Review

Medicinal Chemistry of Annonaceous Acetogenins: Design, Synthesis, and Biological Evaluation of Novel Analogue

Naoto Kojima * and Tetsuaki Tanaka *

Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka, Japan

* Authors to whom correspondence should be addressed; E-Mails: kojima@phs.osaka-u.ac.jp (N.K.); t-tanaka@phs.osaka-u.ac.jp (T.T.); Tel.: +81-6-6879-8210; Fax: +81-6-6879-8214.

Received: 4 August 2009; in revised form: 31 August 2009 / Accepted: 11 September 2009 / Published: 17 September 2009

Abstract: Most Annonaceous acetogenins are characterized by between one and three THF ring(s) with one or two flanking hydroxyl group(s) in the center of a C32/34 fatty acid, and the terminal carboxylic acid is combined with a 2-propanol unit to form an α,β-unsaturated γ-lactone. While many studies have addressed the properties and synthesis of natural acetogenins due to their attractive biological activities and unique structural features, a number of analogues have also been described. This review covers the design, synthesis, and biological evaluation of acetogenin analogues.

Keywords: Annonaceous acetogenins; antitumor activity; analogues; structure–activity relationship; polyketides

1. Introduction

A new class of polyketides, the Annonaceous acetogenins, has been isolated from Annonaceous plants growing in tropical and subtropical regions. Since the isolation of the first acetogenin, uvaricin, more than 400 members of the family have been found and characterized (Figure 1) [1–12].

Most acetogenins are white waxy derivatives of long-chain fatty acids (C32 or C34), and the terminal carboxylic acid is combined with a 2-propanol unit at the C-2 position to form a methyl-substituted α,β-unsaturated-γ-lactone. One of their interesting structural features is a single, adjacent, or nonadjacent tetrahydrofuran (THF) or tetrahydropyran (THP) system with one or two flanking hydroxyl group(s) at the center of a long hydrocarbon chain. Biogenetically, it has been suggested that
the THF or THP cores are generated by polyepoxidation of an unconjugated polyene followed by domino cyclizations.

**Figure 1.** Representative structure of the *Annonaceous* acetogenins.

![Representative structure of the Annonaceous acetogenins.](image)

In addition to their unique chemical structures, much attention has also been paid to acetogenins’ broad range of bioactivity; e.g., their immunosuppressive, antimalarial, insecticidal, antifeedant, and probably most important, antitumor activities. Some acetogenins show growth inhibitory activity against multidrug resistant (MDR) cancer cells [13]. It is generally accepted that the mode of action of acetogenins is the inhibition of NADH–ubiquinone oxidoreductase (complex I) in mitochondria [14]. Inhibition suppresses ATP production, especially for cancer cells with high metabolic levels, leading to apoptosis.

Several strategies for the total synthesis of natural acetogenins and their analogues have been reported, motivated by their unique structural features and attractive biological activities [15–21]. This review focuses on those analogues whose biological activities have been reported previously.

**2. Modification of the Tetrahydrofuran Moiety**

Structural simplification of the tetrahydrofuran moiety, especially the bis-tetrahydrofuran group, is worthwhile because of the limited availability of these complex structures. Grée’s group reported the synthesis and biological activity of a series of acetogenin analogues 1 consisting of ethylene glycol and catechol ethers in place of the bis-tetrahydrofuran core of bullatacinone, which has a ketolactone moiety at the end [22–24]. Their analogues were designed to incorporate various lipophilic side chains in place of an n-alkyl chain. A representative synthetic pathway is given by the preparation of 10a (Scheme 1). The synthesis of the β-hydroxyl ether core was achieved by condensation of the mesylate of solketal 2, ethylene glycol, and epichlorohydrin. After opening of the epoxide with triethylsilylacetylide, the lipophilic side chain was introduced at the opposite end to give the alkyne 6. Sonogashira coupling of 6 with the γ-lactone fragment 7, followed by hydrogenation, afforded the target analogue 10a. Twenty-three analogues were tested for cytotoxicity against L1210 leukemia cells (Table 1). Analogues containing catechol were more effective than the series of ethylene glycol derivatives. No significant differences were observed between the various lipophilic side chain substitutions. The simplified acetogenins showed less cytotoxic activity than the natural acetogenins annonacin, bullatacin, and bullatacinone. However, catechol derivatives showed an interesting effect on cell cycle. Natural acetogenins were equally cytotoxic to each phase of the cell cycle, but analogues 14a, 14c–g, and 15a modified the cell cycle at either phase G1 or G2/M. The target pathway of these analogues may be different from the target of natural acetogenins.
**Scheme 1.** Synthesis of ethylene glycol and catechol analogues by Grée’s group.

**Table 1.** IC$_{50}$ (μM) of synthetic analogues against L1210 cell lines.

| R$^1$          | IC$_{50}$ | R$^1$          | IC$_{50}$ |
|---------------|-----------|---------------|-----------|
| n-C$_{10}$H$_{21}$ (8a) | 3.5       | n-C$_{10}$H$_{21}$ (9a) | 1         |
| Ph (8b)       | 19.8      | Ph (9b)       | 21.6      |
| p-MeOC$_6$H$_4$ (8c) | > 10      | p-MeOC$_6$H$_4$ (9c) | 32        |
| p-CF$_3$C$_6$H$_4$ (8d) | 3.3       | –             | –         |
| 2-Nph (13e)   | 2.1       | 2-Nph (9e)    | 3         |
| Bu$_2$N (8f)  | 3.9       | Bu$_2$N (9f)  | 2.5       |
| Oct$_2$N (8g) | 3.7       | –             | –         |
| N-piperidinyl (8h) | 28.7      | N-piperidinyl (13h) | 23.3      |
| 3-O-cholesteryl (8i) | 2.8       | 3-O-cholesteryl (13i) | 12.2      |
| n-C$_{10}$H$_{21}$ (14a) | 1.0       | n-C$_{10}$H$_{21}$ (15a) | 7.6       |
| Ph (14b)      | 2.0       | –             | –         |
| p-CF$_3$C$_6$H$_4$ (14d) | 2.2       | doxorubicin    | 0.025     |
| 2-Nph (14e)   | 0.7       | annonacin     | 0.042     |
| Bu$_2$N (14f) | 1.3       | bullatacin    | 0.0004    |
| Oct$_2$N (14g) | 2.5       | bullatacinone | 0.016     |

In 2000, Wu’s group reported polyether mimics based on the ionophoric ability [25–27] of the THF moiety in acetogenins [28]. (10RS)-Corossolin was simplified to the diethylene glycol analogue 16, and the corresponding bis-THF acetogenin, bullatin, was simplified to triethylene glycol 17. A representative synthetic pathway is given by the preparation of 16 (Scheme 2). Bis-propargylation of diethyleneglycol 18 with propargyl bromide, followed by monoalkylation with n-octyl bromide,
afforded the polyether 19. The reaction of epoxide 20 with the acetylide of 19 yielded the alcohol 21. Hydrogenation of triple bonds, followed by elimination of the MOM-oxy group, afforded analogue 16. Preliminary screening showed that analogues 16 and 17 had moderate activity against HL-60 and K562 (Table 2).

**Scheme 2.** Synthesis of polyether analogues by Wu’s group.

![Scheme 2](image)

**Table 2.** *In vitro* testing against the HL-60 and K562 cell lines.

| Conc. (µM) | IG% for HL-60 | IG% for K562 |
|-----------|---------------|-------------|
|           | 100 | 10 | 1 | 100 | 10 | 1 |
| 16        | 100 | 50 | 0 | 31  | 18 | 0 |
| 17        | 100 | 65 | 21| 55  | 25 | 22|
| corossolone | 68 | 29 | 0 | 53  | 16 | 2 |
| (10RS)-corossolin | 63 | 56 | 5 | 10  | 2  | 0 |
| solamin   | 24  | 8  | 0 | 59  | 39 | 29|
| bullatacin| 73  | 7  | 0 | 53  | 39 | 27|

Wu’s group reported the synthesis and biological activity of analogues similar to Gree’s, but Wu’s analogues had an α,β-unsaturated-γ-lactone at the end instead of the ketolactone of Gree’s derivatives [29–30]. They completed synthesis of the polyether analogues by a convergent strategy. A representative synthetic pathway is given by the preparation of 22c (Scheme 3). The synthesis of fragment 29 began with the bromo ester 26 prepared from *cis*-erucic acid 25. The Wittig olefination of the phosphonium salt prepared from 26 with (R)-glyceraldehyde acetonide 27 was followed by hydrogenation and removal of the acetonide protective group, yielding fragment 29. The preparation of the other fragment 32 began with the chain extension of (R)-glyceraldehyde acetonide 27. The resulting diol 30 was condensed with 2-benzyloxyethyl iodide via a cyclic stannate intermediate. After protection of the secondary alcohol, followed by deprotection of benzyl ether, the resulting primary hydroxyl group was converted to iodide to give 32. The coupling reaction of fragments 29 and 32 was
achieved by selective etherification with dibutyltin oxide and cesium fluoride. The \(\gamma\)-lactone moiety was introduced by way of the aldol strategy with \(O\)-THP-\((S)\)-lactaldehyde. Deprotection of the MOM ether of 34 gave the polyether analogue 22c. The synthesized samples were evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to measure cytotoxicity against several human solid tumor cell lines (Table 3). All four samples showed potent activities against HCT-8 and HT-29 cell lines, whereas they had no cytotoxicity against normal human cells. Although the \((10R)\)-hydroxyl-substituted analogue 23c showed activity similar to 22c, the introduction of the \((4R)\)-hydroxyl group into 24c raised the potency by a factor of 15 [31]. It was found that most cell death induced by 22c was due to necrosis, and 23c affected mitochondrial complex I [32]. A preliminary antitumor assay in mice (Lewis lung cancer) with 22c showed 60% inhibition of tumor compared with the control.

**Scheme 3.** Synthesis of polyether analogues by Wu’s group.

| Compounds | KB | A2780 | HCT-8 | HT-29 |
|-----------|----|-------|-------|-------|
| 22a       | > 1| > 1   | 6.6 × 10^{-2} | 2.72 × 10^{-1} |
| 22b       | > 1| > 1   | 9.7 × 10^{-2} | 1.12 |
| 22c       | > 1| > 1   | 3.2 × 10^{-2} | 1.1 × 10^{-1} |
| 22d       | > 1| > 1   | 6.5 × 10^{-2} | 7.83 |
| adriamycin| 2.89 × 10^{-3} | 1.02 × 10^{-3} | 4.65 × 10^{-3} | 9.8 × 10^{-4} |

In 2004, Yao and Wu’s group reported preparation of a small library to clarify the structure–activity relationships (SAR) of their polyether analogues [33]. New analogues that had dihydroxyl groups in the vicinity of the ether bonds were prepared by convergent synthesis. A representative synthetic pathway is given by the preparation of 39 (Scheme 4). First, ester 46 was transformed to \(\gamma\)-lactone 47 by a three-step sequence involving an aldol reaction with \(O\)-THP-\((S)\)-lactaldehyde. After epoxidation of the terminal olefin, Jacobsen’s hydrolytic kinetic resolution gave a chiral epoxide 49. The
preparation of the polyether fragment 55 began with the condensation of the mesylate 51 and tetraol derivative 52. O-Alkylation of the alcohol 53 with (R)-epichlorohydrin afforded the epoxide 54. Opening of the epoxide with trimethylsilylacetylide, followed by protection of the resulting secondary alcohol and deprotection of the TMS group, produced the polyether fragment 55. The coupling reaction of the acetylide prepared from 55 and the epoxide 49, followed by reduction of the triple bond and deprotection of the MOM group, yielded the polyether analogue 39. Nearly all new analogues bearing hydroxyl groups in the vicinity of the ether bonds showed no activity against the Bel-7402 cell line, but exhibited good cytotoxicity against HT-29 and HCT-8 cell lines in the low micromolar range (Table 4). It is interesting that the introduction of hydroxyl group and their stereochemistry yielded selectivity among the tumor cell lines.

### Scheme 4. Synthesis by Yao and Wu’s group of polyether analogues with dihydroxyl groups in the vicinity of the ether bonds.

![Scheme 4](image)

### Table 4. Cytotoxicity against human solid tumor cell lines.

| Compounds | IC50 [μM] |
|-----------|-----------|
|           | KB       | Bel-7402 | HT-29 | HCT-8 |
| 35        | 7.65     | 1.99     | 0.099 | 0.11  |
| 36        | 4.02     | > 10     | 1.84  | 3.49  |
| 37        | 13.13    | > 10     | 5.72  | 8.58  |
| 38        | 13.81    | > 10     | 7.19  | 5.71  |
| 39        | 23.30    | > 10     | 9.79  | 10.00 |
Miyoshi et al. noted a structural similarity between the hydroxylated bis-THF moiety of natural acetogenins and the hydroxylated 1,2-cyclopentanediol bis-ether motif, especially the relative spatial positions of the four oxygen atoms (Scheme 5) [34]. Four diastereomeric 1,2-cyclopentanediol cores were synthesized by optical resolution with lipase. A representative synthetic pathway is given by the preparation of 57. Acetylation of (±)-trans-1,2-cyclopentandiol 61, followed by hydrolytic optical resolution, gave the chiral monoacetate 63. After protection of the hydroxyl group, treatment with K_2CO_3 in MeOH afforded 64 in the enantiomerically pure form. Introduction of two remaining secondary hydroxyl groups was performed by a coupling reaction with (2R)-glycidyl tosylate followed by the ring opening of epoxide with acetylide. Sonogashira coupling of alkyn 68 and vinyl iodide 69, followed by selective reduction of the triple bond and enyne, gave the target analogue 57. Inhibitory activities of four mimics against bovine heart mitochondrial complex I were examined (Table 5). All analogues showed potent inhibition at the nanomolar level, being nearly equipotent with bullatacin, which is one of the most potent inhibitors of complex I. It was also shown that the stereochemistry of 1,2-cyclopentanediol bis-ether cores had a slight effect on inhibitory potency.

Yao et al. designed conformationally constrained analogues of the acyclic bis-ether mimics [35]. The 1,2-disubstituted ethylene glycol, tetrahydrofuran-3,4-diol, tetrahydrothiophene-3,4-diol, and bis-amide moieties were conformationally constrained to alter the ether functionality in the lead compound, 22c. A representative synthetic pathway is given by the preparation of 70 and 76 (Scheme 6). Synthesis of the analogue 70 with a 1,2-disubstituted ethylene glycol core was started from O-alkylation of the diol 83 with the mesylate 82, followed by (R)-epichlorohydrin. The introduction of a γ-lactone moiety was achieved by an epoxide opening reaction with the acetylide of 86. Elimination of the secondary hydroxyl group, followed by selective hydrogenation of the resulting enyne moiety and cleavage of the MOM group, yielded the target analogue 70. The bis-amide analogue 76 was synthesized via sequential coupling of the two carboxylic acids (89 and 92) with the diamine fragment 90. The inhibitory activities against human breast cancer cell lines, MDA-MB-435 and MDA-MB-468, and non-cancerous human mammary epithelial cells (HMEC) were examined (Table 6). All analogues, with the exception of 80 and 81, showed low micromolar potencies against MDA-MB-468, whereas they were less active against MDA-MB-435 and displayed satisfactory selectivity for the non-cancerous cell line HMEC. For example, the N,N’-dimethyl bis amide derivative 77 (SI = 69), the most potent analogue in this report, showed better selectivity for the inhibition of MDA-MB-468 and HMEC than did its parent 22c (SI = 14). Moreover, compound 77 exhibited 30 times more potency against MDA-MB-468 cell lines than did 22c. These results indicate that the introduction of conformational constraint was useful for the optimization of this class of anticancer agents.
Molecules 2009, 14 3628

Scheme 5. Synthesis of 1,2-cyclopentanediol bis-ether analogues by Miyoshi’s group.

Table 5. Summary of the inhibitory potencies (IC\textsubscript{50}) of the test compounds.\textsuperscript{a}

| Compounds | IC\textsubscript{50} (nM) |
|-----------|-----------------|
| 56        | 0.83            |
| 57        | 1.9             |
| 58        | 1.0             |
| 59        | 1.4             |
| 60        | 0.90            |
| bullatacin| 0.85            |

\textsuperscript{a} The IC\textsubscript{50} value is the molar concentration needed to reduce the control NADH oxidase activity (0.60–0.65 mmol NADH / min / mg of protein) in submitochondrial particles by half.

Konno and Miyoshi noted that the THF ring acted as a hydrophilic anchor in the mitochondrial membrane. Dihydroxy-cohinbin A 93 was designed to increase the hydrophilicity of cohinbin A, which belongs to a class of non-THF acetogenins (Scheme 7) [36]. The synthesis of dihydroxy-cohinbin A 93 began with (+)-muricatacin 94. The construction of the tetraol moiety was achieved by asymmetric dihydroxylation of the α,β-unsaturated ester with an AD-mix β. Tetraol fragment 97 and γ-lactone fragment 98 were connected by the Sonogashira coupling reaction. After reduction of the enyne moiety, the construction of the α,β-unsaturated-γ-lactone moiety, followed by deprotection of the MOM group, yielded dihydroxy-cohinbin A 93. The inhibitory activities of dihydroxy-cohinbin A 93 and the intermediate 99 against bovine heart mitochondrial complex I were examined (Table 7). The intermediate 99, which has one free and three MOM-protected hydroxyl groups, lost inhibitory
Scheme 6. Synthesis of conformationally constrained polyether analogues by Yao’s group.

Table 6. Bioactivity screening of newly synthesized analogues. a

| Compounds | IC_{50}[μM] |
|-----------|-------------|
|           | MDA-MB-435 b | MDA-MB-468 c | HMEC d |
| 22c e     | > 100        | 5.932        | 82.11  |
| 70        | 6.467        | 0.830        | 14.25  |
| 71        | 4.170        | 1.005        | 10.86  |
| 72        | 5.500        | 0.994        | 13.60  |
| 73        | 11.24        | 1.630        | 17.68  |
| 74        | 25.69        | 2.559        | 20.63  |
| 75        | 18.40        | 3.007        | 17.95  |
| 76        | > 100        | 2.953        | > 100  |
| 77        | > 100        | 0.218        | 15.11  |
| 78        | > 100        | 1.181        | > 100  |
| 79        | > 100        | 61.59        | > 100  |
| 80        | > 100        | 2.019        | > 100  |
| 81        | 12.61        | 0.858        | 70.00  |

a Inhibition of cell growth by the listed compounds for MDA-MB-435, MDA-MB-468, and HMEC cells was determined by WST (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium monosodium salt) assay; b MDA-MB-435: Human breast cancer cell; c MDA-MB-468: Human breast cancer cell; d HMEC: Non-cancerous human mammary epithelial cells; e 22c was used as a positive control.
activity. Dihydroxy-cohinbin A 93 showed potent inhibition at the nanomolar level, although its activity was weaker than that of bullatacin. Konno et al. surmised that bioactivity of dihydro-cohinbin A 93 was diminished by the high degree of flexibility in the tetraol unit compared with the THF unit bearing flanking hydroxyl groups.

Scheme 7. Synthesis of dihydroxy-cohinbin A by Konno and Miyoshi’s group.

Table 7. Summary of the inhibitory potencies (IC₅₀) of the test compounds.

| Compounds | IC₅₀ (nM) |
|-----------|----------|
| 93        | 20       |
| 99        | 4100     |
| bullatacin| 0.8      |

3. Modification of the Hydrocarbon Chain

Miyoshi et al. investigated the role of the hydrophobic alkyl chain that is a feature of natural acetogenins. First, they designed the analogue 101 possessing a methyl group on the left side of the THF ring in place of a long hydrocarbon chain (Scheme 8) [37]. The synthesis of the bis-THF fragment began with condensation of tert-butyl acetate and trans-1,4-dibromo-2-butene, giving the diester 102. Chain extension of 102, followed by Sharpless asymmetric epoxidation, gave the epoxy alcohol 104. After silylation of the primary alcohol, Sharpless asymmetric dihydroxylation followed by treatment with TFA afforded the bis-THF core 106. The bis-THF core 106 was converted to the epoxide 107 in the following sequential reactions: (1) tosylation of secondary alcohols; (2) mono-desilylation of TBDPS group; (3) treatment with K₂CO₃. Opening of epoxide 107 by hydrogenation with Pd–C, followed by treatment with excess TBAF, yielded alcohol 108. After opening epoxide 108 with trimethylsilylacetylide, followed by desilylation, Pd(0)-mediated coupling of alkyne 109 with vinyl iodide 110 afforded the enyne 111. Hydrogenation of 111 and sequential thermal elimination of the sulfide moiety gave the target analogue 101. Although the IC₅₀ of 101 (3.1 nM) against bovine heart mitochondrial complex I was weaker than the inhibitory activity of the model compound 100
(0.9 nM), the analogue 101 retained sufficiently potent inhibitory activity (Table 8). These results indicate that the large hydrophobicity of the left side of the THF ring was not essential for the activity.

Scheme 8. Synthesis of analogues possessing a methyl group on the left side of the THF ring by Miyoshi’s group.

Table 8. Summary of the inhibitory potencies (IC$_{50}$) of the test compounds.

| Compounds | IC$_{50}$ (nM) |
|-----------|---------------|
| 100       | 0.9           |
| 101       | 3.1           |

Miyoshi’s group also reported modification of the alkyl spacer linking the THF and γ-lactone rings [38–40]. They designed a series of derivatives in which the spacer’s length was varied while other structural factors remained the same, or in which the local flexibility of the spacer was specifically reduced by introducing multiple bond(s) into different regions of the spacer. A representative synthetic pathway is given by the preparation of 116 (Scheme 9). The synthesis of the THF core 134 started from the diethyl 2,3-O-isopropylidene-D-tartrate 130 according to Sasaki et al. [41]. After sequential chain extension of 130, Sharpless asymmetric epoxidation of the resulting allyl alcohol 132, followed by treatment with BF$_3$•OEt$_2$, gave the bis-THF core 134. Monomesylation of the secondary alcohol of 134, followed by protection of the remaining alcohol and deprotection of the PNB group, yielded the epoxide 135. Treatment of 135 with nonynyllithium, followed by trimethylsilylacetylide, afforded the diyne 138 via 136 and 137. Connection of the two fragments 138 and 110 was achieved via Sonogashira coupling to give 139. Reduction of 139, followed by the formation of the α,β-unsaturated-γ-lactone moiety, gave the target analogue 116. The inhibitory potency of the synthetic
analalogues was examined (Table 9). The optimal length of the spacer for inhibition was approximately 13 carbon atoms, which corresponds to the length of spacers in most active natural acetogenins, such as bullatacin. Elongating the spacer beyond 13 carbons reduced inhibitory activity more drastically than did shortening the spacer. Inhibitory potency was not influenced by enhancement of the hydrophobicity (128 and 129) or local flexibility of the spacer (119–124). Surprisingly, tetrayne analogues 125–127 still exhibited potent inhibition at nanomolar levels, but the double inhibitor titration of complex I activity suggested that the action site of 126 was not identical to that of common acetogenins.

Scheme 9. Synthesis of analogues possessing modified alkyl spacers linking the THF and γ-lactone rings by Miyoshi’s group.
Molecules 2009, 14

| Compounds | IC_{50} (nM) | Compounds | IC_{50} (nM) |
|-----------|--------------|-----------|--------------|
| 113       | 14           | 122       | 1.0          |
| 114       | 1.6          | 123       | 0.83         |
| 115       | 1.2          | 124       | 0.85         |
| 116       | 0.85         | 125       | 6.2          |
| 117       | 13           | 126       | 1.7          |
| 118       | 271          | 127       | 3.0          |
| 119       | 0.92         | 128       | 1.3          |
| 120       | 1.2          | 129       | 1.2          |
| 121       | 1.1          |           |              |

The inhibitory potency of the mono-THF analogue (144 vs. 150) was drastically reduced, compared with the corresponding bis-THF analogues (116 vs. 126), by the introduction of a tetrasyne structure into the spacer. To clarify the effect of the introduction of tetrasyne, Miyoshi et al. synthesized a new series of tetrasyne analogues 150–155 (Figure 2). The inhibitory activity of this series showed that the flexibility of the spacer region close to the THF ring was more important than the flexibility of the spacer near the γ-lactone (Table 10).

**Figure 2.** Design of analogues possessing modified alkyl spacers linking the THF and γ-lactone rings, by Miyoshi’s group.

| Compounds | IC_{50} (nM) | Compounds | IC_{50} (nM) |
|-----------|--------------|-----------|--------------|
| 140       | 131          | 148       | 1050         |
| 141       | 11           | 149       | 5.2          |
| 142       | 10           | 150       | 280          |
| 143       | 10           | 151       | 72           |
| 144       | 2.3          | 152       | 12           |
| 145       | 16           | 153       | 142          |
| 146       | 34           | 154       | 185          |
| 147       | 117          | 155       | 16           |

To gain further insight into the function of the spacer, Miyoshi et al. designed photoresponsive analogues 156–157 that had an azobenzene moiety in the center of the spacer as a photoresponsive switch (Scheme 10). The azobenzene unit of 156 reversibly trans–cis isomerized by alternating UV-visible irradiation. The NADH oxidase activity of trans-156 was weaker than that of cis-156.
Interestingly, the relative inhibitory effects of the trans and cis-157, which had a longer distance between the THF moiety and the \( \gamma \)-lactone moiety than did 156, were reversed compared with those of 156. As a result of this research, Miyoshi et al. suggested that acetogenins exhibited potent inhibition of complex I only when the THF moiety and the \( \gamma \)-lactone moiety cooperatively bound to the two putative binding sites. One of the two THF rings in bis-THF acetogenins may have served as a pseudospacer to overcome the significant structural disadvantages that arose from the spacer, whereas mono-THF acetogenins could not efficiently adapt to such structural changes.

**Scheme 10.** Photoresponsive analogues possessing an azobenzene moiety in the center of the spacer as a photoresponsive switch.

### 4. Modification of the \( \gamma \)-Lactone Moiety

The \( \gamma \)-lactone moiety in acetogenins was suggested to directly interact with the target site in complex I [42]. To elucidate the role of the \( \gamma \)-lactone moiety, Miyoshi et al. synthesized analogues possessing various lactone moieties in place of the \( \alpha,\beta \)-unsaturated-\( \gamma \)-methyl-\( \gamma \)-lactone in natural acetogenins (Scheme 11) [43]. A representative synthetic pathway is given by the preparation of 160. Jacobsen's hydrolytic kinetic resolution of the racemic epoxide 161 gave the chiral epoxide 161. After the epoxide opening of 161 with the dianion prepared from phenylthioacetic acid, lactonization of the resulting seco acid afforded \( \gamma \)-butyl-\( \gamma \)-lactone 163. Sequential assembly of 163, 1,9-diiodononene, and the THF fragment 165, followed by the usual transformation, gave the target analogue 160. The inhibitory activities of synthetic analogues against complex I were examined (Table 11). The synthetic analogues 158–160 exhibited inhibition that was as potent as that of the parent compound 116, indicating that the inhibitor binding domain in complex I may be the large cavity-like structure.
Scheme 11. Synthesis of analogues possessing various lactones by Miyoshi’s group.

Table 11. Inhibition of mitochondrial complex I.

| Compounds     | IC<sub>50</sub> (nM) |
|---------------|----------------------|
| bullatacin    | 1.2                  |
| 116           | 1.3                  |
| 158           | 1.2                  |
| 159           | 1.3                  |
| 160           | 7.5                  |

Acetogenins were proposed to inhibit the terminal electron transfer step of mitochondrial complex I between the Fe-S cluster N2 and the ubiquinone pool [44–46]. The γ-lactone moiety may bind at the quinone binding site of complex I. To clarify the mode of action of acetogenins, Koert et al. designed quinone-mucocin 166 and quinone-squamocin D 168 in which the γ-lactone moiety was exchanged for the quinone portion of ubiquinone, the natural substrate of complex I (Scheme 12) [47–48]. A representative synthetic pathway is given by the preparation of 166. The ortho-lithiation of 2,3,4,5-tetramethoxytoluene 170, followed by treatment with succinic anhydride, gave the carboxylic acid 171. After reduction with LiAlH<sub>4</sub>, deoxygenation of the resulting benzylic alcohol, followed by Swern oxidation, afforded aldehyde 172. The THF fragment 175 was prepared from the known aldehyde 173. After chain extension of 173 by the Wittig reaction, followed by hydrogenation, the oxygenated moiety was converted into the phosphonium salt to give 175. Wittig reaction of the aldehyde 172 with the phosphonium salt 175 yielded alkene 176. Introduction of the THF fragment was accomplished by stereoselective coupling of the iodide 178 with the aldehyde 177, prepared from 176. Deprotection of the TBS ethers gave the hydroquinone dimethyl ether 167, which was transformed into the target quinone-mucocin 166 by oxidation with CAN. The hybrid analogues (166, 168–169), with the exception of hydroquinone-mucocin 167, were good inhibitors of complex I, and, in particular, quinone-mucocin 166 showed 10 times more potent activity than did mucocin (Table 12). This result indicated that the γ-lactone moiety in natural acetogenins could be exchanged for the quinone of ubiquinone, although it is unclear that the quinone moiety of the hybrid molecule accepted electrons from complex I.
Scheme 12. Synthesis of quinone analogues by Koert’s group.

Table 12. Inhibition of mitochondrial complex I.

| Compounds     | IC\(_{50}\) (nM) |
|---------------|------------------|
| mucocin       | 45               |
| 166           | 4.9              |
| 167           | 163              |
| squamocin D   | 8.7              |
| 168           | 2.3              |
| 169           | 6.2              |
| rotenone      | 1.3              |

Miyoshi et al. also synthesized a quinone analogue 179 using the most potent acetogenin 116 synthesized in their laboratory as the mother compound (Scheme 13) [49]. Analogue 116 had inhibitory potency equal to bullatacin, the most potent acetogenin. The key fragment 182 was prepared from the known quinone 180 [50] by alkylation with 1,9-diiodo-1-nonene and a retro-Diels-Alder reaction followed by methylation of the reduced form of quinone 181. Sonogashira coupling of the THF fragment 183 with the vinyl iodide 182, followed by hydrogenation, gave tetramethoxyltoluene 184, which was transformed into the quinone analogue 179 by oxidation. The inhibitory activity of 179 was comparable with that of the mother compound 116 or bullatacin (Table 13). Miyoshi et al. suggested that the presence of a conjugated carbonyl group may be important for the inhibitory activity of complex I due to the low potency of hydroquinone-dimethyl ether 184. Moreover, the \(^{13}\)C-labeled
quinone acetogenins were also synthesized to examine the binding behavior of the quinone group with complex I [51].

Scheme 13. Synthesis of quinone analogues by Miyoshi’s group.

Table 13. Inhibitory potencies of test compounds.

| Compounds | IC_{50} (nM) |
|-----------|-------------|
| bullatacin | 0.9         |
| 116        | 0.9         |
| 179        | 1.2         |
| 184        | 280         |

Poupon and Susin et al. reported the semisynthesis of quinone analogues 185–189 from natural squamocin (Scheme 14) [52]. A representative synthetic pathway is given by the preparation of 185. Treatment of TBS-protected squamocin 190 with KMnO₄ gave the carboxylic acid 191. The condensation of 191 and thiopyridine-N-oxide with DCC, followed by exposure to light in the presence of the benzoquinone, afforded the thiopyridylquinone derivative 193. After reductive desulfurization of 193 with Raney Ni, the deprotection of the tris-TBS ether yielded the target analogue 185. Screening demonstrated that analogues 185 and 187 possessed a higher pro-apoptotic potential than natural squamocin, whereas the other analogues, 186, 188, and 189, were less effective than squamocin (Table 14). Moreover, quinone analogues 185 and 187 were potent inhibitors of complex I, although the inhibition activity was weaker than that of squamocin.
Scheme 14. Semisynthesis of quinone analogues by Poupon’s group.

Table 14. Inhibition of complex I.

| Compounds | IC₅₀ (nM) |
|-----------|----------|
| squamocin | 1.3      |
| rotenone  | 30       |
| 185       | 15       |
| 186       | 10       |
| 190       | > 3,000  |

Cortes et al. reported a series of semisynthetic analogues, modified at the α,β-unsaturated γ-methyl-γ-lactone moiety (Scheme 15) [53–56]. A representative synthetic pathway is given by the preparation of 200. Translactonization of rolliniastatin-1 by alkaline treatment [57-58] gave the isoacetogenin analogue 198 as a mixture of 2,4-cis and 2,4-trans diastereomers. The carbonyl group of 198 was transformed into the oxime 200 with NH₂OH•HCl in pyridine. The bis-THF acetogenin analogues, with the exception of 202 and 204, indicated more potent inhibitory activity against complex I than the natural compound (Table 15). Annonacin analogues 206–210 were tested against some tumor cell lines. Interestingly, the tetrahydroxyl analogue 207, whose inhibitory potency of complex I was the weakest among the annonacin analogues, was the most potent in the cytotoxicity assays.

Poupon and Brandt et al. reported the synthesis and biological evaluation of β-aminosquamocin 211 (Scheme 16) [59]. One-step transformation into 211 from squamocin was achieved by treatment with sodium azide and zinc bromide in boiling water. β-Aminosquamocin 211 exhibited more potent cytotoxicity against KB3-1 cell lines than did squamocin, despite an inhibitory activity of 211 against complex I that was four times weaker than that of the natural compound (Table 16). Surprisingly, β-aminosquamocin 211 demonstrated inhibitory activity against complex III at nanomolar levels.
Scheme 15. Semisynthesis of analogues modified at the α,β-unsaturated γ-methyl-γ-lactone moiety by Cortes’ group.

Table 15. Inhibitory potency against complex I.

| Compounds       | IC_{50} (nM) | Compounds       | IC_{50} (nM) |
|-----------------|--------------|-----------------|--------------|
| rolliniastatin-1| 0.60         | 202             | 8.47         |
| 194             | 0.42         | 203             | 0.83         |
| 195             | 0.25         | 204             | 2.48         |
| 196             | 0.43         | 205             | 0.54         |
| 197             | 0.21         | annonacin       | 2.3          |
| 198             | 0.33         | 206             | 3.6          |
| 199             | 0.18         | 207             | 21.8         |
| 200             | 0.23         | 208             | 3.3          |
| cherimolin-1    | 1.84         | 209             | 5.8          |
| 201             | 1.22         | 210             | 1.9          |

Scheme 16. Synthesis of β-aminosquamocin by Poupon’s group.

Table 16. Inhibitory activities of 211 and squamocin.

| Compounds | Cytotoxicity (KB3-1)^a (M) | Complex I inhibition^b (nM) | Complex III inhibition^c (nM) |
|-----------|---------------------------|----------------------------|-------------------------------|
| squamocin | 1.6 \times 10^{-13}       | 2                          | inactive                      |
| 211       | < 10^{-14}                | 8                          | 40                            |

^a IC_{50} for human nasopharyngeal epithelioid carcinoma cells; ^b IC_{50} for NADH: n-Decylubiquinone oxidoreductase (bovine submitochondrial particles); ^c IC_{50} for n-decylubiquinol: Cytochrome c oxidoreductase (liposomal reconstitution of bovine enzyme).
A library of heterocyclic analogues of squamocin was semisynthesized by Lewin et al., as heterocycles are commonly found as base-structures of potent complex I inhibitors (Scheme 17) [60–62]. A representative synthetic pathway is given by the preparation of 215 and 227. Ruthenium-catalyzed oxidative degradation of terminal γ-lactone in tris-TBS protected squamocin 190 yielded the α-ketoester 241. The condensation of 241 with 4-methoxy-α-phenylenediamine, followed by deprotection of the TBS ether, afforded the target analogue 215. The γ-ketoamide analogue 227 was synthesized from natural squamocin by treatment with piperidine. The inhibitory activities of heterocyclic analogues 213–221 against complex I indicated that the γ-lactone moiety in the natural acetogenins could be exchanged for heterocycles (Table 17). In particular, the benzimidazole analogue 220 had potent inhibitory activity equal to that of squamocin. The α-ketoamide and α-ketoester derivatives, with the exception of 237, showed potent inhibitory activity against complex I at the nanomolar level. Moreover, they had significant cytotoxic activity against KB 3-1 cell lines, although their activities were weaker than that of squamocin.

Scheme 17. Semisynthesis of squamocin analogues by Lewin’s group.
Table 17. Biological activities of squamocin analogues.

| Complex I Inhibition IC50 (nM) | Cytotoxicity (KB 3-1) IC50 (M) | Complex I Inhibition IC50 (nM) | Cytotoxicity (KB 3-1) IC50 (M) |
|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| NADH oxidase                  | NADH:DB oxidoreductase         | NADH oxidase                  | NADH:DB oxidoreductase         |
| squamocin 0.8–0.9              | 1.3                            | 1.8 × 10−13                   | 227                            |
| nt                             | nt                             | 41                            | nt                             |
| 213                            | 2                              | 7.9                            | nt                             |
| 214                            | nt                             | 229                            | nt                             |
| 215                            | nt                             | 230                            | 52                             |
| 216                            | nt                             | 231                            | nt                             |
| 217                            | 13                             | 232                            | 74                             |
| 218                            | 19                             | 233                            | nt                             |
| 219                            | 8.1                            | nt                             | nt                             |
| 220                            | 0.9                            | nt                             | 13                             |
| 221                            | 14                             | nt                             | 17                             |
| 222                            | 2.3                            | 38                             | 2.8 × 10−7                     |
| 223                            | 2.4                            | 9.2                            | 6.8 × 10−8                     |
| 224                            | nt                             | 3.2 × 10−8                     |
| 225                            | 30                             | nt                             | 3.5 × 10−7                     |
| 226                            | 23                             | nt                             | 3.0 × 10−8                     |

Kojima and Tanaka et al. designed and synthesized heterocyclic analogues of solamin, a simple mono-THF acetogenin (Scheme 18) [63]. A representative synthetic pathway is given by the preparation of 242.

Scheme 18. Synthesis of heterocyclic solamin analogues by Tanaka’s group.

1,2-Diol 247 was converted to the α-TBS-oxyaldehyde 248 via the following sequential reactions: (1) Esterification of the primary alcohol; (2) silylation of the remaining secondary alcohol; (3) deprotection of pivaloyl ester; 4) oxidation of the resulting primary alcohol. The introduction of a
chiral C4-unit 249 was achieved by asymmetric alkynylation with a Zn(OTf)$_2$/Et$_3$N system to give 250 [64]. Reduction of the triple bond and deprotection of the benzylideneacetal, followed by selective sulfonylation of the primary alcohol, gave sulfonate 251. Treatment of 251 with K$_2$CO$_3$, followed by oxidation of the resulting primary alcohol, afforded the THF ring 252. Alkynylation of 252 with 1,11-dodecadiyne proceeded smoothly to give the propargy alcohol 253. After introduction of the aromatic ring by the Sonogashira reaction, hydrogenation of diyne moiety, followed by deprotection, yielded the target analogue 242. Synthetic analogues 242–246 were tested for in vitro antiproliferative activity against a panel of 39 human cancer cell lines [65]. Selected GI$_{50}$ (concentration for 50% inhibition of cell growth relative to control) values are summarized in Table 18. The N-methylpyrazole derivative 242 displayed strong cytotoxicity against NCI-H23 with potencies that were 80 times higher than those of solamin. These results indicate that the γ-lactone moiety could be exchanged for a heterocycle and that antitumor agents more effective than the natural acetogenins could be produced.

Table 18. Selected GI$_{50}$ (M) values of heterocyclic analogues against human cancer cell lines.

| Compounds | MCF-7$^a$ | SF-295$^b$ | HCT-116$^c$ | NCI-H23$^d$ | OVCAR-4$^e$ | MKN7$^f$ | PC-3$^g$ |
|-----------|----------|-----------|-----------|-----------|-----------|--------|--------|
| solamin   | $> 10^{-4}$ | $4.0 \times 10^{-5}$ | $> 10^{-4}$ | $7.3 \times 10^{-5}$ | $> 10^{-4}$ | $1.3 \times 10^{-5}$ | $> 10^{-4}$ |
| 242       | $2.7 \times 10^{-5}$ | $2.4 \times 10^{-5}$ | $9.2 \times 10^{-6}$ | $9.1 \times 10^{-7}$ | $3.7 \times 10^{-5}$ | $6.2 \times 10^{-6}$ | $> 10^{-4}$ |
| 243       | $> 10^{-4}$ | $2.4 \times 10^{-5}$ | $7.0 \times 10^{-6}$ | $1.3 \times 10^{-5}$ | $> 10^{-4}$ | $5.4 \times 10^{-6}$ | $> 10^{-4}$ |
| 244       | $7.9 \times 10^{-5}$ | $> 10^{-4}$ | $> 10^{-4}$ | $2.8 \times 10^{-5}$ | $3.8 \times 10^{-5}$ | $2.7 \times 10^{-5}$ | $6.8 \times 10^{-5}$ |
| 245       | $3.4 \times 10^{-5}$ | $2.5 \times 10^{-5}$ | $2.8 \times 10^{-5}$ | $1.1 \times 10^{-5}$ | $2.2 \times 10^{-5}$ | $6.1 \times 10^{-6}$ | $3.5 \times 10^{-5}$ |
| 246       | $> 10^{-4}$ | $5.6 \times 10^{-5}$ | $> 10^{-4}$ | $3.6 \times 10^{-5}$ | $> 10^{-4}$ | $9.4 \times 10^{-6}$ | $> 10^{-4}$ |

$^a$breast cancer; $^b$central nervous system cancer; $^c$colon cancer; $^d$lung cancer; $^e$ovarian cancer; $^f$stomach cancer; $^g$prostate cancer.

Yao et al. reported the synthesis of new analogues bearing a terminal lactam moiety instead of the γ-lactone in their original polyether mimics 22c (Scheme 19) [66]. A representative synthetic pathway is given by the preparation of 255. Aldol reaction of the enolate from the ester 33 with amino aldehyde 262, removal of the Cbz group, and in situ cyclization with β-elimination gave the lactam 263. Deprotection of the MOM ethers afforded the target compound 255. N-Methyl substituted lactam derivatives 255–257 showed potent cytotoxicity and good selectivity between human cells and tumor cells (Table 19). Unfortunately, analogues 258–260, with different length of the linkers at the nitrogen atom of the terminal lactam, showed dramatically decreased cytotoxic activity compared with 255, and no activity was measured in the fluorescent probe 261.
Scheme 19. Synthesis of lactam-containing analogues by Yao’s group.

Table 19. Cytotoxicity screening of lactam-containing analogues.

| Compounds | IC₅₀ (µM)          |
|------------|--------------------|
|            | Chang B16 BEL-7404 SK-Hepl |
| 22c        | NAᵃ 0.035 0.041 0.065 |
| 254        | NA 0.87 2.20 NA |
| 255        | NA 0.013 0.234 0.589 |
| 256        | NA 0.478 0.845 0.583 |
| 257        | NA 0.168 0.168 0.104 |
| 258        | NA 0.995 1.35 NA |
| 259        | NA NA 3.20 NA |
| 260        | NA NA 2.50 NA |
| 261        | NA NA NA NA |

ᵃ not active.

Miyoshi et al. designed analogues possessing two γ-lactone moieties connected to the bis-THF ring by flexible alkyl chains with the expectation that these analogues would elicit inhibitory activity that was twice as potent as that of ordinary acetogenins [38], because the γ-lactone moiety was suspected to interact directly with the binding site of complex I. A representative synthetic pathway is given by the preparation of 264 (Scheme 20). Mesylation of the secondary alcohols of the bis-THF 134, followed by deprotection of the PNB groups, gave bis-epoxide 270. After epoxide opening of 270 with trimethylsilylacetylide, treatment with TBAF afforded diyne 271. The connection of 271 with the γ-lactone fragment 110 was carried out via the Sonogashira reaction. Reduction of 272, followed by the formation of the α,β-unsaturated-γ-lactone moiety, gave the target analogue 264. The inhibitory activities of the synthetic analogues against complex I were measured (Table 20). The inhibitory activities of analogues 264–267 were identical to the activity of bullatacin. These results indicate that the analogues 264–269 does not work as two mole of inhibitors, although they have two mole of γ-lactone moieties in their one molecule, suggesting in turn that one γ-lactone and one THF moieties act cooperatively on the enzyme.
Scheme 20. Synthesis of analogues possessing two \( \gamma \)-lactone moieties connected to the bis-THF ring by flexible alkyl chains.

### Table 20. Inhibition of complex I.

| Compounds | IC\(_{50}\) (nM) | Compounds | IC\(_{50}\) (nM) |
|-----------|----------------|-----------|----------------|
| 264       | 1.2            | 268       | 2.0            |
| 265       | 1.6            | 269       | 18             |
| 266       | 1.2            | bullatacin | 1.2           |
| 267       | 1.9            |           |                |

Sasaki and Maeda et al. tested the assertion that acetogenins possessed affinity toward metal cations. Such properties may be related to their biological activity, because acetogenins are structurally analogous to known ionophores, such as oligo-tetrahydrofurans [67]. They designed analogues possessing only a bis-THF moiety without the \( \gamma \)-lactone moiety (Scheme 21) [25,26,68]. A representative synthetic pathway is given by the preparation of 282. Epoxide opening of 287 with a Grignard reagent gave the diol 274. Tosylation of two secondary alcohols followed by azidation afforded the azide 288. After reduction of the azide groups, acetylation of the resulting secondary amines yielded the target analogue 282. Complexation properties of natural acetogenins and analogues were investigated by \( ^1 \)H NMR titration (Table 21). Among the bis-THF analogues with flanking hydroxyl groups, it was revealed that some analogues (274 and 277) had selective affinity towards \( \text{Ca}^{2+} \). Very high binding affinity was exhibited by diacetamide 284 to both \( \text{Mg}^{2+} \) and \( \text{Ca}^{2+} \) in the formation of 2:1 ligand-to-metal complexes.
Scheme 21. Synthesis of analogues without the γ-lactone moiety by Sasaki’s group.

Table 21. Binding properties of the bis-THF ligands.

| Compounds     | Metal | Compound/metal | $K_s$ ($10^3$ M$^{-1}$) | Compounds     | Metal | Compound/metal | $K_s$ ($10^3$ M$^{-1}$) |
|---------------|-------|----------------|--------------------------|---------------|-------|----------------|--------------------------|
| bullatacin    | Ca$^{2+}$ | 2:1             | 3.10                     | 280           | Ca$^{2+}$ | 1:1             | 0.08                     |
| asimicin      | Ca$^{2+}$ | 4:1             | 5.50                     | 274           | Mg$^{2+}$ | 1:1             | 0.62                     |
| 273           | Ca$^{2+}$ | 4:1             | 0.15                     | 273           | Mg$^{2+}$ | 4:1             | 0.11                     |
| 273           | K$^+$   | 4:1             | 0.13                     | 273           | K$^+$   | 1:1             | 0.06                     |
| 274           | Ca$^{2+}$ | 4:1             | 1.50                     | 274           | Mg$^{2+}$ | 1:1             | 0.25                     |
| 274           | Mg$^{2+}$ | 4:1             | 0.21                     | 274           | Na$^+$   | 2:1             | 1.20                     |
| 275           | Ca$^{2+}$ | 4:1             | 0.10                     | 275           | Mg$^{2+}$ | 2:1             | 9.60                     |
| 277           | Ca$^{2+}$ | 2:1             | 9.00                     | 277           | Na$^+$   | 4:1             | 3.00                     |
| 277           | Mg$^{2+}$ | 2:1             | 0.04                     | 277           | Na$^+$   | 4:1             | 1.00                     |
| 277           | K$^+$   | –               | –                        | 277           | K$^+$   | 2:1             | 1.60                     |
| 277           | Na$^+$   | –               | –                        | 277           | Na$^+$   | 4:1             | 3.40                     |
| 278           | Ca$^{2+}$ | 2:1             | 0.05                     | 278           | Ca$^{2+}$ | 4:1             | >100                     |
| 278           | Mg$^{2+}$ | 2:1             | 0.06                     | 278           | Mg$^{2+}$ | 4:1             | >100                     |
| 278           | K$^+$   | –               | –                        | 278           | K$^+$   | 4:1             | 1.60                     |
| 278           | Na$^+$   | –               | –                        | 278           | Na$^+$   | 4:1             | 3.40                     |

The GI50 values of their analogues against cancer cell lines are listed in Table 22 [69]. Although the analogues possessing short alkyl chains 277–279, ether chain 280–281, or no chain, 286, did not show activity, the analogues 273–275 with long alkyl chains retained cytotoxicity against P388 cells. The analogues (282–283 and 285) with amino groups instead of hydroxyl groups also showed inhibitory activity against cancer cell lines.
Table 22. GI₅₀ (μM) values against cancer cell lines.

| Compounds | P388ᵃ | PC-6ᵇ | NUGC-³ᶜ | Compounds | P388ᵃ | PC-6ᵇ | NUGC-³ᶜ |
|-----------|-------|-------|---------|-----------|-------|-------|---------|
| bullatacin | 1.04 × 10⁻⁴ | > 0.250 | > 0.250 | 278 | 11.6 | > 50.0 | – |
| asimicin   | 3.51 × 10⁻⁴ | > 0.250 | > 0.250 | 279 | > 25.0 | > 25.0 | > 25.0 |
| 273        | 0.271 | 6.34 | – | 280 | > 50.0 | > 50.0 | > 50.0 |
| enantio-273 | 1.42 | 16.4 | – | 281 | 35.9 | > 50.0 | > 50.0 |
| 274        | 0.111 | 34.6 | – | 282 | > 25.0 | > 25.0 | > 25.0 |
| enantio-274 | 3.10 | > 25.0 | – | 283 | 0.460 | > 2.50 | > 2.50 |
| 275        | 0.140 | 21.9 | – | 284 | > 2.50 | > 2.50 | > 2.50 |
| 276        | > 5.00 | – | – | 285 | 0.610 | 0.484 | 0.722 |
| 277        | > 2.50 | > 25.0 | > 2.50 | 286 | 17.2 | 28.0 | – |

ᵃ mouse leukemia;ᵇ human lung cancer;ᶜ human gastric cancer.

Sasaki et al. tested the possibility that their bis-THF analogues were active DNA binding agents, because a helix-like conformation was suggested in the studies of naturally occurring bis-THF acetogenins [70–71]. The DNA binding affinities of bis-THF analogues were evaluated by Sasaki et al. (Figure 3) [72]. It was revealed that the stereochemistries around the bis-THF moiety and the length of alkyl chains affected the binding affinity. The bis-furan 295 also displayed high affinity against CA12.

Figure 3. Diamino-bis-THF analogues by Sasaki’s group.

Table 23. Comparison of DNA binding affinities.ᵃ

| Compounds | Cₛ₅₀ (μM) | Compounds | Cₛ₅₀ (μM) |
|-----------|-----------|-----------|-----------|
|           | CT12ᵇ     | CA12ᶜ    | CT12ᵇ     | CA12ᶜ    |
| 285       | 7         | 5.5       | 292       | 300       | 330       |
| 287       | 19        | 13        | 293       | 32        | 27        |
| 288       | 30        | 30        | 294       | 490       | 540       |
| 289       | 34        | 23        | 295       | 17        | 4         |
| 290       | 135       | 125       | 296       | > 100     | > 100     |
| 291       | 760       | 880       | distamycin | 19        | > 15      |

ᵃ 1.5 μM DNA and 1.5 μM ETBr were used in the buffer containing 9.4 mM NaCl, 2.0 mM HEPES, 10 mM EDTA, pH 7.0;ᵇ KₑTBr = 2.4 × 10⁶;ᶜ KₑTBR = 7.6 × 10⁶.

Surprisingly, Miyoshi et al. discovered that the analogues that possessed two alkyl tails without a γ-lactone in bis-THF acetogenins showed potent inhibitory activity against mitochondrial complex I (Scheme 22) [73–79]. A representative synthetic pathway is given by the preparation of 320. Tosylation of secondary alcohols of bis-THF cores 335, followed by treatment with TBAF, gave the
Molecules 2009, 14

bis-epoxide 336. Epoxide opening of 336 with the acetylide generated from 337, followed by hydrogenation, afforded the target analogue 320. Inhibitory activities of synthetic analogues against complex I were examined (Table 24). The inhibitory potencies were affected by the length of the alkyl tails, and the analogue 299, possessing unbranched decyl groups, showed the most potent activity among analogues 297–314. Analogues 317–321, possessing a phenol moiety at the end of the alkyl chains, exhibited more potent inhibitory activity. It was revealed that the stereochemistry around the THF ring moiety significantly influenced the inhibitory effect of the analogues. A mode-of-action study suggested that the binding site of the analogues is not identical to the binding site of ubiquinone and is downstream of the binding site of ordinary inhibitors.

**Scheme 22.** Synthesis of analogues possessing two alkyl chains without γ-lactone by Miyoshi’s group.

Based on the structural similarities between the hydroxylated bis-THF moiety and the piperazine ring, Miyoshi et al. designed a series of piperazine derivatives by replacing the bis-THF moiety of their analogues 297–334 with piperazine rings (Scheme 23) [80]. Piperazine analogues (e.g., 344) were easily prepared by the condensation of piperazine and epoxide 356. The inhibitory activities of synthetic analogues against complex I were examined (Table 25). Some piperazine analogues (339, 343–346) showed potent inhibitory activity equal to that of the parent compounds (e.g., 320). Although inhibitory potencies were affected by the length of alkyl chains as well as parent compound,
the presence of two hydroxyl groups was not crucial for activity. Modifying the conformational properties of the piperazine rings did not affect activity. The photoaffinity labeling study of new piperazine derivatives revealed that this analogue bound to the 49 kDa subunit and an unidentified subunit (not ND1) with a frequency of ~1:3, but prevented the specific binding of $[^{125}\text{I}]$(trifluoromethyl)phenyldiazirinyl acetogenin to the ND1 subunit.

**Table 24.** Summary of the inhibitory potencies against complex I.

| Compounds | IC$_{50}$ (nM) | Compounds | IC$_{50}$ (nM) | Compounds | IC$_{50}$ (nM) | Compounds | IC$_{50}$ (nM) |
|-----------|---------------|-----------|---------------|-----------|---------------|-----------|---------------|
|    297    |     4500      |    307    |       620     |    317    |       1.4     |    327    |       39      |
|    298    |      45       |    308    |       7.5     |    318    |       1.1     |    328    |       41      |
|    299    |       1.6     |    309    |       34      |    319    |       0.91    |    329    |       47      |
|    300    |       9.0     |    310    |       410     |    320    |       0.83    |    330    |       38      |
|    301    |      280      |    311    |       27      |    321    |       1.0     |    331    |       16      |
|    302    |       45      |    312    |      1500     |    322    |       150     |    332    |       9.0     |
|    303    |       3.2     |    313    |       870     |    323    |     > 500    |    333    |       5.2     |
|    304    |       5.5     |    314    |       7.5     |    324    |       3.0     |    334    |       3.8     |
|    305    |      330      |    315    |       308     |    325    |     > 1000   |    335    |       0.85    |
|    306    |       14      |    316    |     > 25000   |    326    |       16      |          |                |

**Scheme 23.** Synthesis of analogues possessing piperazines instead of the bis-THF moiety by Miyoshi's group.
### Table 25. Summary of the inhibitory potencies against complex I.

| Compounds | IC$_{50}$ (nM) | Compounds | IC$_{50}$ (nM) |
|-----------|----------------|-----------|----------------|
| 338       | > 22000        | 347       | 1300           |
| 339       | 2.6            | 348       | 12             |
| 340       | 26             | 349       | 2.2            |
| 341       | 110            | 350       | 3.6            |
| 342       | 12             | 351       | 1.7            |
| 343       | 1.7            | 352       | 2.8            |
| 344       | 1.2            | 353       | 670            |
| 345       | 5.9            | 354       | 1100           |
| 346       | 2.3            | 355       | 4700           |

### 5. Modification of the Oxygenated Moiety on Alkyl Chains

McLaughlin et al. reported a semisynthesis of chlorinated analogues, 357–358, of gigantetrocin A (Scheme 24) [81]. Gigantetrocin A was refluxed with PPh$_3$ in CCl$_4$ to give a mixture of 4-chloro-4-deoxygigantetrocin A 357 and dichloro bis-THF derivative 358. Both chlorinated analogues 357–358 showed decreased bioactivity against tumor cell lines compared with gigantetrocin A (Table 26). However, the 4-chloro derivative 357 was selectively cytotoxic to HT-29, and 358 was selectively cytotoxic to PC-3.

**Scheme 24.** Semisynthesis of chlorinated acetogenins by McLaughlin’s group.

### Table 26. Bioactivity data of chlorinated analogues 357–358 (LC$_{50}$ and ED$_{50}$: μg/mL).

| Compounds | BST$^a$ | A-549$^b$ | MCF-7$^c$ | HT-29$^d$ | A-498$^e$ | PC-3$^f$ | PaCa-2$^g$ |
|-----------|--------|----------|----------|----------|---------|---------|----------|
| gigantetrocin A | 2.6   | 2.5 × 10$^{-1}$ | 6.3 × 10$^{-1}$ | 4.1 × 10$^{-5}$ | nt   | nt   | nt       |
| 357       | 54.6   | > 10     | 5.74     | 3.8 × 10$^{-1}$ | > 10 | > 10 | > 10     |
| 358       | 31.9   | 2.39     | > 10     | > 10     | 2.47 | 7.55 × 10$^{-1}$ | 1.16     |
| Adriamycin | nt    | 2.43 × 10$^{-2}$ | 2.09 × 10$^{-1}$ | 3.46 × 10$^{-2}$ | 6.49 × 10$^{-3}$ | 2.62 × 10$^{-2}$ | 7.90 × 10$^{-3}$ |

$^a$Brine shrimp lethality test; $^b$Human lung carcinoma; $^c$Human breast carcinoma; $^d$Human colon adenocarcinoma; $^e$Human kidney carcinoma; $^f$Human prostate adenocarcinoma; $^g$Human pancreatic carcinoma.

The presence of a C4-hydroxyl group in acetogenins is known to influence cytotoxic activity against tumor cell lines. Kojima and Tanaka et al. were interested in whether C4-fluorinated solamin showed similar activity to that of solamin or murisolin (C4-(R)-hydroxyl solamin), because fluorine atoms are known to mimic hydrogen atoms. Substitution of the hydroxyl group with an isoelectronic function such as fluorine in biologically active compounds can produce more potent analogues (Scheme 25) [82]. The synthesis of 359 started from enantioselective α-fluorination [83] of aldehyde 361, followed by reduction of the aldehyde, to give the alcohol 362. After iodination of the primary alcohol of 362, the coupling reaction with the γ-lactone 364 and the sequential thermal elimination of the sulfide moiety afforded α,β-unsaturated γ-lactone 365. Assembly of two fragments, 365 and 366,
prepared by a systematic construction strategy [84–92], with the Sonogashira reaction, followed by selective reduction of endiyne and deprotection of the TBS ether, gave the target fluorinated analogue 359. Synthetic analogues 359–360 were tested for in vitro antiproliferative activity against a panel of 39 human cancer cell lines (Table 27). Selected results are summarized in Table 27. Two fluorinated analogues, 359 and 360, displayed inhibitory potency levels that fell between the potencies of solamin and murisolin. Interestingly, the analogue 359 showed approximately 20 times higher cytotoxicity to MKN7 than did 360, implying that the stereochemistry of the fluorine atom at the C4 position was recognized by cancer cell lines.

Scheme 25. Synthesis of C4-fluorinated solamins by Tanaka’s group.

Table 27. Selected GI50 (M) values of fluorinated analogues against 39 human cancer cell lines.

| Compounds | MCF-7a | SF-295b | HCT-116c | NCI-H23d | DMS114d | MKN7e | PC-3f |
|-----------|--------|---------|----------|----------|---------|--------|-------|
| solamin   | 7.1 × 10^{-5} | 4.0 × 10^{-5} | > 10^{-4} | 7.3 × 10^{-6} | 4.3 × 10^{-6} | 1.3 × 10^{-5} | > 10^{-4} |
| murisolin | 3.9 × 10^{-6} | 7.3 × 10^{-6} | 3.7 × 10^{-6} | 2.2 × 10^{-7} | < 10^{-8} | 7.0 × 10^{-7} | 1.7 × 10^{-5} |
| 359       | 5.6 × 10^{-5} | 3.7 × 10^{-5} | 2.0 × 10^{-5} | 2.3 × 10^{-5} | 2.6 × 10^{-7} | 9.6 × 10^{-7} | > 10^{-4} |
| 360       | 3.7 × 10^{-5} | 3.1 × 10^{-5} | 2.7 × 10^{-5} | 5.1 × 10^{-6} | 4.6 × 10^{-7} | 1.9 × 10^{-6} | 8.4 × 10^{-5} |

a breast cancer; b central nervous system cancer; c colon cancer; d lung cancer; e stomach cancer; f prostate cancer.

Semisynthesis of analogues possessing substituted functionalities in place of the hydroxyl groups were independently reported by Cortes’s group [53, 93–95] and Figadère’s group [96].

A representative synthetic pathway is given by the preparation of 374. Treatment of squamocin with mesyl chloride in pyridine gave the mesylate 371. Azidation of 371, followed by Staudinger reduction, afforded the target analogue 374. The inhibitory potency of analogues against complex I were examined by Cortes [53,93–95]. The oxo and hydroxyimino derivatives showed potent activity, but the activities of acetyl, mesyl, and azide analogues were diminished. The cytotoxicities of analogues against cancer cell lines were evaluated by Figadère et al. [96]. All of the tested squamocin analogues exhibited much lower cytotoxicity than did natural products. On the other hand, Cortes’s group reported that guanacone analogues 381–386 showed more potent cytotoxicity against human cancer cell lines than parent compound [94–95].
Scheme 26. Semisynthesis of acetogenin analogues modified by an oxygenated moiety on the alkyl chains by Cortes’ group and Figadère’s group.

Table 28. Inhibitory potency against complex I and cytotoxicity against cancer cell lines.

| Compounds | IC_{50} (nM) | EC_{50} (μM) | Compounds | IC_{50} (nM) | EC_{50} (μM) |
|-----------|--------------|--------------|-----------|--------------|--------------|
|           | NADH oxidase | KB VERO     |           | NADH oxidase | KB VERO     |
| squamocin | 0.59         | 1.6 × 10^{-5} | 4.8 × 10^{-2} | 0.60         | < 1.6 × 10^{-7} | 1.1 × 10^{-2} |
| 368       | 0.65         | nt           | nt        | 369          | 0.74         | 0.34         | nt           | nt           |
| 370       | nt           | < 1.3 × 10^{-1} | < 1.3 × 10^{-1} | 371          | 14.1         | 1.5 × 10^{-2} | < 10^{-1}    | 372          | 5.0          | 1.46         | nt           | nt           |
| 373       | 18           | < 1.4 × 10^{-1} | < 1.4 × 10^{-1} | 374          | nt           | 2.4 × 10^{-2} | < 1.6 × 10^{-1} | 375          | nt           | 9.7 × 10^{-2} | < 1.6 × 10^{-1} | nt           | nt           |
| 376       | rolliniastatin-1 | 0.60         | nt        | guanacine | 1.52         | nt           | nt           |
| 377       | R^1 = R^2 = R^3 = OH | 378          | 0.34         | 381          | 1.65         | nt           | nt           |
| 379       | R^1 = R^2 = R^3 = OH | 380          | 1.46         | 382          | 0.95         | nt           | nt           |
| 383       | guanacine | 1.52         | nt           | 384          | 12.8         | nt           | nt           |
| 385       | R^1 = R^2 = OH | 386          | 5.5          | 386          | 3.4         | nt           | nt           |
| 387       | R^1 = R^2 = OH | 388          | 1.65         | 389          | 0.95         | nt           | nt           |

Table 28 shows that the transformation of the secondary alcohols into acetate or mesylate led to a disappearance of cytotoxicity, but such derivatives retained strong activity against mitochondrial complex I.
Scheme 27. Semisynthesis of aminoacyl triester of squamocin by Lewin’s group.

To explain this ambiguity, Lewin et al. carried out semisynthesis and evaluation of biological activities of aminoacyl squamocin (Scheme 27) [97]. A representative synthetic pathway is given by the preparation of 393. The esterification of squamocin with the N-boc-protected serine 397 gave the triester 398. Deprotection of tri-benzyl ether using Pd–C/1,4-cyclohexadiene system afforded the target analogue 393. The inhibitory activity against complex I and cytotoxicity against KB 3-1 cell lines were examined (Table 29). Despite enhanced polarity compared with the natural acetogenins, all derivatives showed strongly reduced activities, although their analogues were more strongly active than was the lipophilic trisilyl ether 190.

Table 29. Inhibitory potency against complex I and cytotoxicity against cancer cell lines.

| Compounds | IC_{50} (nM) NADH oxidase | IC_{50} (nM) KB 3-1 | Compounds | IC_{50} (nM) NADH oxidase | IC_{50} (nM) KB 3-1 |
|-----------|--------------------------|---------------------|-----------|--------------------------|---------------------|
| squamocin | 0.8                      | 2.6 × 10^{-14}      | 391       | 247.7                    | 2.0 × 10^{-8}       |
| 372       | 5.0                      | nt                  | 392       | nt                       | 1.1 × 10^{-6}       |
| 190       | > 5000                   | > 10^{-5}           | 393       | nt                       | 5.5 × 10^{-7}       |
| 387       | 625                      | nt                  | 394       | nt                       | > 10^{-5}           |
| 388       | 440.0                    | 6.1 × 10^{-8}       | 395       | nt                       | > 10^{-5}           |
| 389       | nt                       | 2.4 × 10^{-7}       | 396       | nt                       | 1.7 × 10^{-6}       |
| 390       | nt                       | 6.3 × 10^{-8}       |           |                          |                     |

To investigate the influence of aqueous solubility of acetogenins on their cytotoxicity, a series of glycosyl analogues of squamocin was synthesized by Figadère’s group (Scheme 28) [98]. A representative synthetic pathway is given by the preparation of 410. Lewis acid catalyzed glycosylation of squamocin with 1-acetyl-2,3,4,6-tetrabenzyl-α-D-glucopyranose gave a mixture of 399-401. After purification by HPLC, hydrogenation of the compound 399 gave target analogue 410. Cytotoxicity against KB, VERO, and L1210 cell lines were examined (Table 30). Glycosylated analogues showed low activity against the normal cell (VERO) while still active against cancer cell
lines (KB and L1210). The water solubility diminished the cytotoxicity probably because of low lipophilicity to cross the cell membrane. Interestingly, two analogues 401 and 411 have shown significant inhibition of the proliferation of L1210 in the G1 phase, whereas squamocin was not specific.

Scheme 28. Semisynthesis of glycosyl analogues of squamocin by Figadère’s group.

Table 30. Cytotoxic activity of glycosyl analogues of squamocin by Figadère’s group.

| Compounds | EC50 (μM) |   |                 | Compounds | EC50 (μM) |   |                 |
|-----------|-----------|---|-----------------|-----------|-----------|---|-----------------|
| squamocin | 1.6 × 10^-3 | 1.6 × 10^-2 | < 4.0 × 10^-4 | 408       | 1.1 × 10^-2 | 3.4 × 10^-1 | 1.7 × 10^-2 |
| 399       | 4.3 × 10^-1 | 8.7 × 10^-1 | nt             | 409       | 2.4 × 10^-4 | 1.6 × 10^-2 | < 2.5 × 10^-4 |
| 400       | nt         | nt         | 5.0 × 10^-2   | 410       | 1.9 × 10^-3 | 6.0 × 10^-1 | 1.0 × 10^-2 |
| 404       | 9.6 × 10^-4 | 2.0 × 10^-2 | 3.0 × 10^-1   | 411       | nt         | nt         | 8.0 × 10^-2 |
| 406       | 1.05       | 1.05       | 10            | vinblastine | 1.2 × 10^-3 | < 3.7      | nt           |
| 407       | 3.6 × 10^-1 | 1.21       | 50            |           |           |           |               |

* human epidermoid carcinoma cells; * african green monkey kidney epithelial cells; * mouse lymphocytic leukemia cells.

6. Conclusions

Annonaceous acetogenins are a relatively new class of polyketides isolated from Annonaceae species. Much attention has been paid to their unique chemical structures and attractive biological activities, including antitumor activity. To clarify their mode of action, synthesis of fluorescently or photoaffinity-labeled analogues in progress [99–106]. Acetogenins have offered not only a challenging target for total synthesis, but they are also fascinating lead compounds for the development of novel antitumor agents. Acetogenin analogues possibly play an important role in cancer therapy in the near future.

References

1. McLaughlin, J.L. Paw paw and cancer: Annonaceous acetogenins from discovery to commercial products. *J. Nat. Prod.* 2008, 71, 1311-1321.
2. Bermejo, A.; Figadère, B.; Zafra-Polo, M.-C.; Barrachina, I.; Estornell, E.; Cortes, D. Acetogenins from annonaceae. recent progress in isolation, synthesis, and mechanisms of action. *Nat. Prod. Rep.* 2005, 22, 269-303.

3. Johnson, H.A.; Oberlies, N.H.; Alali, F.Q.; McLaughlin, J.L. Thwarting resistance: Annonaceous acetogenins as new pesticidal and antitumor agents. In *Biologically Active Natural Products: Pharmaceuticals*; Cutler, S.J., Cutler, H.G., Eds.; CRC Press: Washington, DC, USA, 1999; pp. 173-183.

4. Alali, F.Q.; Liu, X.X.; McLaughlin, J.L. Annonaceous acetogenins: Recent progress. *J. Nat. Prod.* 1999, 62, 504-540.

5. Zafra-Polo, M.C.; Figadère, B.; Gallardo, T.; Tormo, J.R.; Cortes, D. Natural acetogenins from annonaceae, synthesis and mechanisms of action. *Phytochemistry* 1998, 48, 1087-1117.

6. Cavé, A.; Figadère, B.; Laurens, A.; Cortes, D. Acetogenin from annonaceae. In *Progress in the Chemistry of Organic Natural Products*; Hertz, W., Kirby, G.W., Moore, R.E., Streglich, W., Tamm, Ch., Eds.; Springer-Verlag: New York, NY, USA, 1997; Vol. 70, pp. 81-288.

7. Zeng, L.; Ye, Q.; Oberlies, N.H.; Shi, G.; Gu, Z.-M.; He, K.; McLaughlin, J.L. Recent advances in annonaceous acetogenins. *Nat. Prod. Rep.* 1996, 13, 275-306.

8. Zafra-Polo, M.C.; González, M.C.; Estornell, E.; Sahpaz, S.; Cortes, D. Acetogenins from annonaceae, inhibitors of mitochondrial complex I. *Phytochemistry* 1996, 42, 253-271.

9. Gu, Z.-M.; Zhao, G.-X.; Oberlies, N.H.; Zeng, L.; McLaughlin, J.L. Annonaceous acetogenins: Potent mitochondrial inhibitors with diverse applications. In *Recent Advances in Phytochemistry*; Arnason, J.T., Mata, R., Romeo, J.T., Eds.; Plenum Press: New York, NY, USA, 1995; Vol. 29, pp. 249-310.

10. Ramírez, E.A.; Hoye, T.R. Determination of relative and absolute configuration in the annonaceous acetogenins. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, The Netherlands, 1995; Volume 17, pp. 251-282.

11. Fang, X.P.; Rieser, M.J.; Gu, Z.M.; Zhao, G.X.; McLaughlin, J.L. Annaceous acetogenins: An updated review. *Phytochem. Anal.* 1993, 4, 27-67.

12. Rupprecht, J.K.; Hui, Y.-H.; McLaughlin, J.L. Annonaceous acetogenins: A review. *J. Nat. Prod.* 1990, 53, 237-278.

13. Oberlies, N.H.; Croy, V.L.; Harrison, M.L.; McLaughlin, J.L. The annonaceous acetogenin bullatacin is cytotoxic against multidrug-resistant human mammary adenocarcinoma cells. *Cancer Lett.* 1997, 115, 73-79.

14. Morré, D.J.; de Cabo, R.; Farley, C.; Oberlies, N.H.; McLaughlin, J.L. Mode of action of bullatacin, a potent antitumor acetogenin: Inhibition of NADH oxidase activity of Hela and HL-60, but not liver, plasma membranes. *Life Sci.* 1995, 56, 343-348.

15. Li, N.; Shi, Z.; Tang, Y.; Chen, J.; Li, X. Recent progress on the total synthesis of acetogenins from annonaceae. *Beilstein J. Org. Chem.* 2008, 4, No. 48.

16. Hu, T.S.; Wu, Y.L.; Yao, Z.J. Recent progress on the chemical synthesis of annonaceous acetogenins and their structurally modified mimics. In *Medicinal Chemistry of Bioactive Natural Products*; Liang, X.-T., Fang, W.S., Eds.; John Wiley & Sons, Inc.: West Sussex, UK, 2006; pp. 399-441.

17. Casiraghi, G.; Zanardi, F.; Battistini, L.; Rassu, G. Current advances in the chemical synthesis of annonaceous acetogenins and relatives. *Chemtracts* 1998, 11, 803-827.
18. Marshall, J.A.; Hinkle, K.W.; Hagedorn, C.E. Recent developments in the synthesis of annonaceous acetogenins. *Isr. J. Chem.* **1997**, *37*, 97-107.

19. Figadère, B.; Cavé, A. Total stereoselective synthesis of acetogenins of annonaceae: A new class of bioactive polyketides. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, The Netherlands, 1996; Volume 18, pp. 193-227.

20. Hoppe, R.; Scharf, H.D. Annonaceous acetogenins - synthetic approaches towards a novel class of natural products. *Synthesis* **1995**, 1447-1464.

21. Figadère, B. Syntheses of acetogenins of annonaceae: A new class of bioactive polyketides. *Acc. Chem. Res.* **1995**, *28*, 359-365.

22. Rodier, S.; Huérou, Y.L.; Renoux B.; Doyon, J.; Renard, P.; Pierré, A.; Gesson, J.-P.; Grée, R. New cytotoxic analogues of annonaceous acetogenins. *Anti-Cancer Drug Design* **2001**, *16*, 109-117.

23. Rodier, S.; Huérou, Y.L.; Renoux B.; Doyon, J.; Renard, P.; Pierré, A.; Gesson, J.-P.; Grée, R. Synthesis and cytotoxic activity of acetogenin analogues. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1373-1375.

24. Huérou, Y.L.; Doyon, J.; Grée, R.L. Stereocontrolled synthesis of key advanced intermediates toward simplified acetogenin analogues. *J. Org. Chem.* **1999**, *64*, 6782-6790.

25. Sasaki, S.; Maruta, K.; Naito, H.; Sugihara, H.; Hiratani, K.; Maeda, M. New calcium-selective electrodes based on annonaceous acetogenins and their analogs with neighboring bistetrahydrofuran. *Tetrahedron Lett.* **1995**, *36*, 5571-5574.

26. Sasaki, S.; Maruta, K.; Naito, H.; Maemura, R.; Kawahara, E.; Maeda, M. Novel acyclic ligands for metal cations based on the adjacent bistetrahydrofuran as analogs of natural annonaceous acetogenins. *Tetrahedron* **1998**, *54*, 2401-2410.

27. Peyrat, J.-F.; Mahuteau, J.; Figadère, B.; Cavé, A. NMR studies of Ca$^{2+}$ complexes of annonaceous acetogenins. *J. Org. Chem.* **1997**, *62*, 4811-4815.

28. Yao, Z.J.; Wu, H.-P.; Wu, Y.-L. Polyether mimics of naturally occurring cytotoxic annonaceous acetogenins. *J. Med. Chem.* **2000**, *43*, 2484-2487.

29. Zeng, B.-B.; Wu, Y.; Yu, Q.; Wu, Y.-L.; Li, Y.; Chen, X.-G. Enantiopure simple analogues of annonaceous acetogenins with remarkable selective cytotoxicity towards tumor cell lines. *Angew. Chem. Int. Ed.* **2000**, *39*, 1934-1937.

30. Zeng, B.-B.; Wu, Y.; Jiang, S.; Yu, Q.; Yao, Z.-J.; Liu, Z.-H.; Li, H.-Y.; Li, Y.; Chen, X.-G.; Wu, Y.-L. Studies on mimicry of naturally occurring annonaceous acetogenins: Non-THF analogues leading to remarkable selective cytotoxicity against human tumor cells, *Chem. Eur. J.* **2003**, *9*, 282-290.

31. Jiang, S.; Liu, Z.-H.; Sheng, G.; Zeng, B.-B.; Cheng, X.-G.; Wu, Y.-L.; Yao, Z.-J. Mimicry of annonaceous acetogenins: Enatioselective synthesis of a (4R)-hydroxy analogue having potent antitumor activity. *J. Org. Chem.* **2002**, *67*, 3404-3408.

32. Huang, G.-R.; Jiang, S.; Wu, Y.-L.; Jin, Y.; Yao, Z.-J.; Wu, J.-R. Induction of cell death of gastric cancer cells by a modified compound of the annonaceous acetogenin family. *ChemBioChem* **2003**, *4*, 1216-1221.

33. Jiang, S.; Li, Y.; Chen, X.-G.; Hu, T.-S.; Wu, Y.-L.; Yao, Z.-J. Parallel fragment assembly strategy towards multiple-ether mimicry of anticancer annonaceous acetogenins. *Angew. Chem. Int. Ed.* **2004**, *43*, 329-334.
34. Fujita, D.; Ichimaru, N.; Abe, M.; Murai, M.; Hamada, T.; Nishioka, T.; Miyoshi, H. Synthesis of non-THF analogs of acetogenin toward simplified mimics. *Tetrahedron Lett.* **2005**, *46*, 5775-5779.

35. Liu, H.-X.; Shao, F.; Li, G.-Q.; Xun, G.-L.; Yao, Z.-J. Tuning the acyclic ether moiety of anticancer agent AA005 with conformationary constrained fragments. *Chem. Eur. J.* **2008**, *14*, 8632-8639.

36. Konno, H.; Hiura, N.; Makabe, H.; Abe, M.; Miyoshi, H. Synthesis and mitochondrial complex I inhibition of dihydroxy-cohinbin A, non-THF annonaceous acetogenin analogue. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 629-632.

37. Motoyama, T.; Yabunaka, H.; Miyoshi, H. Essential structural factors of acetogenins, potent inhibitors of mitochondrial complex I. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2089-2092.

38. Kuwabara, K.; Takada, M.; Iwata, J.; Tatsumoto, K.; Sakamoto, K.; Iwamura, H.; Miyoshi, H.; Design syntheses and mitochondrial complex I inhibitory activity of novel acetogenin mimics. *Eur. J. Biochem.* **2000**, *267*, 2538-2546.

39. Abe, M.; Murai, M.; Ichimaru, N.; Kenmochi, A.; Yoshida, T.; Kubo, A.; Kimura, Y.; Moroda, A.; Makabe, H.; Nishioka, T.; Miyoshi, H. Dynamic function of the alkyl spacer of acetogenins in their inhibitory action with mitochondrial complex I (NADH-ubiquinone oxidoreductase). *Biochemistry* **2005**, *44*, 14898-14906.

40. Abe, M.; Kubo, A.; Yamamoto, S.; Hatoh, Y.; Murai, M.; Hattori, Y.; Makabe, H.; Nishioka, T.; Miyoshi, H. Dynamic function of the spacer region of acetogenins in the inhibition of bovine mitochondrial NADH-ubiquinone oxidoreductase (complex I). *Biochemistry* **2008**, *47*, 6260-6266.

41. Naito, H.; Kawahara, E.; Maruta, K.; Maeda, M.; Sasaki S.; The first total synthesis of (+)-bullatacin, a potent antitumor annonaceous acetogenin, and (+)-(15,24)-bisepi-bullatacin. *J. Org. Chem.* **1995**, *60*, 4419-4427.

42. Shimada, H.; Kozlowski, J.F.; McLaughlin J.L.; The localizations in liposomal membranes of the tetrahydrofuran ring moieties of the annonaceous acetogenins, annonacin and sylvaticin, as determined by $^1$H NMR spectoroscopy. *Pharmacol. Res.* **1998**, *37*, 357-364.

43. Takeda, M.; Kuwabara, K.; Nakato, H.; Tanaka, A.; Iwamura, H.; Miyoshi, H. Definition of crucial structural factors of acetogenins, potent inhibitors of mitochondrial complex I. *Biochim. Biophys. Acta* **2000**, *1460*, 302-310.

44. Miyoshi, H.; Ohshima, M.; Shimada, H.; Akagi, T.; Iwamura, H.; McLaughlin, J.L. Essential structural factors of annonaceous acetogenins as potent inhibitors of mitochondrial complex I. *Biochim. Biophys. Acta* **1998**, *1365*, 443-452.

45. Esposti, M.D. Inhibitors of NADH–ubiquinone reductase: An overview. *Biochim. Biophys. Acta* **1998**, *1364*, 222-235.

46. Friedrich, T.; Van Heek, P.; Leif, H.; Ohnishi, T.; Forche, E.; Kunze, B.; Jansen, R.; Trowitzsch-Kienast, W.; Höfle, G.; Reichenbach, H.; Weiss, H. Two binding sites of inhibitors in NADH: Ubiquinone oxidoreductase (complex I): Relationship of one site with the ubiquinone-binding site of bacterial glucose: Ubiquinone oxidoreductase. *Eur. J. Biochem.* **1994**, *219*, 691-698.

47. Hoppen, S.; Emde, U.; Friedrich, T.; Grubert, L.; Koert, U. Natural-product hybrids: Design, synthesis, and biological evaluation of quinone-annonaceous acetogenins. *Angew. Chem. Int. Ed.* **2000**, *39*, 2099-2102.
48. Arndt, S.; Emde, U.; Bäurle, S.; Friedrich, T.; Grubert, L. Koert, U. Quinone-annonomous acetogenins: Synthesis and complex I inhibition studies of a new class of natural product hybrids. *Chem. Eur. J.* 2001, 7, 993-1005.

49. Yabunaka, H.; Abe, M.; Kenmochi, A.; Hamada, T.; Nishioka, T.; Miyoshi, H. Synthesis and inhibitory activity of ubiquinone-acetogenin hybrid inhibitor with bovine mitochondrial complex I. *Bioorg. Med. Chem. Lett.* 2003, 13, 2385-2388.

50. Oshishima, M.; Miyoshi, H.; Sakamoto, K.; Takegami, K.; Iwata, J.; Kuwabara, K.; Iwamura, H. Yagi, T. Characterization of the ubiquinone reduction site of mitochondrial complex I using bulky synthetic ubiquinones. *Biochemistry* 1998, 37, 6436-6445.

51. Ichimaru, N.; Abe, M.; Kenmochi, A.; Hamada, T.; Nishioka, T.; Miyoshi, H. Synthesis of 13C-labeled ubiquinone-acetogenin hybrid inhibitors of mitochondrial complex I. *J. Pestic. Sci.* 2004, 29, 127-129.

52. Derbré, S.; Duval, R.; Roué, G.; Garofano, A.; Poupon, E.; Brandt, U.; Susin, S. A.; Hocquemiller, R. Semisynthesis and screening of a small library of pro-apoptotic squamocin analogues: Selection and study of a benzoquinone hybrid with an improved biological profile. *ChemMedChem* 2006, 1, 118-129.

53. Gallardo, T.; Zafra-Polo, M.C.; Tormo, J.R.; González, M.C.; Franck, X.; Estornell, E.; Cortes D. Semisynthesis of antitumoral acetogenins: SAR of functionalized alkyl-chain bis-tetrahydrofuranic acetogenins, specific inhibitors of mitochondrial complex I. *J. Med. Chem.* 2000, 43, 4793-4800.

54. Tormo, J.R.; Estornell, E.; Gallardo, T.; González, M.C.; Cavé, A.; Granell, S.; Cortes, D.; Zafra-Polo, M.C.; γ-Lactone-functionalized antitumoral acetogenins are the most potent inhibitors of mitochondrial complex I. *Bioorg. Med. Chem. Lett.* 2001, 11, 681-684.

55. Tormo, J.R.; Gallardo, T.; Peris, E.; Bermejo, A.; Cabedo, N.; Estornell, E.; Zafra-Polo, M.C.; Cortes, D. Inhibitory effects on mitochondrial complex I of semisynthetic mono-tetrahydrofuran acetogenin derivatives. *Bioorg. Med. Chem. Lett.* 2003, 13, 4101-4105.

56. Tormo, J.R.; Royo, I.; Gallardo, T.; Zafra-Polo, M.C.; Hernández, P.; Cortes, D.; Peláez, F. In vitro antitumor structure-activity relationships of threo/trans/threo mono-tetrahydrofuran acetogenins: Correlations with their inhibition of mitochondrial complex I. *Oncol. Res.* 2003, 14, 147-154.

57. Duret, P.; Figadère, B.; Hocquemiller, R.; Cavé, A. Epimerization of annonaceous acetogenins under basic conditions. *Tetrahedron Lett.* 1997, 38, 8849-8852.

58. Hoye, T.R.; Hanson, P.R. Assigning the relative stereochemistry between C(2) and C(4) of the 2-acetonyl-4-alkylbutyrolactone substructures of the appropriate annonaceous acetogenins. *J. Org. Chem.* 1991, 56, 5092-5095.

59. Duval, R.A.; Poupon, E.; Brandt, U.; Hocquemiller, R. Remarkable substituent effect: β-Aminosquamocin, a potent dual inhibitor of mitochondrial complex I and III. *Biochim. Biophys. Acta* 2005, 1709, 191-194.

60. Duval, R.A.; Lewin, G.; Hocquemiller, R. Semisynthesis of heterocyclic analogues of squamocin, a cytotoxic annonaceous acetogenin, by unusual oxadative decarboxylation reaction. *Bioorg. Med. Chem.* 2003, 11, 3439-3446.
one the role of the terminal lactone of annonaceous acetogenins. *Biochemistry* 2006, 45, 2721-2728.

62. Duval, R.A.; Poupon, E.; Romero, V.; Peris, E.; Lewin, G.; Cortes, D.; Brandt, U.; Hocquemiller, R. Analogues of cytotoxic squamocin using reliable reactions: New insights into the reactivity and role of the α,β-unsaturated lactone of the annonaceous acetogenins. *Tetrahedron* 2006, 62, 6248-6257.

63. Kojima, N.; Fushimi, T; Maezaki, N.; Tanaka, T.; Yamori, T. Synthesis of hydrid acetogenins, α,β-unsaturated-γ-lactone-free nitrogen-containing heterocyclic analogues, and their cytotoxicity against human cancer cell lines. *Bioorg. Med. Chem. Lett.* 2008, 18, 1637-1641.

64. Frantz, D.E.; Fässler, R.; Carreira, E.M. Facile enantioselective synthesis of propargylic alcohols by direct addition of terminal alkynes to aldehydes. *J. Am. Chem. Soc.* 2000, 122, 1806-1807.

65. Yamori, T.; Matsunaga, A.; Sato, S.; Yamazaki, K.; Komi, A.; Ishizu, K.; Mita, I.; Edatsugi, H.; Matsuba, Y.; Takezawa, K.; Nakanishi, O.; Kohno, H.; Nakajima, Y.; Komatsu, H.; Andoh, T.; Tsuruo T. Potent antitumor activity of MS-247, a novel DNA minor groove binder, evaluated by an in vitro and in vivo human cancer cell line panel. *Cancer Res.* 1999, 59, 4042-4049.

66. Liu, H.-X.; Huang, G.-R.; Zhang, H.-M.; Wu, J.-R.; Yao, Z.-J. Annonaceous acetogenin mimics bearing a terminal lactam and their cytotoxicity against cancer cells. *Bioorg. Med. Chem. Lett.* 2007, 17, 3426-3430.

67. Schultz, W.J.; Etter, M.C.; Pocius, A.V.; Smith, S. A new family of cation-binding compounds: *Threo*-α,ω-poly(cyclooxalkane)diyl. *J. Am. Chem. Soc.* 1980, 102, 7981-7982.

68. Sasaki, S.; Naito, H.; Maruta, K.; Kawahara, E.; Maeda, M. Novel calcium ionophores: Supramolecular complexation by the hydroxylated-bistetrahydrofuran skeleton of potent antitumor annonaceous acetogenins. *Tetrahedron Lett.* 1994, 35, 3337-3340.

69. Sasaki, S.; Maruta, K.; Naito, H.; Maemura, R.; Kawahara, E.; Maeda M. *In vitro* antitumor activities of new synthetic bistetrahydrofuran derivatives as analogs of annonaceous acetogenins. *Chem. Pharm. Bull.* 1998, 46, 154-158.

70. Shimada, H.; Grutzner, J.B.; Kozlowski, J.F.; McLaughlin, J.L. Membrane conformations and their relation to cytotoxicity of asimicin and its analogues. *Biochemistry* 1998, 37, 854-866.

71. Born, L.; Lieb, F.; Lorentzen, J.P.; Moeschler, H.; Nonfon, M.; Söllner, R.; Wendisch, D. The relative configuration of acetogenins isolated from *annona squamosa*: Annnonin I (squamocin) and annonin VI. *Planta Med.* 1990, 56, 312-316.

72. Sasaki, S.; Shibata, T.; Torigoe, H.; Shibata, Y.; Maeda M. Novel class of DNA binding motifs based on bistetrahydrofuran and bisfur an skeleton with long alkyl chains. *Nucleos. Nucleot. Nucleic Acids* 2001, 20, 551-558.

73. Hamada, T.; Ichimaru, N.; Abe, M.; Fujita, D.; Kenmochi, A.; Nishioka, T.; Zwicker, K.; Brandt, U.; Miyoshi, H. Synthesis and inhibitory action of novel acetogenin mimics with bovine heart mitochondrial complex I. *Biochemistry* 2004, 43, 3651-3658.

74. Ichimaru, N.; Murai, M.; Abe, M.; Hamada, T.; Yamada, Y.; Makino, S.; Nishioka, T.; Makabe, H.; Makino, A.; Kobayashi, T.; Miyoshi, H. Synthesis and inhibition mechanism of Δlac-acetogenins, a novel type of inhibitor of bovine heart mitochondrial complex I. *Biochemistry* 2005, 44, 816-825.
75. Ichimaru, N.; Abe, M.; Murai, M.; Senoh, M.; Nishioka, T.; Miyoshi, H. Function of the alkyl side chains of Δlac-acetogenins in the inhibitory effect on mitochondrial complex I (NADH-ubiquinone oxidoreductase). *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3555-3558.

76. Murai, M.; Ichimaru, N.; Abe, M.; Nishioka, T.; Miyoshi, H. Mode of inhibitory action of Δlac-acetogenins, a new class of inhibitors of bovine heart mitochondrial complex I. *Biochemistry* **2006**, *45*, 9778-9787.

77. Murai, M.; Ichimaru, N.; Abe, M.; Nishioka, T.; Miyoshi, H. Synthesis of photolabile Δlac-acetogenin for photoaffinity labeling of mitochondrial complex I. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3555-3558.

78. Ichimaru, N.; Yoshinaga, N.; Nishioka, T.; Miyoshi, H. Effect of stereochemistry of Δlac-acetogenins on the inhibition of mitochondrial complex I (NADH-ubiquinone oxidoreductase). *Tetrahedron* **2007**, *63*, 1127-1139.

79. Miyoshi, H.; Ichimaru, N.; Murai, M. Synthesis and inhibitory action of novel acetogenin mimics Δlac-acetogenins: A new class of inhibitors of mitochondrial NADH-ubiquinone oxidoreductase (complex-I). In *Pesticide Chemistry: Crop Protection, Public Health, Environmental Safety*; Ohkawa, H., Miyagawa, H., Lee, P.W., Eds.; WILEY-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2007; pp. 171-174.

80. Ichimaru, N.; Murai, M.; Kakutani, N.; Kako, J.; Ishihara, A.; Nakagawa, Y.; Nishioka, T.; Yagi, T.; Miyoshi, H. *Biochemistry* **2008**, *47*, 10816-10826.

81. Ye, Q.; Shi, G.; He, K.; McLaughlin, J.L. Chlorinated annonaceous acetogenins and their bioactivities. *J. Nat. Prod.* **1996**, *59*, 994-996.

82. Kojima, N.; Hayashi, H.; Suzuki, S.; Tominaga, H.; Maezaki, N.; Tanaka, T.; Yamori, T. Synthesis of C4-fluorinated solamins and their growth inhibitory activity against human cancer cell lines. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6451-6453.

83. Beeson, T.D.; MacMillan, D.W.C. Enantioselective organocatalytic α-fluorination of aldehydes. *J. Am. Chem. Soc.* **2005**, *127*, 8826-8828.

84. Maezaki, N.; Kojima, N.; Tanaka, T.; Systematic synthesis of diastereomeric THF ring cores and total synthesis of anti-tumor annonaceous acetogenins. *Synlett* **2006**, *993-1003*.

85. Tominaga, H.; Maezaki, N.; Yanai, M.; Kojima, N.; Urabe, D.; Ueki, R.; Tanaka, T. First total synthesis of longimicin D. *Eur. J. Org. Chem.* **2006**, *1422-1429*.

86. Maezaki, N.; Tominaga, H.; Kojima, N.; Yanai, M.; Urabe, D.; Ueki, R.; Tanaka, T.; Yamori, T. Total synthesis of murisolins and evaluation of tumor-growth inhibitory activity. *Chem. Eur. J.* **2005**, *11*, 6237-6245.

87. Maezaki, N.; Tominaga, H.; Kojima, N.; Yanai, M.; Urabe, D.; Ueki, R.; Tanaka, T.; Yamori, T. First total synthesis of murisolin. *Chem. Commun.* **2004**, *406-407*.

88. Kojima, N. Systematic synthesis of antitumor annonaceous acetogenins. *Yakugaku Zasshi* **2004**, *124*, 673-681.

89. Kojima, N.; Maezaki, N.; Tominaga, H.; Yanai, M.; Urabe, D.; Tanaka, T. Stereodivergent and reiterative synthesis of bistetrahydrofuran ring cores of annonaceous acetogenins. *Chem. Eur. J.* **2004**, *10*, 672-680.

90. Kojima, N.; Maezaki, N.; Tominaga, H.; Asai, M.; Yanai, M.; Tanaka, T. Systematic construction of a monotetrahydrofuran-ring library in annonaceous acetogenins by asymmetric alkynylation and stereodivergent tetrahydrofuran-ring formation. *Chem. Eur. J.* **2003**, *9*, 4980-4990.
91. Maezaki, N.; Kojima, N.; Tominaga, H.; Yanai, M.; Tanaka, T. Systematic synthesis of bis-THF ring cores in annonaceous acetogenins. *Org. Lett.* **2003**, *5*, 1411-1414.

92. Maezaki, N.; Kojima, N.; Asai, M.; Tominaga, H.; Tanaka, T. Highly stereoselective and stereodivergent synthesis of four types of THF cores in acetogenins using a C₄-chiral building block. *Org. Lett.* **2002**, *4*, 2977-2980.

93. Gallardo, T.; Saez, J.; Granados, H.; Tormo, J.R.; Velez, I.D.; Brun, N.; Torres, B.; Cortes, D. 10-Oximeguanacone, the first nitrogenated acetogenin derivative found to be a potent inhibitor of mitochondrial complex I. *J. Nat. Prod.* **1998**, *61*, 1001-1005.

94. Tormo, J.R.; DePedro, N.; Royo, I.; Barrachina, I.; Zafra-Polo, M.C; Cuadrillero, C.; Hernández, P.; Cortes, D.; Peláez, F. In vitro antitumor structure–activity relationships of *threo/trans/threo/trans/erythro* bis-tetrahydrofuranic acetogenins: Correlations with their inhibition of mitochondrial complex I. *Oncol. Res.* **2005**, *15*, 129-138.

95. Royo, I.; DePedro, N.; Estornell, E.; Cortes, D.; Peláez, F.; Tormo, J.R. In vitro antitumor SAR of *threo/cis/threo/cis/erythro* bis-THF acetogenins: Correlations with their inhibition of mitochondrial complex I. *Oncol. Res.* **2003**, *13*, 521-528.

96. Duret, P.; Hocquemiller, R.; Gantier, J.-C.; Figadère, B. Semisynthesis and cytotoxicity of amino acetogenins and derivatives. *Bioorg. Med. Chem.* **1999**, *7*, 1821-1826.

97. Duval, R.A.; Duret, P.; Lewin, G.; Peris, E.; Hocquemiller, R. Semisynthesis and biological activity of aminoisocarbonyl triesters of squamocin, an annonaceous acetogenin. *Bioorg. Med. Chem.* **2005**, *13*, 3773-3781.

98. Queiroz, E.F.; Roblot, F.; Duret, P.; Figadère, B.; Gouyette, A.; Laprévote, O.; Serani, L.; Hocquemiller, R. Synthesis, spectroscopy, and cytotoxicity of glycosylated acetogenin derivatives as promising molecules for cancer therapy. *J. Med. Chem.* **2000**, *43*, 1604-1610.

99. Han, H.; Sinha, M.K.; D’Souza, L.J.; Keinan, E.; Sinha, S.C. Total synthesis of 34-hydroxyasimicin and its photoactive derivative for affinity labeling of the mitochondrial complex I. *Chem. Eur. J.* **2004**, *10*, 2149-2158.

100. Derbré, S.; Roué, G.; Poupon, E.; Susin, S.A.; Hocquemiller, R. Annonaceous acetogenins: The hydroxyl groups and THF rings are crucial structural elements for targeting the mitochondria, demonstration with the synthesis of fluorescent squamocin analogues. *ChemBioChem* **2005**, *6*, 979-982.

101. Derbré, S.; Gil, S.; Taverna, M.; Boursier, C.; Nicolas, V.; Demey-Thomas, E.; Vinh, J.; Susin, S.A.; Hocquemiller, R.; Poupon, E. Highly cytotoxic and neurotoxic acetogenins of the annonaceae: New putative biological targets of squamocin detected by activity-based protein profiling. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5741-5744.

102. Fujita, D.; Murai, M.; Nishioka, T.; Miyoshi, H. Light control of mitochondrial complex I activity by a photoresponsive inhibitor. *Biochemistry* **2006**, *45*, 6581-6586.

103. Murai, M.; Ishihara, A.; Nishioka, T.; Yagi, T.; Miyoshi, H. The ND1 subunit constructs the inhibitor binding domain in bovine heart mitochondrial complex I. *Biochemistry* **2007**, *46*, 6409-6416.

104. Liu, H.-X.; Huang, G.-R.; Zhang, H.-M.; Jiang, S.; Wu, J.-R.; Yao, Z.-J. A structure-activity guided strategy for fluorescent labeling of annonaceous acetogenin mimetics and their application in cell biology. *ChemBioChem* **2007**, *8*, 172-177.
105. Maezaki, N.; Urabe, D.; Yano, M.; Tominaga, H.; Morioka, T.; Kojima, N.; Tanaka, T. Synthesis of fluorescent solamin for visualization of cell distribution. *Heterocycles* **2007**, *73*, 159-164.

106. Kojima, N.; Morioka, T.; Yano, M.; Suga, Y. Maezaki, N.; Tanaka, T. Convergent synthesis of fluorescence labelled solamin. *Heterocycles* **2009**, *79*, 387-393.

*Sample availability*: Not available.

© 2009 by the authors; licensee Molecular Diversity Preservation International, Basel, Switzerland. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).