Heterocyclic Analogues of Squamocin as Inhibitors of Mitochondrial Complex I. On the Role of the Terminal Lactone of Annonaceous Acetogenins

ARTICLE *in* BIOCHEMISTRY · MARCH 2006

Impact Factor: 3.02 · DOI: 10.1021/bi051261u · Source: PubMed

9 AUTHORS, INCLUDING:

Romain Duval
Institute of Research for Development
15 PUBLICATIONS **184** CITATIONS
SEE PROFILE

Stefan Dröse
Goethe-Universität Frankfurt am Main
78 PUBLICATIONS **3,693** CITATIONS
SEE PROFILE

Heterocyclic Analogues of Squamocin as Inhibitors of Mitochondrial Complex I. On the Role of the Terminal Lactone of Annonaceous Acetogenins

Romain A. Duval,*[,‡] Guy Lewin,[‡] Eva Peris,[§] Nadia Chahboune,[§] Aurelio Garofano,[||] Stefan Dröse,[||] Diego Cortes,[§] Ulrich Brandt,[||] and Reynald Hocquemiller[‡]

Laboratoire de Pharmacognosie (BioCIS, UMR 8076 CNRS), Faculté de Pharmacie, University Paris-Sud, rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France, Laboratorio de Farmacognosia, Facultad de Farmacia, Universitad de Valencia, Burjassot, Spain, and Zentrum der Biologischen Chemie, Fachbereich Medizin, Universität Frankfurt, Theodor-Stern-Kai 7, Haus 26, D-60590, Frankfurt am Main, Germany

Received July 1, 2005; Revised Manuscript Received December 27, 2005

ABSTRACT: Heterocyclic analogues of squamocin have been semisynthesized by condensation reactions between squamocin-derived α-keto esters and heterodinucleophiles. The strong complex I inhibitory potency of squamocin-benzimidazole, a hybrid derivative, illustrates for the first time the functional analogy existing between the terminal butenolide of annonaceous acetogenins and heteroaromatic substructures of classic inhibitors of the enzyme. This finding supports the categorization of this atypical group of inhibitors as antagonists of the ubiquinone substrates. In addition, competition experiments of squamocin-benzimidazole versus squamocin and rolliniastatin-2 suggest that the binding of this hybrid inhibitor is responsible for a negative allosteric effect at the level of the first ubiquinone-binding site (A site) of mitochondrial complex I. This result supports the existence of a large cooperatively regulated inhibitor/ubiquinone-binding pocket located within the catalytic core of the enzyme, consisting of the association of the previously defined affinity sites A and B.

Acetogenins from Annonaceae exhibit a broad range of biological activities (e.g., cytotoxic, insecticidal, and antiparasitic) (*1*) resulting from potent inhibition of the NADH-ubiquinone oxidoreductase (complex I) as a highly conserved mitochondrial target (*2*, *3*), causing a drop of ATP biosynthesis and cell death by apoptotic mechanisms (*4*). From a mechanistic viewpoint, the precise interaction between these new types of agents, which are structurally unrelated to classic complex I inhibitors, and their enormous enzymatic target is unknown. In particular, the role of the terminal butenolide as a quasi-ubiquitous and necessary moiety in the core of the acetogenins remains a puzzling question, this nucleus being substitutable by various lactonic prints (*5*, *6*) but not by simple carboxylic or ether functions (*7*). For the first time, Hoppen et al. showed that the butenolide of acetogenins was completely substitutable by the ubiquinone ring (*8*), suggesting that this part of the inhibitor was specifically acting at the level of a quinone-reduction site. In marked contrast, Hamada et al. recently introduced lactone-devoid, terminally unsubstituted acetogenins as inhibitors of similar potency than their lactonic counterparts (*9*). Such analogues, however, exhibited no competition with the model compound, suggesting a mode of interaction different from that of natural acetogenins (*10*−*12*). We report here the semisynthesis and biochemical evaluation of a small library of lactone-devoid heterocyclic analogues of squamocin **1** (Figure 1), a potent respiratory inhibitor among this class of compounds (*10*). These derivatives were targeted because of the analogy existing between readily synthesizable substructures and known pharmacophores of complex I inhibitors. The key reaction of this strategy is a preparative ruthenium(VIII)-catalyzed periodic oxidation of the terminal lactone of protected squamocin **1p** (*13*), leading to acetogenin-derived α-keto esters **2p** and **3**, which were in turn condensed with the appropriate 1,2-heterodinucleophiles (Scheme 1).

MATERIALS AND METHODS

Materials. Squamocin **1** was isolated in significant quantity from the seeds of *Annona reticulata*, collected in Vietnam, using a described procedure (*14*). NMR spectra (^{1}H, COSY, HOHAHA, NOE, HMQC, HMBC experiments) were recorded on a Bruker AC-200 (200 MHz) or a Bruker AM-400 (400 MHz) spectrometer. Mass spectra (MS or HRMS) were recorded on a Kratos MS-80 Rf spectrometer. Column chromatography was performed with silica gel 60 (9385 Merck) or Sephadex LH-20 (Pharmacia). TLC were performed on aluminum plates coated with silica gel 60F_{254} (554 Merck) and revealed with vanillin−sulfuric acid reagent. Solvents used in this study were dried and redistilled using described procedures. 2-*n*-Decylubiquinone (DB[1]), rotenone, capsaicin, and myxothiazol were purchased from Sigma; DQA was a generous gift from Aventis-Cropscience (Frank-

* To whom correspondence should be addressed. Tel: +33-1-46835593. Fax: +33-1-46835399. E-mail: romarinduval@hotmail.com.
‡ University Paris-Sud.
§ Universitad de Valencia.
|| Universität Frankfurt.

[1] Abbreviations used: DB, 2-*n*-decylubiquinone; DMSO, dimethyl sulfoxide; DQA, 2-*n*-decyl-4′-quinazolinylamine; ImH, imidazole; MPP^+, 1-methyl-4-phenylpyridinium; Q, ubiquinone (coenzyme Q_{10}); SMP, submitochondrial particles; THF, tetrahydrofuran.

10.1021/bi051261u CCC: $33.50
Published on Web 02/04/2006

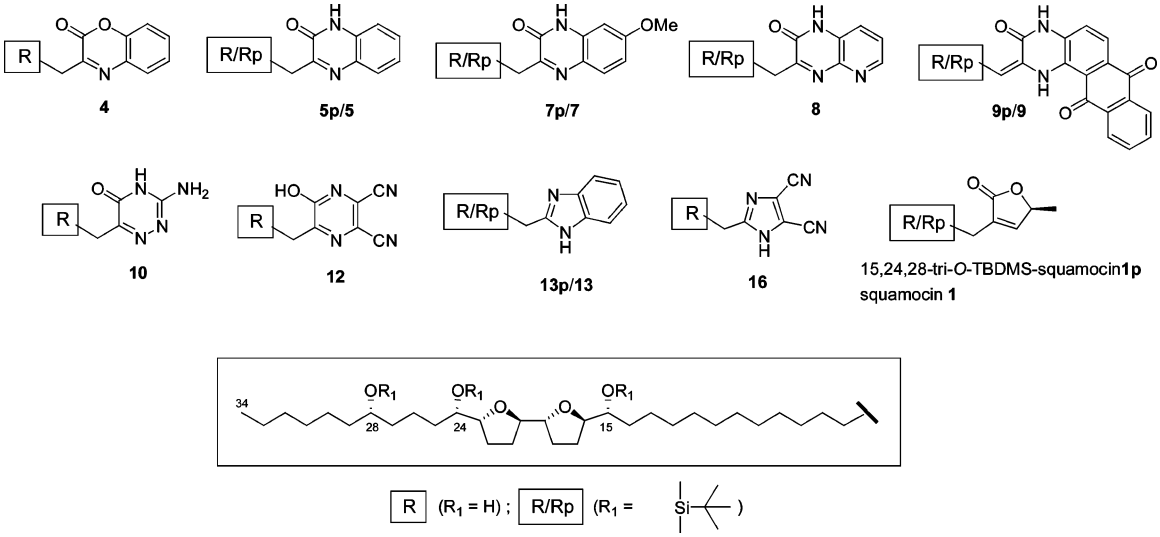
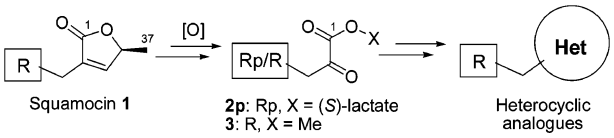


FIGURE 1: Structures of novel squamocin-derived inhibitors of complex I.

Scheme 1



furt am Main, Germany); rolliniastatin-2 and 2-*n*-undecylquinolone **6** were kind gifts from Dr. M. Degli Esposti (School of Biological Sciences, University of Manchester, UK) and Prof. W. Oettmeier (Ruhr-Universität, Bochum, Germany), respectively; 1-*n*-decylbenzimidazole **14** and 1-geranyl-2-methylbenzimidazole **15** were synthesized according to Kuwano et al. (*15*).

Semisynthesis. Compounds **1p**, **2p**, **5p**, **5**, **12**, **13p**, **13**, and **16** were obtained as previously described (*13*).

*Compound **3**.* To a solution of lactic α-keto ester **2p** (1.10 g, 1.09 mmol) in MeOH (19 mL) was added MeOH-washed Amberlyst-15 (2.20 g). The mixture was heated to 60 °C and stirred at that temperature for 5 h. The reaction medium was diluted by EtOAc (19 mL) and filtered over Celite-545. The filtrate was evaporated in vacuo and the residue chromatographed over a column of Sephadex LH-20 (CH_2Cl_2 100%), yielding methyl α-keto ester **3** (438 mg, 66%) as a colorless resin: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.88 (m, 3H, H-34, $J = 6.8$ Hz), 1.60 (m, 4H, H-18, H-21), 1.93 (m, 4H, H-17, H-22), 2.82 (t, 2H, H-3, $J = 8$ Hz), 3.40 (m, 1H, H-15), 3.57 (m, 1H, H-28), 3.82−3.93 (m, 5H, H-16, H-19, H-20, H23, H-24), 3.86 (s, 3H, H-35); ESIMS *m/z* 635 $(M + Na)^+$, 651 $(M + K)^+$.

*Compound **4**.* To an ice-cold solution of α-keto ester **3** (27 mg, 44 μmol) in MeOH (0.8 mL) was added dropwise a solution of *o*-aminophenol (10 mg, 88 μmol) in MeOH (0.4 mL). The mixture was stirred for 1 h at 0 °C then 2 h at room temperature. The reaction medium was evaporated in vacuo, and the residue filtered over a small column of Sephadex LH-20 (CH_2Cl_2/MeOH 97:3 v/v), furnishing benzoxazinone **4** (26 mg, 88%) as a pale-yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3H, H-34, $J = 6.7$ Hz), 1.65 (2H, H-4), 1.61 (m, 4H, H-18, H-21), 1.96 (m, 4H, H-17, H-22), 2.88 (t, 2H, H-3, $J = 7.7$ Hz), 3.39 (m, 1H, H-15), 3.59 (m, 1H, H-28), 3.84 (m, 4H, H16, H-19, H-20, H-23), 3.91 (m, 1H, H-24), 7.28 (dd, 1H, H-39, $J = 8.4$ Hz), 7.35 (td, 1H, H-37, $J = 7.7$ Hz, $J = 7.9$ Hz), 7.46 (td, 1H, H-38, $J = 7.7$ Hz), 7.73 (dd, 1H, H-36, $J = 7.9$ Hz); ESIMS *m/z* 672 $(M + H)^+$, 694 $(M + Na)^+$, 710 $(M + K)^+$; HRESIMS *m/z* $(M + H)^+$ calculated 672.4839, found 672.4846.

*Compound **7p**.* To an ice-cold solution of α-keto ester **2p** (150 mg, 0.15 mmol) in MeOH (3 mL) was added dropwise a solution of 3-methoxy-*o*-phenylenediamine (22.5 mg, 0.163 mmol) in MeOH (1.25 mL). The mixture was stirred for 2 h at 0 °C, diluted by water (3 mL), and extracted by CH_2Cl_2 (3 × 5 mL). The organic phases were gathered, dried (Na_2SO_4), and then evaporated in vacuo. The crude product was chromatographed over a column of silica gel (EtOAc/cyclohexane 30:70 v/v), furnishing 37-methoxyquinoxalinone **7p** (74 mg, 48%) as a pale-yellow solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.02−0.06 (m, 18H, Me-Si), 0.87 (m, 30H, ^{t}Bu-Si, H-34), 1.66 (m, 4H, H-18, H-21), 1.83 (m, 6H, H-4, H-17, H-22), 2.96 (t, 2H, H-3, $J = 8$ Hz), 3.60 (m, 2H, H-15, H-28), 3.76 (m, 1H, H-24), 3.87 (m, 4H, H-16, H-19, H-20, H-23), 3.87 (s, 3H, H-41), 7.10 (dd, 1H, H-37, $J = 8.9$ Hz), 7.20 (s, 1H, H-39), 7.27 (dd, 1H, H-36), 11.64 (s, 1H, NH); ESIMS *m/z* 1043 $(M + H)^+$, 1065 $(M + Na)^+$, 1081 $(M + K)^+$.

*Compound **7**.* To a solution of 37-methoxyquinoxalinone **7p** (30 mg, 28.8 μmol) in MeOH (1 mL) was added MeOH-washed Amberlyst-15 (240 mg). The mixture was heated to 40 °C and stirred at that temperature for 7 h. The reaction medium was diluted with EtOAc (1 mL) and filtered over Celite-545. The filtrate was evaporated in vacuo and the crude product chromatographed over a column of Sephadex LH-20 (CH_2Cl_2 100%), furnishing 37-methoxyquinoxalinone **7** (12 mg, 59%) as a pale-yellow solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (t, 3H, H-34, $J = 6.5$ Hz), 1.64 (m, 4H, H-18, H-21), 1.82 (m, 1H, H-4), 1.95 (m, 4H, H-17, H-22), 2.93 (t, 2H, H-3, $J = 8$ Hz), 3.42 (m, 1H, H-15), 3.62 (m, 1H, H-28), 3.89 (s, 3H, H-41), 3.90 (m, 5H, H-16, H-19, H-20, H-23, H-24), 6.66 (d, 1H, H-36, $J = 2$ Hz), 6.90 (dd, 1H, H-38), 7.72 (d, 1H, H-39, $J = 8$ Hz), 10.78 (s, 1H, NH); ESIMS *m/z* 723 $(M + Na)^+$, 739 $(M + K)^+$; HRESIMS *m/z* $(M + H)^+$ calculated 701.5105, found 701.5110.

*Compound **8**.* To a solution of α-keto ester **3** (40 mg, 65.4 μmol) in toluene (1.2 mL) was added 2,3-diaminopyridine

(9.2 mg, 84.4 μmol) and then imidazole (9.0 mg, 0.13 mmol). The reaction mixture was heated to 45 °C, stirred at that temperature for 3 h, and then evaporated in vacuo. The crude product was chromatographed over a column of Sephadex LH-20 (CH_2Cl_2/MeOH 97:3 v/v), furnishing 39-pyridopyrazinone **8** (27 mg, 61%) as a white amorphous solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (t, 3H, H-34, J = 6.5 Hz), 1.61 (m, 4H, H-18, H-21), 1.88 (m, 1H, H-4), 1.97 (m, 4H, H-17, H-22), 3.02 (t, 2H, H-3, J = 7.6 Hz), 3.39 (m, 1H, H-15), 3.59 (m, 1H, H-28), 3.84 (m, 4H, H-16, H-19, H-20), 3.93 (m, 2H, H-23, H-24), 7.43 (dd, 1H, H-37, J = 4.5 Hz, J = 8.1 Hz), 7.75 (dd, 1H, H-36), 8.65 (dd, 1H, H-38); ESIMS *m/z* 672 $(M + H)^+$, 694 $(M + Na)^+$, 710 $(M + K)^+$; HRESIMS *m/z* $(M + H)^+$, calculated 672.4951, found 672.4957.

*Compound **9p**.* To a solution of α-keto ester **2p** (156 mg, 0.15 mmol) in toluene (2.5 mL) was added 1,2-diaminoanthraquinone (48 mg, 0.2 mmol) and then AcOH (254 μL, 4.62 mmol). The mixture was heated to 70 °C and stirred at that temperature for 15 h. The reaction medium was evaporated in vacuo, the residue retaken in cyclohexane, and the insoluble filtered over Whatman GF/A. The filtrate was evaporated in vacuo, and the crude product chromatographed over a column of silica gel (EtOAc/cyclohexane 30:70 v/v), furnishing anthraquinobenzopiperazinone **9p** (118 mg, 66%) as a dark-violet solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02−0.07 (m, 18H, Me-Si), 0.87 (m, 30H, ^{t}Bu-Si, H-34), 1.65 (m, 4H, H-18, H-21), 1.85 (m, 4H, H-17, H-22), 2.31 (dt, 2H, H-4, J = 7.3 Hz), 3.60 (m, 2H, H-28, H-15/H-24), 3.76 (m, 1H, H-15/H-24), 3.87 (m, 2H, H-19, H-20), 3.90 (m, 2H, H-16, H-23), 5.93 (t, 1H, H-3, J = 7.6 Hz), 7.02 (d, 1H, H-36, J = 8 Hz), 7.64 (dd, 2H, H-41, H-44), 7.68 (d, 1H, H-37), 8.17 (m, 2H, H-42, H-43), 10.09 (s, 1H, amide-NH, NOE H-36/NH), 11.59 (*s*, 1H, enamine-NH, NOE H-3/NH); ESIMS *m/z* 1143 $(M + H)^+$, 1165 $(M + Na)^+$.

*Compound **9**.* To a solution of anthraquinobenzopiperazinone **9p** (57 mg, 50 μmol) in a MeOH/*t*-BuOH/heptane 73:24:3 v/v/v mixture (4 mL) was added MeOH-washed Amberlyst-15 (450 mg). The mixture was heated to 45 °C and stirred at that temperature for 10 h. The reaction medium was diluted by EtOAc (2 mL) and filtered over Celite-545. The filtrate was evaporated in vacuo and the crude product chromatographed over a column of Sephadex LH-20 (CH_2Cl_2/MeOH 97:3 v/v), furnishing anthraquinobenzopiperazinone **9** (34 mg, 85%) as a dark-violet solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (t, 3H, H-34), 1.64 (m, 4H, H-18, H-21), 1.89 (m, 4H, H-17, H-22), 2.32 (dt, 2H, H-4, J = 8 Hz), 3.41 (m, 1H, H-15), 3.61 (m, 1H, H-28), 3.90 (m, 5H, H-16, H-19, H-20, H-23, H-24), 5.95 (t, 1H, H-3, J = 8 Hz), 7.05 (d, 1H, H-36, J = 8 Hz), 7.71 (dd, 2H, H-41, H-44), 7.75 (d, 1H, H-37), 8.26 (m, 2H, H-42, H-43), 9.64 (s, 1H, amide-NH), 11.68 (*s*, 1H, enamine-NH); ESIMS *m/z* 823 $(M + Na)^+$; HRESIMS *m/z* $(M + H)^+$ calculated 801.5054, found 801.5052.

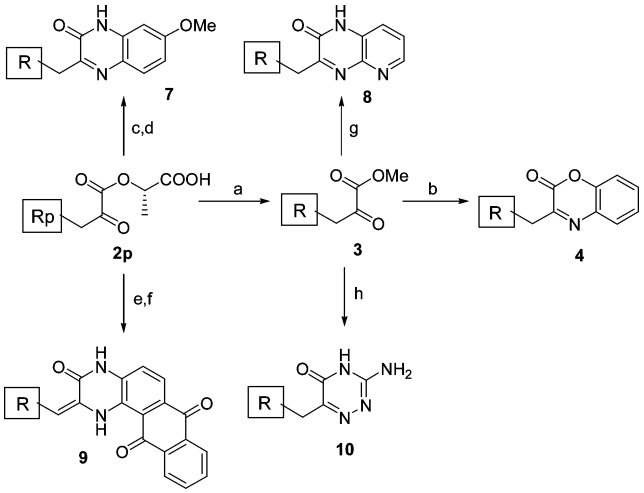
*Compound **10**.* To a solution of α-keto ester **3** (80 mg, 0.13 mmol) in *t*-BuOH (2.5 mL) was added a solution of aminoguanidine·HCl (72 mg, 0.65 mmol) and NaOAc (245 mg, 3.26 mmol) in water (2.5 mL). The biphasic mixture was heated to reflux and stirred for 5 days. The upper organic phase was decanted, and the aqueous phase reextracted by *t*-BuOH (2 × 3 mL). The organic phases were gathered, dried (brine then Na_2SO_4), and then evaporated in vacuo. The residue was twice resuspended in an ice-cold CH_2Cl_2/EtOAc 80:20 v/v mixture and the mother liquor discarded. The crude product was retaken in a CH_2Cl_2/MeOH 50:50 v/v mixture and filtered over cotton. The filtrate was evaporated in vacuo, furnishing triazine **10** (44 mg, 53%) as an amorphous white solid: ^{1}H NMR (200 MHz, $CDCl_3$/MeOD 50:50 v/v) δ 0.87 (t, 3H, H-34), 1.60 (m, 4H, H-18, H-21), 1.94 (m, 4H, H-17, H-22), 2.54 (t, 2H, H-3, J = 7 Hz), 3.39 (m, 1H, H-15), 3.52 (m, 1H, H-28), 3.65 (m, 1H, H-24/H-23), 3.84 (m, 4H, H-24/H-23, H-16, H-19, H-20); ESIMS *m/z* 637 $(M + H)^+$, 659 $(M + Na)^+$, 675 $(M + K)^+$. HRESIMS *m/z* $(M + H)^+$ calculated 637.4895, found 637.4850.

Mitochondrial Complex I Assays. Inhibition of the NADH oxidase activity of complex I from bovine SMP was evaluated as described previously (*12*). Inhibition of the NADH:DB activity was evaluated on a 96-well plate, using a membrane concentration of 100 μg/mL (21 μg SMP per well), in a Tris-HCl buffer (50 mM, pH 7.4) containing 1 mM EDTA, 2 μM antimycin, 2 mM KCN, and 60 μM DB. Inhibitors were added as DMSO solutions, the proportion of which never exceeded 2% and had no influence on the control enzymatic activity. The wells were homogenized by vibrating the plate for 4 × 15 s, and reaction was started by adding NADH (final concentration of 100 μM). Enzymatic activity was measured following NADH oxidation at 340−400 nm (ϵ = 6.22 mM^{-1} cm^{-1}) on a Molecular Devices SPECTRAmax PLUS^{384} spectrophotometer at 23 °C. Each assay was performed in triplicate. Residual enzymatic activity, defined as the membrane NADH oxidase activity not inhibited by a high concentration of inhibitor (1 μM), was subtracted when calculating the I_{50} values (defined as concentrations required for half-maximal inhibition). Residual NADH:DB oxidoreductase activities were as follow: squamocin **1**, 12%; benzimidazole **13**, 28%; quinoxalinone **5**, 21%; benzoxazinone **4**, 26%; imidazole **16**, 25%; 1-geranyl-2-methylbenzimidazole **15**, 13%; rolliniastatine-2, 8%; DQA, 27%; rotenone, 10%. Significant residual activity may be explained by some partial blockade of the access to the active site(s) for Q in the presence of these inhibitors. Competition experiments were performed according to Degli Esposti et al. (*10*).

RESULTS

Inhibitor Design and Semisynthesis. Squamocin **1** was first protected as its tri-*O*-TBDMS silyl ether derivative **1p** and then oxidized into lactic α-keto ester derivative **2p** as described in our previous work (*13*). As the conditions used to desilylate the three secondary alcohols of the acetogenin skeleton (i.e. Amberlyst-15/MeOH) were sometimes found to be incompatible with the neoformed heterocyclic system (methanolysis of compound **4** and irreversible linkage of basic compounds **8** and **10** to the resin), certain analogues were obtained by direct condensation of the corresponding 1,2-heterodinucleophiles with methyl α-keto ester **3** (Scheme 2). Importantly, the initial silylation of squamocin would allow the biological evaluation of protected heterocyclic triether analogues: while acetogenins are known to lose potency upon peracetylation (*5*, *16*), it is noteworthy that the great majority of complex I inhibitors possesses strictly lipophilic side chains (*17*−*21*). Therefore, a significant activity of *O*-silylated heterocyclic analogues of squamocin **1** cannot be excluded.

Scheme 2[a]



[a] Reagents and conditions: (a) Amberlyst-15, MeOH, 60 °C, 5 h, 66%; (b) *o*-aminophenol, MeOH, 0 °C to rt, 3 h, 88%; (c) 5-methoxy-*o*-phenylenediamine, MeOH, 0 °C, 2 h, 48%; (d) Amberlyst-15, MeOH, 40 °C, 7 h, 59%; (e) 1,2-diaminoanthraquinone, AcOH, toluene, 70 °C, 15 h, 66%; (f) Amberlyst-15, MeOH/*t*-BuOH/hexane (73:24:3) v/v/v, 45 °C, 10 h, 85%; (g) 2,3-diaminopyridine, ImH, toluene, 45 °C, 3 h, 61%; (h) aminoguanidine·HCl, NaOAc, *t*-BuOH/H_2O 50:50 v/v, 100 °C, 5 h, 53%.

As explained in the Introduction, the heteroaromatic nuclei tested as substitutes for the natural butenolide of squamocin **1** are structurally analogous to known pharmacophores of complex I inhibitors (*21*). In particular, the structure−activity relationships within this class of agents suggest that (a) 1,3-diazine systems (e.g. pyrimidines, quinazolines, benzimidazoles), as well as heteroatoms engaged in a *para* relationship, constitute privileged patterns for heterocyclic nuclei of inhibitors and (b) the interaction of carbonyl functions (e.g., *p*-quinones, 4-quinolones, chromones), and to a certain extent that of hydroxy groups (4-pyridinols), is superimposable to the one of intracyclic nitrogen atoms (e.g., pyrimidines, quinazolines) with the inhibition sites of the enzyme. Less importance was assigned to the ring position of the polyoxygenated chain of the acetogenin, in view of results in the pyridinol (*22*, *23*) and pyrazole (*24*−*26*) series. Moreover, its atypical structure and strong amphipathy are presumably responsible for a distinct interaction with complex I relative to the apolar chains of classical inhibitors.

Aiming to first semisynthesize a lactonic heterocyclic analogue of squamocin, benzoxazinone **4** was targeted and obtained by condensing *o*-aminophenol with unprotected methyl α-keto ester derivative **3**. Similarly, the protected lactic α-keto ester **2p** was condensed with aromatic *o*-diamines to yield quinoxalinone derivatives **5**, **7**, **8**, and **9** (*27*) (Scheme 2). Acetogenins-derived quinoxalinones are very similar electronically to 2-alkylquinolones, which are classical mitochondrial inhibitors (*17*), an agent such as 2-*n*-undecylquinolone **6** being a moderately potent but specific complex I inhibitor (Figure 2). In addition, the condensation of lactic α-keto ester **2p** with 1,2-diaminoanthraquinone permitted the introduction of a terminal quinone moiety on the squamocin skeleton, furnishing the interesting polycyclic anthraquinobenzopiperazinone derivative **9** as one major regioisomer after deprotection.

We next targeted triazine **10** as a pseudochimeric analogue of squamocin that could be compared to HOE 110779 **11**, a

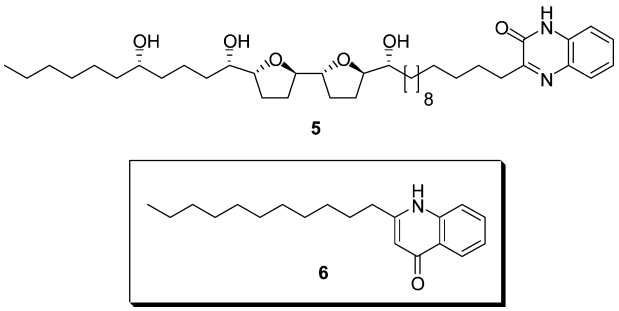


FIGURE 2: Structural analogy between squamocin-quinoxalinone **5** and 2-*n*-undecylquinolone **6**.

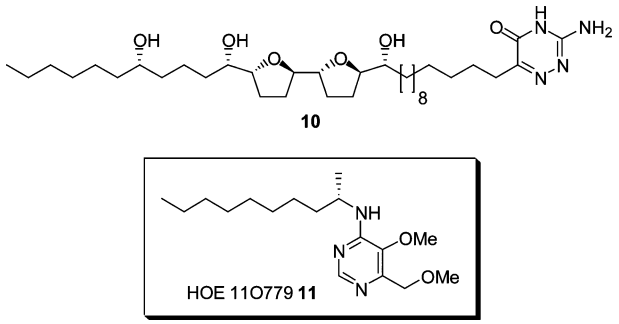


FIGURE 3: Structural analogy between squamocin-triazine **10** and HOE 110779 **11**.

potent synthetic pesticide whose biological target is complex I (*18*, *21*) (Figure 3). Such kind of derivatives also seemed of interest as they feature monocyclic, electron-rich mimics of the natural lactone, in view of the more lipophilic and bulky nuclei introduced above. As explained previously, unprotected triazine **10** was obtained by directly condensing aminoguanidine.HCl on methyl α-keto ester **3** (*27*) (Scheme 2).

As part of this pharmacomodulation of squamocin **1** by heterocyclic semisynthesis, several original analogues, described in our recent paper (*13*), were also of interest (Figure 4). Benzimidazole derivative **13** is a hybrid-type inhibitor, considering that the benzimidazole nucleus is the pharmacophore of synthetic insecticides acting as complex I inhibitors (*19*, *28*, *29*). The dicyanoimidazole nucleus of analogue **16** shows marked steric and electronic analogy with that of tebufenpyrad **17**, a pyrazole pesticide also targeting the mitochondrial enzyme (*18*, *26*). Besides, lipophilic 2-substituted-4,5-dicyanoimidazoles have insecticidal/acaricidal properties (*30*) that could reflect an inhibition of complex I. On the other hand, pyrazinol **12** is featured by a phenolic moiety that is not known to be a pharmacophore of complex I inhibitors and the influence of which on the activity has to be evaluated.

Inhibition of Mitochondrial Complex I. The activity of the obtained heterocyclic derivatives of squamocin **1** was evaluated against complex I from bovine heart submitochondrial particles (SMP), measuring inhibition of NADH oxidase and NADH:DB oxidoreductase activities (Table 1). Benzimidazole **13** was found to be a powerful inhibitor of the enzyme, being of comparable potency to squamocin **1** and 1-geranyl-2-methylbenzimidazole **15**, and 10 times more active than simple 1-*n*-decylbenzimidazole **14** (*10*, *28*, *29*). Similarly, quinoxalinone **5** possessed a high activity that was superior to that of 2-*n*-undecylquinolone **6** (*17*) and showed a loss of potency identical to that of benzimidazole **13** under quasi-

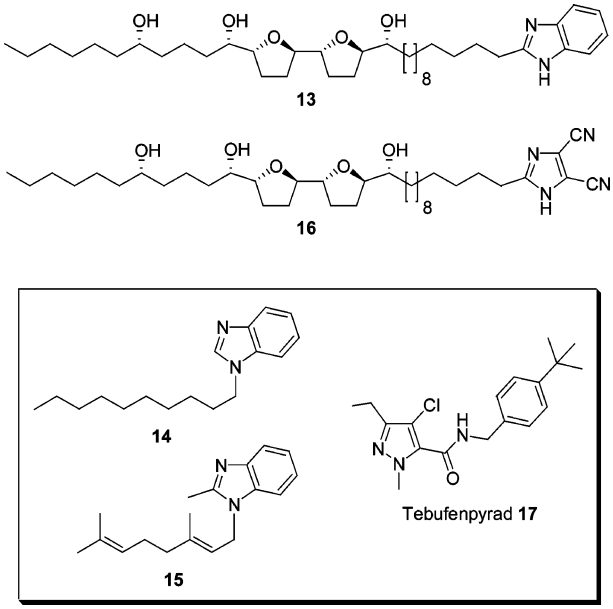


FIGURE 4: Structural analogy between squamocin derivatives **13** and **16** and complex I inhibitors in the benzimidazole and pyrazole series.

Table 1: Inhibitory Activities of Mitochondrial Complex I[a] (I_{50}, nM[b]) of the Described Analogues of Squamocin 1

compound	NADH oxidase	NADH:DB oxidoreductase
squamocin, **1**	0.8	1.3
benzimidazole, **13**	0.9	3.3
quinoxalinone, **5**	2.0	7.9
benzoxazinone, **4**	nt[c]	8.1
37-methoxyquinoxalinone, **7**	nt	10
39-pyridopyrazinone, **8**	nt	17
antraquinobenzopiperazinone, **9**	13	nt
imidazole, **16**	14	41
triazine, **10**	19	nt
pyrazinol, **12**	122	nt
tri-*O*-TBDMS-squamocin, **1p**	>3000	nt
tri-*O*-TBDMS-benzimidazole, **13p**	>3000	nt
tri-*O*-TBDMS-quinoxalinone, **5p**	>3000	nt
1-geranyl-2-methylbenzimidazole, **15**[d]	1.6	6.8
1-*n*-decylbenzimidazole, **14**[d]	11	nt
2-*n*-undecylquinolone, **6**[d]	8.2	nt
DQA[d]	nt	4.9
rolliniastatin-2[d]	nt	1.5
rotenone[d]	4.9	28
myxothiazol[d]	nt	170

[a] Tests performed on bovine heart submitochondrial particles (SMP). [b] Concentrations required for half-maximal inhibition (see Materials and Methods). [c] Not tested. [d] Reference inhibitors.

saturating concentrations of exogenous quinone. Both quinoxalinone **5** and benzimidazole **13** exhibited in fact an inhibitory potency similar to DQA (*31*) tested as a reference for heteroaromatic inhibitors of complex I. Interestingly, benzoxazinone **4** and quinoxalinone **5** identically inhibited the NADH:DB oxidoreductase activity of the enzyme, showing the equivalence of the lactone and lactame functions at this level. This behavior most probably makes benzoxazinone **4** a powerful inhibitor of complex I in limited turnover conditions (NADH oxidase activity). However, substitution of the quinoxalinone nucleus by a methoxy group or an intracyclic nitrogen atom had a limited though unfavorable influence on the inhibitory activity (analogues **7** and **8**). On the other hand, anthraquinobenzopiperazinone **9**, triazine **10**, dicyanoimidazole **16**, and especially pyrazinol **12** appeared to be weaker complex I inhibitors than natural squamocin **1**, exhibiting even lower potencies than mono-THF (type A) acetogenins such as annonacin (*32*). As a way to qualitatively define the mode of action of these novel acetogenin analogues, competition experiments were performed as double-reciprocal and Dixon plots taking squamocin-benzimidazole **13** as representative agent (*10*) (data not shown). This hybrid inhibitor behaved noncompetitively toward *n*-decylubiquinone used as an artificial substrate, a character shared by squamocin **1** as well as synthetic benzimidazoles (*10*, *33*). More surprisingly, squamocin-benzimidazole **13** was found to exhibit mixed competition with squamocin **1** and was exclusive with rolliniastatin-2, despite the fact that squamocin **1** and benzimidazoles seem to occupy the same region (rotenone/B site) within complex I (*10*, *12*, *28*, *33*), while rolliniastatin-2 has been assigned to the first region (A site) of the large inhibitor-binding domain of the enzyme (*10*−*12*). In addition, derivatives **5p** and **13p** were tested as trisilylated representatives and found to be devoid of inhibitory activity at a concentration of 3 μM, identically to tri-*O*-TBDMS-squamocin **1p** (Table 1).

DISCUSSION

Annonaceous Acetogenins as Antagonists of Q Substrates. The great majority of specific complex I inhibitors, acting at the level of the ubiquinone cycle between complexes I and III, exhibit noncompetitive or uncompetitive behavior against the artificial substrate *n*-decylubiquinone (*10*−*12*, *18*, *21*, *32*−*36*). The absence of mutual exclusivity between such inhibitors and ubiquinones, despite their often striking structural similarity and common structure−activity relationships (*17*−*19*, *37*−*39*), represents one major difficulty to identify the actual sites of action of these extremely potent agents and their relation to the Q-reduction sites. It has to be realized, however, that the high potency of such inhibitors (exhibiting apparent stoichiometric binding to the enzyme and subnanomolar K_i values) in comparison to the much weaker affinities of synthetic ubiquinones (K_m in the micromolar range) (*10*, *37*, *40*−*42*) makes competition between these inhibitors and Q substrates very unlikely to be observed. Moreover, it has been suggested that certain inhibitors may in fact act as ubisemiquinones antagonists and thus only indirectly compete with ubiquinone (*21*, *43*, *44*). Interestingly, an action as transition state analogues could explain the high potency of such inhibitors. In a similar manner, inhibitors acting as ubiquinol antagonists do not necessarily compete with ubiquinone (*20*, *33*, *34*). The assumption that many inhibitors of complex I actually occupy Q-reduction sites is supported by their possible action against mitochondrial complex III as well as bacterial photosystems (*10*, *18*, *34*, *35*), in a manner characterized as antagonism toward the Q substrates (*45*, *46*) (see below). At first sight, the structural analogy between ubiquinone species and inhibitors such as the annonaceous acetogenins is rather limited. Nevertheless, Degli Esposti et al. demonstrated that the dual behavior of rolliniastatin-1 and molvizarin was identical to that of piericidin A (*10*, *47*, *48*), which was shown to directly antagonize the binding of endogenous ubiquinone (*42*). Rolliniastatin-2 was later quoted to specifically compete with 10′-hydroxy-*n*-decylubiquinone (idebenone) for the occupancy of the most upstream inhibition site (A site) of the

inhibitor-binding domain of complex I (*10*, *21*, *49*), indicating its equivalence with an ubiquinone-binding site. [The fact that idebenone was nonexclusive with rotenone (binding at the B site) excludes the possibility of indirect competition with rolliniastatin-2 via some negative cooperativity exerted from site B to site A. See the text.] More recently, Hoppen et al. and then Yabunaka et al. showed that the butenolide of several acetogenins was completely substitutable by the ubiquinone ring, as by polymethoxylated phenyl moieties in some cases (*8*, *40*). In the present study, we show that the benzimidazole nucleus of a potent complex I inhibitor was substitutable for the terminal lactone of squamocin **1**. This result illustrates for the first time the analogy existing between this highly conserved substructure of acetogenins and heterocyclic pharmacophores of representative inhibitors acting as Q-mimics. Similarly, we have shown that turning the butenolide of squamocin **1** into captodatives, β-(*E*)-methoxyacrylate-type moieties, was responsible for the co-inhibition of complex III at the level of the Q_o site, indicating an antagonism with ubiquinol and/or semiquinone species (*50*, Duval et al., unpublished material). Taken together, these results support the concept that the common lactone moiety of annonaceous acetogenins acts by mimicking the rings of the Q substrates when binding to respiratory enzymes.

Role of theTerminal Lactone of Annonaceous Acetogenins. The potent, terminally unsubstituted bis-THF acetogenins introduced by Hamada et al. (*9*) do not compete with the model acetogenin and exhibit distinct structure−activity relationships (SAR) and were therefore presented as a new type of complex I inhibitor. It has been shown, however, that (i) acetogenins that only differ by the nature of their lactone nucleus could possess radically distinct binding features to complex I and be mutually nonexclusive (*10*−*12*) and (ii) acetogenins targeting different binding sites of complex I exhibit strikingly divergent SAR (*6*, *51*); reciprocally, the observation of opposite SAR between analogous series of derivatives is a logic presumption for distinct binding modes to the enzyme, as exemplified by tetramethoxyphenyle derivatives of mucocin, squamocin D, and 4-desoxy-24-*epi*-rolliniastatin-2 (*7*, *8*, *40*). In the present study, the distinct behavior of squamocin-benzimidazole **13** relative to squamocin **1** clearly illustrates that the nature of the terminal nucleus considerably influences the binding of acetogenins to complex I. Moreover, the important differences of activity observed between our heterocyclic analogues confirm that the terminal moiety of acetogenins should not be considered an "innocent" structural feature. Regarding the natural inhibitors, some observations suggest that the frequent 4-hydroxylation of the alkyl chain is involved in dual binding of the acetogenin (i.e. affinity for two inhibitor/Q sites) relative to a 4-desoxy analogue (*11*, *12*, *32*). Interestingly, the butenolide of 4-hydroxylated acetogenins (lactonic subtype 1b) can be seen as a distinct pharmacophore from the isolated nucleus (subtype 1a), because of the existence of an intramolecular hydrogen bond (*52*) that notably contributes to its pseudoaromaticity. Altogether, these data show that the terminal lactone of annonaceous acetogenins qualitatively acts as a main kinetic signature, but does not necessarily correspond to a crucial pharmacophore in all cases. This ambiguity fundamentally addresses the role of certain parts of the polyoxygenated chain as obligate Q-mimicking moieties in the case of terminally unsubstituted acetogenins, linked to the possibility for some terminally substituted acetogenins to actually behave as "head−head" chimeric inhibitors (i.e. double action at the level of two inhibitor/Q sites via two tethered pharmacophores) (*50*, Duval et al., unpublished material). At first sight, this behavior of bis-THF acetogenins is opposite to that of its mono-THF counterparts, for which inhibition seems to rely crucially on the presence of a terminal lactone (*53*). Considering that all mono-THF acetogenins tested so far homogeneously behaved as one-site ligands (rotenone/B site) (*10*, *12*, *54*), this suggests that an entire adjacent bis-THF system is required to act as specific Q-mimic in the corresponding acetogenins.

Relationship between Inhibitor/Q-Binding Sites of Mitochondrial Complex I. The kinetic behavior of natural acetogenins as complex I inhibitors is well established relative to those of rotenone and piericidin A (*10*−*12*) and is validated by the observation of several cross-resistance phenomena in *Rhodobacter* (*55*, *56*). Acetogenins acting at the level of the less specific inhibition site (rotenone/B site) like squamocin **1** behave noncompetitively toward *n*-decylubiquinone, whereas acetogenins acting at the level of at least the most upstream inhibitor/Q-binding site (A site) like rolliniastatin-2 systematically exhibit uncompetitive behavior toward this substrate. In this context, the unexpected features of squamocin-benzimidazole **13** against both squamocin **1** and rolliniastatin-2, but its noncompetitive action toward *n*-decylubiquinone, might be explained by a negative allosteric effect induced by the binding of **13** to complex I, underlying its apparent mutual exclusivity with rolliniastatin-2. The putative allosteric action of complex I inhibitors was first observed for 4′-alkyl-MPP^+ derivatives by Ramsay et al. (*48*, *57*) and evidenced more recently (*58*, *59*). It remains to be established if this unusual behavior of squamocin-benzimidazole **13** is common to other heterocyclic squamocin analogues such as the ones described in this study.

CONCLUSION

Besides the interest to elucidate the mechanism of action of annonaceous acetogenins, which comprise the most potent known inhibitors of mitochondrial complex I (*6*, *10*, *16*, *21*), the conception of chimeric agents remains a relevant method for the characterization of inhibition sites and their relation with the physiological Q-reduction sites of the enzyme. In the present work, heteroaromatic moieties such as benzimidazole, benzoxazinone, or quinoxalinone were shown to be substitutable for the butenolide of the acetogenin squamocin **1**, suggesting that this common nucleus possesses a functional analogy with quinone-mimicking pharmacophores of typical complex I inhibitors. Although the heterocyclic moiety of squamocin-benzimidazole **13** quantitatively reproduced the interaction of the natural butenolide, it seemed that this modification was responsible for a distinct binding mode to complex I and for the occurrence of allosteric inhibition presumably at the level of site A. These results support the existence of a large ligand-regulated inhibitor/Q-binding pocket within the ubiquinone-reducing core of the enzyme (*31*, *56*), formed in part by the association of the previously defined affinity sites A and B. These sites possess in fact very little inhibitor specificity (*10*−*12*, *21*, *28*, *29*, *33*, *42*, *47*, *48*, *60*) and seem to be largely overlapping. [In marked contrast, the inhibitor-binding site C of complex I (capsaicin-binding site) is targeted by distinct and specific chemical

families (*28*, *29*, *31*, *33*) and not by any acetogenin up to now, despite possible overlap with site B, as suggested by the binding of a chimeric vanilloid (*31*).] Altogether, our study validates further design of acetogenin-derived tools to dissect the pathway of the final electron-transfer step occurring between complex I and ubiquinone.

ACKNOWLEDGMENT

R.A.D. is indebted to F. Streb and I. Siebels (Zentrum der Biologischen Chemie, Frankfurt Universität, Germany) for valuable experimental assistance. Prof. W. Oettmeier (Ruhr-Universität, Bochum, Germany) is acknowledged for the kind gift of 2-*n*-undecylquinolone. Also many thanks to Dr. M. Degli Esposti (School of Biological Sciences, University of Manchester, UK) and Prof. T. Friedrich (Freiburg Universität, Germany) for their advice and stimulating interest.

REFERENCES

1. Alali, F. Q., Liu, X.-X., McLaughlin, J. L. (1999) Annonaceous acetogenins: Recent progress, *J. Nat. Prod. 62*, 504−540.
2. Londershausen, M., Leicht, W., Lieb, F., Moeschler, H., Weiss, H. (1991) Molecular mode of action of annonins, *Pestic. Sci 33*, 427−438.
3. Ahammadsahib, K. I., Hollingworth, R. M., McGovren, J. P., Hui, Y.-H., McLaughlin, J. L. (1993) Mode of action of bullatacin: A potent antitumor and pesticidal annonaceous acetogenin, *Life Sci. 53*, 1113−1120.
4. Chih, H.-W., Chiu, H.-F., Tang, K.-S., Chang, F.-R., Wu, Y.-C. (2001) Bullatacin, a potent antitumor annonaceous acetogenin, inhibits proliferation of human hepatocarcinoma cell line 2.2.15 by apoptosis induction, *Life Sci. 69*, 1321−1331.
5. Takada, M., Kuwabara, K., Nakato, H., Tanaka, A., Iwamura, H., Miyoshi, H. (2000) Definition of crucial structural factors of acetogenins, potent inhibitors of mitochondrial Complex I, *Biochim. Biophys. Acta 1460*, 302−310.
6. Tormo, J. R., Estornell, E., Gallardo, T., González, M. C., Cavé, A., Granell, S., Cortes, D., Zafra-Polo M. C. (2001) γ-lactone-functionalized antitumoral acetogenins are the most potent inhibitors of mitochondrial Complex I, *Bioorg. Med. Chem. Lett. 11*, 681−684.
7. Arndt, S., Emde, U., Bäurle, S., Friedrich, T., Grubert, L., Koert, U. (2001) Quinone-annonaceous acetogenins: Synthesis and Complex I inhibition studies of a new class of natural product hybrids, *Chem. Eur. J. 7*, 993−1005.
8. Hoppen, S., Emde, U., Friedrich, T., Grubert, L., Koert, U. (2000) Natural product hybrids: Design, synthesis and biological evaluation of quinone-annonaceous acetogenins, *Angew. Chem., Int. Ed. 39*, 2099−2102.
9. Hamada, T., Ichimaru, N., Abe, M., Fujita, D., Kenmochi, A., Nishioka, T., Zwicker, K., Brandt, U., Miyoshi, H. (2004) Synthesis and inhibitory action of novel acetogenin mimics with bovine heart mitochondrial complex I, *Biochemistry 43*, 3651−3658.
10. Degli Esposti, M., Ghelli, A., Ratta, M., Cortes, D., Estornell, E. (1994) Natural substances (acetogenins) from the family Annonaceae are powerful inhibitors of mitochondrial NADH dehydrogenase (Complex I), *Biochem. J. 301*, 161−167.
11. Estornell, E., Tormo, J. R., Cortes, D. (1997) Cherimolin-1, new selective inhibitor of the first energy-coupling site of the NADH: ubiquinone oxidoreductase (complex I), *Biochem. Biophys. Res. Commun. 240*, 234−238.
12. Tormo, J. R., González, M. C., Cortes, D., Estornell, E. (1999) Kinetic characterization of mitochondrial complex I inhibitors using annonaceous acetogenins, *Arch. Biochem. Biophys. 369*, 119−126.
13. Duval, R., Lewin, G., Hocquemiller, R. (2003) Semisynthesis of heterocyclic analogues of squamocin, a cytotoxic annonaceous acetogenin, by an unusual oxidative decarboxylation reaction, *Bioorg. Med. Chem. 16*, 3439−3446.
14. Tam, V. T., Hieu, B. C, Chappe, B. (1993) Squamocin and rolliniastatin-1 from the seeds of *Annona reticulata*, *Planta Med. 59*, 576.
15. Kuwano, E., Sato, N., Eto, M. (1982) Insecticidal benzimidazoles with a terpenoid moiety, *Agric. Biol. Chem. 46*, 6, 1715−1716.
16. Gallardo, T., Zafra-Polo, M. C., Tormo, J. R., González, M. C., Franck, X., Estornell, E., Cortes, D. (2000) Semisynthesis of antitumoral acetogenins: SAR of functionalized alkyl-chain bis-tetrahydrofuranic acetogenins, specific inhibitors of mitochondrial Complex I, *J. Med. Chem. 43*, 4793−4800.
17. Oettmeier, W., Masson, K., Soll, M., Reil, E. (1994) Acridones and quinolones as inhibitors of ubiquinone functions in the mitochondrial respiratory chain, *Biochem. Soc. Trans. 7 22*, 213-216.
18. Lümmen, P. (1998) Complex I inhibitors as insecticides and acaricides, *Biochim. Biophys. Acta 1364*, 287−296.
19. Nakagawa, Y., Kuwano, E., Eto, M., Fujita, T. (1985) Effects of insect-growth-regulatory benzimidazole derivates on cultured integument of the rice stem borer and mitochondria from rat liver, *Agric. Biol. Chem. 49*, 3569−3578.
20. Hollingworth, R. M., Ahammadsahib, K. I., Gadelhac, G., McLaughlin, J. L. (1994) New inhibitors of Complex I of the mitochondrial electron transport chain with activity as pesticides, *Biochem. Soc. Trans. 7 22*, 230−233.
21. Degli Esposti, M. (1998) Inhibitors of NADH-ubiquinone reductase: An overview, *Biochim. Biophys. Acta 1364*, 222−235.
22. Yoshida, S., Nagao, A., Watanabe, N., Takahashi, N. (1980) Structure−activity relationship in piericidins inhibitors on the electron transport system in mitochondria, *Agric. Biol. Chem. 44*, 2921−2924.
23. Chung, K. H., Cho, K. Y., Asami, Y., Takahashi, N., Yoshida, S. (1989) New 4-hydroxypyridine and 4-hydroxyquinoline derivatives as inhibitors of NADH-ubiquinone reductase in the respiratory chain, *Z. Naturforsch. 44C*, 609−616.
24. Okada, I., Okui, S., Takahashi, Y., Fukuchi, T. (1991) Synthesis and acaricidal activity of pyrazole-5-carboxamide derivatives, *Nihon Noyaku Gakkaishi (J. Pestic. Sci.) 16*, 623−629.
25. Motoba, K., Susuki, T., Uchida, M. (1992) Effect of a new acaricide, fenpyroxymate, on energy metabolism and mitochondrial morphology in adult female *Tetranychus urticae* (two-spotted spider mite), *Pestic. Biochem. Physiol. 43*, 37−44.
26. Hollingworth, R. M., Ahammadsahib, K. I. (1995) Inhibitors of respiratory complex I: Mechanisms, pesticidal actions and toxicology, *Rev. Pestic. Toxicol. 3*, 277−302.
27. Labib, G.H., Rahman, M. A., El-Kilany, Y., El-Massry, A. I., El-Ahry, E. S. H. (1988) Synthesis of γ-butyrolactones, quinoxalines, and azauracils from 4-aryl-2-oxobutanoic acid derivatives, *Bull. Chem. Soc. Jpn. 61*, 12, 4427−4430.
28. Friedrich, T., Van Heek, P., Leif, H., Ohnishi, T., Forche, E., Kunze, B., Jansen, R., Trowitzsch-Kienast, W., Höfle, G., Reichenbach, H., Weiss, H. (1994) 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. 219*, 691−698.
29. Friedrich, T., Ohnishi, T., Forche, E., Kunze, B., Jansen, R., Trowitzsch- Kienast, W., Höfle, G., Reichenbach, H., Weiss, H. (1994) Two binding sites for naturally occurring inhibitors in mitochondrial and bacterial NADH:ubiquinone oxidoreductase (complex I), *Biochem. Soc. Trans. 22*, *7*, 226−230.
30. Inter allia: Kawabata, K., Takehana, K., Ikeyama, M., Harada, T. (Nihon Nohyaku Co., Ltd., Japan) (1975) 2-substituted 4,5-dicyanoimidazoles as acaricides, herbicides, and insecticides, *Jpn. Kokai Tokkyo Koho* (patent number JP 50125031).
31. Okun, J. G., Lümmen, P., Brandt, U. (1999) Three classes of inhibitors share a common binding domain in mitochondrial Complex I (NADH:ubiquinone oxidoreductase), *J. Biol. Chem. 274*, *5*, 2625−2630.
32. Tormo, J. R., Gallardo, T., González, M. C., Bermejo, A., Cabedo, N., Andreu, I., Estornell, E. (1999) Annonaceous acetogenins as inhibitors of mitochondrial Complex I, *Curr. Top. Phytochem. 2*, 69−90.
33. Jewess, P. J. (1994) Insecticides and acaricides which act at the rotenone-binding site of mitochondrial NADH:ubiquinone oxidoreductase; competitive displacement studies using a ^{3}H-labeled rotenone analogue, *Biochem. Soc. Trans. 7 22*, 247−251.
34. Degli Esposti, M., Ghelli, A., Crimi, M. (1993) Complex I and Complex III of mitochondria have common inhibitors acting as ubiquinone antagonists, *Biochem. Biophys. Res. Comm. 190*, 3, 1090−1096.
35. Degli Esposti, M., Crimi, M., Ghelli, A. (1994) Natural variation in the potency and binding sites of mitochondrial quinone-like inhibitors, *Biochem. Soc. Trans. 7*, 209−213.

36. Degli Esposti, M., Ghelli, A. (1994) The mechanism of proton and electron transport in mitochondrial complex I, *Biochim. Biophys. Acta 1187*, 116−120.
37. Degli Esposti, M., Ngo, A., McMullen, G., Ghelli, A., Sparla, F., Benelli, B., Ratta, M., Linnane, A. W. (1996) The specificity of mitochondrial Complex I for ubiquinones, *Biochem. J. 313*, 327−334.
38. Miyoshi, H. (1998) Structure−activity relationships of some Complex I inhibitors, *Biochim. Biophys. Acta 1364*, 236−244.
39. Lindell, S. D., Ort, O., Lümmen, P., Klein, R. (2004) The design and synthesis of novel inhibitors of NADH:ubiquinone oxidoreductase, *Bioorg. Med. Chem. Lett. 14*, 511−514.
40. Yabunaka, H., Abe, M., Kenmochi, A., Hamada, T., Nishioka, T., Miyoshi, H. (2003) Synthesis and inhibitory activity of ubiquinone-acetogenin hybrid inhibitor with bovine mitochondrial complex I, *Bioorg. Med. Chem. Lett. 13*, 2385−2388.
41. Fato, R., Estornell, E., Di Bernardo, S., Palotti, F., Parenti, Castelli, G., Lenaz, G. (1996) Steady-state kinetics of the reduction of coenzyme Q analogues by complex I (NADH:ubiquinone oxidoreductase) in bovine heart mitochondria and submitochondrial particles, *Biochemistry 35*, 2705−2716.
42. Gutman, M., Coles, C. J., Singer, T. P., Casida, J. E. (1971) On the functional organization of the respiratory chain at the dehydrogenase-coenzyme Q junction, *Biochemistry 10 11*, 2036−2043.
43. Vinogradov, A. D., Sled, V. D., Burbaev, D. S., Grivennikova, V. G., Moroz, I. A., Ohnishi, T. (1995) Energy-dependent complex I-associated ubisemiquinones in submitochondrial particles, *FEBS Lett. 370*, 83−87.
44. Ohnishi, T. (1998) Iron−sulfur clusters/semiquinones in complex I, *Biochim. Biophys. Acta 1364*, 186−206.
45. Lancaster, C. R. D., Michel, H. (1997) The coupling of light-induced electron transfer and proton uptake as derived from crystal structures of reaction centres from *Rhodopseudomonas viridis* modified at the binding site of the secondary quinone Q_B, *Structure 5*, 1339−1359.
46. Crofts, A. R., Barquera, B., Gennis, R. B., Kuras, R., Guergova-Kuras, M., Berry, E. A. (1999) Mechanism of ubiquinol oxidation by the bc(1) Complex: Different domains for the quinol binding pocket and their role in the mechanism and binding of the inhibitors, *Biochemistry 38*, 48, 15807−15826.
47. Gutman, M., Singer, T. P., Beinert, H., Casida, J. E. (1970) Reaction sites of rotenone, piericidin A and amytal in relation to the non-heme iron complex of NADH dehydrogenase, *Proc. Natl. Acad. Sci. U.S.A. 65*, 3, 763−770.
48. Ramsay, R. R., Krueger, M. J., Youngster, S. K., Singer, T. P. (1991) Evidence that the inhibition sites of the neurotoxic amine 1-methyl-4-phenylpyridinium (MPP^+) and of the respiratory chain inhibitor piericidin A are the same, *Biochem. J. 15*, 273, 481−484.
49. Degli Esposti, M., Ghelli, A. (1999) Ubiquinone and inhibitor sites in complex I: One, two or three? *Biochem. Soc. Trans. 27*, 4, 606−609.
50. Duval, R. A., Poupon, E., Brandt, U., Hocquemiller, R. (2005) Remarkable substituent effect: Beta-aminosquamocin, a potent dual inhibitor of mitochondrial complexes I and III, *Biochim. Biophys. Acta 1709*, 191−194.
51. Tormo, J. R., Gallardo, T., Peris, E., Bermejo, A., Cabedo, N., Estornell, E., Zafra-Polo, M. C., Cortes, D. (2003) Inhibitory effects on mitochondrial complex I of semisynthetic monotetrahydrofuran acetogenin derivatives, *Bioorg. Med. Chem. Lett. 13*, 4101−4105.
52. Cortes, D., Myint, S. H., Harmange, J.-C., Sahpaz, S., Figadère, B. (1992) Catalytic hydrogenation of Annonaceous acetogenins, *Tetrahedron Lett. 33*, 5225−5226.
53. Ichimaru, N., Murai, M., Abe, M., Hamada, T., Yamada, Y., Makino, S., Nishioka, T., Makabe, H., Makino, A., Kobayashi, T., Miyoshi, H. (2005) Synthesis and inhibition mechanism of Δlac-acetogenins, a novel type of inhibitor of bovine heart mitochondrial Complex I, *Biochemistry 44*, 2, 816−825.
54. Tormo, J. R., Gallardo, T., Aragón, R., Cortes, D., Estornell E. (1999) Specific interactions of monotetrahydrofuranic annonaceous acetogenins as inhibitors of mitochondrial Complex I, *Chem.-Biol. Interact. 122*, 171−183.
55. Darrouzet, E., Dupuis, A. (1997) Genetic evidence for the existence of two quinone related inhibitor binding sites in NADH−CoQ reductase, *Biochim. Biophys. Acta 1319*, 1−4.
56. Darrouzet, E., Issrate, J.-P., Lunardi, J., Dupuis, A. (1998) The 49 kDa subunit of NADH-ubiquinone oxidoreductase (Complex I) is involved in the binding of piericidin and rotenone, two quinone-related inhibitors, *FEBS Lett. 431*, 34−38.
57. Ramsay, R. R., Krueger, M. J., Youngster, S. K., Gluck, M. R., Casida, J. E., Singer, T. P. (1991) Interaction of 1-methyl-4-phenylpyridinium ion (MPP^+) and its analogues with the rotenone/piericidin binding site of NADH dehydrogenase, *J. Neurochem. 56*, 4, 1184−1190.
58. Higgins, D. S., Greenamyre, J. T. (1996) [^{3}H]dihydrorotenone binding to NADH:ubiquinone reductase (Complex I) of the electron transport chain: An autoradiographic study, *J. Neurosci. 16*, 12, 3807−3816.
59. Schuler, F., Casida, J. E. (2001) Functional coupling of PSST and ND1 subunits in NADH:ubiquinone oxidoreductase established by photoaffinity labeling, *Biochim. Biophys. Acta 1506*, 79−87.
60. Gluck, M. R., Krueger, M. J., Ramsay, R. R., Sablin, S. O., Singer, T. P., Nicklas, W. J. (1994) Characterization of the inhibitory mechanism of 1-methyl-4-phenylpyridinium and 4-phenylpyridine analogues in inner membrane preparations, *J. Biol. Chem. 269*, 3167−3174.

BI051261U