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Synthesis of new simplified hemiasterlin derivatives with α,β -unsaturated carbonyl moiety



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ABSTRACT

In this Letter, we report a convenient and efficient method for the synthesis of new simplified derivatives of hemiasterlin in which the α,α -dimethylbenzylic moiety A is replaced by α,β -unsaturated aryl groups as Michael acceptor. Most of these derivatives have a strong cytotoxic activity on three human tumor cell lines (KB, Hep-G₂ and MCF₇). Analogs **17b** and **17f** showed a high cytotoxicity against KB and Hep-G₂ cancer cell lines comparable to paclitaxel and ellipticine.

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Hemiasterlins belong to a family of naturally occurring tripeptides from marine sponges.¹ The important derivatives of hemiasterlins are hemiasterlin A, hemiasterlin B, and hemiasterlin C, which were isolated from marine sponge *Auleta* and *Cymbastella* (Fig. 1) and exhibited potent cytotoxicity in vitro against murine leukemia P388 and human breast, ovarian, colon, and lung cancer cell lines.^{2,3} Hemiasterlins suppress microtubule depolymerization presumably by binding to the vinca-alkaloid binding-domain of tubulin and leading to mitotic arrest and cell death.^{4a} The synthetic analog HTI-286 (**2**) displayed especially potent cytotoxicity against paclitaxel (TaxolTM) resistant tumor cell lines in vitro and in vivo and is currently in clinical trials.^{4b}

There are several reports on the synthesis of new derivatives of hemiasterlin in which the indole aromatic ring in the moiety A of the original molecule was replaced by aryl functional groups.^{5,6a} However, asymmetric synthesis of the stereospecific amine group and especially the *gem*-dimethyl moiety were proved to be highly problematic. To overcome this difficulty, several studies explored modifications of segment A in which the *gem*-dimethyl moiety has been eliminated. Some of these derivatives showed promising cytotoxic activity.⁶

In recent years, previous works have shown that the presence of a α,β -unsaturated carbonyl system in peptide derivatives can improve

the biological activity compared to the initial compound.^{7,8} Explanations may include either a limitation of free energy of the peptide by addition of a constrained system or by an electrophilic character of the compound leading to the possibility to form covalent bond with the protein target. Based on the idea that hemiasterlin derivatives containing α,β -unsaturated carbonyl systems could induce a remarkable cytotoxicity, we decided to synthesize new hemiasterlin derivatives in which the α,α -dimethylbenzylic group (fragment A) is replaced by a α,β -unsaturated carbonyl group.

New hemiasterlin derivatives were synthesized via classical peptide coupling reactions between two fragments **7a–h** and **14a,b**. A general procedure for the synthesis of compound **7** is outlined in Scheme 1. Compounds **7** were prepared from glycine in three steps.⁹ The synthesis started by acetylation of glycine with acetic anhydride in water at room temperature following by condensation with aryl aldehydes using sodium acetate in the presence of acetic anhydride at 90 °C for 12 h which afforded azalactones **6a–h** in 72–83% yields.⁹ Finally, azalactones **6a–h** were hydrolyzed in aqueous sodium hydroxide, followed by treatment with hydrochloric acid (12 N) at 100 °C for 4 h to give compounds **7a–h** in 65–88% yields.

Compounds **14a,b** were obtained from Boc-L-valine in 6 steps as depicted in Scheme 2.⁴ In the first step, Boc-L-valines **8a,b** were converted to Weinreb amide **9a,b** in good yields (86% and 81%) by treatment with a mixture of *N,O*-dimethylhydroxylamine hydrochloride, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)

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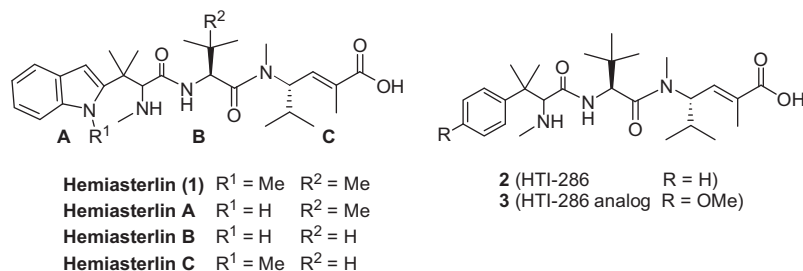
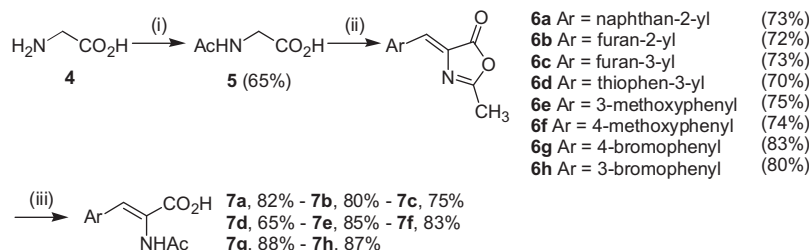


Figure 1. Structures of hemiasterlin derivatives and HTI analogs.



Scheme 1. Reagents and conditions: (i) 2.0 equiv Ac_2O , H_2O , rt, 20 h. (ii) 0.75 equiv ArCHO , 1.0 equiv AcONa , Ac_2O , 90 °C, 12 h. (iii) NaOH (1 N) then HCl (12 N), 100 °C, 4 h.

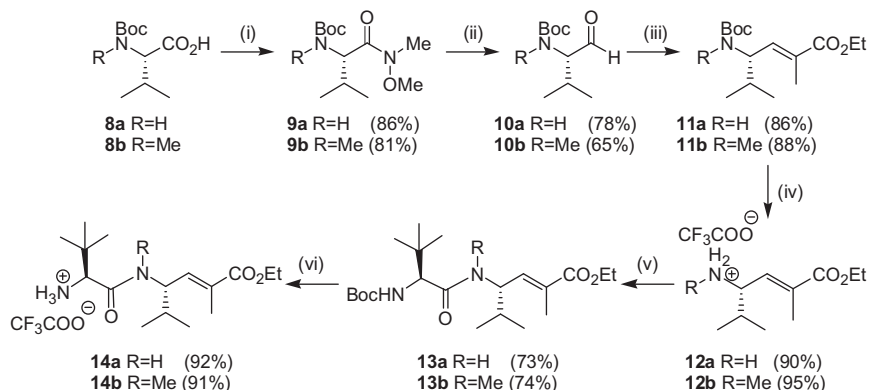
and hydroxybenzotriazole (HOBt) in the presence of *N*-ethylisopropylamine (*i*-PrNH₂) at room temperature for 12 h.¹⁰ Reduction of **9a,b** using LiAlH_4 in THF was carried out at room temperature for 1 h to give aldehydes **10a,b** in 78% and 65% yields, respectively.¹¹ Afterward, Wittig reaction of aldehydes **10a,b** with ethyl 2-(triphenylphosphoranylidene)propanoate was carried out at reflux in CH_2Cl_2 for 6 h to afford the alkenoates **11a** and **11b** in 86% and 88% yields, respectively.^{6,12} Then, removal of the Boc group using trifluoroacetic acid in CH_2Cl_2 at room temperature for 1 h led to **12a,b** in high yields (90% and 95%). The expected compounds **14a,b** are finally obtained after coupling **12a,b** and Boc-L-leucine in the presence of EDC/HOBt in DMF at room temperature for 12 h following by Boc deprotection in classical reaction conditions.

The final compounds **16a–h** were then prepared in two steps after peptide coupling reactions of **14a** with amides **7a–h** following by the saponification of ester using a 1 N lithium hydroxide solution (Scheme 3a). Similarly, the hemiasterlin derivatives **18a–g** were obtained from **14b** (Scheme 3b).

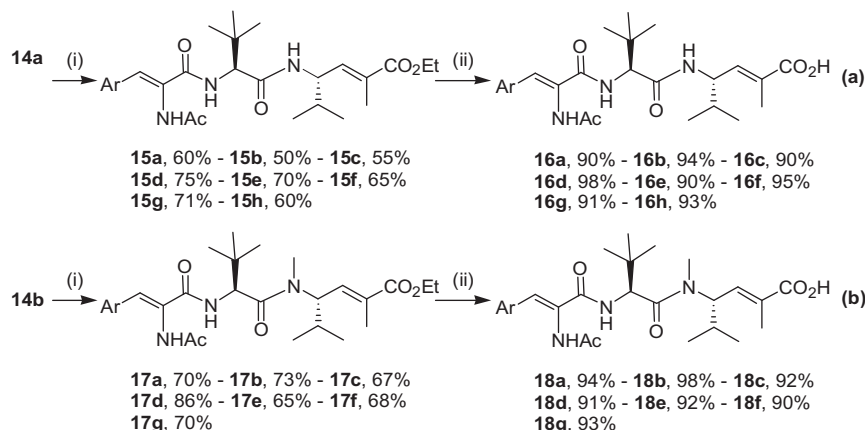
All compounds **15a–h**, **16a–h**, **17a–g** and **18a–g** were evaluated in vitro for their cytotoxic activity against four human tumor cell lines (KB, Hep-G₂, LU and MCF₇) and the results were summarized in Table 1 (in bold are indicated the most significant results of inhibition activities).

Eleven hemiasterlin derivatives showed strong activity against the KB, Hep-G₂ cell line with IC_{50} value below 100 nM. Analogs **16b**, **17b** and **18f** exhibited potent cytotoxicity against the KB cell line with IC_{50} = 8.2, 3.5 and 3.7 nM, respectively. Meanwhile analogs **16b**, **2217c** and **17f** displayed potent cytotoxicity against the Hep-G₂ cell line with IC_{50} value 17.8, 16.3 and 3.7 nM, respectively. Derivatives **15a**, **15e** and **17a** presented a cytotoxic activity against MCF₇ cell line with IC_{50} value 42.3, 60.0 and 67.9 nM. Concerning the last cell line, the LU cell line, the hemiasterlin analogs showed weak activities with IC_{50} values above 269 nM. It is noteworthy to mention that two derivatives **17a** and **17f** present a cytotoxic activity against two cancer cell lines (KB, Hep-G₂) comparable with those of ellipticine and paclitaxel.

In conclusion, a concise synthetic approach for new modified hemiasterlin derivatives was achieved in which the α,α -dimethylbenzylic group and amino NHMe moiety were replaced respectively by a α,β -unsaturated aryl and an amide NH-Ac group avoiding the synthesis of the chiral fragment A. Most of these derivatives possess strong cytotoxic activity on three human tumor cell lines (KB, Hep-G₂ and MCF₇) and two analogs, **17b** and **17f**, showed a comparable cytotoxicity activity to paclitaxel and ellipticine against KB and Hep-G₂ cancer cell lines. Based on previously reported work,^{6b} we can envisage that our hemiasterlin derivatives



Scheme 2. Reagents and conditions: (i) 1.1 equiv $\text{NH}(\text{Me})\text{OMe}\cdot\text{HCl}$, 1.1 equiv EDC, 1.1 equiv HOBt, 2.0 equiv *i*-PrNH₂, DMF, rt, 12 h. (ii) 4.0 equiv LAH, THF, rt, 1 h. (iii) $\text{Ph}_3\text{P}=\text{C}(\text{CH}_3)\text{CO}_2\text{Et}$, CH_2Cl_2 , reflux, 6 h. (iv) TFA, CH_2Cl_2 , rt, 1 h. (v) 1.0 equiv Boc-L-Leucine, 1.1 equiv EDC, 1.1 equiv HOBt, 2.0 equiv *i*-PrNH₂, DMF, rt, 12 h. (vi) TFA, CH_2Cl_2 , rt, 1 h.



Scheme 3. Reagents and conditions: (i) 1.0 equiv **7a–h**, 1.1 equiv EDC, 1.1 equiv HOBt, 2.0 equiv *i*-PrNH₂, DMF, rt, 12 h. (ii) 10 equiv LiOH, MeOH/H₂O (2:1), rt, 10 h.

Table 1
Cytotoxicity evaluation

Entry	Compound	IC ₅₀ (nM)			
		KB	Hep-G ₂	LU	MCF ₇
1	15a	30.8	39.7	>269	42.3
2	15b	160.5	214.2	>269	>269
3	15c	86.2	67.3	>269	>269
4	15d	107.4	57.6	>269	203
5	15e	77.4	47.7	228.4	62
6	15f	>269	>269	>269	>269
7	15g	>269	>269	>269	>269
8	15h	>269	>269	>269	>269
9	16a	63	33.4	>269	>269
10	16b	8.2	17.8	>269	>269
11	16c	155.4	>269	>269	>269
12	16d	>269	>269	>269	>269
13	16e	>269	>269	>269	>269
14	16f	>269	>269	>269	>269
15	16g	>269	>269	>269	>269
16	16h	>269	>269	>269	>269
17	17a	23.0	23.1	>269	67.9
18	17b	3.5	31.4	231.0	178.4
19	17c	24.1	16.3	158	112.8
20	17d	202	15.0	>269	>269
21	17e	>269	>269	>269	>269
22	17f	3.7	3.7	>269	215
23	17g	49.0	>269	>269	>269
24	18a	69.7	13.0	>269	>269
25	18b	188.0	>269	>269	>269
26	18c	>269	149.4	>269	>269
27	18d	234	63.2	>269	>269
28	18e	>269	>269	>269	>269
29	18f	15.8	63.8	>269	>269
30	18g	>269	>269	>269	>269
31	Ellipticine	1.26	1.26	1.82	2.15
32	Paclitaxel	3.9	0.19	—	—

act as tubulin polymerization inhibitors. From our best compounds, more detailed biological studies will be undertaken and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2014.03.091>.

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