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Total synthesis and biological evaluation of apicularen A and synthetic analogs

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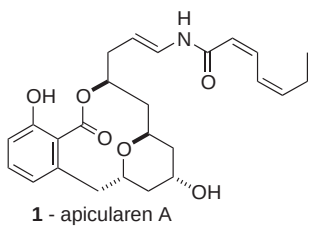
Abstract—The first total synthesis of the potent cytostatic agent apicularen A is described along with the synthesis of the corresponding C_{11}-epimers and side chain modified congeners. Growth inhibition experiments with the human melanoma cancer cell line SK-MEL-5 revealed an important role for the unusual *N*-acyl enamide side chain.

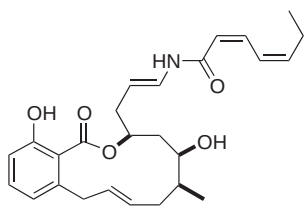
Over the past few years, a variety of macrocyclic salicylate natural products have been isolated from both terrestrial and marine sources based on their ability to induce a particular phenotype in mammalian cells. The bacterial metabolite apicularen A (**1**, Fig. 1) for example, has been shown to display extremely potent cytostatic activity against nine human cancer cell lines ($IC_{50} \sim 0.1$–3 ng/mL), including the multi-drug resistant cervix carcinoma cell line KB-V1 ($IC_{50} \sim 0.4$ ng/mL).[1] 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.[1] Structural relatives include the salicylihalamides[2] and lobatamides,[3] 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 (oximidines)[4] or induce low density lipoprotein (LDL) receptor gene expression (CJ-12,950 and CJ-13,357).[5]

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.[6,7] Herein, we report the first total synthesis of apicularen A and congeners.[8]

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.[6,9] To exploit a similar approach for the synthesis of apicularen A, we had to convert the terminal double bond of our previously derived truncated apicularens[8] to an α,β-unsaturated carboxylic acid (Scheme 1).



1 - apicularen A



2 - salicylihalamide A

Figure 1.

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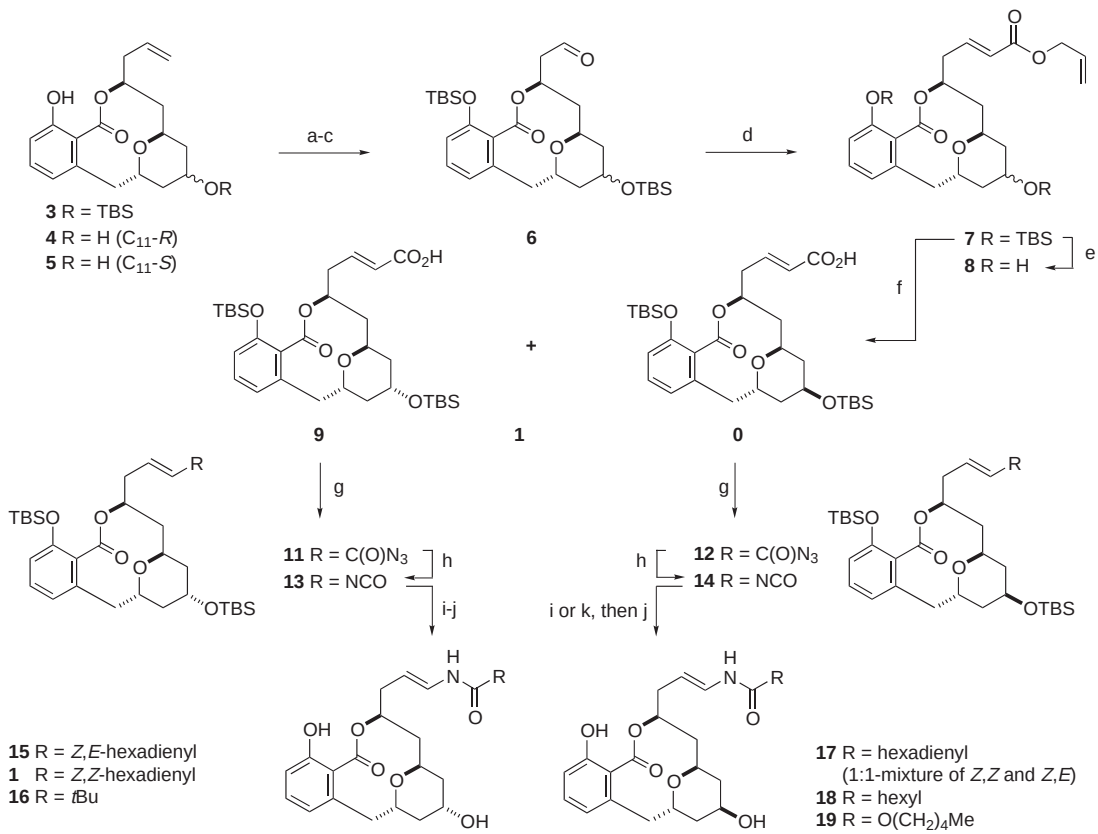
Thus, silylation of phenol **3**[8] (1:1 mixture at C_{11}), oxidative cleavage of the terminal olefin and homologation of the resulting aldehyde **6** with allyl diethylphosphonoacetate provided geometrically pure *E*-allyl ester **7**.[7] Methanolysis of both silyl ethers provided analog **8**, which was set aside for biological testing (vide infra). For the purpose of the total synthesis, **7** was converted to pure carboxylic acids **9** and **10** by Pd-catalyzed deprotection and chromatographic separation of the C_{11}-epimers.[10]

Setting the stage for the introduction of the enamine side chain, acids **9** and **10** were converted in parallel to the corresponding isocyanates **13** and **14** via the intermediacy of acyl azides **11** and **12**.[6] Addition of a 1:1 mixture of 1*Z*,3*Z*- and 1*Z*,3*E*-1-lithio-1,3-hexadiene[6] to **13** and final deprotection afforded a mixture of apicularen A (**1**), the corresponding C_{22}-*E* isomer **15** and *t*-butyl analog **16**.[11,12] These 3 compounds could only be resolved by two consecutive preparative HPLC purification steps. Pure isomer **15** was obtained after elution with 40% acetone in hexanes; apicularen A (**1**) was subsequently separated from **16** with 20% *i*-PrOH in hexanes.[13] In a similar fashion, isocyanate **14** was converted to the C_{11}-epimeric apicularens **17**, which were purified, characterized, and evaluated for biological activity (vide infra) as a 1:1 mixture of geometrical isomers (25% from **12**).

The strategic use of late stage isocyanate intermediates presents opportunities for the generation of a diverse collection of side chain modified congeners. For instance, derivative **18** resulted from addition of hexyllithium to isocyanate **14** followed by deprotection and HPLC purification (40% acetone in hexanes) in 40% yield from **12**, while carbamate **19** was prepared by heating **12** in the presence of *n*-pentanol followed by deprotection (35%, two steps).[12]

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 salicylihalamide 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 C_{11} 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. OsO_4, NMO, acetone–H_2O, rt, 12 h, 85%; (c) $NaIO_4$, CH_2Cl_2, rt, 30 min, 97%; (d) allyl diethylphosphonoacetate, NaH, THF, 0°C, 40 min, 99%; (e) amberlyst-15®, MeOH, rt, 16 h, 80%; (f) cat. $Pd[(PPh_3)]_4$, PPh_3, HCO_2NH_4, THF, rt, 18 h, 65%; (g) $(PhO)_2P(O)N_3$, NEt_3, PhH, rt, 12 h (75–79%); (h) PhH, 82°C, 6 h; (i) 1-bromo-1,3-hexadiene (1:1 mixture of 1*Z*,3*Z* and 1*Z*,3*E* isomers) or 1-bromohexane, *t*-BuLi, Et_2O, −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-5[a]

Compound	GI_{50} (μM)	Compound	GI_{50} (μM)	Compound	GI_{50} (μM)
1	0.006	**8**	>20	**17**	0.45
2	0.03	**15**	0.06	**18**	7.5
4	>20	**16**	0.9	**19**	0.5

[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 GI_{50} 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.

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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 transmetalation 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 Et_2O, freshly prepared from the corresponding bromide by transmetalation 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 (Et_2O), drying (Na_2SO_4) 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 (Na_2SO_4) and concentration. The crude material was purified by normal-phased semi-preparative HPLC (5μ 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**: ^{1}H NMR (400 MHz, acetone-d_6) δ 9.03 (1H, d, J=10.4 Hz), 8.36 (1H, s), 7.64 (1H, dddt, J=1.2, 1.2, 10.8, 15.2 Hz), 7.10 (1H, dd, J=7.6, 8.0 Hz), 6.90 (1H, ddt, 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, ddq, 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), 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 $C_{25}H_{32}NO_6$ (MH^+): 442.2230. Found: 442.2228. Compound **19**: δ 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, dddt, $J=1.2$, 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 $C_{24}H_{34}NO_7$ (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.