KAT6A inhibitor screening cascade to facilitate novel drug discovery

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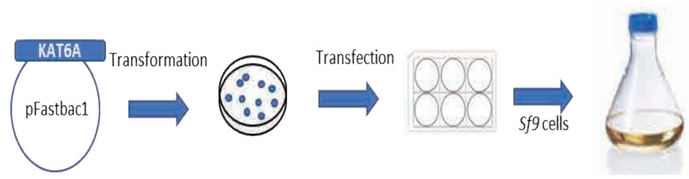
Introduction

Lysine acetyltransferase 6A (KAT6A, also called MOZ and MYST3) is a histone acetyltransferase (HAT) belonging to the MYST family. Other members include MOF(KAT8)、MORF(KAT6B)、TIP60(KAT5) and HBO1(KAT7). The histone substrates of KAT6A identified to date include H3K9, -K14, and -K23. KAT6A has been reported to play a critical role in hematopoietic stem cell maintenance, cell cycle regulation, and cell senescence. In addition, KAT6A is thought to be an oncogene in human cancers, including breast cancer, glioma and leukemia. Here, we have developed KAT6A related biochemical assays, cellular assays and in vivo pharmacology models to support novel KAT6A inhibitor discovery.

KATs protein expression and purification

KAT protein, were expressed in sf9 cells and purified by GST column and followed by size exclusion chromatography. Protein product link is https://protein.ice-biosci.com/search/product?searchText=KAT

A



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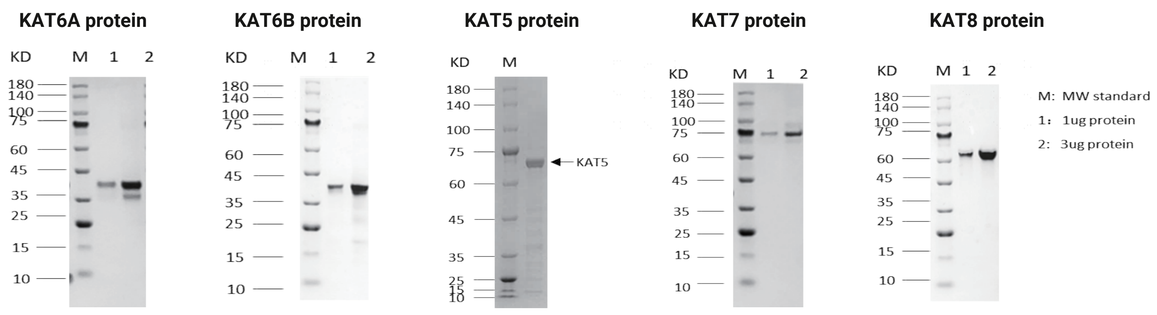
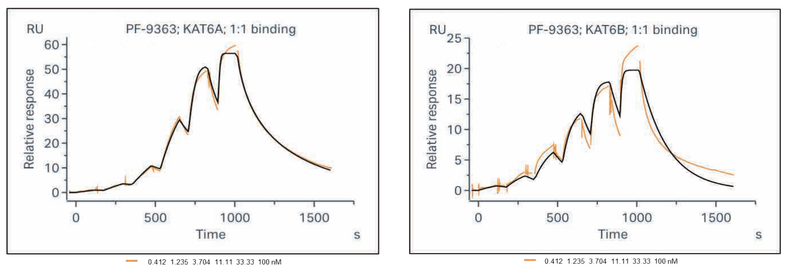


Figure 1. A. Procedure for KATs protein expression. B. SDS-PAGE for KAT protein purification.

KATs SPR assay development



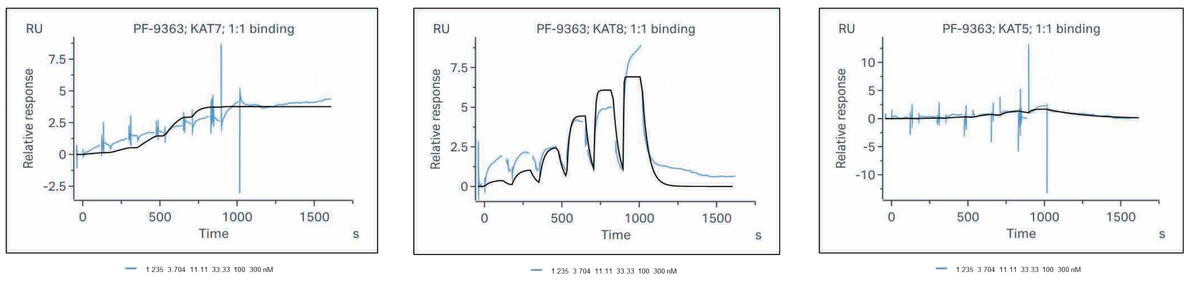


Figure 2. Reporter of PF-9363 binding with KATs protein in SPR assay. We have successfully conducted SPR assays of the KAT family

Immobilized ligand	Injection variables Single cycle kinetics 1 Solution	Kinetics model	1:1 binding ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Quality Kinetics Chi² (RU²)	U-value
KAT6A	PF-9363	1:1binding	2.34E+06	1.24E-02	5.29E-09	59.4	1.03E+00	3
KAT6B	PF-9363	1:1binding	1.05E+06	5.78E-03	5.49E-09	20.8	2.47E+00	5
KAT5	PF-9363	1:1binding	1.46E+05	3.93E-03	2.70E-08	1.80E+00	1.71E-01	1.50E+01
KAT7	PF-9363	1:1binding	2.61E+05	1.99E-07	7.63E-13	4.80E+00	5.58E-01	9.50E+01
KAT8	PF-9363	1:1binding	1.13E+06	2.51E-02	2.22E-08	7.40E+00	8.06E-01	1.20E+01

Table1. The parameter of PF-9363 binding with KATs protein in SPR assay.

KATs HTRF assay development

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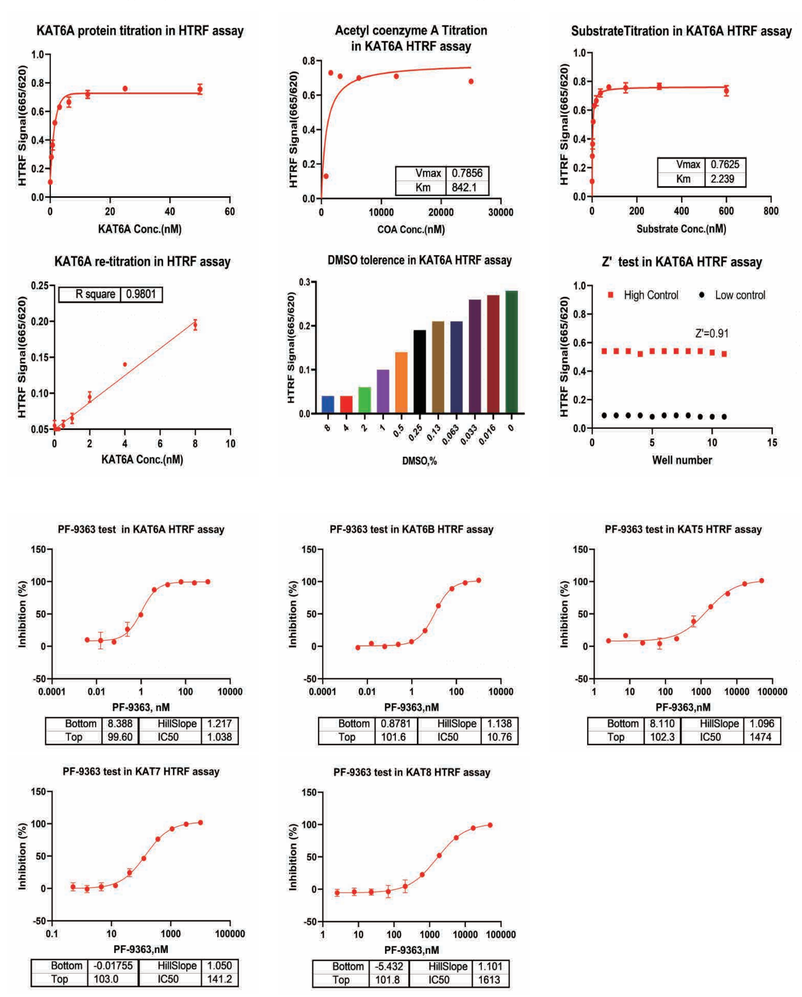

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Figure 3. A.The procedure KAT6A HTRF assay development. B. PF-9363 IC50 test in KAT6A and KAT family target HTRF assay

Cellular Assays

1. Cell proliferation assay

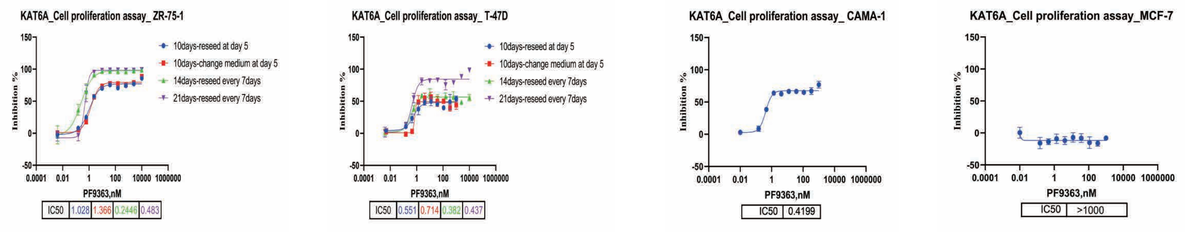
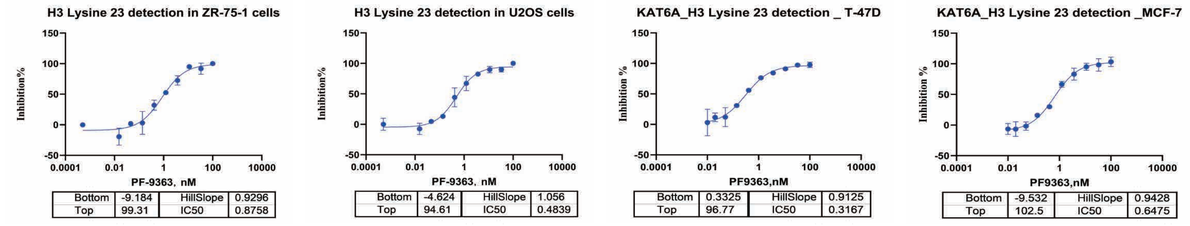


Figure 4. PF-9363 IC50 test in ZR-57-1, T-47D, CAMA-1 and MCF-7 cells at different treatment time

2. In cell western assay

A



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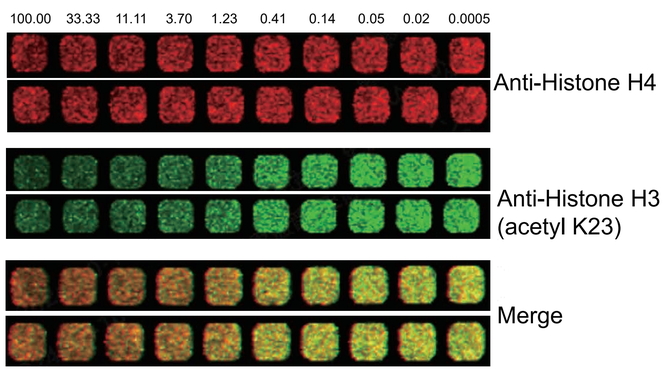
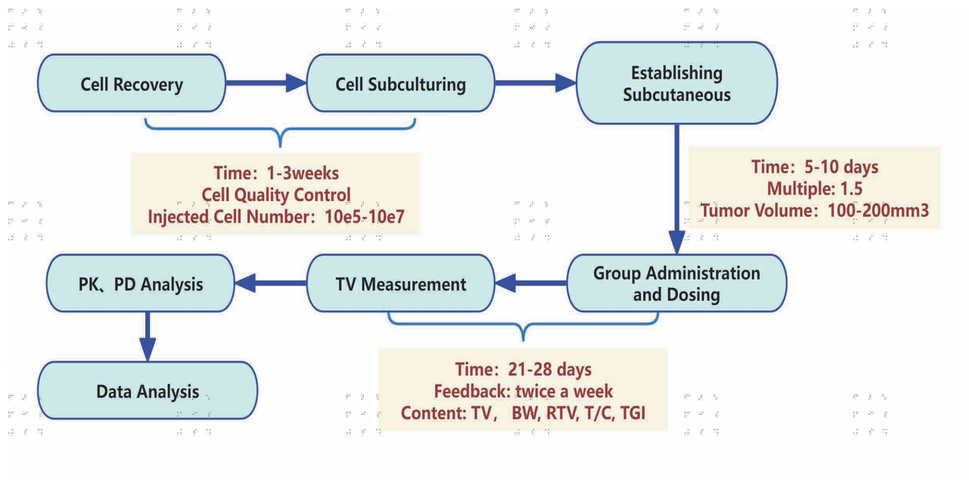
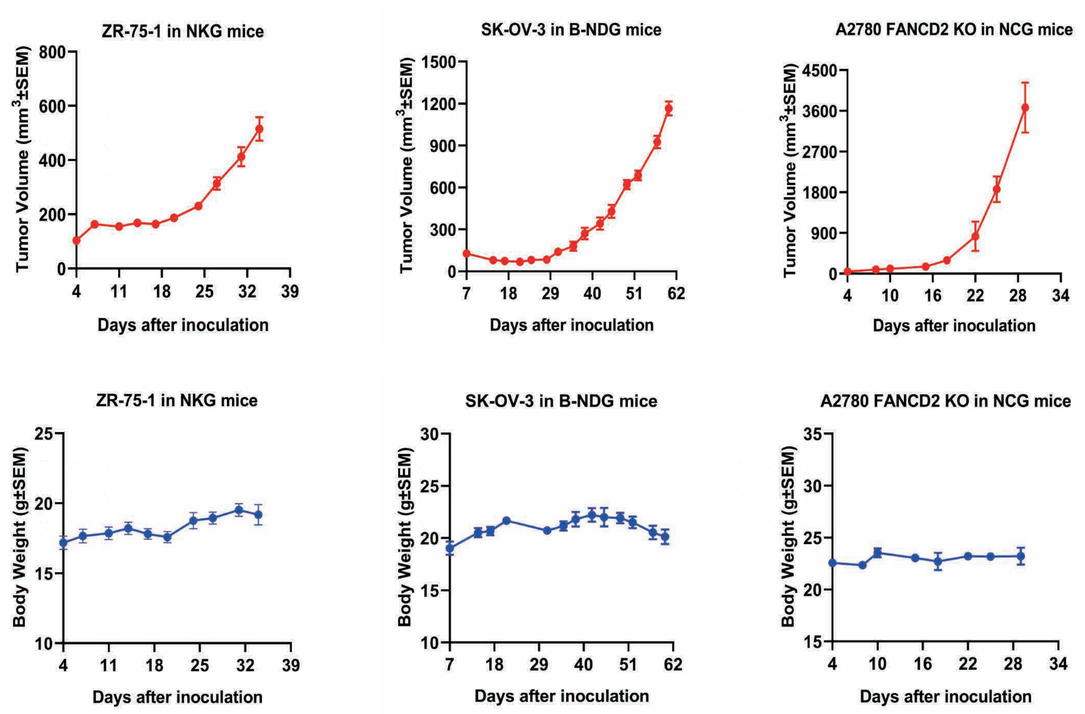


Figure 5. A. IC50 curve of PF-9363 in the acetylation assay in ZR-75-1, U2OS,T-47D and MCF-7 cell. B. Images of ZR-75-1 cells treated with different concentrations of PF-9363 after adding fluorescent antibodies.

Animal Modeling

CDX modeling utilizing different cell lines has been established for the efficacy study as shown below to expand the drug discovery cascade to the in vivo experiments.





Summary

In conclusion, inhibition of KAT6A target could be regarded as a rational approach in Breast cancer or ovarian cancer. we constructed an experimental cascade from in vitro to in vivo, which is composed of protein production, biochemical assays, cellular assays, and animal modeling. Our KAT6A screening cascade can satisfy the mechanism study of KAT6A as well as efficient and comprehensive screen of KAT6A inhibitor, thus accelerate the novel drug discovery.

References

[1] Huang F, Abmayr SM, Workman JL. Regulation of KAT6 Acetyltransferases and Their Roles in Cell Cycle Progression, Stem Cell Maintenance, and Human Disease. Mol Cell Biol. 2016 Jun 29;36(14):1900-7. doi: 10.1128/MCB.00055-16. PMID: 27185879; PMCID: PMC4936061.

[2] Wiesel-Motiuk N, Assaraf YG. The key roles of the lysine acetyltransferases KAT6A and KAT6B in physiology and pathology. Drug Resist Updat. 2020 Dec;53:100729. doi: 10.1016/j.drup.2020.100729. Epub 2020 Oct 7. PMID: 33130515.