Discovery of Potent KAT6 Inhibitors That Demonstrate Anti-Tumor Activity in Preclinical Models of ER+ Breast Cancer

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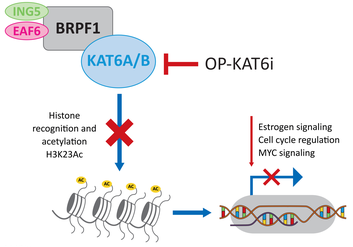
Background

- Overexpression of KAT6 correlates with worse clinical outcome in ER+/HER2− breast cancers[1]
- Dysregulation of KAT6 activity (gene amplification, overexpression, fusion, mutation, etc.) is observed in many cancers including breast cancer[2]
- KAT6 paralogs KAT6A (MOZ; MYST3) and KAT6B (MORF; MOZ2; MYST4) are histone acetyltransferase (HAT) enzymes that acetylate histone H3 and epigenetically regulate gene transcription by altering chromatin structure (**Figure 1**)[3]
- Inhibition of KAT6 reduces breast cancer cell proliferation through downregulation of genes involved in estrogen receptor signaling and other signaling pathways[4] (**Figure 1**)
- In several subtypes of breast cancer, KAT6A is amplified as part of the 8p11-p12 amplicon and is overexpressed in 11 to 15% of the overall breast cancer population[5]
- **RESULTS:** Here we describe the discovery and preclinical characterization of orally bioavailable, potent KAT6A and KAT6B-selective inhibitors (OP Compounds 1, 2, 3, 4 and 5)

Figure 1. Schematic of KAT6 biology and impact of inhibition



i, inhibitor.

Methods

- **Biochemical assays:** Compounds were serially diluted and incubated with KAT proteins. Biotinylated Histone-H4 protein and Acetyl-CoA were added, and samples were further incubated for 60 minutes. Plates were read in TR-FRET mode (Ex: 340 nm, Em:615 and 665 nm) post-addition of the detection mix containing Eu anti-acetyl lysine antibody and streptavidin-APC
- **Cell proliferation assays:** Cells were plated in 96-wp at optimized densities in appropriate complete medium and incubated overnight before being treated with serial dilutions of compounds for 7, 10 or 14-days. Cell number was assessed using CTG and normalized to T=0
- **Drug combination analysis:** Synergism was evaluated using the Zero Interaction Potency (ZIP) model, assuming that the combined effect of two molecules is the sum of their individual responses, without any interaction. Deviations from this predicted combined effect indicate either synergy (greater effect) or antagonism (lesser effect)
- **Cellular pharmacodynamic assays:** Cells were plated at an optimized density in appropriate complete medium and incubated overnight. Compounds were serially diluted and dosed to cells which were incubated at 37 °C, 5% CO_2 for 24-hours or 5-days. Cells were lysed and samples analyzed by western blotting using antibodies specific to H3K23Ac, Histone-H3, ERα and β-actin
- **Xenograft studies:** Female, immune deficient NOD SCID mice were supplemented with estradiol and implanted with an ER+ breast cancer cell line, ZR-75-1, subcutaneously in mammary fat region and were randomized into groups when the tumor volume reached ~150 mm^3. Mice were treated for 25 days with either vehicle or discovery compounds at 0.1, 0.3 or 1 mg/kg
- **Positive control KAT6 inhibitor:** Compound E from patent[6] was used as a comparator in several *in vitro* and *in vivo* studies as noted in Results section

Results

Our KAT6 inhibitors are potent and selective against KAT6A/B

- OP compounds 1-5 were potent against KAT6A and KAT6B (<20 nM) and were comparable to positive control (**Table 1**)
- KAT6 inhibitors showed >250-fold selectivity over other KAT family members, especially the essential proteins KAT5 and KAT8

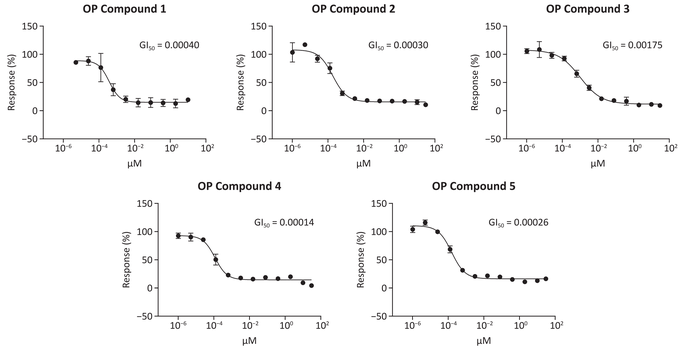
Table 1. Biochemical potencies of KAT6-targeting candidate inhibitors (Assay format: TR-FRET)

	OP Compound 1	OP Compound 2	OP Compound 3	OP Compound 4	OP Compound 5	Positive control
KAT6A IC_{50} (nM)	12	9	13	14	14	7
KAT6B IC_{50} (nM)	2	1	0.5	3	3	1
KAT5 IC_{50} (nM)	8619	6792	>10,000	>10,000	9929	1288
KAT7 IC_{50} (nM)	55	108	711	578	180	88
KAT8 IC_{50} (nM)	>10,000	5444	>10,000	>10,000	3764	1372

KAT6 inhibitors potently inhibit proliferation of KAT6-amplified ZR-75-1, an ER+, breast cancer cell line

- OP compounds 1-5 potently inhibited cell proliferation of the KAT6 amplified ER+, ZR-75-1 breast cancer cells (**Figure 2**)
- GI_{50} values observed were nanomolar or lower

Figure 2. OP KAT6 inhibitors on ZR-75-1 cell proliferation.



Expression of KAT6 confers sensitivity to inhibitors of KAT6, in both *ESR1* wild type (WT) and mutant breast cancer cell lines

- In KAT6 overexpressing ER+ breast cancer cell lines T47D and CAMA-1, OP-2, -4, -5 strongly inhibited cell proliferation. KAT6-low MCF7 cells, were not sensitive to inhibitors (**Table 2**)
- Proliferation of *ESR1* mutant D538G and Y537S CAMA-1 cells was inhibited by OP-2, -4, -5, suggesting that KAT6 inhibitors may be effective in treating *ESR1* mutant breast cancer

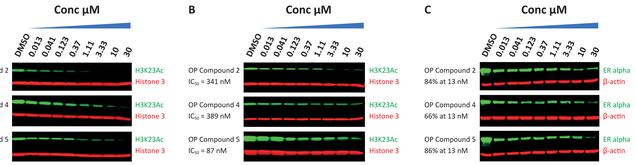
Table 2. Growth inhibition values (GI_{50}) for KAT6 inhibitors on cell proliferation

	OP Compound 2	OP Compound 4	OP Compound 5
T47D GI_{50} (nM)	6.5	11	0.8
CAMA-1 GI_{50} (nM)	4.6	92	5.3
CAMA-1 D538G GI_{50} (nM)	1.8	3.4	3.0
CAMA-1 Y537S GI_{50} (nM)	3.2	0.2	6.6
MCF7 GI_{50} (nM)	>10,000	>10,000	>10,000

KAT6 inhibitors strongly engage histone target in ZR-75-1 and CAMA-1 cells

- OP-2, -4, and -5 demonstrated strong concentration-dependent target engagement leading to inhibition of histone 3 lysine 23 acetylation (H3K23Ac) (**Figure 3A, B**)
- KAT6 inhibitors demonstrated significant reduction in ERα protein levels, showing induction of downstream gene expression changes subsequent to target engagement (**Figure 3C**)

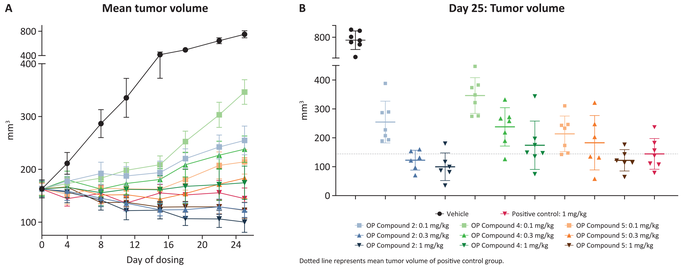
Figure 3. Western blots of OP compounds on H3K23Ac in ZR-75-1 (**A**) and CAMA-1 (**B**) and ZR-75-1 ERα levels (**C**)



KAT6 inhibitors cause dose-dependent tumor regression in a ZR-75-1 breast cancer xenograft model

- KAT6 inhibitor compounds caused dose-dependent tumor growth inhibition and tumor regression either comparable to or better than positive control (**Figure 4A, B**)
- These KAT6 inhibitors were well tolerated, and no significant body weight changes were observed among different treatment groups compared to vehicle

Figure 4. Impact of OP-2, -4, -5 and positive control on ZR-75-1 tumor volume in a 25-day mouse xenograft model



Dotted line represents mean tumor volume of positive control group.

KAT6 inhibitors are orally bioavailable with desirable pharmacokinetics

- Upon oral administration, KAT6 inhibitors demonstrated good oral bioavailability with long half-life and slow clearance (**Table 3**)
- OP-2, -4, and -5 dosed at 1 mg/kg in mice, were calculated to demonstrate >100-fold of the GI_{50} at C_{ave} (free) (**Table 3**)

Table 3. Pharmacokinetic (PK) parameters of OP-2, 4, and 5 in mouse, rat and dog and calculated C_{ave} (free)/GI_{50} in mice

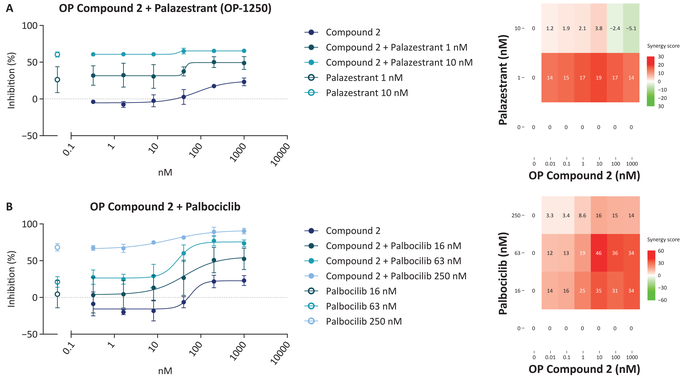
Species Dose (mg/Kg)	CD-1 Mice 10			Wistar Rat 10			Beagle Dog 3	
	OP-2	OP-4	OP-5	OP-2	OP-4	OP-5	OP-2	OP-5
$AUC_{(0-last)}$ (ng*hr/mL)	286800	152726	250519	207906	160531	265421	1071885	906987
$T_{1/2}$ (hr)	6.8	2.9	3.4	2.6	4.4	3.5	22.3 (MRT)	24.3 (MRT)
C_{max} (ng/mL)	29214	22181	34176	32042	12687	29544	29082	23355
% F	62	78	84	100	72	81	~100	~100
C_{ave} (free)/GI_{50} at 1 mg/kg	345	313	108					

MRT, Mean Residence Time.

ERα and CDK4/6 inhibitors synergize with KAT6 inhibitors in ER+ and KAT6-overexpressing cell line, T47D

- In combination with the Complete Estrogen Receptor Antagonist (CERAN) palazestrant (OP-1250), dose-dependent synergism was observed at the 1 nM concentration (**Figure 5A**)
- OP-2 showed synergy in a dose-dependent manner at all concentrations tested with the CDK4/6 inhibitor palbociclib (**Figure 5B**)

Figure 5. T47D cell proliferation of OP-2 alone and in combination with palazestrant (**A**) and palbociclib (**B**)



Conclusions

- OP-1, -2, -3, -4, and -5 are highly potent against KAT6A/B and are highly selective versus KAT5 and KAT8.
- In KAT6-amplified and overexpressing breast cancer cell lines, OP-2, -4, and -5 demonstrated target engagement and efficacy.
- OP-2, -4, and -5 showed comparable tumor regression versus positive control in a KAT6-amplified ER+ breast cancer mouse model.
- In vivo (mouse) assessment of OP-2, -4, and -5 showed high levels of free drug exposure.
- OP-2 showed synergism with CDK4/6 inhibitor (palbociclib) or CERAN (palazestrant; OP-1250).
- Development candidate nomination and IND-enabling studies will begin shortly.

References

1. Yu L, et. al. Identification of MYST3 as a novel epigenetic activator of ERα frequently amplified in breast cancer. Oncogene. 2017 May 18;36(20):2910–2918
2. Wiesel-Motiuk N, et al. The key roles of the lysine acetyltransferases KAT6A and KAT6B in physiology and pathology. Drug Resist Updat. 2020. Dec;53:100729
3. Utley RT, et. al. The MYST family of histone acetyltransferases. Curr Top Microbiol Immunol. 2003;274:203–36
4. Sharma S, et. al. Discovery of a highly potent, selective, orally bioavailable inhibitor of KAT6A/B histone acetyltransferases with efficacy against KAT6A-high ER+ breast cancer. Cell Chemical Biology. 30, 1–20
5. Turner Ivery B, et. al. KAT6A, a chromatin modifier from the 8p11-p12 amplicon is a candidate oncogene in luminal breast cancer. Neoplasia. 2014 Aug;16(8):644–55.
6. Paul AS, et. al. Kat6 inhibitor methods and combinations for cancer treatment. WO2022013369.

Acknowledgments

This study was sponsored by Olema Oncology. Editorial and layout support were provided by Twist Medical, and funded by Olema Oncology.

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Presented at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, 2023; Boston, MA; October 11–15, 2023

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