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

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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 and Crohn's disease. Attempts to design small molecules directed to this cytokine have not led to approved products yet.

In collaboration with Key Organics Limited, we conducted a fragment-based screening study against TNF-α. For this study, a surface plasmon resonance (SPR) "clean screen" of BIONET Premium Fragment Library was performed to remove compounds that bind non-specifically to the biosensor chip surface. A total of 1,149 fragments were screened against a blank dextran surface (reference flow cell) and immobilized TNF-α on the surface (active flow cell) on a CM7 sensor chip at a concentration of 1 mM. The clean screen identified 26 fragments (2.3%) as "sticky" and are to be excluded from future screens. Also, 41 fragments had erratic/irregular sensorgrams (due to solubility issues) and will therefore also be excluded from future screens. In total, 67 (5.8%) of fragments were excluded from binding studies against TNF-α. Binding studies were performed for the remaining fragments. As a complementary approach, molecular modeling for these fragments were performed against TNF-α using Schrödinger software. Docking results of the top-ranked fragments correlates with the SPR binding screen. This study is still ongoing and currently is in "affinity screening" phase to determine the most affinitive compounds in order to develop a structure-activity relationship (SAR) study for TNF-α target.

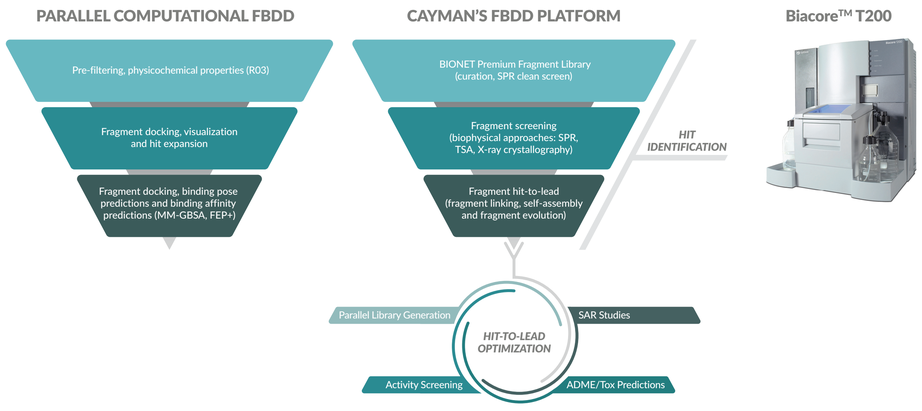
This work outlines our fragment screening workflow which consists of an established integrated Medicinal/Computational Chemistry Platform (CADD) tailored to biophysical screening capabilities such as SPR to obtain better enrichment for drug discovery projects.

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 1H NMR previously for:

- Structure verification
- Purity
- Solubility
- Lack of aggregation

WORKFLOW



For this study, 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).

In the meantime, an SPR clean screen was performed on the BIONET Premium Fragment Library to help identify residual binding of fragments to the biosensor surface. The clean screen is run at a single concentration (1 mM) against a blank dextran surface as well as immobilized target protein. Fragments that show residual binding are removed from subsequent screening steps.

- Fragments were loaded in 96-well PP microplates and screened at 1 mM in PBS-P+ with 1% dimethyl sulfoxide (DMSO).
- Computational modeling was performed for this library against human TNF-α (trimer, PDB ID 1TNF and dimer, PDB ID 2AZ5 structures) using Schrödinger software.

SPR Clean Screen Settings

Parameter	Setting
Inject type	Fast inject
Contact time (s)	30
Dissociation time (s)	0
Fragment concentration (mM)	1.0
Start-up cycles	3
Extra wash	50% DMSO

RESULTS

Computational FBDD

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.

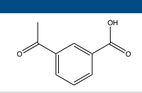
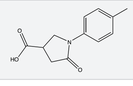
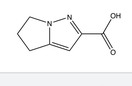
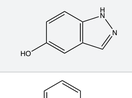
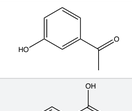
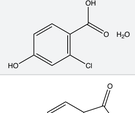
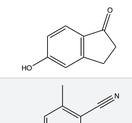
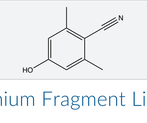
Fragment ID	SMILES	Structures	Glide gscore (kcal/mol)	Glide emodel	MMGBSA dG Bind
PS-3016	O=C(O)C1(=CC(C(=O)C)=CC=C1)		-5.556	-37.481	-20.73
AS-5481	O=C2(N(C1(=CC=C(C)C=C1))CC(C2)C(=O)O)		-5.552	-38.418	-21.3
FS-3146	O=C(O)C1(=NN2(CCCC2(=C1)))		-5.625	-36.612	-17.27
PS-3678	OC2(=CC1(=C(NN=C1)C=C2))		-5.532	-28.201	-22.45
PS-3431	O=C(C1(=CC(O)=CC=C1))C		-5.808	-30.927	-28.26
PS-4320	ClC1(=C(C(=O)O)C=CC(=C1)O).O		-6.132	-37.072	-15.91
PS-3391	O=C2(C1(=C(C=C(O)C=C1)CC2))		-5.575	-24.383	-28.04
PS-4472	OC1(=CC(=C(C#N)C(=C1)C)C)		-5.613	-27.193	-28.91

TABLE 1 – Fragment docking results of BIONET Premium Fragment Library, plate 1 against TNF-α (PDB ID 2AZ5). Top scored fragments (nearly 0.8%) are listed in the table.

RESULTS CONTINUED

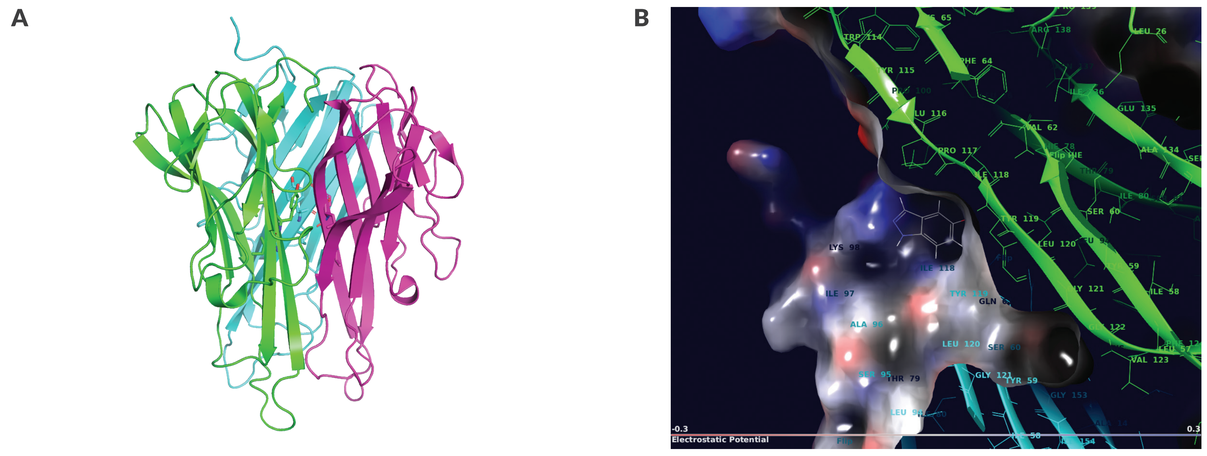


FIGURE 1 – (A) Crystal structure of trimer TNF-α (PDB ID 1TNF). Each chain is color coded. Ile[118] and Tyr[119] were shown in sticks. **(B)** Predicted pose for one of the top scored fragments (PS-3678) docked against dimer TNF-α (PDB ID 2AZ5).

SPR Clean Screen

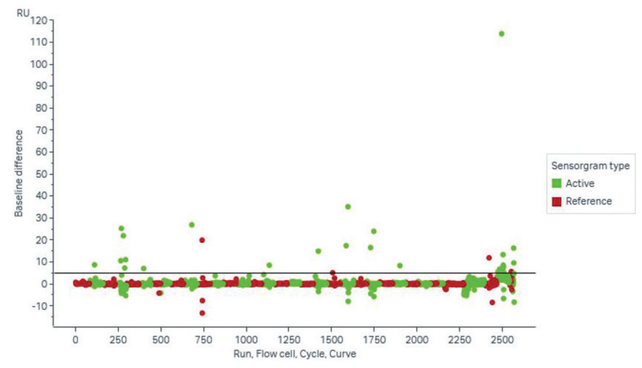


FIGURE 2 – Clean screen plot. A total of 1,149 fragments were screened against dextran surface (reference) and TNF-α immobilized target surface (active) on CM7 sensor chip at a concentration of 1 mM. The clean screen identified 26 fragments (2.3%) as sticky and are excluded from future screens.

- Only 1 fragment showed residual binding to the unmodified surface (reference flow cell).
- 23 fragments showed residual binding to target surface only (2.0%).
- 2 fragments showed residual binding to both the target and reference surface (0.17%).
- 41 fragments (not including 26 above) had erratic/irregular sensorgrams and will therefore also be excluded from future screens—this is most likely due to poor solubility in the running buffer.
  - A total of 67 fragments (5.8%) were excluded from binding studies against TNF-α going forward.

SPR Binding Level Screen

In order to provide deeper insights into binding characteristics, a binding level screen was performed for fragments at a single concentration of 250 µM (excluding 67 fragments mentioned in SPR clean screen).

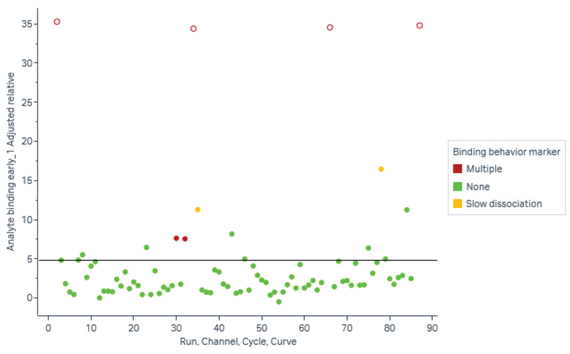


FIGURE 3 – Binding level screen. A total of 79 fragments were screened at a single concentration against the TNF-α immobilized target surface and blank surface on a CM7 sensor chip. 8 fragments above the defined cut-off point with well-behaving binding characteristics (green) were identified. Cycle 47 is related to PS-3678 run. These will be taken forward for subsequent screening.

- Fragments that are above the cut-off point and show typical binding are G7, G12, E5, E11, F3, A12, B8, and E3.
- Atypical binding behavior: yellow is slow dissociation and red is slope (slope during injection instead of expected rapid binding to steady-state level).
- Based on the binding screen on the first plate and modeling data, G7 (PS-3678) is both computational and experimental hit so far.
- This phase is still ongoing. The remaining fragments from BIONET Premium Fragment Library are being screened against TNF-α.

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 TNF-α target.

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.
- Fragment screen workflow was conducted on Biacore™ T200 tailored with computational FBDD. 1,149 fragments were screened.
- This work summarizes our fragment screening workflow which consists of an established integrated Medicinal/Computational Chemistry Platform (CADD) tailored to biophysical screening capabilities such as SPR to obtain more efficient screening hits and enrichment for drug discovery projects.

References

1. Niu, J., Cederstrand, A.J., Eddinger, G.A., *et al*. Trimer-to-monomer disruption mechanism for a potent, protease-resistant antagonist of tumor necrosis factor-α signaling. *J. Am. Chem. Soc.* **144(22)**, 9610-9617 (2022).
2. He, M.M., Smith, A.S., Oslob, J.D., *et al.* Small-molecule inhibition of TNF-α. *Science* **310(5750)**, 1022-1025 (2005).
3. Blevitt, J.M., Hack, M.D., Herman, K.L., *et al*. Structural basis of small-molecule aggregate induced inhibition of a protein–protein interaction. *J. Med. Chem.* **60(8)**, 3511-3517 (2017).
4. Hofmann, D., Salmon, L., and Wider, G. Activity of tumor necrosis factor α is modulated by dynamic conformational rearrangements. *J. Am. Chem. Soc.* **140(1)**, 167-175 (2018).