Synthesis, spectral characterization and biological evaluation of 4H-1,4-benzothiazines, their sulfones and ribofuranosides

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ABSTRACT
Synthesis of heterocyclic compounds like benzothiazines has attracted attention in recent years due to their biological and industrial value. This article reflects up-to-date and comprehensive coverage of biochemical aspects of benzothiazines, their sulfones and ribofuranosides. The nitrogen and sulfur containing heterocycles were prepared by condensation followed by oxidative cyclization of 2-aminobenzenethiol with β-diketones/β-ketoester in dimethylsulfoxide. These compounds were then used as base to prepare ribofuranosides by treating them with sugar (β-D-ribofuranose-1-acetate-2,3,5-tri-O-benzylate). On refluxing with hydrogen peroxide in glacial acetic acid, these substituted dimethyl 4H-1,4-benzothiazines yielded 4H-1,4-benzothiazine-1,1-di-oxides. Antioxidant and antimicrobial activity of these compounds were carried out and structure evaluation was done by spectral and elemental analysis.

1. Introduction

The synthesis of benzothiazines, their sulfones and ribofuranosides has attracted tremendous interest due to wide spectrum of biological activities possessed by these compounds such as antibacterial, CNS depressants, anticancer, antifungal etc. As a part of the ongoing study, we have synthesized some new benzothiazines, their sulfones and ribofuranosides. Substituted benzothiazines were prepared by condensation of 2-aminobenzenethiols with β-diketone/β-ketoester in presence of dimethylsulfoxide through oxidative cyclization. Intermediate bis-(2-aminoethyl) disulfides undergoes cyclization through scission of S-S bond due to high reactivity of alpha position of enaminooleate system towards nucleophilic attack. The structures of these compounds were determined on the basis of spectral data and elemental analysis. These compounds were also screened for biological activity [1-11].

2. Experimental

All the melting points were determined in open capillary tubes but are uncorrected. 1H NMR and 13C NMR were recorded on JEOL AL 300 spectrometer (300 MHz) in DMSO-d6 / CDCl3 using TMS (tetramethyl silane) as an internal standard (Chemical shifts are measured in δ ppm). IR spectra were recorded in KBr on SHIMADZU 8400 S FTIR spectrophotometer. Mass spectra were recorded on JEOL SX 102/DA 600 using Xenon/Argon as FAB (Fast Atom Bombardment) gas. The purity of compounds were checked by thin layer chromatography using silica gel "G" as adsorbent, visualizing these by UV light or in an iodine chamber. Elemental analysis of these compounds was also done.

2.1. General method of synthesis of substituted 4H-1,4-benzothiazine (3a-d)

To a stirred suspension of 0.01 mole of β-diketone/β-ketoester (2a-d) in 5 mL of dimethylsulfoxide was added 0.01 mole of 2-aminobenzenethiol (1) and resulting mixture was refluxed for 20 minutes (Table 1). The reaction mixture was concentrated, cooled down to room temperature. The solid separated out was filtered, washed with petroleum ether and crystallized from methanol (Scheme 1). Characterization data and spectral analysis of each compound (3a-d) is given as:

Ethyl-3-trifluoromethyl-6,8-dimethyl-4H-1,4-benzothiazine-2-carboxylate (3a): Yield: 46%. M.p.: 55 °C. IR (KBr, ν cm⁻¹): 3260 (N-H), 1690 (C=O), 1340, 1160 (C-C str.), 1255, 1015 (–C–O str.). 1H NMR (DMSO-d6, 300 MHz, δ ppm): 8.90 (s, 1H, N-H), 8.08-7.20 (m, 2H, aromatic-H), 4.19 (q, 2H, J = 6.2 Hz, CH₂ of C₂H₅ at C₂), 2.35 (s, 3H, CH₃ at C₆), 2.36 (s, 3H, CH₃ at C₈). 13C NMR (CDCl₃, 300 MHz, δ ppm): 107.2 (C-2), 138.6 (C-3), 113.1 (C-5), 135.2 (C-6), 120.2 (C-7), 139.1 (C-8), 21.2 (CH₃ at C₆), 14.1 (CH₃ at C₈), 165 (C of CO at C₇), 114 (–CF₃ at C₃), 59.2 (CH₂ of COOC₆H₄ at C₆), 13.8 (CH₂ of COOC₆H₄ at C₈). MS (m/z %): 317 (M⁺), 244 (52), 202 (38), 275 (76), 73 (100). Anal. calcd. for C₁₅H₁₄NO₂F₃S: C, 52.99; H, 4.41; N, 4.41. Found: C, 53.25; H, 4.39; N, 4.35%.

2-Trifluoroacetyl-3-trifluoromethyl-6,8-dimethyl-4H-1,4-benzothiazine (3b): Yield: 68%. M.p.: 65 °C. IR (KBr, ν cm⁻¹): 3385 (N-H), 1650 (C=O), 1350, 1180 (C-F str.), 2895 (–CH₃ str.) cm⁻¹. 1H NMR (DMSO-d6, 300 MHz, δ ppm): 8.29 (s, 1H, NH), 8.26-6.86 (m, 2H, aromatic-H), 2.55 (s, 3H, CH₃ at C₆), 2.36 (s, 3H, CH₃ at C₈). 13C NMR (CDCl₃, 300 MHz, δ ppm): 116.2 (C-2), 136.3 (C-3), 1122 (C-5), 136.4 (C-6), 119.2 (C-7), 138.2 (C-8), 15.8 (CH₃ at C₆), 20.1 (CH₃ at C₈), 196.5 (C of CO at C₇), 129.8 (CF₃ at C₆), 114.1 (CF₃ at C₈).
Table 1. The exact reaction times and yields of individual reactions (3a-d, 4a-d, 5a-b).

<table>
<thead>
<tr>
<th>Compound No</th>
<th>R3</th>
<th>R4</th>
<th>Reaction times (min.)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>CF3</td>
<td>OC2H5</td>
<td>22</td>
<td>46</td>
</tr>
<tr>
<td>3b</td>
<td>CF3</td>
<td>CF3</td>
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<td>68</td>
</tr>
<tr>
<td>3c</td>
<td>CH2CH3</td>
<td>OCH3</td>
<td>23</td>
<td>76</td>
</tr>
<tr>
<td>3d</td>
<td>CH2</td>
<td>C2H5(OCH3)2 (o, p)</td>
<td>21</td>
<td>80</td>
</tr>
<tr>
<td>4a</td>
<td>CF3</td>
<td>OC2H5</td>
<td>255</td>
<td>69</td>
</tr>
<tr>
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<td>CF3</td>
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<tr>
<td>4d</td>
<td>CH2</td>
<td>C2H5(OCH3)2 (o, p)</td>
<td>260</td>
<td>82</td>
</tr>
<tr>
<td>5a</td>
<td>CF3</td>
<td>OC2H5</td>
<td>920</td>
<td>84</td>
</tr>
<tr>
<td>5b</td>
<td>CF3</td>
<td>CF3</td>
<td>980</td>
<td>68</td>
</tr>
</tbody>
</table>

**MS (m/z, %):** 341 (M⁺), 244 (58), 202 (29), 327 (78), 97 (100). Anal. calcd. for C13H9NOF6S: C, 45.74; H, 2.63; N, 4.10. Found: C, 45.98; H, 2.65; N, 4.06%.

Methyl-3-ethyl-6,8-dimethyl-4H,1,4-benzothiazine-2-carboxylate (3c): Yield: 76%. M.p.: 115 °C. IR (KBr, ν cm⁻¹): 3405 (NH), 1700 (>C=O), 1240, 1055 (C-O-C str.), 2875 (-CH3 str.). 1H NMR (DMSO-d6, 300 MHz, δ ppm): 8.40 (s, 1H, NH), 7.29–6.38 (m, 2H, aromatic-H), 2.35 (s, 3H, –CH3 at C6), 2.36 (s, 3H, –CH3 at C8), 2.00 (q, 2H, J = 7.01 Hz, CH2 of CH2CH3 at C3), 1.06 (t, 3H, J = 6 Hz, CH3 of CH2CH3 at C3), 3.76 (s, 3H, –CH3 of OCH3 at C2). 13C NMR (CDCl3, 300 MHz, δ ppm): 108.2 (m, 2H, aromatic-H), 2.35 (s, 3H, –CH3 at C6), 2.36 (s, 3H, –CH3 at C8), 2.00 (q, 2H, J = 7.01 Hz, CH2 of CH2CH3 at C3), 1.06 (t, 3H, J = 6 Hz, CH3 of CH2CH3 at C3), 3.76 (s, 3H, –CH3 of OCH3 at C2). MS (m/z, %): 263 (M⁺), 204 (56), 162 (31), 221 (71), 59 (100). Anal. calcd. for C14H17NO2S: C, 63.87; H, 6.46; N, 5.32. Found: C, 64.11; H, 6.40; N, 5.39%.

2-(2',4'-Dimethoxybenzoyl)-3,6,8-trimethyl-4H,1,4-benzothiazine (3d): Yield: 80%. M.p.: 115 °C. IR (KBr, ν cm⁻¹): 3405 (NH), 1700 (>C=O), 1240, 1055 (C-O-C str.), 2875 (-CH3 str.). 1H NMR (DMSO-d6, 300 MHz, δ ppm): 9.09 (s, 1H, NH), 7.16–6.08 (m, 2H, aromatic-H), 1.71 (s, 3H, –CH3 at C3), 3.73 (s, 3H, –OCH3 at para position of –COCH3 (OCH3)2 (o, p) at C2), 3.78 (s, 3H, –OCH3 at para position of –COCH3 (OCH3)2 (o, p) at C2), 2.14 (s, 3H, –CH3 at C6), 2.20 (s, 3H, –CH3 at C8), 7.20–5.95 (m, 3H, aromatic-H of benzoyl group). 13C NMR (CDCl3, 300 MHz, δ ppm): 112.9 (C-2), 138.6 (C-3), 111.9 (C-5), 136.2 (C-6), 118.2 (C-7), 146.2 (C-8), 16.5 (CH3 at C8), 187 (C of CO at C5), 56.8 (C of OCH3 at C2).
of OCH₃ at ortho position -COCH₃ (OCH₃)₂ (o, p) at C₂). MS (m/z, %): 395 (M⁺), 190 (63), 137 (48), 148 (28), 313 (70), 165 (100). Anal. calcd. for C₁₀H₁₁O₃N·H₂O: C, 45.06; H, 5.81; N, 3.94. Found: C, 67.88; H, 5.89; N, 3.89%.

2.2. General method of synthesis of 4H,1,4-benzothiazine, 1,1-dioxides (sulfones) (4a-d)

30% Hydrogen peroxide (5 mL) was added to a solution of 0.01 mole of 4H,1,4-benzothiazine in 20 mL glacial acetic acid and refluxed for 15 minutes at 50-55 °C. Heating was stopped and another lot of 5 mL of 30% Hydrogen peroxide was added. The reaction mixture was again refluxed for 4-5 hrs. The excess of solvent was removed by distillation under reduced pressure and the solution was poured into a beaker containing crushed ice. The yellow residue separated out was filtered and then crystallized from ethanol (Scheme 1). Characterization and spectral data of these compounds (4a-d) is given as:

Ethyl-3-trifluoromethyl-6,8-dimethyl-4H,1,4-benzothiazine-2-carboxylate-1,1-dioxide (4a): Yield: 69%. Mp.: 70 °C (KBr, v, cm⁻¹): 3270 (N-H), 1700 (C=O), 1130 (C-OH), 1085 (C-S str.). 1H NMR (DMSO-d₆, 300 MHz, δ, ppm): 8.28 (s, 1 H, NH), 7.60-6.70 (m, 2H, aromatic-H), 4.20 (q, J = 6.9 Hz, 2H, CH₃ of CH₂C₂H₃ at C₂), 1.29 (t, 3H, J = 6.6 Hz, CH₃ of CH₂C₂H₃ at C₂) 2.35 (s, 3H, -CH₃ at C₆), 2.36 (s, 3H, -CH₃ at C₆). 13C NMR (CDCl₃, 300 MHz, δ, ppm): 120.9 (C-7), 136.4 (C-8), 114.5 (C-9), 114.5 (C-10), 114.5 (C-11), 168 (C of COCH₃). MS (m/z, %): 95.6 (C₂H₅OCH₂CO₂H at C₁₃), 13.7 (CH₃ of COOCH₃ at C₁₃). 13C NMR (CDCl₃, δ, ppm): 9.56 (C₂H₅OCH₂CO₂H at C₁₃), 13.7 (CH₃ of COOCH₃ at C₁₃). MS (m/z, %): 349 (M⁺), 276 (100). Anal. calcd. for C₁₃H₁₉NO₃F₃S: C, 46.12; H, 4.01; N, 3.94.

1.1. Biological activity

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging assay

Radical scavenging activity of the synthesized compounds against stable 1,1-diphenyl-2-picyryl hydrazyl (DPPH) radical was determined spectrophotometrically as described by Cuendet et al. [13]. A stock solution of 1 mg/mL of the compound was prepared in methanol. 50 µL of compounds were added to 5 mL of a 0.004% methanol solution of DPPH. After 30 minutes incubation in dark at room temperature, absorbance was read against a blank at 517 nm (Table 2). Since IC₅₀ Value is inversely related to the antioxidant activity; ascorbic acid, which is a very good antioxidant, shows a lower IC₅₀ value of 17.8 µg/mL. Among all the synthesized compounds, compound 3e shows a value of 23.56 µg/mL which shows its good antioxidant nature.

The assay was carried out in triplicate and the percentage of inhibition was calculated by using the following formula.

\[
\text{Percentage of inhibition} = \frac{\text{AB} - \text{AA}}{\text{AB}} \times 100
\]

where AB = Absorption of blank, AA = Absorption of test, Ascorbic acid (shows antioxidant activity) as a positive control and methanol (no antioxidant activity) as a negative control has been used in this assay.
2.4.1.2. ABTS radical cation decolorization assay

The 2,2-azinobis(3-ethylenethiazoleine-6-sulphonic acid) radical cation (ABTS\(^+\)) decolorization test was carried out using an improved assay of Re et al. [13]. In brief, ABTS\(^+\) was generated by oxidation of ABTS with potassium persulphate. For this purpose, ABTS was dissolved in ionized water at concentration of 7 mM, and potassium persulphate was added to a concentration of 2.45 mM. The reaction mixture was left at room temperature overnight (12-15 hours), in the dark before use; the ABTS solution then was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. After addition of 1 mL of the diluted ABTS solution to 10 mL of compound and mixing, absorbance readings were taken at 30 °C at intervals of exactly 1-6 min. The experiment was carried out in triplicate (Table 3 and Figure 1). We have used ascorbic acid as a positive control and ethanol as a negative control in this assay.

2.4.2. Antimicrobial activity

The antimicrobial assay of the synthesized compounds was carried out by using paper disc method of Gould et al. [13] against some bacteria and fungi at 100 μg per disc concentration using Vancomycin, Gatifloxacin as reference compounds against bacteria (Coagulase negative Staphylococci, Coagulase positive Staphylococci, Enterobacter) and Fluconazole against fungus (Candida albicans). Paper disc method includes preparation of plates by pouring molten media into sterile petriplates which was then allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and allowed to dry for 5 min. The compound discs prepared were then placed over the plates and incubated for 37 °C for 24 hrs. At the end inhibition zones were measured with ruler in millimetre. These microorganisms were obtained from Microbiology Department, Swai Man Singh Medical College, Jaipur (Table 4).

3. Results and discussion

2-Aminobenzethiol (1) and \(\beta\)-diketones / \(\beta\)-ketoesters (2a-d) were refluxed in dimethyl sulphoxide which involves condensation and oxidative cyclization. A bis-(2-aminophenyl) disulfide (1) was obtained by oxidation of 2-aminobenzene thiol which cyclizes to form 4H-1,4-benzothiazines (3a-d) by cleavage of sulfur-sulfur bond due to high reactivity of \(\alpha\)-position of enaminoketone system towards nucleophilic attack. Compound (3a-d) on treatment with 30% hydrogen peroxide in glacial acetic acid were converted into their corresponding sulfones (4a-d). Treatment of (3a-b) in toluene with \(\beta\)-D-ribofuranose-1-acetate-2,3,5-tri benzoate in vacuum gave the corresponding ribofuranosides (5a-b) (Scheme 1). The structures of synthesized compounds are well supported by spectral data and elemental analysis.
showed a singlet due to N-H proton in the region δ 9.12-8.29 ppm. Peak due to N-H proton was found to be absent in compounds (5a-b) due to ribosylation. In ribofuranosides, C1'-H proton showed multiplet in region δ 4.43-4.80 ppm, C2'-H and C3'-H protons appeared in region δ 4.51-5.90 ppm as multiplet and C4'-H proton appeared as doublet at δ 6.30-6.40 ppm.

3.1.3. Mass spectra

The molecular ion peaks of 4H-1,4-benzothiazines were in accordance with their molecular weights. In all the cases side chain at C2 appears as a base peak which is obtained by its fission (Scheme 2).

![Scheme 2](image)

3.2. Biological activity (Antioxidant and Antimicrobial)

All the synthesized compounds (3a-d), (4a-d), and (5a-b) were screened for their antioxidant activity by DPPH free radical scavenging assay and (ABTS•+) radical cation decolorization assay. The synthesized compounds were also screened for antimicrobial activity (antibacterial and antifungal) by paper disc method. The present study demonstrated that these compounds showed mixed activity in DPPH and ABTS•+ assay.

1. Compounds (3b, 3c, 3d, 4b, 5a, 5b) showed strong radical scavenging activity in DPPH assay that have DPPH % inhibition >50.
2. Compounds (3a, 4d) showed moderate activity in DPPH assay that have DPPH % inhibition >30.
3. Compounds (4a, 4c) showed mild activity (<30) in DPPH assay.
4. Compounds (3b, 3c, 3d, 4b, 4d, 5a, 5b) were active in ABTS•+ assay.

All these compounds were found to be moderately active against various bacteria such as (Coagulase negative Staphylococci, Coagulase positive Staphylococci, Enterobacter) and fungi (Candida albicans). Compounds (3a, 3c, 3d, 5b) showed good activity against Coagulase negative Staphylococci and compounds (3a, 3b, 3c and 4d) shows good activity against Candida albicans.

4. Conclusion

The structures of synthesized compounds are well supported by spectral data and elemental analysis. The synthesized compounds were also screened for antioxidant activity (DPPH assay and ABTS•+ assay) and antimicrobial activity (antibacterial and antifungal). The present study demonstrated that these compounds showed mixed activity in DPPH and ABTS•+ assay. Compounds 3c, 3d showed excellent antioxidant activity in DPPH assay. Compounds 5a, 5b showed much better activity in DPPH assay than compounds 3a, 3b which are the precursors of 5a and 5b. It shows that the ribofuranosides 5a and 5b showed much better antioxidant activity than their phenothiazine bases (3a, 3b). This is due to the replacement of H by the sugar moiety (ribosylation). Compounds 3b, 3c, 3d, 4b, 5a, 5b showed good activity in both DPPH and ABTS assays.

The present paper is focused on the synthesis of novel heterocyclic compounds as possible antibacterial and antifungal agents. Compounds 3c and 3d showed antibacterial activity against Coagulase negative Staphylococci which is comparable to vancomycin. Compound 3a is much better than

3.1. Spectral analysis

3.1.1. IR spectra

Compounds (3a-d) showed peaks in region 3385-3260 cm⁻¹ due to N-H stretching vibrations and 1700-1650 cm⁻¹ due to >C=O stretching vibrations which gets shifted to higher frequencies to 3390-3270 cm⁻¹ and 1710-1680 cm⁻¹, respectively, in compounds (4a-d). Compounds (4a-d) also exhibited two intense peaks in region 1560-1540 cm⁻¹ and 1180-1140 cm⁻¹ due to asymmetric and symmetric stretching vibrations of sulfonyl group. Compounds (4a-d) also showed C=S stretching vibrations in region 1095-1077 cm⁻¹.

Absence of stretching vibrations due to >N-H group in compounds (5a-b) showed site of ribosylation, further in compounds (5a-b) bands due to C=O-C linkage of sugar appeared in the region 1170-1165 cm⁻¹.

3.1.2. 1H NMR spectra

All compounds showed multiplet in region δ 8.52-6.06 ppm due to aromatic protons and compounds (3a-d) and (4a-d) also
vancomycin in antibacterial activity against Coagulase negative Staphylococci. All these compounds were found to be moderately active against various bacteria such as (Coagulase negative Staphylococci, Coagulase positive Staphylococci, Enterobacter) and fungi (Candida albicans). Compounds (3a, 3c, 3d, 5b) showed good activity against Coagulase negative Staphylococci and compounds (3a, 3b, 3c and 4d) shows good activity against Candida albicans.

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References