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Synthesis, Radiolabeling and Preliminary Biological Evaluation of Radiolabeled 5-Methyl-6-nitroquipazine, a Potential Radioligand for the Serotonin Transporter

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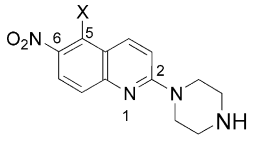
Abstract—5-Methyl-6-nitroquipazine, a novel analogue of the potent and selective serotonin transporter inhibitor 6-nitroquipazine was synthesized and radiolabeled with tritium and the positron emitter carbon-11. [^{3}H]5-methyl-6-nitroquipazine was found to have a $K_d = 51 \pm 7$ pM. The high affinity and the facile labeling of [^{11}C]5-methyl-6-nitroquipazine makes it a promising radioligand for visualization of the serotonin transporter with positron emission tomography.

Neuronal uptake of serotonin (5-HT) occurs via a serotonin transporter protein (5-HTT) which plays a major role in the regulation of synaptic 5-HT levels. There are several arguments in favor of alterations of the 5-HTT in various neurological and psychiatric disorders such as Parkinson's and Alzheimer's disease and depression. In addition, the 5-HTT is the target of several antidepressant drugs. PET examination of the 5-HTT would be of great value in order to better understand the pathophysiological mechanisms of neurodegenerative and mental illness, as well as to quantify 5-HTT-occupancy in relation to antidepressant drug treatment. Several structurally diverse compounds such as [^{11}C]McN5652, [^{123}I]ADAM, [^{11}C]DASB and [^{11}C]RTI-357 have been suggested as potential radioligands for visualization of the 5-HTT with PET and SPECT (single photon emission computed tomography).[1—4]



1, X = H
2, X = ^{123}I
3, X = ^{76}Br
4, X = CH_3

6-Nitroquipazine (**1**) is one of the most selective and potent 5-HTT inhibitors known.[5–7] Compound **1** has low affinity for non-serotonergic transporter systems and other pre- and postsynaptic receptor sites. The incorporation of the positron emitter carbon-11 ($t_{1/2} = 20.4$ min) into the carbon framework of **1** is, however, not easily performed. The 5-position has been shown to be suitable for halogen substitution yielding halogenated analogues equipotent to **1**.[8] Iodine-123 labeled [^{123}I]5-iodo-6-nitroquipazine (**2**) and bromine-76 labeled [^{76}Br]5-bromo-6-nitroquipazine (**3**) have been reported as potential SPECT and PET radioligands, respectively.[9,10] Dehalogenation has been found to be a metabolic route for **2** in non-human primates. Consequently, SPECT images recorded with **2** are obscured by the presence of free iodine-123 in the primate brain.[9]

We hypothesized that a carbon-11 labeled and methylated analogue **4** would retain the affinity for the 5-HTT and that the absence of a halogen might be advantageous from a metabolic point of view. Furthermore, being less lipophilic, the non-halogenated [^{11}C]**4** might be expected to give less non-specific binding in vivo than the halogenated counterparts **2** and **3**.

In this paper we report the synthesis and radiolabeling with tritium and carbon-11 of **4**. The binding affinity for

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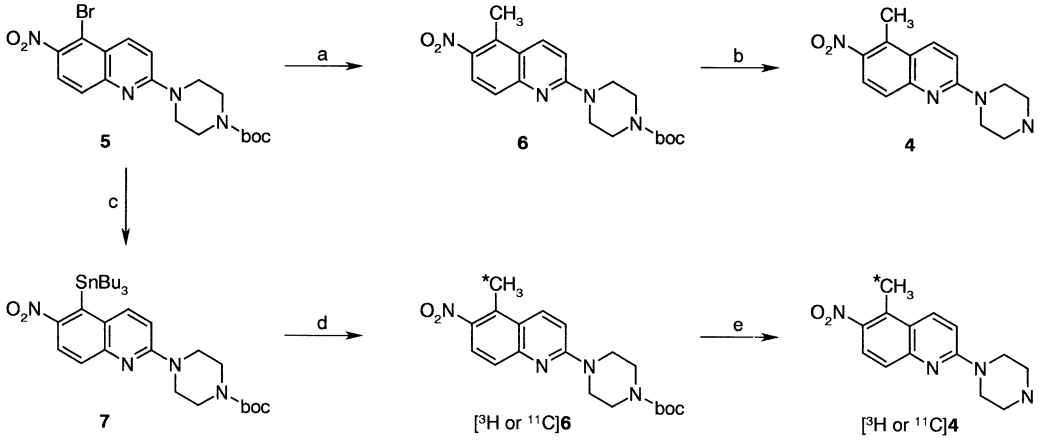

Scheme 1. Reagents and conditions: (a) $(CH_3)_4Sn$, Pd_2dba_3, DMF, 70 °C, 24 h, 70%; (b) TFA, 70 °C, 60 min, 95%; (c) *n*-BuLi, Bu_3SnCl, THF, −100 °C, 2 h, 50%; (d) **7** (2.0 mg), [^{3}H/^{11}C]MeI, Pd_2dba_3 (0.9 mg), P(*o*-$CH_3C_6H_4)_3$ (1.2 mg), DMF (350 μL), 130 °C, 4 min, 80%; (e) TFA (150 μL), 130 °C, 4 min, 95%. * Position of label.

the 5-HTT was measured using rat frontal cortex homogenates and [^{3}H]**4**.

Chemistry

Compound **5** was prepared according to a procedure reported previously.[11] **6** was synthesized in a palladium promoted cross-coupling reaction between tetramethyltin and **5** (Scheme 1). Removal of the BOC-group with TFA yielded **4**.[12] Compound **7** was synthesized from **5** by lithiation and subsequent addition of tributyltinchloride.

Radiochemistry

[^{3}H]**4** Was prepared from **7** in a two step procedure (Scheme 1). The first step was a palladium-promoted cross-coupling reaction between **7** and [^{3}H]methyl iodide.[13] The specific radioactivity of the [^{3}H]methyl iodide used was 3.15 GBq/μmol. Removal of the BOC-group was performed with TFA. Before purification by HPLC the reaction mixture was neutralized with aq/NaOH. Purification by HPLC (Column C-18 μ-Bondapak 300×7.8 mm; eluent methanol/water/triethylamine 63:37:0.2) yielded the final compound in 60% yield. The radioactive fraction containing [^{3}H]**4** was collected and after evaporation of the mobile phase [^{3}H]**4** was dissolved in ethanol and stored at −20 °C before use. Under these storage conditions no radiochemical degradation could be observed by HPLC analysis after 4 months.

The same protocol was applied for the radiolabeling with [^{11}C]methyl iodide. The [^{11}C]methyl iodide was produced from cyclotron produced [^{11}C]carbon dioxide using a GE MeI system.[14] The reaction mixture was purified by HPLC and the radioactive fraction containing [^{11}C]**4** was collected and after evaporation of the mobile phase the residue was dissolved in sterile phosphate-buffered saline (pH = 7.4). The isolated yield of [^{11}C]**4** was 60% (decay corrected, counted from the amount of [^{11}C]methyl iodide). The total synthesis time was 40 min and the specific radioactivity was 25 GBq/μmol (range 19–30 GBq/μmol, $n = 4$) at the end of the synthesis. The obtained radioligand was analyzed by HPLC showing a purity better than 99%.

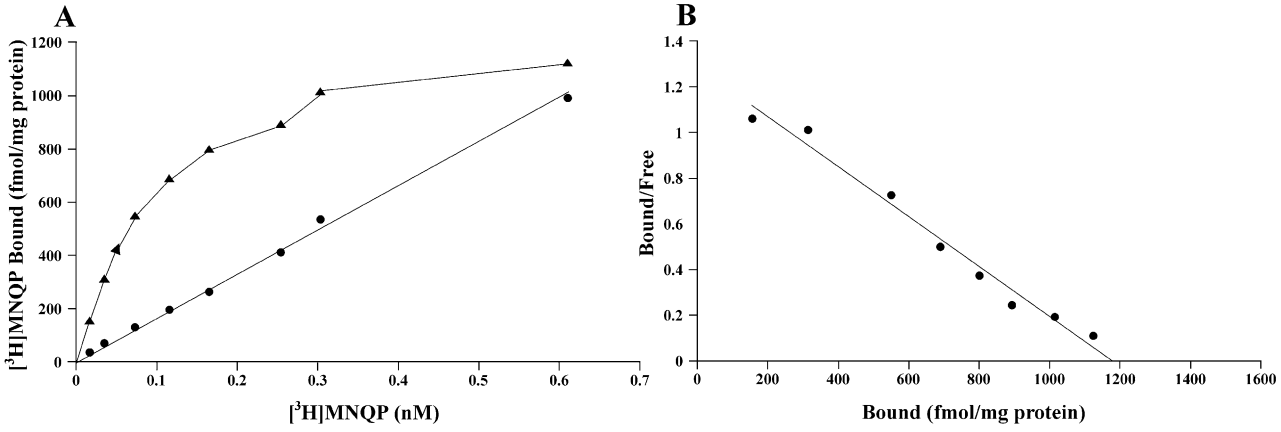

Figure 1. Saturation experiments of [^{3}H]MNQP binding in the rat prefrontal cortex. Membranes were incubated in Tris–HCl buffer with various concentrations of [^{3}H]MNQP (30–1000 pM) for 90 min at 22 °C. Non-specific binding was estimated in the presence of 1 μM paroxetine. The results shown are from a typical experiment and the values are the mean of duplicate determinations. (A) The saturation binding isotherm shows specific binding (▲) and non-specific binding (●). (B) Scatchard plot analysis of binding of [^{3}H]MNQP resulted in a K_d of 51 ± 7 pM and a B_{max} of 1530 ± 395 fmol/mg protein.

Results and Discussion

The binding assay was performed essentially as described by Bonnet et al.[15] Figure 1A shows a saturation curve of [^{3}H]**4** to rat frontal cortex homogenate. The linearity of the Scatchard plot in Figure 1B is indicative of the presence of a single class of binding sites with a $K_d = 51 \pm 7$ pM and $B_{max} = 1530 \pm 395$ fmol/mg protein ($n = 3$, independent experiments each performed as duplicates). The dissociation constant of **4** is in the same range as for **1**,[6] thus **4** and **1** are equipotent.

We recently reported a positron emission tomography examination of [^{11}C]**4** in cynomolgus monkey brain.[16] The regional distribution of radioactivity was in accordance with the known distribution of 5-HTT, and pretreatment with the selective 5-HTT inhibitor citalopram indicated that [^{11}C]**4** binds specifically to 5-HTT in vivo in the primate brain. The high binding constant, specific in vivo binding, and the facile labeling makes [^{11}C]**4** a potential radioligand for visualization of the 5-HTT with PET. Furthermore, the low non-specific binding observed in vitro with [^{3}H]**4** renders this ligand a promising radioligand for in vitro studies of the 5-HTT.

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12. Characterization of compound **4**. Yellow solid, mp 122–125 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.75 (br s, 1H, NH), 2.79 (s, 3H, CH_3), 3.02 (t, $J = 4.6$ Hz, 4H, CH_2), 3.80 (t, $J = 4.6$ Hz, 4H, CH_2), 7.06 (d, $J = 9.5$ Hz, 1H, H3), 7.54 (d, $J = 9.2$ Hz, 1H, H8), 8.00 (d, $J = 9.2$ Hz, 1H, H7), 8.22 (d, $J = 9.5$ Hz, 1H, H4).
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