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# Tetra and Pentapeptide Derivatives of Hemiasterlin. Synthesis and Activity Studies

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## Introduction

Hemiasterlin **1** (Fig. 1) is a natural tripeptide toxin first isolated from the marine sponge *Hemiasterella minor* [1] and later from a number of other sponges. Hemiasterlin acts as an extremely potent inhibitor of tubulin polymerization and is active against leukemia, ovarian carcinoma, and breast cancer cells [2,3].

We investigated a very active hemiasterlin analog **2** (Fig. 1) [4] as a toxin in a receptor-mediated, enzyme-dependent, drug delivery approach. Since the lysosomal processing of a receptor-targeted peptide prodrug adds 1 or 2 amino acid residues to the C-terminus of the toxin, it was necessary to select residues that would lead to the most active final products.

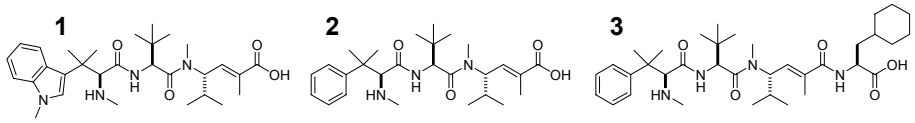


*Fig. 1. Chemical structures of hemiasterlin (**1**), HTI-286 (**2**), and HTI-286 extended on the C-terminus by cyclohexylalanine (**3**).*

## Results and Discussion

We generated a small library of hemiasterlin tetra- and pentapeptide derivatives using both natural and unnatural amino acids in the fourth and fifth positions and determined their inhibitory activities in MTT cytotoxicity and tubulin polymerization assays.

Hemiasterlin analog **2** (HTI-286) was synthesized according to a slight modification of the method of Andersen *et al.* [4]. All tetra- and pentapeptide derivatives of **2** were synthesized on solid support by coupling Boc protected **2** to the appropriate amino acid or dipeptide attached to a resin.

Cytotoxicity was determined by the MTT assay [5] using NIH 3T3 cells. Inhibition of tubulin polymerization was determined as described earlier [6]. Purified tubulin from bovine brain tissue was used for this study.

All elongated hemiasterlin derivatives showed much lower activities than **2** in the MTT cytotoxicity assay. Even the most active tetrapeptide **3** (Fig. 1), $IC_{50}$ = 8 nM, was three orders of magnitude less active than **2**, $IC_{50}$ = 3 pM (Table 1). Surprisingly, no significant differences were observed between **2** and any of the tetra- or pentapeptides in their abilities to inhibit tubulin polymerization. All 25 compounds had $IC_{50}$'s between 1 and 3 μM in the tubulin assay, as compared to the value of 1.4 μM obtained for **2**. Results in the tubulin assay corresponded very well to previously reported values for different hemiasterlins and dolastatins [4].

The lack of correlation between cytotoxicities of the newly synthesized hemiasterlin derivatives and the activities of those compounds in the tubulin polymerization assay suggests that other factors must be critical for the extraordinary cell toxicity of the most potent hemiasterlin derivatives. One possibility could derive from differences in cell penetration of the various compounds or, once taken up by the cells, differences in intracellular drug distribution. However, other factors that are independent of the ability of the molecules to bind to tubulin may also be important determinants of toxicity.

*Table 1. Activities of hemiasterlin derivatives in MTT and tubulin assays*

| Name | Residue | $IC_{50}$ [μM] MTT, 3T3 | $IC_{50}$ [μM] Tubulin |
|---|---|---|---|
| HTI-286 | — | 0.000003 | 1.4±0.07 |
| MD039 | Gly | 2.3 | 2.8±0.09 |
| MD040 | Asp | 0.59 | 1.6±0.04 |
| MD041 | Lys | 1.0 | 2.1±0.16 |
| MD042 | Phe | 0.066 | 1.6±0.06 |
| MD044 | Ser | 0.70 | 1.7±0.05 |
| MD047 | Trp | 0.38 | 1.2±0.01 |
| MD049 | Bip | 0.50 | 1.4±0.03 |
| MD051 | 1-Nal | 0.028 | 1.1±0.07 |
| MD052 | Hfe | 0.078 | 1.2±0.09 |
| MD053 | Tic | 0.39 | 1.1±0.05 |
| MD054 | Cha | 0.008 | 1.8±0.13 |
| MD055 | 2-Nal | 0.052 | 1.2±0.04 |
| MD056 | Phe(4-F) | 0.084 | 1.3±0.07 |
| MD057 | Chg | 0.049 | 1.3±0.10 |
| MD058 | Phe(4-CN) | 0.50 | 1.2±0.11 |
| MD059 | Tyr | 0.60 | 1.2±0.10 |
| MD046 | Phe-Ala | 1.1 | 1.2±0.07 |
| MD091 | Cha-Asp | 0.45 | 1.7±0.08 |
| MD092 | Cha-Phe | 0.45 | 1.4±0.07 |
| MD093 | Cha-Lys | 0.10 | 1.5±0.13 |
| MD094 | Cha-Gly | 2.3 | 1.3±0.07 |
| MD095 | Cha-Leu | 0.42 | 1.4±0.02 |
| MD096 | Cha-Ser | 2.9 | 1.3±0.01 |
| MD097 | Cha-Cha | 0.079 | 1.6±0.21 |

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