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γ-Lactone-Functionalized Antitumoral Acetogenins are the Most Potent Inhibitors of Mitochondrial Complex I

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Abstract—To study the relevance of the terminal α,β-unsaturated γ-methyl-γ-lactone moiety of the antitumoral acetogenins of Annonaceae for potent mitochondrial complex I inhibition, we have prepared a series of semisynthetic acetogenins with modifications only in this part of the molecule, from the natural rolliniastatin-1 (**1**) and cherimolin-1 (**2**). Some of the hydroxylated derivatives (**1b, 1d** and **1e**) in addition to two infrequent natural β-hydroxy γ-methyl γ-lactone acetogenins, laherradurin (**3**) and itrabin (**4**), are more potent complex I inhibitors than any other known compounds.

Introduction

The acetogenins of Annonaceae (ACG) are very interesting compounds because they have well-known biological effects such as potent cytotoxic, antitumor, parasitic and insecticide activities.[1] All these diverse biological activities are explained by the inhibition of the enzyme NADH:ubiquinone oxidoreductase or complex I of the mitochondrial respiratory chain.[2] Although complex I is probably the first enzyme regarding number of inhibitors,[3] some natural ACG are the most potent inhibitors known to date.[1,4]

ACG are members of a class of polyketide derivatives characterized by the presence of one or two tetrahydrofuranic (THF) rings in the center of a long alkyl chain bearing a terminal γ-lactone.[1,4] Therefore, they have longer and more flexible structures than most complex I inhibitors, an unusual characteristic that has hindered the unambiguous establishment of a structure–activity relationship.[5,6] Recent studies have shown that the two functional units, the central THF system flanked by hydroxyl groups and the terminal α,β-unsaturated γ-lactone, and also the alkyl chain that acts as a spacer between them, play significant roles in inhibiting the enzyme.[6,7]

It is thought that this THF system mainly mimics the quinone head of the natural substrate of the complex I, the ubiquinone, whereas the γ-lactone moiety allows a second binding point if it is properly positioned by the alkyl spacer and its oxygenated groups.[6,7d,e] However, the relevance of the γ-lactone moiety itself for potent complex I inhibition has not been studied yet. With this aim, we have prepared two series of semisynthetic ACG with modifications only in the terminal α,β-unsaturated γ-lactone moiety and the close 4-hydroxy group of the alkyl chain spacer. The first series was obtained from the adjacent bis-THF ACG, rolliniastatin-1 (**1**), isolated from *Rollinia membranacea* seeds.[7c] The second series was obtained from the non-adjacent bis-THF ACG, cherimolin-1 (**2**), isolated from *Annona cherimolia* seeds.[8a] The inhibitory potency of the new derivatives was additionally compared with laherradurin (**3**) and itrabin (**4**), also isolated from *A. cherimolia* seeds,[8b] which present a scarce β-hydroxylated saturated γ-lactone moiety. We have found that some of these lactone-modified ACG are more potent complex I inhibitors than any other known compound.

Chemistry

When the catalytic hydrogenation of the 4-hydroxylated α,β-unsaturated γ-lactone ACG was performed in a MeOH solution with 10% Pd/C a 1:1 mixture of two diastereoisomers (2,36-*cis* and -*trans*) was obtained, due

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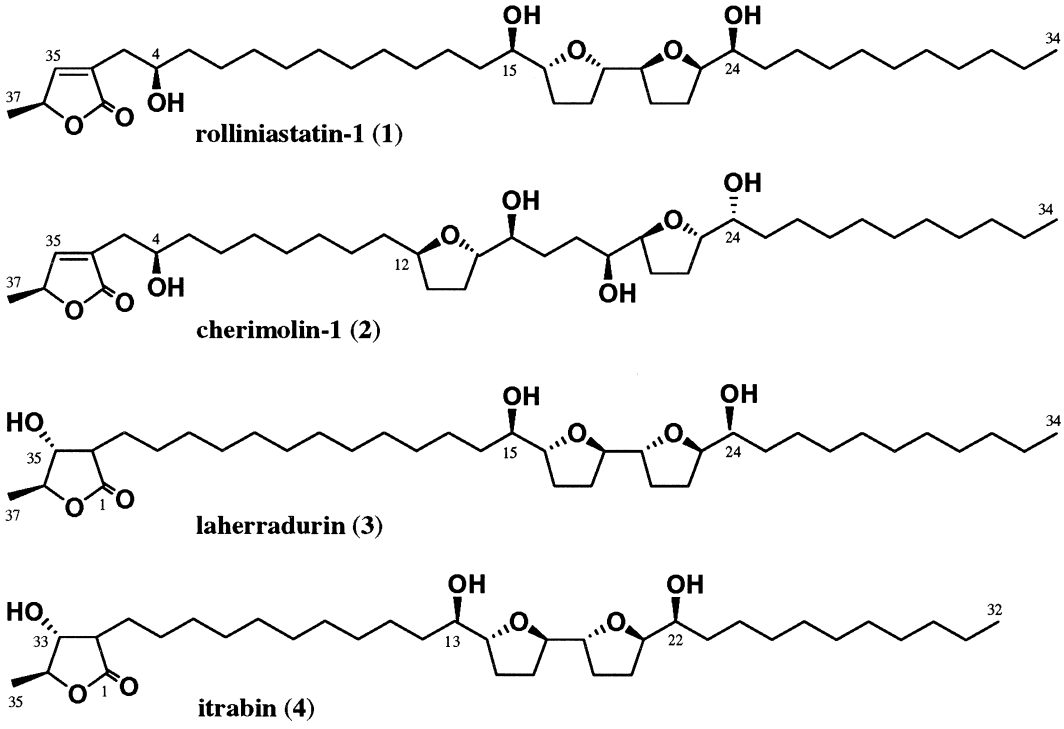
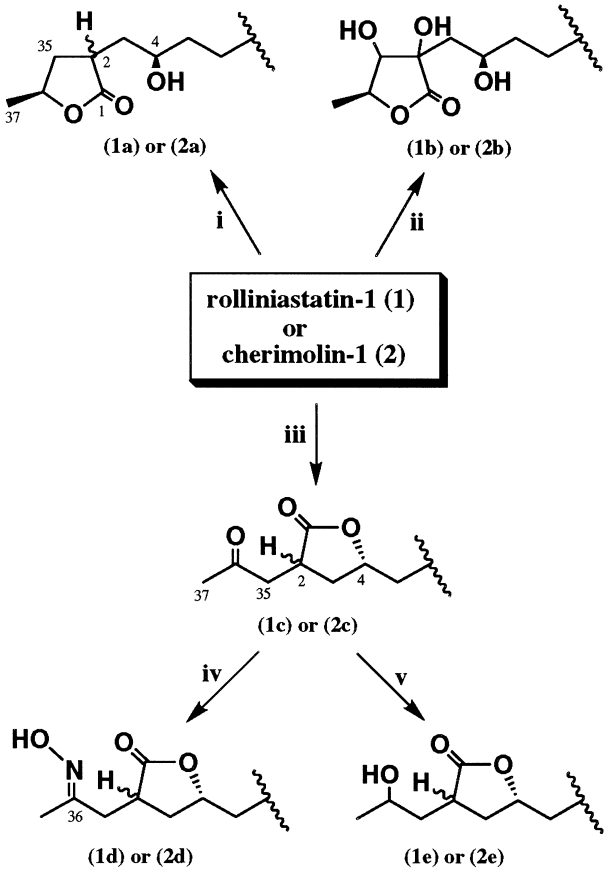


to a stabilization of the molecules by H-bonding that allows both faces to be similarly accessible.[9] In these conditions the 2,35-dihydrogenated derivatives, **1a** and **2a**,[10] were prepared in quantitative yields from **1** and **2** (Scheme 1).

The implementation of two hydroxyl groups in the α,β-unsaturated γ-methyl-γ-lactone was carried out by treatment of **1** and **2** with osmium tetroxide and *N*-methylmorpholine *N*-oxide monohydrate to regenerate the oxidizing agent,[11] affording **1b** (75%) and **2b** (60%),[12] respectively, as mixtures of the corresponding *cis*-dihydroxylated derivatives.

It has been well established that the alkaline treatment of α,β-unsaturated γ-methyl-γ-lactone ACG hydroxylated at 4 position, affords in high yields the translactonized 2-acetonyl saturated γ-lactone derivatives, as a mixture of 2,4-*cis* and 2,4-*trans* diastereoisomers usually called 'isoacetogenins'.[13] Thus, the alkaline treatment (DEA) of a MeOH solution of **1** and **2** and further purification of the crude by preparative HPLC (CH_3CN/H_2O 80:20) at a specific wavelength (263 nm), afforded **1c** and **2c** in a good yield (≈85%).[14]

Recently, we have reported that the hydroxylimino derivatives prepared from ketonic bis-THF ACG in which a nitrogen atom is inserted in the molecule, increase the complex I inhibitory potency.[7a,e] Therefore the ketonics **1c** and **2c** were employed as starting material for preparing the corresponding 36-deoxo-36-hydroxylimino derivatives, **1d** and **2d**,[15] respectively, obtained as a mixture of 2,4-*cis* and 2,4-*trans* diastereoisomers in an ≈80% yield.



Scheme 1. Reagents and conditions: (i) H_2, 10% Pd/C, 1 atm, rt, 2 h; (ii) NMO/OsO_4, rt, 24 h, followed by 2% $NaHSO_3$, rt, 1 h; (iii) DEA, reflux, 24 h; (iv) $H_2NOH{\cdot}HCl$/Pyr, reflux, 1.5 h; (v) $NaBH_4$/MeOH, rt, 1 h.

Table 1. Inhibitory potency of natural and semisynthetic ACGs

Compound		IC_{50} (nM)		IC_{50} DB/Ox ratio	Relative potency[a]
		NADH oxidase	NADH:DB oxidoreductase		
1	Rolliniastatin-1	0.60±0.04	0.74±0.04	1.2	3.3
1a	2,35-Dihydrorolliniastatin-1	0.43±0.07	0.57±0.06	1.3	2.4
1b	2,35-Dihydro-2,35-dihydroxy-rolliniastatin-1	0.21±0.05	0.56±0.02	2.7	1.2
1c	Isorolliniastatin-1	0.33±0.02	1.21±0.23	3.7	1.8
1d	36-Deoxo-36-hydroxylimino-isorolliniastatin-1	0.23±0.06	0.22±0.04	1.0	1.3
1e	36-Dihydroisorolliniastatin-1	0.18±0.01	0.21±0.04	1.2	**1.0**
2	Cherimolin-1	1.84±0.27	5.18±0.63	2.8	10.2
2a	2,35-Dihydrocherimolin-1	1.22±0.28	3.32±1.22	2.7	6.8
2b	2,35-Dihydro-2,35-dihydroxy-cherimolin-1	8.47±1.31	38.3±8.1	4.6	47.1
2c	Isocherimolin-1	0.83±0.38	2.88±0.04	3.5	4.6
2d	36-Deoxo-36-hydroxylimino-isocherimolin-1	0.54±0.11	1.42±0.13	2.6	3.0
2e	36-Dihydroisocherimolin-1	2.48±0.32	4.64±0.44	1.9	13.8
3	Laherradurin	0.18±0.02	0.29±0.01	1.6	**1.0**
4	Itrabin	0.21±0.03	0.72±0.10	3.4	1.2

[a]Relative potency compared with the NADH oxidase IC_{50} of the most potent compounds **1e** and **3** (the lowest value, the highest potency).

The reduction of the ketonic group of the isoacetogenins was prepared to elucidate the influence in the inhibitory potency of the 2-acetonyl group. $NaBH_4$ reduction of **1c** and **2c** produced 36-dihydrogenated derivatives (**1e** and **2e**)[16] in good yields (Scheme 1).

Bioactivity

The inhibitory potency of the natural and semi-synthetic ACG was evaluated against the integrated NADH oxidase activity of beef heart open submitochondrial particles as well as against the specific complex I NADH:ubiquinone oxidoreductase activity using decylubiquinone (DB) as artificial ubiquinone-like substrate (NADH:DB oxidoreductase activity),[6,17] which is a good indicator of tight binding of the inhibitor to the enzyme.[3,6,18] Results are shown in Table 1. All rolliniastatin-1 derivatives (**1a**–**1e**) were more potent than the natural compound (**1**). Isoacetogenin derivatives (**1c**–**1e**) were more potent probably because of a shorter distance between the THF system and the terminal γ-lactone in accordance with previous results obtained with rollimembrin,[19] an ACG with 2 C shorter as **1**. Moreover, the presence of a hydroxylimino group (**1d**) increased the potency, according to previous results with other ACG series.[7a,e] It is worth noting that 36-dihydro-isorolliniastatin-1 (**1e**) gave the strongest inhibitory potency ever found for a complex I inhibitor. It seems that the presence of hydroxyl groups in the core of the lactone itself gives an extreme potency in these compounds.

For that reason we decided to assay the β-hydroxylated γ-lactone ACG, laherradurin (**3**) and itrabin (**4**). These compounds also showed an extremely high potency, confirming the relevance of the hydroxyl group placed in the terminal saturated γ-lactone moiety, especially at a proper length from the THF system.

Nevertheless, compounds **1b** and **1c** gave IC_{50} DB/Ox ratios higher than the remaining derivatives of this series. This is an indication of their weaker binding to the enzyme and easier displacement by the substrate analogue. Therefore, it reveals that the binding site of the terminal lactone moiety of the ACG is also related with an ubiquinone binding site in the complex I.[6,18]

Three out of five cherimolin-1 derivatives (**2a**, **2c** and **2d**) were also more potent than their head of series (**2**). But all of them were less potent than rolliniastatin-1 derivatives. The different THF system and the consequent different length of the alkyl spacer between this and the terminal lactone moiety were then probably less appropriate for potent inhibition.

In conclusion, we have found that some γ-lactone-functionalized ACG derivatives as well as two natural compounds with an uncommon β-hydroxylated saturated γ-lactone moiety are the most potent complex I inhibitors known up to date. Moreover, we have shown that the terminal lactone of the long and flexible ACG structure contributes significantly for tight binding to the enzyme and potent inhibitory activity. Therefore, our results are highly interesting for an optimal design of antitumoral drugs with this new mechanism of action.

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References and Notes

1. (a) Zafra-Polo, M. C.; Figadère, B.; Gallardo, T.; Tormo, J. R.; Cortes, D. *Phytochemistry* **1998**, *48*, 1087. (b) Alali, F. Q.; Liu, X. X.; McLaughlin, J. L. *J. Nat. Prod.* **1999**, *62*, 504.
2. Londershausen, M.; Leicht, W.; Lieb, F.; Moeschler, H.; Weiss, H. *Pestic. Sci.* **1991**, *33*, 427.
3. Degli Esposti, M. *Biochim. Biophys. Acta* **1998**, *1364*, 222.
4. Tormo, J. R.; Gallardo, T.; González, M. C.; Bermejo, A.; Cabedo, N.; Andreu, I.; Estornell, E. *Curr. Top. Phytochem.* **1999**, *2*, 69.

5. Miyoshi, H.; Ohsima, M.; Shimada, H.; Akagi, T.; Iwamura, H.; McLaughlin, J. L. *Biochim. Biophys. Acta* **1998**, *1365*, 443.
6. Tormo, J. R.; Gallardo, T.; Aragón, R.; Cortes, D.; Estornell, E. *Chem.–Biol. Interact*. **1999**, *122*, 171.
7. (a) Gallardo, T.; Sáez, J.; Granados, H.; Tormo, J. R.; Vélez, I. D.; Brun, N.; Torres, B.; Cortes, D. *J. Nat. Prod.* **1998**, *61*, 1001. (b) Shimada, H.; Grutzner, J. B.; Kozlowski, J. F.; McLaughlin, J. L. *Biochemistry* **1998**, *37*, 854. (c) González, M. C.; Lavaud, C.; Gallardo, T.; Zafra-Polo, M. C.; Cortes, D. *Tetrahedron* **1998**, *54*, 6079. (d) Kuwabara, K.; Takada, M.; Iwata, J.; Tatsumoto, K.; Sakamoto, K.; Iwamura, H.; Miyoshi, H. *Eur. J. Biochem*. **2000**, *267*, 2538. (e) Gallardo, T.; Zafra-Polo, M. C.; Tormo, J. R.; González, M. C.; Franck, X.; Estornell, E.; Cortes, D. *J. Med. Chem*. **2000**, *43*, 4793.
8. (a) Cortes, D.; Myint, S. H.; Dupont, B.; Davoust, D. *Phytochemistry* **1993**, *32*, 1475. (b) Cortes, D.; Myint, S. H.; Leboeuf, M.; Cavé, A. *Tetrahedron Lett*. **1991**, *32*, 6133.
9. Cortes, D.; Myint, S. H.; Harmange, J. C.; Sahpaz, S.; Figadère, B. *Tetrahedron Lett*. **1992**, *33*, 5225.
10. 2,35-Dihydrogenated ACG derivatives. Compound **1a**: $C_{37}H_{68}O_7$; $[\alpha]_D$ +20° (*c* 3.1, EtOH); LSIMS *m*/*z* 647 $M+Na^+$, 625 MH^+. Compound **2a**: see ref 8a. The elucidations were made by EIMS, COSY 45, DEPT and HMQC.
11. Elomri, A.; Mitaku, S.; Michel, S.; Skaltsounis, A. L.; Tillequin, F.; Koch, M.; Pierré, A.; Guilbaud, N.; Léonce, S.; Kraus-Berthier, L.; Rolland, Y.; Atassi, G. *J. Med. Chem*. **1996**, *39*, 4762.
12. 2,35-Dihydroxylated ACG derivatives: relevant values. Compound **1b**: $[\alpha]_D$ +30° (*c* 0.1, EtOH); HRLSIMS *m*/*z* 657.495061 MH^+ (calcd 657.4941 for $C_{37}H_{69}O_9$); ^{1}H NMR* ($CDCl_3$, 400 MHz) δ 4.70 and 4.10 (2m, H-4), 4.36 and 3.95 (2m, H-36), 3.77 and 3.75 (2d, *J* = 8, 6.4 Hz, H-35), 2.26, 2.18, 2.10 and 1.77 (4dd, *J* = 6, 14 Hz, H-3), 1.47 and 1.35 (2d, *J* = 6.8, 6.0 Hz, H-37); ^{13}C NMR* ($CDCl_3$, 100 MHz) δ 176.9 and 175.2 (C-1), 80.2 and 75.0 (C-2), 79.7 and 69.6 (C-36), 79.2 and 68.9 (C-4), 77.9 and 73.4 (C-35), 40.5 and 36.4 (C-3), 18.0 and 20.5 (C-37). Compound **2b**: $[\alpha]_D$ +6° (*c* 3.3, EtOH); HRLSIMS *m*/*z* 673.492257 MH^+ (calcd 673.489074 for $C_{37}H_{69}O_{10}$).*The assignments were made by COSY 45, DEPT, TOCSY, HMQC and HMBC.
13. (a) Hoye, T. R.; Hanson, P. R. *J. Org. Chem*. **1991**, *56*, 5092. (b) Duret, P.; Laurens, A.; Hocquemiller, R.; Cortes, D.; Cavé, A. *Heterocycles* **1994**, *39*, 741.
14. (a) Abreo, M. J.; Sneden, A. T. *J. Nat. Prod*. **1990**, *53*, 983. (b) Duret, P.; Gromek, D.; Hocquemiller, R.; Cavé, A.; Cortes, D. *J. Nat. Prod*. **1994**, *57*, 911.
15. 36-Hydroxylimino isoacetogenin derivatives: relevant values. Compound **1d**: $[\alpha]_D$ +31° (*c* 0.3, EtOH); HRLSIMS *m*/z 638.498581 MH^+ (calcd 638.499579 for $C_{37}H_{68}O_7N$); ^{1}H NMR* ($CDCl_3$, 400 MHz) δ 4.50 (m, H-4 *trans*), 4.35 (m, H-4 *cis*), 3.01–2.94 (m, H-2 *trans*), 2.92–2.87 (m, H-2 *cis*), 2.83 (dd, *J* = 3.4, 16 Hz, H-35b *cis*), 2.72 (m, H-35b *trans*), 2.53–2.47 (m, H-3b *cis*), 2.34 (dd, *J* = 10.4, 16.0 Hz, H-35a *trans*), 2.26 (dd, *J* = 10.4, 16.0 Hz, H-35a *cis*), 2.09–2.04 (m, H-3b *trans*), 1.88 (s, H-37), 1.55–1.40 (m, H-3a); ^{13}C NMR* ($CDCl_3$, 100 MHz) δ 178.7 (C-1 *trans*), 178.2 (C-1 *cis*), 155.5 (C-36 *cis*), 155.3 (C-36 *trans*), 79.2 (C-4 *cis*), 79.0 (C-4 *trans*), 38.2 (C-2 *cis*), 36.6 (C-2 *trans*), 36.5, 35.4, 35.3 (C-3, C-5 and C-35 *cis*), 35.5, 35.2, 32.9 (C-3, C-5 and C-35 *trans*), 13.8 (C-37 *cis*), 13.7 (C-37 *trans*). Compound **2d**: HRLSIMS *m*/*z* 654.494133 MH^+ (calcd 654.494494 for $C_{37}H_{68}O_8N$). *The assignments were made by COSY 45, DEPT and HMQC.
16. 36-Dihydrogenated isoacetogenin derivatives. Compound **1e**: $[\alpha]_D$ +27° (*c* 3.1, EtOH); HRLSIMS *m*/*z* 647.484177 (calcd 647.486275 for $C_{37}H_{68}O_7+Na$). Compound **2e**: $[\alpha]_D$ +8.4° (*c* 3.2, EtOH); HRLSIMS *m*/z 641.496251 (calcd 641.499245 for $C_{37}H_{69}O_8$). The elucidations were made by EIMS, COSY 45, DEPT, TOCSY, HMQC and HMBC.
17. Estornell, E.; Fato, R.; Palotti, F.; Lenaz, G. *FEBS Lett*. **1993**, *332*, 127.
18. Tormo, J. R.; González, M. C.; Cortes, D.; Estornell, E. *Arch. Biochem. Biophys*. **1999**, *369*, 119.
19. González, M. C.; Tormo, J. R.; Bermejo, A.; Zafra-Polo, M. C.; Estornell, E.; Cortes, D. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1113.