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Preliminary communication

New aldehyde and vinylsulfone proteasome inhibitors for targeted melanoma therapy

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ABSTRACT

The proteasome is a promising target in cancer therapy. However, it is ubiquitous and its inhibitors cause side effects. To target melanoma cells we synthesized new peptide aldehyde and vinylsulfone inhibitors of the proteasome conjugated to the melanin-targeting ligand (MTL) derived from radiotracer [^{123}I]-*N*-(2-diethylaminoethyl)benzamide ([^{123}I]BZA) or [^{125}I]-*N*-(4-dipropylaminobutyl)-4-iodobenzamide ([^{125}I]BZ18). Influence on the cytotoxicity of the benzamide alkyl side chain length and the composition of the amino acid sequence was assessed. Among the conjugates evaluated, compound **16** and **22** presented the highest cytotoxicity (IC_{50}, 0.71 and 0.64 μM respectively), which persisted in the presence of an MTL derived from *N*-(dialkylaminoalkylenyl)benzamide residue.

1. Introduction

The proteasome is a multicatalytic complex that has five proteolytic activities (chymotrypsin-like, trypsin-like, post-glutamyl peptide hydrolyzing, branched-chain preferring activity and small neutral amino acids preferring activity) which gives it a critical role in several events such as inflammatory response, metabolic pathway regulation….[1–3] Proteasome inhibition is thus a target of interest in cancer therapeutic strategies. One proteasome inhibitor, the dipeptidyl boronic acid bortezomib (Velcade®, Fig. 1), is commercialized for its activity against several hematologic malignancies (particularly multiple myeloma) and solid tumors [4,5]. However, the proteasome is ubiquitous and its inhibitors cause side effects including fatigue, nausea, sensory neuropathy, diarrhea and thrombocytopenia [6]. To overcome this limitation, a strategy for targeting cancer tissue would be available [7] (Fig. 2).

A class of radiopharmaceuticals has been developed by Moreau et al. [6] for scintigraphic imaging of melanoma. These radiotracers e.g. [^{123}I]-*N*-(2-diethylaminoethyl)benzamide ([^{123}I]BZA, Fig. 1) share the same *N*-(dialkylaminoalkylenyl)benzamide residue, which binds specifically to melanin expressed in more than 92% of melanoma lesions [8–11]. Also, a lengthening of the alkyl side chain length of these radiotracers has been correlated with an enhanced and/or long-lasting tumor uptake: melanotic tumor retention of [^{125}I]-*N*-(4-dipropylaminobutyl)-4-iodobenzamide ([^{125}I]BZ18, Fig. 1), which has a longer alkyl chain than BZA, was observed with values of 3.2 ± 0.6% activity/g 72 h in B16 melanoma after injection in melanoma-bearing mice (versus 0.8 ± 0.3% activity/g for BZA) [9].

In previous works, we used these melanin-targeting ligands (MTLs) derived from radiotracer [^{123}I]BZA to target toward melanotic tumor proteasome inhibitors, such as peptide aldehyde inhibitors such as MG132 (Fig. 1), which represent a major members of this drug class [12]. Aldehyde inhibitors have the advantage of non-covalent binding with proteasome making its action rapidly reversible. The substitution of the benzyloxycarboxyl *N*-terminal protective group of MG132 by *N*-(2-diethylaminoethyl)

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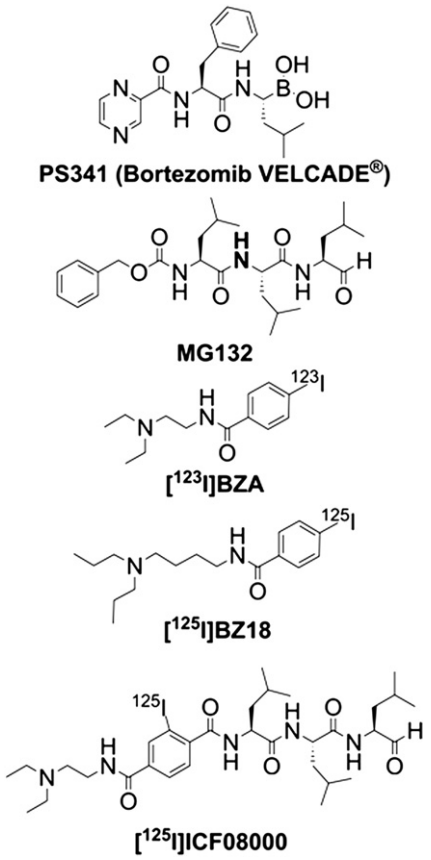


Fig. 1. Compounds discussed in the text.

benzamide residue as MTL does not affect cytotoxicity on human ocular melanoma IPC227F cells. Unfortunately, the aldehyde compounds tested are rapidly eliminated *in vivo* [13,14]. As an example, the drug biodistribution of [^{125}I]ICF08000 (Fig. 1) in a subcutaneous melanoma-bearing mice showed a radioactive accumulation of 2.7% of the injected activity/g in B16 tumors at 15 min, with 1.2% of radioactivity remaining in the tumor at 3 h and 6 h after administration, thus giving a tumor/blood ratio greater than or equal to 1.

Based on these results and with the aim of enhancing both tumor uptake and biological stability of these conjugates, structural modifications of the inhibiting function, peptide composition and melanin-targeting moieties were undertaken.

We also report on the synthesis and cytotoxicity assessment for IPC227F cells of these new proteasome inhibitor-MTL conjugates.

2. Chemistry

Firstly, we initiated two trileucine aldehyde inhibitors bearing long alkyl side chain of the vector. The melanin-targeting moieties

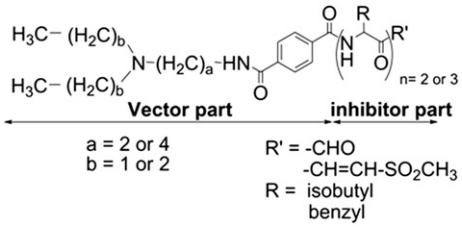


Fig. 2. Pharmacomodulated proteasome inhibitors.

were synthesized according to Schemes 1 and 2. Alkylation of the dipropylamine with chloroacetonitrile ($n = 2$) or γ-bromobutyronitrile ($n = 4$) followed by the nitrile reduction of compounds **4** and **5** with lithium aluminium hydride ($LiAlH_4$) gave amines **6** and **7** with excellent overall yields (88% and 78%, respectively) (Scheme 1).

Reaction of **6** and **7** with dimethyl terephthalate in the presence of trimethylaluminium afforded benzamides **9** and **10**, respectively with the concomitant formation of the disubstituted derivatives (not shown), which were easily separated by flash chromatography. Saponification of methyl esters **9** and **10** with lithium hydroxide gave compounds **11** and **12** in good yields (87% and 97% respectively, Scheme 2).

The coupling reaction between the lithium carboxylates **11** or **12** and amine **13**, which has the same peptidic sequence as MG132, were undertaken in the presence of DCC and HOBt as coupling reagents leading to the Weinreb amides **14** and **15** with moderate yields (34% and 17%, respectively). The Weinreb amides thus obtained were reduced at −80 °C with $LiAlH_4$ to give corresponding aldehydes **16** and **17** (Scheme 3).

Secondly, three vinylsulfone proteasome inhibitors were developed to enhance proteasome inhibition and cellular uptake. Vinylsulfones are less reactive than aldehydes and C-terminal leucine vinyl sulfone appears to be able to interact covalently with the proteasome catalytic threonine increasing the residence time of these compounds in cells compared with the aldehyde. A general synthetic pathway to obtain vinylsulfone peptides is presented in Scheme 4.

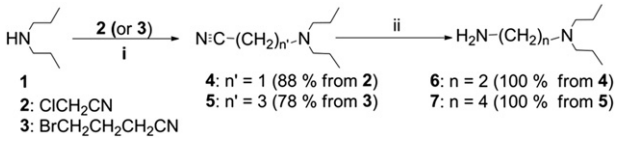
In the first step, commercially available diethyl methylsulfanylmethylphosphate **18** was oxidized in the presence of acetic acid and hydrogen peroxide to give sulfone **19** in acceptable yield (59%). The vinylsulfones were obtained by reaction of **19** with the previously formed aldehydes **20**, **22** and **24** [13] in the presence of NaH at −70 °C and in anhydrous tetrahydrofuran with yields ranging from 25% to 42%.

3. Pharmacology

The cytotoxicity of these compounds was tested *versus* MG132 control on human ocular melanoma IPC227F cells. IPC227F cells were cultured for 24 h on a microplate and then exposed for 48 h to increasing concentrations of the aldehydes **16**, **17**, **20**, **22** or **24** or vinylsulfone **21**, **23** or **25** derivatives. The effect of these compounds on cell growth (Table 1) was evaluated by assay with Hoechst dye 33342.

4. Results and discussion

We elected to modify the length of the alkyl side chain of the vector on trileucine aldehyde inhibitors (same peptidic sequence as reference MG132) to take advantage (i) of the potential melanotic tumor retention of these MTLs, and (ii) of the inhibitory function by vinylsulfone. Peptides possessing a vinylsulfone moiety represent another class of proteasome inhibitors: they covalently modify the catalytic β-subunits of proteasome, but are less reactive than aldehydes [12].



Scheme 1. reagents: (i) KI, K_2CO_3, 80-85 °C, CH_3CN; (ii) LiAlH4, -78 °C, ether.