SHORT COMMUNICATION

Synthesis of the 4’-desmethoxy analogue of RU79115*

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Abstract: The synthesis, and biological activity in vitro of the 4’-desmethoxy analogue (3) of RU 79115 (2) is described. Comparison of the biological activity of the two analogues clearly indicated the importance of the 4’-methoxy group in conferring good gyrase B inhibitory activity as well as antibacterial activity.

Keywords: structure-activity, inhibitor, gyrase B, antibacterial, sugar, L-arabinose, coumarin.

INTRODUCTION

In a previous report from these laboratories¹ the synthesis and structure–activity relationship of a series of coumarin inhibitors (1) of DNA gyrase B bearing various 5’,5’-dialkylnoviose, and in particular, the most potent derivative RU79115 (2) having 5’,5’-spirocyclopentyl moiety were described. So far, the role and importance of the 4’-methoxy substituent in the noviose moiety in the binding of coumarin drugs to the active site of gyrase B and its influence regarding antibacterial properties have not been studied. Early crystallographic structures of novobiocin and clorobiocin in a

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* Dedicated to Professor Živorad Čeković on the occasion of his 70th brithday
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complex with 24 kDa N-terminal fragment of gyrase B indicated that the 4’-methoxy group is involved in hydrophobic interactions with the surrounding amino acid residues of the gyrase B protein, as well as in hydrogen bonding to the side chain of Asn-46.\textsuperscript{2,3} Further, the 5,5-dimethylcyclohexyl noviose mimic gave some indications regarding the importance of the 4’-methoxy substituent.\textsuperscript{4} In order to obtain unambiguous answers to the role of the 4’-methoxy substituent, it was decided to prepare the 4’-desmethoxy analogue of RU79115 and compare directly their activities.

CHEMISTRY

The silyl protected 3,4-\textit{O}-isopropylidene-L-arabino-1,5-lactone (7) was chosen as a key intermediate that could provide access to a 4-desmethoxy noviose or to a potential 4-hydroxy and 4-alkyloxy noviose series. So far, two synthetic approaches toward lactone 8 have been described.\textsuperscript{5} However, neither of them was suitable for scale-up synthesis. Finally, a five-step synthetic sequence with good overall yield was established (Scheme 1). Starting from L-arabinose (4), the corresponding benzyl glycoside was protected as acetonide according to a literature procedure.\textsuperscript{6} Silylation of the remaining hydroxyl group under standard conditions provided the fully protected arabinose 6. Catalytic hydrogenation removed quantitatively the benzyl group and the corresponding lactol was subjected to Swern oxidation to provide the desired silyl-protected lactone 7. Deprotection of the silyl group was performed under the usual conditions with Bu\textsubscript{4}NF to give the hydroxy lactone 8.

The free hydroxy group of the desilylated lactone 8 (Scheme 2) was then converted to the triflate 9, which was subjected to reductive conditions with lithium iodide trihydrate\textsuperscript{7} to afford the 2-deoxylactone 10 in moderate yield. Lactone opening of 10 with a Grignard reagent of 1,4-dibromobutane provided the diol 11 which was oxidized with two equivalents of Py·SO\textsubscript{3} directly to the corresponding lac-
This was followed by reduction with DIBALH to provide the lactol 12 which was ready for coupling with the coumarin part. In general, the two-step sequence from diol 11 to lactol 12 gave better yields than the one step oxidation of diol 11 with one equivalent of oxidant. Glycosylation of lactol 12 with 7-hydroxy-8-methyl-4-benzhydryloxycoumarin (13) was effected under Mitsunobu conditions in DMF to give exclusively the α-anomer 14. Deprotection of the benzhydryl group of 14 by catalytic reduction gave the free 4-hydroxycoumarin derivative in quantitative yield. This readily underwent C-acetylation at the 3-position with acetic anhydride and in the presence of DMAP to afford the corresponding coumarin methyl ketone 15. The acetonide of 15 was easily deprotected with trifluoroacetic acid/water and the diol was subsequently converted to the carbonate 17 with 1,1'-carbonyldimidazole. The predominantly regioselective opening of the carbonate with O-propargylhydroxylamine in the presence of lithium trifluoromethanesulfonate and accompanied with transoximation of the keto functionality afforded a regio-meric mixture of 3'- and 2'-N-propargyloxycarbamates in the proportion 3.5:1. This mixture was not separated but was subjected once again to transoximation at the 3-acetyl group with an excess of O-methyl hydroxylamine in ethanol. Finally, the 4'-desmethoxy analogue of RU79115 (2) was separated from its 2'-N-propargyloxycarbamate regioisomer by chromatography on silica gel utilising a mixture CH2Cl2-EtOAc-AcOH in the ratio 80:20:1 as eluent.

**BIOLOGICAL RESULTS**

Table I shows the inhibition in the supercoiling activity of *S. aureus* DNA gyrase by novobiocin, RU79115 (2) and the corresponding 4'-desmethoxy analogue 3. Clearly, the absence of the 4'-methoxy group in the noviose part leads to loss of inhibitory DNA gyrase B supercoiling activity and to a loss of the antibacterial properties by two orders of magnitude. Not only the hydrophobic/hydrogen bonding interactions of 4'-methoxy group with the surrounding amino acid residues of gyrase B protein are important in supercoiling inhibition of DNA gyrase, but also the 4'-methoxy substituent plays an important role in intracellular uptake of the coumarin analogues.

**TABLE I. In vitro inhibitory activity of 2 and 3 against *S. aureus* DNA gyrase B supercoiling activity (IC50),a and selected in vitro antibacterial activity (MIC).b**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Novobiocin</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 nov/IC50 comp</td>
<td>1</td>
<td>2.6</td>
<td>0.33</td>
</tr>
<tr>
<td>MICb  <em>S. aureus</em> 011HT3</td>
<td>≤ 0.04</td>
<td>≤ 0.04</td>
<td>1.2</td>
</tr>
</tbody>
</table>

a) IC50 was determined for gyrase B of *S. aureus* against novobiocin (0.5 μg/mL) as reference. For the details see Ref. 6; b) MIC, Minimal Inhibitory Concentrations (μg/mL) were measured by using a twofold broth microdilution after 24 h incubation.

In conclusion a synthetic route that leads to 4'-desmethoxy derivatives of noviose analogues has been developed. Silyl lactone 7 could also be a useful inter-
mediate allowing access to 4’-hydroxy or 4’-O-alkyls substituted noviose series. Furthermore, future design of novobiocin type inhibitors possessing noviose or noviose mimics should include the 4’-methoxy group or the corresponding hydrophobic isostere in the noviose part in order to confer good antibacterial properties of the analogues.

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IZVOD

СИНТЕЗА 4’-ДЕМЕТОКСИ-АНАЛОГА RU79115

БРАНИСЛАВ МУШИЋКИ, АНА МАРИЈА ПЕРИЋ, НИКОЛ ТЕСО, МИШЕЛ КЛИШ

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У раду је описана синтеза и биолошка активност 4’-деметоксис аналога (3) једињења RU 79115 (2). Упоређивање биолошке активности ова два једињења јасно показује на важност и утицај 4’-метоксис групе у погледу њихове инхибиторске активности као и антибактеријске активности.

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REFERENCES


