Novel Strategy for Proteasome Inhibition: Enhanced Specificity and Cellular toxicity

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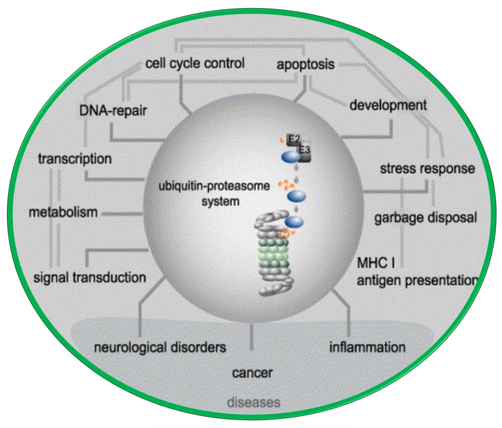
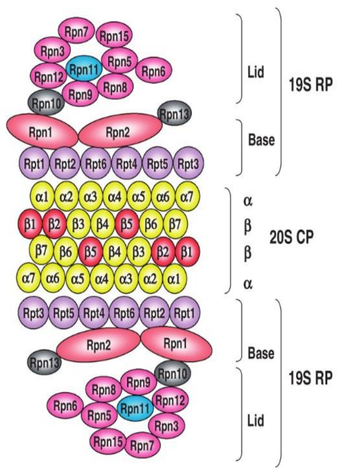
Introduction

Proteasome is a multi-catalytic subunit which degrade unwanted or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds.

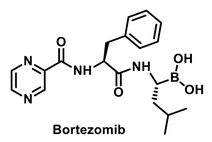


Function of 26S proteasome

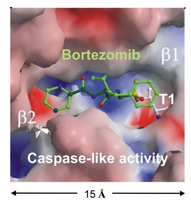


Structure of 26S proteasome

FDA approved drug for Blood Cancer

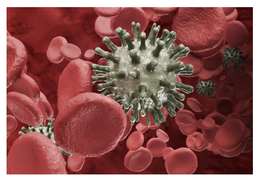


Bortezomib

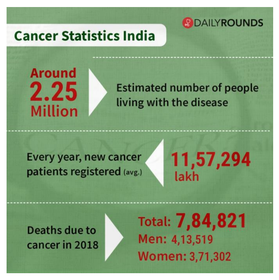


- **Bortezomib is a proteasome inhibitor, used for blood cancer.(Approved by FDA in 2004)**
- **PI prevent this targeted decomposition of protein, which can affect multiple signaling cascades within the cell.**
- **This disruption of normal homeostatic mechanisms can lead to cell death.**
- **PI can be used to treat multiple myeloma and certain types of lymphoma**

Cancer



Why Cancer?



Drawbacks with Proteasome Inhibitors

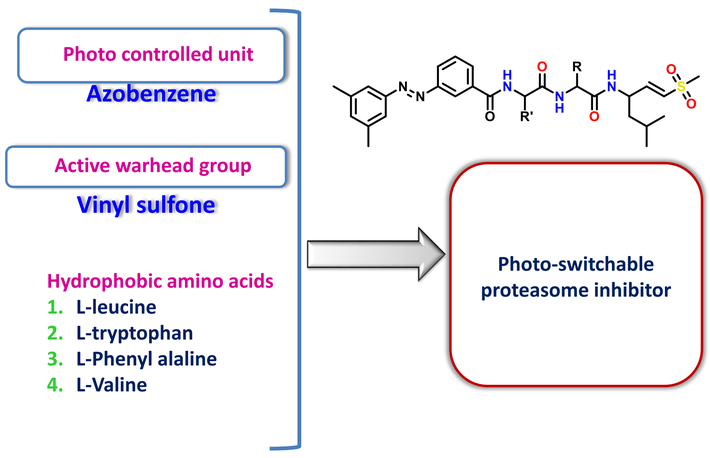
- **Non-specific cytotoxicity towards healthy tissue**
- **Cause severe side effects.**
- **Metabolic Instability.**

For example, delanzomib, and oprozomib etc. it shows limited efficacy in the treatment of other due to non-specific toxicity towards healthy tissue of cancer. It displays severe side effects also.

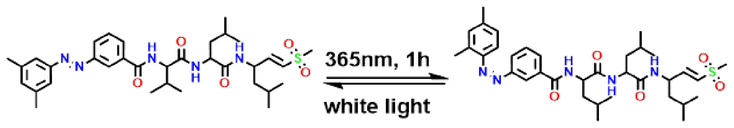
Our Target

The design and development of proteasome inhibitors and their biological activity which can be dynamically and externally controlled using external stimuli such as light.

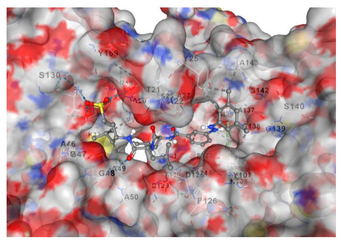
Design Strategy



Photoisomerization of 1



Molecular docking of 2



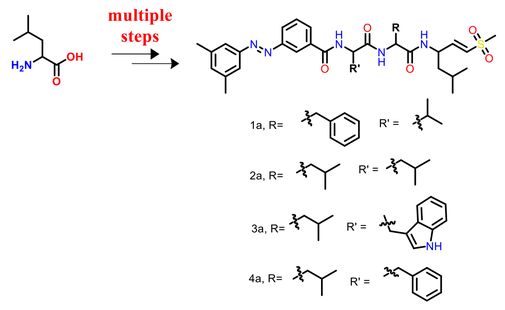
Docking Score	Cavity Size(Å^3)	Binding Pocket
-9.20	2764	β1-β5
-8.90	1009	β3-β7
-8.70	4440	β2-β5
-6.80	951	β6-β7
-5.90	1490	β4-β6

- ✓ **All the Compounds show significant binding with the active site residues in the intersubunit pocket of β1-β5.**

Compound	MM-GBSA(kcal/mol)					
	ΔG_{bind}	ΔG_{bind}Lipo	ΔG_{bind}vdW	ΔG_{bind} Coulomb	ΔG_{bind} SolvGB	ΔG_{bind} Covalent
❑ 2	-93.9681 ± 5.30	-29.91162	-76.1109	-0.09006	10.4353	5.35558
❑ 2	-99.2460 ± 4.58	-32.18054	-80.15599	0.71843	11.0364	4.793811

- ✓ **The binding free energy calculation of the protein–ligand docking complex was calculated by using the Prime-MM/GBSA and OPLS2005 force field.**
- ✓ **2b is showing high binding energy than the 2a.**

Synthesis



UV spectra

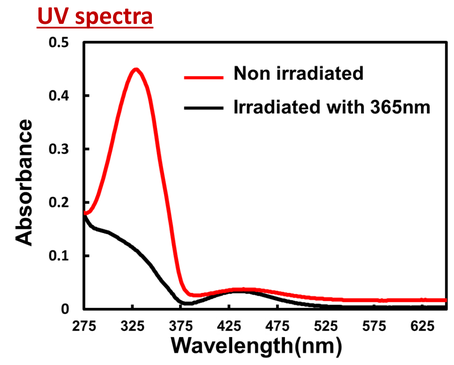
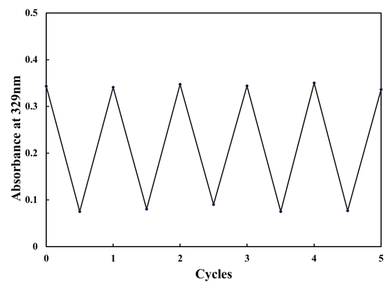
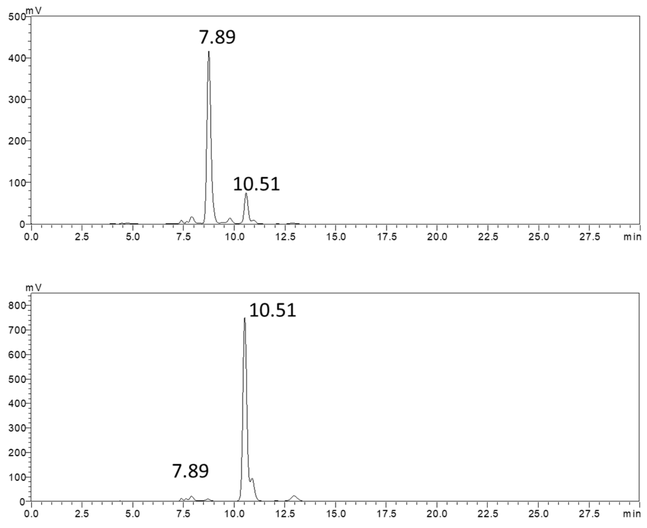


Photo-reversibity

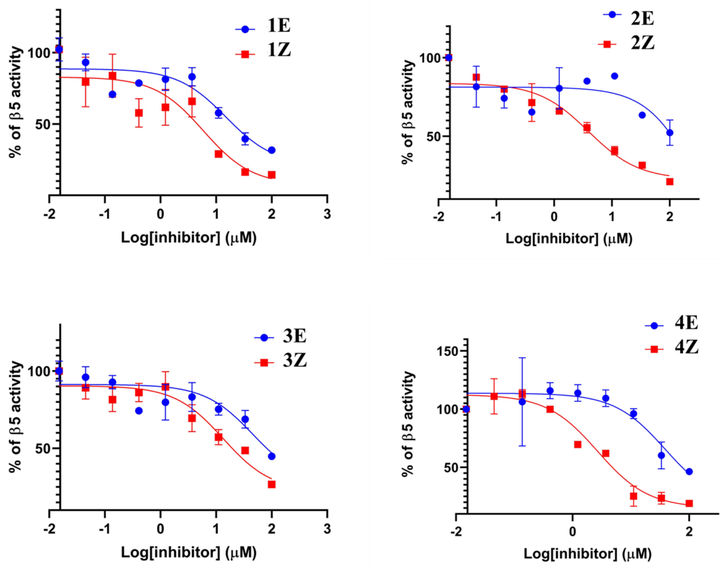


All the Compounds show Photoisomerization as well as photoreversibity.

RP-HPLC of E and Z isomer of compound 1 before and after irradiation with 365nm light

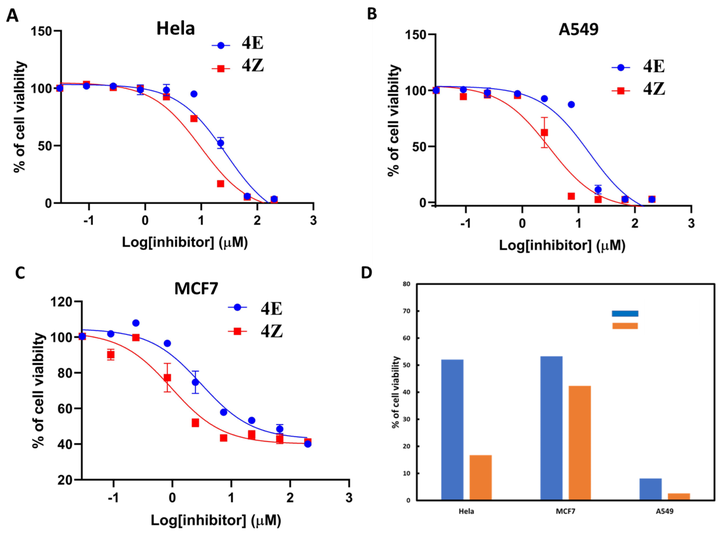


Proteasome β5 inhibition activity of compound 1-4 before and after irradiation with 365nm light



Proteasome activity is taken by incubating human 20S proteasome with different concentration of compound(both E and Z isomer) and activity was checked by changed fluorescent intensity of AMC based substrate .

Cell viability of compound 4 before and after irradiation with 365nm light for different cancer cell lines



Cell viability was checked by performing cell tire blue assay. Different concentration of compounds (both E and Z isomer) was treated to adherent cells and followed by addition of the cell tire blue reagent after 48hr of incubation time, fluorescence was measured, and activity was checked by changed fluorescent intensity of the substrate

Table1. % of E and Z isomer of compound 1-4 before and after irradiation with 365nm light

Compound	Before Irradiation(Z:E)	After Irradiation with 365nm (Z:E)
1	1.62:78.4	73.6:12.7
2	15.5:76.2	75.5:13.9
3	2.60:83.4	63.1:26.0
4	2.2:80.0	70.4:12.3

- ✓ **All the four-compound showed more than 60% of photoconversion from E to Z isomer after irradiation with 365nm light.**

Table2. Ratio of IC_{50} Value of E and Z isomers of Proteasome β5 inhibition activity of compound 1-4

Compound	IC_{50} value of E isomer(μM)	IC_{50} Value of Z isomer(μM)
1	15.12	5.98
2	--	3.9
3	46.8	13.40
4	38.64	2.6

- ✓ **Z isomer is better inhibitor of proteasome, and this result is translating in cytotoxicity.**

Table 3. IC_{50} of Cell viability of compound 1-4 before and after irradiation for different cancer cell lines

IC_{50}(μM)	1E	1Z	2E	2Z	3E	3Z	4E	4Z
Hela	32	16	14.2	2.2	-	-	9.8	2.83
A549	-	-	-	112	37.18	3.3	15.42	2.9
MCF7	25.25	0.08	-	1.16	6.9	2.9	3.09	0.962

- ✓ **Z isomers are more cytotoxic for different cancer cell line for all the 4 compounds than the E isomers**

Summary

- ✓ **We successfully designed and synthesized a series of peptides comprised of war-head vinyl-sulfone and a photo-switchable azobenzene moiety.**
- ✓ **Protein-small molecule docking studies revealed that these molecules binds to the β1-β5. catalytic site of the proteasome.**
- ✓ **MM-GBSA calculation is showing that Z isomer has high binding energy than the E isomer for the compound 2.**
- ✓ **The synthesized peptides show good cell permeability and photo-switchability.**
- ✓ **Biological assay studies suggest that Z isomer is more cytotoxic to cancer cells than the E isomer for all the compounds.**
- ✓ **These results collectively imply that one can remotely modulate the proteasome activity with an external stimuli like light.**

References:

1. M. Groll, C. R. Berkers, L. Ploegh, and H. Ovaa, *Structure.*, **2006**, 14, 451–456
2. Tanaka, *Proc. Jpn. Acad. Ser B.*, **2009**, 85, 112-36
3. Sambit Pradhan, Surajit Sarker Thilagar Pakkirisamy Unpublished work.

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