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Synthesis and antibacterial evaluation of anziaic acid and analogues as topoisomerase I inhibitors

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Abstract

Naturally occurring anziaic acid was very recently reported as a topoisomerase I inhibitor with antibacterial activity. Herein total synthesis of anziaic acid and structural analogues is described and the preliminary structure-activity relationship (SAR) has been developed based on topoisomerase inhibition and whole cell antibacterial activity.

Introduction

DNA topoisomerases are involved in the processes of DNA replication and transcription by controlling the topology of DNA.^{1, 2} Topoisomerases can be divided into types I and II subclasses according to the number of strands cut in the mechanism.³ As topoisomerases are essential for normal cellular processes and cell proliferation, topoisomerase inhibitors can lead to cell death by binding and stabilizing cleavable topoisomerase-DNA complexes and preventing subsequent religation of the cleaved DNA strand. In this regard, topoisomerases represent attractive targets in antibacterial and anticancer drug discovery.⁴⁻⁷ Clinically, semisynthetic camptothecin derivatives topotecan and irinotecan inhibit human type IB topoisomerases and have been used for the treatment of various cancers.^{8, 9} In addition, the human type IIA topoisomerases are targets for anticancer drug classes of podophyllotoxins⁸ (etoposide and teniposide) and anthracyclines.¹⁰ Fluoroquinolones, one of the most successful antibiotic classes, exert their antibacterial activity by inhibiting type IIA topoisomerases (DNA gyrase and topoisomerase IV).¹¹

The continuing emergence and prevalence of multidrug resistant bacterial pathogens, such as methicillin resistant *Staphylococcus aureus*,¹² extensively drug resistant tuberculosis,¹³ fluoroquinolone resistant *Pseudomonas aeruginosa*,¹⁴ and carbapenem resistant Enterobacteriaceae¹⁵ have become an alarming and serious public health threat. Therefore, the new chemotype antibacterial agents with novel targets and mode of action are highly needed to combat pathogenic and drug resistant microorganisms. Bacterial topoisomerase I belonging to the type IA topoisomerase subfamily has emerged as a promising target for developing new antibiotics and notably, currently no clinically used antibiotics target type

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[†]Electronic Supplementary Information (ESI) available: Experimental synthetic procedures and copies of ¹H, ¹³C NMR spectra and HPLC chromatographs of synthetic intermediates, anziaic acid, and its analogues. See DOI: 10.1039/b000000x/

IA topoisomerase enzyme.^{4, 6, 16} In this context, new, potent, and selective type IA topoisomerase inhibitors may be developed as effective antibacterial agents with therapeutic potential for bacterial pathogens resistant to current antibiotics.

Natural product has been one of the most important and successful sources of novel antibacterial agents.^{17–20} Naturally occurring depsides which commonly exist in lichens showed promising and diverse biological activities. As examples, atranorin (Fig. 1) from lichen *Parmelia reticulata* was reported to exhibit antifungal activity against *S. roffsii* (ED₅₀ = 39.70 μg/mL).²¹ Jaboticabin from the fruit of jaboticaba (*Myrciaria cauliflora*) showed antioxidant and anti-inflammatory activities with therapeutic potential for the treatment of chronic obstructive pulmonary disease, as well as anticancer activity.^{22, 23} Anziaic acid,^{24–26} a depside isolated from lichen *Hypotrachyna* sp., was very recently found to exhibit inhibitory activity against bacterial (*Y. pestis* and *E. coli*) topoisomerase I and human topoisomerase II with IC₅₀ values of 14–19 and 35 μM, respectively.²⁷ This natural product also demonstrated whole cell antibacterial activity against *Bacillus subtilis* and a membrane permeable strain (BAS3023) of *Escherichia coli* with minimum inhibitory activity (MIC) values of 6 and 12 μg/mL, respectively.²⁷

In our continued effort to discover novel chemotype antibacterial agents, we have employed emerging natural product leads as medicinal chemistry starting points, guided by whole cell activity-driven approach and followed by target deconvolution and identification.^{28, 29} Recently, we initiated this topoisomerase I target-driven antibacterial research program. In particular, the promising bacterial topoisomerase I inhibition and whole cell antibacterial activity and the symmetrical and dimeric structural features of anziaic acid attracted our interest toward its resynthesis and biological evaluation of its structural analogues. Here the synthesis of anziaic acid and analogues is described and the preliminary SAR is reported.

Results and discussion

Chemistry

Structurally, anziaic acid is a dimer of 2,4-dihydroxy-6-*n*-pentylbenzoic acid (**4**), and was previously synthesized by Asahina and Hiraiwa³⁰ and Elix.³¹ In this work, a convergent synthesis of anziaic acid was achieved from commercially available starting material (Scheme 1). First, **2** was obtained from olivetol (**1**) *via* Vilsmeier-Haack reaction in moderate yield. The two phenol hydroxyl groups were protected with benzyl groups using potassium carbonate as the base, followed by oxidization with NaClO₂ to yield the phenol-protected acid product **3** in 61% yield. The acid-protected phenol (**5**) was synthesized following two sequential steps of oxidation of **2** and selective benzyl ester protection of carboxylic acid in **4**.³¹ Compound **4** was also prepared by debenzoylation of **3** with higher yield and purity. Once the two key intermediates are in hand, subsequent selective condensation of **3** and **5** was performed³² to afford benzyl protected dimeric precursor **6** in 64% yield by using trifluoroacetic acid anhydride as a condensation reagent. Finally, the benzyl groups were removed under palladium on carbon (Pd/C) and hydrogen atmosphere to give the synthetic anziaic acid in 95% yield.

Next, to investigate the effect of different substituents of the anziaic acid scaffold, such as the metal chelating salicylic acid motif and two lipophilic *n*-pentyl alkyl groups, a series of anziaic acid analogues (Scheme 2) were designed and synthesized following the same synthetic strategy as that used in the synthesis of anziaic acid. Briefly, the ester condensation of the acid-protected phenol monomer **5**, **7**, **8**, or **12** and the phenol-protected acid monomer **3** or **9** yielded the benzyl protected dimers **10a-d** or **13**, which could be transformed into the corresponding acid-phenol products **11a-d** or **14** following final debenzoylation reaction.

In addition, to further expand the chemical diversity of existing anziaic acid derivatives and to investigate if this dimeric scaffold possesses any tractable topoisomerase inhibition and antibacterial activity, a focused compound collection with structural similarity to anziaic acid and with diverse linkers (e.g., amide or reverse amide in **15–17**, the azo N=N linkage in **18** and **19**, ester linker in **20** and **21**, C=C bond in **22** and **23**, -OCH₂- linkage in **24** and **25** and -NHCH₂- in **26**) was procured from Sigma-Aldrich (purity 97%, Fig. 2) and included in our screening. Structurally, these compounds possess a wide array of chemical functionalities and linkers, thus the screening data of these selected compounds may provide additional insights toward probing the specificity of structural features (e.g., the metal chelating motif, the dimeric scaffold, and the ester linker) of anziaic acid and establishing the preliminary SAR for enzyme and whole cell activity.

Biological studies

Thus synthesized anziaic acid and its analogues were evaluated for their ability to inhibit topoisomerase enzyme (representative results shown in Fig. 3) and the growth of whole cell bacteria. The results are summarized in Table 1. In the *E. coli* topoisomerase I inhibition assay, our synthetic sample of anziaic acid exhibited reproducible topoisomerase I inhibition and antibacterial activity (IC₅₀ = 17.7 μM; MIC = 12.5 μM) as the reported natural product sample isolated from lichen *Hypotrachyna* sp.²⁷ In contrast, the monomer of anziaic acid, 2,4-dihydroxy-6-pentylbenzoic acid (**4**), had no inhibitory activity against topoisomerase I and gyrase as well as whole cell bacteria. This data demonstrates that the dimeric scaffold bearing both phenyl rings is required for topoisomerase inhibition and antibacterial activity, ruling out the hydrolysis product **4** of anziaic acid being responsible for the activity.

In addition, compared with the prototype anziaic acid, benzyl fully protected compound **6** and acetal protected variant **14** were inactive against *E. coli* topoisomerase I and DNA gyrase; compound **11d** with the absence of the carboxylic acid group showed very weak activity (IC₅₀ = 250–500 μM). Taken together, these results demonstrate that the absent or masked carboxylic acid group of anziaic acid had a detrimental effect on topoisomerase I and gyrase inhibition. This suggests that interaction of the acidic carboxylate with divalent metal ions at the active site might be required for inhibition of these types IA and IIA topoisomerase activities that require divalent ions for their catalytic activity.²⁷

In terms of the effects of the *n*-pentyl lipophilic substituents of anziaic acid, removal of either one *n*-pentyl alkyl group in anziaic acid (compounds **11a** and **11b**) significantly reduced the inhibitory activities against topoisomerase I and DNA gyrase (IC₅₀ = 250–500 μM), which is about 11–28 fold decrease relative to anziaic acid (IC₅₀ = 17.7–23.6 μM). Compound **11c**, with both *n*-pentyl groups removed, led to the complete loss of both enzyme inhibition and antibacterial activities. This data illustrates that the lipophilic alkyl groups significantly enhance both enzyme inhibitory and whole cell antibacterial activity and may play an important role in hydrophobic interactions with topoisomerase enzyme binding.

Interestingly, *in vitro* antibacterial evaluation revealed that **11d** with the carboxylic acid group absent (MIC = 3.12 and 25 μM against *B. subtilis* and a membrane permeable strain of *E. coli*, respectively) and the acetal protected anziaic acid **14** (MIC = 50 μM against *B. subtilis*) exhibited moderate to good antibacterial activity, despite very weak or no activity (IC₅₀ = 500 and >1000 μM for **11d** and **14**, respectively) in the topoisomerase I enzyme assay. These data indicate that these two compounds may inhibit bacterial growth by a mechanism independent of topoisomerase inhibition, which does not require the presence of the carboxylic acid group.

Most of the analogues **15–26** with diverse linkers were not active in the test assays except that compounds **18** and **19** with the azo linker exhibited weak to moderate topoisomerase inhibition ($IC_{50} = 62.5\text{--}250\ \mu\text{M}$) and antibacterial activity ($MIC = 200\text{--}400\ \mu\text{M}$).

Finally, these active compounds were also found to inhibit human topoisomerase II α enzyme in the same pattern as DNA gyrase and topoisomerase I. Further work is warranted to systematically optimize and evaluate advanced anziaic acid analogues in an effort to discover more selective bacterial topoisomerase I inhibitors with improved potency and specificity profiles and antibacterial therapeutic potential.

On the basis of these data, a preliminary SAR has been obtained and is shown in Fig. 4.

Conclusions

In summary, anziaic acid and its analogues were synthesized and evaluated against topoisomerases, DNA gyrase, and *Bacillus subtilis* as well as a membrane permeable strain of *E. coli*. Preliminary SAR studies demonstrate that the dimeric scaffold and the free carboxylate group of anziaic acid are essential in both topoisomerase and gyrase inhibition. However, the carboxylic acid functionality is not required for whole cell antibacterial activity. Furthermore, the lipophilic *n*-pentyl alkyl groups significantly enhance both topoisomerase enzyme inhibition and antibacterial activity.

Experimental section

Chemistry

General—All reagents and solvents were obtained from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Hanover Park, IL) and were used without further purification. Reactions were monitored either by thin-layer chromatography (TLC) or by reverse-phase HPLC with a Shimadzu LC-20A series HPLC system. TLC was performed using glass plates pre-coated with silica gel (0.25 mm, 60-Å pore size, 230–400 mesh, Sorbent Technologies, GA) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV). Debenzylation reactions were done using domnick hunter NITROX UHP-60H hydrogen generator, USA. Flash column chromatography was performed using a Biotage Isolera One system and a Biotage SNAP cartridge. Proton and carbon nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were recorded employing a Bruker AM-400 spectrometer. Chemical shifts were expressed in parts per million (ppm), *J* values were in Hertz. Mass spectra were recorded on a Varian 500-MS IT mass spectrometer using ESI. High-resolution mass spectra (HRMS) were recorded with a BioTOF II ESI mass spectrometer. The purity of compounds was determined by analytical HPLC using a Gemini, 3 μm , C18, 110Å column (50 mm \times 4.6 mm, Phenomenex) and a flow rate of 1.0 mL/min. Gradient conditions: solvent A (0.1% trifluoroacetic acid in water) and solvent B (acetonitrile): 0–2.00 min 100% A, 2.00–7.00 min 0–100% B (linear gradient), 7.00–8.00 min 100% B, 8.00–9.00 min 0–100% A (linear gradient), 9.00–10.00 min 100% A, UV detection at 254 and 220 nm.

Representative procedure for the synthesis of dimeric precursors

To a stirred solution of benzyl 2,4-dihydroxy-6-pentylbenzoate (**5**) (94.3 mg, 0.3 mmol) and 2,4-bis(benzyloxy)-6-pentylbenzoic acid (**3**) (121.4 mg, 0.3 mmol) in dry toluene (3 mL) was slowly added trifluoroacetic acid anhydride (486 μL , 3.45 mmol) at room temperature. The mixture was stirred overnight. The solvent was then removed under reduced pressure. The residue was purified by flash column chromatography (hexane: ethyl acetate = 97: 3) to give **6** as a solid (135 mg, 64%). ^1H NMR (400 MHz, CDCl_3 , ppm) δ 11.45 (s, 1H), 7.44–7.23 (m, 15H), 6.63 (d, *J* = 2.0 Hz, 1H), 6.50 (d, 2.4 Hz, 1H), 6.48 (d, *J* = 2.0 Hz, 1H), 6.38

(d, $J = 2.4$ Hz, 1H), 5.36 (s, 2H), 5.06 (s, 2H), 5.05 (s, 2H), 2.70–2.66 (m, 4H), 1.66–1.62 (m, 2H), 1.35–1.31 (m, 6H), 1.14–1.10 (m, 2H), 1.02–1.01 (m, 2H), 0.88 (t, $J = 7.2$ Hz, 3H), 0.79 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100.5 MHz, CDCl_3 , ppm) δ 171.09, 166.06, 164.41, 161.02, 157.66, 155.27, 148.32, 143.94, 136.49, 136.35, 134.79, 129.10, 128.83, 128.78, 128.71, 128.57, 128.22, 128.11, 127.59, 127.54, 116.13, 115.62, 109.56, 108.78, 107.46, 98.24, 70.70, 70.20, 67.81, 36.84, 33.95, 31.92, 31.86, 31.71, 31.06, 22.60, 22.57, 14.08, 14.04; MS (ESI⁻): m/z 699.6 [M-H]⁻; HRMS (ESI⁺) Calcd for $\text{C}_{45}\text{H}_{48}\text{O}_7$ (M⁺): 701.3473, Found: 701.3472; HPLC purity: 100% (254 nm), t_R : 8.91 min; 100% (220 nm), t_R : 8.91 min.

Representative procedure for debenzoylation of dimers and synthesis of anziaic acid

A solution of benzyl 4-((2,4-bis(benzyloxy)-6-pentylbenzoyl)oxy)-2-hydroxy-6-pentylbenzoate (**6**) (135 mg, 0.19 mmol) in ethyl acetate (5 mL) was treated with 10% Pd/C (38 mg). The mixture was stirred at room temperature under 1 bar of H_2 atmosphere for ca. 2 h. The reaction was stopped once it was complete (monitored by HPLC). The mixture was filtered through Celite and washed with ethyl acetate. The combined organic layer was evaporated under reduced pressure (the water bath temperature was kept below 30 °C) to give anziaic acid as a white solid (78.6 mg, 95%). ^1H NMR (400 MHz, CD_3OD , ppm) δ 6.62 (d, $J = 2.4$ Hz, 1H), 6.56 (d, $J = 2.0$ Hz, 1H), 6.27 (d, $J = 2.0$ Hz, 1H), 6.22 (d, $J = 2.4$ Hz, 1H), 2.92 (t, $J = 7.2$ Hz, 2H), 2.86 (t, $J = 7.6$ Hz, 2H), 1.62–1.60 (m, 4H), 1.34–1.32 (m, 8H), 0.91–0.86 (m, 6H); ^{13}C NMR (100.5 MHz, CD_3OD , ppm) δ 173.89, 170.41, 166.05, 164.34, 164.14, 154.90, 149.36, 149.04, 116.22, 113.16, 112.23, 109.03, 105.22, 102.01, 37.73, 36.79, 33.21, 33.13, 32.99, 32.62, 23.60, 23.46, 14.44, 14.38; MS (ESI⁻): m/z 429.3 [M-H]⁻; HRMS (ESI⁺) Calcd for $\text{C}_{24}\text{H}_{30}\text{O}_7$ (M+Na⁺): 453.1884, Found: 453.1887; HPLC purity: 100% (254 nm), t_R : 7.54 min; 100% (220 nm), t_R : 7.54 min.

The spectroscopic data of synthetic anziaic acid are consistent with those of isolated anziaic acid natural product from lichen *Hypotrachyna* sp.²⁷

Synthesis of other structural analogues of anziaic acid is described in the supporting information.

Topoisomerase inhibition assays

The topoisomerase assays were performed as previously described.²⁷ The IC_{50} values were determined from average of experiments repeated at least twice. Briefly, inhibition of relaxation activity of 10 ng of *E. coli* topoisomerase I was assayed with 250 ng of supercoiled pBAD/Thio plasmid DNA substrate purified by CsCl gradient. The relaxation reaction was carried out in 20 μL of 10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.1 mg/mL gelatin with 0.5 mM MgCl_2 . After 30 min at 37 °C, the reactions were terminated and analyzed by agarose gel electrophoresis.

Human topoisomerase II α (from TopoGen) relaxation assay and *E. coli* DNA gyrase (from New England BioLab) supercoiling assay were carried out as recommended by the suppliers. Relaxed plasmid DNA substrate for DNA gyrase was purchased from New England BioLabs.

Antibacterial testing

The MICs of compounds against different bacterial strains grown in cation-adjusted Mueller-Hinton Broth were measured with standard microdilution procedures.²⁷ Complete growth inhibition was recorded after 24 h in a 37 °C incubator.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

1. Wang JC. *Nat Rev Mol Cell Biol.* 2002; 3:430–440. [PubMed: 12042765]
2. Vos SM, Tretter EM, Schmidt BH, Berger JM. *Nat Rev Mol Cell Biol.* 2011; 12:827–841. [PubMed: 22108601]
3. Deweese JE, Osheroff N. *Nucleic Acids Res.* 2009; 37:738–748. [PubMed: 19042970]
4. Pommier Y. *ACS Chem Biol.* 2013; 8:82–95. [PubMed: 23259582]
5. Pommier Y, Leo E, Zhang H, Marchand C. *Chem Biol.* 2010; 17:421–433. [PubMed: 20534341]
6. Tse-Dinh YC. *Nucleic Acids Res.* 2009; 37:731–737. [PubMed: 19042977]
7. Tse-Dinh YC. *Infect Disord Drug Targets.* 2007; 7:3–9. [PubMed: 17346206]
8. Hartmann JT, Lipp HP. *Drug Saf.* 2006; 29:209–230. [PubMed: 16524321]
9. Pommier Y. *Nat Rev Cancer.* 2006; 6:789–802. [PubMed: 16990856]
10. Bailly C. *Chem Rev.* 2012; 112:3611–3640. [PubMed: 22397403]
11. Hooper DC. *Clin Infect Dis.* 2000; 31:S24–S28. [PubMed: 10984324]
12. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. *Lancet.* 2006; 368:874–885. [PubMed: 16950365]
13. Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis, 2011 Update. Geneva: World Health Organization; 2011.
14. Hsu DI, Okamoto MP, Murthy R, Wong-Beringer A. *J Antimicrob Chemother.* 2005; 55:535–541. [PubMed: 15728150]
15. Schwaber MJ, Carmeli Y. *J Am Med Assoc.* 2008; 300:2911–2913.
16. Nagara V, Siker D, Pain J. *Curr Pharm Des.* 2002; 8:1995–2007. [PubMed: 12171525]
17. Abreu AC, McBain AJ, Simões M. *Nat Prod Rep.* 2012; 29:1007–1021. [PubMed: 22786554]
18. Lewis K. *Nat Rev Drug Discov.* 2013; 12:371–387. [PubMed: 23629505]
19. Clardy J, Fischbach MA, Walsh CT. *Nat Biotech.* 2006; 24:1541–1550.
20. Cragg GM, Newman DJ. *Biochim Biophys Acta.* 2013; 1830:3670–3695. [PubMed: 23428572]
21. Goel M, Dureja P, Rani A, Uniyal PL, Laatsch H. *J Agric Food Chem.* 2011; 59:2299–2307. [PubMed: 21351753]
22. Reynertson KA, Wallace AM, Adachi S, Gil RR, Yang H, Basile MJ, D'Armiento J, Weinstein IB, Kennelly EJ. *J Nat Prod.* 2006; 69:1228–1230. [PubMed: 16933884]
23. Wu SB, Wu J, Yin Z, Zhang J, Long C, Kennelly EJ, Zheng S. *J Agric Food Chem.* 2013; 61:4035–4043. [PubMed: 23597039]
24. Asahina Y, Hiraiwa M. *Ber Dtsch Chem Ges.* 1935; 68:1705–1708.
25. Elix JA, Wardlaw JH. *Aust J Chem.* 1997; 50:1145–1150.
26. Fox CH, Huneck S. *Phytochemistry.* 1970; 9:2057.
27. Cheng B, Cao S, Vasquez V, Annamalai T, Tamayo-Castillo G, Clardy J, Tse-Dinh YC. *PLoS ONE.* 2013; 8:e60770. [PubMed: 23593306]
28. Sun D, Hurdle JG, Lee R, Lee R, Cushman M, Pezzuto JM. *ChemMedChem.* 2012; 7:1541–1545. [PubMed: 22847956]
29. Shen L, Maddox MM, Adhikari S, Bruhn DF, Kumar M, Lee RE, Hurdle JG, Lee RE, Sun D. *J Antibiot.* 2013; 66:319–325. [PubMed: 23549356]
30. Asahina Y, Hiraiwa M. *Ber Dtsch Chem Ges.* 1937; 70:1826–1828.

31. Elix JA. Aust J Chem. 1974; 27:1767–1779.
32. Wang P, Zhang Z, Yu B. J Org Chem. 2005; 70:8884–8889. [PubMed: 16238322]

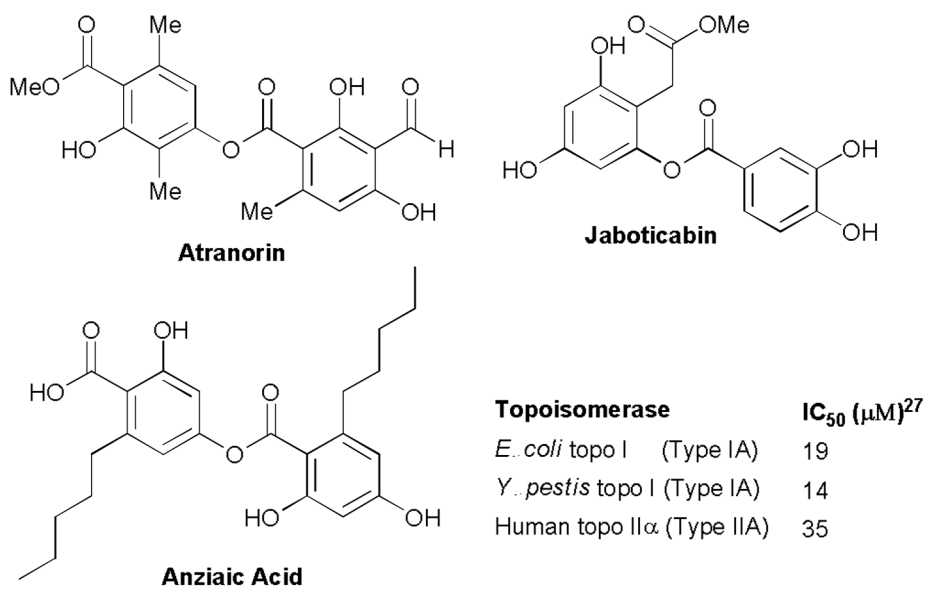


Fig. 1. Structures of selected naturally occurring bioactive depsides.

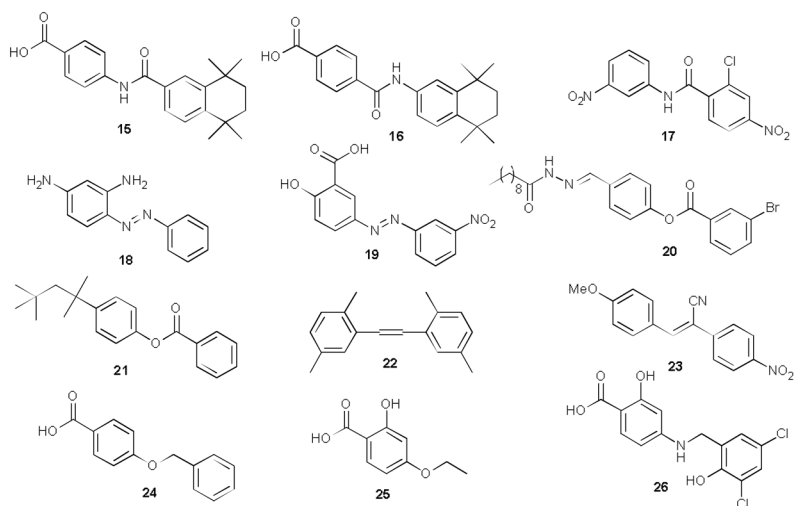


Fig. 2.
Structural analogues **15-26** of anziaic acid included in the screen.

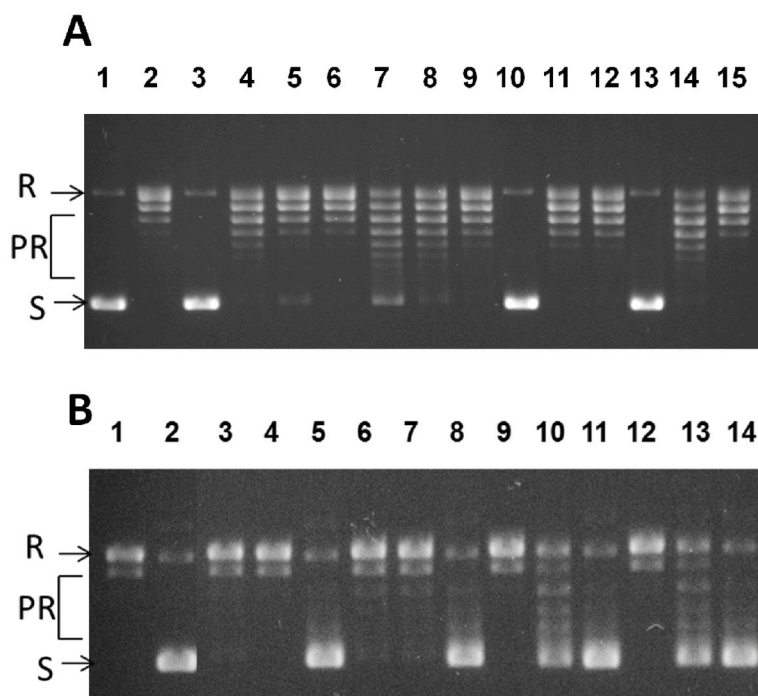
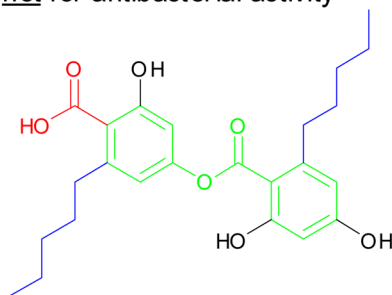


Fig. 3. Representative results of topoisomerase inhibition assays. **(A)** *E. coli* topoisomerase I inhibition assays with supercoiled plasmid DNA. (S) substrate. Lane 1: no enzyme; Lane 2: DMSO control; Lanes 3–6: 31.2, 23.6, 17.7, 13.2 μM (**anziaic acid**); Lanes 7–9: 1000, 500, 250 μM (**11d**); Lanes 10–12: 1000, 500, 250 μM (**11b**); Lanes 13–15: 1000, 500, 250 μM (**11a**). R: relaxed DNA; PR: Partially relaxed DNA. **(B)** *E. coli* gyrase inhibition assays with relaxed plasmid DNA. Lane 1: no enzyme; Lane 2: DMSO control; Lanes 3–5: 62.5, 31.2, 23.6 μM (**anziaic acid**); Lanes 6–8: 1000, 500, 250 μM (**11d**); Lanes 9–11: 500, 250, 125 μM (**11b**); Lanes 12–14: 500, 250, 125 μM (**11a**).

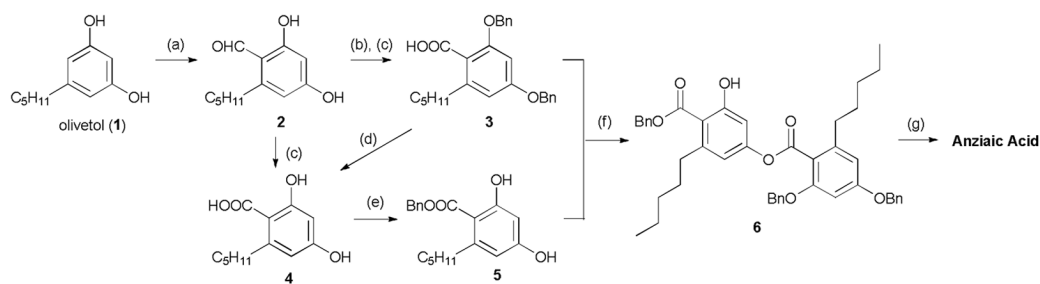
Free carboxylic acid functionality is essential
for both topoisomerase and gyrase inhibition
not for antibacterial activity



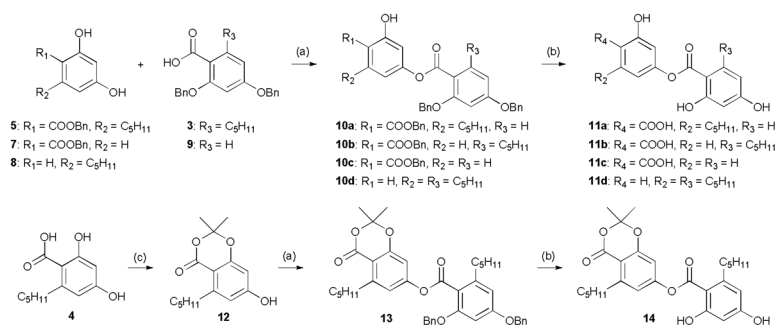
The dimeric scaffold is required
for both topoisomerase and gyrase inhibition
and antibacterial activity

Lipophilic *n*-pentyl groups enhance
topoisomerase I and gyrase inhibition
as well as antibacterial activity

Fig. 4.
Preliminary SAR.

**Scheme 1.**

Total synthesis of anziaic acid: *Reagents and conditions*: (a) DMF, POCl₃, 0 °C to rt, 56%; (b) BnBr, K₂CO₃, acetone, reflux, 70%; (c) NaClO₂, NaH₂PO₄, DMSO/H₂O, 61% (3) and 50% (4); (d) Pd/C, H₂, ethyl acetate, 97%; (e) BnBr, KHCO₃, DMF, 92%; (f) (CF₃CO)₂O, toluene, 64%; (g) Pd/C, H₂, ethyl acetate, 95%.

**Scheme 2.**

Synthesis of anziaic acid analogues **11a-d** and **14**: *Reagents and conditions:* (a) **3** (for **13**), $(\text{CF}_3\text{CO})_2\text{O}$, toluene, 57–85%; (b) Pd/C, H_2 , ethyl acetate, 80–100%; (c) SOCl_2 /acetone/DMAP, 1,2-dimethoxyethane, 0 °C to rt, 39%.

Table 1

Evaluation of anziaic acid and analogues against topoisomerases and whole cell bacteria^a

Compound	MW (g/mol)	cLogP ^b	<i>E. coli</i> Topo I inhibition IC ₅₀ (μM)	Human Topo I inhibition IC ₅₀ (μM)	Human Topo IIα inhibition IC ₅₀ (μM)	<i>E. coli</i> DNA gyrase IC ₅₀ (μM)	<i>B. subtilis</i> MIC (μM)	<i>E. coli</i> MIC (μM)
4	224.25	3.34	1000	>1000	>1000	>1000	>200	>200
6	700.86	-	>1000	>1000	>1000	>1000	>200	>200
Anziaic acid (isolated natural product) ²⁷	430.49	7.77	19	35	19	19	14	28
Anziaic acid (synthetic sample)	430.49	7.77	17.7	35	23.6	23.6	12.5	100
10c	560.59	9.25	>1000	>1000	>1000	>1000	>200	>200
11a	360.36	5.15	500	250	250	250	50	200
11b	360.36	6.05	250	125	250	250	50	>200
11c	290.23	3.44	>1000	>1000	>1000	>1000	>200	>200
11d	386.48	7.92	500	250	250	250	3.12	25
14	470.55	9.42	>1000	500	>1000	>1000	50	>200
15	351.44	6.34	>250	n.d.	n.d.	n.d.	50–100	200
16	351.44	6.38	>250	n.d.	n.d.	n.d.	100	400
17	321.67	2.75	>250	n.d.	n.d.	n.d.	>800	>800
18	212.25	2.35	125–250	n.d.	n.d.	n.d.	400	200–400
19	287.23	4.27	62.5–125	n.d.	n.d.	n.d.	200	200
20	473.40	8.04	>250	n.d.	n.d.	n.d.	>800	>800
21	310.43	7.30	>250	n.d.	n.d.	n.d.	>800	>800
22	236.35	6.83	>250	n.d.	n.d.	n.d.	>800	>800
23	280.28	3.12	>250	n.d.	n.d.	n.d.	>800	>800
24	228.24	3.79	>250	n.d.	n.d.	n.d.	>400	>400
25	182.17	2.76	>250	n.d.	n.d.	n.d.	>400	>400
26	328.15	4.02	>250	n.d.	n.d.	n.d.	200	200

^a n.d.-not determined.^b The *n*-octanol/water partition coefficient (LogP) of compounds was calculated using ChemBioOffice® Ultra version 12.0 from CambridgeSoft Corporation.