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Structure–activity relationships of ustiloxin analogues

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Dedicated to Professor Harry Wasserman on the occasion of his 90th birthday.

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ABSTRACT

Four novel ustiloxin D analogues were synthesized focusing on the size of the macrocyclic core, the stereochemistry at the bridgehead ether, and the enantiomer of ustiloxin D. All four were subjected to biological evaluation testing the inhibition of tubulin polymerization. Only 2,2-dimethyl-ustiloxin D retained any activity.

1. Introduction

The ustiloxins A–F (Fig. 1) are a class of antimitotic natural products isolated from the water extracts of false smut balls, caused by the parasitic fungus *Ustilaginoidea virens*.[1] These compounds universally inhibit the assembly of the α,β-tubulin dimer into microtubules, thus resulting in mitotic arrest of eukaryotic cells. Many antimitotic agents are known, ranging from the vinca alkaloids and taxoids to chalcones,[2] but the mechanism of action of the naturally occurring peptides that inhibit tubulin polymerization is still not fully elucidated.[3]

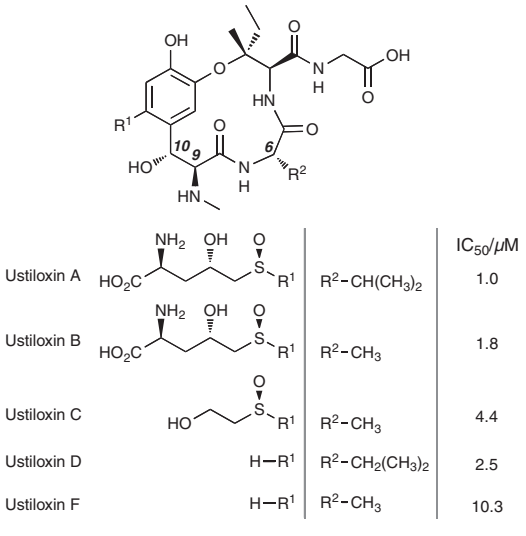

Figure 1. Ustiloxins A–F with IC_{50} values based on inhibition of porcine brain microtubule assembly.

Recent syntheses of ustiloxin D[4] and F[5] have been published, and the syntheses of eight analogues focusing on structural varia-

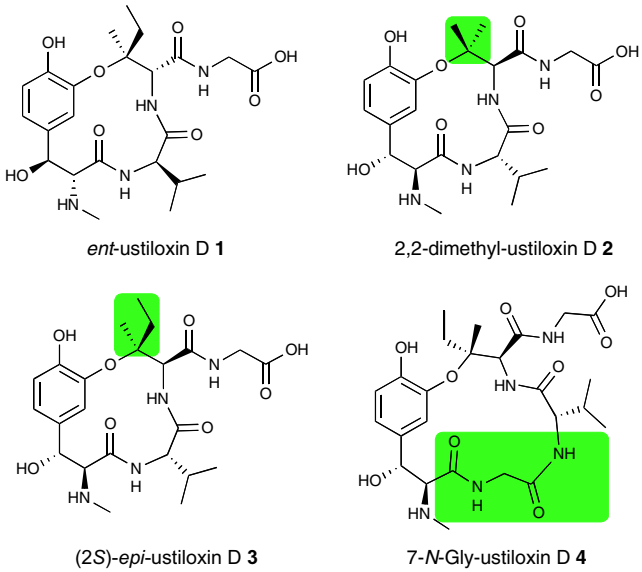

Figure 2. Four ustiloxin D analogues.

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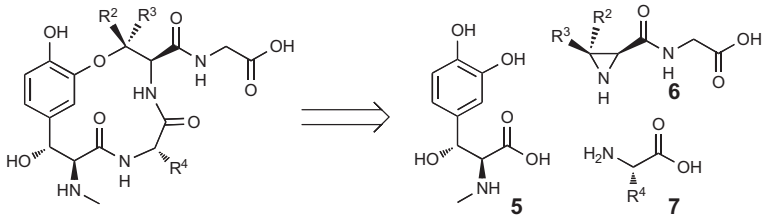


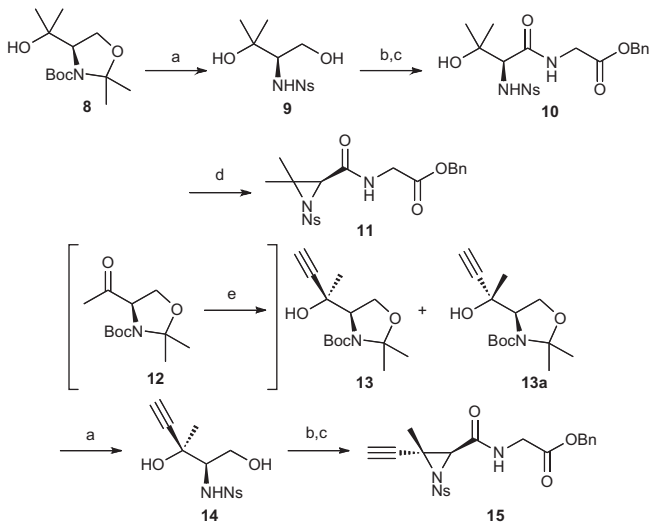
Figure 3. Retrosynthetic analysis of the ustiloxins.

tions at R^2, stereochemistry at C9 and C10, and methylation of the phenol were also reported by our group.[6] To complete the SAR analysis we wished to probe the stereochemistry and the substituents at the tertiary aryl-ether bridge, the size of the macrocyclic core, and the enantiomer of ustiloxin D. The four analogues are shown in Figure 2.

2. Synthesis of ustiloxin D analogues

The convergent synthesis of the ustiloxins developed in our laboratory allowed for expeditious synthesis of the four ustiloxin analogues. In a retrosynthetic manner, the ustiloxins can be divided into three constituent parts: β-hydroxytyrosine **5**, aziridine **6,** and commercially available amino acid **7** (Fig. 3).

The aziridine **6** and β-hydroxytyrosine moiety 5 can be quickly accessed from D-valine,[4e] and a (*S*)-salen-aluminium catalyzed aldol[7] reaction, respectively. This convergent route allows for rapid derivation of any of the three components.



Scheme 1. Synthesis of aziridines **11** and **15**. Reagents and conditions: (a) HCl, THF, 0 °C, 1 h, then NsCl, Na_2CO_3, THF/H_2O, rt, 16 h, 58% for **9**, 90% for **14**; (b) TEMPO, $NaClO_2$, NaClO, Na_2HPO_4, MeCN, 40 °C, 18 h; (c) H-Gly*O*Bn·HCl, EDCI, HOBt, $NaHCO_3$, DMF, 12 h, rt, 95% for **10**, 90% for alkynyl precursor; (d) DIAD, PPh_3, CH_2Cl_2, 18 h, rt, 77% for **11**, 68% for **15**; (e) ethynylMgBr, THF, rt, 80% ds = 11:1 (13a:13).

To access 2,2-dimethyl ustiloxin D, oxazolidine **8** was utilized to form the requisite dimethylaziridine **11** (Scheme 1).

Thus, the 2,2-dimethyl aziridine was synthesized by treatment of the tertiary alcohol **8** with aqueous HCl and subsequent *ortho*-nitrobenzenesulfonamide protection of the amine afforded diol **9**. TEMPO oxidation, followed by EDCI-mediated coupling of glycine benzyl ester gave the precursor to aziridine **10**, and a Mitsunobu reaction afforded the dimethyl aziridine **11** in good yield. The (2*S*,3*R*)-ethynyl aziridine **15** was synthesized by Grignard addition to ketone **12** which resulted in a 11:1 diastereomeric ratio; the minor diastereomer (**13**) being utilized within this route. The synthesis of **15** was continued supra vida to afford the second aziridine.

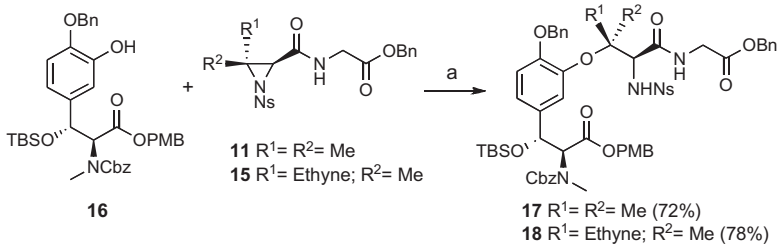
The requisite β-hydroxytyrosine moiety (**16**) was synthesized as previously reported[6] and coupled to aziridines **11** and **15** via a regioselective 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) mediated ring opening reaction developed in our laboratory,[8] to afford the nosyl protected amines **17** and **18** (Scheme 2).

The synthesis of *ent*-ustiloxin D followed the previous[6] synthesis of ustiloxin D and will not be reported in detail here. An (*R*)-salen-aluminium catalyzed aldol reaction afforded the β-hydroxytyrosine, L-serine provided the aziridine and D-valine was used to complete the protected linear precursor. The synthesis of 7-*N*-Gly-ustiloxin D (4) also followed the previous synthesis to give the nosyl protected amine **21** (Scheme 3).

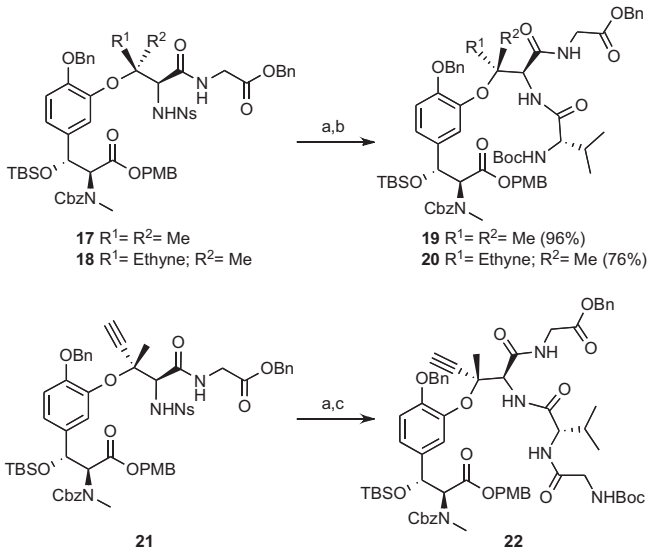
Removal of the nosyl protecting group on each of the three analogues with benzene thiol proceeded without incident to provide the free amine. EDCI-mediated coupling of tripeptides **17** and **18** with *N*-Boc-D-valine gave compounds **19** and **20**, respectively. Coupling of amine **21** with *N*-Boc-glycine-valine afforded **22** (Scheme 3).

With all three analogues and the *ent*-ustiloxin D protected linear precursor in hand (not shown), the synthesis was concluded with a deprotection/macrocyclization/deprotection sequence (Scheme 4).

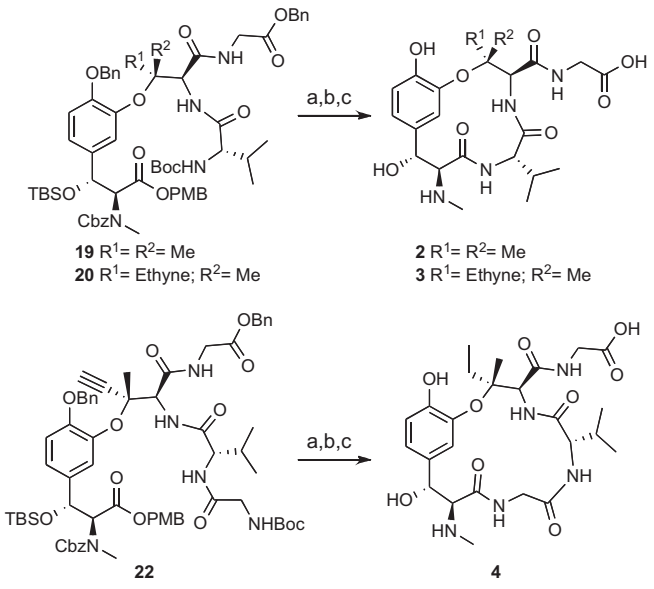
Treatment of the linear precursors with TFA/Et_3SiH afforded the TFA salts, which were subsequently converted into the hydrochloride salts. Subjection of each amino acid salt to EDCI-mediated macrocyclization and concomitant Pd/C catalyzed hydrogenolysis gave *ent*-ustiloxin D (**1**), 2,2-dimethyl ustiloxin D (**2**), (2*S*)-*epi*-ustiloxin D (**3**), and 7-*N*-Gly-ustiloxin D (**4**).



Scheme 2. Ring opening of aziridines **11** and **15**. Reagents and conditions: (a) TBD, toluene, 0 °C to rt, 3 h.



Scheme 3. Deprotection and coupling of amino acid residues. Reagents and conditions: (a) PhSH, Cs_2CO_3, DMF, 0 °C, 4 h; (b) *N*-Boc-Gly, EDCI, HOBt, $NaHCO_3$, DMF, 12 h, rt; (c) *N*-Boc-Val-Gly, EDCI, HOBt, $NaHCO_3$, DMF, 12 h, 0 °C to rt, 79%.



Scheme 4. Reagents and conditions: (a) TFA, Et_3SiH, CH_2Cl_2, 0 °C, 4 h; (b) EDCI, HOBt, $NaHCO_3$, DMF, 0 °C to rt, 24 h, 46% for $R^1 = R^2 = Me$, 30% for $R^1 = Me$ R^2 = ethyne, 34% for N-Gly macrocycle; (c) Pd/C, H_2, THF/H_2O, 24 h, 80% for **2**, 60% for **3**, and 55% for **4**.

3. Bioactivity

The effect of the changes present in the four analogues on the polymerization of purified tubulin was investigated using the IC_{50} value of ustiloxin D (2.5 μM) as the benchmark. Biological evaluation of *ent*-ustiloxin D (**1**) showed no inhibition with an IC_{50} value >40 μM. Similarly, 7-*N*-Gly-ustiloxin D (**4**) showed no inhibition (IC_{50} >40 μM), suggesting that the naturally occurring ustiloxin D macrocycle size is optimum. (2*S*)-*epi*-Ustiloxin D (**3**) exhibited a very slight inhibitory effect, but overall had an IC_{50} >40 μM. Interestingly, removal of the stereogenic carbon at the ether bridge afforded 2,2-dimethyl ustiloxin D (**2**) with an $IC_{50} = 9.2 \pm 2$ μM.

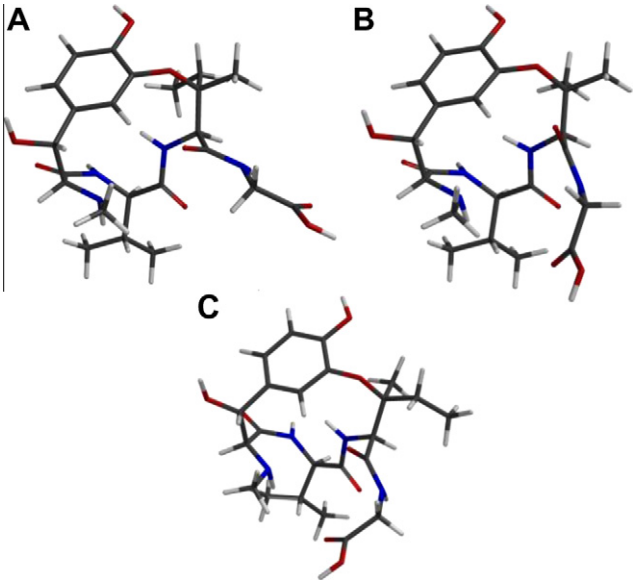


Figure 4. Spartan®'03 energy minimized molecular models at HF/AM1 level of theory. Ustiloxin D (**A**), 2,2-dimethyl-ustiloxin D (**B**), and (2*S*)-*epi*-ustiloxin D (**C**).

The difference in inhibitory activity of ustiloxin D, (2*S*)-*epi*, and the dimethyl species was investigated using molecular modeling (Fig. 4). Comparison of ustiloxin D (**A**) to 2,2-dimethyl ustiloxin (**B**) and (2*S*)-*epi*-ustiloxin (**C**) quite clearly shows distortion of the glycine sidechain, progressively pushing it toward the valine residue. The out-of-plane glycine sidechain may lessen the ability of the molecule to bind at this position, but it may also block the binding ability of the valine residue, which was shown to play an important role in the ustiloxins inhibitory effect.[6,9]

4. Conclusions

Novel ustiloxin analogues were synthesized to investigate the effect of changes at the C2 center, the macrocycle size, and the enantiomeric series on tubulin inhibition. The structural modifications produced significant changes in the biological activity suggesting the importance of the valine residue in the binding process.

Acknowledgments

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