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Investigation of an F-18 oxytocin receptor selective ligand via PET imaging

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ABSTRACT

The compound 1-(1-(2-(2-(2-fluoroethoxy)-4-(piperidin-4-yloxy)phenyl)acetyl)piperidin-4-yl)-3,4-dihydroquinolin-2(1*H*)-one (**1**) was synthesized and positively evaluated in vitro for high potency and selectivity with human oxytocin receptors. The positron emitting analogue, [F-18]**1**, was synthesized and investigated in vivo via PET imaging using rat and cynomolgus monkey models. PET imaging studies in female Sprague–Dawley rats suggested [F-18]**1** reached the brain and accumulated in various regions of the brain, but washed out too rapidly for adequate quantification and localization. In vivo PET imaging studies in a male cynomolgus monkey suggested [F-18]**1** had limited brain penetration while specific uptake of radioactivity significantly accumulated within the vasculature of the cerebral ventricles in areas representative of the choroid plexus.

Oxytocin was the first endocrine system hormone to be synthesized in the laboratory, and has been studied extensively for its role in the induction of labor and the milk ejection reflex.[1] More recently, research foci have been directed toward the role of oxytocin and the oxytocin receptor (OTR) in the regulation of psychosocial behavior.[2–10] In addition to being implicated in modulating maternal nurturing and mother–infant bonding, pair bonding, social recognition, increased eye-to-eye contact, trust and empathy, the oxytocin system has also been investigated in relation to disorders characterized by disruption in social behavior, including autism spectrum disorders.[11–17] While the bulk of the research conducted with the oxytocin system in humans has been limited to experimental paradigms involving administration of oxytocin followed by behavior monitoring, methodologies implemented for investigating OTR within the brain have been limited to post-mortem receptor autoradiography, in situ hybridization, and gene association studies.[12,13,18–21] The development of a methodology permitting the quantification of OTR in the living brain would represent a significant advance in this area of research. Our goal is to develop a positron-emitting small molecule with high affinity and selectivity for the human and primate OTR to enable non-invasive correlations between neural OTR densities and behavior via in vivo positron emission tomography (PET). In addition to this goal, we are simultaneously seeking to discover new candidate pharmaceuticals which may prove useful in reaching OTR specifically within the brain or the periphery for the purpose of potentially alleviating and/or investigating symptoms associated with social behavior disorders. We report here the synthesis, radiosynthesis, and PET imaging evaluation of one of our lead candidate compounds meeting these criteria.

We have recently reported progress in the development of OTR selective ligands bearing radioactive isotopes with some bearing structural similarity to the lead candidate described here.[22,23] These molecules were found to either not penetrate the blood–brain barrier or have high affinity for the p-glycoprotein pump which prevented their retention within the brain. We concluded that the lack of a hydrogen bond donor may have been the cause for lack of brain penetration or retention. Therefore, we proceeded to modify the structure by eliminating the methyl sulfonyl group of our former lead candidate, **2**, to generate a compound bearing a free amine to act as a hydrogen bond donor, **1** (Fig. 1). The synthetic route was derived from a precursor in our previously reported synthetic pathway, **3**, and is outlined in Scheme 1[22] The free amine of **3** was protected using di-*tert*-butyl dicarbonate to generate **4**. The phenol position of **4** was then alkylated using

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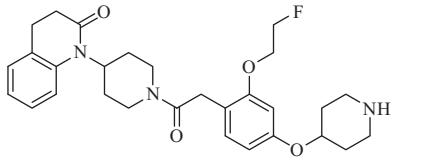

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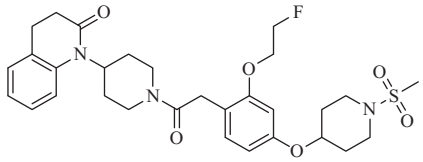

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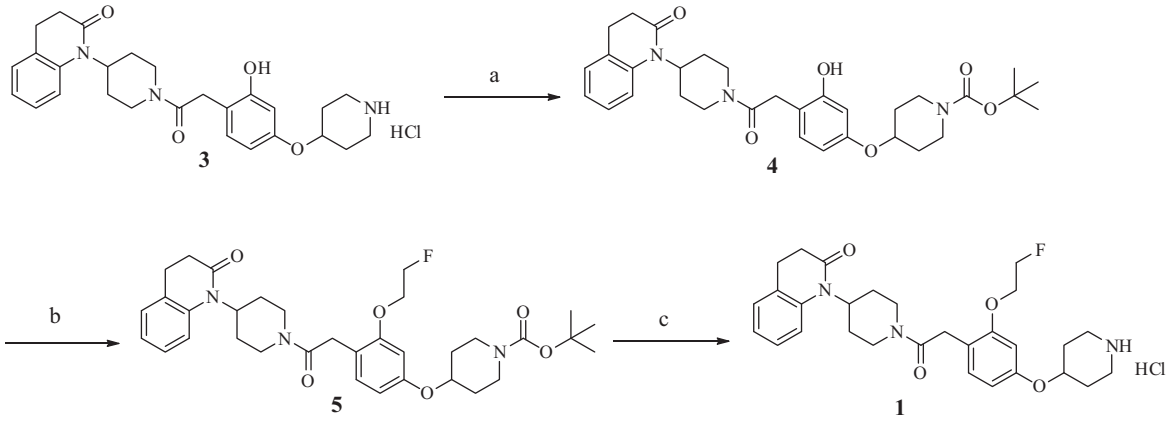
Figure 1. Structures of OTR selective compounds **1** and **2**.

the binding affinity of **1** for the V1aR slightly increased, the binding affinity for the V2R decreased significantly in comparison to **2**, thus making it slightly more selective overall. Further in vitro binding assays were also performed on 88 other human G-protein coupled receptors (GPCR) in a screen to determine if **1** had affinity for these other receptors. Table 2 contains the results of the screen in which 7 other GPCR had modest to mild affinity for **1**, none of which would be expected to display significant uptake during a PET scan. The most notable binding affinity was found in the alpha-1A subtype of the adrenergic receptor and the 6 subtype of the 5-hydroxytryptophan receptor (5-HT6) having a K_i values of 82 nM and 187 nM, respectively. Although these K_i values for the non-target receptors are not believed to be optimal for PET imaging, they would present an issue if **1** was to be considered for pharmaceutical development.

Having good binding affinity and selectivity, the F-18 analogue



Scheme 1. Synthetic route to obtain the target molecule **1**. Reagents and conditions: (a) Boc_2O, dioxane, 1 N NaOH, rt, 48%; (b) 1-bromo-2-fluoroethane, Cs_2CO_3, DMF, rt, 97%, (c) (1) 2 M HCl in ether, rt, 96%. Further reaction details and characterizations are provided in the Supplementary data.

1-bromo-2-fluoroethane to provide the fluoroethoxy moiety of **5**. A deprotection using 2 M HCl in ether then resulted in the target molecule, **1**, as a hydrochloride salt. Further synthetic details and characterizations are provided in the Supplementary data.

To determine if the structural modification resulted in any loss of biological properties, **1** was subjected to in vitro competitive binding assays using cell lines expressing human oxytocin and all known vasopressin receptor subtypes (V1aR, V1bR, and V2R). The results of these assays are displayed in Table 1 with data for **2** obtained from our previously reported study.[22] These data indicate the removal of the methyl sulfonyl group actually resulted in a slight improvement of the biological properties. Having a K_i value of 15 nM for the OTR, **1** matched the binding affinity of **2**. While

Table 1
In vitro K_i (nM) determinations of **1**–**2** from competition assays with human OTR and vasopressin receptors (3 subtypes)[a]

Compound	OTR	V1aR	V1bR	V2R
2	16	8700	>10,000	1800
1	15	5600	>10,000	8700

For experimental details please refer to the PDSP web site http://pdsp.med.unc.edu/ and click on 'Binding Assay' or 'Functional Assay' on the menu bar.

[a] K_i Values were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda MD, USA.

of **1** was generated for further investigation. The synthetic route to [^{18}F]**1** is outlined in Scheme 2. The Boc-protected amine **4** was alkylated with ethane-1,2-diyl bis(4-methylbenzenesulfonate) to generate our tosylate precursor **6**. Radiolabeling was then conducted in a CTI-Seimens Chemistry process Control Unit (CPCU) to perform S_N2 substitution using the cryptand ligated $K_{222}{}^{18}F$ followed by removal of the Boc-protecting group with 1 M HCl. The crude radioactive mixture was eluted from the CPCU unit with a 7 ml solution of ethanol:water:triethylamine (50:50:0.1). Solid phase extraction was then performed to remove excess acid prior to HPLC purification. The extracted mixture was then purified via HPLC and concentrated into a dose form (15 ml solution of 10% ethanol in saline) via solid phase extraction and passed through 1 and 0.2 μm filters using high argon pressure. A dose would normally be prepared in 120 min from the start of synthesis and result in approximately 19% uncorrected yields of [^{18}F]**1** with a specific activity greater than 10 Ci/μmol. Radiochemical purity was greater than 99.9%. A representative analytical HPLC graph is shown in Figure 2.

To gauge the probability of brain penetration, the log $P_{7.4}$ was determined to be 1.57 using previously reported methods. This lipophilicity measurement is within the range of 1–3 estimated to be the calculated optimal range for penetration of the blood brain barrier.[24] To evaluate brain penetration in vivo, PET studies were first performed using female 180–200 g Sprague–Dawley rats ($n = 4$) in the same manner as our previously reported study.[22,25] Figure 3 contains representative time activity curves generated