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STRUCTURE-ACTIVITY RELATIONSHIP IN TWO SERIES OF AMINOALKYL SUBSTITUTED COUMARIN INHIBITORS OF GYRASE B

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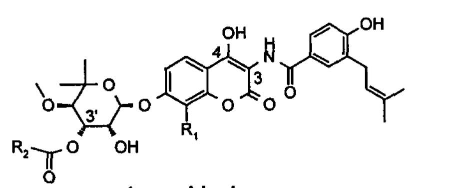
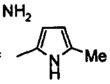
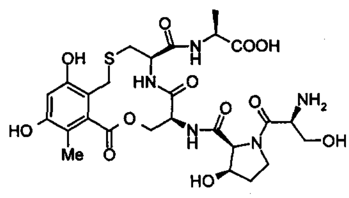
Abstract: Two series of aminosubstituted coumarins were synthesised and evaluated *in vitro* as inhibitors of DNA gyrase and as potential antibacterials. Novel novobiocin-like coumarins, 4-(dialkylamino)-methylcoumarins and 4-((2-alkylamino)ethoxy)coumarins, were discovered as gyrase B inhibitors with promising antibacterial activity *in vitro*.

Introduction: DNA gyrase and DNA topoisomerase IV, the essential enzymes in prokaryotes, are targets of synthetic quinolones,[1] as well as of diverse classes of naturally occurring antibiotics, like coumarins (novobiocin **1**, chlorobiocin **2**)[2] and cyclothialidines **3**.[3] While quinolones have been used successfully in the last decades in the clinical practice for the treatment of antibacterial infections,[4] coumarin drugs have failed to find widespread use in clinics. In contrast to the quinolones which are broad spectrum antibiotics, coumarins are active mostly against Gram-positive bacteria, are not very soluble in water, and display some toxicity in eukariotes.[5] However better understanding of the mechanism of action of this class of antibiotics at the molecular level, that was made possible through X-ray crystallographic studies of 24 kDa N-terminal sub-domain of gyrase B protein with different coumarin and cyclothialidine inhibitors,[6,7] has prompted design of newer, improved antibacterial agents.[8]



1 novobiocin : R_1 = Me, R_2 = NH_2

2 clorobiocin : R_1 = Cl, R_2 = 



3 cyclothialidine

As we had developed an efficient method for glycosylation of noviose with the coumarin fragment,[8d] we could explore the effect of the basic, polar, substituents in the coumarin part of the molecule. We hoped that the introduction of the basic groups would lead to novel class of gyrase B inhibitors with improved physicochemical properties, especially better aqueous solubility. This led to two series of alkylaminocoumarins:

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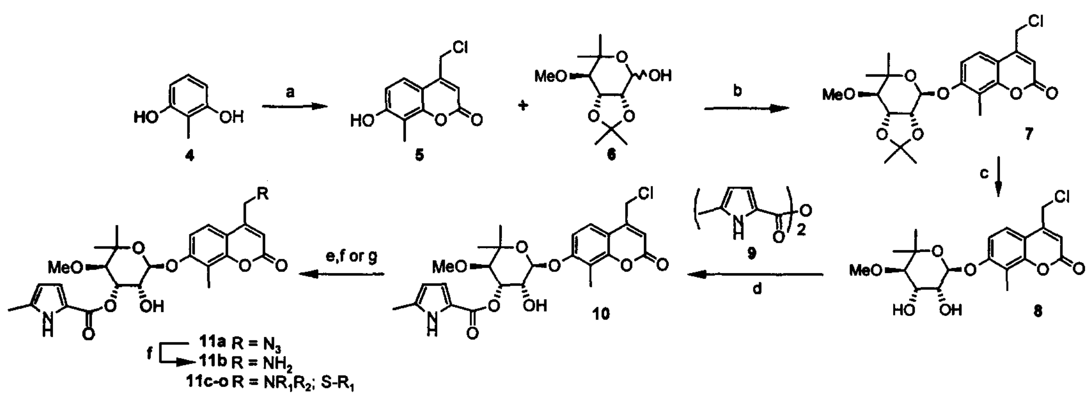
4-(dialkylamino)methylcoumarins **11b-o** (Table 1), and 4-((2-alkylamino)ethoxy)coumarins **21b-m** and **22a-c** (Table 2), which displayed both inhibitory effects on negative supercoiling of DNA gyrase and promising antibacterial properties *in vitro*.

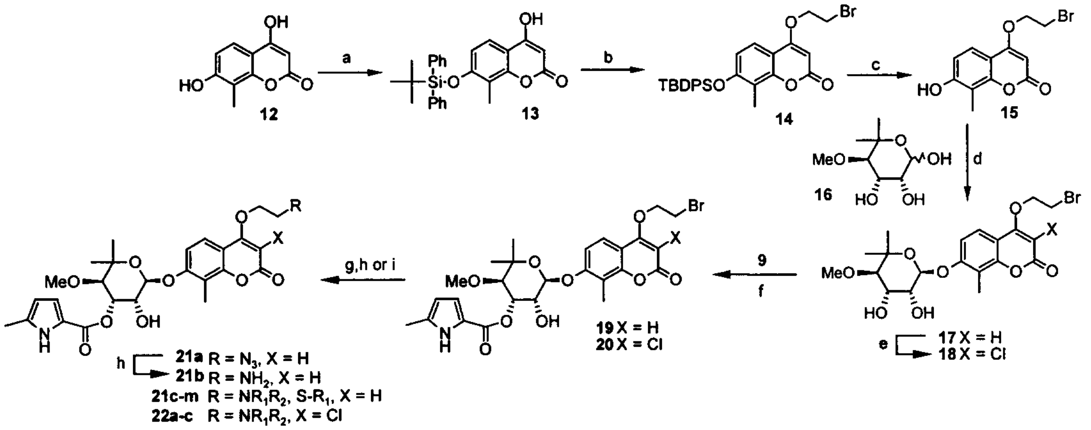
Chemistry: The series of 4-alkylaminomethylcoumarins were prepared as outlined in Scheme 1. Classical Pechmann condensation[9] of 2-methylresorcinol (**4**) with ethyl 4-chloroacetoacetate in concentrated sulfuric acid provided 4-chloromethylcoumarin **5**. This was then coupled with noviose acetonide **6**[8d] under Mitsunobu's conditions in DMF to afford α-glycoside **7** in 46% yield after chromatographic separation. Hydrolysis of the acetonide was easily realised in trifluoroacetic acid to provide the diol **8**. Esterification of the diol **8** with 5-methylpyrrole-2-carboxylic anhydride **9** was achieved in MeCN in the presence of anhydrous $CoCl_2$[10] to yield 26% of the desired 3'-(5-methylpyrroyl)ester **10** along with 17% of 2'-regioisomer (readily separable by column chromatography) and 29% of recovered diol **8**. With the exception of the aminomethyl derivative **11b**, that was prepared from azido intermediate **11a**, a variety of 4-alkylaminomethylcoumarins **11c-o** were obtained by direct reaction of **10** with amines in DMF.



Scheme 1: *Reagents and conditions*: (a) $ClCH_2COCH_2CO_2Et$, H_2SO_4 conc, rt, 58%; (b) PPh_3, $iPrO_2CN{=}NCO_2iPr$, DMF, 0°C, 46%; (c) CF_3CO_2H-H_2O, 0°C, 88%; (d) $CoCl_2$, MeCN, reflux, 26%; (e) NaN_3, DMF, rt, 45%; (f) Pd-C/10%, H_2, THF, rt. 54%; (g) HNR_1R_2, or HSR_1, DMF, rt.

The synthetic approach to 4-((2-alkylamino)ethoxy)coumarin inhibitors **21b-m** and **22a-c** is illustrated by Scheme 2. Silyl protected 4-hydroxycoumarin derivative **13**,[11] prepared from 4,7-dihydroxy-8-methyl-coumarin (**12**), was converted to 2-bromoethoxy intermediate **14** by Mitsunobu coupling with 2-bromoethanol. Deprotection of the *t*-butyldiphenylsilyl group was accomplished with the mixture $HF/KF/H_2O$, and the resulting phenol **15** was used in glycosylation step with noviose triol **16** under Mitsunobu's conditions to afford α-glycoside **17** as a major product in 48% yield. The diol **17** was converted to pyrrole ester **19** applying the same esterification conditions as described for the analogue **8** in Scheme 1. The mixture of 3'- and 2'-pyrrole esters were separated by chromatography. The reaction of **19** with different amines in DMF and the presence of catalytic amount of Bu_4NI provided desired 4-((2-alkylamino)ethoxy)coumarins **21b-m**.

Introduction of chlorine at the C-3 position of the coumarin portion was effected by chlorination of **17** with Cl_2 to afford chloro derivative **18**. Again, following described methodology, amino analogues **22a-c** (Table 2) were prepared.



Scheme 2: *Reagents and conditions*: (a) TBDMSCl, Et_3N, THF, rt, 85%; (b) $BrCH_2CH_2OH$, PPh_3, $EtO_2CN{=}NCO_2Et$, CH_2Cl_2, rt,; (c) HF, KF, H_2O, rt, 82% (from **13**); (d) PPh_3, $EtO_2CN{=}NCO_2Et$, CH_2Cl_2, rt, 48%; (e) Cl_2, THF, AcOH, rt, 72%; (f) $CoCl_2$, MeCN, reflux, 26% (for **19**); (g) NaN_3, DMF, Bu_4NI cat, rt, 71%; (h) Pd-C/10%, H_2, THF, rt, 80%; (i) HNR_1R_2, DMF, Bu_4NI cat, or HSR_1, DMF, rt.

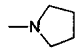
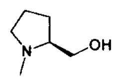
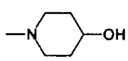
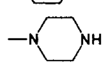
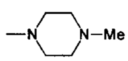
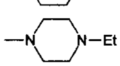
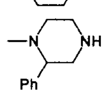
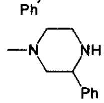
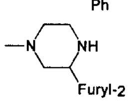
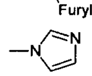
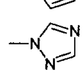
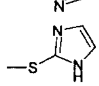
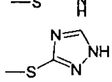
Biological results: In Table 1 and Table 2 are presented the results for inhibition of the supercoiling activity of *E. coli* DNA gyrase by novobiocin, clorobiocin and aminocoumarin inhibitors along with their antibacterial activity. In general, compared to series of 4-hydroxy-3-COR- or 4-hydroxy-3-C(=N-OR)R'-coumarins,[8d] the two classes of aminocoumarins were less potent as inhibitors of negative supercoiling of DNA gyrase. The exceptions are analogues possessing a primary amino group **11b**, **21b** or a non-hindered secondary amino group **11f**, **21c**.

In terms of antibacterial activity, the compounds were active against Gram-positive bacteria including oxacillin-resistant isolates. With few exceptions, relatively low activity was observed against *Enterococcus faecium* or novobiocin-resistant staphylococci. A promising series was that of thio derivatives **11n,o** and **21m,** that displayed a well-balanced activity spectrum against different species and different phenotypes of resistant bacteria.

In the quinolone series, introduction of a halogen atom (F, Cl) at the 6-position ortho to the alkylamino function at C-7 resulted in improvement of the biological activities.[12] We therefore prepared a number of 3-chlorocoumarins **22a-c** (Table 2). However, a decrease in the inhibitory activity on supercoiling of DNA gyrase by factor ~1.5 was reflected directly in the lower MIC values.

In conclusion, we succeeded in reversing the acidic character[13] of coumarin antibiotics by introducing basic functional groups into the molecule and at the same time preserving inhibition of supercoiling of DNA gyrase and their antibacterial properties. Most of the basic analogues, in form of their acid salts, displayed improved aqueous solubility. Among the aminocoumarins described, 2-thioimidazo derivatives **11n** and **21m** and 3-thiotriazo derivative **11o** were found to be the most promising because of their uniformly good antibacterial activity.

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

Compound	R	Ratio $IC_{50}nov^a$ / $IC_{50}comp$	MIC (μg/mL) *S.aureus* 011HT3	*S. aureus* 011GO76 OfloOxaEry-R	*S. aureus* 011HT1 Nov-R	*S.epidermidis* 012GO39 OxaTei-R	*S.pyogenes* 02A1UC1	*E.faecium* 02D31P2 VanTeiEry-R
Novobiocin		1	≤ 0.04	≤ 0.04	20	0.08	0.15	0.3
Clorobiocin		1.7	≤ 0.04	≤ 0.04	0.15	≤ 0.04	≤ 0.04	ND
11b	$-NH_2$	1.8	10	40	> 40	5	40	> 40
11c		0.5	2.5	5	40	2.5	10	> 40
11d		0.3	0.6	1.2	10	1.2	2.5	20
11e		0.22	0.6	1.2	20	0.6	2.5	20
11f		2.3	10	20	> 40	5	20	> 40
11g		0.46	5	5	40	2.5	10	> 40
11h		1.0	5	10	> 40	2.5	20	> 40
11i		0.17	1.2	5	5	0.6	1.2	5
11j		0.28	1.2	5	10	1.2	2.5	10
11k		0.33	0.6	10	10	0.6	1.2	10
11l		1.0	1.2	5	> 40	1.2	5	> 40
11m		0.4	1.2	2.5	40	0.6	2.5	40
11n		0.35	0.6	10	20	0.6	2.5	20
11o		0.40	0.3	0.6	10	0.3	0.6	2.5

a) IC_{50} was determined for gyrase B of *E. coli* against novobiocin (0.25 μg/ml) as reference. For the details see ref. 8d.

b) MIC, Minimum Inhibitory Concentrations (μg/mL) were measured by using a twofold broth microdilution after 24 hours incubation.

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

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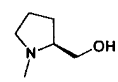
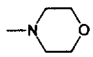
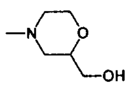
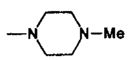
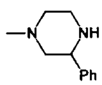
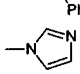
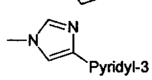
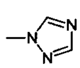
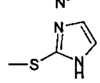
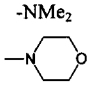
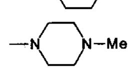
Clorobiocin		1.7	≤ 0.04	≤ 0.04	0.15	≤ 0.04	≤ 0.04	ND
21b	$-NH_2$	0.81	2.5	5	20	1.2	5	20
21c	-NHMe	2.3	2.5	2.5	20	1.2	5	> 40
21d	$-NMe_2$	0.57	2.5	5	40	2.5	10	> 40
21e		0.66	0.6	ND	40	2.5	5	20
21f		0.22	0.3	0.6	10	0.15	2.5	20
21g		0.25	1.2	> 40	40	0.6	10	> 40
21h		0.5	1.2	2.5	20	1.2	10	40
21i		0.33	0.3	10	5	0.6	1.2	5
21j		0.22	0.6	1.2	>20	0.3	1.2	10
21k		0.63	0.6	> 40	> 40	0.08	1.2	> 40
21l		0.57	0.3	10	20	≤ 0.04	1.2	10
21m		0.33	0.08	5	2.5	≤ 0.04	0.6	5
22a	$-NMe_2$	0.33	10	20	> 40	5	40	> 40
22b		0.17	1.2	2.5	> 40	2.5	5	> 40
22c		0.33	5	10	> 40	5	10	> 40

a) IC_{50} was determined for gyrase B of *E. coli* against novobiocin (0.25 μg/mL) as reference. For the details see ref. 8d.
b) MIC, Minimum Inhibitory Concentrations (μg/mL) were measured by using a twofold broth microdilution after 24 hours incubation.
c) 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.

Acknowledgement: We are grateful to the Analytical Department (HMR, Romainville) for performing the spectral measurements.

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