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SYNTHESIS AND *IN VITRO* EVALUATION OF NOVEL HIGHLY POTENT COUMARIN INHIBITORS OF GYRASE B

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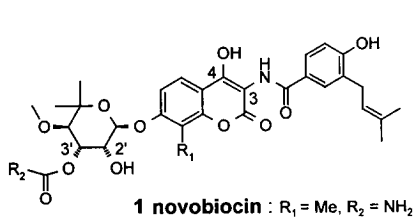
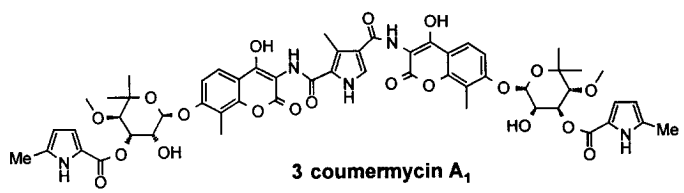
Abstract: The design, synthesis, and *in vitro* biological activity of a series of novel coumarin inhibitors of gyrase B is presented. Replacement of the 3-acylamino residue (3-NHCOR) of coumarin drugs with reversed isosteres C(=O)R, C(=N-OR)R', COOR, CONHR and CONHOR leads to highly potent analogues which displayed excellent inhibition of the negative supercoiling of the relaxed DNA and antibacterial activity.

Introduction: Coumarin-containing antibiotics such as novobiocin (**1**), clorobiocin (**2**) and coumermycin A_1 (**3**), produced by number of Streptomyces species, have been known for more than 40 years.[1] These antibiotics have not found successful pharmaceutical application so far for several reasons: i) poor water solubility and oral bioavailability, ii) low activity against Gram-negative bacteria, iii) toxicity and side effects, iv) rapid emergence of coumarin-resistant bacterial strains. However, renewed interest for this class of antibiotics came after the discovery that these antibiotics are potent inhibitors of bacterial DNA gyrase[2] and topoisomerase IV.[3] DNA gyrase belongs to a class of enzymes known as topoisomerases and is made up of two subunits A and B which form a tetramer A_2B_2. These enzymes play a crucial role in many vital cellular processes such as replication, transcription, translation and recombination.[4] While nalidixic acid and fluoroquinolones are subunit A inhibitors, coumarins are inhibiting the gyrase B subunit that is involved in ATP driven negative supercoiling of relaxed DNA.[5] Renewed interest in coumarin antibiotics came also from their potent Gram-positive antibacterial activity and especially against methicilin-resistant strains of staphylococci species (MRSA and MRSE) which are currently one of the major concerns in treatment of bacterial infections.



1 novobiocin : R_1 = Me, R_2 = NH_2

2 clorobiocin : R_1 = Cl, R_2 = Me



3 coumermycin A_1

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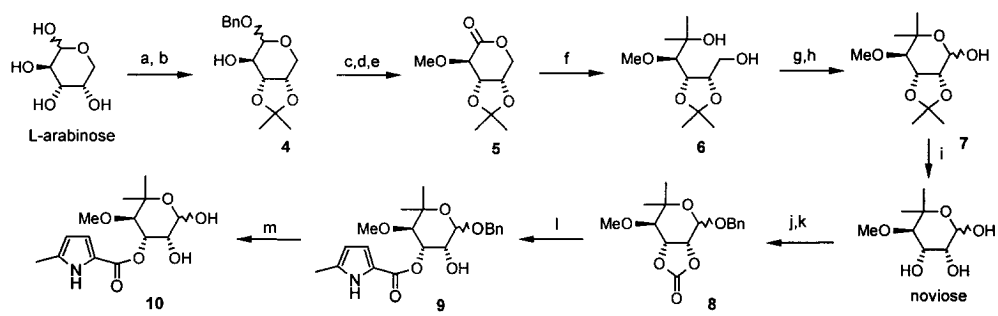
Extensive chemical modifications that have been performed on coumermycin A_1 were all based on semi-synthetic approaches, starting with the convenient intermediates obtained by degradation of coumermycin A_1 isolated by fermentation.[6] The positions mostly exploited were the C-3 amino-position of the coumarin and to the smaller extent C-3' of the sugar noviose.[7] As a part of our program directed toward developing novel antibacterial agents, we decided to look more closely into structure-activity relationship (SAR) of these coumarins. In the light of the recent results obtained by X-ray crystal structure determinations of 24 kDa N-terminal subdomain of the DNA gyrase B with coumarin antibiotics,[8,9] detailed SAR of these inhibitors should lay out a basis for rational drug design of structurally diverse analogues with improved pharmacological profile and physico-chemical properties. In order to achieve this goal, it was necessary to develop an efficient total synthesis of coumarin inhibitors that will enable modifications of the noviose part of the molecule, as well as different positions of the coumarin, to be prepared.

At the beginning of our program, we focussed our efforts on replacing the acylamino function at C-3 position of coumarin with isosteres. It is known that 3-acylamino side chains in **1-3** are not crucial for the inhibition of gyrase B,[10] but contribute to the intracellular uptake of the drug. High lipophilicity of these side chains is probably responsible for the poor aqueous solubility and unfavourable pharmacokinetics of this class of antibiotics. Isosteric replacement of 3-acylamino group of coumarin with C(=O)R, C(=N-OR)R', COOR, CONHR and CONHOR leads to highly potent gyrase inhibitors possessing potent *in vitro* antibacterial activity.

Chemistry: Although several syntheses of noviose have been described,[11] none of them was sufficiently flexible to allow possible functional modifications at the different positions. The efficient synthesis of noviose, starting from L-arabinose, was achieved in 9 steps, with the overall 20% yield and is outlined in Scheme 1.

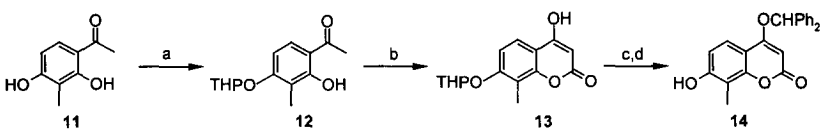


Scheme 1: *Reagents and conditions*: (a) BnOH, HCl gas, rt, 69%; (b) 2,2-Dimethoxypropane, acetone, TosOH cat, rt, quant; (c) *t*-BuOK, Me_2SO_4, THF, rt, quant; (d) H_2, Pd-C/10%, EtOAc, quant; (e) PCC, CH_2Cl_2, rt, 87%; (f) MeMgBr (2 eq), THF, 0 °C, 86%; (g) PCC, CH_2Cl_2, rt, 66%; (h) DIBAH, THF, 0°C, 63%; (i) H_2SO_4, H_2O, 65 °C, 95%; (j) BnOH, HCl gas, rt, 90%; (k) Im_2CO, $(CH_2Cl)_2$, reflux, 82%; (l) 2-methylpyrrole, MeMgBr, toluene, 0°C, 49%; (m) H_2, Pd-C/10%, EtOH, 60°C, quant.

Glycosylation of L-arabinose with excess benzyl alcohol in the presence of gaseous HCl provided the corresponding benzyl glycoside, which was easily converted to acetonide **4** under standard conditions. The free hydroxyl group could be methylated quantitatively either with MeI or Me_2SO_4 in the presence of *t*-BuOK. Hydrogenolysis of the benzyl group proceeded smoothly to afford the lactol, which was oxidised with PCC to

the corresponding lactone **5**. The introduction of gem-dimethyl group of noviose was accomplished by addition of two equivalents of MeMgBr to lactone **5**. The diol **6** could be converted to lactol **7** by employing one equivalent of the oxidant, however, better yields for this transformation were achieved by performing first oxidation of **6** to the lactone followed by reduction with DIBAH. Hydrolysis of acetonide **7** in 0.05M H_2SO_4 at 65°C afforded noviose in 95% yield. Elaboration of noviose to arrive at 3'-(5-methylpyrroyl-2-)-noviose **10** was initiated by benzyl glycosylation as described for L-arabinose. The resulting diol was converted to a carbonate **8** with 1,1'-carbonyldiimidazole in dichloroethane at reflux. Opening of the carbonate with a corresponding pyrrole-Grignard reagent afforded pyrrole ester **9** in 49% yield after chromatographic separation. Subsequent hydrogenolysis of benzyl group in **9** gave rise the diol **10** in quantitative yield.

A suitably protected 4-hydroxycoumarin intermediate **14** was prepared in 4 steps in 12% overall yield starting from the commercially available 2,4-dihydroxy-3-methylacetophenone (**11**) as summarised in Scheme 2.

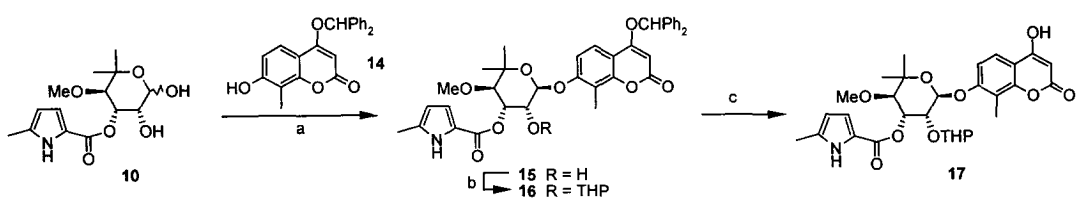


11 12 13 14

Scheme 2: *Reagents and conditions*: (a) DHP, TosOH cat, Et_2O, rt, 83%; (b) NaH, $CO(OEt)_2$, toluene, reflux, 91%.; (c) Ph_2CN_2, DMF, 65°C, 55%.; (d) HCl, MeOH, CH_2Cl_2, rt, 90%.

Selective introduction of dihydropyran in **11** afforded mono-protected THP derivative **12** which was converted with NaH and diethylcarbonate in refluxing toluene directly to 4-hydroxycoumarin **13**. Introduction of the benzhydryl group *via* diphenyldiazomethane onto the 4-OH of coumarin proceeded with a moderate yield. This group proved to be a good choice among the plethora of hydroxyl protective groups. Final deprotection of the THP ether yielded coumarin building block **14**.

With the synthesis of **10** and **14** in hand, we turned to the crucial glycosylation step. This was successfully realised using Mitsunobu's conditions[12] when **10** and **14** were exposed to PPh_3 and DEAD in dichloromethane at room temperature (Scheme 3).

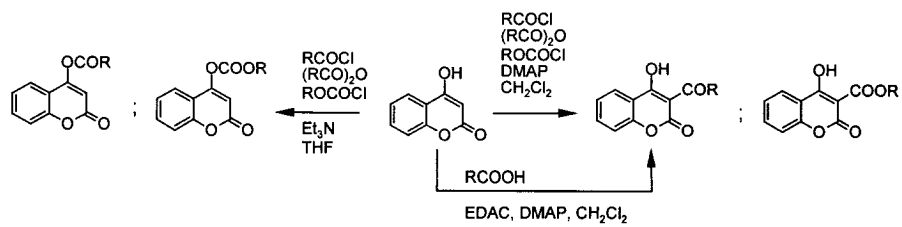


Scheme 3: *Reagents and conditions*: (a) PPh_3, $EtO_2CN{=}NCO_2Et$, CH_2Cl_2, rt, 54%; (b) DHP, TosOH cat, Et_2O, rt, 98%; (c) H_2, Pd-C/10%, THF, quant.

The desired α-glycoside **15** was formed as a major product (α:β = 4:1) and could be easily separated by chromatography from the β-glycoside. Free 2'-hydroxyl group of the noviose fragment was conveniently

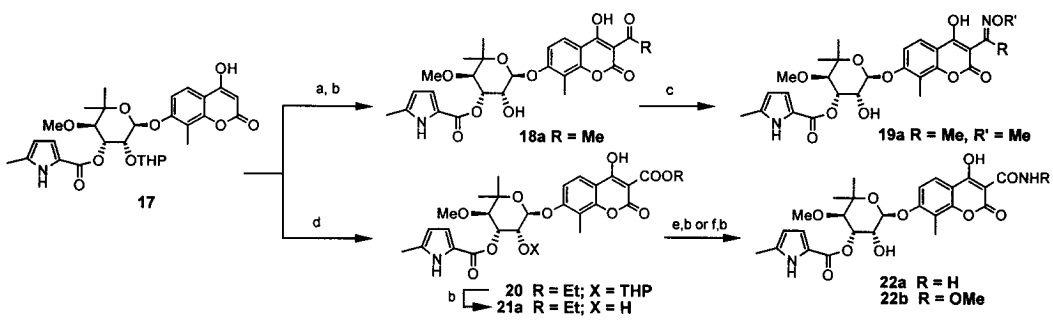
protected as THP ether **16**, while hydrogenolysis of benzhydryl group under standard hydrogenation conditions afforded the coumarin intermediate **17** ready for further functional elaboration.

Two methods were utilised for introduction of COR or COOR groups at C-3 position of 4-hydroxycoumarin (Scheme 4). Reaction of 4-hydroxycoumarin with activated carboxylic acid derivatives (chlorides, anhydrides) or chloroformates in THF in the presence of Et_3N provided the corresponding 4-acyloxy derivatives or carbonates, respectively. However, the same reaction performed in dichloromethane in the presence of DMAP lead directly to the C-3 rearranged products: 4-hydroxy-3-COR or 4-hydroxy-3-COOR coumarins. Moreover, coupling of different acids with 4-hydroxycoumarin in dichloromethane in the presence of DMAP and EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) afforded also corresponding 4-hydroxy-3-COR coumarins.



Scheme 4

Application of the above methodology to 4-hydroxycoumarin intermediate **17** is illustrated by the synthesis of gyrase B inhibitors **18-22** as outlined in Scheme 5 (Table 1). Ketocoumarin **18a** was easily transformed to oxime derivative **19a**[13] with NH_2OMe in ethanol. Further transformation of the coumarin ester **21a** to amide **22a** or hydroxamate **22b** was performed by reaction of **20** with excess of ammonia in THF or hydroxylamine in pyridine, respectively. Deprotection of THP ether was smoothly accomplished in all cases with catalytic amount of *p*-toluenesulfonic acid in methanol at room temperature.

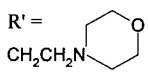
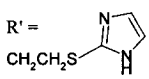


Scheme 5: *Reagents and conditions*: (a) Ac_2O, DMAP, CH_2Cl_2, rt, quant; (b) TosOH cat, MeOH, rt, 95%;(c) $HCl{\cdot}NH_2OMe$, EtOH, KOAc, rt, 73%. (d) $ClCO_2Et$, DMAP, CH_2Cl_2, rt, 82%; (e) NH_3 gas, THF, rt, 95%; (f) $HCl{\cdot}NH_2OMe$, Py, rt, 80%.

Biological results: Table 1 shows the inhibition of the supercoiling activity of *E. coli* DNA gyrase by novobiocin, clorobiocin and the novel coumarin inhibitors and their antibacterial activity. Most of the novel analogues exhibited more potent inhibitory activity than novobiocin. Oximinoderivatives (**19a-d**) generally

displayed excellent inhibition. Introduction of substituents of diverse polarity in novel isosteres (**19b, 19c, 22d, 22e**) were well tolerated. In terms of MICs, all the compounds were active against staphylococci, including MRSA and MRSE and *Streptococcus pyogenes*. With the exception of the phenyl-substituted derivative **22c**, the compounds were active against vancomycin and teicoplanin-resistant *Enterococcus*. Amide and hydroxamate derivatives (**22a**, **22d**) displayed the best antibacterial activity, particularly against novobiocin-resistant strains.

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 $\frac{IC_{50}nov^a}{IC_{50}comp}$	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
18a	Me	2	≤ 0.04	0.15	5	0.08	0.15	2.5
18b	CH_2SMe	1.6	≤ 0.04	≤ 0.04	5	0.08	0.3	5
18c	CH_2OCH_2Me	1.3	≤ 0.04	0.08	20	0.08	0.3	5
19a	Me; R'= Me	3.3	0.15	5	40	0.6	0.3	10
19b	Me R' = 	2	0.15	0.3	40	0.3	1.2	10
19c	Me R' = 	2.8	0.3	0.3	40	0.3	0.3	10
19d	COOEt; R' = Me	1.3	≤ 0.04	ND	10	ND	0.15	5
21a	Et	3.1	0.3	2.5	> 20	0.15	0.3	5
21b	*i*-Bu	0.67	0.08	2.5	10	0.08	ND	5
22a	H	1.67	≤ 0.04	≤ 0.04	2.5	ND	0.15	2.5
22b	OMe	4.2	≤ 0.04	0.08	5	≤ 0.04	0.15	2.5
22c	Ph	2	≤ 0.04	0.08	1.2	≤ 0.04	0.6	> 40
22d	CH_2CH_2OH	1	≤ 0.04	≤ 0.04	2.5	≤ 0.04	0.15	2.5
22e	$CH_2CH_2NMe_2$	2	0.15	0.3	20	ND	1.2	10

a) Supercoiling assay using purified DNA Gyrase from *Echerichia coli*. The enzyme was purified as described in ref. 14 with minor modifications: cells were lysed in the presence of proteases inhibitors and 45% $(NH_4)_2SO_4$ fractions were directly purified on novobiocin-Sepharose affinity column. Relaxed DNA was prepared from pBR322 plasmid with calf thymus topoisomerase I (GIBCO-BRL), 1 hour at 37°C. The DNA concentration was determined by spectrophotometric measurements. Supercoiling assay was performed on 40 μl assay mixture containing 40mM Tris-HCl pH 7.3, 20mM KCl, 4mM $MgCl_2$, 0.5mM ATP, 2mM spermidine-HCl, 0.8μg tRNA, 50ng relaxed DNA and 1 unit of DNA-gyrase. 1 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 (0.25 μg/mL) as reference.

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 noviobiocin, Tei for teicoplanin, Van for vancomycin. Otherwise, strains were fully susceptible.

In conclusion, we have developed an efficient methodology for the preparation of a series of novel coumarin inhibitors of gyrase B. The novel coumarins displayed potent *in vitro* supercoiling inhibitory activity of DNA gyrase and antibacterial activity. With most of the analogues, good antibacterial activity against vancomycin and teicoplanin-resistant *Enterococcus* was observed. Amide derivatives were also active against novobiocin-resistant strains.

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References and Notes:

1. (a) Hinman, J. W.; Hoeksema, H.; Caron, E. L.; Jackson, W. G. *J. Am. Chem. Soc.* **1956**, *78*, 1072-1074. (b) Kawaguchi, H.; Tsukiura, H.; Okanishi, M.; Miyaki, T.; Ohmori, T.; Fujisawa, K.; Koshiyama, H. *J. Antibiotics, Ser A* **1965**, *18*, 1-10.
2. Gellert, M.; O'dea, M. H.; Itoh, T; Tomizawa, J. I. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4474-4478. For a review of gyrase structure and function see: Reece, R. J.; Maxwell, A. *Crit. Rev. Biochem. Mol. Biol.* **1991**, *26*, 335-375.
3. Peng, H.; Marians, K. J. *J. Biol. Chem.* **1993**, *268*, 24481-24490.
4. (a) Watt, P. M.; Hickson, I. D. *Biochem. J.* **1994**, *303*, 681-695. (b) Poljak, L.; Kas, E. *Trends Cell Biol.* **1995**, *5*, 348-354.
5. (a) Drlica, K.; Franco, R. J. *Biochemistry* **1988**, *27*, 2253-2259. (b) Levine, C.; Hiasa, H.; Marians, J. K. *Biochem. Biophys. Acta* **1998**, *1400*, 29-43.
6. (a) Keil, J. G.; Hooper, I. R.; Cron, M. J.; Fardig, O. B.; Nettleton, D. E.; O'Herron, F. A.; Ragan, E. A.; Rousche, M. A.; Schmitz, R. H.; Scheiber, R. H.; Godfrey, J. G. *J. Antibiotics* **1968**, *21*, 551-556. (b) Schmitz, H.; DeVault, R. L.; McDonnell, C. D.; Godfrey, J. G. *J. Antibiotics* **1968**, ***21***, 603-610. (c) Ueda, Y.; Chuang, J. M.; Crast, L. B. Jr.; Partyka, R. A. *J. Org. Chem.* **1988**, *53*, 5107-5133. (d) Ueda, Y.; Chuang, J. M.; Crast, L. B. Jr.; Partyka, R. A. *J. Antibiotics* **1989**, *42*, 1379-1989. (e) Bell, W.; Block, M. H.; Cook, C.; Grant, A.; Timms, D. *J. Chem. Soc., Perkin Trans.* **1997**, *1*, 2789-2801.
7. Ueda, Y.; Chuang, J. M.; Fung-Tomc, J.; Partyka, R. A. *Bio. Med. Chem. Lett.* **1994**, *4*, 1623-1628.
8. (a) Wigley, D. B.; Davies, G. J.; Dodson, E. J.; Maxwell, A.; Dodson, G. *Nature* **1991**, *351*, 624-629. (b) Lewis, R. J.; Singh, O. M. P.; Smith, C. V.; Maxwell, A.; Skarzynsky, T.; Wonacott, A. J.; Wigley, D. B. *J. Mol. Biol.* **1994**, *241*, 128-130. (c) Lewis, R. J.; Singh, O. M. P.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B. *EMBO J.* **1996**, *15*, 1412-1420. (d) Tsai, F. T. F.; Singh, O. M. P.; Skarzynski, T.; Wonacott, J. A.; Weston, S.; Tucker, A.; Pauptit, R. A.; Breeze, A.; Poyser, J. P.; O'Brien, R.; Ladbury, J. E.; Wigley, D. B. *Proteins: Struct. Funct. and Genet.* **1997**, *28*, 41-52.
9. We successfully solved X-ray crystal structures of several coumarin derivatives with 24 kDa N-terminal fragment of gyrase B in collaboration with P. Oudet and D. Moras at the University of Louis Pasteur, Illkirch, France, unpublished results.
10. (a) Reusser, F.; Dolak, L. A. *J. Antibiotics* **1986**, *39*, 272-274. (b) Althaus, I. W.; Dolak, L. A.; Reusser, F. A. *J. Antibiotics* **1988**, *41*, 373-376.
11. (a) Kiss, J.; Spiegelberg, H. *Helv. Chim. Acta* **1964**, *47*, 398-407. (b) Achmatowicz, Jr., O.; Grynkiewicz, G.; Szechner, B.; *Tetrahedron* **1976**, *32*, 1051-1054. (c) Klemer, A; Waldmann, M. *Liebigs Ann. Chem.* **1986**, *2*, 221-225. (d) Pankau, W. M.; Kreiser, W. *Tetrahedron Lett.* **1998**, *39*, 2089-2090.
12. Mitsunobu, O. *Synthesis* **1981**, 1-28.
13. In all cases **19a-d**, only the isomer with *E*-geometry was observed, as confirmed by NOE experiments. This could be attributed to the strong intramolecular hydrogen bond between the hydrogen of the 4-OH of coumarin and N-atom of the oxime.
14. Staudenbauer, W. L.; Orr, E. *Nucleic Acids Res.* **1981**, *9*, 3589-3603.