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Research paper

Improved synthesis and comparative analysis of the tool properties of new and existing D-ring modified (*S*)-blebbistatin analogs

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ABSTRACT

(*S*)-Blebbistatin is a widely used research tool to study myosin II, an important regulator of many motility based diseases. Its potency is too low to be of clinical relevance, but identification of analogs with enhanced potency could deliver leads for targeted pharmacotherapeutics. This, however, requires a profound insight into the structure-activity relationship of the (*S*)-blebbistatin scaffold. Therefore, new D-ring modified (*S*)-blebbistatin derivatives were prepared to extend the existing small library of analogs. These molecules were obtained *via* an improved synthesis pathway and their myosin II inhibitory properties were evaluated *in vitro*. Finally, all new and known D-ring modified (*S*)-blebbistatin analogs were compared and the most potent ones underwent a screening of their physicochemical properties.

1. Introduction

Myosins are actin-based molecular motors of the cell that convert the energy released by ATP hydrolysis into force and movement in an ATPase cycle [1]. Myosin II is part of the complex cellular processes driving cell division and cell movement. In recent years, interest in the role of myosin II in aberrant cellular function has strongly grown [2]. It has been identified as an important regulator in motility-based diseases, e.g. viral infections [3–6], bacterial infections [7] and invasion in malignant disease [8–14]. The central role of myosin II in these diseases was revealed with the aid of the small-molecule research tool (*S*)-blebbistatin (*S*)-**1**. This molecule is the best known probe for myosin II: it is a micromolar, cell membrane permeable, uncompetitive inhibitor of the protein's ATPase activity [15]. (*S*)-Blebbistatin (*S*)-**1** is an important research tool, but it carries a number of physicochemical deficiencies (e.g. photosensitivity upon blue light irradiation, low water solubility and ensuing interference of precipitates in read-outs) and improved analogs have been proposed in this context [16–19]. Novel myosin II inhibitors with significantly enhanced potency could also have the potential to serve as leads for targeted pharmacotherapeutics. Thus, new (*S*)-blebbistatin derivatives are necessary to provide supplemental information to the hitherto established structure-activity relationships [16–21].

Analysis of the co-crystal structure of (*S*)-blebbistatin (*S*)-**1** bound to the metastable state of *Dictyostelium discoideum* myosin II (PDB: 1YV3) [22] indicated that space for extra (hydrophobic) substituents is present in the binding pocket surrounding ring D, in particular near the 3'- and 4'-positions (Fig. 1). We therefore investigated the tolerance of: (i) substituents of varying size in the 3'-position, ranging from allyloxy in analog (*S*)-**2** to diallylamino in compound (*S*)-**7**, (ii) ring fusion at the 3',4'-position, with a conformationally restrained monofunctionalized 3'-amino group in indoline (*S*)-**8**, a lipophilic naphthyl group incorporated in structure (*S*)-**9** and a more polar indole in analog (*S*)-**10** and (iii) functional groups of varying size in the 4'-position, i.e. hydroxy, allyloxy and benzyloxy (derivatives (*S*)-**11**–**13**) (Fig. 2).

Abbreviations: 2-mTHF, 2-methyltetrahydrofuran; A-B, apical-to-basolateral; B-A, basolateral-to-apical; CV, column volume; DMAP, 4-(dimethylamino)pyridine; DMEM, Dulbecco's modified Eagle medium; EGTA, ethylene glycol-bis(β-amino-ethyl ether)-*N,N,N',N'*-tetraacetic acid; GFP, green fluorescent protein; HBSS, Hank's balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MOE, molecular operating environment; N.D., not determined; P_{app}, apparent permeability; PBS, phosphate-buffered saline; s.d., standard deviation; $t_{1/2}$, photodegradation half-life; TBAB, tetra-*n*-butylammonium bromide.

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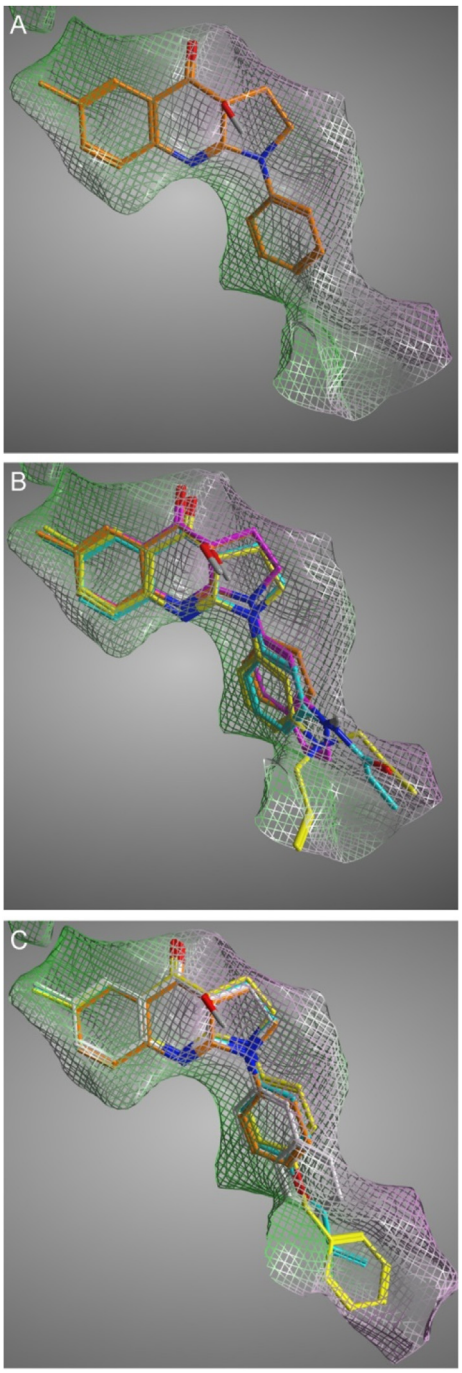

Fig. 1. (A) Binding mode of (*S*)-blebbistatin (*S*)-**1** (orange) with *Dictyostelium discoideum* myosin II (PDB structure: 1YV3) [22]. (B) Predicted binding poses of molecules (*S*)-**5** (cyan), (*S*)-**7** (yellow) and (*S*)-**10** (fuchsia). (C) Predicted binding poses of compounds (*S*)-**9** (white), (*S*)-**12** (cyan) and (*S*)-**13** (yellow). Interaction surfaces (onset of Van der Waals clash) are shown: pink indicates polar areas on the receptor surface, green indicates greasy areas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Results and discussion

2.1. Chemistry

The synthesis of (*S*)-3'-allyloxyblebbistatin (*S*)-**2**, (*S*)-3'-(diallylamino)blebbistatin (*S*)-**7**, (*S*)-3'-hydroxyblebbistatin (*S*)-**17** and (*S*)-3'-aminoblebbistatin (*S*)-**18** has been reported previously by us (Scheme 1) [19]. In that sequence, amidines **14a**–**c** underwent intramolecular cyclization upon treatment with LiHMDS, resulting in isolation of quinolones **15a**–**c**. α-Hydroxy ketones (*S*)-**1**, (*S*)-**2** and (*S*)-**7** were synthesized *via* deprotonation of quinolones **15a**–**c** using LiHMDS and Davis' oxaziridine methodology. Allyl deprotection of enantiopure (*S*)-3'-allyloxyblebbistatin (*S*)-**2** and (*S*)-3'-(diallylamino)blebbistatin (*S*)-**7** afforded (*S*)-3'-hydroxyblebbistatin (*S*)-**17** and (*S*)-3'-aminoblebbistatin (*S*)-**18**, respectively.

We have meanwhile developed a more convenient one-pot synthesis of α-hydroxy ketones from amidines, without isolation of the intermediate quinolones (Scheme 1). In this way, amidine **14a** was converted to α-hydroxy ketone (*S*)-**1** (step (c)) in higher yield (quant vs 83%) and enantiomeric excess (86% vs 75%) than the two-pot procedure. This is an important enhancement of the synthesis of the blebbistatin scaffold.

(*S*)-3'-Hydroxyblebbistatin (*S*)-**17** and (*S*)-3'-aminoblebbistatin (*S*)-**18** were used as starting points for the synthesis of derivatives (*S*)-**3**, (*S*)-**4**, (*S*)-**5** and (*S*)-**6** (Scheme 2). Selective esterification of phenol (*S*)-**17** (step (a)) was obtained by using stoichiometric amounts of Cs_2CO_3 and a small excess of acryloyl chloride or propionyl chloride, resulting in (*S*)-3'-acryloxyblebbistatin (*S*)-**3** (61%, ee >99%) and (*S*)-3'-propionyloxyblebbistatin (*S*)-**4** (99%, ee 99%), respectively. (*S*)-3'-Acrylamidoblebbistatin (*S*)-**5** (73%, ee 99%) and (*S*)-3'-propionylamidoblebbistatin (*S*)-**6** (91%, ee >99%) were prepared through a procedure adopted from Jahani et al. [23]. Selective monoamidation of aniline (*S*)-**18** (step (b)) was achieved using stoichiometric amounts of acrylic [24] or propionic anhydride and guanidine hydrochloride. In both sequences, reactions conducted with acryloyl reagents required chromatographic purification, thereby lowering the isolated yields.

The synthesis of (*S*)-benzo[*c'*]blebbistatin (*S*)-**9** was performed using 2-bromonaphthalene (**19**) as starting material (Scheme 3). CuI-catalyzed *N*-arylation of 2-pyrrolidinone (**20**) with tris(3,5-dimethyl-1*H*-pyrazol-1-yl)methane [25,26] and K_2CO_3 [27,28] as a ligand and base, respectively, only gave trace amounts of pyrrolidinone **21a**. In contrast, use of *N*,*N'*-dimethylethylenediamine and K_2CO_3 provided pyrrolidinone **21a** in excellent yield (step (a), 98%). Amidine **14d** was obtained in moderate yield (step (b), 48%), which was completely converted to α-hydroxy ketone (*S*)-**9** through our improved two-step one-pot procedure (step (c), 50%, ee 72%). Isolation of compound (*S*)-**9** by acid-base extraction proved more difficult than for (*S*)-**1** due to its more hydrophobic nature, resulting in a moderate yield. Recrystallization from CH_3CN afforded enantiopure (*S*)- benzo[*c'*]blebbistatin (*S*)-**9** (ee >99%).

Preparation of (*S*)-(2,3-dihydro-1*H*)-pyrrolo[2,3-*c'*]blebbistatin (*S*)-**8** and (*S*)-(1*H*)-pyrrolo[2,3-*c'*]blebbistatin (*S*)-**10** started from 6-bromoindole (**23**) (Scheme 4), which was selectively protected to yield allylated indole **24** (step (a), 99%) [29]. The feasibility to remove the allyl protecting group from the indole moiety was tested using a first generation Grubbs' carbene catalyst [30], but this attempt failed. As successful allyl deprotection of aliphatic amines had been reported previously by us [19], we opted to reduce allylated indole **24** to indoline **19b** (step (b), 80%) [29] and reoxidize it further down the line. Pyrrolidinone **21b** was synthesized from indoline **19b** with *N*,*N'*-dimethylethylenediamine and K_2CO_3 (step (c), 99%). Next, the amidine **14e** synthesis proved difficult (step (d), 42%) due to the electron withdrawing amino group (protonated under the reaction conditions used) present on the aryl amide. Subsequent one-pot intramolecular ring closure and enantioselective hydroxylation (step (e), 51%, ee 80%) afforded a mixture containing 86% of compound (*S*)-**25** and 14% of oxidation product (*S*)-**26**, which was not the result of oxidized impurities present in the starting material. The moderate yield was caused by the tough isolation *via* acid-base extraction. Recrystallization from CH_3CN