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SYNTHESIS OF GPR119 INTERACTING DRUGS FOR TREATMENT OF TYPE II DIABETES

A Thesis

by

TIEN M. TRAN

Submitted in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

Major Subject: Chemistry

The University of Texas Rio Grande Valley

May 2022

SYNTHESIS OF GPR119 INTERACTING DRUGS FOR TREATMENT OF TYPE II DIABETES

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May 2022

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ABSTRACT

Tran, Tien M., Synthesis of GPR119 Interacting Drugs for Treatment of Type II Diabetes. Master of Science (MS), May, 2022, 21 pp., 20 figures, references, 8 titles.

Type II Diabetes is one of the leading causes of death in the United States and is categorized by high blood glucose levels, insulin deficiencies, and inhibition. Located in the pancreas and intestine, the G-Protein Receptor 119 (GPR119) was noted to have an important effect on insulin secretion. Previous studies found that when the receptor is activated, insulin secretion was upregulated which is vital for type II diabetes treatment. Compounds AR437735 and AR437948 are the proposed agonist and inverse agonist pair that will be synthesized and tested for bioactivity to the receptor. It is theorized that the different double bonded atoms on thetwo compounds will help determine which ligand on the receptor should be targeted for drug binding to help promote insulin secretion. The synthesis of compounds AR437735 and AR437948 failed, however, a new pair of agonists and inverse agonist were synthesized using the base structure of the two initial compounds. The agonist version of the compound had an overall yield of 18.051%, while its counterpart had an overall yield of 6.259%. All compounds are characterized by Nuclear Magnetic Radiation Spectroscopy (NMR).

DEDICATION

The completion of my master's degree would not have been possible without the motivation and support of my family and friends. My mother, Spring Nguyen, who financially supported me and offered me her wisdom. My father, Tung M. Tran, for motivating me to be a better person. My best friend, Abigail M. Zepeda, who worked alongside me and offered advicewhen I was stuck.

ACKNOWLEDGMENTS

I am grateful to Dr. Shizue Mito, chair of my dissertation committee, for her mentorship and advice. From funding, procedural changes, and NMR confirmation, to textual editing, she encouraged me to complete my program with kindness and guidance. My thanks go to my dissertation committee members: Dr. Evangelia Kotsikorou, Dr. Dean Frank, and Dr. Jose J. Gutierrez. Their advice was integral in ensuring that the quality of my thesis was up to par. I would also like to thank Dr. Jason Parsons for the use of the Irradiation Radiation Microwave and HPLC measurement to determine the purity of the compounds.

I would also like to thank my colleagues in the research lab who contributed to the project: Derek Rodriguez and Alejandro Palacios. I would also like to thank Mathew Rosales, who is a research student in Dr. Evangelia Kotsikorou's lab, for doing the theoretical bioactivity analysis of the compounds and providing Figure 2. Finally, I would like to thank the Department of Chemistry at the University of Texas Rio Grande Valley for its generous support provided by a Departmental Grant from the Robert A. Welch Foundation (Grant No. BX-0048).

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CHAPTER I

INTRODUCTION

Type II Diabetes is the most common form of diabetes and occurs when blood glucose levels are too high. Normally, insulin – a hormone produced in the pancreas – helps lower the blood glucose level by transporting the sugar to cells throughout the body, which is then used as asource of energy. However, for individuals with type II diabetes, their body either does not respondto the insulin, this term is coined insulin resistant, or their body is incapable of producing enough insulin to remove the glucose from the bloodstream.[3]

In recent years, studies have been conducted on the protein, G-Protein Receptor 119 (GPR119), as a new alternative route for treatments of Type II Diabetes as opposed to traditional methods and medications. The GPR119 is a protein that is found in both the intestine and the pancreas, and studies have shown that modification of this protein can lead to higher stimulationsof insulin release from pancreatic ß-cell and glucagon-like-peptide-1 (GLP-1) in the intestine, dueto the accumulation of cAMP.[8] GLP-1 is known for upregulating both the insulin-releasing and appetite-suppressing hormone, and it was found that analogs of this compound can lower blood glucose levels and help manage body weight. The importance of GLP-1 in type II diabetes treatments is because type II diabetes is characterized as the body's malfunction of its intestinal GLP-1 and pancreatic ß-cell.[2]

Using the prototype GPR119 agonist, AR231453, it was possible to narrow down the number of possible binding conformations, but upon further studies using analogs of AR231453, it was found that there was a strong correlation to a specific binding conformation that results in the activation of GPR119.[5] To gather more data on the binding conformation of GPR119, a knownagonist and its inverse agonist are introduced to the protein to determine what section of the molecule responds to the endogenous ligand of the receptor that helps activate it; the agonist and inverse agonist that was used for this study are AR437735 and AR437948.

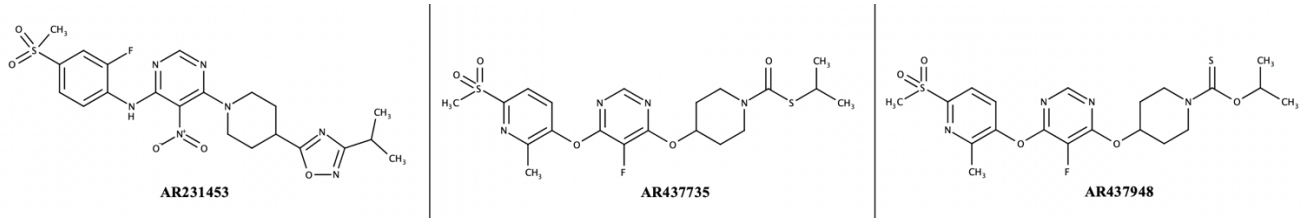


Figure 1. Structures of AR231453, AR437735, and AR437948.

At the docking site on the GPR119 protein, the receptor has tryptophan and two leucine residues.[6] It is theorized that the residue(s) that has the most effect on the receptor's activation arethe two leucine residues located at the 5th and 6th transmembrane helices. To test this theory, the synthesized agonist and inverse agonist, AR437735 and AR437948 respectively, were used as experimental samples to determine their bioactivity and binding conformation. However, synthesis of AR437735 and AR437948 failed due to issues with replacing the iodine molecule with the methylsulfonyl structure. Instead, an alternative set of agonist and inverse agonist pair were synthesized by keeping the iodine molecule and completing the piperidine-1-carbothioate.

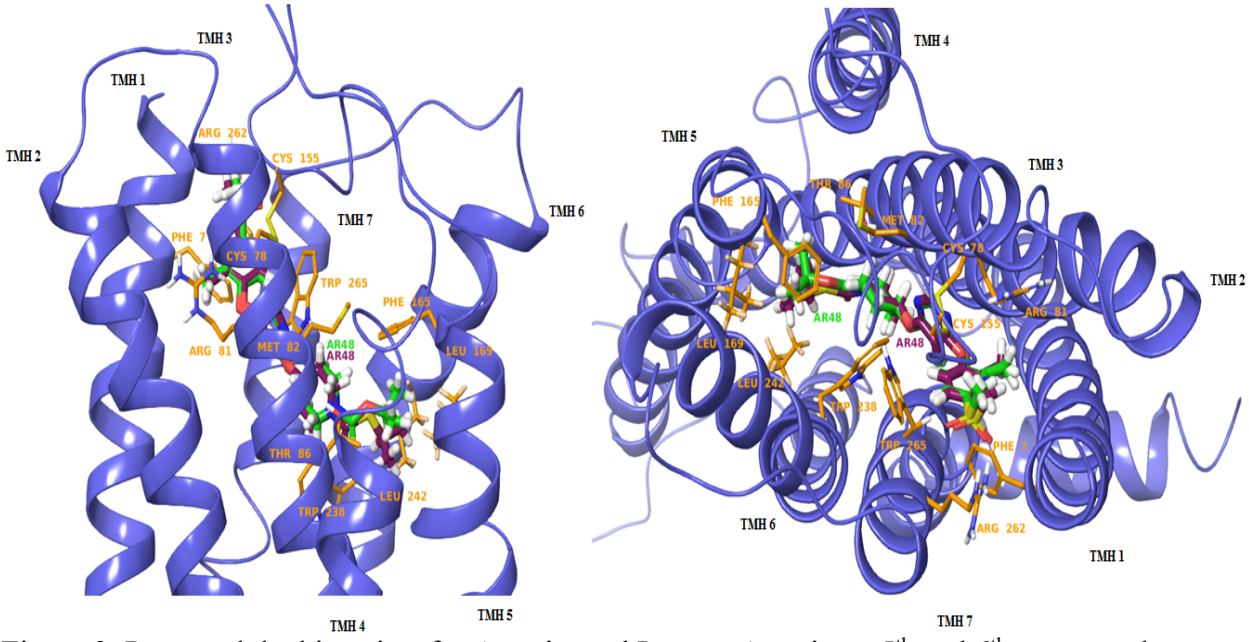


Figure 2. Proposed docking sites for Agonist and Inverse Agonist at 5th and 6th transmembrane helices.

CHAPTER II

REVIEW OF LITERATURE

As of 2016, the CDC estimates that approximately 23 million U.S. citizens have been diagnosed with one of the two forms of diabetes.[4] Type 1 Diabetes (T1D) is characterized by the body's inability to produce insulin and requires the hormone to survive. Two common treatments for type 1 diabetes are daily insulin shots or routinely used insulin pumps. People with type 2 diabetes (T2D) can be characterized by either low insulin production or insulin resistant.[4] Unlike with T1D, people with T2D can still produce insulin but at a much lower rate in comparison to a healthy adult. Those who are insulin resistant are unable to use the hormone to transport glucose to different parts of the body as a source of energy, which results in higher concentrations of blood glucose. Currently, the treatment for T2D includes glucose-lowering effectors, insulin, insulin promoters for the pancreas, and α-glucosidase inhibitors for the gut.[8] Unfortunately, these treatments have various side effects such as weight gain, gastrointestinal problems, and hypoglycemia. Another issue with current treatment is that the body may build a resistance to medication after long-term exposure.[8]

In 1999, the Human Genome Project discovered a receptor that is predominately found in the pancreas and gastrointestinal tract in humans that may play a role in future T2D treatments.[8] This receptor, the G-Protein Receptor 119 (GPR119), was found to be a point of interest in insulin production regulation and is present in various mammalian species such as mice, rate, chimpanzees, humans, etc.[8] Numerous *in vitro* experiments were conducted in various

mammalian systems, and it was found that the receptor, when activated, would help maintain glucose homeostasis by upregulating insulin production to stimulate glucose transportation.[7] This is key for future T2D treatment because it can simultaneously counter the two main factors that are associated with T2D; low insulin production and insulin resistance. Promoting insulin production directly from the pancreatic ß-cells eliminates the need to use insulin shots or other insulin resources that need to be entered into the body. The activation of GPR119 also helps combat the insulin resistance of patients because by having an excess amount of insulin in the body, some of the hormones will be able to bypass the body's resistance to help transport the blood glucose to various parts of the body to be used as energy. Another benefit of activating this receptor, in comparison to current market treatment for T2D, is that there will not be additional side effects to the treatment such as weight gain, gastrointestinal problems, or hypoglycemia.

The GPR119 receptor has been classified as a Class A (rhodopsin-type) G-protein-coupled receptor that is found primarily in the pancreas, fetal liver, and gastrointestinal tract in the human system.[1] Further investigations show that GPR119 mRNA was heavily expressed in the pancreas and fetal liver while in other species, such as rodents, were more expressive in most of its tissues like the gastrointestinal tract, small intestine, pancreas, and even in certain regions of the brain.[8] During the investigation of GPR119 receptor isoforms, there is conflicting data on the length size between rat and human receptors; one study claims that rats' have 133 more amino acids to their receptor while another study claims that the length size between rat and human receptors are the same.[8]

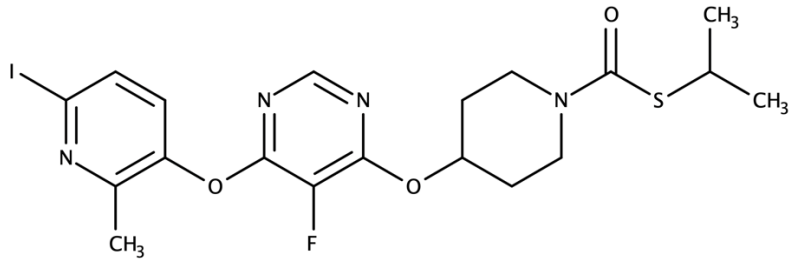
As previously states, the GPR119 is located in the pancreas and gastrointestinal tract, and when activated it also activates an enzyme called adenylate cyclase. The activation of the enzyme leads to an increase in cAMP production, which is used to amplify insulin production via

the activation of K^+ channels in the ß-cell of the pancreas. The insulin would then be used as a transporter of glucose to different parts of the body to be used as a source of energy. A potent agonist was synthesized by Arena Pharmaceuticals, Inc., titled AR231453. The initial studies on this agonist focused on proving whether or not it was dependent on the GPR119 receptor, and it was confirmed when it was tested on rats that carried the receptor and those that were GPR119 deficient.[8] The results showed that there was an increase in insulin production in the rats that were injected with the agonist and had the receptor, while those without the receptor showed no insulin production despite being injected with the agonist. However, studies show that when AR231453 is administered *in vivo*, there are toxic effects after continuous treatment in various mammalian systems.[7] However, using the base structure of AR231453 there have been various modifications to reduce the toxicity of the compound while maintaining its dependency on the GPR119 for binding and activation.

CHAPTER III

METHODOLOGY AND RESULTS

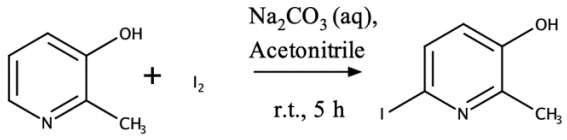
Method 1: Synthesis of Agonist S-isopropyl 4-(5-fluoro-6-(2-methyl-6-(iodo) pyridine-3-yloxy) pyrimidin-4-yloxy) piperidine-1-carbothioate



Agonist

Figure 3. Structure of proposed agonist compound.

Reaction 1.1: Synthesis of 6-iodo-2-methylpyridine-3-ol (Compound 1)



Compound **1**

Figure 4. Reaction 1.1

A mixture of 2-methylpyridine-3-ol (1.976 g, 18.1 mmol), iodine (5.809 g, 22.89 mmol), and sodium carbonate (4.048 g, 38.19 mmol) were dissolved in D.I. water (62 mL) and acetonitrile (30 mL), and left to stir for 5 hours at room temperature. The reaction was checked for completion by TLC and concentrated by vacuum to remove the acetonitrile. Afterwards, the mixture was extracted with EtOAc, dried over anhydrous Na_2SO_4, and filtered.

The crude oil was a dark brown, and purified by column chromatography (silica gel, Hex/EtOAc = 30:70) and gave an off-white solid that was NMR confirmed to be compound **1** (3.475 mg, 14.79 mmol, 94.95%).

Reaction 2.1: Synthesis of tert-butyl 4-(6-chloro-5-fluoropyrimidin-4-yloxy) piperidine-1-carboxylate (Compound 2)

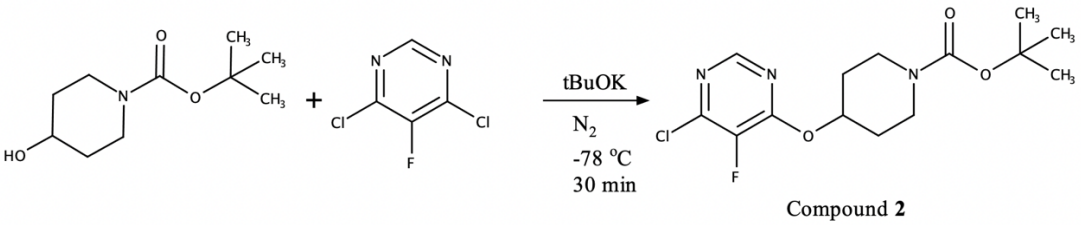


Figure 5. Reaction 2.1

To a solution of 1 M potassium tert-butoxide (1.216 g, 10.8 mmol) in dry THF (26 mL), under nitrogen, tert-butyl 4-hydroxypiperidine-1-carboxylate (2.436 g, 12.1 mmol) was added and left to stir until the mixture was white and homogenous with no clumps. The round-bottom flask was then transferred to a dry ice and acetone bath, at -78 °C, and 4,6-dichloro-5-fluoropyrimidine (2.154 g, 12.9 mmol) was slowly added in; gradually turning into a bright yellow. The reaction was left to stir for 30 minutes and checked for completion using TLC plates. The reaction mixture was quenched with water and extracted with EtOAc, and left to dry over anhydrous Na_2SO_4 before being filtered and rinsed with EtOAc. The organic layer was then concentrated by vacuum which yielded a bright yellow oil. The oil was then purified by column chromatography (silica gel, Hex/EtOAc = 80:20) to yield a white solid that was NMR confirmed to be compound **2** (3.125 g, 9.44 mmol, 77.97%).

Reaction 3.1: Synthesis of S-isopropyl 4-(6-chloro-5-fluoropyrimidin-4-yloxy) piperidine-1-carbothioate (Compound 3)

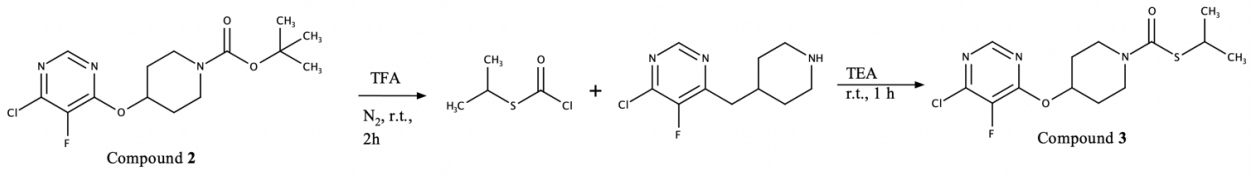


Figure 6. Reaction 3.1

The second reaction is a two-step process. Compound **2** (188 mg, 0.568 mmol) was dissolved in an excess amount of DCM (11 mL) and TFA (4.6 mL), and left to stir for 1 hour under nitrogen. The reaction did not complete and was left to stir for an additional 4 hours to ensure all starting materials reacted. It was then concentrated by vacuum which yielded the crude compound 4-chloro-5-fluoro-6-(piperidin-4-yloxy) pyrimidine hydrochloride, which appeared as an off-white solid. The crude compound was then dissolved in DCM (35 mL) and reacted with S-isopropyl chlorothioformate (0.06 mL, 0.483 mmol) and TEA (4 mL). It was left to stir for 1 hour and checked for completion, before extracted with DCM, dried over anhydrous Na_2SO_4, and filtered. The organic layer was then concentrated by vacuum, which yielded a light brown oil, that was then purified by column chromatography (silica gel, DCM/MeOH = 99:1). A white solid was obtained and NMR confirmed that it was Compound **3** (185.1 mg, 0.555 mmol, 98.0%).

Reaction 4.1: Synthesis of Agonist

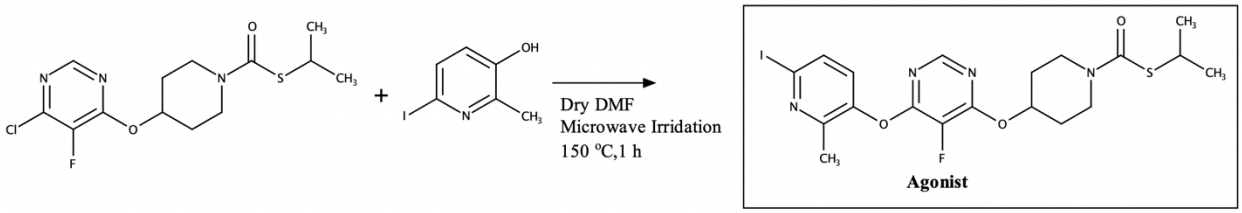
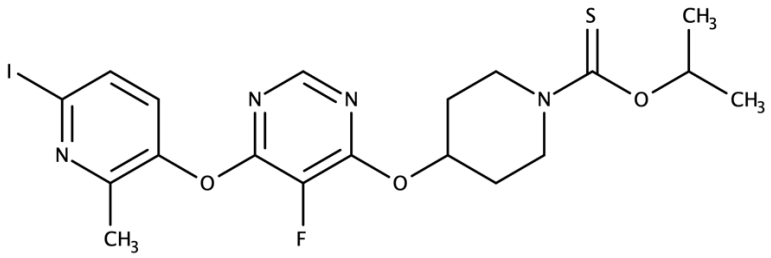


Figure 7. Reaction 4.1

To a suspension of compound **3** (166 mg, 0.498 mmol), compound **1** (117.3 mg, 0.499 mmol) was added alongside dry DMF (15 mL) and potassium carbonate (187 mg, 1.35 mmol). The reaction is under microwave irradiation at 150 ^{o}C for 1 hour, and then checked for completion. The mixture was then filtered to remove inorganic solids, and extracted with EtOAc, dried over anhydrous Na_2SO_4, and filtered again. The organic layer was concentrated by vacuum to yield a brown oil, that was purified by column chromatography (silica gel, Hex/EtOAc = 75:25) to afford a white solid that was NMR confirmed to be the agonist product (66 mg, 0.124 mmol, 24.88%).

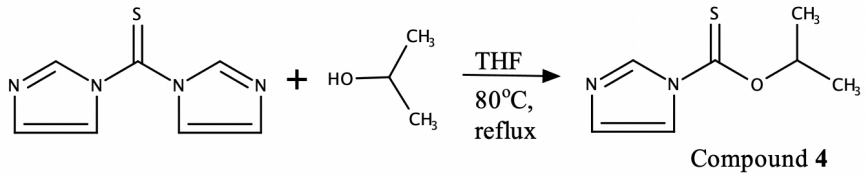
Method 2: Synthesis of Inverse Agonist O-isopropyl 4-(5-fluoro-6-(2-methyl-6-(iodo) pyridine-3-yloxy) pyrimidin-4-yloxy) piperidine-1-carbothioate



Inverse Agonist

Figure 8. Structure of proposed inverse agonist.

Reaction 1.2: Synthesis of O-isopropyl 1H-imidazole-1-carbotioate (Compound 4)



Compound **4**

Figure 9. Reaction 1.2

To a solution of 2-propanol (8 mL), N,N'-Thiocarbonyldiimidazole (997 mg, 5.59 mmol), and dry THF (60 mL) was added and refluxed at 88^{o}C for 1 hour under nitrogen. After the reaction

was checked for completion by TLC, it was quenched with sat. NaCl and extracted with EtOAc. The mixture was then dried over anhydrous Na_2SO_4, filtered, and evaporated to remove the excess solvent which yielded a brown oil. The crude oil was purified by column chromatography (silica gel, Hex/EtOAc = 60:40) to afford a yellow oil that was NMR confirmed to be Compound **4** (509 mg, 2.99 mmol, 53.38%).

Reaction 2.2: Synthesis of O-isopropyl 4-(6-chloro-5-fluoropyrimidin-4-yloxy) piperidine-1-carbothioate (Compound 5)

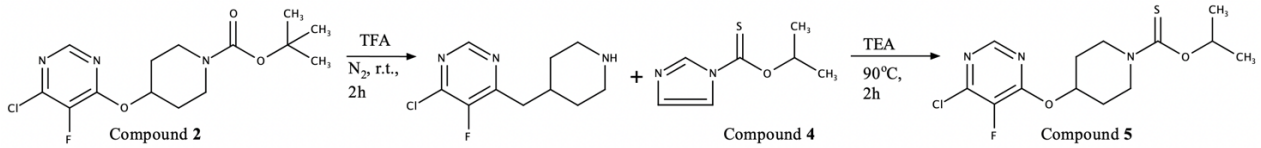


Figure 10. Reaction 2.2

Compound **2** (397 mg, 1.20 mmol) was dissolved in an excess amount of DCM (22 mL) and TFA (10 mL), and left to stir for 2 hours under nitrogen at room temperature. The reaction was TLC checked for completion, and evaporated to remove the solvent and the acid at 50°C. Compound **4** (0.2 mL, 1.36 mmol), THF (70 mL), and TEA (8 mL) was added to the reaction flask and left to reflux for 2 hours at 90°C under nitrogen, this caused the reaction to turn a bright yellow. After the reaction was TLC checked for completion, it was extracted with DCM, dried over anhydrous Na_2SO_4, and filtered. The organic layer was then concentrated by vacuum, which yielded a light brown oil, that was then purified by column chromatography (silica gel, DCM/MeOH = 98:2) to afford an off-white solid. The NMR confirmed that the product was Compound **5** (304 mg, 0.912 mmol, 76.08%).

Reaction 3.2: Synthesis of Inverse Agonist

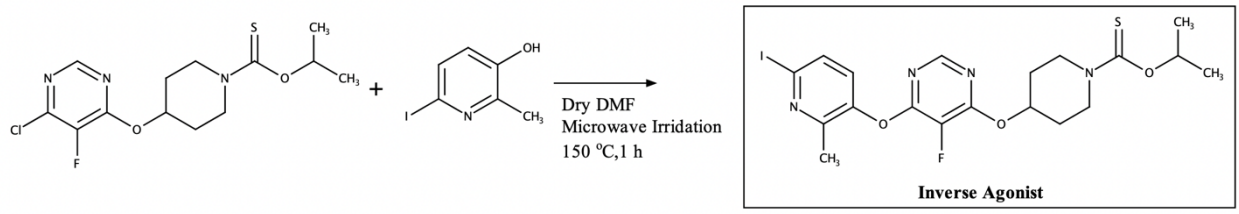


Figure 11. Reaction 3.2

To a reaction flask with compound **5** (304 mg, 0.912 mmol), compound **1** (251 mg, 1.07 mmol) was added alongside dry DMF (20 mL) and potassium carbonate (365 mg, 2.64 mmol). The reaction was under microwave irradiation at 150 °C for 1 hour, and then checked for completion. The mixture was then filtered to remove inorganic solids, and extracted with EtOAc, dried over anhydrous Na_2SO_4, and filtered again. The organic layer was concentrated by vacuum to yield a dark brown oil, that was purified by column chromatography (silica gel, Hex/EtOAc = 70:30) to afford a white solid that was NMR confirmed to be the inverse agonist product (101 mg, 0.190 mmol, 20.82%).

CHAPTER IV

DISCUSSION AND CONCLUSION

Substitution of Iodine and Methylsulfonyl

Reaction 1.1 was originally supposed to yield 2-methyl-6-(methylsulfonyl) pyridine-3-ol which would be used as the methylsulfonyl attachment for compounds AR437735 and AR437948. However, the substitution between the iodine and the methylsulfonyl group never went through, and the 6-iodo-2-methylpyridine-3-ol (compound **1**) remained very stable despite being treated with sodium methansulfonate, CuI, and L-proline at 110°C for 8 hours; all under a nitrogen atmosphere. Due to its stability, compound **1** was used instead to proceed with the rest of the reactions, the percent yield after purification was 94.95%. For future work, sodium methansulfonate will be replaced with sodium methansulfinate to see if the reaction will yield the desired product. It is theorized that because sodium methansulfinate has one less oxygen bond attached to the sulfur, the reaction will go through because there won't be any steric hindrance to interfere with the formation of 2-methyl-6-(methylsulfonyl) pyridine-3-ol.

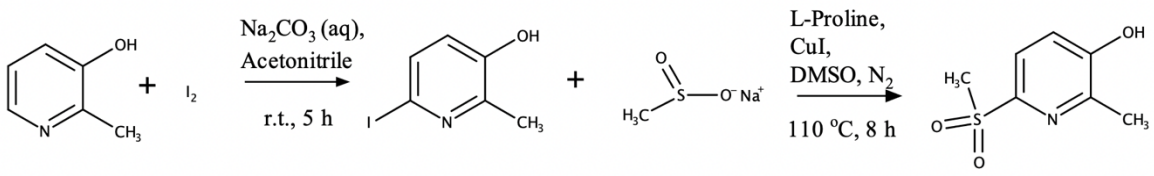


Figure 12. Proposed reaction to get methylsulfonyl product.

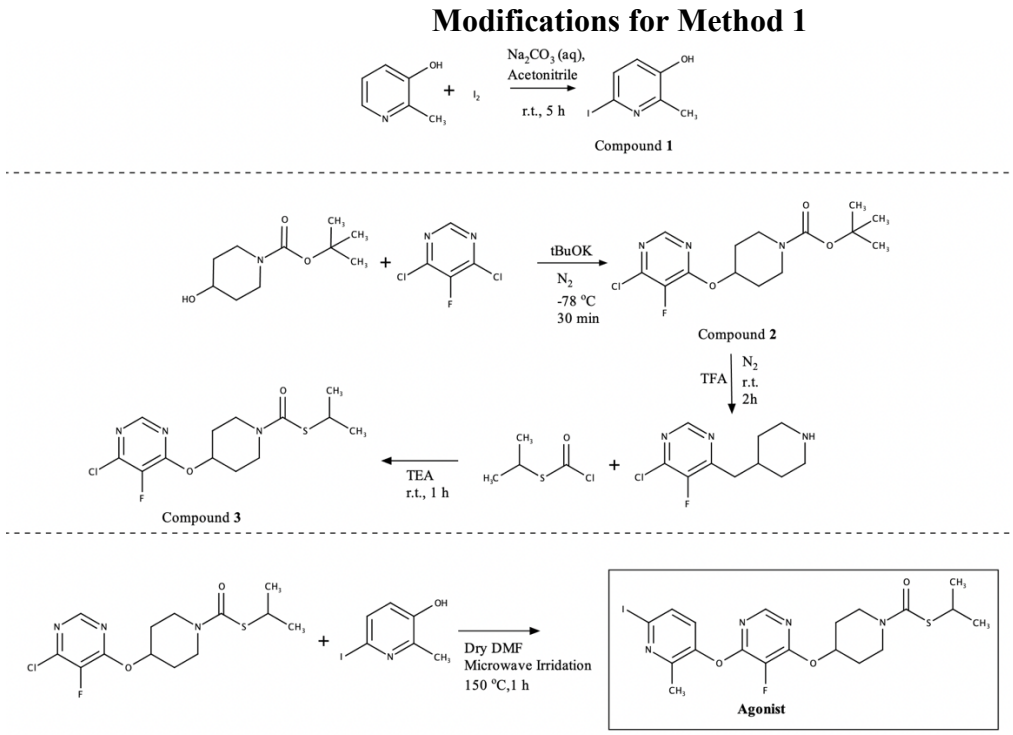


Figure 13. Overall reaction scheme of Method 1.

During the research process, modifications were made to the reference procedure to optimize the percent yield. Initially, the reaction for compound **2** called for a solution of tert-butyl 4-hydroxypiperidine-1-carboxylate and 4,6-dichloro-5-fluoropyrimidine, with the tert-butoxide solution slowly being added at -78 ºC. This gave a low yield of roughly 14%, thus the reaction was repeated but this time using the modified instructions (Reaction 2.1) with the addition of a nitrogen balloon and a flame-dried round bottom flask. These modifications and additional measures resulted in a percent yield of 77.97%, it was theorized that some products may have been lost during the purification process.

Modifications were also made for the synthesis of compound **3**. According to the reference procedures, 4N HCl in Dioxane was used to deprotect compound **2** by cleaving its Boc protection group. However, the reaction failed and none of compound **2** used for the reaction could be salvaged. To successfully remove the Boc protection group on compound **2**, the 4N

HCl in Dioxane was replaced with equal amounts of TFA, which proved successful in the deprotection reaction. No further modifications were made to the initial set of procedures, and the percent yield for compound **3** was 98.0%.

For the final reaction in Method 1 (reaction 4.1), there were no significant changes to the reference procedure. The only modification made to the procedure was the use of excess potassium carbonate and dry DMF, both of which acted as drying agents to limit water contamination, in the reaction. Otherwise, the reaction followed the same instructions on time and temperature and get a percent yield of 24.88%. The modified compound was submitted for bioactivity testing to see if the structure would act as an agonist to the GPR119 receptor, and has since been confirmed to be an agonist by Dr. Dean's lab.

Modifications for Method 2

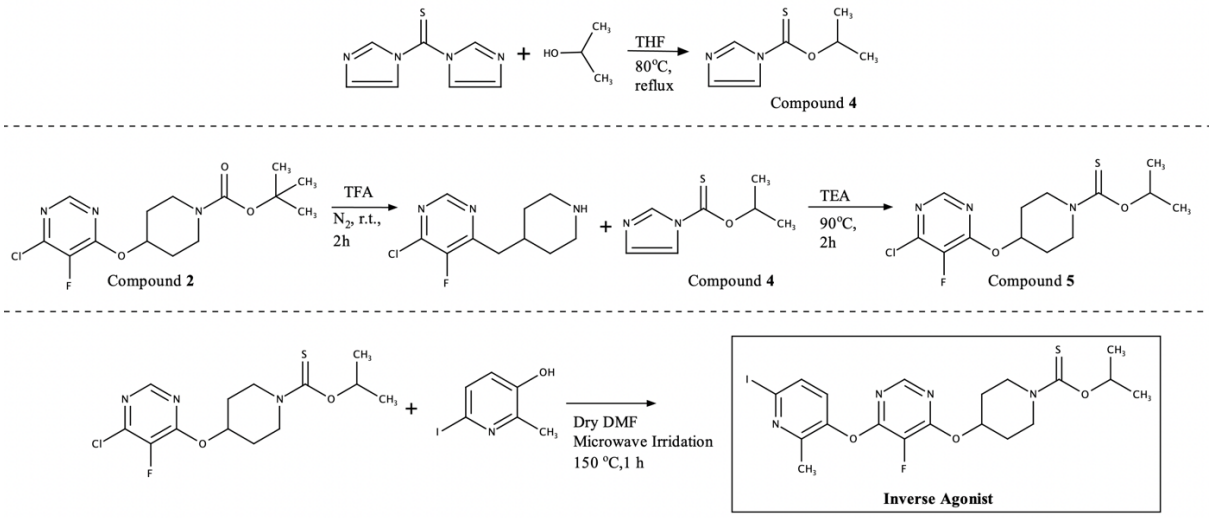


Figure 14. Overall reaction scheme of Method 2.

The reference procedures for synthesizing the potential inverse agonist had to undergo major modifications. Unlike Method 1, where the procedures focused on forming the compound structure by attaching compounds from the right to the left, Method 2 did the reactions in reverse. However, for reaction 1.2 the reference procedure did not use microwave

irradiation to attach compound **1** to compound **2**, which failed because the two individual compounds are too structurally stable and need extreme conditions to react. To combat the issues with the reference procedures, the reactions for Method 2 were altered to follow similar baseline procedure from Method 1.

The first step in the modified procedures was the synthesis of compound **4**, which had to be kept in a refrigerator to prevent the decomposition of its structure. Immediately after compound **4** was confirmed by NMR with a percent yield of 53.38%. Compound **4** was attached to compound **2** using the same procedures that used TFA to cleave the Boc protection group, which synthesized compound **5** and gave a percent yield of 76.08%. Once the base skeleton of the inverse agonist compound was confirmed, it underwent microwave irradiation and followed the same steps as the agonist compound for purification and confirmation. The percent yield of the compound was 20.82% and was sent to Dr. Dean's lab to have its bioactivity tested.

NMR

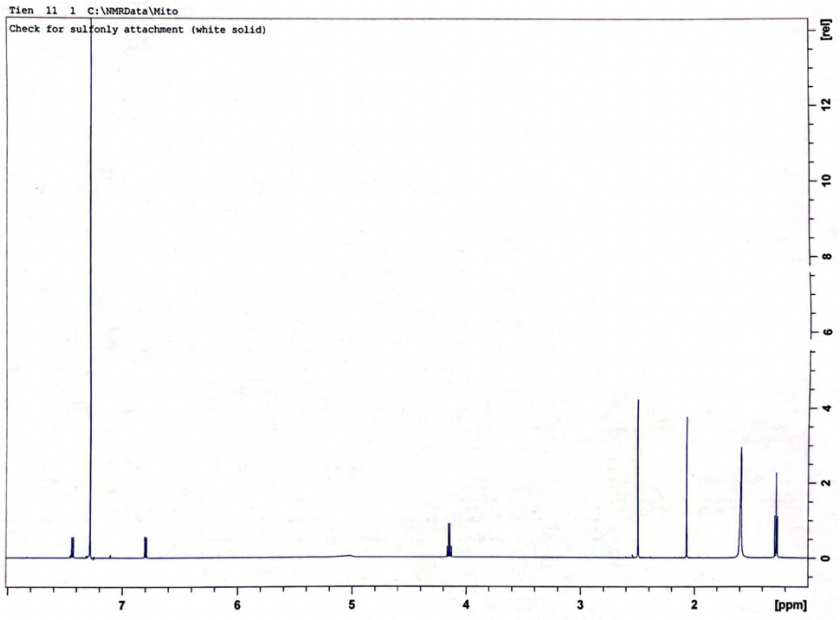


Figure 15. Experimental NMR of Compound **1**.

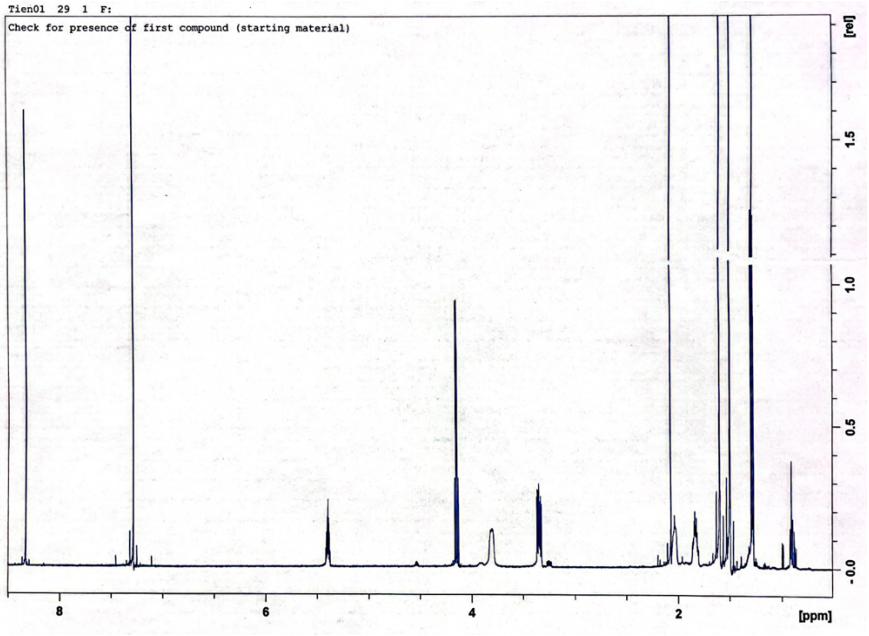


Figure 16. Experimental NMR of Compound **2**.

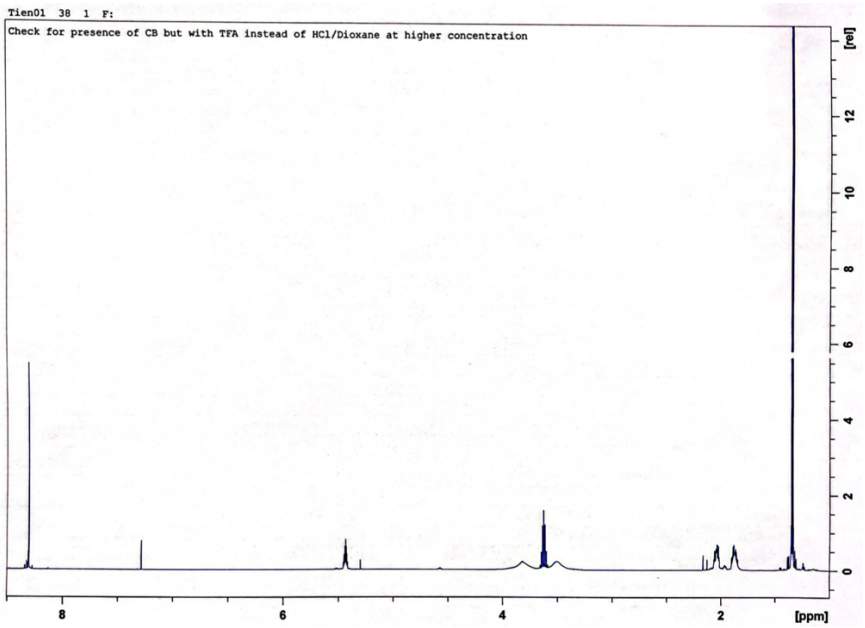


Figure 17. Experimental NMR of Compound **3**.

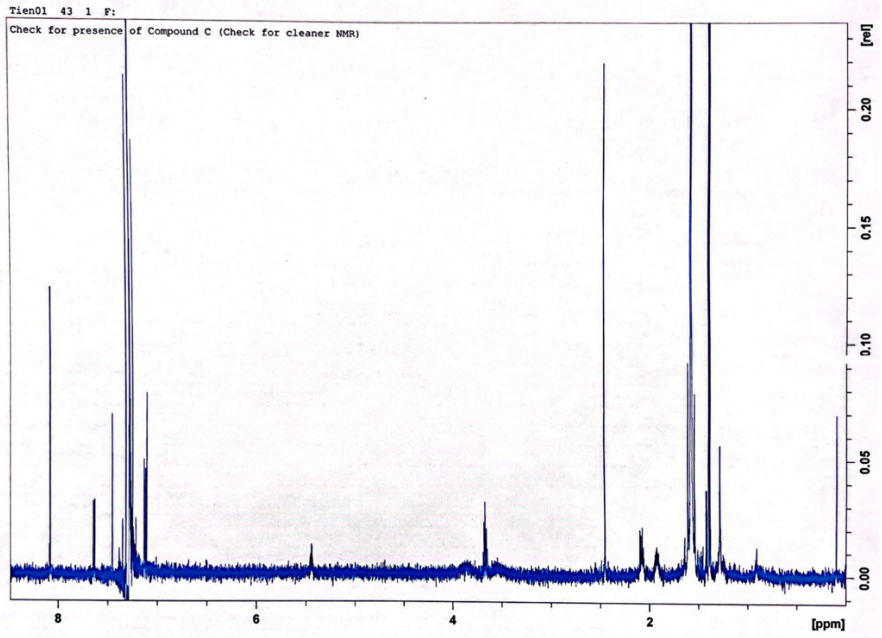


Figure 18. Experimental NMR of proposed Agonist compound.

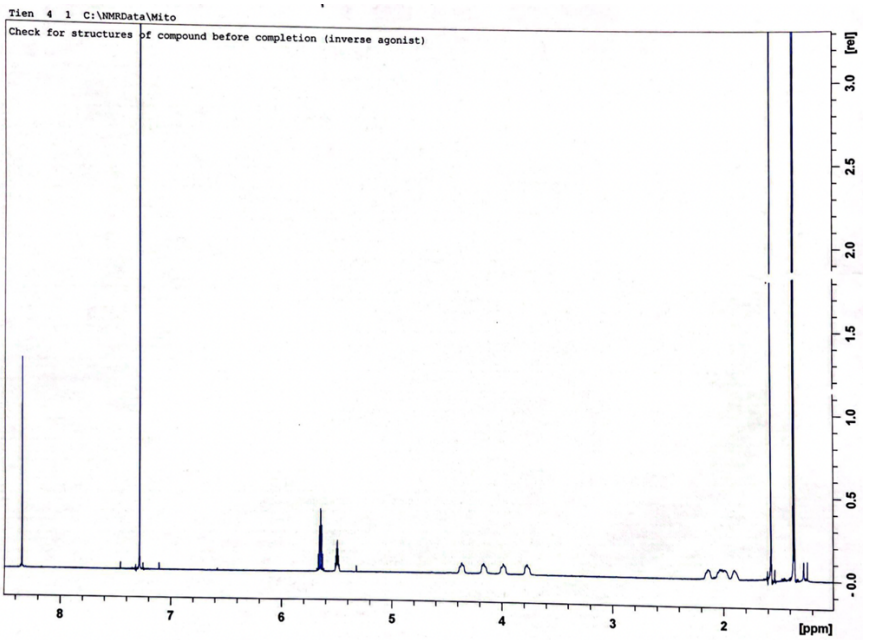


Figure 19. Experimental NMR of Compound **5**.

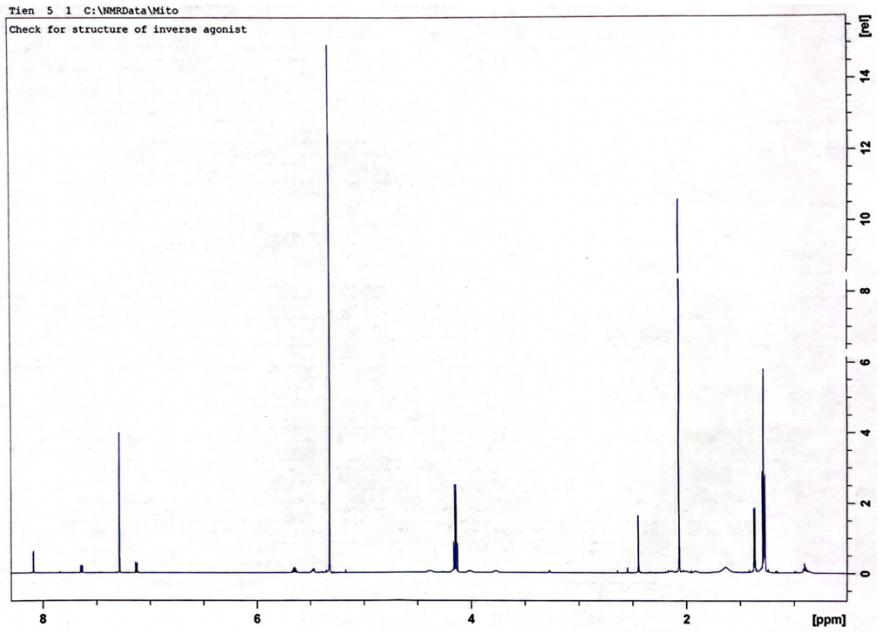


Figure 20. Experimental NMR of the proposed Inverse Agonist compound.

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BIOGRAPHICAL SKETCH

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