Case Study: Design of LTA4H Inhibitors – CSS Power in Turning Fragments to Leads

LTA4H was screened with a library of ~1,300 fragments[1], and two fragment-binding pockets were identified. The first pocket contained a series of fragments consisting of two aromatic rings with variable 2-3 bond linkers; the second pocket unvaryingly contained an acetate ion (Figure 1).

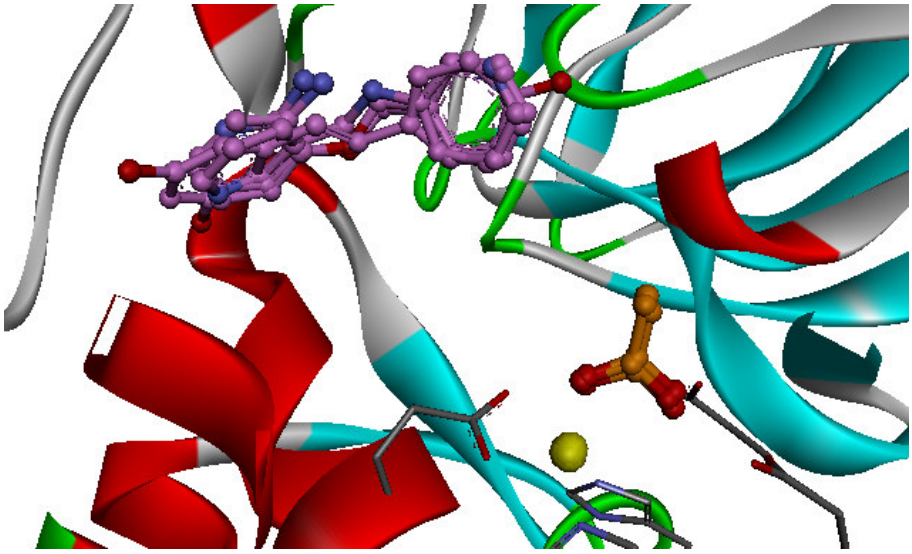


Figure 1: Example fragment hits. Pink fragments (with oxygen atoms in red and nitrogen in blue) depict a series of fragments containing two aromatic rings, connected by various 2 and 3 bond linkers. Orange fragments depict acetate ions from multiple screens. The yellow atom depicts a zinc ion, present in all screens in the same spatial position. These fragments are a representative set of those used to construct the model used by CSS

The 2-ring fragments were tested for enzymatic inhibition of LTA4H, and demonstrated IC_{50}s in the range of ~200μM to >1000μM[1].

Turning Fragments into drug-like molecules using CSS

Our goal was to design drug-like molecules containing two-ring fragments in the first pocket with a carboxylate group spatially oriented in the position of the acetated ion observed in the fragment screens in the second pocket. We generated models consisting of either (i) the exact fragments identified in the fragment screens, (ii) fragments containing two aromatic rings in the position identified in the screens, connected by linkers that are chemically similar to those identified in the screens, or (iii) structures which contain the fragments identified in the screens as sub-structures. Screening of our "super molecule" library with CSS identified 1000s of candidate compounds that contain either the identified fragments, or highly similar fragments in the correct orientation, with a correctly positioned carboxylate group in the position of the acetate ion. These compounds were ranked using an empirical energy function, and clustered by chemical similarity.

Examples of high-ranking compounds suggested by CSS, overlaid on the original fragment hits are depicted in Figure 2.

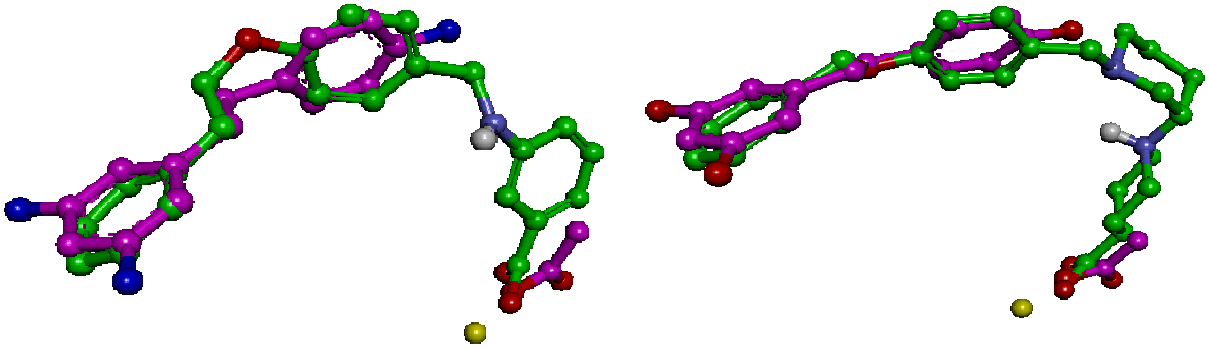
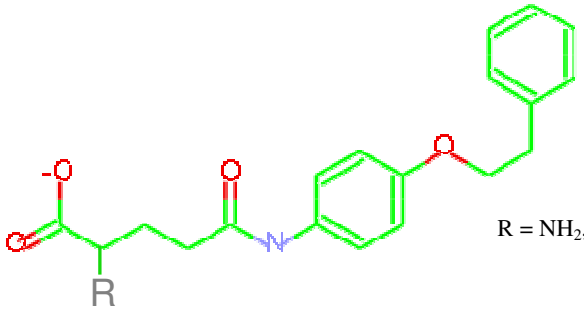


Figure 2: Example of CSS "hits" overlaid on two fragments identified by X-ray crystallography. Fragments are colored pink (with oxygen atoms in red, nitrogen in blue and zinc yellow); suggested CSS "hits" are colored in green (with oxygen and nitrogen atoms as for fragments).

A screen of the scientific literature demonstrated that several of the CSS "hits" are highly similar to known LTA4H inhibitors. An example of such a pair is depicted in Figure 3.



R = NH_2, Kirkland et al. (2008)[2], IC_{50}=46nM

R = H, CSS "hit"

Figure 3: Example of CSS "hit" similar to know LTA4H inhibitor.

Additional "hits" with chemical similarity, and strong potential for structural similarity to known compounds were also found. An example of such a "hit" can be seen in Figure 4. The linker in the known compound[3], which has an IC_{50} of 2nM, is comprised of two rings, a benzene ring and a piperidine, whereas in the CSS "hit" this region is comprised of two piperidine rings.

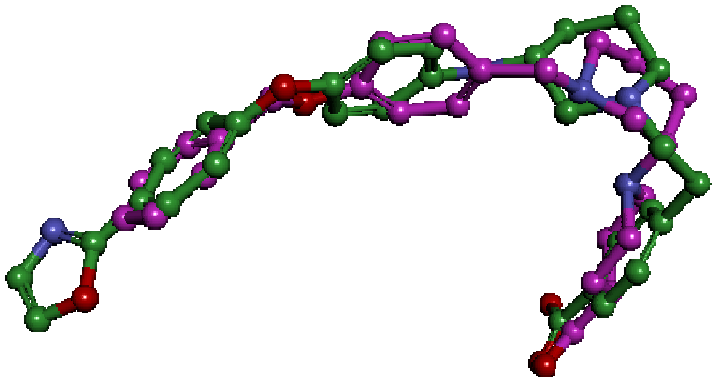
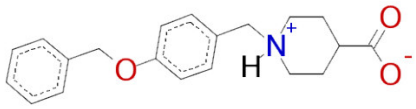
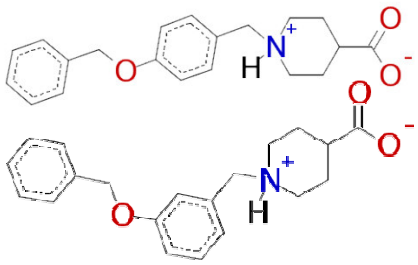
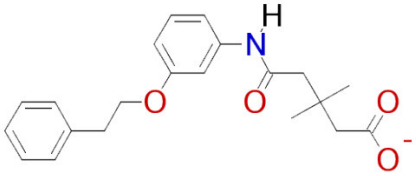
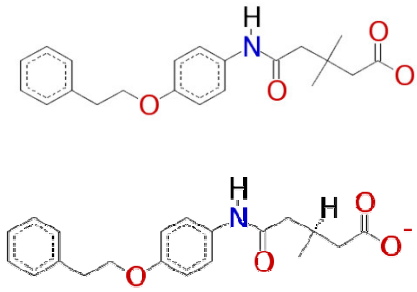
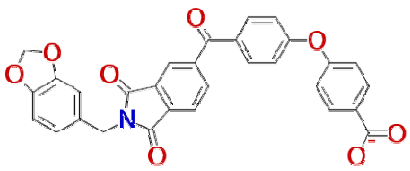
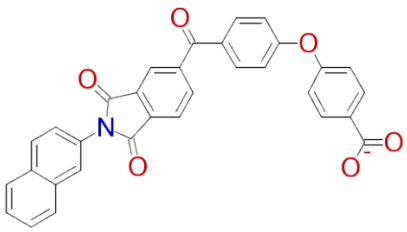
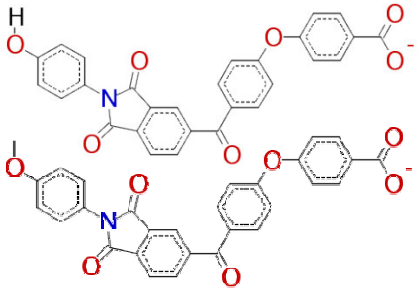
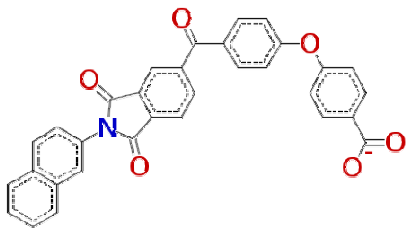


Figure 4: Example of CSS "hit" similar to known inhibitor. CSS "hit in pink (with oxygen atoms in red and nitrogen in blue), known inhibitor from Khim et al. (2008)[3], in green with oxygen and nitrogen as for CSS "hit". IC_{50} of known hit is 2nM

Comparing all "hits" from our top scoring clusters to a database of commercially available compounds, we identified 7 purchasable compounds, either identical or chemically similar to our "hits", representing 3 different clusters. These compounds were tested for inhibition, and the results are summarized in Table I.

Table I: Inhibition of peptidase activity of LTA4H* by analogs of CSS "hits"

CSS "hit"	Commercially available analog	Cluster	IC_{50}(μM)
		3	0.9 15.9
		2	45.9 211.3
 	 	5	2.5 1.7 2.0

*enzyme inhibition assays were performed as in reference 1.

The hit from cluster 3 represents a nearly exact match to experimentally observed fragments (the experimental fragment contained a pyridine in place of the central benzene ring, and an amine group branching from this ring); the linker is identical to the linker reported in Rao et al.[4] The CSS hit, and a close analog in which the rightmost ring is connected via the meta position were both tested. The exact CSS hit demonstrated an IC_{50} of 0.9□M, more than 100-fold higher than the original fragment used for the computational screen (308□M)[1].

Cluster 2 contained a series of compounds in which the linkers in the two-ring fragments contained a linker that was one bond longer than the linkers in the experimentally identified fragments. Here also we acquired a compound identical to the CSS hit, and a close analog, lacking one of the methyl groups in the linker. Both were active.

Cluster 5 contained compounds in which the experimentally identified fragments were sub-structures of larger ring systems. The first CSS hit represented compounds that contain benzodioxole overlapping the leftmost ring of the fragments. The most similar commercially available analogs contained either phenol or anisole in this position. Both were acquired, and demonstrated high levels of inhibition. The second CSS hit from this cluster contained a naphthalene ring in this position, and was commercially available. Its IC_{50} was 2.0□M.

These results demonstrate the power of CSS to design compounds that properly connect distant fragments identified by fragment-based technologies, and provide super-additivity in binding affinity – one of the toughest challenges in FBDD today. Despite the limiting constraint of commercial availability, all tested compounds were active, with the most active more than two orders of magnitude higher than the binding of the highest affinity fragments.

References

1) Davies et al. (2009) Discovery of Leukotriene A4 Hydrolase Inhibitors Using Metabolomics Biased Fragment Crystallography. **J. Med. Chem**. 52, 4694.
2) Kirkland et al. (2008) Synthesis of glutamic acid analogs as potent inhibitors of leukotriene A4 hydrolase. **Bioorg. Med. Chem.** 16, 4963.
3) Khim et al. (2008) Discovery of novel and potent aryl diamines as leukotriene A4 hydrolase inhibitors. **Bioorg. Med. Chem. Lett.** 18, 3895.
4) Rao et al. (2007) Anti-Inflammatory Activity of a Potent, Selective Leukotriene A4 Hydrolase Inhibitor in Comparison with the 5-Lipoxygenase Inhibitor Zileuton. **The Journal of Pharmacology and Experimental Therapeutics** 321, 1154.