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Semisynthesis of new tetrahydrofuranic alkyl ester and furano-pyrone derivatives as inhibitors of the mitochondrial complex I

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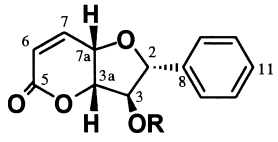
Abstract—Methoxymethylation of altholactone (**1**) led to the corresponding *O*-methoxymethyl derivative (**3**) in addition to the unexpected 6,7-dihydro-7-methoxy analogue (**4**), and two original tetrahydrofuranic (THF) alkyl esters (**5**,**6**). Moreover, when we accomplished a new method for the preparation of the furano-pyrone goniofupyrone (**7**) through 7-hydroxylation of **1** in acid medium, a minor compound (**8**) with an identical skeleton to that of compounds **5** and **6** was identified. Careful examination of the published spectral data of the reported styryl-lactones with an heptolide skeleton reveals that those structures possess also a THF alkyl ester skeleton. The revision of those structures was confirmed by chemical correlation. All altholactone derivatives assayed proved to be specific inhibitors of the mitochondrial complex I.

1. Introduction

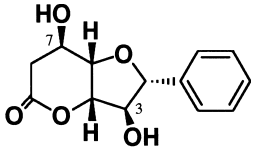
Styryl-lactones represent a class of natural and synthetic compounds, with significant cytotoxicity against several human tumor cell lines.[1] We have recently showed that the mechanism of cytotoxicity of furano-pyrones, altholactone (**1**), *O*-acetylaltholactone (**2**)[2] and other related styryl-lactones[3] isolated from *Goniothalamus arvensis*, is based on the inhibition on mammalian mitochondrial respiratory chain. These results stimulated us to prepare several semisynthetic altholactone analogues in order to establish their structure–activity relationship as inhibitors of the cellular respiration.[2–4]

There is evidence that several protecting groups affect the potency of cytotoxic activity.[5] Thus, the introduction in **1** of a methoxymethyl group (MOM) led to the corresponding *O*-methoxymethyl derivative (**3**), in addition to the unexpected 6,7-dihydro-7-methoxy analogue (**4**) and two original tetrahydrofuranic (THF) alkyl esters (**5**,**6**). Moreover, when we accomplished a new method for the preparation of the furano-pyrone goniofupyrone (**7**) through 7-hydroxylation of altholactone (**1**) in acid medium, we identified a minor compound (**8**), which presents an identical skeleton to those of compounds **5** and **6**, and the natural goniothalesdiol (**9**).[6,7] The careful examination of the published spectral data of styryl-lactones with an heptolide skeleton (gonioheptolide-A[8] and almuheptolide-A[3]) revealed that those structures possess also a THF alkyl ester skeleton. The revision of those structures was confirmed by chemical correlation.

In this work we also report the activity of several prepared altholactone derivatives and accomplished the structure–activity relationship as specific inhibitors of mitochondrial complex I.



1: R= H: altholactone
2: R= $COCH_3$: *O*-acetylaltholactone



7: goniofupyrone

Keywords: tetrahydrofuran alkyl esters; furano-pyrones; styryl-lactones; mitochondrial complex I inhibitors.
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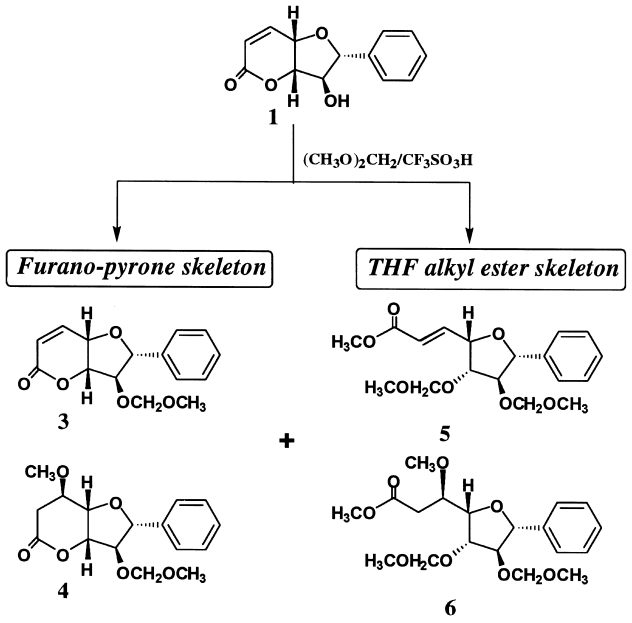
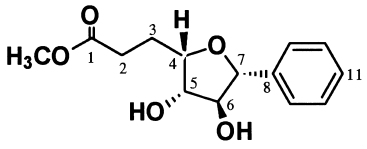


Scheme 1. Semisynthesis of compounds **3**–**6** from altholactone (**1**).



9: goniothalesdiol

2. Results and discussion

We envisaged that introduction of MOM group to the C-3 position of the furano-pyrone (+)-altholactone (**1**) would increase the cytotoxicity of this class of bioactive compounds.[5] The methoxymethylation of the secondary alcohol in **1** was carried out with dimethoxymethylether and trifluoromethane sulfonic acid at room temperature during 6 h, under nitrogen atmosphere.[9] The expected *O*-MOM derivative (**3**) was obtained in good yield. However when the reaction conditions were modified (refluxing for 24 h), three minor compounds (**4**–**6**) were obtained, in addition to compound **3** (Scheme 1).

Inspection of their mono- and bi-dimensional NMR spectra revealed that **3** and **4** present a furano-pyrone skeleton (altholactone type). The most significant structural difference between these two compounds appears in the saturation degree of the δ-lactone ring. Similar to **1**, compound **3** presents an α,β-unsaturated δ-lactone moiety, whereas **4** has in a 7-methoxy-saturated δ-lactone. The mechanism of 7-alkoxylation of unsaturated furano-pyrones was described as a Michael-type addition of an alcohol molecule (as nucleophilic agent) in acid medium, across the α,β-unsaturated δ-lactone double bond from the less electron dense position (β of the carbonyl group).[3,10] So, the 7-methoxy-saturated δ-lactone (**4**) is probably generated from **3** in the procedure of methoxymethylation.

The two other minor compounds (**5** and **6**) were consistent with an original structure without lactone moiety and they are characterised by the presence of a methyl-ester alkyl chain linked to a tetrasubstituted THF ring. Compound **5** was obtained as yellow oil. The molecular formula $C_{18}H_{24}O_7$ was established by the ion observed at *m/z* 353.161374 $[MH]^+$ in the HRLSIMS, and by liquid chromatography with mass spectrometry detection (LC–MS). Inspection of the 1D (^1H and ^{13}C NMR spectra), 2D homonuclear (^1H–^1H COSY 45), 2D heteronuclear correlation spectroscopy (HMQC and HMBC) and NOE experiments, revealed the existence of a THF ring substituted by two MOM groups, an olefinic chain, and a phenyl moiety. Moreover, an IR absorption band at 1721 cm^{-1} suggested a methyl ester function which was confirmed by the NMR signals at δ_H 3.75 ($COOCH_3$) and δ_C 166.0 ($COOCH_3$).

The NMR spectral data of **5** revealed the presence of a THF ring with four oxygen-linked methines. The more deshielded proton at δ_H 5.55 (HMQC, δ_C 79.2) is correlated in COSY 45 with both a THF methine proton at δ_H 4.51 (H-5), and an olefinic proton at δ_H 6.56 (H-3). Moreover the carbon at δ_C 79.2 possesses a ^3J correlation (HMBC) with the other methine olefinic proton at δ_H 5.99 (H-2). All those results allowed to place the olefinic chain over one of the oxygen-bearing carbons (position 4) on the THF ring. Indeed, the HMBC correlations observed between the carbonyl of the terminal ester function (C-1) with both the methyl ester protons and one of the olefinic protons (H-3), were consistent with a THF alkyl ester skeleton for the compound **5**, identical to that described for the natural goniothalesdiol (**9**) (see Fig. 1 and Table 1).[6]

The combined analysis of HMQC, HMBC and NOE's experiments allowed the placement in the THF system, of two *O*-MOM groups, and a phenyl ring at 5–7 positions, respectively (Fig. 1).

The relative stereochemistry of the chiral centres in **5** was deduced from both ^1H-coupling constant values and NOEDIFF experiments. The NOEs observed between H-4/H-7 and H-4/H-5 were in agreement with a *cis*-relationships for these three protons. The relative configuration of **5** is therefore postulated on the basis of the known configuration of the starting furano-pyrone altholactone (**1**) as 4,5-*cis*, 5,6-*trans*, and 6,7-*trans*. In conclusion, the novel compound **5** was identified as 3-(5,6-dimethoxymethyl-7-phenyl)-tetrahydrofuranyl acrylic methyl ester.

Compound **6** was also obtained as a yellow oil. Its molecular formula $C_{19}H_{28}O_8$ was deduced from the HRLSIMS by a peak at *m/z* 385.18643 $[MH]^+$ and by LC–MS (API-ES) in positive mode which showed an intensive positive ion at *m/z* 407 corresponding to $[M+Na]^+$. Inspection of the NMR and mass spectral data indicated the existence in **6** of similar THF substituents to those of **5**, two *O*-MOM groups, a phenyl ring, and a methyl ester terminal chain. An additional methoxyl group on the saturated alkyl chain, was evidenced in HRLSIMS by a fragment ion peak at *m/z* 353 $[M-OCH_3]^+$ and confirmed by 1D and 2D NMR experiments, where a proton resonance at δ_H 3.57 correlated with the carbon signal at δ_C 59.7, was observed. Compound

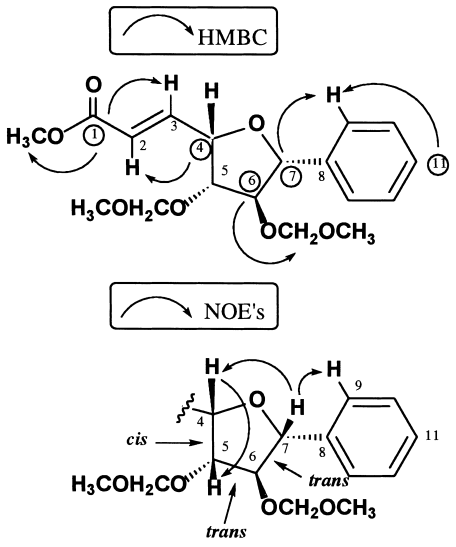


Figure 1. $^{1}H-^{13}C$ long-range correlations (^{3}J) established from HMBC, and NOE of **5**.

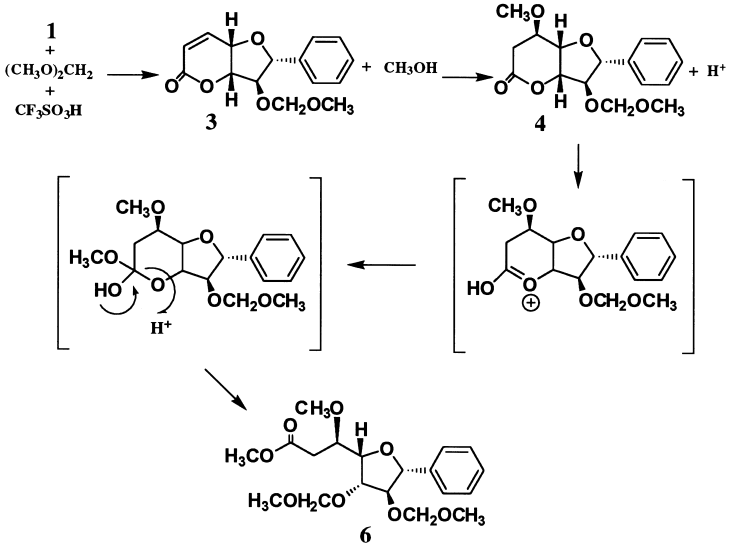
6 was identified as 3-(5,6-dimethoxymethyl-7-phenyl)-tetrahydrofuranyl-3-methoxy propionic methyl ester, and an identical stereochemistry to that of **5** was established.

In order to explain the mechanism operating in the formation of the minor THF alkyl ester compounds (**5** and **6**) during this reaction, we propose that the generation of those compounds could be produced from the corresponding furano-pyrone precursors **3** and **4**.

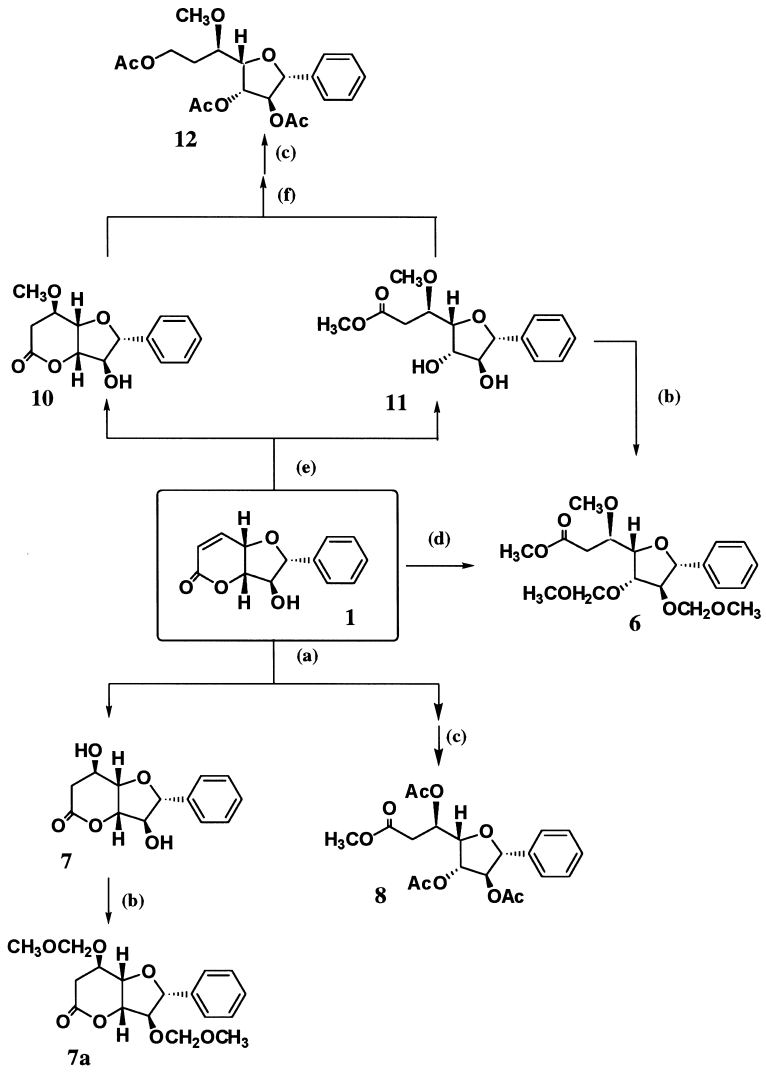
In the hypothesis depicted in Scheme 2, we suggest that the mechanism operating in the formation of THF alkyl ester compounds would be considered as follows; i.e. the acidic medium (CF_3SO_3H, $CH_2(OCH_3)_2$) liberaties CH_3OH that opens up the lactone ring and subsequently gives rise to methoxymethyl etherification. Because the presence of an olefinic chain in **5** is consistent with the α,β-unsaturated-δ-lactone in **3**, compound **5** could be generated via this furano-pyrone. In this way, compound **4**, resulting of the methoxylation by a Michael-type addition of **1**, seems to be the

Table 1. 1D and 2D NMR experiments of **5** ($CDCl_3$, 600 MHz)

Position	^{1}H NMR and COSY 45		^{13}C NMR/DEPT, HMQC and HMBC		
	δ_H (J Hz)	$^{1}H-^{1}H$ COSY 45	δ_C (multiplicity)	HMQC (^{1}J)	HMBC (^{3}J)
1	–	–	166.0 (C)		H-3, OCH_3
2	5.99 dd (11.7; 1.6)	H-3	120.3 (CH)	H-2	H-4
3	6.56 dd (11.7; 6.6)	H-2, H-4	146.2 (CH)	H-3	–
4	5.55 ddd (6.6; 3.9; 1.6)	H-3, H-5	79.2 (CH)	H-4	H-2
5	4.51 dd (3.9; 0.7)	H-4, H-6	83.1 (CH)	H-5	OCH_2 of 5-OMOM
6	4.16 dd (3.9; 0.7)	H-5, H-7	87.9 (CH)	H-6	OCH_2 of 6-OMOM
7	4.80 d (3.9)	H-6	86.2 (CH)	H-7	H-9, H-13
8	–	–	140.3 (C)	–	H-10, H-12
9,13	7.47 d (7.6)	H-10, H-12	126.3 (2 CH)	H-9, H-13	Ar-H
10,12	7.34 t (7.6)	H-9, H-11, H-13	128.4 (2 CH)	H-10, H-12	Ar-H
11	7.27 d (7.6)	H-9, H-10, H-12, H-13	127.6 (CH)	H-11	H-9, H-13
1-OCH_3	3.75 s	–	51.4 (CH_3)	OCH_3	–
5-OMOM	CH_2: 4.61 d/4.53 d (6.8)	–	95.7 (CH_2)	CH_2 of 5-OMOM	H-5, CH_3 of 5-OMOM
	CH_3: 3.34, s	–	55.5 (CH_3)	CH_3 of 5-OMOM	CH_2 of 5-OMOM
6-OMOM	CH_2: 4.77 d/4.68 d (6.7)	–	95.7 (CH_2)	CH_2 of 6-OMOM	H-6, CH_3 of 6-OMOM
	CH_3: 3.19, s	–	55.5 (CH_3)	CH_3 of 6-OMOM	CH_3 of 6-OMOM



Scheme 2. Proposed mechanism for THF alkyl esters formation.



Scheme 3. (a) H_2O–dioxane, H_2SO_4, reflux; (b) $(CH_3O)_2CH_2$, CF_3SO_3H, CH_2Cl_2, rt; (c) Ac_2O, pyr, rt; $(CH_3O)_2CH_2$, CF_3SO_3H, CH_2Cl_2, reflux; (e) MeOH, H_2SO_4, reflux; (f) $NaBH_4$, MeOH, reflux.

Table 2. 1D and 2D NMR experiments of **8** ($CDCl_3$, 600 MHz)

Position	^{1}H NMR and COSY 45		^{13}C NMR/DEPT, HMQC and HMBC		
	δ_H (J Hz)	^{1}H–^{1}H COSY 45	δ_C (multiplicity)	HMQC (^1J)	HMBC (^2J and ^3J)
1	–	–	170.3 (C)		OCH_3, H-2a, H-2b
2a	2.77 dd (16.1; 5.3)	H-2b, H-3	35.0 (CH_2)	H-2a, H-2b	–
2b	2.67 dd (16.1; 7.2)	H-2a, H-3			–
3	5.57 dt (7.2; 5.3; 5.2)	H-2a, H-2b, H-4	68.3 (CH)	H-3	H-2a, H-2b
4	4.52 t (5.4; 5.2)	H-3, H-5	79.0 (CH)	H-4	–
5	5.39 dd (5.4; 3.8)	H-4, H-6	76.1 (CH)	H-5	H-4, H-6
6	5.20 dd (5.2; 3.8)	H-5, H-7	81.7 (CH)	H-6	H-5, H-7
7	4.90 d (5.2)	H-6	83.3 (CH)	H-7	H-9, H-13
8	–	–	138.4 (C)	–	H-10, H-12
9,13	7.47 d (7.6)	H-10, H-12	126.1 (2 CH)	H-9, H-13	H-11, H-9
10,12	7.35 t (7.6)	H-9, H-11, H-13	128.4 (2 CH)	H-10, H-12	Ar-H
11	7.29 d (7.6)	H-9, H-10, H-12, H-13	128.1 (CH)	H-11	H-9, H-13
1-OCH_3	3.69 s	–	52.0 (CH_3)	OCH_3	
Oac	2.12[a] s	–	21.1 (CH_3)	CH_3	
		–	170.0 (C)		
Oac	2.10[a] s	–	20.8 (CH_3)	CH_3	
		–	169.8 (C)		
Oac	1.90[a] s	–	20.6 (CH_3)	CH_3	
			169.6 (C)		

[a] Assignments for three acetyl exchangeable groups.

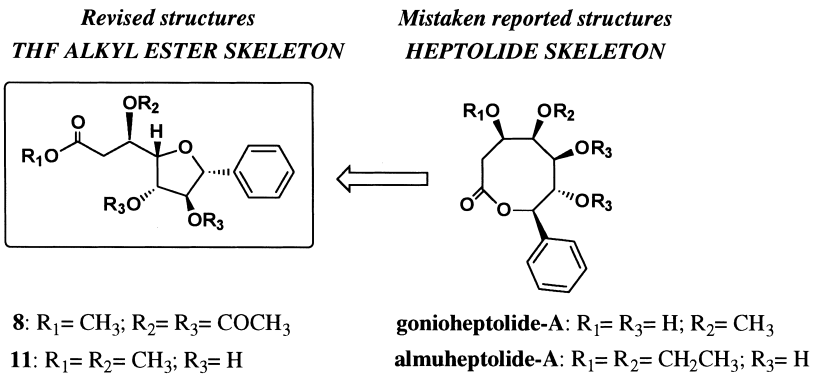


Figure 2. Revised structure of heptolide-type compounds.

precursor of **6**. This procedure constitutes a new method for preparing this class of compounds.

The reaction, in both cases, proceeded in low yields but with high stereoselectivity because no other lactone-ring-opened products were observed. Thus, **5** and **6** were prepared with complete retention of the stereochemical configuration of the starting phenyl-THF centres of altholactone (**1**).

In view of the good results obtained in the preparation of 7-alkoxylated furano-pyrone derivatives,[3,10] we decided to apply this procedure to the synthesis of goniofupyrone (**7**) an 7-hydroxylated analogue isolated in 1991 from *G. giganteus*.[11] So, we semisynthesised **7** from **1** by a single-step method through selective hydroxylation at C-7 position in concentrated acid medium, together with a not isolated intermediate, which was acetylated to led to compound **8**. Spectral and physical data of **7** were in all identical to those previously reported for the natural goniofupyrone (Scheme 3).[11]

Inspection of the spectral data revealed that structure of **8** was closely related to that of **5** and **6**. A THF alkyl ester skeleton was established for **8** by careful examination of the 2D homonuclear (COSY 45) and 2D heteronuclear correlation (HMQC and HMBC) NMR spectra (see Table 2). Comparison of the spectral data of **8** with those of the previously reported triacetyl-gonioheptolide-A[8] suggested, as it has been recently described,[12] that the heptolide-type skeleton (α-oxocanone) proposed for gonioheptolide-A and related natural and semisynthetic heptolides,[3,8] should now be revised to their corresponding THF alkyl ester ones (Scheme 3 and Table 2). Thus compound **8** was identified as 3-(5,6-di-*O*-acetyl-7-phenyl)-tetrahydrofuranyl-3-*O*-acetyl propionic methyl ester.

To confirm this new structural hypothesis, we carried out the synthesis of 6,7-dihydro-7-methoxyaltholactone (**10**) from **1**, under the same previously described conditions (conc. H_2SO_4/MeOH).[3,13] The minor compound obtained in this reaction (**11**), previously considered as a heptolide,[3,13] showed the same spectral features that were observed for compounds **5**, **6** and **8** described above. The chemical correlation between compounds **11** and **6** (see Scheme 3), allowed us to confirm unambiguously a THF alkyl ester skeleton for the related natural and synthetic heptolides (Fig. 2). Finally, a supplementary proof that the THF alkyl ester compounds have not lactone function, but a methyl ester, was reported by reduction of both compound **10** (a saturated δ-lactone) and **11** (a THF alkyl ester) with $NaBH_4$ in refluxing MeOH. The identification of the acetylated reductive derivatives (**12**) corroborates this structural hypothesis.

The inhibitory activity of these compounds against mammalian respiratory chain was systematically assayed to identify their mode of action as described in Section 3. Cytochrome oxidase activity (complex IV) and succinate/cytochrome c reductase activity (integrated complexes II and III activity) were not affected by these compounds. Instead, NADH oxidase activity (integrated complexes I, III and IV activity) and NADH/cytochrome c reductase activity (integrated complexes I and III activity) were inhibited. Therefore, compounds were specific inhibitors of the mitochondrial complex I. It was assessed by inhibition of the NADH/ubiquinone oxidoreductase activity, the isolated complex I activity, measured as described in Section 3. However, as potency criterion IC_{50} was taken against the integrated NADH oxidase activity of beef heart open submitochondrial particles (SMP),[14] which evaluates the complex I activity in a more physiological environment. Results are shown in Table 3.

Table 3. Inhibitory potency of styryl-lactones against NADH oxidase

Inhibitors	CI_{50} (μM)
Furano-pyrone skeleton	
1	24.80±7.5
2	4.70±1.6
3	11.22±3.32
7	18.17±3.39
7a	9.82±2.42
THF alkyl ester skeleton	
5	12.77±3.91
6	15.07±4.44

All derivatives were complex I inhibitors more potent that the starting product, altholactone (**1**). It is noteworthy that protection of the hydroxyl groups increased the inhibitory potency lowering the IC_{50} of the compounds. The acetylated derivative **2** was found to be the most potent inhibitor of this series. Taking into account the hydrophobic nature of the inhibitor binding site (s) of complex I, likely placed inside

the inner mitochondrial membrane or near the interface, and in accordance with previous studies with other complex I inhibitors,[14] it is thought that protection of the hydroxyl groups improves the access of the inhibitor to the site of action in the mitochondrial complex I.

3. Experimental

3.1. General instrumentation

Optical rotations were determined with a Perkin–Elmer 241 polarimeter. IR spectra were run in film using NaCl plates on a Perkin–Elmer 1750 FTIR spectrometer. EIMS, LSIMS, HREIMS and HRLSIMS were recorded on a VG Auto Spec Fisons spectrometer instruments. Liquid chromatography with mass spectrometry detection (LC–MSD) with API (atmospheric pressure ionisation) source configurated as APCI (atmospheric pressure chemical ionisation) or API-ES (electrospray ionisation) in positive mode, were determined on a Hewlett–Packard (HP-1100). ^{1}H and ^{13}C NMR spectra were recorded with $CDCl_3$ as solvent on a Bruker AC-400, Bruker AC-600, Varian-Unity-300 or Varian-Unity-400. Multiplicities of ^{13}C NMR resonances were assigned by DEPT experiments. NOEDIFF irradiations, COSY 45, HMQC, HSQC and HMBC correlations were recorded at 400 or 600 MHz. All reactions were monitored by analytical TLC with silica gel 60 F_{254} (Merck 5554). The residues were purified through 60H silica gel column (5–40 μm, Merck 7736) or by flash chromatography (230–400 μm, Merck 9385).

3.2. Natural starting styryl-lactones

Altholactone (**1**) and 3-*O*-acetylaltholactone (**2**), were isolated from *G. arvensis* Scheff (Annonaceae) stem bark.[2,15]

3.3. General procedure for O-methoxymethylation

3.3.1. Semisynthesis of 3-*O*-methoxymethylaltholactone (3). To a solution of altholactone (**1**, 130 mg, 0.56 mmol) in dry dichloromethane (30 mL) and $(CH_3O)_2CH_2$ (30 mL, 3.39×10^{-4} mmol), were added 4 drops of trifluoromethane sulfonic acid (CF_3SO_3H). The reaction mixture was stirred at rt for 6 h under nitrogen atmosphere. The excess CF_3SO_3H was destroyed by dropwise addition of NH_4Cl 15% aq (4 mL), and the resulting solution was concentrated and extracted with CH_2Cl_2, washed with brine and H_2O, and dried. The residue was subjected to column chromatography on silica gel 60H, eluting with hexane–EtOAc (70:30), to give 80 mg of *3-O-methoxymethylaltholactone* (**3**, 52%). $C_{15}H_{16}O_5$; $[\alpha]_D=+48°$ (*c* 1.0, EtOH); IR ν_{max} (film) cm^{-1}: 3064, 3033, 2950, 2894, 2826, 1736, 1640, 1495, 1454, 1368, 1246, 1152, 1101, 1038, 970, 918, 763, 700; EIMS *m/z* (%): 232 $[M-OCH_2OCH_3]^+$ (4), 231 (20), 214 (88), 170 (23), 141 (100), 107 (75), 97 (67), 77 (27); LC–MSD API-ES negative mode, *m/z* 275 $[M-H]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.35 (m, 5H, H-9-13), 7.00 (dd, *J*=9.8, 5.1 Hz, 1H, H-7), 6.25 (d, *J*=9.8 Hz, 1H, H-6), 4.98 (dd, *J*=4.9, 1.7 Hz, 1H, H-3a), 4.83 (d, *J*=5.1 Hz, 1H, H-2), 4.76 and 4.66 (2d, *J*=6.8 Hz, 2H, OCH_2OCH_3), 4.57 (t, *J*=4.9, 5.1 Hz, 1H, H-7a), 4.39 (dd, *J*=5.1, 1.7 Hz, 1H, H-3), 3.31 (s, 3H, OCH_2OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.9 (C-5), 139.5 (C-7), 138.1 (C-8), 128.6 (C-10,12), 128.4 (C-11), 126.3 (C-9,13), 124.3 (C-6), 96.3 (OCH_2OCH_3), 88.1 (C-3a), 85.6 (C-2), 84.5 (C-3), 68.7 (C-7a), 55.8 (OCH_2OCH_3).

3.4. Semisynthesis of compounds 4–6

Using a similiar procedure as described earlier for preparation of **3**, but refluxing in this case for 24 h, afforded **4** (12 mg, 7%), **5** (20 mg, 10%) and **6** (8 mg, 4%).

3.4.1. 6,7-Dihydro-7-methoxy-3-*O*-methoxymethylaltholactone (4). $C_{16}H_{20}O_6$; $[\alpha]_D=+20°$ (*c* 1.0, EtOH); IR ν_{max} (film) cm^{-1}: 2936, 2895, 2828, 2359, 2341, 1744, 1496, 1455, 1371, 1236, 1200, 1152, 1103, 1034, 973, 918, 761, 700; LC–MSD API-ES mode positive *m/z*: 331 $[M+Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.35 (m, 5H, H-9-13), 4.92 (dd, *J*=4.2, 1.6 Hz, 1H, H-3a), 4.78 (d, *J*=5.5 Hz, 1H, H-2), 4.74 and 4.63 (2d, *J*=6.6 Hz, 2H, OCH_2OCH_3), 4.34 (t, *J*=4.2, 3.9 Hz, 1H, H-7a), 4.25 (dd, *J*=5.5, 1.6 Hz, 1H, H-3), 3.95 (m, 1H, H-7), 3.47 (s, 3H, OCH_3), 3.28 (s, 3H, OCH_2OCH_3), 2.87 (dd, *J*=16.4, 3.9 Hz, 1H, H-6a), 2.72 (dd, *J*=16.4, 5.8 Hz, 1H, H-6b); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.2 (C-5), 138.1 (C-8), 128.7 (C-10,12), 128.3 (C-11), 126.3 (C-9,13), 96.4 (OCH_2OCH_3), 87.6 (C-3a), 85.5 (C-2), 84.5 (C-3), 76.1 (C-7a), 74.6 (C-7), 57.2 (OCH_3), 55.7 (OCH_2OCH_3), 32.6 (C-6).

3.4.2. 3-(5,6-Dimethoxymethyl-7-phenyl)-tetrahydrofuranyl acrylic methyl ester (5). $C_{18}H_{24}O_7$; $[\alpha]_D=+179°$ (*c* 1.0, EtOH); IR ν_{max} (film) cm^{-1}: 2950, 2891, 2824, 2360, 2341, 1721, 1439, 1203, 1151, 1032, 919, 827, 761, 700; HRLSIMS *m/z*: 353.161374 $[MH]^+$ (calcd 353.160028); ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 1.

3.4.3. 3-(5,6-Dimethoxymethyl-7-phenyl)-tetrahydrofuranyl-3-methoxy propionic methyl ester (6). $C_{19}H_{28}O_8$; $[\alpha]_D=+34°$ (*c* 1.0, EtOH); IR ν_{max} (film) cm^{-1}: 2947, 2893, 2826, 2359, 2340, 1739, 1439, 1151, 1104, 1030, 917, 761, 700; HRLSIMS *m/z*: 385.186432 $[MH]^+$ (calcd 385.186243), 353 $[M-OCH_3]^+$, 229 $[M-OCH_3-2\times HOCH_2OCH_3]^+$; LC–MSD API-ES mode positive *m/z*: 407 $[M+Na]^+$; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.45 (d, *J*=7.5 Hz, 2H, H-9,13), 7.33 (t, *J*=7.5 Hz, 2H, H-10,12), 7.26 (d, *J*=7.5 Hz, 1H, H-11), 4.84 (d, *J*=3.6 Hz, 1H, H-7), 4.72 and 4.67 (2d, *J*=6.8 Hz, 2H, OCH_2OCH_3-6), 4.64 and 4.57 (2d, *J*=7.2 Hz, 2H, OCH_2OCH_3-5), 4.10 (m, 4H, H-3-6), 3.72 (s, 3H, $COOCH_3$), 3.57 (s, 3H, OCH_3), 3.34 (s, 3H, OCH_2OCH_3-6), 3.21 (s, 3H, OCH_2OCH_3-5), 2.69 (dd, *J*=15.4, 3.2 Hz, 1H, H-2a), 2.57 (dd, *J*=15.4, 8.1 Hz, 1H, H-2b); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.8 (C-1), 140.6 (C-8), 128.2 (C-10,12), 127.5 (C-11), 126.2 (C-9,13), 96.0 (OCH_2OCH_3-6), 95.8 (OCH_2OCH_3-5), 87.0 (C-6), 86.3 (C-7), 83.7 (C-5), 82.2 (C-4), 77.4 (C-3), 59.7 (OCH_3), 56.1 (OCH_2OCH_3-6), 55.5 (OCH_2OCH_3-5), 51.7 ($COOCH_3$), 36.6 (C-2).

3.5. General procedure for hydroxylation

3.5.1. Semisynthesis of goniofupyrone (7) and 3-(5,6-di-*O*-acetyl-7-phenyl)-tetrahydrofuranyl-3-*O*-acetyl-propionic methyl ester (8). To a solution of altholactone (**1**,

100 mg, 0.43 mmol) in dioxane (2 mL) and H_2O (13.5 mL), was added dropwise concentrated H_2SO_4 (2 mL). After the mixture was stirred and refluxed for 9 h, water was added and the reaction mixture was extracted with EtOAc. The organic solution after usual workup was purified by silica gel 60H column chromatography (eluting with CH_2Cl_2–EtOAc 50:50), affording goniofupyrone (**7**, 54 mg, 50%) and a minor polyhydroxylated compound. This last compound was *O*-acetylated (Ac_2O/pyridine), and purified by silica gel 60H column chromatography (hexane–EtOAc 60:40), affording the corresponding triacetate **8** (15 mg, 11%).

3.5.2. Goniofupyrone (7). Spectral and physical data of compound **7** were identical to those reported for the natural goniofupyrone.[11]

3.5.3. 3-(5,6-Di-*O*-acetyl-7-phenyl)-tetrahydrofuranyl-3-*O*-acetyl propionic methyl ester (8). $C_{20}H_{24}O_9$; $[\alpha]_D$=+20° (*c* 0.5, EtOH); HRLSIMS *m/z* (%): 431 $[M+Na]^+$ (18), 409.150309 $[MH]^+$ (calcd 409.149858) (100), 349 $[MH-CH_3COOH]^+$ (20), 289 $[MH-2\times CH_3COOH]^+$ (5), 229 $[MH-3\times CH_3COOH]^+$ (15), 154 $[MH-3\times OCOCH_3-Ph]^+$ (58); EIMS *m/z* (%): 348 $[M-CH_3COOH]^+$ (2), 288 $[M-2\times CH_3COOH]^+$ (20), 228 $[M-3\times CH_3COOH]^+$ (100), 197 $[M-HOCH_3-3\times CH_3COOH]^+$ (3), 173 (39), 161 (29), 120 $[M-Ph-OCH_3-3\times CH_3COOH]^+$ (7), 107 (10), 105 (12), 98 (3), 91 (13), 77 (5); ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 2.

3.5.4. Di-*O*-methoxymethylation of 7. Goniofupyrone (**7**, 95 mg, 0.38 mmol) were treated using the same procedure described above for **1**. After applied silica gel 60 H column chromatography (hexane–CH_2Cl_2–EtOAc 60:5:35), afforded *3,7-di-O-methoxymethyl-goniofupyrone* (**7a**, 10 mg, 8%). $C_{17}H_{22}O_7$; EMIE *m/z* (%): 293 $[M-CH_2OCH_3]^+$ (100), 276 (25), 247 (3), 214 (6), 187 (38), 151 (40), 144 (75), 115 (14), 105 (20), 77 (10), 51 (2); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.32 (m, 5H, H-9-13), 5.00 (dd, *J*=4.4, 1.6 Hz, 1H, H-3a), 4.80 (d, *J*=5.4 Hz, 1H, H-2), 4.75 (m, 2H, OCH_2OCH_3), 4.72 (m, 1H, OCH_2OCH_3), 4.62 (m, 1H, OCH_2OCH_3), 4.35 (m, 1H, H-7a), 4.31 (m, 1H, H-7), 4.25 (dd, *J*=5.4, 1.6 Hz, 1H, H-3), 3.40 and 3.30 (2s, 6H, OCH_2OCH_3), 2.90 (dd, *J*=16.8, 3.6 Hz, 1H, H-6a), 2.00 (dd, *J*=16.8, 4.5 Hz, 1H, H-6b); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.9 (C-5), 138.1 (C-8), 128.7 (C-10, 12), 128.4 (C-11), 126.2 (C-9,13), 96.2 and 95.9 ($2\times OCH_2OCH_3$), 87.8 (C-3), 84.8 (C-3a), 85.5 (C-2), 75.6 (C-7), 70.9 (C-7a), 55.8 and 55.7 ($2\times OCH_2OCH_3$), 33.3 (C-6).

3.6. General procedure for alkoxylation

3.6.1. Semisynthesis of 6,7-dihydro-7-methoxy-altholactone (10) and 3-(5,6-dihydroxy-7-phenyl)-tetrahydrofuranyl-3-methoxy propionic methyl ester (11). To a solution of altholactone (**1**, 60 mg, 0.258 mmol) in MeOH (10 mL), was added dropwise at 0°C concentrated H_2SO_4 (1.5 mL). After the mixture was stirred and refluxed for 3 h, water was added and the reaction mixture was extracted with CH_2Cl_2. The organic solution after usual workup was purified by silica gel 60 H column chromatography (eluting with CH_2Cl_2–EtOAc 70:30), affording **10** (20 mg, 28%) and **11** (34 mg, 40%).

3.6.2. 6,7-Dihydro-7-methoxyaltholactone (10). $C_{14}H_{16}O_5$; $[\alpha]_D$=−69.2° (*c* 1.3, EtOH); EIMS *m/z*: 264 $[M]^+$ (see Refs. 3,13).

3.6.3. 3-(5,6-Dihydroxy-7-phenyl)-tetrahydrofuranyl-3-methoxy propionic methyl ester (11). $C_{15}H_{20}O_6$; $[\alpha]_D$=+15.0° (*c* 0.8, EtOH); LSIMS *m/z*: 297 $[MH]^+$ (see Refs. 3,13).

3.7. Chemical correlations

3.7.1. Di-*O*-methoxymethylation of compound 11 (semisynthesis of 6 from 11). Compound **11** (4 mg, 0.0135 mmol) was reacted using the same procedure described above for **1** ($CH_2(OCH_3)_2$/CF_3SO_3H), to afford the corresponding 5,6-dimethoxymethyl derivative, which was found to be in all identical respects to the previously prepared compound **6**.

3.7.2. Reduction of compounds 10 and 11 (semisynthesis of compound 12). To a solution of **10** (40 mg, 0.15 mmol) in dry MeOH (2 mL) was added an excess of $NaBH_4$ (3 mg). After the reaction mixture was stirred for 3 h at 90°C, water was added and the resulting solution was concentrated to dryness. The residue obtained was *O*-acetylated using dry pyridine (1 mL) and Ac_2O (2 mL) to yield after usual workup the triacetylated derivative **12** (42.4 mg, 73.6%). The same procedure was used starting from **11** (5 mg, 0.016 mmol) to afford compound **12** (5 mg, 76.5%). $C_{20}H_{26}O_8$; LC–MSD API-ES mode positive *m/z*: 417 $[M+Na]^+$, 395 $[MH]^+$; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.42 (m, 5H, H-9-13), 5.24 (dd, *J*=3.4, 1.3 Hz, 1H, H-5), 5.03 (dd, *J*=3.0, 1.3 Hz, 1H, H-6), 4.92 (d, *J*=3.0 Hz, 1H, H-7), 4.23 (m, 3H, CH_2-1, H-4), 3.70 (m, 1H, H-3), 3.58 (s, 3H, OCH_3), 2.13, 2.07 and 1.94 (3s, 9H, $3\times OCOCH_3$), 1.78 (m, 2H, CH_2-2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7 and 169.7 ($3\times OCOCH_3$), 138.9 (C-8), 128.1 (C-10,12), 127.7 (C-11), 126.0 (C-9,13), 85.3 (C-7), 83.8 (C-4), 82.8 (C-6), 76.9 (C-3), 76.6 (C-5), 60.6 (C-1), 59.5 (OCH_3), 30.1 (C-2), 20.9, 20.8 and 20.5 ($3\times OCOCH_3$).

3.8. Biochemical methods

3.8.1. Styryl-lactone derivatives and other reagents. Styryl-lactone derivatives were either isolated and purified from the plant material, or prepared by semisynthesis as described earlier. Rotenone, antimycin-A, decylubiquinone and other biochemical reagents were purchased from Sigma Chemical Co. Stock solutions (15 mm in absolute ethanol) of compounds were prepared and kept in the dark at −20°C. Appropiate dilutions (3–6 mm) were made before the experiments.

3.8.2. Preparation of beef-heart submitochondrial particles. Inverted SMP from beef heart were obtained by extensive ultrasonic disruption of frozen-thawed mitochondria in such a way to produce open membrane fragments where permeability barriers to substrates were lost.[16] After ultracentrifugation they were finally resuspended in 250 mm sucrose, 10 mm Tris–HCl buffer, pH 7.4, and treated with 0.3 mm NADH to activate complex I before starting experiments.[17]

NADH oxidase was measured as the aerobic oxidation of 75 μM NADH in the absence of the quinone substrate and other inhibitors of the respiratory chain. NADH/ubiquinone oxidoreductase was measured with 75 μM NADH and 30 μM decylubiquinone (DB) as soluble short-chain ubiquinone analogue in the presence of 2 μM antimycin and 2 mm potassium cyanide to block any reaction downstream of complex I.[18] Cytochrome c reduction by both NADH and succinate, and the cytochrome c oxidase activity were measured as previously described.[19,20]

Inhibitor titrations were made as previously described in detail.[4,17,21] The inhibitory concentration 50 (IC_{50}) was taken as the final compound concentration in the assay cuvette that yielded 50% of the initial activity. Data from four titrations were used to assess the means and standard deviations.

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References

1. Blázquez, M. A.; Bermejo, A.; Zafra-Polo, M. C.; Cortes, D. *Phytochem. Anal.* **1999**, *10*, 161–170.
2. Peris, E.; Estornell, E.; Cabedo, N.; Cortes, D.; Bermejo, A. *Phytochemistry* **2000**, *54*, 311–315.
3. Bermejo, A.; Tormo, J. R.; Cabedo, N.; Estornell, E.; Figadère, B.; Cortes, D. *J. Med. Chem.* **1998**, *41*, 5158–5166.
4. Gallardo, T.; Zafra-Polo, M. C.; Tormo, J. R.; González, M. C.; Franck, X.; Estornell, E.; Cortes, D. *J. Med. Chem.* **2000**, *43*, 4793–4800.
5. Tsubuki, M.; Kanai, K.; Nagase, H.; Honda, T. *Tetrahedron* **1999**, *55*, 2493–2514.
6. Cao, S-G.; Wu, X-H.; Sim, K-Y.; Tan, B. K. H.; Pereira, J. T.; Goh, S.-H. *Tetrahedron* **1998**, *54*, 2143–2148.
7. Yoda, H.; Shimojo, T.; Takabe, K. *Synlett* **1999**, *12*, 1969–1971.
8. Fang, X.-P.; Anderson, J. E.; Qiu, X-X.; Kozlowski, J. F.; Chang, C-J.; McLaughlin, J. L. *Tetrahedron* **1993**, *49*, 1563–1570.
9. Groziak, M. P.; Koohang, A. *J. Org. Chem.* **1992**, *57*, 940–944.
10. Bermejo, A.; Blázquez, M. A.; Serrano, A.; Zafra-Polo, M. C.; Cortes, D. *J. Nat. Prod.* **1997**, *60*, 1338–1340.
11. Fang, X-P.; Anderson, J. E.; Chang, C-J.; McLaughlin, J. L. *Tetrahedron* **1991**, *47*, 9751–9758.
12. Mukai, C.; Yamashita, H.; Hirai, S.; Hanaoka, M.; McLauglin, J. L. *Chem. Pharm. Bull.* **1999**, *47*, 131–132.
13. Bermejo, A.; Léonce, S.; Cabedo, N.; Andreu, I.; Caignard, D. H.; Atassi, G.; Cortes, D. *J. Nat. Prod.* **1999**, *62*, 1106–1109.
14. Tormo, J. R.; Gallardo, T.; Aragón, R.; Cortes, D.; Estornell, E. *Chem.-Biol. Interact.* **1999**, *122*, 171–183.
15. Bermejo, A.; Lora, M. J.; Blázquez, M. A.; Rao, K. S.; Cortes, D.; Zafra-Polo, M. C. *Nat. Prod. Lett.* **1995**, *7*, 117–122.
16. Fato, R.; Estornel, E.; Di Bernardo, S.; Palloti, F.; Parenti-Castelli, G.; Lenaz, G. *Biochemistry* **1996**, *35*, 2705–2716.
17. Degli Esposti, M.; Ghelli, A.; Ratta, M.; Cortes, D.; Estornell, E. *Biochem. J.* **1994**, *301*, 161–167.
18. Estornell, E.; Fato, R.; Palloti, F.; Lenaz, G. *FEBS Lett.* **1993**, *332*, 127–131.
19. Zafra-Polo, M. C.; Figadère, B.; Gallardo, T.; Tormo, J. R.; Cortes, D. *Phytochemistry* **1998**, *48*, 1087–1117.
20. Estornell, E.; Fato, R.; Catelluccio, C.; Cavazzoni, M.; Parenti-Castelli, G.; Lenaz, G. *FEBS Lett.* **1992**, *311*, 107–109.
21. González, M. C.; Tormo, J. R.; Bermejo, A.; Zafra-Polo, M. C.; Estornell, E.; Cortes, D. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1113–1118.