Importance of a 4-Alkyl Substituent for Activity in the Englerin Series

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Supporting Information

ABSTRACT: The ring closing metathesis/transannular etherification approach to the englerin nucleus was adapted to provide two key intermediates for analogue synthesis: the 4-desmethyl $\Delta^{5,6}$ tricycle and the 4-oxo $\Delta^{5,6}$ tricycle. The former was elaborated to 4-desmethyl englerin A and the latter served as a common precursor for englerin A, 4-ethyl englerin A, and 4-isopropyl englerin A. 4-Desmethyl englerin A was less active than the natural product by an order of magnitude, but the 4-ethyl and 4-isopropyl analogues were comparable in activity to englerin A. These results are consistent with the premise that the 4-alkyl group enforces the binding conformation of the cinnamoyl ester substituent. Furthermore, they suggest that 4-alkyl englerin structures may prove to be useful tool compounds.

KEYWORDS: Englerin, NCI 60 screen, etherification, SAR

Englerin A (**1a**, Figure 1), a sesquiterpene diester isolated from an East African *Phyllanthus* species, exhibits potent

Figure 1. Englerins and C-4 synthetic analogues.

and impressively selective inhibition of kidney tumor cell growth. This was first demonstrated by the isolation team in the NCI 60 screen.¹ In an independent study, englerin A was shown to reduce the viability of renal cancer cells (UO-31 and A-498) with IC_{50} values in the mid nanomolar range, while its effect on the viability of two noncancerous cell lines (immortalized HEK 293 cells, derived from human embryonic kidney cells and renal proximal tubule cells) was barely detectable at much higher concentrations (low micromolar and molar, respectively).²

In vivo experiments show the potential of englerin A as a drug lead and the difficulties associated with drug delivery. When administered to mice bearing the renal cancer xenograft 786–O, an intraperitoneal (ip) dosage protocol led to reduced tumor growth along with effects on phosphorylation of two kinase targets in tumor tissue.³ However, rats and nude mice,

subjected to a different ip dosing protocol, did not tolerate the compound.⁴ Intravenous injection of englerin A has generally proven to be rapidly lethal and oral administration ineffective.⁵

Englerin A could be detected in mouse serum following ip dosing; however, it could not be detected in serum when the maximum tolerated dose was administered orally (by gavage). It has been shown to be unstable over time in rodent serum, being converted to the inactive englerin B (**2**).⁴ The picture that emerges is one in which the glycolate ester of englerin A is cleaved by gastric acid or, more slowly, by serum esterases.

Nonetheless, in the mere eight years since its discovery, the englerin series has reached important milestones on the path to drug development and remains an area of active research.^{5–11} Total syntheses based on several different strategies have been reported. These and more classical medicinal chemistry have yielded active analogues and contributed to the development of a structure–activity relationship (SAR). The issue of *in vivo* hydrolysis was addressed with the preparation of a moderately active, orally available aza analogue, which has improved serum stability. Studies of the mechanism of action of englerin A have implicated several protein targets and are now leading to proposals for drug combination studies and applications in diseases other than cancer. Much of this progress is summarized in the recent review on englerins.⁵

Seeking to contribute to the developing SAR for englerin A and also to devise potent and selective “tool compounds” for mechanism studies, we contemplated the requirements for the cinnamic acid and glycolic acid ester substituents at C-6 and C-

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9.^{12–15} We postulated that the methyl groups adjacent to these appendages might be important for maintaining side chain conformations that are required for binding to the target protein; indeed, we note that truncation of the C-7 isopropyl group to an ethyl or methyl group results in reduced activities.¹⁴ We assumed that larger alkyl groups at C-4 would not be detrimental to activity and that, if functionalized, they would prove useful for affinity binding studies. Here we describe the synthesis and activity profiles in the NCI 60 panel of 4-desmethyl englerin A¹⁶ and two analogues that bear extended alkyl groups at C-4. We note that a recent total synthesis paper includes reports of three C-4 alkyl extended englerins; however, no biological testing was disclosed.⁶

Initially it appeared that the synthesis of the 4-desmethyl englerin (1b) would be simpler than that of englerin A or another 4-alkyl structure by our ring closing metathesis/transannular etherification approach.¹⁷ Thus, we imagined that the desmethyl englerin core 3 would be obtained from the metathesis product 5 (Scheme 1) by modifying the oxy-

Scheme 1. Retrosynthetic Analysis of 4-Desmethyl Englerin A

mercuration product 4 and that the carbonate 6 would be obtained from the readily available 7. Therefore, we began our SAR study with the preparation of analogue 1b.

Our synthesis of the metathesis substrate 6 from known alcohol 7¹⁸ through the chiral epoxide 8¹⁹ (Scheme 2) followed our prior art as described in our formal synthesis of englerin A.¹⁷ Regioselective opening of the epoxide ring gave the 1,2-diol 9 and oxidation gave the expected aldehyde 10. We were able to favor the desired homoallylic alcohol 11 by employing the B-allyldiisopinocampheylborane reagent prepared according to Singaram.²⁰ Treatment with carbonyl diimidazole then gave the metathesis substrate.

Continuation of the synthesis paralleled our ring closing metathesis/transannular etherification englerin approach. Double ring closure gave bicyclic 12, and functional group modification gave the key intermediate 5. Oxymercuration allowed isolation of the mercurial 4, and oxidative demercuration led to a mixture of alcohols 14 and olefin 15. However, reductive demercuration converted mercurial 4 cleanly to olefin 15.

We were able to adapt Ma's 4-step net hydroxylation sequence²¹ to this intermediate to obtain the allylic alcohol 18. As expected,¹² hydrogenation with the iridium Crabtree catalyst²² gave us the *trans*-hydroazulene 3 (Scheme 3). Application of Echavarren's 4-step sequence for englerin A (O-6 esterification, O-9 deprotection, and esterification, glycolate deprotection)²³ gave the 4-desmethyl analogue (1b).

Scheme 2. Synthesis of RCM Substrate for 4-Desmethyl Englerin A

Although we preferred that the oxidative demercuration of mercurial 4 give alcohol 18 directly, we noted the potential of the alcohol mixture 14a,b as the synthetic equivalent of ketone 19 (Scheme 4), an attractive common precursor for 4-alkyl englerin systems. Indeed addition of MeMgBr to ketone 19 gave a mixture of alcohols 20; a similar mixture of alcohols 20 has been separated by the Echavarren group who then converted alcohol 20a to englerin A.²³ Therefore, this variant of our approach also affords a formal total synthesis of the natural product.

We noted that if this chemistry were to prove general, it would provide access to englerin analogues that bear C-4 "methyl extensions."²⁴ Such compounds are attractive targets, not only because they would provide data for SAR construction but also because appropriately functionalized, active derivatives could be employed in protein labeling and imaging studies. In order to test the premise that extended and/or locally bulky C-4 substituents would be tolerated, we set out to prepare both 4-ethyl and 4-isopropyl englerin A (Scheme 5).

Some exploration of reactions and conditions proved necessary but ultimately successful. Treatment of ketone 19 with EtMgBr gave the 1,2-addition product 21 exclusively. However, the isopropyl alcohol mixture (22a,b) was obtained in useful yields only when *i*-PrMgCl was accompanied by a lanthanum salt additive.²⁵ From this point the completion of the synthesis of the C4 substituted nuclei required transposition of the allylic alcohol functionality. To accomplish this in their englerin synthesis, Echavarren and co-workers had treated allylic alcohol 20a with Collins reagent (CrO₃·py₂) or CrO₃/2,5-dimethylpyrazole and then deoxygenated the resulting epoxy alcohol with Sharpless's low valent tungsten reagent (from WCl₆/2*n*-BuLi).^{23,26,27} Following this protocol, we oxidized alcohol 21 with Collins reagent, obtaining epoxy alcohol 23a in 62% yield. However, attempts to apply the deoxygenation protocol to epoxide 23a gave us a complex mixture that did not appear to contain the desired 25a.

However, treatment of alcohol 21 with PDC gave enone 24a along with epoxide 23a. Borohydride reduction of the enone followed by Nicolaou's Crabtree hydrogenation¹² gave the

Scheme 3. Desmethyl Englerin A by a Modified RCM Transannular Etherification Sequence

Scheme 4. New Formal Synthesis of Englerin A

expected 26a. Application of the four-step side chain introduction sequence gave 4-ethyl englerin A (**1c**). A parallel set of procedures applied to alcohol **22a** gave 4-isopropyl englerin A (**1d**).

Compounds **1b**, **1c**, and **1d** were tested in the NCI 60 human tumor cell line screen (Table 1). The data from all three compounds were highly correlated with those for englerin A (Table S-1, Supporting Information). While the 4-desmethyl compound **1b** was notably less potent than englerin A in nearly

Scheme 5. Synthesis of C4-Englerin Analogues **1c** and **1d** Table 1. NCI 60 Test Results: Potency of 4-Alkyl Englerin Analogues in Sensitive Cell Lines, GI₅₀ Values in nM

compd	A498	ACHN	UO-31	HS 578T
1a (<i>n</i> = 3) ^a	10	17	15	10
1b (<i>n</i> = 1)	123	162	182	31
1c (<i>n</i> = 1)	38	13	19	4.1
1d (<i>n</i> = 1)	8.1	8.3	11	3.2

^a*n* = the number of five-dose experiments used to calculate the GI₅₀ values.

all sensitive cell lines, extension of the 4-substituent to ethyl and isopropyl groups yielded analogues that were approximately as potent as the parent.

The SAR data shown, while limited to the natural product and three analogues, are consistent with the original hypothesis. The apparent tolerance of the englerin A binding site for substituents larger than methyl at C-4 offers opportunities for further analogue development and for the introduction of biochemical probes.^{28,29}

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.7b00161.

Original NCI 60 charts and graphs. Experimental procedures and characterization data for all new compounds. Also Table S-1. Pearson correlation coefficients³⁰ among Englerin analogues at GI₅₀ level of response(PDF)

Compound 14b X-ray structure (CIF)

Compound 26a X-ray structure (CIF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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