Design and Synthesis of reversible covalent inhibitors for MAGL and ERO1 for the treatment of Cancer

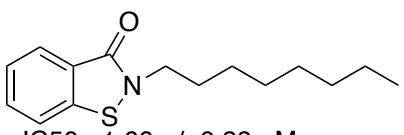
Covalent Inhibitors, play an important role in medicinal chemistry. Generally, they covalently modify the enzyme and consequently deactivate it irreversibly, but in some cases, they can act reversibly. Small bioactive molecule that covalently bind irreversibly to their target have several advantages over conventional reversible inhibitors such as an increased duration of action, less-frequent drug dosing, reduced pharmacokinetic sensitivity, and the potential to target binding sites normally difficult to reach. Despite this, the key challenges of irreversible covalent drugs are their potential for off-target toxicities and immunogenicity risks. The idea is to target different enzymes developing reversible covalent inhibitors, these are not permanently bound and can be released from off-target proteins reducing toxicity.
This project is based on two enzymes to which the pharmaceutical world is paying special attention, that could be targeted as discussed before, in particular monoacylglycerol lipase (MAGL) and Endoplasmic reticulum oxidoreductin 1 (ERO1).

MAGL is a serine hydrolase that cleaves medium- and long-chain monoacylglycerols into fatty acid and glycerol, with the highest rate of arachidonic acid derivatives.
MAGL expression is widespread in the body. The main role of MAGL in the central nervous system is to catalyze the hydrolytic deactivation of 2-arachidonoyl-sn-glycerol (2-AG), an endogenous antagonist of CB1 and CB2 cannabinoid receptors.
In addition to central actions, 2-AG acts on peripheral CB1 and CB2 receptors to mediate immunomodulation and inhibition of cancer cell growth, among other effects.

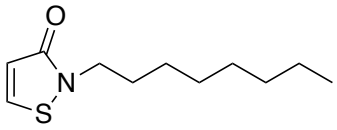
The enzyme ERO1 is involved in the process of functional protein synthesis within the endoplasmic reticulum. Protein folding is an oxidative process that involves the formation of disulfide bonds by the action of two main enzymes: PDI or protein disulfide isomerase, which is responsible for the formation, breaking and isomerization of disulfide bonds; and ERO1 or RE oxidoreductin 1, an FAD-containing oxidative enzyme that enables the reoxidation of PDI. Since the byproduct that originates at the end of each oxidative cycle constitutes ROS (free oxygen radical), it is necessary to modulate ERO1 activity to avoid unnecessary oxidative cycling. There are several factors that go into the oxidative stress of RE, among them we have protein misfolding that activates the UPR (misfolded protein response).

Due to the interesting role of the described targets, this project will be focused on the design and synthesis of selective covalent reversible inhibitors to, on the one hand, counteract the growth of cancer cells by targeting MAGL and, on the other hand, promote, for already formed cancer cells, the activation of the UPR, which would lead to the initiation of the apoptotic cell death program with the inhibition of ERO1.

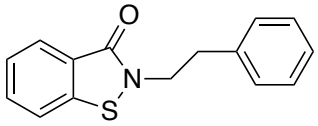
Some studies on the MAGL enzyme have shown that substances characterized by the presence of an isothiazolinone/benzoisothiazolinone scaffold turn out to be interesting enzyme inhibitors capable of interacting with the Cys201/Cys208 regulatory cysteines located near the catalytic site.



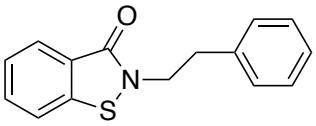
IC50= 1.66 +/- 0.38 μM



IC50= 1.09 +/- 0.19 μM



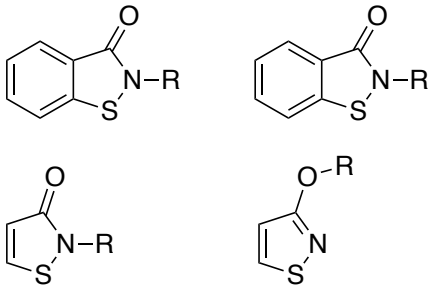
IC50= 34 +/- 2 μM



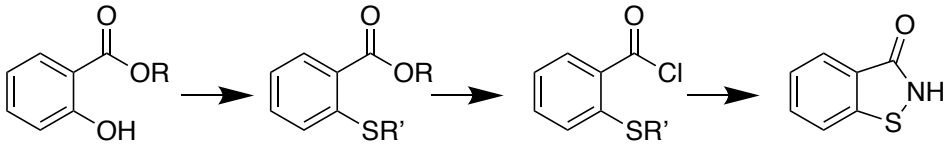
% inhibition (10-3M)= 55% +/- 5

These results demonstrate that compounds with (benz)isothiazolinone structure can be considered good candidates for the identification of other covalent and reversible MAGL inhibitors. In particular, the development of a class of "mechanism-based MAGL inhibitors" endowed with greater selectivity than derivatives developed to date would reduce the possibility of interfering with other enzyme systems or with cellularly available thiols such as glutathione.

The goal could be achieved by inserting into the isothiazolinone/benzoisothiazolinone scaffold, residues and decorations that can both favor recognition by MAGL over generic esterases and modulate the inhibitory potency of these new derivatives.

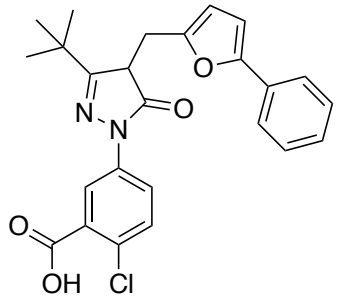


A possible synthetic approach might be as follows:



Scheme: salicylic acid ester will be used as starting material, sulfur will be introduced by nucleophilic substitution followed by acyl chloride formation via thionyl chloride. The scaffold will be finally synthesized by cyclization.

The first inhibitor that has been discovered for ERO1 is EN460; identified by the high throughput screening (HTS) technique.

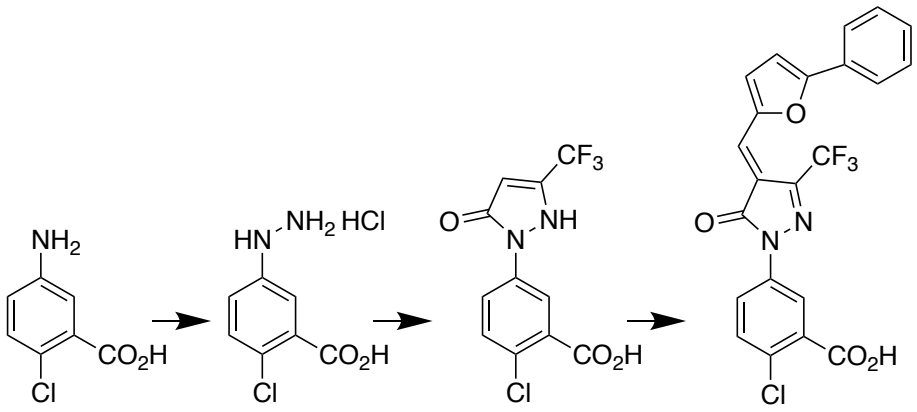


Although its high selectivity toward ERO1, it demonstrated several critical issues:

- Not selective toward free thiols
- Low potency in vivo
- Uncertain toxicity profile

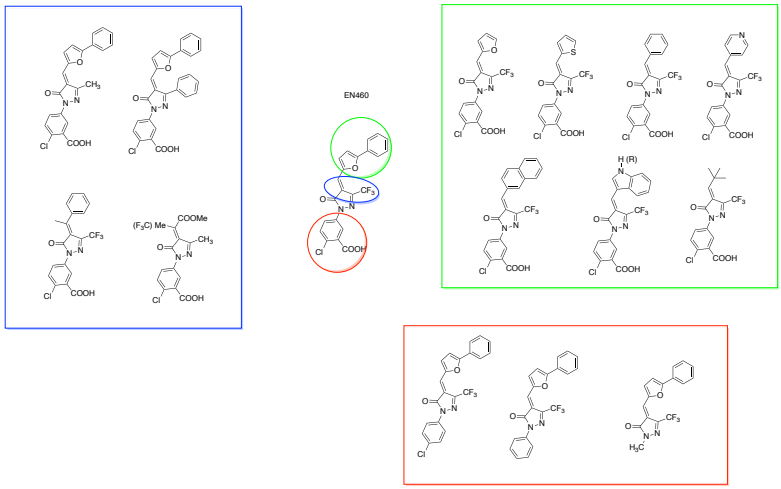
This shows the need for further studies to arrive at safer and more selective inhibitors than EN460. The goal could be achieved by taking EN460 itself as the scaffold, inserting or removing residues and decorations to try to obtain molecules that can inhibit the enzyme of interest and at the same time eliminate the criticality of the scaffold.

The first goal is to obtain a safe synthetic process for obtaining our scaffold. One possible synthetic approach is:



Scheme: Using 5-amino-2-chlorobenzoic acid as the starting agent, we form 2-chloro-5-hydrazinobenzoic acid hydrochloride by exploiting the attack of the amine on a nitro group and the subsequent reduction of the latter in an acidic environment. We then exploit the reactivity of hydrazine to form a ketone condensation and subsequent lactamation to form pyrazolone. In the last step we exploit an aldolic condensation to obtain EN460.

Once the first goal is hit, we can focus on structural modification of the compound. One possible approach is to divide the scaffold into 3 zones with different functional groups and modify one portion at a time. This is critical to realize a precise structure-activity relationship study that will permit the identification of functional groups tolerated by the enzyme, and give a correct decoration to the scaffold to have minimum off-target effects.



Organization of the research period:

Year 1: Synthesis model for starting preparation. Design and synthesis of a series of compounds capable of inhibiting ERO1 and MAGL. Evaluate and select the best ones in terms of yields, purity and stability.

Year 2: Study the ability of new compounds to inhibit the desired enzyme in vitro. Deepen the study of structure-activity relationships of new compounds.

Year 3: Drug design and synthetic strategies to optimize the metabolic stability of selected compounds. Final thesis writing.

References

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3. (1) Hayes, K.; Batsomboon, P.; Chen, W.; Johnson, B. Bioorganic & Medicinal Chemistry 27 (2019) 1479-1488.
4. (2) Blais, J.; Chin, K-T.; Zhang, Y.; Heldman, N. The Journal of Biological Chemistry Vol.285, NO.27, pp.20993–21003, July 2, 2010

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