Fragment-Based Drug Discovery (FBDD) Approach for TNF-α

Raniya Abdel-Haq[1], Zahra Assar[1], Kendall M. Muzzarelli[1], Andrew Lowerson[2], and Stephen D. Barrett[1]

[1]Cayman Chemical Company, Ann Arbor, Michigan, United States, [2]Key Organics Limited, Camelford, Cornwall, UK

KEY FINDING

Integrated computational and biophysical approaches improve screening efficiency and finding hits in FBDD.

ABSTRACT

Tumor necrosis factor-α (TNF-α) is a cytokine and key mediator in autoimmune diseases such as rheumatoid arthritis, Crohn's disease, and ulcerative colitis. Attempts to design small molecule TNF-α inhibitors have not yet led to approved products due to their low potency and/or high cytotoxicity. In this study, we followed a fragment-based screening approach to provide insight into designing small molecule inhibitors directly targeting TNF-α.

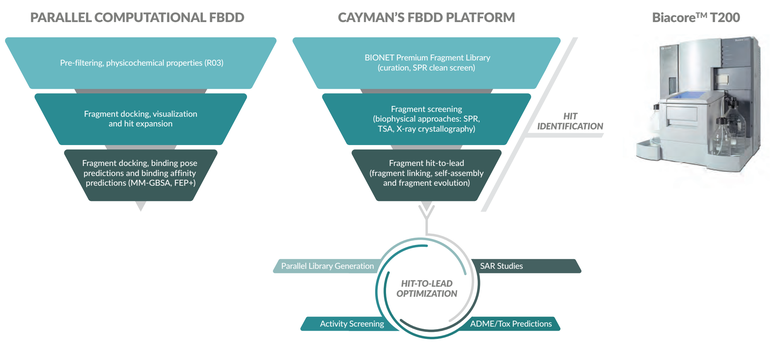
In collaboration with Key Organics Limited, we conducted a fragment-based screening study against TNF-α using our Integrated Medicinal/Computational Chemistry Platform. In the first round of the study, a surface plasmon resonance (SPR) "clean screen" of BIONET Premium Fragment Library was run to identify and remove fragments that bind non-specifically to the Biacore CM7 Sensor Chip. Out of the 1,149 fragments screened, 26 fragments (2.3%) showed residual binding to the biosensor surface and were omitted from subsequent screens. Additionally, 41 other fragments had irregular sensorgrams due to poor solubility in the assay buffer, eliminating them from subsequent screens. After this initial clean-up step, "binding level screens" were carried out for the remaining 1,082 fragments to identify binders against the target protein and exclude fragments with atypical binding behavior. Top hits from the binding level screens will then be validated by "affinity screens" to verify binders and estimate affinity (K_D). In parallel, molecular modeling and X-ray crystallography for these fragments against TNF-α are currently underway in order to develop a structure-activity relationship (SAR) study for TNF-α.

OBJECTIVE

In collaboration with Key Organics Limited, 2nd Generation BIONET Premium Fragment Library was selected to be tested and screened against TNF-α using SPR. All 1,149 fragments in the 2nd Generation Premium Fragment Library were analyzed by ^{1}H NMR previously for:

- Structure verification
- Purity
- Solubility
- Lack of aggregation

WORKFLOW



EXPERIMENTAL SETUP

- Human soluble TNF-α (Cayman Chemical Item No. 32020) was immobilized to a single flow cell of Series S Sensor Chip CM7 (Cytiva) through amine coupling (~8,000 RU).
- SPR experiments were run on a Biacore™ T200 instrument.
- SPR clean screen was run at a single concentration against a blank dextran surface and target surface. Fragments were loaded in 96-well polypropylene microplates and screened at 1 mM in PBS-P$^+$ with 1% dimethyl sulfoxide (DMSO).
- SPR binding screen was run at a single concentration against a blank dextran surface and target surface. Fragments were loaded in 96-well polypropylene microplates and screened at 250 µM in PBS-P$^+$ with 0.5% DMSO. Solvent correction cycles and positive and negative controls were included.
- Computational modeling was performed for this library against human TNF-α (trimer, PDB ID 1TNF and dimer PDB ID 2AZ5 structures) using Schrödinger software.
- SPD-304 (Cayman Chemical Item No. 10008012), a potent TNF-α small molecule inhibitor, was used as a positive control in both biophysical (SPR, X-ray crystallography) and computational experiments.

Parameters	Clean Screen	Binding Screen
Inject Type	Fast inject	High performance
Contact time(s)	30	30
Dissociation time(s)	0	15
Fragment Concentration (mM)	1.0	0.25
Start-up Cycles	3	3
Extra Wash	50% DMSO	50% DMSO
Solvent correction, positive controls, negative controls	No	Yes

RESULTS

Objectives

In order to pre-filter and visualize any fragments in the TNF-α binding site, docking was performed (Glide followed by MM-GBSA for better enrichment) against both trimer (PDB ID 1TNF) and dimer (PDB ID 2AZ5, **Table 1** and **Figure 1**). Top ranked compounds are predicted to bind in the binding pocket of TNF-α near the surface and potentially disrupt the TNF-α quaternary structure, which is required for signal transduction.

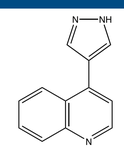
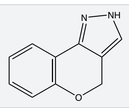
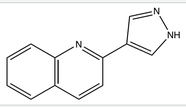
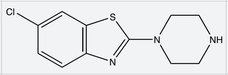
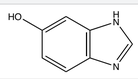
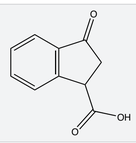
Fragment ID	Structure	Glide gscore (kcal/mol)	Glide emodel	MMGBSA dG Bind
FS-1423		-6.13	-39.00	-39.92
5X-0922		-5.91	-33.47	-37.45
FS-1424		-6.64	-43.34	-37.4
PS-3094		-5.93	-41.27	-35.26
PS-4150		-5.49	-26.00	-27.32
SS-3073		-5.89	-31.08	-24.81

TABLE 1 – Fragment docking results of BIONET Premium Fragment Library against TNF-α (PDB ID 2AZ5). Top scored fragments are listed in the table.

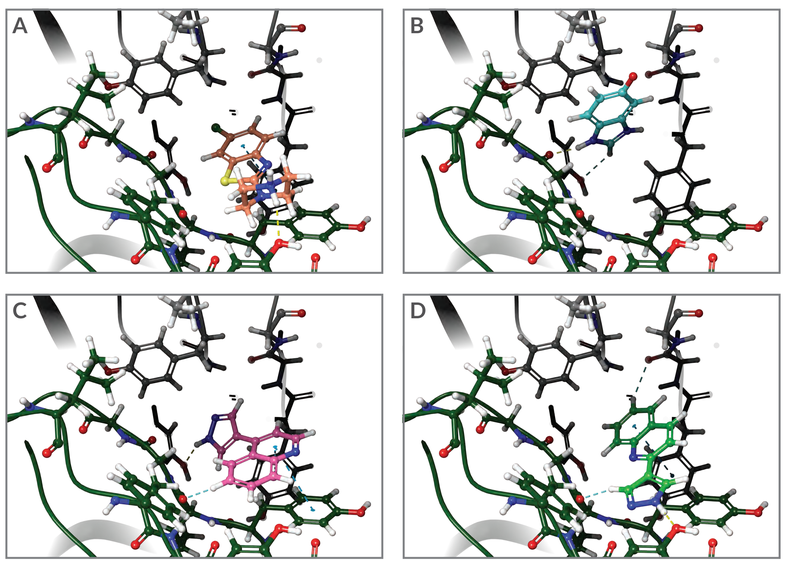


FIGURE 1 – Predicted poses for six of the top scored fragments docked against dimer TNF-α (PDB ID 2AZ5). Chain A and B are colored grey and green, respectively. **(A)** PS-3094, **(B)** PS-4150, **(C)** FS-1423, **(D)** FS-1424, **(E)** 5X-0922, **(F)** SS-3073

SPR Clean Screen

A clean screen of the fragment library was first carried out at a single concentration of 1 mM to identify and remove fragments that show residual binding to the biosensor surface.

A total of 26 fragments (2.3%) showed residual binding against the CM7 sensor chip and were therefore excluded from future screens.

- 1 fragment showed residual binding to the reference surface only (0.09%).
- 23 fragments showed residual binding to the target surface only (2.0%).
- 2 fragments showed residual binding to both the target and reference surfaces (0.17%).

41 fragments (not including 26 above) had erratic/irregular sensorgrams and were therefore excluded from subsequent screens—this is most likely due to poor solubility in the running buffer.

Therefore, a total of 67 fragments (5.8%) were excluded from binding screens against TNF-α.

RESULTS CONTINUED

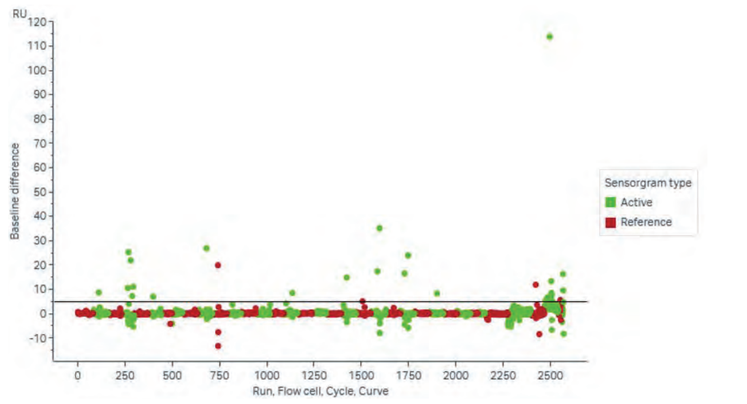


FIGURE 2 – Clean screen plot. A total of 1,149 fragments were screened at a single concentration against dextran surface (reference) and TNF-α immobilized target surface (active) on CM7 sensor chip. Clean screen identified 26 fragments (2.3%) as sticky and were omitted from subsequent screens.

SPR Binding Level Screen

In order to provide deeper insight into the binder characteristics of each fragment, a binding level screen was carried out for fragments at a single concentration of 250 µM (excluding 67 fragments mentioned under SPR Clean Screen).

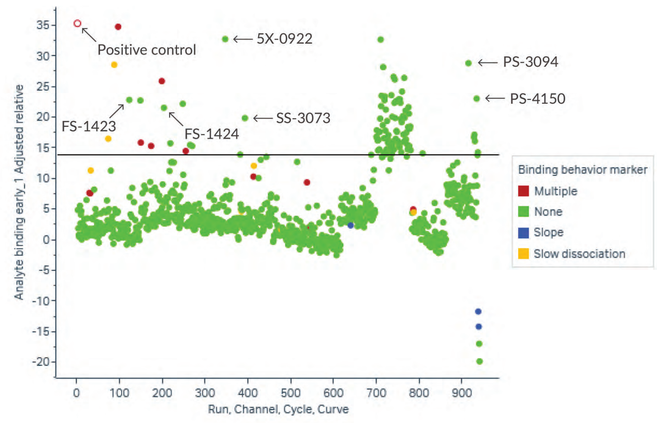


FIGURE 3 – Binding level screen. A total of 1,082 fragments were screened at a single concentration against dextran surface (reference) and TNF-α immobilized target surface (active) on a CM7 sensor chip.

- 91 fragments identified above the cut-off point with well-behaved binder characteristics (shown in green).
- Based on the binding level screens and modeling data, FS-1423, 5X-0922, FS-1424, PS-3094, PS-4150, and SS-3073 are both computational and experimental hits.
- Top hits from binding level screen will then be validated by "affinity screens" to verify binders and estimate affinity (K_D).

SPR Affinity Screen

This study is still ongoing and currently in the affinity screening phase to determine the most affinitive compounds in order to develop a SAR study for the TNF-α target.

Protein Crystallization

Large-scale protein production of TNF-α is currently underway for the purpose of crystallizing TNF-α complexed with top fragment hits from affinity screening.

SUMMARY

- TNF-α signaling is associated with many inflammatory diseases such as rheumatoid arthritis. A possible mechanism of antagonism is to disrupt the trimeric TNF-α quaternary structure, which is required for signal transduction.
- A fragment screen workflow was conducted on a Biacore™ T200 instrument against TNF-α in combination with computational FBDD. 1,149 fragments from BIONET Premium Fragment Library were screened.
- This work outlines our fragment screening workflow which consists of an established Integrated Medicinal/Computational Chemistry Platform tailored to biophysical screening capabilities such as SPR to obtain better enrichment for drug discovery projects.

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References

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