HHS Public Access

Author manuscript

Bioorg Med Chem Lett. Author manuscript; available in PMC 2017 June 01.

Published in final edited form as:

Bioorg Med Chem Lett. 2016 June 1; 26(11): 2641–2644. doi:10.1016/j.bmcl.2016.04.016.

Synthesis of a Stable and Orally Bioavailable Englerin Analogue

David M. Fash[a], **Cody J. Peer**[c], **Zhenwu Li**[a], **Ian J. Talisman**[a], **Sima Hayavi**[f], **Florian J. Sulzmaier**[b], **Joe W. Ramos**[b], **Carole Sourbier**[e], **Leonard Neckers**[e], **W. Douglas Figg**[c], **John A. Beutler**[d,*], and **William J. Chain**[a,b,*,g]

[a]Department of Chemistry, University of Hawaii at Manoa, 2545 McCarthy Mall, Honolulu, Hawaii 96822, United States

[b]The University of Hawaii Cancer Center, 701 Ilalo Street, Honolulu, Hawaii 96813, United States

[c]Genitourinary Malignancies Branch, National Cancer Institute, Frederick, Maryland 21702, United States

[d]Molecular Targets Laboratory, National Cancer Institute, Frederick, Maryland 21702, United States

[e]Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute, Frederick, Maryland 21702, United States

[f]Developmental Therapeutics Program, National Cancer Institute, Frederick, Maryland 21702, United States

Abstract

Synthesis of analogues of englerin A with a reduced propensity for hydrolysis of the glycolate moiety led to a compound which possessed the renal cancer cell selectivity of the parent and was orally bioavailable in mice.

Graphical Abstract

[*]**Corresponding Authors**. beutlerj@mail.nih.gov, wchain@udel.edu.

[g]Present address: Department of Chemistry and Biochemistry, University of Delaware, 163 The Green, Newark, Delaware 19716.

ASSOCIATED CONTENT

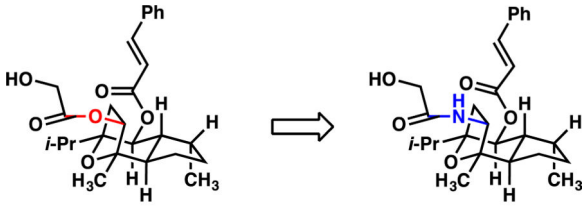
Supplementary Information

Experimental procedures, characterization data, and ^{1}H and ^{13}C spectra for all new compounds. Bioloigical evaluation data for compounds **1**, **2**, and **17**–**20**.

Author Contributions

W.J.C., J.A.B., L.N., and W.D.F. designed research; W.D.F., C.P., S.H., Z.L., C.S., I.T., and F.J.S. performed research; J.A.B., W.J.C., L.N., and W.D.F. analyzed data, and W.J.C. and J.A.B. wrote the manuscript. All authors have given approval to the final version of the manuscript.

The authors declare no competing financial interest.



Renal cancer is an ongoing and critical medical problem in the United States, with alarming annual increases in incidence and death.[1] In 2015 alone, it is estimated that the U.S. will see 61,560 new patients and 14,080 deaths resulting from renal cancer.[2] Patients with nonresectable or metastatic tumors are faced with several chemotherapy options, including immunomodulatory therapies, and target-based approaches that owe their success to the discovery of the von Hippel-Lindau (VHL) tumor suppressor gene. The target-based approaches include receptor tyrosine kinase-based therapies that interrupt or block VEGF or TGF-α signaling or related pathways, and are collectively a multi-billion dollar annual health burden in the United States alone. Unfortunately, these treatments can have serious adverse side effects,[3] and provide only modest survival advantages,[4] driving intense interest in effective new chemotherapeutic drug leads for the treatment of renal cancer.

We isolated the guaiane sesquiterpene ester englerin A from an extract of the Tanzanian plant *Phyllanthus engleri* Pax on the basis of its high potency and selectivity for inhibiting renal cancer cell growth.[5] This compound has stimulated wide interest and activity in both the synthetic chemistry[6] and cancer biology[7] communities. We report here a stable and orally bioavailable analogue of the natural product englerin A.[8]

Initial intraperitoneal administration of englerin A to mice was done in DMSO solution.[7b] We developed more suitable vehicles for both parenteral and oral administration. A cosolvent approach with hydroxypropyl-β-cyclodextrin for parenteral use provided a clear and refrigerator-stable preparation with a concentration of up to 5.7 mg/mL (see Supplemental Information for details). An oral formulation utilizing Labrasol™ was able to dissolve englerin A directly at 20 mg/mL at room temperature.

Using the above mentioned vehicles, we attempted to determine maximum tolerated doses (MTD) in mice by intraperitoneal (i.p.), intravenous (i.v.), and oral (p.o.) routes. The i.p. MTD for englerin A was approximately 10 mg/kg. Intravenous administration was rapidly lethal,[7e] with an estimated MTD of 50 μg/kg. Mice died almost instantaneously at doses from 1–10 mg/kg. In contrast, p.o. administration of englerin A by gavage was tolerated up to 100 mg/kg. While englerin A was detected in mouse serum after i.p. injection, the oral route did not produce detectable levels of the compound (see Supplemental Information for details). We reasoned that the glycolate moiety of englerin A was hydrolyzed by gastric acid, and thus sought to prepare a more stable series of analogues.

The incorporation of nitrogen at C9 of the natural product was a straightforward process requiring minimal modification to the synthesis of englerin A described by our laboratory in 2011.[6f] The production of aza-englerin analogues employed the hydroxyketone **3** as a

starting point. The C6 hydroxyl function was acylated using the Yamaguchi protocol or commercially available acid chloride under standard conditions to give esters **5**–**8** in 41–93% yield. Reduction of the C9 ketone functions to the corresponding alcohols **9**–**12** under the action of methanolic sodium borohydride proceeded as previously described in 67–99% yield. Conversion of the alcohols to the imidizolium sulfonates was similarly straightforward, and proceeded under the action of lithium bis(trimethylsilyl)amide and sulfuryl diimidazole in 55–100% yield. In each case, the nitrogen atom was introduced in the form of azide under standard substitution conditions (NaN_3 in warm DMF) and gave the azides **13**–**16** cleanly in 75–99% yield. Chemoselective reduction of the azides to the corresponding primary amines was achieved under hydrogenative conditions or under the action of zinc powder and ammonium chloride in methanol (See Supplementary Information for details). In each case, the primary amines were acylated directly without further purification utilizing glycolic acid or fluoroacetic acid and *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride to give a series of C9-aza-englerin A analogues **2** and **17**–**20** in 29–72% yield (two steps).

Five analogues **2**, **17**, **18**, **19**, and **20** were evaluated in the NCI 60 cell screening assay.[9] Compound **20** was inactive in preliminary screening at 10^{-5}M, and was not tested further. The results are shown in Tables 1, 2, and 3, and in the Supplemental Information.

Even the most active englerins (e.g., **1**) display relatively modest average growth inhibition at 10^{-5}M in the one dose initial tests (Table 1), since only 8 cell lines of 60 display substantial sensitivity. The range of response at 10^{-5}M gives a better estimate of potency; by this criterion, none of the aza-englerin analogues is as potent as **1**. Compounds **2**, **17** and **19** come the closest to englerin A. Inspecting the response of the cell lines which are most sensitive to englerin A (A498 and HS 578T), the same conclusion can be reached. One dose data is of limited precision compared to the five dose data, therefore we tested the five compounds in the latter format.

The five dose tests support the compound potency estimates (Table 2). Compound **2** shows substantial selectivity for kidney cancer cell lines, but is clearly at least 15-fold weaker than englerin A. In the breast cancer cell line HS 578T, which is very sensitive to englerin A, **2** is ~80-fold less potent. Compounds **17** and **19** are 5–10-fold weaker than **2**, while **18** is another several-fold weaker.

Examination of the patterns of selectivity confirm that all of the more potent analogues share the renal selectivity profile of the natural product (Table 3). Compound **18** was not included due to the limited dynamic range of its data.

The most potent analogue **2** was chosen for preliminary pharmacokinetic evaluation in mice, using the oral route. The compound was administered in Labrasol vehicle at 50 mg/kg, and was well tolerated. Analysis of serum samples taken at different time points demonstrated that **2** was bioavailable, and serum levels were maintained much longer and at higher concentrations than they were with englerin A given i.p. (Figure 2).

This result supports the theory that englerin A is not orally bioavailable due to cleavage of the glycolate ester in the acid conditions of the stomach, which would produce englerin B, which we[5] and others[6c] have previously shown to have no activity on cancer cell growth. Furthermore, recent data show that englerin B can be detected in serum of rats after oral administration of englerin A.[7e]

While **2** is bioavailable by the oral route, its intrinsic activity in cellular systems such as the NCI 60 assay is much weaker than englerin A, as noted above. Further evidence for its weaker intrinsic activity was obtained with a panel of 4 VHL-deficient kidney cancer cell lines, in which the IC_{50} values ranged from 10 to 100 μM (see Supplemental Information) and in stimulation of generic PKC activity in lysates of 786-0 cells, where **2** had no activity at 1 μM, while **1** was active at 10 nM, and 2'-chloro-englerin A[12] and tonantzitlolone[13] were active at 1 μM (see Supplementary Information). It is not clear why analogues **17**–**19** are even weaker than **2**, since replacement of the cinnamate with alternative ester groups has been reported by others to give high cellular potency in the glycolate series.[10,11] Although purely speculative at this time, it is possible that the amide N-H presents a sufficiently different hydrogen bonding landscape relative to the natural product to alter its conformation (perhaps due to internal hydrogen bonding with the endocyclic oxygen atom) or otherwise disrupt the nature of its binding to the cellular target, which remains unknown as of this writing. Current efforts are focused on design and synthesis of aza-englerin analogues with improved cell growth inhibition that address this other structural speculation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

J.A.B., C.J.P., C.S., L.N., and W.D. F. are supported by the Intramural Program of the National Cancer Institute (1ZIABC01147003, 1ZIABC01068311, and 1Z1CSC00653621). W.J.C. gratefully acknowledges financial support from the National Institutes of Health (R01CA163287), the University of Hawaii, the University of Hawaii Cancer Center, and the University of Delaware. Some data were acquired at UD on instruments obtained with the assistance of NSF and NIS funding (NSF CHE0421224, CHE1229234, and CHE0840401; NIH P20GM103541, P20GM104316, P30GM110758, S10RR02692, and S10OD016267). W.J.C. gratefully acknowledges the assistance of Michael F. Wisthoff and Senzhi Zhao in the preparation of the Supplemental Information and spectral data.

References and Notes

1. Weir HK, Thompson TD, Soman A, Moller B, Leadbetter S. Cancer. 2015; 121:1827. [PubMed: 25649671]
2. US National Cancer Institute. [accessed 02-29-2016] Surveillance, Epidemiology, and End Results Program (SEER). http://seer.cancer.gov
3. Launay-Vacher V, Aapro M, De CG Jr, Cohen E, Deray G, Dooley M, Humphreys B, Lichtman S, Rey J, Scotte F, Wildiers H, Sprangers B. Ann. Oncol. 2015; 26:1677. [PubMed: 25735315]
4. Macleod LC, Tykodi SS, Holt SK, Wright JL, Lin DW, Tretiakova MS, True LD, Gore JL. Urology. 2015; 86:262. [PubMed: 26199164]
5. Ratnayake R, Covell DG, Ransom TT, Gustafson KR, Beutler JA. Org. Lett. 2009; 11:57. [PubMed: 19061394]
6. (a) Willot M, Radtke L, Könning D, Fröhlich R, Gessner VH, Strohmann C, Christmann M. Angew. Chem. Int. Ed. Engl. 2009; 48:9105. [PubMed: 19882614] (b) Molawai K, Delpont N, Echavarren AM. Angew. Chem. Int. Ed. Engl. 2010; 49:3517. [PubMed: 20544903] (c) Nicolaou KC, Kang Q,

Ng SY, Chen DY. J. Am. Chem. Soc. 2010; 132:8219. [PubMed: 20496885] (d) Xu J, Caro-Diaz EJ, Theodorakis EA. Org. Lett. 2010; 12:3708. [PubMed: 20669919] (e) Zhou Q, Chen X, Ma D. Angew. Chem. Int. Ed. Engl. 2010; 49:3513. [PubMed: 20544902] (f) Li Z, Nakashige M, Chain WJ. J. Am. Chem. Soc. 2011; 133:6553. [PubMed: 21476574] (g) Lee J, Parker KA. Org. Lett. 2012; 14:2682. [PubMed: 22564056] (h) Takahashi K, Komine K, Yokoi Y, Ishihara J, Hatakeyama SJ. Org. Chem. 2012; 77:7364.(i) Zahel M, Kessberg A, Metz P. Angew. Chem. Int. Ed Engl. 2013(j) Zhang J, Zheng S, Peng W, Shen Z. Tetrahedron Lett. 2014; 55:1339.

7. (a) Sulzmaier FJ, Li Z, Nakashige ML, Fash DM, Chain WJ, Ramos JW. PLoS. One. 2012; 7:e48032. [PubMed: 23144724] (b) Sourbier C, Scroggins BT, Ratnayake R, Prince TL, Lee S, Lee JM, Trepel JB, Beutler JA, Linehan WM, Neckers LM. Cancer Cell. 2013; 23:228. [PubMed: 23352416] (c) Williams RT, Yu AL, Diccianni MB, Theodorakis EA, Batova AJ. Exp. Clin. Cancer Res. 2013; 32:1.(d) Akbulut Y, Gaunt HJ, Muraki K, Ludlow MJ, Amer MS, Bruns A, Vasudev NS, Radtke L, Willot M, Hahn S, Seitz T, Ziegler S, Christmann M, Beech DJ, Waldmann H. Angew. Chem. Int Ed Engl. 2015; 54:3787. [PubMed: 25707820] (e) Carson C, Raman P, Tullai J, Xu L, Henault M, Thomas E, Yeola S, Lao J, McPate M, Verkuyl JM, Marsh G, Sarber J, Amaral A, Bailey S, Lubicka D, Pham H, Miranda N, Ding J, Tang HM, Ju H, Tranter P, Ji N, Krastel P, Jain RK, Schumacher AM, Loureiro JJ, George E, Berellini G, Ross NT, Bushell SM, Erdemli G, Solomon JM. PLoS One. 2015; 10:e0127498. [PubMed: 26098886] (f) López-Suárez L, Riesgo L, Bravo F, Ransom TT, Beutler JA, Echavarren AM. Chem Med Chem. 2016 In press. (g) Caropreso V, Darvishi E, Turbyville TJ, Ratnayake R, Grohar PJ, McMahon JB, Woldemichael G. J. Biol. Chem. 2016 In press.
8. Chain, WJ.; Beutler, JA.; Fash, DM.; Figg, WD.; Li, Z.; Peer, CJ.; Ramos, JW.; Sulzmaier, FJ. Aza-epoxy-guaiane Derivatives and Treatment of Cancer. U. S. Patent Application. PCT/US2015/014601. 2015 Feb 5.
9. Shoemaker RH. Nat. Rev. Cancer. 2006; 6:813. [PubMed: 16990858]
10. Chan KP, Chen DY. Chem Med Chem. 2011; 6:420. [PubMed: 21360817]
11. Radtke L, Willot M, Sun H, Ziegler S, Sauerland S, Strohmann C, Frohlich R, Habenberger P, Waldmann H, Christmann M. Angew. Chem. Int. Ed Engl. 2011; 50:3998. [PubMed: 21472928]
12. Akee RK, Ransom TT, Ratnayake R, McMahon JB, Beutler JA. J. Nat. Prod. 2012; 75:45.
13. Sourbier C, Scroggins B, Mannes PZ, Liao PJ, Siems K, Wolf D, Beutler JA, Linehan WM, Neckers L. Oncotarget. 2015; 6:29963. [PubMed: 26298773]

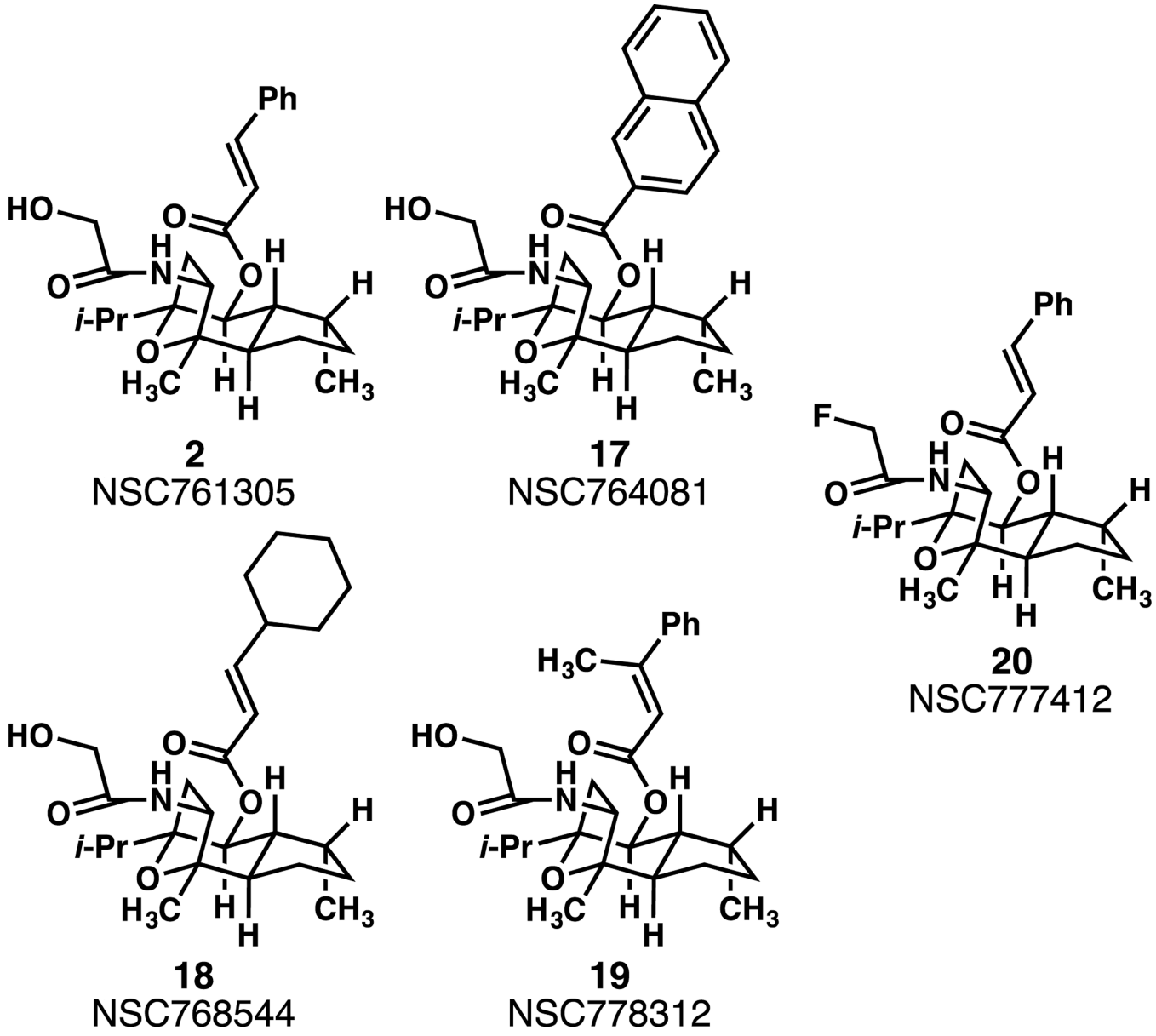


Figure 1.
Structures of target aza-englerins.

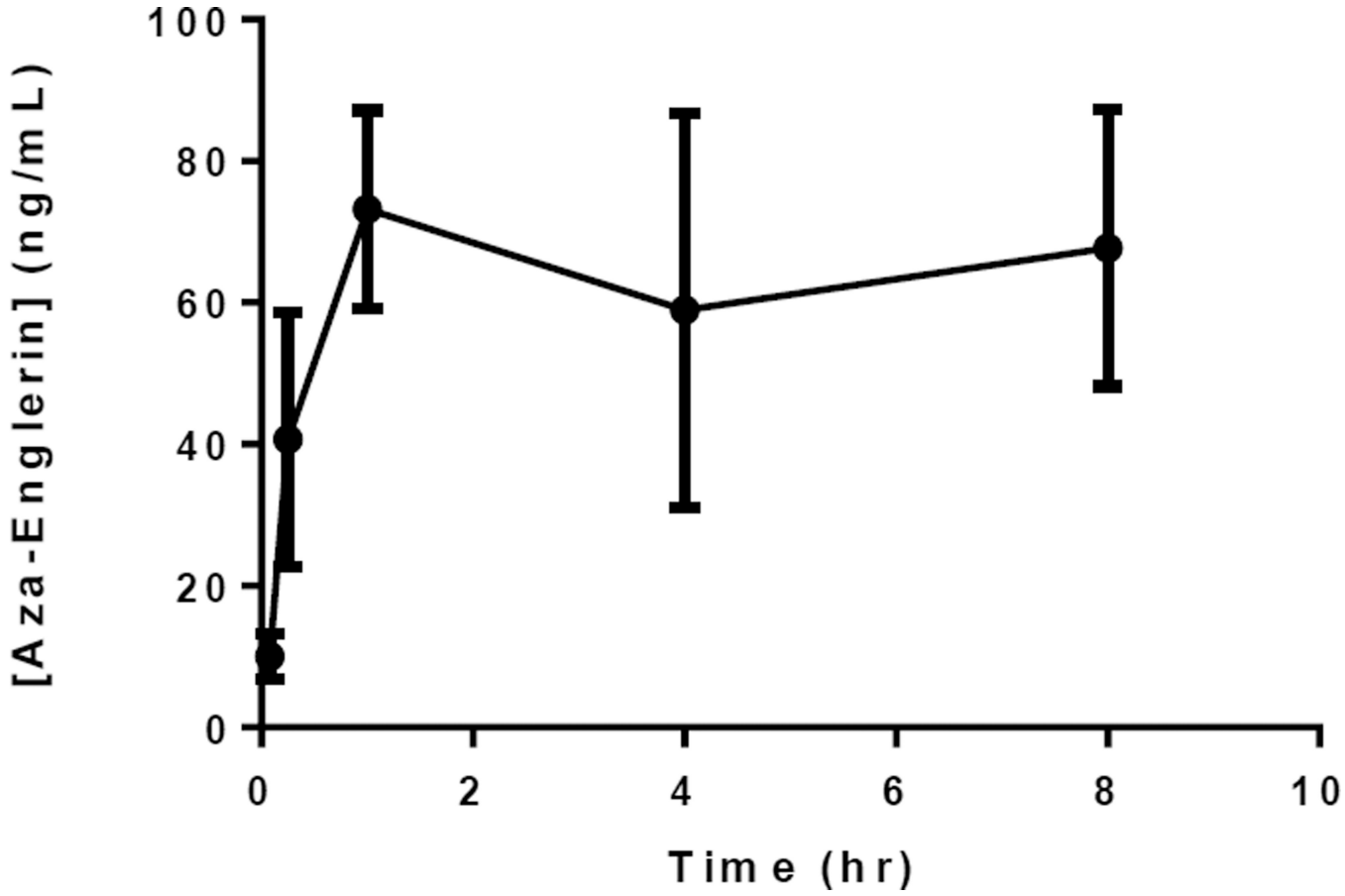
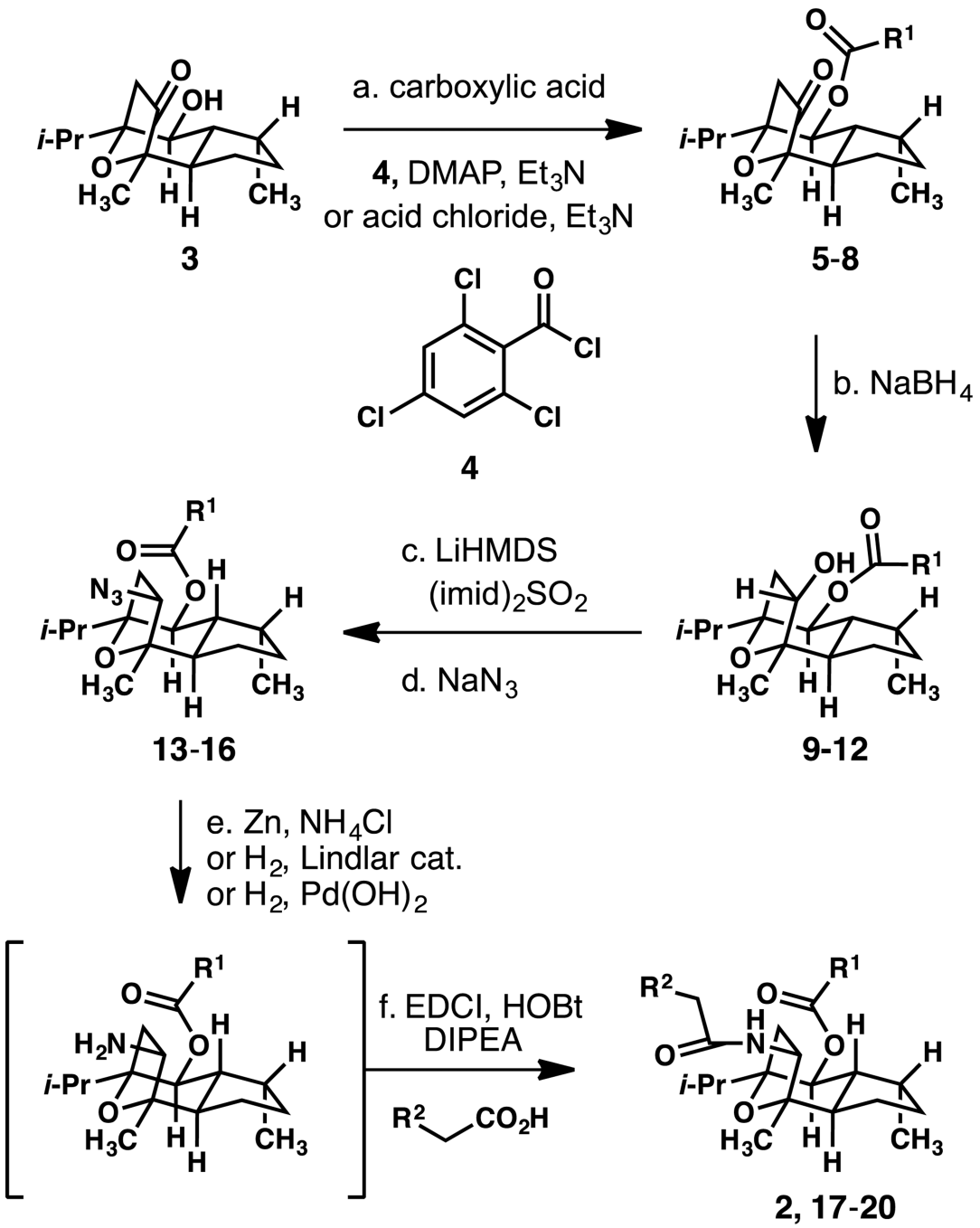


Figure 2.
Serum levels of 2 after oral administration at 50 mg/kg in mice (n=3) measured by HPLC-MS



Scheme 1.

Synthesis of Aza-englerin analogues.

Conditions: (a) carboxylic acid, 2,4,6-trichlorobenzoyl chloride, DMAP, Et_3N, toluene, 23 °C or carboxylic acid chloride, DMAP, Et_3N, toluene, 23 °C. (b) $NaBH_4$, CH_3OH, 0 °C. (c) LiHMDS, $(imid)_2SO_2$, THF, −10 → 23 °C. (d) NaN_3, DMF, 80 °C. (e) Zn dust, NH_4Cl, CH_3OH, 23 °C or H_2, Lindlar catalyst, EtOH, 23 °C or H_2, $Pd(OH)_2$, CH_3OH, 23 °C (f)

$R^2CH_2CO_2H$, EDCI, HOBt, *i*-Pr_2NEt, DMF, 23 °C. See Supplementary Information for detailed procedures.

Table 1

NCI 60 data one dose data for aza-englerins at 10^{-5}M.

Compound	One Dose Mean Pct	One Dose Range Pct	A498 Pct	HS 578T Pct
1	81	236	39	29
2	84	127	−15	9
17	79	128	−15	−18
18	100	92	41	73
19	93	150	−25	−9
20	99	41	83	93

Table 2

NCI 60 data five dose data for aza-englerins.

Compound	Mean GI_{50} μM	Range log_{10} units	A498 GI_{50} nM	HS 578T GI_{50} nM
1	3.7	3.30	10	10
2	13	2.49	162	832
17	14	1.10	1620	2230
18	39	0.9	5620	6170
19	27	1.94	1150	2240

Table 3

Matrix COMPARE of selected analogues versus englerin A at the GI_{50} level, Pearson correlation coefficients.

Compound	1	2	17	19
1	1	0.731	0.748	0.833
2	0.731	1	0.856	0.846
17	0.748	0.856	1	0.782
19	0.833	0.846	0.782	1