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Azobenzene-containing photoswitchable proteasome inhibitors with selective activity and cellular toxicity

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ABSTRACT

A series of azobenzene-containing peptidic boronate esters was prepared and the activity of the thermally adapted states (TAS), enriched in *trans* isomer, and the photostationary states (PSS), enriched in *cis* isomer, for each compound were evaluated against $\beta 5$ and $\beta 1$ proteasome subunits. Compounds with a sterically demanding phenyl-substituted azobenzene at P2 (**4c**), and a less sterically demanding unsubstituted azobenzene at the *N*-terminus (**5a**), showed the greatest difference in activity between the two states. In both cases, the more active *trans*-enriched TAS had activity comparable to bortezomib and delanzomib. Furthermore, *cis*-enriched **4c** inhibited tumor growth in both breast and colorectal carcinoma cell lines. Significantly, the initial *trans*-enriched TAS of **4c** was not cytotoxic against the non-malignant MCF-10A cells.

1. Introduction

The 26S proteasome is a supramolecular protein assembly that plays a pivotal role in the degradation of proteins that regulate the cell cycle.[1] Its over activity is associated with the development and progression of some cancers and as such it has recently been identified as an attractive target for anticancer drugs, particularly for the treatment of multiple myeloma (MM).[2] Its substrates are degraded at three sites located within the inner cavity of the component 20S proteasome, i.e., chymotrypsin-like ($\beta 5$), trypsin-like ($\beta 2$), and caspase-like ($\beta 1$) subunits.[3–6] The clear link between the proteasome and the development of a number of human diseases has encouraged the development of a range of inhibitors and an evaluation of their therapeutic potential.[7–15] In fact, the FDA has now approved three such inhibitors (bortezomib (**1**), carfilzomib and ixazomib) for the treatment of multiple myeloma and refractory mantle cell lymphoma, with others in development.[16–18] Several other proteasome inhibitors have entered clinical trials, including delanzomib (**2**) and oprozomib.[19] While delanzomib[20] is reported to overcome resistance reported for bortezomib *in vitro*,[21] it shows limited efficacy in the treatment of other types of cancer. It also displays severe side effects due to non-specific cytotoxicity towards healthy tissue (Fig. 1).[22,23]

There is a clear need to develop new proteasome inhibitors with improved safety and efficacy profiles. One approach described herein is to develop inhibitors that undergo specific activation at the site of action, e.g., through the action of light.[24] Light is ideally suited to control the activity of a pharmacophore as it can be delivered with very high spatiotemporal precision.[25] With this in mind, Feringa et al.[26] recently reported an analogue of bortezomib containing an *N*-terminal azobenzene, see structure **3** in Fig. 2. A photostationary state (PSS) of **3** enriched in the *cis* isomer proved to be moderately (two- to three-fold) more active than a thermally adapted state (TAS) enriched in the *trans* isomer, with the magnitude of difference showing some dependence on the nature of the component azobenzene. Related studies have been carried out on other proteases.[27–29] We now report studies on an extended series of photoswitchable proteasome inhibitors with a number of different azobenzenes at the *N*-terminus (see **5**) and at P2 (see **4**), to further investigate the effect of azobenzene substitution on activity and also to explore the S2 binding site[30,31] as an alternative site for modification. All compounds contain a boronate ester, rather than the boronic acid of bortezomib and delanzomib, with this prodrug presenting similar potency while being easier to prepare.[32,33] The P2 positioned azobenzene of **4** replaces the phenylalanine of bortezomib, where this site is known to accommodate larger groups.[5] The *N*-terminal azobenzene-based peptide

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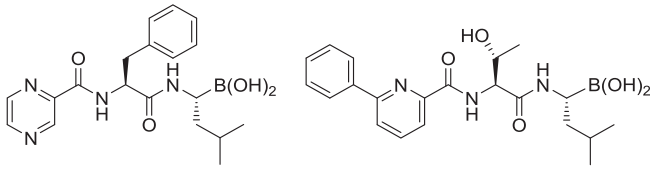

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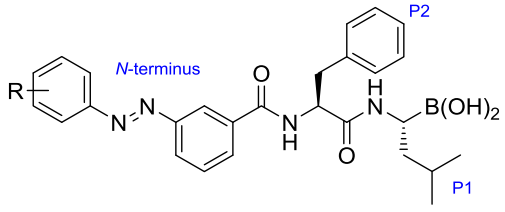
Fig. 1. Chemical structures of proteasome inhibitors bortezomib (**1**) and delanzomib (**2**).



3

R = Ph, *o*-Me, *m*-Me, *p*-Me, *p*-OMe and 2,6-Me

Fig. 2. Photoswitchable proteasome inhibitors **3**. Residues are designated P1, P2 etc from the boronic acid, where these groups interact with corresponding proteasomal specificity pockets according to the nomenclature of *Schechter and Berger*.34.

boronates **5** provide an opportunity to investigate the influence of an alternative backbone sequence with a threonine at P2 as found in delanzomib. Azobenzene substituents were chosen to investigate potential steric effects and to expand on earlier studies.[26] Synthetic *trans*-enriched azobenzenes **4** and **5** were photochemically isomerized to give an alternative PSS enriched in the *cis* isomer. All states were assayed against the *β5* and *β1* subunits of rabbit 20S proteasome (Fig. 3).

2. Results and discussion

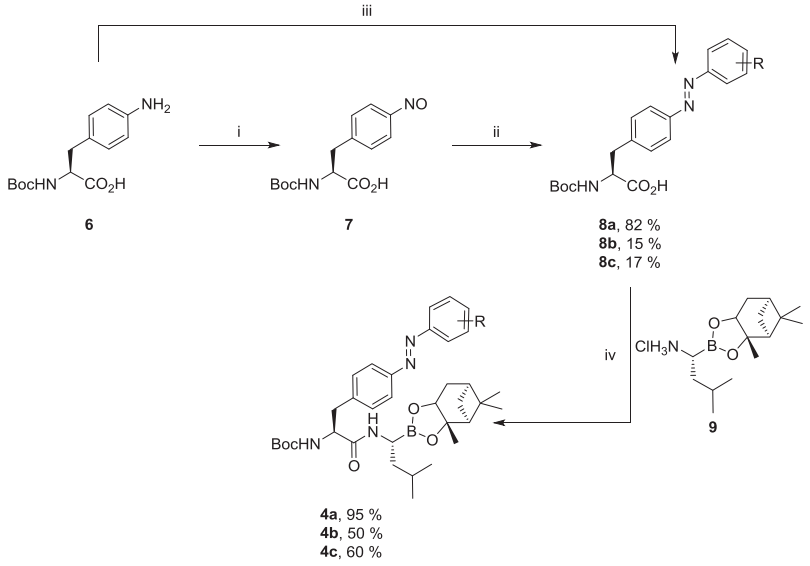
2.1. Chemistry

The bortezomib analogues **4a–c** were prepared as shown in Scheme 1. The key intermediates **8b** and **8c** were prepared by oxidation of the aniline **6** to the nitroso derivative **7** using Oxone® followed by condensation with the appropriate aniline. The other key intermediate **8a** was synthesized by condensation of **6** directly with commercially available nitrosobenzene. The proposed inhibitors **4a–c** were then prepared by coupling of **8a–c** with **9**[35,36] using *N,N,N′,N′*-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU) as the coupling agent. The alternative delanzomib derivatives **5a–c**, containing an *N*-terminal azobenzene, were synthesized as outlined in Scheme 2. The key azobenzenes **12b** and **12c** were prepared by oxidation of the aniline **10** to the nitroso **11**[37] using Oxone® followed by condensation with the corresponding aniline. The remaining key azobenzene **12a** was prepared by condensation of **10** directly with commercially available nitrosobenzene. TBTU mediated coupling of **12a–c** with **13**[38] then gave the desired boronate esters **5a–c** as shown.

2.2. Proteasome inhibitory activity

^{1}H NMR analysis of compounds **4–5** in DMSO-d_6 revealed an initial TAS strongly enriched in the *trans* isomer, with the results shown in Table 1a. A solution of each compound in DMSO-d_6 (~1 mg/mL) was then irradiated with UV light using a UVP BL6SV lamp (λ = 365 nm) for 1 h to give the corresponding *cis*-enriched PSS of **4–5**. ^{1}H NMR again defined the *trans/cis* compositions, with the results also shown in Table 1a. All compounds analysed after irradiation gave a PSS strongly enriched in the *cis* isomer (>76%), with the exception of **5a** with its relatively small *N*-terminal azobenzene (46% *cis*). Compound **5c**, with its sterically large *N*-terminal azobenzene, gave the highest enrichment of *cis* isomer (92%). In comparison, compounds with a less sterically demanding azobenzene substituent (as in **4a** and **5a**) give the highest isomer differential prior to UV irradiation, with the *trans* isomer being the major in this case.

Both states of **4** and **5** were then evaluated for inhibitory activity against *β5* and *β1* proteasome subunits and the results are shown in Table 1b. IC_{50} values were determined graphically according to Dixon methodology and as detailed in Supplementary data.[39] Potency data is also included in Table 1b for bortezomib and delanzomib for comparison. All azobenzene derivatives inhibited the *β5* and *β1* active sites, with IC_{50} values ranging from low nanomolar to micromolar. Compounds were generally more potent



Scheme 1. Synthesis of photoswitchable inhibitors **4**. Reagents and conditions: i) Oxone®, DCM:water, RT, 3 h; ii) $ArNH_2$, DMSO, HOAc, 60 °C, O/N; iii) Nitrosobenzene, HOAc, RT, O/N; iv) **9**, TBTU, DIPEA, DMF, 0 °C to RT, O/N.