Optimized Fatty Acid Binding Protein Inhibitors: Augmenting the Viability of Novel Pain-Relief Mechanism

by Alby J. Joseph

Abstract—The risks and side-effects associated with current pain treatments have prompted the investigation of novel pain-relief mechanisms. This study focused on the inhibition of fatty acid binding proteins (FABPs), such as FABP5, as a novel therapeutic pathway. Although previous studies have named SB-FI-26 as a potent FABP inhibitor, the compound has yet to be optimized to create a more practical drug candidate. In this study, a novel drug candidate (SB-FI-95) with greater binding affinity and selectivity to FABP5 was developed *in silico*, synthesized, and evaluated *in vitro*. Computational and *in vitro* results ultimately pointed to a new class of mono-amide α-truxillic acids with fused ring moieties as potential next-generation pain-relieving agents.

I. INTRODUCTION

Chronic pain has become a very prevalent public health issue, affecting over 100 million people in the United States alone. [1] The numerous side-effects associated with the use of over-the-counter (OTC) medications for pain-relief have brought about the need for novel therapeutic targets. Contemporary research has sought to selectively increase concentrations of anandamide (AEA), an endogenous neurotransmitter that exhibits analgesic properties. To do so, studies have sought to selectively inhibit FABPs, which serve as intracellular carriers that transport AEA to its catabolic enzyme. This mechanism of relieving pain and inflammation would provide a much more natural and localized approach that could potentially avoid the risk of systemic side effects.

Previous studies named SB-FI-26, a mono-ester α-truxillic acid, as a potent inhibitor, citing the compound's anti-nociveptive and anti-inflammatory effects *in vivo* due to its inhibitory activity against FABP5 (epidermal FABP). [2] As such, research has focused on mono-ester α-truxillic acids, with minimal research on mono-amide α-truxillic acids. Nonetheless, SB-FI-26 must be optimized in terms of its binding affinity and selectivity to FABP5 in order to become a more practical analgesic drug candidate.

II. MATERIALS AND METHODS

A four step approach was employed to develop a novel therapeutic drug candidate. First, novel FABP5 inhibitors were computationally designed. This library of novel inhibitors featured mono-esters, mono-amides, and different stereoisomers. Binding affinities of these compounds were then analyzed *in silico* using grid-based molecular docking via AutoDock Vina. Subsequently, the lead compound from this computational study was chemically synthesized and then evaluated *in vitro* through a fluorescence displacement assay to determine inhibitory activity. [3]

III. RESULTS AND DISCUSSION

After analyzing over 250 potential inhibitors *in silico*, 70 hit compounds with greater binding affinity than SB-FI-26 were identified and one lead compound, SB-FI-95, was chosen. Computational results indicated that >75% of the hit compounds were mono-amide α-truxillic acids, such as SB-FI-95, and that >80% of the 'hit compounds' contained a fused ring moiety, such as SB-FI-26 and SB-FI-95. Although stereoisomeric effects were significant for each individual compound, no particular stereoisomer exhibited greater binding affinity overall. SB-FI-95 scored much higher in binding affinity to FABP5 than SB-FI-26 and showed greater selectivity to FABP5 *in silico*.

TABLE I. BINDING AFFINITIES OF SB-FI-26 AND SB-FI-95 TO FABP5

Compound	AutoDock Vina Binding Energy Scores (kcal/mol)		
	R,R Enantiomer	*S,S* Enantiomer	γ stereoisomer
SB-FI-26	8.0	7.8	8.4
SB-FI-95	9.0	9.5	9.3

SB-FI-95 also exhibited a very similar binding geometry as SB-FI-26, giving promise to SB-FI-95 as a potential FABP5 inhibitor. Although not better than SB-FI-26, SB-FI-95 demonstrated good inhibitory activity *in vitro* with a Ki value of 3.4 μM against FABP5.

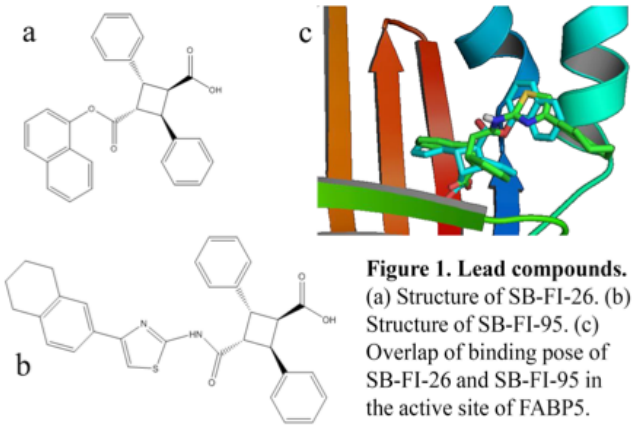

Figure 1. Lead compounds. (a) Structure of SB-FI-26. (b) Structure of SB-FI-95. (c) Overlap of binding pose of SB-FI-26 and SB-FI-95 in the active site of FABP5.

IV. CONCLUSION AND FUTURE WORK

Practically, SB-FI-95 may not be effective enough to serve as an FABP5 inhibitor in its current form; however, further *in vitro* testing should be performed to verify its activity against FABP7, another therapeutic target. The data supports the conclusion that a greater focus should be placed on mono-amide α-truxillic acid derivatives, especially those containing a fused ring moiety, due to these specific compounds' demonstrated affinity for FABP5 *in silico* and *in vitro*. The continued use of computational methods for lead optimization can help narrow the search for more potent, yet selective, FABP inhibitors and thereby a new class of next-generation analgesic agents.

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VI. REFERENCES

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