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Tubulin inhibitors. Synthesis and biological activity of HTI-286 analogs with B-segment heterosubstituents

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Abstract—Modifications of the B-segment of HTI-286 (**2**) produced a class of analogs incorporating heteroatom-substituents. The structure–activity relationship was studied. Analogs bearing methylsulfide and fluoride groups exhibited potency comparable to that of the parent compound HTI-286 and to paclitaxel in cytotoxicity assays against KB-3-1 cell lines. These analogs were more potent than paclitaxel against P-glycoprotein expressing KB-8-5 and KB-V1 cell lines. Several analogs showed strong inhibition of tubulin polymerization.

It is well known that taxanes and Vinca alkaloids are the two classes of tubulin inhibitors clinically used as anticancer drugs. Despite their successes, a major challenge to the efficacy of these drugs is inherent and acquired resistance by tumors during clinical use.[1] The recently discovered natural product hemiasterlin (**1**, Fig. 1),[2] isolated from marine sponges, is an antimitotic agent that effectively inhibits tubulin polymerization by binding to the Vinca-peptide site.[3] It also exhibits potent cytotoxicity against human breast, ovarian, colon, and lung cancer cell lines.[4] The synthetic analog HTI-286 (**2**),[5] in which a phenyl group has replaced the *N*-methylindole ring of **1**, was found to be a potent cytotoxin and mitotic blocker like **1**, vincristine and paclitaxel (Taxol™).[6] Compound **2** exhibited especially potent activity against paclitaxel-resistant cell lines, in vitro and in vivo. These potent effects of HTI-286 (**2**) on tubulin make it very attractive as a drug candidate. Currently HTI-286 is in clinical trials.[7]

Broad SAR studies of HTI-286 were conducted in our laboratories, concentrating on the systematic investigation of each of the designated A, B, C, D segments.[8] The synthetic analogs were evaluated for inhibition of tubulin polymerization and for cellular cytotoxicity against a panel of epidermoid carcinoma cell lines with varying levels of P-glycoprotein expression (KB-3-1, KB-8-5, KB-V1). In vivo studies in tumor xenograft models identified several potent analogs, including one

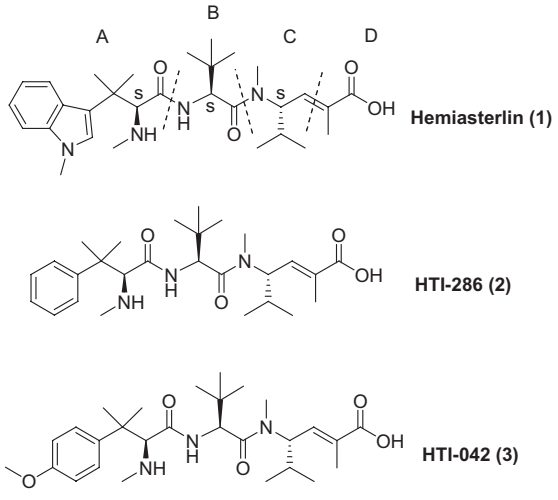

Figure 1.

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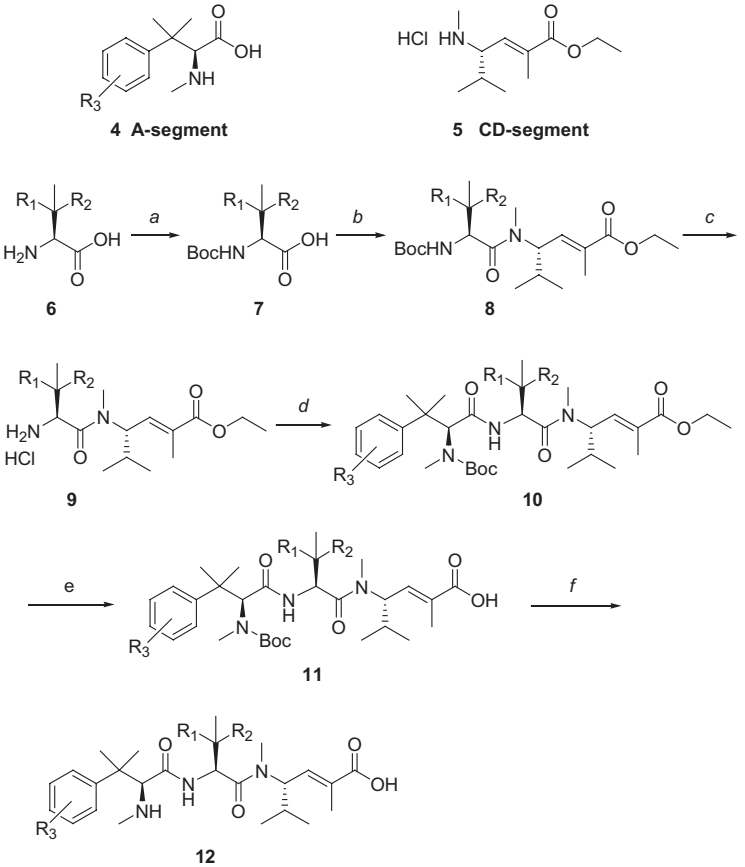
superior analog, HTI-042 (**3**), which has been selected as a development candidate. This paper describes the synthesis and biological results of novel analogs of HTI-286, focusing on the heteroatom containing B segment analogs as potential inhibitors of tubulin polymerization.

The total synthesis of hemiasterlin has been reported in the literature.[4] This synthetic approach was also used for synthesis of HTI-286 and other analogs.[8] The general synthesis for the purposes of this paper, involved preparing amino acids as B-segments, followed by coupling to the CD-segment (**5**) and the A-segment (**4**).[8] Deprotection of the amino and carboxyl groups provided the desired analogs (**12**, Scheme 1).

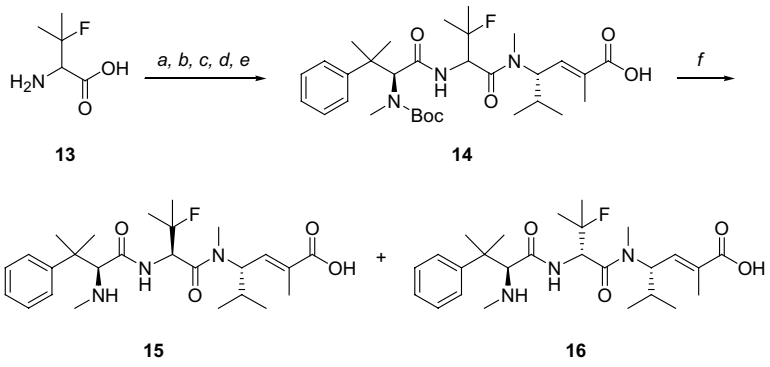
Commercially available (*S*)-amino acid **6**, containing either O or S heteroatoms as well as other functional groups, was treated with di-*tert*-butyl dicarbonate to give the *N*-Boc-protected amino acids **7**.[9] A few of the latter compounds **7** were also commercially available. Intermediate **7** was then coupled to optically pure CD-segment **5**, producing the *N*-Boc-BCD segment **8**. Removal of the Boc protecting group in **8** with 4 N HCl in dioxane gave compound **9**. Coupling of **9** to the A-segment **4**, in the presence of PyBOP and *N*,*N*-diisopropylethylamine,[10] produced the protected tripeptide **10**. Hydrolysis of ester **10** with 1 N lithium hydroxide solution gave the corresponding acid **11**. Final deprotection of the Boc protecting group in **11**, under acidic conditions, provided the optically pure tripeptides **12a**–**k**.

The synthesis of B-piece analogs incorporating a fluorine atom is outlined in Scheme 2. Tripeptide **14**, as a mixture of two diastereoisomers, was synthesized start-



12a[8] $R_1 = CH_3$, $R_2 = SCH_3$, $R_3 = H$
12b $R_1 = CH_3$, $R_2 = SOCH_3$, $R_3 = H$
12c $R_1 = CH_3$, $R_2 = SO_2CH_3$, $R_3 = H$
12d $R_1 = CH_3$, $R_2 = SCH_2C_6H_4OCH_3$-*p*, $R_3 = H$
12e[8] $R_1 = CH_3$, $R_2 = C_6H_4OCH_3$-*p*, $R_3 = H$
12f $R_1 = H$, $R_2 = OH$ (*R*), $R_3 = H$
12g $R_1 = H$, $R_2 = OH$ (*S*), $R_3 = H$
12h $R_1 = H$, $R_2 = OCH_3$ (*R*), $R_3 = H$
12i $R_1 = CH_3$, $R_2 = OH$, $R_3 = H$
12j $R_1 = CH_3$, $R_2 = SCH_2C_6H_4OCH_3$-*p*, $R_3 = OCH_3$
12k $R_1 = CH_3$, $R_2 = SCH_3$, $R_3 = OCH_3$

Scheme 1. Reagents and conditions: (a) Boc_2O, $NaHCO_3$, H_2O; (b) **5**, PyBOP, DIEA, CH_2Cl_2; (c) 4 N HCl/dioxane; (d) **4**, PyBOP, DIEA, CH_2Cl_2; (e) 1 N LiOH, H_2O, CH_3OH; (f) 4 N HCl/dioxane or TFA, CH_2Cl_2.



Scheme 2. Reagents and conditions: a, b, c, d, e refer to Scheme 1; (f) (i) TFA, CH_2Cl_2; (ii) HPLC.

ing from racemic fluorovaline (**13**), following the procedure described in Scheme 1. Removal of the Boc protecting group, using trifluoroacetic acid, followed by preparative HPLC separation, gave the (*S*,*S*,*S*) diastereoisomer **15** and the (*S*,*R*,*S*) isomer **16**.

The analogs thus prepared were evaluated for their antimitotic activities by measuring their inhibition of tubulin polymerization, as well as their cytotoxicity against KB cell lines (KB-3-1, KB-8-5, and KB-V1, Table 1). Most of the analogs showed moderate to strong inhibition of MAP-rich tubulin polymerization in comparison with the inhibition by the lead compound HTI-286 (average 88%). The sulfur containing analogs, **12a**,[8] **12c**, **12d**, and **12k**, oxygen containing analogs **12g**, **12h**, and **12i**, as well as the fluoro-substituted analog **15**, strongly inhibited tubulin polymerization, comparable to HTI-286. In the cellular assays, the KB-3-1 cell line does not express P-glycoprotein (Pgp), while the paclitaxel-resistant cell lines KB-8-5 and KB-V1 express moderate to very high levels of Pgp, respectively. HTI-286 and paclitaxel were used as reference compounds. Both analogs **12a** and **12k**, incorporating methylsulfide, showed potent activity against the KB-3-1 cell line, as well as the paclitaxel resistant cell lines KB-8-5 and KB-V1. The sulfoxide, **12b**, showed no activity against the KB cell lines below 3 μM concentration. However, the corresponding sulfone, **12c**, retained moderate potency against KB-3-1 and KB-8-5 cell lines. Analog **15**, having one methyl group of the L-*tert*-leucine moiety substituted with fluorine, retained good potency against KB cell lines. However, when one methyl group of the L-*tert*-leucine moiety was replaced with a hydroxyl group, the resulting analog **12i** showed moderate potency against KB-3-1 and KB-8-5 cell lines, and was inactive versus the KB-V1 cell line at or below 3 μM concentration. Replacing the L-*tert*-leucine moiety with L-threonine gave analog **12f**, which exhibited very poor inhibition of tubulin polymerization and lost activity under the given assay conditions. Interestingly, replacing the L-*tert*-leucine moiety with L-*allo*-threonine provided analog **12g**, which showed excellent inhibition of tubulin polymerization and moderate potency in KB-3-1 and KB-8-5 cells. With O-methyl L-threonine in the place of the L-*tert*-leucine moiety, the analog **12h** strongly inhibited tubulin polymerization and showed moderate activity versus KB-3-1 cell line. Analogs **12d** and **12j**, containing the bulky functional group $SCH_2C_6H_4OCH_3$-*p*, maintained good activity against the KB cell lines.

Table 1. In vitro potency and inhibition of tubulin polymerization of hemiasterlin analogs versus paclitaxel

Compound	Inhibition of tubulin polymerization (%)[a]	IC_{50} (nM)		
		KB-3-1	KB-8-5	KB-V1
12a	95	1.2	2.2	144
12b	43	>3000	>3000	>3000
12c	87	177.8	588.8	>3000
12d	94	58.8	93.7	2291
12e	56	25.5	54.9	1794
12f	9	>3000	>3000	>3000
12g	91	275.4	691.8	>3000
12h	95	870.9	1737	>3000
12i	84	57.9	95.0	>3000
12j	77	15.8	43.7	2089
12k	88	0.642	1.8	62.4
15	91	1.6	3.5	117.3
Paclitaxel	Promotes tubulin polymerization	2–6	30–80	>3000
HTI-286 (**2**)	88[b]	0.6–2	1.2–5	40–140

[a] % Inhibition of MAP-rich tubulin polymerization at 0.3 μM.
[b] Average over 10 runs.

The pharmaceutical profiling of oxygen containing analogs **12c**, **12g**, and **12h** revealed that these polar analogs exhibited very poor permeability[11] at pH 7.4. Presumably, these compounds cannot penetrate into the cell sufficiently to achieve good potency even though they are good inhibitors of cell-free tubulin polymerization.

SAR studies in our laboratories had confirmed that the *S*-configuration and branching on the β-carbon of the B-segment are necessary for potent activity.[8] The SSS isomer, **2**, exhibited high potency against KB cell lines, but the SRS isomer was much less active. The same trend was seen in the fluorinated analogs **15** and **16**. Variation of the *tert*-butyl group revealed that potency was related to the branching, with the order in potency as $(CH_3)_3C > (CH_3)_2CH > CH_3CH_2 > CH_3$. Enlargement of the *tert*-butyl group, via incorporation of a *p*-methoxyphenyl functional group, gave analog **12e**,[8]

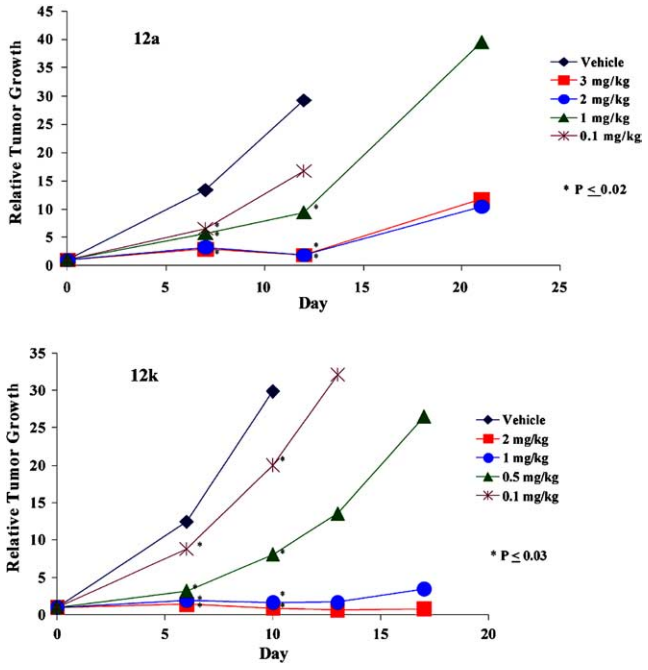


Figure 2. Effect of **12a** and **12k** on growth of human LOX melanoma xenografts in athymic mice.

which showed good activity against KB cell lines in vitro.

The two compounds **12a** and **12k** selected as possible backup candidates of HTI-286 were further evaluated in in vivo assays, using standard pharmacological test procedures, which measure the ability to inhibit the growth of human tumor xenografts.[12] Both the minimum effective dose (MED) and the maximum tolerated dose (MTD) were determined. The MED of **12a** was 0.5 mpk (mg per kilogram) to inhibit the LOX melanoma xenograft model and its MTD value was between 3 and 10 mpk. The MED and MTD values of analog **12k** were determined to be 0.5 and 4 mpk, respectively. Zero tumor growth was achieved with analog **12k** at 2 mg/kg dose in the LOX melanoma xenograft model (Fig. 2). Compound **12k** was also active in vivo against the paclitaxel-resistant cell line DLD1.

In summary, we have synthesized a series of analogs of HTI-286, containing O, S, and F hetero-substituents in the B-segment. Most of these compounds strongly inhibited tubulin polymerization. Tolerance to a free hydroxyl group on the β-carbon of the B-segment was shown to be strongly dependent on the configuration at this carbon atom, as evidenced by the difference in inhibition of tubulin polymerization by diasteroisomers **12f** versus **12g**. Several compounds showed potent cellular cytotoxicity against KB cell lines in vitro. The in vivo assays revealed that analogs **12a** and **12k** also effectively inhibited the growth of human tumor xenografts in athymic mice, including tumors resistant to paclitaxel.

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