A novel non-covalent and rapidly reversible proteasome inhibitor for multiple myeloma and various solid cancers

Won-Gyun Ahn[2], Yeejin Jeon[2], Yeong-In Yang[2], Jaeseung Kim[2], Seung-Joo Lee[2], Uwe Koch[1,3], Gunther Zischinsky[3], Axel Choidas[3], Ayesha Pasha[3], Bert Klebl[3], Robert Huber[1,4], Michael Hamacher[1,3] and Kiyean Nam[1, 2]

[1]QLi5 Therapeutics GmbH, [2]Qurient Co. Ltd., [3]Lead Discovery Center GmbH, [4]Emeritus Group Structure Research, Max Planck Institute, Germany

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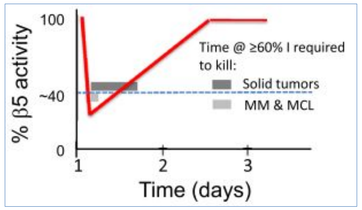
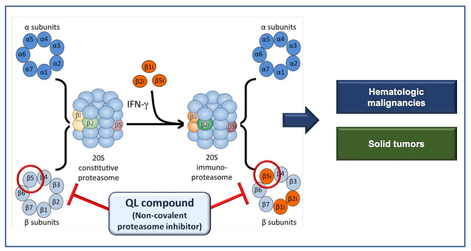
INTRODUCTION

Protein homeostasis plays an important role in maintaining normal cellular metabolism and proteasomes have a critical role in regulating this process. Although essential for normal cell regulation, proteasomal activity, in particular, is pivotal for the proliferation of cancer cells. It has been reported that the levels and activity of proteasomes are >90% higher in primary tumors compared with normal tissues[1], and it is widely accepted that high levels of proteasome activity provide tumor cells in continuous proliferation[2,3]. For this reason, a variety of improved PIs have been developed, but all of them are covalent-based compounds and are associated with undesirable side effects, mainly hematologic toxicity and peripheral neuropathy. Furthermore, none of these have been approved by the Food and Drug Administration (FDA) for the treatment of solid cancers that require high therapeutic doses due to serious side effects.

Actually, solid tumor malignancy is far from hematological malignancies. The solid tumor cells were intrinsically more resistant to proteasome inhibitors (PIs) and had lower expression of the β1i, β2i, β5i, and β2 subunits, compared to the neoplastic B cells[3]. Therefore, anti-tumor activity against solid tumors requires stronger and longer proteasome inhibition than multiple myeloma with improved PK properties for higher tumor distribution[4].



Strategy of QL series proteasome inhibitors



Here, we report a non-covalent and rapidly reversible PIs as a potential anti-tumor agent against solid tumors as well as multiple myeloma. These novel inhibitors, called QL compounds, are non-covalent chymotrypsin-like selective PIs that can be taken orally. QL compounds show stronger anti-tumor activity against multiple myeloma and solid tumors than current FDA-approved proteasome inhibitors due to improved PK properties, different patterns of selectivity and inhibitory potencies for proteasome subunits, along with reduced side effects.

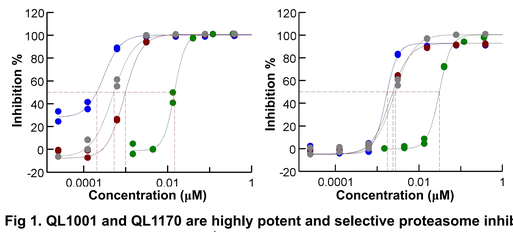
RESULTS

QL compounds has better selectivity and anti-cancer activity for multiple myeloma

A

Compounds		Enzymatic activity [IC_{50}, μM]					
		β5i	β5c	β1i	β1c	β2i	β2c
QL compounds	QL1001	**0.001**	**0.002**	5.28	>10	>10	>10
	QL1170	**0.001**	**0.002**	5.28	>10	>10	>10
1st generation PIs	Ixazomib	**0.002**	**0.003**	0.001	0.019	>10	>10
	Carfilzomib	**0.004**	**0.002**	0.042	0.266	0.057	0.021

B



C

Compounds	MM1.S	PRMI8226
	IC_{50} (μM)	
QL1001	0.0002	0.002
QL1170	0.001	0.002
Ixazomib	0.015	0.031
Carfilzomib	0.001	0.003

Fig 1. QL1001 and QL1170 are highly potent and selective proteasome inhibitor. **(A)** The 20S proteasome subunit inhibition profile of QL compounds and 1st generation PIs was determined by measuring enzymatic activity values. **(B)** Representative multiple myeloma cell lines, MM1.S and RPMI8226 were treated with QL compounds or 1st generation PIs for 48 hours and analyzed for cell viability inhibition. Cell viability was assessed using CellTiter-Glo® Cell Viability Assay. **(C)** Summary of IC_{50} for each compound in MM1.S and RPMI8226.

QL compounds induces potent anti-tumor activity by compared to 1st generation PIs

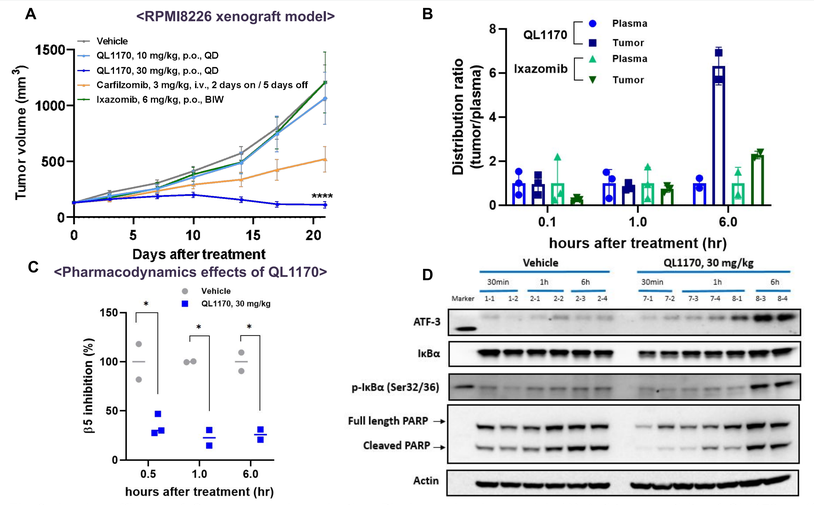


Fig 2. *In vivo* efficacy of QL compounds and other PIs in an *in vivo* model. (A) Anti-tumor activity of QL1170 or 1st generation PIs in a mouse xenograft model bearing human multiple myeloma cells. RPMI8226 ($10X10^6$) were inoculated subcutaneously in BALB/c nude mice. When tumor size reached 131 mm^3, respectively, mice (n= 8 / group) were treated with each compounds (****, p< 0.0001). **(B)** The concentration ratio of tumor/plasma of QL1170 (30 mg/kg, p.o.) or Ixazomib (6 mg/kg, p.o.) after 0.1 h, 1 h, and 6 h of oral dosing in mouse xenograft models of multiple myeloma using human RPMI8226. **(C)** Inhibition of chymotrypsin-like proteasome (β5) activity in tumor samples from RPMI8226 efficacy study (*, p< 0.05). **(D)** The protein expression level of NF-κB related apoptosis pathway in tumor samples was accessed by western blot assay. Each tumor sample was collected from RPMI8226 efficacy study.

QL compound easily moves from whole blood into plasma compartment

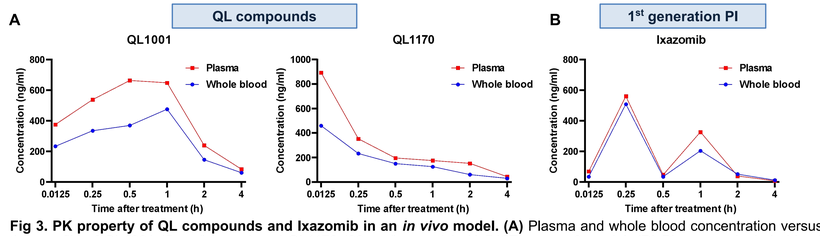


Fig 3. PK property of QL compounds and Ixazomib in an *in vivo* model. (A) Plasma and whole blood concentration versus time profile of QL1001 (5 mg/kg, s.c.) and QL1170 (30 mg/kg, p.o.) in Hs746T xenograft model. **(B)** Plasma and whole blood concentration versus time profile of Ixazomib in Hs746T xenograft model following an oral administration at 6 mg/kg. Each sample was collected at times indicated on the figures from the Hs746T efficacy study.

Select solid tumors are sensitive to proteasome inhibition

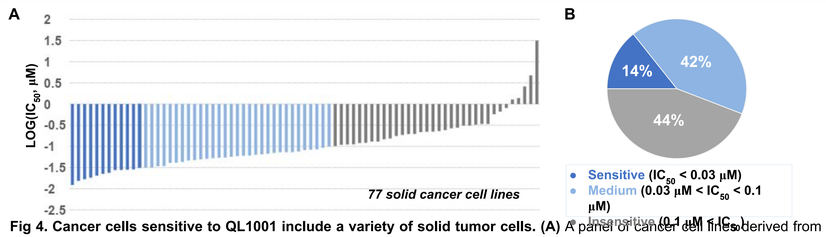
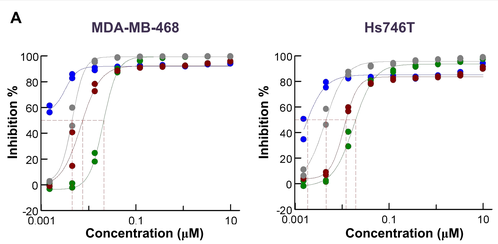


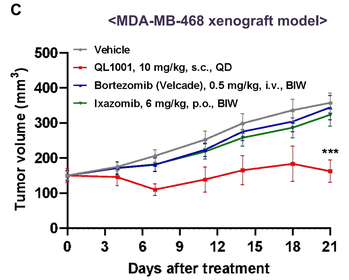
Fig 4. Cancer cells sensitive to QL1001 include a variety of solid tumor cells. (A) A panel of cancer cell lines derived from different solid tumor types was screened by cell viability assay condition with 4 h pulse treatment. Each IC_{50} was measured at 44 h after 4 h treatment QL1001 and washout. **(B)** Cell lines with IC_{50} < 0.03 μM were classified as sensitive, 0.03 μM< IC_{50} < 0.1 μM as medium, whereas IC_{50} > 0.1μM as insensitive.

Anti-tumor activity of QL compounds in solid tumor models with sufficient tumor distribution



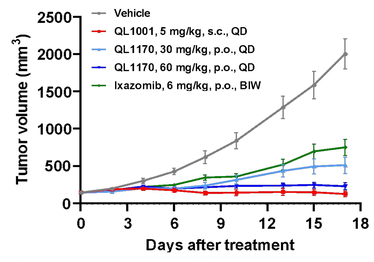
B

Compounds	MDA-MB-468 (breast cancer)	Hs746T (gastric cancer)
	IC_{50} (μM)	
QL1001	0.002	0.002
QL1170	0.007	0.012
Ixazomib	0.04	0.04
Bortezomib	0.004	0.005



D

Compounds	Time (h)	Plasma (ng/ml)	Tumor (ng/g)	T / P
QL1001	**0.5**	1558	12861	8.3
	1	698	827	1.2
Bortezomib	**0.5**	108	35.4	0.3
	1	13.6	ND	-
Ixazomib	**0.5**	66.6	ND	-
	1	129	ND	-



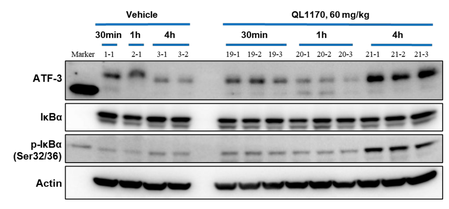


Fig 5. Anti-tumor activity of QL compounds in solid tumor *in vivo* models. (A) MDA-MB-468 and Hs746T cells were treated with QL compounds or 1st generation PIs for 48 hours and then analyzed for cell growth inhibition. Cell growth inhibition was assessed using CellTiter-Glo® Cell Viability Assay. **(B)** Summary of IC_{50} for each compounds in MDA-MB-468 and Hs746T. **(C and E)** MDA-MB-468 ($10X10^6$) and Hs746T ($2X10^6$) were inoculated subcutaneously in BALB/c nude mice. When tumor size reached 150 mm^3 and 148 mm^3, respectively, mice (n= 8 / group) were treated with each compounds (***, p< 0.001). **(C)** Anti-tumor activity of QL1001 in a mouse xenograft model with human breast cancer. **(D)** Plasma and tumor distribution of QL1001 and 1st generation PIs in the MDA-MB-468 xenograft model. **(E)** Anti-tumor activity of QL1001 and QL1170 in a mouse xenograft model with human gastric cancer. **(F)** The protein expression level of NF-κB related pathway in tumor samples was accessed by western blot assay. Each tumor sample was collected from the efficacy study.

SUMMARY

- **QL compounds effectively inhibited tumor growth in multiple myeloma xenograft models through improved PK properties, especially compound distribution between plasma and whole blood.**
- **Moreover, improved PK properties of QL compounds allowed for sufficient distribution outside the blood compartment and induced tumor growth inhibition in some types of solid cancer xenograft models that were sensitive to proteasome inhibition.**
- **These results indicate that non-covalent and rapidly reversible proteasome inhibitors are ideal strategy for drugs for multiple myeloma and potential agents for solid tumors.**

Reference

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(4) Deshaies RJ. Proteotoxic crisis, the ubiquitin-proteasome system, and cancer therapy. BMC Biology 2014, 12:94

Contact Info: E-mail : info@qurient.com Web site : www.*qurient.com*