SYNTHESIS OF NOVEL POTENTIAL PROTEASOME INHIBITORS BASED ON TRIPEPTIDE BACKBONE

Jan Dušek[1], Aleš Imramovský[1], Radek Jorda[2,3], Eva Řezníčková[2], Vladimír Kryštof[2], Karel Pauk[1]

[1]*Institute of Organic Chemistry and Technology, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 532 10 Pardubice, Czech Republic, jan.dusek87@gmail.com, ales.imramovsky@upce.cz, karel.pauk@seznam.cz*

[2] *Laboratory of Growth Regulators, Faculty of Science, Palacký University & Institute of Experimental Botany, Šlechtitelù 11, 78371 Olomouc, Czech Republic*

[3] *Regional Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute, Žlutý kopec 7, 656 53,Brno, Czech Republic*

ABSDTRACT

The inhibition of proteasome via blocking his protein recycling function is one of promising ways to treat tumor cells or multiple myeloma. In the present days a series of compounds like MG132, marizomib, CEP-18770, MLN-9708 or ONX-0912 are clinically used or in clinical trials and some of them like carfilzomib or bortezomib are already approved and available for the public. *O*-Benzyl-5-chlorsalicyl-tripeptide aldehydes and oxiranes are goind to be tested due their very similar constitution suitable for the same purpose as above mentioned compounds.

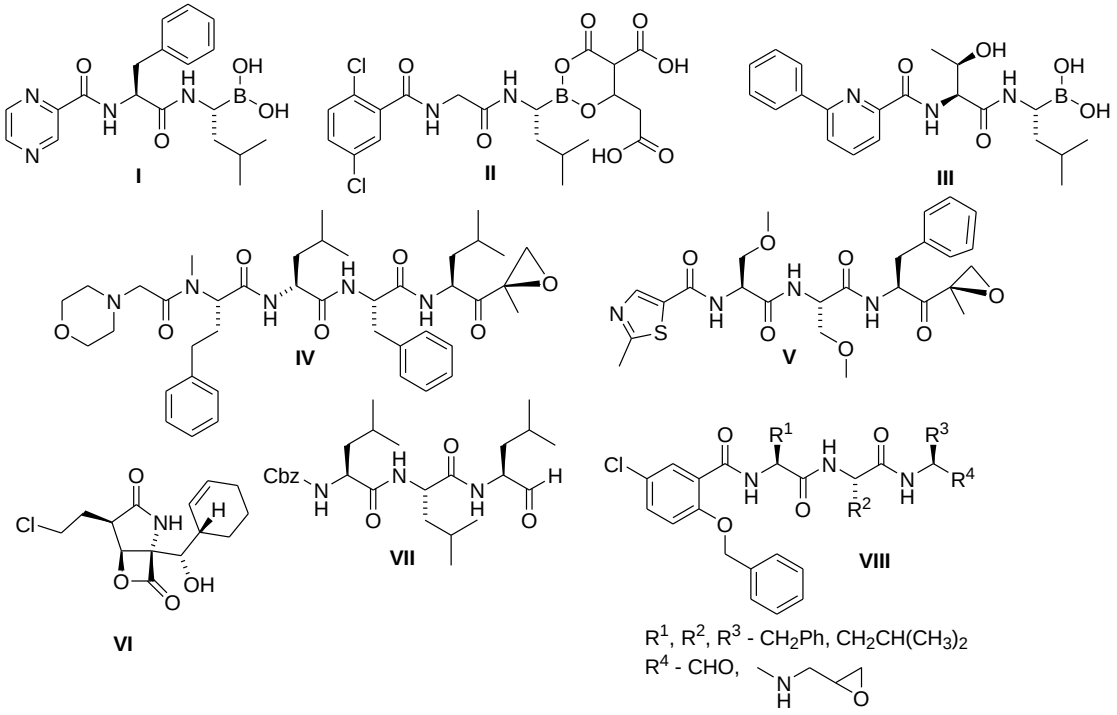
KEY WORDS

proteasome, inhibitors, tripeptide, bortezomib, carflizomib, MG132, CEP-18770, carbodiimides, DCC, EDCI·HCl

INTRODUCTION

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. Tumor cells are damaged and not all of their regulatory functions are working well. Especially the systems responsible for regulation of peptide synthesis are malfunctioning and the amount of misfolded proteins is quite high. 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.

Many discoveries have been made on the field of proteasome inhibitors in the last decade. Several new groups of inhibitors have been discovered (aldehydes, boronates, epoxyketones, α-ketoaldehydes, vinyl sulfones or surbactines) and X-ray structures of all major classes have been solved. From the most perspective compounds we can name 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), carfilzomib (**IV**, clinical trials: phase III, FDA filed as Kyprolis®), oprozomib (**V**, ONX-0912, clinical trials: phase I), marizomib (**VI**, clinical trials: phase I) or MG132 (**VII**). For structures see **Scheme 1**.

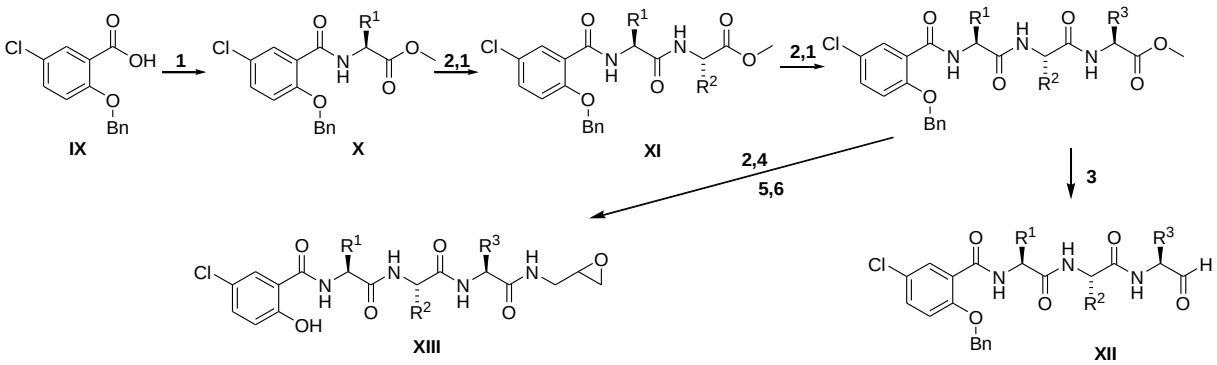


Scheme 1: Overview of promising (**I-VII**) and potential (**VIII**) proteasome inhibitors

The derivates designed by our scientific group are sharing very similar di-/tripeptide backbone and aldehyde or oxirane functional group as above mentioned compounds and should work also as covalently bounded proteasome inhibitors.

EXPERIMENTS

The multiple-step synthesis (**Scheme 2**) 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 step procedure is repeated twice since the tripeptide backbone is completed. Afterwards the end of the tripeptide is further modified to form either aldehyde (**XII**) or oxirane (**XIII**) derivate.



Scheme 2: Synthetical approach design. **1** amidation in the presence of carbodiimide; **2** deprotection of the carboxylic group by a strong base cleavage; **3** reduction; **5** deprotection of the phenolic hydroxyl group; **6** amidation with allylamine in the presence of carbodiimide; **7** oxidation of the double bond.

After joining the second amino acid the molecule (**XI**) became a diasteroisomeric and partial racemization was observed in ^{1}H NMR spectrum, although only optically pure amino acids were used. The assumptions were made, that the racemization is directly caused by the type of carbodiimide, its equivalent ratio and the type of the basis. Large amount of time and effort was spent to adjust reagents ratios and various reaction conditions that way so the synthesis runs without any racemization and strictly stereoselective.

RESULTS AND DISCUSSION

The multi-step synthesis was not 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 the used carbodiimide. While using DCC, the ratio of diasteroisomers was absolutely converted (**XI a**) 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 to cause racemization we carried on by using it during formation of amide bond. To compare the results we also used DIPEA according to the advices from elder and more experienced colleagues. Surprisingly, worse ee [%] was observed at products prepared by using "anti-racemization agent" DIPEA. The outcome was that, regardless to the type of base we used, the racemization is still present. So we totally removed the need of base to be present in the reaction mixture by using different reagent. We used methylester amino acid instead of methylester amino acid hydrochloride. There was no need to liberate the amino group.

The third assumption was about possibility the excess of EDCI·HCl may causethe racemization. The amount was reduced from 1,4 to 0,95 molar equivalent.

For results see **Table 1.**

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

Table 1: Overview of achived results

CONCLUSIONS

The set of reagent types and ratios was found to yield the product with 92 % ee, which is significantly better than any other result we obtained. Still there is place for improvement. Reactions are going to be repeated to get as good results as possible. The final set of reagent types and ratios is going to be incorporated in the general procedure and the synthesis of the final tripeptide aldehydes and oxiranes will continue.

ACKNOWLEDGEMENTS

We wish to thank to the Students Grant Projects of the Faculty of Chemical Technology, University of Pardubice (SG FCHT 002) for the financial support.

REFERENCES

[1] Kisselev, A. F.; van der Linden, W. A.; Overkleeft, H. S.: *Chem. Biol.* **2012**, *19*, 99-115.

[2] Berkers, C. R.; Leestemaker, Y.; Schruurman, K. G.; Ruggeri, B.; Jones-Bolin, S.; Williams, M.; Ovaa, H.: Mol. Pharmaceutics **2012**, *9*, 1126-1135.

[3] Frankland-Searby, S.; Bhaumik, R. S.: *Biochim. Biophys. Acta* **2012**, *1825*, 64–76.

[4] Wua, W. K. K.; Cho, C. H.; Lee, C. W.; Wud, K.; Fan, D; Yu, J.; Sung, J. J. Y.: *Cancer Lett.* **2010**, *293*, 15–22.