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Coumarin Inhibitors of Gyrase B with *N*-Propargyloxy-carbamate as an Effective Pyrrole Bioisostere

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Abstract—The synthesis and biological profile in vitro of a series of coumarin inhibitors of gyrase B bearing a *N*-propargyloxy-carbamate at C-3′ of noviose is presented. Replacement of the 5-methylpyrrole-2-carboxylate of coumarin drugs with an *N*-propargyloxycarbamate bioisostere leads to analogues with improved antibacterial activity. Analysis of crystal structures of coumarin antibiotics with the 24 kDa N-terminal domain of the gyrase B protein provides a rational for the excellent inhibitory potency of C-3′ *N*-alkoxycarbamates.

Introduction

As part of our continuing structure–activity relationship (SAR) study of coumarin antibiotics (**1**–**3**),[1–5] we describe our efforts aimed at modification of 5-methylpyrrole-2-carboxylate moiety linked to the C-3′ position of the sugar noviose. Coumarin antibiotics are inhibitors of the catalytic functions of DNA gyrase that are dependent on ATP hydrolysis (i.e. DNA supercoiling and decatenation), by competitive inhibition of ATP binding[6] (for a review on structure and function of DNA gyrase, see refs 7–10). Family members of coumermycin antibiotics (**3**–**6**) produced by *Streptomyces* species differ by the presence of 5-methylpyrrole-2-carboxylate or pyrrole-2-carboxylate moiety in the molecule.[11] The comparative studies of antibacterial activity of this group of coumarin analogues indicated that the superior antibacterial profile of coumermycin A_1 could be attributed to the 5-methyl group of the 5-methylpyrrole-2-carboxylate. Similarly, Berger and Batcho[12] pointed out that 5-methylpyrrole-2-carboxylate, and not the replacement of the C-8 methyl group of the coumarin by a chlorine atom, is the principal reason for higher antibacterial potency of clorobiocin **2** compared to novobiocin **1**. As clorobiocin displays 2-fold higher inhibitory activity in negative supercoiling of DNA gyrase with respect to novobiocin, whereas 10-fold higher antibacterial potency, it appears that 5-methypyrrole-2-carboxylate contibutes to higher hydrophobicity and higher membrane permeability.

In order to develop effective bioisosteres of 5-methylpyrrole-2-carboxylate[13] it was necessary to fulfil two criteria. Firstly, isosteric replacement of 5-methylpyrrole-2-carboxylate should maintain similar, or provide stronger inhibitory potency of negative supercoiling of DNA gyrase to that of clorobiocin or novobiocin. Secondly, as DNA gyrase is an intracellular target, isosteric replacement should confer membrane permeability to the analogue. Since X-ray crystal structures of several coumarin drugs with 24 kDa N-terminal domain of gyrase B of *E. coli* have been solved,[14–17] these results were an obvious starting point for our research.

Examination of the binding site of the noviose portion of clorobiocin bearing at C-3′ 5-methylpyrrole-2-carboxylate moiety revealed that the pyrrole ring is surrounded by hydrophobic amino acid residues Val-43, Val-71, Val-120, Val-167, and Ile-78 (hydrophobic pocket; Fig. 1). Besides these hydrophobic contacts, there is an important hydrogen-bonding interaction between the N–H of the pyrrole and the side chain of Asp-73, and the hydrogen bonding that links the carbonyl group of the pyrrole-2-ester, an ordered water molecule and the side chain of Thr-165 and Gly-77. As will be seen by the results of biological activity (*vide infra*) of coumarin analogues, both hydrophobic

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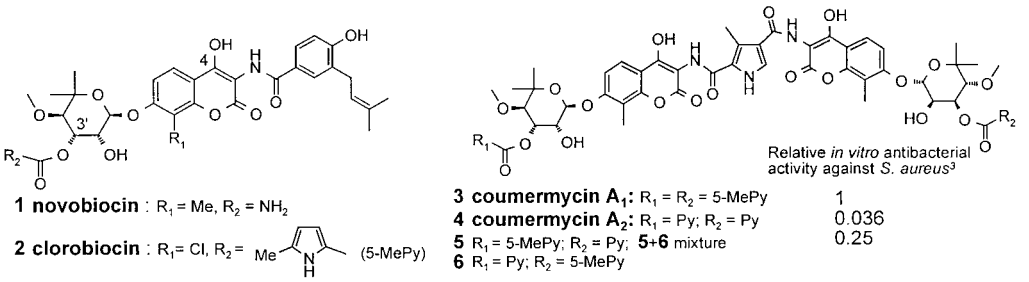


interactions as well as *s-cis* orientation of H_a–N and C=O groups around the amide bond are crucial in achieving a bioisostere with good biological activity.

Chemistry

The standard synthetic route that could be used to introduce potential pyrrole isosters consists of opening the conveniently α-glycosylated noviose 2′,3′-carbonate **7** with nucleophiles (Scheme 1).[13,19,20] However, it is known that the opening of the corresponding carbonate under thermodynamic control leads to a mixture of 3′-substituted **8** and 2′-substituted **9** derivatives. Although thermodynamic equilibrium favours regioisomer **8** (from 4:1 to 1:1 depending on nucleophile), in many cases separation of the regioisomers is troublesome. In order to avoid testing the mixtures **8** and **9**, we wanted to develop a synthetic approach that would enable regioselective introduction of potential bioisosteres at the C-3′ position of noviose.

Previously, we have described the short synthesis of a valuable intermediate for the construction of coumarin drugs: 7-hydroxy-8-methyl-4-benzhydryloxycoumarin (**10**).[3] The preparation of the equally useful coumarin building block **13** starting with THP protected derivative **11** is depicted in Scheme 2.

As in the case of the Mitsunobu's coupling of compound **10** with noviose **14** (Scheme 4), coupling of the noviose **14** with coumarin **13** under same conditions provided an α-glycoside **15** as the major product after chromatographic separation (Scheme 3). Having prepared diol **15**, we investigated regioselective protection of 3′-OH with Et_3Si, MEM ($MeOCH_2CH_2OCH_2$-), and BOM ($PhCH_2OCH_2$-) protecting groups. In all cases the major regioisomers formed **16a–c** were at 3′-OH and could be easily separated by chromatography from 2′-regioisomers **17a–c**. In general, deprotection of silyl groups is relatively easy and we decided to continue our synthetic scheme with this protecting group. Dihydropyranylation of **16a** afforded a diastereomeric mixture of THP derivatives (~1:1) **18** that was smoothly desilylated and hydrogenolysed to furnish intermediate **19**. The free 3′-OH of the noviose derivative **19** could be selectively transformed under standard conditions (RCOCl, DMAP, Py or EDAC, DMAP, CH_2Cl_2) into 3′-noviose esters. Similarly, intermediate **19** could be selectively converted by the reaction of its *p*-nitrophenylcarbonate activated form with amines into 3′-carbamates **20a**, or, by the reaction with hydroxylamines into 3′-*N*-alkoxycarbamates **20b**. Once the potential pyrrole bioisosteres were introduced at C-3′, the intermediates were subjected to hydrolytic conditions with TosOH cat. in MeOH to remove tetrahydropyranyl group and afford 3′-carbamates **21a–d** or 3′-*N*-alkoxycarbamates **22a–g** of the coumarin-3-carboxy series. Alternatively, the 3-ester group in **20a,b** could be first exchanged by ammonia, amines or hydroxylamines to provide coumarin-3-carboxamide **24a**, *N*-substituted coumarin-3-carboxamides **24b–d** or coumarin-3-hydroxamate derivatives **25a–c**, respectively, after subsequent THP deprotectection.

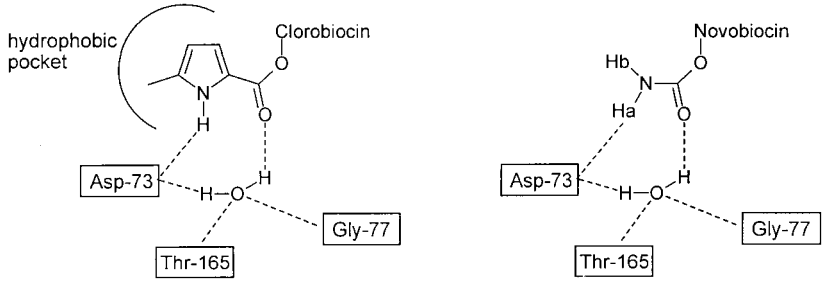
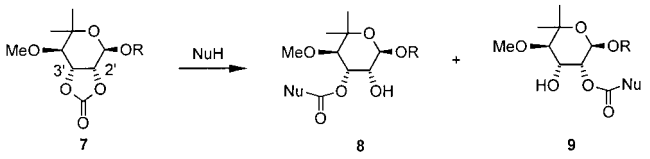
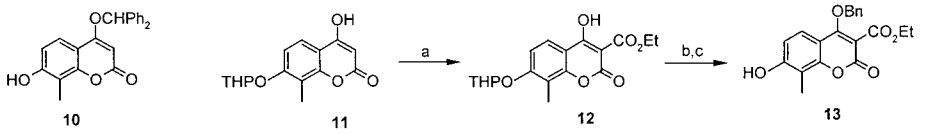


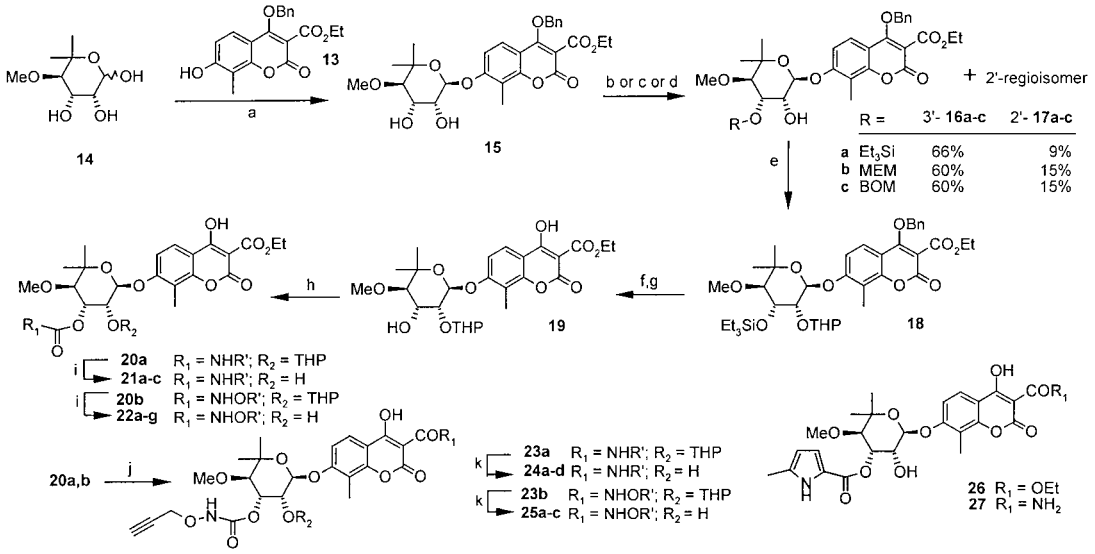
Figure 1. Interactions between the 24 kDa N-terminal protein fragment of gyrase B of *E. coli* with clorobiocin and novobiocin.[16,18]



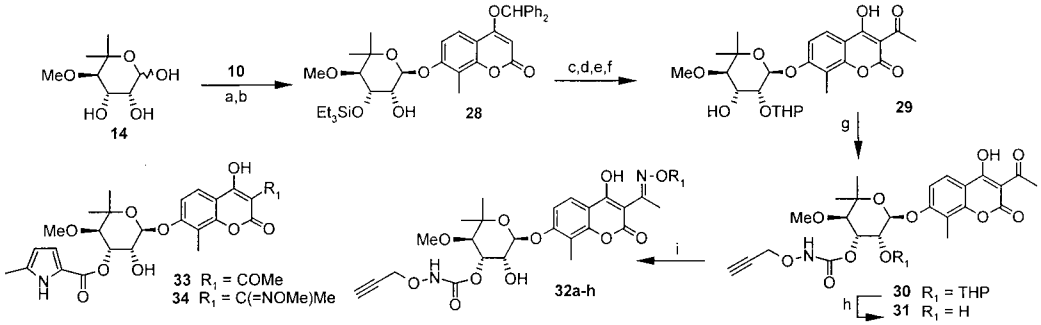
Scheme 1.



Scheme 2. Reagents and conditions: (a) ClCOOEt, DMAP, CH_2Cl_2, rt, 55%; (b) BnOH, PPh_3, $EtO_2CN=NCO_2Et$, CH_2Cl_2, rt, 49%; (c) HCl (1 M), THF, rt, 89%.



Scheme 3. Reagents and conditions: (a) PPh_3, $EtO_2CN=NCO_2Et$, CH_2Cl_2, rt, 54%; (b) Et_3SiCl, DIPEA, Im, CH_2Cl_2, rt, 66%; (c) MEMCl, DIPEA, Im, CH_2Cl_2, rt, 60%; (d) BOMCl, DIPEA, Im, CH_2Cl_2, rt, 60%; (e) DHP, TosOH cat, CH_2Cl_2, rt, 79%; (f) H_2, Pd-C/10%, THF, rt; (g) Bu_4NF, THF, rt, (81% from **16a**); (h) (i) *p*-$NO_2C_6H_4OCOCl$, DMAP, CH_2Cl_2, 0 °C; (ii) R′NH_2 or R′ONH_2, DMAP, DMF, rt; (i) TosOH cat, MeOH, rt, (40–60% from **19**); (j) NH_3, R′NH_2, THF, rt; or R′ONH_2, Py, rt, (k) TosOH cat, MeOH, rt. (30–60% from **19**).



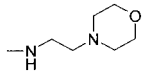
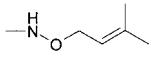
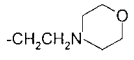
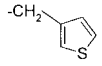
Scheme 4. Reagents and conditions: (a) **10**, PPh_3, *i*$PrO_2CN=NCO_2$*i*Pr, CH_2Cl_2, rt, 67%; (b) Et_3SiCl, DIPEA, Im, CH_2Cl_2, rt, 70%; (c) DHP, TosOH cat, CH_2Cl_2, rt; (d) H_2, Pd-C/10%, THF, rt; (e) Ac_2O, CH_2Cl_2, 0 °C; (f) Bu_4NF, THF, rt, (70% from **28**); (g) (i) *p*-$NO_2C_6H_4OCOCl$, DMAP, CH_2Cl_2, 0 °C; (ii) $HC{\equiv}CCH_2ONH_2{\cdot}HCl$, DMAP, DMF, rt; (h) TosOH cat, MeOH, rt, (67%, from **28**); (i) $RONH_2HCl$, KOAc, EtOH, rt, 80%-quant.

A similar synthetic approach to that described, but starting with coumarin intermediate **10** provided access to a 3-acetylcoumarin **31** and a series of corresponding oxime analogues **32a**–**h** bearing the a 3′-*N*-propargyloxycarbamate moiety (Scheme 4).

Biological Results

Table 1 shows the inhibition in the supercoiling and antibacterial activity of *E. coli* or *S. aureus* DNA gyrase by novobiocin, clorobiocin and the coumarin-3-carbethoxy inhibitors possessing 3′-carbamates **21a**–**c** or 3′-*N*-alkoxycarbamates **22a**–**g** and a series of diverse coumarin analogues with 3′-*N*-propargyloxycarbamate on the sugar noviose. For the comparative assessment, in the Table 1 results are also presented showing the biological activity of some pyrrole counterparts (**26**, **27,** Scheme 3 and **33**, **34**, Scheme 4). A carbamoyl derivative **21a** gave similar inhibition in negative supercoiling of DNA gyrase as novobiocin. With *N*-alkyl substitution, a diminution of the inhibitory potency was observed (**21b**–**d**). In the series of *N*-alkoxycarbamates **22a**–**g,** an inverse effect dominated. Increasing the

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

Compound	R_1	Ratio $\frac{IC_{50}nov^{a,b}}{IC_{50}comp}$	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[a,b]	≤0.04	≤0.04	10	≤0.04	0.3	0.6
Clorobiocin		1.7[a]	≤0.04	ND	0.6	ND	≤0.04	≤0.04
Scheme 3								
21a	$-NH_2$	1.6[a]	>40	ND	>40	ND	>40	>40
21b	-NH-Allyl	0.38[a]	>40	>40	>40	20	>40	>40
21c	-NH-Propargyl	0.38[a]	40	>40	>40	20	>40	>40
21d	-NH*i*Pr	0.025[a]	>40	>40	>40	>40	>40	>40
22a	-NHOMe	0.66[a]	>40	>40	>40	>40	>40	>40
22c	-NHOEt	2.0[a]	20	>40	>40	>40	40	>40
22d	-NHO*i*Pr	2.0[a]	>40	>40	>40	>40	>40	>40
22e	-NHO-Allyl	2.0[a]	10	20	>40	20	20	>40
22f	-NHO-Propargyl	5.6[a]	1.2	2.5	>40	0.3	20	10
22g	-NHO-But-2-ynyl	0.5[b]	10	40	>40	10	>40	>40
24a	$-NH_2$	1.0[a]	≤0.04	≤0.04	0.3	≤0.04	0.6	0.08
24b	-NH-3-Pyridyl	0.67[b]	≤0.04	≤0.04	1.2	≤0.04	0.08	0.6
24c		2.6[b]	≤0.04	0.08	1.2	0.08	1.2	2.5
24d	$-NHCH_2CH_2OH$	2.6[b]	≤0.04	0.08	1.2	≤0.04	0.6	1.2
25a	-NHOMe	11.1[b]	0.08	0.6	10	0.08	0.6	2.5
25b	-NHOEt	5.6[b]	≤0.04	0.08	1.2	0.6	0.15	0.6
25c		2.0[b]	≤0.04	≤0.04	0.6	0.15	0.08	0.3
26		3.1[a]	0.3	ND	>20	ND	0.3	5
27		1.67[a]	≤0.04	ND	2.5	ND	0.15	2.5
Scheme 4								
31		8.1[b]	≤0.04	0.6	2.5	≤0.04	0.3	1.2
32a	-Me	12.5[b]	0.15	0.15	5	0.08	0.6	2.5
32b	-Et	8.3[b]	0.08	0.15	2.5	0.15	0.6	1.2
32c		4.0[b]	0.15	0.6	5	0.15	1.2	20
32d	$-CH_2O(CH_2)_2OMe$	4.0[b]	0.08	0.6	10	1.2	0.6	5
32e		1.3[b]	≤0.04	≤0.04	2.5	≤0.04	0.15	0.6
33		2[a]	≤0.04	ND	5	ND	0.3	2.5
34		3.3[a]	0.15	ND	40	ND	0.3	10

[a]IC_{50} was determined for gyrase B of *E. coli* against novobiocin (0.25 μg/mL) as reference. For details see ref 3.
[b]Supercoiling assay using purified DNA Gyrase from *S. aureus*: the enzyme was purified from a crude extract of *S. aureus* E34159. The frozen cells were suspended in TGED buffer (pH 7.5) supplemented with 9 mM dithiothreitol, 20 mM EDTA, 0.4% Brij 58, 60 μg of lysostaphin per mL, proteases inhibitors and incubated for 30 min at 20 °C and 25 min at 37 °C. After the addition of 0.4 M KCl, incubation was repeated 30 min at 20 °C. The cell suspension were sonicated, centrifuged at 100,000 g for 45 min. The supernatant was treated with 4% streptomycin for the removal of DNA. After centrifugation, the supernatant was supplemented with 65% $(NH_4)_2SO_4$. The precipitate was dissolved and dialysed in TGED buffer. The dialysate was directly purified on novobiocin–Sepharose affinity column. The gyrase A was eluted with TGED buffer containing 1 M KCl and the gyrase B was eluted with TGED buffer containing 1 M KCl and 5 M urea. After dialyse, the proteins were concentrated with PEG 2000. Relaxed DNA was prepared from pBR322 plasmid with calf thymus topoisomerase I (GIBCO-BRL), 1 h at 37 °C. The DNA concentration was determined by spectrophotometric measurements. Supercoiling assay was performed on 40 μL assay mixture containing 40 mM Tris–HCl pH 7.3, 20 mM KCl, 4 mM $MgCl_2$, 2 mM ATP, 2 mM spermidine-HCl, 0.8 μg tRNA, 50 ng relaxed DNA and 1 unit of DNA-gyrase. One unit of gyrase was defined as the amount of activity that supercoiled 50 ng of relaxed pBR322 in 60 min at 37 °C. The reaction was stopped by addition of protease K and separated by electrophoresis. IC_{50} was determined for inhibitors against novobiocin (0.5 μg/mL) as reference.
[c]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.

length of the substituent (up to a 3-carbon substituent) produced an increase in inhibitory potency. The poor activity of the carbamate series (**21b–d**) could be a reflection of their preferred *s-trans* conformation **35b** around the amide bond (Fig. 2).[21,22] This conformation is placing N–H and C=O bonds in the opposite direction to that observed by the X-ray model in Figure 1.

In the case of *N*-alkoxycarbamates (**22a–g**), *s-cis* conformer **36b** prevails, directing at the same time the alkyl

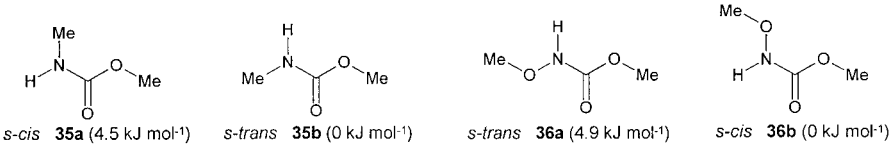


Figure 2. Calculated relative conformational energies of carbamate (**35a,b**) and alkoxycarbamate (**36a,b**) derivatives.

group to the hydrophobic pocket. However, this hydrophobic domain is not large enough to accommodate the alkyl chains containing more than three carbon atoms, and an important loss of activity was observed with analogue **22g**.

Comparison of the analogues possessing *N*-propargyloxycarbamate moiety and 5-methylpyrrole-2-carboxy group: **22f** and **26** (Scheme 3), **24a** and **27** (Scheme 3), **31** and **33** (Scheme 4), **32** and **34** (Scheme 4), clearly indicates improved inhibitory potency of the first. Thus, *N*-propargyloxycarbamate has fulfilled the first criteria imposed for a good bioisostere of 5-methylpyrrole-2-carboxylate: superior inhibitory potency to analogues possessing 5-methylpyrrole-2-carboxylate. While three *N*-alkoxycarbamates **22d–f** display almost the same inhibitory activity, a dramatic difference between the three in MIC values is observed. While **22d** is devoid of antibacterial activity, **22f** displays similar antibacterial spectrum to pyrrole analogue **26**. In this way, *N*-propargyloxycarbamate has fulfilled the second criteria of the good bioisostere: it conferred the membrane permeability that is prerequisite for a good antibacterial activity.

Different coumarin classes having 3′-*N*-propargyloxycarbamate: coumarin-3-esters (**22f**), -3-amides (**24a**), -3-*O*-alkylhydroxamates (**25a**), -3-ketones (**31**) and -3-alkoxyimino (**32a**) derivatives, were highly active against all the Gram-positive strains tested including *Enteroccus* and multi-resistant staphylococci. In general, improved anti-Enterococcal activity was observed with the novel series compared to pyrrole analogues. Amide and hydroxamate derivatives **24a** and **25c**, respectively, displayed the best antibacterial activity, particularly against novobiocin-resistant strains.

In conclusion, based on the results of crystallographic determination of the active site of 24 kDa N-terminal subdomain of gyrase B with coumarin antibiotics, we succeeded in designing *N*-propargyloxycarbamate as an effective 5-methylpyrrole-2-carboxylate bioisostere. This leads to analogues with improved in vitro inhibitory potency of the negative supercoiling of DNA gyrase as well as improved antibacterial activity. Among the candidates, amido **24a–d** and hydroxamate **25a–c** derivatives with the uniformly good antibacterial activity seem to be the most promising ones.

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