Isolation and Viability of Potential Anti-Cancer Compound "Sakura"

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Introduction

Histone Deacetylases (HDACs) are enzymes that removes an acyl group from DNA histones. When the acetyl group is removed, DNA tightens and becomes inaccessible to transcription factors. In recent years, HDACs have been heavily researched to treat many different genetic conditions because of its ability inhibit transcription of affected cells. Suberolyanilide hydroxamic acid, or SAHA, is an HDAC inhibitor currently used as a chemotherapeutic treatment. The role of SAHA is to facilitate transcription of genes that result in apoptosis, differentiation and growth arrests, and has been shown to have beneficial results predominantly in lymphomas.

Cell lines are invaluable tools for scientific research due to their ability to replicate rapidly, allowing research to largely circumvent human patients. However, many labs are ill-equipped or under funded to obtain and maintain cells. However, many programs have emerged to allow researchers to dive into theoretical biology even at the cellular level. Two such programs are PyRx™ and Toxtree™. PyRx™ is an inexpensive program the displays how a ligand may bind to a substrate, as well as the numerical binding affinity. Toxtree is a free program that assesses a variety of compound characteristics including, ability to bind to DNA or proteins, and potential reactions the ligand may have intra and extracellularly.

Methods

Purification **(Previously synthesized and stored by McMurry Alumni Mukundwa K. Gael)**
Solvents were created to find the best Rf Value
Column Chromatography
GC Mass Spectrometry

Cytotoxicity Assay **(Cytotoxicity assays test ligand toxicity in vitro)**
Performed by Nicole Doyle, Lara Meyers, and Ernesto Valle using MDA-MB-231 cells.

Synthesis **(A Williamson Synthesis Reaction was performed)**
0.5g of phenol, 1.43g of bromo-6-hexanoate, 0.88g of potassium carbonate and 15ml of acetone was added in a microwaveable reaction vessel with a stir bar. The concoction was then microwaved for 30 minutes at 350W and 100°C. Acetone was evaporated on a rotavap and mass spectrometry confirmed the presence of the product -

PyRx™ **(PyRx™ is a program that shows theoretical docking of ligands onto substrates)**
A skeletal structure was formed using ChemDraw™ than converted to 3D using Chemdraw 3D Pro™. Our substrate, HDAC1-5ICN, was obtained from the Protein Data Base (PDB) Another program, PyRx™, was used to remove previously attached ligands from the HDAC as well as water from the environment. Finally, the Autodock Vena tool in PyRx™ was used to show docking of "Sakura" to HDAC1- 5ICN.

Toxtree™ **(Toxtree™ is a program that uses three different decision trees to predict how toxic a compound may be to human cells)**
The name, Smiles Code, and CAS# were enter into the program. Toxtree™ than gave the overall results and answer to each question back on an excel sheet.

Is the Ligand 6-phenoxyhexonate theoretically viable as a potential chemotreatment for breast cancer?

Physical Data

Purification

Solvent	RF Values
2:2:1 Chloroform: Hexanes: methanol	none
5:2:1 Chloroform: Hexanes: methanol	none
50:50 Methanol: Acetone	0.17
2:3:0.04 acetonitrile: water: formic acid	0.21
3:4 hexanes dichloromethane	0.53

Table 1. solvents and resulting Rf values

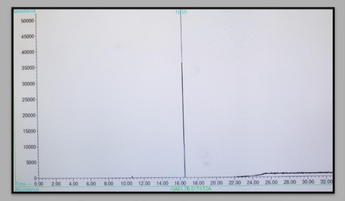

Figure 1. GC Mass Spectrometry analysis

Cytotoxicity Assay

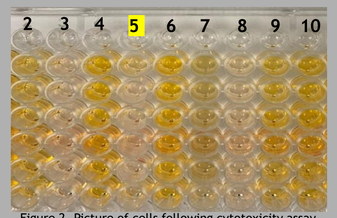

Figure 2. Picture of cells following cytotoxicity assay

MBA MB 231 Cell Count			
2 DMSO	3 TSA	4 Nothing	5 "Sakura" (Non-diluted)
0.017	0.014	0.021	0.015
.662	0.143	1.729	0.332
.589	0.109	1.548	0.118
.713	0.128	1.401	0.188
.773	0.203	2.261	0.196
.82	0.133	1.697	0.118
0.808	0.136	1.702	0.368

Table 2. Cellular death count following assay

Theoretical Data

PyRx™

Sakura Binding Affinity $\left(\frac{Kcal}{mol}\right)$	SAHA Binding Affinity $\left(\frac{Kcal}{mol}\right)$	TSA Binding Affinity $\left(\frac{Kcal}{mol}\right)$
-6.4	-6.8	-7.7
-6.0	-6.8	-7.4
-6.6	-6.7	-7.3
-6.5	-6.6	-7.1
-5.8	-6.6	-7.0
-5.4	-6.6	-6.8
-4.7	-6.5	-6.8

Table 3. Binding affinities of ligands onto HDAC1-5ICN

Toxtree™

Name	Cramer rules	Kroes TTC decision tree	Revised CDT	Verhaar scheme
6 phenoxy Hexanoate "Sakura"	Low (Class I)	Negligible risk (life-time cancer risk probably lower than 1 in 10^6)	Intermediate (Class II)	Class 1 (narcosis or baseline toxicity)
SAHA	Low (Class I)	No Safety Concerns Expected	Intermediate (Class II)	Class 5 (Scheme can not classify)
Capsaicin	Low (Class I)	No Safety Concerns Expected	Intermediate (Class II)	Class 5 (scheme can not classify)

Table 4. Results of each decision tree based on Toxtree™

Name	Acyl Transfer agent	Michael Acceptor	SN1	SN2	SNAr	Schiff base formation	Can QSAR Calculation Be applied	DNA & Protein Binding alerts	alerts for S. typhimurium mutagenicity	QSAR6 applicable?	Structural S. typhimurium mutagenicity
6 phenoxy hexanoate	NO	YES	NO	NO	NO	YES	NO	YES	YES	NO	YES
SAHA	YES	YES	YES	NO	NO	NO	NO	YES	YES	YES	YES
Capsaicin	NO	YES	NO	YES	NO	NO	NO	YES	NO	NO	NO

Table 5. Different Traits assessed by Toxtree™

Conclusion

- The Cytotoxicity Assay shows that Sakura proved to be lethal to the MDA-MB-231 cell line. The effectiveness of the ligand even rivals that of Trichostatin A, or TSA.
- PyRx results show that that optimal confirmations may result in beneficial binging to the substrate. However, Sakura does not have as consistent confirmation as SAHA or TSA.
- Toxtree results were came back as expected. Each decision tree showed the same results for both Sakura and SAHA, except for two.
- The Kroes TTC tree is known to detect "negligible risk" for many compounds including foods that may trigger allergic reaction. It largely cites this as a concentration or extreme exposure risk
- Since, like SAHA, Sakura is expected to induce apoptosis, it is expected that to have triggered some form of toxicity detection.

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