Development of a novel PET tracer for detecting early stage neuroinflammation

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Introduction

Alzheimer's Disease: Current Challenges

- Alzheimer's disease is the most common type of dementia in older adults, but little is known about the causes of the disease and methods for an early diagnosis are currently not available (Sun et al., 2018).

Chronic Neuroinflammation and Alzheimer's Disease

- Chronic neuroinflammation is a significant contributor to the development of neurodegenerative diseases like Alzheimer's.
- Chronic neuroinflammation is a result of overactive microglia, immune cells that are responsible for neuroinflammatory responses and maintaining homeostasis in the central nervous system (Perry and Teeling, 2013).
- In Alzheimer's disease, initial activation of the microglia can be beneficial by promoting clearance of amyloid-β plaques (Hickman et al., 2008). However, in later stages of the disease, persistent neuroinflammation was shown to encourage disease progression (Hickman et al., 2008).

Role of Kv1.3 in Neuroinflammation

- Microglia are regulated by Kv1.3, a voltage-gated potassium ion channel, which is highly expressed in the brain tissue of patients with Alzheimer's disease (Rangaraju et al., 2015).
- It has been observed that the high expression of Kv1.3 was limited to the microglia (Maezawa et al., 2018).

PET Imaging as a Potential Tool for Diagnosing Alzheimer's Disease

- PET is a non-invasive technique that uses radioactive tracers to show images of organ and tissue function. Unlike other diagnostic techniques, such as CT and MRI scans, PET scans have the ability to detect the presence of cellular abnormalities before any physical damage has occurred.
- The concept of microglia-targeted therapy through Kv1.3 inhibition could be a potential treatment for Alzheimer's disease (Maezawa et al., 2018).
- Based on this concept, we hypothesized that targeting activated microglia with a Kv1.3 inhibiting PET tracer could be an effective method for detecting chronic neuroinflammation during the early stages of Alzheimer's.

Compound Selection & Aim

QMC-7003

- Lead target candidate
- Fluorinated version of QMC-7005
- Represents the structure of the requisite [18]F] PET tracer for detecting neuroinflammation.

QMC-7005

- Displayed potent inhibitory activity against Kv1.3 (IC_{50} = 5 nM) in previous study (Miao et al., 2003).

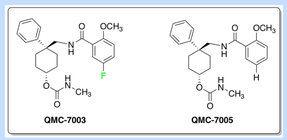


Figure 1. Test candidate QMC-7003 and the previously reported Kv1.3 inhibitor QMC-7005.

Aim

- Synthesize a non-radiolabeled variant of QMC-7003 to investigate its biology and chemistry.

Materials & Methods

Chemical Synthesis of Compound QMC-7003 and QMC-7005

Step 1 (Compound B). To a 0 °C solution of **A** in THF, $Li(O^tBu)_3AlH$ was slowly added. After confirming completion of the reaction by TLC, the solution was warmed to 20 °C and $LiAlH_4$ was slowly added. The mixture was then heated to 65 °C and was left to stir for at least 15 hours. Afterwards, the reaction was quenched with water, 10% NaOH, then water once again. The reaction was then stirred vigorously for a minimum of 3 hours, then anhydrous sodium sulfate was added. The mixture was filtered, washed with THF, and concentrated in vacuo. Precipitation out of DCM then yielded a white powder (574.6 mg; 93%).

Step 2 (Compound D/E). To 0 °C slurry of **B** in DCM, triethylamine was added, then **C** was added dropwise. The mixture was then warmed to 20 °C and monitored by TLC. When the reaction was complete, the reaction was quenched with 1M HCl. The organic layers were washed with 1M HCl and saturated $NaHCO_3$, and dried over sodium sulfate, filtered and concentrated. Finally, the crude mixture was purified by column chromatography, yielding a thick oil (54.4 mg; 5%).

Step 3 (QMC-7003/7005). 4-nitrophenyl chloroformate and triethylamine was added to a solution of **D/E** in DCM. The reaction was then stirred for a minimum of 3 hours. When the reaction was complete, methylamine was added and the reaction was stirred for at least 2 hours. Upon completion, the reaction mixture was concentrated and the residue was purified by column chromatography yielding a white foam (15.8 mg; 26%).

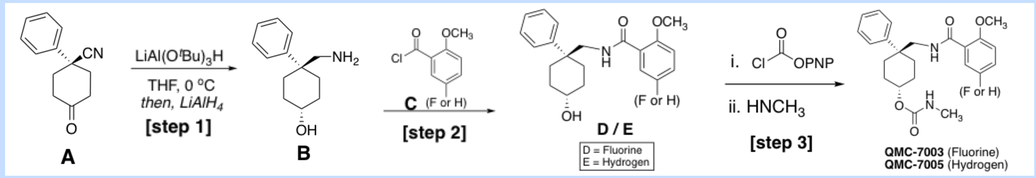
Kv1.3 Inhibition Assay of QMC-7003 and QMC-7005

Cell Culture. HEK293 cells stably transfected with human Kv1.3 that was cultured in a DMEM medium containing 10% fetal bovine serum, and G418 (500 μg/ml). Cells were kept at 37°C under 95% air and 5% CO_2 conditions.

Electrophysiology. The patch clamp experiments were performed in a whole-cell configuration using EPC-9. Kv1.3 currents were elicited by a step protocol from a holding potential of -80 mV to +40 mV with a pulse duration of 20 ms and an interpulse interval of 10 sec. Data was normalized to the current before compound application. All values were given as a mean ± standard error of mean (S.E.M).

Results

Chemical Synthesis of QMC-7003 and QMC-7005



Scheme 1. Chemical Synthesis of QMC-7003 and QMC-7005.

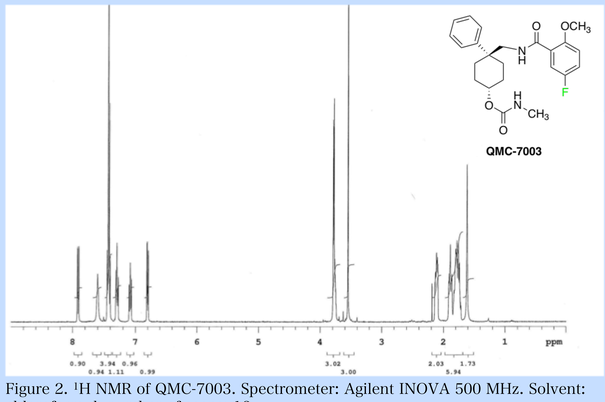


Figure 2. ^{1}H NMR of QMC-7003. Spectrometer: Agilent INOVA 500 MHz. Solvent: chloroform-d; number of scans: 16.

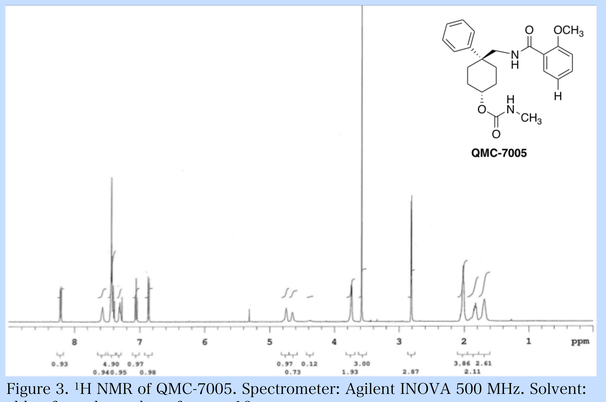


Figure 3. ^{1}H NMR of QMC-7005. Spectrometer: Agilent INOVA 500 MHz. Solvent: chloroform-d; number of scans: 16.

Kv1.3 Inhibition Assay of QMC-7003, QMC-7005, and PAP-1

Raw Data

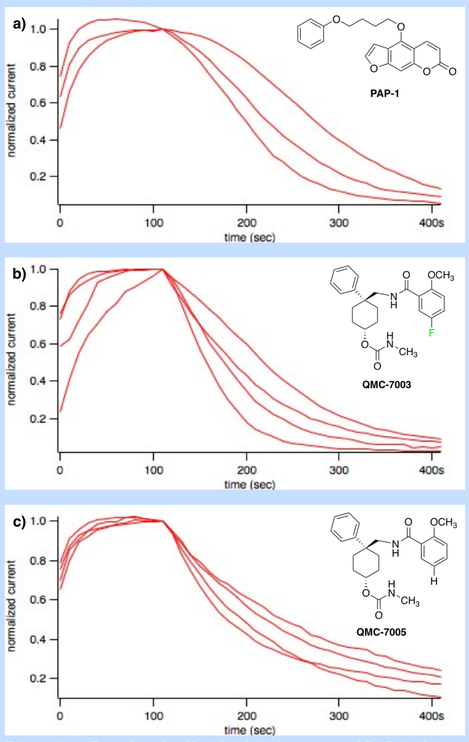


Figure 4. a) Raw data for Kv1.3 inhibition assay of PAP-1.; b) Raw data for Kv1.3 inhibition assay of QMC-7003.; c) Raw data for Kv1.3 inhibition assay of QMC-7005.

Overall Data

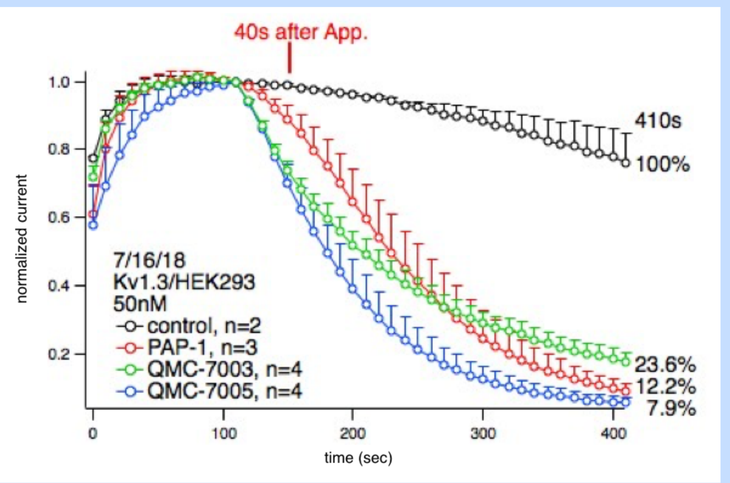


Figure 5. Kv1.3 inhibition assay of QMC-7003 (green), QMC-7005 (blue), and PAP-1 (red). Whole-cell currents were elicited by a step protocol from a holding potential of -80 mV to +40 mV with a pulse duration of 20 ms and an interpulse interval of 10 sec. Inhibitors were examined at a final concentration of 50 nM. All data were normalized to the control (black; no drug) at the time of application.

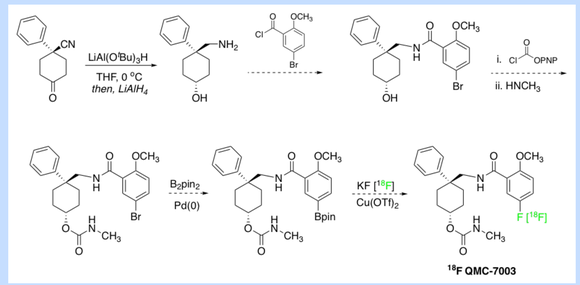
	40s after App. (Inh.)		410s (Inh.)	
control	100%	(0%)	100%	(0%)
PAP-1	86.6%	(13.4%)	12.2%	(87.8%)
QMC-7003	67.2%	(32.8%)	23.6%	(76.4%)
QMC-7005	62.1%	(37.9%)	7.9%	(92.1%)

Table 1. The inhibition of Kv1.3 by the test compound QMC-7003 and two known Kv1.3 inhibitors, PAP-1 and QMC-7005. The results are reported as percent reduction of the Kv1.3 current 40 sec. and 410 sec. after drug application.

Discussion

- The results of this study show that the novel test compound QMC-7003 is an effective inhibitor of Kv1.3.
- The addition of the aromatic fluorine did not adversely affect the potency of Kv1.3 inhibition, as QMC-7003 was able to block Kv1.3 channels with similar effectiveness to that of QMC-7005 and PAP-1.
- While QMC-7003 did exhibit a slightly lower Kv1.3 inhibition (76.4%) compared to that of QMC-7005 (92.1%) and PAP-1 (87.8%), this difference was not substantial, which encouraged the team to pursue further development of this compound.

Conclusion and Future Direction



Scheme 2. Proposed synthetic route to the [18]F] QMC-7003 PET tracer.

- QMC-7003 is a promising Kv1.3 inhibitor and candidate for further development into a [18]F] PET tracer for detecting neuroinflammation.
- In future studies, the [18]F] QMC-7003 PET tracer will be synthesized (Scheme 2) and tested on animals to determine its Kv1.3 binding specificity in a living system. This test will also determine if [18]F] QMC-7003 has the ability to permeate the blood-brain barrier and bind to any Kv1.3 present in the brain, allowing for the detection and visual imaging of neuroinflammation.
- This summer research project provided the necessary data to support the continued development of this novel Kv1.3 inhibitor into a PET tracer, introducing the idea of a new method for the early diagnosis of Alzheimer's and other neurodegenerative diseases.

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For Further Information

Please contact *acullen@queens.org*. More information on this and related projects can be obtained at *www.qcbr.jabsom.hawaii.edu*.