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Analogues of cytotoxic squamocin using reliable reactions: new insights into the reactivity and role of the α,β-unsaturated lactone of the annonaceous acetogenins

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Abstract—A small library of squamocin analogues has been prepared and screened biologically (cytotoxicity, inhibition of mitochondrial complex I and complex III). To centre diversity on a crucial part of the molecule (i.e., the α,β-unsaturated lactone), an original and reliable lactone opening reaction has been discovered and exploited among other efficient reactions.

1. Introduction

In the search for efficient principles to improve not only the number of compounds in libraries but also, and mainly, their quality in term of 'drug-likeness', new methodologies have to be designed. In this context, natural products with validated biological activities constitute ideal starting templates and drug researchers are increasingly embracing natural-product-like libraries. Concomitantly, among the new methodologies allowing a rapid access to original chemical entities developed in the recent years, 'click chemistry' constitutes an exciting direction.[1] As a way of making quickly large numbers of molecules, it offers an attractive route for developing compound libraries. In the search for original bioactive molecules, we believe that a high number of ready-to-test molecules can be prepared easily when combining the pre-existing complexity of natural products and selected reliable reactions. We want to use the set of these reactions, the essence of the concept, to synthesize natural-product-based libraries of molecules. The selectivity of the reactions is of first importance to enable the rapid assembly of molecules without the use of any protecting group.

In this paper, we wish to describe the first results of our explorations. We set on studying α,β-unsaturated lactones, a common substructure found in many natural products. Acetogenins from Annonaceae constitute interesting and privileged skeletons containing such a moiety because of their especially intense biological activities.[2] They are a structurally unique, broad group of polyketides, which are only found, so far, in several genuses of the Annonaceae. These compounds exhibit a wide range of potent biological activities (cytotoxic, antiparasitic, pesticidal) resulting from strong inhibition of the mitochondrial NADH–ubiquinone oxidoreductase (complex I) as the main target, with a dramatic drop of ATP production and cell death by apoptotic mechanisms. The exact structural basis of the interaction between the acetogenins and this respiratory target is unknown. They have also, recently, been suspected of being implicated in atypical Parkinsonism syndromes in conjunction with the consumption of annonaceous-derived edible products or traditional medicines.[3] Therefore, there is a great need for studies directed towards a better understanding of acetogenin molecular mechanisms of action.

In particular, the role of the terminal α,β-unsaturated γ-methylbutyrolactone as a quasi-ubiquitous moiety in the core of these inhibitors remains unclear. Since only a limited number of synthetic modifications have been described at this level (because of the scarcity of these compounds and

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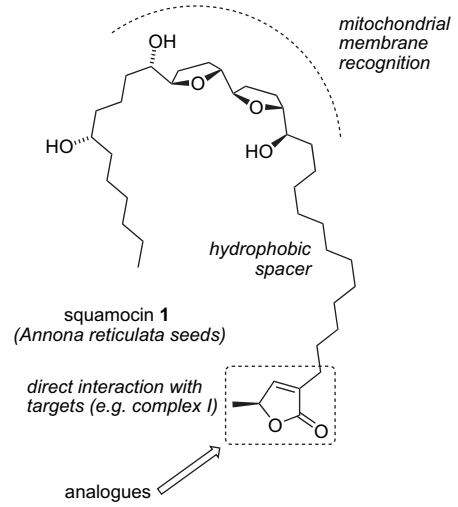


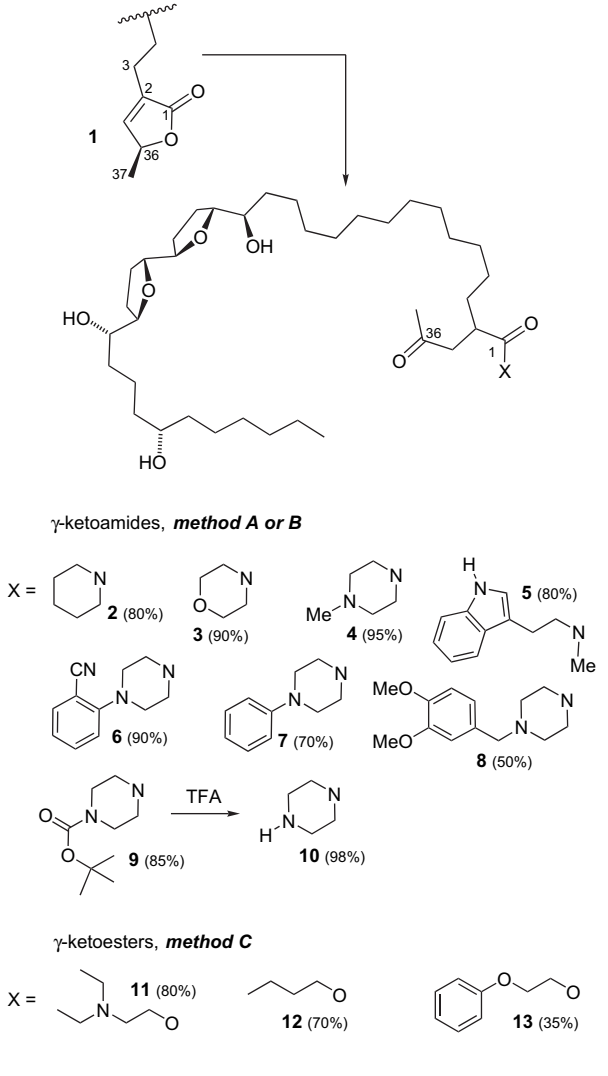
Figure 1. Squamocin **1** and fundamental pharmacophores.

the difficulties encountered in their total synthesis[4,5]), it is still uncertain whether this structural part has to be considered crucial for the inhibition.[6,7] Our aim was to develop simple, efficient and versatile transformation procedures of the lactone core of those rather complex natural products, allowing access to a certain degree of molecular diversity in this class of bioactive secondary metabolites. Isolated from *Annona reticulata*, squamocin **1** (Fig. 1) was selected as a lead compound for our studies for its exhibited properties. It also constituted a great underlying challenge to be able to modify **1**, considering the physicochemical issues inherent to the lipid-like behaviour of acetogenins, which make these molecules difficult to manipulate and purify.[†] Two types of modifications of the lactone will be described: a nucleophilic ring opening and a reduction into a furane suitable for Diels–Alder reactions.

2. Chemistry[‡]

2.1. An efficient lactone ring opening

With our natural substrate in hand, we explored its properties and discovered an original reactivity of the terminal butenolide with nucleophiles. Our conditions led to γ-ketoamides (**2**–**10**), γ-ketoesters (**11**–**13**) or γ-ketoacid (**14**) derivatives of squamocin,[§] these semisynthetic analogues being obtained in quasi-quantitative yields and high purity (Scheme 1). Few examples of such addition reactions even on simple α,β-unsaturated butyrolactones are described. Furthermore, in addition to an increase in the efficiency of those transformations, it must be mentioned that these have never been deliberately and widely applied to complex natural products.[8] A careful study of reaction conditions was carried out to understand these unusual transformations and define their scope and limitations.



Scheme 1. Lactone opening. Reagents and conditions: *Method A*, neat conditions, nucleophile (1.0 equiv to excess), 100–110 °C; *Method B*, boiling water, nucleophile (2.0–3.0 equiv); *Method C*, nucleophile+base, 100–110 °C. Average yields are indicated for compound purity >97% (simple work-up or purification if needed, see Section 5).

Reactions were especially practical when simple volatile secondary amines were used in excess and served as solvent but they ran well with only 1 equiv of nucleophile in neat conditions. Most interestingly, these reactions proceeded readily in boiling water despite the insolubility of the acetogenin (see below).[9] Apart from γ-ketoamides, the most striking example in this context corresponded to the high-yielding semisynthesis of a completely water-soluble sodium salt of acid **14** by simple dispersion of squamocin in boiling 1 N aqueous NaOH. As far as the obtention of γ-ketoesters is concerned, the reacting primary alcohol needed to be used as solvent for the reaction to occur, the amount of which being reducible to 3 equiv. Amines rarely required

[†] Concerning natural acetogenins, they are generally present in small to minute amounts as complex mixtures of isomers.

[‡] All compounds (**2**–**23**) were fully characterized by NMR (^{1}H, ^{13}C, HMQC, HMBC, NOESY and, when needed, HOHAHA experiments), IR and MS. See Section 5 for details.

[§] As can be expected, these adducts were obtained as equimolar mixtures of epimers, as evidenced by HPLC analysis. Most of the time, the diastereoisomers were undistinguishable by spectroscopic methods, this behaviour being similar to the one observed for γ-epimerized natural acetogenins (see Ref. 10).

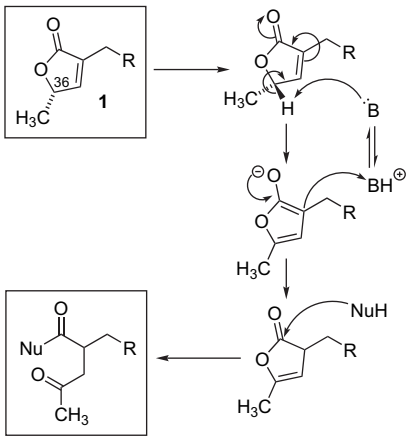


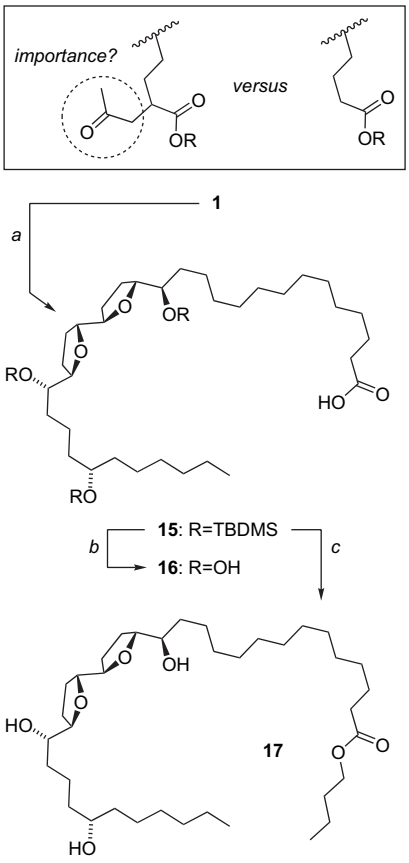
Figure 2. Plausible mechanism for the lactone opening.

any addition of base (easy to handle K_2CO_3 could be used efficiently, giving similar results with marked increase in reaction rates) whereas it was the case for primary alcohols. Among the numerous bases tested, DBU gave the best results. Importantly, it has to be mentioned here that secondary amines and primary alcohols of low melting points (below 100 °C) were also suitable for such reactions, allowing the introduction of more sophisticated substructures in the acetogenin skeleton (Schemes 1 and 3). Primary amines exhibited a different reactivity towards the butenolide nucleus and afforded Michael 1,4-adducts, while the tested secondary alcohols failed to furnish the desired γ-ketoesters.

On the basis of these results, we propose the following mechanistic scheme to explain this unusual nucleophilic opening of the lactone. A possible pathway might be initiated by a deprotonation of the acidic proton at C-36 on the butenolide system, leading to an increase of the electrophilicity of the lactone coupled to an irreversible ring opening (Fig. 2).[10]

As a way to evaluate the contribution of the α-acetonyl side chain to the biological activity of such derivatives, we synthesized two linear analogues of α-acetonylacid **14** and α-acetonyl-*n*-butylester **12**, respectively (Scheme 2). Acid **16** was obtained from its tri-*O*-TBDMS precursor **15** as described previously.[6a,6d,6e] On the other hand, *n*-butylester **17** was prepared by a one-pot desilylation/esterification reaction of **15** in a hot Amberlyst®-15/*n*-BuOH system, with an excellent yield.

The described reactions,[¶] involving only carbon–heteroatom bond formation, are highly selective with no or very few by-products. Having secured the reaction with various nucleophiles, our attention shifted to the possibility of gaining further complexity by constructing new molecular architectures. With this aim, dimeric structure **18** was targeted and obtained when reacting squamocin **1** with 0.5 equiv of dipiperidinyl propane, demonstrating as stated above the feasibility of the reactions with a stoichiometric amount of

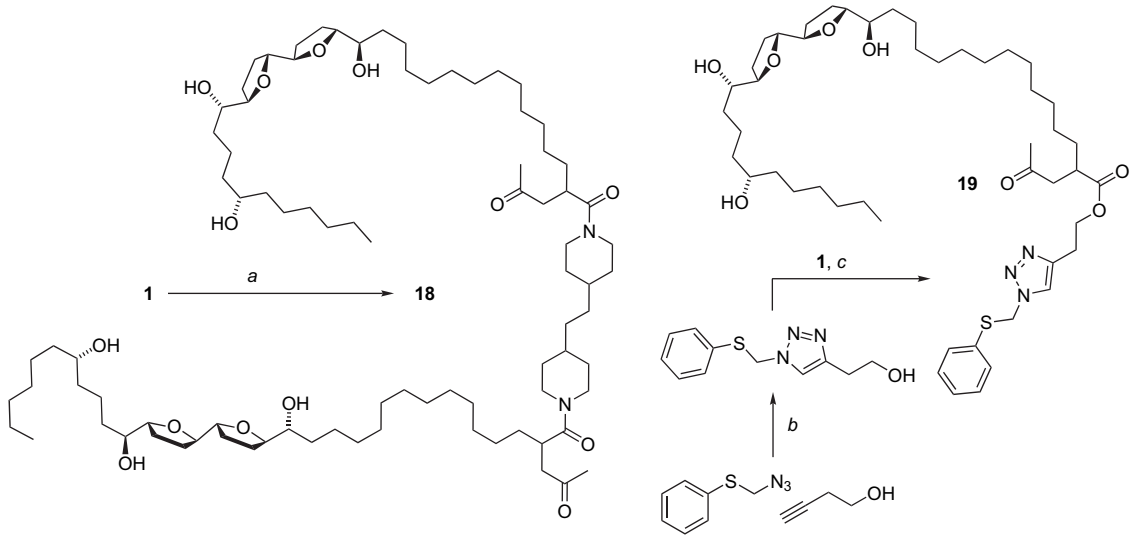


Scheme 2. Synthesis of acid **16** and ester **17** for SAR comparisons. Reagents and conditions: (a,b) Refs. 6a, 6d, 6e; (c) Amberlyst 15®, *n*-BuOH, 65 °C, 15 h (90%). TBDMS=*tert*-butyldimethylsilyl.

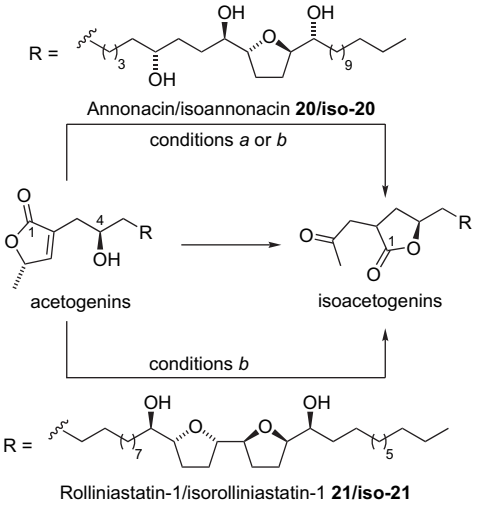
nucleophile in the absence of solvent (Scheme 3). Other developments were strongly influenced by a few of the seminal 'good reactions' in click chemistry, e.g., cycloadditions reactions to form triazoles. A two-step sequence was employed for the formation of derivative **19**. A copper(I)-catalyzed regioselective ligation of thiophenylazido-methane and butyn-1-ol via Huisgen dipolar cycloaddition in a simple Cu^{II}/ascorbate aqueous system[11] gave rise to an alcohol which was added to squamocin in the conditions described in Scheme 1, yielding highly functionalized compound **19** (Scheme 3).

Finally, the nucleophilic ring opening can be envisioned in an intramolecular way. Encouraged by our success in using water as a favourable medium for clean reactions between lipophilic partners, we discovered an original way to transform a type of natural compounds into another. Annonacin, a known annonaceous acetogenin from *Annona muricata*,[12a,b] was thus totally converted into isoannonacin *iso*-**20**[12c] by simple dispersion in boiling water. As in the case of the semisynthesis of γ-ketoacid salt of **14**, isoannonacin of high purity was simply recovered by extraction from water. Such a reaction failed in the case of rolliniastatin-1,[13] another 4-hydroxyacetogenin from *Rollinia mucosa*, probably because of its higher melting point. However, both acetogenins could be converted into their isoacetogenin counterparts by simple heating at 150 °C under argon (Scheme 4).

[¶] When using 1 equiv of nucleophile, the process is a 'fusion reaction', i.e., the combined formulae of the reactants equal the formula of the product.



Scheme 3. Two-step sequences to compounds **18** and **19**. Reagents and conditions: (a) 1,3 (4-piperidinyl)propane (0.5 equiv), K_2CO_3, 110 °C, 4 h (90%); (b) $CuSO_4$ (1 mol %), ascorbic acid (10 mol %), H_2O/*t*-BuOH (1:1), 20 °C, 48 h (90%); (c) 1 (0.3 equiv, excess alcohol easily recoverable), DBU (0.25 equiv), 110 °C, 4 h (70%). DBU=1,8-diazabicyclo[5.4.0]undec-7-ene.



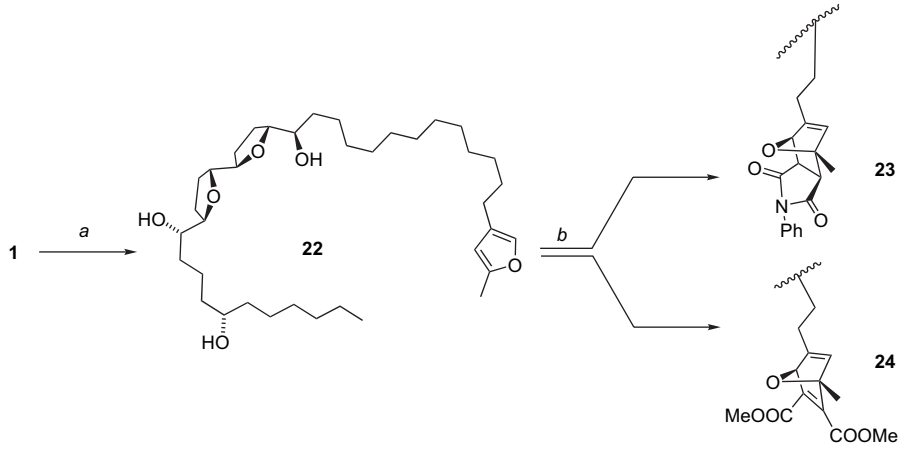
Scheme 4. Conversion of acetogenins into isoacetogenins: conditions (a) dispersion in boiling water, 12 h (*iso*-**20**: 90%); conditions (b) 150 °C, Ar (*iso*-**20**: 60%, *iso*-**21**: 40%).

2.2. Diels–Alder reactions

Reduction of squamocin **1** with DIBAL–H furnished, with an excellent yield, furane derivative **22** (Scheme 5), an ideal candidate for simple Diels–Alder reactions with symmetric dienophiles. Indeed, reaction with *N*-phenylmaleimide provided bicyclic compound **23** with 90% yield. A similar result was obtained with acetylenedicarboxylic dimethylester giving cycloadduct **24**. These reactions could alternatively be performed without any solvent or in boiling water (Scheme 5).

3. Biological activities

Compounds were evaluated for their cytotoxicity against KB 3-1 cells (human nasopharyngeal epidermoid carcinoma) in vitro (Table 1), as well as their complex I inhibitory activities (inhibition of the NADH oxidase and NADH-*n*-decylubiquinone oxidoreductase activity, measured on submitochondrial particles from sonicated beef heart mitochondria—Table 1). A selection of molecules was also tested as inhibitors of mitochondrial complex III.



Scheme 5. Reduction into furane and Diels–Alder reactions. Reagents and conditions: (a) DIBAL–H, CH_2Cl_2/$PhCH_3$, −78 °C (87%); (b) *N*-phenylmaleimide (1.0 equiv) or methyl acetylenedicarboxylate (1.0 equiv), 100–110 °C, no solvent or boiling water (**23**: 92%, **24**: 90%).

Table 1. Biological activities of the semisynthetic derivatives: complex I inhibition and cytotoxicity

Compound	Complex I inhibition[b] IC_{50} (nM)		Cytotoxicity IC_{50} (M)[a]
	NADH oxidase	NADH–DB oxidoreductase	
Squamocin **1**	0.9	1.3	1.8×10^{-13}
α-Acetonylpiperazinamide **2**	41	nt	4.0×10^{-7}
α-Acetonylmorpholinamide **3**	22		3.6×10^{-7}
α-Acetonyl-*N*-methylpiperazinamide **4**	nt[c]	nt	4.0×10^{-7}
α-Acetonyl-N_{ω}-methyltryptamide **5**	52	nt	3.9×10^{-7}
α-Acetonyl-*N*-phenylpiperazinamide **7**	nt	nt	1.5×10^{-8}
α-Acetonyl-*N*-(3′,4′-dimethoxybenzyl)-piperazinamide **8**	74	154	1.2×10^{-8}
α-Acetonyl-*N*-(Boc)piperazinamide **9**	nt	nt	4.1×10^{-8}
α-Acetonylpiperazinamide **10**	nt	nt	5×10^{-7}
α-Acetonyl-(*N*,*N*′-diethylamino)-2-ethyl ester **11**	13	nt	7.5×10^{-10}
α-Acetonyl-*n*-butylester **12**	17	nt	4.6×10^{-10}
α-Acetonyl-2-phenoxyethyl ester **13**	>3000	nt	8.1×10^{-8}
α-Acetonylacid **14**	12	nt	1.3×10^{-9}
Acid **16**	6.2	38	2.8×10^{-7}
n-Butylester **17**	2.3	9.2	6.8×10^{-8}
Bis(α-acetonyl-1,3-(4-piperidinamide)propane) **18**	26	nt	2.7×10^{-8}
Triazole ester **19**	12	nt	5.1×10^{-9}
Furane **22**	2.4	nt	3.2×10^{-8}
Cycloadduct **23**	30	nt	3.5×10^{-7}
Cycloadduct **24**	23	nt	3.0×10^{-8}
Doxorubicin[d]	—	—	2.4×10^{-9}
Rotenone[d]	4.9	28	—
DQA[d]	nt	4.9	—

[a] KB 3-1 cells.
[b] Mitochondrial complex I (NADH–ubiquinone oxidoreductase), submitochondrial particles from beef heart.
[c] Not tested.
[d] References; DQA=2-*n*-decyl-4-quinazolinyl amine.

Acetonylamides (**2–10**, **18**) showed collapsed, though still very significant, cytotoxic activities in comparison with squamocin **1**. Interestingly, all obtained α-acetonylamides exhibited very similar cytotoxicities despite the introduction of different and more or less complex moieties. Concerning α-acetonylesters (**11–13**) and α-acetonylacid (**14**), these analogues exhibited decreased though significantly more similar cytotoxicities relatively to squamocin **1** and compared to amide derivatives. The two linear analogues **16** and **17** exhibited a 100 times diminished cytotoxicity in comparison to their α-acetonyl counterparts, suggesting that the 1,4-dicarbonylated part of the above adducts from nucleophilic opening was responsible for some of their strong cytotoxic activity. Paradoxically, linear *n*-butylester **17** appeared to be a seven times more potent complex I inhibitor than its α-acetonylated counterpart **12**. Ester **17** is indeed a powerful mitochondrial inhibitor, exhibiting an activity close to the one of squamocin **1**. Furane analogue **22** exhibited a collapsed cytotoxicity in comparison with squamocin **1**, despite an important enzymatic inhibitory potency, which was only three times weaker than that of the natural acetogenin. This observation is an echo to the fact that the butenolide of squamocin **1** was completely substitutable by a benzimidazole nucleus,[2b] and by other electron-rich aromatic moieties.[6b] None of the compounds tested at concentrations of 1–3 μM (derivatives **5**, **8**, **16**, **17**, **19** and **21**) was found to inhibit the *n*-decylubiquinol/cytochrome *c* oxidoreductase activity of complex III from bovine heart mitochondria reconstituted into liposomes.

4. Conclusion

This study has led to the discovery of novel pharmacophores in the annonaceous acetogenins series (Fig. 3), both at the complex I inhibition and the cytotoxicity levels. But the results revealed obvious discrepancies between the cellular and enzymatic inhibitory activities of the squamocin analogues. Cytotoxicities are decreased but still in a 10^{-7} M range for the less active derivatives, placing the modified acetogenins on an average among cytotoxic agents used as antitumor drugs and giving new insights into the potential development of acetogenins for chemotherapy.

As far as synthetic organic chemistry is concerned, a single molecular skeleton was exposed to different reaction conditions to achieve original and efficient transformations. The α,β-unsaturated butyrolactone nucleus appeared to be a modular chemical block capable of driving spontaneous and irreversible linkage reactions. As part of chemically viable tools and methods, simple reaction conditions, especially the ones

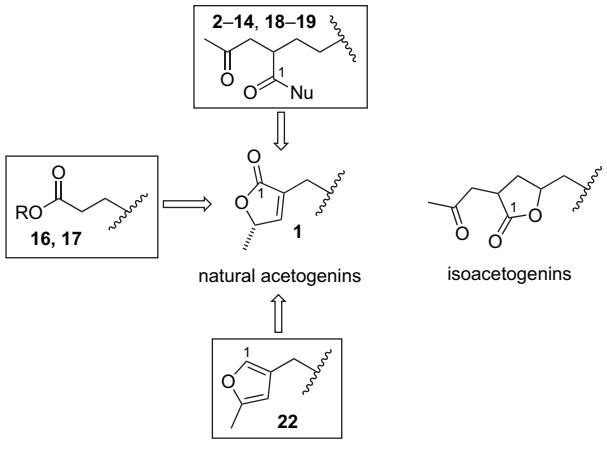


Figure 3. Novel terminal pharmacophores in the squamocin series.

ran in a boiling aqueous medium, allowed the easy preparation of original squamocin analogues. Moreover, our results demonstrated the usefulness of selecting reliable reactions for modifying with good to quantitative yields small amounts of natural products without the need for protecting groups.[‖] In this context, the set of powerful reactions proposed by Sharpless et al. has been an interesting source of inspiration. Although nucleophilic opening of a lactone was not part of the 'good reactions' toolbox,[1] this reaction was of great interest to us in the sense that it permitted, in one efficient operation, to considerably modify the pharmacophore of an active natural product while incorporating complexity. Moreover, Diels–Alder reactions and Huisgen cycloadditions are qualified as 'click reactions'. This is the first small discrete library of acetogenin derivatives with diversity centred on a crucial part of the natural substrate. Inherent reactivity combined with the efficiency of the selected reactions gave immediate access to the analogues in one step. As far as acetogenins are concerned, such efficient reactions as the ones described here may have important biological significance, as one can imagine a similar nucleophilic trapping and opening of the lactone ring by intracellular targets, explaining thereby part of their activity. This new postulate is currently being studied in our laboratory.

5. Experimental

5.1. General methods

^{1}H NMR spectra were recorded on a Bruker AM-400 (400 MHz) at room temperature unless specified otherwise, ^{13}C NMR spectra were recorded on a Bruker AC-200 (50 MHz) or on a Bruker AM-400 (100 MHz) spectrometer at room temperature and were calibrated with the residual undeuterated solvent as an internal reference. All compounds were fully characterized by NMR (^{1}H, ^{13}C, COSY, HMQC, HMBC and, when needed, NOESY and HOHAHA experiments). Mass spectra (MS or HRMS) were recorded at the 'Laboratoire de Spectrométrie de Masse' de l'Institut de Chimie des Substances Naturelles de Gif-sur-Yvette, France. Optical rotations were measured using a Schmidt–Haensch polarimeter E at 589 nm. IR spectra were recorded on a Perkin–Elmer 257 FTIR spectrometer and samples were analyzed as a film from a solution in the indicated solvent. All reagents were purchased at the highest commercial quality and used without further purification. Column chromatography was performed with silica gel 60 (9385 Merck) in the 'flash' mode or Sephadex® LH-20 (Pharmacia). Reactions were monitored by TLC carried out on aluminium plates coated with silica gel 60F254 (E Merck) using UV light as a visualizing agent and sulfuric vanillin and heat as a developing agent. Squamocin **1** was isolated from the seeds of *A. reticulata*, collected in Viet-Nam.

5.2. Lactone openings

5.2.1. General procedures (see Scheme 1 for individual yields). *Method A*: squamocin **1** (10 mg, 16 μmol) and the amine (1.1–20 equiv) were stirred at 100–110 °C until completion of the reaction followed by TLC (CH_2Cl_2/MeOH 9:1), dry K_2CO_3 (1 equiv *m*/*m*) could be added to increase reaction rate. The resulting mixture was evaporated or diluted with CH_2Cl_2 and washed 3 times with water. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure to give the desired compound after chromatography on silica gel (CH_2Cl_2/MeOH 95:5).

Method B: squamocin **1** (10 mg, 16 μmol) and the amine (2–3 equiv) were vigorously stirred in boiling water until completion of the reaction followed by TLC (CH_2Cl_2/MeOH 9:1). The cooled mixture was extracted with CH_2Cl_2 (3 times). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to give the desired compound after chromatography on silica gel (CH_2Cl_2/MeOH 95:5).

Synthesis of acid **14**: squamocin **1** (10 mg, 16 μmol) was dispersed under vigorous stirring in boiling 1 N aqueous NaOH (1 mL) for 15 h. After cooling of the reaction medium and elimination of the aqueous supernatant, the organic residue was partitioned between THF and brine, and the aqueous phase re-extracted with THF. The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was triturated in ice-cold EtOAc then filtered, leading to the sodium salt of acid **14** as a white powder. The free acid form was obtained by acidification of an aqueous solution of sodium salt (1 N AcOH) and extraction by EtOAc followed by drying (Na_2SO_4) and evaporation under reduced pressure.

Method C: squamocin **1** (10 mg, 16 μmol) and the primary alcohol (1–3 equiv) were stirred at 100–110 °C with DBU (1 equiv) until completion of the reaction followed by TLC (CH_2Cl_2/MeOH 9:1). The mixture was then diluted with CH_2Cl_2 and washed with H_2O (3 times), dried (Na_2SO_4) and concentrated under reduced pressure after chromatography on silica gel (CH_2Cl_2/MeOH 95:5).

5.2.2. Data for compounds.

5.2.2.1. α-Acetonylpiperidinamide (2). Colourless resin; $C_{42}H_{77}NO_7$; R_f=0.3 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J=6.5 Hz), 1.36 (m, 5H), 1.39 (m, 2H), 1.48 (m, 5H), 1.58 (m, 4H), 1.63 (m, 2H), 1.95 (m, 4H), 2.09 (s, 3H), 2.41 (dd, 1H, J=3.9 Hz, J=17.6 Hz), 2.98 (dd, 1H, J=9.5 Hz, J=17.6 Hz), 3.13 (m, 1H), 3.34 (m, 1H), 3.48 (dd, 2H, J=4.9 Hz, J=10.3 Hz), 3.50 (dd, 2H, J=4.9 Hz, J=10.3 Hz), 3.55 (m, 1H), 3.78 (m, 2H), 3.79 (m, 1H), 3.87 (m, 2H); ^{13}C NMR (CD_3OD, 50 MHz): δ 14.4, 23.1, 23.7, 25.6, 26.9, 27.5, 28.0, 29.2, 29.5, 29.7, 30.7, 33.0, 33.6, 34.2, 34.6, 38.4, 44.3, 72.3, 74.0, 74.9, 83.3, 83.7, 84.2, 175.9, 210.2; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 1715, 1620. ESIMS *m*/*z* 708 [M+H]$^+$, 730 [M+Na]$^+$, 746 [M+K]$^+$, 1438 [2M+Na]$^+$; HRMS (ES) *m*/*z* [M+Na]$^+$ calcd for $C_{42}H_{77}NO_7Na$ 730.5598, found 730.5602.

5.2.2.2. α-Acetonylmorpholinamide (3). Colourless resin; $C_{41}H_{75}NO_8$; R_f=0.35 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J=6.9 Hz), 1.39 (m, 5H), 1.42 (m, 2H), 1.51 (m, 1H), 1.63 (m, 4H), 1.97 (m, 4H), 2.13 (s, 3H), 2.46 (dd, 1H, J=14.8 Hz, J=5 Hz), 3.09 (dd, 1H, J=14.8 Hz, J=9.8 Hz), 3.11 (m, 1H), 3.39

[‖] Typical scale for the described reactions was 10 mg of **1** with similar results when tested on 100 mg as in a few cases (**3**, **14**, **19**).

(m, 1H), 3.56 (m, 1H, H-38), 3.57 (m, 2H), 3.67 (m, 2H), 3.68 (m, 1H), 3.71 (m, 1H), 3.79 (m, 2H), 3.84 (m, 2H), 3.86 (m, 1H), 3.91 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.1, 22.0, 22.6, 24.9, 25.6, 27.1, 28.4, 28.9, 29.6, 30.0, 31.8, 32.6, 33.2, 35.6, 37.2, 37.5, 42.3, 46.5, 46.4, 66.8, 66.9, 71.5, 71.7, 74.0, 82.1, 82.5, 82.8, 83.2, 174.1, 207.8; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 1715, 1635; ESIMS *m/z* 732 [M+Na]$^+$, 748 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{41}H_{75}NO_8Na$ 732.5390, found 732.5393.

5.2.2.3. α-Acetonyl-*N*-methylpiperazinamide (4). Colourless resin; $C_{42}H_{78}N_2O_7$; R_f=0.2 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, *J*=7.0 Hz), 1.38 (m, 5H), 1.41 (m, 2H), 1.48 (m, 1H), 1.60 (m, 4H), 1.96 (m, 4H), 2.12 (s, 3H), 2.31 (s, 3H), 2.38 (m, 2H), 2.44 (dd, 1H, *J*=17.5 Hz, *J*=3.2 Hz), 2.52 (m, 2H), 3.06 (dd, 1H, *J*=9.7 Hz, *J*=17.5 Hz), 3.13 (m, 1H), 3.37 (m, 1H), 3.57 (m, 3H), 3.72 (m, 2H), 3.83 (m, 3H), 3.91 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.6, 30.0, 35.6, 37.4, 45.5, 45.8, 46.0, 54.7, 55.0, 71.3, 71.6, 74.0, 82.1, 82.4, 82.7, 83.1, 173.7, 207.8; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 1715, 1638; ESIMS *m/z* 723 [M+H]$^+$, 745 [M+Na]$^+$, 761 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{42}H_{78}N_2O_7Na$ 745.5707, found 745.5701.

5.2.2.4. α-Acetonyl-*N*-methyltryptamide (5). Pale-yellow resin; $C_{48}H_{80}N_2O_7$; R_f=0.3 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (DMSO-d_6, 400 MHz, 390 K): δ 0.87 (t, 3H, *J*=6.7 Hz), 1.2–1.3 (m, ΣCH_2), 1.32 (m, 4H), 1.34 (m, 3H), 1.39 (m, 1H), 1.63 (m, 4H), 1.85 (m, 4H), 2.03 (s, 3H), 2.43 (dd, 1H, *J*=5.0 Hz, *J*=17.2 Hz), 2.75 (m, 1H), 2.89 (m, 2H), 2.95 (br s, 3H), 3.06 (m, 1H), 3.30 (m, 1H), 3.40 (m, 1H), 3.58 (m, 2H), 3.64 (m, 1H), 3.71 (m, 2H), 3.76 (m, 1H), 3.83 (m, 1H), 6.97 (dd, 1H, *J*=7.8 Hz, *J*=7.1 Hz), 7.05 (dd, 1H, *J*=7.1 Hz, *J*=8 Hz), 7.09 (s, 1H), 7.33 (d, 1H, *J*=8.0 Hz), 7.55 (d, 1H, *J*=7.8 Hz), 10.33 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz, equilibrium of rotamers): δ 14.1, 22.6, 23.2, 30.1, 34.0, 36.2, 36.3, 36.5, 37.3, 37.5, 45.8, 46.2, 49.2, 50.9, 71.5, 71.7, 74.1, 82.2, 83.3, 111.1, 111.3, 112.4, 113.1, 118.4, 118.8, 119.2, 119.4, 121.8, 122.0, 122.2, 122.3, 127.3, 127.5, 136.3, 136.4, 175.2, 175.4, 208.0, 208.1; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 3408, 2924, 2854, 1715, 1621; ESIMS *m/z* 797 [M+H]$^+$, 819 [M+Na]$^+$, 835 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{48}H_{80}N_2O_7Na$ 819.5863, found 819.5860.

5.2.2.5. α-Acetonyl-*N*-cyanophenylpiperazinamide (6). Pale-yellow resin; $C_{48}H_{79}N_3O_7$; R_f=0.4 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, *J*=6.7 Hz), 1.15–1.45 (m, ΣCH_2), 1.45–1.70 (m, 8H), 1.75–2.0 (m, 6H), 2.13 (s, 3H), 2.47 (dd, 1H, *J*=17.4 Hz, *J*=2.4 Hz), 3.08 (dd, 1H, *J*=17.4 Hz, *J*=9 Hz), 3.18 (m, 2H), 3.29 (m, 2H), 3.37 (m, 1H), 3.56 (m, 2H), 3.70 (m, 1H), 3.82 (m, 2H), 3.90 (m, 2H), 3.97 (m, 1H), 4.09 (br d, 1H, *J*=13 Hz), 6.98–7.07 (m, 2H), 7.49 (t, *J*=7.8 Hz, 1H), 7.57 (d, *J*=7.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.0, 22.0, 22.6, 24.8, 25.6, 27.1, 28.3, 28.9, 29.3, 29.4, 29.6, 29.7, 30.0, 31.8, 32.5, 32.6, 33.2, 35.8, 37.2, 37.4, 41.9, 46.1, 46.2, 51.2, 52.1, 71.4, 71.7, 74.1, 81.7, 82.1, 82.5, 82.8, 83.3, 106.5, 118.1, 119.0, 122.4, 133.8, 134.3, 155.2, 174.0, 207.9; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 3420, 2246, 1717, 1632, 1600; ESIMS *m/z* 810 [M+H]$^+$, 832 [M+Na]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{48}H_{79}N_3O_7Na$ 832.5816, found 832.5814.

5.2.2.6. α-Acetonyl-*N*-phenylpiperazinamide (7). Pale-yellow resin; $C_{47}H_{80}N_2O_7$; R_f=0.25 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, *J*=6.7 Hz), 1.2–1.3 (m, ΣCH_2), 1.38 (m, 2H), 1.40 (m, 1H), 1.42 (m, 4H), 1.50 (m, 1H), 1.62 (m, 4H), 1.94 (m, 4H), 2.14 (s, 3H), 2.48 (dd, 1H, *J*=17.7 Hz, *J*=2.5 Hz), 3.10 (dd, 1H, *J*=9.7 Hz, *J*=17.7 Hz), 3.16 (m, 2H), 3.19 (m, 2H), 3.20 (m, 1H), 3.39 (m, 1H), 3.59 (m, 1H), 3.71 (m, 2H), 3.82 (m, 1H), 3.84 (m, 2H), 3.87 (m, 2H), 3.92 (m, 2H), 6.89 (t, 1H, *J*=7.2 Hz), 6.93 (dd, 2H, *J*=8.0 Hz), 7.28 (dd, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.8, 30.0, 35.7, 37.2, 41.7, 45.7, 46.1, 49.2, 49.4, 71.4, 71.6, 74.0, 82.1–83.2, 116.4, 120.2, 129.1, 173.8, 207.8; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 3421, 2925, 2854, 1715, 1633, 1600; ESIMS *m/z* 785 [M+H]$^+$, 807 [M+Na]$^+$, 823 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{47}H_{80}N_2O_7Na$ 807.5863, found 807.5859.

5.2.2.7. α-Acetonyl-*N*-(dimethoxybenzyl)piperazinamide (8). Pale-yellow resin; $C_{50}H_{86}N_2O_9$; R_f=0.25 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, *J*=6.8 Hz), 1.2–1.3 (m, ΣCH_2), 1.38 (m, 7H), 1.50 (m, 1H), 1.60 (m, 4H), 1.95 (m, 4H), 2.12 (s, 3H), 2.41 (m, 2H), 2.43 (dd, 1H, *J*=2.6 Hz, *J*=17.5 Hz), 2.52 (m, 2H), 3.06 (dd, 1H, *J*=9.6 Hz, *J*=17.5 Hz), 3.13 (m, 1H), 3.38 (m, 1H), 3.45 (s, 2H), 3.51 (m, 2H), 3.55 (m, 1H), 3.69 (m, 2H), 3.83 (m, 2H), 3.86 (m, 1H), 3.86 (s, 3H), 3.87 (s, 3H), 3.90 (m, 2H), 6.78–6.83 (m, 2H), 6.89 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 22.0, 22.6, 24.8, 25.6, 27.1, 28.3, 28.9, 29.3, 29.4, 29.6, 29.7, 30.1, 31.8, 32.5, 32.6, 33.2, 33.4, 35.7, 37.2, 37.4, 41.8, 45.8, 46.1, 52.9, 53.1, 55.9, 62.5, 71.4, 71.7, 74.1, 82.2, 82.5, 82.8, 83.1, 83.3, 110.8, 112.1, 121.3, 130.3, 148.2, 148.9, 173.7, 208.0; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 2925, 2854, 1715, 1627, 1515, 1463; ESIMS *m/z* 859 [M+H]$^+$, 881 [M+Na]$^+$, 897 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{50}H_{86}N_2O_9Na$ 881.6231, found 881.6225.

5.2.2.8. α-Acetonyl-*N*-(Boc)piperazinamide (9). Colourless resin; $C_{46}H_{84}N_2O_9$; R_f=0.3 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.84 (t, 3H, *J*=6.9 Hz), 1.10–1.5 (m, ΣCH_2), 1.44 (s, 9H), 1.58 (m, 4H), 1.93 (m, 4H), 2.10 (s, 3H), 2.44 (d, 1H, *J*=15.0 Hz), 3.05 (m, 1H), 3.09 (m, 1H), 3.42 (m, 1H), 3.50 (m, 4H), 3.56 (m, 1H), 3.68 (m, 4H, H-39), 3.81 (m, 3H), 3.89 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.9, 21.9, 22.5, 24.8, 25.5, 27.0, 28.3, 28.8, 29.2, 29.4, 29.9, 31.7, 32.5, 33.1, 35.7, 37.1, 41.6, 43.6, 45.6, 46.1, 71.3, 74.0 (C-15), 80.0, 82.1, 82.4, 82.7, 83.2, 154.5, 174.0, 207.7; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 2925, 2855, 1696, 1636, 1458, 1417; ESIMS *m/z* 831 [M+Na]$^+$, 848 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{46}H_{84}N_2O_9Na$ 831.6075, found 831.6074.

5.2.2.9. α-Acetonyl-piperazinamide (10). Compound **9** (20 mg, 29 μmol) was diluted with CH_2Cl_2 (1 mL). Trifluoroacetic acid (0.5 mL) was added dropwise to the solution at 0 °C. After 1 h of stirring, an aqueous saturated solution of $NaHCO_3$ (5 mL) was introduced and the resulting mixture stirred at rt for 2 h. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to yield

10 (21 mg, 98%). Colourless resin; $C_{41}H_{76}N_2O_9$; R_f=0.3 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, J=6.5 Hz), 1.15–1.4 (m, ΣCH_2), 1.4–1.6 (m, 4H), 1.6–1.7 (m, 2H), 1.75–1.9 (m, 2H), 1.96 (br s, 4H), 2.11 (s, 3H), 2.51 (dd, 1H, J=23.6 Hz, J=8.4 Hz), 3.06 (m, 2H), 3.38 (m, 4H), 3.57 (m, 1H), 3.8 (m, 4H), 3.9–3.15 (m, 5H), 4.32 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.9, 21.5, 22.0, 22.5, 25.1, 25.5, 26.8, 28.6, 29.2, 29.6, 31.7, 32.4, 33.2, 33.5, 35.4, 37.0, 37.3, 43.3, 46.5, 71.5, 71.8, 74.3, 82.0, 82.6, 83.2, 174.4, 208.3; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 1714, 1620; ESIMS m/z 709 [M+H]$^+$, 731 [M+Na]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{41}H_{76}N_2O_7Na$ 731.5550, found 731.5555.

5.2.2.10. α-Acetonyl-*N*,*N*′-diethylaminoethylester (11). Yellow resin; $C_{43}H_{81}NO_8$; R_f=0.35 (AcOEt/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, J=6.8 Hz), 1.20 (t, 6H, J=7.2 Hz), 1.20–1.35 (m, ΣCH_2), 1.39 (m, 2H), 1.41 (m, 4H), 1.47 (m, 1H), 1.56 (m, 1H), 1.61 (m, 4H), 1.95 (m, 4H), 2.14 (s, 3H), 2.53 (dd, 1H, J=3.7 Hz, J=17.7 Hz), 2.78 (m, 1H), 2.89 (dd, 1H, J=17.7 Hz, J=7 Hz), 2.93 (q, 4H, J=7 Hz), 3.05 (m, 2H), 3.38 (m, 1H), 3.59 (m, 1H), 3.83 (m, 3H), 3.92 (m, 2H), 4.27 (m, 1H), 4.42 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 9.4, 13.9, 21.5, 21.9, 22.4, 24.8, 25.5, 26.9, 28.3, 28.8, 29.2, 29.4, 31.7, 32.3, 33.0, 37.1, 37.3, 39.9, 44.9, 46.5, 49.5, 59.9, 71.4, 71.6, 74.0, 82.0, 82.4, 82.7, 83.1, 175.2, 206.9; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 2923, 2854, 1718, 1459, 1403, 1370, 1266, 1160, 1058; ESIMS m/z 740 [M+H]$^+$, 762 [M+Na]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{43}H_{81}NO_8Na$ 762.5860, found 762.5863.

5.2.2.11. α-Acetonyl-*n*-butylester (12). Amorphous white solid; $C_{41}H_{76}O_8$; R_f=0.4 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J=6.8 Hz), 0.93 (t, 3H, J=7.4 Hz), 1.20–1.30 (m, ΣCH_2), 1.37 (m, 6H), 1.40 (m, 2H), 1.49 (m, 1H), 1.53 (m, 1H), 1.58 (m, 6H), 1.96 (m, 4H), 2.15 (s, 3H), 2.47 (dd, 1H, J=4.5 Hz, J=19.6 Hz), 2.84 (m, 1H), 2.89 (dd, 1H, J=19.6 Hz, J=9.6 Hz), 3.39 (m, 1H), 3.59 (m, 1H), 3.85 (m, 4H), 3.92 (m, 1H), 4.07 (m, 2H, J=6.6 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.4, 14.0, 19.1, 22.0, 22.6, 24.8, 25.6, 27.0, 28.4, 28.9, 29.4, 29.6, 30.0, 30.6, 31.0, 32.5, 33.3, 37.3, 37.5, 40.2, 45.0, 64.3, 71.4, 71.7, 74.1, 82.2, 82.5, 82.8, 83.3, 176.2, 206.8; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 2924, 2854, 1720, 1635, 1459, 1404, 1364; ESIMS m/z 720 [M+Na]$^+$, 736 [M+K]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{41}H_{76}O_8Na$ 719.5483, found 719.5479.

5.2.2.12. α-Acetonyl-phenoxyethylester (13). Colourless resin; $C_{45}H_{76}O_9$; R_f=0.55 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.81 (t, 3H), 1.10–1.30 (m, ΣCH_2), 1.31 (m, 2H), 1.33 (m, 4H), 1.43 (m, 1H), 1.55 (m, 1H), 1.58 (m, 4H), 1.90 (m, 4H), 2.07 (s, 3H), 2.43 (dd, 1H, J=21.5 Hz, J=8.4 Hz), 2.82 (m, 2H), 3.33 (m, 1H), 3.52 (m, 1H), 3.78 (m, 2H), 3.80 (m, 1H), 3.85 (m, 2H), 4.09 (m, 2H), 4.36 (m, 2H), 6.83 (dd, 2H, J=8.7 Hz, J=1 Hz), 6.89 (br t, 1H, J=7.3 Hz), 7.21 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.9, 21.9, 22.5, 24.7, 25.5, 26.8, 28.3, 28.9, 29.3, 29.5, 29.8, 31.8, 32.4, 33.1, 37.2, 37.4, 40.0, 44.9, 62.6, 65.7, 71.3, 71.6, 74.0, 76.3, 81.7, 82.1, 82.4, 87.7, 83.2, 114.5, 121.0, 129.3, 158.4, 175.2, 206.6; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 2927, 2855, 1736, 1653, 1541, 1492, 1254; ESIMS m/z 783 [M+Na]$^+$, 799 [M+K]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{45}H_{76}O_9Na$ 783.5387, found 783.5390.

5.2.2.13. α-Acetonylacid 14. Colourless resin; $C_{37}H_{68}O_8$; R_f=0.25 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, J=7.0 Hz), 1.20–1.35 (m, ΣCH_2), 1.39 (m, 2H), 1.42 (m, 4H), 1.49 (m, 1H), 1.61 (m, 5H), 1.96 (m, 4H), 2.15 (s, 3H), 2.47 (dd, 1H, J=7.6 Hz, J=20.4 Hz), 2.86 (m, 3H), 3.40 (m, 1H), 3.59 (m, 1H), 3.84 (m, 3H), 3.93 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.9, 21.9, 22.5, 24.7, 25.5, 26.8, 28.3, 28.8, 29.3, 29.5, 31.7, 32.3, 33.0, 37.1, 37.3, 39.8, 71.4, 71.7, 74.0, 76.3, 82.0, 82.4, 82.7, 83.1, 178.6, 207.7; IR (film, CH_2Cl_2, sodium carboxylate form) ν_{max} cm^{-1}: 2925, 2854, 1714, 1570, 1459, 1406, 1365, 1317, 1066; ESIMS m/z 663 [M+Na]$^+$, 679 [M+K]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{37}H_{68}O_8Na$ 663.4812, found 663.4814.

5.2.2.14. Butylester 17. Compound **15** (100 mg, 0.109 mmol) was dissolved in *n*-BuOH (2 mL) with Amberlyst® 15 (500 mg). The reaction was stirred and heated at 65 °C for 15 h. After filtration, the solvent was evaporated under reduced pressure and the residue purified by flash chromatography (AcOEt) to afford **17** (65 mg, 90%). Colorless oil; $C_{37}H_{70}O_7$; R_f=0.25 (AcOEt); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.91 (t, 3H, J=6.8 Hz), 0.95 (t, 3H, J=8 Hz), 1.65 (m, 4H), 1.96 (m, 2H), 1.99 (m, 2H), 2.30 (t, 2H, J=8 Hz), 3.43 (m, 1H), 3.63 (m, 1H), 3.89 (m, 5H), 4.08 (t, 2H, J=7 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.5, 13.6, 17.1, 19.0, 21.9, 22.5, 24.7, 24.9, 25.5, 28.3, 28.8, 29.0, 29.1, 29.3, 29.5, 30.6, 31.7, 32.4, 33.2, 34.3, 37.2, 37.4, 63.9, 71.3, 71.6, 74.0, 82.1, 82.4, 82.7, 83.2, 174.0; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 1736; ESIMS m/z 649 [M+Na]$^+$, 665 [M+K]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{37}H_{70}O_7Na$ 649.5019, found 649.5036.

5.2.2.15. Bis-[α-acetonyl-1,3-(4-piperidinamide)propane] (18). Colourless resin; $C_{87}H_{158}N_2O_{14}$; R_f=0.3 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 6H, J=7.0 Hz), 1.0–2.0 (m, ΣCH_2), 1.94 (m, 8H), 2.10 (s, 3H), 2.12 (s, 3H), 2.41 (dd, 2H, J=16.0 Hz, J=2.2 Hz), 2.53 (m, 2H), 3.03 (m, 14H), 3.15 (m, 2H), 3.39 (m, 2H), 3.57 (m, 2H), 3.75 (m, 4H), 3.84–3.94 (m, 10H), 4.02 (m, 2H), 4.55 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.0, 25.0, 25.3, 25.6, 27.0, 28.4, 28.8, 29.3, 29.4, 29.6, 29.7, 30.1, 31.8, 32.1, 32.6, 33.1, 33.4, 36.0, 36.5, 37.3, 37.5, 42.2, 42.5, 46.0, 46.1, 46.4, 71.6, 71.7, 74.1, 82.1, 82.4, 82.8, 83.3, 173.4, 173.5, 207.9; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 3428, 2922, 2853, 1716, 1619, 1458, 1366, 1266, 1165, 1058. ESIMS m/z 1478 [M+Na]$^+$.

5.2.2.16. Triazole 19. Colourless oil; $C_{41}H_{79}N_3O_7S$; R_f=0.4 (silica gel, CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.9 (t, 3H, J=7 Hz), 1.15–2.05, 2.12 (s, 3H), 2.48 (dd, 1H, J=17 Hz, J=3.5 Hz), 2.8 (m, 1H), 2.87 (dd, 1H, J=17 Hz, J=9.7 Hz), 3.04 (t, 2H, J=6.5 Hz), 3.4 (m, 1H), 3.6 (m, 1H), 3.8–3.95 (m, 5H), 4.2–4.3 (m, 1H), 4.33–4.42 (m, 1H), 5.6 (s, 2H), 7.25–7.35 (m, 5H), 7.53 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.0, 22.0, 22.6, 25.0, 25.6, 27.0, 28.4, 28.9, 29.3, 29.4, 29.6, 29.7, 29.9, 31.8, 31.9, 32.6, 33.4, 37.3, 37.6, 40.2, 45.1, 53.7, 63.1, 71.6, 71.8, 74.1, 82.1, 82.4, 82.8, 83.2, 121.3, 128.6,

129.4, 132.1, 144.8, 175.2, 206.6; IR (film, $CHCl_3$) ν_{max} cm^{-1}: 3451, 2926, 2854, 2360, 2341, 1718, 1650, 1460, 1365, 1226; ESIMS *m/z* 864 $[M+Na]^+$; HRMS (ES) *m/z* $[M+Na]^+$ calcd for $C_{48}H_{79}N_3O_7SNa$ 864.5536, found 864.5531.

5.3. Diels–Alder adducts

5.3.1. Furane 22. To a vigorously stirred solution of **1** (116 mg, 0.186 mmol) in CH_2Cl_2 (2.5 mL) cooled to −78 °C a 1 M DIBAL–H solution in toluene (2.2 mL, 2.2 mmol) was added. The mixture was stirred for 4 h and then quenched by glacial AcOH (3.5 mL). The reaction mixture was allowed to warm up to rt, and stirring was continued for 1.5 h. The resulting gel was partitioned between EtOAc (50 mL) and water (35 mL). The organic phase was washed with water (2×30 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified over a short column of silica gel (toluene/EtOAc/EtOH 30:70:1 to 30:70:4), furnishing furane **22** (99 mg, 87%). White amorphous solid; $C_{37}H_{66}O_6$; R_f=0.45 (silica gel, CH_2Cl_2/MeOH 9:1); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.91 (t, 3H, *J*=6 Hz), 1.2–1.35 (m, ΣCH_2), 1.63 (m, 4H), 1.97 (m, 4H), 2.27 (s, 3H), 2.36 (t, 2H, *J*=7.6 Hz), 3.43 (m, 1H), 3.63 (m, 1H), 3.89 (m, 5H), 5.86 (s, 1H), 7.04 (s, 1H); IR (film, CH_2Cl_3) ν_{max} cm^{-1}: 2926, 2855, 1764, 1458, 1065; ESIMS (16,25,29-tri-(*O*-TBDMS) derivative) *m/z* 971 $[M+Na]^+$, 966 $[M+NH_4]^+$; HRMS (ES) *m/z* $[M+Na]^+$ calcd for $C_{37}H_{66}O_6Na$ 729.4757, found 729.4756. $[\alpha]_D^{20}$ +2 (*c* 0.8, CH_2Cl_2).

5.3.2. Diels–Alder adducts 23 and 24. Furane **22** (15 mg, 25 μmol) and *N*-phenylmaleimide (4.3 mg, 25 μmol, 1 equiv) or methyl acetylenedicarboxylate (3.5 mg, 25 μmol, 1 equiv) were mixed in water (0.2 mL). The mixture was boiled for 6 h under vigorous stirring. Extraction with CH_2Cl_2 (2×10 mL), drying (Na_2SO_4) and concentration under reduced pressure yielded adduct **23** (17 mg, 92%) or **24** (16 mg, 90%). **23**: Colourless oil; $C_{47}H_{73}NO_8$; R_f=0.4 (CH_2Cl_2/MeOH 9:1); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, *J*=7 Hz), 1.2–1.55 (m, ΣCH_2), 1.62 (m, 4H), 1.56–1.70 (m, 5H), 1.74 (s, 3H), 1.96 (m, 4H), 2.20 (m, 2H), 2.90 (d, 1H, *J*=6.5 Hz), 3.09 (d, 1H, *J*=6.5 Hz), 3.40 (m, 1H), 3.60 (m, 1H), 3.8–3.95 (m, 5H), 5.06 (s, 1H), 5.88 (s, 1H), 7.28–7.48 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.9, 15.9, 21.9, 22.5, 24.8, 25.5, 27.1, 27.3, 28.3, 28.8, 29.2, 29.5, 31.7, 32.4, 33.2, 37.2, 37.4, 50.3, 51.1, 71.4, 71.7, 74.0, 82.1, 82.4, 82.7, 83.2, 83.4, 89.3, 126.5, 128.5, 129.0, 131.8, 132.5, 152.7, 174.1, 175.5; IR (film, $CHCl_3$) ν_{max} cm^{-1}: 3439, 2924, 2853, 1711, 1500, 1458, 1382, 1189, 1058, 947 cm^{-1}; ESIMS *m/z* 802 $[M+Na]^+$; HRMS (MALDI) calcd for $C_{47}H_{73}NO_8Na$ $[M+Na]^+$ 802.5234, found 802.5240. **24**: Colourless oil; $C_{43}H_{72}O_{10}$; R_f=0.4 (CH_2Cl_2/MeOH 9:1); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, *J*=6.9 Hz), 1.2–1.3 (m, ΣCH_2), 1.35–1.55 (m, 17H), 1.55–1. 7 (m, 5H), 1.73 (s, 3H), 1.97 (m, 4H), 2.24 (m, 2H), 3.39 (m, 1H), 3.59 (m, 1H), 3.78 (s, 3H), 3.84 (s, 3H), 3.75–4.00 (m, 5H), 5.34 (s, 1H), 6.36 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.4, 15.8, 22.4, 23.0, 25.4, 26.0, 27.4, 28.8, 29.2, 29.4, 29.6, 29.7, 29.9, 30.0, 30.1, 32.2, 33.0, 33.8, 37.7, 37.9, 52.4, 52.5, 72.0, 72.2, 74.5, 82.5, 82.8, 83.2, 83.6, 86.3, 94.8, 136.3, 150.8, 157.9, 162.0; ESIMS *m/z* 771 $[M+Na]^+$; HRMS (ES) *m/z* $[M+Na]^+$ calcd for $C_{43}H_{72}O_{10}Na$ 771.5023, found 771.5029.

5.4. Biological activities

5.4.1. Cytotoxicity. Cytotoxic activities were colorimetrically evaluated on KB 3-1 cells according to previously described procedures.[6d]

5.4.2. Complex I inhibition. The inhibitory activities against bovine mitochondrial complex I from submitochondrial particles (SMP) (i.e., NADH oxidase and NADH/*n*-decylubiquinone oxidoreductase activities) of the described compounds were measured as described elsewhere.[14]

5.4.3. Complex III inhibition. Purification and reconstitution of complex III from bovine heart mitochondria, as well as measurement of *n*-decylubiquinol/cytochrome *c* oxidoreductase activity was performed as described earlier.[15]

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