This is a pre- or post-print of an article published in
Altendorfer, M., Irschik, H., Menche, D.
Design, synthesis and biological evaluation of simplified
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(2012) Bioorganic and Medicinal Chemistry Letters, 22
(17), pp. 5731-5734.
Design, synthesis and biological evaluation of simplified side chains of the macrolide antibiotic etnangien

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Article info: Novel simplified side chains of the potent RNA polymerase inhibitor etnangien were designed, synthesized and evaluated for antibacterial activity against Gram-positive bacteria and one Gram-negative bacterium.

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Keywords: Etnangien Analogues RNA-Polymerase Structure-activity data Antibiotics

The labile polyketide macrolide antibiotic etnangien (1, Figure 1) presents a highly potent antibiotic against bacteria, isolated from various strains of the myxobacterium Sorangium cellulosum So ce750 and So ce1045.1,2 It is a structurally unique type of a particularly efficient RNA polymerase inhibitor in vitro and in vivo with average IC50 values in the submicromolar scope.3 In detail, it is effective against a broad panel of Gram-positive bacteria, especially those belonging to the actinomycetes.3 Notably, etnangien (1) shows no cross-resistance to rifampicine, which presents the only curative useful RNA-polymerase inhibitor so far. This qualifies the bacterial RNA polymerase as one of the rare validated, but underexploited, targets for broad-spectrum antibiotics.4 Furthermore, etnangien (1) also retains activity against retroviral DNA polymerase and shows low cytotoxicity against mammalian cells, which add to its attractiveness for further investigation.3 The notorious instability, of the side chain embodying a polyunsaturated hexaene subunit demands the development of stable and more readily available analogues to further advance this highly promising macrolide antibiotic.

The full relative and absolute stereochemistry of etnangien (1) has been determined in our group in a cooperation with the group of Rolf Müller by a combination of extensive high-field NMR-studies, modeling, chemical derivatization and an innovative bioinformatics approach.5 Furthermore, a first and so far only total synthesis of etnangien (1) and its equipotent methyl ester derivative (2) have been accomplished in our group.6,7 In connection with the total synthesis a few simplified etnangien analogues have been obtained and biologically evaluated against a range of Gram-positive and negative bacteria8

Figure 1. Etnangien (1) and its methyl ester (2).

Herein, we report the synthesis of the first side chain analogues of etnangien and their evaluation for antibacterial activity against Gram-positive bacteria and one Gram-negative bacterium.

Inspired by various polyene antibiotics,9 we initiated a synthetic programme developed towards polyene side chain

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analouges of etnangien in a rationale to understand the biological importance of this part of the etnangien structure. One focus was placed on the design and synthesis of modified side chains, lacking the notoriously labile polyene subunit. As shown in Scheme 1, our concept relied on a late stage 3-fragment cross coupling strategy, involving a Stille/Suzuki-Miyaura coupling sequence bases on a central bis-metallated reagent 5. Notably, this sequence avoids a labile precursor molecule by installing the polyene fragment at a very late stage. Also, replacement of the central bis-metallated fragment enables a rapid access to various analogues.

**Scheme 1.** Late stage cross coupling strategy of simplified etnangien side chains.

As a first target, we decided to synthesize dienyl iodide 7. This was available from the commercially available acid 8 in a six step reaction sequence (Scheme 2), involving acid catalyzed esterification, cross metathesis with crotonaldehyde using Hoveyda Grubbs II catalyst, Brown allylation of the terminal allylic aldehyde, TBS protection, another second cross metathesis croton aldehyde and a subsequent Takai homologation. This sequence afforded vinyl iodide 7 in high stereoselectivities and good yields (45% yield, 6 steps). Light sensitive dienyl iodide 7 was then immediately connected to the bis-metallated central building block 5 by a Stille coupling. Accordingly, treatment of iodide 7 and diene 5a with PdCl2(CH2CN)2 in degassed DMF afforded the desired pinacolborane 8a in good yield. The analogous Stille coupling of stannane 5b and dienyl iodide 7 under same conditions proceeded in moderate yield generating methyl ester 8b. To complete the coupling sequence we next focused on the construction of western fragments 6. The primary alcohol function of 9 was initially protected both as a silyl and a PMB ether. Starting from aldehyde 9a we advanced towards (E)-vinyl iodide 6a via asymmetric alkyn addition of TMS-acetylene to aldehyde 9a as a first key step. After TBS protection of the selectively installed hydroxy group TMS was removed and the terminal alkyne underwent a hydrozirconation reaction followed by iodolysis to obtain bis-TBS ether 6a (55% yield, 4 steps). Using the same reaction conditions PMB ether 9b was readily transformed into vinyl iodide 6b in 47% yield over 4 steps. Subsequently, pinacolboranes 8 and iodides 6 underwent a Suzuki-Miyaura coupling applying mild reactions conditions with Pd(dppf)Cl2 as catalyst and Ba(OH)2:8H2O as a base.

In total four different types of protected side chains could be prepared demonstrating the general usefulness of our approach. With the highly unsaturated polyenes 10a, 10b, 11a and 11b in hand, efforts were then directed towards the challenging deprotection of these polyenes. After considerable experimentation, it was found that best results in terms of yield and practicability were obtained with a protocol reported by Kishi, which involves treatment with TBAF and anhydrous work-up procedure. In detail, methyl esters 10a, 10b, 11a and 11b were treated with 1 M TBAF and stirred at room temperature over night, resulted complete and clean conversion. Finally, after working up with CaCO3, DOWEX and methanol the desired target compounds 3a, 3b, 4a and 4b could be isolated in good yields.

**Scheme 2.** Synthesis of novel simplified etnangien side chains 3 and 4.

Reagents and conditions: (a) H2SO4, MeOH, reflux, 3 h, 72%; (b) crotonaldehyde, Hoveyda Grubbs II, DCM, reflux, 2 h, 93%; (c) -)(Ipc)2B(allyl), Et3O, -100 °C, 90%, 96% ee; (d) TBSOTf, 2,6-Lutidine, DCM, -78 °C, 1 h, 99%; (e) crotonaldehyde, Grubbs II, DCM, reflux, 2 h, 86%; (f) CH3I, CrCl3, THF/Dioxane (1:6), 0 °C to rt, 87%, E/Z 7:1; (g) PdCl2(CH2CN)2, DMF, rt, 12 h, 80% for 8a and 68% for 8b; (h) Et3Zn, TMS-acetylene, toluene, reflux, 1 h then Ti(O-Pr)4, (R)-BINOL, Et3O, rt, 12 h, d/r = 8:1 using 9a and d/r = 9:1 using 9b; (i) TBSOTf, 2,6-Lutidine, DCM, -78 °C, 1 h; (j) K2CO3, MeOH, rt, 1 h; (k) ZrCp2Cl2, DIBAL-H, THF, 30 min, -78 °C to rt then I2, -78 °C, 30 min, 55% over 4 steps for 6a and 47% for 6b; (l) Pd(dppf)Cl2, Ba(OH)2:8H2O, DCM, rt, 4 h, 49% for 10a, 47% for 10b, 77% for 11a and 83% for 11b; (m) TBAF, THF, rt, 12 h then CaCO3,
Nocardia coralline belonging to the suborder of Corynebacterineae, such as their methyl ester derivative (1), 27% for 4a and 61% for 4b.

Based on the syntheses of the simplified etnangien side chains reported above, we decided to apply this 3-fragment coupling concept also for the construction of the original side chain 12 of etnangien. Again, we were focusing on avoiding unstable precursors which allow an assembling of the labile hexaene subunit in an efficient late stage cross coupling sequence.

**Scheme 3.** Late stage cross coupling strategy of the original etnangien side chain.

The synthesis of the identical etnangien side chain 12 could again be realized in a similar way to the above reported construction of simplified side chain derivatives 3 and 4, demonstrating again the usefulness of this approach. Starting from aldehyde 9b asymmetric addition\(^1\) of propyne afforded the desired propargylic alcohol 16 in an anti Felkin-Anh preference \(d_r = 12:1\). After TBS protection of the installed secondary hydroxyl group, the triple bond was transformed selectively into a stannylated olefin via Palladium-catalyzed hydrostannylation.\(^9\) Finally, metal/halogen exchange\(^9\) afforded vinyl iodide 14 in 27% over 4 steps. Subsequent Stille coupling\(^12\) of western fragment 14 with stannane 13\(^9\) gave pentaene 17 in good yields considering the lability of this compound (67%). Next, the eastern fragment 15 was obtained in reliable fashion by a 12 step procedure starting from commercially available alcohol 18 (6.0% over 12 steps).\(^6\) As illustrated in Scheme 4, pinacolborane 17 and eastern fragment 15 underwent a Suzuki-Miyaura reaction \(^16,17\) to obtain bis-TBS ether 19 in high yield (83%). Global deprotection using the established Kishi protocol\(^18\) afforded the desired methyl ester 12 in 56% yield.\(^19\)

The potent antimicrobial activity of etnangien motivated us to likewise analyse the developed and more readily available side chain analogues for their antibiotic potential. Table 1 presents their inhibitory qualities against several microorganisms, in direct comparison to the authentic natural product etnangien (1) and its methyl ester derivative (2). As previously reported, bacteria belonging to the suborder of Corynebacterineae, such as Nocardia coralline and some Mycobacteria, were particularly sensitive to (1) and (2).

**Scheme 4.** Synthesis of the original etnangien side chain 11. Reagents and conditions: (a) EtZn, propyne, toluene, reflux, 1 h then Ti(O-iPr)\(_4\), (R)-BINOL, Et\(_2\)O, rt, 12 h, 60%, \(d_r = 12:1\); (b) TBSOTf, 2,6-Lutidine, DCM, -78 °C, 1 h, 96%; (c) Pd(Ph)\(_2\)Cl\(_2\), n-BuSnH, THF, rt, 30 min, 68%; (d) I\(_2\), DCM, 0 °C, 15 min, 98%; (e) PdCl\(_2\)(CH\(_3\)CN), DMF, rt, 12 h, 67%; (f) Pd(dppf)Cl\(_2\), Bu(OH)\(_2\), H\(_2\)O, DMF, rt, 4 h, 83%; (g) TBAF, THF, rt, 12 h then CaCO\(_3\), DOWEX, MeOH, rt, 1 h, 58%.

However, yeast and Gram-negative Escherichia coli proved to be rather resistant. Presumably, this may be arised from the Gram-negative character of myxobacteria.\(^1,3\) In agreement with these data for the parent natural product 1 and its methyl ester 2, the side chain analogues 3a, 3b, 4a, 4b and 12 likewise showed no activity against Gram-negative E. Coli and the yeast Saccharomyces cerevisiae. However, a certain activity of triol 3b was observed against Bacillus subtilis, with a similar potency as the parent natural product 1 and its methyl ester derivative 2.

Also a certain inhibition against Corynebacterium mediolanum was observed for 3b. All other analogues, including the original side chain 12, showed no or very low degrees of activities, suggesting a critical importance of the macroyclic part for full biological potency.

**Table 1.** Antibiotic activity of etnangien analogues (3, 4, 12) in comparison to etnangien (1) and its methyl ester (2)\(^2\).

<table>
<thead>
<tr>
<th>Test organism</th>
<th>1</th>
<th>2</th>
<th>3a</th>
<th>3b</th>
<th>4a</th>
<th>4b</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>2.5</td>
<td>&gt;80</td>
<td>80</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;40</td>
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<tr>
<td>Bacillus subtilis</td>
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<td>20</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;80</td>
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</tr>
<tr>
<td>C. glutanicum</td>
<td>0.03</td>
<td>0.24</td>
<td>&gt;80</td>
<td>80</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;40</td>
</tr>
<tr>
<td>C. mediolanum</td>
<td>0.06</td>
<td>n. d.</td>
<td>&gt;80</td>
<td>40</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Mycobacterium phlei</td>
<td>0.12</td>
<td>n. d.</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>0.39</td>
<td>0.06</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;40</td>
</tr>
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</table>
In conclusion, we developed a highly concise first synthesis of side chain analogues of the etnangiens enabling a first biological evaluation of this structural part of these potent antibiotics. The synthesis of these compounds was enabled by a highly modular and convergent 3-fragment coupling strategy. Most side chain analogues demonstrated little or no antibacterial activity, suggesting that the macrocyclic core and the side chain are critical parts of the pharmacophore. However, a certain degree of activity was also observed for simplified side chain analogue 3b, suggesting that the parent natural structure may still be simplified with retention of activity.

Acknowledgments

Generous financial support from the ‘Deutsche Forschungsgemeinschaft’ (Me 2756/4) is most gratefully acknowledged.

References and notes


21. All final compounds reported in the paper were chromatographically purified and characterized by NMR and HRMS. Characterization data for compound 3a: 1H NMR (500.132 MHz, CDCl3): δ = 0.85 (d, J = 6.9 Hz, 2 H), 1.65 (br. s., 3 H), 1.82 (ddt, J = 14.5 Hz, J = 10.8 Hz, J = 7.3 Hz, J = 3.3 Hz, 1 H), 2.34 (m, 10 H), 3.65 (m, 1 H), 3.68 (s, 3 H), 3.75 (dd, J = 10.8 Hz, J = 3.4 Hz, 1 H), 4.05 (t, J = 7.7 Hz, 1 H), 4.13 (dt, J = 11.9 Hz, J = 5.8 Hz, 1 H), 5.63 (m, 6 H), 6.12 (m, 6 H); 13C NMR (125.76 MHz, CDCl3): δ = 13.6, 27.4, 32.4, 33.6, 40.5, 41.0, 51.6, 67.6, 71.8, 78.6, 128.8, 129.7, 129.8, 130.6, 130.9, 131.9, 132.3, 132.3, 133.4, 133.9, 133.9, 134.9, 173.4; HR-MS (ESI): found m/z = 427.2459 ([C20H22O2Na]+), calculated m/z = 427.2455. Compound 12: 1H NMR (600.130 MHz, CDCl3): δ = 0.85 (d, J = 7.0 Hz, 3 H), 1.62 (br. s., 1 H), 1.70 (s, 3 H), 1.82 (s, 3 H), 1.94 (m, 1 H), 2.33 (m, 4 H), 2.45 (m, 2 H), 3.41 (br. s., 1 H), 3.46 (t, J = 8.5 Hz, 1 H), 3.59 (dd, J = 9.2 Hz, J = 4.0 Hz, 1 H), 3.67 (s, 3 H), 3.81 (s, 3 H), 4.41 (m, 2 H), 4.47 (s, 2 H), 5.22 (d, J = 8.6 Hz, 1 H), 5.48 (d, J = 8.9 Hz, 1 H), 5.68 (m, 1 H), 6.02 (m, 1 H), 6.25 (m, 8 H), 6.89 (d, J = 8.4 Hz, 2 H), 7.26 (d, J = 8.4 Hz, 2 H); 13C NMR (150.90 MHz, CDCl3): δ = 13.0, 13.4, 16.7, 32.5, 34.3, 39.2, 41.2, 51.6, 55.3, 68.0, 72.8, 73.1, 74.5, 113.8, 127.6, 128.7, 129.4, 129.8, 130.0, 132.0, 132.7, 132.9, 133.0, 133.1, 133.4, 133.8, 133.8, 136.1, 137.1, 137.4, 159.3, 173.6; HR-MS (ESI): found m/z = 573.3189 ([C28H1406Na]+), calculated m/z = 573.3187.

22. MIC values were determined by serial dilution, for further information see: Irschik, H.; Jansen, R.; Gerth, K.; Höffle, G.; Reichenbach, H. J. Antibiot. 1987, 40, 7.