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Synthesis, Radiolabelling and Biological Characterization of (D)-7-Iodo-*N*-(1-phosphonoethyl)-5-aminomethylquinoxaline-2,3-dione, a Glycine-Binding Site Antagonist of NMDA Receptors

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Abstract—(D)-7-Iodo-*N*-(1-phosphonoethyl)-5-aminomethylquinoxaline-2,3-dione (I-PAMQX), is a potent, in vivo active antagonist acting at the glycine binding site of the NMDA receptor complex. Radioiodinated [^{131}I]I-PAMQX was prepared with good yields and high specific activity from its 7-bromo analogue. Biodistribution studies of [^{131}I]I-PAMQX in mice showed a relatively slow clearance from the blood. The uptake of radioactivity was highest in the kidneys, moderate in the heart, lung, liver and bones, and low in the brain.

Introduction

L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). *N*-Methyl-D-aspartate (NMDA)-preferring glutamate receptors, a subtype of ionotropic glutamate receptors, are widespread throughout the brain.[1] They are involved in synaptic transmission, synaptic plasticity, and, during ontogeny, contribute to CNS development.[2] It has been shown that the expression of NMDA receptors is altered in some pathological states,[3] and they are thought to be directly involved in many neurological diseases such as the neurodegeneration following stroke[4] or traumatic brain injury,[5] epilepsy,[6] or Huntington's disease.[7] As a result there has been great interest in developing radioligands that could be used to visualize the distribution of NMDA receptors in living human brain using non-invasive tomographic imaging.

Recently, the synthesis and in vivo evaluation of a series of 5-aminomethylquinoxaline-2,3-diones with high affinity for the glycine binding site of the NMDA receptor complex have been reported.[8] PAMQX (**1**) is the most selective and potent compound from this series. In vitro, PAMQX exhibited an IC_{50} value of 5 nM at the glycine binding site of the NMDA receptor complex. In comparison to previously published analogues[10,11] PAMQX also showed improved activity in the maximal electroshock test in mice. Interestingly, binding affinity was shown to be fairly insensitive to the nature of the substituent on C(7), suggesting the possibility of introducing a radiolabel in this position (Table 1).

In this article, we report on the synthesis, radiolabelling, pharmacological characterization and biodistribution of (D)-7-iodo-*N*-(1-phosphonoethyl)-5-aminomethylquinoxaline-2,3-dione (I-PAMQX, **7**).

Chemistry

Alkylation of *N*-benzyloxycarbonyl-(D)-phosphoalanine dimethyl ester **3** with protected quinoxaline-2,3-dione **2**[11] yielded **4**, the starting material for the introduction of iodine (Scheme 1). An iododebromination exchange

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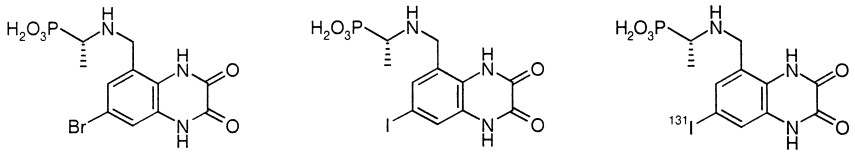


1 (PAMQX) **7 (I-PAMQX)** **7'**

reaction[17] failed to give any synthetically useful yields, therefore, iodine was introduced via stannyl intermediate **5**. The use of tributylstannyl group in radiohalogenations is a general method that permits regioselective electrophilic substitution.[18] Heating **4** with hexabutyl distannane in refluxing dioxane, using $Pd(PPh_3)_4$ as a catalyst, allowed the convenient preparation of **5**. Iododestannylation was achieved by treating **5** in chloroform with iodine at room temperature. Finally, hydrolysis of **6** with HCl in dioxane yielded I-PAMQX (**7**). All compounds were characterized by ^1H NMR (250 MHz) and LC-MS with electrospray as interface.

Radiochemical Synthesis of ^{131}I-labelled I-PAMQX

For practical reasons, ^{131}I was used for radiolabelling. ^{131}I is well suited for therapeutic applications because of its high energy. However, for diagnostic imaging, ^{123}I would be preferred. Starting from **5** the radioiodinated intermediate **6′** was obtained by treatment with [^{131}I]NaI and hydrogen peroxide at room temperature (Scheme 1). Much lower radiochemical yields were obtained when chloramine-T was used as an oxidizing agent. Removal of the protecting groups was accomplished by heating **6′** with 20% HCl for 1 h at 65°C. The final product was purified by HPLC using a reversed phase semi-preparative μ-Bondapak column and a mixture of 8% ethanol and 92% 0.01 M phosphoric acid as solvent. A retention capacity k' of 5.0 was observed for both [^{131}I]I-PAMQX and an authentic sample. The radiochemical purity of **7′** was higher than 98% and specific activity was greater than 2000 Ci/mmol, with an overall radiochemical yield ranging from 60 to 90%.

The distribution coefficient (log D) of [^{131}I]I-PAMQX was determined in the water/octanol system at different pH values using the shake-flask method (Table 2).

Table 1. Lack of influence of C(7) substitution on affinity for the glycine binding site[b,c]

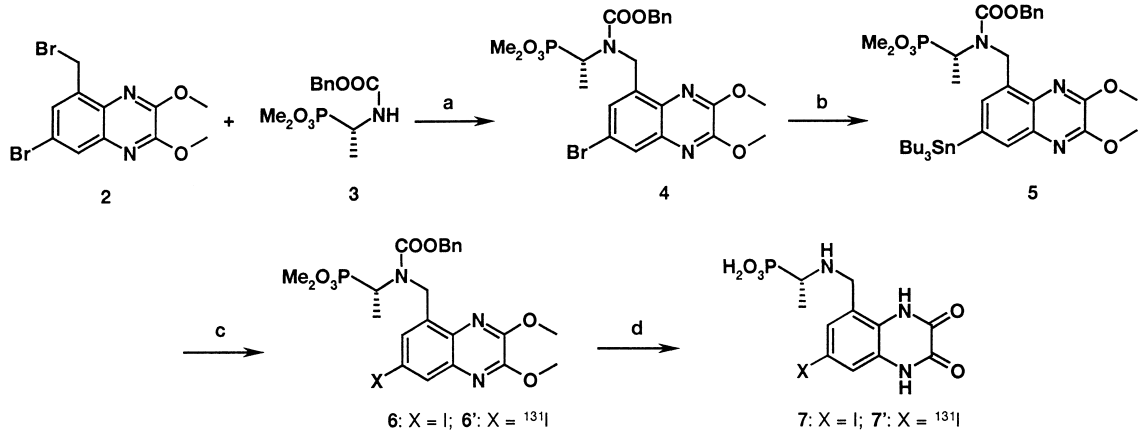
Substituent on C(7)	F	Cl[d]	Br	CN[d]	CF_3	NO_2
IC_{50} [nM][a]	9	6	5	76	8	32

[a]IC_{50}s obtained from 6 or 12 concentrations of compound, average of at least two independent experiments run in triplicate.
[b]All compounds were synthesized as described in refs 8 and 10.
[c][^{3}H]MDL-105519 binding assay.[12]
[d]Racemic mixture.

Table 2. Octanol/water distribution coefficients of [^{131}I]I-PAMQX

pH	3	4	4.8	5.5	6.5	7.5	7.4*
log D[a]	−0.8	−1.2	−1.5	−1.6	−1.7	−1.8	−2.5

[a]The log D values were determined in citric acid/disodium hydrogen phosphate buffer or (*) in PBS.



Scheme 1. Reagents and conditions: (a) DMF, Cs_2CO_3, 50% (b) $(SnBu_3)_2$, $Pd(PPh_3)_4$, dioxane, reflux, 6 h, 30%; (c) $CHCl_3$, I_2 or [^{131}I]NaI, H_2O_2, MeOH, 45 min; (d) 12N HCl, 60°, 3 h, 40 or 20% HCl, 65°C, 1 h, 60–90% based on ^{131}I.

Table 3. IC_{50}[a] or % inhibition at 10 μM in radioligand binding assays

	NMDA (glycine)[b]	AMPA[c]	Kainate[d]	NMDA (glutamate)[e]	NMDA (channel)[f]
1	5 nM	3 μM	2.7 μM	≥10 μM	≥10 μM
7	8 nM	340 nM	49%	≥10 μM	≥10 μM

[a]IC_{50}s obtained from 6 or 12 concentrations of each compound, average of at least two independent experiments run in triplicate.
[b][^{3}H]-(Z)-2-carboxy-4,6-dichloroindole-3-(2′-phenyl-2′-carboxy)-ene ([^{3}H]MDL-105519) binding assay.[12]
[c][^{3}H]AMPA binding assay.[13]
[d][^{3}H]kainate binding assay.[14]
[e][^{3}H]CGP39653 binding assay.[15]
[f][^{3}H]MK-801 binding assay[16] (measured after 1h preincubation with the radioactive ligand to saturate all channel sites).

Pharmacological Characterization

The in vitro profile was determined using radioligand binding assays for ionotropic glutamate receptors (Table 3). PAMQX (**1**) shows an excellent selectivity for the glycine-binding site of NMDA receptors. I-PAMQX (**7**) also binds to AMPA receptors, but with a 42-fold lower affinity.

In in vitro binding assays, I-PAMQX exhibited an IC_{50} value of 8 nM at the glycine binding site, indicating that the replacement of the bromine atom with an iodine atom in position 7 of the quinoxaline-2,3-dione moiety did not affect in vitro potency. In vivo, PAMQX and I-PAMQX had a potent anticonvulsant effect in the maximal electroshock test in mice[9] after intravenous injection and 30 min pre-treatment time, with ED_{50} values of 5 and 10 mg/kg respectively (Table 4).

Biodistribution Studies

The tissue biodistribution of [^{131}I]I-PAMQX was performed using female Balb/C mice. Between 700 and 800 kBq of [^{131}I]I-PAMQX were injected into the tail vein of the female mice. The mice were sacrificed 0.5, 2.5 or 5 h after injection of radioactivity (three animals per time point), dissected, and the radioactivity contents of various organs were measured in a well counter (Table 5). The highest uptake of radioactivity was observed in kidneys, which is not surprising in view of the high water solubility of I-PAMQX and the rapid clearance from the blood pool. All other organs showed low or even very low radioactivity uptakes. At 30 min post-injection, the brain showed only 0.03% of the injected dose and decreased to 0.01% at 2.5 and 5 h post-injection. This decline of brain concentrations is also reflected in the time-course of anticonvulsant activity in mice where a higher protection rate was noted after a pretreatment period of 0.5 h as compared to one of 1 h. It is likely that the low extraction of [^{131}I]I-PAMQX by mouse brain is due to its high polarity and low log *D* value at physiological pH. A recently published experiment using ^{11}C-labeled 6,7-dichloroquinoxaline-2,3-dione (DCQX) revealed that less than 2% of the radioligand was extracted by rat brain.[19] For imaging purposes, therefore, it appears that quinoxaline-2,3-dione derivatives may not be ideal candidates for in vivo NMDA receptor imaging. Additional studies linking structure–activity, lipophilicity and blood-brain barrier penetration are warranted in order to develop an improved glycine-site antagonist of NMDA receptors that may be useful for imaging.

Table 4. ED_{50} [mg/kg][a] or % protection in the maximal electroshock test in mice,[b] after i.v. injection

Pre-treatment time	30 min	60 min
1, PAMQX	5	60% at 10 mg/kg
7, I-PAMQX	10	20% at 10 mg/kg

[a]ED_{50} values calculated according to Spearman-Kaerber.
[b]Five mice per dose.

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Table 5. Biodistribution of [^{131}I]I-PAMQX in female Balb/C mice: % injected dose/gram tissue ± SEM, $n=3$

Organ	0.5 h	2.5 h	5 h	Organ	0.5 h	2.5 h	5 h
Blood	1.10 ± 0.52	0.54 ± 0.41	0.49 ± 0.07	Intestines	0.44 ± 0.21	0.22 ± 0.17	0.20 ± 0.03
Heart	1.34 ± 1.45	0.93 ± 0.07	0.82 ± 0.69	Liver	0.92 ± 0.54	0.49 ± 0.27	0.43 ± 0.15
Lung	0.96 ± 0.25	0.40 ± 0.50	0.38 ± 0.13	Brain	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Spleen	0.46 ± 0.24	0.23 ± 0.15	0.21 ± 0.05	Muscle	0.40 ± 0.23	0.21 ± 0.12	0.19 ± 0.17
Kidneys	6.68 ± 3.19	3.29 ± 2.47	2.98 ± 0.45	Bone	1.46 ± 1.30	0.92 ± 0.11	0.78 ± 0.60
Stomach	0.58 ± 0.11	0.23 ± 0.33	0.22 ± 0.11				

the radioligand binding assays, and to C. Portet and A. Jeker for the anticonvulsion tests.

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