Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2881–2886

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND BIOLOGICAL EVALUATION OF COUMARINCARBOXYLIC ACIDS AS INHIBITORS OF GYRASE B. L-RHAMNOSE AS AN EFFECTIVE SUBSTITUTE FOR L-NOVIOSE

Didier Ferroud,[a] Jeannine Collard,[a] Michel Klich,[a] Claudine Dupuis-Hamelin,[b] Pascale Mauvais,[b] Patrice Lassaigne,[b] Alain Bonnefoy,[b] and Branislav Musicki*[a]

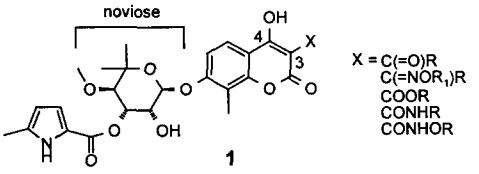
Medicinal Chemistry,[a] Infectious Disease, [b] Hoechst Marion Roussel, 102 route de Noisy, 93235 Romainville Cedex, France

Received 23 June 1999; accepted 2 September 1999

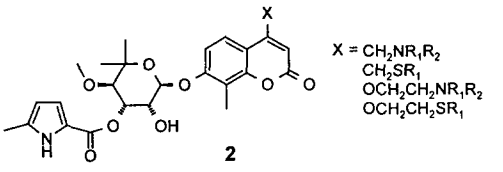
Abstract: A series of novobiocin-like coumarincarboxylic acids has been prepared bearing the L-rhamnosyl moiety as the sugar portion of the molecule. The similar DNA gyrase inhibitory activity of the novel class of coumarins to that of novobiocin demonstrates that L-rhamnose can effectively replace L-noviose. Introduction of alkyl side-chains at C-5 of coumarin leads to improved *in vitro* antibacterial properties in the novel series.

Introduction: For some time, gyrase B as an active component of the tetrameric A_2B_2 complex of DNA gyrase, has attracted considerable attention as a potential antibacterial target since it has no direct counterpart in eucaryotes.[1-3] Previously we have reported two novel series of coumarin inhibitors of DNA gyrase: 4-OH-coumarins with reversed 3-aminoacyl isosteres **1**,[1f] and aminocoumarins **2**,[1e,g] in which the 4-OH group of the coumarin portion was replaced with the basic substituents. We were surprised that the latter analogues proved to be potent inhibitors of DNA gyrase and that structure-activity relationship (SAR) was in contrast to the established knowledge on coumarin inhibitors that emphases 4-OH functionality as indispensable for the potent inhibition of the enzyme.[4]

The X-ray crystallographic studies of several complexes of coumarin inhibitors and the 24 kDa N-terminal fragment of gyrase B indicated that one of the very important interactions was the hydrogen bonding between Arg-136 and the ester (1-position) and carbonyl (2-position) oxygens of the coumarin.[5,6] However, the exact relevance of the interaction of acidic 4-OH of coumarin[7] with gyrase B remained unclear. This could be attributed to the conformational flexibility of the protein loop in gyrase B[5,6] comprising amino acids ~100-120 that was supposedly interacting with acidic 4-OH. This prompted us to prepare a series of coumarinic acids with a carboxyl group at different positions of the coumarin. Moreover, these acids would contain different sized spacers separating the coumarin and carboxyl group.



1



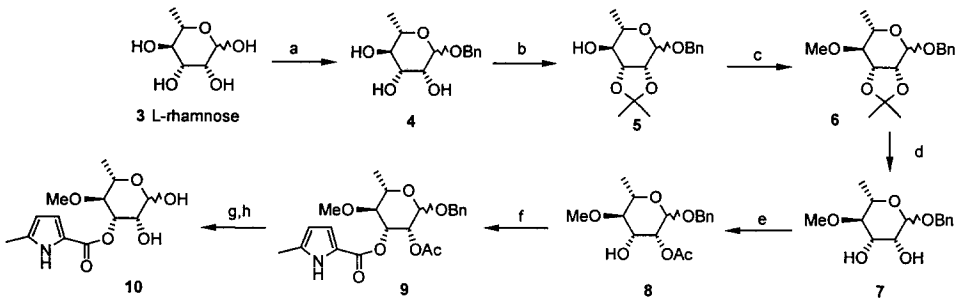
2

*E-mail: branislav.musicki@hmrag.com Fax: 33 1 49 91 50 87

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00493-X

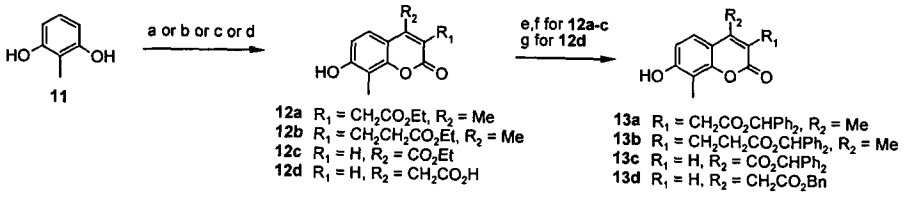
In this report we also describe the development of a complementary series of coumarin drugs based on L-rhamnose that evolved concurrently. It occured to us that already established synthetic sequence for preparation of L-noviose containing analogues starting from L-arabinose[1f] could be applied with minor modifications to L-rhamnose, a comercially available product. Considering that L-rhamnose is structurally very similar with hydrogen replacing axial 5'-methyl group of noviose, this novel series could provide further SAR of this part of the molecule.

Chemistry: Sequential glycosylation of L-rhamnose **3** with excess benzyl alcohol and the protection of the corresponding triol **4** as an acetonide gave the intermediate **5**. The free hydroxyl group in **5** underwent methylation quantitatively with Me_2SO_4 in the presence of *t*-BuOK to give methyl ether **6**. Removal of the isopropylidene acetal in **6** with a mixture of trifluoroacetic acid-H_2O afforded the diol **7** in quantitative yield, that was then protected at the 2'-position as acetate **8** by the method of Pinto M. B. et al.[8] using trimethyl-orthoacetate. Esterification of 3'-position was readily performed with anhydride of (5-methylpyrrole)-2-carboxylic acid[1f] in the presence of $CoCl_2$ in MeCN at reflux[9] to provide the ester **9**. Hydrolysis of acetate **9** was achieved with DBU in MeOH. Finally, hydrogenolysis of the benzyl group proceeded quantitatively to afford the lactol **10**, a suitable intermediate for glycosylation.



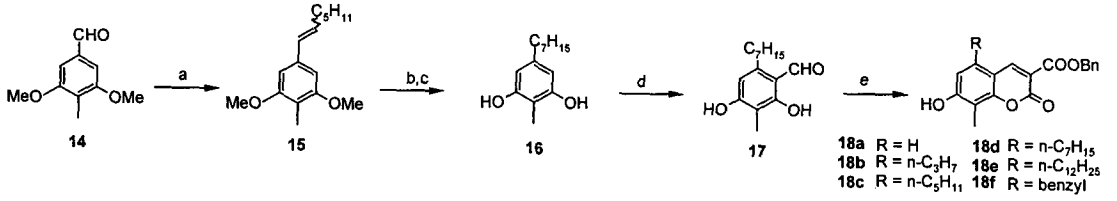
Scheme 1: *Reagents and conditions*: (a) BnOH, HCl gas, rt, 82%; (b) 2,2-Dimethoxypropane, TosOH cat, rt, 96%; (c) *t*-BuOK, Me_2SO_4, THF, rt, quant.; (d) CF_3CO_2H-H_2O 4:1, rt, quant.; (e) $MeC(OMe)_3$, TosOH cat., MeCN, then AcOH-H_2O 4:1, rt, 78%; (f) (5-methylpyrroyl)-2-carboxyl anhydride, $CoCl_2$, MeCN, reflux, 84%; (g) DBU, MeOH, rt. 75%; (h) H_2, Pd-C/10%, EtOH, 50°C, quant.

Most of the coumarincarboxylic acids were prepared by the classical Pechmann condensation[10] of the 2-methylresorcinol **11** with various β-ketocarboxylic esters as outlined in Scheme 2. The isolated ethyl esters **12a-c** were transformed into benzhydryl esters **13a-c** by saponification with NaOH and subsequent esterification with diphenyldiazomethane in DMF. Coumarin-4-acetic acid **12d** underwent selective benzylation at carboxyl group using benzylbromide in the presence of $NaHCO_3$ in DMF to give benzyl ester **13d**.



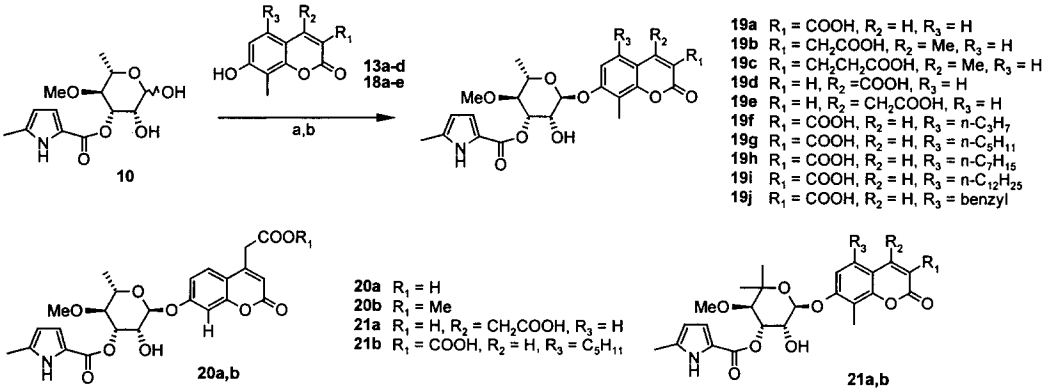
Scheme 2: *Reagents and conditions*: (a) $MeCOCH(CO_2Et)CH_2CO_2Et$, H_2SO_4, 23%; (b) $MeCOCH(CO_2Et)CH_2CH_2CO_2Et$, H_2SO_4, 52%; (c) $EtO_2CCOCHNaCO_2Et$, EtOH, reflux, 13%; (d) $HO_2CCH_2COCH_2CO_2H$, H_2SO_4, 75%; (e) NaOH 2N, rt, 60-90%; (f) Ph_2CN_2, DMF, 50°C, 50-60%; (g) BnBr, $NaHCO_3$, DMF, rt, 55%.

The synthesis of 5-alkylsubstituted coumarin-3-carboxylic acids is illustrated in Scheme 3, by the preparation of C-5 heptyl analogue **18d**. Wittig olefination of aldehyde **14**, itself readily available from 3,5-dimethoxybenzaldehyde,[11] with n-pentyltriphenylphosphoranylidene afforded a *Z/E* mixture of olefins **15** in good yield. Hydrogenation of the double bond in **15** followed by bis-demethylation with TMSI in chloroform at reflux provided resorcinol **16** in 75% yield. Introduction of the aldehyde group was achieved under classical Gattermann formylation[12] with $Zn(CN)_2$ and gaseous HCl. Condensation of the aldehyde **17** with dibenzyl-malonate was readily accomplished in DMF in the presence of pyrrolidine to give coumarin-3-carboxylic acid **18d** in 92% yield, protected as benzyl ester.



Scheme 3: *Reagents and conditions*: (a) $C_5H_{11}CH_2PPh_3Br$, BuLi, THF, 0°C, 78%; (b) H_2, Pd-C, THF, rt., quant. (c) Me_3SiI, $CHCl_3$; reflux, 75%; (d) $Zn(CN)_2$, HCl gas, Et_2O, rt, 60%; (e) $CH_2(COOBn)_2$, pyrrolidine, DMF, 65°C, 92%.

Having prepared coumarincarboxylic acids as protected benzyl or benzhydryl esters (**13a-d** and **18a-f**), we used the Mitsunobu conditions[1f,g] for their glycosylation with the rhamnose intermediate **10** (Scheme 4). In all cases the desired α-glycosides were formed as the major products and could be easily separated from the minor β-glycosides by chromatography. Free carboxyl groups were liberated by hydrogenolysis under standard hydrogenation conditions to provide the coumarincarboxylic acids **19a-j**.



Scheme 4: *Reagents and conditions*: (a) PPh_3, $EtO_2CN{=}NCO_2Et$, CH_2Cl_2, rt. 40-50%; (b) H_2, Pd-C/10%, THF, quant.

In order to compare L-rhamnose and L-noviose series, as well the series of coumarin acids lacking the 8-methyl group in the coumarin part, several analogues **20a,b** and **21a,b** (Scheme 4) were prepared by Mitsunobu coupling of the described coumarins with pyrrole-rhamnose **10** or pyrrole-noviose,[1f] respectively.

Biological results: Table 1 shows the results for the inhibition of the supercoiling activity of *E. coli* DNA gyrase by novobiocin, clorobiocin and the novel coumarincarboxylic acids and their antibacterial activity. Comparison of the inhibitory activities of free acid **20a** and its methyl ester **20b** clearly indicates that the caboxyl group provides an important contribution in stabilising the complex of inhibitor with the protein. The position of the carboxyl group may not be crucial as similar activities were observed in the analogues **19a,b**, **19d** and **19e**. Insertion of the two carbon atoms in between the carboxyl group and coumarin skeleton (**19c**) resulted only in minor diminution of inhibition.

Replacement of the 5'-axial methyl group of the noviose moiety (**21**) by hydrogen in rhamnose (**19e**) resulted in five-fold reduction of inhibition of supercoiling of DNA gyrase. This diminution of activity is not surprising considering the results provided by the analysis of crystal structures of coumarin drugs and gyrase B of *E. coli*.[5,6] The 5',5'-dimethyl group of the noviose in these complexes is surrounded by the hydrophobic amino acids and substitution of methyl group for hydrogen reflects a partial loss of these hydrophobic interactions. Nevertheless, the series of coumarinic acids containing rhamnose did retain similar inhibitory activity on DNA gyrase to that of novobiocin.

The same hydrophobic effect could be invoked to explain three-fold diminution of DNA gyrase inhibition that resulted from the lack of C-8 methyl group of coumarin (comparison between **20a** and **19e**).

Although coumarincarboxylic acids showed good inhibition of supercoiling of DNA gyrase, they were not as active against whole bacterial cells. This could be rationalised by highly hydrophilic nature of these compounds. It was necessary to make them more lipophilic through introduction of the alkyl chains. The most suitable position was the C-5 of the coumarin. The optimum length of the aliphatic chain with respect to antibacterial activity was seven for the rhamnose series (**19h**), and five for the noviose series (**21b**). Replacing

rhamnose moiety (**19g**) by noviose (**21b**) resulted in ten-fold increase in the antibacterial activity. The derivative **21b** was highly active against all the strains tested, including multi-resistant staphylococci and vancomycin-resistant *E. faecium*.

We were also interested to see the effect of isosteric replacement (-$CONHSO_2$-)of carboxyl group in coumarins **19a-j**. The *N*-coumaroylbenzensulfonamide **22** was prepared from acid **19h** by coupling with benzensulfonamide in the presence of *N,N*-diisopropylcarbodiimide and DMAP in CH_2Cl_2. As demonstrated by measurements of IC_{50}, *N*-acylsulfonamides can effectively replace the carboxyl group, preserving at the same time uniformly good antibacterial properties whatever the resistance phenotype of the strains studied.

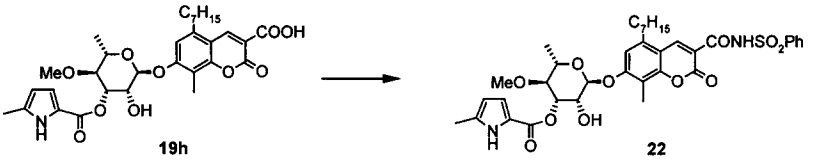


Table 1. *In vitro* activity of coumarin inhibitors against *E. coli* DNA gyrase supercoiling (IC_{50}),[a] and selected *in vitro* antibacterial activity (MIC).[b,c]

Compound	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
19a	0.33	>40	>40	>40	>40	40	>40
19b	0.5	>40	>40	>40	>40	40	>40
19c	0.33	>40	>40	>40	>40	40	>40
19d	0.5	>40	>40	>40	>40	>40	>40
19e	1	10	40	>40	10	>40	>40
19f	0.5	10	40	>40	10	>40	>40
19g	0.5	0.6	2.5	20	0.6	1.2	2.5
19h	0.5	0.08	0.3	5	0.3	0.3	0.6
19i	1.3	0.08	5	>40	40	0.3	>40
19j	0.5	2.5	5	>40	2.5	5	20
20a	0.33	>40	>40	>40	>40	>40	>40
20b	0.031	>40	>40	>40	>40	>40	>40
21a	5.3	2.5	5	>40	2.5	10	>40
21b	0.66	≤ 0.04	0.15	2.5	≤ 0.04	≤ 0.04	0.3
22	1	0.15	0.6	2.5	0.6	0.3	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 1f.

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.

In conclusion, the described series of coumarincarboxylic acids exerted similar inhibition in the negative supercoiling of DNA gyrase to novobiocin. Antibacterial activity in the series was conferred by introduction of the alkyl chains at the 5-position of the coumarin. Although some loss of the inhibitory and antibacterial activity was observed on replacement of noviose by rhamnose, the latter remained an effective substitute for noviose. The best candidate **21b** displayed excellent antibacterial activity against all the strains including the novobiocin-resistant strain.

Acknowledgement: We would like to thank the Analytical Department (HMR Romainville) for performing the spectral analysis.

References and Notes

1. For the recent work on novobiocin-like inhibitors of DNA gyrase see: (a) Ueda, Y.; Chuang, J. M.; Crast, L. B. Jr.; Partyka, R. A. *J. Antibiotics* **1989**, *42*, 1379-1392. (b) Ueda, Y.; Chuang, J. M.; Fung-Tomc, J; Partyka, R. A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1623-1628. (c) Bell, W.; Block, M. H.; Cook, C.; Grant, A.; Timms, D. *J. Chem. Soc., Perkin Trans.* **1997**, *1*, 2789-2801. (d) Klich, M.; Laurin, P.; Musicki, B.; Schio, L. WO 9747634, 1998; *Chem. Abstr.* **1998**, *128*, 75634. (e) Chartreaux, F.; Klich, M.; Schio, L. EP 894805, 1999; *Chem. Abstr.* **1999**, *130*, 125344. (f) Laurin, P; Ferroud, D.; Klich, M; Dupuis-Haelin, C.; Mauvais, P.; Lassaigne, P.; Bonnefoy, A.; Musicki, B. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2079-2084. (g) Laurin, P; Ferroud, D.; Schio, L.; Klich, M; Dupuis-Haelin, C.; Mauvais, P.; Lassaigne, P.; Bonnefoy, A.; Musicki, B. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2875.
2. For the family of cyclothialidine inhibitors of DNA gyrase see: (a) Arisawa, M.; Gotsshi, E. Kamiyama, T.; Masciadri, R.; Shimada, H.; Watanabe, J.; Hebeisen, P.; Link, H. WO 9218490, 1992; *Chem. Abstr.* **1992**, *119*, 117285; (b) Nakada, N.; Shimada, H.; Hirata, T.; Aoki, Y.; Kamiyama, J.; Watanabe, J.; Arisawa, M. *Antimicrob. Agents Chemother.* **1993**, *37*, 2656-2661. (c) Goetschi, E.; Angehrn, P.; Gmünder, H; Hebeisen, P.; Link, H.; Masciadri, R.; Nielsen, *J. Pharmacol. Ther.*, **1994**, *60*, 367. (d) Watanabe, J.; Nakada, N.; Sawaira, S.; Shimada, H.; Ohshima, S.; Kamayma. T.; Arisawa, M. *J. Antibiot.* **1994**, *47*, 32-36. (e) Kamiyama, T.; Schimma, N.; Ohtsuka, T; Nakayama, N.; Itezono, Y.; Nakada, N.; Watabane, J.; Yokose, K. *J. Antibiot.* **1994**, *47*, 37-45. (f) Nakada, N.; Gmünder, Hirata, T.; Arisawa, M. *Antimicrob. Agents Chemother.* **1994**, *38*, 1966-1973. (g) Goschi, E.; Hebeisen, P.; Link, H. Lubbers, T. EP 675122, 1995; *Chem. Abstr.* **1995**, *124*, 146213. (h) Yamaji, K.; Masubuchi, M.; Kawahara, F.; Nakamura, Y.; Nishio, A.; Matsukuma, S.; Fujimori, M.; Nakada, N.; Watanabe, J.; Kamiyama, T. *J. Antibiotics* **1997**, *50*, 402-411.
3. For the novel class of triazine inhibitors of DNA gyrase see: Poyser, J. P.; Telford, B.; Timms, D.; Block, M. H.; Hales, N. J. WO 9901442, 1999; *Chem. Abstr.* **1999**, *130*, 125099.
4. Godfrey, J. C.; Price, K. E. *Adv. Appl. Microbiol.* **1972**, *15*, 231-296.
5. (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.
6. We successfully determined the 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.
7. The pKa of the 4-OH of the coumarin part of novobiocin was determined as 4.3. Hoeksema, H.; Johnson, J. L.; Hinman, J. W. *J. Am. Chem. Soc.* **1955**, *77*, 6710-6711.
8. Pinto. M. B.; Morissete, D. G. *J. Chem. Soc. Perkin Trans. I* **1987**, 9-14.
9. Iqbal, J.; Srivastava, R. R. . *J. Org. Chem.* **1992**, *57*, 2001-2007.
10. Sethna, S.; Phadke, R. In *Organic Reactions*; John Willey & Sons; New York, 1953; Vol 7, pp 1-58.
11. Comins, D. L.; Brown, J. D. *J. Org. Chem.* **1984**, *49*, 1078-1083
12. Truce, W. E. In *Organic Reactions*; John Willey & Sons; New York, 1957; Vol 9 , pp 37-72.