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Improved Antibacterial Activities of Coumarin Antibiotics Bearing 5′,5′-Dialkylnoviose: Biological Activity of RU79115

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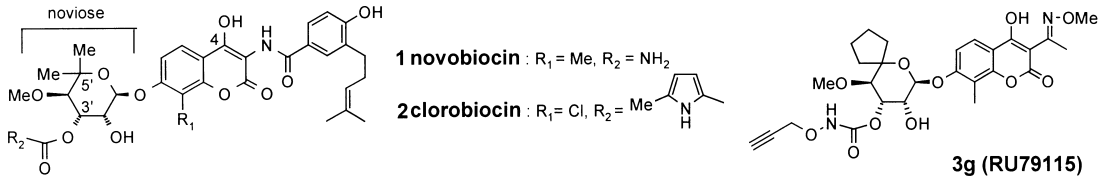
Abstract—A new series of coumarin inhibitors of DNA gyrase B bearing a *N*-propargyloxycarbamate at C-3′ of various 5′,5′-dialkylnoviose, including RU79115, were synthesised and their antibacterial activities have been delineated. Introduction of dialkyl substituents at 5′5′-position of noviose leads to coumarin analogues with improved in vitro and in vivo antibacterial activity.

Introduction

In the past several years we have seen intensive efforts by several research groups, including ours,[1] in finding potential inhibitors of bacterial DNA gyrase, a DNA topoisomerase that catalyses a variety of interconversions between topological isomers of DNA.[2] Specifically, the subunit of DNA gyrase, gyrase B, was identified as a target for two structurally different classes of natural products: coumarins (novobiocin **1**, clorobiocin **2**)[3] and cyclothialidines,[4] as well as synthetic triazines.[5] Our work in this field has been directed toward the coumarin antibiotics and their structural modification. Recently, we disclosed the marked influence of 5′,5′-dimethyl group of noviose in determining the antibacterial spectrum of coumarin antibiotics.[1d] Furthermore, comparison of gyrase B aminoacid sequences from twelve different Gram-positive strains[6] indicated that enterococcal and streptococcal gyrase B activity could be more influenced by the 5′,5′-dialkyl group of noviose compared to that of gyrase B of *E. coli*. (Fig. 1). Their hydrophobic pockets, consisting of Val 94 and Phe 95, are slightly larger compared to that of gyrase B of *E. coli*.[7,8] As we have developed efficient stereoselective synthetic approaches to 5′-monoalkyl and 5′,5′-dialkylnoviose derivatives,[9] these provided us with a useful tool to probe structure–activity relationships for this part of the molecule.

In this report we disclose the synthesis, in vitro and in vivo biological activities of a novel series of coumarin congeners incorporating various 5′,5′-dialkylnoviose. Also, the pharmacological profile of RU79115, the most potent candidate within the series is reported.



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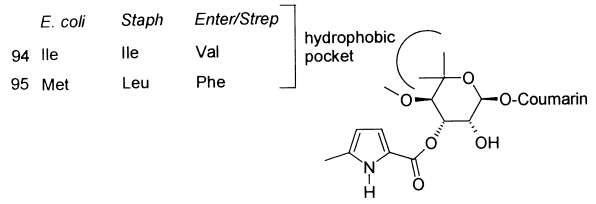


Figure 1. Comparison of the amino acid sequences of the hydrophobic pocket surrounding 5′,5′-dimethyl group of noviose part in different gyrase B.

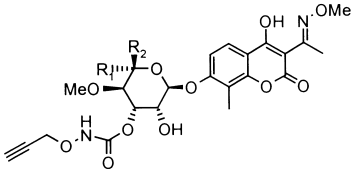
Chemistry

General synthetic route towards 5′,5′-dialkylnoviosyl coumarin antibiotics is illustrated by the preparation of 5′,5′-spirocyclopentylnoviose analogue **3g** (RU79115) (Scheme 1). The noviose derivative[9] **4** was transformed to the corresponding 2′,3′-noviose carbonate **5** with 1,1′-carbonyldiimidazole in dichloroethane at reflux in 67% yield. The carbonate was then coupled with coumarin building block **6**[1e] under Mitsunobu conditions to afford the α-glycoside **7** as the major product (80% yield), easily separable by chromatography from β-glycoside (7% yield). The benzhydryl protecting group in **7** was readily removed by hydrogenolysis and the 3-acetyl functionality in 4-hydroxycoumarin was introduced by the base catalysed C-3 acylation of coumarin part in dichloromethane in the presence of DMAP to afford **8** in quantitative yield.[1c] Opening of the carbonate **8** under thermodynamic conditions with excess *O*-propargylhydroxylamine in pyridine in the presence of lithium perchlorate or lithium triflate, provided a mixture of 3′- **9a** and 2′-*N*-propargyloxycarbamate **9b** in the ratio 3:1. The regioisomers were separated by chromatography using CH_2Cl_2:EtOAc:AcOH 80:20:1 as eluent. The opening of the carbonate was accompanied by formation of *O*-propargyloxime at C-3 acetyl group of the coumarin. This oxime could be easily exchanged for methoxy **3** by reaction with excess of *O*-methylhydroxylamine in ethanol at room temperature.

Biological Results and Discussions

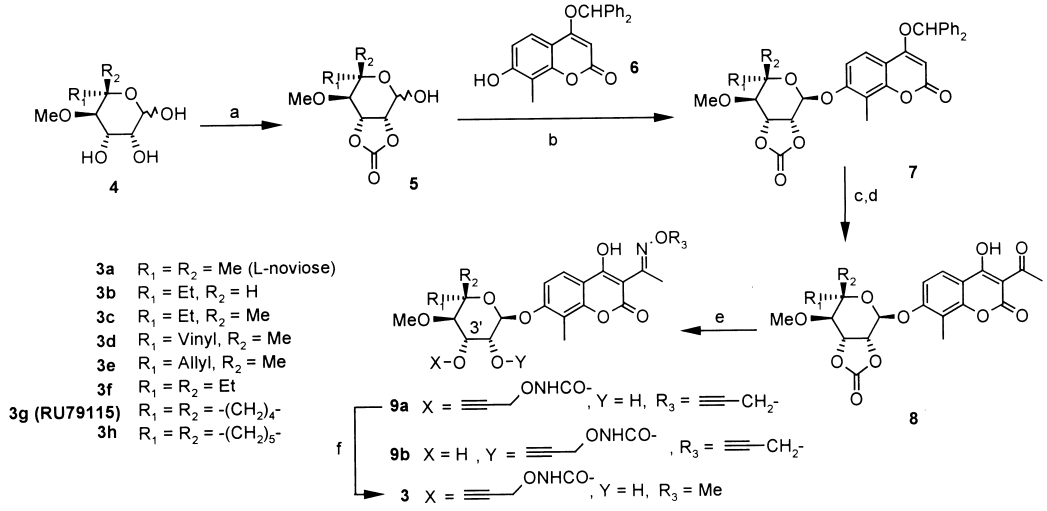
The in vitro profiles of the inhibition of the supercoiling and antibacterial activities of *S. aureus* DNA gyrase by novobiocin, clorobiocin and the novel series of coumarin inhibitors **3b–h** possessing 3′-*N*-propargyloxycarbamate on the modified sugar noviose are shown in Table 1. Comparison of the inhibitory potencies between the noviose analogue (**3a**)[1f] with those containing 5′,5′-dialkylnoviose (**3c–h**), reveals that the introduction of bulkier alkyl substituents at the C-5′ of noviose does not produce significant change in their supercoiling inhibitory activities. The dialkyl derivative (**3c**) seems to display better inhibition of the negative supercoiling of gyrase B than 5′-monoalkyl counterpart (**3b**).

3a $R_1 = R_2 =$ Me (L-noviose)
3b $R_1 =$ Et, $R_2 = H$
3c $R_1 =$ Et, $R_2 =$ Me
3d $R_1 =$ Vinyl, $R_2 =$ Me
3e $R_1 =$ Allyl, $R_2 =$ Me
3f $R_1 = R_2 =$ Et
3g $R_1 = R_2 = -(CH_2)_4-$
3h $R_1 = R_2 = -(CH_2)_5-$



Regarding the antibacterial properties, improved anti-enterococcal activity was observed with the 5′,5′-dialkylnoviose series. Low antibacterial activity of analogue **3b** (MIC from 0.3–40 μg/mL) lacking the C-5′ axial methyl group compared to **3c** (MIC from ≤0.04–0.3 μg/mL) confirms our previous findings observed within the rhamnose series[1e] that the presence of a C-5′ axial substituent is important in conferring good antibacterial activity.

Further in vitro and in vivo pharmacological activities of **3g** (RU79115) are presented in Tables 2–5. Against staphylococci (Table 2), whatever their phenotype of resistance, RU79115 showed MIC_{50}'s 7.5 times lower than eperezolid[10] and far lower than vancomycin (0.08, 0.6 and 1.2 μg/mL, respectively).



Scheme 1. Reagents and conditions: (a) Im_2CO, $ClCH_2CH_2Cl$ reflux, 67%; (b) PPh_3, $EtO_2CN=NCO_2Et$, DMF, rt, 80%; (c) H_2, Pd-C/10%, THF, rt, quant; (d) Ac_2O, DMAP, CH_2Cl_2, 0 °C, quant; (e) $HC{\equiv}CCH_2ONH_2$-HCl, $LiClO_4$, rt, quant; (f) $MeONH_2$-HCl, KOAc, EtOH, rt, 90%.

Table 1. In vitro activity of coumarin inhibitors against *S. aureus* DNA gyrase supercoiling (IC_{50}),[a] and selected in vitro antibacterial activity (MIC)[b]

Compound	Ratio $\frac{IC_{50}nov}{IC_{50}comp}$ [a]	MIC (μg/mL)					
		S.aureus 011HT3	*S. aureus* 011GO64 OfloOxaEry-R	*S. aureus* 011HT1 Nov-R	*S. epidermidis* 012GO42 Oxa-R	*S. pyogenes* 02A1UC1	*E. faecium* 02D31P2 VanTeiEry-R
Novobiocin	1	≤0.04	≤0.04	10	≤0.04	0.3	0.6
Clorobiocin	1.7	≤0.04	ND	0.6	ND	≤0.04	≤0.04
3a	12.5	0.16	0.16	5	0.08	0.6	2.5
3b	2.6	0.3	0.6	20	0.3	2.5	40
3c	11.1	≤0.04	≤0.04	0.3	≤0.04	0.08	0.15
3d	2.6	≤0.04	0.3	2.5	0.15	1.2	1.2
3e	2.6	≤0.04	≤0.04	1.2	≤0.04	0.3	0.6
3f	4.0	≤0.04	0.08	2.5	≤0.04	0.6	0.6
3g (RU79115)	2.6	≤0.04	≤0.04	1.2	≤0.04	0.15	0.15
3h	2.6	≤0.04	0.15	0.6	≤0.04	0.6	0.15

[a]IC_{50} was determined for gyrase B of *S. aureus* against novobiocin (0.5 μg/mL) as reference. For the details see ref 1f.
[b]MIC, Minimum Inhibitory Concentrations (μg/mL) were measured by using a 2-fold broth microdilution after 24 h incubation. Particular phenotype of resistance (-R) of the tested bacterial strains were mentioned: Oflo for ofloxacin, Oxa for oxacillin, Ery for erythromycin, Nov for novobiocin, Tei for teicoplanin, Van for vancomycin. Otherwise, strains were fully susceptible.

Table 2. In vitro anti-staphylococcal activity of RU79115 (MIC μg/mL)

	VAN	Eperezolid	RU79115
S. spp[a]	[91][d]	[91]	[91]
GM[b]	1.15	0.74	0.099
MIC90	2.5	1.2	0.15
S. spp Oxa-R	[66]	[66]	[66]
GM	1.16	0.73	0.097
MIC90[c]	2.5	1.2	0.15
S. spp Oflo-R	[59]	[59]	[59]
GM	1.2	0.71	0.082
MIC90	2.5	1.2	0.15
S. spp Oxa-R Oflo-R	[47]	[47]	[47]
GM	1.21	0.72	0.081
MIC90	2.5	1.2	0.15

[a]*S.*: *staphylococcus*; Oxa: oxacillin; Oflo: ofloxacin; R: resistant.
[b]GM: geometric mean.
[c]MIC's were determined using a 2-fold agar-dilution (μg/mL).
[d][ ]: number of isolates.

Against streptococci (Table 3), including penicillin-resistant pneumococci, RU79115, vancomycin and eperezolid displayed very similar activities with MIC_{50}'s ranging from 0.15 to 0.6 μg/mL. In septicemic mice infected by these species (Table 5), the efficacy of RU79115 was close to those of the reference compounds, with PD_{50}'s[10] from 1 to 37.6 mg/kg.

Against enterococci (Table 4), RU79115 exhibited similar activities against vancomycin-susceptible and resistant strains (MIC_{50}: 0.15–0.3 μg/mL). In comparison, eperezolid was twice less active. In vivo (Table 5), in 12 systemic infections induced in mice by various Gram+ cocci, including vancomycin resistant enterococci, PD_{50}'s for RU79115 ranged from 10 to 77 mg/kg, i.e., at a higher level than that of eperezolid and linezolid.

In vitro, RU79115 bactericidal activity against *E. faecium* (Fig. 2) and *S. aureus* (not shown) was time dependent and similar to that of vancomycin in the case of *S. aureus*.

Table 3. In vitro anti-streptococcal activity of RU79115 (MIC μg/mL)

	VAN	Eperezolid	RU79115
St. spp (groupable)[a]	[38][c]	[38]	[38]
GM	0.35	0.52	0.47
MIC90	0.6	0.6	1.2
St. spp (viridans)	[25]	[25]	[25]
GM	0.4	0.39	0.59
MIC90	0.6	1.2	1.2
P. Pen-R[b]	[29]	[29]	[29]
GM	0.24	0.25	0.12
MIC90	0.3	0.3	0.3

[a]St.: streptococci.
[b]P.: pneumococci; Pen.: penicilin; R: resistant.
[c][ ]: number of isolates.

Table 4. In vitro anti-enterococcal activity of RU79115 (MIC μg/mL)

	VAN	Eperezolid	RU79115
E. spp VAN-S	[26][b]	[26]	[26]
GM	0.75	0.51	0.26
MIC90	0.6	0.6	1.2
E. spp VAN-R	[65]	[65]	[65]
GM	23.7	0.72	0.26
MIC90	>20	1.2	0.6
E. spp VAN-R TEI-R[a]	[17]	[17]	[17]
GM	36.9	0.43	0.16
MIC90	>20	1.2	0.3

[a]VAN: vancomycin; TEI: teicoplanin; R: resistant; E.:enterococci.
[b][ ] : number of isolates.

Pharmacokinetic properties of RU79115 (Table 6) were in accordance with the pharmacological profile.

In summary, our current work demonstrates that the novel series of coumarin inhibitors of gyrase B bearing 5′,5′-dialkylsubstituted noviose displays improved in vitro and in vivo antibacterial properties compared to natural series possessing noviose. Improvement of the anti-

Table 5. In vivo anti-bacterial activity of RU79115

Strains	Reference	Phenotype	Antibiotic	MIC (μg/mL)	PD_{50}[a] (mg/kg)
S.a.[b]	HT18		VAN[c]	1.2	7.8
			eperezolid (SC)[d]	0.6	20
			RU79115	< = 0.04	5.6
	GR56	OXA-R[e]	VAN	1.2	4
			eperezolid (SC)	1.2	5
			RU79115	0.08	1
	HT17		linezolid (SC)	1.2	5.8
			RU79115	0.15	2.8
	GO3		linezolid (SC)	2.5	8.7
			RU79115	0.3	7.1
S.pn[b]	PW3		linezolid (SC)	1.2	3
			RU79115	1.2	8.5
S. py.[b]	A1UC1		VAN	0.6	< 1.5
			eperezolid (SC)	< = 0.15	22.8
			RU79115	0.15	37.6
S. ag.[b]	B1HT3		VAN	0.3	17.2
			eperezolid (SC)	0.6	>50
			RU79115	—	27.7
E. fs.[b]	D2HT6		VAN	2.5	16.7
			eperezolid (SC)	0.6	6.4
			linezolid	—	4.2
			RU79115	0.3	10
E. fm.[b]	D3HT12	VAN-R[e]	VAN	>40	>50
			eperezolid (SC)	0.3	1.5
			linezolid	—	1.8
			RU79115	0.15	40
	D3HM4	VAN-R	VAN	>40	>50
			eperezolid (SC)	0.3	27.2
			linezolid	—	8.6
			RU79115	0.6	77
	D3AP9	VAN-R	VAN	>40	>50
			eperezolid (SC)	1.2	8.7
			linezolid	—	2.3
			RU79115	0.6	44
	D3HM3	VAN-R	VAN	>40	>50
			eperezolid (SC)	—	2.8
			linezolid	—	2.1
			RU79115	0.08	32
	D3HT7		VAN	2.5	8.7
			eperezolid (SC)	0.6	3
			linezolid	——	2.4
			RU79115	0.15	112

[a]PD_{50} were determined in a murine intraperitoneally-induced septicaemia model and calculated by using the probit method.
[b]*S.a.: S. aureus; S. pn.: S. pneumoniae; S. py.: S. pyogenes; S. ag.: S. agalactiae; E. fs.: E. faecalis; E. fm.: E. faecium.*
[c]All vancomycin administrations were done SC. If not mentioned, the compounds were administered orally.
[d]SC: subcutaneous.
[e]OXA: oxacillin; VAN: vancomycin; R: resistant.

Table 6. Pharmacokinetic parameters of RU79115

Mouse	Dose 10 mg/kg	T_{max}	C_{max}	Half life	F%
	IV			2.5 H	
	PO	0.5 H	5.4 μg/ml		62%

bacterial spectrum is notably pronounced in the anti-enterococcal activity. Among the newly synthesised inhibitors 5′,5′-spirocyclopentylnoviose analogue **3g** (RU79115) was identified to be most potent representative of the new family of coumarin antibiotics and is currently undergoing further pharmacological evaluation.

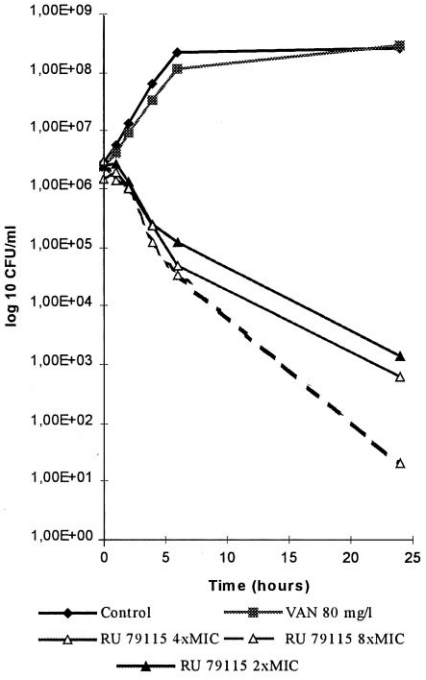


Figure 2. In vitro bactericidal activity of RU 79115 against *E. faecium* D3AP9. Time killing curves were determined using a micromethod in broth.

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