Total Synthesis and Biological Evaluation of Potent Apratoxin S4 Based Anticancer Agents

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Introduction

Apratoxins are cytotoxic natural products originally isolated from marine cyanobacteria that act by preventing cotranslational translocation early in the secretory pathway to downregulate receptor levels and inhibit growth factor secretion, leading to potent antiproliferative activity. Through total synthesis of the apratoxin A/E hybrid apratoxin S4 (**1a**) we have previously improved the antitumor activity and tolerability in vivo and demonstrated the therapeutic potential of this mechanism of action.

Experimental

NMR spectra were recorded on a Varian Mercury 400 MHz, Bruker Avance III 600 MHz or Agilent VNMR 600 MHz spectrometers. 600 MHz spectrometers were equipped with a 5 mm TXI cryogenic probe.

Results and Discussion

Apratoxin S4 (**1a**) and newly designed analogues apratoxins S7-S9 (**1b-d**) with various degrees of methylation at C34 (**1b,c**) or epimeric configuration at C30 (**1d**) were efficiently synthesized utilizing improved procedures (Figure 1). Apratoxins S7 (**1b**) and S8 (**1c**) lack the C34 stereocenter and this reduction of structural complexity did not affect the mutidimensional assay profile, viz. antiproliferative activity against HCT116 colorectal cancer cell growth in vitro, downregulation of receptor tyrosine kinase MET and inhibition of VEGF-A secretion as an indicator of target engagement (Figure 2). Apratoxin S9 (**1d**) – the C30 epimer of apratoxin S4 (**1a**) – exhibited increased activity in all three assays with sub-nanomolar activity and represents the most potent compound of this class to date. Compounds **1a-d** showed excellent aqueous, plasma and cellular stability, but were rapidly metabolized by microsomes to uncharacterized biotransformation products that potentially retain activity. Dehydration is hypothesized to be a deactivation pathway of the apratoxins. Unlike other apratoxins, apratoxin S8 (**1c**) lacks the propensity to dehydrate. Compound **1c** showed efficacy in a dose-dependent manner using a HCT116 xenograft mouse model (Figure 3).

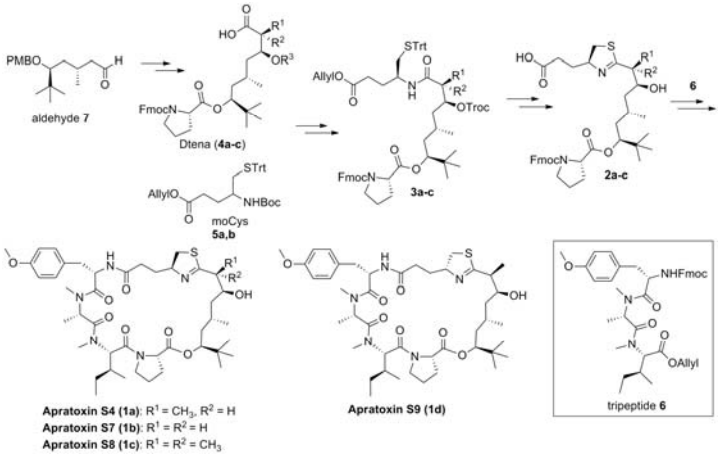
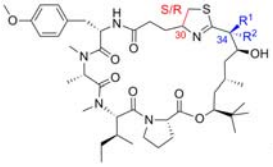


Figure 1. General synthetic route for apratoxin S4 and analogues.



Apratoxin	IC_{50} (nM) cell viability	IC_{50} (nM) VEGF-A secretion
S4 (30*S*,34-Me)	1.43	0.32
S7 (30*S*,34-H_2)	1.25	0.30
S8 (30*S*,34-Me_2)	1.99	0.47
S9 (30*R*,34-Me)	0.69	0.12

Figure 2. Activities of synthetic apratoxins on HCT116 cell viability and VEGF-A secretion.

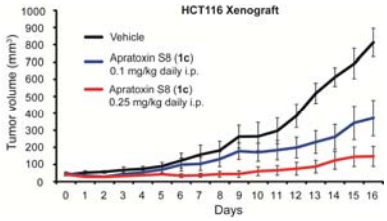


Figure 3. In vivo efficacy of apratoxin S8 (**1c**).

Conclusions

SAR studies demonstrated that **1b** and **1c** retain activity compared with **1a** and identified apratoxin S9 (**1d**) as the most potent apratoxin to date, exhibiting 2-3 fold improved in vitro activity. Overall our study further corroborates that the development of this class of anticancer agents is warranted.

Acknowledgements

Bankhead-Coley Cancer Research Program, grants 1BG07 and 4BB07; NIH, grant R01CA172310; and J.R. Rocca.

References

[1] Chen, Q.-Y., *et al.*, Journal of Medicinal Chemistry, **57**, 3011-3029 (2014).