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Syntheses and Biological Evaluation of B-Ring-Modified Analogs of Dafachronic Acid A

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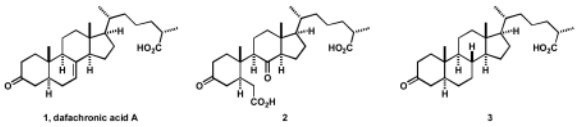
Abstract



Synthesis and testing of dafachronic acid A (1) and its derivatives 2 and 3 have revealed that 1, and not a further oxidation product, is the natural ligand for the DAF-12 receptor of *C. elegans*.

Remarkably, the life span of the nematode *C. elegans* can be increased significantly by loss of function of a handful of genes that affect endocrine function. Amongst them, the daf-9 gene encodes a cytochrome P450 enzyme which is responsible for the biosynthesis of the bile acid-like steroid, dafachronic acid A (**1**). Based on various analytical techniques, it has been recently proposed by Mangelsdorf and Antebi that **1** is the major ligand for the nuclear receptor DAF-12, which in its ligand bound form regulates genes that prevent entry into the dauer stage, a long lived quiescent mode.[1] However, synthesis of the proposed ligand remained elusive until a later work, in which the 25-(*S*) structure of **1** and its 25-(*R*)-diastereomer were made.[2,3]

In this research we address the question of whether dafachronic acid A is the true ligand for the nuclear hormone receptor DAF-12 or just a precursor of a further biooxidation product which is the actual ligand. We were intrigued by the fact that dafachronic acid A, with its Δ^7-olefinic linkage, might be further oxidized biologically to a seco acid structure resembling that of glycinoeclepin A,[4,5] a potent hatching factor for the eggs of the nematode *Heterodera*

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glycines. Consequently, we became interested in exploring the biological activity of the *β*-seco dafachronic acid A derivative **2**, as an analog of glycinoeclepin A, which might even be a more active metabolite of **1**. In this letter we describe the synthesis and biological evaluation of **2**. For comparaison, we have also synthesized the 7,8-dihydro derivative of dafachronic acid A, **3**, which would be expected to be devoid of activity if the seco acid **2** were the real ligand for DAF-12, rather than dafachronic acid A (**1**).

The synthesis of the diketo diacid **2** started with the previously reported 6-keto steroid **4**.[2] Baeyer-Villiger oxidation of **4** with trifluoroperacetic acid ($(CF_3CO)_2O$, H_2O_2, 0 °C, $CHCl_3$) afforded the desired 7-membered lactone **5** in 94% yield and as a sole regioisomer. Lactone **5** was cleaved to a ketoacid intermediate by treatment with Jones' reagent (2 equiv, 23 °C, acetone) which was esterified by diazomethane (CH_2N_2, Et_2O) to give ketoester **6** in essentially quantitative yield over two steps. Saponification of the 3*β*-acetate, oxidation of the resulting alcohol to the ketone, and hydrolysis gave the diketo diacid **2** in 52% overall yield (three steps, Scheme 1). Our initial strategy for the synthesis of **2** involved the oxidation of the Δ^7-olefinic linkage in **1** by various methods. Surprisingly, all attempts to directly oxidize the Δ^7 bond to the diketo diacid **2** using O_3 then H_2O_2, $KMnO_4$, NBu_4MnO_4 and $RuCl_3$-$NaIO_4$ were unsuccessful.

To synthesize the 7,8-dihydro analog **3**, we have also used an intermediate from our synthesis of **1**.[2] Thus, the Δ^5-double bond in **7** was reduced (H_2, 1 atm, Pd-C, EtOAc) to give the fully saturated steroid and the same three steps as above were performed to give analog **3** in 33% overall yield for the four steps. It should also be mentioned that the hydrogenation of **1** to **3** failed under several conditions.[4]

Next, samples of the synthetic dafachronic acid A **1**, the seco-diacid **2,** and 7,8-dihydrodafachronic acid A **3** were evaluated for their bioactivity. First, the ability of synthetic ligands to rescue daf-9 hormone biosynthetic mutants from the dauer state was measured. Consistent with **1** being a natural ligand for DAF-12, dafachronic acid A rescued dauer formation in the nanomolar range, with half maximal activity of 18.5 nM (Figure 2). Similarly, the 7,8-dihydrodafachronic acid A also gave substantial rescue with half maximal rescue at 292 nM. By contrast, the seco-diacid **2** was found not to rescue *C. elegans* from the dauer state, indicating that it is not a ligand. Second the ability of synthetic ligands to activate DAF-12 in transcriptional assays on a target gene, lit-1, was measured. To do this, plasmid constructs containing the daf-12 gene and the lit-1 gene fused to a luciferase reporter were co-transfected into human embryonic kidney cells (HEK293T), treated with various doses of the compounds, and luciferase induction measured by light emission.[1] In accord with the dauer rescue results, **2** showed no activity even at 100 μM concentration (Figure 3), whereas 7,8-dihydrodafachronic acid A (**3**) showed similar activity as dafachronic acid A (**1**). Specifically, measurement of the dose response revealed EC_{50} values for daf-12 activation to be: for 7,8-dihydrodafachronic acid A, 114 nM and for dafachronic acid A, 26 nM. These results taken together allow the following conclusions: (1) dafachronic acid A is a natural ligand for DAF-12 nuclear receptor (2) in contrast to the soybean nematode case, ring B oxidative cleavage products are not the active agents, for gene activation of *C. elegans* DAF-12 and (3) $\Delta^{7,8}$ double bond is not essential for dafachronic acid activity on *C. elegans*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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2. Giroux S, Corey EJ. J Am Chem Soc 2007;129:9866–9867. [PubMed: 17658816]
3. Giroux S, Corey EJ. Org Lett 2008;10:801–802. [PubMed: 18247628]
4. To the best of our knowledge, no succesful hydrogenation of isolated Δ^7 double bonds have been reported in the literature.
5. Glycinoeclepin A, a natural product that is released into soil from the roots of the soybean plant, is active at 10^{-12} g/mL as hatching factor for H. glycines, see: (a) Fukuzawa A, Furusaki A, Ikura M, Masamune T. J Chem Soc Chem Commun 1985;221–222:748. (b) Masamune T, Anetai M, Takasugi M, Katsui N. Nature 1982;297:495–496.
6. For the syntheses of glycinoeclepin A, see: (a) Murai A, Tanimoto N, Sakamoto N, Masamune T. J Am Chem Soc 1988;110:1985–1986. (b) Mori K, Watanabe H. Pure Appl Chem 1989;61:543–546. (c) Corey EJ, Houpis IN. J Am Chem Soc 1990;112:8997–8998.

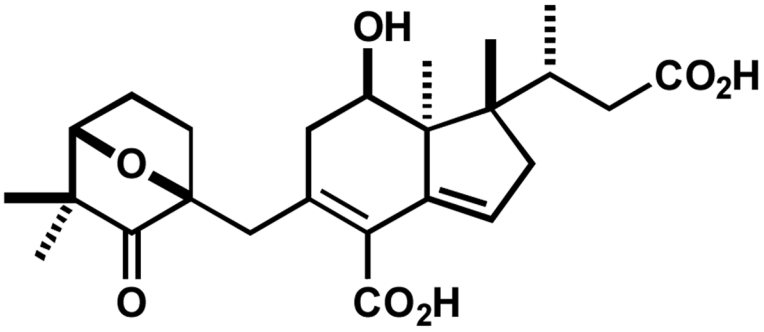


Figure 1.
Structure of glycinoeclepin A

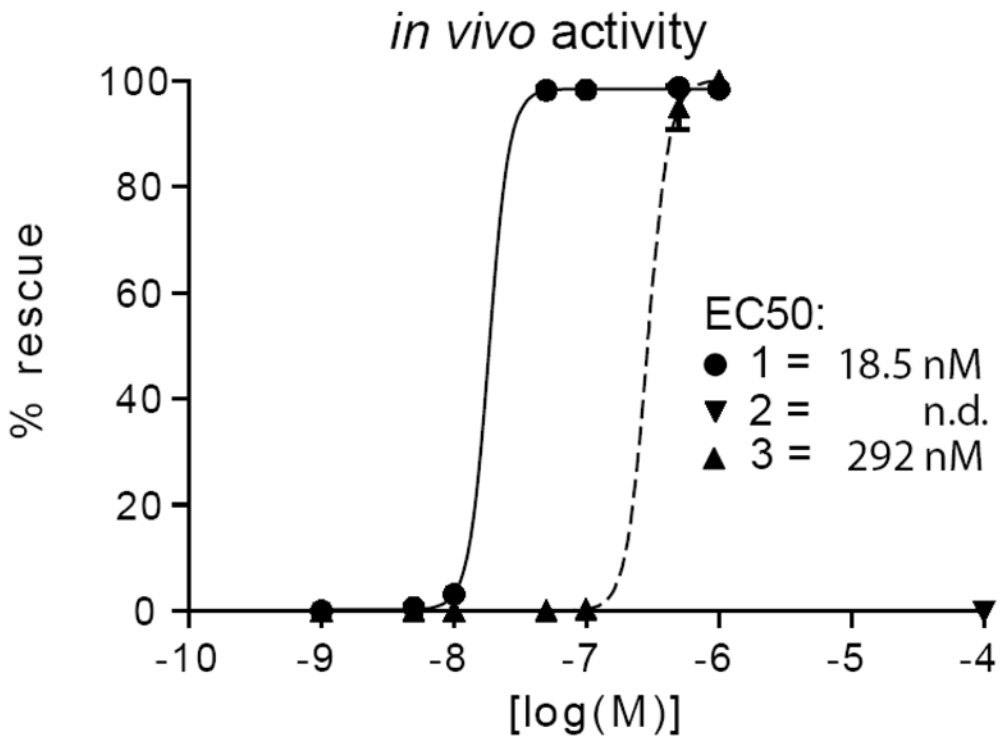


Figure 2.
In vivo activity of sterols **1, 2,** and **3** measured as the percentage of rescue of *daf-9(dh6)* null worms from dauer to wild-type gravid adults.

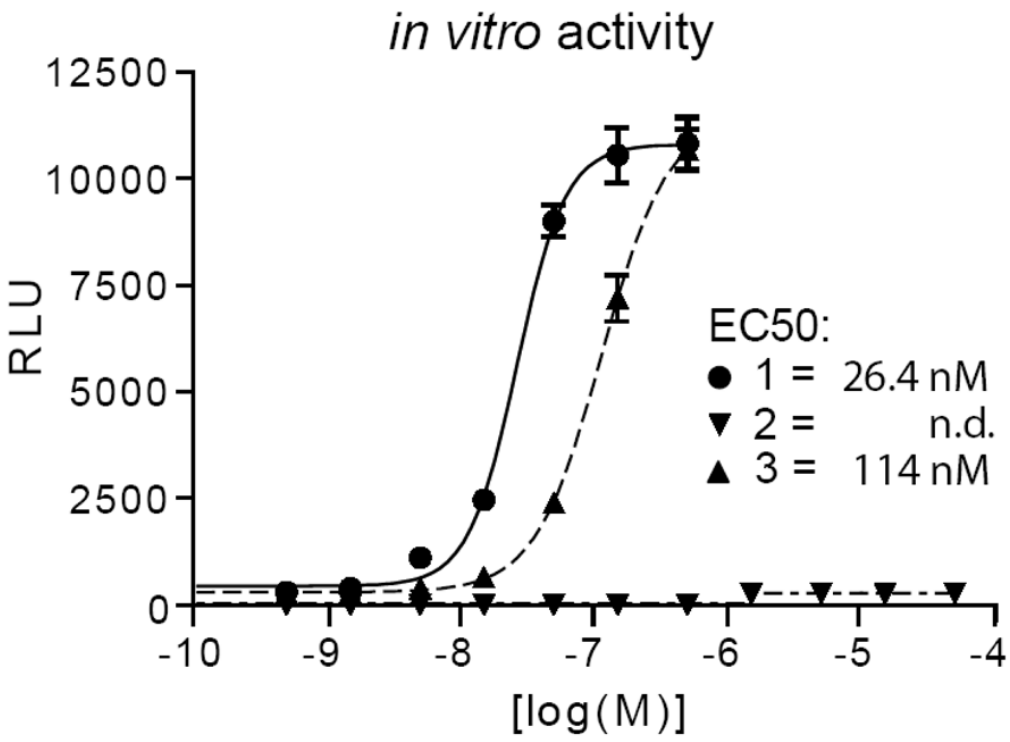
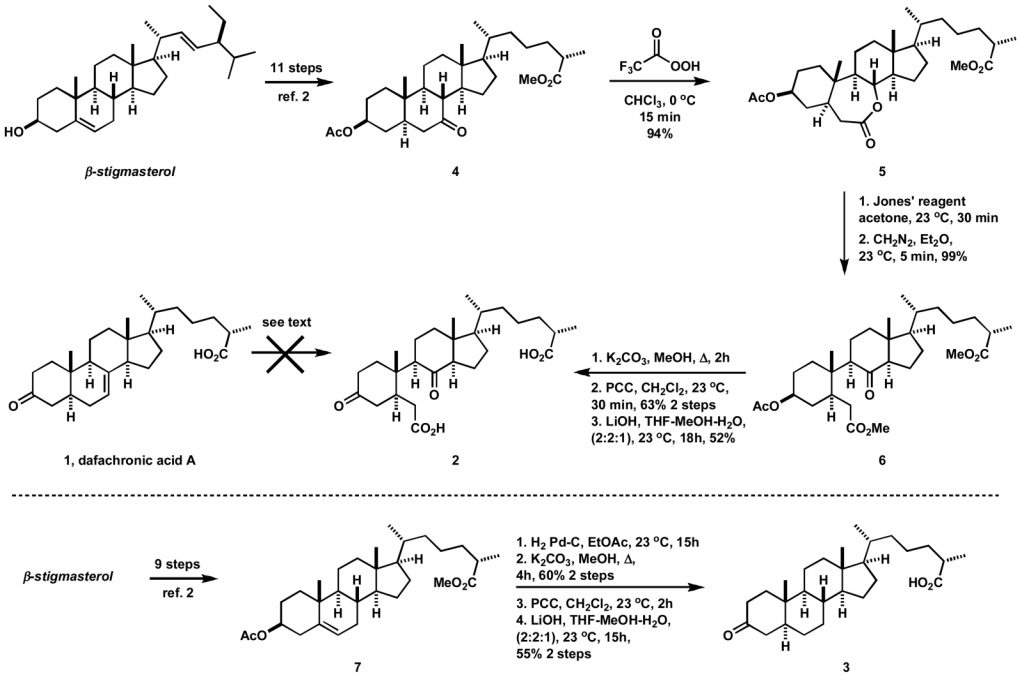


Figure 3.
Transcriptional activation of DAF-12 by **1, 2** and **3** on lit-1::ptk-luciferase reporter constructs, measuring relative luciferase units with and without ligand (RLU) vs concentration.



Scheme 1.
Synthesis of analogs **2** and **3** from *β*-stigmasterol