A novel benzophenanthridine alkaloid, 6ME, targets acute myeloid leukemia (AML) involving peroxisome proliferator-activated receptors (PPARs)

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Background

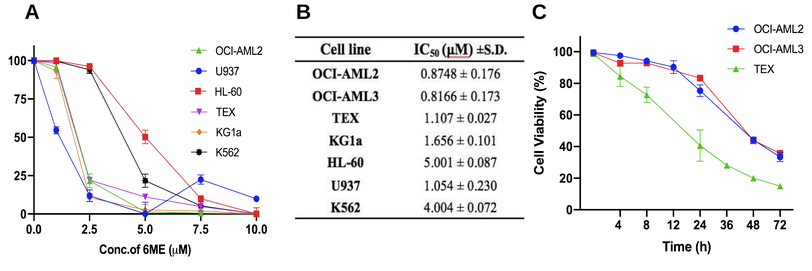
Acute myeloid leukemia (AML) is a devastating hematological malignancy characterized by uncontrolled proliferation and accumulation of undifferentiated myeloid precursors (myeloblasts) in the peripheral blood, bone marrow, and/or other tissue leading to impaired hematopoiesis and bone marrow failure. Existing treatment strategies for AML therapy (e.g., chemotherapy) can induce remission but are associated with relapse while also posing serious adverse effects on the healthy/normal cells and individual's health. Therefore, alternatives are needed to improve patient outcomes. A high throughput flow-cytometry based screen, in multiple leukemia cell lines, identified a novel benzophenanthridine alkaloid, 6ME, as a novel and selective anti-AML drug. Thus, this research project aims to explore how 6ME exerts anti-AML activity.

Methods

Advanced theoretical and experimental approaches including *in vitro* was conducted to investigate the anti-AML efficacy of 6ME. The 7-aminoactinomycin (7-AAD) assay was used to determine IC_{50} values, and clonogenic growth assays were and will be used in primary patient-derived AML cells and normal peripheral blood stem cells (PBSC) from healthy donors. Meanwhile, Published computational methods predicted potential targets of 6ME and identified peroxisome proliferator-activated receptors (PPARs) as the potential target, and molecular docking studies predict 6ME to bind to PPARs via structural interaction. To study the role of PPARs in 6ME's anti-AML activity, molecular and cellular techniques including co-immunoprecipitation (coIP) and immunoblotting were employed to evaluate the level of PPARs in 6ME-treated AML cells. Moreover, as Leukemic stem cells (LSCs) are uniquely reliant on mitochondrial oxidative phosphorylation (OXPHOS) for the production of high-energy compounds while tightly control and maintain a low reactive oxygen species (ROS) level, high resolution respirometry was conducted to assess cellular oxygen consumption and predict how 6ME affect mitochondrial metabolism.

Results

6ME reduces Viability in a Panel of Leukemic Cell Lines



Cell line	IC_{50} (μM) ±S.D.
OCI-AML2	0.8748 ± 0.176
OCI-AML3	0.8166 ± 0.173
TEX	1.107 ± 0.027
KG1a	1.656 ± 0.101
HL-60	5.001 ± 0.087
U937	1.054 ± 0.230
K562	4.004 ± 0.072

Fig 1. 6-methoxydihydroavicine (6ME), a benzophenanthridine alkaloid imparts cytotoxicity in leukemic cell lines: (A) 7-AAD staining, and flow cytometry analysis of cell viability reveal potent, cytotoxic effects of 6ME in a panel of leukemia cell lines after 72-hour treatment. (B) IC_{50} values range from approximately 0.8-5.1μM. (C) OCI-AML2, OCI-AML3, and TEX cells were treated with 1μM 6ME, and the cell viability was assessed at different time points by flow cytometry.

Results

6ME impairs Clonogenic Growth of Primary AML

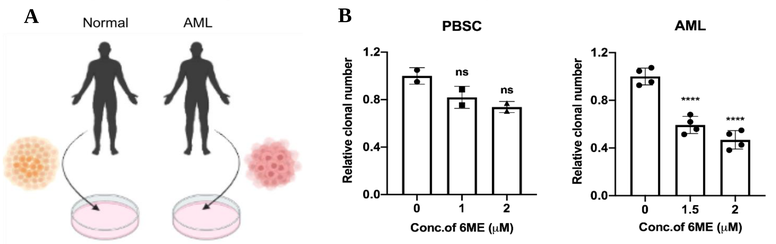


Fig 2. 6ME selectively reduces clonogenic growth in primary AML cells: (A) Normal peripheral blood stem cells (10^4 cells/dish) and AML primary cells (10^5 cells/dish) were seeded as single cells in Melthocult H4034 semi-solid media, in the presence or absence of 6ME. **(B)** Following a 14-day incubation, colonies consisting of ≥50 cells were enumerated and normalized to the untreated group. Data presented as mean ± S.D., ns not significant, ****p<0.0001 using an unpaired, one-way ANOVA calculated in GraphPad Prism 8.0.

PPARs are the Potential Targets of 6ME

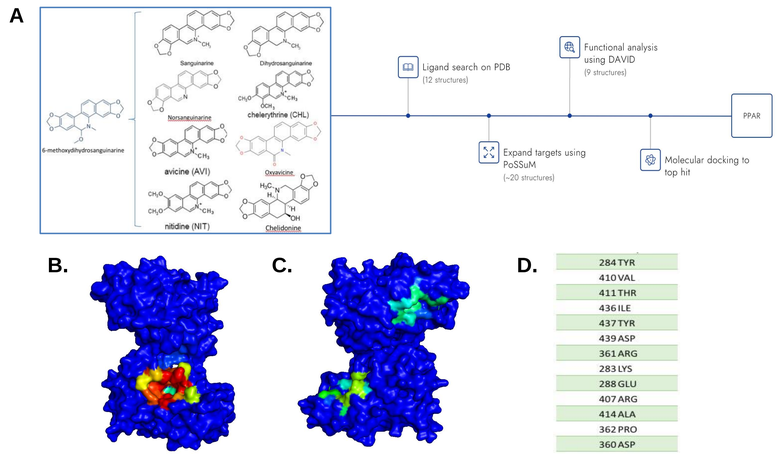


Fig 3. PPARs are the predicted molecular target of 6ME. (**A**) A flow chart outlining the structural bioinformatics approach for prediction and analysis of 6ME-binding proteins from the PDB. Molecular docking experiments using the Autodock Vina program were performed to explore the binding interactions between 6ME and PPARδ. (**B**) shows the major docked poses of 6ME interacting with the chain B of PPARδ, and other interactions are shown as (**C**). Moreover, (**D**) lists the notably involved residues in the binding interactions between 6ME and PPARδ.

Summary

- 6ME exhibits impressive and selective cytotoxicity against AML cells according to the results of 7AAD assay and clonogenic growth assays with primary cells.
- PPARs might play a role in 6ME's anti-AML activity; it indicated 6ME significantly lowered the expression levels of PPARα and PPARδ in tested AML cell lines by coIP and immunoblotting analysis.
- 6ME alters mitochondrial respiration in AML cells via inhibiting FAO-supported intact cell respiration.
- 6ME may be a novel molecule with selectivity and potency at eliminating AML via PPARs-mediated pathway.

6ME lowers the expression level of PPARα and PPARδ in AML cells

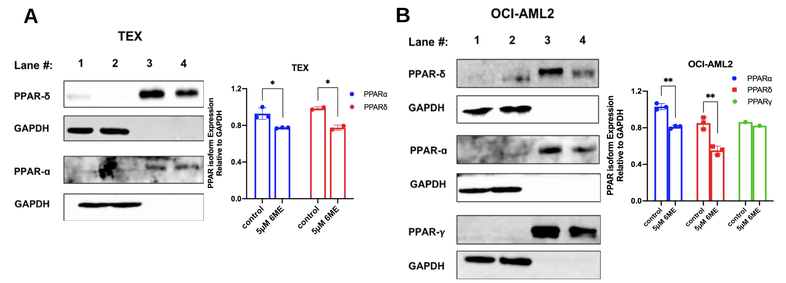


Fig 4. Co-immunoprecipitation (co-IP) was performed to pull down the PPARs in 5 μM-6ME-treated OCI-AML2 and TEX cells. Magnetic separation produces two fractions from the lysate: 1. the flow-through fraction, containing all other cellular components except PPARs, and 2. The PPAR-enriched fraction, containing exclusively PPARs. To determine fraction purity, both fractions underwent immunoblotting to confirm PPARs pull-down. Immunoblotting shows PPAR isolation from the flow-through fractions (lane #1 and #2) into PPAR-enriched fractions (lane #3 and #4) following separation by magnetic co-IP. Specifically, 6ME significantly lowered the expression levels of PPARα and PPARδ in **(A)** TEX cells and **(B)** OCI-AML2 cells.

6ME Inhibits FAO-supported Intact AML Cell Respiration

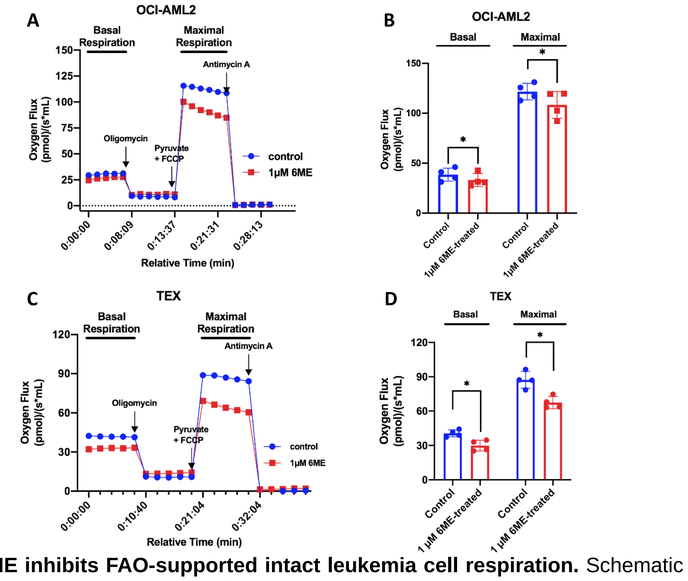


Fig 5. 6ME inhibits FAO-supported intact leukemia cell respiration. Schematic showing oxygraphy trace quantifying basal and maximal mitochondrial oxygen respiration of intact OCI-AML2 **(A)** and TEX cells **(C)**. Briefly, cells exhibited a basal rate of respiration after injected into the high resolution respirometer (HRR). The injection of ATP synthase inhibitor oligomycin (OLI) uncoupled ATP synthesis from mitochondrial respiration. Maximal respiration was stimulated with an injection of a chemical uncoupler (FCCP) and pyruvate (PYR). All mitochondrial oxygen consumption was then inhibited with the addition of the complex III inhibitor antimycin A (AA). **(B)** FAO-supported intact cell basal and maximal respiration of untreated- and 1hr-6ME-treated OCI-AML2 cells, as well as that of **(D)** untreated- and 1hr-6ME-treated TEX cells. For **(B, D),** data presented as mean ± S.D., * p≤0.05, using a paired t-test calculated in GraphPad Prism 8.0.