Lead Optimization of Tetrahydropyridines as Potential Breast Cancer Agents

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ABSTRACT

Objective: While successful strides have been made towards developing antiestrogens for estrogen receptor positive (ER+) breast cancer (ie., tamoxifen), they are limited by adverse effects and acquired resistance. To address this gap, previously, we reported the synthesis of a novel group of compounds, known as tetrahydropyridines (THPs) where their pharmacological activity depends on the position and nature of the substituent on the THP ring structure. Preliminary studies demonstrated that THP derivatives exhibited antiproliferative activity in Ishikawa, MCF-7, and MDA-MB-231 cells. Here, the overall objective is to use lead optimization, synthesis, and biological evaluation of a series of novel THP derivatives in hormone-dependent and tamoxifen-resistant breast cancer cells. We hypothesized that novel N-substituted [benzoylamino]-5-ethyl-1,2,3,6-tetrahydropyridine will have lower IC_{50} values and higher ER binding affinities compared to the lead compound N-[4-Iodobenzylamino]-5-ethyl-1,2,3,6-tetrahydropyridine (EH4) thus improving the biological activity.

Methods: To calculate the molecular properties, drug-likeness and predict the bioactivity score, Molinspiration Cheminformatics was utilized. The synthetic scheme of the THP derivatives is: 3-Ethylpyridine reacted with O-mesitylenesulfonylhydroxylamine (O-MSH) to furnish N-amino-3-ethylpyridinium mesitylenesulfonate. The reaction of the N-amino-3-ethylpyridinium mesitylenesulfonate with substituted acid chlorides yielded stable crystalline pyridinium ylides. A sodium borohydride reduction of ylides furnished the target compounds, N-substituted [benzoylamino]-5-ethyl-1,2,3,6-tetrahydropyridines. Molecular modeling studies were performed using SYBYL-X 2.1 to mimic the behavior of the THPs docked to the active site of the estrogen receptor alpha (ERα).

Results: The THP derivatives were synthesized, purified, and characterized. Biological evaluation studies using ER+ and endocrine resistant cells lines are currently underway.

Conclusions: Based on the preliminary characterization results, it is predicted that the novel derivatives will have improved biological activity compared to the parent compound.

OBJECTIVE

The ***overall objective*** is to use lead optimization, synthesis, and biological evaluation of a series of novel THP derivatives (**Figure 1**) in hormone-dependent and tamoxifen resistant breast cancer cells.

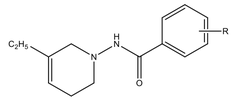


Figure 1: General Structure of N-Substituted[Benzoylamino]-5-Ethyl1,2,3,6-Tetrahydropyridine

HYPOTHESIS

Our ***central hypothesis*** is the novel N-substituted [benzoylamino]-5-ethyl-1,2,3,6-tetrahydropyridine will have lower IC_{50} values and higher ER binding affinities compared to the lead compound N-[4-Iodobenzylamino]-5-ethyl-1,2,3,6-tetrahydropyridine (EH4) which will translate into improved biological activity.

INTRODUCTION

- Approximately 80% of breast cancer is estrogen receptor positive (ER+).
- While successful strides have been made towards developing antiestrogens for ER+ breast cancer (ie., tamoxifen), they are somewhat limited by adverse effects and the development of resistance due to alteration in ER expression and ER mutations.[1]
- Once resistance develops, metastasis ensues, and treatment options are limited leading to morbidity and mortality. As such, synthesizing compounds with less adverse effects and prolonged sensitivities are required.
- Previously, Redda et al., and Ardley et al., reported the synthesis a novel group of compounds, known as tetrahydropyridines (THPs).[2-4] Several reports demonstrate that the pharmacological activity of these agents depends on the position and nature of the substituent on the THP ring structure.[2-5]
- The research project will examine the antiproliferative activity of novel THP derivatives in two ER positive breast cancer cell lines (MCF-7 and tamoxifen-resistant MCF-7 cells) and one triple-negative breast cancer cell line (SUM 159).

RESULTS

Specific Aim 1. Synthesize and characterize novel N-Substituted [Benzoylamino]-5-Ethyl-1,2,3,6-Tetrahydropyridines as potential breast cancer agents.

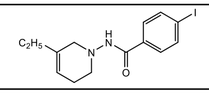
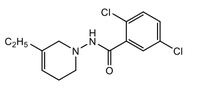
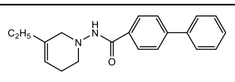
Aim 1a. To evaluate the drug-like properties of the novel N-Substituted [Benzoylamino]-5-Ethyl-1,2,3,6-Tetrahydropyridines.

Table 1: Molecular Properties and Bioactivity Scores of THPs for Some Drug Targets

Compound Name	miLog P	TPSA	natoms	MW	nOH	nOHNH	nviolations	nrotb	volume	Bioactivity Score
EH4	2.67	32.34	18	356.21	3	1	0	3	252.55	GPCR (0.09) Ion (-0.28) Kinase (-0.23) Nuclear (-0.43) Protease (-0.29) Enzyme (-0.12)
EDH 3	2.88	32.34	19	299.20	3	1	0	3	255.63	GPCR (0.12) Ion (-0.27) Kinase (-0.26) Nuclear (-0.39) Protease (-0.26) Enzyme (-0.11)
EDH 8	3.39	32.34	23	306.41	3	1	0	4	299.96	GPCR (0.26) Ion (-0.18) Kinase (0.04) Nuclear (-0.12) Protease (0.07) Enzyme (0.05)

Aim 1b. To synthesize and characterize a series of novel N-Substituted [Benzoylamino]-5-Ethyl-1,2,3,6-Tetrahydropyridines.

Table 2: Structures of THPs

Compound Names	STRUCTURE
EH4	
EDH 3	
EDH 8	

Specific Aim 2. To evaluate the novel tetrahydropyridine derivatives cytotoxicity in breast cancer cell lines.

Aim 2a. To determine whether the novel THPs dock to ERα.

Table 3: Docking Scores of THPs to ERα

Compounds	Glide Docking Scores
EH4	-10.57
EDH 3	-8.01
EDH 8	-7.91

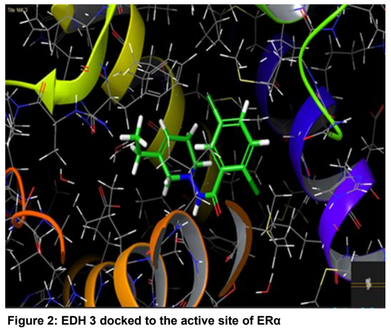


Figure 2: EDH 3 docked to the active site of ERα

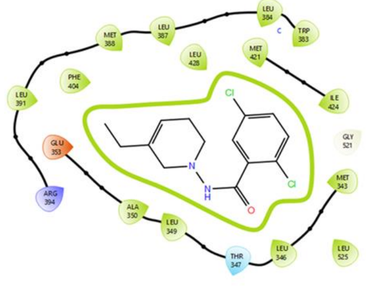
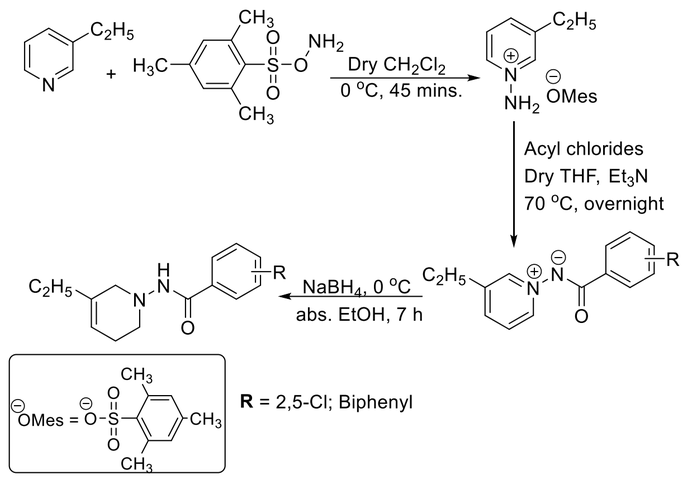


Figure 3: 2-D view of EDH 3 docked to the active site of ERα

METHODS



CONCLUSIONS

Molinspiration Cheminformatics calculated the molecular properties of the two novel N-substituted [benzoylamino]-5-ethyl-1,2,3,6- tetrahydropyridines, which determined these THPs have potential drug-like properties. The two THP derivatives were synthesized using the synthetic scheme and purified using column chromatography. Proton nuclear magnetic resonance (^{1}H-NMR) spectra, mass spectrometry, and high-performance liquid chromatography (HPLC) were done to characterize the compounds. Elemental analysis (C,H,N,Cl), carbon nuclear magnetic resonance (^{13}C-NMR), and melting point determinations will be done to further characterize and confirm the expected structures. Based on the docking scores, the THP derivatives have a low binding affinity to the ERα. The cytotoxicity and antiproliferative studies for these novel tetrahydropyridines on MCF-7, MCF-7 TAM, and SUM 159 breast cancer cell lines are currently underway.

ACKNOWLEDGEMENTS

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