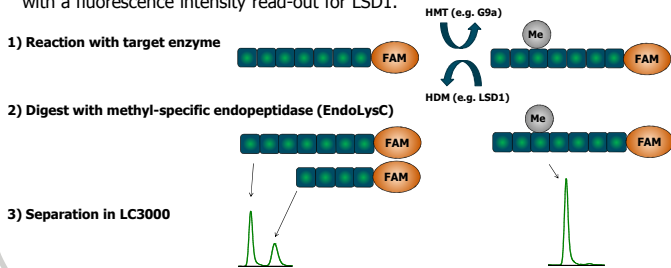


Introduction

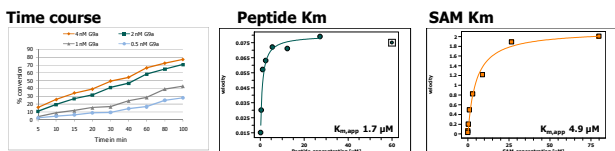
Epigenetic targets have been recognized to be implicated in cancer, inflammatory, neurodegenerative and metabolic diseases. Histone methyltransferases (HMTs) and demethylases are two of several target classes of regulatory enzymes in the epigenetics field which are in the focus of drug discovery research.

Mobility shift assays (MSA) for the HMT G9a and the histone demethylase LSD1 were set up using the Caliper LabChip 3000 microfluidics system (Wigle et al.). For assay validation, potencies of tool compounds (SAH, Sinefungin, BIX-01294 for G9a and Tranylcypromine for LSD1) were determined. In parallel, orthogonal G9a and LSD1 assays were established - namely isotopic FlashPlate and fluorescence lifetime (FLT) assays for G9a and a formaldehyde detection assay with a fluorescence intensity read-out for LSD1.

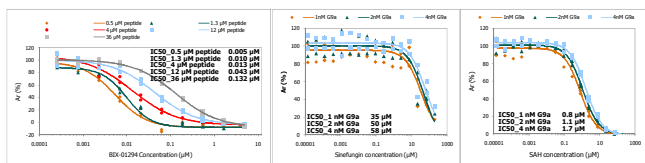


LC3000 HMT and HDM mobility shift assay principle

LC3000 G9a assay development



Reaction linearity at 5 μ M SAM, histone H3 peptide $K_{m,app}$, SAM $K_{m,app}$ and DMSO tolerance (not shown) were determined. Optimized reaction conditions were 5 μ M SAM, 1 nM G9a, 1.3 μ M G9a-FAM peptide, 60 min reaction time, stop by excess of SAH, and > 1h EndoLysC digest.

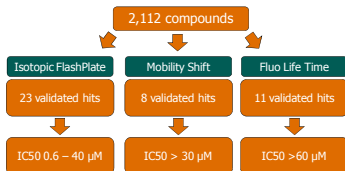


The G9a HMT assay was validated by reference compound titrations. Competitive binding of BIX-01294 to the G9a histone binding site was confirmed by titration against different peptide concentrations. For SAH and sinefungin tight binding inhibition was determined when titrating against different G9a concentrations.

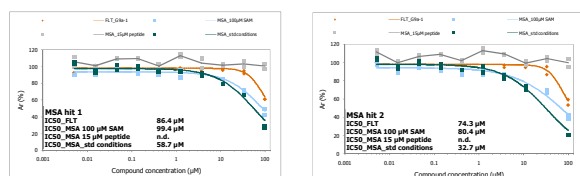
2,112 compounds out of the entire BioFocus compound collection comprising ~870,000 compounds were selected using complementary virtual screening (VS) approaches (Ahrens et al.). The compounds were screened in the G9a LC3000 MSA assay, an orthogonal fluorescence lifetime (FLT) assay and an orthogonal FlashPlate assay.

Results G9a screen

Plate #	Z'	Mean % inhibition control 3 μ M SAH
1	0.84	51
2	0.82	53
3	0.82	48
4	0.83	48
5	0.82	48

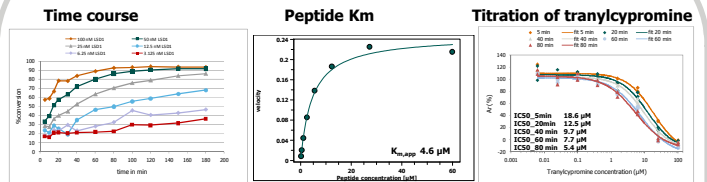


Assay performance in G9a miniscreen and hit validation



For one series of LC3000 G9a hits activity was confirmed in an orthogonal Fluorescence lifetime assay. Experimental evidence suggests that these compounds are competitive for the histone binding pocket of G9a. Inhibition is competed by excess peptide and not SAM. The histone substrate strongly influences the inhibitory potency of this compound series.

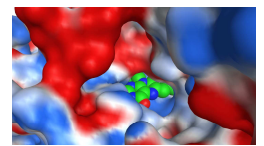
LC3000 LSD1 assay development



Reaction linearity, peptide $K_{m,app}$ and DMSO tolerance were determined. Final assay conditions were 15 nM LSD1 (BPS Bioscience), 2 μ M peptide and a reaction time of 60 min. Reactions were stopped by heat shock, EndoLysC digest was carried out o/n. Reference inhibitor tranylcypromine was titrated and a preincubation time dependent IC50 shift observed. 30 min preincubation time was chosen for screening.

Compound selection: virtual screening (VS) tailored towards LSD1

- No ligand-based VS was applied since known ligands represent structures with highly undesired properties
- Selection based on docking using (Glide; Schrödinger Inc.) the entire diverse BioFocus compound collection (~870,000 compounds) into the flavin cofactor binding pocket
- 25,000 top ranked poses were evaluated using consensus scoring and the BioFocus VS Toolkit which assesses, for example, the snugness of fit for all the poses
- Since only few virtual hits with high ranking was identified in the post-processing of the poses, a second set of fragment-like compounds was added to the selection



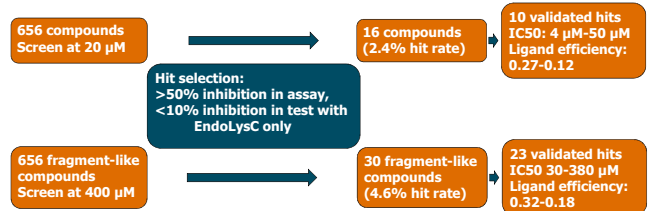
LSD1 binding site with flavin cofactor deeply buried in its binding pocket

Results LSD1 screen

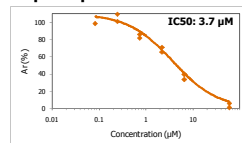
Assay performance in LSD1 miniscreen

Plate #	Z'	Mean % inhibition control 10 μ M tranylcypromine
1 (cpd)	0.50	79
2 (cpd)	0.88	77
3 (fragment-like)	0.81	80
4 (fragment-like)	0.65	90

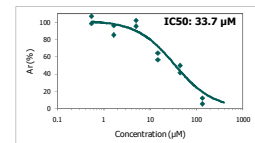
Screening cascade



Top compound



Top fragment-like compound



Conclusions

- Medium throughput MSA assays for detection of G9a and LSD1 activity have been developed and were validated by IC50 determination of reference compounds
- Subsets of the BioFocus library were specifically selected for G9a and LSD1 by virtual screening approaches (G9a and LSD1) or fragment-like properties (LSD1), and selections were screened in the MSA assays
- In both screens active compounds were found and validated in dose response experiments and by compound purity determinations
- Initial mode of binding analysis is presented for G9a hits
- Concurrent validations of compounds in orthogonal assays during the hit finding process provide important additional information and are in line with BioFocus' plans to further add cellular assays and selectivity panels for hit identification with epigenetic targets

References

- Wigle et al. (2010), *Chemistry and Biology*, 17, 695-704
- Ahrens et al. (2011), *Journal of Biomolecular Screening*, in press