Faculty Research Grant 2019

"Halogenation of amino acids as a route to neuroinflammation treatment precursors"

Final Report

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Restatement of problem researched or creative activity

The PI's scholarly efforts focus on development of molecules capable of inhibiting Xc- transport protein, which is overexpressed by cancer cells. This project focused on synthesis of a novel molecule, diiodohistidine (DIH), which was previously identified *in silico* research (PI) as a potentially biologically active on the Xc- transport protein. Well known Xc- inhibitor, quisqualate, displays remarkable activity on the transport protein, and the molecule proposed here was modeled based on quisqualate structure, Fig. 1.

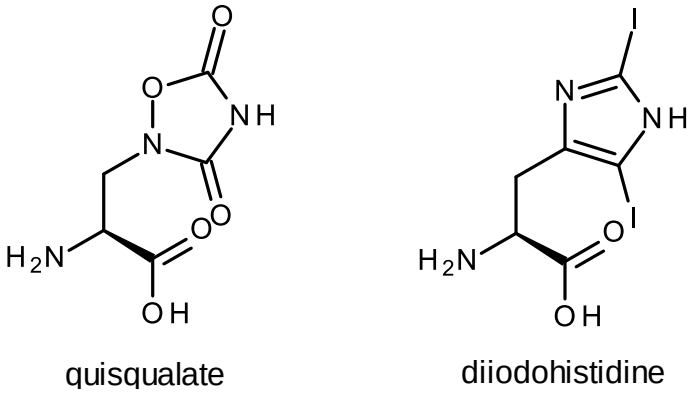

Figure 1. Quisqualate vs. Diiodohistidine

This project involved design of the synthetic pathway leading to the desired product, synthesis itself, characterization of the product and preliminary screening in vitro for biological activity, in collaboration with specialists from UAMS.

Brief review of the research procedure utilized

The PI spent significant time modeling the target molecule using computational methods (SPARTAN 16), to obtain a promising candidate for the new inhibitor. After identification of the target, the PI designed a method of its synthesis, acquired necessary chemicals and supplies, and executed the synthesis. The process yielded ~80% of the product. The method included solution

of natural amino acid, L-histidine (His) in basic conditions and iodination of the amino acid by slow addition of iodine solution in hexane, with constant vigorous mechanical stirring, Fig. 2.

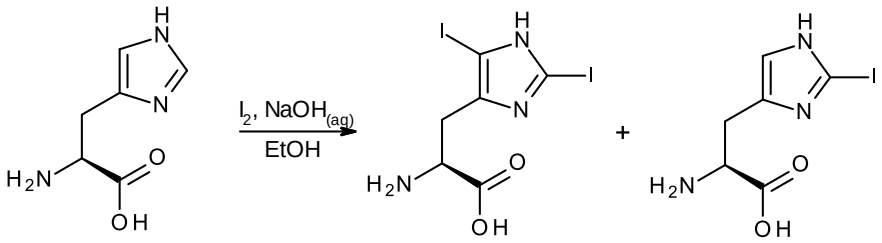

Figure 2. Iodination of histidine

After isolation of the byproducts, the solution was brought to higher pH with concentrated ammonium hydroxide to convert the product to a zwitterion, which is poorly soluble in water and precipitates out. The product obtained this way was dissolved in diluted hydrochloric acid and again brought to higher pH with ammonia to precipitate it. The final product obtained this way was of very high purity (~98% by LC-MS; analysis at Fayetteville State Wide Mass Spectrometry Facility), Fig. 3.

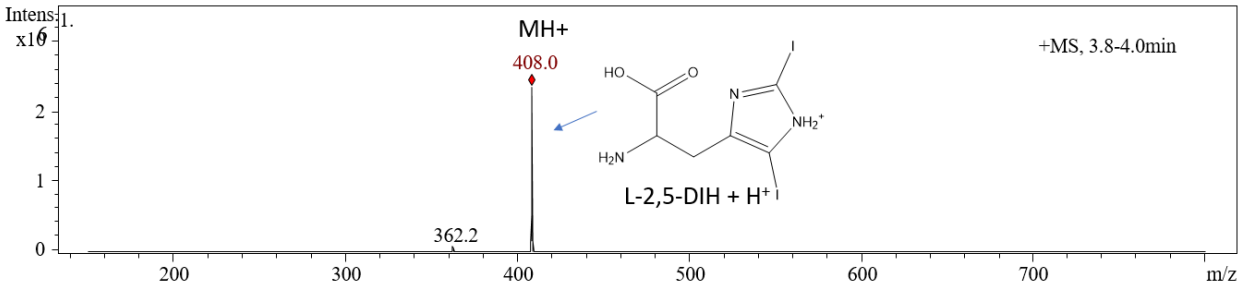

Figure 3. DIH LC-MS Analysis

After the characterization, the substance was tested for biological activity at the facilities at UAMS.

Biological activity screens

The molecule was tested *in vitro* on rat glial Xc- transporter for its ability to modulate the function of this protein. There was a residual activity observed, however much lower than that displayed

by the PIs other molecules of interest. Even though not very active, the molecule provided more insight into the binding site and laid grounds for speculation regarding that site's architecture. This in turn will support future design of molecules with further improved properties.

Summary of findings

Design and synthesis of the proposed molecule were successful. Analytical characterization proved not only the identity of the product abut also its high purity. Biological testing showed residual inhibitory activity displayed by DIH on Xc- transporter. We speculate that modification of the pKa of the proton on heterocyclic nitrogen might change the outcome. New derivatives are currently under development. Fig. 4. shows the results of the biological activity screen of DIH (named here CPD10) against one of our more active compounds, CPD5. As evident, DIH does not inhibit the Xc- system across a broad range of concentrations.

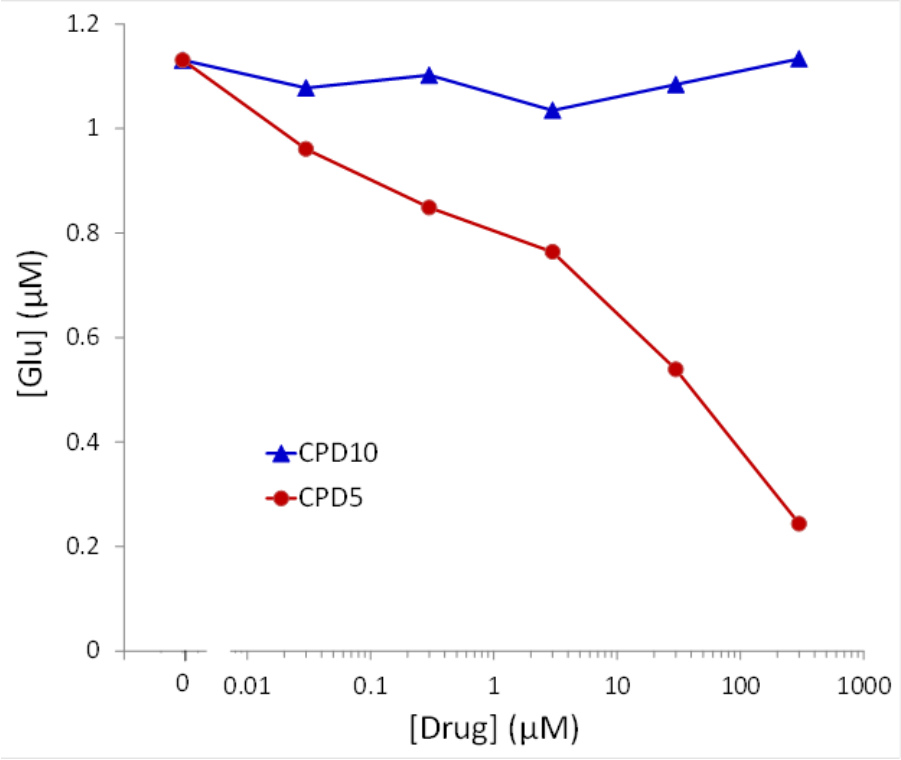

Figure 4. DIH (CPD10) activity vs. CPD5 on Xc- protein

Conclusions and recommendations

In conclusions, as evidenced by mass spectrometry (analysis performed on a charge-per-sample base at the University of Arkansas in Fayetteville Mass Spectrometry Facility), the target molecule was successfully synthesized and purified to a very high degree. Analytical methods proved its identity and high purity. In a collaborative effort (Dr. Steve Barger, UAMS), the compound was then screened for biological activity. It was found that the molecule does not display substantial inhibitory activity on the Xc- transport protein. However, its similarity to quisqualate and the fact that it is not active, provided the PI much information and subtle details of the binding site of the Xc- transport protein. Future inhibitors will be designed in accordance with this data, increasing the chances of success of discovery of another major class of Xc- inhibitors, potentially useful in therapy in broadly defined neuroinflammation. The results of this project greatly enhanced the outcome of the externally sponsored project (AR INBRE) conducted this summer (2019) by the PI and one ATU undergrad student.