Synthesis of MC1 for Toxicology Study, Preceding Evaluation of [^{11}C]MC1 as a PET Radioligand for Imaging Neuroinflammation in Humans

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INTRODUCTION

Cyclooxygenases COX-1 and COX-2 are enzymes responsible for the production of pro-inflammatory prostaglandins from arachidonic acid (Figure 1). Both enzymes are naturally found in animal kidneys, reproductive organs, and the brain tissues;[1] however, COX-2 is found sparingly in healthy bodies, only becoming significantly overexpressed in response to inflammatory illnesses (e.g. neurodegenerative diseases, cancer).[2] Exploiting this conditional inducement by developing a PET radioligand that is highly selective for COX-2 in the human brain could advance research regarding neuroinflammation.

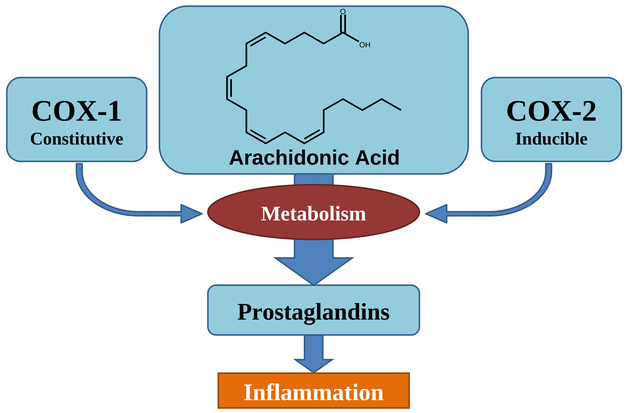

Figure 1. Mechanism of Action of Cyclooxygenase

[^{11}C]MC1 (Figure 2) is a potential COX-2 radioligand planned to be evaluated in PET imaging for the detection of neuroinflammation in humans.

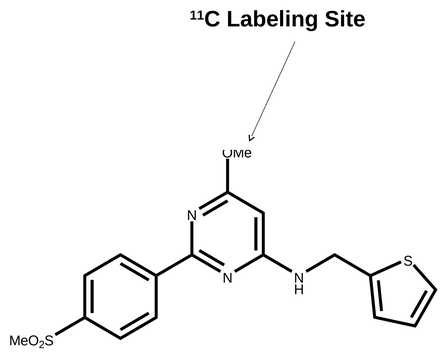

Figure 2. 6-methoxy-2-(4-(methylsulfonyl)phenyl)-N-(thiophen-2-ylmethyl)pyrimidin-4-amine [MC1]

OBJECTIVES

- ❑ **Synthesize MC1, a COX-2 ligand, to be used for toxicology studies**
- ❑ **Test blocking ability of MC1 on COX-2 in PET studies of neuroinflammation using [^{11}C]MC1 as the radioligand**

METHODS

Synthesis

MC1 was prepared by a seven step process modified from a published synthetic pathway (Figure 3).[3] Purification by HPLC was performed after each of the final three steps, with two additional rounds of purification for the final product. This product was analyzed for purity with HPLC and chemical composition with ^{1}H and ^{13}C NMR spectroscopy.

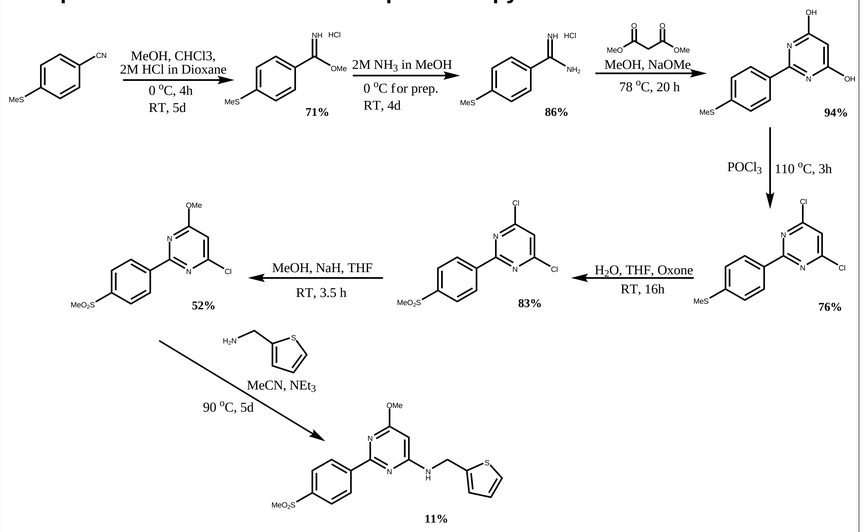

Figure 3. Reaction Pathway for MC1 Synthesis

RESULTS

MC1 (104 mg) was obtained in a 2.1% overall yield. The product was identified as the expected MC1 and fully characterized as pure by ^{1}H NMR (Figure 4), ^{13}C NMR (Figure 5), and HPLC (Figure 6).

^{1}H NMR

^{1}H NMR spectroscopy showed ten significant peaks, with integration revealing a total of seventeen hydrogens in the compound (Figure 4). Two methyl groups in highly electronegative conditions and three pairs of identically marked hydrogens are represented in addition to five single-proton peaks. This spectrum matches the predicted analysis for MC1.

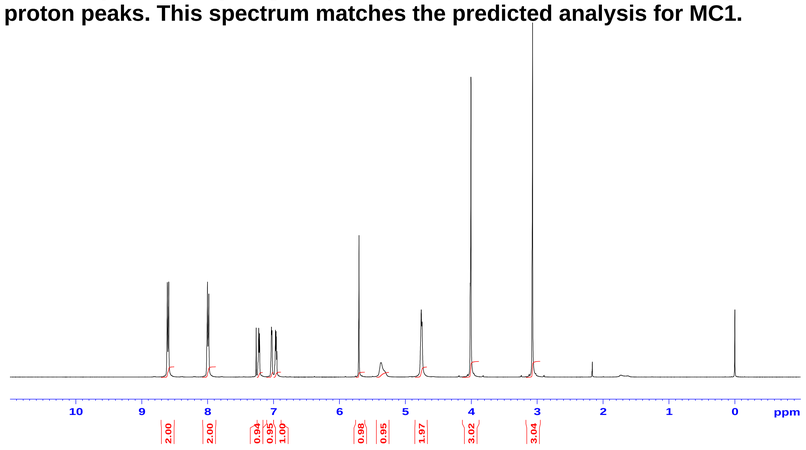

Figure 4. ^{1}H NMR Spectrum of Synthesized MC1 in $CDCl_3$

RESULTS CONTD.

^{13}C NMR

^{13}C NMR spectroscopy showed fifteen significant peaks, two of which were notably taller than the others (Figure 5). This spectrum also bears a similar appearance to the predicted analysis for MC1.

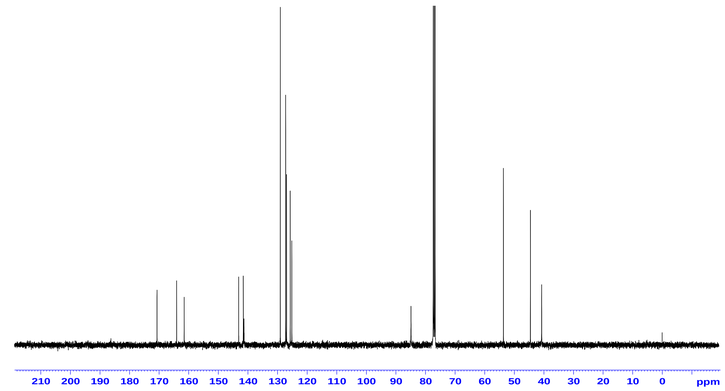

Figure 5. ^{13}C NMR Spectrum of Synthesized MC1 in $CDCl_3$

HPLC

HPLC analysis of the purified product reported 98.24% purity of the analyzed compound.

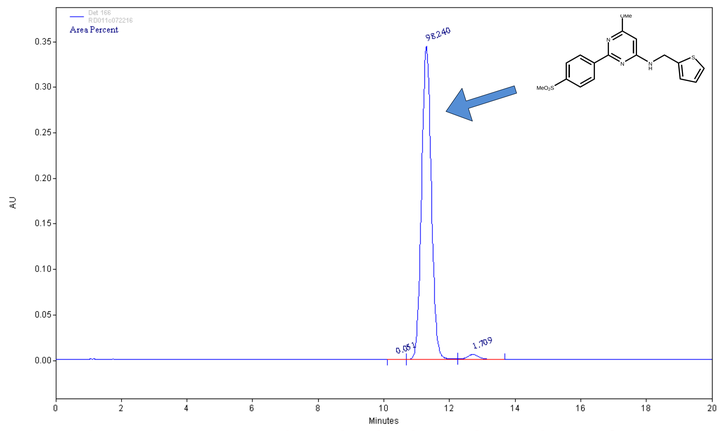

Figure 6. Spectrum of Synthesized MC1 for Purity Analysis

CONCLUSIONS AND FUTURE DIRECTIONS

- ❑ **The end product of the synthesis is 98.24% pure MC1.**
- ❑ **Synthesized MC1 will be sent to an external lab for toxicological testing.**
- ❑ **MC1 will continue to be tested for viability as a blocker for use in PET neuroinflammation studies.**
- ❑ **Additional methodology will be applied to intermediate steps in an attempt to increase overall yield.**

References

[1] Tietz O, et al. (2013) *Org. Biol. Chem*. 8052-64.
[2] Zarghi A, et al. (2011) *Iranian J. Pharm. Res*. 10, 655-83.
[3] Orjales A, et al. (2008) *Bioorg. Med. Chem*., 16, 2183-99.

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