SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF THIADIAZOLE DERIVATIVES

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ABSTRACT

A new series of novel analogues of 1, 3, 4-thiadiazole were synthesized. These analogues were identified on the basis of melting point range, Rf values, IR, $^1$H NMR and mass spectral analysis. The analogues were screened for anti-mycobacterial and antibacterial activities. The analogues exhibited significant to moderate anti-mycobacterial and antibacterial activities.

KEYWORDS: Thiadiazole, anti-mycobacterial activity, antibacterial activity.

INTRODUCTION

Thiadiazole contains the five-membered diunsaturated ring structure having molecular structure formula $C_2H_2N_2S$. The ending azole designates a five membered ring system with two or more heteroatoms, one of which is Nitrogen. Thiadiazoles are associated with diverse biological activity probably by virtue of $\text{–N=C=S}$ grouping. Literature reveals that compounds having thiadiazole nucleus have wide spectrum of pharmacological activities such as antimicrobial$^{[1]}$, antitubercular$^{[2]}$, ulcerogenic$^{[3]}$, anti-inflammatory$^{[4]}$, analgesic$^{[5]}$, CNS depressant$^{[6]}$, anticonvulsant$^{[7]}$, anticancer$^{[8]}$, antioxidant$^{[9]}$, antiviral$^{[10]}$, antiepileptic$^{[11]}$ etc. Research in this area is still unexplored, therefore the present study is directed towards the synthesis of newer analogues of 1, 3, 4-thiadiazole with good yield and enhance anti-mycobacterial and antibacterial activities.
MATERIALS AND METHODS

All the chemicals procured from Central Drug House (P.) Ltd, New Delhi. The melting points were determined in open glass capillaries and were uncorrected. Thin Layer Chromatography using silica gel G (E. Merck) plates were used to access the reaction and purity of synthesized compounds. The IR spectra were recorded on Perkin-Elmer FTIR/FTFIR system in KBr pellets and noted the absorption levels (cm⁻¹) were listed. ¹H NMR spectra were run on Bruker DPX400 in DMSO-d₆ as solvent and TMS as an internal standard. The Mass spectra were recorded on EI ionization mode on a JEOL JMS600H EI mass spectrometer.

STEP 1: Synthesis of N-phenyl thiosemicarbazide

- From aromatic amines

Aniline (0.01mol) was dissolved in ethanol and ammonia (25ml). Carbon disulfide (0.01mol) was added drop wise and stirred for 30 minutes. To this mixture, hydrazine hydrate (0.01mol) was added, Reaction mixture was refluxed on water bath for 9-12 hrs. Completion of reaction was checked by TLC. After reaction, reaction mixture was allowed to cool to room temperature, kept overnight in freezing condition to get solid product. Separated solid product was filtered and dried. Recrystalized from ethanol-water mixture (4:1 ratio) to yield white shining crystals. Yield: 62.33% W/W.

- From phenylisothiocyanate

Phenylisothiocyanate (0.01mol), hydrazine hydrate (0.01mol) in ethyl alcohol (25ml) were taken and subjected to microwave irradiation for 6-12 minutes at 245-350W power. In between, the completion of reaction was checked by TLC. After that reaction mixture was slowly poured into crushed ice and kept overnight. Separated solid was filtered, washed with water and dried. Solid was then purified by recrystallisation from ethanol-water mixture (4:1 ratio) to yield desired compounds.

STEP 2: Synthesis of 5-aryl-N-phenyl-1,3,4-thiadiazole-2-amine.

N-phenyl thiosemicarbazide (0.01mol), aromatic acid (0.01mol) and sulfuric acid in DMF (25ml) were taken and subjected to microwave irradiation for 6-12 minutes at 245-350W power. In between, the completion of reaction was checked by TLC. After that reaction mixture was slowly poured into crushed ice and kept overnight. Separated solid was filtered, washed with water and dried. Solid was then purified by recrystallisation from ethanol-water mixture (4:1 ratio) to yield desired compounds [TD1-TD8] respectively.
Anti-mycobacterial screening

Antimycobacterial study was performed by using Alamar Blue Assay method. Resazurin, an oxidation reduction indicator, has been used to access viability and bacterial contamination and to test for antimicrobial activity. Alamar blue was used as resazurin in cell cytotoxicity studies.
Protocol for Anti-TB activity using Alamar Blue Dye

1) The antimycobacterial activity of analogues were assessed against M. tuberculosis using Micro plate Alamar Blue Assay (MABA).

2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

3) Briefly, 200μl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation.

4) The 96 wells plate received 100 μl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.

5) The final drug concentrations tested were 100 to 0.2 μg/ml.

6) Plates were covered and sealed with paraffin and incubated at 37ºC for five days.

7) After this time, 25μl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.

8) A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

9) The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Antibacterial activity[14][15]

Antibacterial activity of the synthesized analogues was screened using the disc diffusion method against selected pathogens such as Staphylococcus aureus, Escherichia coli.. The analogues were dissolved in DMSO and sterilized by filtering through 0.45 μm millipore filter. Nutrient agar (anti bacterial activity) was prepared and sterilized by an autoclave (121º C and 15 lbs for 20 min) and transferred to previously sterilized petridishes (9 cm in diameter). After solidification, petriplates were inoculated with bacterial organisms in sterile nutrient agar medium at 45 ⁰C Sterile whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized analogues at a concentration of 500µg/ml, 300µg/ml and 150 µg/ml(E.coli) and 150 µg/ml, 100 µg/ml and 50 µg/ml(S. aureu.) were placed in the organism impregnated petri plates under sterile condition. The plates were left for 30 min to allow the diffusion of analogues at room temperature. Antibiotic discs of Ertapenam-10 mcg/disc, Netilmycin-30 mcg/disc and Streptomycin-100 μg/ml was used as positive control, while DMSO used as negative control. Then the plates were incubated for 24 H at 37 ± 1. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no microbial growth around the each disc.
RESULTS AND DISCUSSION
The melting points of all synthesized compounds were found in open capillary tubes and readings were uncorrected. The structures of the synthesized analogues were supported by physical data and following spectral analysis.

N-phenyl thiosemicarbazide
MF: C7H9N3S, MW: 167.23 AMU, m.p: 120-123 °C, Rf: 0.77 (ethanol : ethyl acetate ;9:1), IR(υ cm⁻¹) : 3414.32 (NH-NH2), 3051.37 (aromatic C-H str),1648.11(primary amino N-H str), 1591.27 (N-H bend), 1490.97, 1425.40 (aromatic C=C ring str), 1233.87 (C=S), 755.38 (C6H5), LC-MS: m/z 167 (M⁺).

5-(4-chlorophenyl)-N-phenyl-1,3,4-thiadiazole-2-amine [TD1]
MF: C14H10ClN3S, MW: 287.77 AMU, m.p: 233-235 °C, Rf: 0.86 (ethyl acetate:n Hexane; 1:1), IR(υ cm⁻¹) : 3051.39 (aromatic C-H str), 1573.91 (N-H bend), 1490.97 (C6H5), 1425.40 (aromatic C=C ring str), 1321.24 (secondary aromatic C-N str), 1111.00 (N-N=C), 1091.71(C-Cl str), 1014.56 (N-N str), 852.54(C6H4), 761.88 (C6H5), 682.80 (C-S-C), ¹H NMR(DMSO- d6) δ: 13.201 singlet, -NH (1H)7.563-7.953- m, Ar (9H), LC-MS: m/z 287.66 (M⁺).

5-(4-nitrophenyl)-N-phenyl-1,3,4-thiadiazole-2-amine [TD2]
MF: C14H10N4O2S, MW: 298.32 AMU, m.p: 230-232 °C, Rf: 0.87 (ethyl acetate: n Hexane; 1:1), IR(υ cm⁻¹) : 3035.71 (aromatic C-H str), 1686.41(asymmetric aromatic NO2 str),1604.99 (N-H bend), 1426.19 (aromatic C=C ring str), 1349.58(symmetric aromatic NO2 str), 1310.55 (secondary aromatic C-N str), 1109.23 (N-N=C), 1014.04 (N-N str), 887.97(aromatic nitro C-N str), 800.45(C₆H₄), 715.31 (C₆H₅), 671.45 (C-S-C), ¹H NMR(DMSO- d6) δ: 13.692 singlet, -NH (1H)8.163-8.342- m, Ar (9H), LC-MS: m/z 298.3 (M⁺).

4(5-(phenyl amino)-1,3,4-thiadiazole-2-yl) phenol [TD3]
MF: C14H11N3OS, MW: 269.329 AMU, m.p: 168-171 °C, Rf: 0.75 (ethyl acetate: n Hexane; 1:1), IR(υ cm⁻¹) : 3380.88 (OH str), 3085.72 (aromatic C-H str), 1600.57(N-H bend),1574.46, 1487.82,1455.51, 1418.21 (aromatic C=C ring str), 1298.57 (secondary aromatic C-N str), 1200.14(phenolic C-O str), 1181.76 (N-N=C), 1032.53 (N-N str), 771.51(C6H4), 746.84 (C6H5), 686.08 (C-S-C), 606.83(O-H out of plane bend), LC-MS: m/z 269.3 (M⁺).
5-(3-aminophenyl)-N-phenyl-1,3,4-thiadiazole-2-amine [TD4]
MF: C14H12N4S, MW: 268.34 AMU, m.p: 165-167 °C, Rf: 0.79 (ethyl acetate: n Hexane; 1:1), IR(ν cm⁻¹) : 3030.31 (aromatic C-H str), 1598.29(N-H bend), 1545.71,1477.21,1442.96 (aromatic C=C ring str), 1310.49 (secondary aromatic C-N str), 1252.29( primary aromatic C-N str) 1100.88 (N-N=C), 1011.31 (N-N str), 825.13(C6H4), 745.00 (C6H5), 692.84 (C-S-C), LC-MS: m/z 268.34 (M⁺).

3(5-(phenyl amino)-1,3,4-thiadiazole-2-yl) phenol [TD5]
MF: C14H11N3OS, MW: 269.329 AMU, m.p: 163-165 °C, Rf: 0.73 (ethyl acetate: n Hexane; 1:1), IR(ν cm⁻¹) : 3186.16(O-H str), 303 0.85 (aromatic C-H str), 1598.17 (N-H bend), 1546.99 (C6H5), 1470.98,1442.65 (aromatic C=C ring str), 1310.00 (secondary aromatic C-N str), 1251.65, 1170.15(phenolic C-O str), 1189.39 (N-N=C),1024.77 (N-N str), 833.26(C6H4), 743.88 (C6H5), 692.53 (C-S-C), 603.93(out of plane O-H bend), LC-MS: m/z 269.32 (M⁺).

N-phenyl-5-p tolyl-1,3,4-thiadiazole-2-amine [TD6]
MF: C15H13N3S, MW: 267.357 AMU, m.p: 143-145 °C, Rf: 0.76 (ethyl acetate: n Hexane; 1:1), IR(ν cm⁻¹) : 2970.43 (aliphatic C-H str), 1601.49 (N-H bend), 1501.35 (C6H5), 1455.72, 1417.97 (aromatic C=C ring str), 1280.17 (secondary aromatic C-N str), 1115.69 (N-N=C), 771.92(C6H4), 747.50 (C6H5), 686.95 (C-S-C) ¹ H NMR(DMSO- d6) δ: 12.794- singlet, -NH (1H)6.981-7.846- m, Ar (9H)2.371- s, -CH3(3H), LC-MS: m/z 267.35 (M⁺).

5-(5-(phenyl amino)-1,3,4-thiadiazole-2-yl) benzene 1,2,3 triol [TD7]
MF: C14H11N3O3S, MW: 301.328 AMU, m.p: 167-169 °C, Rf: 0.78 (ethyl acetate: n Hexane; 1:1), IR(ν cm⁻¹) : 3393.43 (secondary aromatic N-H str) 3030.51 (aromatic C-H str), 3242.74(O-H str), 1601.11 (N-H bend), 1496.41, 1455.68, 1422.31(aromatic C=C ring str), 1299.00 (secondary aromatic C-N str), 1200.42(phenolic C-O str), 1082.15(N-N=C), 1058.84 (N-N str), 893.34(C6H3), 745.58 (C6H5), 687.88 (C-S-C), 606.94 (O-H out of plane bend), LC-MS: m/z 301.3 (M⁺).

2(5-(phenyl amino)-1,3,4-thiadiazole-2-yl) phenol [TD8]
MF: C14H11N3OS, MW: 269.329 AMU, m.p: 231-233 °C, Rf: 0.8 (ethyl acetate: n Hexane; 1:1), IR(ν cm⁻¹) : 3186.18(O-H str), 3031.01 (aromatic C-H str), 1598.87 (N-H bend), 1548.49,1495.67, 1471.68 (aromatic C=C ring str), 1310.95 (secondary aromatic C-N str),
1252.13 (phenolic C-O str), 1189.61 (N-N=C), 1024.73 (N-N str), 743.78 (C6H4), 692.41 (C-S-C), 603.93 (O-H out of plane bend), LC-MS: m/z 269.32 (M+).

All the synthesized analogues were tested for their activity against *Mycobacterium tuberculosis*. As the potency of the newly synthesized analogues was unknown, various concentrations were used in this study. The concentrations of the standards (Pyrazinamide and Streptomycin) were also the same as that of test analogues. MIC of the tested analogues was shown in table 1 & 2. Among eight synthesized analogues, analogue TD7 showed significant inhibitory activity with MIC of 6.25 μg/ml against *Mycobacterium tuberculosis* H37Rv strain. It is interesting to note that the activity ratio is decreased in the synthesized structures when electron withdrawing groups such as chloro and nitro group in the phenyl system. The promising activities of the analogues are mainly attributed with the presence of electron donating group such as hydroxyl group in the phenyl system. Analogues TD4 and TD5 showed considerable activity with an MIC of 25 μg/ml. MIC of analogue TD6 was found to be 50 μg/ml. All other compounds showed an MIC of 100 μg/ml.

**Table 1: Antimycobacterial screening of analogues with different concentrations**

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**Standards:** Pyrazinamide - 3.125 μg/ml, Streptomycin - 6.25 μg/ml
Table 2: Antimycobacterial activity results of analogues

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<th>CODE</th>
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S – Sensitive, R – Resistant

Microplate showing the result of test analogues

Microplate of standards- Pyrazinamide and Streptomycin

Figure 1.

The analogue TD2 was screened for antibacterial activity against Gram –ve organism (E.coli) and Gram +ve organism (S. aureus) using agar diffusion method. 500µg/ml, 300µg/ml and 150 µg/ml concentrations of test analogue were prepared in DMSO for screening antibacterial activity against E.coli. 150 µg/ml, 100 µg/ml and 50 µg/ml concentrations of test analogue were used for S. aureus. The results were compared with the standard drugs Ertapenam-10 mcg/disc, Netilmycin-30 mcg/disc and Streptomycin-100 µg/ml concentration and DMSO as the control. The test analogue i.e. analogue TD2 was found to be moderately active towards
Gram +ve and Gram –ve organism as it showed the zone of inhibition. The antibacterial screening results is shown in table 3.

**Table 3: Antibacterial screening results of analogue TD2.**

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<th>GROUP</th>
<th>MEAN ZONE OF INHIBITION (mm)</th>
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<td><em>Staphylococcus aureus</em></td>
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<td>50μg/ml</td>
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<tr>
<td>TEST(TD2)</td>
<td>13</td>
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<tr>
<td>Ertapenam-10 mcg/disc</td>
<td>36</td>
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<tr>
<td>Netilmicin-30 mcg/disc</td>
<td>24</td>
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<tr>
<td>Streptomycin-100 μg/ml</td>
<td>22</td>
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<tr>
<td>DMSO</td>
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</table>

CONCLUSION

The research work was oriented towards the finding of newer analogues of 1, 3, 4-thiadiazole with enhance anti-mycobacterial and antibacterial activities. The different analogues were synthesized. The synthesized analogues showed very good anti-
mycobacterial and antibacterial activities against previously reported analogues of 1, 3, 4-thiadiazole.

ACKNOWLEDGEMENT
The authors are thankful to Narayan Institute Of Pharmacy, Sasaram, Jamuhar, Bihar, India for providing research facilities and encouragement and to our friends those helped us to complete this research.

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