Non-Steroidal Anti-Inflammatory Drugs and Their Ability to Inhibit Aggregation of IAPP

Nicole Pepe
Korina Sandoval
Alex Santiago
In Rachel Wang
Mentor: Dr. David A. Moffet, Department of Chemistry and Biochemistry
Loyola Marymount University

Introduction

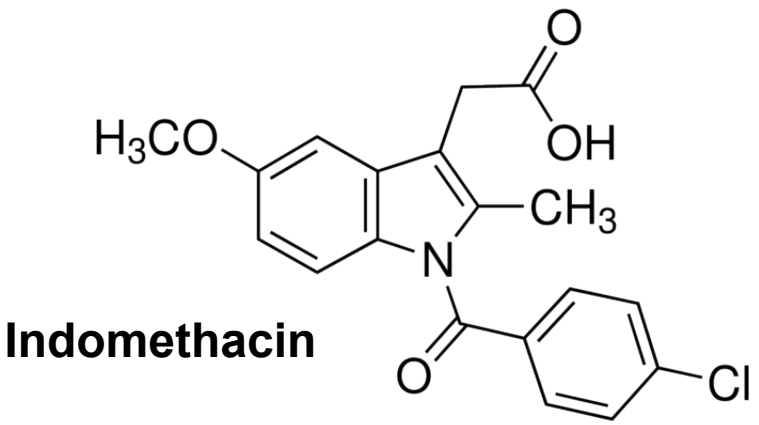
The aggregation of Islet Amyloid Polypeptide (IAPP, amylin), a 37-amino acid polypeptide, has been thought to correlate with the loss of pancreatic B-islet cells that are necessary for the secretion of insulin (1). These effects are thought to contribute to Type II Diabetes, and for this reason, the inhibition of the aggregate proteins can be vital for the discovery of potential therapeutic drugs (2). With the use of Thioflavin T binding assays, six non-steroidal anti-inflammatory drugs (NSAIDs) were tested and analyzed for their ability to prevent the aggregation of amyloidogenic IAPP in order to evaluate their viability for possible therapeutic uses.

Methods

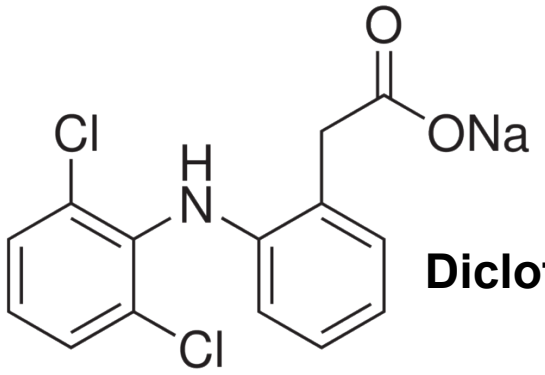
- Pipet 375 µL of thawed IAPP into glass culture tubes (one per sample)
- Use the speed vacuum (for ten minutes) to isolate IAPP from hexafluoroisopropanol (HFIP)
- For each sample, add 15 µL of NSAID to 135 µL of Tris buffer (pH=7.4) in an eppendorf tube for a total solution of 150 µL
- Add each solution to the IAPP glass culture tubes
- Incubate for ten to fifteen minutes
- During incubation, obtain 0.0008 g Thioflavin T (ThT) and mix with 40.0 mL of Tris buffer in falcon tube

  Thioflavin T is a fluorescent dye that is known to bind to structures that are rich in beta sheets in which it exhibits an enhanced fluorescence when read from a fluorescence spectrophotometer. This analysis is useful in determining the compounds that are most effective at inhibiting IAPP, since a large shift in the emission spectrum is to be expected from IAPP aggregation, while a subtle or absent shift is to be expected from protein inhibition.

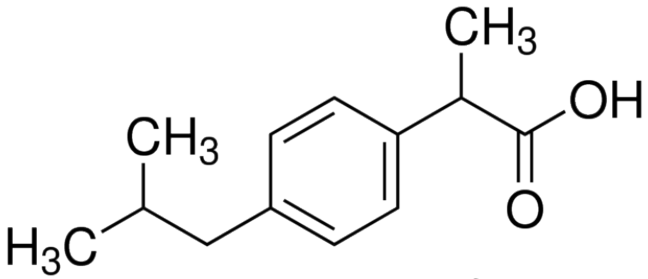
- Mix 663 µL ThT solution with 17 µL of the samples in new glass culture tubes
- Keep covered in dark for 2-3 minutes then quickly place each sample in the cuvette (one at a time) and run samples through fluorescence spectrophotometer machine (record second peak)



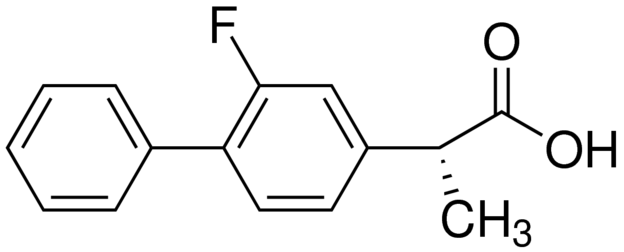
Indomethacin



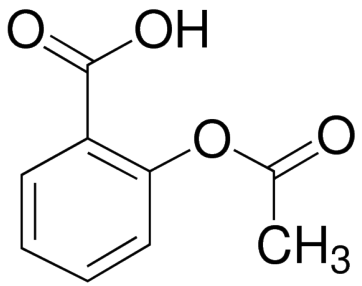
Diclofenac



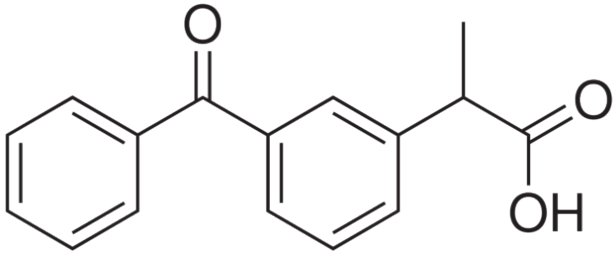
Ibuprofen



R-2-fluoro-a-methyl-4-biphenylacetic acid



Aspirin



Ketoprofen

Figure 1: Structures of the six NSAIDS tested

Results

- Based on ThT assays, the six NSAIDs tested appear not to be viable compounds for inhibiting IAPP aggregation
- If the NSAIDS were successful in inhibiting the aggregation of IAPP, the data would have shown lower fluorescence emission than that of the IAPP control
- Binding of IAPP to the NSAIDs would have decreased the values to below the IAPP control, representing the inhibition of IAPP aggregation

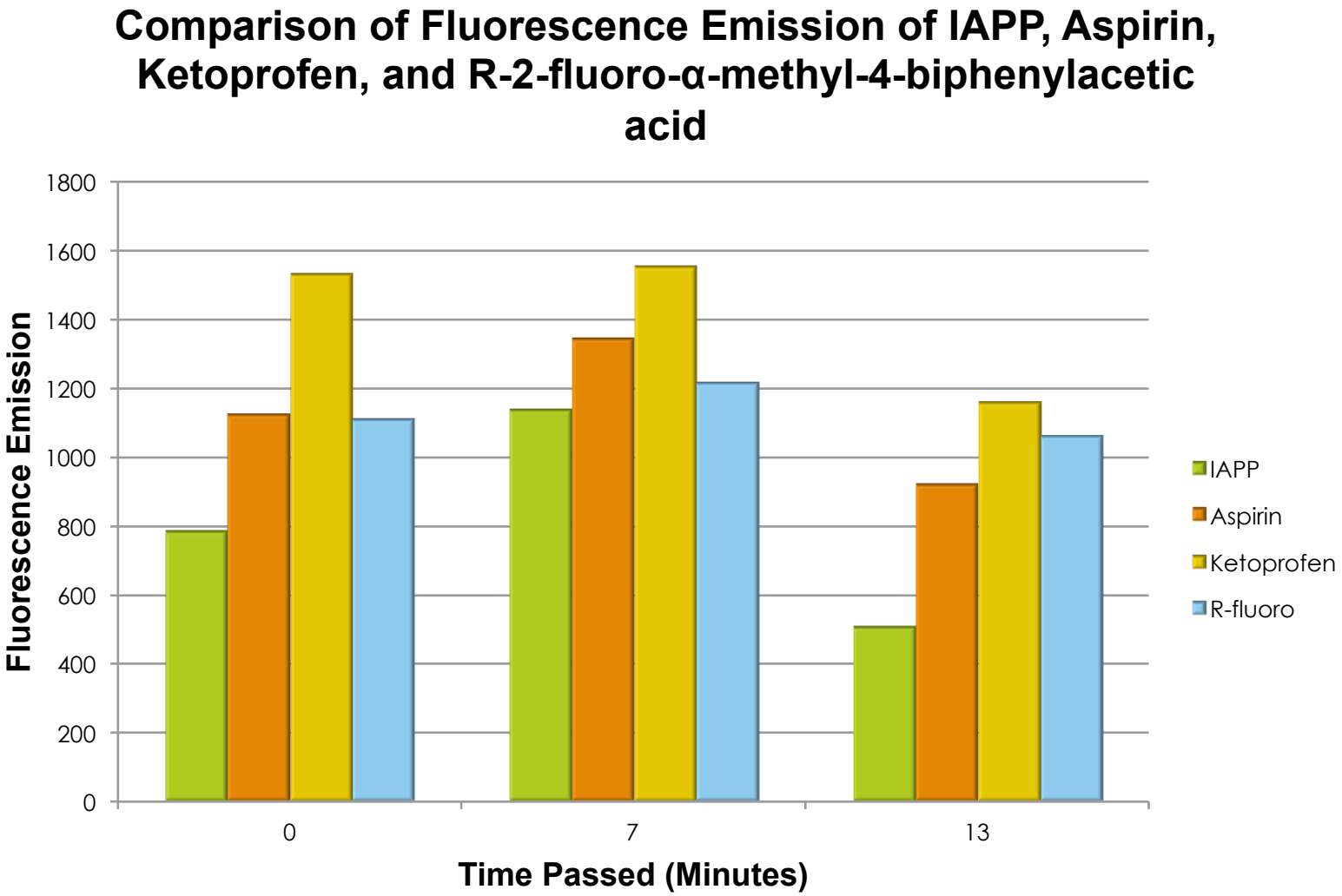


Figure 2: Graph comparing IAPP against the first three NSAIDS tested

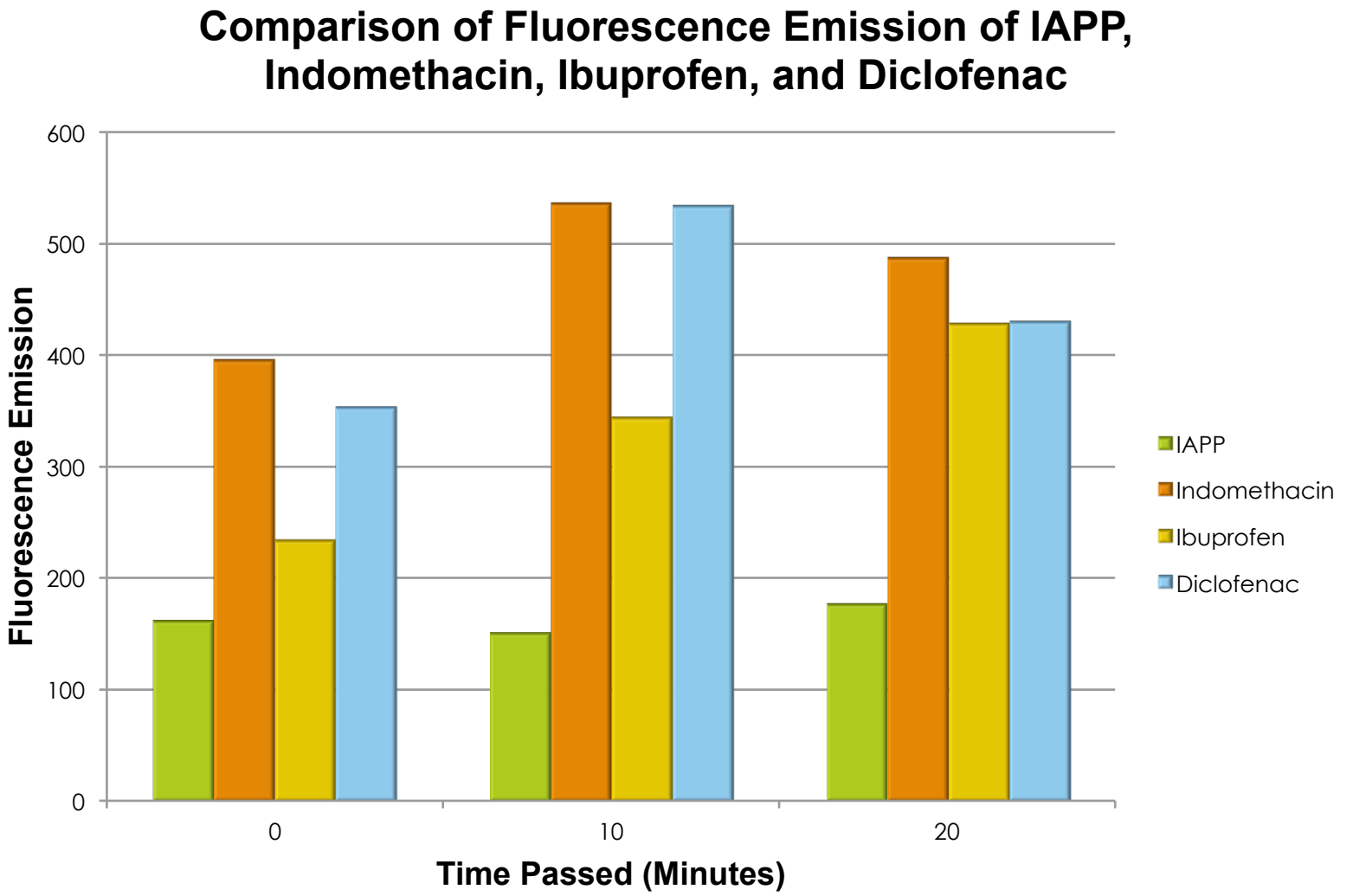
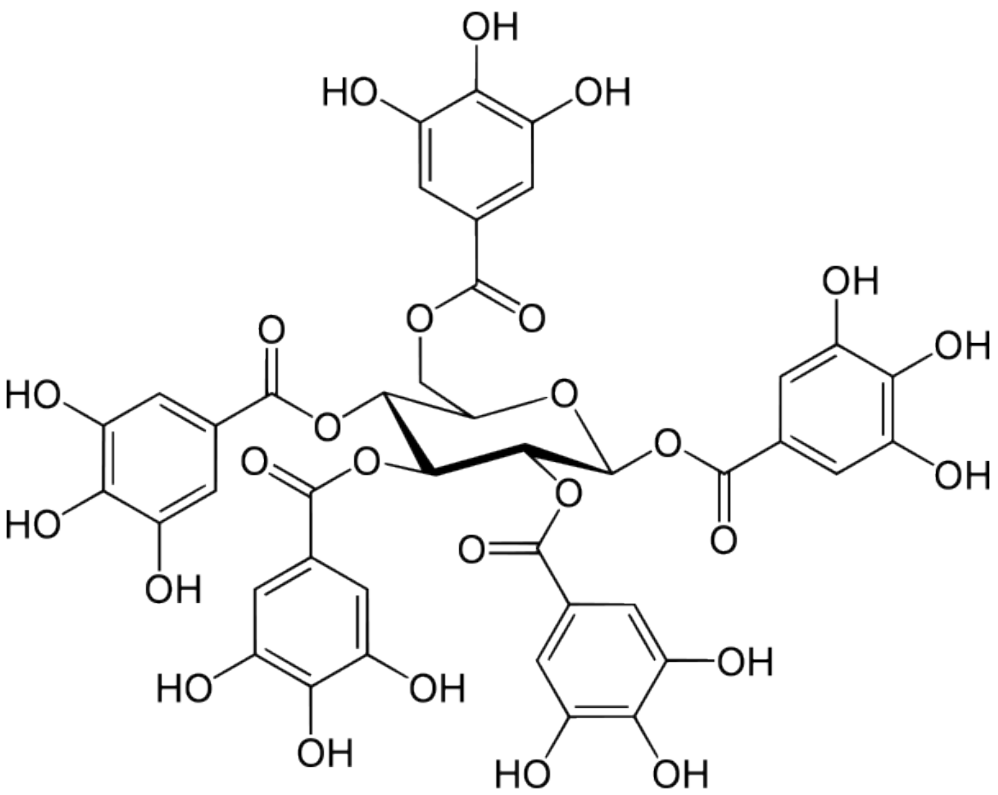


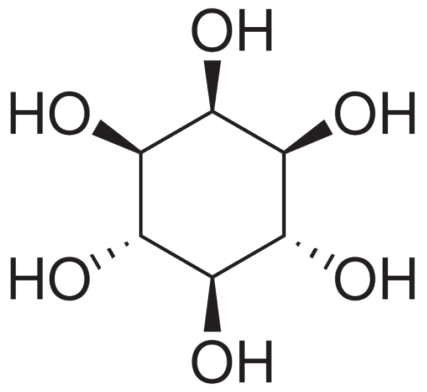
Figure 3: Graph comparing IAPP against the second three NSAIDS tested

Future Work

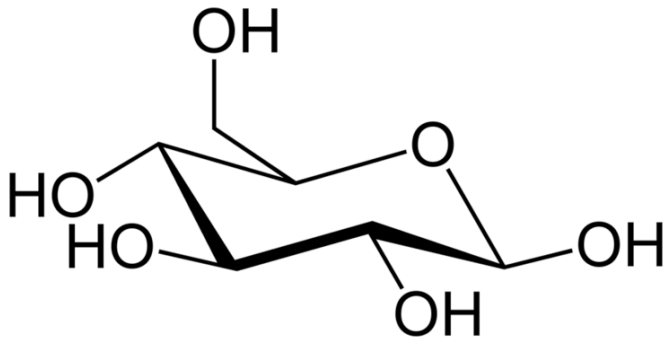
In a recent study for IAPP, it was shown that the molecule PGG greatly inhibited IAPP aggregation and was far superior to tannic and gallic acids, which are known inhibitors of this protein (3). The structure of PGG is a gallic acid attached to the hydroxyls of glucose. Based on the fact that PGG is a much stronger inhibitor of IAPP than gallic acid is on its own, future research includes attaching NSAIDS to glucose in hopes of coming up with similar results. NSAIDS will also be attached to inositol, a molecule that has a similar structure to glucose.



PGG
(1,2,3,4,6-penta-O-galloyl-beta-D-glucose)



Inositol



Glucose

Figure 4: Structures of PGG, inositol, and glucose

References & Acknowledgements

(1) Bradley, Neddenriep, Calciano Anastasia, Daniel Conti, Erin Suave, Marissa Paterson, Edward Bruno, and David A. Moffet. "Short Peptides as Inhibitors of Amyloid Aggregation." *The Open Biotechnology Journal* 5 (2011): 39-46. Print.

(2) Bruno, Edward, Catalina Pereira, Karla P. Roman, Marisa Takiguchi, Pei-Yu Kao, Luiza A. Nogaj, and David A. Moffet. "IAPP Aggregation and Cellular Toxicity are Inhibited by 1,2,3,4,6-penta-O-galloyl-B-D-glucose." *Amyloid* (2013): 1-5. Print.

(3) Moffet, D. A., A. Fox, T. Snollaerts, C. Errecart Casanova, A. Calciano, and L. A. Nogaj. "Selection for Nonamyloidogenic Mutants of Islet Amyloid Polypeptide (IAPP) Identifies an Extended Region for Amyloidogenicity." *Biochemistry* 49 (2010): 7783-789. Print.

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Dr. Luiza Nogaj and Mount Saint Mary's College