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Formal Synthesis of (–)-Englerin A and Cytotoxicity Studies of Truncated Englerins

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Abstract

An efficient formal synthesis of (–)-englerin A (**1**) is reported. The target molecule is a recently isolated guaiane sesquiterpene that possesses highly potent and selective activity against renal cancer cell lines. The developed strategy proceeds in an enantioselective manner by constructing the BC ring system of **1** via a Rh(II)-catalyzed [4+3] cycloaddition and subsequently attaching the A ring via an intramolecular aldol condensation reaction. As such, this strategy allows the synthesis of truncated englerins. Evaluation of these analogues in A498 renal cancer cell line suggests that the A ring of englerin is crucial to its antiproliferative activity. Moreover, evaluation of these analogues led to the identification of potent growth inhibitors of CEM cells with GI₅₀ values ranging from 1–3 μM.

Keywords

total synthesis; natural product; cycloaddition; renal cancer; leukemia

Introduction

The need to identify new chemical motifs as potential drug leads has spurred the screening of plant extracts used in traditional African, Ayurvedic (Indian) and Chinese medicines.^[i] In particular, South Africa has a remarkable botanical diversity with over 30,000 flowering species, from which more than 3,000 are used for medicinal purposes throughout the country.^[ii] Among them, plants of the genus *Phyllanthus* (Euphorbiaceae) are widely distributed and have long been used in African folk medicine to treat kidney and urinary tract infections.^[iii] With this in mind, the Beutler laboratory has been screening extracts of the Tanzanian plant *Phyllanthus engleri* against renal cell carcinoma (RCC) and has recently reported the isolation of two novel bioactive sesquiterpenes, named englerin A (**1**) and englerin B (**2**) (Figure 1).^[iv]

Preliminary biological investigations^[4] have shown that **1** possesses very potent growth inhibitory activity (GI₅₀ = 1–87 nM) against RCC with approximately 1,000-fold tissue selectivity as compared to other carcinomas. These findings are of particular significance since RCC: (a) is among one of the ten leading cancer types in the US;^[v] (b) is characterized by a lack of early warning signs, has diverse clinical manifestations and shows resistance to

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radiation,^[vi] and (c) cannot be effectively treated with current chemotherapeutic agents, leaving surgical procedures as the only treatment option.^[vii]

Biogenetically, the englerins belong to the family of guaiane natural products in which a common bicyclic sesquiterpene skeleton has undergone a sequence of oxygenations and oxo-cyclizations.^[viii]

Further decoration at the periphery of this core produces the structures of **1** and **2**. Due to the combination of uncommon structural architecture, potent and selective cytotoxicity against RCC and promising pharmacology, the englerins have received the attention of the chemical community. Since their isolation in 2009, five total syntheses^[ix] and many strategies and studies^[x] have been reported. These studies also led to the identification of synthetic englerin analogues, such as **3**, **4** and **5**, products of various esterifications of the common tricyclic core. Several recent reviews nicely summarize the current status of englerins.^[xi] Our continuous interest in exploring natural products from ethnomedicine as medicinal leads^[xii] has prompted us to design an enantioselective strategy toward englerins.^[xiii] In this account we describe in detail the results of our efforts and the biological evaluation of structurally simplified englerin analogues.

Results and Discussion

Retrosynthetic analysis

Our retrosynthetic strategy is shown in Scheme 1. We anticipated that a sequence of reactions including: inversion of the C₆ stereocenter, hydrogenation of the C₄-C₅ alkene (englerin numbering) and selective esterifications at C₆ and C₉ hydroxyl groups would form englerin (**1**) from diol **6**. Compound **6** could be derived from enone **7** following a C₃ deoxygenation and a regioselective hydroboration of the C₈-C₉ double bond. We theorized that the cyclopentene moiety of **7** could be constructed from an intramolecular aldol condensation, while an α -hydroxylation would produce the C₆ hydroxyl moiety. Disconnection along these lines led us to target the construction of bicyclic motif **8** possessing the BC ring scaffold of englerins. We hypothesized that compound **8** could be formed in enantiomerically pure form by exploring a Rh(II)-catalyzed [4+3] cycloaddition between readily available furan **9**, representing the C ring of **1**, and chiral pantolactone-derived diazoester **10**.

[4+3] Rh-catalyzed cycloadditions

Rhodium-triggered cyclization reactions have been widely applied in synthetic chemistry.^[xiv] In 1996, Davies *et al.* reported an elegant enantioselective Rh(II)-catalyzed [4+3] cycloaddition between furans and chiral diazoesters.^[xv] Selection of the appropriate pantolactone enantiomer, as the chiral auxiliary, allows diastereo-control of the produced oxy-bridged adduct. The transfer of chirality, observed in this reaction, can be rationalized by considering that the carbonyl moiety of pantolactone interacts with the Rh-carbenoid as shown in Scheme 2. This interaction blocks one of the two faces of the carbene **10** leading to a selective *si*-face attack by the 2-methyl-5-isopropyl-furan (**9**). The stereochemical outcome is consistent with a tandem cyclopropanation/Cope rearrangement (see intermediate [**11**]). Cyclopropanation of the achiral furan **9** was expected to occur in a regioselective manner at the less substituted and thus less hindered C₉-C₁₀ double bond. The potential of this cycloaddition to construct bicyclic structure **8** was of great interest to our strategy, as it provided an efficient, rapid and enantioselective access to the englerin core. Notably, both of starting materials **9**^[xvi] and **10**^[15a,xvii] can be readily prepared in more than 50 gram-scale from commercially available materials in 3 steps.

To the best of our knowledge, 2,5-disubstituted furans have not been evaluated as substrates in the Rh(II)-catalyzed [4+3] cycloaddition. With this in mind, we initially evaluated the regioselectivity of this reaction using achiral diazoester **14**^[xviii] as a model system (Scheme 3). The synthesis of furan **9** is highlighted in Scheme 3. Commercially available 2-methyl furan (**12**) was treated with *n*-BuLi and acetone followed by the dehydration (Ac₂O/KOAc) to afford alkene **13**. A regioselective hydrogenation of **13** proceeded smoothly by controlling the reaction time to 1 hour and the sequential distillation delivered the desired 2,5-disubstituted furan **9**. Notably, this 3-step preparation of **9** can be done in more than 50 gram-scale and only one distillation is needed for purification. Refluxing of **9** and diazoester **14** in hexane in the presence of catalytic amounts of rhodium(II) octanoate (2 mol%), afforded oxacyclic product ($\pm$)-**15** as the only regio-isomer in excellent yields (95%). Cleavage of the ester auxiliary proved to be more challenging than anticipated. In fact, the reported LiAlH₄ reductive cleavage led only to decomposition.^[15a] However, treatment of ester **15** with DIBAL-H yielded the labile β -hydroxy silyl enol ether **16** that, upon immediate treatment with stoichiometric amounts of BF₃•Et₂O, underwent rearrangement^[xix] to afford exocyclic enone ($\pm$)-**17** in good yield (81%).

Encouraged by these results, we synthesized chiral diazoester **10** departing from (*R*)-pantolactone.^[15a,17] The Rh(II)-catalyzed [4+3] cycloaddition of **10** with **9** proceeded efficiently with Rh₂(OOct)₄ to yield **8** in good yield (90%) albeit in moderate diastereoselectivity (dr 3:1, calculated via ¹H-NMR) (Scheme 4). It should be noted that, under the same conditions, use of Rh₂(OAc)₂ led only to decomposition of the starting materials. Moreover, attempts to increase the d.r. by performing this reaction at lower temperature or by using less catalyst loading led to a significant decrease in the yield without any significant enhancement of diastereoselectivity. Table 1 highlights our efforts to optimize this cycloaddition. The diastereomeric mixture of **8** could be separated via a silica gel column chromatography. Our previously established reductive cleavage conditions were applied to **8** to produce optically active enone ($\pm$)-**17**, although the yield was significantly lowered (59%). This decrease of yield may be due to the presence of the two reactive carbonyl moieties in **8** that require extended reaction times and excess of DIBAL-H (7.5 equiv) as compared to reduction of ($\pm$)-**15** (2.5 equiv), leading to partial decomposition of the sensitive silyl enol ether moiety.

The next attempts of installing a hydroxyl group on the C₆ carbon of ($\pm$)-**17** with Davis oxaziridines^[xx] proved unsuccessful. However, Rubottom oxidation^[xxi] provided the desired hydroxy enone **19** in good yield (36%; 87% brsm), although with the inverse stereochemistry as compared to the englerin C₆ hydroxyl moiety. The absolute stereochemistry of hydroxyl enone **19** was established by a single crystal X-ray analysis,^[xxii] which simultaneously confirmed the absolute stereochemistry of oxy-bridged ester **8**. We were pleased to observe that the Rubottom oxidation proceeded regioselectively at the more electronically rich TMS enol ether without affecting any other alkenes in this molecule. The stereochemical outcome of this reaction was also satisfactory since it proceeded exclusively from the top face of the intermediate TMS-enolate [**18**] suggesting that this face is less hindered. We predicted that this selectivity would allow us to have complete substrate control during the following steps and invert the C₆ stereochemistry at a later stage in our synthesis.

Formation of the A ring via olefin metathesis

The next stage of the synthesis involved constructing the tricyclic core of englerin from compound **19**. We envisioned to accomplish this cyclization by the means of a Grubbs ring closing metathesis (RCM)^[xxiii] of precursor **21** (Scheme 5) that after oxidation of C₆ hydroxyl group would reveal a conjugate system for 1,4-addition of methyl nucleophile at

the C₄ site. To this end, a Lewis acid-promoted conjugate allylation with allyltrimethylsilane afforded the corresponding ketone **20** in moderate yield (68%) as a single diastereomer. The ensuing olefination of C₅, however, proved to be unexpectedly challenging. After attempting many different methods including Wittig reaction, Peterson olefination,^[xxiv] Petasis^[xxv] and Tebbe olefination^[xxvi] etc., only the TiCl₄-assisted Nysted olefination^[xxvii] gave low yields (15-36%) of trialkene **21**. Despite the inefficient formation of **21** we attempted the RCM. We were pleased to see that this reaction proceeded smoothly in almost quantitative yields to give allylic alcohol, which after TPAP/NMO oxidation,^[xxviii] formed α,β -unsaturated ketone **22**. Unfortunately, all efforts to add methyl nucleophiles to **22** via conjugate addition were unsuccessful. To overcome this issue, we sought to perform the RCM on substrate **24** that contains the challenging C₁₁ methyl group. Consequently, we allylated **19** with methylallyltrimethyl silane. While we were successful in producing **24**, olefination of this precursor at C₅ resulted in irreproducible results. This prompted us to abandon this approach and seek an alternative strategy for the formation of the 5-membered ring from **19**.

Formation of the 5-membered ring (A-ring) via an intra-molecular aldol condensation and completion of the synthesis

Based on the above results, we pursued an alternative strategy for the construction of the tricyclic core of englerin based on an intramolecular aldol condensation. Initially, we explored the feasibility of this reaction using non-hydroxylated enone **17** as a model system. To this end, treatment of propanal with thiazolium salt **26** and enone **17** under Stetter conditions^[xxix] produced diketone **27** (Scheme 6). To our delight, the intramolecular aldol reaction of **27** proceeded under mild conditions (KOH/EtOH, rt, 24 hours) to afford **28** in good yield (76%).

Motivated by this result, we converted **19** to the C₆ silyl ether **29** and treated this product with propanal under the previously established Stetter conditions. This reaction proceeded efficiently under basic conditions to furnish diketone **30** in good yield (75% over two steps) as a single diastereomer. However, application of previously successful KOH condensation procedure only resulted in deprotection of the TBS ether with no further reaction. This prompted an extensive investigation on this intramolecular condensation, with different C₆ protecting groups (H-, MOM-, TES-, etc.), various bases (*t*-BuOK, KOH, NaOMe, LDA, etc.) and several reaction conditions. Many failed attempts of the aldol condensation to provide the tricyclic motif proved this reaction to be unexpectedly difficult. Eventually, treatment of diketone **30** with NaHMDS afforded the corresponding aldol addition product, which underwent sequential dehydration process (NaOMe/MeOH, heat) to produce the key tricyclic core **7** in an acceptable yield (36%; 43% brsm). NaBH₄ reduction of enone **7** yielded allylic alcohol **31** (99%) as a single diastereomer that, without further purification, was protected as the benzyl ether to furnish **32** in good yield (71%). Treatment of **32** with BH₃•THF followed by H₂O₂ oxidation afforded regio- and stereoselectively alcohol **33** (60%). Silylation of **33** followed by deprotection of the C₃ benzyl ether yielded **35** via **34** in 99% overall yield. (Scheme 7). Our efforts to hydrogenate the tetra-substituted alkene of **35** using different catalysts and H₂ pressure were unsuccessful presumably due to the steric hindrance of the double bond (C₄-C₅). This prompted us to deoxygenate the C₃ hydroxyl group under Barton-McCombie conditions^[xxx] (40% yield). In our efforts to improve this transformation, we discovered that dehydration of **35** with Burgess reagent^[xxxi] followed by standard hydrogenation improved significantly the yield to 90% over the two steps. Deprotection of the di-TBS ether **36** with TBAF gave poor results, however, we were pleased to find that a microwave accelerated reaction under similar conditions (TBAF/THF, 80 °C) made diol **6** in a quick and high yielding conversion (45 min, 93%).

To access the natural product from diol **6**, we would need to achieve stereoselective saturation of the tetra-substituted bond followed by esterification of the C₉ hydroxyl group, inversion of C₆ stereocenter by oxidation/reduction sequence and finally esterification in the presence of cinnamic acid. An alternative synthesis of (+)-**6** was reported by Ma et. al.^[9b] Our synthetic route to (+)-**6** provides another novel and facile method for the construction of englerin A and related compounds. More significantly, the Rh-catalyzed [4+3] annulation/intramolecular aldol condensation sequence presents a very useful, practical and general method for the preparation of the guaiane sesquiterpene core present in englerin related compounds.

The intriguing bioactivity of englerin has prompted several groups to perform SAR studies.^[9f,10e] These studies have focused exclusively on modifications of the C₆ and C₉ side chains. An advantage of our strategy is that it can produce in an efficient and stereoselective manner compound **19**, which represents the BC ring system of englerins. In other words, this approach could provide information on the biological significance of the A ring of englerin. With this in mind, we synthesized truncated englerins **37**, **38** and **39** (Scheme 8). To further expand such SAR studies, we designed a new approach towards compound **46** representing the nor-A-ring englerin A. The synthesis of **46** is highlighted in Scheme 9 and is inspired by Wender's pioneering [5+2] cycloaddition reactions.^[xxxii,9d] 2-Methyl-5-furfural (**40**) was first alkylated under Grignard conditions and the resulting alcohol was converted to hemiacetal **41** in 75% overall yield. Treatment of **41** with excess acrylonitrile (*i*Pr₂NEt/MsCl, 100 °C, 14 h) under gave us the desired bicyclic product **42** in 32% yield. Moreover, we found that this reaction could be accelerated under microwave conditions with improved yield (*i*Pr₂NEt/MsCl, 150 °C, 4 h, 45%). To the best of our knowledge, this is the first example of microwave accelerated this type of [5+2] cycloaddition reaction. The Luche reduction^[xxxiii] followed by hydrogenation provided compound **43**^[xxxiv] in almost quantitative yield. The transformation from **43** to **44** was accomplished in a 3-step sequence that included: (a) oxidative cleavage of the cyanide group to the corresponding ketone; (b) esterification with cinnamic acid under Yamaguchi condition;^[xxxv] (c) hydride reduction of C₉ carbonyl moiety. The last two steps were performed following the reported method^[9b] to furnish **46**. Moreover, the acetate analogue **45** was readily prepared from acetylation of **44**.

Cytotoxicity studies of truncated englerins

The cytotoxicity of the truncated englerins was evaluated in both A498 renal cancer cells and CEM T-cell acute lymphoblastic leukemia (T-ALL) cells using a ³H-thymidine incorporation assay. In our initial study, we compared the growth inhibitory activity of englerin A to that of truncated englerins **44** and **46**. We found that englerin A inhibited the growth of A498 renal cancer cells with a GI₅₀ of 45 nM which is in agreement with previous findings (Figure 2).^[4,9d,9f,10e] However, compounds **44** and **46** did not have any effect on the growth of A498 cells even at concentrations of 100 nM or greater (Figure 2). These results suggest that the A ring is essential for the growth inhibitory activity of englerin A in renal cancer cells.

We then evaluated the antiproliferative activity of englerin analogues in CEM cells, a cell line in which englerin A has little activity with a reported GI₅₀ of 20.4 μM.^[4] Of all analogues tested, **17**, (±)-**17**, and **19** had significant cytotoxicity with GI₅₀ values of 3.3, 1.8, and 2.4 μM, respectively (Figure 3, Table 2). It is likely that these cytotoxicities are due to the exocyclic enone moiety that acts as a conjugate electrophile with bionucleophiles.^[xxxvi] In contrast, englerin A and analogues containing an additional ring, such as **7**, had little or no cytotoxicity at concentrations as high as 20 μM. These results suggest that the single ring

analogues can target leukemia cells effectively and the addition of structural complexity may result in a loss of cytotoxicity to leukemia cells.

Conclusion

We have accomplished an efficient and enantioselective formal synthesis of englerin A (**1**), a potent and selective growth inhibitor of renal cancer cells. The synthetic approach to intermediate **6** proceeds in 15 steps from readily available compounds **9** and **10** in 5% overall yield. Key to our strategy is the enantioselective formation of the BC ring of **1** via a Rh(II)-induced enantioselective [4+3] cycloaddition, followed by the construction of the A ring via an intramolecular aldol condensation. Inspired by this sequence, we have also synthesized a small family of truncated englerins and have evaluated their growth inhibitory activities against certain renal cancer and leukemia cell lines. These studies suggest that the A-ring of englerin A plays an important role in its bioactivity and tissue selectivity. Interestingly, compounds (–)-**17**, (±)-**17** and (–)-**19** have shown significant growth inhibitory activity against CEM cell lines at low micromolar concentrations (GI_{50} = 1–3 μ M). Consequently, these compounds may represent new lead structures for the development of small molecule therapeutics against leukemia.

Experimental Section

(1*R*,5*S*)-(*R*)-4,4-Dimethyl-2-oxotetrahydrofuran-3-yl3-[(*tert*-butyldimethylsilyl)oxy] -5-isopropyl-1-methyl-8-oxabicyclo[3.2.1]octa-2,6-diene-2-carboxylate (**8**)

A solution of **10** (11.2 g, 31.6 mmol) in dry hexanes (750 mL) was added dropwise over 5.5 hours to a refluxing solution of **9** (7.85 g, 8.8 mL, 63.2 mmol) and rhodium(II) octanoate dimer (492 mg, 0.63 mmol) in anhydrous hexanes (750 mL). The reaction mixture was stirred for an additional 30 minutes, at which time TLC showed no starting material remaining. The reaction mixture was then allowed to cooled down to room temperature, filtered through a silica plug and concentrated. Crude product purification using flash column chromatography (100:1 to 9:1, slow gradient Hexanes: EtOAc) afforded 8.1 g of bicyclic ester **8** as colorless thick oil (57%). $[\alpha]_D^{23} = +36.40$ (c 1.0, $CHCl_3$). 1H NMR (500 MHz, $CDCl_3$) δ : 6.38 (dd, $J = 5.7, 0.5$ Hz, 1H), 5.72 (d, $J = 5.7$ Hz, 1H), 5.40 (s, 1H), 4.04 (q, $J = 8.9$ Hz, 2H), 2.41 (d, $J = 17.4$ Hz, 1H), 1.95 – 1.83 (m, 2H), 1.62 (s, 3H), 1.24 (s, 3H), 1.17 (s, 3H), 1.00 (d, $J = 6.9$ Hz, 3H), 0.95 (d, $J = 6.8$ Hz, 3H), 0.92 (s, 9H), 0.19 (s, 3H), 0.18 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 164.2, 157.1, 141.6, 126.8, 117.9, 88.5, 82.8, 76.3, 75.0, 40.2, 37.1, 34.3, 25.8, 23.3, 21.5, 20.6, 18.4, 17.2, 17.1, –3.4, –3.5. HRMS (FAB) m/z : calcd for $C_{24}H_{38}O_6SiNa(M+Na^+)$ 473.2330, found: 473.2331.

(1*R*,5*S*)-5-isopropyl-1-methyl-2-methylene-8-oxabicyclo[3.2.1]oct-6-en-3-one (**17**)

To a solution of **8** (20.7 g, 45.9 mmol) in dry CH_2Cl_2 (459 mL) was added quickly dropwise via addition funnel a 1.0 M solution of DIBAL-H in heptanes (344.3 mmol, 344.3 mL) at –78°C. After completion of addition, the reaction was stirred for 15 min at which time TLC showed no starting material. The reaction mixture was quenched with saturated Rochelle salt solution (300 mL), was allowed to reach room temperature and was stirred for 1 hour. The reaction mixture was filtered through Celite and washed with CH_2Cl_2 until TLC showed no more crude product remaining in the filter cake. The filtered mixture was separated, extracted with CH_2Cl_2 (2 $\times$ 150 mL), washed with brine (300 mL) dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to 500 mL of CH_2Cl_2 . This solution was flushed with Argon and treated directly with $BF_3 \cdot Et_2O$ (68.9 mmol, 8.7 mL) dropwise at –30 °C. 5 min after addition TLC showed no starting material. The reaction mixture was further diluted with CH_2Cl_2 (250 mL), quenched with saturated $NaHCO_3$ solution (350 mL) and extracted with CH_2Cl_2 (2 $\times$ 200 mL). The combined organic layers were washed with

brine (300 mL), dried over Na₂SO₄, filtered, and concentrated under vacuum. Crude product purification using flash column chromatography afforded 5.2 g of ketone **17** as a yellow oil (59%). $[g=a]_D^{23} = +103.01$ (c 1.0, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ: 6.05 (d, *J* = 5.8 Hz, 1H), 5.96 (s, 1H), 5.92 (d, *J* = 5.7 Hz, 1H), 5.24 (s, 1H), 2.56 (d, *J* = 17.7 Hz, 1H), 2.46 (d, *J* = 17.7 Hz, 1H), 2.00 – 1.89 (m, 1H), 1.61 (s, 3H), 0.99 (d, *J* = 4.9 Hz, 3H), 0.98 (d, *J* = 4.7 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 197.8, 147.3, 136.3, 133.4, 115.3, 89.8, 84.9, 45.9, 33.7, 19.8, 17.3, 17.3. HRMS (FAB) *m/z*: calcd for C₁₂H₁₆O₂Na (M+Na⁺) 215.1043, found: 215.1045.

(1*S*,2*S*,5*R*)-2-hydroxy-1-isopropyl-5-methyl-4-methylene-8-oxabicyclo[3.2.1]oct-6-en-3-one (19)

n-Butyllithium (77.6 mL, 124.2 mmol, 1.6 M in hexane) was added dropwise to a solution of dry diisopropylamine (19 mL, 135.0 mmol) in dry THF (800 mL) at –78 °C. The reaction mixture was stirred for 15 minutes, and then a solution of **17** (5.2 g, 27.0 mmol) in dry THF (500 mL) was added quickly dropwise to the reaction mixture. The reaction mixture temperature was raised from –78 °C to 0 °C over 45 minutes and stirred for 1 hour at 0 °C. The reaction mixture was cooled down to –78 °C and TMSCl (15.8 mL, 124.2 mmol) was added dropwise. The reaction mixture temperature was raised from –78 °C to 0 °C over 45 minutes and stirred for 1 hour at 0 °C at which time TLC showed no starting material. The reaction mixture was diluted with hexane (650 mL) quenched with 5% NaHCO₃ solution (500 mL), washed with brine (300 mL), dried over Na₂SO₄, filtered, and concentrated under vacuum. To a solution of the crude enol ether product in CH₂Cl₂ (250 mL) a solution of NaHCO₃ (250 mL, 10%) was added all at once at 0 °C. Then a solution of *m*CPBA (5.2 g, 30.0 mmol) in CH₂Cl₂ (60 mL) was added slowly while vigorous stirring of the reaction mixture. The reaction was closely monitored by TLC. When traces of enol ether (<5%) was observed the reaction was further diluted in CH₂Cl₂ (200 mL), quenched with saturated NaHSO₃ solution (300 mL), allowed to reach room temperature, extracted with CH₂Cl₂ (2 × 200 mL), washed with brine (300 mL), dried over Na₂SO₄, filtered, and concentrated under vacuum to approximately 250 mL. To this solution of crude epoxide product was added a solution of (COOH)₂ (15.6 g, 124.2 mmol) in MeOH (150 mL). After 30 min of stirring at room temperature TLC showed no epoxide product remaining. The reaction mixture was slowly quenched with saturated K₂CO₃ solution until neutral pH was achieved and extracted with CH₂Cl₂ (2 × 200 mL). The combined organic layers were washed with brine (300 mL), dried over Na₂SO₄, filtered, and concentrated under vacuum. Crude product purification using flash column chromatography (100:1 to 4:1, hexanes: EtOAc) afforded **17** (2.7 g) and α-hydroxy ketone **19** as a crystalline solid (2.2 g, 36%; 87% b.r.s.m). Recrystallization from hexanes afforded high purity crystals for X-Ray characterization. $[g=a]_D^{23} = +101.00$ (c 1.3, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ 6.08 (dd, *J* = 5.8, 0.9 Hz, 1H), 6.00 (d, *J* = 5.9 Hz, 1H), 5.97 (s, 1H), 5.28 (s, 1H), 3.81 (d, *J* = 6.4 Hz, 1H), 2.37 – 2.28 (m, 1H), 1.62 (s, 3H), 1.05 (d, *J* = 6.9 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 199.0, 146.9, 140.2, 130.1, 116.5, 93.4, 85.5, 73.3, 28.4, 19.4, 17.7, 17.0. HRMS (ESI) *m/z*: calcd for C₁₂H₁₆O₃Na (M+Na⁺) 231.0992, found: 231.0993.

(1*S*,2*S*,5*R*)-1-isopropyl-5-methyl-4-methylene-3-oxo-8-oxabicyclo[3.2.1]oct-6-en-2-yl acetate (37)

To a solution of **19** (5 mg, 0.024 mmol) in pyridine (0.25 mL) was added 4-DMAP (0.6 mg, 0.005 mmol) and Ac₂O (0.011 mL, 0.12 mmol). The reaction was heated to 60 °C for 3 hours. The reaction was cooled down to room temperature quenched with saturated NaHCO₃ and extracted 3 times with ethyl acetate. The combined organic layers were dried over MgSO₄, concentrated and the residue was purified by preparatory plate chromatography to yield **37** as a white foam (5.7 mg, 95%). ¹H NMR (400 MHz, CDCl₃) δ: 6.16 (d, *J* = 5.9 Hz, 1H), 6.02 (d, *J* = 5.9 Hz, 1H), 6.00 (s, 1H), 5.30 (s, 1H), 5.27 (s, 1H),

2.16 (s, 3H), 2.16 (m, 1H), 1.65 (s, 3H), 0.95 (d, $J = 6.9$ Hz, 3H), 0.92 (d, $J = 6.9$ Hz, 3H); ^{13}C NMR (126 MHz, CDCl_3) δ : 194.1, 170.0, 146.6, 141.2, 129.3, 116.9, 92.6, 85.7, 71.7, 29.9, 28.8, 19.5, 17.4, 17.1. HRMS (ESI) m/z : calcd for $\text{C}_{14}\text{H}_{18}\text{O}_4 \text{Na}$ ($\text{M}+\text{Na}^+$) 273.1097, found: 273.1098.

(1S,2S,5R)-1-isopropyl-5-methyl-4-methylene-3-oxo-8-oxabicyclo[3.2.1]oct-6-en-2-yl benzoate (38)

To a stirred mixture of benzoic acid (7.3 mg, 0.06 mol) and **19** (6.5 mg, 0.026 mol) in dry toluene (0.55 mL) were added successively NEt_3 (15 μL , 0.11 mol) and 2,4,6-trichlorobenzoyl chloride (9 μL , 0.075 mol). The reaction mixture was stirred for 10 min, then catalytic amount of 4-DMAP (1 crystal) was added. After 16 h of stirring at room temperature, the reaction was diluted with ethyl acetate, and the organic phase was washed successively with aqueous HCl solution (1M), saturated sodium bicarbonate solution, and brine. The organic phase was dried over MgSO_4 and concentrated in vacuum after filtration. The residue was purified by flash chromatography to afford the benzoic keto-ester **38** (9.1 mg, 90%) as a colorless oil: ^1H NMR (400 MHz, CDCl_3) δ 8.10 (m, 2H), 7.57 (m, 1H), 7.24 (m, 2H), 6.21 (dd, $J = 5.8, 0.9$ Hz, 1H), 6.09 (d, $J = 5.8$ Hz, 1H), 6.02 (s, 1H), 5.51 (s, 1H), 5.32 (s, 1H), 2.25 (m, 1H), 1.69 (s, 3H), 0.96 (d, $J = 6.9$ Hz, 3H), 0.93 (d, $J = 6.9$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 193.8, 165.5, 146.7, 141.1, 133.4, 130.1, 129.4, 128.4, 92.8, 85.6, 72.0, 28.9, 19.6, 17.4, 17.1. HRMS (ESI) m/z : calcd for $\text{C}_{19}\text{H}_{20}\text{O}_4 \text{Na}$ ($\text{M}+\text{Na}^+$) 335.1254, found: 335.1256.

(1S,2S,5R)-1-isopropyl-5-methyl-4-methylene-3-oxo-8-oxabicyclo[3.2.1]oct-6-en-2-yl cinnamate (39)

To a stirred mixture of cinnamic acid (8 mg, 0.053 mol) and **19** (5.5 mg, 0.026 mol) in dry toluene (0.5 mL) were added successively NEt_3 (11 μL , 0.08 mol) and 2,4,6-trichlorobenzoyl chloride (7 μL , 0.07 mol). The reaction mixture was stirred for 10 min, then catalytic amount of 4-DMAP (1 crystal) was added. After 16 h of stirring at room temperature, the reaction was diluted with ethyl acetate, and the organic phase was washed successively with aqueous HCl solution (1M), saturated sodium bicarbonate solution, and brine. The organic phase was dried over MgSO_4 and concentrated in vacuum after filtration. The residue was purified by flash chromatography to afford compound **39** (7.8 mg, 88%) as a colorless oil: ^1H NMR (500 MHz, CDCl_3) δ 7.70 (d, $J = 15.9$ Hz, 1H), 7.52 (m, 2H), 7.39 (m, 3H), 6.52 (d, $J = 16.0$ Hz, 1H), 6.19 (d, $J = 5.8$ Hz, 1H), 6.06 (d, $J = 5.8$ Hz, 1H), 6.03 (s, 1H), 5.42 (s, 1H), 5.33 (s, 1H), 2.22 (m, 1H), 1.68 (s, 3H), 0.96 (t, $J = 6.9$ Hz, 6H); ^{13}C NMR (126 MHz, CDCl_3) δ 194.1, 166.0, 146.5, 141.2, 134.3, 130.7, 129.4, 129.0, 128.4, 117.2, 116.9, 92.8, 85.7, 71.7, 28.8, 19.6, 17.5, 17.1. HRMS (ESI) m/z : calcd for $\text{C}_{21}\text{H}_{22}\text{O}_4\text{Na}(\text{M}+\text{Na}^+)$ 361.1416, found: 361.1417.

(1R,5S,6S)-1-isopropyl-5-methyl-2-oxo-8-oxabicyclo[3.2.1]oct-3-ene-6-carbonitrile (42)

To a solution of **41** (1.6 g, 11.0 mmol) in acrylonitrile (60 mL) was added diisopropylethylamine (2.3 mL, 13 mmol), followed by methanesulfonyl chloride (1.0 mL, 13 mmol). The resulting solution was microwave heated at 150°C for 4 hours. After cooling to room temperature, the reaction mixture was filtered through a plug of Celite, and the filtrate was concentrated *in vacuo*. Flash chromatography of the residue (20% ethyl acetate in hexanes) provided the cycloadduct **42** (880 mg, 45%) as colorless oil. ^1H NMR (500 MHz, CDCl_3) δ : 6.93 (d, $J = 9.7$ Hz, 1H), 6.01 (d, $J = 9.8$ Hz, 1H), 3.06 (dd, $J = 9.2, 2.9$ Hz, 1H), 2.52 (dd, $J = 14.3, 2.9$ Hz, 1H), 2.30 (p, $J = 7.0$ Hz, 1H), 2.19 (dd, $J = 14.3, 9.3$ Hz, 1H), 1.74 (s, 3H), 1.08 (d, $J = 6.9$ Hz, 3H), 1.04 (d, $J = 6.9$ Hz, 3H). ^{13}C NMR (126 MHz,

CDCI₃) δ: 196.7, 151.8, 128.1, 118.8, 100.0, 90.4, 37.4, 35.2, 30.0, 21.6, 17.6, 16.5. HRMS (ESI) *m/z*: calcd for C₁₂H₁₅NNaO₂ (M+Na⁺) 228.0995, found: 228.0996.

³H-Thymidine incorporation assay

CEM cells were plated at 10–20 × 10³ cells/well and A498 cells at 3,500 cells/well in 96-well plates in RPMI supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 u/ml penicillin/streptomycin (complete medium). Englerin A or its analogues were added to the cells at increasing concentrations and 0.1% DMSO was added to control cells. Cells were incubated for 48 hours and then pulsed with ³H-thymidine for 6 hours. Incorporation of ³H-thymidine was determined in a scintillation counter (Beckman Coulter Inc., Fullerton, CA) after cells were washed and deposited onto glass microfiber filters using a cell harvester M-24 (Brandel, Gaithersburg, MD).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Representative structures of natural and synthetic englerins

Figure 2.
Effect of englerin A and analogues **44**, **46** on the proliferation in of A498 renal cancer cells.

Figure 3. Dose-dependent inhibition of cell proliferation by selected englerin analogues in T-ALL cells.

Figure 3.
Dose-dependent inhibition of cell proliferation by selected englerin analogues in T-ALL cells.

Scheme 1.
Retrosynthetic analysis of (-)-englerin A (1).

Scheme 2.

Rationale to stereochemical consequences of the Davies Rh-catalyzed [4+3] cycloaddition.

Scheme 3.

Reagents and conditions: (a) *n*-BuLi (0.94 equiv), Et₂O, -20 °C, reflux; then -20 °C, acetone (1.1 equiv), reflux; then Ac₂O (1.5 equiv), KOAc (0.63 equiv), 110 °C; (b) Pd/C (catalytic), H₂ (1 bar), 1 h; (c) Rh₂(OOct)₄ (2 mol%), hexanes, reflux, 95%; (d) DIBAL-H (2.5 equiv), CH₂Cl₂, -78 °C; (e) BF₃•Et₂O (1.5 equiv), CH₂Cl₂, -30°C, 81%.

Scheme 4.

Reagents and conditions: (a) **9** (2.0 equiv), $\text{Rh}_2(\text{OOct})_4$ (2 mol%), hexanes, reflux, 95% for $(\pm)\text{-16}$, 90% for $(-)\text{-16}$ (dr 3:1); (b) DIBAL-H (7.5 equiv), CH_2Cl_2 , -78°C , then $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (1.5 equiv), CH_2Cl_2 , -30°C , 81% for $(\pm)\text{-17}$, 59% for $(-)\text{-17}$; (c) LDA (4.6 equiv), TMSCl (4.6 equiv), -78°C to 0°C ; (d) *m*CPBA (1.1 equiv), NaHCO_3 , CH_2Cl_2 , 0°C , then $(\text{COOH})_2$ (4.6 equiv), 36%; 87% brsm.

Scheme 5.

Reagents and Conditions: (a) TiCl₄ (1.2 equiv), allyltrimethylsilane (3 equiv), CH₂Cl₂, -78 °C, 68%; (b) Nysted reagent (10 equiv), TiCl₄ (10 equiv), NaHCO₃ (7 equiv), -78 °C, 36%, (c) Grubbs 2nd gen. (5 mol%), CH₂Cl₂, rt, 99%; (d) NMO (3 equiv), TPAP (0.1 equiv), CH₂Cl₂, rt, 72%; (e) various methyl cuprate reagents; (f) TiCl₄ (1.1 equiv), trimethyl(2-methylallyl)silane (5 equiv), CH₂Cl₂, -78 °C, 71%.

Scheme 6.

Reagents and conditions: (a) propanal (4 equiv), **26** (20 mol%), Et₃N (1.2 equiv), 80 °C, 75%; (b) KOH (5 equiv), EtOH, rt, 76%.

Scheme 7.

Reagents and conditions: (a) TBSOTf (2 equiv), NEt₃ (5 equiv), CH₂Cl₂, 0 °C to r.t. (b) propanal (4 equiv), **26** (20 mol%), Et₃N, 80 °C, 75% over 2 steps; (c) NaHMDS (5 equiv), THF, 0 °C; (d) NaOMe (1.3 equiv), MeOH, 65 °C, 36%; 43% brsm for 2 steps; (e) NaBH₄ (5 equiv), MeOH, rt; (f) NaH (4 equiv), BnBr (8 equiv), DMF, 60 °C, 71% for 2 steps; (g) BH₃•THF (3 equiv), THF, rt then 3N NaOH/30% H₂O₂, 60%; (h) TBSOTf (2 equiv), Et₃N (4 equiv), CH₂Cl₂, rt, 97%; (i) H₂ (1 atm), Pd(OH)₂ (catalytic), MeOH, rt, 99%; (j) Burgess reagent (5 equiv), PhMe, 80 °C, 90%; (k) H₂ (1 atm), Pd/C (catalytic), MeOH, rt, 99%; (l) TBAF (3 equiv), THF, 80 °C, microwave, 45 min, 93%.

Synthesis of truncated englerin analogues

Scheme 8.

Reagents and conditions: for **37**: Ac₂O (1.5 equiv), pyridine, 60 °C, 95%; for **38**: benzoic acid (2.0 equiv), 2,4,6-trichlorobenzoyl chloride (2.5 equiv), Et₃N (3.0 equiv), 4-DMAP (catalytic), toluene, 90%; for **39**: cinnamic acid (2.0 equiv), 2,4,6-trichlorobenzoyl chloride (2.5 equiv), Et₃N (3.0 equiv), 4-DMAP (catalytic), toluene, 88%.

Scheme 9.

Reagents and conditions: (a) $i\text{Pr}_2\text{MgCl}$ (1.5 equiv), Et_2O , $-10\text{ }^\circ\text{C}$; (b) $m\text{CPBA}$ (1.0 equiv), CH_2Cl_2 , 75% over two steps; (c) $i\text{Pr}_2\text{NEt}$ (1.2 equiv), MsCl (1.2 equiv), acrylonitrile (80 equiv), $100\text{ }^\circ\text{C}$, microwave, 45%; (d) NaBH_4 (1.0 equiv), $\text{CeCl}_3\cdot\text{H}_2\text{O}$ (1 equiv), MeOH , $0\text{ }^\circ\text{C}$, 98%; (e) H_2 , Pd/C (catalytic), EtOH , 99%; (f) LDA (2 equiv), THF , $-78\text{ }^\circ\text{C}$, then O_2 , then $\text{SnCl}_2\cdot\text{HCl}$ (30 equiv), $0\text{ }^\circ\text{C}$, 40%; (g) cinnamic acid (2.0 equiv), 2,4,6-trichlorobenzoyl chloride (2.5 equiv), Et_3N (3.0 equiv), 4-DMAP (catalytic), toluene, 95%; (h) NaBH_4 (1.0 equiv), MeOH , $0\text{ }^\circ\text{C}$, 100%; (i) Ac_2O (1.5 equiv), Et_3N (4.0 equiv), 4-DMAP (0.5 equiv), CH_2Cl_2 , r.t., 80%; (j) LiHMDS (1.3 equiv), Im_2SO_2 (1.3 equiv), THF , $0\text{ }^\circ\text{C}$ to r.t., 99%; (k) Cesium hydroxy acetate (5 equiv), 18-crown-6 (5 equiv), toluene, $110\text{ }^\circ\text{C}$, 74%.

Table 1

Optimization of the key [4+3] cycloaddition reaction between 9 and 10. n.r.= no reaction. ND = not determined

Furan 9	Catalyst (mol%)	Temp.	Yield (dr)
10 equiv	Rh ₂ (OOct) ₄ (2 mol%)	reflux	91% (3:1)
2 equiv	Rh ₂ (OOct) ₄ (2 mol%)	reflux	90% (3:1)
2 equiv	Rh ₂ (OOct) ₄ (1 mol%)	reflux	73% (3:1)
2 equiv	Rh ₂ (OOct) ₄ (2 mol%)	−78 °C	n.r.
2 equiv	Rh ₂ (OOct) ₄ (2 mol%)	−40 °C	n.r.
2 equiv	Rh ₂ (OOct) ₄ (2 mol%)	0 °C	n.r.
5 equiv	Rh ₂ (OOct) ₄ (2 mol%)	30 °C	31% (ND)
5 equiv	Rh ₂ (OAc) ₄ (2 mol%)	reflux	n.r.

Table 2

Inhibition of cell proliferation by (–)-englerin A and analogues in T-ALL cells (ND: not determined)

Compound	% of Control at 20 μ M	GI ₅₀ (μ M)
(–)-englerin A	81.4 $\pm$ 2.5	> 20
44	74.6 $\pm$ 2.6	> 20
45	75.4 $\pm$ 4.5	> 20
46	88.7 $\pm$ 4.3	> 20
7	98.7 $\pm$ 4.0	> 20
17	0.2 $\pm$ 0	3.3
($\pm$)– 17	ND	1.8
19	0.4 $\pm$ 0.1	2.4
37	0.3 $\pm$ 0.3	ND
38	0.2 $\pm$ 0.1	ND
39	0.1 $\pm$ 0	ND