Design and Synthesis of Endocannabinoid Enzyme Inhibitors for Potential Peripherally Selective Glaucoma Treatments

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Abstract

Glaucoma – an eye disease characterized by increased intraocular pressure (IOP) – is the second leading cause of blindness internationally. Patients experience acute damage to the optic nerve causing blindness, however, lowering the intraocular pressure of the eye can allow for less progressive retinal ganglion loss. Cannabinoid receptors 1 and 2 (CB1 and CB2) are G protein coupled receptors that are found in the glaucoma affected areas in the eye. These endocannabinoid receptors are activated by agonists anandamide (AEA) and 2-arachyidonoylglycerol (2-AG), which according to literature contain promising retinal ganglion cell protective properties. Endocannabinoids have been observed to increase blood flow to the optic nerve head which could potentially be vasoprotective and benefit the altered hemodynamics seen in glaucoma. AEA and 2-AG are broken down by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), which leads to increased IOP, therefore, the primary goal of this project is to design and synthesize peripherally selective (unable to cross the blood brain barrier) FAAH and MAGL inhibitors. Our research involves novel synthetic methods to synthesize sulfonamide compounds to inhibit the breakdown of the agonists. The compounds made were tested for confirmation of inhibitive properties via a FAAH kit, which utilized a which utilized a known inhibitor to compare efficacy. Increasing the number of cannabinoids by way of enzyme inhibition would be expected to lower intraocular pressure in glaucoma patients, which is the preferred outcome.

Background

The endocannabinoid system is a neuromodulatory system that has been shown to suppress pain, relieve nausea, modulate synaptic plasticity, and enhance wound healing. This system is popular for being stimulated by the psychoactive cannabinoid, tetrahydrocannabinol (THC). The activation of the endocannabinoid system occurs through the interaction of endocannabinoids with the CB1 and CB2 receptors. It has been demonstrated that drugs with peripheral selectivity that stimulate activity of these receptors have a range of therapeutic benefits, including enhanced healing of eye wounds. The CB1 receptor, which is ubiquitous in the human body, can be stimulated by biomolecules known as endocannabinoids. While there are multiple endogenous cannabinoid, the two most well characterized and abundant are AEA and 2-AG. Despite their abundance, the enzymes FAAH and MAGL rapidly hydrolyze them. In this project, FAAH and MAGL were targeted for therapeutic inhibition to enhance CB1 activation.

Introduction

Increased AEA and 2-AG levels in vivo have been shown to be of therapeutic benefit. This can be achieved through the dual inhibition of the hydrolase enzymes, FAAH and MAGL. For this purpose, dual FAAH/MAGL inhibitors were designed to be peripherally selective in order to increase AEA and 2-AG levels in the periphery. Sulfonamide derivatives of JZL195 containing carbamate functionalities in the southern region of the inhibitor compounds were produced using novel carbamate exchange reactions. Polar functionalities were introduced to increase their high topological surface area (TPSA) to prevent the crossing of these inhibitors through the blood-brain barrier (BBB), which in turn prevents any potential adverse effects on the central nervous system. The potency of each compound was measured using inhibitor screening assay kits for FAAH and MAGL.

Methods

The inhibitors were synthesized using carbamoylation reaction using carbamoylimidazolium salts, as depicted in Scheme 1. Carbamoylimidazolium salts were initially synthesized and this was reacted with wither a pyridine or quinoline to form the desired compound. After the compounds were synthesized, they were purified using radial preparative layer chromatography (RPLC). Liquid chromatography-mass spectrometry (LCMS) and nuclear magnetic resonance (NMR) were used to confirm the desired product. The potency of each compound was measured using inhibitor screening assay kits for FAAH and MAGL, utilizing JZL195 as standard, a well-characterized dual inhibitor of FAAH and MAGL.

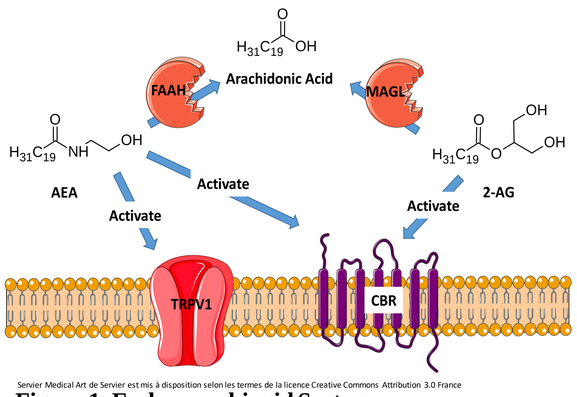
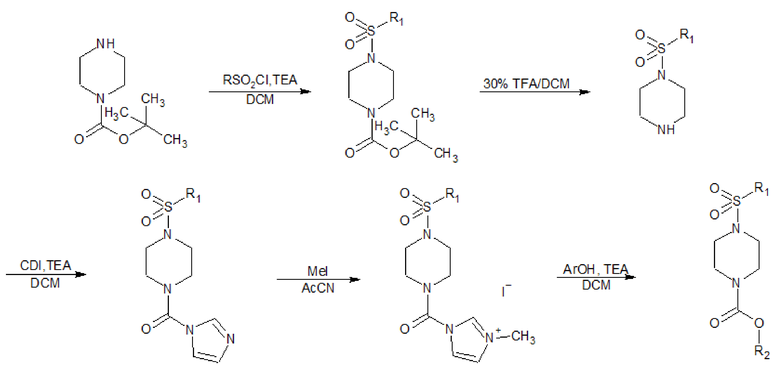


Figure 1. Endocannabinoid System



Scheme 1. (fill in name for reactions above)

Figure 2. Dual FAAH/MAGL Inhibitor to be Modified

(data to be added)

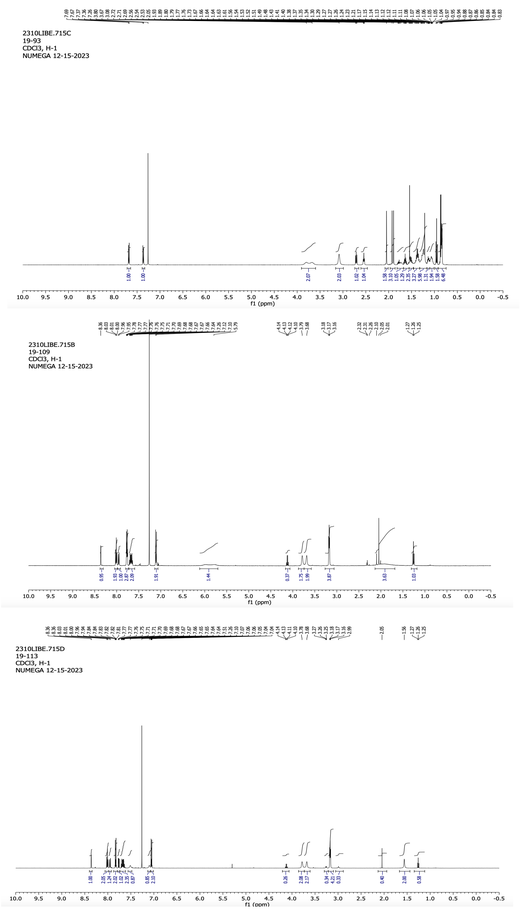


Figure 3. NMR identification of the desired compounds.

Figure 4. Graph pad data to be added

Table 1. FAAH and MAGL Assay Results

Type	Northern Region	Southern Region	IC50 FAAH (μM)	IC50 MAGL (μM)
1	A			
2	A			
3	B			
4	B			

Results and Conclusion

The compounds tested using enzyme inhibitor assays were all found to be potent inhibitors of FAAH and MAGL. Though potent for both, compounds showed greater efficacy against FAAH in the dose-response curves. Further optimization of the structures along with an additional characterization of compounds has been planned, including further work with FAAH and MAGL using advanced activity-based protein profiling assays.

Future Work

Our research has focused on synthesizing and evaluating the effectiveness of FAAH and MAGL inhibitors. Our ongoing research is focused on modifying the southern region of previously identified sulfonamides. Future plans include evaluating the efficacy of the synthesized compounds to inhibit MAGL via a kit of similar nature.

Acknowledgments

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