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Syntheses and Binding Affinities of 6-Nitroquipazine Analogues for Serotonin Transporter: Part 3.† A Potential 5-HT Transporter Imaging Agent, 3-(3-[^{18}F]Fluoropropyl)-6-nitroquipazine

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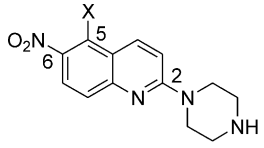
Abstract—3-(3-[^{18}F]Fluoropropyl)-6-nitroquipazine ([^{18}F]FPNQ) as a 5-HT transporter imaging agents was designed, synthesized, and evaluated. FPNQ was selected due to its potent in vitro biological activity ($K_i = 0.32$ nM) in rat brain cortical membranes. The ^{18}F-labeled FPNQ was prepared by reaction of the propyl mesylate as a precursor with tetra-*n*-butylammonium [^{18}F]fluoride generated under NCA conditions. The precursor mesylate was synthesized from commercially available hydrocarbostyril in nine steps in 21% overall yield. The specific activity of the [^{18}F]FPNQ determined by radioreceptor assay was 27.0 GBq/μmol. Tissue distribution studies in mice showed the highest uptake in the frontal cortex (5.79 %ID/g) at 60 min post-injection.

Introduction

Development of radiopharmaceuticals for positron emission tomography (PET) and single photon emission computed tomography (SPECT) as diagnostic imaging tools, especially for the central nervous system, is an active and emerging field in nuclear medicine.[2] Many dopamine transporter (reuptake site) imaging agents, such as cocaine derivatives β-CFT,[3–5] β-CIT,[6–10] IPT,[11] and TRODAT,[12–15] enabled one to gain a better understanding of Parkinson's disease and to help in staging patients with Parkinson's disease. Serotonin (5-hydroxyltryptamine or 5-HT) transporter which is related to depression and other psychiatric diseases in the mammalian brain has also been studied extensively using radiotracers such as [^{3}H]imipramine, [^{3}H]paroxetine, and [^{3}H]citalopram. Most of these agents, however, did not reach the suitable target-to-non-target uptake ratios in vivo for serotonin transporter imaging. On the other hand, [^{11}C]McN-5652,[16] 5-[^{123}I]iodo-6-nitroquipazine (**1a**),[17–20] and 5-[^{76}Br]bromo-6-nitroquipazine (**1b**),[21] showed somewhat encouraging results. The recently developed ADAM, ODAM and IDAM series[22–24] are also attractive lead compounds in the development of new PET and SPECT radioligands for the understanding of dysfunction in psychiatric diseases as well as pathological studies of 5-HT neurotransmission.



1, X=H
1a, X=^{125}I
1b, X=^{76}Br

Although 6-nitroquipazine (**1**, 6-NQ) has been known as one of the most potent and selective inhibitors for the 5-HT transporter (SERT) in vitro[25,26] and in vivo,[27,28] the structure–activity relationship of its derivatives has not been extensively studied. Recently, we reported the first structure–activity relationship[29] of 6-NQ derivatives and the first regioselective synthetic route[30] to 6-NQ.

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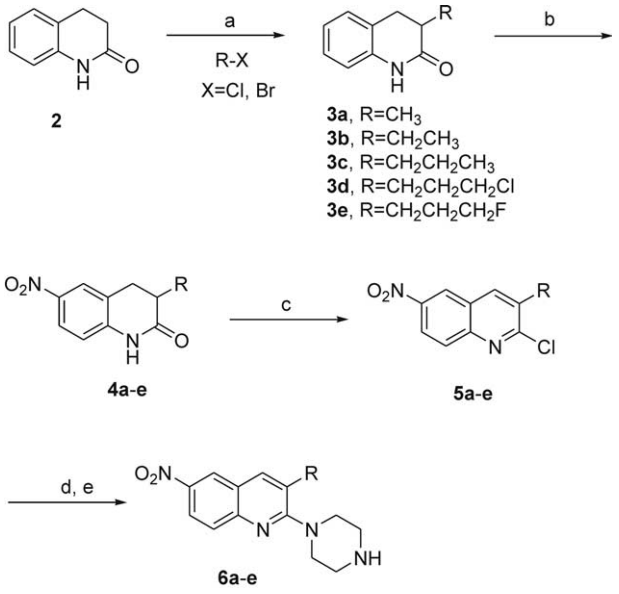
In this paper, we synthesized 3-alkyl-6-NQ derivatives and measured their biological activities for the 5-HT transporter. Among these derivatives, 3-(3-fluoropropyl)-6-NQ (**6e**) was selected for ^{18}F-labeling and tissue distribution in mice.

Results and Discussion

Synthesis of 3-alkylated 6-nitroquipazines 6a–e

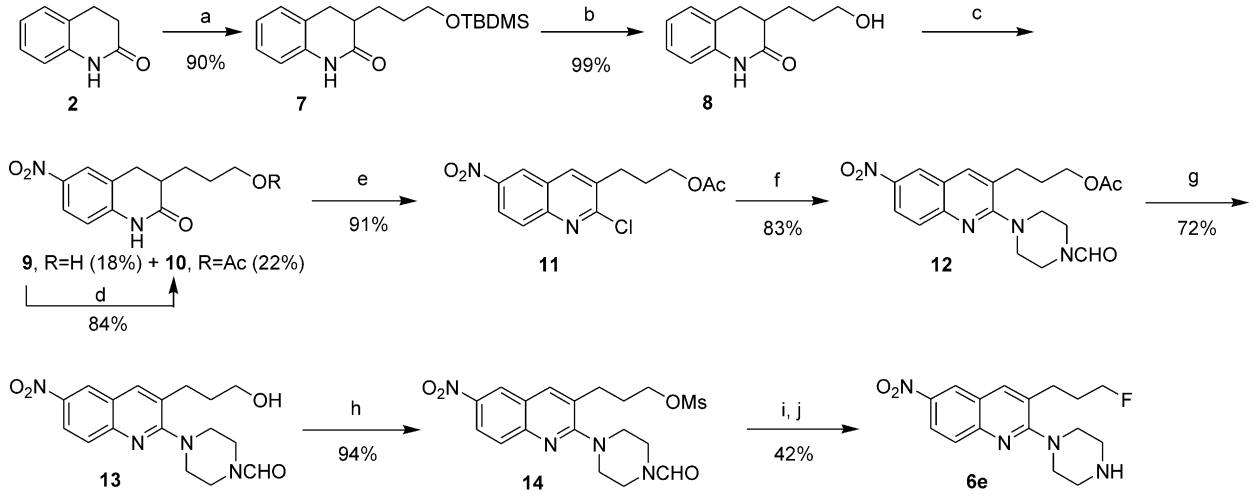
We reported the regioselective synthetic route of 6-nitroquipazine from commercially available hydrocarbostyril (**2**).[30] Five, 3-alkylated 6-NQ derivatives **6a–e** were prepared by applying this route as shown in Scheme 1. Several alkyl groups were introduced to C3 position of 6-nitroquipazine by the reaction of dianion of hydrocarbostyril and alkyl halides using lithiumdiisopropylamide (LDA) as a base at −78 °C. When *n*-butyllithium was used instead of LDA, many side products that lowered the reaction yield were formed. The nitration of **3a–e** gave two isomers, 6-nitro as a major and 8-nitro as a minor compound. Compounds **5a–e** were obtained by the reaction of **4a–e** with one equivalent of DDQ and three equivalents of phosporous oxychloride in DMF under very mild conditions (rt, 3 h). Nucleophilic aromatic substitution of the chloride on C2 position with 1-piperazinecarboxaldehyde provided piperazin-1-yl quinoline analogues. Without further purification, direct deformylation under acidic conditions provided yellow solids **6a–e** which were further treated with 4 N NaOH until basic.



Scheme 1. Reagents and conditions: (a) LDA, R-X, THF, −78 °C; (b) c. H_2SO_4, c. HNO_3, 0 °C; (c) $POCl_3$, DDQ, DMF, rt; (d) 1-piperazinecarboxaldehyde, DMF, 80 °C; (e) 4 M H_2SO_4, 80 °C.

Synthesis of the mesylate precursor 14

The mesylate **14** was used as the precursor for the synthesis of ^{18}F-labeled quipazine derivative **6e**. The synthetic route for **14** is shown in Scheme 2. Although **14** was synthesized via the same synthetic route shown in Scheme 1, several steps were modified for the protection of the alcohol group due to the introduction of 3′-hydroxyl group. As the nitration reaction of **7** failed to give the desired product, an additional removal step of the *t*-butyldimethylsilyl group was necessary. To introduce a chlorine atom selectively on C2 position, the propyl alcohol had to be protected. The nitration of **8** followed by acetylation gave the compound **10**. In early attempts, the nitrated compound **9** was obtained in 40% yield, because TLC analysis of the nitration reaction showed the multiple spots at the beginning of the reaction. The number of spots were, however, reduced as the reaction mixture was allowed to stand overnight, which also improved the yield up to 55%. Treatment of **12** with methanolic potassium carbonate furnished compound **13** that was further reacted with methanesulfonyl chloride to give the corresponding mesylate **14**. FPNQ was prepared by fluorination of **14** with tetra-*n*-butylammonium fluoride hydrate followed by direct deformylation under acidic conditions.



Scheme 2. Reagents and conditions: (a) LDA, $Br(CH_2)_3OTBDMS$, THF, −78 °C; (b) *n*-Bu_4NF, THF, rt, 12 h; (c) c. H_2SO_4, c. HNO_3, 0 °C, 10 min; (d) Ac_2O, pyridine, rt, 12 h; (e) $POCl_3$, DDQ, DMF, rt, 3 h; (f) 1-piperazinecarboxaldehyde, DMF, 100 °C, 3 h; (g) K_2CO_3, $MeOH/CH_2Cl_2$, rt, 15 h; (h) MsCl, Et_3N, CH_2Cl_2, 0 °C, 1 h; (I) *n*Bu_4NF, CH_3CN, reflux, 1 h; (j) 4 M H_2SO_4, 100 °C, 2 h.

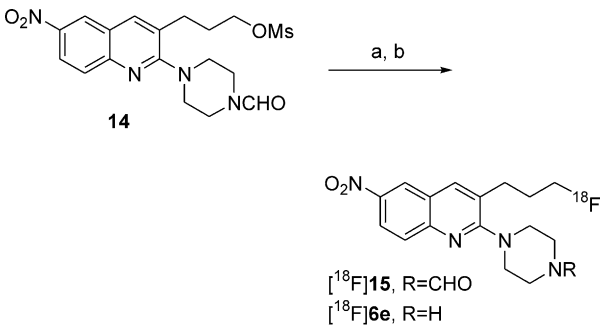
Radiochemical synthesis of [^{18}F]FPNQ

The [^{18}F]FPNQ was prepared by fluoride ion displacement reaction of the mesylate **14** (2.0 mg, 5.0 μmol) at 130 °C for 30 min with tetra-*n*-butylammonium [^{18}F]fluoride generated under no-carrier-added (NCA) conditions, followed by direct deformylation of [^{18}F]**15** under acidic conditions at 130 °C for 15 min as shown in Scheme 3. Resolubilization of tetra-*n*-butylammonium [^{18}F]fluoride prepared from the [^{18}F]fluoride ion and tetra-*n*-butylammonium hydroxide (40% aq, 2.6 μL, 4.0 μmol) was obtained in 70–90% yield and took 10–12 min.

The isolated radiochemical yield of [^{18}F]**15** and [^{18}F]**6e** were 16–28 and 85–89% (decay-corrected), respectively. After workup and removal of the solvent, [^{18}F]FPNQ was purified by a semipreparative HPLC column before testing its specific activity, binding affinity, and biodistribution in mice.

In vitro serotonin transporter bindings studies

Table 1 shows the in vitro binding affinities, expressed as the inhibition binding constant, of **6a–e** for the 5-HT transporter including 6-NQ, fluoxetine, and paroxetine. In comparison with 6-nitroquipazine, the binding affinity of 3-methyl-6-nitroquipazine (**6a**) decreased by approximately 50-fold, but was slightly higher than that of 3-bromo-6-nitroquipazine. The methyl group itself donates electrons to the quinoline ring, consequently decreasing the binding affinity. However, in cases of 3-ethyl- (**6b**) and 3-propyl-6-nitroquipazine (**6c**), the values of the inhibition binding constant dramatically increased to those similar to 6-nitroquipazine. This significant improvement in the binding affinities indicates that alkyl chains longer than a methyl group have a favorable lipophilic interaction and thus compensates for electron-donating effects by the alkyl group on C3 position of 6-nitroquipazine derivatives. It is likely that there are London forces between 3-alkyl chains of 6-nitroquipazine derivatives and the corresponding residue in the binding pocket of the 5-HT transporter. The target compound, 3-(3-fluoropropyl)-6-nitroquipazine, has the binding affinity similar to **6c**. These results suggest that [^{18}F]**6e** can be a good imaging agent for the 5-HT transporter.



Scheme 3. Reagents and conditions: (a) *n*-$Bu_4N[^{18}F]F$, CH_3CN, 130 °C, 20 min; (b) 4 M H_2SO_4, 130 °C, 10 min.

Table 1. Binding affinities (K_i) for 5-HT transporter[a]

Compd	K_i (nM)
6a	8.45±0.62
6b	0.36±0.02
6c	0.26±0.01
6d	1.08±0.17
6e	0.31±0.01
3-Bromo-6-nitroquipazine	12.62±1.44
Fluoxetine	22.13±1.77
Paroxetine	0.53±0.08
6-Nitroquipazine	0.17±0.03

[a]The inhibition of [^{3}H]citalopram binding was determined at 1 nM [^{3}H]citalopram for the various compounds listed. Binding data are the means of three independent experiments. Eleven concentrations of displacer were used for each determination. The K_d value of [^{3}H]citalopram measured by Scatchard analysis of the equilibrium-saturation experiment was 1.12 nM.

Specific activity and in vivo tissue distribution studies of [^{18}F]6e

The [^{18}F]**6e** collected from HPLC was dissolved in 10% ethanol-saline and the specific activity determined by radioreceptor assay was 27.0±4.4 GBq/μmol (Fig. 1). The [^{18}F]**6e** solution was injected into mice (25–30 g, $n=4$). Tissue distribution data were obtained at 5, 15, 30, 60, 90 and 120 min post-injection and are shown in Figure 2 and Table 2. The brain uptake of this radioligand reached 1.8 %ID/g at 60 min similar to [^{125}I]5-iodo-6-nitroquipazine (1.9 %ID/g at 5 min). The [^{18}F]**6e** showed the highest uptake (5.79 %ID/g) in the frontal cortex and low uptake (2.73 %ID/g) in the cerebellum at 60 min

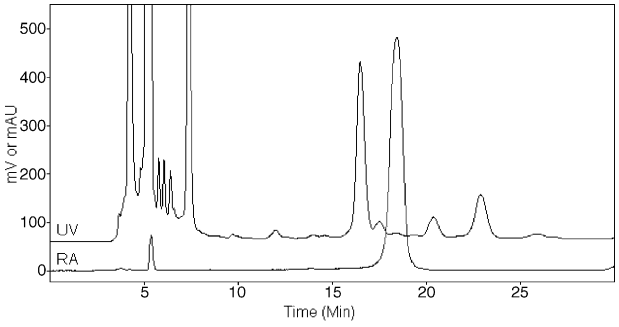


Figure 1. HPLC profiles of the reaction mixture containing [^{18}F]FPNQ.

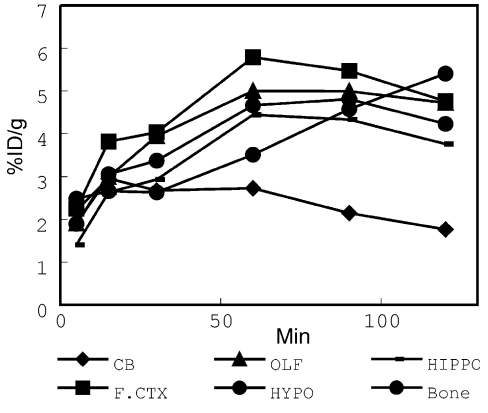


Figure 2. Tissue distribution of [^{18}F]FPNQ in mouse brain ($n=4$). CB, cerebellum; F. Ctx, frontal cortex; Olf, Olfactory tubercle; Hypo, hypothalamus; Hippo, hippocampus.

Table 2. Tissue distribution (% ID/g) of [^{18}F]FPNQ in mouse brain and bone ($n=4$)[a]

Min	CB	F. Cox	Olf	Hypo	Hippo	Str	Ctx	S. Colli	I. Colli	Thal	Bone
5	2.21±0.33	2.25±0.52	1.90±0.87	1.89±0.61	1.40±0.45	2.05±0.30	2.29±0.38	2.26±0.63	1.97±0.46	2.02±0.48	2.49±0.20
15	2.95±0.36	3.82±0.54	2.99±0.60	3.06±0.68	2.63±0.37	3.00±0.43	3.31±0.41	3.79±0.46	3.27±0.32	3.57±0.67	2.66±0.21
30	2.68±0.69	4.04±1.22	3.95±1.21	3.37±0.94	2.93±0.88	3.21±0.82	3.54±0.80	3.64±0.97	3.44±0.94	3.75±0.94	2.63±0.42
60	2.73±0.21	5.79±0.41	5.00±0.64	4.66±0.48	4.45±0.50	4.24±0.42	4.30±0.58	4.36±0.62	4.65±0.31	4.17±2.16	3.51±0.44
90	2.14±0.16	5.47±0.73	4.99±0.99	4.81±1.04	4.33±0.72	4.12±0.76	4.24±0.84	4.03±1.08	4.70±1.60	5.12±1.17	4.58±0.76
120	1.76±0.17	4.76±0.35	4.72±0.33	4.23±0.38	3.76±0.30	3.34±0.27	3.08±0.15	3.32±0.30	3.80±0.33	4.17±0.45	5.41±0.64

[a]CB, cerebellum; F. Cox, frontal cortex; Olf, Olfactory tubercle; Hypo, hypothalamus; Hippo, hippocampus; Str, striatum; Ctx, cortex; S. Colli:, I. Colli:, Thal: thalamus.

Table 3. In vivo blocking studies at 60 min postinjection. Ratio of %ID/g of organ/cerebellum [^{18}F]FPNQ in mouse brain ($n=4$)

	Saline	Paroxetine	Fluoxetine	GBR12909	Cold FPNQ
F. Ctx	2.20	1.57	1.72	1.95	1.55
Olf	1.92	1.24	1.42	1.72	1.06
Hypo	1.75	1.17	1.23	1.66	1.00
Hippo	2.01	1.41	1.43	1.42	1.19
Str	1.63	1.27	1.30	1.53	1.17
Ctx	1.63	1.47	1.10	1.58	1.39

postinjection, although the region-to-cerebellum ratios increased as a function of time. The temporal distribution pattern of this radioligand in brain regions was quite different from that of [^{125}I]5-iodo-6-nitroquipazine which was taken up quickly to the brain (~1.5 %ID/g in the prefrontal cortex at 5 min post-injection) and washed out over time.[18] Bone uptake slowly increased over time, ranging from 2.49 %ID/g at 5 min, 3.51 %ID/g at 60 min to 5.41 %ID/g at 120 min, suggesting slow defluorination in vivo.

In vivo blocking studies

The [^{18}F]**6e** was injected alone or with various drugs to mice to investigate the effects of these drugs on the in vivo binding of this radioligand. Displacement of the binding of this radioligand was measured at 60 min postinjection, the time point when a maximal uptake to the cortex and relatively low level of bone uptake were observed (Table 3). Both fluoxetine and paroxetine, serotonin reuptake blockers, inhibited the in vivo binding of the [^{18}F]**6e** in the frontal cortex by 11–31%, although higher levels of inhibition of [^{125}I]5-iodo-6-nitroquipazine by these two blockers has been reported.[18] The difference in the level of inhibition may be explained by the different uptake patterns between these two radioligands.[18] In contrast, no inhibition was detected in the cerebellum where low level of serotonin reuptake sites is located.

Coadministration of the unlabeled **6e** showed higher level of inhibition than paroxetine in all regions studied. Meanwhile, a dopamine reuptake blocker, GBR-12909 did not inhibit the in vivo binding of [^{18}F]**6e**. This study demonstrates specific binding of [^{18}F]**6e** to the 5-HT transporter in vivo. At this point, it is not clear whether [^{18}F]**6e** has the selective binding toward SERT or not, because SERT presents high similarities to dopamine transporter as well as norepinephrine transporter.[31]

Conclusions

We synthesized five, high affinity ligands for the 5-HT trnasporter, 3-alkyl-6-nitroquipazine (**6a–e**) and one ^{18}F-labeled analogue [^{18}F]FPNQ ([^{18}F]**6e**) for tissue distribution studies in vivo. The [^{18}F]FPNQ was prepared by ^{18}F-labeling of the propyl mesylate **14** followed by deformylation in high radiochemical yield. Tissue distribution studies of this radioligand in mice showed the highest uptake (5.79 %ID/g) in the frontal cortex at 60 min post-injection, although bone uptake increased slowly over time. This result suggested that the investigation of this radioligand at 60 min post-injection may be suitable for 5-HT imaging in mice. Further studies on the syntheses and biological studies of fluoroalkylated 6-nitroquipazines at different positions such as C4, C5 and C7 are in progress in our laboratories to develop radioligands with lower bone uptake.

Experimental

General procedure for the synthesis of compounds 3a–3e

Under N_2 atmosphere, to a solution of hydrocarbostyril (**2**, 1.0 g, 6.79 mmol) in 15 mL of sodium-dried THF was dropwise added 2.0 M LDA solution (7.47 mL, 14.95 mmol) at −78 °C. The mixture was stirred for 30 min at −78 °C and the cooling bath was removed. The mixture was stirred for additional 1 h at rt, and then cooled to −78 °C. Alkyl halide (7.13 mmol) was added dropwise to the mixture at −78 °C, which was stirred for 90 min from −78 °C to rt. The reaction was quenched by adding of 1 mL of methanol and 200 mL of water in succession. The organic compounds were extracted with 70 mL of EtOAc three times. The combined extract was dried over Na_2SO_4 and concentrated with 1.0 g of silica gel. The product was isolated by flash column chromatography with 20% EtOAc/hexane and recrystallized from EtOAc/hexane as a white solid.

3 - Methyl - 3,4 - dihydro - 2(1*H*) - quinolinone (3a). 60%, white solid, mp 85–87 °C; ^{1}H NMR ($CDCl_3$) δ 8.38 (br s, 1H), 7.13–7.20 (m, 2H), 6.93–7.01 (m, 1H), 6.78 (d, $J=7.6$ Hz, 1H), 2.62–3.02 (m, 3H), 1.29 (d, $J=6.6$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.0, 135.6, 126.3, 125.8, 121.9, 121.2, 113.5, 33.3, 31.8, 13.7; MS (EI) m/z 161 (M^+, 100), 132, 118, 92. Anal. calcd for $C_{10}H_{11}NO$: C, 74.50; H, 6.88; N, 9.93. Found: C, 74.23; H, 6.99; N, 10.21.

3-Ethyl-3,4-dihydro-2(1*H*)-quinolinone (3b). 61%, white solid, mp 79–81 °C; ^{1}H NMR ($CDCl_3$) δ 9.09 (br s, 1H), 7.12–7.19 (m, 2H), 6.92–7.00 (m, 1H), 6.82 (d, J = 7.8 Hz, 1H), 3.04 (dd, J = 15.8, 6.0 Hz, 1H), 2.75 (dd, J = 15.6, 8.6 Hz, 1H), 2.40–2.55 (m, 1H), 1.79–1.99 (m, 1H), 1.40–1.62 (m, 1H), 1.02 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.2, 135.4, 126.5, 125.7, 121.6, 121.2, 113.4, 39.9, 28.5, 21.0, 9.8; MS (EI) m/z 175 (M^+), 146 (100), 132, 118, 106. Anal. calcd for $C_{11}H_{13}NO$: C, 75.39; H, 7.48; N, 8.00. Found: C, 75.15; H, 7.80; N, 8.31.

3-Propyl-3,4-dihydro-2(1*H*)-quinolinone (3c). 44%, white solid, mp 105–107 °C; ^{1}H NMR ($CDCl_3$) δ 8.74 (br s, 1H), 7.12–7.20 (m, 2H), 6.96 (td, J = 7.2, 1.3 Hz, 1H), 6.80 (d, J = 7.6 Hz, 1H), 3.11 (dd, J = 15.6, 3.0 Hz, 1H), 2.73 (dd, J = 15.8, 8.5 Hz, 1H), 2.52–2.59 (m, 1H), 1.74–1.88 (m, 1H), 1.37–1.58 (m, 3H), 0.93 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.6, 135.4, 126.5, 125.7, 121.6, 121.2, 113.3, 38.2, 30.0, 29.0, 18.5, 12.3; MS (EI) m/z 191 (M^+, 100), 161, 145, 130, 117. Anal. calcd for $C_{12}H_{15}NO$: C, 76.15; H, 7.99; N, 7.40. Found: C, 76.05; H, 8.12; N, 7.41.

3-(3-Chloropropyl)-3,4-dihydro-2(1*H*)-quinolinone (3d). 75%, white solid, mp 97–100 °C; ^{1}H NMR ($CDCl_3$) δ 9.13 (br s, 1H), 7.13–7.21 (m, 2H), 6.97 (td, J = 7.3, 1.2 Hz, 1H), 6.83 (d, J = 8.0 Hz, 1H), 3.54–3.60 (m, 2H), 3.05 (dd, J = 15.4, 6.0 Hz, 1H), 2.76 (dd, J = 15.3, 9.1 Hz, 1H), 2.53–2.61 (m, 1H), 1.86–2.04 (m, 3H), 1.62–1.71 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 172.1, 140.7, 135.2, 126.5, 125.9, 121.4, 113.5, 43.1, 37.8, 29.4, 28.5, 25.5; MS (EI) m/z 225 ($M^+ + 2$), 223 (M^+), 188, 160, 146 (100), 132. Anal. calcd for $C_{12}H_{14}ClNO$: C, 64.43; H, 6.31; N, 6.26. Found: C, 64.35; H, 6.52; N, 6.17.

3-(3-Fluoropropyl)-3,4-dihydro-2(1*H*)-quinolinone (3e). 61%, white solid, mp 92–93 °C; ^{1}H NMR ($CDCl_3$) δ 8.44 (br s, 1H), 7.14–7.21 (m, 2H), 6.98 (t, J = 7.5 Hz, 1H), 6.78 (d, J = 7.6 Hz, 1H), 4.47 (dt, J = 47.1, 5.7 Hz, 2H), 3.05 (dd, J = 15.6, 5.6 Hz, 1H), 2.77 (dd, J = 15.4, 9.4 Hz, 1H), 2.55–2.63 (m, 1H), 1.73–2.00 (m, 3H), 1.58–1.66 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 172.1, 135.2, 126.5, 125.9, 121.4, 121.4, 113.5, 82.2 (d, J = 164 Hz), 38.0, 29.3, 26.3 (d, J = 19 Hz), 24.0 (d, J = 5 Hz); MS (EI) m/z 207 (M^+), 146 (100), 132, 117, 106. Anal. calcd for $C_{12}H_{14}FNO$: C, 69.55; H, 6.81; N, 6.76. Found: C, 69.61; H, 6.97; N, 6.75.

General procedure for the synthesis of compounds 4a–4e

To a solution of **3a** (586 mg, 3.64 mmol) in 10 mL of concd H_2SO_4 and 2 mL of water at 0 °C was dropwise added 61% HNO_3 (0.30 mL, 4.00 mmol). After stirred for 5 min at 0 °C, the reaction mixture was carefully poured into 400 mL of ice-water. The resulting precipitate was filtered and washed with 300 mL of water and dried under suction for 3 h. The solid was dissolved in 250 mL of EtOAc, which was concentrated with 1.0 g of silica in vacuo. The product **4a** (582 mg, 78%) was obtained by flash column chromatography with 20% EtOAc/hexane as a white solid.

3-Methyl-6-nitro-3,4-dihydro-2(1*H*)-quinolinone (4a). Mp 213–214 °C; ^{1}H NMR ($CDCl_3$) δ 8.92 (br s, 1H), 8.09–8.12 (m, 2H), 6.90 (d, J = 9.0 Hz, 1H), 3.12 (dd, J = 15.0, 5.2 Hz, 1H), 2.66–2.90 (m, 2H), 1.32 (d, J = 6.6 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 170.1, 122.0, 121.0, 120.9, 112.3, 112.3, 95.5, 31.6, 30.2 12.0; MS (EI) m/z 206 (M^+, 100), 178, 131, 117. Anal. calcd for $C_{10}H_{10}N_2O_3$: C, 58.24; H, 4.89; N, 13.59. Found: C, 58.46; H, 4.70; N, 13.91.

3-Ethyl-6-nitro-3,4-dihydro-2(1*H*)-quinolinone (4b). 50%, white solid, mp 191–192 °C; ^{1}H NMR ($CDCl_3$) δ 9.35 (br s, 1H), 8.10 (br s, 2H), 6.91 (d, J = 9.2 Hz, 1H), 3.14 (dd, J = 16.2, 6.2 Hz, $1H_0$, 2.86 (dd, J = 16.2, 9.0 Hz, 1H), 2.47–2.61 (m, 1H), 1.83–1.96 (m, 1H), 1.43–1.65 (m, 1H), 1.04 (t, J = 7.5 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 171.4, 143.3, 140.6, 123.2, 122.6, 113.8, 39.4, 28.0, 21.2, 10.1; MS (EI) m/z 220 (M^+) 191 (100), 177, 145, 130, 117. Anal. calcd for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 60.03; H, 5.58; N, 12.49.

3-Propyl-6-nitro-3,4-dihydro-2(1*H*)-quinolinone (4c). 84%, white solid, mp 207–208 °C; ^{1}H NMR ($CDCl_3$) δ 9.07 (br s, 1H), 8.08–8.11 (m, 2H), 6.89 (d, J = 0.2 Hz, 1H), 3.15 (dd, J = 16.2, 5.8 Hz, 1H), 2.84 (dd, J = 16.1, 8.5 Hz, 1H), 2.59–2.66 (m, 1H), 1.79–1.85 (m, 1H), 1.39–1.55 (m, 3H), 0.95 (t, J = 6.8 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 172.3, 141.5, 141.0, 122.5, 122.2, 122.2, 113.3, 37.6, 30.0, 28.7, 18.4, 12.2; MS (EI) m/z 234 (M^+), 191 (100), 161, 145, 130, 117. Anal. calcd for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 6.02; N, 11.96. Found: C, 61.05; H, 6.17; N, 11.92.

3-(3-Chloropropyl)-6-nitro-3,4-dihydro-2(1*H*)-quinolinone (4d). 89%, white solid, mp 206–208 °C; ^{1}H NMR (DMSO-d_6) δ 10.69 (br s, 1H), 8.11 (s, 1H), 8.05 (d, J = 8.8 Hz, 1H), 6.98 (d, J = 8.8 Hz, 1H), 3.64 (t, J = 6.1 Hz, 2H), 3.13 (dd, J = 15.9, 6.1 Hz, 1H), 2.79 (dd, J = 16.2, 10.2 Hz, 1H), 2.48–2.60 (m, 1H), 1.77–1.91 (m, 3H), 1.37-1.52 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 176.3, 148.2, 145.6, 128.1, 127.6, 127.6, 118.8, 49.2, 41.9, 33.5, 33.4, 30.6; MS (EI) m/z 270 ($M^+ + 2$), 268 (M^+), 233, 205, 191 (100), 177, 145, 130, 117. Anal. calcd for $C_{12}H_{13}ClN_2O_3$: C, 53.64; H, 4.88; N, 10.43. Found: C, 53.28; H, 4.95; N, 10.34.

3-(3-Fluoropropyl)-6-nitro-3,4-dihydro-2(1*H*)-quinolinone (4e). 77%, white solid, mp 208–209 °C; ^{1}H NMR ($CDCl_3$) δ 8.65 (br s, 1H), 8.10–8.14 (m, 2H), 6.88 (d, J = 9.2 Hz, 1H), 4.40 (dt, J = 47.1, 5.5 Hz, 2H), 3.71 (dd, J = 15.8, 5.8 Hz, 1H), 2.88 (dd, J = 18.5, 9.8 Hz, 1H), 2.05–2.73 (m, 1H), 2.05–1.57 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 171.3, 143.2, 140.6, 123.1, 122.6, 122.5, 113.9, 82.6 (d, J = 161 Hz), 37.1, 28.5, 26.2 (d, J = 19 Hz), 24.0 (d, J = 6 Hz); MS (EI) m/z 252 (M^+), 232, 205, 191 (100), 177, 145, 130, 117. HRMS (FAB) m/z $C_{12}H_{14}FN_2O_3$ (MH^+) calcd: 253.0988; found: 253.0988.

General procedure for the synthesis of compounds 5a–5e

To a solution of **4a** (586 mg, 2.84 mmol) and DDQ (724 mg, 3.13 mmol) in 15 mL of DMF was dropwise added

phosphorus oxychloride (0.41 mL, 4.26 mmol) at rt. The mixture was stirred for 2 h at rt and then poured into ice-water. The resulting precipitate was filtered and washed with 50 mL of water and dried under suction for 3 h. The solid was dissolved in EtOAc and the concentrated with 1.0 g of silica gel in vacuo. The product **5a** (609 mg, 96%) was obtained by flash column chromatography with 10% EtOAc/hexane as a white solid.

2-Chloro-3-methyl-6-nitroquinoline (5a). Mp 145–147 °C; ^{1}H NMR ($CDCl_3$) δ 8.72 (d, J = 2.2 Hz, 1H), 8.44 (dd, J = 9.3, 2.3 Hz, 1H), 8.15 (s, 1H), 8.11 (d, J = 10.0 Hz, 1H), 2.60 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 156.9, 146.7, 144.1, 137.2, 131.2, 128.3, 124.7, 121.8, 121.3, 18.5; MS (EI) m/z 224 (M^+ + 2), 222 (M^+, 100), 192, 176, 164, 155, 140, 113. Anal. calcd for $C_{10}H_7ClN_2O_2$: C, 53.95; H, 3.17; N, 12.59. Found: C, 54.01; H, 3.50; N, 12.52.

2-Chloro-3-ethyl-6-nitroquinoline (5b). 75%, white solid, mp 141–142 °C; ^{1}H NMR ($CDCl_3$) δ 8.75 (d, J = 2.6 Hz, 1H), 8.44 (dd, J = 9.1, 2.5 Hz, 1H), 8.14 (s, 1H), 8.11 (d, J = 9.0 Hz, 1H), 2.96 (q, J = 7.5 Hz, 2H), 1.40 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 153.9, 146.5, 144.1, 136.5, 135.7, 128.2, 124.8, 122.0, 121.3, 24.8, 11.4; MS (EI) m/z 238 (M^+ + 2), 236 (M^+, 100), 221, 206, 191, 175, 155, 140, 127. Anal. calcd for $C_{11}H_9ClN_2O_2$: C, 55.82; H, 3.83; N, 11.84. Found: C, 55.80; H, 4.07; N, 11.63.

2-Chloro-3-propyl-6-nitroquinoline (5c). 95%, white solid, mp 133–134 °C; ^{1}H NMR ($CDCl_3$) δ 8.74 (d, J = 2.6 Hz, 1H), 8.44 (dd, J = 9.5, 2.5 Hz, 1H), 8.12 (s, 1H), 8.13 (d, J = 9.5 Hz, 1H), 2.90 (t, J = 7.7 Hz, 2H), 1.71–1.89 (m, 2H), 1.06 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 154.0, 146.6, 144.1, 136.7, 135.1, 128.3, 124.7, 122.0, 121.4, 33.6, 20.5, 12.0; MS (EI) m/z 252 (M^+ + 2), 250 (M^+, 100), 236, 220, 192, 176, 155. Anal. calcd for $C_{12}H_{11}ClN_2O_2$: C, 57.50; H, 4.42; N, 11.17. Found: C, 57.17; H, 4.57; N, 11.22.

2-Chloro-3-(3-chloropropyl)-6-nitroquinoline (5d). 99%, white solid, mp 137–138 °C; ^{1}H NMR ($CDCl_3$) δ 8.76 (d, J = 2.6 Hz, 1H), 8.46 (dd, J = 9.2, 2.2 Hz, 1H), 8.20 (s, 1H), 8.13 (d, J = 10.0 Hz, 1H), 3.62 (t, J = 6.3 Hz, 2H), 3.13 (t, J = 7.5 Hz, 2H), 2.17–2.31 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 153.6, 146.8, 144.2, 137.3, 133.4, 128.3, 124.6, 122.0, 121.7, 42.1, 29.7, 28.9; MS (EI) m/z 288 (M^+ + 4), 286 (M^+ + 2), 284 (M^+), 256, 221 (100), 175, 140. Anal. calcd for $C_{12}H_{10}Cl_2N_2O_2$: C, 50.55; H, 3.54; N, 9.82. Found: C, 49.98; H, 3.58; N, 9.67.

2-Chloro-3-(3-fluoropropyl)-6-nitroquinoline (5e). 80%, white solid, mp 117–119 °C; ^{1}H NMR ($CDCl_3$) δ 8.75 (d, J = 2.6 Hz, 1H), 8.46 (dd, J = 9.2, 2.6 Hz, 1H), 8.18 (s, 1H), 8.12 (d, J = 9.6 Hz, 1H), 4.55 (dt, J = 47.1, 5.7 Hz, 2H), 3.09 (t, J = 7.7 Hz, 2H), 2.03–2.29 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 153.6, 146.8, 144.2, 137.2, 133.8, 128.3, 124.7, 122,0, 121.7, 80.9 (d, J = 166 Hz), 27.9 (d, J = 24 Hz), 27.7; MS (EI) m/z 270 (M^+ + 2), 268 (M^+), 238, 221 (100), 191, 175, 155, 140. Anal. calcd for $C_{12}H_{10}ClFN_2O_2$: C, 53.65; H, 3.75; N, 10.43. Found: C, 53.29; H, 3.66; N, 10.43.

General procedure for the synthesis of compounds 6a–6e

The mixture of **5a** (609 mg, 2.74 mmol) and 1-piperazinecarboxaldehyde (1.00 mL, 8.21 mmol) in 30 mL of DMF was stirred for 3 h at 100 °C and then cooled to rt. The reaction was quenched by adding 100 mL of water. The resulting precipitate was filtered, washed with 50 mL of water and dried under suction for 1 h. The solid was dissolved in 4 M H_2SO_4, which was stirred for 3 h at 80 °C and then cooled to rt. After adding of 50 mL of water into mixture, aqueous 4 N NaOH was slowly added to the mixture until basic to litmus. The resulting precipitate was filtered, washed with 50 mL of water and dried under suction for 1 h to give **6a** (402 mg, 56%) as a yellow solid.

3-Methyl-6-nitro-2-(piperazin-1-yl)quinoline (6a). Mp 164–166 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (d, J = 1.8 Hz, 1H), 8.29 (dd, J = 9.2, 1.8 Hz, 1H), 7.87 (s, 1H), 7.83 (d, J = 10.2 Hz, 1H), 3.40–3.44 (m, 4H), 3.04–3.09 (m, 4H), 2.46 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 161.5, 147.3, 141.6, 137.6, 126.5, 125.0, 122.0, 121.5, 120.5, 48.8, 44.4, 18.0; MS (CI) m/z 273 (M^+ + 1), 257, 243 (100), 173. Anal. calcd for $C_{14}H_{16}N_4O_2$: C, 61.78; H, 5.93; N, 20.59. Found: C, 61.91; H, 6.26; N, 20.52.

3-Ethyl-6-nitro-2-(piperazin-1-yl)quinoline (6b). 66%, yellow solid, mp 130–131 °C; ^{1}H NMR ($CDCl_3$) δ 8.60 (d, J = 2.6 Hz, 1H), 8.30 (dd, J = 9.2, 2.2 Hz, 1H), 7.94 (s, 1H), 7.85 (d, J = 9.2 Hz, 1H), 3.35–3.40 (m, 4H), 3.04–3.09 (m, 4H), 2.80 (q, J = 7.2 Hz, 2H), 1.61 (br s, 1H), 1.37 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.6, 147.0, 141.6, 135.4, 131.3, 126.6, 122.4, 121.8, 120.5, 49.5, 44.4, 22.8, 12.3; MS (CI) m/z 287 (M^+ + 1), 271, 257 (100), 187. Anal. calcd for $C_{15}H_{18}N_4O_2$: C, 62.92; H, 6.34; N, 19.57. Found: C, 63.25; H, 6.08; N, 19.63.

3-Propyl-6-nitro-2-(piperazin-1-yl)quinoline (6c). 54%, yellow solid, mp 140–141 °C; ^{1}H NMR ($CDCl_3$) δ 8.58 (d, J = 2.4 Hz, 1H), 8.30 (dd, J = 9.1, 2.5 Hz, 1H), 7.92 (s, 1H), 7.84 (d, J = 9.2 Hz, 1H), 3.35–3.39 (m, 4H), 3.04–3.09 (m, 4H), 2.72 (t, J = 7.7 Hz, 2H), 1.72–1.83 (m, 2H), 1.67 (br s, 1H), 1.01 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.8, 147.1, 141.8, 136.0, 129.9, 126.7, 122.3, 121.8, 120.5, 49.6, 44.4, 32.1, 21.4, 12.4; MS (CI) m/z 301 (M^+ + 1), 285, 271 (100), 201. Anal. calcd for $C_{16}H_{20}N_4O_2$: C, 62.18; H, 6.14; N, 17.07. Found: C, 62.08; H, 6.41; N, 17.26.

3-(3-Chloropropyl)-6-nitro-2-(piperazin-1-yl)quinoline (6d). 42%, yellow solid, 132–133 °C; ^{1}H NMR ($CDCl_3$) δ 8.58 (d, J = 2.6 Hz, 1H), 8.30 (dd, J = 9.2, 2.6 Hz, 1H), 7.93 (s, 1H), 7.85 (d, J = 9.2 Hz, 1H), 3.59 (t, J = 6.3 Hz, 2H), 3.34–3.41 (m, 4H), 3.05–3.10 (m, 4H), 2.91–2.99 (m, 2H), 2.17–2.24 (m, 2H), 1.98 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 161.6, 147.2, 142.0, 136.6, 128.5, 126.9, 122.3, 121.8, 120.8, 49.6, 44.3, 42.7, 30.7, 27.6; MS (CI) m/z 337 (M^+ + 3), 335 (M^+ + 1), 285, 271, 257 (100). Anal. calcd for $C_{16}H_{19}ClN_4O_2$: C, 57.40; H, 5.72; N, 16.74. Found: C, 57.46; H, 6.08; N, 16.72.

3-(3-Fluropropyl)-6-nitro-2-(piperazin-1-yl)quinoline (6e). 53%, yellow solid, mp 136–138 °C; ^{1}H NMR ($CDCl_3$) δ

8.58 (d, $J=2.4$ Hz, 1H), 8.31 (dd, $J=9.2$, 2.6 Hz, 1H), 7.93 (s, 1H), 7.85 (d, $J=9.2$ Hz, 1H), 4.52 (dt, $J=47.4$, 5.7 Hz, 2H), 3.35–3.40 (m, 4H), 3.05–3.10 (m, 4H), 2.92 (t, $J=7.9$ Hz, 2H), 2.32 (br s, 1H), 2.13 (dm, $J=26.0$ Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 161.6, 147.2, 141.9, 136.4, 128.8, 126.8, 122.3, 121.8, 120.8, 81.5 (d, $J=165$ Hz), 49.5, 44.2, 28.8 (d, $J=20$ Hz), 26.1. HRMS (FAB) m/z $C_{16}H_{20}FN_4O_2$ (MH^+) calcd: 319.1578; found: 319.1574.

3 - (3 - *tert* - Butyldimethylsilyloxypropyl) - 3,4 - dihydro - 2(1*H*)-quinolinone (7). Under N_2 atmosphere, to a solution of hydrocarbostyril (3.0 g, 20.38 mmol) in 60 mL of sodium-dried THF was dropwise added 2.0 M LDA solution (22.42 mL, 44.85 mmol) at −78 °C. The mixture was stirred for 30 min at −78 °C and cooling bath was removed. The mixture was stirred for additional 1 h at rt, and then cooled to −78 °C. (3-Bromopropoxy)-1-*tert*-butyldimethylsilane (5.42 g, 21.40 mmol) was dropwise added to the mixture at −78 °C· which was stirred for 90 min at −78 °C to rt. The reaction was quenched by adding of 3 mL of MeOH and 500 mL of water in succession. The organic compounds were extracted three times with 100 mL of EtOAc. The combined extract was dried over Na_2SO_4 and concentrated with 5.0 g of silica gel in vacuo. The product was isolated by flash column chromatography with 20% EtOAc/hexane to give **7** (5.86 g, 90%) of as a white amorphous solid: ^{1}H NMR ($CDCl_3$) δ 9.00 (s, 1H), 7.19–7.12 (m, 2H), 6.95 (t, $J=7.3$ Hz, 1H), 6.81 (d, $J=7.2$ Hz, 1H), 3.63 (t, $J=6.2$ Hz, 2H), 3.05 (dd, $J=15.4$, 6.0 Hz, 1H), 2.75 (dd, $J=15.4$, 8.8 Hz, 1H), 2.63–2.52 (m, 1H), 1.94–1.81 (m, 1H), 1.74–1.46 (m, 3H), 0.86 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 172.6, 135.4, 126.5, 125.7, 121.6, 121.2, 113.4, 61.3, 38.2, 29.1, 28.6, 24.4, 24.3, 16.6, −7.0. HRMS (FAB) m/z $C_{18}H_{30}NO_2Si$ (MH^+) calcd: 320.2030; found: 320.2038.

3-(3-Hydroxypropyl)-3,4-dihydro-2(1*H*)-quinolinone (8). The mixture of **7** (3.31 g, 10.37 mmol) and tetrabutylammonium fluoride hydrate (4.07 g, 15.55 mmol) in 65 mL of THF was stirred for 12 h at rt and then washed with 100 mL of water twice, dried over Na_2SO_4, concentrated in vacuo. The product **8** (2.11 g, 99%) was obtained by flash column chromatography with EtOAc only as a white amorphous solid; ^{1}H NMR ($CDCl_3$) δ 8.49 (br s, 1H), 7.13–7.20 (m, 2H), 6.97 (t, $J=7.5$ Hz, 1H), 6.78 (d, $J=7.4$ Hz, 1H), 3.66 (t, $J=5.6$ Hz, 2H), 3.03 (dd, $J=15.4$, 5.8 Hz, 1H), 2.77 (dd, $J=15.4$, 9.6 Hz, 1H), 2.67–2.57 (m, 1H), 2.24 (br s, 1H), 1.95–1.58 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 172.8, 135.2, 126.5, 125.8, 121.6, 121.4, 113.5, 60.6, 38.0, 29.3, 28.3, 24.2. HRMS (FAB) m/z $C_{12}H_{16}NO_2$ (MH^+) calcd: 206.1181; found: 206.1182.

3-(3-Hydroxypropyl)-6-nitro-3,4-dihydro-2(1*H*)-quinolinone (9). To a solution of **8** (1.56 g, 7.59 mmol) in 30 mL of concd H_2SO_4 and 6 mL of water was dropwise added 61% HNO_3 (0.68 mL, 9.10 mmol) at 0 °C. After stirring for 12 h at rt, the reaction mixture was carefully poured into 500 mL of ice-water and neutralized with potassium carbonate to pH 3. Organic compounds were extracted from aqueous phase two times with 70 mL of CH_2Cl_2. The combined extract was dried over Na_2SO_4 and concentrated with 2.0 g of silica gel in vacuo. Flash column chromatography with 70% EtOAc/hexane gave **9** (1.032 g, 55%) as a white solid: mp 157–158 °C; ^{1}H NMR ($CDCl_3$) δ 8.36 (br s, 1H), 8.10 (br s, 2H), 6.86 (d, $J=9.0$ Hz, 1H), 3.65–3.74 (m, 2H), 3.16 (dd, $J=16.0$, 6.0 Hz, 1H), 2.89 (dd, $J=15.9$, 9.7 Hz, 1H), 2.71–2.63 (m, 1H), 2.04–1.61 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 171.6, 143.3, 140.5, 123.1, 122.7, 122.6, 113.8, 59.6, 37.5, 28.7, 28.5, 24.9. Anal. calcd for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.47; H, 5.77; N, 11.23.

3-(3-Acetoxypropyl)-6-nitro-3,4-dihydro-2(1*H*)-quinolinone (10). To a solution of **9** (362 mg, 1.47 mmol) in 7 mL of pyridine was dropwise added acetic anhydride (0.42 mL, 4.41 mmol) at rt. The mixture was stirred for 12 h at rt and poured into 50 mL of ice-water. 4 M H_2SO_4 was added to the mixture until acidic to litmus. The resulting precipitate was filtered, washed with water and dried under suction for 1 h to give **10** (362 mg, 84%) as a white solid: mp 143 °C; ^{1}H NMR ($CDCl_3$) δ 8.14–8.10 (m, 3H), 6.85 (d, $J=9.2$ Hz, 1H), 4.10 (t, $J=6.2$ Hz, 2H), 3.16 (dd, $J=15.8$, 5.8 Hz, 1H), 2.87 (dd, $J=15.9$, 9.7 Hz, 1H), 2.59–2.67 (m, 1H), 2.04 (s, 3H), 2.00–1.54 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 171.9, 169.4, 141.5, 140.8, 122.4, 122.4, 122.0, 113.5, 62.3, 37.4, 28.9, 24.5, 24.4, 19.3. HRMS (FAB) m/z $C_{14}H_{17}N_2O_5$ (MH^+) calcd: 293.1129; found: 293.1133.

2-Chloro-3-(3-acetoxypropyl)-6-nitroquinoline (11). The mixture of **10** (547 mg, 1.87 mmol), DDQ (433 mg, 1.87 mmol) and phosphorus oxychloride (0.27 mL, 2.81 mmol) in 10 mL of DMF was stirred for 3 h at rt and then poured into 100 mL of ice-water. The resulting precipitate was filtered, washed with water and dried under suction for 1 h. The solid was dissolved in 100 mL of EtOAc, concentrated with 1.0 g of silica gel in vacuo. The product **11** (525 mg, 91%) was obtained by flash column chromatography with 10% EtOAc/hexane as a white solid: mp 114 °C; ^{1}H NMR ($CDCl_3$) δ 8.75 (d, $J=2.6$ Hz, 1H), 8.46 (dd, $J=9.2$, 2.6 Hz, 1H), 8.17 (s, 1H), 8.12 (d, $J=9.0$ Hz, 1H), 4.19 (t, $J=6.4$ Hz, 2H), 3.01 (t, $J=7.9$ Hz, 2H), 2.04–2.15 (m, 2H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.3, 153.7, 146.7, 144.1, 137.0, 133.9, 128.3, 124.6, 122.0, 121.4, 61.6, 28.3, 26.2, 19.3. Anal. calcd for $C_{14}H_{13}ClN_2O_3$: C, 54.47; H, 4.24; N, 9.07. Found: C, 54.11; H, 4.09; N, 8.89.

3-(3-Acetoxypropyl)-6-nitro-2-(4-formylpiperazin-1-yl)-quinoline (12). The mixture of **11** (436 mg, 1.41 mmol) and 1-piperazinecarboxaldehyde (0.51 mL, 2.12 mmol) in 9 mL of DMF was stirred for 3 h at 100 °C and then cooled to rt, poured into 100 mL of ice-water. The resulting precipitate was filtered, washed with 50 mL of water and dried under suction for 1 h. The solid was dissolved in 100 mL of CH_2Cl_2, concentrated with 1.0 g of silica gel in vacuo. The product **12** (452 mg, 83%) was obtained by flash column chromatography with 3% MeOH/CH_2Cl_2 as a yellow solid: mp 142 °C; ^{1}H NMR ($CDCl_3$) δ 8.58 (d, $J=2.6$ Hz, 1H), 8.29 (dd, $J=9.2$, 2.6 Hz, 1H), 8.13 (s, 1H), 7.99 (s, 1H), 7.85 (d, $J=9.2$ Hz, 1H), 4.13 (t, $J=6.4$ Hz, 2H), 3.72–3.74 (m, 2H), 3.57–3.62 (m, 2H), 3.40–3.47 (m, 2H), 3.30–3.35 (m, 2H), 2.85 (t, $J=7.9$ Hz, 2H), 1.99–2.16 (m, 2H), 2.03 (s,

3H); ^{13}C NMR ($CDCl_3$) δ 169.2, 161.0, 159.3, 146.8, 142.3, 136.7, 128.7, 127.1, 122.7, 121.8, 120.9, 62.0, 48.7, 48.1, 43.8, 38.3, 27.0, 26.5, 19.2. HRMS (FAB) *m*/*z* $C_{15}H_{13}N_2O_3$ (MH^+) calcd: 387.1656; found: 387.1662.

3-(3-Hydroxypropyl)-6-nitro-2-(4-formylpiperazin-1-yl)-quinoline (13). The mixture of **12** (380 mg, 0.98 mmol) and K_2CO_3 (408 mg, 2.95 mmol) in 8 mL of MeOH and 8 mL of CH_2Cl_2 was stirred for 15 h at rt. The reaction was quenched by adding 100 mL of water. Organic compounds were extracted from aqueous phase three times with 30 mL of CH_2Cl_2. The combined extract was dried over Na_2SO_4 and concentrated with 0.5 g of silica gel in vacuo. The product **13** (242 mg, 72%) was obtained by flash column chromatography with 3% MeOH/CH_2Cl_2 as yellow solid: mp 131–132 °C; ^{1}H NMR ($CDCl_3$) δ 8.59 (d, J = 2.4 Hz, 1H), 8.30 (dd, J = 9.2, 2.6 Hz, 1H), 8.12 (s, 1H), 8.01 (s, 1H), 7.87 (d, J = 9.2 Hz, 1H), 3.78–3.56 (m, 6H), 3.41–3.48 (m, 2H), 3.32–3.37 (m, 2H), 2.91 (t, J = 7.7 Hz, 2H), 2.25 (br s, 1H), 1.92–2.06 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 161.1, 159.4, 146.7, 142.2, 136.8, 129.6, 127.1, 122.8, 121.8, 120.7, 59.8, 48.8, 48.1, 43.8, 38.3, 31.0, 26.0. HRMS (FAB) *m*/*z* $C_{17}H_{21}N_4O_4$ (MH^+) calcd: 345.1562; found: 345.1555.

3-(3-Methanesulfonyloxypropyl)-6-nitro-2-(4-formylpiperazin-1-yl)quinoline (14). To a solution of 13 (206 mg, 0.60 mmol) and triethylamine (0.13 mL, 0.90 mmol) in 5 mL of CH_2Cl_2 was added dropwise methane sulfonyl chloride (0.05 mL, 0.66 mmol) at 0 °C. After stirring for 1 h at 0 °C, the mixture was washed with 10 mL of water twice and dried over Na_2SO_4. The product **14** (238 mg, 94%) was obtained by short column chromatography with 3% MeOH/CH_2Cl_2 as a yellow solid: mp 145–151 °C; ^{1}H NMR ($CDCl_3$) δ 8.63 (d, J = 2.6 Hz, 1H), 8.36 (dd, J = 9.6, 2.6 Hz, 1H), 8.14 (s, 1H), 8.01 (2, 1H), 7.90 (d, J = 9.0 Hz, 1H), 4.28 (t, J = 6.0 Hz, 2H), 3.75–3.80 (m, 2H), 3.59–3.64 (m, 2H), 3.45–3.32 (m, 4H), 3.02 (s, 3H), 2.97 (t, J = 8.0 Hz, 2H), 2.18–2.26 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 161.0, 159.3, 146.9, 142.4, 136.8, 128.1, 127.2, 122.6, 121.9, 121.1, 67.3, 48.8, 48.1, 43.7, 38.2, 35.9, 27.6, 26.0. HRMS (FAB) *m*/*z* $C_{18}H_{23}N_4O_6S$ (MH^+) calcd: 423.1338; found: 423.1340.

3-(3-Fluoropropyl)-6-nitro-2-(piperazin-1-yl)quinoline (6e). The mixture of **14** (80 mg, 0.19 mmol) and tetrabutylammonium fluoride hydrate (59 mg, 0.23 mmol) in 2 mL of acetonitrile was heated under reflux for 1 h and then cooled to rt. The reaction was quenched by adding 100 mL of water. The resulting precipitate was filtered, washed with 50 mL of water and dried under suction for 1 h. The solid was dissolved in 4 M H_2SO_4, which was stirred for 3 h at 80 °C and then cooled to rt. After adding of 50 mL of water to the mixture, aqueous 4 N NaOH was slowly added to the mixture until basic to litmus. The resulting precipitate was filtered, washed with 50 mL of water and dried under suction for 1 h to give product **6e** (402 mg, 42%) as a yellow solid.

3-(3-[^{18}F]Fluoropropyl)-6-nitro-2-(piperazin-1-yl)quinoline ([^{18}F]6e). [^{18}F]Fluoride ion (1.91 GBq) produced on a medical cyclotron was transferred to a Vacutainer® containing tetra-*n*-butylammonium hydroxide (40% aq., 2.6 μL, 4.0 μmol). Three azeotropic distillations were carried out each time with 100–200 μL of CH_3CN at 90 °C (oil bath) under a gentle stream of N_2. The radioactivity was resolublized and transferred to a reaction vial containing a magnetic stirrer and **14** (2.0 mg, 5.0 μmol) using 2×100 μL of CH_3CN. The mixture was stirred at 130 °C for 20 min and then cooled. The solvent was removed under a gentle stream of N_2 at 90 °C (water bath). To the residue was added H_2SO_4 (4 M, 0.3 mL), and the reaction mixture was stirred at 130 °C for 10 min. The reaction was quenched by adding 0.1 mL of water to the mixture, prior to the addition of NaOH (4 N) until basic to litmus. The organic compounds were extracted from aqueous phase with CH_2Cl_2 (1.0 mL×3). The combined extracts (396 MBq; not decay-corrected) was dried over sodium sulfate and concentrated (N_2 flow). The residue was redissolved in 1 mL of CH_2Cl_2 and injected onto a semipreparative HPLC column (Alltech Econosil silica gel, 10 μ, 10×250 mm), which was eluted with a 95:5 mixture of CH_2Cl_2 and MeOH (1% NH_4OH) at a flow 4 mL/min. HPLC purification of [^{18}F]**6e** (t_R = 17–19 min) and removal of the solvents gave the desired compound (241 MBq) in 17.3% radiochemical yield (decay-corrected). Authenticity of [^{18}F]FPNQ was confirmed by coinjection with unlabeled standard FPNQ on HPLC. The overall yield was 14–25%, based on the initial radioactivity, and total time including HPLC purification was 120 min.

In vitro serotonin transporter bindings studies

Binding of [^{3}H]citalopram to the 5-HT transporter was measured according to the method of Pähkla et al.[32] with slight modifications using crude synaptic membranes prepared from the rat cerebral cortex.[33] Saturation binding assays were performed to measure the equilibrium dissociation constant (K_d) and the density of binding sites (B_{max}) using 15 concentrations of [^{3}H]citalopram between 0.01 and 30 nM. Nonspecific binding was defined as that determined in the presence of 10 μM fluoxetine. Scatchard analysis of the saturation binding data indicated a single population of binding sites with a K_d of 1.12±0.01 nM and a B_{max} of 704±47 fmol/mg protein (n = 3). Competition binding assays were performed to measure the concentrations of test compounds which inhibited the specific binding by 50% (IC_{50} values) using 1 nM [^{3}H]citalopram and 11 concentrations of the unlabeled compounds between 10^{-11} and 10^{-5} M. IC_{50} values were determined from the competition binding data using computer-assisted curve fitting with the GraphPad Prism 3.0 program. Inhibition binding constant (K_i) values were subsequently calculated from IC_{50} values using the Cheng–Prusoff equation.[34]

Specific activity and in vivo tissue distribution studies of [^{18}F]6e

The radioligand collected from HPLC was concentrated, redissolved in ethanol, and diluted with saline to give a final solution of 10% ethanol-saline. The specific activity of [^{18}F]**6e** was determined by radioreceptor assay as described by Fowler et al.[35] In brief, aliquots of 200 μL

solution of [^{18}F]**6e** were assayed for radioactivity with a NaI(TI) well counter. After allowing the F-18 to decay, successive dilutions of this solution were made in incubation buffer just before aliquoting for determination of the concentration of **6e** in each dilution. The competitive binding of each dilution of the [^{18}F]**6e** solution with 1 nM [^{3}H]citalopram was determined in duplicate and compared with that of a standard competitive binding curve made up of a series of concentrations of non-radioactive **6e**. Only those dilutions producing 20–80% inhibition of the specific binding were used for calculation, since they represent the most accurate values. The concentration of **6e** in each of those dilutions was determined from the standard competitive binding curve using computer-assisted curve fitting with the GraphPad Prism 3.0 program. Together with the original radioactivity determinations of the batch preparation, the average of three separate determinations was used to determine the specific activity of the batch. Male ICR mice (25–30 g, four mice per time points) were injected with the radioligand (1.5–1.9 MBq) in 0.2 mL of 10% ethanol–saline via tail vein. At the indicated time points (5, 15, 30, 60, 90 and 120 min), the mice were sacrificed by cervical dislocation, and samples of bone and brain tissue (striatum, frontal cortex, hypothalamus, hippocampus, olfactory tubercle, cerebellum, and the rest of the brain) were removed, weighed, and counted. The data were expressed as percent injected dose per gram of tissue (%ID/g).

In vivo blocking studies

The mice ($n=4$) were injected with the radioligand [^{18}F]**6e** alone or with the following compounds: paroxetine (2 mg/kg), fluoxetine (5 mg/kg), GBR-12909 (RBI, 2 mg/kg) or unlabeled **6e** (2 mg/kg). These compounds were dissolved in saline prior to the injection, except the unlabeled **6e** that was dissolved in 2% HCl (0.1 N)-saline due to the insolubility in saline alone. The mouse brain tissues were dissected at 60 min postinjection as previously described. The data were expressed as percent injected dose per gram of tissue (%ID/g).

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