Fragment-Based Drug Discovery (FBDD) Approach for Acetylcholinesterase

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KEY FINDING A biophysical approach to finding hits in FBDD

ABSTRACT

Acetylcholinesterase (AChE) is an enzyme that has been identified in Alzheimer's disease (AD). It is thought to accelerate Aβ aggregation by a rapid increase in hydrolysis of acetylcholine leading to a reduction in cholinergic receptor stimulation and memory loss. Design of small molecule AChE inhibitors has been successful but with limited clinical benefits due to extensive side effects. In this study, we followed a fragment-based drug discovery (FBDD) screening approach to provide insight into designing small molecule inhibitors directly targeting AChE.

In collaboration with Liverpool ChiroChem (LCC), we conducted a fragment-based screening study against AChE using surface plasmon resonance (SPR)-based fragment screening. In the first round of the study, an SPR "clean screen" of LCC Fragment Collection was run to identify and remove fragments that bind non-specifically to the Cytiva Series S Sensor Chip CM5. Out of the 960 fragments screened, 89 fragments (9.3%) showed residual binding to the biosensor surface and were omitted from subsequent screens. After the initial clean screen, "binding level screens" were carried out for the remaining 871 fragments to identify binders against the target protein and exclude fragments with atypical binding behavior. Top hits from the binding level screens were then validated by "affinity screens" to verify binders and estimate affinity (K_D).

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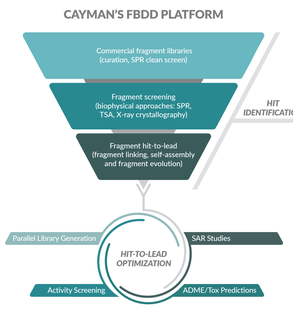

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EXPERIMENTAL PARAMETERS

Parameters	Clean Screen	Binding Screen	Affinity Screen
Inject type	Fast inject	High performance	High performance
Contact time (s)	30	30	30
Dissociation time (s)	0	15	30
Fragment concentration (mM)	1.0	0.25	0.0039-0.5
Start-up cycles	3	3	3
Extra wash	50% DMSO	50% DMSO	50% DMSO
Solvent correction, positive controls, negative controls	No	Yes	Yes - R_{max} control included

EXPERIMENTAL SETUP

- Human soluble AChE was immobilized to a Series S Sensor Chip CM5 (Cytiva) through amine coupling (~12,500 RU).
- SPR experiments were run on a Biacore™ 8K instrument.
- SPR clean screen and binding screen were run at a single concentration, 1 mM and 250 µM, respectively, against a blank dextran surface and target surface. PBS-P+ with 2% DMSO was used as running buffer.
- SPR affinity screen was run with an 8-point concentration range (59 µM to 1,000 µM) against a blank dextran surface and target surface. Solvent correction cycles and positive (**phenserine; Item No. 21060**) and negative controls were included. PBS-P+ with 2% DMSO was used as running buffer.
- Active binders in **Table 1** were modeled using Schrödinger software.

RESULTS

SPR Clean Screen

A clean screen at a concentration of 1 mM was carried out to remove fragments that show reference or target residual binding.

A total of 89 fragments (9.3%) showed residual binding against the CM5 sensor chip and were therefore excluded from future screens.

- 0 fragments showed residual binding to the reference surface only (0.0%)
- 78 fragments showed residual binding to the target surface only (8.12%)
- 11 fragments showed residual binding to both the target and reference surfaces (1.15%)

A total of 871 fragments (90.7%) were included for binding screens.

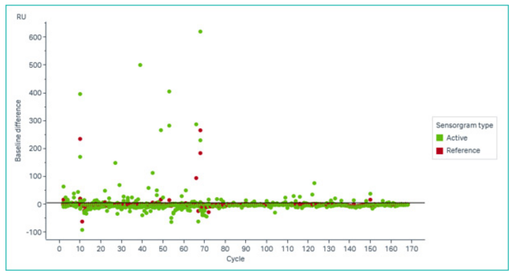


FIGURE 1 – Clean screen plot. A total of 960 fragments were screened at a single concentration against dextran surface (reference) and AChE immobilized target surface (active) on CM5 sensor chip. Clean screen identified 89 fragments (9.3%) as sticky and were omitted from subsequent screens.

SPR Binding Level Screen

In order to provide deeper insight into the binding characteristics of each fragment, a binding level screen was carried out for fragments at a single concentration of 250 µM (excluding 89 fragments mentioned under SPR Clean Screen).

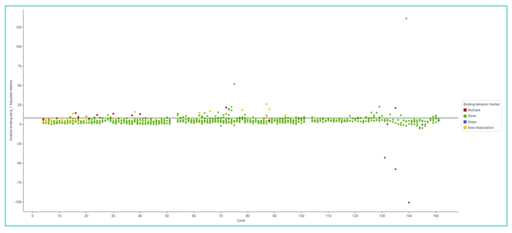


FIGURE 2 – Binding level screen. A total of 871 fragments were screened at a single concentration against the reference and active surface.

AChE SPR Affinity Screen Results

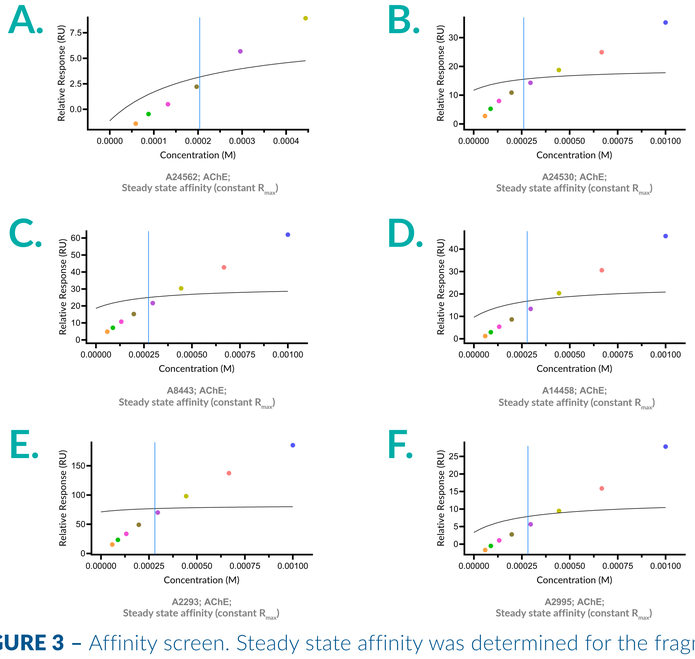


FIGURE 3 – Affinity screen. Steady state affinity was determined for the fragment binding screen hits using an 8-point dose response against the reference and active surface.

RESULTS CONTINUED

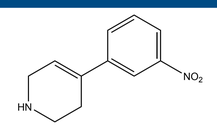
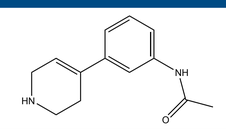
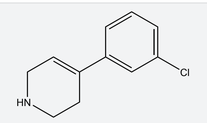
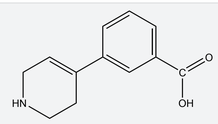
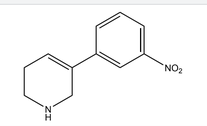
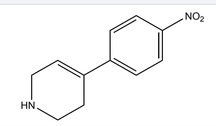
Active (Binders)	Inactive (Non-binders)
	
	
	

TABLE 1 – Initial SAR identified in SPR fragment screen against AChE.

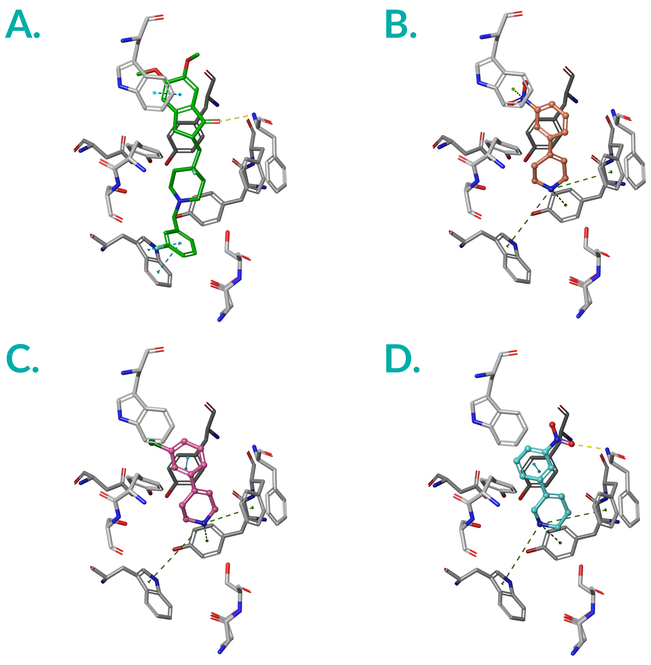


FIGURE 4 – Predicted poses for three of the top scored fragments docked against AChE (7QD9.pdb). AChE is colored grey. **(A)** 7QD9 bound ligand (Control, green) **(B-D)** Active binders from **Table 1**; Top (B, salmon), Middle (C, pink), Bottom (D, cyan).

SUMMARY

- Increase in AChE enzymatic activity is associated with AD. Design of small molecule AChE inhibitors to disrupt rapid enzymatic activity could lead to an improvement of AD symptoms.
- A fragment screen workflow was conducted on a Biacore™ 8K instrument against AChE. 960 fragments from LCC Fragment Collection were screened.
- This work outlines our fragment screening workflow which showcases Cayman's biophysical screening capabilities such as SPR to obtain better enrichment for drug discovery projects.

References

1. Zhou, Y., Fu, Y., Yin, W., *et al.* Kinetics-driven drug design strategy for next-generation acetylcholinesterase inhibitors to clinical candidate. *J. Med. Chem.* **64(4)**, 1844-1855 (2021).
2. Patil, D.N., Patil, S.A., Sistla, S., *et al.* Comparative biophysical characterization: A screening tool for acetylcholinesterase inhibitors. *PLoS One* **14(5)**, e0215291 (2019).

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