Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 3439–3446

BIOORGANIC & MEDICINAL CHEMISTRY

Semisynthesis of Heterocyclic Analogues of Squamocin, a Cytotoxic Annonaceous Acetogenin, by an Unusual Oxidative Decarboxylation Reaction

R. Duval, G. Lewin* and R. Hocquemiller

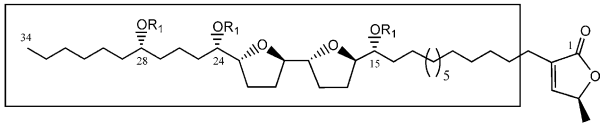
Laboratoire de Pharmacognosie (BioCis, UMR 8076 CNRS), Faculté de Pharmacie, rue J. B. Clément, 92296 Châtenay-Malabry Cedex, France

Received 28 February 2003; revised 22 April 2003; accepted 6 May 2003

Abstract—In addition to two expected pyrazin derivatives, two imidazole analogues of squamocin **1** have been semisynthetised from squamocin derived α-ketoesters/α-ketoacid, via an unusual condensation-oxidative decarboxylation reaction with 1,2 diamines in presence of acetic acid and oxygen as the key step. Some of these analogues exhibited potent, although significantly reduced cytotoxicities relatively to squamocin **1**. In addition, benzimidazole **8** possessed in comparison with the natural acetogenin some interesting cell cycle effects.

Introduction

Annonaceous acetogenins exhibit a wide range of biological activities (cytotoxic, antiparasitic, insecticide...)[1] resulting from the inhibition of the mitochondrial NADH-ubiquinone oxidoreductase (Complex I) as the main target.[2] Despite their often spectacular cytotoxicity against various cell lines in vitro, natural acetogenins suffer from having an inhibiting action independent towards the cell cycle,[3] resulting in the absence of selectivity at a cellular level, and excessive toxicity.[4,5] Furthermore, from a mechanistic point of view, the role of the terminal α,β-unsaturated γ-methyl butyrolactone as a quasi-ubiquitous moiety in the core of the acetogenins remains unclear, since very different lactonic prints can be highly effective Complex I pharmacophores.[6–8] Recently, the first example of chimeric acetogenins, in which the lactone part was replaced by the dimethoxybenzoquinone moiety of ubiquinone,[9] showed how new insights into the structural dynamic of the enzyme could be made with such inhibitors possessing mixed binding properties. We wish to report here a short and simple procedure to introduce a potentially broad choice of heterocyclic systems in place of this terminal lactone, via usual or unusual condensation reactions of lactone-derived α-ketoesters/α-ketoacid with 1,2 diamines. Aromatic heterocycles are commonly found as base-structures of potent Complex I inhibitors.[10–12] In consequence, the enzymatic inhibition by such chimeric or pseudo-chimeric acetogenins could be strongly influenced by the heterocyclic pharmacophore, and be very different from the acetogenin-type inhibition in term of competition behaviour and subsites affinities. Also, in addition to a possible mechanistic change underlying the cytotoxicity of those compounds, the acetogenin-type profile of action on the cell cycle may not be conserved in all cases. The chosen natural acetogenin to be modified was squamocin **1**, a highly cytotoxic and potent respiratory inhibitor among this class of compounds.[13–17]



1 R (R_1 = H)

1a R_S (R_1 = TBDMS)

Results and Discussion

Semisynthesis

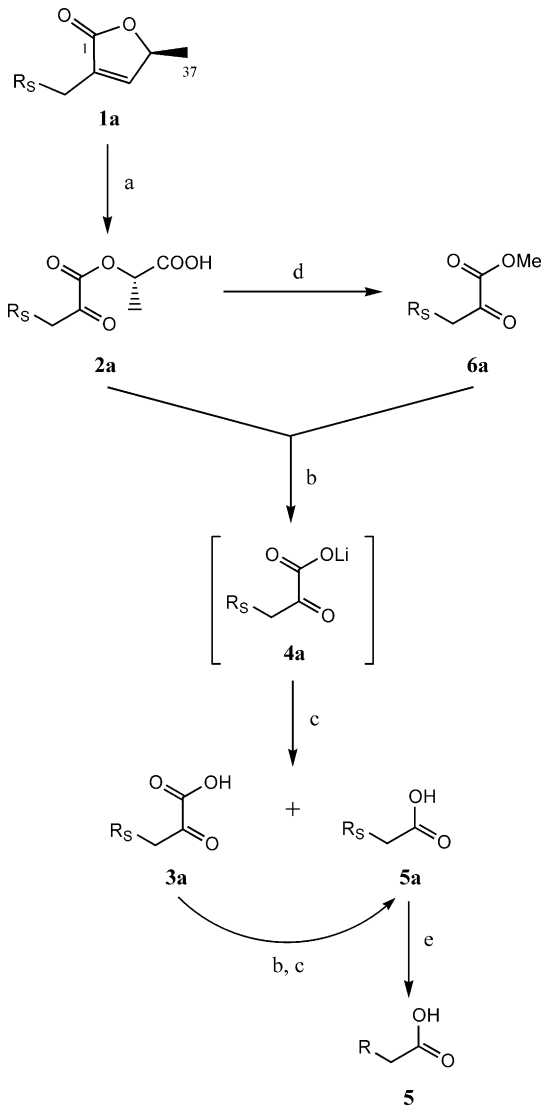
Ruthenium-catalysed periodic oxidation of the terminal lactone of squamocin **1**, commonly used analytically to determine the C-36 absolute configuration of this type

*Corresponding author. Tel.: +33-1-4683-5593; fax: +33-1-4683-5399; e-mail: guy.lewin@cep.u-psud.fr

0968-0896/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0968-0896(03)00306-7

of acetogenins through the obtained lactate,[18–21] seemed to be a good entry into heterocyclic transformation of α,β-unsaturated lactones, since the expected lactic α-ketoester/α-ketoacid derivatives are very effective electrophiles in condensation reactions with 1,2-diamines.[22–25] Squamocin **1** was first protected as its tri-TBDMS silylether derivative **1a** (TBDMSOTf, pyridine, 0 °C, 2 h, 93%). This precaution appeared necessary since oxidative conditions with catalytic ruthenium chloride and excess sodium periodate led to significant degradation of unprotected squamocin, presumably through clivages of the polyoxygenated system.[13,17,26] Protected squamocin **1a** was oxidized in a 50:50 THF/water solvent system, found to make the reaction work cleaner than the traditionnal acetonitrile/carbon tetrachloride/water biphasic system.[27,28] This oxidative degradation reaction, brought up to a preparative scale (several grams of **1a**), enabled us to obtain in a excellent yield of 86% the lactic α-ketoester derivative **2a** (Scheme 1).

Our first observation of an unusual oxidative decarboxylation pathway for such α-dicarbonyl compounds came from the finding that saponification of **2a** (1N aqueous LiOH, 1,4-dioxane, rt then 1N aqueous HCl, 0 °C) furnished the expected α-ketoacid **3a** with a disappointing yield of 58% due to its concomitant degradation to acid **5a**, isolated in a 9% yield after purification (Scheme 1). It has to be pointed out that this degradation only occurred when the lithium α-ketocarboxylate intermediate **4a** was treated with aqueous HCl. Such an oxidative degradation of α-ketoacids has been described with diverse oxidizing agents,[29–31] and could be due, in our case, to residual traces of oxidants in the lactic α-ketoester derivative **2a**. However, this explanation seems unlikely as purified α-ketoacid **3a** was found to be partially redecay into acid **5a** when resubmitted to saponification conditions. Furthermore, methyl α-ketoester **6a** from transesterification of **2a** (cat. 2,3-diaminopyridine, MeOH, 40 °C, 4 h, 57%), purified by flash-chromatography then saponified as described, exhibited the same behaviour than **2a** (Scheme 1).



Scheme 1. Reagents and conditions: (a) $RuCl_3$, $NaIO_4$, THF/H_2O, 50 °C, 2 h; (b) 1N LiOH, 1,4-dioxane, rt, 15 min; (c) 1N HCl, H_2O, 0 °C; (d) cat. 2,3-diaminopyridine, MeOH, 40 °C, 4 h; (e) TBAF 1M, THF, 50 °C, 6 h.

We were then interested to analyse the heterocyclization behaviour of α-ketoester **2a** or **6a** versus α-ketoacid **3a** in presence of *o*-phenylenediamine (OPD).[23] Compounds **2a** and **6a** furnished the expected quinoxalinone derivative **7a** with a 70–79% yield (OPD, MeOH, 0 °C, 2 h), but compound **3a** was found to yield 62% benzimidazole **8a** and 13% acid **5a** when reacted in the same conditions (Scheme 2). In order to expand the synthetic potential of this unusual reactivity and to unambiguously characterize a condensation-oxidative decarboxylation pathway, the reaction of **2a** or **6a** with OPD was carried out in two steps: (a) saponification to lithium α-ketocarboxylate intermediate **4a** (1N aqueous LiOH, *tert*-BuOH, rt, 10 min); (b) condensation of **4a** with OPD (MeOH, 4.5 eq. AcOH, 0 °C, 1.5 h). The course of the reaction appeared to strictly depend on experimental conditions of the second step (Scheme 2, Table 1): an 'open' reaction cleanly and solely afforded benzimidazole **8a** while continuous bubbling of argon into the reaction medium led to a quinoxalinone **7a**/benzimidazole **8a** mixture in a 23/77 molar ratio, turning to a ratio of 57/43 when both addition solution of OPD and reaction mixture were to be degased with argon (Table 1).

The same heterocyclisation behaviour of **2a** or **6a** was observed when *o*-phenylenediamine was replaced by diaminomaleonitrile (DAMN),[32] a strongly electron deficient 1,2 diamine. Hence, pyrazinol **9a** was obtained by directly condensing lactic or methyl α-ketoester **2a** or **6a** with DAMN (imidazole, toluene, 45 °C, 18 h, 61%), MeOH being here an inadequate solvent for the reaction. The phenolic structure of **9a** (rather than its pyrazinone tautomer form) was established by comparison of its IR spectral data (weak band at 1683 cm^{-1}) with the ones of quinoxalinone **7a** (very strong carbonyl band at 1666 cm^{-1}). Furthermore, it has to be mentionned that pyrazinol **9a** was obtained as its imidazolium phenate salt when directly purified from the reaction medium in the absence of acidic work-up.

On the other hand, using DAMN in the two-steps condensation procedure via the saponification intermediate **4a** led to dicyanoimidazole **10a** (DAMN, imidazole, AcOH, *n*-BuOH, 90 °C, 2 h, 38%) and acid **5a** (7%), pyrazinol **9a** being undetectable by TLC in the reaction mixture (Scheme 3). Replacement of toluene by *n*-BuOH and the high temperature used for this last reaction gave a better heterocycle/acid ratio than conditions used for the formation of pyrazinol **9a**.

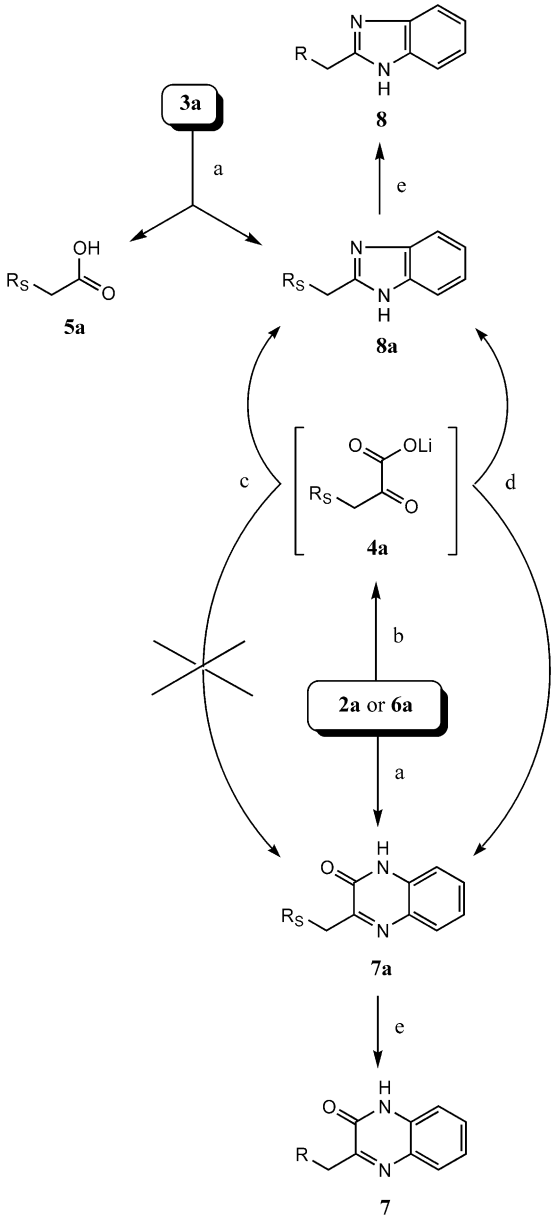
In a last step, all compounds were deprotected by the use of TBAF/THF (for acid **5a**) or Amberlyst®15/MeOH system (for heterocyclic derivatives) to furnish with good to excellent yields (57–95%) the squamocin analogues **5** and **7–10**.

The formation of acid **5a** and heterocyclic compounds **8a** and **10a** corresponds to the loss of a monocarbon fragment. The study of this two-steps reaction of **2a** or **6a** via **4a** with OPD showed that this formal decarbonylation, leading to benzimidazole **8a** instead of quinoxalinone **7a**, was directly linked to the presence of oxygen at the second step (formation of benzimidazole **8a** under presumably anaerobic conditions could be attributed to the recapture of oxygen by the OPD stock solution before its addition, since these semisynthetic experiments were conducted on a little scale (50–100

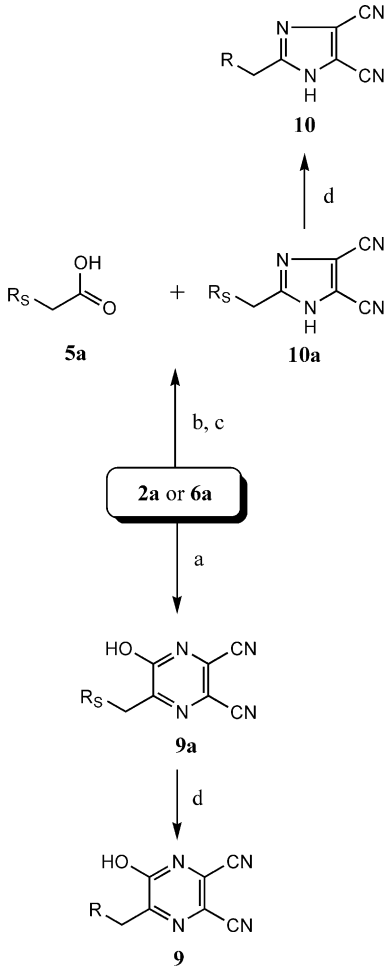
Table 1. Influence of oxygen in the heterocyclisation of **2a** or **6a** via **4a** with OPD

Reaction conditions		Quinoxalinone **7a** (%)	Benzimidazole **8a** (%)
OPD sol.	React. mixt.	0	58
OPD sol.	Ar-purged react. mixt.	23	77
Ar-purged OPD sol.	Ar-purged react. mixt.	57	43

Reagents and conditions: (i) **2a** or **6a** 1 eq., 1N LiOH 12 equiv, *tert*-BuOH, rt, 10 min, then AcOEt, rt, 15 min; (ii) OPD 1,1 equiv, AcOH 4.5 equiv, +/− air, MeOH, 0 °C, 1 h 30 min.



Scheme 2. Reagents and conditions: (a) OPD, MeOH, 0 °C, 2 h; (b) 1N LiOH, *tert*-BuOH, rt, 10 min, then AcOEt, rt, 15 min; (c) OPD, AcOH, air, MeOH, 0 °C, 1.5 h; (d). OPD, AcOH, Ar, MeOH, 0 °C, 1.5 h, cf: **7a**/**8a** ratios, Table 1; (e) Amberlyst-15®, MeOH, 40 °C, 7 h.



Scheme 3. Reagents and conditions: (a) DAMN, imidazole, toluene, 45 °C, 18 h; (b) 1N LiOH, rt, 10 min then AcOEt, rt, 15 min; (c) DAMN, AcOH, imidazole, air, *n*-BuOH, 90 °C, 2 h; (d) Amberlyst-15®, MeOH, 40 °C, 7 h or rt, 15 h.

μmol of α-ketocarboxylate **4a**), making difficult the complete elimination of oxygen from the medium). These experiments are clearly in favour of an oxidative decarboxylation process, albeit we can not explain it at that stage. However, the recovery of acid **5a** in media containing unreacted 1,2-diamines (OPD or DAMN) demonstrates that **5a** is not the reacting species and that the decarboxylation step occurs after the condensation one.

Biological Evaluation

Cytotoxicity

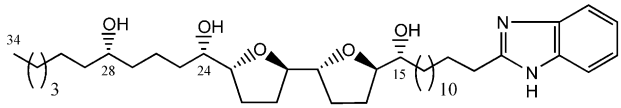
The obtained heterocyclic compounds, in the form of their tri-TBDMS triethers as well as free trihydroxy derivatives, were tested on KB 3-1 cells (human nasopharyngeal epidermoid carcinoma) in vitro, and their cytotoxicity evaluated (Table 2). None of the protected derivatives was cytotoxic at a concentration of 10^{-5} M (data not shown), thus showing that no additional activity was gained by turning the natural α,β-unsaturated butyrolactone into the described heterocycles, since the IC_{50} of triprotected squamocin **1a** was also above 10^{-5} M. Some deprotected semisynthetic derivatives exhibited significant though strongly reduced cytotoxicities in comparison with squamocin **1**, benzimidazole **8** being the most potent analogue with a nM IC_{50}. Pyrazinol **9** and dicyanoimidazole **10** were found to be much weaker cytotoxic agents, as was acid **5**. However, it is to be noted that derivatives **8** and **10**, obtained through the condensation-oxidative decarboxylation pathway, were 20–30 times more potent than the corresponding 'normal' analogues **7** and **9**.

Cell cycle effect

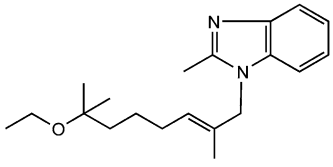
Benzimidazole **8** induced a 61% accumulation in the G_1 phase of the KB 3-1 cell cycle at concentrations of 1–5 nM, with apoptosis above 10 nM. In comparison, squamocin **1** showed a complete absence of specificity, in accordance with previous results by Queiroz et al.[33]

Conclusion

Two couples of heterocyclic analogues of squamocin **1** were obtained by condensing squamocin-derived α-ketoesters **2a** or **6a** with *o*-phenylenediamine (OPD) or diaminomaleonitrile (DAMN), through a direct reaction or via a controlled condensation-oxidative decarboxylation pathway. This last reaction can apparently be performed with structurally different 1,2-diamines, allowing the access to a potentially broad family of five-membered ring analogues of natural acetogenins. In particular, this condensation-oxidative decarboxylation process appears to be a good alternative for the synthesis of benzimidazole nuclei in very mild, weakly acidic conditions. This original modulation in the acetogenins chemistry also seems of interest considering the promising cell cycle effect of benzimidazole derivative **8** in comparison with all tested natural or artefactual acetogenins.[3,33] In the field of Complex I inhibition, benzimidazole **8** can truly be considered as a chimeric analogue of squamocin **1** and pesticidal benzimidazole **11**, the last also being a very potent inhibitor of the enzyme.[34–36] (Scheme 4).



8



11

Scheme 4. Structural analogy between chimeric squamocin analogue **8** and pesticidal benzimidazole **11**.

Many potent inhibitors of Complex I are structurally characterized by a long aliphatic chain attached to an aromatic heterocyclic nucleus. The ruthenium-catalysed oxidation of the terminal butenolide of natural acetogenins, described here as the key reaction of our pharmacomodulation strategy, seems therefore to be a simple and versatile synthetic entry towards new exploratory tools of such a multi-enzymatic system.

The determination of the Complex I inhibitory activities of all heterocyclic derivatives presented in this study is currently underway.

Table 2. Cytotoxicity (KB 3-1) of compounds **5** and **7**–**10**

Compd	IC_{50} (M)
5	1.7×10^{-6}
7	6.1×10^{-8}
8	2.2×10^{-9}
9	6.5×10^{-6}
10	3.7×10^{-7}
Squamocin **1**	10^{-13}
Doxorubicin[a]	2.4×10^{-9}
Etoposide[a]	1.3×10^{-7}

[a]Compounds of reference.

Experimental

Infra-red (IR) spectra were recorded on a Perkin–Elmer 257 apparatus. ^1H and ^{13}C NMR spectra were recorded with a Bruker AC-200 (200 MHz) and a Bruker AM-400 (400 MHz). Mass spectra (MS) were recorded on Kratos MS-80 R_f. Chromatography was performed with silica gel 60 (9385 Merck) or Sephadex® LH-20 (Pharmacia). TLC were performed on aluminium plates coated with silica gel $60F_{254}$ (554 Merck) and revealed with sulfuric vanillin reagent. Solvents used in this study were simply redistilled before use.

Extraction

Squamocin **1** was isolated in significant quantities from the seeds of Annona reticulata, collected in Vietnam, using a described procedure.[14] Its chemical identity was determined by extensive ^{1}H and ^{13}C NMR experiments, and comparison with an authentic sample.

Semisynthesis

Tri-TBDMS silylether derivatives. Compound 1a. To a ice-cooled solution of 2.94 g squamocin **1** (4.72 mmol) and 5 mg (0.041 mmol) of 4-DMAP in 16 mL of pyridine were added dropwise 5 g (18,9 mmol) of *tert*-butyldimethylsilyl triflate. The ice bath was removed and the reaction medium stirred at room temperature for 2 h. The obtained biphasic mixture was treated with water (11 mL) then diluted by Et_2O (300 mL). The organic phase was washed by 10% aqueous $CuSO_4$ solution (3×100 mL) then water (3×100 mL). The ethereal layer was dried (Na_2SO_4), filtered and evaporated under reduced pressure. The crude product was purified by flash-chromatography (SiO_2, cyclohexane/AcOEt 9:1), yielding 4,26 g (93%) of triether **1a** as a colourless viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.03–0.08 (m, 18H, Me-Si), 0.88 (m, 30H, *t*-Bu-Si and H-34, $J=6.8$ Hz), 1.25 (m, H-13), 1.28 (m, H-14 and H-33), 1.39 (m, H-27 and H-29), 1.40 (d, 3H, H-37, $J=6,9$ Hz), 1.55 (m, 2H, H-4), 1.67 (m, 4H, H-18 and H-21), 1.87 (m, 4H, H-17 and H-22), 2.26 (t, 2H, H-3, $J=7.8$ Hz), 3.60 (m, 2H, H-28 and H-15/H-24), 3.75 (m, 1H, H-15/H-24), 3.87 (m, 2H, H-19 and H-20), 3.91 (m, 2H, H-16 and H-23), 4.99 (dq, 1H, H-36, $J=1.4$ Hz, $J=6.9$ Hz), 6.98 (d, 1H, H-35, $J=1.6$ Hz). IR (film, ν_{max} cm^{-1}): 1761. ESIMS *m/z* 965 $[M+H]^+$, 987 $[M+Na]^+$. $[\alpha]_D=+15$ ($c=1$, CH_2Cl_2).

Compound 2a. To an emulsion of 2,18 g (2.26 mmol) **1a** in 109 mL of THF/water (1:1) mixture was added 7.27 g (33,9 mmol) $NaIO_4$ then 60 mg (0,292 mmol) $RuCl_3$. The mixture was warmed to 50 °C and vigourously stirred at that temperature for 2 h. The reaction mixture was filtered over Celite-545 and the precipitate washed by CH_2Cl_2 (350 mL). The organic phase was partitioned with water (350 mL), and the aqueous layer reextracted by CH_2Cl_2 (5×100 mL). The organic phases were gathered, dried (Na_2SO_4), filtered and evaporated under reduced pressure to yield a yellowish liquid residue. The last was repeatly chromatographied over a column of Sephadex® LH-20 impregnated with 100% CH_2Cl_2, to furnish 1.97 g (86%) of **2a** as a colourless viscous oil. Rem: this quite unstable product was conserved at −20 °C or readily turned into its more stable α-keto methylester analogue **6a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.03–0.064 (m, 18H, Me-Si), 0.88 (m, 30H, *t*-Bu-Si and H-34, $J=7$ Hz), 1.24 (m, H-26), 1.37 (m, H-27 and H-29), 1.63 (m, 2H, H-4), 1.64 (d, 3H, H-37, $J=7.1$ Hz), 1.66 (m, 4H, H-18 and H-21), 1.84 (m, 4H, H-17 and H-22), 2.84 (t, 2H, H-3, $J=7.3$ Hz), 3.61 (m, 2H, H-28 and H-15/H-24), 3.77 (m, 1H, H-15/H-24), 3.88 (m, 2H, H-19 and H-20), 3.92 (m, 2H, H-16 and H-23), 5.23 (q, 1H, H-36, $J=7.1$ Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm −4.5 (Me–Si), 13.7 (C-34), 16.8 (C-37), 22.5 (C-4), 26.2 (*t*-Bu–Si), 38.8 (C-3), 69.4 (C-36), 71.6 (C-15/C-28), 73.5 (C-24), 75.0 (C-15/C-28), 81.2-82.1 (C16, C-19, C-20, C-23), 172.8 (C-35), 193.7 (C-2). IR (film, ν_{max} cm^{-1}): 1733. ESIMS *m/z* 1013 $[M+H]^+$, 1036 $[M+Na]^+$. $[\alpha]_D=0$ ($c=1$, CH_2Cl_2).

Compound 6a. To a solution of 460 mg (0.454 mmol) lactic α-ketoester **2a** in 8 mL MeOH were added 5 mg (45.4 μmol) 2,3-DAP. The mixture was warmed to 40 °C and stirred at that temperature for 4 h. The reaction mixture was evaporated under reduced pressure and the crude product purified by chromatography (SiO_2, cyclohexane/AcOEt 94:6). 247 mg (57%) of the methanolytic product **6a** were obtained as a colourless resin. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.03–0.064 (m, 18H, Me–Si), 0.88 (m, 30H, *t*-Bu–Si and H-34), 1.36 (m, H-27 and H-29), 1.68 (m, 4H, H-18 and H-21), 1.86 (m, 4H, H-17 and H-22), 2.85 (t, 2H, H-3, $J=7.3$ Hz), 3.62 (m, 2H, H-28 and H-15/H-24), 3.78 (m, 1H, H-15/H-24), 3.88 (s, 3H, H-35), 3.88 (m, 2H, H-19 and H-20), 3.92 (m, 2H, H-16 and H-23). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm −4.2 (Me-Si), 13.9 (C-34), 21.0 (C-26), 25.7 (*t*-Bu–Si), 29.1-37.3 (C-14, C-17, C-18, C-21, C-22, C-25, C-27, C-29), 39.1 (C-3), 52.6 (C-35), 72.1 (C-28), 73.5 (C-15/C-24), 74.7 (C-15/C-24), 81.2-82.1 (C-16, C-19, C-20, C-23), 161.5 (C-1), 194.1 (C-2). IR (film, ν_{max} cm^{-1}): 1734. ESIMS *m/z* 955 $[M+H]^+$, 977 $[M+Na]^+$, 993 $[M+K]^+$. $[\alpha]_D=+9$ ($c=1$, CH_2Cl_2).

Compounds 3a and **5a**. To a solution of 1.35 g (1,38 mmol) lactic α-ketoester **2a** in 47 mL 1,4-dioxane at room temperature were added 12 mL (12 mmol) 1N aqueous LiOH. The mixture was stirred for 15 min and diluted by 350 mL water. The obtained mixture was cooled to 0 °C and 8.5 mL of 1N aqueous HCl were added dropwise until pH 3–4. The reaction mixture was extracted by 4×200 mL CH_2Cl_2, the organic phases were gathered, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was chromatographied over a column of Sephadex® LH-20 impregnated with CH_2Cl_2, yielding by order of elution 293 mg of contaminated acid **5a** then 725 mg (58%) of pure α-keto-acid **3a** as a colourless resin. Acid **5a** was further purified by chromatography (SiO_2, cyclohexane/AcOEt 1:1), furnishing 103 mg (9%) of pure product as a viscous colourless oil. **3a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.03–0.065 (m, 18H, Me–Si), 0.88 (m, 30H, *t*-Bu–Si and H-34, $J=7.1$ Hz), 1.27 (m, H-14), 1.37 (m, H-27 and H-29), 1.65 (m, 4H, H-18 and H-21), 1.67 (m, 2H, H-4), 1.84 (m, 4H, H-17 and H-22), 2.92 (t, 2H, H-3, $J=7.2$ Hz), 3.61 (m, 2H, H-28 and H-15/H-24), 3.77 (m, 1H, H-15/H-24), 3.87 (m, 2H, H-19 and H-20), 3.92 (m, 2H, H-16 and H-23). IR (film, ν_{max} cm^{-1}): 1728. ESIMS *m/z* 963 $[M+Na]^+$, 979 $[M+K]^+$. $[\alpha]_D=+8$ ($c=1$, CH_2Cl_2). **5a** :^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.03–0.065 (m, 18H, Me–Si), 0.88 (m, 30H, *t*-Bu–Si and H-33), 1.28 (m, H-13), 1.40 (m, H-26 and H-28), 1.64 (m, 4H, H-17 and H-20), 1.86 (m, 4H, H-16 and H-21), 2.34 (t, 2H, H-2, $J=7.4$ Hz), 3.61 (m, 2H, H-27 and H-14/H-23), 3.76 (m, 1H, H-14/H-23), 3.87 (m, 2H, H-18 and H-19), 3.90 (m, 2H, H-15 and H-22). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm −4.6 (Me–Si), 13.9 (C-33), 25.2 (*t*-Bu–Si), 32.4–37.4 (C-13, C-16, C-17, C-20, C-21, C-24, C-26,

C-28), 33.9 (C-2), 72.2 (C-27), 73.6 (C-14/C-23), 74.7 (C-14/C-23), 81.3–82.1 (C-15, C-18, C-19, C-22), 179.2 (C-1). IR (film, ν_{max} cm^{-1}): 1711. ESIMS *m/z* 913 $[M+H]^+$, 935 $[M+Na]^+$, 951 $[M+K]^+$. $[\alpha]_D = +5$ ($c = 1$, CH_2Cl_2).

Compound 7a. To a solution of 310 mg (0.306 mmol) lactic α-ketoester **2a** in 6.5 mL ice-cold MeOH was added dropwise a solution of 39 mg (0.361 mmol) *o*-phenylenediamine in 3 mL MeOH. The mixture was stirred for 2 h at 0° C then evaporated under reduced pressure. The residue was filtered over a small column of SiO_2 with a cyclohexane/AcOEt 4:1 mixture, yielding 247 mg (79%) **7a** as a pale yellow resin. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 0.03–0.08 (m, 18H, Me–Si), 0.88 (m, 30H, *t*-Bu–Si and H-34), 1.29 (m, H-14), 1.40 (m, H-27 and H-29), 1.65 (m, 4H, H-18 and H-21), 1.80 (m, 2H, H-4, $J = 7.7$ Hz), 1.84 (m, 4H, H-17 and H-22), 2.93 (t, 2H, H-3, $J = 7.7$ Hz), 3.55 (m, 1H, H-15/H-24), 3.64 (m, 1H, H-28), 3.72 (m, 1H, H-15/H-24), 3.88 (m, 4H, H-16, H-19, H-20, H-23), 7.27 (dd, 1H, H-39, $J = 8.1$ Hz), 7.31 (*td*, 1H, H-37, $J = 7.7$ Hz), 7.47 (*td*, 1H, H-38, $J = 7.6$ Hz), 7.78 (dd, 1H, H-36, $J = 8$ Hz), 11.27 (*s*, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm −5.0 (Me–Si), 13.7 (C-34), 25.6 (*t*-Bu–Si), 33.2 (C-3), 72.0 (C-28), 73.2 (C-15/C-24), 74.5 (C-15/C-24), 80.9-83.1 (C-16, C-19, C-20, C-23), 115.1 (C-39), 123.6 (C-37), 128.4 (C-36), 129.3 (C-38), 130.8 (C-40), 132.6 (C-35), 155.4 (C-2), 161.8 (C-1). IR (film, ν_{max} cm^{-1}): 1666 (strong). ESIMS *m/z* 1013 $[M+H]^+$, 1035 $[M+Na]^+$, 1051 $[M+K]^+$. $[\alpha]_D = -5$ ($c = 0.8$, CH_2Cl_2).

Compound 8a. To a solution of 162 mg (0.160 mmol) lactic α-ketoester **2a** in 6 mL *tert*-BuOH at room temperature were added dropwise 1.6 mL (1.6 mmol) aqueous 1N LiOH. The mixture was stirred for 10 min then 12 mL AcOEt were added dropwise. After stirring for 15 min, the mixture was filtered over cotton and the filtrate evaporated under reduced pressure. The residue was suspended in 2.7 mL MeOH, the reaction mixture was cooled to 0 °C, and 43 μL (0.72 mmol) AcOH followed by 19 mg (0.176 mmol) *o*-phenylenediamine in 1.6 mL MeOH were added. The resulting mixture was stirred for 1.5 h in a ice-bath, then diluted by 1 mL water and extracted by 4×5 mL cyclohexane. The organic phases were gathered, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was chromatographied on column (SiO_2, cyclohexane/AcOEt 3:2) yielding 91 mg (58%) **8a** as a colourless resin. ^1H NMR (200 MHz, $CDCl_3$) δ ppm 0.023–0.062 (m, 18H, Me–Si), 0.88 (m, 30H, *t*-Bu–Si and H-34), 1.39 (m, H-4), 1.69 (m, 4H, H-18 and H-21), 1.84 (m, 4H, H-17 and H-22), 2.91 (t, 2H, H-3, $J = 7.8$ Hz), 3.61 (m, 2H, H-28 and H-15/H-24), 3.76 (m, 1H, H-15/H-24), 3.89 (m, 4H, H-16, H-19, H-20, H-23), 7.20 (*ddd*, 2H, H-37 and H-38), 7.54 (m, 2H, H-36 and H-39, $J = 6$ Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm −4.3 (Me–Si), 14.0 (C-34), 18.2 (*t*-Bu–Si), 25.9 (*t*-Bu–C), 29.5 (C-3), 72.2 (C-28), 73.6 (C-15/C-24), 74.7 (C-15/C-24), 81.4-82.2 (C-16, C-19, C-20, C-23), 122.0 (C-37 and C-38), 138.3 (C-36 and C-39), 155.3 (C-2). IR (film, ν_{max} cm^{-1}): 1541. ESIMS *m/z* 985 $[M+H]^+$, 1007 $[M+Na]^+$. $[\alpha]_D = +8$ ($c = 1$, CH_2Cl_2).

Compound 9a. To a solution of 182 mg (0.180 mmol) α-lactic ketoester **2a** in 3 mL toluene were added 25 mg (0.231 mmol) diaminomaleonitrile then 24 mg (0.353 mmol) imidazole. The reaction mixture was heated to 45 °C and stirred at that temperature for 18 h, then evaporated under reduced pressure. The residue was redissolved in 50 mL CH_2Cl_2 and the organic phase successively washed by 20 mL water acidified by 1N aqueous HCl (pH 4–5), by water (20 mL) and brine (20 mL). The organic phase was dried (Na_2SO_4), filtered, and the filtrate evaporated. The crude product was chromatographied over a column of Sephadex® LH-20 impregnated with a 97:3 CH_2Cl_2/MeOH mixture, furnishing 111 mg (61%) **9a** as a pale yellow resin. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 0.021–0.049 (m, 18H, Me–Si), 0.88 (m, 30H, *t*-Bu–Si and H-34), 1.71 (m, 4H, H-18 and H-21), 1.90 (m, 4H, H-17 and H-22), 2.83 (t, 2H, H-3, $J = 7$ Hz), 3.62 (m, 2H, H-15 and H-28), 3.75 (m, 1H, H-24), 3.99 (m, 4H, H-16, H-19, H-20, H23). IR (film, ν_{max} cm^{-1}): 2224, 1683 (weak). ESIMS *m/z* 1011 $[M-H]^-$. $[\alpha]_D = +2$ ($c = 1$, CH_2Cl_2).

Compound 10a. To a solution of 33 mg (32.61 μmol) lactic α-ketoester **2a** in 1.2 mL *tert*-BuOH at room temperature was added dropwise 0.3 mL (0.3 mmol) aqueous 1N LiOH. The mixture was stirred for 10 min then 2.5 mL AcOEt were added dropwise. After stirring for 15 min, the mixture was filtered over cotton and the filtrate evaporated under reduced pressure. The residue was suspended in 0.3 mL *n*-BuOH, and 10 μL (0.167 mmol) AcOH were added. The mixture was heated to 90 °C, and a solution of 5.5 mg (81 μmol) imidazole and 5.3 mg (49.1 μmol) diaminomaleonitrile in 0.2 mL hot *n*-BuOH was added dropwise. The reaction mixture was stirred for 2 h at that temperature, then evaporated under reduced pressure. The residue was chromatographed on column (SiO_2, cyclohexane/AcOEt 4:1) yielding 12 mg (38%) **10a** and 2 mg (7%) acid **5a** as colorless resins. ^1H NMR (200 MHz, $CDCl_3$) δ ppm 0.018–0.063 (m, 18H, Me–Si), 0.87 (m, 30H, *t*-Bu–Si and H-34), 1.66 (m, 4H, H-18 and H-21), 1.88 (m, 4H, H-17 and H-22), 2.75 (t, 2H, H-3, $J = 8$ Hz), 3.58 (m, 2H, H-28 and H-15/H-24), 3.67 (m, 1H, H-15/H-24), 3.89 (m, 2H, H-19 and H-20), 3.96 (m, 2H, H-16 and H-23). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm −4.9 (Me–Si), 13.9 (C-34), 25.8 (^{t}Bu–Si), 28.4 (C-3), 72.1 (C-28), 73.6 (C-15/C-24), 74.1 (C-15/C-24), 81.7–82.4 (C16, C-19, C-20, C-23), 154.3 (C-2). IR (film, ν_{max} cm^{-1}): 2240. ESIMS *m/z* 985 $[M+H]^+$, 1007 $[M+Na]^+$, 1002 $[M+NH_4]^+$. $[\alpha]_D = +6$ ($c = 0.6$, CH_2Cl_2).

Deprotected derivatives. Compound 5. 172 mg (0.188 mmol) 15,24,28 tri-(*O-ter*-butyldimethylsilyl) acid squamocin **5a** were dissolved in 19 mL (19 mmol) of a 1 M TBAF solution in THF. The mixture was heated to 50 °C and stirred at that temperature for 6 h, then quenched with water (35 mL) and extracted by AcOEt (5×50 mL). The organic phases were gathered, washed with water (1×80 mL) and brine (1×80 mL), then evaporated under reduced pressure. The crude product was chromatographied over a column of Sephadex® LH-20 eluted with a 97:3 CH_2Cl_2/MeOH mixture, yielding 85 mg (79%) of deprotected acid **5** as a white resin. ^1H

NMR (200 MHz, $CDCl_3$) δ ppm 0.91 (m, 3H, H-33, $J=6.8$ Hz), 1.66 (m, 4H, H-17, H-20), 1.97 (m, 2H, H-16/H-21), 2.0 (m, 2H, H-16/H-21), 2.36 (t, 2H, H-2, $J=7.2$ Hz), 3.43 (m, 1H, H-14), 3.64 (m, 1H, H-27), 3.89 (m, 5H, H-15, H-18, H-19, H22, H-23). ^{13}C RMN (50 MHz, $CDCl_3$) δ ppm 13.9 (C-33), 32.2–37.2 (C-13, C-16, C-17, C-20, C-21, C-24, C-26, C-28), 37,0 (C-2), 71.4 (C-23), 71.7 (C-14/C-27), 74.0 (C-14/C-27), 82.0-83.1 (C-15, C-18, C-19, C-22), 177.6 (C-1). IR (film, ν_{max} cm^{-1}): 3393, 1739. ESIMS m/z 593 $[M+Na]^+$, 588 $[M+NH_4]^+$ (in positive mode); 569 $[M-H]^-$, 551 $[M-H-H_2O]^-$ (in negative mode). HRMS: calcd for $C_{33}H_{61}O_7$ $[M-H]^-$: 569.4417, found: 569.4406. $[\alpha]_D=-4$ ($c=0.45$, CH_2Cl_2).

Compound 7. To a solution of 130 mg (0.128 mmol) quinoxalinone **7a** in 3 mL MeOH was added 1 g of methanol-washed Amberlyst® −15. The mixture was warmed to 40 °C and stirred at that temperature for 7 h. The reaction mixture was diluted by 3 mL AcOEt and filtered over Celite. The filtrate was evaporated under reduced pressure and the residue chromatographied over a column of Sephadex® LH-20 impregnated with 100% CH_2Cl_2, furnishing 52 mg (60%) **7** as a pale yellow resin. ^1H NMR (200 MHz, $CDCl_3$) δ ppm 0.88 (t, 3H, H-34, $J=6.8$ Hz), 1.64 (m, 4H, H-18 and H-21), 1.94 (m, 4H, H-17 and H-22), 3.0 (t, 2H, H-3, $J=8$ Hz), 3.40 (m, 1H, H-15), 3.61 (m, 1H, H-28), 3.89 (m, 5H, H-16, H-19, H-20, H-23, H-24), 7.28 (dd, 1H, H-39), 7.33 (*td*, 1H, H-37, $J=8$ Hz), 7.49 (*td*, 1H, H-38, $J=8$ Hz), 7.90 (dd, 1H, H-36, $J=8$ Hz). IR (film, ν_{max} cm^{-1}): 3393, 1663 (strong). ESIMS m/z 671 $[M+H]^+$, 693 $[M+Na]^+$. HRMS: calcd for $C_{40}H_{67}N_2O_6$ $[M+H]^+$: 671.4999, found: 671.4990. $[\alpha]_D=0$ ($c=0.5$, CH_2Cl_2).

Compound 8. To a solution of 85 mg (86,4 μmol) benzimidazole **8a** in 2 mL MeOH was added 615 mg of methanol-washed Amberlyst® −15. The mixture was warmed to 40 °C and stirred at that temperature for 7 h. The reaction mixture was filtered, and the resin washed by MeOH (2 mL) then CH_2Cl_2 (2 mL). The resin was resuspended in 2 mL satd. aqueous $NaHCO_3$, and pH adjusted to 9–10 by dropwise addition of 3 N aqueous NaOH solution. The mixture was extracted by 3×3 mL THF, the organic phases were gathered, dried (Na_2SO_4), filtered and evaporated under reduced pressure, yielding 53 mg (95%) **8** as a white amorphous solid. ^1H NMR (400 MHz, CD_3OD) δ ppm 0.89 (t, 3H, H-34, $J=6.7$ Hz), 1.64 (m, 4H, H-18 and H-21), 1.83 (m, 2H, H-4), 1.95 (m, 4H, H-17 and H-22), 2.87 (t, 2H, H-3, $J=7.6$ Hz), 3.37 (m, 1H, H-15), 3.51 (m, 1H, H-28), 3.57 (m, 1H, H-24), 3.84 (m, 4H, H-16, H-19, H-20, H-23), 7.16 (t, 1H, H-37, $J=6.2$ Hz), 7.17 (t, 1H, H-38, $J=6.6$ Hz), 7.48 (dd, 2H, H-36 and H-39, $J=6.7$ Hz). ^{13}C NMR (50 MHz, CD_3OD) δ ppm 14.4 (C-34), 29.3 (C-3), 72.4 (C-24), 74.1 (C-28), 74.9 (C-15), 83.3-84.2 (C-16, C-19, C-20, C-23), 123.1 (C-37 and C-38), 157.0 (C-2). IR (film, ν_{max} cm^{-1}): 3393. ESIMS m/z 643 $[M+H]^+$. HRMS: calcd for $C_{39}H_{67}N_2O_5$ $[M+H]^+$: 643.5050, found: 643.5038. $[\alpha]_D=+19$ ($c=0.8$, MeOH).

Compound 9. See preparation of **7** (chromatography over Sephadex® LH-20 with CH_2Cl_2/MeOH 95:5). 42 mg (41,5 μmol) pyrazinol **9a** gave 16 mg (57%) **9** as a colourless resin. ^1H NMR (200 MHz, $CDCl_3$) δ ppm 0.86 (t, 3H, H-34, $J=6.8$ Hz), 1.67 (m, 4H, H-18 and H-21), 1.94 (m, 4H, H-17 and H-22), 2.88 (t, 2H, H-3, $J=7$ Hz), 3.58 (m, 3H, H-15, H-24 and H-28), 3.95 (m, 4H, H-16, H-19, H-20, H-23). IR (film, ν_{max} cm^{-1}): 3394, 2223, 1685, 1541. ESIMS m/z 671 $[M+H]^+$, 693 $[M+Na]^+$. HRMS: calcd for $C_{38}H_{63}N_4O_6$ $[M+H]^+$: 671.4747, found: 671.4738. $[\alpha]_D=-3$ ($c=0.35$, CH_2Cl_2).

Compound 10. See preparation of **7** (reaction at rt for 15 h; chromatography over Sephadex® LH-20 with CH_2Cl_2/MeOH 95:5). 38 mg (38.6 μmol) imidazole **10a** gave 19 mg (77%) **10** as a colourless resin. ^1H NMR (400 MHz, CD_3OD) δ ppm 0.88 (t, 3H, H-34, $J=7$ Hz), 1.66 (m, 4H, H-18 and H-21), 1.72 (m, 2H, H-4), 1.96 (m, 4H, H-17 and H-22), 2.74 (t, 2H, H-3, $J=8$ Hz), 3.38 (m, 1H, H-15), 3.50 (m, 1H, H-28), 3.58 (m, 1H, H-24), 3.85 (m, 4H, H-16, H-19, H-20, H-23). ^{13}C NMR (50 MHz, CD3OD) δ ppm 14.4 (C-34), 28.7 (C-3), 72.3 (C-24), 73.9 (C15/C-28), 74.9 (C-15/C-28), 83.3–84.9 (C-16, C-19, C-20, C-23), 111.5 (C-35 and C-36), 156.4 (C-2). IR (film, ν_{max} cm^{-1}): 3399, 2237, 1516. ESIMS m/z 643 $[M+H]^+$, 665 $[M+Na]^+$. HRMS: calcd for $C_{37}H_{63}N_4O_5$ $[M+H]^+$: 643.4798, found: 643.4793. $[\alpha]_D=-2$ ($c=0.5$, CH_2Cl_2).

Cytotoxicity

Cytotoxicities were colorimetrically evaluated through a 96-well plate assay after 72 h cellular exposure to the acetogenins. Cells were of the KB 3-1 line (nasopharyngeal epidermoïd carcinoma). Cells were cultivated in Dullbecco's MEM ≪glutamax≫ (1 g/L glucose) enriched with 10% FCS, in a moist atmosphere containing 5% CO_2. Each well of the test plate was inseminated at t_0 with 100 μL of a $1.3.10^5$ cell/mL cellular suspension as the cytotoxicities were to be recorded after 72 h, taking into account the slow cytotoxic action of the acetogenins in comparison with classical antitumour agents (e.g. Taxol).

Homogeneous dilutions were performed from a 10^{-3} M solution of the acetogenin in DMSO, a 100 μL aliquote of this solution being diluted in 5.90 mL of the culture media. This obtained 2.10^{-5} M solution was further and extensively diluted by an order of three with 2% DMSO precontaining culture media. After 24 h cell growth, 100 μL of each of the dilutions were as triplicates administrated into the wells (the final well concentation of acetogenin being therefore divided by two), and homogeneisation was ensured by 5 min agitation on a microtitration plate.

After 72 h contact, the test plates were emptied and directly treated with 200 μL of a 2,5 g/L methylene blue solution in MeOH/water 50:50 v/v. After 40 min contact, the plates were emptied and the wells quickly rinsed under a very mild flow of cold tap water. The plates were air-dried (50 °C) and each well treated with 100 μL of 0.1 N HCl. Absorbances were recorded after 30 min incubation at 37 °C and homogeneisation of the plates, at 620 nm and under a blank of 100 μL of 0.1 N

HCl. IC_{50} were expressed as the extrapolated concentrations allowing 50% cell survival in comparison with untreated controls.

References and Notes

1. Rupprecht, J. K.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237.
2. Londershausen, M.; Leicht, W.; Lieb, F.; Moeschler, H.; Weiss, H. *Pestic. Sci.* **1991**, *33*, 427.
3. Rodier, S.; Le Huérou, Y.; Renoux, B.; Doyon, J.; Renard, P.; Pierré, A.; Gesson, J.-P.; Grée, R. *Anti-Cancer Drug Des.* **2001**, *16*, 109.
4. Holschneider, C. H.; Johnson, M. T.; Knox, R. B.; Rezai, A.; Ryan, W. J.; Montz, F. J. *Cancer Chemother. Pharmacol.* **1994**, *34*, 166.
5. Duret, P.; Hocquemiller, R.; Gantier, J.-C.; Figadère, B. *Bioorg. Med. Chem.* **1999**, *7*, 1821.
6. Takada, M.; Kuwabara, K.; Nakato, H.; Tanaka, A.; Iwamura, H.; Miyoshi, H. *Biochim. Biophys. Acta* **2000**, *1460*, 302.
7. Tormo, J. R.; Estornell, E.; Gallardo, T.; González, M. C.; Cavé, A.; Granell, S.; Cortes, D.; Zafra-Polo, M. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 681.
8. Cortes, D.; Myint, S. H.; Lebœuf, M.; Cavé, A. *Tetrahedron Lett.* **1991**, *32*, 6133.
9. Arndt, S.; Emde, U.; Bäurle, S.; Friedrich, T.; Grubert, L.; Koert, U. *Chem. Eur. J.* **2001**, *7*, 993.
10. Lümmen, P. *Biochim. Biophys. Acta* **1998**, *1364*, 287.
11. Degli Esposti, M. *Biochim. Biophys. Acta* **1998**, *1364*, 222.
12. Miyoshi, H. *Biochim. Biophys. Acta* **1998**, *1364*, 236.
13. Fujimoto, Y.; Eguchi, T.; Kakinuma, K.; Ikekawa, N.; Sahai, M.; Gupta, Y. K. *Chem. Pharm. Bull.* **1988**, *36*, 4802.
14. Vu, T.; Tam, B. C.; Hieu Chappe, B. *Planta Med.* **1993**, *59*, 576.
15. Degli Esposti, M.; Ghelli, A.; Ratta, M.; Cortes, D.; Estornell, E. *Biochem. J.* **1994**, *301*, 161.
16. Cavé, A.; Figadère, B.; Laurens, A.; Cortes, D. In *Progress in the Chemistry of Organic Natural Products*; Hertz, W; Kirby, G. W; More, R. E.; Steglich, W.; Tamm, C. Eds. Springer Wien New York 1997, pp 81–288.
17. Gallardo, T.; Zafra-Polo, M. C.; Tormo, J. R.; González, M. C.; Franck, X.; Estornell, E. *J. Med. Chem.* **2000**, *43*, 4793.
18. Jolad, S. D.; Hoffmann, J. J.; Cole, J. R.; Barry, C. E.; Bates, R. B.; Linz, G. S.; Konig, W. A. *J. Nat. Prod.* **1985**, *48*, 644.
19. Ramirez, E. A.; Hoye, T. R. In *Studies in Natural Products Chemistry, vol. 17*, Atta ur-Rahman Ed., Elsevier Science, B.V., 1995, pp 251–282.
20. Sahai, M.; Singh, S.; Singh, M.; Gupta, Y. K.; Akashi, S.; Yuji, R.; Hirayama, K.; Asaki, H.; Araya, H.; Hara, N.; Eguchi, T.; Kakinuma, K.; Fujimoto, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1163.
21. Duret, P.; Waechter, A.-I.; Figadère, B.; Hocquemiller, R.; Cavé, A.; Piérard, C.; Pérès, M. *Tetrahedron Lett.* **1996**, *37*, 7043.
22. Biekert, E.; Hoffmann, D.; Meyer, F. J. *Chem. Ber.* **1961**, *94*, 1664.
23. Labib, G. H.; Rahman, M. A.; El Kilani, Y.; El Massry, A. I.; El Ashry, E. S. H. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4427.
24. Kolar, P.; Risler, M. *J. Heterocycl. Chem.* **1993**, *30*, 1253.
25. Kaup, G.; Naimi-Jamal, M. R. *Eur. J. Org. Chem* **2002**, 1368.
26. Cecil, A. R. L.; Brown, R. C. D. *Org. Lett.* **2002**, *4*, 21 3715.
27. Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.
28. Harmange, J.-C.; Figadère, B.; Hocquemiller, R. *Tetrahedron: Asymmetry* **1991**, *2*, 347.
29. Nwaukwa, S. O.; Keehn, P. M. *Tetrahedron Lett.* **1982**, *23*, 3135.
30. Gottlieb, L.; Kellner, D.; Lowenthal, H. J. E. *Synth. Comm.* **1989**, *19*, 2987.
31. Morrow, N.; Ramsden, C. A.; Sargent, B. J.; Wallet, C. D. *Tetrahedron* **1998**, *54*, 9603.
32. Tada, M.; Tsuzuki, K. *J. Heterocycl. Chem.* **1986**, *23*, 5 1299.
33. Queiroz, E. F.; Roblot, F.; Duret, P.; Figadère, B.; Gouyette, A.; Laprévote, O.; Serani, L.; Hocquemiller, R. *J. Med. Chem.* **2000**, *43*, 1604.
34. Nakagawa, Y.; Kuwano, E.; Eto, M.; Fujita, T. *Agric. Biol. Chem.* **1986**, *49*, 3569.
35. Hollingworth, R. M.; Ahammadsahib, K. I.; Gadelhac, G.; McLaughlin, J. L. *Biochem. Soc. Trans.* **1994**, *22*, 230.
36. Friedrich, T.; Ohnishi, T.; Forche, E.; Kunze, B.; Jansen, R.; Trowitzsch, W.; Höfle, G.; Reichenbach, H.; Weiss, H. *Biochem. Soc. Trans.* **1994**, *22*, 226.