Synthesis and microbial assay of maltosyl tetrazino benzothiazoles

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ABSTRACT

A new series of 3-oxo-4-hepta-O-benzoyl-β-D-maltosyl-(1,2,4,5)-tetrazino-(2,1b)-benzothiazoles by the oxidative cyclisation of 1-hepta-O-benzoyl-β-D-maltosyl-4-benzothiazolyl semicarbazides. The identities of these newly synthesized compounds have been established on the basis of usual chemical transformations and IR, 1H NMR, 13C NMR and Mass spectral studies and these compounds were screened for their antibacterial activity against pathogens like E. coli, S. aureus, P. vulgaris, S. typhi, K. pneumoniae, Ps. aeruginosa and for antifungal activity against T. harzianum and Verticillium species to get potent bioactive molecule.

Keywords: Maltosyl benzothiazololyl semicarbazides, maltosyl tetrazino benzothiazoles, oxidative cyclisation, antimicrobial activity.

INTRODUCTION

In recent years, we have witnessed significant improvements in the field of synthetic carbohydrate chemistry[1]. It seems, reasonable to support that an exhaustive investigation of N-maltosides and related compounds might result in the discovery of wide range of biological activity[2]. Isocyanates of sugars are one of the versatile reagents in the field of synthetic carbohydrate chemistry. Many of these derivatives have been found to possess wide applications in industry as carbohydrate base detergent and in medicine as anticancer and antifungal agents. The maltosylated derivatives show great potential in biological process and in medicinal chemistry. They act as bacteriostatic agent[3], antifungal agent[4] and antitumour agent[5]. Heterocycles bearing thiazole, sulphur and nitrogen moieties constitute the core structure of a number of pharmacologically and biologically active interesting compounds. Semicarbazides are valuable building blocks for the synthesis of heterocycles. Biologically active semicarbazide derivatives use as antibacterial[6], antifungal[7], anticonvulsant[8], antimicrobial[9] and antitumor agents[10]. Tetrazines also possess a wide range of antiviral and antitumor properties and have been widely used as pesticides and herbicides[11].

These findings encouraged us to explore the synthesis and to examine antibacterial and antifungal properties of new synthesized maltosyl tetrazino benzothiazoles in a trial to obtain new derivatives with higher activity than that of the parent compounds.

MATERIALS AND METHODS

General
The reagent grade chemicals were obtained from commercial sources and purified by either distillation or recrystallization before use. Melting points of all synthesized compounds were determined using open capillary tube on Mac digital melting point apparatus and were uncorrected. IR spectra were recorded in solid phase KBr disks on SHIMADZU IR affinity-1 FTIR spectrometer and 1H NMR spectra in CDCl3 on Bruker DRX-300 of NMR spectrometer 300 MHz. The Mass spectra were recorded on Waters UPLC-TQD Mass Spectrometer. Optical
rotations were measured on Equip-Tronics EQ 800 Digital Polarimeter in CHCl₃. Purity of synthesized compounds has been checked by thin layer chromatography. It was performed on E. Merck precoated silica gel plates.

Starting material

**Synthesis of 1-hepta-O-benzoyl-β-D-maltosyl-4-benzothiazolyl semicarbazides**

A acetonitrile solution of hepta-O-benzoyl-β-D-maltosyl isocyanate (0.0025M, 2.73g in 20 ml) was mixed with acetonitrile solution of 2-hydravazo benzothiazole (0.0025M, 0.41g in 10 ml) and mixture after shaking for sometime was refluxed on water bath for 3.30 h. Acetone was distilled off to obtained sticky residue. This residue was triturated several times with petroleum ether to afford a light coloured solid.

**General Procedure for Synthesis of 3-oxo-4-hepta-O-benzoyl-β-D-maltosyl(1,2,4,5)-tetrazio(2,1b)-benzothiazole (3a-g)**

Slurry of 1-heptaoxybenzoyl-β-D-maltosyl-4-benzothiazolyl semicarbazide (1a) (2.52g, 0.002 M in 2 mL) was mixed with acetone solution of 2-hydrazino benzothiazole (0.0025M, 2.73g in 20 ml) and mixture after shaking for sometime was kept at room temperature for 24 h. The separated solid was crystallized from ethanol. It was acetic acid to litmus and on determination of equivalent weight, found to be dihydrobromide (2a). It on basification with dilute ammonium hydroxide solution afforded a free base (3a). It was recrystallized from aqueous ethanol (Scheme-I).

**RESULTS AND DISCUSSION**

All products were crystallized from ethanol before recording the physical data (Table-1). The purity of compounds was checked by TLC. The spectral analysis 13-15 IR, 1H NMR, 13C NMR and Mass spectra of the product were observed. Optical rotation of the product was also recorded. All the compounds have been screen for both antimicrobial and antifungal activity using cup plate agar diffusion method 14-15 by measuring the inhibition zone in mm. Tetracycline (100 µg/ml) was used as standard for antibacterial activity and Fluconazole (100 µg/ml) as standard for antifungal activity. Antibacterial studies of these compounds indicated that compounds 3a and 3d were found to be active against *E.coli* and rest of were found to be moderately active. Compound 3a, 3b and 3g exhibited most significant activity against *S.aureus* and compound 3a, 3b and 3d towards *Pseudomonas*. All the other compounds exhibited low to moderate activity. The results of antifungal activities are also tabulated in Table2. Almost all compounds are most effectively active against *Thrichodermora harzianum* and actively inhibited *Verticillium species*. (Table-2).

**Spectral Data**

3a) IR (KBr, cm⁻¹) : ν 3064 (Ar-H stretch), 2958 (Aliphatic C-H stretch), 1730 (C=O), 1598 (C=N), 1450 (C-N), 1174 (C-O), 1097 & 937 (characteristic of maltose unit), 711 (C-S); 1H NMR (CDCl₃, ppm): δ 8.113-7.760 (39H, m, Ar-H), 6.276-3.959 (14H, m, maltosyl ring protons), 5.540 (s, 1H, NH); 13C NMR (300 MHz, CDCl₃): δ 165.86 (C=O), 133.38-129.36 (7COC₆H₄), 128.29-119.35 (6 Ar-C benzothiazole), 96.97 (C=N), 78.15-62.85 (maltosyl ring-C); Mass (m/z): M⁺ 1258, 1053, 579, 353, 151 (100%); Anal. Calcd. for C₁₈₀H₁₄₀N₁₈S : C, 65.81; H, 4.29; N, 4.45; S, 2.41%. Found : C, 65.78; H, 4.26; N, 4.41; S, 2.52%.

3b) IR (KBr, cm⁻¹) : ν 3064 (Ar-H stretch), 2958 (Aliphatic C-H stretch), 1732 (C=O), 1598 (C=N), 1448 (C-N), 1174 (C-O), 1099 & 937 (characteristic of maltose unit), 711 (C-S), 686 (C-Cl); 1H NMR (CDCl₃, ppm): δ 8.033-7.188 (38H, m, Ar-H), 6.181-3.841 (14H, m, maltosyl ring protons), 5.530 (s, 1H, NH); 13C NMR (300 MHz, CDCl₃): δ 165.87 (C=O), 133.38-129.87 (7COC₆H₄), 129.69-120.76 (6 Ar-C benzothiazole), 96.98 (C=N), 78.16-62.85 (maltosyl ring-C); Mass (m/z): M⁺ 1292, 1054, 1011 (100%), 579, 243; Anal. Calcd. for C₁₈₀H₁₄₀N₁₈SOCI : C, 64.06; H, 4.10; N, 4.33; S, 2.47. Found : C, 64.01; H, 4.04; N, 4.41; S, 2.41%.

3e) IR (KBr, cm⁻¹) : ν 3064 (Ar-H stretch), 2956 (Aliphatic C-H stretch), 1730 (C=O), 1598 (C=N), 1450 (C-N), 1174 (C-O), 1097 & 937 (characteristic of maltose unit), 711 (C-S); 1H NMR (CDCl₃, ppm): δ 8.110-7.199 (38H, m, Ar-H), 6.274-3.958 (14H, m, maltosyl ring protons), 5.541 (s, 1H, NH), 1.256 (s, 3H, CH₃); 13C NMR (300 MHz, CDCl₃): δ 165.88 (C=O), 133.37-129.69 (7COC₆H₄), 128.54-128.30 (6 Ar-C benzothiazole), 90.07 (C=N), 78.23-62.85 (maltosyl ring-C), 23.11 (CH₃); Mass (m/z): M⁺ 1272, 1053, 1011 (100%), 579, 206; Anal. Calcd. for C₁₈₀H₁₄₀N₁₈S : C, 66.03; H, 4.40; N, 4.40; S, 2.51. Found : C, 65.98; H, 4.35; N, 4.38; S, 2.49%.
Scheme for synthesis shown as follows:

\[
\text{1-hepta-O-benzoyl-} \beta \text{-D-maltosyl-4-benzothiazolyl semicarbazides 1(a-g)}
\]

\[
\text{3-Oxo-4-hepta-O-benzoyl-} \beta \text{-D-maltosyl-(1,2,4,5)-tetrazino-(2,1b)-benzothiazole hydrobromides 2(a-g)}
\]

\[
\text{3-Oxo-4-hepta-O-benzoyl-} \beta \text{-D-maltosyl-(1,2,4,5)-tetrazino-(2,1b)-benzothiazoles 3(a-g)}
\]

Scheme-I

Where, Bz = COC₆H₅, R = a) Hydrogen, b) 4-Chloro, c) 5-Chloro, d) 6-Chloro, e) 4-methyl, f) 5-methyl, g) 6-methyl.

Table 1: Physial characterisation of 3-oxo-4-hepta-O-benzoyl-β-D-maltosyl-(1,2,4,5)-tetrazino(2,1b)-benzothiazoles (3a-g)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Compd.</th>
<th>Yield (g %)</th>
<th>m. p. (°C)</th>
<th>Elemental Analysis Found (Required)</th>
<th>[α]D²⁸ (c, CHCl₃)</th>
<th>Rf Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3a</td>
<td>2.03 (81)</td>
<td>134-136</td>
<td>N 4.41 (4.45) S 2.52 (2.54)</td>
<td>+90° (0.50)</td>
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<td>2.</td>
<td>3b</td>
<td>2.01 (78)</td>
<td>141-142</td>
<td>N 4.28 (4.33) S 2.43 (2.47)</td>
<td>+102° (0.53)</td>
<td>0.30</td>
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<tr>
<td>3.</td>
<td>3c</td>
<td>1.93 (75)</td>
<td>138-139</td>
<td>N 4.30 (4.33) S 2.45 (2.47)</td>
<td>-89° (0.51)</td>
<td>0.27</td>
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<tr>
<td>4.</td>
<td>3d</td>
<td>2.06 (80)</td>
<td>133-135</td>
<td>N 4.26 (4.33) S 2.41 (2.47)</td>
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<tr>
<td>5.</td>
<td>3e</td>
<td>1.96 (77)</td>
<td>145-146</td>
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<td>-98° (0.52)</td>
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<td>6.</td>
<td>3f</td>
<td>1.85 (73)</td>
<td>148-149</td>
<td>N 4.34 (4.40) S 2.47 (2.51)</td>
<td>+127° (0.50)</td>
<td>0.38</td>
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<tr>
<td>7.</td>
<td>3g</td>
<td>2.18 (86)</td>
<td>143-144</td>
<td>N 4.37 (4.40) S 2.44 (2.51)</td>
<td>+119° (0.51)</td>
<td>0.34</td>
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</table>

Table 2: Antimicrobial activities of newly synthesized 3-oxo-4-hepta-O-benzoyl-β-D-maltosyl-(1,2,4,5)-tetrazino(2,1b)-benzothiazoles (3a-g).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>E. coli</th>
<th>S. Aureus</th>
<th>P. vulgaris</th>
<th>P. aeruginosa</th>
<th>Klebsiella species</th>
<th>T. harzianum</th>
<th>Verticillium species</th>
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<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>30</td>
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</table>

**Including the well diameter of 6 mm. Zone of inhibition in mm (15 or less) resistant (16-20 mm) moderate and (more than 20 mm) sensitive.**
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REFERENCES