NOVEL POTENTIAL PROTEASOME INHIBITORS BASED ON TRIPEPTIDE SCAFFOLD

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Abstract

This work is aimed to prepare new compounds (*O*-benzyl-5-chlorosalicyl-tripeptide aldehydes, epoxides and boronic acids) which should join to a large group of proteasome inhibitors (bortezomib (Velcade®), ixazomib (clinical trials phase I-II), delanozomib (clinical trials phase I-II), carfilzomib (Kyprolis®). Due to a very similar constitution of mentioned they are expected to bear very similar properties and activities as well. Inhibition of proteasome via blocking its protein recycling function is one of promising ways to treat tumor cells or multiple myeloma.

During the synthesis a partial racemization occurred and diasteroisomers were formed. To avoid the racemization, to get exact stereospecific synthesis and defined product are the aims, which are achieved by suitable adjustment of used reagents and their ratios. The details are discussed in the following contribution.

Prepared compounds are going to be tested as proteasomal and protein kinase inhibitors, for the type of caused apoptosis and antimicrobial activity.

Key words: *inhibitor; tripeptide; proteasome; bortezomib.*

Introduction

Proteasome

The proteasome is a protein complex and can be found in all eukaryotes and archea, and in some bacteria. The main function of the proteasome is to degrade unneeded, misfolded or damaged proteins by breaking the peptide bond. The process of degradation yields shorter peptides, which are further degraded into amino acids or reused in protein synthesis.

The tumor cell systems responsible for regulation of peptide synthesis are malfunctioning and the amount of misfolded proteins is quite high. That makes tumor cells very sensitive on proteasome working faultless. Inhibition of the main proteasome function leads to accumulation of these undesired proteins, which brings the cellular metabolism to the edge of collapse and death.

This theory is proved by many substances. The inhibition mechanism works both on covalent and noncovalent bonds. The functional moiety is covalently linked to oxygen atom of threonine, which works as the cleavage tool on active sites. The peptide scaffold is noncovalently linked to the walls of the core particle. [1, 2]

Inhibitors

Many important discoveries were made on the field of proteasome inhibitors in the last decade. Several new groups of inhibitors were discovered (aldehydes, boronates, epoxyketones, α-ketoaldehydes, vinyl sulfones or surbactines) and X-ray structures of all major classes were solved. From the most perspective compounds for example bortezomib (**I**, approved as Velcade®), ixazomib (**II**, MLN-9708, clinical trials: phase I-II), delanozomib (**III**, CEP-18770, clinical trials: phase I-II), marizomib (**IV**, clinical trials: phase I), carfilzomib (**V**, clinical trials: phase III, FDA filed as Kyprolis®), oprozomib (**VI**, ONX-0912, clinical trials: phase I) or

MG132 (**VII**) can be named. In additon carfilzomib and bortezomib are the most interesting because they are approved and they are already available for the public. For structures see **Fig. 1**. [4, 5, 6]

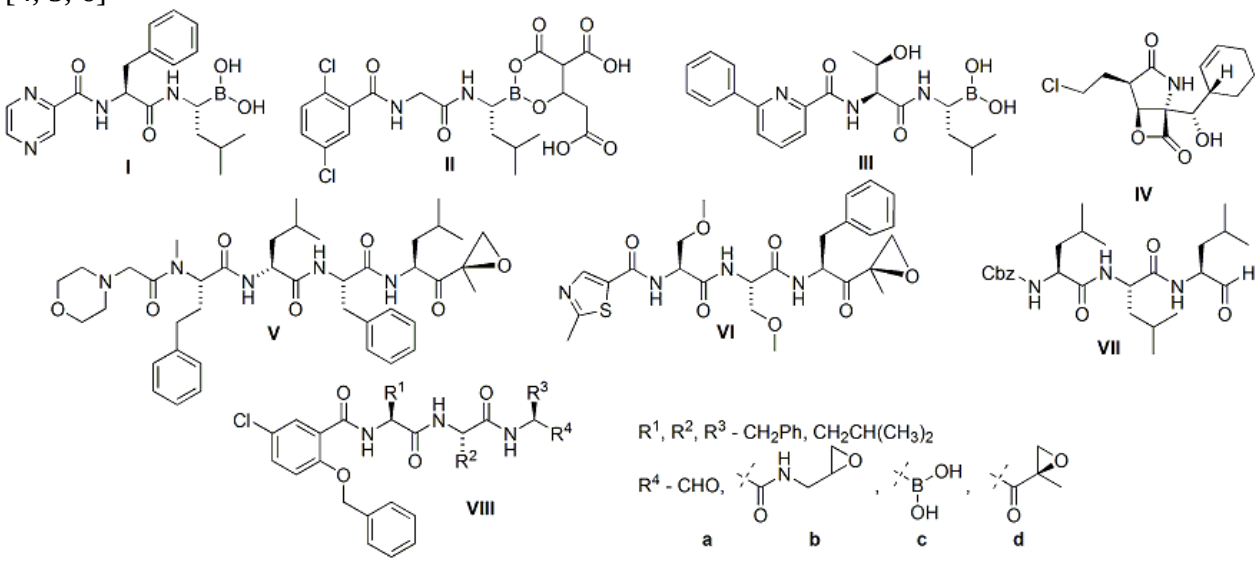
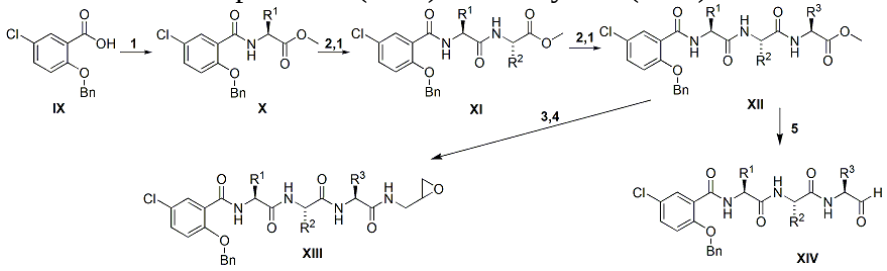


Fig. 1 Overview of promising (**I-VII**) and potential (**VIII**) proteasome inhibitors

The derivates (**VIII**) designed in my work are carrying very similar di-/tripeptide scaffold and aldehyde (**a**), epoxide (**b**,**d**) or boronic acid (**c**) functional group as compounds mentioned above and they should act similarly as proteasome inhibitors.

Experimental section

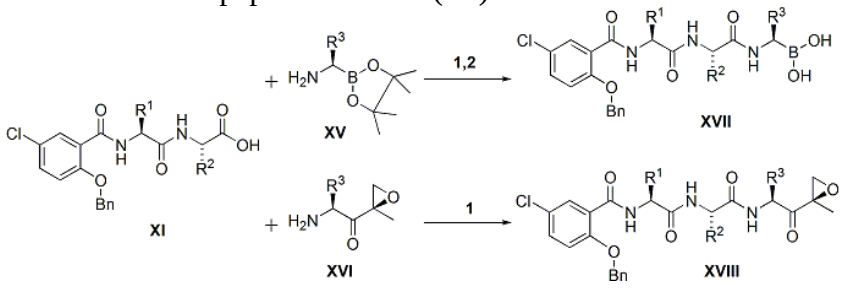
The multi-step synthesis (**Scheme 1**) begins with connection of *O*-benzyl-5-chloro-salicylic acid (**IX**) to methylester amino acid hydrochloride by forming amide bond in the presence of carbodiimides, acyltransfer agent and a base to liberate the amino group. In the next step the deprotection of the carboxylic acid via strong base cleavage is proceeded. This two steps are repeated twice until the tripeptide scaffold is completed. Afterwards the end of the tripeptide is further modified to form either epoxide **b** (**XIII**) or aldehyde **a** (**XIV**) derivate.



Scheme 1 Synthetic approach of aldehyde **a** and epoxide **b** derivates. **1** methylester amino acid or methylester amino acid hydrochloride, carbodiimide, HOBt, CH_2Cl_2, rt, 1 h; **2** LiOH, H_2O, 1,4-dioxane, rt, 24 h; **3** allylamine, carbodiimide, HOBt, CH_2Cl_2, rt, 1 h; **4** *m*-CPBA, CH_2Cl_2, 2 h at 0 °C, 72 h at rt; **5** DIBAL, THF, -78 °C, 20 h.

For boronic acid **c** and epoxide **d** derivates there is a slightly different synthetical approach more suitable. Functional groups **c** and **d** are more sensitive and difficult to prepare and the rest

of large molecule makes the common manipulation like column chromatography complicated. The functional groups are synthesized on the last amino acid separately and then the block (**XV**, **XVI**) is connected to the rest of the peptide scaffold (**XI**) as shown at **Scheme 2**.



Scheme 2 Synthetical approach for boronic acids **c** and epoxide **d** derivates. **1** carbodiimide, HOBt, CH_2Cl_2, rt, 1 h; **2** *i*$BuB(OH)_2$, hex, MeOH, 1M $HCl_{(aq)}$

After addition of the second amino acid the molecule (**XI**) became a diasteroisomeric and partial racemization was observed in ^{1}H NMR spectrum, while optically pure amino acids were used. The assumptions were made, that the racemization was directly caused by the type of carbodiimide, its equivalent ratio and the type of the basis. Long period of time was spent to adjust reagents ratios and various reaction conditions to improve the synthesis to work without any racemization and strictly stereoselective.

Results and discussion

The review work for the block synthesis (**XV**, **XVI**) is successfully done. The first experiments are currently ongoing. The main multi-step synthesis of peptide scaffold (**Scheme 1**) is not going to be carried out to the final compounds till the racemization issues are not sufficiently dealt with.

The first assumption was made that the racemization occurs due to used carbodiimide. While using DCC, the ratio of diasteroisomers was absolutely converted (**XI i**) in disadvantage for our desired product, so the reactions were then proceeded with using EDCI·HCl instead of DCC. The second assumption dealt with the type of used base. Despite the knowledge about TEA causing racemization both TEA and DIPEA were used as amidation agents to compare of the results. Surprisingly, worse ee [%] was observed in using "anti-racemization agent" DIPEA. Regardless to the type of base the racemization was still present. Methylester amino acid was used instead of methylester amino acid hydrochloride. There was no need to liberate the amino group. The third assumption was about possibility of the excess EDCI·HCl which may cause the racemization. The amount was reduced from 1,4 to 0,95 molar equivalent.

With all these assumptions the final and the most suitable conditions for amidation was determined to EDCI·HCl (0,95 mol. eq.), HOBt (1 eq.) CH_2Cl_2, rt, 1 h. For results see **Table 1.**

Table 1 Overview of achieved results

Compound	Carbodiimide Equivalent	Base Equivalent	Ratio of diasteroisomers	ee [%]
XI i	DCC, 1,1	DMAP,catalyst amount	40:60	-20
XI ii	EDCI·HCl, 1,4	TEA, 1	90:10	+80
XI iii	EDCI·HCl, 1,4	DIPEA,1	78:22	+56
XI iiii	EDCI·HCl, 0,95	-	96:4	+92

Conclusion

The set of reagent types and ratios was found yielded the product with 92 % ee, which is significantly better than any other results we obtained. There is still place for improvement. Reactions are going to be repeated to get as good results as possible. The final set of reagent types and ratios (EDCI·HCl (0,95 mol. eq.), HOBt (1 eq.) CH_2Cl_2, rt, 1 h) is going to be incorporated in the general procedure and the synthesis of the final di-/tripeptide aldehyde, epoxide and boronic acid derivates will continue.

Acknowledgement

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Table 2 List of Abbreviations

Bn	benzyl
*i*BuB(OH)$_2$	isobutylboronic acid
Cbz	carboxybenzoyl
m-CPBA	*meta*-chloroperoxybenzoic acid
DDC	dicyclohexylcarbodiimide
DIBAL	diisobutylaluminium hydride
DIPEA	diisopropylethylamine
EDCI·HCl	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ee	enantiomeric excess [%]
hex	hexane
HOBt	1-hydroxybenzotriazole
MeOH	methanol
rt	room temperature, 25 °C
TEA	triethylamine