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HALOHYDRIN ANALOGUES OF CRYPTOPHYCIN 1: SYNTHESIS AND BIOLOGICAL ACTIVITY

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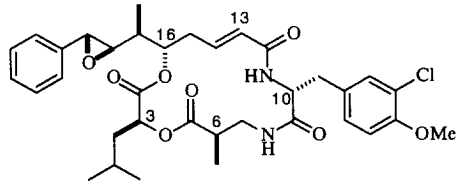
Abstract: The chloro-, bromo-, and iodo-derivatives **2–4** of the antimitotic drug cryptophycin 1 were synthesized by opening the epoxide ring. The biological activities of the compounds were tested in an in vitro microtubule assembly and a cell proliferation assay. The chloro-derivative **2** showed lower activity in the tubulin assay compared to **3** and **4**, but they all showed similar inhibition in the proliferation assay.

Cryptophycin 1 (**1**), a depsipeptide from the cyanobacterium *Nostoc* sp., was first described as a strong antifungal agent.[1–3] Subsequently it was shown to have potent antiproliferative properties in mammalian cell cultures, and to have excellent antitumor activities. [4–7]



Cryptophycin 1 (**1**)

Cryptophycin 1 is an antimitotic compound with unusually high activity; it has a 100- to 1000-fold greater potency than paclitaxel and vinblastine and induces cell death by apoptosis.[8] Of particular interest is the fact that cryptophycin 1 does not appear to be a substrate for the *p*-glycoprotein multi-drug transporter.[4] Cryptophycin 1 binds to tubulin, the protein subunit of microtubules at a site distinct from the colchicine site but one that may overlap with the vinblastine site.[6,9–11] In vitro cryptophycin 1 dampens microtubule dynamics at very low concentrations, perhaps by binding to microtubule ends.[12]

A limited amount of SAR information is available from the known properties of naturally occurring analogues of cryptophycin 1 and from synthetic approaches.[7,13,14] These studies demonstrated that replacement of the epoxide group with a diol or a double bond resulted in loss of in vivo activity but activity was retained when the epoxide was converted to a chloro- or bromohydrin.[7] In fact, the activity of the chlorohydrin in vivo was greater than the activity of cryptophycin 1.[7] To test the possibility that the halohydrins revert to the epoxide under cell culture conditions, we synthesized the chloro-, bromo-, and iodohydrins (**2–4**) of cryptophycin 1 and examined their stabilities in solution as well as their activities in a tubulin assembly assay and as cytotoxic agents.

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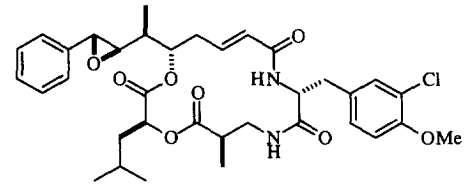
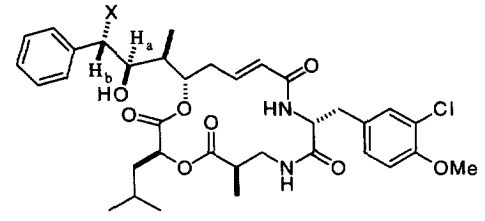
Treatment of cryptophycin 1 with one equivalent of the trimethylsilylhalide in the presence of triphenyl phosphine gave the corresponding halohydrins in almost quantitative yields (Scheme).[15] The halohydrins were purified by flash column chromatography over silica gel and were found to be stable.[16]

Scheme



1

TMSX, Ph_3P, $CHCl_3$,
25 °C, 20 min,
95-100 % yield



2 X = Cl
3 X = Br
4 X = I

When tested in the microtubules assembly assay the bromo (**3**)- and iodo (**4**)-derivatives had activities similar to that of the parent compound but the chlorohydrin (**2**) was substantially less active (Table). On the other hand, all three derivatives had activities similar to cryptophycin 1 in a cell proliferation assay (Table). Since the cell proliferation assay lasts for several days as opposed to the 15 min assembly assay, we considered the possibility that the halohydrins were reverting to cryptophycin 1 during the incubation periods. Further studies were done to test the stability of the halohydrin derivatives under conditions similar to those used in the biological assays. The compounds were incubated in phosphate buffer at pH 7.4 or in tubulin assembly buffer at pH 6.9, and samples were analyzed by HPLC as a function of time. The results showed that the iodo compound very rapidly converted to cryptophycin 1 (Table). The bromo derivative was also converted to the parent compound with a short half-time, while the chlorohydrin was much more stable. The derivatives were less stable in assembly buffer than in phosphate buffer. The half-lifes were much longer in ethanol:water (35:65). For example, the half-time of the reaction with the bromohydrin increased to 86 min in this solvent. The relative stability of the three halohydrins is consistent with the known properties of the chloro-, bromo-, and iodo-functions as leaving groups.

The fact that the activity of the chlorohydrin derivative was much lower in the assembly assay indicates that the halohydrins have reduced or no biological activity, and their observed activity is due to the conversion to the parent compound.

Table. Biological properties and stability of cryptophycin 1 halohydrin derivatives

Compound	MT assembly, ED_{50}, mM[a]	Cell Toxicity, ED_{50}, nM[b]	Half-life, pH 7.4[c]	Half-life, PEM buffer[d]
1	1.0	1.3	-----	-----
2	7.5	1.4	11 h	75 min
3	0.5	0.8	11 min	3.7 min
4	0.6	1.4	2.5 min	<1 min

[a]Tubulin at 1.5 mg/mL was assembled at 37 °C for 15 min in the presence of PEM buffer and 0.5 mM GTP. Microtubules were pelleted and the protein remaining in the supernatant determined. The IC_{50} value is the concentration that reduces the amount of pelleted protein by 50%.
[b]The IC_{50} value is the concentration that inhibits the proliferation of B16 melanoma cells by 50% after 72 h of growth.
[c]The compounds were incubated at room temperature at a concentration of 0.7 to 1.8 mM in 0.01 M phosphate buffer containing 0.15 M NaCl and 35% ethanol with an apparent pH of 7.4.
[d]The compounds were incubated at a concentration of 3.7 to 6.2 mM in PEM buffer (0.1 M 1,4-piperazinediethanesulfonic acid, 1 mM ethyleneglycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid, 1 mM $MgSO_4$), pH 6.9, at 37 °C.

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16. The 1H NMR data for chlorohydrin **2** were identical to that reported for **2** in reference 5. The ^{1}H NMR data for bromohydrin **3** and iodohydrin **4** were closely related to the data for **2**. The ^{1}H NMR resonances for the epoxide protons in cryptophycin 1 are found at 2.92 ppm (dd; 7.5 and 2.0 Hz), and 3.69 ppm (d; 2.0 Hz).[5] For the halohydrins a downfield shift of approximately 1 ppm and an increase in coupling constants were observed for the corresponding halohydrin protons. Compound **2**: 4.01 (Ha, dd; 9.6 and 1.9 Hz), 4.60 (Hb, d; 9.9 Hz). Compound **3**: 4.19 (Ha, d; 9.8 Hz), 4.73 (Hb, d; 9.9 Hz). Compound **4**: 4.32 (Ha, d; 10 Hz), 4.88 (Hb, d; 10 Hz).