Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 2385–2388

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Inhibitory Activity of Ubiquinone–Acetogenin Hybrid Inhibitor with Bovine Mitochondrial Complex I

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Received 7 March 2003; accepted 13 April 2003

Abstract—To elucidate the inhibitory action of acetogenins, the most potent inhibitors of mitochondrial complex I, we synthesized an acetogenin analogue which possesses a ubiquinone ring (i.e., the physiological substrate of complex I) in place of the α,β-unsaturated γ-lactone ring of natural acetogenins, and named it Q-acetogenin. Our results indicate that the γ-lactone ring of acetogenins is completely substitutable with the ubiquinone ring. This fact is discussed in light of the inhibitory action of acetogenins.

Acetogenins have very potent and diverse biological effects such as antitumor, antimalarial, pesticidal and antifeedant activities.[1,2] The inhibitory effects of acetogenins on mitochondrial NADH-ubiquinone oxidoreductase (complex I) are of particular note as the diverse biological activities are thought to be attributable to this effect.[1,2] In fact, some acetogenins, such as bullatacin (=rolliniastatin-2, Fig. 1) and rolliniastatin-1, are the most potent inhibitors of this enzyme identified to date.[3–6] Although the acetogenins are thought to act at the terminal electron transfer step of complex I,[5–6] there is still no hard experimental evidence to verify whether the inhibitors bind to the ubiquinone reduction site. Additionally, there are few structural similarities between the acetogenins and ordinary complex I inhibitors such as piericidin A and rotenone. Thus, considering the unusual structural characteristics as well as the very strong inhibitory potency of acetogenins, a detailed analysis of the inhibitory actions of these inhibitors is important to elucidate the structural and functional features of the terminal electron transfer step of complex I.

Recently, Hoppen et al. indicated that the α,β-unsaturated γ-lactone ring of acetogenins, one of the most common structural features of a large number of natural acetogenins, is substitutable with a ubiquinone ring (i.e., 2,3-dimethoxy-5-methyl-1,4-benzoquinone) using mucocin and squamocin D as the mother compounds.[7] Taking into consideration the fact that ubiquinone is a physiological substrate of complex I, this finding is very interesting to elucidate the mode of action of acetogenins. Unfortunately, mucocin and squamocin D are about 300- and 80-fold less potent, respectively, than the most potent acetogenins like bullatacin.[8] Moreover, it seems somewhat confusing that mucocin showed rather less activity than the quinone-mucocin hybrid inhibitor. Therefore, it remains to be verified whether the ubiquinone ring is generally substitutable for the γ-lactone ring. In the present study, we synthesized an acetogenin analogue which possesses a ubiquinone ring in place of the α,β-unsaturated γ-lactone ring, named Q-acetogenin (Fig. 1), using the most potent acetogenin (compound **1**) synthesized in our laboratory as the mother compound. The inhibitory action of Q-acetogenin was examined with bovine heart mitochondrial complex I.

Synthesis

The synthetic procedures are outlined in Scheme 1. The vinyl iodide **5** was prepared by methylation of the reduced form of quinone **4**, which was synthesized by reacting **3** and (*E, Z*)-1,9-diiodo-1-nonene and a sequential retro-Diels–Alder reaction.[9] Synthetic procedures of compound **3** were described in ref 9.

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0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00439-6

The key intermediate **6** was synthesized as described previously.[10] Treatment of **6** with 0.5 equiv TsCl (repeated twice) and sequential MOM ether protection afforded **7**. Desilylation of **7** with TBAF, and the opening of epoxide **8** with 1-lithium-1-nonyne in the presence of BF_3 etherate[11] provided **9**. After acetyl protection, deprotection of the MOM ether and sequential treatment with TsCl afforded **11**. Hydrolysis of the acetyl groups gave epoxide **12**. The opening of epoxide **12** with lithium (trimethylsilyl)acetylide in the presence of BF_3 etherate provided **13**, ^1H and ^{13}C NMR data and the optical rotation of **13** were identical to those reported.[12] Pd(0)-catalyzed coupling[13] of alkyne **13** with vinyl iodide **4** gave the corresponding eneyne in poor yield. Therefore, vinyl iodide **5** converted from **4** was used as a coupling partner to obtain eneyne **14**.[7] Catalytic hydrogenation of **14** with Wilkinson's catalyst and sequential oxidation by CAN afforded Q-acetogenin.[14]

Compounds **1**, **2** and DB were the same samples as those used previously.[15]

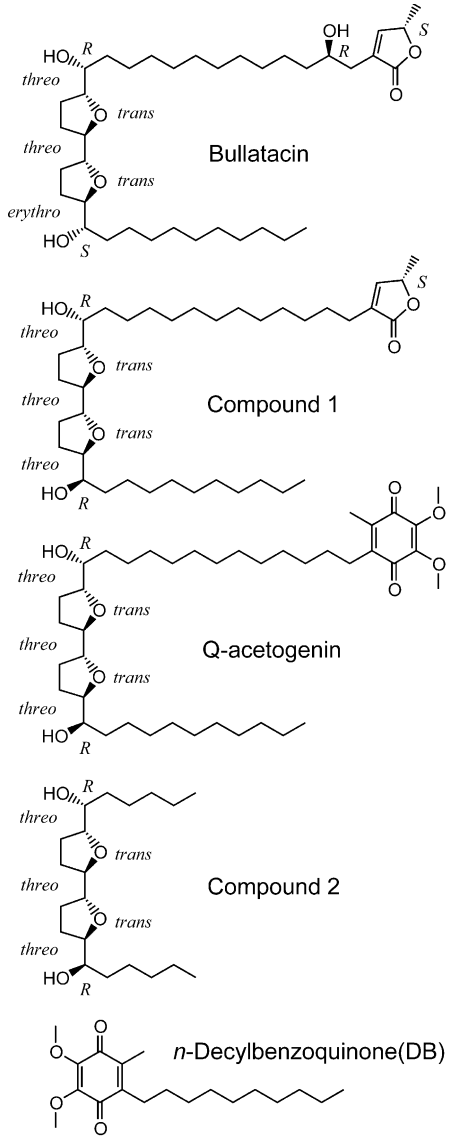


Figure 1. Structures of acetogenins and substructures examined in this study.

Bioactivity

The inhibition of complex I activity was determined by NADH oxidase assay using bovine heart submitochondrial particles.[16] Previous studies indicated that the inhibitory potency of compound **1** is comparable to that of bullatacin, one of the most potent natural acetogenins.[17,18] The inhibitory potencies of **1** and Q-acetogenin in terms of IC_{50}, that is, the molar concentration needed to halve the control NADH oxidase activity, were 0.9 and 1.2 nM, respectively, in the present study. Complete inhibition by Q-acetogenin was attained at about 4 nM. We confirmed that Q-acetogenin has no effect on the enzyme activities downstream of complex I. Thus, the ubiquinone ring is almost completely substitutable for the γ-lactone ring. This result supports not only the result of the earlier work by Hoppen et al.,[7] but also our previous proposal that the α,β-unsaturated γ-lactone is not an essential structural factor required to elicit potent activity of acetogenins (Table 1).[15,18]

The IC_{50} value of the precursor of Q-acetogenin (compound **15**) was 280 nM, indicating a significant contribution of the ubiquinone ring to the inhibition. Considering the structural similarity between α,β-unsaturated γ-lactone and ubiquinone rings, the presence of a conjugated carbonyl group may be required for this moiety.

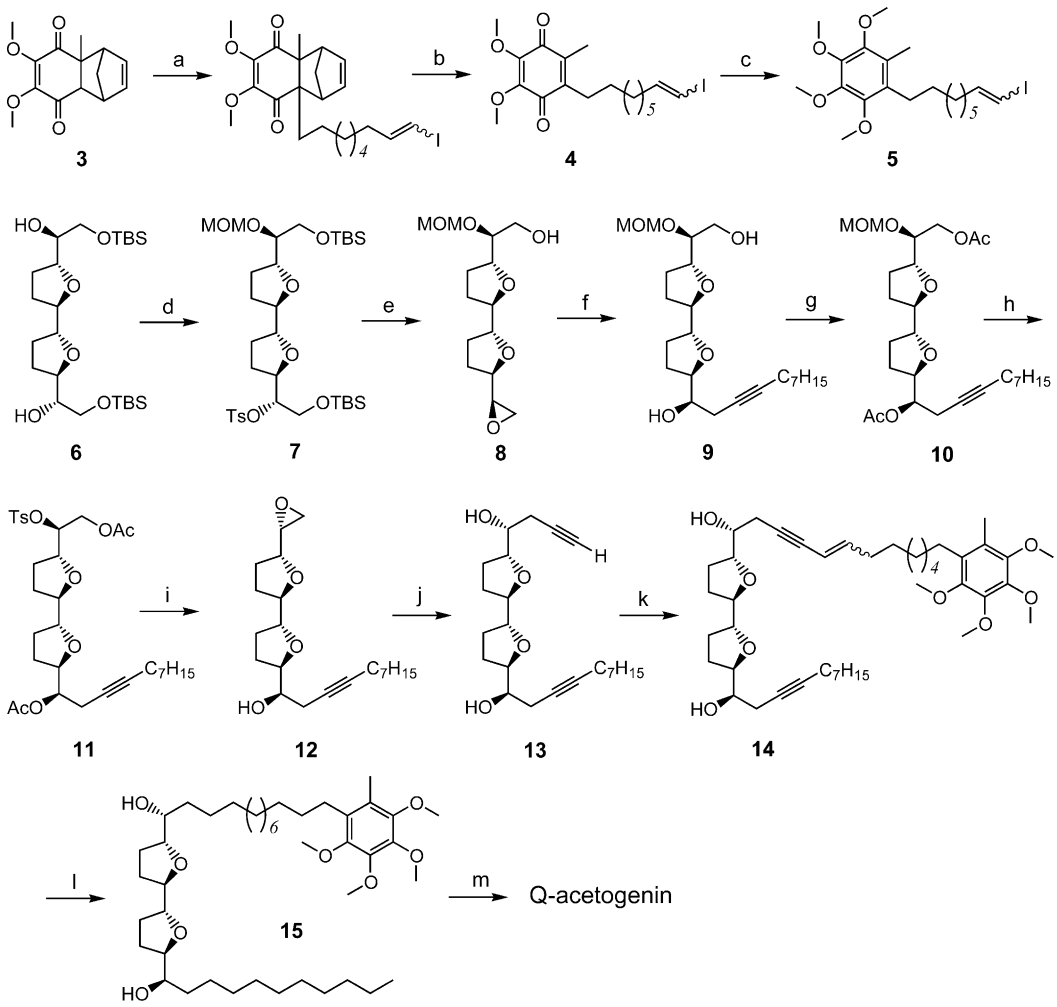
We previously showed that acetogenin acts as a potent inhibitor only when the γ-lactone and the THF ring moieties are directly linked by an alkyl spacer.[15] To confirm whether this is also the case for Q-acetogenin, we examined the inhibition by combined use of two substructures (i.e., compound **2** and DB) at various molar ratios, from 0.01 to 100. Under the experimental conditions, the IC_{50} of compound **2** was 4.5 μM.[19] No synergistic enhancement was observed, indicating that the ubiquinone ring and the THF ring moieties must be directly linked by an alkyl spacer to elicit potent inhibition.

Discussion

Recent photoaffinity labeling studies using pyridaben[20] and fenpyroximate[21] indicated that PSST, ND5, and IP 49 kDa subunits contribute to the inhibitor binding domain in bovine heart mitochondrial complex I. Nevertheless, the binding subunit(s) of acetogenin, which is significantly different from the two inhibitors structurally, is still unknown. The present study along with the earlier work[7] indicated that the ubiquinone ring is almost completely substitutable for the γ-lactone

Table 1. Inhibitory potencies of test compounds

Compd	IC_{50} (nM)
Bullatacin	0.9
Compound **1**	0.9
Q-acetogenin	1.2
Compound **2**	4500
Compound **15**	280



Scheme 1. (a) KOBu-*t*, THF/DMF (9:1) at −20 °C, and 1,9-diiodo-1-nonene, −20 °C to 0 °C, 3 h, 65%; (b) toluene, 90 °C, 2 h, 96%; (c) (i) $Na_2S_2O_4$, *n*-hexane/H_2O (1:1), rt, (ii) CH_3I, K_2CO_3, acetone, 40 °C, 16 h, 75%; (d) (i) TsCl (0.5 equiv), DMAP, Et_3N, CH_2Cl_2, rt, (repeated twice, 55%), (ii) MOMCl, $(i\text{-}Pr)_2NEt$, 97%; (e) TBAF, THF, rt, 88%; (f) 1-nonyne, *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C, 0.5 h, 69%; (g) AcCl, DMAP, CH_2Cl_2, 0 °C to rt, 96%; (h) (i) $BF_3{\cdot}Et_2O$, Me_2S, −20 °C, 1.5 h, 94%, (ii) TsCl, DMAP, Et_3N, CH_2Cl_2, 55 °C, 3 h, 95%; (i) KOH, MeOH, rt, 0.5 h, 92%; (j) (i) lithium (trimethylsilyl)acetylide, $BF_3{\cdot}Et_2O$, −78 °C, 15 min, (ii) K_2CO_3, MeOH, rt, 5 h, 69%; (k) $(Ph_3P)_4Pd$, CuI, Et_3N, rt, 5 h, 72%, (e) H_2, $(Ph_3P)_3RhCl$, benzene, rt, 1 day, 91%; (m) $Ce(NH_4)_2(NO_3)_6$, pyridine-2,6-dicarboxylic acid, CH_3CN/H_2O (1:1), 0 °C, 4 h, 86%.

ring of natural acetogenins. This result is important to elucidating the inhibitory action of acetogenins since ubiquinone is the physiological substrate of complex I. Actually, the so-called DB (*n*-decylbenzoquinone) has been widely used in complex I assays as an electron accepting substrate. Therefore, at first sight, Q-acetogenin and consequently natural acetogenins seem to bind to the ubiquinone reduction site of the enzyme.

However, this possibility can be ruled out for the following two reasons. Firstly, a Lineweaver–Burk plot for NADH-DB (or Q_1) oxidoreductase activity in the presence of Q-acetogenin or compound **1** suggested that the inhibition by these inhibitors is noncompetitive against DB and Q_1 (data not shown, cf. ref 17). Secondly, since the K_m of DB and the K_d of potent acetogenin differ by three orders of magnitude,[22] it is unlikely that the ubiquinone moiety of Q-acetogenin binds tightly to the ubiquinone reduction site. Therefore, the ubiquinone ring of quinone-acetogenin hybrid inhibitors synthesized here and earlier[7] may serve merely as a substitute of the α,β-unsaturated γ-lactone ring of natural acetogenins. Although we cannot exclude the possibility that the ubiquinone ring of the hybrid inhibitors accepts electrons from complex I, this effect would not be responsible for their inhibitory action since the hybrid inhibitors elicited inhibition at concentrations far lower than the concentration of NADH, an electron donor, under the experimental conditions. The mode of action of acetogenins including their active conformation remains to be elucidated. Nevertheless, the fact that Q-acetogenin is a good mimic of potent acetogenins may open a new experimental approach to the study of ligand-complex I interaction, for example, the redox-reaction induced FTIR spectroscopic technique used for other electron-transfer enzymes in combination with [1- or 4-$^{13}C_1$]-ubiquinones.[23,24]

Acknowledgements

We thank Dr. Kazuhiro Irie (Kyoto Univ.) for his help with the measurement of HRMS spectra.

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