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Noviose mimics of the coumarin inhibitors of gyrase B

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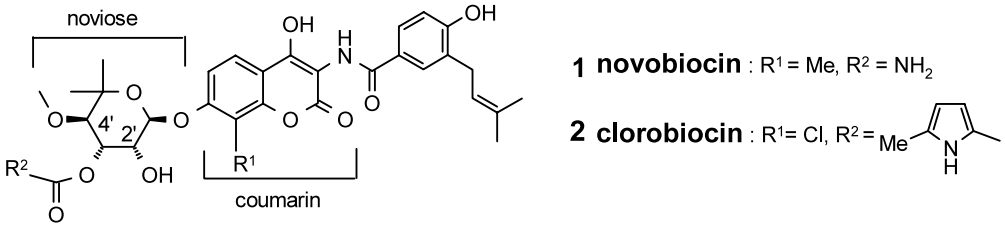
Abstract—The design, synthesis, and biological activity in vitro of modified coumarin inhibitors of gyrase B are presented. Noviose, the sugar portion of coumarin antibiotics, was replaced by simplified mimics, a 5′,5′-dimethylcyclohexane or piperidine.

In previous reports from these laboratories[1] we have described the synthesis and structure–activity relationships of different series of novobiocin-(**1**)- or clorobiocin-(**2**)-like coumarin inhibitors of DNA gyrase, a bacterial enzyme that participates in the regulation of DNA topology in cells.[2] So far, we have been performing structural modifications on the coumarin part of the analogues. In this report we describe attempts to replace the noviosyl part of coumarin drugs with simplified mimics. Our objective was to explore alternative, simpler synthetic strategies, compared to the relatively complex synthesis of noviose,[1f] trying at the same time to improve pharmaco-chemical properties of the coumarin drugs, especially their bioavailability and aqueous solubility.

In the search for simplified mimics of the noviosyl part of coumarin antibiotics, our first choice was a cyclohexyl mimic **3** bearing the 5′,5′-dimethyl group and lacking the 4′-MeO group of noviose (Fig. 1).[3] Obviously, replacement of the tetrahydropyran ring of the sugar with a 5′,5′-dialkylcyclohexane is accompanied by the absence of the stabilising anomeric effect associated with the more stable axial (α) orientation of the coumarin moiety in coumarin drugs.[4] The calculation of the relative energies of two possible conformations, the 'active' one **3** and the 'inactive' one **4**, indicated an energy difference of 3.1 kJ mol^{-1}.[5] Considering the absence of solvation effects in the calculations and the inherent limitations of the computational model, this small energy difference in favour of the 'inactive' conformation did not discourage us from pursuing the synthesis of the cyclohexyl target.

Our second choice for the noviosyl mimic was the piperidine moiety **5**, with a nitrogen atom replacing the 5′,5′-dimethyl group of noviose (Fig. 1). The presence of the nitrogen atom could serve as a convenient handle for diverse structural modifications of this part of the molecule. As in the first target, the piperidine ring in **5** could not provide the necessary anomeric stabilisation and the results of calculations of relative energies gave the preference to the 'inactive' conformation **6** over the 'active' one **5** by 2.7 kJ mol^{-1}.



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(±)-5,5-Dimethylcyclohex-2-en-1-ol (**8**)[6] seemed to be an ideal starting material for the preparation of structurally simplified cyclohexyl inhibitors (Scheme 1). Previously[1d] we described a short synthesis of a useful coumarin building block: 4-benzyloxy-3-carbethoxycoumarin **7**. As in the case of noviose, coupling the cyclohexenol **8** with the coumarin **7** was effected in the presence of PPh_3 and DEAD (Mitsunobu conditions) in dichloromethane to afford **9** in 51% yield after chromatographic separation. *cis*-Hydroxylation of the olefinic bond in **9** was realised in the presence of catalytic OsO_4/4-methylmorpholine *N*-oxide to afford exclusively the diol **10**. Regioselective silylation of diol **10** at the 3′-position was carried out with Et_3SiCl in the presence of diisopropylethylamine and imidazole. Tetrahydropyranylation of the 2′-hydroxy group under standard conditions provided the THP derivative **11** that was desilylated smoothly with Bu_4NF to afford the alcohol **12**.

The free 4-OH group of the coumarin was recovered in quantitative yield by hydrogenolysis in the presence of a Pd catalyst. Introduction of the *N*-propargyloxycarbamate (a 5-methylpyrrole-2-carboxylate bioisostere) in **13** at C-3′ was carried out using *p*-nitrophenyl chloroformate and *O*-propargylhydroxylamine as described in Ref. 1d. The ester group (CO_2Et) of **14** was converted to an amide ($CONH_2$) with gaseous NH_3 in THF. In the last step, the THP group was easily deprotected with TsOH in MeOH to give the corresponding cyclohexyl target **15**.

The synthesis of the racemic piperidine mimic was performed similarly to the cyclohexyl target and is outlined in Scheme 2. The starting piperid-4-en-3-ol **17** protected as its Cbz derivative was prepared according to Ref. 7. The introduction of the 5-methylpyrrolecarboxylate at the free 5′-hydroxyl group in **21** was achieved with EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-HCl) and DMAP in dichloromethane to afford intermediate ester **22**. Catalytic hydrogenation of **22** in THF at room temperature provided an easily separable mixture of **23a** and **23b** in 70% and 30% yields, respectively. These were smoothly deprotected in MeOH with TsOH to furnish the corresponding free 4′-alcohols, **24** and **25**, respectively, **25** being isolated as a *p*-toluenesulfonic acid salt. Further alkylation of the secondary amino group in **25** under the usual conditions provided the *N*-allyl and *N*-*i*Pr analogues **26** and **27**.

Biological results: Table 1 shows the inhibition in the supercoiling activity of *E. coli*/*S. aureus* DNA gyrase by novobiocin, clorobiocin and coumarin inhibitors with noviose mimics. In Table 1 also included are the corresponding results of the reference compounds incorporating sugar noviose: **16**[1d] (Scheme 1) and **28** (Scheme 2).[1c]

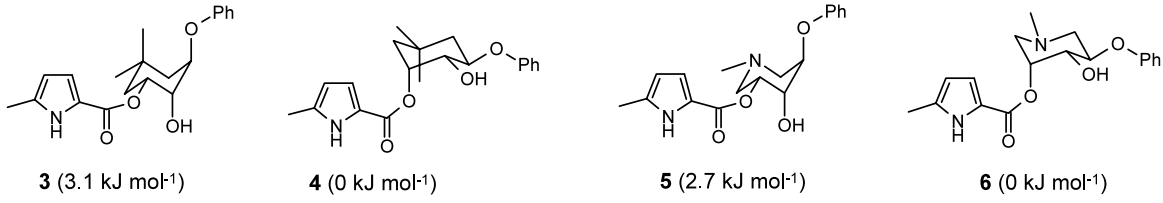
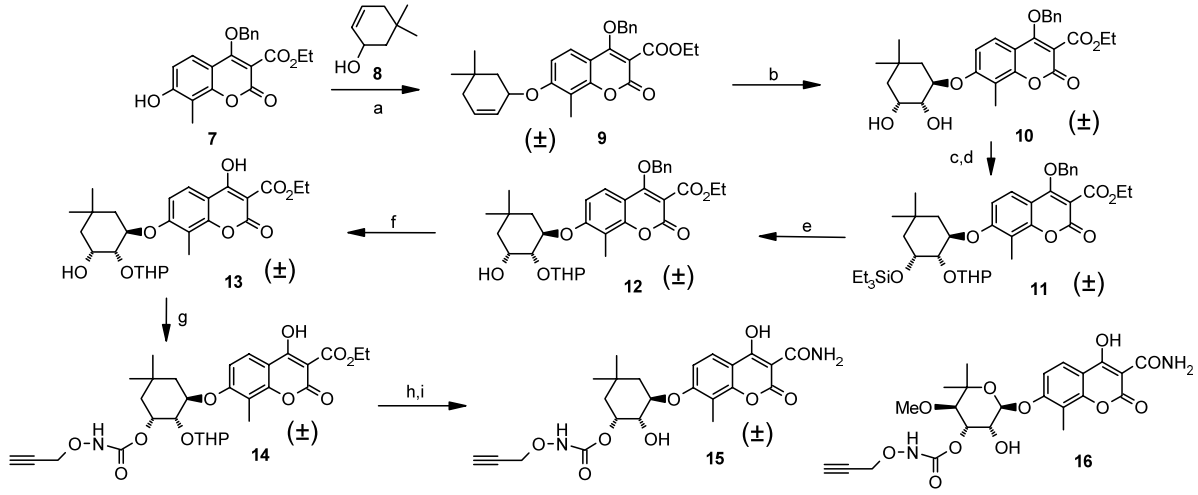
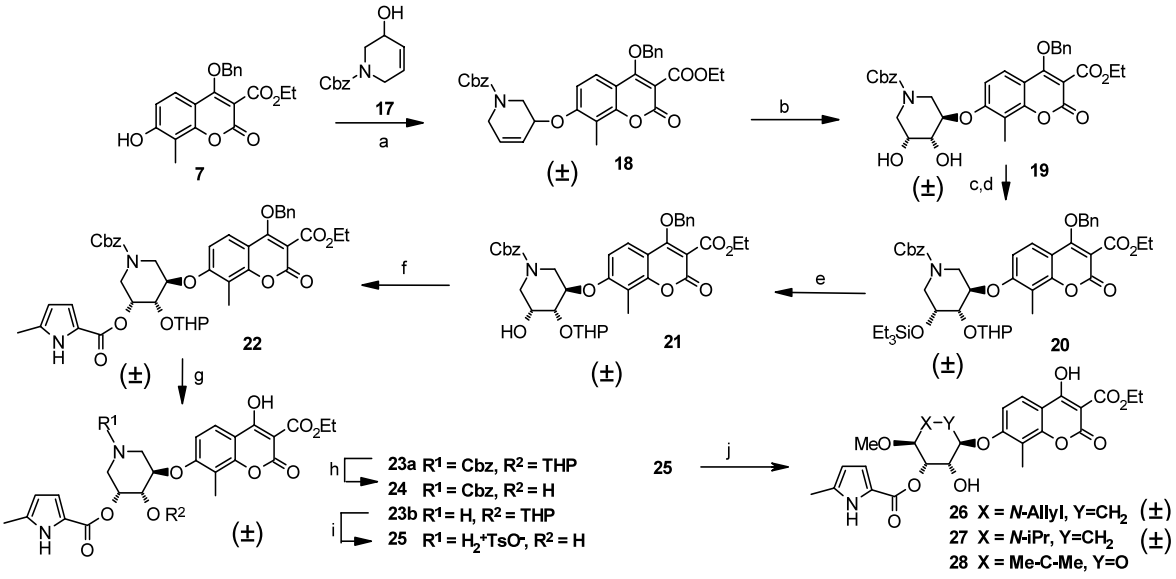


Figure 1. Calculated relative conformational energies of cyclohexyl mimics **3** and **4** and *N*-methylpiperidine mimics **5** and **6**.



Scheme 1. *Reagents and conditions*: (a) PPh_3, $EtO_2CN{=}NCO_2Et$, CH_2Cl_2, rt, 51%; (b) OsO_4, 4-methylmorpholine *N*-oxide, *t*BuOH, THF, H_2O, rt, 91%; (c) Et_3SiCl, DIPEA, Im, CH_2Cl_2, 0°C, 64%; (d) DHP, TsOH cat, CH_2Cl_2, rt, 66%; (e) Bu_4NF, THF, rt, 75%; (f) H_2, Pd–C/10%, THF, rt, quant; (g) i. $pNO_2C_6H_4OCOCl$, DMAP, CH_2Cl_2, 0°C; ii. $HC{\equiv}CCH_2ONH_2HCl$, NaH, DMAP, DMF, 0°C; (h) NH_3, THF, rt; (i) TsOH cat, MeOH, CH_2Cl_2, rt, 54% overall for (g)–(i).



Scheme 2. *Reagents and conditions*: (a) PPh_3, $EtO_2CN{=}NCO_2Et$, CH_2Cl_2, rt, 51%; (b) OsO_4, 4-methyl morpholine-*N*-oxide, *t*BuOH, THF, H_2O, rt, 92%; (c) Et_3SiCl, DIPEA, Im, CH_2Cl_2, rt, 77%; (d) DHP, TsOH cat, CH_2Cl_2, rt, quant.; (e) Bu_4NF, THF, rt, 96%; (f) 5-methylpyrrole-2-carboxylic acid, EDAC, DMAP, CH_2Cl_2, 63%; (g) H_2, Pd–C/10%, THF, rt.; (h) TsOH cat, MeOH, CH_2Cl_2, rt, 39%.; (i) TsOH cat, MeOH, rt, 39%; (j) Allyl-Br, 27% or *i*PrI, 20%, K_2CO_3, DMF, rt.

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

Compound	Novob	**15**	**16**	**24**	**25**	**26**	**27**	**28**
IC_{50}nov[a,b]/IC_{50}comp	1[a,b]	0.34[b]	1[a]	0.06[b]	0.25[b]	0.16[b]	0.06[b]	3.1[b]
MIC *S. aureus* 011HT3	≤0.04	2.5	≤0.04	>40	>40	>40	>40	0.3

[a] IC_{50} was determined for gyrase B of *E. coli* against novobiocin (0.25 μg/mL) as reference. For the details see Ref. 1c.
[b] IC_{50} was determined for gyrase B of *S. aureus* against novobiocin (0.5 μg/mL) as reference. For the details see Ref. 1d.
[c] MIC, minimal inhibitory concentrations (μg/mL) were measured by using a twofold broth microdilution after 24 h incubation.

Proton NMR spectra of the final targets **15** and **24**–**27** confirmed the calculation results that predicted the more stable conformations **4** and **6**. However, the small difference in inhibition in supercoiling of analogue **15** compared to **16**, could be attributed to the absence of the 4′-MeO group in these molecules rather than to prevalence of the more favourable 'inactive' conformations for **15**. As revealed by X-ray studies of complexes of coumarin drugs and the 24 kDa N-terminal fragment of gyrase B,[8,9] the 4′-MeO group of noviose 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.[10] Cyclohexyl mimics with a 5′,5′-dimethyl group proved to be better inhibitors in supercoiling activity of DNA gyrase than the corresponding piperidine counterpart, probably due to the more effective interaction of the *gem*-dimethyl group with the surrounding hydrophobic pocket. Introduction of the *N*-alkyl groups in **25** did not improve the inhibition of the series (**26**, **27**), whereas *N*-carbamate (**24**) substitution more or less abolished the inhibition.

In conclusion, we successfully realised the synthesis of cyclohexyl and piperidine mimics of noviose. However, these simplified targets showed only weak inhibitory activity and moderate in vivo inhibition (MIC values). We believe that the absence of the 4′-MeO substituent of the noviose mimics is most probably responsible for the diminution, by one order of magnitude, of the inhibition of the supercoiling activity of DNA gyrase.

Acknowledgements

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