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Tubulysin analogs incorporating desmethyl and dimethyl tubuphenylalanine derivatives

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Abstract—A series of tubulysin analogs in which one of the stereogenic centers of tubuphenylalanine was eliminated were synthesized. All compounds were tested for antiproliferative activity towards ovarian cancer cells and for inhibition of tubulin polymerization. The dimethyl analogs were generally more active than the desmethyl analogs, and four analogs have tubulin polymerization IC_{50} values similar to combretastatin A4 and the hemiasterlin analog HTI-286. © 2008 Elsevier Ltd. All rights reserved.

The tubulysins, represented by tubulysins A and D (1 and 2), have emerged as important anticancer lead compounds.¹ They possess impressive activity against multidrug-resistant cancer cells that overexpress P-gly-coprotein.^{1b,2} Mechanistically, tubulysins inhibit tubulin polymerization³ and also have antiangiogenic effects.² Their potential for the treatment of drug-resistant cancers has stimulated a flurry of research into the chemistry and biology of these natural products.

Limited availability of tubulysins from the producing myxobacteria by fermentation¹ has led to recent disclosures for their total synthesis⁴ and the synthesis of novel analogs.⁵ Tubulysins are mixed nonribosomal-polyketide tetrapeptides that, in addition to isoleucine (IIe), contain three unique amino acids: *N*-methylpipecolinic acid (Mep), tubuvaline (Tuv), and tubuphenylalanine (Tup). We recently reported the synthesis and initial biological activity of simplified tubulysin analogs that lack the *N*,*O*-acetal, and have the acetate group of tubuvaline replaced with a ketone.^{5d} Systematic modification of the *N*-methyl group, stereochemistry, and ring size of Mep revealed preliminary structural requirements. Here we extend these structure–activity studies by reporting the

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synthesis and biological activity of tubulysin analogs **3** containing desmethyl and dimethyl tubuphenylalanine derivatives. Both of these modifications result in the elimination of a stereogenic center that further simplifies their synthesis.



Synthesis of the key tripeptide intermediates commenced with opening of lactams 4^{5d} and 5^{5d} and esterification to give benzyl esters 6^6 and 7 (Scheme 1). Amine deprotec-

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Scheme 1. Synthesis of tripeptide intermediates 11 and 12.

tion and coupling with tubuvaline fragment 8^{5d} afforded dipeptides 9 and 10. Further deprotection and coupling with *N*-Boc-L-Ile produced tripeptides 11 and 12.

Desmethyl tripeptide 11 was deprotected at the N-terminus, coupled with *N*-methyl amino acids 13a–d, and deprotected to afford tubulysin analogs 14a–d (Scheme 2). Amino group deprotection and coupling of tripeptide 11 with *N*-Boc protected amino acids 13e–h gave protected tetrapeptides 14e–h. Removal of the Boc groups and benzyl esters then gave *N*-desmethyl tubulysin analogs 15a–d.

At this stage, antiproliferative and tubulin inhibition screening established that tubulysin analogs lacking the *N*-methyl group of Mep, or that have the unnatural L-configuration at this residue were dramatically less potent.^{5d} Therefore, the full complement of Mep analogs in the dimethyl tubuphenylalanine series was not synthesized. Deprotection of tripeptide **12**, followed by coupling with amino acids **13a** and **13b** and removal of the benzyl esters afforded tubulysin analogs **16a** and **16b** (Scheme 3). Saponification of the benzyl esters required heating at reflux due to the increased substitution at the α -position.

All tubulysin analogs were screened for antiproliferative activity and inhibition of tubulin polymerization using combretastatin A4 and the hemiasterlin analog HTI-286/SPA110 as positive controls (Table 1). Their antiproliferative activity in 1A9 ovarian cancer cells and the tubulin polymerization assay was conducted under standard conditions as previously described.^{5d,7}

The antiproliferative data closely adhere to the structure-activity relationship (SAR) trends found in our previous series of tubulysin analogs.^{5d} Within the desmethyl series (**14a-d** and **15a-d**), it is clear that the *N*-methyl group and the natural D-configuration of the



Scheme 2. Synthesis of desmethyl tubulysin analogs 14a-d and 15a-d.



Scheme 3. Synthesis of dimethyl tubulysin analogs 16a and 16b.

various Mep analogs are required for activity. The activity of analog **14a** demonstrates that the ring size of Mep can be changed to *N*-Me-D-Pro, although this is accompanied with a slight loss of activity. Since these data are consistent with other tubulysin analogs,^{5d} we did not synthesize the complete complementary series of dimethyl analogs. Comparison of the antiproliferative activity of tubulysin analogs **16a** and **16b** again shows that while *N*-Me-D-Pro can be substituted for Mep, there is a loss of potency. New SAR trends for the Tup fragment are also revealed here. Modification to Tup by the incorporation of a geminal dimethyl group (**16a** and **16b**) results in comparable levels of antiproliferative activity to the corresponding mono-methyl analogs.^{5d} However, unsubstituted Tup variants lacking

Table 1. Antiproliferative activity of tubulysin analogs in 1A9 ovarian cancer cells and inhibition of tubulin polymerization



Me				
Compound	\mathbb{R}^1	R ^{2a}	Antiproliferative activity IC_{50}^{b} (μM)	Tubulin Inhibition $IC_{50}{}^{c}$ (μM)
14a (FT-032)	Н	N-Me-D-Pro	24	2.3
14b (FT-030)	Η	N-Me-D-Pip	4	0.6
14c (FT-033)	Н	N-Me-L-Pro	50	11
14d (FT-031)	Н	N-Me-L-Pip	>50	13
15a (FT-028)	Н	D-Pro	>50	>20
15b (FT-026)	Н	D-Pip	>50	4.4
15c (FT-029)	Н	L-Pro	>50	>20
15d (FT-027)	Н	L-Pip	>50	>20
16a (FT-034)	Me	N-Me-D-Pro	3.2	2.7
16b (FT-035)	Me	N-Me-D-Pip	0.3	1.5
Combretastatin A4		•	3.6 nM	1.1
HTI-286 (SPA110)			0.3 nM	0.7

^a Pro, proline; Pip, pipecolinic acid; all compounds were isolated as their HCl salts.

^b Values are means of two independent IC₅₀ determinations against 1A9 ovarian cancer cells with a maximum drug concentration of 50 μM in DMSO (See Supplementary Data).

^c In vitro inhibition of tubulin polymerization (see Supplementary Data).

an α -methyl group are an order of magnitude less active (compare 14a and 14b to 16a and 16b).

Results from the in vitro tubulin polymerization inhibition assay closely mirror the antiproliferative data (Table 1). In general, the most active tubulysin analogs are also the most potent tubulin polymerization inhibitors. Notably, all compounds that retain the natural D-configuration and the N-methyl group of Mep found in the natural products (14a, 14b, 16a, and 16b) are the most potent, with low or sub-micromolar IC₅₀ values that are comparable to combretastatin A4 and HTI-286. Comparison of analogs 14a and 14b to 16a and 16b suggests that the lower antiproliferative activity of 14a and 16a is due to decreased inhibition of tubulin polymerization caused by the change from N-Me-D-Pip to N-Me-D-Pro. Alkylation of the α -position of Tup also influences the biological activity of tubulysin analogs. While the tubulin polymerization inhibition $IC_{50}s$ of the dimethyl substituted analogs 16a and 16b are comparable to the unsubstituted 14a and 14b, 16a and 16b are an order of magnitude more active against 1A9 cells. This suggests that the increased antiproliferative activity of 16a and 16b is due to their higher lipophilicity which enables better cell membrane permeability. The only compound that does not follow these general trends is analog 15b. It is unclear why 15b is not antiproliferative despite its effective inhibition of tubulin polymerization. One possible explanation is that the unsubstituted D-Pip residue decreases its lipophilicity and is unable to effectively cross the cell membrane.

In summary, we have synthesized and evaluated a new series of simplified tubulysin analogs that differ at the Mep and Tup residues. Many functional groups in the natural products were found to be unnecessary for antiproliferative activity and tubulin inhibition: (1) the stere-

ogenic α -methyl group of tubuphenylalanine, and the (2) N,O-acetal and (3) stereogenic acetate groups of tubuvaline. Our previously published route to tubulysin analogs enabled their efficient synthesis. Clear SAR trends emerged with regard to antiproliferative activity and inhibition of tubulin polymerization. Four of these analogs (14a, 14b, 16a, and 16b) inhibit tubulin polymerization comparable to the clinical agents combretastatin A4 and HTI-286. Their inhibition of tubulin polymerization is also similar to the reported value for tubulysin A $(IC_{50} = 0.75 \,\mu M)$, although differences in assay conditions preclude direct comparison of these values.³ The reasons for the decreased antiproliferative activity of these tubulysin analogs relative to combretastatin A4 and HTI-286 are unknown at this time. Further examination of the promising activity of these and other tubulysin analogs will be reported in due course.

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Supplementary data

Experimental procedures, spectral data, and copies of ¹H and ¹³C NMR spectra for compounds 6, 7, 9–12, 14a–h, 15a–d, 16a, and 16b. Supplementary data associ-

ated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.046.

References and notes

- (a) Sasse, F.; Steinmetz, H.; Heil, J.; Höfle, G.; Reichenbach, H. J. Antibiot. 2000, 53, 879; (b) Steinmetz, H.; Glaser, N.; Herdtweck, E.; Sasse, F.; Reichenbach, H.; Höfle, G. Angew. Chem. Int. Ed. 2004, 43, 4888.
- Kaur, G.; Hollingshead, M.; Holbeck, S.; Schauer-Vukasinovic, V.; Camalier, R. F.; Dömling, A.; Agarwal, S. *Biochem. J.* 2006, 396, 235.
- 3. Khalil, M. W.; Sasse, F.; Luensdorf, H.; Elnakady, Y. A.; Reichenbach, H. Chem. Biol. Chem. 2006, 7, 678.
- (a) Peltier, H. M.; McMahon, J. P.; Patterson, A. W.; Ellman, J. A. J. Am. Chem. Soc. 2006, 128, 16018; (b)

Dömling, A.; Beck, B.; Eichelberger, U.; Sakamuri, S.; Menon, S.; Chen, Q.-Z.; Lu, Y.; Wessjohann, L. A. Angew. Chem. Int. Ed. 2006, 45, 7235; (c) Dömling, A.; Beck, B.; Eichelberger, U.; Sakamuri, S.; Menon, S.; Chen, Q.-Z.; Lu, Y.; Wessjohann, L. A. Angew. Chem. Int. Ed. 2007, 46, 2347; (d) Sani, M.; Fossati, G.; Huguenot, F.; Zanda, M. Angew. Chem. Int. Ed. 2007, 46, 3526.

- (a) Wipf, P.; Wang, Z. Org. Lett. 2007, 9, 1605; (b) Wang, Z.; McPherson, P. A.; Raccor, B. S.; Balachandran, R.; Zhu, G.; Day, B. W.; Vogt, A.; Wipf, P. Chem. Biol. Drug Des. 2007, 70, 75; (c) Patterson, A. W.; Peltier, H. M.; Sasse, F.; Ellman, J. A. Chem. Eur. J. 2007, 13, 9534; (d) Raghavan, B.; Balasubramanian, R.; Steele, J. C.; Sackett, D. L.; Fecik, R. A. J. Med. Chem. 2008, 51, 1530–1533.
- 6. Misiti, D.; Zappia, G.; Delle Monache, G. Synthesis 1999, 873.
- 7. Hamel, E. Cell Biochem. Biophys. 2003, 38, 1.