PREPARATION OF PSEUDOPEPTIDE ALDEHYDES AND VINYL SULFONES AS A POTENCIONAL PROTEASOME INHIBITORS

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Introduction:

Proteasome is 2,5 MDa large multi-subunit complex responsible for the protein degradation in cells. Inhibition of this process is one of strategies for suppression of uncontrolled proliferation of multiple myeloma or leukemia cancer cell lines.[1]

Proteasome regulates the number of important cellular processes either directly or indirectly. Changing this regulation can lead to the development of cancer. Therefore it concentrates precisely on proteasomes especially the proteasome 26S and its proteolytically active sites. Proteasome 26S consists two parts—the control unit (19S) and the core (20S), where the core contains 28 subunits which are composed of 4 circles (2 outer and 2 internal). β circles contains threonines proteases namely β1 unit (caspase), β2 unit (trypsin) and β5 unit (chymotrypsin).[2]

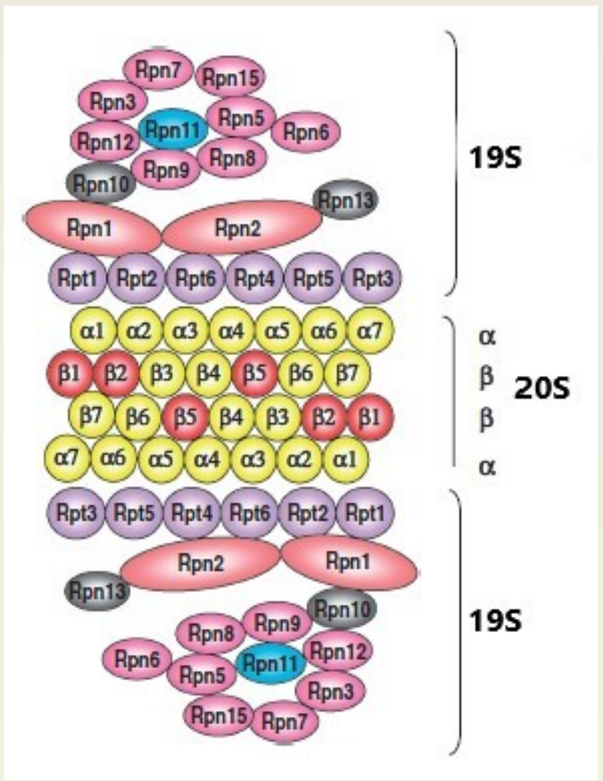

Figure 1: 26S proteasome [1]

Our research team:

Recently and outgoing research:

Our research group developed *O*-benzyl salicylamide-based proteasome inhibitors built from *L*-leucine and *L*-phenylalanine.[3,4] These compounds contain various functional groups such as aldehydes or weinreb amides or epoxyketones at the C-end of molecule. Our ongoing research investigated other functional groups with higher stability than aldehyde group. We also investigated influence of amino acid sequence in peptide chain on the inhibition activity.

To follow our studies we synthetized and fully characterized a new series of *O*-benzylsalicylamides having tripeptide chain containing optically pure aminoacids (*L*-Leu, *L*-Trp, *L*-NLeu, *L*-CHA, *L*-Pro, *L*-Phe) and terminated with aldehyde or vinyl sulfone warhead. All novel salicylamides were tested for their antiproliferative properties against various cancer cell lines derived from hematopoietic malignancies and the resulting data will be presented.

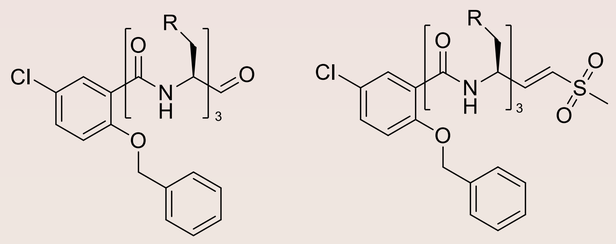

Fig.1: prepared compounds with aldehyde or vinyl sulfone functional group

Antiproteasomal activities of prepared salicylamides :

	Integrated amino acids			Warhead	MCF-7 cell line			MCF-7	U2OS-PI-GFP
					Caspase (β1)	Trypsin (β2)	Chymotrypsin (β5)	Chymotrypsin	
					20 μM			IC_{50} (μM)	c (μM)
	R_1	R_2	R_3		% of ctrl± SD	% of ctrl± SD	% of ctrl± SD	data±SD	data
6a	L-Leu	L-Leu	L-Leu	aldehyde	45.2±1.0	91.1±1.0	10.0±0.1	0.37±0.03	1.6
6b	L-Leu	L-Leu	L-Trp	aldehyde	63.5±0.1	94.4 ±1.4	11.1±0.9	2.22±0.06	>5
6c	L-Leu	L-Leu	L-ChA	aldehyde	82.7±1.1	≥100	35.8±1.7	7.58±0.08	>5
6d	L-Leu	L-Leu	L-NorL	aldehyde	86.5±1.3	≥100	16.2±0.3	3.27±0.07	>5
6e	L-Leu	L-Leu	L-Pro	aldehyde	95.4±3.8	96.6±6.8	≥100	>20	>5
6f	L-Leu	L-Leu	L-Phe	aldehyde	≥100	≥100	30.8±1.3	6.22±0.60	>5
6g	L-Leu	L-Trp	L-Leu	aldehyde	26.2±1.4	87.6±1.5	7.0±0.2	0.61±0.02	1.6
6h	L-Leu	L-ChA	L-Leu	aldehyde	48.3±1.1	87.6±2.4	8.7±0.1	2.34±0.00	3.2
6i	L-Leu	L-NorL	L-Leu	aldehyde	40.0±0.7	96.1±1.6	7.3±0.0	0.27±0.02	1.6
6j	L-Leu	L-Pro	L-Leu	aldehyde	≥100	≥100	90.6±4.3	>20	>5
6k	L-Phe	L-Leu	L-Leu	aldehyde	75.4±3.6	≥100	9.4±0.1	1.75±0.01	4
12a	L-Leu	L-Leu	L-Leu	vinylsulfone	76.7±1.3	≥100	11.2±0.1	2.27±0.10	2.5
12b	L-Leu	L-Leu	L-Trp	vinylsulfone	54.8±0.2	88.1± 1.6	7.9±0.2	1.77±0.33	3.2
12c	L-Leu	L-Leu	L-ChA	vinylsulfone	74.9±2.3	96.2± 0.4	69.6±2.7	>20	>5
12d	L-Leu	L-Leu	L-NorL	vinylsulfone	70.2±3.4	≥100	11.2±0.5	3.97±0.14	4
12e	L-Leu	L-Leu	L-Pro	vinylsulfone	≥100	≥100	94.8±0.1	>20	>5
12f	L-Leu	L-Leu	L-Phe	vinylsulfone	52.5±0.8	89.5±0.4	5.9±0.3	1.71±0.01	2.5
12g	L-Leu	L-Trp	L-Leu	vinylsulfone	73.9±0.3	95.7±1.9	19.0±0.3	5.31±0.30	5
12h	L-Leu	L-ChA	L-Leu	vinylsulfone	75.8±2.2	93.8±4.3	44.2±1.3	5.58±0.57	>5
12i	L-Leu	L-NorL	L-Leu	vinylsulfone	63.1±1.0	89.1±0.7	8.7±0.2	3.26±0.07	3.2
12j	L-Leu	L-Pro	L-Leu	vinylsulfone	≥100	≥100	93.0±1.2	>20	>5
12k	L-Phe	L-Leu	L-Leu	vinylsulfone	77.5±0.9	≥100	21.0±0.5	3.38±0.03	5
bortezomib 1000 nM					27.5±6.5	85.9±2.0	7.3±1.2	0.008±0.00	n.t.

Table 1: Results of all 22 compounds with highlighted two most active aldehydes and vinylsulfones

References:

1. S. Frankland-Searby, S. R. Bhaumik. *Biochimica et Biophysica Acta.* **2012**,1825, 64-76
2. Tanaka, Keiji. *Proc. Jpn. Acad., Ser. B.* **2009**, 85, 12-36
3. R. Jorda, J. Dušek, E. Řezníčková, K. Pauk, P. Magar, A. Imramovský, V. Kryštof. *Eur. J. Med. Chem*, **2017**, 135, 142-158
4. R. Jorda, P. Magar, D. Hendrychová, K. Pauk, M. Dibus, E. Pilařová, A. Imramovský, V. Kryštof. *Eur. J. Med. Chem,* **2020**, 188, 1-13.

The authors wish to acknowledge the financial support to the Faculty of Chemical Technology, University of Pardubice as well as to the project GA 18-03847S.