Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 69–74

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL CRYPTOPHYCIN ANALOGS WITH MODIFICATION IN THE β-ALANINE REGION

Chuan Shih,* Lynn S. Gossett, Joseph M. Gruber, C. Sue Grossman, Sherri L. Andis, Richard M. Schultz, John F. Worzalla, Tom H. Corbett and James T. Metz
Lilly Research Laboratories, Indianapolis, IN 46285, U. S. A. and
Karmanos Cancer Institute, Wayne State University, Detroit, MI 48202, U. S. A.

Received 25 August 1998; accepted 18 November 1998

Abstract: Structure modification of the β-alanine region (fragment C) of the potent antimitotic agent cryptophycin was investigated. This includes: (1) introduction of substituents at the previously unsubstituted C7 position of the macrolide ring and (2) replacement of the (2*R*)-3-amino-2-methyl-propanoic acid (β-alanine) with various (l)-amino acids to give the corresponding 15-membered unnatural cryptophycin analogs.

Cryptophycins are potent tumor-selective depsipeptides isolated from the terrestrial blue-green algae (cyanobacteria) Nostoc sp. ATCC 53798[1] and Nostoc sp. GSV224.[2] Studies have demonstrated that cryptophycins are highly active against a broad spectrum of murine solid tumors and human tumor xenografts. Cryptophycins are highly active against resistant tumors that express multiple drug resistant (MDR and MRP) phenotypes. A total of 26 naturally occurring cryptophycins have been isolated from Nostoc sp. GSV 224, with Cryptophycin 1 (Crypto 1, **1**) as the major component.[2] Cryptophycins are peptolides with a 16-membered macrolide ring structure, in which the peptolide ring is connected by two ester bonds and two amide linkages. Retrosynthetic disconnection of cryptophycin showed that it can be assembled through the four major fragments A through D (Figure 1).[3] From this novel depsipeptide series, a synthetic cryptophycin analog (LY355703, Crypto 52, **2**) has recently been selected for phase I clinical development for the treatment of solid tumors.[4]

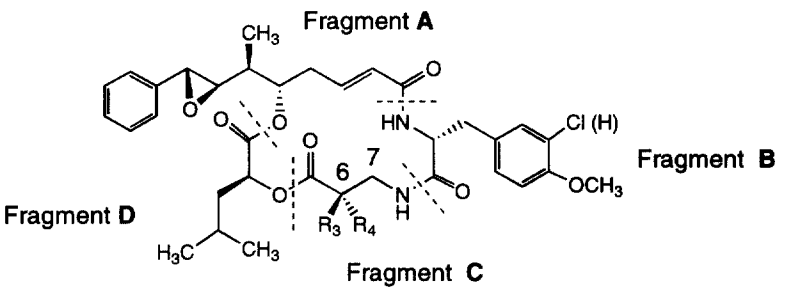
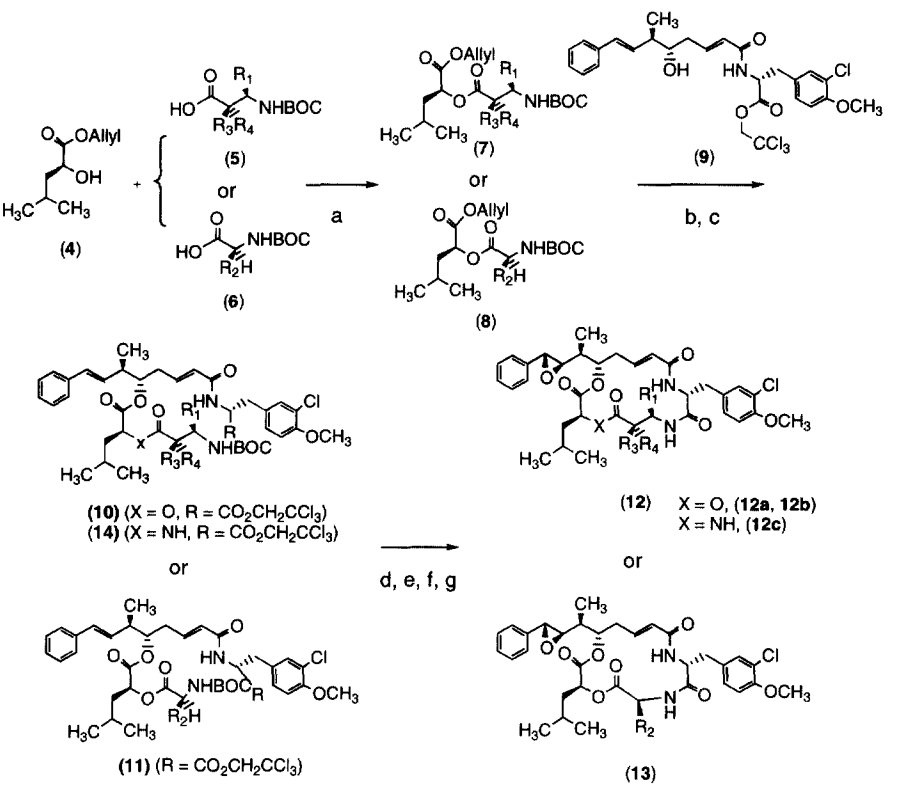


Figure 1. (**1**), Crypto 1 ($R_3 = CH_3$, $R_4 = H$)
(**2**), Crypto 52 ($R_3 = R_4 = CH_3$)
(**3**), Arenastatin A ($R_3 = R_4 = H$, deschloro in fragment B)

PII: S0960-894X(98)00682-9

The majority of the naturally occurring cryptophycins possess the (2*R*)-3-amino-2-methyl-propionic acid unit (fragment C) as the building block with a methyl substituent at the C6 position of the peptolide ring. No substitution, however, has been observed at the C7 position of the natural cryptophycins and arenastatins,[5] a closely related potent cytotoxic natural product series isolated from Japanese sponge *Dysidea arenaria*. This report describes our efforts of introducing substituents specifically into the C7 position (with an *R* configuration similar to that of the methyl group of Crypto 1) of cryptophycins. We have also prepared a number of the unnatural 15-membered cryptophycin analogs by replacing the β-alanine fragment (fragment C) with various (l)-amino acids, for biological evaluation.

Scheme 1. Synthesis of Cryptophycin Analogs with Modification in β-Alanine Region

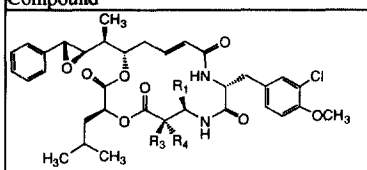
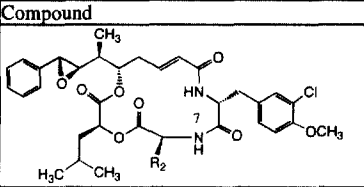
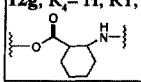
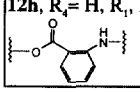


(a) DCC, DMAP, CH_2Cl_2, 0-25 °C, 88-91%; (b) $Pd(PPh_3)_4$, N-methylmorpholine, THF, 25 °C, 85-93%; (c) DCC, DMAP, CH_2Cl_2, 0-25 °C, 88-95%;(d) Zn, CH_3COOH; (e) CF_3COOH, neat, 25 °C, 72-78% (two steps); (f) FDPP, DIEA, DMF, 25 °C, 87%; (g) MCPBA, CH_2Cl_2, 70-80%, reverse phase HPLC separation of β-epoxides.

The general synthetic sequence for the C7 substituted and the 15-membered cryptophycin analogs is shown in Scheme 1.[3] For the preparation of C7 substituted analogs (methyl, isobutyl, phenyl and benzyl) with an R configuration, various (3*R*)-3-butyloxycarbonylamino-3-substituted-propionic acids[6] (**5**)were used as the starting materials. These can be readily coupled (DCC, DMAP) with O-allyl-

(l)-leucic acid (**4**)[7] to give the protected C-D fragments in good yields. The allyl group of compounds (**7**) was removed by treating with tetrakistriphenylphosphinepalladium/N-methylmorphine, and the resulting free carboxylic acids were then coupled (DCC, DMAP) with the A-B fragment (**9**)[7] to give the fully protected noncyclized A-B-C-D fragments (**10**). Removal of the trichloroethyl group by zinc followed by deprotection of the BOC group (neat TFA) gave the seco compounds, which upon treatment with FDPP/diisopropylethylamine gave the cyclized macrolides in good overall yields. Epoxidation of the styrene olefin of (**10**) was accomplished by using MCPBA (CH_2Cl_2), which gave a 2.5 to 1 mixture of β vs. α epoxides. The two epoxides then can be readily separated by reverse-phase HPLC (CH_3CN/H_2O) to give the pure β epoxides (**12**) for biological evaluation.[8] The β-epoxides can be further transformed into the corresponding chlorohydrin derivatives by treating compounds (**12**) with TMSCl at -60 °C (80%). The same sequence was used for the preparation of 15-membered ring analogs (compounds **13a-e**, started with various N-BOC-amino acids **6**) and analogs with both C6 and C7 positions tied up in cyclic structures (compounds **12g** and **12h**). A C-D amide bond analog (**12c**) with C7-methyl substitution was also prepared to examine the effect of the bioisosteric replacement of the C-D ester bond. This amide analog was synthesized via a slightly different approach.[9] The A-B fragment (**9**) was first coupled with N-BOC-(l)-leucine (DCC, DMAP, 80%) to give the fully protected A-B-D fragment. The BOC group of the (l)-leucine was then removed (TFA, quantitative) and the resulting amine was coupled with (3R)-3-butyloxycarbonylamino-3-methyl-propionic acids (EDAC, HOBT, 90%) to give the corresponding C-D amide (**14**). Compound (**14**) was then transformed into the final β-chlorohydrin derivative (**12c**) by following the same synthetic sequence for the preparation of compound (**12b**) as outlined in Scheme 1.

Table 1. Cytotoxicity of Cryptophycin Analogs with Modification in the β-Alanine Region (C6, C7 and C-D ester bond)

Compound	Cells/IC_{50} GC3/(nM)	Compound	Cells/IC_{50} GC3/(nM)
			
1, R_1= H, R_3= CH_3, R_4= H (Crypto 1)	0.24		
2, R_1= H, R_3=R_4= CH_3 (Crypto 52)	0.10-0.20		
12a, R_1= CH_3, R_3=R_4= H	0.40	**13a**, R_2= CH_3	13.00
12b, R_1= CH_3, R_3=R_4= H (β-chlorohydrin)	0.03	**13b**, R_2= $CH(CH_3)_2$	306.00
12c, R_1= CH_3, R_3=R_4= H (C-D amide, X=NH, β-chlorohydrin)	0.06	**13c**, R_2= isobutyl	320.00
12d, R_1= isobutyl, R_3=R_4= H	3.00	**13d**, R_2= benzyl	1000.00
12e, R_1= phenyl, R_3=R_4= H	0.94	**13e**, R_2= $(CH_2CH_2CH_2\text{-}N_2)$ (proline)	>1500.00
12f, R_1= benzyl, R_3=R_4= H	5.20		
12g, R_4= H, R1, R_3= $(CH_2)_4$ 	1.30		
12h, R_4= H, R_1, R_3= (CH=CH–CH=CH) 	870.00		

The antiproliferative effect of these new cryptophycin analogs was evaluated by the 72 h MTT cell viability assay[10] using human colon carcinoma cells (GC3). Crypto 52 (LY355703) was used as a reference compound in all the studies and the IC_{50}s are summarized in Table 1. The in vitro results indicate that moving the (*R*) methyl substituent from the C6 to the C7 position of the peptolide ring does not significantly affect the antiproliferative effect of the compound (IC_{50}: **12a**, 0.4 nM vs 0.24 nM of Crypto 1). The antiproliferative activity of **12a** is also very much comparable to that of Crypto 52. It is interesting to observe that the β-chlorohydrin derivative (**12b**, IC_{50} = 0.03 nM) is more potent than the corresponding epoxide; replacement of the C-D ester bond of chlorohydrin **12b** with an amide also produces a very active analog in vitro (**12c**, IC_{50} = 0.06 nM). The introduction of substituents larger than the methyl group (e.g., isobutyl, phenyl and benzyl) into the C7 position, however, leads to a decrease of the antiproliferative activity, compounds **12d-f** tend to be more lipophilic and less soluble when compared with analogs with smaller substituents at C6 and/or C7 positions (**12a** and Crypto 52). Two examples were prepared to examine the effect of tie up substituents at both the C6 and C7 positions. In both cases, the C6 and C7 carbons were part of the cyclic *cis*-1,2-substituted cyclohexyl (nonchiral) (**12g**) or 1,2-substituted phenyl ring structure (**12h**). The in vitro testing results, however, indicated that this type of modification led to a major decrease of activity, especially when both C6 and C7 carbons were part of the phenyl ring (**12g**, IC_{50}: 870 nM). To further examine the structure-activity relationship of the β-alanine fragment of the cryptophycin molecule, various substituted (l)-amino acids (l-alanine, valine, leucine, phenylalanine, proline) were used in replacing the (2*R*)-3-amino-2-methyl-propionic acid unit of Crypto 1. These unnatural 15-membered cryptophycin analogs were found to be significantly less active than the corresponding 16-membered compounds. The most active alanine derivative (**13a**) is ca. 30- to 50-fold less active when compared to that of Crypto 1 or **12a**. It was also observed in this series that larger substituents at R_2 led to the less active compounds.

In order to apprehend the major decrease of activity of the unnatural 15-membered cryptophycins, molecular modeling and conformational analysis were conducted for both Crypto 1 and compound (**13a**). Analysis of the low energy conformations[11] of the two compounds has revealed conformational changes along the macrolide ring as well as on the side chains. This was particularly evident when two structures were overlapped (Figure 4) and the electrostatic potential molecular surface of both Crypto 1 and compound (**13a**) were compared (Figure 5). The deletion of one carbon atom in the macrolide ring has caused it to adopt a more strained conformation especially around the B-C fragments region.

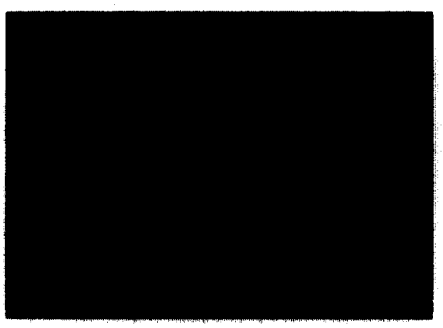


Figure 2. Calculated low energy conformation of Crypto 1

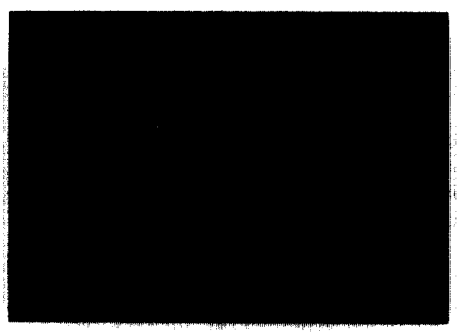


Figure 3. Calculated low energy conformation of compound **13a**

A closer analysis of the B-C amide bond of the 15-membered macrolide ring also suggested that it can adopt a different conformation in relative to the A-B amide and C-D ester bonds of the corresponding 16-membered conformer (Crypto 1). As a result of this conformational change, movements of the side chains (methylchlorotyrosine of the fragment B and the styrylepoxide of the fragment A, Figure 4) occurred and significant difference of electrostatic potential surface can be observed in the styrylepoxide region (upper left, Figure 5) between Crypto 1 and compound (**13a**). A higher electron negativity (darker blue/purple) was observed for the natural 16-membered macrolide in the styrylepoxide region than the corresponding 15-membered analog. It is quite likely that unique conformation and electrostatic potential surface changes like these are responsible for the decreased recognition and interaction of these unnatural cryptophycin molecules with their molecular target (i.e., microtubules).

Figure 4. Overlap of Crypto 1 (green) and compound **13a** (purple)

Figure 5. Electrostatic potential molecular surface comparison between Crypto 1 (top) and compound **13a** (bottom)

Several of these new analogs were also evaluated in animal (murine pancreatic adenocarcinoma PO3 model)[12] for their antitumor efficacy. Although compounds (**12a**) and (**12b**) were found highly potent in the cell based assay, their antitumor activity in vivo was disappointing. A T/C of ca. 50 % was observed at maximal tolerated doses (MTD) for these new analogs (compared to T/C of <5 % for Crypto 1 and 52). It was reported earlier that the hydrolytic instability of the C-D ester bond may attribute to the poor antitumor activity of arenastatin and some cryptophycin molecules (Crypto 21)[13] in vivo. It was rationalized that substitution(s) α (position C6) to the C-D ester bond protects the carbonyl group from the nucleophilic attack and may contribute significantly to the overall physiological/metabolic stability of cryptophycin molecules. The current study on compounds (**12a**), and (**12b**) has provided further evidence to support the hypothesis that a shielded C-D ester bond is critically important toward the in vivo stability and thus antitumor efficacy of the cryptophycin molecules.

References

1. Schwartz, R. E.; Hirsch, C. F.; Sesin, D. F.; Flor, J. E.; Chartrain, M.; Fromcling, R. E.; Harris, G. H.; Salvatore, M. J.; Liesch, J. M.; Yudin, K. *J. Ind. Microbiol.* **1990**, *5*, 118.
2. Golakoti, T.; Ohtani, I.; Patterson, G. M .L; Moore, R. E.; Corbett, T. H.; Valeriote, F. A.; Demchik, L. *J. Am. Chem. Soc.* **1994**, *116*, 4729.

3. Barrow, R. A.; Hemscheidt, T.; Liang, J., Paik, S.; Moore, R. E.; Tius, M. A. *J. Am. Chem. Soc.*, **1995**, *117*, 2479.
4. Worzalla, J. F.; Cao, J.; Ehlhardt, W. J.; Harrison, S. D.; Law, K. L.; Martinelli, M .J.; Self, T. D.; Starling, J. J.; Shih, C.; Theobald, K. S.; Toth, J. E.; Zimmermann, J. L.; Corbett, T. H. *Proc. Am. Assoc. Cancer Res.* **1997**, *38*, Abstract 1516. Corbett, T.; Valeriote, F.; Simpson, C.; Moore, R.; Tius, M.; Barrow, R.; Hemscheidt, T.; Liang, J.; Paik, S.; Polin, L.; Pugh, S.; Kushner, J.; Harrison, S.; Shih, C.; Martinelli, M. *Proc. Am. Assoc. Cancer Res.* **1997**, *38*, Abstract 1515.
5. Kobayashi, M.; Aoki, S.; Ohyabu, N.; Kurosu, M.; Wang, W.; Kitagawa, I. *Tetrahedron Lett.* **1994**, *35*, 7969. Kobayashi, M.; Kurosu, M.; Ohyabu, N.; Wang, W.; Fujii, S.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2196.
6. (3*R*)-3-Butyl-oxycarbonylamino-3-substituted-propionic acids were prepared in two steps: (a) TFA, (b) di-*t*-butyl-dicarboxylate, NaOH, from the commercially available *t*-butyl-(3*R*)-3-substituted-3-aminopropionic acids (Oxford Asymmetry, UK).
7. Compounds **4** and **9** were prepared according to the routes/methods described in ref 3. We are greatly indebted to Dr. Mike Martinelli's group at Chemical Process R&D Division of Lilly Research Laboratories for the supply of these valuable intermediates.
8. The α-epoxides of these new analogs were found significantly less active (10- to 60-fold) than the corresponding β isomers, an observation that is consistent with previous reports.
9. A more detailed description on the preparation of the C-D amide cryptophycin analogs will appear in the future publication: Norman, B.H.; Hemscheidt, T.; Schultz, R.M.; Andis, S.L., *J. Org. Chem.*, **1998**, submitted for publication.
10. The antiproliferative activity of the cryptophycin analogs was determined by using a 72h MTT based assay. The GC3 human colon carcinoma cells were kindly supplied by Dr. Janet Houghton of St. Jude Children's Hospital, Memphis, TN.
11. The X-ray structure of Crypto 52 (Martinelli, M., Moher E. and Stephenson G., unpublished result, Lilly Research Laboratories) was used as the basis for molecular modelling effort. The coordinates of Crypto 52 were imported into the QUANTA97, all hydrogens were added and atomic charges were assigned using the molecular editor. Initial structures for Crypto 1 and **13a** were generated from the X-ray structure of Crypto 52. The C6 (S) methyl and C7 methylene group was removed in each case, respectively, all hydrogens were added, and atomic charges were assigned using the molecular editor. The modified structure was then subjected to 1000 steps of ABNR minimization. The electrostatic potential surfaces of Crypto 1 and **13a** were generated and displayed using QUANTA. The color mapping for each electrostatic potential surface was "normalized" by assigning blue (electro-negative) and red (electro-positive) to represent the same extreme of the potentials for both molecules (-20.0 and +20.0).
12. Corbett, T.; Valeriote, F.; Lorusso, P.; Polin, L.; Panchapor, C. *Int. J. Pharmacognosy.* **1995**, *33* (SUPPL.):102.
13. Golakoti, T.; Ogino, J.; Heltzel, C. E.; Le Husebo, T.; Jensen, C.M.; Larsen, L. K.; Patterson, G. M. L.; Moore, R. E.; Mooberry, S. L.; Corbett T. H.; Valeriote, F. A. *J. Am. Chem. Soc.* **1995**, *117*, 12030.