Synthesis and antimicrobial activities of novel 10H-pyrido[3,2-b][1,4]benzo-[b]thiazine ribofuranosides

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The present communication describes the synthesis of 10-acetyl-9-chloro-9-bromo-9,9-dichloro-3-nitro-pyrido[3,2-b][1,4]-benzo[b]thiazines 4, 10H-9-chloro-9-bromo-8,9-dichloro-3-nitro-pyrido[3,2-b][1,4]-benzo[b]thiazines 5, 10H-9-chloro-9-bromo-8,9-dichloro-3-nitro-pyrido[3,2-b][1,4]-benzo[b]thiazine-5-oxide 6. Compounds 5, on refluxing with β-D-ribofuranose-1-acetate-2,3,5-tribenzoate, afford N-(2',3',5'-tri-0-benzoyl-β-D-ribofuranosyl)-9-chloro-9-bromo-8,9-dichloro-3-nitro-pyrido[3,2-b][1,4]-benzo[b]thiazines 7. Compounds 4-7 have been characterized by 1H NMR and elemental analysis and screened for antimicrobial activity.

Perusal of the literature on pharmaceutical studies have been shown to be of immense chemotherapeutic importance and exhibit antihistaminic1, antitiussive2 analgesic3, anticonvulsant4 and antileukemic5 properties. On account of variety of therapeutic applications, a great amount of work has been carried out on the synthesis of 10H-pyrido[3,2-b][1,4]-benzo[b]thiazines6,9. Thus, we thought of synthesizing new 10H-pyrido[3,2-b][1,4]-benzo[b]thiazine and subjected these compounds for antimicrobial screening.

Zinc mercaptide of 2-amino-3-chloro-3-bromo-3,4-dichlorobenzethanol 1 and 2-chloro-3,5-dinitropyridine were refluxed in ethanol, in the presence of anhydrous sodium acetate, to yield 2-amino-3-chloro-3-bromo-3,4-dichlorophenyl-2'-(3',5'-dintio)pyrydylsulphide 2. Compound 2 on condensation with acetic anhydride in the presence of pyridine afforded 2-acetamino-3-chloro-3-bromo-3,4-dichlorophenyl-2'- (3',5'-dintio)pyrydylsulphide 3. When 3 was treated with potassium hydroxide in 1:2 molar ratio, afforded 10H-9-chloro-9-bromo-8,9-dichloro-3-nitro-pyrido[3,2-b][1,4]-benzo[b]thiazine 4, while the same reaction in 1:1 molar ratio gave 10-acetyl-9-chloro-9-bromo-8,9-dichloro-3-nitro-pyrido[3,2-b][1,4]-benzo[b]thiazine 5. Compound 5 on treatment with hydrogen peroxide in ethanol-acetone yielded 10H-9-chloro-9-bromo-8,9-dichloro-3-nitro-pyrido[3,2-b][1,4]-benzo[b]thiazine-5-oxide 6. The pasty mixture of 5 in toluene, on stirring with sugar viz. β-D-ribofuranose-1-acetate-2,3,5-tribenzoate at 155-60°C for 10 hr, in vacuo, gave corresponding ribofuranosides 7 (Scheme 1).

The IR spectral data of 2 and 3 are consistent with their structures. The characteristic absorption band at 675-655 cm⁻¹ in IR spectra of 4-7 was ascribed to C=S-C linkage in 4-7. The carbonyl group absorbed at higher frequency (1710-1700 cm⁻¹) in 4. Compounds 5 and 6 showed a band in the region 3300-3280 cm⁻¹ due to >NH stretching vibrations. The sharp and strong absorption bands appeared between 1590-1575 and 1410-1380 cm⁻¹, were due to asymmetric and symmetric stretching vibrations of -NO₂ group, respectively, in 4-7.

In compounds 7, the >NH band vanished completely, suggesting its ribosylation. The band due to C=O and C-O-C appeared at 1750-1745 cm⁻¹ and 1185-1020 cm⁻¹, respectively.

The 1H NMR spectra of 2 and 3 are consistent with their structures. In 1H NMR spectra the -COCH₃ protons in 4 appeared at δ 3.53-3.57 as a singlet. In compounds 4-6, the multiplet observed in the range δ 6.31-7.64 corresponded to phenyl protons, and protons in pyridine nucleus resonated in the region δ 8.25-8.91. The >NH proton appeared at δ 8.07-8.20 in 1H NMR spectra of 5 and 6, the same signal disappeared in 7, establishing its ribosylation. The aromatic protons in ribofuranosides 7 appeared as a multiplet at δ 6.89-8.91. C (׳)-H and >CH₂ protons of sugar moiety gave multiplet in the region δ 4.45-4.82, while C (׳)-H and C (׳)-H signals appeared in the region δ 5.69-5.51 as multiplet. The singlet at δ 6.43 is attributed to C (׳)-H.

Antimicrobial activity

The antimicrobial activity assay was carried out by the paper disc method¹ against different bacteria and fungi at the conc. of 100 µg per disc using Streptomycin and Mycostatin, respectively, as the reference compounds. All the compounds were found to be moderately active against various bacteria, such as,
Escherichia coli (gram-negative) and Staphylococcus aureus (gram-positive) and fungi (Aspergillus niger, Aspergillus flavus and Fusarium oxysporum). A close look, on the activity indices reveals that the ribofuranosides are better antimicrobial agents than their parent bases.

Experimental Section
Melting points of all the compounds were determined on an electrothermal apparatus (capillary method) and are uncorrected. IR spectra were recorded on a NICOLET MEGNA FT-IR 550 spectrometer using potassium bromide pellets; and $^1$H NMR spectra on a FX 90Q JEOL type spectrometer in CDCl$_3$/DMSO-$d_6$ using TMS as internal standard (chemical shifts in $\delta$ ppm). TLC were performed using silica gel “G” and the spots were visualized by exposure to iodine vapours.

Synthesis of 2-amino-3-chloro/3-bromo/3,4-dichlorophenyl-2’-(3’,5’-dinitro) pyridylsulphides 2a-c. A mixture of zinc mercaptide of substituted benzethiol (0.005 mole), 2-chloro-3,5-dinitropyridine (0.01 mole) and anhydrous sodium acetate (0.025 mole) in absolute ethanol (1.5 mL), was refluxed for 4 hr on a water-bath. The solid obtained on cooling, was filtered, washed with water, dried and recrystallized from ethanol.

Synthesis of 2-acetylamino-3-chloro/3-bromo/3,4-dichlorophenyl-2’-(3’,5’-dinitro) pyridylsulphides 3a-c. Compound 2 (0.005 mole) in pyridine (0.4 mL) and acetic anhydride (4.8 mL) were refluxed on a water-bath for 3 hr. The reaction contents were cooled to obtain the product. It was filtered, washed with water, dried and recrystallized from benzene, to give 3. The characterization data of 3a-c are given Table I.

Synthesis of 10-acetyl-9-chloro/9-bromo/8,9-dichloro-3-nitro-pyrido[3,2-b][1,4]benzothiazines 4a-c. In stirred ethanolic solution of potassium hydroxide (0.28 g), acetone (10 mL) was added under nitrogen atmosphere, followed by addition of
compound 3 (0.0005 mole). The mixture was heated on a water-bath until the original volume was reduced to half (5 mL), and then water (5 mL) was added. The yellow solid product thus obtained was collected by filtration, which was washed with water, dried and recrystallized from isopropanol to give 4. The characterization data of 4a-c are given in Table I.

**Synthesis of 10H-9-chloro/9-bromo/8,9-dichloro-3-nitropyrido[3,2-b][1,4]benzo[b]thiazines 5a-c.**

To a stirred mixture of compound 3 (0.0005 mole) in acetone (6.5 mL), potassium hydroxide (0.62 g) was added and it was refluxed for 3 hr. Acetone was distilled off and water (7.0 mL) was added to the residue. The product thus obtained was collected by filtration, washed with water, dried and recrystallized from benzene to give 5. The characterization data of compounds 5a-c are given in Table I.

**Synthesis of 10H-9-chloro/9-bromo/8,9-dichloro-3-nitropyrido[3,2-b][1,4]benzo[b]thiazin-5-oxides 6a-c.**

To a solution of compound 5 (0.002 mole) in warm solution of ethanol (7.5 mL) and acetone (15 mL), H₂O₂ (30% w/v; 0.0021 mole) was added and mixture was refluxed for 3 hr. The colour of the solution darkened during the refluxing. The solvent was removed by distillation and the product was recrystallized from ethanol to give 6. The characterization data of 6a-c are given in Table I.

**Synthesis of N-(2',3',5'-tri-O-benzoyl-2,3,5,6-tetrahydro-5H-pyran-3-yl)-9-chloro/9-bromo/8,9-dichloro-3-nitropyrido[3,2-b][1,4]benzo[b]thiazin-5-oxides 7a-c.**

To a solution of 5 (0.002 mole) in minimum toluene, β-D-ribofuranose-1-acetate-2,3,5-tri-60°C, 15 min. The vacuo was removed and the reaction mixture was protected from moisture by fitting guard tube. Stirring was further continued for 10 hr and vacuum was applied for 10 min at every hour. The viscous mass thus obtained was dissolved in methanol, boiled for 10 min and cooled to room temperature. The reaction mixture was filtered. The methanol was removed by distillation under reduced pressure. The viscous residue thus obtained was dissolved in ether, filtered, concentrated and kept in a refrigerator overnight to get crystalline ribofuranosides 7. The characterization data of 7a-c are given in Table I.

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**Table I — Characterization data of compounds 4a-c, 5a-c, 6a-c and 7a-c**

<table>
<thead>
<tr>
<th>Compd</th>
<th>R²</th>
<th>R³</th>
<th>m.p. °C</th>
<th>Yield (%)</th>
<th>¹H NMR (δ from TMS)</th>
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<tbody>
<tr>
<td>4a</td>
<td>Cl</td>
<td>H</td>
<td>203</td>
<td>69</td>
<td>6.40-7.41 (3H, m, P-H), 3.54 (3H, COCH₃), 8.31, 8.77 (2H, s, s, Py-H)</td>
</tr>
<tr>
<td>4b</td>
<td>Br</td>
<td>H</td>
<td>200</td>
<td>65</td>
<td>6.32-7.39 (3H, m, Ph-H), 3.53 (3H, COCH₃), 8.28, 8.69 (2H, s, s, Py-H)</td>
</tr>
<tr>
<td>4c</td>
<td>Cl</td>
<td>Cl</td>
<td>225</td>
<td>66</td>
<td>6.41-7.49 (2H, 2d, Ph-H), 3.57 (3H, COCH₃), 8.36, 8.89 (2H, s, s, Py-H)</td>
</tr>
<tr>
<td>5a</td>
<td>Cl</td>
<td>H</td>
<td>271</td>
<td>70</td>
<td>6.79-7.38 (3H, m, Ph-H), 8.11 (1H, &gt;NH), 8.43, 8.87 (2H, s, s, Py-H)</td>
</tr>
<tr>
<td>5b</td>
<td>Br</td>
<td>H</td>
<td>304</td>
<td>71</td>
<td>6.60-7.35 (3H, m, Ph-H), 8.07 (1H, &gt;NH), 8.37, 8.83 (2H, s, s, Py-H)</td>
</tr>
<tr>
<td>5c</td>
<td>Cl</td>
<td>Cl</td>
<td>312</td>
<td>69</td>
<td>6.45-7.53 (2H, 2d, Ph-H), 8.11 (1H, &gt;NH), 8.45, 8.91 (2H, s, s, Py-H)</td>
</tr>
<tr>
<td>6a</td>
<td>Cl</td>
<td>H</td>
<td>325</td>
<td>63</td>
<td>6.36-7.52 (3H, m, Ph-H), 8.13 (1H, &gt;NH), 8.33, 8.78 (2H, s, s, Py-H)</td>
</tr>
<tr>
<td>6b</td>
<td>Br</td>
<td>H</td>
<td>315</td>
<td>71</td>
<td>6.31-7.42 (3H, m, Ph-H), 8.09 (1H, &gt;NH), 8.25, 8.71 (2H, s, s, Py-H)</td>
</tr>
<tr>
<td>6c</td>
<td>Cl</td>
<td>Cl</td>
<td>332</td>
<td>68</td>
<td>6.40-7.64 (2H, m, 2d, Ph-H), 8.29 (1H, &gt;NH), 8.37, 8.80 (2H, s, s, Py-H)</td>
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<tr>
<td>7a</td>
<td>Cl</td>
<td>H</td>
<td>165</td>
<td>63</td>
<td>6.91-8.79 (20H, m, Ph &amp; Py-H)</td>
</tr>
<tr>
<td>7b</td>
<td>Br</td>
<td>H</td>
<td>181</td>
<td>67</td>
<td>6.89-8.91 (20H, m, Ph &amp; Py-H)</td>
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<tr>
<td>7c</td>
<td>Cl</td>
<td>Cl</td>
<td>177</td>
<td>71</td>
<td>7.02-8.89 (19H, m, Ph &amp; Py-H)</td>
</tr>
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</table>

The elemental analyses (C, H and N) of all these compounds were found to be in reasonable agreement with the calculated values.