

Discovery of Novel Inhibitors of Serine Palmitoyltransferase (SPT) by Mass Spectrometry-Based High-Throughput Screening (HTS)

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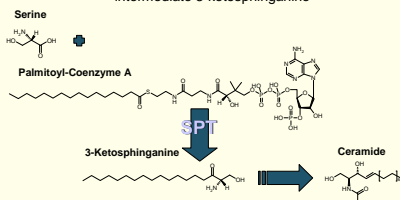
Overview

- Obesity has been linked with increased ceramide accumulation and resultant impaired insulin sensitivity.
- SPT may represent an attractive enzyme to target within the ceramide synthetic pathway in order to treat type 2 diabetes.
- A mass spectrometry based HTS approach to identify inhibitors of mammalian SPT was used to screen a library of >250,000 small molecules.
- Screening identified potent SPT inhibitors from multiple chemotypes.

Introduction

Objective: Develop HTS assay against SPT, an enzyme involved in an early and rate-limiting step in ceramide synthesis

Figure 1: Condensation of palmitoyl-CoA (PCoA) and serine catalyzed by SPT to form the intermediate 3-ketosphinganine



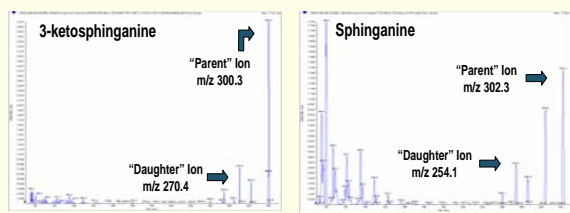
•SPT inhibition is challenging to screen by conventional HTS, however it is well suited to mass spectrometry based detection methods.

Methods

Detection:

The combination of parent molecular mass and fragmentation pattern afforded by triple quadrupole mass spectrometry allows very selective quantitation of label-free SPT product and internal standard with reduced crosstalk from matrix or library compounds.

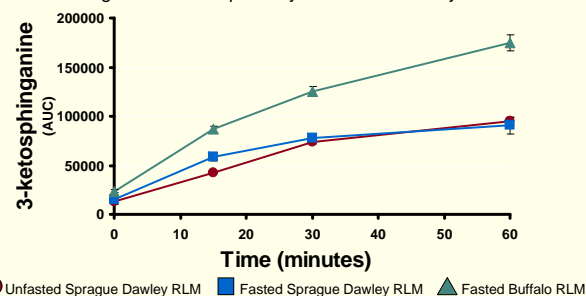
Figure 2: Daughter ion scans of product, 3-ketosphinganine and internal standard, sphinganine



Selection Of Microsomes

- Microsome preps were evaluated for enzymatic activity.

Figure 3: Serine palmitoyltransferase activity in LM's.

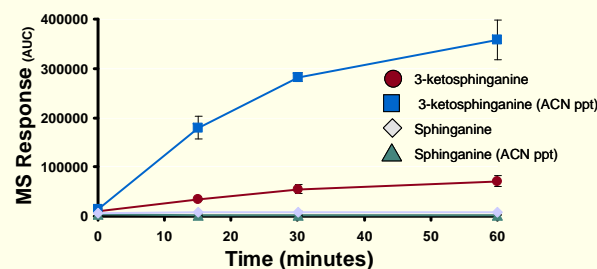


- Buffalo rat liver microsomes were used for subsequent assay development

Optimization of Signal Window

- Limited signal window could be the result of low solubilization of 3-ketosphinganine in reaction mix.
- Hydrophobic interactions with microsome membrane bilayers could render analyte inaccessible to online purification system.
- An acetonitrile precipitation of the reaction mix liberates 3-ketosphinganine and markedly increases signal window.

Figure 4: Effect of ACN precipitation on 3-ketosphinganine response.

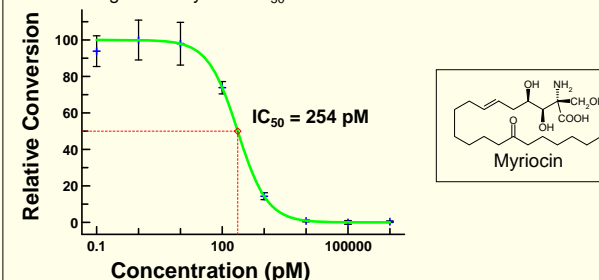


NOTE: The formation of sphinganine, a downstream product, is not observed in this system, indicating that it is a suitable internal standard for the assay

Assay Validation Using Reference Compound

- Myriocin, a known SPT inhibitor, was used to evaluate assay performance.

Figure 5: Myriocin IC₅₀ determination



- The determined IC₅₀ (254pM) value agrees well with published reports (300pM)¹

HTS

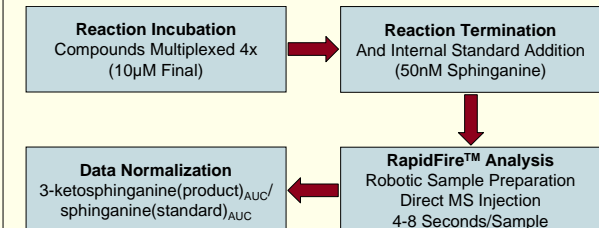
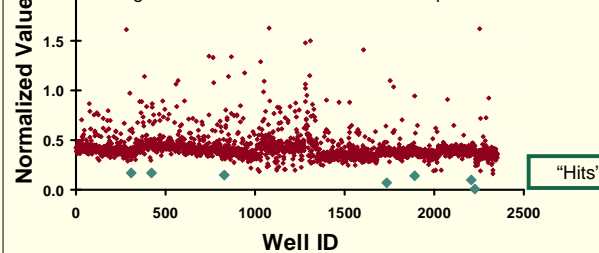


Figure 6: Normalized raw data from 7 plate HTS run



- HTS and confirmation completed in under 4 months.
- Wells detected that demonstrate >-50% inhibition. Possible causes:
 - Library compounds of similar MW
 - Matrix components affecting ionization of product and/or internal std.

Results

Screening Statistics

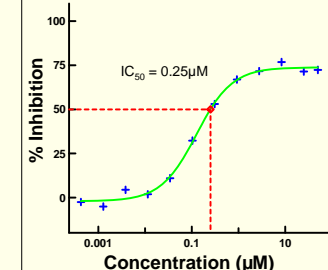
Compounds Tested	252,742
Pass Rate	98.9%
Unconfirmed Hit Rate	1.1%
Z' Factor	>0.5

Confirmation

Compounds Tested	2771
Confirmed Activity	347
IC ₅₀ Determinations	117

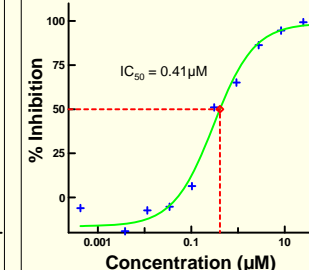
Series A:

- >20 closely related molecules with IC₅₀ values from 100nM to 18µM.
- MW's from 378 – 443.
- All exhibit incomplete inhibition which may reflect 2 distinct pools of SPT activity or allosteric inhibition.



Series B:

- Single molecule in series.
- Complete inhibition of SPT activity observed.
- 4 other molecules also exhibited >90% inhibition with IC₅₀ values from 470nM to 2.15µM.



Conclusions

•A mass spectrometry-based HTS approach was developed to identify inhibitors of SPT, an enzyme that was previously intractable to conventional screening methods.

•The screen successfully identified potent inhibitors of mammalian SPT activity across several distinct chemotypes.

References

1. Yamaji-Hasegawa, A., Takahashi, A., Tetsuka, Y., Senoh, Y. and Kobayashi, T. *Biochemistry* 44, 268-277 (2005)