Synthesis of Aza-Amanitins to Enhance Cytotoxicity for Targeted Cancer Therapeutics

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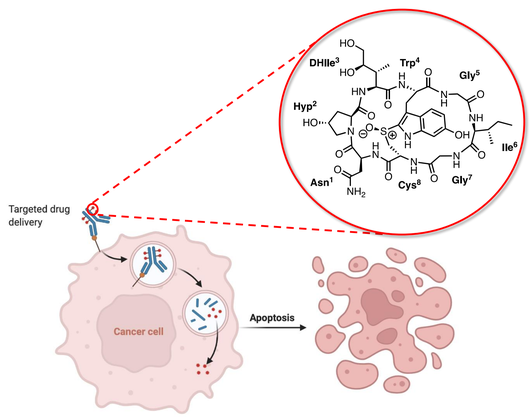
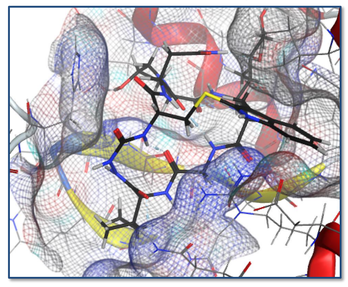
Abstract

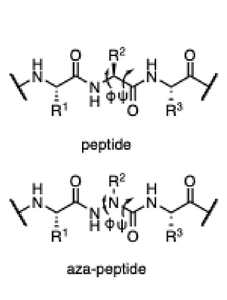
- The development of cancer therapies in which cancer-specific agents can be used to deliver a cytotoxic agent to cancer cells is at the forefront of drug research[1,2]
- There has been more research into antibody drug conjugates (ADCs) as a method of targeted cancer therapy[3]
- α-amanitin, a highly selective inhibitor of RNA polymerase II (Pol II), produced by the death-cap mushroom targets tumors in a cell-cycle independent manner[4]
- α-amanitin as a drug payload for ADCs enhances therapeutic potential and specificity

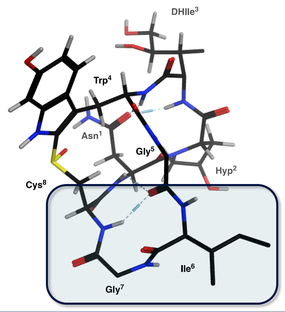


Introduction

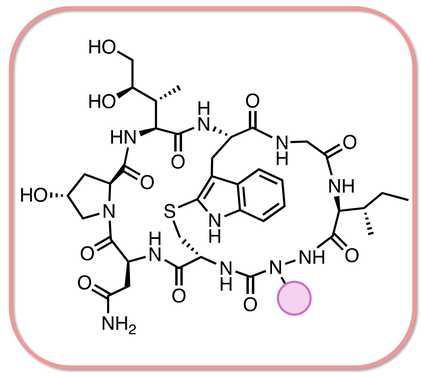
- Rationally designed amanitin analogs have been shown to influence cytotoxicity on various cell lines[5]
- Replacing glycine at position 7 with an aza-glycine amino acid results in increased cytotoxicity on CHO, HEK293 and HeLa cells
- May enhance aza-peptide stability to enzymatic and chemical degradation
- Constraints on the φ and Ψ dihedral angles → favor turn geometry
- α-amanitin contains a β - turn at Ile[6]







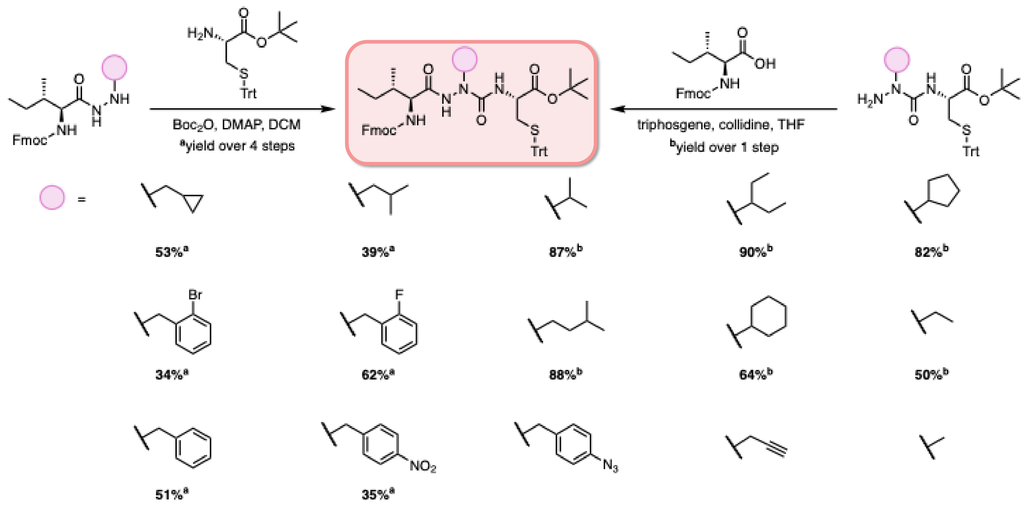
Objectives



- **Accessing aza-amino acids for amanitin analogs**
- **Probe the structure activity relationship**
- **Discover aza-amanitin analogs with enhanced cytotoxicity**

Synthesis

Aza-Amino Acid Substrate Scope



Synthesis of Aza-Amanitin Analogs

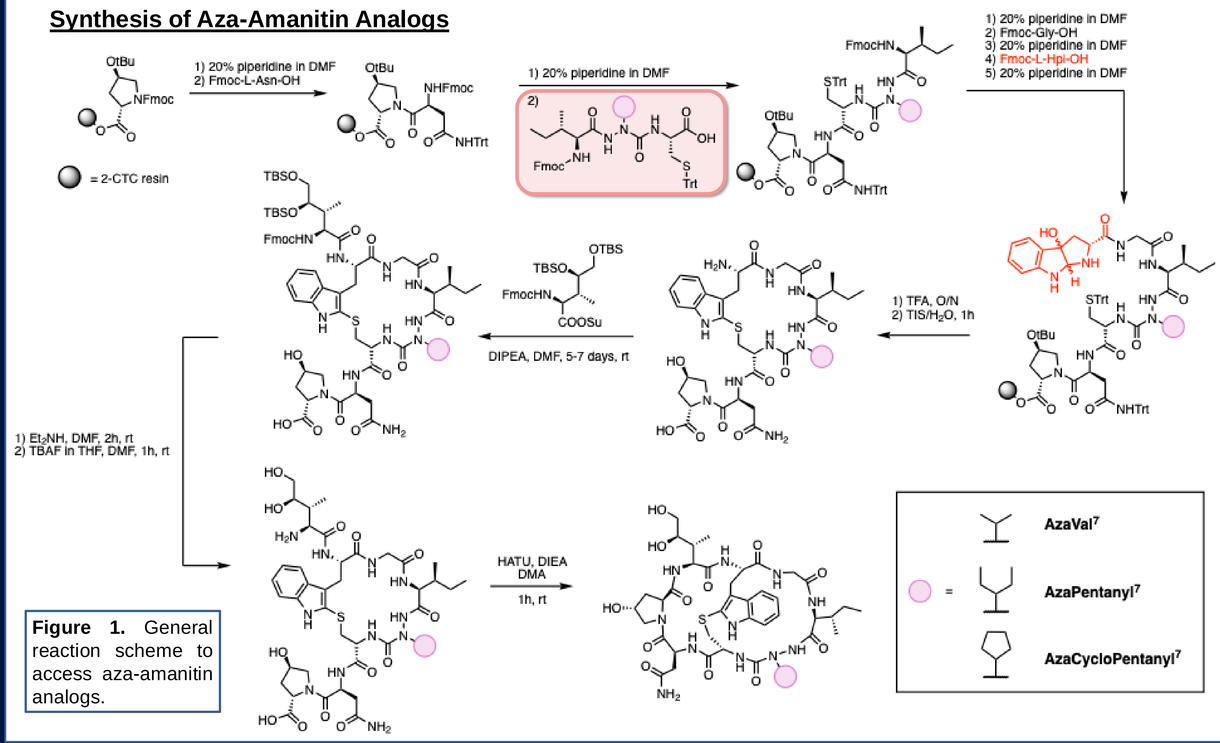
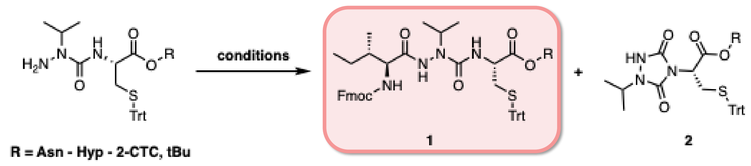


Figure 1. General reaction scheme to access aza-amanitin analogs.

Optimization

Optimization of Aza-Amino Acid Synthesis



Entry	Phase	Amino Acid	Conditions (coupling agent, base, solvent)	Percent Conversion (%)[d,e]	
				1	2
1	SPPS	Fmoc-Ile-OH[a]	Triphosgene (2.5 eq), 2,4,6-collidine (15 eq), THF	30	56
2	SPPS	Fmoc-Ile-OH[a]	DIC (7.5 eq), HOBt (7.5 eq), DIPEA (15 eq), DMF	5	35
3	SPPS	Fmoc-Ile-OH[a]	PyBOP (7.5 eq), DIPEA (15 eq), DMF	9	32
4	SPPS	Fmoc-Ile-OH[a]	HATU (7.5 eq), DIPEA (15 eq), DMF	31	32
5	SPPS	Fmoc-Ile-OH[a]	COMU (7.5 eq), DIPEA (15 eq), DMF	37	45
6	Solution	Fmoc-Ile-OSu[b]	2,4,6-collidine (3 eq), DMF	0	0
7	Solution	Fmoc-Ile-OH[b]	DCC (3 eq), DMAP (3 eq), DCM	0	0
8	Solution	Fmoc-Ile-OH[c]	Triphosgene (3 eq), 2,4,6-collidine (15 eq), THF	87[f]	0

[a] 7.5 eq; [b] 3 eq; [c] 9 eq; [d] Conversion determined by HPLC; [e] Remaining material was determined to be unconverted starting material; [f] Isolated yield

Cytotoxicity Assays

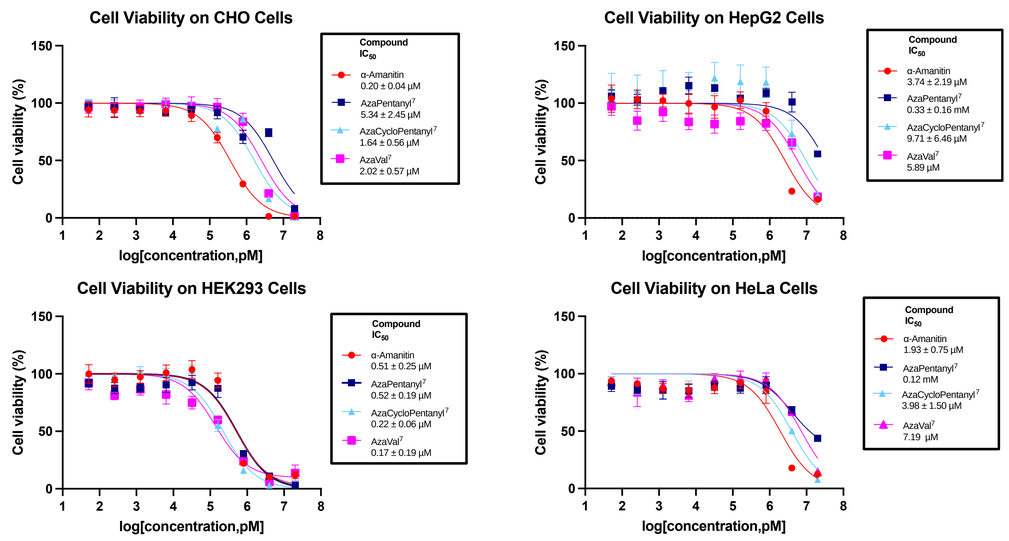
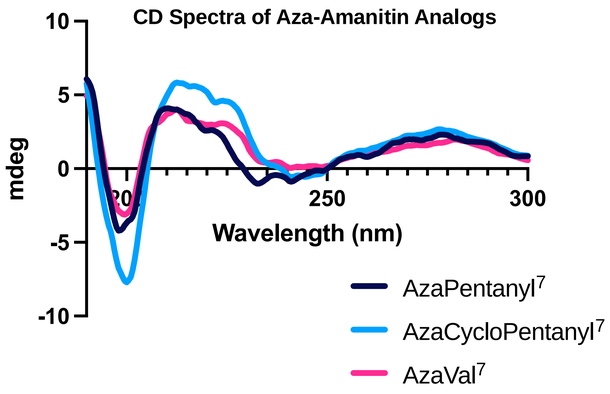


Figure 2. In vitro cytotoxicity of aza-amanitin analogs and α-amanitin. CHO, HepG2, HEK293, and HeLa cells were treated with various concentrations of toxins. Cell viability was determined by MTT assays.

Circular Dichroism



Conclusions & Future Directions

Conclusions

- Route to access aza-amino acids was determined
- New amanitin analogs were synthesized and cytotoxicity was evaluated
- AzaVal[7] and AzaCycloPentanyl[7] were more cytotoxic than natural α-amanitin in HEK293 cells
- AzaPentanyl[7] had comparable cytotoxicity in HEK293 cells

Future Directions

- Synthesis and biological assays of more aza-amanitin analogs to provide further insight into structure activity relationship

References

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Acknowledgments

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