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REPURPOSING PHENFORMIN AS A GAMMA-SECRETASE INHIBITOR:

A MACHINE LEARNING AND IN VITRO INVESTIGATION

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Abstract

Introduction: Gamma-secretase plays a crucial role in the pathogenesis of many diseases, including Alzheimer's disease and various cancers, by regulating essential signaling pathways. Recent studies highlight its involvement in lysosomal acidification, a key process in cellular homeostasis and disease progression. Thus, the development of inhibitors targeting gamma secretase represents a promising therapeutic strategy.
Methods: We performed an *in silico* screening of 3,537 ligands against gamma secretase with known IC50 to develop a machine learning (ML) model predicting molecular descriptors carrying the inhibitory effect. The top candidate, phenformin, was selected and anchored to gamma secretase subunits (PSEN1, PSEN2, NCSTN, PEN-2). *In vitro*, phenformin (0.5 mM) was tested on colorectal cancer (CRC) cell lines HCT-116 to assess its impact on lysosomal acidification, using lysosensor green DND, and on cell viability, using MTT assay.
Results: The ML model identified phenformin as a potent gamma secretase inhibitor with a predicted IC50 = 1.1 µM. Molecular docking revealed preferential binding to the PEN-2 subunit (- 6.3 kcal/mol), which is overexpressed in CRC. *In vitro*, phenformin effect on Gamma-secretase was confirmed by reducing lysosomal acidity by >30% ($P < 0.01$). This resulted in a decrease in HCT116 viability by 71% ($P < 0.01$).
Conclusion: This combination of *in silico* and *in vitro* experimentation demonstrates the ability of phenformin to disrupt lysosomal function and impair cancer cell survival through inhibition of gamma-secretase. Given gamma secretase's role in multiple diseases, phenformin represents a promising therapeutic candidate that deserves further investigation.

Keywords : *Colorectal cancer, Gamma-secretase, lysosomes, machine learning, phenformin*

1-INTRODUCTION

Gamma-secretase is a multi-subunit intramembrane protease complex (comprising PSEN1, PSEN2, NCSTN, and PEN-2), best known for its role in the cleavage of amyloid precursor protein (APP) and Notch receptors. Aberrant gamma-secretase activity is implicated in the pathogenesis of Alzheimer's disease, due to the generation of neurotoxic amyloid-beta peptides, and in various cancers, where it modulates Notch signaling to promote tumor growth and survival [1, 2]. Recent studies have expanded our understanding of gamma-secretase, revealing its involvement in lysosomal acidification—a process essential for autophagy, protein degradation, and cellular homeostasis [3]. Dysregulation of lysosomal pH is increasingly recognized as a hallmark of cancer cells, contributing to their survival and resistance to therapy [4]. Therefore, targeting gamma-secretase not only disrupts oncogenic signaling but may also impair lysosomal function, offering a dual mechanism for therapeutic intervention. In this study, we employed a machine learning-driven *in silico* screening approach, followed by *in vitro* validation, to identify novel gamma-secretase inhibitors with potential anti-cancer activity.

2-MATERIALS AND METHODS

In Silico Screening and Machine Learning:

A library of 3,537 ligands with known gamma-secretase inhibitory activity (IC50 values) was curated from public databases. Molecular descriptors were calculated for each compound and used to train a supervised machine learning model to predict inhibitory potency. Multiple FDA-approved were tested for screened to predict their IC50 and repurpose them as gamma-secretase inhibitor. Top candidate, Phenformin, was further evaluated by molecular docking using AutoDock Vina, focusing on binding affinity to gamma-secretase subunits (PSEN1, PSEN2, NCSTN, PEN-2).

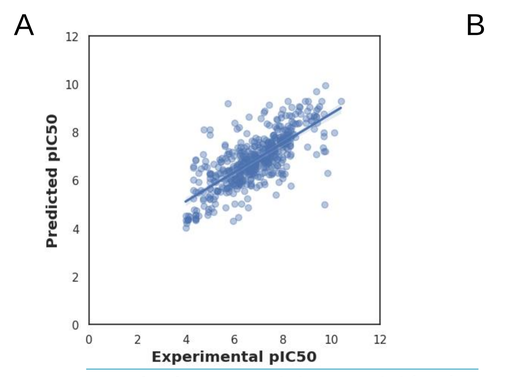
Lysosomal Acidification and Cell viability :

Human colorectal cancer HCT-116 cells were cultured under standard conditions. Phenformin was administered at a concentration of 0.5 mM for all in vitro assays.
Lysosomal pH was assessed using Lysosensor Green DND-189 dye. Fluorescence intensity was measured by flow cytometry to quantify changes in lysosomal acidity following phenformin treatment.

3-RESULTS AND DISCUSSION

Our machine learning model, trained on a diverse set of gamma-secretase inhibitors, enabled the rapid screening of thousands of compounds and identified phenformin—a biguanide previously used as an anti-diabetic agent—as a promising candidate. Notably, phenformin's predicted IC50 (1.1 µM) suggests a strong inhibitory effect, comparable to or exceeding that of established gamma-secretase inhibitors. Molecular docking studies revealed that phenformin exhibits preferential binding to the PEN-2 subunit, a critical component for gamma-secretase assembly and activity, and one that is often upregulated in colorectal cancer (CRC) cells.

The functional consequences of gamma-secretase inhibition by phenformin were evaluated in HCT-116 CRC cells. Phenformin treatment led to a significant reduction in lysosomal acidity, as measured by lysosensor green DND fluorescence. This finding aligns with recent reports that gamma-secretase activity is required for the maintenance of lysosomal pH, possibly through the regulation of v-ATPase assembly or trafficking. The observed >30% decrease in lysosomal acidity is particularly relevant, as impaired acidification can disrupt autophagic flux and promote cell death in cancer cells that rely on autophagy for survival under metabolic stress.



Molecule	pIC50	IC50 (mM)
Phenformin	5.95	1.1 mM

ID	RMSD L.B.	RMSD U.B.	SCORE
1.1	0,000	0,000	-6,1

Figure 1. Computational Prediction and Molecular Docking of Phenformin as a Gamma Secretase Inhibitor

(A) Scatterplot of experimental vs. predicted IC50 and pIC50 values for gamma secretase inhibitors using a random forest model, highlighting the predicted IC50 of phenformin. (B) Molecular docking of phenformin with PEN-2, showing the predicted binding pose and docking score.

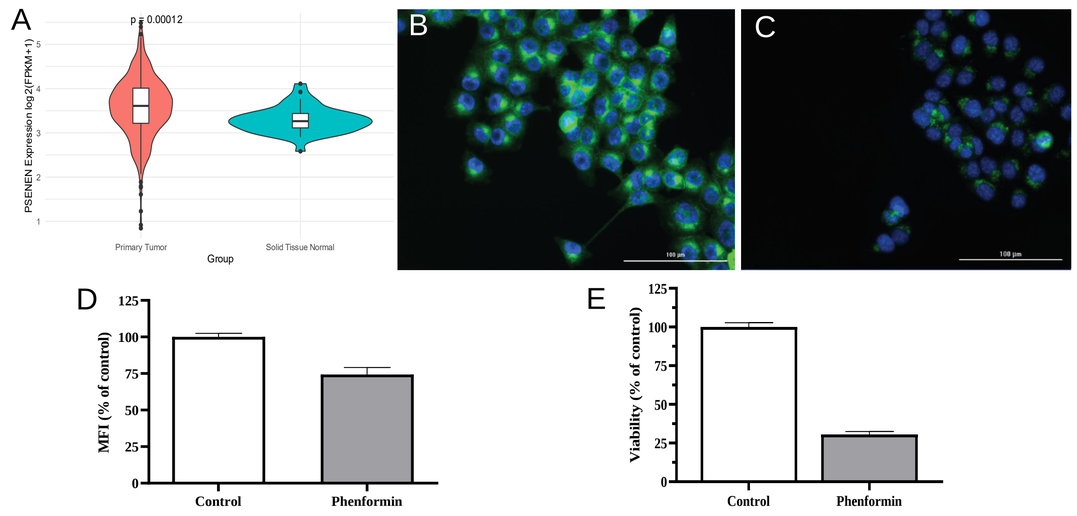


Figure 2. PEN-2 Expression, Lysosomal Acidity, and Cell Viability in Response to Phenformin in Colorectal Cancer Cells

(A) PEN-2 mRNA expression in TCGA-COAD tumor versus normal tissues. (B-D) Fluorescence microscopy images and quantification of lysosomal acidity in control and phenformin-treated HCT116 cells. (E) Cell viability of HCT116 cells following phenformin treatment.

4- CONCLUSION

Our study demonstrates that phenformin, identified through a machine learning-based in silico screen, is a potent inhibitor of gamma-secretase with a unique mechanism of action involving disruption of lysosomal acidification. The dual targeting of oncogenic signaling and lysosomal function represents a promising therapeutic strategy, particularly for cancers such as CRC that exhibit high gamma-secretase activity and dependence on autophagy. These findings not only highlight the potential for drug repurposing in cancer therapy but also underscore the value of integrating computational and experimental approaches in drug discovery. Further studies are warranted to elucidate the precise molecular mechanisms and to evaluate the efficacy of phenformin in *in vivo* cancer models and other gamma-secretase-related diseases.

References

1.McCaw TR, Inga E, Chen H, Jaskula-Sztul R, Dudeja V, Bibb JA, Ren B, Rose JB. Gamma Secretase Inhibitors in Cancer: A Current Perspective on Clinical Performance. Oncologist. 2021 Apr;26(4):e608-e621. doi: 10.1002/onco.13627. Epub 2021 Jan 2. PMID: 33284507; PMCID: PMC8018325.
2.Hur JY. γ-Secretase in Alzheimer's disease. Exp Mol Med. 2022 Apr;54(4):433-446. doi: 10.1038/s12276-022-00754-8. Epub 2022 Apr 8. PMID: 35396575; PMCID: PMC9076685.
3. Hung COY, Livesey FJ. Altered γ-Secretase Processing of APP Disrupts Lysosome and Autophagosome Function in Monogenic Alzheimer's Disease. Cell Rep. 2018 Dec 26;25(13):3647-3660.e2. doi: 10.1016/j.celrep.2018.11.095. PMID: 30590039; PMCID: PMC6315085.
4. Rodriguez R, Cañeque T, Baron L, Müller S, Carmona A, Colombeau L, Versini A, Sabatier M, Sampaio J, Mishima E, Picard-Bernes A, Solier S, Zheng J, Proneth B, Thoidingjam L, Gaillet C, Grimaud L, Fraser C, Szylo K, Bonnet C, Charafe E, Ginestier C, Santofimia P, Dusetti N, Iovanna J, Sa Cunha A, Pittau G, Hammel P, Tzanis D, Bonvalot S, Watson S, Stockwell B, Conrad M, Ubellacker J. Activation of lysosomal iron triggers ferroptosis in cancer. Res Sq [Preprint]. 2024 Apr 8:rs.3.rs-4165774. doi: 10.21203/rs.3.rs-4165774/v1. Update in: Nature. 2025 May 7. doi: 10.1038/s41586-025-08974-4. PMID: 38659936; PMCID: PMC11042398.