

Bridgehead Modifications of Englerin A Reduce TRPC4 Activity and Intravenous Toxicity but not Cell Growth Inhibition

Zhenhua Wu^{a,f}, Jean-Simon Suppo^{b,f}, Sarka Tumova^{c,f}, Jonathan Strobe^{d,f}, Fernando Bravo^b, Melody Moy^a, Ethan S. Weinstein^a, Cody J. Peer^d, William D. Figg^d, William J. Chain^a, Antonio M. Echavarren^b, David J. Beech^c and John A. Beutler^{e*}

^aDepartment of Chemistry & Biochemistry, University of Delaware, 163 The Green, Newark DE 19716 USA

^bInstitute of Chemical Research of Catalonia (ICIQ), 43007 Tarragona Spain

^cSchool of Medicine, University of Leeds, Leeds, LS2 9JT UK

^dGenitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892

^eMolecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702 USA

^fThese authors contributed equally to this work.

Abstract

Modifications at the bridgehead position of englerin A were made to explore the effects of variation at this site on the molecule for biological activity, as judged by the NCI 60 screen, in which englerin A is highly potent and selective for renal cancer cells.

Replacement of the isopropyl group by other, larger substituents yielded compounds which displayed excellent selectivity and potency comparable to the natural product. Selected compounds were also evaluated for their effect on the ion channel TRPC4 as well as for intravenous toxicity in mice, and these had lower potency in both assays compared to englerin A.

Keywords: Englerin, kidney cancer, ion channels, , TRPC4, mice

*correspondence: beutlerj@mail.nih.gov; 301-846-1942

Englerin A (**1**) was discovered in extracts of the root and stem bark of the Tanzanian tree *Phyllanthus engleri* on the basis of its nanomolar activity against most renal cancer cell lines and concomitant inactivity against most other cell types in the NCI 60 cell screen.¹ Total syntheses of the molecule established its absolute configuration and have provided multiple synthetic pathways to the natural product.²⁻⁶ Further work in several groups has explored structure activity relationships.⁷⁻¹⁵ Plausible molecular mechanisms of action for englerin A have been proposed, including agonism of protein kinase C θ ,¹⁶ and agonism of the ion channels transient receptor potential canonical (TRPC) 4 and 5.¹⁷⁻¹⁸ In the case of TRPC4/5, it appears that englerin A may bind to an allosteric site on the ion channel complex, thereby facilitating entry of sodium and/or calcium ions into the cell.¹⁹⁻²¹ Two recent reviews have summarized progress.²²⁻²³ One barrier to development of englerin A as a drug candidate has been its potent intravenous toxicity and lethality, observed by both our group and the Novartis group.¹⁷ A recent report identified TRPC4 and TRPC5 ion channels as mediating this adverse response to englerin A.²⁰

Modifications of the bridgehead position at C-7 of englerin A have not been extensively explored. A single study found that trimming the isopropyl group to ethyl or methyl led to a drastic loss of potency in cancer cell growth inhibition.⁹ We report here on a program to examine the effects of expanding the size of the isopropyl group on biological activity, including NCI 60 cell growth inhibition and selectivity, TRPC4/5 agonism, and intravenous toxicity. We have previously reported only NCI 60 data for the initial members of this class, compounds **2**, **3**, **4** and **5** (Table 1, Figure 1).¹⁰ Recently, we evaluated these and other englerin analogues in an assay¹⁸ for TRPC4 agonism. While compound **3**, the cyclopropyl bridgehead analogue of englerin A, was very similar to englerin A in its TRPC4 agonist properties, the cyclohexyl and phenyl bridgehead analogues **4** and **5**, showed much reduced ability to open the ion channel.²⁴⁻²⁵

Thus, we embarked on a collaborative program of analogue synthesis and biological testing using the NCI 60 assay as the primary measure. The synthesis protocols were based on the total syntheses published by the Chain^{6, 11} and Echavarren groups.^{3, 10}

Analogues **2-5** had been prepared following a simplified route that provides 50% of an unwanted and unreactive diastereomer, and which was therefore less efficient in terms of overall yield, but simplified the scale up of the chemical steps. Those analogues possessed an unnatural 4,5 double bond,¹⁰ therefore going forward we chose to prepare the corresponding saturated natural analogues **6** and **7** by the route in Scheme 1. Compound **6** showed particularly good potency. The synthesis of **6** and **7** by the previously described protocols⁶ was straightforward but suffered diminished yields of most steps of the overall sequence due to the increased size of the bridgehead C7-substituent (diversity point **R**¹, Scheme 1). For example, the Michael adducts **19** and **26** resulting from the combination of the furanones **17** and **25** with the aldehyde **18** were isolated in 68% and 49% yield, respectively. The diastereoselectivity of these reactions (1:1.1 dr and 1:2.2 dr [desired:Σ others], respectively) was modest but did not present a problem for analogue production (see Supporting Information).⁶ Later, the samarium(II) iodide-mediated reductive cyclization to give the ketoalcohols **20** and **27** proceeded in 19% and 7% yield, respectively (see Supporting Information).⁶ Fortunately, steps downstream of these events were not significantly impacted; acylations to give a variety of ester substitution at C6 (diversity point **R**², Scheme 1) proceeded in 48–99% yield. The resultant analogues **8-11** with modifications of the cinnamate moiety also possessed the desired potency and selectivity (Table 1). In all cases, the final three-step reduction and glycolate installation sequences were straightforward and proceeded in 15–52% overall yield.

Table 1. NCI 60 selected renal cancer cell growth inhibition data for englerin A (**1**) and analogues (GI_{50} values in nM). n is the number of NCI 60 five dose tests.

Compound	n	Cell lines			Pearson Correlation to Englerin A
		A498	UO-31	RXF 393	
1 ¹	3	11	15	59	1.00
2 ¹⁰	2	34	40	132	0.94
3 ¹⁰	2	100	631	14	0.87
4 ¹⁰	2	21	355	29	0.87
5 ¹⁰	2	95	617	39	0.87
6	1	19	15	78	0.84
7	1	191	155	251	0.80
8	1	21	29	--- ^b	0.84
9	1	16	62	79	0.86
10	1	19	40	71	0.86
11	1	17	407	166	0.75
12	0 ^a	---	---	---	n/a
13	0 ^a	---	---	---	n/a
14	1	2,200	48	3,300	0.78
15	0 ^a	---	---	---	n/a

^aCompound did not pass one dose NCI 60 test. See SI Section VII for one dose data.

^bAssay failed for this cell line.

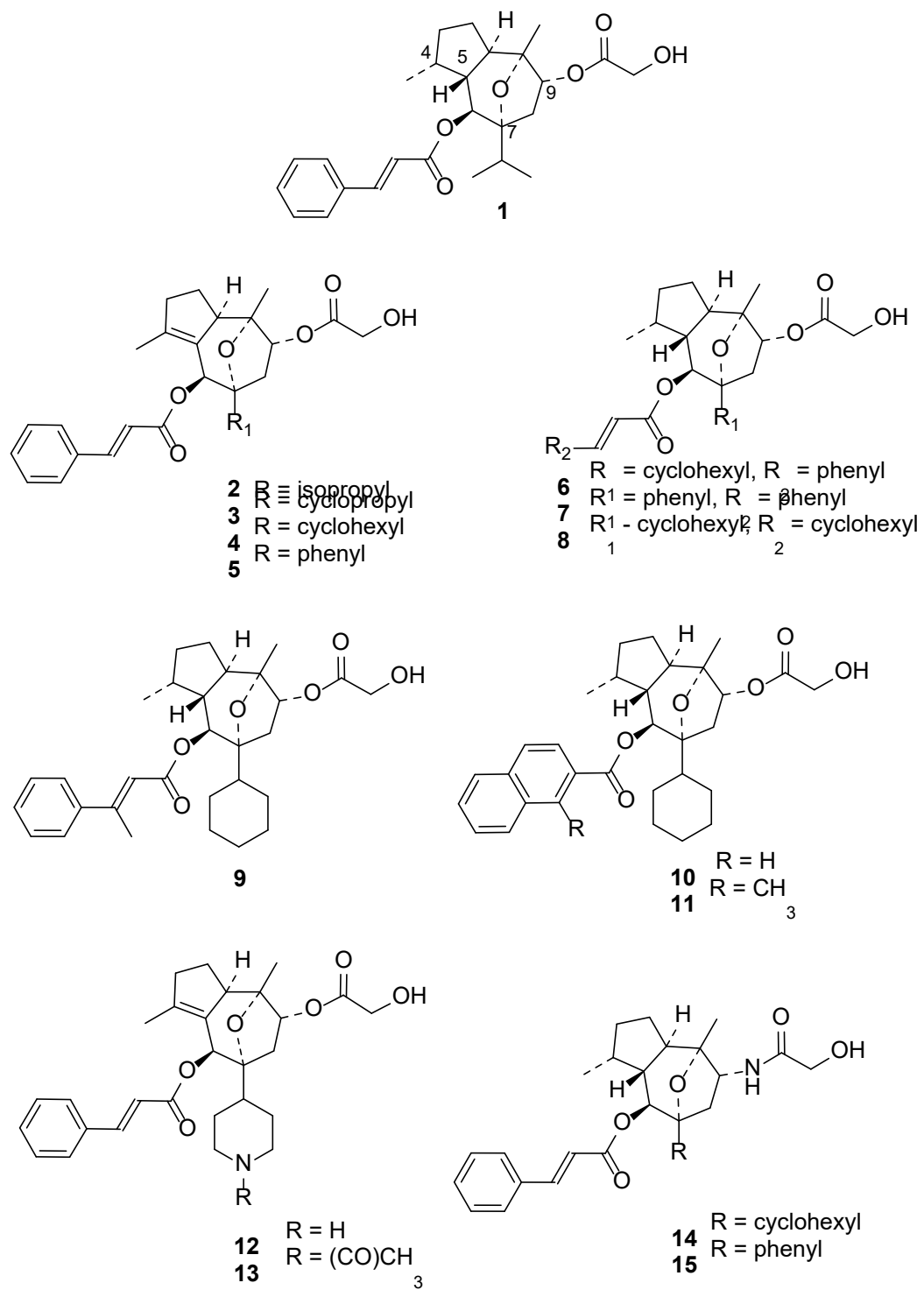
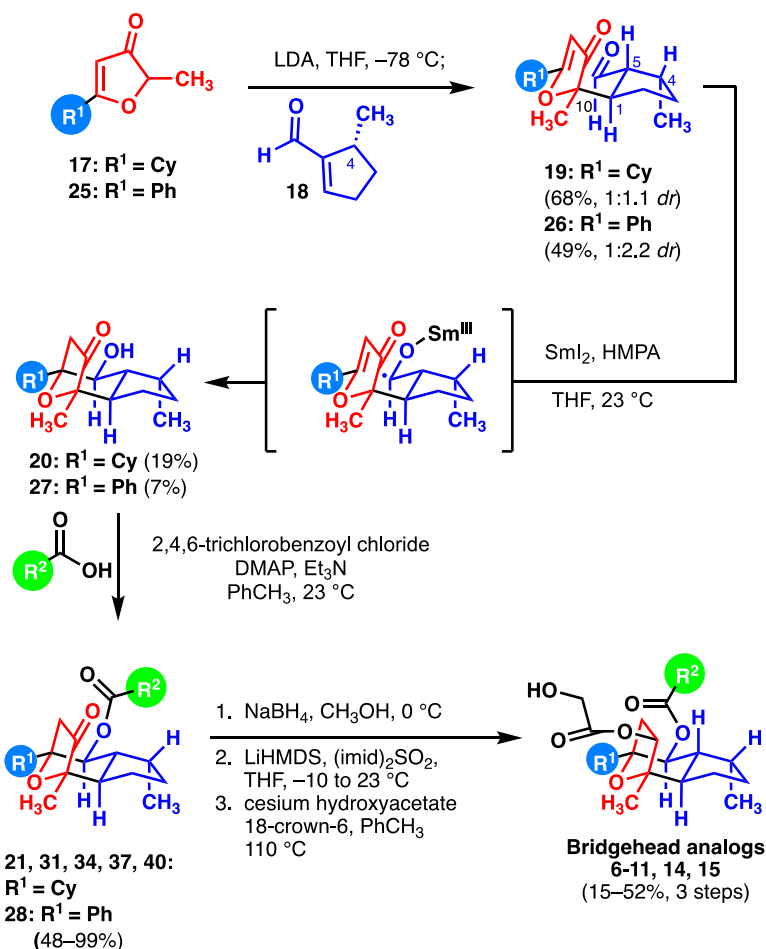
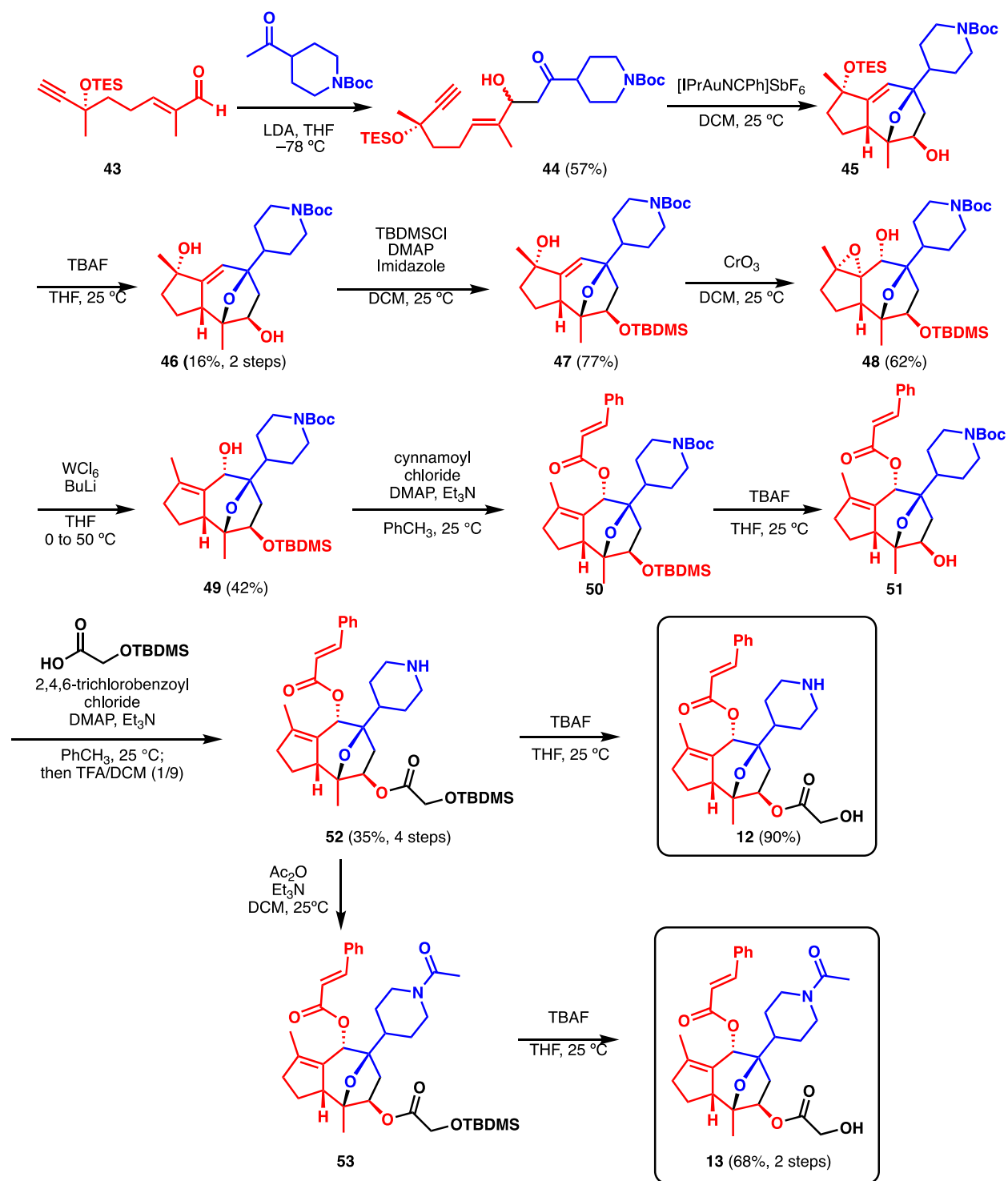


Figure 1. Structures of engerlin A and analogues tested in this work.



Scheme 1.

Next, to determine if nitrogen could be accommodated in the bridgehead cyclohexyl, compounds **12** and **13** were synthesized by means of a gold(I)-catalyzed cascade reaction of 1,6-enyne **44**, which was prepared by an aldol reaction between the enolate *N*-Boc protected 1-(piperidin-4-yl)ethan-1-one with aldehyde **43** (Scheme 2).^{3,10} The gold(I)-catalyzed cyclization of **44** (as a 1:1 mixture of epimeric alcohols), followed by desilylation, gave rise to key intermediate **46** in low yield, although as a single stereoisomer. After protection of the secondary alcohol, **47** was converted into **48** by allylic rearrangement and subsequent epoxidation.^{3,10} Reduction of **48** with WCl₆ and *n*-BuLi provided **49**, which furnished **52** by stepwise esterification of both secondary alcohols and removal of the *N*-Boc protecting group. Finally, intermediate **52** was transformed into **12** and **13** by standard procedures.



Scheme 2.

While slight renal selectivity was seen in piperidine **12** in the NCI 60 single dose test (Supplemental information), the piperidine acetamide **13** was essentially inactive. Thus, no further testing was conducted on the pair.

Finally, recapitulation of our previous strategy to replace the glycolate ester with an amide¹¹ led to the bridgehead cyclohexyl aza-analogue **14** and bridgehead phenyl aza-analogue **15** as shown in Scheme 1. These scaffolds were prepared as described above from ketoesters **21** and **28**, and incorporation of the nitrogen atom proceeded exactly as previously described¹¹ with no modifications required and no loss of reaction efficiency. Compound **14** was weakly active and renal selective, while **15** was nearly inactive. This and the results for the piperidine analogues **12** and **13** may indicate that the englerin pharmacophore does not tolerate nitrogen in the context of either a glycolamide or a bridgehead piperidine.

Based on all of the above results, we chose to carefully test compounds **1**, **4**, **5**, **6**, and **10** in a cellular TRPC4-TRPC1 assay utilizing Tet⁺ HEK 293 cells with an endpoint of Ca⁺² response via Fura-2.¹⁷⁻¹⁸ These selected compounds had excellent NCI-60 potency as seen in Table 1, and were available in amounts sufficient for further testing in mice. Their Pearson correlations to englerin A were all >0.8, indicating a very similar pattern of cell line selectivity to the natural product.

In the TRPC4-TRPC1 assay, however, all of these analogues had lower potency at the ion channel when compared to **1**. At a concentration of 1 μ M, the order of potency was **5** < **4** < **6** < **10** < **1** (Figure 2A). At 100 nM, only **1** displayed any detectable activity (Figure 2B). This is quantified in Figure 3.

Furthermore, all responses were completely inhibited by the TRPC4/5 antagonist Pico 145 (Figure S1A).²⁶ Experiments with the same set of compounds in a similar TRPC5 assay at 10 nM concentration confirmed **1** to be highly active but only compound **10** of the four analogues fully opened the channel (Figure S1B).

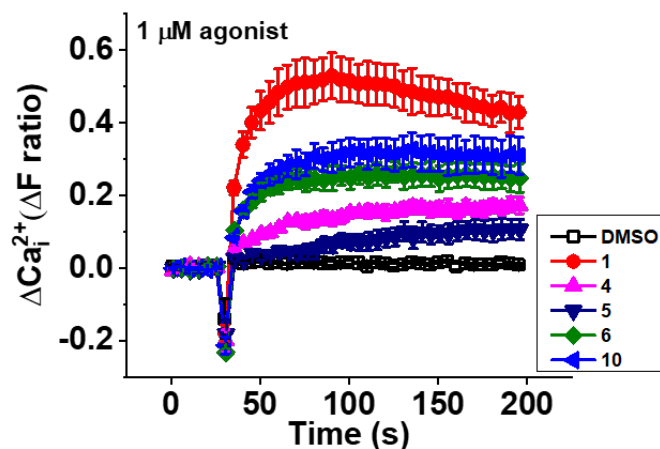
The same four analogues were tested in mice to estimate the maximum tolerated intravenous dose using the “Up-and-Down” protocol, which is designed to minimize the number of mice used for both ethical and economic reasons.²⁷ As reported previously,¹¹ **1** was tolerated only at the very low dose of 50 μ g/kg. Compounds **4**, **6** and **10** were

tolerated at 500 $\mu\text{g}/\text{kg}$, while **5** was tolerated at 200 $\mu\text{g}/\text{kg}$. Hence, **4**, **6**, and **10** are essentially equipotent with **1** in the NCI 60 screen, but 10-fold weaker in both the TRPC4 assay and intravenous toxicity. Detailed results are found in the Supplemental Information.

Figure 2.

Activation of TRPC4/TRPC1 channels. (a, b) The $[\text{Ca}^{2+}]$ responses were measured in TRPC4/TRPC1 expressing HEK293 Tet cells after stimulation with compound **1**, **4**, **5**, **6** or **10** at 1 μM (a) or 0.1 μM (b) concentration. The traces represent mean responses \pm SEM obtained from (n/N=4/22) measurements for all compounds in (a), or (n/N=4/20) measurements in (b), except for the compound **4** (n/N=3/18). Vehicle control was 0.1 % DMSO.

A



B

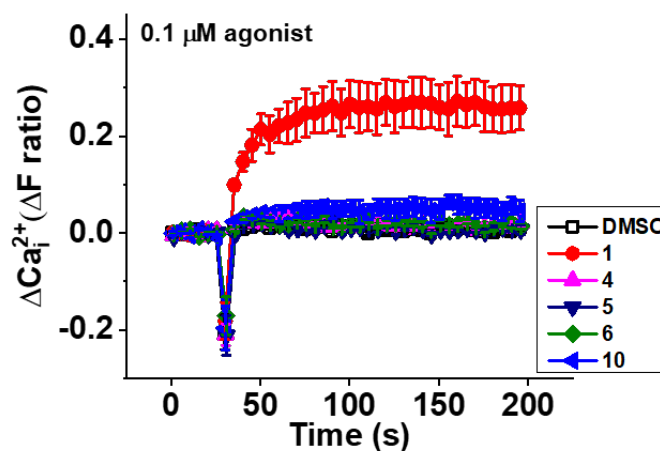
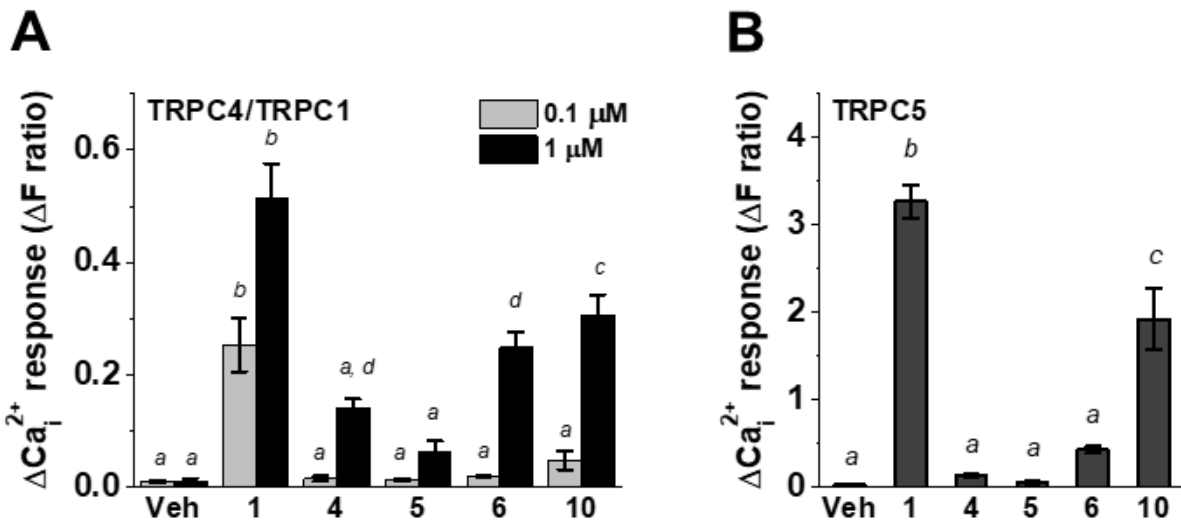


Figure 3:

Quantification of the $[Ca^{2+}]$ responses triggered by the different compounds. The maximum response was measured in TRPC4/TRPC1 (A) and TRPC5 (B) cells after addition of vehicle or compound at final concentration of 0.1 or 1 μ M (A) or 10 nM (B). The values were calculated as mean \pm standard error of mean from the data presented in figure 1 and S1B. At each concentration (0.1 μ M and 1 μ M for TRPC4/TRPC1 and 10 nM for TRPC5), the differences between the effects of the compounds 1, 4, 5, 6 and 10 were assessed by one-way ANOVA followed by post-hoc Bonferroni test for means comparison. The lowercase letters above the bars indicate statistical significance within each group; the means labelled with different letters are significantly different from each other ($p < 0.05$ in the Bonferroni test) while those denoted with common letter are not significantly different ($p > 0.05$).



It has been established through experiments in knockout models that TRPC4 and TRPC5 are both involved in the intravenous lethality and morbidity of englerin A in the mouse.²⁰ Here we have sought to identify chemical modifications which would prevent the TRPC-mediated lethal effect. TRPC channels, including TRPC4 and TRPC5, are known to form heterotetrameric complexes, with an impact on their pharmacological properties and functionality²⁸⁻²⁹ Because of its ubiquitous expression, TRPC1 is expected to participate widely in the heteromer formation in vivo; indeed heteromeric channels containing combinations of TRPC1, TRPC4 and TRPC5 subunits have been detected,³⁰ and formation of novel TRPC channels by complex subunit interactions has been detected in embryonic brain.³⁰⁻³¹ For englerin A, TRPC4/TRPC1 channels in

particular have been shown to mediate the toxicity in synovial sarcoma cells;¹⁹ therefore we have used a cell line expressing a TRPC4-TRPC1 concatemer to determine the compound's ability to activate this specific channel. The results were further validated in TRPC5 expressing cells to confirm the effects.

We have shown here that modification of the 7-isopropyl substituent of the englerin core can substantially reduce the TRPC4 and TRPC5 agonism of the compounds, while having a limited impact on potency and selectivity of cell growth inhibition, as measured by the NCI 60 assay. Furthermore, the best analogues have a 10-fold reduced liability for acute intravenous toxicity in mice.

These modifications may provide a template for development of englerins as human cancer therapeutics. It is important to consider that many oncology drugs are administered by continuous intravenous infusion to prevent adverse effects. However, the acute mouse tail vein model we utilized is only partially informative about the potential clinical hazards of englerins, where slow infusion would likely reduce the acute effects. Furthermore, it is possible that the antitumor activity we observed for englerin A in kidney and prostate cancer xenografts¹⁶ may not be due to either PKC θ or TRPC4/5 agonism. Indeed, a compound with parallel activity against both targets, tonantzitlolone A³²⁻³³ has been found to also affect kinesin-5 function.³⁴ Englerin A has not been tested for this activity.

Recently obtained cryo-EM structures of TRPC4 may also inform the relationship between englerin A, its analogues, and the ion channels.³⁵⁻³⁶ Coupled with data from mutational analysis of TRPC5 function in relation to englerin agonism,²¹ it may eventually be possible to rationally design englerin analogues with reduced clinical liabilities.

Acknowledgments

Thanks to Jason Evans for the COMPARE analysis. Research supported in part by the Intramural Program of the Center for Cancer Research, National Cancer Institute, under

1Z1A BC011470-07 (J.A.B.) and 1ZIABC010547-17 (W.D.F.). Support for the Echavarren lab was from MINECO/FEDER, UE (CTQ2016-75960-) and AGAUR (2017 SGR 1257). S.T. was funded by a Medical Research Council Confidence in Concept Award to D.J.B. W.J.C. gratefully acknowledges financial support from the University of Delaware and the National Institutes of Health (R01CA163287 and P20GM104316). Spectral data at UD was acquired on instruments obtained with the assistance of NSF and NIH funding (NSF CHE0421224, CHE0840401, CHE1229234, CHE1048367; NIH S10 OD016267-01, S10 RR026962-01, P20GM104316, P30GM110758).

Supplementary Material

Detailed synthetic procedures, NMR spectra, ion channel, NCI 60 and mouse toxicity details and data.

Abbreviations

GI₅₀, Growth Inhibition 50 Percent; TRPC1, Transient Receptor Canonical 1; TRPC4, Transient Receptor Canonical 4; TRPC5, Transient Receptor Canonical 5.

References and notes

1. Ratnayake, R.; Covell, D. G.; Ransom, T. T.; Gustafson, K. R.; Beutler, J. A., Englerin A, a Selective Inhibitor of Renal Cancer Cell Growth, from *Phyllanthus engleri*. *Org. Lett.* **2009**, *11*, 57-60.
2. Willot, M.; Radtke, L.; Könnig, D.; Fröhlich, R.; Gessner, V. H.; Strohmann, C.; Christmann, M., Total Synthesis and Absolute Configuration of the Guaiane Sesquiterpene Englerin A. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 9105-9108.
3. Molawai, K.; Delpont, N.; Echavarren, A. M., Enantioselective Synthesis of (-)-Englerins A and B. *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 3517-3519.
4. Xu, J.; Caro-Diaz, E. J.; Theodorakis, E. A., Enantioselective Formal Synthesis of (-)-Englerin A via a Rh-Catalyzed [4 + 3] Cycloaddition Reaction. *Org. Lett.* **2010**, *12*, 3708-3711.
5. Zhou, Q.; Chen, X.; Ma, D., Asymmetric, Protecting-Group-Free Total Synthesis of (-)-Englerin A. *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 3513-3515.
6. Li, Z.; Nakashige, M.; Chain, W. J., A Brief Synthesis of (-)-Englerin A. *J. Am. Chem. Soc.* **2011**, *133*, 6553-6556.
7. Xu, J.; Caro-Diaz, E. J.; Batova, A.; Sullivan, S. D.; Theodorakis, E. A., Formal Synthesis of (-)-Englerin A and Cytotoxicity Studies of Truncated Englerins. *Chem. Asian J.* **2012**, *7*, 1052-1060.
8. Ushakov, D. B.; Navickas, V.; Ströbele, M.; Maichle-Mössmaer, C.; Sasse, F.; Maier, M. E., Total Synthesis and Biological Evaluation of (-)-9-Deoxy-Englerin A. *Org. Lett.* **2011**, *13*, 2090-2093.

9. Radtke, L.; Willot, M.; Sun, H.; Ziegler, S.; Sauerland, S.; Strohmam, C.; Frohlich, R.; Habenberger, P.; Waldmann, H.; Christmann, M., Total Synthesis and Biological Evaluation of (-)-Englerins A and B: Synthesis of Analogues with Improved Activity Profile. *Angew. Chem. Int. Ed. Engl.* **2011**, *50*, 3998-4002.
10. López-Suárez, L.; Bravo, F.; Riesgo, L.; Ransom, T. T.; Beutler, J. A.; Echavarren, A. M., Synthesis and Biological Evaluation of New (-)-Englerin Analogues. *ChemMedChem* **2016**, *11*, 1003-1007.
11. Fash, D. M.; Peer, C. J.; Li, Z.; Talisman, I. J.; Hayavi, S.; Ramos, J. W.; Figg, W. D.; Beutler, J. A.; Chain, W. J., Synthesis of a Stable and Orally Bioavailable Englerin Analogue. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2641-2644.
12. Elliott, D. C.; Beutler, J. A.; Parker, K. A., The Importance of a 4-Alkyl Substituent for Activity in the Englerin Series. *ACS Med. Chem. Lett.* **2017**, *8*, 746-750.
13. Dong, L.; Jiao, X. Z.; Liu, X. Y.; Tian, C. S.; Li, X. Y.; Yao, Y. Y.; Xie, P., Total Synthesis of (+/-)-4-Demethylenglerin A. *J. Asian Nat. Prod. Res.* **2014**, *16*, 629-639.
14. Chan, K. P.; Chen, D. Y., Chemical Synthesis and Biological Evaluation of the Englerin Analogues. *ChemMedChem* **2011**, *6*, 420-423.
15. Acerson, M. J.; Bingham, B. S.; Allred, C. A.; Andrus, M. B., Design and Synthesis of Terpene Based Englerin A Mimics Using Chromium Oxide Mediated Remote CH₂ Oxidation. *Tet. Lett.* **2015**, *56*, 3277-3280.
16. Sourbier, C.; Scroggins, B. T.; Ratnayake, R.; Prince, T. L.; Lee, S.; Lee, J. M.; Trepel, J. B.; Beutler, J. A.; Linehan, W. M.; Neckers, L. M., Englerin a Stimulates PKC theta to Inhibit Insulin Signaling While Simultaneously Activating Hsf1: A Case of Pharmacologically Induced Synthetic Lethality. *Cancer Cell* **2013**, *23*, 228-237.
17. Carson, C.; Raman, P.; Tullai, J.; Xu, L.; Henault, M.; Thomas, E.; Yeola, S.; Lao, J.; McPate, M.; Verkuyl, J. M.; Marsh, G.; Sarber, J.; Amaral, A.; Bailey, S.; Lubicka, D.; Pham, H.; Miranda, N.; Ding, J.; Tang, H. M.; Ju, H.; Tranter, P.; Ji, N.; Krastel, P.; Jain, R. K.; Schumacher, A. M.; Loureiro, J. J.; George, E.; Berellini, G.; Ross, N. T.; Bushell, S. M.; Erdemli, G.; Solomon, J. M., Englerin A Agonizes the TRPC4/C5 Cation Channels to Inhibit Tumor Cell Line Proliferation. *PLoS One* **2015**, *10*, e0127498.
18. Akbulut, Y.; Gaunt, H. J.; Muraki, K.; Ludlow, M. J.; Amer, M. S.; Bruns, A.; Vasudev, N. S.; Radtke, L.; Willot, M.; Hahn, S.; Seitz, T.; Ziegler, S.; Christmann, M.; Beech, D. J.; Waldmann, H., (-)-Englerin A Is a Potent and Selective Activator of TRPC4 and TRPC5 Calcium Channels. *Angew. Chem. Int. Ed. Engl.* **2015**, *54*, 3787-3791.
19. Muraki, K.; Ohnishi, K.; Takezawa, A.; Suzuki, H.; Hatano, N.; Muraki, Y.; Hamzah, N.; Foster, R.; Waldmann, H.; Nussbaumer, P.; Christmann, M.; Bon, R. S.; Beech, D. J., Na(+) Entry through Heteromeric TRPC4/C1 Channels Mediates (-)Englerin A-Induced Cytotoxicity in Synovial Sarcoma Cells. *Sci. Rep.* **2017**, *7*, 16988.
20. Cheung, S. Y.; Henrot, M.; Al-Saad, M.; Baumann, M.; Muller, H.; Unger, A.; Rubaiy, H. N.; Mathar, I.; Dinkel, K.; Nussbaumer, P.; Klebl, B.; Freichel, M.; Rode, B.; Trainor, S.; Clapcote, S. J.; Christmann, M.; Waldmann, H.; Abbas, S. K.; Beech, D. J.; Vasudev, N. S., TRPC4/TRPC5 Channels Mediate Adverse Reaction to the Cancer Cell Cytotoxic Agent (-)-Englerin A. *Oncotarget* **2018**, *9*, 29634-29643.
21. Jeong, S.; Ko, J.; Kim, M.; Park, K. C.; Park, E. Y. J.; Kim, J.; Baik, Y.; Wie, J.; Cho, A. E.; Jeon, J. H.; So, I., Englerin A-Sensing Charged Residues for Transient Receptor Potential Canonical 5 Channel Activation. *Korean Journal of Physiology and Pharmacology* **2019**, *23*, 191-201.
22. Wu, Z.; Zhao, S.; Fash, D. M.; Li, Z.; Chain, W. J.; Beutler, J. A., Englerins: A Comprehensive Review. *J. Nat. Prod.* **2017**, *80*, 771-781.
23. Hagihara, S.; Hanaya, K.; Sugai, T.; Shoji, M., Syntheses of Englerin A, a Potent Renal Cancer Inhibitor. *Asian J. Org. Chem.* **2019**, *8*, 48-62.

24. Beutler, J. A.; Echavarren, A. M.; Chain, W. J.; Beech, D. J.; Wu, X.; Suppo, J.-S.; Bravo, F.; Rubaiy, H. N.; Ransom, T. T. Compounds for Treating Cancer. U.S. Pat. Appl. 62/529,063.
25. Beutler, J. A.; Echavarren, A. M.; Lopez, L.; Bravo, F.; Riesgo, L.; Ransom, T. T. Epoxyazulene Derivatives Useful for Treating Cancer and Diabetes U.S. Pat. 10,287,297, May 14, 2019.
26. Rubaiy, H. N.; Ludlow, M. J.; Bon, R. S.; Beech, D. J., Pico145 - Powerful New Tool for TRPC1/4/5 Channels. *Channels* **2017**, *11*, 362-364.
27. Bruce, R. D., An up-and-Down Procedure for Acute Toxicity Testing. *Fundam. Appl. Toxicol.* **1985**, *5*, 151-7.
28. Minard, A.; Bauer, C. C.; Wright, D. J.; Rubaiy, H. N.; Muraki, K.; Beech, D. J.; Bon, R. S., Remarkable Progress with Small-Molecule Modulation of TRPC1/4/5 Channels: Implications for Understanding the Channels in Health and Disease. *Cells* **2018**, *7*.
29. Kim, J.; Ko, J.; Myeong, J.; Kwak, M.; Hong, C.; So, I., TRPC1 as a Negative Regulator for TRPC4 and TRPC5 Channels. *Pflügers Archiv* **2019**, *471*, 1045-1053.
30. Bröker-Lai, J.; Kollwe, A.; Schindeldecker, B.; Pohle, J.; Nguyen Chi, V.; Mathar, I.; Guzman, R.; Schwarz, Y.; Lai, A.; Weissgerber, P.; Schwegler, H.; Dietrich, A.; Both, M.; Sprengel, R.; Draguhn, A.; Kohr, G.; Fakler, B.; Flockerzi, V.; Bruns, D.; Freichel, M., Heteromeric Channels Formed by TRPC1, TRPC4 and TRPC5 Define Hippocampal Synaptic Transmission and Working Memory. *EMBO J.* **2017**, *36*, 2770-2789.
31. Strübing, C.; Krapivinsky, G.; Krapivinsky, L.; Clapham, D. E., Formation of Novel TRPC Channels by Complex Subunit Interactions in Embryonic Brain. *J. Biol. Chem.* **2003**, *278*, 39014-9.
32. Sourbier, C.; Scroggins, B.; Mannes, P. Z.; Liao, P. J.; Siems, K.; Wolf, D.; Beutler, J. A.; Linehan, W. M.; Neckers, L., Tonantzitolone Cytotoxicity toward Renal Cancer Cells Is PKC theta- and Hsf1-Dependent. *Oncotarget* **2015**, *6*, 29963-29974.
33. Rubaiy, H. N.; Wolf, D.; Beutler, J. A.; Beech, D. J., Tonantzitolone Is a Nanomolar Potency Activator of TRPC1/4/5 Channels. *Br. J. Pharmacol.* **2018**, *175*, 3361-3368.
34. Pfeffer, T. J.; Sasse, F.; Schmidt, C. F.; Lakamper, S.; Kirschning, A.; Scholz, T., The Natural Diterpene Tonantzitolone A and Its Synthetic Enantiomer Inhibit Cell Proliferation and Kinesin-5 Function. *Eur. J. Med. Chem.* **2016**, *112*, 164-70.
35. Vinayagam, D.; Mager, T.; Apelbaum, A.; Bothe, A.; Merino, F.; Hofnagel, O.; Gatsogiannis, C.; Raunser, S., Electron Cryo-Microscopy Structure of the Canonical TRPC4 Ion Channel. *Elife* **2018**, *7*.
36. Duan, J.; Li, J.; Zeng, B.; Chen, G. L.; Peng, X.; Zhang, Y.; Wang, J.; Clapham, D. E.; Li, Z.; Zhang, J., Structure of the Mouse TRPC4 Ion Channel. *Nat. Commun.* **2018**, *9*, 3102.