Highly selective, orally available CDK7 inhibitor for cancer therapy

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Abstract

Cyclin-dependent kinase 7 (CDK7) has dual functions in cell; 1) CDK–activating kinase (CAK) that regulates cell cycle progression by phosphorylation of CDK1, CDK2, and CDK4/6, 2) A component of transcription factor II H (TFIIH) that regulates transcriptional initiation through phosphorylation of RNA polymerase II C-terminal domain (CTD). Here we present orally available selective CDK7 inhibitors that show distinct mechanism of action in different cancer cell lines. In some cell lines, compounds exert anti-CAK activity leading to cell cycle arrest, and in others, show transcription inhibitor activity leading to apoptosis. The differential roles of CDK7 seem to be a basis for good specificities of selective CDK7 inhibitors in cancer cell line panel, showing big difference in cytotoxic activity between responding and non-responding cells. Lead compounds have been optimized for drug-like properties, high potency, and good selectivity and desirable pharmacokinetic profile to achieve anti-tumor activity. The lead compound show good activity in mouse models through oral administration without gross adverse findings. Final optimization is underway for candidate nomination.

Background
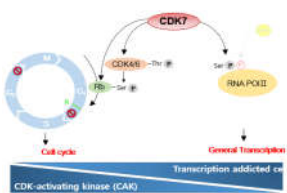
Recently, CDK7 selective inhibition has emerged as a new potential cancer therapy through cancer specific transcriptional regulation. Many cancer cells are 'transcriptionally addicted' such that cancer cell's survival heavily depend on massive transcription of certain genes (myc, mcl-1 etc.). CDK7, being a part of transcriptional factor IIH (TFIIH), modulates transcriptional initiation and its inhibition leads to effective anti-cancer activities.

CDK7 has also been known as CDK Activating Kinase (CAK), regulating cell cycle by activating cyclin-CDK complex. However, the effect on cell cycle by the CDK7 selective inhibition has not been reported extensively. QS series compounds are highly selective CDK7 reversible inhibitors and demonstrates anti-cancer activity through transcriptional regulation as well as cell cycle control depending on cancer cell type.

Results

### QS compounds are potent and selective CDK7 inhibitor

a)

Compounds	Selectivity score [at 1μM]	Number of 50% inhibited kinase
Q1138	0.039	16
Q1189	0.046	19

b)

Enzymatic assay [IC50, μM]	QS1138	QS1189	THZ-1
Km ATP			
CDK1	0.453	0.322	0.208
CDK2	0.276	0.129	0.849
CDK5	0.066	0.035	0.156
CDK7	0.003	0.004	0.019
1mM ATP			
CDK2	4.92	4.89	3.82
CDK5	>10	>10	>10
CDK7	<0.003	0.007	0.376

a) In vitro enzyme inhibition assays were performed against 410 kinases including 29 CDKs to determine the selectivity of QS lead compounds at 1μM. QS1138 and QS1189 show high selectivity with selectivity score less than 0.05. Out of 29 CDKs titrated, only three CDKs (CDK2, CDK5 and CDK16) showed selectivity less than 30-fold. In addition, newly optimized QS1274 shows much improved selectivity against all tested kinases (selectivity score; 0.012) as well as CDK2 and CDK5 showed selectivity over 300-fold.

b) QS lead compounds maintain enzyme inhibition activity and show greater selectivity in presence of a 1mM ATP.

Differential response for tumor cell lines by QS compounds
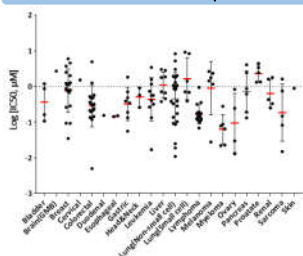
Cancer cell viability assay was performed against 161 cancer cell lines from the 22 lineages. Representative QS1138 show wide range of cytotoxic effect depending on cancer cell lines and lineages.

QS compounds show cytotoxic effect for specific cancer cell lines

Cell lines	Q1138	QS1189	THZ-1
	[IC50, μM]		
RPMI-8226	0.024	0.122	0.116
NCI-H460	0.026	0.036	0.13
MM1.S	0.038	0.055	0.008
MV4-11	0.022	0.074	0.006
hPBMC	2.927	1.687	0.158

A 3 day in vitro cell viability assay was performed using 'Celltierglo' and a 50% survival inhibition value determined for QS lead compounds. QS lead compounds show a stronger cytotoxic effect against cancer cell than against hPBMC, compared to reference CDK7 selective covalent inhibitor, THZ-1.

G1 Cell Cycle Arrest Activity (H460, NSCLC)

1. QS compounds induce G1 cell cycle arrest in H460
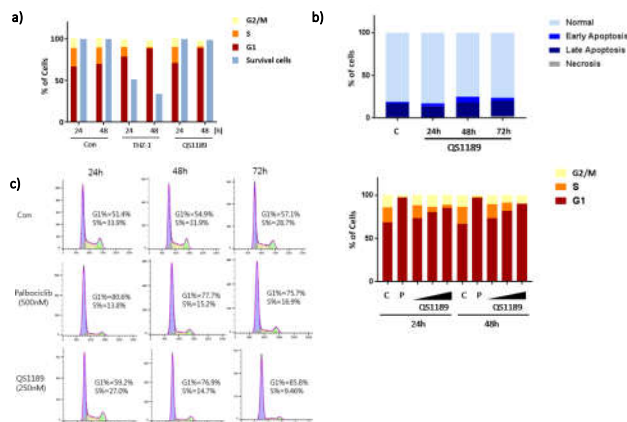
a) QS1189 was tested in H460 cells at IC90 for cell cycle analysis. QS1189 induces strong G1 cell cycle arrest over time, while maintaining live cell count.

b) No increase of apoptosis observed by QS1189 treatment at IC90.

c) QS1189 was compared with CDK 4/6 inhibitor Palbociclib. Palbociclib shows onset of G1 cell cycle arrest at 24hr, while QS 1189 shows gradual onset of G1 cell cycle arrest over time.

2. QS 1189 inhibits tumor growth in H460 mouse xenograft model
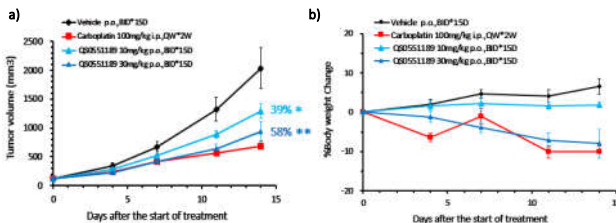
a) QS1189 was able to inhibit tumor growth dose dependently.

b) Body weight for all of animals was comparable throughout the study. Moderate body weigh loss observed only in high dose group.

Transcriptional Regulation Activity (RPMI-8226, multiple myeloma)

1. QS 1189 inhibits RNAPII CTD phosphorylation and induces apoptosis in RPMI-8226
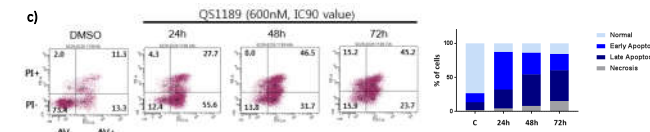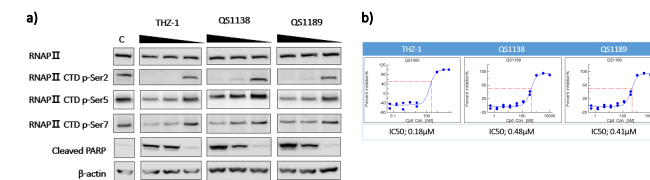
a) RNAPII CTD phosphorylation inhibition and cleaved PARP level increased in RPMI-8226 cells upon QS lead compounds treatment.

b) Total mRNA synthesis is inhibited by both QS lead compounds and THZ-1.

c) Rapid induction of apoptosis by QS1189 was confirmed by FACS analysis.

2. QS 1189 inhibits tumor growth in RPMI-8226 mouse xenograft model
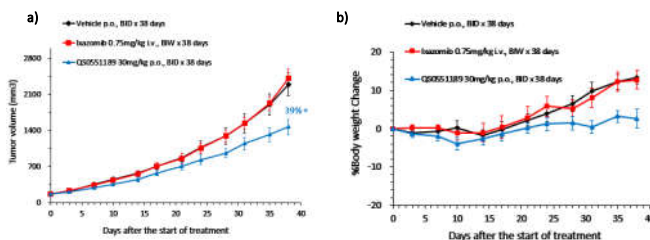
a) QS1189 shows better tumor growth inhibition than Ixazomib.

b) No significant body weight change by QS1189 treatment.

Conclusion

- QS compounds are high selective and potent CDK7 inhibitors
- QS compounds show big difference in cytotoxic activity between responding and non-responding cells
- Cytotoxicity of QS compounds shows correlation with p-RNAPII modulation as in RPMI-8226 cells
- G1 cell cycle arrest seems to be a main mechanism of anti-tumor activity by QS compounds as in H460 cells
- Lead optimization is underway to improve properties and selectivity

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