4-22-2020

Structure activity relationship (SAR) studies of neurotoxin quinoline-derivatives

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An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Chemistry

By

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Abstract

Structure activity relationship (SAR) studies are performed in order to identify the core structure that is responsible for the biological activity of an organic molecule. Recently, we have synthesized a drug prototype which contains several functional groups, such as an alcohol, an ester, a fluorine, and an aromatic ring. While studying in vivo toxicity of this molecule in zebrafish (Danio rerio) embryo, we observed that it has a unique biological activity that causes a sudden inactivity in embryo movement. Continued investigation revealed that this molecule blocks sodium channels in neurons causing a temporary anesthesia in Danio rerio embryo. The biological activity in zebrafish was performed with Dr. Sittaramane. We have also observed that after transferring the embryo to fresh water, the embryo resumed normal behavior. As our next step, we would like to synthesize a variety of structural analogs and determine their activity. The ultimate goal of this project was to develop effective methods of synthesizing various molecules that have one of the functional groups removed in order to identify its role in biological activity. All products and intermediates will be fully analyzed using NMR, IR and Mass spectrometer analysis. Further structural modifications may be required depending on the activity findings. After successful synthesis of the proposed molecules, Dr. Sittaramane and I performed in vivo activity determination.

Thesis Mentor:_____

Dr. Abid Shaikh

Honors Director:_____

Dr. Steven Engel

Acknowledgements

I would like to first thank Dr. Abid Shaikh and Dr. Vinoth Sittaramane for their assistance in performing this research. I would also like to thank my peers and research partners, specifically Ashley Williams for her assistance in learning to work with zebrafish. Thank you to Georgia Southern University's Honors Program for motivating me to extend my education beyond the classroom to undertake this research. Special thanks to the College Office of Undergraduate Research for financial support in completing this research. Finally, thank you to my friends and family for your constant encouragement throughout my education.

Introduction

Quinolines are naturally occurring structures that typically act as structural subunits of much larger, more complex molecules[1]. Further, quinoline derivatives are known to exhibit many medical properties. For instance, quinoline derivatives are currently being utilized as a method to treat and prevent malaria[2].

There are many compounds that are known to possess sedative properties. Specifically, anesthetics are compounds which prevent nerves from communicating any sensations that they are exposed to. These anesthetics are known to alter how the blood-brain barrier (BBB) functions[4]. The BBB, endothelial tight junction between capillaries and the CNS, is essential in maintaining homeostasis throughout the central nervous system[5]. One known method of interfering with the BBB is to affect the function sodium channels, as seen in lidocaine[6]. This works because sodium channels are known to initiate and propagate action potentials in muscles; if no action potential is initiated or propagated, then no sensation will be registered by the brain[7].

Structure-activity relationship studies are utilized to determine the role between the structure of a specific compound and its biological activity. In this case, we have a compound, 4,4,4-trifluoro-3-hydroxy-3-(quinolin-8-chloro-2-ylmethyl)butanoate, which is biologically active. This was discovered while studying in vivo toxicity of this molecule in zebrafish (Danio rerio) embryo, we observed that it has a unique biological activity that causes a sudden inactivity in embryo movement. Danio rerio was chosen as a model organism due to its genetic similarity to humans and its similar motor system[3]. Continued investigation revealed that this molecule blocks sodium channels in neurons causing a temporary anesthesia in Danio rerio embryo. We have also observed that after transferring the embryo to fresh water, the embryo resumed normal behavior.

The goal of this project is to determine which aspects of the chemical structure of our compound are biologically active and if we can alter it to make it more effective. In order to accomplish this, various analogs will be synthesized by altering key functional groups. Specifically, the changes will involve the removal of an ethyl, the removal of a chlorinated aromatic ring, the addition of chlorine, or the addition of fluorine. These newly synthesized compounds will then be tested on Danio rerio embryo and the results will be statistically evaluated.

Results and discussion

Our original goal was to determine which functional groups on **JF-1-1** contributed to the sedative phenotype which was characterized in Danio rerio. In order to accomplish this, our study synthesized structural analogs of **JF-1-1** to help determine which functional groups played a role in the phenotype. To begin this test, five distinct compounds (**JF-1-1**, compound **1**, compound **3**, compound **6**, and compound **8**) were synthesized and isolated using varying techniques.

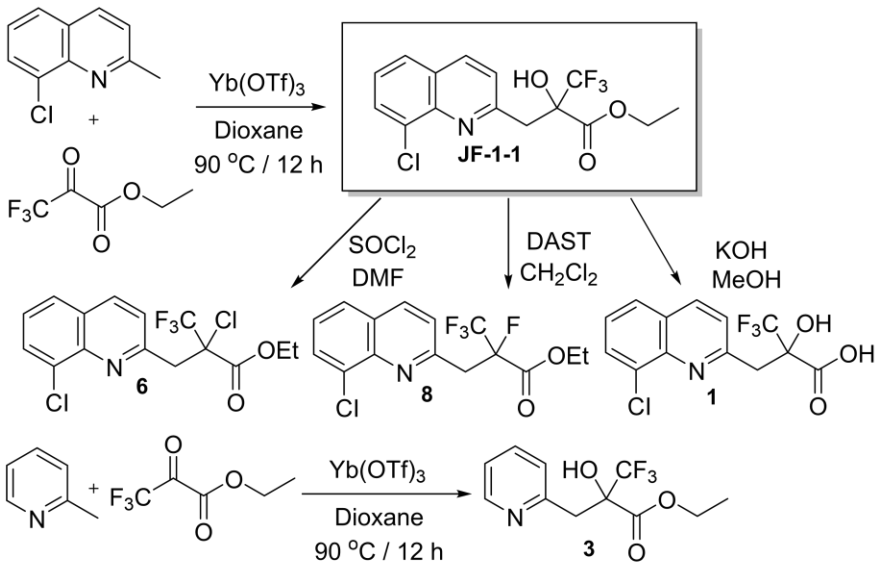


Figure 1. Shows the pathway for the synthesis of JF-1-1,DS-1-1, DS-1-3, DS-1-6, and DS-1-8

1 altered **JF-1-1** by replacing an Ethyl with an OH group, **3** altered **JF-1-1** by removing a chlorobenzene, **6** altered **JF-1-**1 by replacing a hydroxide with a chlorine, and **8** altered **JF-1-1** by replacing a hydroxide with a fluorine. Over the course of the next several weeks, various assays were conducted on Danio rerio. For these assays, Danio rerio were exposed to the control condition (1µM DMSO suspended in embryonic medium (E3)) or the experimental condition (6.7 µM of compound in DMSO suspended E3) for twelve hours prior to data collection. Data were collected using a DanioVision in conjunction with EthoVision XT software to monitor the total distance moved (mm) by Danio rerio and the mean amount of activity (moving parts of body without physical distance traveled) in two cycles, a cycle exposed to white light and a cycle exposed to no light. Only compounds **6** and **JF-1-1** (6.7 µM in DMSO suspended E3) were statistically different compared to the control (1µM DMSO in E3).

Conclusion

In summary, our experimentation yielded that compound **6** (4,4,4-trifluoro-3-chloro-3-(quinolin-8-chloro-2-ylmethyl)butanoate) and **JF-1-1** (4,4,4-trifluoro-3-hydroxy-3-(quinolin-8-chloro-2-ylmethyl)butanoate) were statistically significant in terms of producing a sedative phenotype in Danio rerio at a dosage of 6.7 µM. From this, it can be inferred that functional groups such as 4-chlorobenzene, EtOH, CF_3, etc. were all crucial for producing the observed phenotype. Future experimentation can build upon this framework to further determine which functional groups play the largest role in the effectiveness of these quinoline derivatives. Further testing needs to be done with compound **6** to ensure that it is statistically significant at producing the sedative phenotype with a large sample size. This experimentation paves the way for further investigation

into quinoline derivatives being used as painkillers or sedatives rather than using the traditional medication of today.

References

1. Chu, X.-M.; Wang, C.; Liu, W.; Liang, L.-L.; Gong, K.-K.; Zhao, C.-Y.; Sun, K.-L., Quinoline and quinolone dimers and their biological activities: An overview. European Journal of Medicinal Chemistry 2019, 161, 101-117.
2. Nayak, N.; Ramprasad, J.; Dalimba, U., Synthesis and antitubercular and antibacterial activity of some active fluorine containing quinoline–pyrazole hybrid derivatives. Journal of Fluorine Chemistry 2016, 183, 59-68.
3. Guo, S., Linking genes to brain, behavior and neurological diseases: what can we learn from zebrafish?. Genes, Brain and Behavior 2003, 3: 63-74.
4. Tanobe,; K. Nishikawa, K.; Hinohara, H.; Okamoto, T.; Saito, S.; Goto, F., Blood-Brain barrier and general anesthetics. Japanese Journal of Anesthesiology 2003, 52, 840-845.
5. Fan, K.; Jia, X.; Zhou, M.; Wang, K.; Conde, J.; He, J.; Tian, J.; Yan, X., Ferritin Nanocarrier Traverses the Blood Brain Barrier and Kills Glioma. ACS Nano 2018, 12, 4105-4115.
6. Oakes, V.; Domene, C., Capturing the Molecular Mechanism of Anesthetic Action by Simulation Methods. Chemical Reviews 2019,119, 5998-6014.
7. Cronin, N.; O'Reilly, A.; Duclohier, H.; Wallace, B., Effects of Deglycosylation of Sodium Channels on Their Structure and Function. Biochemistry 2005, 44, 441-449.
8. Sittaramane, V.; Padgett, J.; Salter, P.; Williams, A.; Luke, S.; McCall, R.; Arambula, J.F.; Graves, V.B.; Blocker, M.; Van Leuven, D.; Bowe, K.; Heimberger, J.; Cade, H.C.; Immaneni, S.; Shaikh, A., Discovery of Quinoline-Derived Trifluoromethyl Alcohols,

Determination of Their in vivo Toxicity and Anticancer Activity in a Zebrafish Embryo Model 2015, 10, 1802-1807.

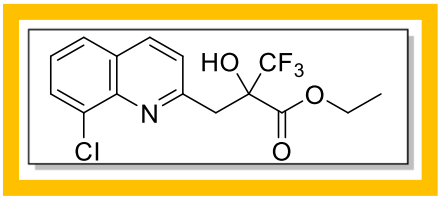
Appendix: Experimental Procedure and Product Characterization

Experimental Procedure

JF-1-1 (4,4,4-trifluoro-3-hydroxy-3-(quinolin-8-chloro-2-ylmethyl)butanoate) (35mg) was added to a pressure tube along with 2 mL of methanol, potassium hydroxide (56 mg) and 500µL of water. The reaction was then left to stir for 12 hours at room temperature. After the reaction was complete, the product was added to a separatory funnel along with ethyl acetate to isolate the organic layer. The resulting mixture was then passed over anhydrous sodium sulfate before utilizing a Buchi RotaVapor R-200 to concentrate product. This product was then allowed to sit under a vacuum overnight to allow for further concentration. The product was then verified by using ^{13}C and ^{1}H Nuclear Magnetic Resonance Spectroscopy.

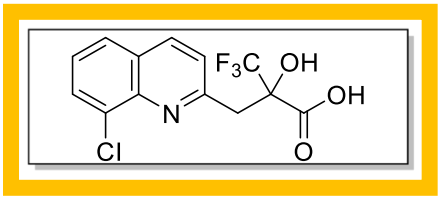
Product characterization

JF-1-1



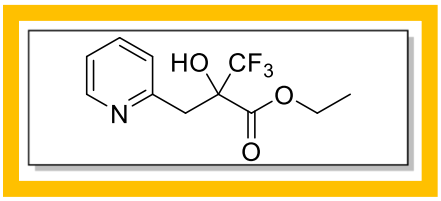
^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm) 8.16 (d, *J* = 8.35 Hz, 1H), 7.81 (d, *J* = 7.50 Hz, 1H), 7.72 (d, *J* = 7.50 Hz, 1H),7.45 (t, *J* = 8.35 Hz, 1H), 7.38 (d, *J* = 8.35 Hz, 1H), 7.07 (s, 1H), 4.22 (quart, *J* = 7.11 Hz, 2H), 3.82 (d, *J* = 15.72 Hz, 1H), 3.55 (d, *J* = 15.72 Hz, 1H), 1.17 (t, *J* = 7.21 Hz, 3H)

DS-1-1



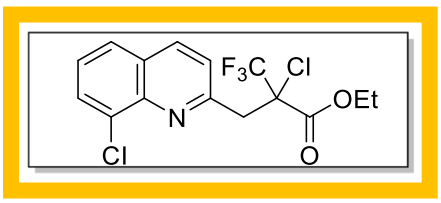
^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm) 8.29 (d, *J* = 8.56 Hz, 1H), 7.88 (d, *J* = 7.69 Hz, 1H), 7.79 (d, *J* = 8.56 Hz, 1H), 7.55-7.46 (m, 2H), 3.70 (quart, *J* = 18.28 Hz, 3H)

DS-1-3



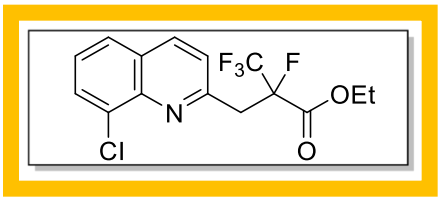
^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm) 8.43 (d, *J* = 4.86 Hz, 1H), 7.65 (t, *J* = 8.04 Hz, 1H), 7.19 (m, 2H), 6.52 (s, 1H), 4.21 (quart, *J* = 7.04 Hz, 2H), 3.51 (d, *J* = 14.80 Hz, 1H), 3.32 (d, *J* = 14.80 Hz, 1H), 1.18 (m, 4H)

DS-1-6



^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm) 8.48 (d, *J* = 9.0 Hz, 1H), 7.86 (t, *J* = 8.0, 1H), 7.74 (m, 1H), 7.55 (m, 2H), 4.18 (quart, *J* = 7.1 Hz, 2H), 3.09 (s, 2H), 1.30 (t, *J* = 7.1 Hz, 3H)

DS-1-8



^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm) 8.18 (d, *J* = 8.40 Hz, 1H), 7.86 (d, *J* = 7.55 Hz, 1H), 7.80-7.67 (m, 4H), 87.56 (d, *J* = 7.92 Hz, 1H), 3.94 (quart, *J* = 7.47 Hz, 2H), 2.81 (s, 1H)