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Synthesis of a Fluorine-18-labelled Derivative of 6-Nitroquipazine, as a Radioligand for the In Vivo Serotonin Transporter Imaging with PET

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Abstract—Considerable efforts have been engaged in the design, synthesis and pharmacological characterization of radioligands for imaging the serotonin transporter, based on its implication in several neuropsychiatric diseases, such as depression, anxiety and schizophrenia. In the 5-halo-6-nitroquipazine series, the fluoro derivative has been designed for positron emission tomography (PET). The corresponding 5-iodo-, 5-bromo- and 5-chloro *N*-Boc-protected quipazines as labelling precursors, as well as 5-fluoro-6-nitroquipazine as a reference compound have been synthesized. 5-[^{18}F]Fluoro-6-nitroquipazine has been radiolabelled with fluorine-18 (positron-emitting isotope, 109.8 min half-life) by nucleophilic aromatic substitution from the corresponding *N*-Boc protected 5-bromo- and 5-chloro-precursors using K[^{18}F]F-K_{222} complex in DMSO by conventional heating (145 °C, 2 min) or microwave activation (50 W, 30–45 s), followed by removal of the protective group with TFA. Typically, 15–25 mCi (5.5–9.2 GBq) of 5-[^{18}F]fluoro-6-nitroquipazine (1–2 Ci/μmol or 37–72 GBq/μmol) could be obtained in 70–80 min starting from a 550–650 mCi (20.3–24.0 GBq) aliquot of a cyclotron [^{18}F]F^- production batch (2.7–3.8% non decay-corrected yield based on the starting [^{18}F]fluoride). Ex vivo studies (biodistribution in rat), as well as PET imaging (in monkey) demonstrated that 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) readily crossed the blood brain barrier and accumulated in the regions rich in 5-HT transporter (frontal- and posterial cortex, striata). However, the low accumulation of the tracer in the thalamus (rat and monkey) as well as the comparable displacement of the tracer observed with both citalopram, a reference 5-HT re-uptake inhibitor and maprotiline, a norepinephrine re-uptake inhibitor (rat), indicate that 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) does not have the suggested potential for PET imaging of the serotin transporter (SERT).

Introduction

Based on the implication of the serotoninergic system in several neuropsychiatric diseases, such as depression, anxiety and schizophrenia, considerable efforts have been engaged in the design, synthesis and pharmacological characterization of single photon emission computed tomography (SPECT) and positron emission tomography (PET) radioligands for imaging this neurotransmitter system. In recent years, much interest has been shown in the visualization and quantification of the serotonin transporter (SERT), which plays a key role in the regulation of synaptic serotonin (5-hydroxytryptamine, 5-HT) levels. Postmortem studies have reported decreased SERT densities in cortical and subcortical regions of Parkinsonian patients,[1] depressed subjects[2] and suicide victims.[3] Therefore, SERT is the primary target of the widely prescribed serotonin system re-uptake inhibitors (SSRIs). The majority of antidepressant agents reinforce monoaminergic transmission by blocking neuronal re-uptake of 5-HT (and/or norepinephrine) via actions at the serotonin transporter [and the norepinephrine transporter (NET)].[4] Given the large use of these drugs in depression treatment, there is a strong incentive to explore their pharmacology in patients non-invasively using imaging techniques such as SPECT and PET.

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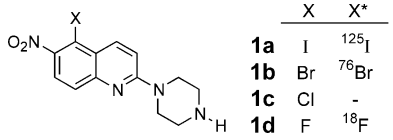


	X	X*
1a	I	^{125}I
1b	Br	^{76}Br
1c	Cl	-
1d	F	^{18}F

Figure 1. Chemical structures of the 5-halo-6-nitroquipazine derivatives **1a–d**.

Several putative SPECT and PET radioligands of this transporter have been suggested and reported, but none proved to be the ultimate agent for the imaging of the 5-HT transporter. For example, in the aryl-nortropane series, radiolabelled 2β-carbomethoxy-3β-(4′-iodophenyl)nortropane (nor-β-CIT) has been developed and used in PET and SPECT,[5,6] in spite of its considerable undesirable affinity for the dopamine transporter. Currently, a promising diphenylsulfide series of ligands for both PET and SPECT is under development in different laboratories and structure–activity relationship studies are in progress in order to improve the selectivity and pharmacokinetics of these new tracers, and at least two potent candidates, ADAM ([2-(2-amino-4-iodophenylthio)benzyl]dimethylamine)[7,8] and DSAB ([2-(2-amino-4-cyanophenylthio)benzyl]dimethylamine)[9,10] have been reported.

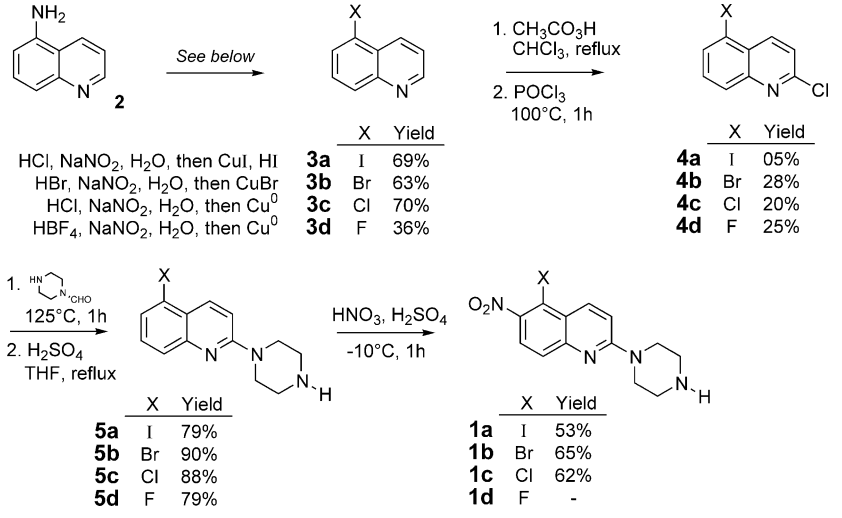
Another class of 5-HT transporter ligands is based on halogenated derivatives of 6-nitroquipazine (6-nitro-2-piperazin-1-ylquinoline).[11–14] The 5-iodo-analogue (**1a**) and the 5-bromo-analogue (**1b**) have been synthesized and characterized (Fig. 1). Both compounds show similar affinity for the serotonin transporter: K_i of 0.19 and 0.13 nM for **1a** and **1b**, respectively.[15] 5-Iodo-6-nitroquipazine (**1a**) has already been labelled both with iodine-125 and iodine-123[16]: [^{125}I]-**1a** has been demonstrated to be selective for the 5-HT transporter[17–19] and [^{123}I]-**1a** has been used to image the 5-HT transporter by SPECT.[20,21] 5-Bromo-6-nitroquipazine (**1b**) has also been labelled with the positron-emitting isotope bromine-76 (half-life: 16.2 h) and [^{76}Br]-**1b** has been partially characterized as a radioligand for PET imaging of the 5-HT transporter.[22] The 5-fluoro-derivative, 5-fluoro-6-nitroquipazine (**1d**), labelled with fluorine-18 ([^{18}F]-**1d**, another positron-emitting isotope of 109.8 min half-life) has been suggested as a potential radioligand for PET imaging, based on its high affinity for the serotonin transporter (K_i of 0.25 nM).[15] This radioligand would be the first one in this series labelled with fluorine-18.

We report herein (1) the synthesis of 5-fluoro-6-nitroquipazine (**1d**, 5-fluoro-6-nitro-2-piperazin-1-ylquinoline) and the corresponding *N*-Boc-protected 5-iodo-, 5-bromo- and 5-chloro-6-nitro-2-piperazin-1-ylquinoline, (2) its comparative radiolabelling by nucleophilic aromatic fluorination with fluorine-18 from the corresponding 5-halo-derivatives using both conventional heating and microwave activation, and (3) the preliminary pharmacological evaluation, including PET imaging.

Results and Discussion

Chemistry and radiochemistry

5-Aminoquinoline (**2**) was used as starting material for the preparation of all 5-halo-6-nitroquipazine derivatives (**1a–d**) (Scheme 1). 5-Haloquinolines were synthesized from **2** via the corresponding diazonium salts.[23] Treatment of **2** with sodium nitrite in water containing hydrochloric acid, followed by addition of copper iodide and hydriodic acid gave 5-iodoquinoline (**3a**) in 69% yield. Treatment of **2** with sodium nitrite in water containing hydrobromic acid, hydrochloric acid or fluoboric acid,[24] gave, after addition of copper (Cu^I or Cu^0), the corresponding 5-bromo-, 5-chloro- and 5-fluoro-quinoline (**3b–d**) in 63, 70 and 36% yield, respectively. Subsequent reaction of **3a–d** with peroxyacetic acid, followed by treatment of the corresponding *N*-oxides[25] with phosphorus oxychloride gave the desired 2-chloro-5-haloquinolines **4a–d** in 5 to 28% non-optimized yield. Condensation of the 2-chloro-5-haloquinolines **4a–d** with the *N*-formyl-protected piperazine (commercially available) at 125 °C for 1 h



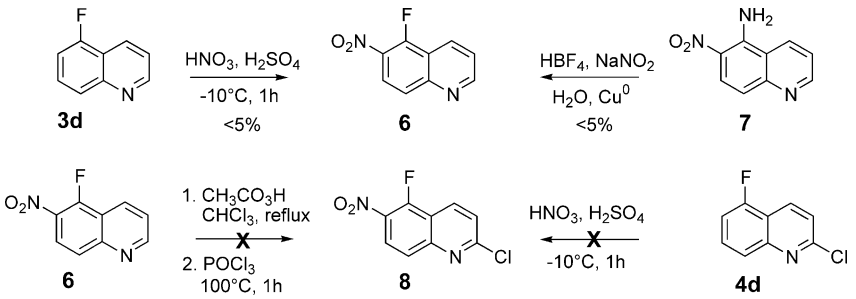
	X	Yield
3a	I	69%
3b	Br	63%
3c	Cl	70%
3d	F	36%

	X	Yield
4a	I	05%
4b	Br	28%
4c	Cl	20%
4d	F	25%

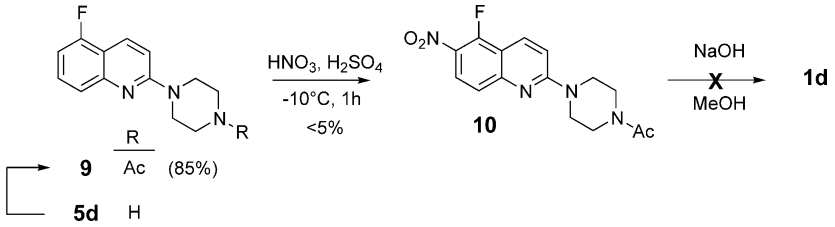
	X	Yield
5a	I	79%
5b	Br	90%
5c	Cl	88%
5d	F	79%

	X	Yield
1a	I	53%
1b	Br	65%
1c	Cl	62%
1d	F	-

Scheme 1. Preparation of 5-chloro-, 5-bromo- and 5-iodo-6-nitroquipazine (**1a–c**).



Scheme 2. Attempted preparation of 2-chloro-5-fluoro-6-nitroquinoline (**8**).

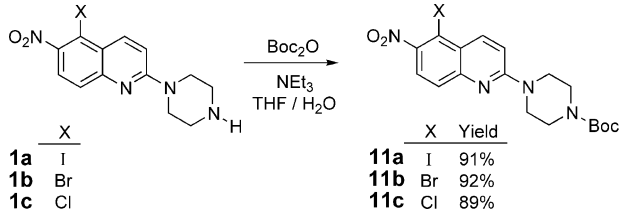


Scheme 3. Preparation of *N*-acetyl-5-fluoro-6-nitroquipazine (**10**).

gave, after deprotection using sulfuric acid the corresponding quipazine derivatives **5a**–**d** in 79 to 90% yield. Nitration of the quinolinyl ring system of the iodo-, bromo- and chloro- derivatives **5a–c** using nitric acid in sulfuric acid at low temperature cleanly gave the 5-halo-6-nitroquipazines **1a**–**c** in 53 to 65% yield.[26,16] The same conditions were used to nitrate the 5-fluoro analogue **5d**, without success. We observed complete decomposition of the starting material without isolation of the desired 5-fluoro-6-nitroquipazine (**1d**).

In order to synthesize **1d**, alternative routes to the 5-fluoro-6-nitroquinoline ring system were investigated. Nitration of the fluoro derivative **3d** (Scheme 2) using conditions similar to those used for the preparation of the 5-halo-6-nitroquipazines **1a**–**c** (nitric acid in sulfuric acid at low temperature) afforded **6** in very low yields (<5%). Fluorination of commercially available 5-amino-6-nitroquinoline **7** with sodium nitrite, copper (0) and aqueous fluoboric acid, also gave **6**, but again in low yields (<5%). Nevertheless, derivative **6** was treated with peroxyacetic acid and phosphorus oxychloride using the conditions described for the preparation of the 2-chloro analogues **4a**–**d**. The desired quinoline **8** could not be observed or isolated. Attempts to nitrate the chlorofluoroquinoline **4d** with conditions similar to those used above were unsuccessful and derivative **8** could not be isolated.

On the other hand, derivative **5d** was cleanly acetylated using acetic anhydride (room temperature, in dichloromethane containing an excess of triethylamine), giving **9** in 85% yield (Scheme 3). *N*-Acetylated fluoroquipazine **9** was nitrated, in again poor yield (<5%), but gave **10** which could be isolated. Unfortunately, we were not able to remove the acetyl protective group without complete degradation of starting material and the desired fluoronitroquipazine **1d** could not be observed.



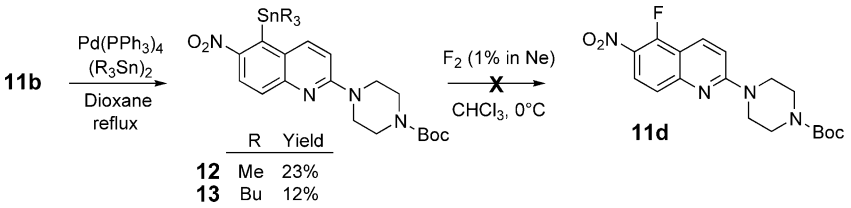
Scheme 4. Preparation of the *N*-Boc-protected 5-chloro-, 5-bromo- and 5-iodo-6-nitroquipazine (**11a**–**c**).

As stable precursors for fluorine-18 labelling, the *N*-Boc-protected 5-iodo-, 5-bromo- and 5-chloro-6-nitroquipazines **11a**–**c** were easily prepared from **1a**–**c** using *tert*-butoxydicarbonate in a mixture of THF and aqueous sodium hydroxide in 89 to 92% yield (Scheme 4).

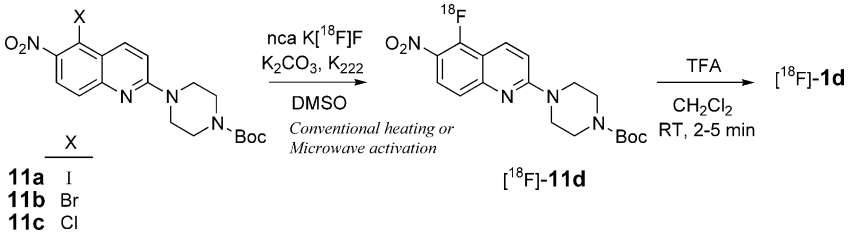
Another approach to the desired 5-fluoro-6-nitroquipazine **1d** was investigated at this time. The *N*-Boc-protected bromonitroquipazine **11b** was stannylated in dioxane at reflux containing *tetrakis*-triphenylphosphine palladium (0) and the appropriated hexaalkylditin to give derivatives **12** and **13**, in 23 and 12% yield, respectively (Scheme 5). Both compounds were then treated in chloroform with fluorine (in conditions routinely used in our laboratory for the preparation of 6-[^{18}F]fluoro-L-DOPA)[27] without showing the expected *N*-Boc-protected fluoroderivative **11d**. Again, we observed complete decomposition of the starting material.

Radiolabelling of 5-fluoro-6-nitroquipazine **1d** with fluorine-18 was nevertheless investigated starting from the *N*-Boc-protected chloro-, bromo- and iodo-nitroquipazine **11a**–**c** (Scheme 6).

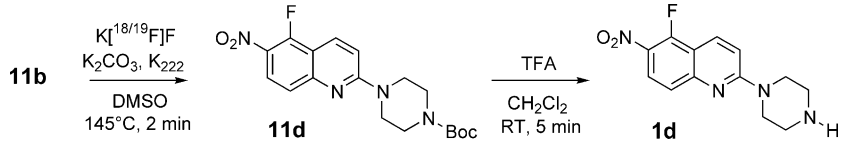
The radiosynthesis can be decomposed in two steps: (1) nucleophilic aromatic halo-to-fluoro substitution,



Scheme 5. Preparation of the *N*-Boc-protected 5-trialkylstannyl-6-nitroquipazine (**12**, **13**).



Scheme 6. Radiosynthesis of 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**).



Scheme 7. Preparation of 5-fluoro-6-nitroquipazine (**1d**) as an analytical reference.

followed by (2) removal of the *tert*-butoxycarbonyl protective group with TFA. Introduction of the fluorine-18 atom was attempted in dimethylsulfoxide as the solvent with the activated K[^{18}F]F-Kryptofix® K_{222} complex[28] as the no-carrier-added radiofluorinating reactant (K_{222}: 4, 7, 13, 16, 21, 24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) by either conventional heating (non-stirred sealed reactor, placed in a heating block at 80 to 250 °C for 0.5 to 10 min) or microwave activation (non-stirred non-sealed reactor, placed in a dedicated microwave oven at 50, 100 or 200 W, for 0.5 to 5 min).

The iodo precursor **11a** was completly unreactive towards the fluoro exchange whatever the conditions used. No incorporation of fluorine-18 could be detected for 0.5 to 20 min at 80 °C or higher temperatures. The same observation was made for the use of microwave activation (at 50, 100 or 200 W, for 0.5 to 5 min). Both the *N*-Boc-protected chloro- and bromo-nitroderivative, **11c** and **11b**, were reactive in the same conditions that are described above: using conventional heating, 25–30% of fluorine-18 incorporation could be observed at 145 °C for 1.5 to 3 min for **11c** (10–20% for **11b**). Using microwave activation, up to 20% of incorporation of fluorine-18 was observed for 50 W (30–45 s) for both **11c** and **11b**. A typical decomposition pattern was observed for both chloro- and bromo- derivatives when longer reaction times, higher temperatures or higher power (100 and 200 W) were used.

After Sep-pak separation, the reaction mixture obtained from either **11b** or **11c** was purified by SiO_2 semi-preparative HPLC. One major radioactive compound, representing about 85% of the total radioactivity injected onto the column, could be isolated. It co-elutes in our SiO_2 HPLC system (with dichloromethane and ethyl acetate as eluent) with the chloro-, bromo- and iodo-*N*-Boc-protected nitro-derivatives **11a–c** and was therefore assigned as the *N*-Boc-protected [^{18}F]fluoronitro-derivative [^{18}F]-**11d**. Removal of the *tert*-butoxycarbonyl function in a 10:1 mixture of dichloromethane/trifluoroacetic acid at room temperature for 2 min was quantitative. Final C-18 semi-preparative HPLC purification gave pure 5-[^{18}F]fluoro-6-nitroquipazine **1d**. Typically, 15–25 mCi (5.5–9.2 GBq) of 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**, 1–2 Ci/μmol or 37–72 GBq/μmol) could be obtained in 70–80 min starting from a 550–650 mCi (20.3–24.0 GBq) aliquot of a cyclotron [^{18}F]F^- production batch (2.7–3.8% non decay-corrected yield based on the starting [^{18}F]fluoride). Formulation of labelled product for iv injection was effected as follows: the HPLC solvents were removed by evaporation and the residue was re-dissolved in physiological saline. The solution was then filtered on a 0.22 μm Millipore filter. As demonstrated by HPLC analysis, the radiopharmaceutical preparation was found to be >95% chemically and >97% radiochemically pure and was radiochemically stable for at least 120 min.

Finally, on a micromolar scale, we succeeded in preparing derivative **1d** as an analytical reference only, using experimental conditions similar to those used and developed for the radiosynthesis of the fluorine-18 labelled analogue (Scheme 7). Treatment of the *N*-Boc-protected bromoquipazine **11b** with potassium fluoride in dimethylsulfoxide containing potassium carbonate and Kryptofix® K_{222} for 2 min at 145 °C gave the *N*-Boc-protected fluoronitroquipazine **11d** after HPLC

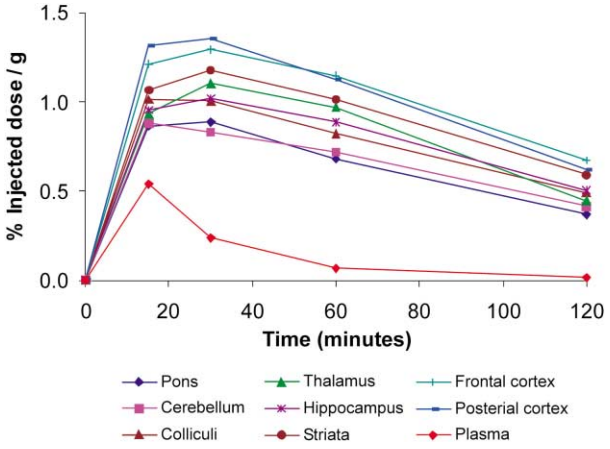


Figure 2. Time course of 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) in Sprague–Dawley rats for 0–120 min. Twelve adult male Sprague–Dawley rats (weight 250–300 g) were i.v. injected in the tail vein with 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**, 22–26 μCi or 0.81–0.96 MBq). Animals were sacrificed 15, 30, 60 and 120 min after injection of the radiotracer ($n=3$ per time point). The brain was quickly removed and dissected. Samples of cerebellum, pons, colliculi, thalamus, hippocampus, striata, frontal cortex, posterial cortex and plasma were obtained for each animal, weighed and their radioactivity measured in a γ-counter (Cobra Quantum, Packard). Tissue concentration were expressed as percent injected dose per gram tissue (% I.D. /g).

purification. The reaction was conducted with a few milliCi of fluorine-18 labelled potassium fluoride added to the previous mixture, permitting therefore an easier monitoring of the reaction and a clarification of the HPLC purification.

Derivative **11d** was immediately deprotected using trifluoroacetic acid in dichloromethane at room temperature and 5-fluoro-6-nitroquipazine **1d** could then be isolated using preparative HPLC. About 0.5 mg of **1d** could be isolated and accumulated from several consecutive preparations, permitting therefore classical chemical characterization after decay of the fluorine-18. Mass spectroscopy and ^{1}H NMR were in accordance with the proposed structure. Moreover, the ^{1}H NMR spectrum was superimposable (chemical shift and coupling constants of the aromatic quinolinyl ring) with that observed with *N*-acetylated 5-fluoro-6-nitroquipazine **10**. Note that fluorination of the *N*-Boc-protected bromoquipazine **11b** was also attempted at the millimolar scale but never the *N*-Boc-protected fluoroquipazine **11d** could be isolated from the complex reaction mixture.

Pharmacology

Tissue distribution studies in rat were performed with 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) to evaluate its capability of mapping the SERT in the brain. Ex vivo studies demonstrated that [^{18}F]-**1d** readily crossed the blood brain barrier and accumulated in the regions rich in 5-HT transporter, such as frontal- and posterial cortex, striata and thalamus (Fig. 2). At 60 min post-injection, the highest uptake was found in the cortex (1.048 and 1.041% injected dose per gram tissue (% I.D./g)) and the striata (1.010% I.D./g) when compared to cerebellum (0.71% I.D./g), a region which possesses low density of SERT in the rat. Only slightly higher accumulations compared to cerebellum were found in the hippocampus, thalamus and colliculi (0.811, 0.825 and 0.814% I.D./g, respectively). No specific uptake was found in the pons (0.684% I.D./g). From 30 to 120 min there was a parallel decrease of the radioactivity in all brain areas, showing a ratio of the radioactivities in SERT-rich over poor regions of 1.48. These results are different from those obtained with the bromine-76-labelled analogue [^{76}Br]-**1b** [22] where only the cerebellar radioactivity decreased with time while the radioactivity in the other cerebral structures remained constant.

In the competition studies with citalopram (Table 1), a reference 5-HT re-uptake inhibitor, reduced accumulation of the tracer by 20–30% only in the thalamus. For all other brain areas (for example frontal- and posterial cortex or striata), the accumulation of the tracer was reduced by 10 to 20%. Competition studies did not show any change following GBR 12909 (a dopamine re-uptake inhibitor) administration. By contrast, co-injection with maprotiline (a norepinephrine re-uptake inhibitor) induced in all brain regions a reduction of 10 to 20% of the accumulation of the tracer, comparable to the reduction observed with the citalopram competition studies. These results could be explained by the potential affinity of the tracer both the SERT and the NET in vivo. No in vitro data have been reported about the specificity of this tracer for the SERT.

PET imaging

A PET study of the brain distribution of 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) was carried out in an adult male (weight: 4 kg) *Macaca fascicularis*. Two hours before the PET acquisition the animal received intramuscular ketamine (10 mg/kg). After being intubated, the animal ventilated spontaneously and was maintained anaesthetized on ketamine and xylazine. A separate MRI examination was performed with a 1.5 Tesla system (GE) in order to provide anatomical images corresponding to PET slices. PET experiments were performed with a CTI HR + Exact positron tomograph (CTI PET Systems, Knoxville, TN, USA). This scanner allowed simultaneous acquisition of 63 slices every 2.2 mm with a spatial and axial resolution of 4.5 mm. The baboon's head was positioned in the tomograph using a custom-designed stereotaxic headholder. All the cerebral regions studied (cortex, diencephalon, cerebellum) were contained in axial cross sections parallel to the orbito-meatal anatomical line of reference.

The time course of 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) in the monkey brain is shown in Figure 3. The ligand entered rapidly the brain: 0.02% I.D. at 5 min post-injection in most of the cerebral structures such as frontal and parietal cortex, cerebellum, striata and thalamus. Twenty minutes after injection of the tracer, radioactivity in the brain remained rather constant, with the highest concentrations in the frontal and parietal

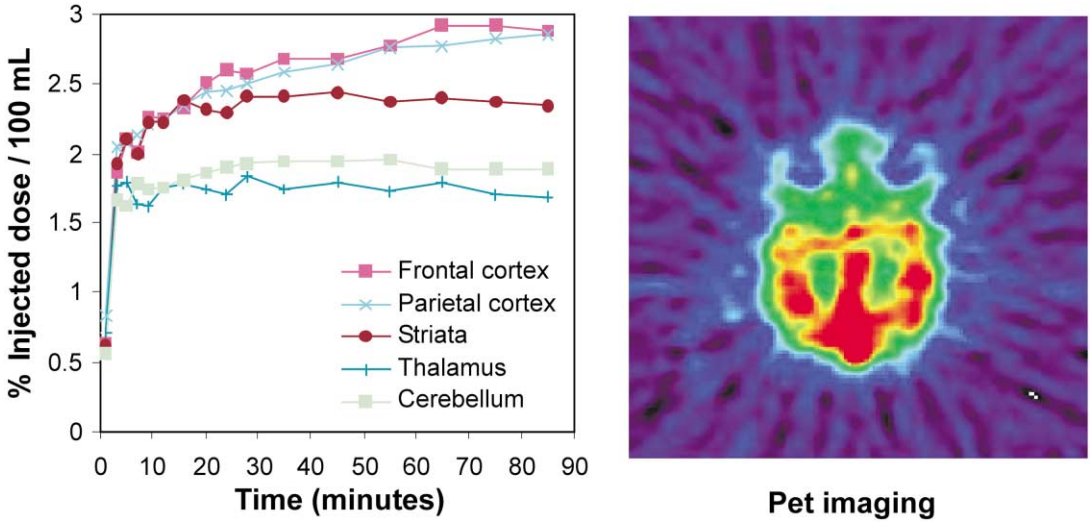


Figure 3. PET imaging of 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) in Macaca fascicularis. Monkey was i.v. injected with 2.6 mCi (96.2 MBq) of 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) and imaged for 90 min. The scanning protocol consisted of 16 images (5×2 min, 5×4 min, 6×10 min). During PET acquisition, arterial blood samples were withdrawn from a femoral artery at designated times. For data analysis, regions of interest (ROI) were placed on the frontal and parietal cortex, the cerebellum, the striata and the thalamus. The correct anatomical localization of the ROIs were checked on the MRI images. The radioactivity measured in each ROI was corrected for attenuation and fluorine decay and expressed as percent injected dose per 100 mL of tissue (% I.D./100 mL).

cortices. In the cerebellum, the 5-HT-poor region, the radioactivity concentration was slightly lower, the ratio of radioactivity in the frontal cortex over the cerebellum was 1.53 at 80 min post-injection (the ratio of radioactivity in the striata over the cerebellum was 1.25 at the same time). These results were comparable with those obtained with 5-[^{76}Br]bromo-6-nitroquipazine ([^{76}Br]-**1b**)[22] in *Papio papio* baboons. However, in the thalamus, no specific accumulation of the tracer could be found, compared to the cerebellum.

Conclusion

The development of radiolabelled tracers suitable as PET and SPECT ligands for imaging the 5-HT transporter has been an important goal in recent years. In the 5-halo-6-nitroquipazine series, the fluoro derivative has been designed for PET. The corresponding 5-iodo-, 5-bromo- and 5-chloro *N*-Boc-protected quipazines (**11a–c**) as labelling precursors, as well as 5-fluoro-6-nitroquipazine (**1d**) as a reference compound have been synthesized. 5-Fluoro-6-nitroquipazine (**1d**) has been radiolabelled with fluorine-18 (positron-emitting isotope, 109.8 min half-life) by nucleophilic aromatic substitution from the corresponding *N*-Boc protected 5-bromo- and 5-chloro-precursors (**11b**/**11c**) using K[^{18}F]F-K_{222} complex in DMSO by conventional heating (145 °C, 2 min) or microwave activation (50 W, 30–45 s), followed by removal of the protective group with TFA. Typically, 15–25 mCi (5.5–9.2 GBq) of 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**, 1–2 Ci/μmol or 37–72 GBq/μmol) could be obtained in 70–80 min starting from a 550–650 mCi (20.3–24.0 GBq) aliquot of a cyclotron [^{18}F]F^- production batch (2.7–3.8% non-decay-corrected yield based on the starting [^{18}F]fluoride). Ex vivo studies (biodistribution in rat), as well as PET imaging (in monkey) demonstrated that 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) readily crossed the blood brain barrier and accumulated in the regions rich in 5-HT transporter (frontal and posterial cortex, striata). However, the low accumulation of the tracer in the thalamus (rat and monkey) as well as the comparable displacement of the tracer observed with both citalopram, a reference 5-HT re-uptake inhibitor and maprotiline, a norepinephrine re-uptake inhibitor (rat), indicates that 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) does not have the suggested potential for PET imaging of the SERT.

Table 1. Competition studies for 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**) with maprotiline, GBR 12909 and citalopram

	% Injected dose/g ($n = 3$[a]), mean (±SD)			
	Control	Maprotiline	GBR 12909	Citalopram
Cerebellum	0.710 (0.105)	0.614 (0.048)	0.727 (0.101)	0.639 (0.064)
Pons	0.684 (0.120)	0.578 (0.069)	0.704 (0.103)	0.524 (0.080)
Colliculi	0.814 (0.105)	0.701 (0.082)	0.859 (0.075)	0.674 (0.038)
Thalamus	0.825 (0.102)	0.694 (0.034)	0.809 (0.065)	0.589 (0.063)
Hippocampus	0.811 (0.102)	0.704 (0.068)	0.824 (0.109)	0.733 (0.072)
Striata	1.010 (0.106)	0.797 (0.075)	0.961 (0.110)	0.824 (0.094)
Front. cortex	1.048 (0.112)	0.867 (0.069)	1.077 (0.163)	0.870 (0.069)
Post. cortex	1.041 (0.102)	0.883 (0.083)	0.999 (0.073)	0.891 (0.059)
Plasma	0.088 (0.019)	0.080 (0.018)	0.079 (0.014)	0.069 (0.013)

Nine adult male Sprague–Dawley rats (weight 250–300 g) were iv co-injected in the tail vein with [^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**, 20–29 μCi or 0.74–1.07 MBq) and maprotiline (a norepinephrine reuptake inhibitor, 5 mg/kg) or GBR 12909 (a dopamine reuptake inhibitor, 5 mg/kg) or citalopram (a 5-HT reuptake inhibitor, 1 mg/kg). Animals were sacrificed 60 min after the injection of the radiotracer ($n = 3$ per time point). The brain was quickly removed and dissected. Samples of cerebellum, pons, colliculi, thalamus, hippocampus, striata, frontal cortex, posterial cortex and plasma were obtained for each animal, weighed and their radioactivity measured in a γ-counter (Cobra Quantum, Packard). Tissue concentration were expressed as percent injected dose per gram tissue (% I.D./g).
[a]Per compound.

Experimental

General

Chemicals, TLCs and HPLCs. Chemicals were purchased from standard commercial sources (Aldrich, Fluka or Sigma France) and were used without further purification unless stated otherwise. TLCs were run on pre-coated plates of silicagel $60F_{254}$ (Merck). The compounds were localized (1) when possible at 254 nm using a UV-lamp and/or (2) by iodine staining and/or (3) by dipping the TLC-plates in a 1% ethanolic ninhydrin solution (or in a 1% aqueous $KMnO_4$ solution) and heating on a hot plate. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyzer. HPLCs: **HPLC A**: Equipment: Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481 and 486 UV-multiwavelength detectors; the effluent was also monitored for radioactivity with a Geiger-Müller counter; column: semi-preparative SiO_2 Hewlett Packard Zorbax Rx-SIL (250×9.4 mm); porosity: 5 μM; conditions: isocratic elution: eluent: dichloromethane/EtOAc 95:5; flow rate: 5.0 mL/min; temperature: rt; UV detection at λ: 254 nm; **HPLC B**: Equipment: Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481 and 486 UV-multiwavelength detectors; the effluent was also monitored for radioactivity with a Geiger-Müller counter; column: semi-preparative SymmetryPrep® C-18, Waters (300×7.8 mm); porosity: 7 μm; conditions: isocratic elution with: H_2O/CH_3CN/TFA: 80:20:0.15 (v:v:v); flow rate: 8.0 mL/min; temperature: rt; UV detection at λ: 254 nm; **HPLC C**: Equipment: Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481 and 486 UV-multiwavelength detectors; the effluent was also monitored for radioactivity with a Geiger-Müller counter; column: semi-preparative SiO_2 Hewlett Packard Zorbax Rx-SIL (250×9.4 mm); porosity: 5 μm; conditions: isocratic elution: eluent: heptane/EtOAc 95:5; flow rate: 6.0 mL/min; temperature: rt; UV detection at λ: 254 nm; **HPLC D**: Equipment: Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18, Waters (4.6×50 mm, microcolumn); porosity: 5 μm; conditions: isocratic elution with: H_2O/CH_3CN/TFA: 85:15:0.1 (v/v/v); flow rate: 2.0 mL/min; temperature: 35 °C; UV detection at λ: 254 nm.

Spectroscopies. NMR spectra were recorded at 298.0 K on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuteriated solvents (DMSO-d_6, δ 2.50 ppm; CD_2Cl_2, δ 5.32 ppm; CD_3OD, δ 4.78; CD_3CN, δ 1.93 ppm) and/or TMS as internal standards for ^1H NMR as well as the deuteriated solvents (DMSO-d_6, δ 39.5 ppm; CD_2Cl_2, δ 53.8 ppm; CD_3OD, δ 49.3 ppm) and/or TMS as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, dd, q, b for singlet, doublet, triplet, doublet of doublet, quadruplet and broad respectively). The mass spectra (MS), were measured on a Quadripolair Finnigan 4600 instrument (DCI/NH_4^+) and a LCQ-duo [trap-ion] Thermo-Finnigan (Electrospray).

Isotope availability. No-carrier-added aqueous [^{18}F]fluoride ion was produced on a CGR-MeV 520 cyclotron by irradiation of a 2 mL water target using a 20 MeV proton beam on 95% enriched [^{18}O]water by the $[^{18}O(p,n)^{18}F]$ nuclear reaction and was transferred to the appropriate hot cell. Typical production: 550–650 mCi (20.3–24.0 GBq) of $[^{18}F]F^-$ at the end of bombardment for a 20 μA, 30 min (36,000 μC) irradiation. A complete description of the target hardware and operation can be found in refs 29 and 30.

Miscellaneous. Radiosyntheses using fluorine-18, including the semi-preparative HPLC purifications were performed in a 7.5 cm-lead shielded cell using a computer assisted Zymate robot system (Zymark Corporation, USA). Microwave activation was performed with a MicroWell 10 oven (2.45 GHz), Labwell AB, Sweden. Specific radioactivity was determined as follows: the area of the UV absorbance peak corresponding to the radiolabelled product was measured on the HPLC chromatogram and compared to a standard curve relating mass to UV absorbance.

Animal subjects. All animal-use procedures were in strict accordance with the recommendations of the EEC (86/609/CEE) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

Chemistry

5-Iodoquinoline (3a). To a solution of 5-aminoquinoline (**2**, 1.0 g, 6.9 mmol, 1 equiv) in HCl (36%, 3 mL, excess) in H_2O (20 mL), cooled to −10 °C, was added $NaNO_2$ (0.8 g, 11.6 mmol, 1.67 equiv) dissolved in H_2O (6 mL). The mixture was stirred at −10 °C for 10 min and copper (I) iodide (1.6 g, 8.4 mmol, 1.21 equiv) dissolved in H_2O (20 mL) and aq HI (57%, 10 mL) was added. The mixture was heated at 60 °C for 30 min, poured into ice-water, basified with aq NaOH (4 M) to a final pH of ∼10, and extracted with EtOAc. The organic layer was dried (Na_2SO_4). The solvent was removed and the residue was chromatographed on silica gel (eluent: heptane/EtOAc: 90:10 to 50:50) to give 1.22 g of 5-iodoquinoline (**3a**) as a yellow solid (69%). R_f (EtOAc/heptane: 50:50): 0.3. ^1H NMR (CD_2Cl_2): δ 8.84 (bd, J = 3.0 Hz, 1H); 8.31 (d, J = 8.7 Hz, 1H); 8.12 (d, J = 7.5 Hz, 1H); 8.08 (d, J = 7.8 Hz, 1H); 7.46 (dd, J = 8.7 and 4.2 Hz, 1H); 7.42 (t, J = 7.5 Hz, 1H). The 2 protons at 8.12 and 8.08 may also appear like a t, J_{app} ∼7.5 Hz, 2H). ^{13}C NMR (CD_2Cl_2): δ 151.5 [CH]; 149.1 [C]; 140.3 [CH]; 138.0 [CH]; 130.9 [CH]; 130.6 [CH]; 130.5 [C]; 123.1 [CH]; 98.7 [C]. MS (DCI/NH_4^+), C_9H_6NI, 256 [$M+H^+$].

5-Bromoquinoline (3b). To a solution of 5-aminoquinoline (**2**, 6.4 g, 44.4 mmol, 1 equiv) in aq HBr (48%, 16 mL, excess), cooled to −10 °C, was added $NaNO_2$ (3.5 g, 50.7 mmol, 1.14 equiv) dissolved in H_2O (30 mL). The mixture was stirred at 0 °C for 10 min and copper bromide (7.4 g, 51.6 mmol, 1.16 equiv) dissolved in H_2O

(84 mL) and aq HBr (48%, 25.5 mL, excess) was added. The mixture was heated at 60 °C for 30 min, poured into ice-water, basified with aq NaOH (4M) to a final pH of ~10, and extracted with EtOAc. The organic layer was dried (Na_2SO_4). The solvent was removed and the residue was chromatographed on silica gel (eluent: heptane/EtOAc: 90:10 to 50:50) to give 5.80 g of 5-bromoquinoline (**3b**) as a white solid (63%). R_f (EtOAc/heptane: 50:50): 0.55. ^{1}H NMR (CD_2Cl_2): δ 8.90 (bs, 1H); 8.52 (d, J = 8.4 Hz, 1H); 8.06 (d, J = 8.4 Hz, 1H); 7.82 (d, J = 7.5 Hz, 1H); 7.56 (t, J_{app} ~8.1 Hz, 1H); 7.49 (dd, J = 8.4 and 4.2 Hz, 1H). Analytical data were in accordance with those already published.[16]

5-Chloroquinoline (3c). To a solution of 5-aminoquinoline (**2**, 5.0 g, 34.7 mmol, 1 equiv) in aq HCl (36%, 60 mL, excess), cooled to −10 °C, was added $NaNO_2$ (7.2 g, 104.4 mmol, 3.01 equiv) dissolved in H_2O (20 mL). The mixture was stirred at −10 °C for 10 min, and copper dust (1.3 g, 20.5 mmol, 0.59 equiv) was added. The mixture was heated at 85 °C for 2 h, poured into ice-water, basified with aq NaOH (4M) to a final pH of ~10, filtered and extracted with EtOAc. The organic layer was dried (Na_2SO_4). The solvent was removed and the residue was chromatographed on silica gel (eluent: heptane/EtOAc: 90:10 to 50:50) to give 3.98 g of 5-chloroquinoline (**3c**) as a yellow solid (70%). R_f (EtOAc/heptane: 50:50): 0.4. ^{1}H NMR (CD_2Cl_2): δ 8.93 (bd, J = 2.4 Hz, 1H); 8.55 (d, J = 8.4 Hz, 1H); 8.02 (t, J = 4.5 Hz, 1H); 7.63 (bd, J = 4.5 Hz, 2H super-imposed); 7.52 (dd, J = 8.4 and 4.2 Hz, 1H). Analytical data identical with those obtained from now commercially available 5-chloroquinoline (**3c**, Fluka, 1999).

5-Fluoroquinoline (3d). To a solution of 5-aminoquinoline (**2**, 3.0 g, 20.8 mmol, 1 equiv) in aq HBF_4 (48%, 150 mL, excess), cooled to −10 °C, was added $NaNO_2$ (15 g, 217.4 mmol, 10.44 equiv) dissolved in H_2O (20 mL). The mixture was stirred at 0 °C for 15 min and copper dust (0.75 g, 11.8 mmol, 0.56 equiv) was added. The mixture was heated at 100 °C for 1 h, poured into ice-water, basified with aq NaOH (2M) to a final pH of ~10, and extracted with EtOAc. The organic layer was dried (Na_2SO_4). The solvent was removed and the residue was chromatographed on silica gel (eluent: heptane/EtOAc: 90:10 to 50:50) to give 1.10 g of 5-fluoroquinoline (**3d**) as a green liquid (36%). R_f (EtOAc/heptane: 50:50): 0.45. ^{1}H NMR (CD_2Cl_2): δ 8.90 (bd, J = 3.0 Hz, 1H); 8.36 (d, J = 8.4 Hz, 1H); 7.88 (d, J = 8.7 Hz, 1H); 7.59 (q, J_{app} ~8.8 Hz, 1H); 7.39 (dd, J = 8.5 and 4.2 Hz, 1H); 7.18 (t, J_{app} ~8.7 Hz, 1H). ^{13}C NMR (CD_2Cl_2): δ 158.0 [C, d, J^1_{F-C}: 253 Hz]; 151.4 [CH]; 149.3 [C]; 129.0 [CH]; 128.9 [CH, d, J^3_{F-C}: 8 Hz]; 125.7 [CH]; 121.4 [CH]; 119.0 [C, d, J^2_{F-C}: 17 Hz]; 110.1 [CH, d, J^2_{F-C}: 20 Hz]. MS (DCI/NH_4^+), C_9H_6NF, 148 [M + H$^+$].

General procedure for the chlorination of 5-haloquinoline giving the corresponding 2-chloro-5-haloquinoline (4a–d)

To a solution of 5-haloquinoline (**3a**–**d**, 1–5 g scale, 1 equiv) in $CHCl_3$ (20–60 mL) was added dropwise peroxyacetic acid (32%, 3–12 equiv). The mixture was refluxed for 3–4 h and poured into ice-water followed by the addition of aq NaOH (10 M) to a final pH of ~10. The *N*-oxide, if precipitated, was filtered-off. Any non-precipitated *N*-oxide was extracted with EtOAc from the reaction mixture. The organic layers were combined, dried (Na_2SO_4) and concentrated to dryness. *N*-oxide solids were combined and treated with cooled (5 °C) $POCl_3$ (15–50 equiv) and heated at 100 °C for 1 h. The mixture was poured into ice-water, diluted with aq NaOH (10 M) to a final pH of ~8, filtered and extracted with CH_2Cl_2. Evaporation of the solvent afforded a brown oil that was chromatographed on silica gel (eluent: heptane/EtOAc: 95:5 to 70:30).

2-Chloro-5-iodoquinoline (4a). The procedure described above was used with 5-iodoquinoline (**3a**, 1.0 g), $CHCl_3$ (20 mL) and peroxyacetic acid (10 mL) and afforded, after chromatography, 61 mg of 2-chloro-5-iodoquinoline (**4a**) as a yellow oil (5%). R_f (EtOAc/heptane: 50:50): 0.6. ^{1}H NMR (CD_2Cl_2): δ 8.30 (d, J = 9.0 Hz, 1H); 8.10 (d, J = 7.5 Hz, 1H); 7.96 (d, J = 8.4 Hz, 1H); 7.43 (d and t super-imposed, J_{app} (t) ~8.1 Hz and J_{app} (d) ~8.7 Hz, 2H). ^{13}C NMR (CD_2Cl_2): δ 152.1 [C]; 148.4 [C]; 143.5 [CH]; 138.6 [CH]; 131.8 [CH]; 130.9 [C]; 130.0 [CH]; 124.4 [CH]; 98.2 [C].

5-Bromo-2-chloroquinoline (4b). The procedure described above was used with 5-bromoquinoline (**3b**, 2.5 g), $CHCl_3$ (40 mL) and peroxyacetic acid (20 mL) and afforded, after chromatography, 820 mg of 5-Bromo-2-chloroquinoline (**4b**) as a white solid (28%). R_f (EtOAc/heptane: 50:50): 0.6. ^{1}H NMR (CD_2Cl_2): δ 8.47 (d, J = 9.0 Hz, 1H); 7.96 (d, J = 8.4 Hz, 1H); 7.84 (d, J = 7.5 Hz, 1H); 7.60 (t, J_{app} ~7.9 Hz, 1H); 7.47 (d, J = 9.0 Hz, 1H). ^{13}C NMR (CD_2Cl_2): δ 152.0 [C]; 148.9 [C]; 138.8 [CH]; 131.2 [CH]; 131.1 [CH]; 128.9 [CH]; 126.9 [C]; 123.9 [CH]; 122.1 [C].

2,5-Dichloroquinoline (4c). The procedure described above was used with 5-chloroquinoline (**3c**, 3.3 g), $CHCl_3$ (60 mL) and peroxyacetic acid (25 mL) and afforded, after chromatography, 810 mg of 2,5-dichloroquinoline (**4c**) as a white solid (20%). R_f (EtOAc/heptane: 50:50): 0.6. ^{1}H NMR (CD_2Cl_2): δ 8.50 (d, J = 9.0 Hz, 1H); 7.90 (dd, J = 7.2 and 1.8 Hz, 1H); 7.65 (m, 2H super-imposed); 7.47 (d, J = 8.8 Hz, 1H). ^{13}C NMR (CD_2Cl_2): δ 151.6 [C]; 148.6 [C]; 135.9 [CH]; 131.4 [C]; 130.5 [CH]; 127.7 [CH]; 127.2 [CH]; 125.2 [C]; 123.3 [CH].

2-Chloro-5-fluoroquinoline (4d). The procedure described above was used with 5-fluoroquinoline (**3d**, 1.2 g), $CHCl_3$ (25 mL) and peroxyacetic acid (12 mL) and afforded, after chromatography, 365 mg of 2-chloro-5-fluoroquinoline (**4d**) as a white solid (25%). R_f (EtOAc/heptane: 50:50): 0.6. ^{1}H NMR (CD_2Cl_2): δ 8.35 (d, J = 9.0 Hz, 1H); 7.79 (d, J = 8.4 Hz, 1H); 7.67 (q, J_{app} ~8.7 Hz, 1H); 7.43 (d, J = 9.0 Hz, 1H); 7.24 (t, J_{app} ~8.7 Hz, 1H). ^{13}C NMR (CD_2Cl_2): δ 157.4 [C, d, J^1_{F-C}: 254 Hz]; 152.2 [C]; 149.0 [C]; 132.5 [CH]; 130.7 [CH, d, J^3_{F-C}: 8 Hz]; 124.8 [CH]; 122.9 [CH]; 117.9 [C, d, J^2_{F-C}: 16 Hz]; 111.1 [CH, d, J^2_{F-C}: 19 Hz].

General procedure for the preparation of the 5-halo-2-piperazin-1-ylquinoline (5a–d). A stirred mixture of

2-chloro-5-haloquinoline (**4a**–**d**, 0.2–1.0 g scale, 1 equiv) and 1-piperazinecarboxaldehyde (25–85 equiv) was heated at 125 °C for 30–90 min under argon. The mixture was then cooled and diluted with satured aq $NaHCO_3$. The aqueous phase was extracted with ether and the combined extracts were dried (Na_2SO_4). Evaporation of the solvent yielded a solid which was immediately dissolved in THF (5–30 mL) and 4M aq H_2SO_4 (10–20 equiv). The resulting solution was brought to reflux and stirred for 1–2 h. The solution was cooled and poured into 1 M aq NaOH. The resulting basic suspension was extracted twice with ether, and the ether extracts were dried (Na_2SO_4). The solvent was removed and the residue was chromatographed on silica gel (eluent: CH_2Cl_2/MeOH/NH_4OH: 95:5/0.1 to 80:20:0.1)

5-Iodo-2-piperazin-1-ylquinoline (5a). The procedure described above was used with 2-chloro-5-iodoquinoline (**4a**, 0.2 g) and afforded, after chromatography, 180 mg of 5-iodo-2-piperazin-1-ylquinoline (**5a**) as a yellow solid (79%). R_f (CH_2Cl_2/MeOH/NH_4OH: 70:30:1): 0.5. ^{1}H NMR (CD_2Cl_2): δ 8.02 (d, J = 9.3 Hz, 1H); 7.71 (d, J = 7.5 Hz, 1H); 7.60 (d, J = 7.8 Hz, 1H); 7.18 (t, J = 7.8 Hz, 1H); 6.94 (d, J = 9.6 Hz, 1H); 3.67 (bt, J = 5.6 Hz, 4H); 2.93 (bt, J = 5.7 Hz, 4H). ^{13}C NMR (CD_2Cl_2): δ 158.2 [C]; 148.8 [C]; 141.5 [CH]; 133.3 [CH]; 130.7 [CH]; 127.8 [CH]; 125.2 [C]; 111.3 [CH]; 98.4 [C]; 46.5 [2 CH_2]; 46.4 [2 CH_2]. Additional ^{13}C NMR data of quipazine derivatives could be found in ref 31.

5-Bromo-2-piperazin-1-ylquinoline (5b). The procedure described above was used with 5-bromo-2-chloroquinoline (**4b**, 0.6 g) and afforded, after chromatography, 650 mg of 5-bromo-2-piperazin-1-ylquinoline (**5b**) as a yellow solid (90%). R_f (CH_2Cl_2/MeOH/NH_4OH: 70:30:1): 0.5. ^{1}H NMR (CD_2Cl_2): δ 8.19 (d, J = 9.3 Hz, 1H); 7.59 (d, J = 8.4 Hz, 1H); 7.44 (d, J = 7.2 Hz, 1H); 7.34 (t, J_{app} ~7.9 Hz, 1H); 7.03 (d, J = 9.3 Hz, 1H); 3.70 (bt, J = 3.0 Hz, 4H); 2.96 (bt, J = 3.0 Hz, 4H). ^{13}C NMR (CD_2Cl_2): δ 158.1 [C]; 149.3 [C]; 136.7 [CH]; 130.0 [CH]; 126.7 [CH]; 126.0 [CH]; 122.5 [C]; 121.9 [C]; 111.0 [CH]; 46.6 [2 CH_2]; 46.5 [2 CH_2].

5-Chloro-2-piperazin-1-ylquinoline (5c). The procedure described above was used with 2,5-dichloroquinoline (**4c**, 1.0 g) and afforded, after chromatography, 1.1 g of 5-chloro-2-piperazin-1-ylquinoline (**5c**) as a yellow solid (88%). R_f (CH_2Cl_2/MeOH/NH_4OH: 70:30:1): 0.5. ^{1}H NMR (CD_2Cl_2): δ 8.25 (d, J = 9.6 Hz, 1H); 7.54 (d, J = 8.4 Hz, 1H); 7.41 (dd, J = 8.4 and 7.2 Hz, 1H); 7.24 (d, J = 7.2 Hz, 1H); 7.05 (d, J = 9.6 Hz, 1H); 3.68 (bt, J = 3.0 Hz, 4H); 2.95 (bt, J = 3 Hz, 4H). ^{13}C NMR (CD_2Cl_2): δ 158.1 [C]; 149.3 [C]; 134.1 [CH]; 131.3 [C]; 129.6 [CH]; 126.0 [CH]; 122.4 [CH]; 121.1 [C]; 110.7 [CH]; 46.5 [2 CH_2]; 46.4 [2 CH_2].

5-Fluoro-2-piperazin-1-ylquinoline (5d). The procedure described above was used with 2-chloro-5-fluoroquinoline (**4d**, 1.0 g) and afforded, after chromatography, 1.0 g of 5-fluoro-2-piperazin-1-ylquinoline (**5d**) as a yellow solid (79%). R_f (CH_2Cl_2/MeOH/NH_4OH: 70:30:1): 0.5. ^{1}H NMR (CD_2Cl_2): δ 8.10 (d, J = 9.3 Hz, 1H); 7.41 (AB^{19}F system, b, 2H); 6.97 (d, J = 9.3 Hz, 1H); 6.86 (m, 1H); 3.65 (bt, J = 6 Hz, 4H); 2.93 (bt, J = 6 Hz, 4H). ^{13}C NMR (CD_2Cl_2): δ 159.2 [C, d, J^1_{F-C}: 250 Hz]; 158.2 [C]; 149.6 [C]; 130.4 [CH]; 129.3 [CH, d, J^3_{F-C}: 8 Hz]; 122.6 [CH]; 113.2 [C, d, J^2_{F-C}: 15 Hz]; 109.9 [CH]; 106.1 [CH, d, J^2_{F-C}: 20 Hz]; 46.5 [4 CH_2].

General procedure for the preparation of the 5-halo-6-nitro-2-piperazin-1-ylquinoline (1a–c). To a −10 °C solution of 5-halo-2-piperazin-1-ylquinoline (**5a**–**d**, 50 mg to 2.0 g scale, 1 equiv) in concentrated H_2SO_4 (excess) was added dropwise concentrated HNO_3 (5 equiv). The mixture was stirred for 30–60 min at −10 to 0 °C, poured into ice, and diluted with 1 M aq NaOH until basic (pH ~9). The mixture was filtered, extracted with CH_2Cl_2, and the combined organic layers were dried (Na_2SO_4). Evaporation of the solvent afforded the desired product which could be used in the next step without further purification. If wished, the product was chromatographed on silica gel (eluent: CH_2Cl_2/MeOH: 95:5 to 90:10).

5-Iodo-6-nitro-quipazine or 5-iodo-6-nitro-2-piperazin-1-ylquinoline (1a). The procedure described above was used with 5-iodo-2-piperazin-1-ylquinoline (**5a**, 80 mg) and afforded 48 mg of 5-iodo-6-nitro-2-piperazin-1-ylquinoline (**1a**) as a yellow-orange oily residue (53%). R_f (CH_2Cl_2/MeOH: 90:10): 0.2. ^{1}H NMR (CD_2Cl_2): δ 8.32 (d, J = 9.6 Hz, 1H); 7.84 (d, J = 9.0 Hz, 1H); 7.60 (d, J = 9.1 Hz, 1H); 7.07 (d, J = 9.6 Hz, 1H); 3.75 (bt, J = 5.0 Hz, 4H); 2.96 (bt, J = 5.1 Hz, 4H). ^{1}H NMR (CD_3OD): δ 8.47 (d, J = 9.1 Hz, 1H); 7.86 (d, J = 8.4 Hz, 1H); 7.72 (d, J = 8.3 Hz, 1H); 7.38 (d, J = 9.2 Hz, 1H); 3.80 (t, J = 6.0 Hz, 4H); 2.98 (t, J = 6.0 Hz, 4H). ^{13}C NMR (CD_2Cl_2):: δ 157.3 [C]; 150.0 [C]; 147.9 [C]; 143.6 [CH]; 127.3 [CH]; 125.1 [C]; 124.0 [CH]; 112.1 [CH]; 90.9 [C]; 46.3 [2 CH_2]; 42.6 [2 CH_2].

5-Bromo-6-nitroquipazine or 5-bromo-6-nitro-2-piperazin-1-ylquinoline (1b). The procedure described above was used with 5-bromo-2-piperazin-1-ylquinoline (**5b**, 1.6 g) and afforded 1.2 g of 5-bromo-6-nitro-2-piperazin-1-ylquinoline (**1b**) as a yellow-orange solid (65%). R_f (CH_2Cl_2/MeOH: 90:10): 0.2. ^{1}H NMR (CD_2Cl_2): δ 8.39 (d, J = 9.6 Hz, 1H); 7.91 (d, J = 9.0 Hz, 1H); 7.59 (d, J = 9.0 Hz, 1H); 7.13 (d, J = 9.6 Hz, 1H); 3.80 (bt, J = 6.0 Hz, 4H); 2.98 (bt, J = 6.0 Hz, 4H). ^{13}C NMR (CD_2Cl_2): δ 158.7 [C]; 150.7 [C]; 144.5 [C]; 138.4 [CH]; 126.8 [CH]; 125.5 [CH]; 122.3 [C]; 116.2 [C]; 112.4 [CH]; 46.4 [2 CH_2]; 46.0 [2 CH_2].

5-Chloro-6-nitroquipazine or 5-chloro-6-nitro-2-piperazin-1-ylquinoline (1c). The procedure described above was used with 5-chloro-2-piperazin-1-ylquinoline (**5c**, 145 mg) and afforded 106 mg of 5-chloro-6-nitro-2-piperazin-1-ylquinoline (**1c**) as an orange-brown solid (62%). R_f (CH_2Cl_2/MeOH: 90:10): 0.2. ^{1}H NMR (CD_2Cl_2): δ 8.39 (d, J = 9.4 Hz, 1H); 7.97 (d, J = 9.0 Hz, 1H); 7.54 (d, J = 9.0 Hz, 1H); 7.14 (d, J = 9.4 Hz, 1H); 3.78 (bt, J = 4.0 Hz, 4H); 2.96 (bt, J = 4.0 Hz, 4H). ^{13}C NMR (DMSO-d_6): δ 157.6 [C]; 149.3 [C]; 141.8 [C]; 135.3 [CH]; 130.5 [C]; 126.0 [CH]; 125.3 [CH]; 120.0 [C]; 112.9 [CH]; 42.6 [2 CH_2]; 41.4 [2 CH_2].

General procedure for the preparation of the *N*-Boc derivatives of 5-halo-6-nitro-2-piperazin-1-ylquinoline (11a–c). To a solution of 5-halo-6-nitro-2-piperazin-1-ylquinoline (**1a–c**, 50–500 mg, 1 equiv) in dioxane (5–25 mL) and water (1–5 mL) was added Et_3N (5–10 equiv) and Boc_2O (1.5–2.0 equiv). The mixture was stirred at room temperature for 2 h and poured into water. The mixture was then extracted with ether, the combined ether extracts were washed with water and dried (Na_2SO_4). Evaporation of the solvent afforded the desired product as a yellow solid, that was chromatographed on preparative SiO_2–HPLC (eluent: CH_2Cl_2/EtOAc: 90:10).

5-Iodo-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline (11a). The procedure described above was used with 5-iodo-6-nitro-2-piperazin-1-ylquinoline (**1a**, 48 mg) and afforded 55 mg of 5-iodo-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline (**11a**) as a yellow solid (91%). R_f (EtOAc/heptane: 50:50): 0.6. ^{1}H NMR (CD_2Cl_2): δ 8.33 (d, $J=9.6$ Hz, 1H); 7.83 (d, $J=9.0$ Hz, 1H); 7.60 (d, $J=9.0$ Hz, 1H); 7.05 (d, $J=9.6$ Hz, 1H); 3.83 (bt, $J=6.0$ Hz, 4H); 3.58 (bt, $J=5.9$ Hz, 4H); 1.48 (s, 9H). ^{13}C NMR (CD_2Cl_2): δ 158.6 [C]; 154.9 [C]; 149.8 [C]; 149.2 [C]; 144.4 [CH]; 128.0 [CH]; 125.4 [CH]; 125.2 [C]; 112.8 [CH]; 93.6 [C]; 80.2 [C]; 44.9 [2 CH_2]; 43.8 [2 CH_2]; 28.5 [3 CH_3].

5-Bromo-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)-quinoline (11b). The procedure described above was used with 5-bromo-6-nitro-2-piperazin-1-ylquinoline (**1b**, 360 mg) and afforded 430 mg of 5-bromo-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline (**11b**) as a yellow solid (92%). R_f (EtOAc/heptane: 50:50): 0.6. ^{1}H NMR (CD_2Cl_2): δ 8.44 (d, $J=9.6$ Hz, 1H); 7.92 (d, $J=9.0$ Hz, 1H); 7.63 (d, $J=9.0$ Hz, 1H); 7.13 (d, $J=9.6$ Hz, 1H); 3.82 (bt, $J=5.0$ Hz, 4H); 3.56 (bt, $J=5.0$ Hz, 4H); 1.47 (s, 9H). ^{13}C NMR (CD_2Cl_2): δ 158.5 [C]; 154.9 [C]; 150.4 [C]; 144.8 [C]; 138.7 [CH]; 127.0 [CH]; 125.5 [CH]; 122.4 [C]; 116.1 [C]; 112.4 [CH]; 80.2 [C]; 44.9 [2 CH_2]; 44.7 [2 CH_2]; 28.5 [3 CH_3].

5-Chloro-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)-quinoline (11c). The procedure described above was used with 5-chloro-6-nitro-2-piperazin-1-ylquinoline (**1c**, 100 mg) and afforded 120 mg of 5-chloro-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline (**11c**) as a yellow solid (89%). R_f (EtOAc/heptane: 50:50): 0.6. ^{1}H NMR (CD_2Cl_2): δ 8.43 (d, $J=9.6$ Hz, 1H); 7.98 (d, $J=9.0$ Hz, 1H); 7.57 (d, $J=9.1$ Hz, 1H); 7.15 (d, $J=9.6$ Hz, 1H); 3.82 (bt, $J=6.0$ Hz, 4H); 3.57 (bt, $J=6.0$ Hz, 4H); 1.48 (s, 9H). ^{13}C NMR (CD_2Cl_2): δ 158.5 [C]; 154.9 [C]; 150.7 [C]; 135.8 [CH]; 130.5 [C]; 126.2 [CH]; 125.5 [CH]; 121.2 [C]; 120.5 [C]; 112.0 [CH]; 80.2 [C]; 44.9 [4 CH_2]; 28.5 [3 CH_3].

5-Fluoro-6-nitroquinoline (6). From 5-amino-6-nitroquinoline (**7**): To a solution of 5-amino-6-nitroquinoline (**7**, 1.0 g, 5.3 mmol, 1 equiv) in aq HBF_4 (48%, 40 mL, excess), cooled to-10 °C, was added $NaNO_2$ (4.0 g, 58.0 mmol, 11.0 equiv) dissolved in H_2O (5 mL). The mixture was stirred at 0 °C for 15 min and copper dust (0.2 g, 3.15 mmol, 0.6 equiv) was added. The mixture was heated at 100 °C for 1 h, poured into ice-water, basified with aq NaOH (2M) to a final pH of ~10, and extracted with EtOAc. The organic layer was dried (Na_2SO_4). The solvent was removed and the residue was chromatographed on silica gel (eluent: heptane/EtOAc: 70:30 to 30:70) to give 30 mg of 5-fluoro-6-nitroquinoline (**6**) as a brown oily residue (3% at best). R_f (EtOAc/heptane: 60:40): 0.6. ^{1}H NMR (CD_3OD): δ 9.07 (d, $J=3.8$ Hz, 1H); 8.75 (d, $J=8.6$ Hz, 1H); 8.17 (t, $J=9.1$ Hz, 1H); 8.04 (d, $J=9.0$ Hz, 1H); 7.67 (dd, $J=8.5$ and 4.2 Hz, 1H). MS (DCI/NH_4^+), $C_9H_5N_2O_2F$, 193 [$M+H^+$].

From 5-fluoroquinoline (**3d**): To a −10 °C solution of 5-fluoroquinoline (**3d**, 1.0 g, 1 equiv) in concentrated H_2SO_4 (excess) was added dropwise concentrated HNO_3 (5 equiv). The mixture was stirred for 60 min at −10 to 0 °C, poured into ice, and diluted with 1 M aq NaOH until basic (pH ~9). The mixture was filtered, extracted with CH_2Cl_2, and the combined organic layers were dried (Na_2SO_4). The solvent was removed and the residue was chromatographed on silica gel (eluent: heptane/EtOAc: 70:30 to 30:70) to give 26 mg of 5-fluoro-6-nitroquinoline (**6**) as a brown oily residue (2% at best).

1-[4-(5-Fluoroquinolin-2-yl)piperazin-1-yl]ethanone (9). To a solution of 5-fluoro-2-piperazin-1-ylquinoline (**5d**, 600 mg, 1 equiv) in CH_2Cl_2 (100 mL) was added Et_3N (2 equiv) and Ac_2O (2 equiv). The mixture was refluxed for 15 h and poured into water. The mixture was then extracted with CH_2Cl_2, the combined CH_2Cl_2 extracts were washed with water and dried (Na_2SO_4). Evaporation of the solvent afforded 600 mg of 1-[4-(5-fluoroquinolin-2-yl)piperazin-1-yl]ethanone (**9**) as a yellow solid. R_f (CH_2Cl_2/MeOH: 90:10): 0.3. ^{1}H NMR (CD_2Cl_2): δ 8.16 (d, $J=9.3$ Hz, 1H); 7.44 (AB^{19}F system, b, 2H); 7.02 (d, $J=9.3$ Hz, 1H); 6.89 (m, 1H); 3.85–3.55 (b, 8H); 2.10 (s, 3H). ^{13}C NMR (CD_2Cl_2): δ 169.3 [C]; 158.7 [C, d, J^1_{F-C}: 251 Hz]; 157.7 [C]; 149.4 [C]; 130.8 [CH]; 129.6 [CH, d, J^3_{F-C}: 7 Hz]; 122.7 [CH]; 113.5 [C, d, J^2_{F-C}: 15 Hz]; 109.9 [CH]; 106.6 [CH, d, J^2_{F-C}: 20 Hz]; 46.2 [CH_2]; 45.2 [CH_2]; 45.0 [CH_2]; 41.3 [CH_2]; 21.5 [CH_3].

1-[4-(6-Nitro-5-fluoroquinolin-2-yl)piperazin-1-yl]ethanone (10). To a −10 °C solution of 1-[4-(5-fluoroquinolin-2-yl)piperazin-1-yl]ethanone (**9**, 0.5 g, 1 equiv) in concentrated H_2SO_4 (excess) was added dropwise concentrated HNO_3 (5 equiv). The mixture was stirred for 30 min at −10 °C to 0 °C, poured into ice, and diluted with 1 M aq NaOH until basic (pH ~9). The mixture was filtered, extracted with CH_2Cl_2, and the combined organic layers were dried (Na_2SO_4). The solvent was removed and the residue was chromatographed on silica gel (eluent: heptane/EtOAc: 50:50 to 100% EtOAc) to give 23 mg of 1-[4-(6-nitro-5-fluoroquinolin-2-yl)piperazin-1-yl]ethanone (**10**) as a yellow oil (4%). R_f (EtOAc/MeOH: 90:10): 0.4. ^{1}H NMR (CD_2Cl_2): δ 8.27 (d, $J=9.6$ Hz, 1H); 8.12 (t, J_{app} ~9.0 Hz, 1H); 7.42 (d, $J=9.1$ Hz, 1H); 7.10 (d, $J=9.6$ Hz, 1H); 3.95–3.55 (b, 8H); 2.11 (s, 3H). ^{13}C NMR (CD_2Cl_2): δ 169.3 [C]; 158.8 [C]; 153.5 [C, d, J^1_{F-C}: 255 Hz]; 152.4 [C]; 132.4 [CH, d, J^3_{F-C}: 6 Hz]; 129.7 [C]; 125.0 [CH]; 122.6 [CH]; 112.6 [C, d, J^2_{F-C}: 15 Hz]; 111.0 [CH]; 46.0 [CH_2]; 44.8 [CH_2]; 44.6 [CH_2]; 41.1 [CH_2]; 21.5 [CH_3].

General procedure for the preparation of the 5-trialkylstannyl-6-nitro-2-piperazin-1-ylquinoline (*N*-Boc derivatives) (12, 13). To a solution of 5-bromo-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline (**11b**, 50 mg scale, 1eq) in anhydrous 1,4-dioxane (5 mL) at room temperature were added 5 mg of *tetrakis* triphenylphosphine palladium (0) (0.05 equiv) and 2–3 equiv of the corresponding hexaalkylditin derivative. The mixture was refluxed for 5 h, cooled to room temperature and then filtered. The filtrate was then diluted with EtOAc, washed with water and brine, dried (Na_2SO_4) and concentrated to dryness. The residue was rapidly chromatographed on silica gel (eluent: ether/heptane 75:25 to 50:50).

5-Trimethylstannyl-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline (12). The procedure described above was used with hexamethylditin and afforded 14 mg of 5-trimethylstannyl-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline (**12**) as a brown oil (23%). ^{1}H NMR was then immediately recorded and the residue was engaged in the fluorination step (fluorine in chloroform, see ref 27). R_f (ether/heptane: 50:50): 0.6. ^{1}H NMR (CD_2Cl_2): δ 7.93 (d, J = 9.6 Hz, 1H); 7.77 (d, J = 9.1 Hz, 1H); 7.40 (d, J = 9.0 Hz, 1H); 7.08 (d, J = 9.6 Hz, 1H); 3.75 (bt, J = 6.0 Hz, 4H); 3.55 (bt, J = 5.9 Hz, 4H); 1.48 (s, 9H); 0.46 (s, 9H). MS (DCI/NH_4^+): $C_{21}H_{30}N_4O_4Sn$: 519, 520, 521, 522, 523, 525, 527 [M + H$^+$]; 614, 615, 616, 617, 618, 620, 622 [M + H$^+$] for Sn isotopes of 116, 117, 118, 119, 120, 122 and 124 respectively.

5-Tributylstannyl-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline (13). The procedure described above was used with hexabutylditin and afforded 9 mg of 5-tributylstannyl-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline (**13**) as a brown oil (12%). R_f (ether/heptane: 70:30): 0.5. ^{1}H NMR was then immediately recorded and the residue was engaged in the fluorination step (fluorine in chloroform, see ref 27). Analytical data were in accordance with those already published.[16]

5-Fluoro-6-nitroquipazine or 5-fluoro-6-nitro-2-piperazin-1-ylquinoline (1d). Kryptofix® K_{222} (4, 7, 13, 16, 21, 24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, 16.0 mg), potassium carbonate (4.5 mg) and potassium fluoride (2.0 mg) were dissolved in 0.9 mL of water. Aqueous [^{18}F]fluoride (0.1 mL, 50 mCi or 1.85 MBq) was then added and the resulting solution was then gently concentrated to dryness at 145–150 °C under a nitrogen stream for 10 min to give carrier-added K[$^{18/19}$F]F-K_{222} complex as a white semi-solid residue. Freshly distilled DMSO (600 μL) containing 20 mg of the labelling bromo-precursor **11b** were directly added into the tube containing the dried K[$^{18/19}$F]F-K_{222} complex. The tube (not sealed) was then placed in a heating block at 145 °C for 2 min. The reaction mixture was diluted with 1 mL of water and transferred on a C18 Sep-pak cartridge (Waters). The tube was rinsed twice with 1 mL of water which was also transferred and added to the diluted reaction mixture on the cartridge. The whole was then passed through the cartridge. The cartridge was washed with 3 mL of water and partially dried for 0.5 min by applying a nitrogen stream. The *N*-Boc-protected quipazine derivatives (**11b** and **11d**) were eluted from the cartridge with 3 mL of dichloromethane. The mentioned dichloromethane solution was concentrated to dryness and the residue was then dissolved in 0.5 mL of dichloromethane. The crude was injected onto HPLC. Isocratic elution [HPLC A] gave a non-separated mixture of the *N*-Boc-protected 5-bromo-6-nitro-2-piperazin-1-ylquinoline (**11b**, starting material) and 5-fluoro-6-nitroquipazine (**11d**, visualized via its fluorine-18), retention time: 11–13 min. The above HPLC-collected mixture was concentrated to dryness and the residue was dissolved in 10 mL of dichloromethane/trifluoroacetic acid (4:1: v/v). The mixture was again concentrated to dryness, the residue was re-dissolved in 2 mL of CH_2Cl_2 and concentrated again to dryness to minimize TFA presence and finally purified on semi-preparative ^{18}C HPLC. Isocratic elution [HPLC B] gave pure 5-fluoro-6-nitroquipazine (**1d**), retention time: 8–9 min, well separated from the 5-bromo-6-nitro-2-piperazin-1-ylquinoline (**1b**), retention time: 17–18 min. R_f (CH_2Cl_2/MeOH: 90:10): 0.2. ^{1}H NMR (CD_3CN): δ 8.07 (d, J = 9.4 Hz, 1H); 7.87 (t, J_{app}: J ~9.0 Hz, 1H); 7.17 (d, J = 9.0 Hz, 1H); 7.01 (d, J = 9.4 Hz, 1H); 3.82 (bt, J < 3 Hz, 4H); 3.01 (bt, J < 3 Hz, 4H). MS (ES, positive mode): $C_{13}H_{13}FN_4O_2$: 277 [M + H$^+$].

Radiochemistry

K[^{18}F]F-K_{222} complex. In order to recover and recycle the [^{18}O]water target, the 2 mL of aqueous [^{18}F]fluoride from the target were passed through an anion exchange resin (AG1X8, Bio-Rad, 100–200 mesh). See refs 29 and 30 for more practical details. The [^{18}F]fluoride ion was then eluted from the resin using 1.0 mL of a 4.5 mg/mL aq K_2CO_3 solution. After addition of 11.0 to 15.0 mg of Kryptofix® K_{222} (4, 7, 13, 16, 21, 24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane), the resulting solution was then gently concentrated to dryness at 145–150 °C under a nitrogen stream for 10 min to give no-carrier-added K[^{18}F]F-K_{222} complex as a white semi-solid residue.

5-[^{18}F]Fluoro-6-nitroquipazine or 5-[^{18}F]fluoro-6-nitro-2-piperazin-1-ylquinoline ([^{18}F]-1d). Freshly distilled DMSO (600 μL) containing 10–20 mg of the labelling precursor (**11a**, **11b** or **11c**) were directly added into the tube containing the dried K[^{18}F]F-K_{222} complex. The tube (not sealed) was then placed in a heating block (at 80 to 250 °C for 0.5 to 10 min) or in a dedicated microwave oven (at 50, 100 or 200 W, for 0.5 to 5 min). The tube was then cooled using an ice/water bath and the remaining radioactivity was measured. Eighty-five to 95% of the initial activity placed in the vessel was still present. The resulting dark-coloured reaction mixture was then analyzed by radiochromatography. The incorporation yields were calculated from the TLC-radiochromatogram and defined as the *N*-Boc-protected 5-[^{18}F]fluoro-6-nitroquipazine over total fluorine-18 activity area ratio (SiO_2–TLC, eluent: heptane/EtOAc: 50:50, R_f 5-[^{18}F]fluoro-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline ([^{18}F]-**11d**): 0.65 and R_f

[^{18}F]fluoride ion: 0.0). The reaction mixture was diluted with 1 mL of water and transferred on a C18 Sep-pak cartridge (Waters). The tube was rinsed twice with 1 mL of water which was also transferred and added to the diluted reaction mixture on the cartridge. The whole was then passed through the cartridge. The cartridge was washed with 3 mL of water and partially dried for 0.5 min by applying a nitrogen stream. The *N*-Boc-protected 5-[^{18}F]fluoro-6-nitroquipazine derivative [^{18}F]-**11d** was eluted from the cartridge with 3 mL of dichloromethane. Twice 1 mL of dichloromethane was used to wash the cartridge and to completely transfer [^{18}F]-**11d** (10% of the total radioactivity amount engaged in the fluorination process was left on the cartridge). The mentioned dichloromethane solution was concentrated to dryness (at 60–80 °C under a gentle nitrogen stream for 4–6 min). The residue was then dissolved in 0.5 mL of dichloromethane and the crude was injected onto HPLC. Isocratic elution [HPLC C] gave pure labelled 5-[^{18}F]fluoro-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline ([^{18}F]-**11d**), retention time: 14–15 min.

The above HPLC-collected 5-[^{18}F]fluoro-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline ([^{18}F]-**11d**) was concentrated to dryness (at 60–80 °C under a gentle nitrogen stream for 4–6 min) and the residue was dissolved in 2 mL of dichloromethane/trifluoroacetic acid (9:1: v/v). The mixture was allowed to stand without stirring at room temperature for 2 min and was then concentrated to dryness (at 60–80 °C under a gentle nitrogen stream for 4–6 min). The yield of deprotection was quantitative: No *N*-Boc-protected quipazine derivative [^{18}F]-**11d** could be detected by radiochromatography (SiO_2–TLC, eluent: heptane/EtOAc: 50:50, R_f 5-[^{18}F]fluoro-6-nitro-2-(*N*-*tert*-butylcarboxypiperazin-1-yl)quinoline ([^{18}F]-**11d**): 0.65 and R_f 5-[^{18}F]fluoro-6-nitroquipazine ([^{18}F]-**1d**): 0.0). The above residue was redissolved in 2 mL of CH_2Cl_2 and concentrated again to dryness to minimize TFA presence (at 60–80 °C under a gentle nitrogen stream for 4–6 min). Finally, the residue was redissolved in 2 mL of the HPLC solvent used for purification and the crude was injected onto HPLC. Isocratic elution [HPLC B] gave pure labelled 5-[^{18}F]Fluoro-6-nitroquipazine ([^{18}F]-**1d**), retention time: 8–9 min.

Formulation and Quality Control

Formulation of labelled product for iv injection was effected as follows: (1) HPLC solvent removal by evaporation; (2) taking up the residue, while heating gently (45 °C), in 5 mL of physiological saline; (3) sterile filtration on a 0.22 μm Millipore filter. Injection in animal experiments was done within 15 min after end of synthesis.

As demonstrated by HPLC analysis [HPLC D], the radiolabelled product was found to be >95% chemically and radiochemically pure (retention time: 3.9–4.0 min). The preparation was shown to be free of non-radioactive precursor and radiochemically stable for at least 120 min.

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