between 35-48%. The IR spectrum of final derivatives showed absorption at 1820-1660 cm\(^{-1}\) confirming the carbonyl group. The aromatic stretch was shown at 3116.80 cm\(^{-1}\) and the other C-H stretching was indicated at 2918.83 cm\(^{-1}\).

The presence of nitro group was confirmed by the absorption at 1511.25, 1344.29 cm\(^{-1}\) region. \(^1\)HNMR spectrum of the final derivatives showed singlet of one proton at δ 8.01 confirming the presence of CH at 5- position and singlet at δ 2.15 for three methyl protons. The mass spectrum showed molecular ion peak at m/z 319.2 which is in agreement with the molecular formula C\(_7\)H\(_7\)N\(_2\)O\(_2\)S.
Antimicrobial activity

All the synthesized derivatives exhibited very good antimicrobial activity (Table 2) when compared to standard especially with Gram negative Escherichia coli (MTCC 40). The synthesized compound 6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (6) and 6-(4-methoxyphenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazoles (5b) had moderate antibacterial activity especially with Gram negative Escherichia coli (MTCC 40) where 6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (5a) and 2,6-bis(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazoles (5f) had good antibacterial activity. All the compounds were found to be inactive against Pseudomonas aeruginosa. All these compounds were also tested against fungal strains. In this study the compounds 6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (5a) and 5-bromo-6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole (5e) were active against F. oxysporium (Table 2).

Table 2 In vitro antimicrobial activity of synthesized compounds

<table>
<thead>
<tr>
<th>C. No.</th>
<th>Zone of inhibition (mm)</th>
<th>Gram+ve bacteria</th>
<th>Gram-ve bacteria</th>
<th>Fungal strains</th>
<th>F. solani</th>
<th>F. oxysporium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B. subtilis</td>
<td>S. aureus</td>
<td>E. coli</td>
<td>C. albicans</td>
<td>F. solani</td>
</tr>
<tr>
<td>5a</td>
<td>15±2*</td>
<td>13±3*</td>
<td>6±1*</td>
<td>19±2*</td>
<td>17±2*</td>
<td>19±2*</td>
</tr>
<tr>
<td>5b</td>
<td>11±1*</td>
<td>11±1*</td>
<td>8±2*</td>
<td>10±3*</td>
<td>21±2*</td>
<td>25±3*</td>
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<tr>
<td>5c</td>
<td>NA</td>
<td>9±6*</td>
<td>9±3*</td>
<td>11±3*</td>
<td>20±3*</td>
<td>15±2*</td>
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<tr>
<td>5d</td>
<td>NA</td>
<td>5±3*</td>
<td>8±2*</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5e</td>
<td>NA</td>
<td>NA</td>
<td>2±4*</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>5f</td>
<td>17±2*</td>
<td>12±2*</td>
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<td>21±1*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>13±1*</td>
<td>NA</td>
<td>7±2*</td>
<td>17±2*</td>
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<td>9±3*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td>8±1*</td>
<td>6±1*</td>
<td>11±1*</td>
<td>18±3*</td>
</tr>
<tr>
<td>St.</td>
<td>23±1*</td>
<td>18±2*</td>
<td>11±1*</td>
<td>28±2*</td>
<td>24±3*</td>
<td>33±2*</td>
</tr>
</tbody>
</table>

St., standard (Ciprofloxacin and Fluconazole); *± SD (n=3) mean of zone of inhibition in mm; NA, No activity.

CONCLUSION

The imidazo[2,1-b][1,3,4] thiadiazole was successfully substituted at 2, 5 and 6 positions to form various imidazo-thiadiazole 6-phenyl-2-p-substituted imidazo[2,1-b][1,3,4] thiadiazoles, 6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde,5-Nitroso-6-phenyl-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole. All the synthesized compounds were characterized using the spectral techniques and subjected to antimicrobial activity against various bacterial and fungal strains. From the data observed for antimicrobial activity it may be concluded that the synthesized derivatives have potential to act as antimicrobial agents and activity of the various compounds can be increased by substituting these with electron withdrawing groups. The synthesized derivatives can be further explored by studying the structure activity relationship.

Acknowledgement

The authors are thankful to SAIF, Panjab University, Chandigarh, for providing spectral data of synthesized compounds. We are also grateful to IMTECH, Chandigarh for providing help in carrying out the antimicrobial screening and Management, ASBASJS JM College of Pharmacy for providing the necessary facilities.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES


