ABSTRACT

Objective: The major objective of the present study was to design, synthesize some Mannich base derivative of 2-substituted-5-amino thiadiazoles with primary amines/phthalimide and formaldehyde anticipating effective as antimicrobial agents.

Methods: The efficient syntheses of substituted thiadiazole were done simply by reaction of substituted benzoic acid with thiosemicarbazide in presence of only conc. sulphuric acid. The isolated products were further subjected for Mannich reaction and evaluated for antibacterial activity.

Results: Three substituted thiadiazoles (BA-1 to BA-3) were synthesized by solvent less effic ient synthesis and their Mannich base derivates were also prepared with phthalimide, p-nitro/methyl/chloro-aniline. All characterized products were screened for antibacterial and antifungal activities. Among these compounds having nitro group (3,7,13) showed maximum activity followed by phthalimide, chloro and methyl groups.

Conclusion: Thiadiazole-Mannich bases derivatives exhibited better antimicrobial activities then their starting components (BA-1 to BA-3) indicated joining different pharmacophore in the same molecule have increased activity.

Keywords: Thiadiazole Mannich bases derivatives, Characterization, Antimicrobial activities.

INTRODUCTION

Heterocycles are widely used in the development of several pharmaceutically important compounds. The nitrogen and sulphur heterocyclic systems are very interesting because of their physicochemical properties with relevance to the design of new drugs. The compounds containing thiadiazole ring play a prominent role in natural processes and also found in numerous biologically active natural and synthetic compounds. According to the literature survey, thiadiazoles were reported to possess various pharmacological activities [1-7]. Anti-microbial activities of substituted thiadiazoles are well established because it posses (S–C=N) toxophoric unit. They have enhanced lipid solubility with hydrophilicity and can be easily metabolized by routine biochemical reactions.

Mannich reaction is one of the versatile and convenient approaches to powerful C-C bond formation. It has wide application in organic synthesis and many drug molecules. However, the classical Mannich reaction has some limitations and to overcome these limitations, modern variants of this reaction came into the form of various publications [8-9]. Several medicinally useful Mannich base has been reviewed by various scientist [10-12]. Fanciated by various bioactivity of above mentioned compounds along with structure-based pharmacophore approaches in drug discovery and design, we have planned to design and synthesize thiadiazole-Mannich base analogues by using substituted aminothiadiazole, phthalimide, p-nitro/methyl/chloro-aniline in presence of formaldehyde with a hope to develop new, potent, less toxic antimicrobial agents. In continuation of our research we studied the antimicrobial action of the resultant molecules against gram negative bacteria Escherichia coli, Pseudomonas aeruginosa and gram positive bacteria Staphylococcus aureus, Bacillus subtilis and along with a fungus Candida albicans.

MATERIALS AND METHODS

Chemicals and instrumentation

Reagents such as thiosemicarbazide, phenyl propiolic acid/o-hydroxy benzoic/cinnamic acid, phthalimide, p-nitro aniline, p-methyl aniline, p-chloro aniline and formaldehyde were purchased from Across Ltd and used as it is. All the solvents were of analytical grade and were distilled before use. Melting points were uncorrected. The solid IR spectra were recorded in KBr on Perkin Elmer FTIR spectrophotometer at GITAM University, Visakhapatnam; however 1H and 13C NMR spectra were taken on Bruker 400 MHz NMR at Biocon Limited electronic city Bangalore, in DMSO-d6 using TMS as internal reference. Elemental analysis and mass spectra were recorded at Micro Analytical centre at Andhra University, Visakhapatnam.

Synthesis of 2-amino-5-sustituted thiadiazole (BA-1 to BA-3)

Substituted amino thiadiazoles were prepared by the conventional method as outlined in Scheme 1. A mixture of carboxylic acid (phenyl propiolic/o-OH-benzoic/cinnamic acid, 0.01 moles, in separate reactions) and thiosemicarbazide (0.012 mole) was mixed thoroughly. The reaction mixture was heated under reflux (Scheme 1) in the presence of only conc. H2SO4 without any solvent for 6-8 h [13]. The reaction mixture was cooled to room temperature and neutralized with ammonia solution. The solid were filtered and washed with water followed by few drops of ether. The products were re-crystallized from ethanol and characterized by various spectral methods.

Scheme 1

where B1 = phenyl propiolic acid
B2=R1= O-H-benzoic acid
B3=R1= cinnamic acid

Scheme 1

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Synthesis of BA1a/1b/1c to BA-3a/3b/3c

A methanolic solution of BA-1/BA-2/BA-3 (0.01 mol) was taken in separate reaction in a round bottom flask equipped with a stirrer and dropping funnel. The solution was stirred to dissolve it completely. To this methanolic solution of formaldehyde (3.0 ml) was added drop wise during 15-20 min. The resulting mixture was stirred during half an hour to complete the reaction. To this reaction mixture the methanolic solution of phthalimide/p-N2/p-CH3/p-Cl aniline (0.01 mol) was added drop wise with stirring in about half an hour at 30 °C temperature and refluxed for 2 h at 65-75 °C (Scheme 2). It was allowed to cool and poured in cold water. The solid obtained was filtered off washed thoroughly with hot water and air dry. All products were characterized by IR, NMR and were found consistent with an expected structure as shown in table 1.

Scheme 2

2-[5-amino-1,3,4-thiadiazol-2-yl] phenyl acetylene (BA-1)

(C10H17N3S): IR(γ max cm−1): 3465 (NH2), 3368 (Ar-H), 2106 (C=C), 1627, 1519 (C=N); 1HNMR (ppm): 7.5-7.9 (Ar-H), 3.5(NH), 13CNMR (ppm): 84.5, 75.5 (Ar-C), 126.1, 128.3, 129.3, 130.2 (Ar-C), 157, 152 (thiazole carbons); m/e: 203; Analysis: Calcd C, 59.12; H, 4.06; N, 19.72; Found: C, 59.12; H, 4.07; N, 19.74.

2-[{5-[phenyl acetylene]-1,3,4-thiadiazol-2-yl} amino] methyl-1-H-isindole-1,3(2H)-dione (BA-1a)

(C17H13N5O2S): IR(γ max cm−1): 3463(OH), 3333 (NH2), 3006 (Ar-H), 1674 (HC=CH), 3.7 (NH); 1HNMR (δppm): 7.12 (O-H); 6.8-7.7 (Ar-H), 4.9 (C=H), 13CNMR (δppm): 9.76 (O-H); 6.6-7.3 (Ar-H), 3.94 (NH); 13CNMR (ppm): 115, 117, 127, 131 (Ar-C), 154, 150, 151 (thiazole carbons); m/e: 343; Analysis: Calcd C, 52.47; H, 3.79; N, 20.40; Found: C, 52.52; H, 3.75; N, 20.36.

2-[{[5-(4-nitro phenyl) amino]methyl} amino]-1,3,4-thiazol-2-yl Phenol (BA-2a)

(C15H13N5O3S): IR(γ max cm−1): 3463(OH), 3333 (NH2), 3006 (Ar-H), 1624, 1515.6 (C=N); 1HNMR (ppm): 9.74 (O-H); 6.6-7.3 (Ar-H), 3.94 (NH); 13CNMR (ppm): 115, 118, 128, 132.7 (Ar-C), 156, 151.6 (thiazole carbons); m/e: 343; Analysis: Calcd C, 52.47; H, 3.79; N, 20.40; Found: C, 52.52; H, 3.75; N, 20.36.

2-[{[5-(4-chloro phenyl) amino] methyl] amino]-1,3,4-thiazol-2-yl Phenol (BA-2c)

(C15H13N4OSCl): IR(γ max cm−1): 3463(OH), 3333 (NH2), 3006 (Ar-H), 1624, 1515.6 (C=N); 1HNMR (ppm): 9.72 (O-H); 6.8-7.6 (Ar-H), 3.92 (NH); 13CNMR (ppm): 114, 117, 127, 131 (Ar-C), 154, 150, 151 (thiazole carbons); m/e: 343; Analysis: Calcd C, 52.47; H, 3.79; N, 20.40; Found: C, 52.52; H, 3.75; N, 20.36.

2-[{[5-(4-chloro phenyl) amino] amino} methyl] amino]-1,3,4-thiazol-2-yl Phenol (BA-2d)

(C15H13N5O3S): IR(γ max cm−1): 3463(OH), 3333 (NH2), 3006 (Ar-H), 1624, 1515.6 (C=N); 1HNMR (ppm): 9.72 (O-H); 6.8-7.6 (Ar-H), 3.92 (NH); 13CNMR (ppm): 114, 117, 127, 131 (Ar-C), 154, 150, 151 (thiazole carbons); m/e: 343; Analysis: Calcd C, 52.47; H, 3.79; N, 20.40; Found: C, 52.52; H, 3.75; N, 20.36.

2-[{[5-(4-nitro phenyl) amino]methyl] amino]-1,3,4-thiazol-2-yl Phenol (BA-3a)

(C15H13N5O3S): IR(γ max cm−1): 3463(OH), 3333 (NH2), 3006 (Ar-H), 1624, 1515.6 (C=N); 1HNMR (ppm): 9.72 (O-H); 6.8-7.6 (Ar-H), 3.92 (NH); 13CNMR (ppm): 114, 117, 127, 131 (Ar-C), 154, 150, 151 (thiazole carbons); m/e: 343; Analysis: Calcd C, 52.47; H, 3.79; N, 20.40; Found: C, 52.52; H, 3.75; N, 20.36.
2-[[4-(Chloro phenyl) amino] methyl] amino]-1,3,4-thiadiazol-2-yl] phenyl ethylene (BA-3d)

$C_{17}H_{15}N_4SCl$:

IR ($\gamma$ max cm$^{-1}$): 3350 (NH), 3090 (Ar -H), 1669 (HC=CH), 1515, 1455 (C=N); $^1$HNMR (δ ppm): 7.0-7.9 (Ar-H), 4.8-5.8 (=C-H), 3.9.5 (NH); $^{13}$CNMR (δ ppm): 114.7, 122.6, 125, 127 (Ar-C), 150 (C=C), 147, 157 (thiazole carbons); m/e: 342.5; Analysis: Calcd: C, 59.56; H, 4.37; N, 16.35; Found: C, 59.59; H, 4.34; N, 16.38.

Antimicrobial assay

The synthesized compounds (1–15) were assessed for antimicrobial assay against gram positive bacteria BS- Bacillus subtilis (MTCC 441), SA-Staphylococcus aureus (MTCC 96), and gram negative bacteria EC-Escherichia coli (MTCC 1687), PA-Pseudomonas aeruginosa (MTCC 424) along with a fungal strain CA-Candida albicans using the well diffusion method[14]. The compounds were dissolved in DMSO and activity was determined using serial dilution method. The whole procedure was carried out as reported by us earlier [15]. Ciprofloxacin and ketoconazole were used as antibacterial and antifungal reference drugs, respectively. The minimum inhibitory concentration (MIC, 10 μg ml$^{-1}$) was determined for each compound in triplicate experiments; the values were averaged and are presented in Table 2.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Cmpds.</th>
<th>Structure</th>
<th>M. pt °C/Yield (%)</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
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<td>BA-1</td>
<td></td>
<td>174/69</td>
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<tr>
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<tr>
<td>7</td>
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<td>138/71</td>
<td>Brown</td>
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<tr>
<td>8</td>
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<td></td>
<td>152/70</td>
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<td>15</td>
<td>BA-3d</td>
<td></td>
<td>128/71</td>
<td>Pale yellow</td>
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</tbody>
</table>
RESULTS AND DISCUSSION

Synthesis of 1, 3, 4-thiadiazole analogs

Synthesis of novel 1, 3, 4-thiadiazole analogs were achieved by following the procedure as depicted in Scheme 1 and 2. The Stoichiometric ratio of thiosemicarbazide and (phenyl propiolic/p-0H-benzoic/cinnamic acid) was grinded thoroughly in the mortar and pestle. The reaction mixture was further refluxed [14] in the presence of only conc. H2SO4, which was used as cyclising agent. The isolated and characterized substituted thiadiazole, while C-S-C absorption stretches of compounds (1-5, 10-15) were further condensed with phthalimide/phthalimide/base derivatives. TLC was run throughout the reaction to optimize the reaction for purity and completion.

Designed series of molecules (1-15; BA-1 to BA-3, BA-1a/b/c/d to BA-3a/b/c/d) was characterized by spectral techniques before evaluating for antimicrobial studies. Two strong absorption bands appeared in the range between 3340 and 3200 cm\(^{-1}\) in the IR spectra of compounds (1,6,11) was attributed to NH\(_2\) group[16] of thia diazole ring, while C-S-C absorption stretches (thiadiazole ring)[17] from 1604 to 1623 cm\(^{-1}\). The other major peaks observed in the IR of these compounds are 3130-3050; 1550-1520 cm\(^{-1}\) could be assigned to aromatic hydrogen and C=C of the ring. The additional peak observed in the IR of 16; BA-3 at 1623 cm\(^{-1}\) was attributed to CH=CH of cinnamic acid. The disappearance of NH\(_2\) peaks of substituted thia diazoles (compound no.1,6,11) in the IR of target compounds (2-5, 7-10 and 12-15) are clearly indicated the condensation of it with formaldehyde and various substituted aniline/phthalimide which was further confirmed by their NMR spectra. The additional peak observed at the range of 1770-1735 cm\(^{-1}\) in compound no.2,7,12 was attributed to C=O of phthalimide ring[18]. Other peaks observed in the IR spectrum of all the compounds were fitted well as expected structure shown in table 1.

The major proton NMR spectral peaks appeared at δ 3.2-3.9 and 6.7-7.8 ppm in the compounds (1, 6, 11) was assigned to NH\(_2\) and aromatic. In the \(^1^C\) NMR spectra, the signals in the range δ 110-135 ppm are due to aromatic carbons and signals in the region δ 150-170 ppm are due to thiadiazole carbons[19]. The disappearance of NH\(_2\) and carbonyl peak in the \(^1^H\) NMR spectra of compounds (2-5,7,10-12, 15) were further supportive of condensation of amino thiadiazoles with formaldehyde and various substituted aniline/phthalimide. The additional peaks at δ 4.2-4.5 ppm were attributed to CH\(_2\) of Mannich base derivatives [20]. The observed signals in \(^1^C\) NMR spectra of compounds (2,5,7,10 and 12-15) in the range of δ 33.41-38.55 ppm are due the formation of N-CH\(_2\)-N-of derivatives [21] of thiadiazole. Other proton and carbon peaks were found to be well consistent with the expected structures. All the compounds showed a single peak in their mass spectra suggesting their purity.

All synthesized and characterized compounds (1–15) were evaluated for their antibacterial activity against gram positive bacteria-Bacillus subtilis, Staphylococcus aureus and gram negative bacteria Escherichia coli, Pseudomonas aeruginosa together with a fungus Candida albicans using well diffusion method.and compared with well-known antibacterial/antifungal drug, Ciprofloxacin/ketoconazole. The minimum inhibitory concentration (MIC, 10μg ml\(^{-1}\)) was determined for each compound in triplicate experiments. The values were averaged and reported in table 2 while graphically shown in fig. 1.

The observed values reveal that type of substituent directly attached to 1, 3, 4-thiadiazole ring has a significant impact on the antibacterial/antifungal activities of these novel analogs. In particular, compound no.3, 7, 13 showed the activity to a greater extent due to the presence of nitro group [22]. However, the chloro [23] containing compounds 5, 10, and 15 showed significant activity against all five microorganisms. In light of the various available literature describing biological activities of compounds containing phthalimides, we also noticed the compounds 2,7,12 showed appreciable activity. Additionally, unsaturation [24] plays some role in activity since compounds having triple bond (1-5) showed better activity then compound having the double bond (11-15). However, the trend observed in all synthesized compounds containing nitro, chloro and methyl is in decreasing order (BA-1, BA-2, BA-3) and well consistent with literature.

Table 2: The MIC* values of the synthesized compounds for antibacterial and antifungal activity at 10 μg/ml concentration

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Bacteria</th>
<th>Fungus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PA</td>
<td>BS</td>
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<tr>
<td>1</td>
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<td>BA-1a</td>
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<td>17</td>
</tr>
<tr>
<td>3</td>
<td>BA-1b</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>BA-1c</td>
<td>12</td>
<td>10</td>
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<tr>
<td>17</td>
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</tbody>
</table>

* MIC = minimum inhibitory concentration (Mean of triplicate experiments)

NA-Not active, EC-Escherichia coli, PA-Pseudomonas aeruginosa, BS-Bacillus subtilis, SA-Staphylococcus aureus, CA-Candida albicans

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CONCLUSION
The present work provides a convenient and efficient route for the preparation of new substituted thiadiazole (BA-1, BA-2, BA-3) and their Mannich base derivatives. All the newly synthesized compounds have been screened for their antimicrobial activities. Most of the tested compounds exhibited activities against the strains used. Our present results demonstrated that the presence of nitro group has the significant effect on activity, which was followed by chloro, methyl groups. However, phthalimide had showed appreciable activity in all three cases.

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CONFLICT OF INTERESTS
The authors have no conflict of interest in publication of this article.

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