Total synthesis and biological evaluation of apicularen A and synthetic analogs

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Abstract—The first total synthesis of the potent cytostatic agent apicularen A is described along with the synthesis of the corresponding C₁₁-epimers and side chain modified congeners. Growth inhibition experiments with the human melanoma cancer cell line SK-MEL-5 revealed an important role for the unusual N-acyl enamide side chain. © 2001 Elsevier Science Ltd. All rights reserved.

Over the past few years, a variety of macrocyclic salicylate natural products have been isolated from both terrestrial and marine sources based on their ability to induce a particular phenotype in mammalian cells. The bacterial metabolite apicularen A (1, Fig. 1) for example, has been shown to display extremely potent cytostatic activity against nine human cancer cell lines (IC₅₀ 0.1–3 ng/mL), including the multi-drug resistant cervix carcinoma cell line KB-V1 (IC₅₀ ~ 0.4 ng/mL). The same report mentions anecdotally that apicularen A causes several abnormal effects including the formation of mitotic spindles with multiple spindle poles and clusters of bundled actin from the cytoskeleton. Structural relatives include the salicylihalamides and lobatamides, which are of interest because of their unique signature in the National Cancer Institute 60-cell panel, as well as compounds that selectively inhibit the growth of oncogene-transformed cell lines (oximidiines) or induce low density lipoprotein (LDL) receptor gene expression (CJ-12,950 and CJ-13,357). Beginning to understand the molecular basis for these distinct activities will require structure–function correlation studies and the development of synthetic chemistry in this area. Our laboratory has pioneered the first total synthesis of salicylihalamide A (2, Fig. 1), which resulted in a revision of its absolute configuration, as well as a variety of analogs for structure–activity relationship studies. Herein, we report the first total synthesis of apicularen A and congeners.

Apicularen A shares an identical, acid labile, N-acyl enamine side chain with salicylihalamide A. Our solution for its introduction hinged upon the addition of 1-lithio-1,3-hexadiene to an α,β-unsaturated isocyanate, derived from the corresponding α,β-unsaturated carboxylic acid. To exploit a similar approach for the synthesis of apicularen A, we had to convert the terminal double bond of our previously derived truncated apicularen to an α,β-unsaturated carboxylic acid (Scheme 1).

Figure 1.
Thus, silylation of phenol 3 (1:1 mixture at C11), oxidative cleavage of the terminal olefin and homologation of the resulting aldehyde 6 with allyl diethylphosphonoacetate provided geometrically pure E-allyl ester 7. Methanolation of both silyl ethers provided analog 8, which was set aside for biological testing (vide infra). For the purpose of the total synthesis, which was set aside for biological testing (vide infra).

Setting the stage for the introduction of the enamine side chain, acids 9 and 10 were converted in parallel to pure carboxylic acids 9 and 10 by Pd-catalyzed deprotection and chromatographic separation of the C11 epimers.10

Table 1 shows the growth inhibitory properties of synthetic apicularens against the melanoma cell line SK-MEL-5. Interestingly, apicularen A (1) is about 5-fold more potent than the structurally related compound salicylhalamide A (2) against this cell line, although it is unclear at this point if they share a similar mode-of-action. While some variations including C11 epimers (17), geometrical isomers (15), N-pivaloyl- (16), N-hexanoyl- (18), and N-carbamoyl- (19) enamine derivatives can be accommodated without serious loss of activity, the inability of compounds 4 and 8 to arrest cell-growth indicates that there might be a specific role for the N-acyl enamine functionality.

Scheme 1. Reagents and conditions: (a) TBSCl, imidazole, cat. DMAP, DMF, rt, 12 h, 99%; (b) cat. OsO4, NMO, acetone–H2O, rt, 12 h, 85%; (c) NaIO4, CH2Cl2, rt, 30 min, 97%; (d) allyl diethylphosphonoacetate, NaH, THF, 0°C, 40 min, 99%; (e) amberlyst-15, MeOH, rt, 16 h, 80%; (f) cat. Pd[(PPh3)4], PPh3, HCO2NH4, THF, rt, 18 h, 65%; (g) (PhO)2P(O)N3, NEt3, PhH, rt, 12 h (75–79%); (h) PhH, 82°C, 6 h; (i) 1-bromo-1,3-hexadiene (1:1 mixture of 1Z,3Z and 1Z,3E isomers) or 1-bromohexane, t-BuLi, Et2O, –78°C, then add 13 or 14, –78°C, 1 h; (j) HF/pyridine, pyridine–THF, 10% for 15, 10% for 1, 10% for 16, 25% for 17, 40% for 18 (three steps from 11 or 12); (k) 1-pentanol, PhH, 82°C, 7 h, then step j, 35% from 12 (three steps).
Table 1. Growth inhibitory properties of selected compounds against the human melanoma cell line SK-MEL-5a

<table>
<thead>
<tr>
<th>Compound</th>
<th>GI50 (µM)</th>
<th>Compound</th>
<th>GI50 (µM)</th>
<th>Compound</th>
<th>GI50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.006</td>
<td>8</td>
<td>&gt;20</td>
<td>17</td>
<td>0.45</td>
</tr>
<tr>
<td>2</td>
<td>0.03</td>
<td>15</td>
<td>0.06</td>
<td>18</td>
<td>7.5</td>
</tr>
<tr>
<td>4</td>
<td>&gt;20</td>
<td>16</td>
<td>0.9</td>
<td>19</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*a Growth inhibition was determined 2 days after the addition of the compounds by the MTT assay (Mosmann, T. J. Immunol. Methods 1983, 65, 55–63). The GI50 values (drug concentrations required to inhibit growth by 50%) were calculated based on triplicate assays at four different concentrations of the drug.

While less potent than the parent compound, all the active analogs could be derived from a common isocyanate precursor. This observation is important for it will allow us to access a much larger collection of apicularen derivatives through the late stage addition of a wide variety of commercially available nucleophiles to a common isocyanate 13. We are now focussing our efforts towards a more practical and stereoselective synthesis of key intermediate isocyanate 13 in order to achieve these goals.

Acknowledgements

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References

11. These reactions were performed on a small scale. Excess t-BuLi might have been present due to an imperfect control of stoichiometry or an incomplete consumption during the transmetallation step, explaining the formation of 16 by the addition of t-BuLi to isocyanate 13.
12. General experimental procedure for compounds 1 and 15–19: Isocyanate 13 or 14 was obtained by heating the corresponding acyl azide 11 or 12 in benzene for 6 h at 80°C. After evaporation of the solvent, the crude isocyanate was dissolved in diethyl ether (~0.014 M) and cooled to ~78°C. To this solution, the organolithium compound (1.3 equiv., ~0.15 M in Et2O, freshly prepared from the corresponding bromide by transmetallation with 2.05 equiv. of t-BuLi) was added dropwise and stirring was continued for 1 h. Addition of pH 7.0 phosphate buffer, extraction (Et2O), drying (Na2SO4) and concentration gave a residue that was transferred to a polyethylene vial. To this residue was added an aliquot of a solution prepared from 0.5 g commercial HF-pyridine in 1.25 mL pyridine and 6.75 mL THF (100 µL/mg isocyanate). After stirring for 6–12 h at rt, the reaction was quenched by the addition of pH 7.0 phosphate buffer followed by extraction (EtOAc), drying (Na2SO4) and concentration. The crude material was purified by normal-phase semi-preparative HPLC (5 µm Luna silicagel; 250×10 mm column). See Scheme 1 and text for yields and mobile phases. Carbamate 19 (1 mg) was obtained by heating acyl azide 12 (5 mg, 0.008 mmol) and 1-pentanol (8.7 µL, 0.08 mmol) in benzene (1 mL) at 80°C for 6 h, followed by evaporation of the solvent, deprotection with HF-pyridine as described above, workup and HPLC purification (40% acetone in hexanes). All compounds were characterized by NMR and HRMS. Compound 15: 1H NMR (400 MHz, acetone-d6) δ 9.03 (1H, d, J=10.4 Hz), 8.36 (1H, s), 7.64 (1H, ddd, J=12.1, 12.0, 10.8, 15.2 Hz), 7.10 (1H, dd, J=7.6, 8.0 Hz), 6.90 (1H, ddd, J=1.5, 10.4, 14.4 Hz), 6.77 (1H, d, J=8.0 Hz), 6.70 (1H, d, J=7.6 Hz), 6.46 (1H, app,t, J=11.2), 6.04 (1H, app,dt, J=6.8, 15.2 Hz), 5.63 (1H, d, J=11.2 Hz), 5.38–5.46 (1H, m), 5.24 (1H, app,dt, J=7.6, 14.4 Hz), 4.22–4.30 (1H, m), 3.95–4.03 (1H, m), 3.84–3.90 (1H, m), 3.76 (1H, d, J=4.0 Hz), 3.34 (1H, dd, J=10.0, 14.8 Hz), 2.44 (1H, dd, J=1.6, 14.8 Hz), 2.34 (2H, ddd, J=1.2, 6.4, 6.4 Hz), 2.19 (2H, ddd, J=1.6, 7.6, 7.6 Hz), 1.93 (1H, ddd, J=4.4, 4.4, 13.2 Hz), 1.83 (1H, ddd, J=10.8, 10.8, 14.8 Hz).
Hz), 1.68 (1H, ddd, \( J = 5.2, 7.2, 12.8 \) Hz), 1.51 (1H, ddd, \( J = 4.8, 7.6, 12.4 \) Hz), 1.49 (1H, ddd, \( J = 8.4, 8.4, 12.8 \) Hz), 1.03 (3H, t, \( J = 7.6 \) Hz); HRMS (FAB) calcd for \( \text{C}_{25}\text{H}_{32}\text{NO}_6 (\text{MH}^+): \) 442.2230. Found: 442.2228. Compound \( 19: \) \( \delta \) 8.38 (1H, s), 8.25 (1H, d, \( J = 10.4 \) Hz), 7.13 (1H, dd, \( J = 7.6, 8.0 \) Hz), 6.78 (1H, d, \( J = 8.0 \) Hz), 6.76 (1H, d, \( J = 7.6 \) Hz), 6.56 (1H, d, t, \( J = 1.2, 1.2, 9.6, 11.6 \) Hz), 5.40–5.48 (1H, m), 5.15 (1H, app.dt, \( J = 7.2, 14.4 \) Hz), 3.86–4.08 (5H, m), 3.74 (1H, d, \( J = 4.4 \) Hz), 3.33 (1H, dd, \( J = 11.6, 13.6 \) Hz), 2.34 (1H, dd, \( J = 1.6, 13.6 \) Hz), 2.30 (2H, ddd, \( J = 1.2, 6.8, 6.8 \) Hz), 1.50–1.92 (8H, m), 1.27–1.38 (4H, m), 0.89 (3H, t, \( J = 6.8 \) Hz); HRMS (FAB) calcd for \( \text{C}_{24}\text{H}_{34}\text{NO}_7 (\text{MH}^+): \) 448.2335. Found: 448.2324.

13. Synthetic 1 was identical to an authentic sample of the natural product as judged by NMR, MS, HPLC and TLC analyses and growth inhibitory properties against the SK-MEL-5 cell line.