

Screening LpxC (UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc deacetylase) using BioTrove RapidFire HTS Mass Spectrometry



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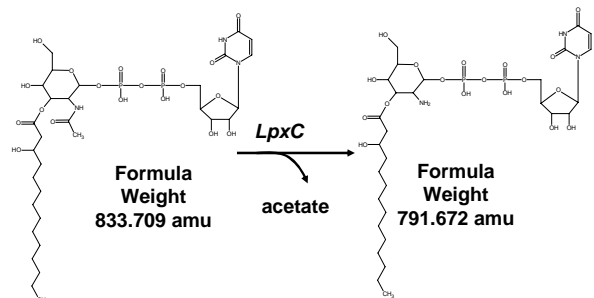
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ABSTRACT

We have developed a high throughput enzymatic assay to detect compounds that inhibit LpxC (UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc deacetylase), an essential enzyme for lipid A biosynthesis in gram negative bacterial pathogens. The system utilizes high throughput mass spectrometry to quantitate the enzymatic deacetylation of the lipid substrate. The process involved the timed reaction of enzyme with substrate, followed by stopping the reaction with acid and freezing the resulting 384-well plates. The plates were then sent to BioTrove Inc, Woburn, MA for quantitation of substrate and product. We demonstrate that this process resulted in a highly robust screening assay, with the mean z'-factor reaching 0.74. Approximately 500 hits were identified by mixture screening of the SPRI compound library – confirmation testing of these compounds is under way. Our results suggest that mass spectrometry-based screening is a valuable tool for detecting inhibitors of enzymatic assays involving difficult to detect reactions.

INTRODUCTION

- LpxC: Essential Target in Lipid A Biosynthesis¹
- Essential for viability of gram-negative bacteria
- Chemically validated: hydroxamate inhibitors have antibacterial activity¹
- Conserved among important gram-negative pathogens
 - >60% identity in the active site
- **THE PROBLEM:** Enzyme difficult to screen using conventional technologies
- **THE SOLUTION:** High Throughput Mass Spectrometry²



BioTrove

<http://www.biotrove.com>

METHODS

- Reaction started by adding 20 μ l LpxC enzyme to compounds (10 μ l) plus substrate (20 μ l) using PLATEMATE PLUS
- After 60 minutes at RT, HCl added (20 μ l 0.25 N) to stop reaction
- Plates sealed, then placed at -80°C (minimum 2 hours)
- Plates shipped to BioTrove, Inc. Woburn, MA for analysis

RESULTS

Figure 1. BioTrove Rapid Fire technology accurately measured LpxC enzymatic activity.

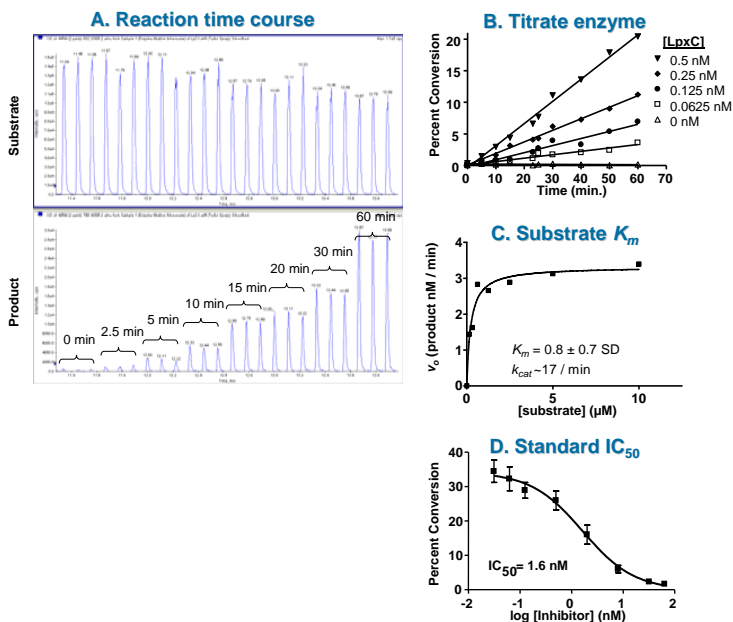


Figure 2. BioTrove Rapid Fire technology was robust in HTS - median z'-factor for mixture screening was 0.74

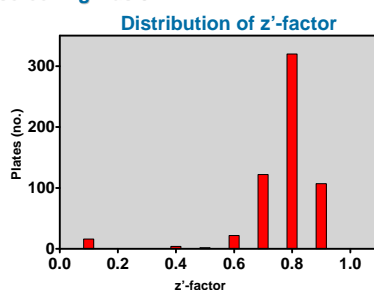


Figure 3. BioTrove Rapid Fire HTS screening campaign identified ~500 hits from mixture screening.

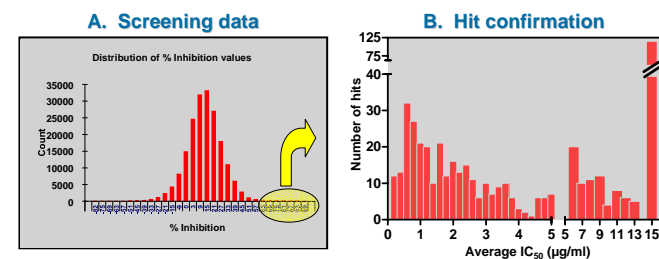


Table 1. Selected hits from the BioTrove Rapid Fire HTS screening campaign were found to bind to LpxC.

Correlation of LpxC IC₅₀ and K_d values

Compound no.	IC ₅₀ (µM)	K _d (µM)
1	0.44 ± 0.01	0.60
2	1.0 ± 0.4	0.90
3	1.6 ± 0.7	1.3
4	3.7 ± 0.7	3.6
5	4.5 ± 0.3	4.3
6	5 ± 1	5.2
7	6 ± 1	5.4

CONCLUSION AND DISCUSSION

- LpxC is an attractive target for antibacterial drug discovery however the reported assay methods were not convenient for high throughput screening.
- The BioTrove RapidFire HTS Mass Spectrometry provided a robust method to screen LpxC using the native reaction substrate.
- Selected hits from the BioTrove screen were confirmed by biophysical analysis having K_d values comparable to the biochemical IC₅₀ values.

BioTrove RapidFire HTS Mass Spectrometry provides a useful alternate screening strategy where native biochemical substrates can be used.

REFERENCES

1. Onishi, HR., et al. 1996. Antibacterial agents that inhibit lipid A biosynthesis. Science. 274(5289):980-982.
2. Ozbal, CC., et al (2004) High throughput screening via mass spectrometry: a case study using acetylcholinesterase. Assay Drug Dev Technol. 2(4): 373-381.