SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF THIAZOLE DERIVATIVES CONTAINING TRIAZOLE MOIETY USING LiBr AS A CATALYST

Chetna Ameta\(^1\), Diwaker Sitha\(^1\), Rajat Ameta\(^2\), and Suresh C. Ameta* \\
\(^1\)Synthesis Laboratory, Department of Chemistry, M. L. Sukhadia University, Udaipur (Raj.)-313 001, India \\
\(^2\)Cadila Zyfine, Ahmedabad (Guj.) India

Received April 20, 2010; Accepted September 2, 2010

ABSTRACT

Synthesis of triazole derivatives from 2-mercaptobenzothiazole has been achieved in four steps under microwave irradiation. LiBr potentially replace solvents and corrosive acids in this reaction scheme. The products of triazole derivatives have shown antimicrobial activity.

Keywords: LiBr, Microwave irradiation, Solvent free condition, Basic alumina

INTRODUCTION

At the beginning of this century, organic solvent free reactions have attracted considerable interest due to increase of awareness about environmental problems in chemical research and industry [1]. Organic solvent-free reactions have many advantages such as reduced pollution, lower costs and simplicity. Another aspect, which is receiving increasing attention, is the water-accessible Lewis acid catalysts in many chemical transformations [2]. Water, as the reaction medium, is generally considered as inexpensive, safe and environmentally benign alternative to synthetic solvents [3]. This prompted the systemic investigation into the feasibility of organic solvent – less catalyzed reactions under microwave irradiations. Microwave irradiation has been used for variety of applications including synthetic organic chemistry [4]. Benzothiazole derivatives have been studied extensively because of their broad spectrum of biological activities [5-8]. Similarly triazoles display a number of antimicrobial activities like antifungal [9-11], anti-mycobacterial [12], antibacterial [13-15] and variety of industrial applications [16]. Benzothiazole derivatives bearing triazole moiety are widely investigated for their pharmacological properties [17-18]. The Knoevenagel condensation has been a subject of considerable interest and to effect this condensation efficiently, large number of catalyst have been explored e.g. sodium acetate in glacial acetic acid, piperidinium benzate in refluxing toluene, zeolite, tetrabutylammonium bromide, refluxing reactants in toluene at 110 °C for 3 days, etc. Certainly, these processes are not facile as they require long reaction times, high temperature and products are obtained in low yields. LiBr can potentially replace solvents and conventional corrosive acids in many of their applications [19-24].

EXPERIMENTAL SECTION

Materials

All reagents were obtained commercially and used after purification. Basic alumina, 4-nitrobenzaldehyde and 2-, 3- or 4-chlorobenzaldehyde were obtained from Himedia. 2-, 3- or 4-hydroxybenzaldehydes, benzaldehyde, dichloromethane, hydrazine hydrate and ethylchloroacetate, were obtained from CDH. All other chemicals like lithium bromide and carbon disulphide were obtained from Merck, 2- or 4-methoxybenzaldehyde & 2-mercapto benzothiazole were obtained from SRL and Otto Kemi, respectively.

Instrumentation

All reactions were carried out in a domestic microwave oven (Kenstar, Model No. OM-26 EGO). Melting points are uncorrected and determined in open capillaries. Reactions were monitored by thin layer chromatography using silica gel-G as adsorbent using ethyl acetate: n-hexane (7: 3) as eluent and products were detected by iodine vapors. IR spectra (KBr pellets) were recorded on Perkin-Elmer 1800 (FTIR) spectrometer. \(^1\)H NMR spectra (DMSO-d\(_6\)) were taken on a Bruker DRX spectrometer (300MHz FT NMR) using TMS as internal standard and chemical shift were expressed in \(\delta\). Mass spectra were taken on a Jeol SX-102/PA-6000 (EI) spectrometer and elemental analysis was carried out on a C, H, N analyzer (Elemental Vario Carlo Alba 1108). The results were found to be in good agreement with the calculated values (\(\pm\) 0.2%). The starting compounds were prepared according to reported method.

Chetna Ameta et al.
**Procedure**

**Microwave induced synthesis of ethyl (1, 3-benzothiazol-2-ylsulfanyl) acetate (1)**

2-Mercaptobenzothiazole (0.01 mole) and ethyl chloroacetate (0.01 mole) adsorbed over basic alumina were placed in a conical flask and exposed to microwave irradiation at 800 W for 4.0 min. After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature. Then dichloromethane (20 mL) was added and the mixture was stirred for 5 min, filtered, evaporated and recrystallized from ethanol to afford compound 1.

Found: C 52.14, H 4.39, N 5.32, S 25.30% Calc. for C_{11}H_{12}NS_{2}O: C 52.19, H 4.30, N 5.38 S 25.39% IR (KBr cm^{-1}): 3023 (C-H str., Ar-H), 1180 (C-O-C str.), 1221 (C-S str.), 1723 (C=O str.), 1614 (C=N str.), 1601 (C=C str.) and 2915 (CH_{3} str.).

^1H NMR (DMSO - d_{6}, ppm): 1.23 (t, 3H, CH_{3}), 4.13 (q, 2H, CH_{2}), 2.62 (s, 2H, S-CH_{2}) and 6.73-7.87 (m, 4H, Ar-H).

**Conventional synthesis of ethyl (1, 3-benzothiazol-2-ylsulfanyl) acetate (1)**. An equimolar solution of 2-mercaptobenzothiazole (0.01 mole) and ethyl chloroacetate (0.01 mole) in dry acetone 4 mL in the presence of anhydrous K_{2}CO_{3} (1 g) was refluxed on a water bath for 16 h. The solvent was removed by vacuum distillation and the residue was recrystallized from ethanol to afford compound 1.

**Microwave induced synthesis of 2-(1,3-benzo thiazol-2-ylsulfanyl) acetohydrazide (2)**

Compound 1 (0.01 mole) and hydrazine hydrate (0.01 mole) adsorbed over basic alumina was placed in a conical flask and exposed to microwave irradiation at 350 W for 3.5 min. After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature. Then dichloromethane (20 mL) was added and the mixture was stirred for 5 min, filtered, evaporated and recrystallized from ethanol to afford compound 2.

Found: C 45.19, H 3.80, N 17.60, S 26.79% Calc. for C_{10}H_{12}N_{2}O_{2}: C 45.14, H 3.72, N 17.51, S 26.70% IR (KBr cm^{-1}): 3030 (C-H str., Ar-H), 3362, 3378, 3289 (-NHNH_{2} str.), 1222 (C-S str.), 1602 (C=C str.), 1665 (C=O) and 1615 (C=N str.).

^1H NMR (DMSO - d_{6}, ppm): 2.01 (s, 2H, NH_{2}), 7.88 (s, 1H, CONH), 2.61 (s, 2H, S-CH_{2}) and 6.80-7.88 (m, 4H, Ar-H).

**Conventional synthesis of 2-(1,3-benzo thiazol-2-ylsulfanyl) acetohydrazide (2)**. Compound 1 (0.01 mole) and hydrazine hydrate (0.01 mole) in ethanol (20 mL) was refluxed about 5 h on a steam bath. After cooling the resulting solid was filtered, dried and recrystallized from ethanol to obtain compound 2.

**Microwave induced synthesis of 3-[(1,3-benzo thiazol-2-ylsulfanyl)methyl]-4H-1,2,4-triazole-4-amine (3)**

A mixture of compound 2 (0.01 mole) and CS_{2} in ethanol (20 mL) in the presence of KOH (0.5 g) were stirred for 12-15 hr. to obtain potassium salt of phenyl carboxy-hydrazide. Now this potassium salt (0.01 mole), hydrazine hydrate (0.01 mole) and LiBr (10 mole %) were placed in small conical flask and mixed with glass rod at room temperature. The mixture was then exposed to microwave radiation at 720 W for 6.5 min. After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature. Water (15 mL) was then added to reaction mixture and stirred for 5 min. The solid thus obtained was filtered, dried and recrystallized from ethanol to afford compound 3.

Found: C 58.85, H 4.01, N 12.81, S 19.57% Calc. for C_{10}H_{12}N_{2}S_{2}: C 58.69, H 3.95, N 12.85, S 19.51% IR (KBr cm^{-1}): 3031 (C-H str., Ar-H), 3376, 3280 (-NH_{2} str.), 1220 (C-S str.), 1604 (C=C str.), 1616 (C=N str.), 2559 (S-H str.), 1051 (N-N str.), 1256 (C-N str.) and 2962 (CH_{2} str.).

^1H NMR (DMSO - d_{6}, ppm): 2.03 (s, 2H, NH_{2}), 2.60 (s, 2H, S-CH_{2}), 2.65 (s, 1H, SH) and 6.78-7.84 (m, 4H, Ar-H).
Conventional synthesis of 3-[(1,3-benzothiazol-2-ylsufanyl)methyl]-4H-1,2,4-triazole-4-amine (3).

Compound 2 (0.01 mole) and benzaldehyde (0.01 mole) and LiBr (10 mole %) were placed in small conical flask and mixed with glass rod at room temperature. The mixture was then exposed to microwave radiations at 840W for 5-6 min. After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature. Water (15 mL) was then added to reaction mixture and then stirred for 5 min. The solid thus obtained was filtered, dried and recrystallized from ethanol to afford compound 4a.

Found: C 53.25, H 3.41, N 48.29, S 25.05% Calc. for C\(_{75}\)H\(_{3}\)N\(_{3}\)S\(_{3}\): C 53.20, H 3.4, N 48.20, S 25.10% IR (KBr cm\(^{-1}\): 3032 (C-H str., Ar-H), 1224 (C-S str.), 1257 (C-N str.), 1625 (C-N str.), 2960 (-CH\(_2\) str.), 3065 (C-H str., Ar-CH), 1052 (N-N str.), 2560 (S-H str.) and 1605 (C=C str.).

\(^1\)H NMR (DMSO - d\(_6\): \(6\) ppm: 4.71 (s, 1H, =CH-Ar), 2.61 (s, 2H, S-CH\(_2\)), 2.66 (s, 1H, SH) and 7.28 - 7.94 (m, 9H, Ar-H).

Compounds 4b-j were prepared similarly by treating 3 with various aromatic aldehydes.

3-[(1,3-Benzothiazol-2-ylsufanyl)methyl]N-[2]-[4-nitrophenyl]methylidene]-4H-1,2,4-triazole-4-amine (4b).

Found: C 47.67, H 2.63, N 19.60, S 22.42% Calc. for C\(_{75}\)H\(_{3}\)N\(_{3}\)S\(_{3}\): C 47.60, H 2.77, N 19.67, S 22.49% IR (KBr cm\(^{-1}\): 3033 (C-H str., Ar-H), 1225 (C-S str.), 1255 (C-N str.), 1628 (C-N str.), 2962 (-CH\(_2\) str.), 3068 (C-H str., Ar-CH), 1053 (N-N str.), 2561 (S-H str.) and 1604 (C=C str.).

\(^1\)H NMR (DMSO - d\(_6\): \(6\) ppm: 4.73 (s, 1H, =CH-Ar), 2.64 (s, 2H, S-CH\(_2\)), 2.68 (s, 1H, SH) and 7.30 - 7.96 (m, 8H, Ar-H).

3-[(1,3-Benzothiazol-2-ylsufanyl)methyl]N-[2]-[2-chlorophenyl]methylidene]-4H-1,2,4-triazole-4-amine (4c).

Found: C 48.87, H 2.66, N 16.78, S 23.03% Calc. for C\(_{75}\)H\(_{3}\)N\(_{3}\)S\(_{3}\): C 48.80, H 2.90, N 16.70, S 23.07% IR (KBr cm\(^{-1}\): 3034 (C-H str., Ar-H), 1226 (C-S str.), 1254 (C-N str.), 1625 (C-N str.), 2964 (-CH\(_2\) str.), 3068 (C-H str., Ar-CH), 1057 (N-N str.), 2565 (S-H str.) and 1607 (C=C str.).

\(^1\)H NMR (DMSO - d\(_6\): \(6\) ppm: 4.77 (s, 1H, =CH-Ar), 2.66 (s, 2H, S-CH\(_2\)), 2.72 (s, 1H, SH) and 7.33 - 7.99 (m, 8H, Ar-H).

Chetna Ameta et al.
Table 1. Physical data of synthesized compounds

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R</th>
<th>Mol. Formula</th>
<th>Mol. Weight</th>
<th>M.P. °C</th>
<th>Yield (^a) [Time] (h)</th>
<th>Yield (^b) [Time] (min)</th>
<th>Yield (^c) [Time] (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>C(<em>{11}H</em>{14}NS_2O_2)</td>
<td>253</td>
<td>58</td>
<td>66 [16]</td>
<td>69 [8]</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>C(<em>{16}H</em>{22}OS_2)</td>
<td>239</td>
<td>192</td>
<td>70 [5]</td>
<td>75 [5]</td>
<td>78</td>
</tr>
<tr>
<td>4a</td>
<td>-</td>
<td>C(<em>{9}H</em>{12}S_2)</td>
<td>383</td>
<td>188</td>
<td>72 [6]</td>
<td>89 [4.5]</td>
<td>89</td>
</tr>
<tr>
<td>4b</td>
<td>-NO(_2)</td>
<td>C(<em>{11}H</em>{15}NS_2O_2)</td>
<td>428</td>
<td>160</td>
<td>77 [6]</td>
<td>87 [4.5]</td>
<td>87</td>
</tr>
<tr>
<td>4c</td>
<td>-ClC(_2)</td>
<td>C(<em>{11}H</em>{14}NS_2Cl)</td>
<td>418</td>
<td>165</td>
<td>77 [6]</td>
<td>85 [4.5]</td>
<td>85</td>
</tr>
<tr>
<td>4d</td>
<td>-ClC(_2)</td>
<td>C(<em>{11}H</em>{14}NS_2Cl)</td>
<td>418</td>
<td>172</td>
<td>77 [6]</td>
<td>87 [4.5]</td>
<td>87</td>
</tr>
<tr>
<td>4e</td>
<td>-ClC(_2)</td>
<td>C(<em>{12}H</em>{16}NS_2Cl)</td>
<td>418</td>
<td>180</td>
<td>77 [6]</td>
<td>86 [4.5]</td>
<td>86</td>
</tr>
<tr>
<td>4f</td>
<td>-OCH(_2)</td>
<td>C(<em>{11}H</em>{14}NS_2O)</td>
<td>399</td>
<td>214</td>
<td>77 [6]</td>
<td>88 [4.5]</td>
<td>88</td>
</tr>
<tr>
<td>4g</td>
<td>-OCH(_2)</td>
<td>C(<em>{11}H</em>{14}NS_2O)</td>
<td>399</td>
<td>211</td>
<td>77 [6]</td>
<td>85 [4.5]</td>
<td>85</td>
</tr>
<tr>
<td>4h</td>
<td>-OCH(_2)</td>
<td>C(<em>{11}H</em>{14}NS_2O)</td>
<td>399</td>
<td>226</td>
<td>77 [6]</td>
<td>80 [4.5]</td>
<td>80</td>
</tr>
<tr>
<td>4i</td>
<td>-OCH(_2)</td>
<td>C(<em>{11}H</em>{14}NS_2O)</td>
<td>413</td>
<td>219</td>
<td>77 [6]</td>
<td>86 [4.5]</td>
<td>86</td>
</tr>
<tr>
<td>4j</td>
<td>-OCH(_2)</td>
<td>C(<em>{11}H</em>{14}NS_2O)</td>
<td>413</td>
<td>272</td>
<td>79 [6]</td>
<td>90 [6]</td>
<td>90</td>
</tr>
</tbody>
</table>

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-N-[(Z)-2-methoxyphenyl]methylidene] 4H-1,2,4-triazole-4-amine (4l). Found: C 52.30, H 3.68, N 16.92, S 23.28% Calc. for C\(_{11}H_{12}N_2S_2O_2\): C 52.21, H 3.60, N 16.99, S 23.20% IR (KBr cm\(^{-1}\)): 3035 (C-H str., Ar-H), 1227 (S-H str.), 1261 (C-N str.), 1630 (C=N str.), 2970 (-C=H str.), 1054 (N-N str.), 3072 (C-H str., Ar-CH), 1107 (C=O-CH, str.), 2569 (S-H str.), and 1607 (C=C str.).

\(^1\)H NMR (DMSO - d\(_6\)): 8.75 (s, 1H, =CH=Ar), 2.66 (s, 2H, S-CH), 3.33 (s, 3H, -OCH,), 2.71 (s, 1H, SH) and 7.33 - 7.97 (m, 8H, Ar-H).

3-[(1,3-Benzothiazol-2-ylsulfanyl)methyl]-N-[(4-methoxyphenyl)methylidene] 4H-1,2,4-triazole-4-amine (4j). Found: C 52.29, H 3.69, N 16.91 S 23.29% Calc. for C\(_{11}H_{12}N_2S_2O_2\): C 52.23, H 3.62, N 16.95, S 23.21% IR (KBr cm\(^{-1}\)): 3037 (C-H str., Ar-H), 1229 (S-H str.), 1263 (C-N str.), 1632 (C=N str.), 2973 (-C=H str.), 1056 (N-N str.), 3075 (C-H str., Ar-CH), 1109 (C=O-CH, str.), 2571 (S-H str.) and 1610 (C=C str.).

\(^1\)H NMR (DMSO - d\(_6\)): 8.75 (s, 1H, =CH=Ar), 2.68 (s, 2H, S-CH), 3.35 (s, 3H, -OCH,), 2.73 (s, 1H, SH) and 7.34 - 7.99 (m, 8H, Ar-H).

Conventional synthesis of 5-[(1,3-benzothiazol-2-ylsulfanyl)methyl]-4-[(phenylmethylidene)amino]-4H-1,2,4-triazole-3-thiol (4a)

Compound 4a (0.01 mole) and benzaldehyde (0.01 mole) and 2-3 drops of glacial acetic acid in ethanol (25 mL) was refluxed on water bath for about 4 hr. The solvent was removed and residue was recrystallized from ethanol to yield compound 4a. Compounds 4b-j were prepared similarly by treating 3 with various aromatic aldehydes.

RESULT AND DISCUSSION

2-Mercapto benzothiazole treated with ethyl chloroacetate adsorbed over basic alumina yielded ethyl (1,3-benzothiazol-2-ylsulfanyl)acetate 1. Its structure was confirmed by the IR bands at 1180 cm\(^{-1}\) due to C-O-C and 1723 cm\(^{-1}\) due to C=O of ester. It is supported by the presence of quartet and triplet of CH\(_2\)CH\(_3\) at \& 1.23 and 4.13 ppm in \(^1\)H NMR. The compound 1 was treated with hydrazine hydrate to give 2-(1,3-benzothiazol-2-ylsulfanyl) acetoxyhidrazide 2. Compound 2 was confirmed by the disappearance of CH\(_2\)CH\(_3\) in \(^1\)H NMR and appearance of 3352, 3378 of NH\(_2\) and 3289, NH in IR region. Compound 2 was then
Table 2. Antimicrobial activity of some synthesized compounds (500 ppm)

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Antifungal activity (Activity index)</th>
<th>Antibacterial activity (Activity index)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. fumigatus</td>
<td>C. albicans</td>
</tr>
<tr>
<td>4a</td>
<td>41</td>
<td>24</td>
</tr>
<tr>
<td>4b</td>
<td>43</td>
<td>32</td>
</tr>
<tr>
<td>4c</td>
<td>42</td>
<td>33</td>
</tr>
<tr>
<td>4f</td>
<td>40</td>
<td>29</td>
</tr>
<tr>
<td>C1</td>
<td>Nil</td>
<td>39</td>
</tr>
<tr>
<td>C2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Activity index = Inhibition area of the sample / inhibition area of the standard.

| Standard: C1 = Flucanazole, C2 = Ciprofloxacin |

reacted with CS2 in the presence of KOH to obtain potassium salt of phenyl carboxy-hydrazide. Compound 3-[(1,3-benzothiazol-2-ylsulfanyl)methyl]-4H-1,2,4-triazole-4-amine 3 were synthesized by the treatment of potassium salt of phenyl carboxy-hydrazide with hydrazine hydrate in the presence of catalytic amount of LiBr. It was confirmed by the appearance of a band due to SH group at 2559 cm⁻¹ in IR spectra and singlet of SH at δ 2.65 in ¹H NMR. The disappearance of singlet of – CONH moiety in ¹H NMR and band at 1665 cm⁻¹ due to C=O in IR spectra also supports this structure. Compound 4 possesses NH₂ moiety, which undergoes condensation with various aromatic aldehydes in the presence of LiBr to give 5-[(1,3-benzothiazol-2-ylsulfanyl)methyl]-4-[(substituted phenylimethylidene)amino]-4H-1, 2, 4-triazole-3-thiol 4a-j. The formation of compounds 4a-j was confirmed by the disappearance of an intense peak of NH₂ group in compounds 3 due to condensation with substituted benzaldehydes and appearance of a new singlet at 4.71 ppm due to arylidine proton (=CH-AR) of chalcone moiety.

ANTIMICROBIAL ACTIVITY

Pure culture of pathogenic bacteria and pathogenic fungi used for antimicrobial activity was produced from RNT Medical College and Microbial Research Laboratory, Department of Botany, Mohanlal Sukhadia University, Udaipur. Four compounds were screened in vitro for their antibacterial activities against four strains of bacteria (Escherichia coli, Bacillus subtilis, Klebsiella pneumonia and Pseudomonas aeruginosa) and two strains of fungi (Aspergillus fumigates and Candida albicans) using the Cup or Well method.

Growth medium for fungi

Sabourand agar medium was used to culture bacteria. The composition of Sabourand agar medium was as follows: Glucose: 20 g, Peptone: 10 g, Agar-Agar: 1.5 g, Distilled water: 1000 mL.

Cup or Well Method

Nutrient agar medium was autoclaved at 15 psi and 121°C for 15 min. Sterilized Petri dishes were placed in laminar flow bench. One end of the lid of each Petri dish was lifted and approximately 15-20 mL of molten agar medium was poured into it and left for solidification. These were then inoculated with 0.2 mL suspension of organism by spread plate method. With the help of sterile borers, four wells were made in the medium and filled with 500 ppm solution of synthesized compounds. Similarly, other wells were made for standard drug. These Petri dishes were sealed with paraffin and incubated at 37 °C in an incubator. The Petri dishes were examined for zone of inhibition after 24-28 h. Same method is used for antifungal activity. Commercial antibacterial ciprofloxacin and antifungal fluconazole were used as standard drugs.

The results have been tabulated in the form of inhibition zones and activity index in Table 2. The results revealed that all tested compounds exhibit moderate to strong activity against both fungi and E. coli. Compounds 4a, 4b, 4c and 4f shows considerable potency against A. fumigatus while against C. albicans they are moderately active. Similarly, compounds 4a, 4b, 4c and 4f are found to show excellent activity against E. coli while against P. aeruginosa, they exhibited moderate activity.

CONCLUSION

From the above studies we can advocate this method as facile, efficient and environ-economic for the synthesis of 5-[(1,3-benzothiazol-2-ylsulfanyl)methyl]-4-
[(phenyl substituted)amino]-4H-1, 2, 4-triazole-3-thiol 4a-j with catalytic amount of LiBr using basic alumina as inorganic solid support.

ACKNOWLEDGEMENT

Authors are thankful to the Head, Department of Chemistry, M.L. Sukhadia University, Udaipur for providing laboratory facilities and to the Director, SAIF, CDRI, Lucknow, India for providing spectral and analytical data. Authors are grateful to Antimicrobial Research Laboratory, particularly Dr. Kanika Sharma, Department of Botany, M.L. Sukhadia University for evaluating anti-microbial activity.

REFERENCES