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Insights into the myosin II inhibitory potency of A-ring-modified (*S*)-blebbistatin analogs

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ABSTRACT

Myosin II is an interesting target for therapeutic intervention, as it is involved in a large number of motility-based diseases. (*S*)-Blebbistatin is a known micromolar inhibitor of this protein. A new series of (*S*)-blebbistatin derivatives with a modified A-ring was synthesized and the myosin II inhibitory properties were evaluated *in vitro*. In this way, we gained insight into the influence of structural modifications in this part of the scaffold on myosin II inhibitory potency. Our results indicate there are few possibilities for potency enhancement *via* ring A modification of the blebbistatin scaffold.

(*S*)-Blebbistatin (*S*)-**1** (Fig. 1) is a well-known and widely used micromolar inhibitor of myosin II.[1] Given the multiple roles of this protein in a diverse range of motility-based diseases,[2–7] it is a promising target for therapeutic intervention. We have previously reported on the feasibility of improving (*S*)-blebbistatin's myosin II inhibitory potency and physicochemical properties.[8] We have also generated and analyzed SAR data on D-ring-modified analogs.[9] These studies have shown that D-ring modification enables fine-tuning of (*S*)-blebbistatin's physicochemical properties, but potency enhancement cannot be pursued in this manner.[8–12]

In the present report, our search for improved activity focused on the impact of structural changes in ring A. Analysis of the co-crystal structure of (*S*)-blebbistatin (*S*)-**1** bound to the metastable state of *Dictyostelium discoideum* myosin II (PDB: 1YV3)[15] shows that the residues in closest proximity to rings AB are Tyr261, Thr474, Tyr634, Gln637 and Leu641 (Fig. 2). Extending the (aromatic) ring system in this part of the molecule therefore has potential to improve binding affinity through additional hydrophobic interactions and π-π stacking with Tyr261. Lucas-Lopez et al. previously reported that small-sized substituents are of little influence at the C^5, C^6 and C^7 positions, but are undesired at the C^8 position.[13,14] In this study, we incorporated larger cyclic substituents fused at positions C^6 and C^7. Analog (*S*)-**2** was envisioned to

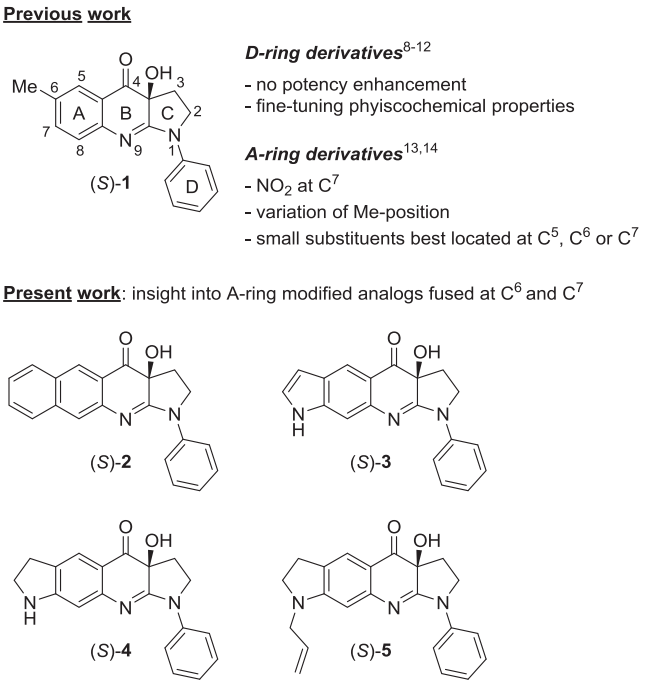

Fig. 1. State-of-the-art on SAR-information of the (*S*)-blebbistatin scaffold and present work.[8–14]

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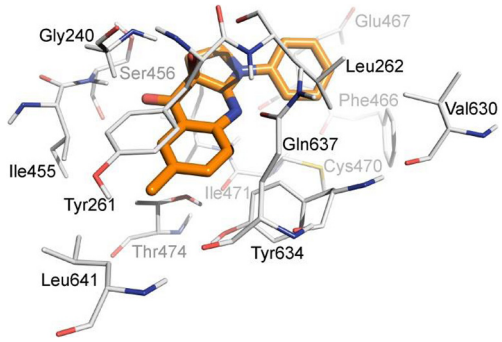
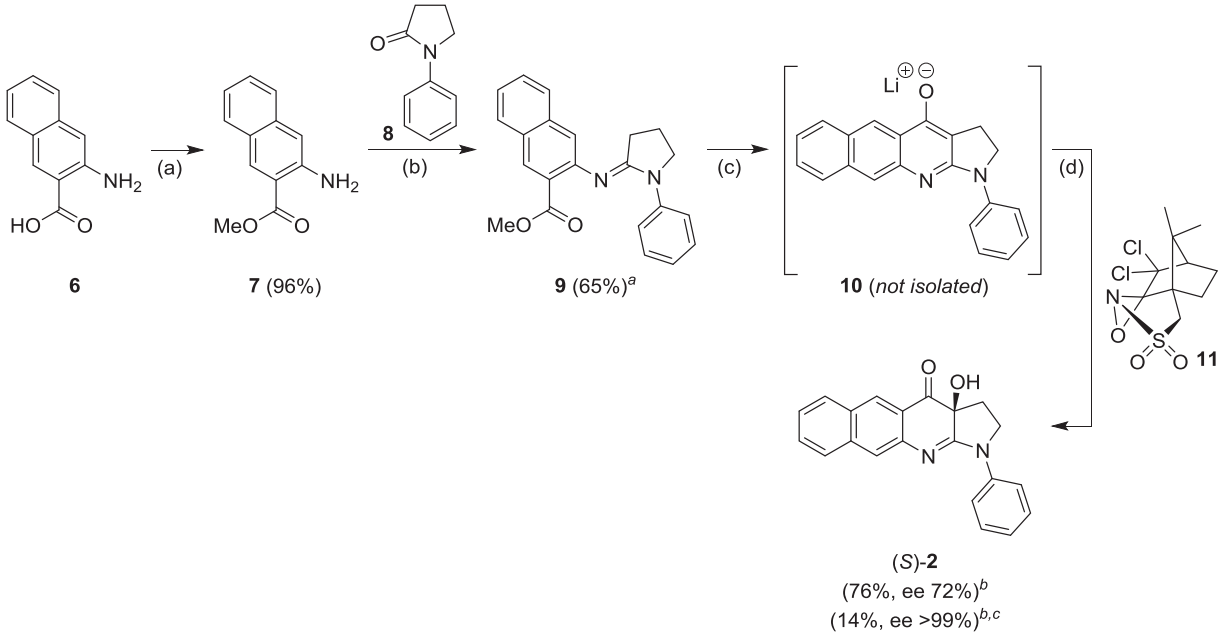


Fig. 2. Co-crystal structure of (*S*)-blebbistatin (*S*)-**1** bound to the metastable state of *Dictyostelium discoideum* myosin II (PDB: 1YV3)[15] indicates possible additional π-π stacking interactions with Tyr261 by extending the aromatic system in ring A of the scaffold.

accommodate π-π stacking with Tyr261. To overcome potential solubility issues associated with the latter compound, a more polar analog (*S*)-**3** was also prepared. Indoline (*S*)-**4** and *N*-allyl protected synthetic intermediate (*S*)-**5** were included as aliphatic counterparts.

(*S*)-Benzo[*h*]blebbistatin (*S*)-**2** was prepared *via* a route that was optimized previously by us.[8,9] The synthesis started from commercially available 3-amino-2-naphthoic acid (**6**) (Scheme 1), which was converted to methyl ester **7** upon treatment with sulfuric acid in MeOH (step (a), 96%). Reaction of pyrrolidinone **8** with $POCl_3$ and amine **7** resulted in a 75% conversion to amidine **9** and isolated yield of 65% (step (b)). Intramolecular ring closure of the latter compound was induced after deprotonation by LiHMDS (step (c)). Asymmetric α-hydroxylation of intermediate **10** using Davis' oxaziridine methodology yielded analog (*S*)-**2** (step (d), 76%, ee 72%). A single recrystallization from CH_3CN afforded enantiopure (*S*)-benzo[*h*]blebbistatin (*S*)-**2** (ee > 99%).

The synthesis of (*S*)-(*N*-allyl-2,3-dihydro-1*H*)-pyrrolo[3,2-*h*] blebbistatin (*S*)-**5**, (*S*)-(2,3-dihydro-1*H*)-pyrrolo[3,2-*h*]blebbistatin (*S*)-**4** and (*S*)-(1*H*)-pyrrolo[3,2-*h*]blebbistatin (*S*)-**3** required the preparation of precursor **16**, which was synthesized in an analogous way as described by Showalter et al. (Scheme 2).[16] In short, nitration of methyl 3-methyl-4-nitrobenzoate (**12**) resulted in a 85:15 mixture of methyl 5-methyl-2,4-dinitrobenzoate (**13**) and its isomer methyl 3-methyl-2,4-dinitrobenzoate. Isolation of compound **13** out of this mixture of isomers proved difficult. Efforts to purify it *via* normal phase and reversed phase automated flash chromatography both failed and initial recrystallization attempts in 2-propanol were not successful either. Recrystallization parameters (solvent volume, timing) appeared to be crucial and eventually we were able to isolate methyl 5-methyl-2,4-dinitrobenzoate (**13**) in 55% yield (step (a)). Condensation with reagent **14** gave enamine **15** (step (b)), which was reductively cyclized to precursor **16** (step (c), 81%) without intermediate isolation. Subsequent protection with allyl bromide yielded a crude mixture of unreacted starting material and mono- and diallylated regioisomers, out of which indole **17** was purified *via* reversed phase automated flash chromatography (step (d), 71%). Reduction with $NaCNBH_3$ in glacial acetic acid yielded indoline **18** (step (e), 92%). The synthesis of amidine **19** proved difficult due to the rather low nucleophilic propensity of aniline **18** (step (f), 48%). One-pot intramolecular cyclization (step (g)) and enantioselective hydroxylation (step



Scheme 1. Synthesis of (*S*)-benzo[*h*]blebbistatin (*S*)-**2**. Reagents and conditions: (a) H_2SO_4, MeOH, reflux, 48 h; (b) (1) **8**, $POCl_3$, CH_2Cl_2, rt, 24 h, (2) **7**, CH_2Cl_2, 35 °C, 48 h; (c) LiHMDS, THF, 0 °C, 1.5 h; (d) **11**, THF, −15 °C, 16 h. [a]The reaction mixture initially consisted of 25 mol% of **8** and 75 mol% of **9**. [b]Determination of ee *via* chiral HPLC analysis. [c]After recrystallization from CH_3CN.