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Syntheses and binding affinities of 6-nitroquipazine analogues for serotonin transporter. Part 4: 3-Alkyl-4-halo-6-nitroquipazines☆

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Abstract—On the basis of the structure–activity relationship (SAR) of 4-chloro-6-nitroquipazine (K_i = 0.03 nM) and 3-fluoropropyl-6-nitroquipazine (K_i = 0.32 nM), 3-alkyl-4-halo-6-nitroquipazines were synthesized and tested for their potential abilities in vitro to displace [^{3}H]citalopram binding to the rat cortical membranes. Binding affinities of **3b** and **4d** were $K_i = 2.70 \pm 0.32$ and 2.23 ± 0.46 nM, respectively. The syntheses of 3-alkyl-4-halo-6-nitroquipazine, their in vitro binding affinities, and the SAR of C3, C4 position in 6-nitroquipazine are described.

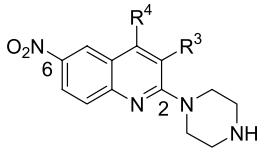
1. Introduction

The inhibition of the reuptake of serotonin (5-hydroxytryptamine or 5-HT) results in the increase of 5-HT concentration in the neuronal synaptic cleft, affording to the enhancement of signal transduction by 5-HT. For this reason, a class of compounds called serotonin-specific reuptake inhibitors (SSRIs) have been researched for the treatment of serotonin-related psychiatric diseases such as schizophrenia, anxiety, neurodegenaratives, emesis, and depression.[2,3] As the 5-HT concentration in the brain is highly related to depression, the selective inhibitors toward serotonin transporter (SERT) mainly show the therapeutic effect for depression. Among the many SERT inhibitors, it was reported that 6-nitroquipazine (6-NQ) has a subnanomolar binding affinity (K_i = 0.17 nM) toward SERT.[4,5]

Recently, our continued interest in 6-NQ derivatives led to the synthesis of 4-chloro-6-NQ (K_i = 0.03 nM),[4] which is more potent than 6-NQ itself, and 3-fluoropropyl-6-NQ (3-FPNQ, K_i = 0.32 nM),[6] which was labeled by F-18 radioisotope with short half-life ($t_{1/2}$ = 110 min) for positron emission tomography (PET) (Fig. 1). The biodistribution of 3-[^{18}F]FPNQ was investigated using four mice each time (5, 15, 30, 60, 90, 120 min) postadministration. 3-[^{18}F]FPNQ was localized in frontal cortex, hippocampus, hypothalamus, and olfactory tissues of murine brain at higher concentration than the rest of the brain, showing results similar to that of other SSRIs. Herein, we report the synthesis and binding affinities of the compounds with both substituents, chlorine and bromine, at the C3 position and with alkyl group at the C4 position.

2. Chemistry

Compounds **4a**–**f** were synthesized by the reaction of 4-nitroaniline and excess diethyl alkylmalonates (more than 10 equiv) without other solvent at 180 °C for



1, R^3 = H, R^4 = H Ki = 0.17 nM
2, R^3 = H, R^4 = Cl Ki = 0.03 nM
3, R^3 = $CH_2CH_2CH_2F$, R^4 = H Ki = 0.32 nM

Figure 1. Three compounds that have good binding affinities toward serotonin transporter.

Keywords: 6-Nitroquipazine; Serotonin transporter; 3-Alkyl-4-halo-6-nitroquipazine; Structure–activity relationship.

☆ For Part 3, see Ref. 1.

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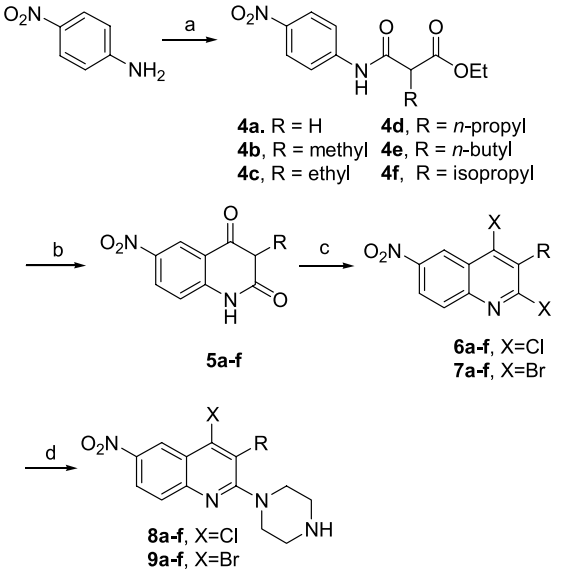
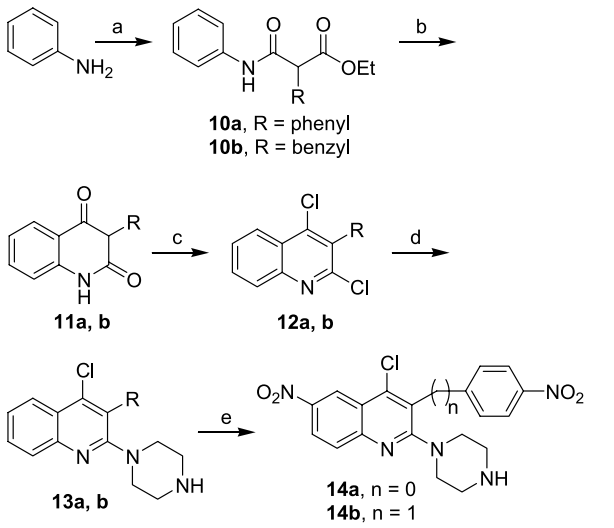
16 h, followed by the removal of excess residue of diethyl alkylmalonates by vacuum distillation. The ring formations of **4a**–**f** were carried out in polyphosporic acid (PPA) at 120 °C for 2.5 h to give **5a**–**f** in moderate yield. The reaction of **5a**–**f** and phosphorus oxychloride gave corresponding dichloro compounds **6a**–**f** on heating at 80 °C for 2.5 h. Dibromo compounds **7a**–**f** were obtained by bromination of **5a**–**f** with phosphorus oxybromide in dioxane under reflux. 3-Alkyl-4-chloro-6-NQs, **8a**–**f**, and 3-alkyl-4-bromo-6-NQs, **9a**–**f**, were synthesized by the replacement of the halogen atom at the C2 position with piperazine.

When the R group of compound **4** was either phenyl or benzyl, compound **5** could not be synthesized. Compounds **4** (R = phenyl and benzyl) were returned to 4-nitroaniline by hydrolysis of the amide bond under the same conditions as in step b of Scheme 1. Thus, 3-phenyl- and 3-benzyl-4-chloro-6-NQs **14a** and **b** were prepared by another synthetic route shown in Scheme 2.

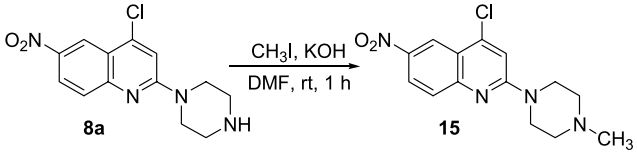
The condensation of aniline and excess diethyl phenyl- and diethyl benzylmalonate provided compounds **10a** and **b**, respectively, in the presence of 1.5 equiv of pyridine under refluxing toluene for 18 h. The ring-closed compounds **11a** and **b** were obtained by intramolecular Friedel–Crafts acylation-type reaction in the presence of aluminum chloride in chlorobenzene solvent. The same reaction in other solvents such as toluene, nitrobenzene, did not give the desired compounds **11a** and **b**. Compounds **13a** and **b** were prepared by the reaction of **11a** and **b** and phosphorous oxychloride followed by the introduction of piperazine at the C2 position on **12a** and **b** using 1-piperazine carboxaldehyde followed by deprotection of *N*-formyl moiety under acidic conditions without purification. Compounds **14a** and **b** gave nitro compounds **14a** and **b** preferably under



Scheme 1. Reagents and conditions: (a) diethyl alkylmalonates, 180 °C, 16 h, 57–72%; (b) PPA, 120 °C, 2.5 h, 62–69%; (c) $POCl_3$, 80 °C, 2.5 h, 35–62% (X = Cl); $POBr_3$, 1,4-dioxane, 100 °C, 3 h, 32–48% (X = Br); (d) piperazine, DMF, 0 °C, 1 h, 94–97% (X = Cl); 89–97% (X = Br).



Scheme 2. Reagents and conditions: (a) diethyl phenylmalonate (for **10a**), diethyl benzylmalonate (for **10b**), pyridine, toluene, reflux, 18 h, 47 and 52%, respectively; (b) $AlCl_3$, chlorobenzene, 120 °C, 2 h, 90%, 97%; (c) $POCl_3$, 80 °C, 2.5 h, 59%, 65%; (d) (i) 1-piperazinecarboxaldehyde, 120 °C, 4 h, (ii) 4 M H_2SO_4, THF, 50 °C, 1 h, 65%, 61%; (e) conc. HNO_3, conc. H_2SO_4, 0 °C, 30 min, 68%, 72%.



Scheme 3.

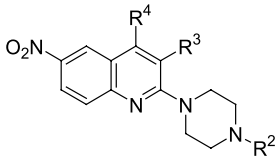
normal nitration condition. As nitration was preformed in the last step, the phenyl ring on C3 position was also nitrated in the para position.

Furthermore, N-methylation of **8a** using iodomethane gives **15**, which might be a potential C-11 ($t_{1/2}$ = 20 min) labeled PET imaging agent as shown in Scheme 3.

3. Binding studies

According to the method of our previous study[4,6] using crude synaptic membranes prepared from the cerebral cortex of male Sprague–Dawley rats.[7] Competition binding assays were performed to measure the concentrations of test compounds that 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. Nonspecific binding was defined as that determined in the presence of 10 μM fluoxetine. IC_{50} values were determined from the competition binding data using computer-assisted curve fitting with GraphPad Prism 3.0 program. Inhibition binding constant (K_i) values were subsequently calculated from IC_{50} values using the Cheng–Prusoff equation.[8] Table 1 illustrates the structures and the in vitro binding affinities of 15 4-substituted derivatives of 6-NQ for the 5-HT transporter, including 6-NQ, fluoxetine, and paroxetine used as reference compounds.

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



Compound	R^4	R^3	R^2	K_i (nM)
3a	Cl	H	H	0.03 ± 0.01
3b	Cl	Methyl	H	2.70 ± 0.32
3c	Cl	Ethyl	H	5.56 ± 0.54
3d	Cl	*n*-Propyl	H	3.97 ± 0.53
3e	Cl	*n*-Butyl	H	42.88 ± 6.98
3f	Cl	*i*-Propyl	H	321.24 ± 5.56
4a	Br	H	H	0.37 ± 0.03
4b	Br	Methyl	H	3.21 ± 0.03
4c	Br	Ethyl	H	5.85 ± 0.32
4d	Br	*n*-Propyl	H	2.23 ± 0.46
4e	Br	*n*-Butyl	H	35.72 ± 1.87
4f	Br	*i*-Propyl	H	485.73 ± 34.07
14a	Cl	4-Nitro phenyl	H	685.07
14b	Cl	4-Nitro benzyl	H	330.86
15	Cl	H	CH_3	17.28 ± 2.33
1	H	H	H	0.17 ± 0.03
Fluoxetine				22.13 ± 1.77
Paroxetine				0.53 ± 0.08

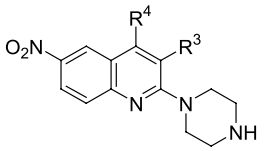
[a] The inhibition of [^{3}H]citalopram binding was determined at 1 nM [^{3}H]citalopram for the various compounds listed. Binding data are the means of three independent experiments. Eleven concentrations of displacer were used for each determination. The K_d value of [^{3}H]citalopram, measured by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

4. Discussion

Fifteen 6-NQ derivatives were synthesized via 3-substituted quinoline-2,4-diones **5a**–**f** and **11a**–**b** from 4-nitroaniline or aniline. Table 1 shows the binding affinities of these 15 6-NQ derivatives toward SERT measured by the replacing ability of [^{3}H]citalopram. Regardless of whether it was 4-chloro or 4-bromo in 6-NQ, the bulkier substituents at the C3 position showed relatively lower binding affinities. As reported in a previous study, 4-chloro-6-NQ is 12 times more potent than 4-bromo-6-NQ. It is thought that the size of the halogen atom influences the nonpolar interaction between quinoline ring and the nonpolar residue of SERT. Bringing the two counterparts as close as possible will enhance the interaction. Therefore, the chlorine atom in 6-NQ must be situated between the two groups. When located at an appropriate distance between the two groups, the chlorine atom induces additional interaction. The bromide atom seems to play a role in weakening the attraction by steric interaction with the nonpolar residue of SERT. It is thought that the size of derivatives at the C4 position influences the binding affinity (see Table 2).

The C3 position of 6-NQ appears to have a space that is associated with the nonpolar residue of SERT, because the binding affinity showed moderate decrease from **3a** (H) to **3d** (*n*-propyl) at C3 position of quinoline ring. Although 4-bromo-6-NQ (**4a**) did not have good activity compared to 4-chloro-6-NQ (**3a**), the binding affinities of 4-chloro- and 4-bromo-6-NQs with increasing alkyl chains at the C3 position were similar for each analogue. It is thought that only 6-NQ derivatives with halides have an influence on property related to the size of the halide. It might have narrow tolerance and change position on C4 position at the binding site of the 5-HT reuptake site. But, when 6-NQ with halogen adds to alkyl chain in the C3 position it seems to compensate for the activity that would be associated with the correct binding position of the alkyl chain.

Table 2. Trends of binding affinities (K_i) for 5-HT transporter by increasing number of carbons on C3 position with halo derivatives on C4 position



R^3	R^4(nM)		
	H	Cl	Br
H	0.17	0.03	0.37
Methyl	8.45	2.70	3.21
Ethyl	0.36	5.56	5.85
n-Propyl	0.26	3.97	2.23
n-Butyl	0.55	42.88	35.72
i-Propyl	ND	321.24	485.73

In summary, the substitutent alkyl chain derivatives with halide at the C4 position show increased binding affinity and help to correct binding at 5-HT reuptake site. On the basis of this result, alkyl chains provide a steric tolerance at the C3 position against 5-HT reuptake site.

5. Experimental

5.1. 2-Methyl-*N*-(4-nitrophenyl)malonamic acid ethyl ester (4b)

The mixture of 4-nitroaniline (4.0 g, 29.0 mmol) and diethyl methylmalonate (15 mL) was stirred at 180 °C for 18 h. After the reaction mixture was cooled, excess malonate was removed by vacuum distillation. The residue was diluted with 100 mL of water and extracted with ethyl acetate (100 mL × 3). The combined organic layer was dried (Na_2SO_4). The product **4b** was obtained as a yellow solid (5.5 g, 71%) by flash column chromatography (50% CH_2Cl_2/hexane): ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.34 (br s, NH), 8.18 (d, J = 9.2 Hz, 2H), 7.79 (d, J = 9.2 Hz, 2H), 4.26 (q, J = 7.2 Hz, 2H), 3.54 (q, J = 7.3 Hz, 1H), 1.57 (d, J = 7.4 Hz, 3H), 1.32 (t, J = 7.2 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 172.2, 167.7, 143.5, 124.9, 119.3, 62.0, 47.4, 15.0, 13.9; MS (ESI) 267 (MH^+). HRMS (EI): Calcd for $C_{12}H_{14}N_2O_5$ (M^+) 266.0903. Found: 266.0906.

5.2. 2-Ethyl-*N*-(4-nitrophenyl)malonamic acid ethyl ester (4c)

This compound was prepared by the same method as for **4b**. **4c**: 67%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$)

δ: 9.40 (br s, NH), 8.20 (d, J = 9.0 Hz, 2H), 7.77 (d, J = 9.0 Hz, 2H), 4.22–4.34 (m, 2H), 3.36 (t, J = 7.1 Hz, 1H), 2.08 (qt, 2H), 1.33 (t, J = 7.1 Hz, 3H), 1.03 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 172.7, 167.1, 143.4, 125.0, 119.4, 62.0, 54.7, 25.3, 14.0, 11.6.; MS (ESI) m/z: 281 (MH^+). HRMS (EI): Calcd for $C_{13}H_{16}N_2O_5$ (M^+) 280.1059. Found: 280.1058.

5.3. 2-Propyl-*N*-(4-nitrophenyl)malonamic acid ethyl ester (4d)

This compound **4d** was prepared by the same method as for **4b**. **4d**: 64%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.36 (br s, NH), 8.10 (d, J = 7.4 Hz, 2H), 7.72 (d, J = 7.4 Hz, 2H), 4.12–4.24 (m, 2H), 3.40 (t, J = 7.4 Hz, 1H), 1.87–2.00 (m, 2H), 1.20–1.40 (m, 5H), 0.88 (t, J = 7.4 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 171.9, 167.4, 143.5, 124.8, 119.3, 61.8, 53.3, 33.1, 20.3, 13.9, 13.4; MS (ESI) m/z: 295 (MH^+). HRMS (CI): Calcd for $C_{14}H_{19}N_2O_5$ (MH^+) 295.1294. Found: 295.1303.

5.4. 2-*n*-Butyl-*N*-(4-nitrophenyl) malonamic acid ethyl ester (4e)

This compound was prepared by the same method as for **4b**. **4e**: 69%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.36 (br s, NH), 8.20 (d, J = 9.2 Hz, 2H), 7.78 (d, J = 9.2 Hz, 2H), 4.22–4.32 (m, 2H), 3.42 (t, J = 7.3 Hz, 1H), 2.00–2.01 (m, 2H), 1.28–1.37 (m, 7H), 0.87–0.94 (m, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 172.4, 167.3, 143.5, 124.9, 119.3, 61.9, 53.5, 31.2, 29.2, 22.1, 14.0, 13.6; MS (ESI) m/z: 309 (MH^+). HRMS (EI): Calcd for $C_{15}H_{20}N_2O_5$ (M^+) 308.1372. Found: 308.1363.

5.5. 2-Isopropyl-*N*-(4-nitrophenyl) malonamic acid ethyl ester (4f)

This compound was prepared by the same method as for **4b**. **4f**: 61%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.40 (br s, NH), 8.21 (d, J = 9.2 Hz, 2H), 7.79 (d, J = 9.2 Hz, 2H), 4.20–4.36 (m, 2H), 3.18 (d, J = 9.2 Hz, 1H), 2.36–2.47 (m, 1H), 1.33 (t, J = 7.1 Hz, 3H), 1.07 (t, J = 6.7 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 172.2, 166.6, 143.4, 124.8, 119.3, 61.8, 60.9, 32.1, 20.3, 13.9; MS (ESI) m/z: 295 (MH^+). HRMS (CI): Calcd for $C_{14}H_{19}N_2O_5$ (MH^+) 295.1294. Found: 295.1295.

5.6. 3-Methyl-6-nitro-1*H*-quinoline-2,4-dione (5b)

2-Methyl-*N*-(4-nitrophenyl)malonamic acid ethyl ester (**4b**, 10.0 g, 39.6 mmol) was added to 30 g of polyphosphoric acid and stirred for 2.5 h at 120 °C. The reaction mixture was poured in ice water and the precipitate was filtered and then washed with 100 mL of ethyl acetate. The product **5b** was obtained as a white solid (5.5 g, 65%) by flash column chromatography (80% EtOAc/hexane) or crystallization with methanol: ^{1}H NMR (200 MHz, DMSO-d_6) δ: 11.95 (br s, NH), 8.72 (d, J = 2.6 Hz, 1H), 8.29 (dd, J = 9.2 and 2.6 Hz, 1H), 7.39 (d, J = 9.0 Hz, 1H), 2.02 (s, 3H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 162.2, 154.8, 139.7, 139.3, 122.6, 117.3, 114.0, 113.4, 107.0, 7.5; MS (ESI) m/z: 221 (MH^+). HRMS (EI): Calcd for $C_{10}H_8N_2O_4$ (M^+) 220.0484. Found: 220.0487.

5.7. 3-Ethyl-6-nitro-1*H*-quinoline-2,4-dione (5c)

This compound was prepared by the same method as for **5b**. **5c**: 66%, white solid; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 11.86 (br s, NH), 10.68 (br s, OH), 8.68 (d, J = 2.6 Hz, 1H), 8.23 (dd, J = 9.0 and 2.6 Hz, 1H), 7.34 (d, J = 9.2 Hz, 1H), 2.55 (q, J = 7.3 Hz, 2H), 0.98 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 161.7, 154.3, 139.9, 139.3, 122.7, 117.5, 114.0, 113.5, 113.2, 14.6, 11.1; MS (ESI) m/z: 235 (MH^+). HRMS (EI): Calcd for $C_{11}H_{10}N_2O_4$ (M^+) 234.0641. Found: 234.0651.

5.8. 3-Propyl-6-nitro-1*H*-quinoline-2,4-dione (5d)

This compound was prepared by the same method as for **5b**. **5d**: 62%, white solid; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 11.83 (br s, NH), 8.66 (d, J = 2.6 Hz, 1H), 8.21 (dd, J = 8.8 and 2.6 Hz, 1H), 7.31 (d, J = 8.8 Hz, 1H), 3.49 (br s, OH), 2.49 (q, 2H), 1.38 (p, 2H), 0.87 (t, J = 7.4 Hz, 3H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 161.7, 154.7, 139.9, 139.3, 122.6, 117.5, 113.9, 113.4, 111.6, 23.2, 19.4, 12.1; MS (ESI) m/z: 249 (MH^+). HRMS (EI): Calcd for $C_{12}H_{12}N_2O_4$ (M^+) 248.0797. Found: 248.0804.

5.9. 3-*n*-Butyl-6-nitro-1*H*-quinoline-2,4-dione (5e)

This compound was prepared by the same method as for **5b**. **5e**: 69%, white solid; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 11.80 (br s, NH), 10.52 (br s, OH), 8.65 (d, J = 2.6 Hz, 1H), 8.18 (dd, J = 8.8 and 2.6 Hz, 1H), 7.30 (d, J = 8.6 Hz, 1H), 2.48–2.55 (m, 2H), 1.31–1.33 (m, 4H), 0.84 (t, J = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 161.9, 154.5, 139.9, 139.3, 122.6, 117.4, 113.9, 113.4, 111.9, 28.3, 21.0, 20.4, 12.1; MS (ESI) m/z: 263 (MH^+). HRMS (EI): Calcd for $C_{13}H_{14}N_2O_4$ (M^+) 262.0954. Found: 262.0959.

5.10. 3-Isopropyl-6-nitro-1*H*-quinoline-2,4-dione (5f)

This compound was prepared by the same method as for **5b**. **5f**: 61%, white solid; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 11.74 (br s, NH), 8.75 (d, J = 2.6 Hz, 1H), 8.20 (dd, J = 9.2 and 2.6 Hz, 1H), 7.31 (d, J = 8.8 Hz, 1H), 3.34–3.41 (m, 1H), 1.23 (d, J = 7.0 Hz, 6H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 161.4, 154.2, 140.0, 139.3, 122.7, 117.8, 117.0, 113.8, 113.6, 22.6, 18.0; MS (ESI) m/z: 249 (MH^+). HRMS (EI): Calcd for $C_{12}H_{12}N_2O_4$ (M^+) 248.0797. Found: 248.0785.

5.11. 2,4-Dichloro-3-methyl-6-nitroquinoline (6b)

3-Methyl-6-nitro-1*H*-quinoline-2,4-dione (**5b**, 3.0 g, 13.9 mmol) was added to 20 mL of phosphorus oxychloride and stirred for 2.5 h at 80 °C. The mixture was cooled and evaporated. The product **6b** was obtained as a white solid (1.0 g, 45%) by flash column chromatography (10% EtOAc/hexane): ^{1}H NMR (200 MHz,

$CDCl_3$) δ: 9.09 (d, J = 2.2 Hz, 1H), 8.48 (dd, J = 9.2 and 2.2 Hz, 1H), 8.12 (d, J = 9.2 Hz, 1H), 2.73 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 155.5, 150.1, 147.9, 144.0, 135.7, 130.9, 130.6, 125.2, 123.8, 121.3, 18.0; MS (ESI) m/z: 257 (MH^+). HRMS (CI): Calcd for $C_{10}H_7Cl_2N_2O_2$ (MH^+) 256.9885. Found: 256.9884.

5.12. 2,4-Dichloro-3-ethyl-6-nitroquinoline (6c)

This compound was prepared by the same method as for **6b**. **6c**: 39%, white solid; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.09 (d, J = 2.6 Hz, 1H), 8.48 (dd, J = 9.2 and 2.6 Hz, 1H), 8.11 (d, J = 9.2 Hz, 1H), 3.18 (q, J = 7.5 Hz, 2H), 1.33 (t, J = 7.5 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 155.1, 148.0, 146.4, 143.8, 136.0, 123.8, 121.3, 25.3, 12.1; MS (ESI) m/z: 271 (MH^+). HRMS (CI): Calcd for $C_{11}H_9Cl_2N_2O_2$ (MH^+) 271.0041. Found: 271.0035.

5.13. 2,4-Dichloro-3-propyl-6-nitroquinoline (6d)

This compound was prepared by the same method as for **6b**. **6d**: 49%, white solid; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.13 (d, J = 2.6 Hz, 1H), 8.49 (dd, J = 9.2 and 2.6 Hz, 1H), 8.12 (d, J = 9.2 Hz, 1H), 3.08–3.17 (m, 2H), 1.69–1.80 (m, 2H), 1.11 (t, J = 7.5 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 153.0, 145.6, 143.9, 141.78, 132.4, 128.2, 122.9, 121.5, 119.1, 31.3, 11.1, 11.7; MS (ESI) m/z: 285 (MH^+). HRMS (CI): Calcd for $C_{12}H_{11}Cl_2N_2O_2$ (MH^+) 285.0198. Found: 285.0204.

5.14. 2,4-Dichloro-3-*n*-butyl-6-nitroquinoline (6e)

This compound was prepared by the same method as for **6b**. **6e**: 43%, white solid; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.12 (d, J = 2.6 Hz, 1H), 8.48 (dd, J = 9.2 and 2.2 Hz, 1H), 8.62 (d, J = 9.2 Hz, 1H), 3.10–3.18 (m, 2H), 1.48–1.72 (m, 4H), 1.02 (t, J = 7.1 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 155.4, 148.0, 146.5, 144.0, 135.2, 130.6, 125.4, 123.8, 121.4, 31.6, 30.1, 22.8, 13.7; MS (ESI) m/z: 299 (MH^+). HRMS (CI): Calcd for $C_{13}H_{13}Cl_2N_2O_2$ (MH^+) 299.0354. Found: 299.0352.

5.15. 2,4-Dichloro-3-isopropyl-6-nitroquinoline (6f)

This compound was prepared by the same method as for **6b**. **6f**: 43%, white solid; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.18 (d, J = 2.2 Hz, 1H), 8.49 (dd, J = 9.2 and 2.6 Hz, 1H), 8.11 (d, J = 9.2 Hz, 1H), 4.05–4.19 (m, 1H), 1.55 (d, J = 7.0 Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 147.7, 146.5, 144.1, 138.5, 130.5, 125.7, 123.9, 121.6, 118.1, 31.5, 19.3; MS (ESI) m/z: 285 (MH^+); HRMS (CI): Calcd for $C_{12}H_{11}Cl_2N_2O_2$ (MH^+) 285.0198. Found: 285.0191.

5.16. 2,4-Dibromo-3-methyl-6-nitroquinoline (7b)

3-Methyl-6-nitro-1*H*-quinoline-2,4-dione (**5b**, 390 mg, 1.57 mmol) and phosphorus oxybromide (1.35 g, 4.71 mmol) were added to 30 mL of dried 1,4-dioxane or chloroform and stirred for 3 h at 100 °C. The mixture was cooled and added to 30 mL of ethyl acetate and then neutralized with saturated sodium bicarbonate solution. The solution was extracted with ethyl acetate and dried over sodium sulfate. The product **7b** was obtained as a pale yellow solid (260 mg, 45%) by flash column chromatography (10% EtOAc/hexane): ^1H NMR (200 MHz, $CDCl_3$) δ: 9.12 (d, J = 2.6 Hz, 1H), 8.47 (dd, J = 9.2 and 2,6 Hz, 1H), 8.14 (d, J = 9.2 Hz, 1H), 2.85 (s. 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 148.6, 148.5, 146.8, 136.6, 135.25, 130.8, 127.1, 124.3, 123.8, 24.0; MS (ESI) m/z: 347 (MH^+). HRMS (CI): Calcd for $C_{10}H_7Br_2N_2O_2$ (MH^+) 346.8854. Found: 346.8873.

5.17. 2,4-Dibromo-3-ethyl-6-nitroquinoline (7c)

This compound was prepared by the same method as for **7b**. **7c**: 36%, pale yellow solid; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.11 (d, J = 2.2 Hz, 1H), 8.46 (dd, J = 9.2 and 2.2 Hz, 1H), 8.12 (d, J = 9.2 Hz, 1H), 3.27 (q, J = 7.6 Hz, 2H), 1.32 (t, J = 7.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 148.6, 148.0, 146.7, 140.0, 136.2, 130.8, 127.2, 124.3, 123.8, 30.7, 12.2; MS (ESI) m/z: 361 (MH^+). HRMS (CI): Calcd for $C_{11}H_9Br_2N_2O_2$ (MH^+) 360.9011. Found: 360.9008.

5.18. 2,4-Dibromo-3-*n*-propyl-6-nitroquinoline (7d)

This compound was prepared by the same method as for **7b**. **7d**: 39%, pale yellow solid; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.11 (d, J = 2.4 Hz, 1H), 8.45 (dd, J = 9.2 and 2.6 Hz, 1H), 8.12 (d, J = 9.2 Hz, 1H), 3.15–3.23 (m, 4H), 1.68–1.80 (m, 2H), 1.34 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 148.6, 148.3, 146.7, 138.9, 136.5, 130.8, 127.2, 124.3, 123.8, 38.9, 21.6, 14.2; MS (ESI) m/z: 375 (MH^+). HRMS (CI): Calcd for $C_{12}H_{11}Br_2N_2O_2$ (MH^+) 374.9167. Found: 374.9169.

5.19. 2,4-Dibromo-3-*n*-butyl-6-nitroquinoline (7e)

This compound was prepared by the same method as for **7b**. **7e**: 39%, pale yellow solid; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.10 (d, J = 2.2 Hz, 1H), 8.45 (dd, J = 9.6 and 2.6 Hz, 1H), 8.11 (d, J = 9.6 Hz, 1H), 3.20 (t, J = 7.8 Hz, 2H), 1.50–1.71 (m, 4H), 1.04 (t, J = 7.2 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 148.6, 148.3, 146.7, 139.1, 136.4, 130.8, 127.2, 124.3, 123.7, 36.9, 30.1, 22.8, 13.7; MS (ESI) m/z: 389 (MH^+). HRMS (CI): Calcd for $C_{13}H_{13}Br_2N_2O_2$ (MH^+) 388.9324. Found: 388.9319.

5.20. 2,4-Dibromo-3-isopropyl-6-nitroquinoline (7f)

This compound was prepared by the same method as for **7b**. **7f**: 48%, pale yellow solid; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.20 (d, J = 2.8 Hz, 1H), 8.45 (dd, J = 9.2 and 2.6 Hz, 1H), 8.11 (d, J = 9.2 Hz, 1H), 4.15–4.24 (m, 1H), 1.57 (d, J = 7.2 Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 148.2, 146.7, 141.9, 130.6, 128.8, 124.6 (m), 123.8, 35.7, 19.3; MS (ESI) m/z: 375 (MH^+). HRMS (CI): Calcd for $C_{12}H_{11}Br_2N_2O_2$ (MH^+) 374.9167. Found: 374.9167.

5.21. 4-Chloro-3-methyl-6-nitroquipazine (8b)

2,4-Dichloro-3-methyl-6-nitroquinoline (**6b**, 540 mg, 2.10 mmol) in DMF (15 mL) solution was added to

piperazine (905 mg, 10.5 mmol) in DMF (3 mL) solution at 0 °C and stirred for 30–60 min and then quenched with excess water. After the precipitate was filtered, it was dissolved in dichloromethane, then washed by water and dried (Na_2SO_4). After removal of solvent, the product **8b** was obtained as a pale yellow solid (620 mg, 96%) by flash column chromatography (10% EtOAc/hexane): ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.96 (d, J = 2.2 Hz, 1H), 8.32 (dd, J = 9.2 and 2.2 Hz, 1H), 7.83 (d, J = 9.2 Hz, 1H), 3.37–3.42 (m, 4H), 3.06–3.11 (m, 4H), 2.50 (s, 3H), 1.81 (br s, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.0, 146.2, 141.4, 141.1, 126.4, 122.5, 120.5, 120.0, 118.5, 48.6, 43.5, 15.1; MS (ESI) m/z: 307 (MH^+). HRMS (CI): Calcd for $C_{14}H_{16}ClN_4O_2$ (MH^+) 307.0962. Found: 307.0969.

5.22. 4-Chloro-3-ethyl-6-nitroquipazine (8c)

This compound was prepared by the same method as for **8b**. **8c**: 94%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.94 (d, J = 2.2 Hz, 1H), 8.29 (dd, J = 9.2 and 2.2 Hz, 1H), 7.82 (d, J = 9.2 Hz, 1H), 3.36–3.40 (m, 4H), 3.07–3.12 (m, 4H), 2.96 (q, J = 7.5 Hz, 2H), 1.95 (br s, NH) 1.35 (t, J = 7.5 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 163.6, 148.5, 144.0, 143.2, 131.4, 129.0, 122.9, 122.8, 120.9, 51.9, 45.9, 23.3, 12.9; MS (ESI) m/z: 321 (MH^+). HRMS (CI): Calcd for $C_{15}H_{18}ClN_4O_2$ (MH^+) 321.1118. Found: 321.1123.

5.23. 4-Chloro-3-propyl-6-nitroquipazine (8d)

This compound was prepared by the same method as for **8b**. **8d**: 97%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.04 (d, J = 2.6 Hz, 1H), 8.36 (dd, J = 9.2 and 2.6 Hz, 1H), 7.88 (d, J = 9.2 Hz, 1H), 3.30–3.38 (m, 4H), 3.06–3.11 (m, 4H), 2.87–2.94 (m, 2H), 1.71–1.80 (m, 2H), 1.03 (t, J = 7.4 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.5, 146.2, 141.7, 140.9, 128.0, 126.6, 120.7, 120.5, 118.8, 49.5, 43.6, 29.8, 19.5, 12.0; MS (ESI) m/z: 335 (MH^+). HRMS (CI): Calcd for $C_{16}H_{20}ClN_4O_2$ (MH^+) 335.1275. Found: 335.1281.

5.24. 4-Chloro-3-*n*-butyl-6-nitroquipazine (8e)

This compound was prepared by the same method as for **8b**. **8e**: 94%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.99 (d, J = 2.6 Hz, 1H), 8.33 (dd, J = 9.2 and 2.6 Hz, 1H) 7.86 (d, J = 9.2 Hz, 1H), 3.34–3.39 (m, 4H), 3.07–3.12 (m, 4H), 2.92 (t, J = 7.8 Hz, 2H), 2.32 (br s, NH), 1.61–1.76 (m, 2H), 1.36–1.54 (m, 2H), 0.99 (t, J = 7.2 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 163.9, 148.6, 144.2, 143.2, 130.6, 129.1, 123.2, 122.9, 121.1, 51.9, 45.9, 30.6, 29.9, 22.9, 13.7; MS (ESI) m/z: 349 (MH^+). HRMS (CI): Calcd for $C_{17}H_{22}ClN_4O_2$ (MH^+) 349.1431. Found: 349.1437.

5.25. 4-Chloro-3-isopropyl-6-nitroquipazine (8f)

This compound was prepared by the same method as for **8b**. **8f**: 96%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.02 (d, J = 2.2 Hz, 1H), 8.30 (dd, J = 9.2 and 2.2 Hz, 1H), 7.84 (d, J = 9.2 Hz, 1H), 3.60–3.67 (m, 1H), 3.30–3.67 (m, 4H), 3.08–3.13 (m, 4H), 2.39 (br s, NH), 1.54 (d, J = 7.0 Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 164.5, 148.2, 144.1, 142.8, 134.4, 129.0, 123.8, 122.9, 120.7, 52.1, 45.7, 29.3, 19.7; MS (ESI) m/z: 335 (MH^+). HRMS (CI): Calcd for $C_{16}H_{20}ClN_4O_2$ (MH^+) 335.1275. Found: 335.1275.

5.26. 4-Bromo-3-methyl-6-nitroquipazine (9b)

This compound was prepared by the same method as for **8b**. **9b**: 94%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.87 (d, J = 2.2 Hz, 1H), 8.24 (dd, J = 9.2 and 2.2 Hz, 1H), 7.76 (d, J = 9.2 Hz, 1H), 3.36–3.41 (m, 4H), 3.06–3.11 (m, 4H), 2.52 (s, 3H), 1.98 (br s, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 163.1, 148.5, 143.9, 137.4, 128.8, 127.5, 123.8, 123.4, 122.7, 51.0, 45.9, 20.9; MS (ESI) m/z: 351 (MH^+). HRMS (CI): Calcd for $C_{14}H_{16}BrN_4O_2$ (MH^+) 351.0457. Found: 351.0464.

5.27. 4-Bromo-3-ethyl-6-nitroquipazine (9c)

This compound was prepared by the same method as for **8b**. **9c**: 97%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.99 (d, J = 2.6 Hz, 1H), 8.31 (dd, J = 9.0 and 2.6 Hz, 1H), 7.84 (d, J = 9.0 Hz, 1H), 3,34–3.39 (m, 4H), 2.96–3.12 (m, 6H), 1.85 (br s, NH), 1.33 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 163.6, 148.6, 144.3, 136.9, 134.1, 129.1, 124.7, 123.8, 122.9, 52.1, 46.0, 26.2; MS (ESI) m/z: 365 (MH^+). HRMS (CI): Calcd for $C_{15}H_{18}BrN_4O_2$ (MH^+) 365.0613. Found: 365.0619.

5.28. 4-Bromo-3-propyl-6-nitroquipazine (9d)

This compound was prepared by the same method as for **8b**. **9d**: 95%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.99 (d, J = 2.6 Hz, 1H), 8.30 (dd, J = 9.0 and 2.6 Hz, 1H), 7.84 (d, J = 9.2 Hz, 1H), 3.32–3.37 (m, 4H), 3.06–3.11 (m, 4H), 2.90–2.98 (m, 2H), 2.02 (br s, NH), 1.67–1.79 (m, 2H), 1.04 (t, J = 7.4 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.4, 146.2, 141.9, 134.5, 130.6, 126.7, 122.3, 121.5, 120.4, 49.6, 43.6, 32.5, 19.8, 11.8; MS (ESI) m/z: 379 (MH^+). HRMS (CI): Calcd for $C_{16}H_{20}BrN_4O_2$ (MH^+) 379.0770. Found: 379.0775.

5.29. 4-Bromo-3-*n*-butyl-6-nitroquipazine (9e)

This compound was prepared by the same method as for **8b**. **9e**: 94%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.01 (d, J = 2.2 Hz, 1H), 8.32 (dd, J = 9.2 and 2.2 Hz, 1H), 7.86 (d, J = 9.2 Hz, 1H), 3.32–3.37 (m, 4H), 3.09–3.11 (m, 4H), 2.97 (t, J = 7.9 Hz, 2H), 1.86 (br s, NH), 1.60–1.72 (m, 2H), 1.40–1.50 (m, 2H), 0.99 (t, J = 7.2 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 163.9, 148.7, 144.4, 136.9, 133.3, 129.2, 124.8, 123.9, 122.9, 52.2, 46.0, 32.7, 30.9, 22.9, 13.7; MS (ESI) m/z: 393 (MH^+). HRMS (CI): Calcd for $C_{17}H_{22}BrN_4O_2$ (MH^+) 393.0926. Found: 393.0930.

5.30. 4-Bromo-3-isopropyl-6-nitroquipazine (9f)

This compound was prepared by the same method as for **8b**. **9f**: 96%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.12 (d, J = 2.6 Hz, 1H), 8.32 (dd, J = 9.2 and

2.6 Hz, 1H) 7.84 (d, J = 9.2 Hz, 1H), 3.66–3.80 (m, 1H), 3.28–3.33 (m, 4H), 3.07–3.12 (m, 4H), 2.24 (br s, NH), 1.55 (d, J = 7.0 Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 164.7, 148.3, 144.5, 136.8, 135.3, 129.2, 125.4, 123.7, 123.0, 51.3, 45.8, 30.5, 20.0; MS (ESI) m/z: 379 (MH^+). HRMS (CI): Calcd for $C_{16}H_{20}BrN_4O_2$ (MH^+) 379.0770. Found: 379.0764.

5.31. 2,*N*-Diphenylmalonamic acid ethyl ester (10a)

Aniline (3.0 g, 32.2 mmol), diethyl phenyl malonate (11.4 g, 48.3 mmol), and pyridine (5.2 mL, 64.4 mmol) were added to 50 mL of toluene and stirred for 18 h at 120 °C. The solvent of reaction mixture was evaporated and the desired product **10a** was obtained as a brown solid (4.2 g, 47%) by flash column chromatography (10% EtOAc/hexane): ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.16 (br s, NH), 7.42–7.51 (m, 4H), 7.20–7.35 (m, 5H), 7.05 (t, J = 7.3 Hz, 1H), 4.67 (s, 1H), 4.14–4.26 (m, 2H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 170.6, 165.3, 137.5, 133.9, 128.9, 128.7, 128.2, 124.4, 120.0, 61.9, 59.0, 13.8; MS (ESI) m/z: 284 (MH^+). HRMS (EI): Calcd for $C_{17}H_{17}NO_3$ (M^+) 283.1208. Found: 283.1203.

5.32. 2-Benzyl-*N*-phenylmalonamic acid ethyl ester (10b)

This compound was prepared by the same method as for **10a**. **10b**: 52%, brown solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.70 (br s, NH), 7.44–7.49 (m, 2H), 7.03–7.29 (m, 8H), 4.09 (q, J = 5.3 Hz, 2H), 3.68 (t, J = 7.5 Hz, 1H), 3.29 (q, 2H), 1.12 (t, J = 7.1 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 171.1, 165.9, 137.5, 137.4, 128.8, 128.4, 126.7, 124.4, 120.1, 61.5, 55.4, 36.5, 13.8; MS (ESI) m/z: 298 (MH^+). HRMS (EI): Calcd for $C_{18}H_{19}NO_3$ (M^+) 297.1365. Found: 297.1376.

5.33. 3-Phenyl-1*H*-quinoline-2,4-dione (11a)[9]

2,*N*-Diphenylmalonamic acid ethyl ester (**10a**, 3.7 g, 13.1 mmol) was dissolved in 50 mL chlorobenzene and aluminum chloride (5.2 g, 39 mmol) was added in three portions. The mixture was heated to 120 °C and stirred for 2 h. The reaction mixture was quenched by ice water and extracted with ethyl acetate, and dried (Na_2SO_4). The product **11a** was obtained as a pale yellow solid (3.02 g, 97%) by flash column chromatography (10% MeOH/CH_2Cl_2): ^{1}H NMR (200 MHz, CD_3OD) δ: 7.45–7.52 (m, 4H), 7.06–7.10 (m, 5H), 6.96–6.99 (m, 1H), 4.89 (s, 1H); ^{13}C NMR (50 MHz, CD_3OD) δ: 177.1, 171.8, 139.1, 137.9, 129.7, 129.5, 128.3, 125.6, 121.7, 62.1.

5.34. 3-Benzyl-1*H*-quinoline-2,4-dione (11b).[10]

Compound was prepared by the same method as for **11a**. **11b**: 90%, pale yellow solid; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 10.16 (br s, NH), 7.54 (d, J = 7.6 Hz, 2H), 7.11–7.32 (m, 6H), 7.03 (t, J = 7.3 Hz, 1H), 4.27 (br s, OH), 3.79 (t, J = 7.5 Hz, 1H), 3.12 (d, J = 7.2 Hz, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 168.8, 165.1, 137.2, 137.0, 127.0, 126.9, 126.4, 124.4, 121.7, 117.6, 52.7, 32.5.

5.35. 2,4-Dichloro-3-phenylquinoline (12a)

This compound was prepared by the same method as for **6b**. **12a**: 59%, white solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.25 (d, J = 8.2 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.77–7.85 (m, 1H), 7.64–7.71 (m, 1H), 7.48–7.56 (m, 3H), 7.26 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 150.4, 147.0, 143.2, 135.9, 133.3, 131.1, 129.7, 128.9, 128.7, 128.5, 128.1, 125.7, 124.9; MS (ESI) m/z: 274 (MH^+). HRMS (CI): Calcd for $C_{15}H_{10}Cl_2N$ (MH^+) 274.0190. Found: 274.0186.

5.36. 3-Benzyl-2,4-dichloroquinoline (12b)

This compound was prepared by the same method as for **6b**. **12b**: 65%, white solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.14 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.70 (t, J = 7.5 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.17–7.26 (m, 5H), 4.47 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 151.7, 146.4, 143.8, 137.2, 130.6, 128.7, 128.4, 125.8, 124.5, 36.9; MS (ESI) m/z: 288 (MH^+). HRMS (CI): Calcd for $C_{16}H_{12}Cl_2N$ (MH^+) 288.0347. Found: 288.0343.

5.37. 4-Chloro-3-phenylquipazine (13a)

In a pressure tube, 2,4-dichloro-3-phenylquinoline **12a** (900 mg, 3.3 mmol) was added to 1-piperazinecarboxaldehyde (5 mL) and stirred for 4 h at 120 °C. The reaction mixture was cooled and diluted with sodium bicarbonate solution and then extracted with ethyl acetate. The organic layer was evaporated and the residue was dissolved in THF (20 mL). The solution was added to 4 M H_2SO_4 (20 mL) and stirred for 1 h at 50 °C. The reaction mixture was poured in ice water (100 mL) and basified with 1 N NaOH and then extracted with dichloromethane. The organic layer was dried with sodium sulfate and evaporated. The product **13a** was obtained as a yellow oil (692 mg, 65%) by flash column chromatography (10% MeOH/CH_2Cl_2): ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.13 (d, J = 8.4 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.63 (t, J = 6.9 Hz, 1H), 7.37–7.47 (m, 6H), 3.10–3.15 (m, 4H), 2.64–2.69 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 159.7, 146.7, 141.7, 136.7, 130.3, 129.9, 128.2, 127.7, 127.6, 127.2, 124.6, 123.3, 50.3, 45.5; MS (ESI) m/z: 324 (MH^+). HRMS (CI): Calcd for $C_{19}H_{19}ClN_3$ (MH^+) 324.1268. Found: 324.1262.

5.38. 3-Benzyl-4-chloroquipazine (13b)

This compound was prepared by the same method as for **13a**. **13b**: 61%, white solid; ^{1}H NMR (200 MHz, CD_3OD) δ: 8.07 (d, J = 8.4 Hz, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.64 (t, J = 7.4 Hz, 1H), 7.43 (t, J = 7.5 Hz, 1H), 7.07–7.24 (m, 5H); ^{13}C NMR (50 MHz, CD_3OD) δ: 162.9, 147.6, 145.0, 140.1, 131.0, 129.5, 129.0, 128.9, 127.9, 127.2, 127.0, 125.5, 125.0, 51.6, 46.0, 36.2; MS (ESI) m/z: 338 (MH^+). HRMS (CI): Calcd for $C_{20}H_{21}ClN_3$ (MH^+) 338.1424. Found: 338.1426.

5.39. 4-Chloro-6-nitro-3-(4-nitrophenyl)quipazine (14a)

Concentrated HNO_3 (14.1 mmol, 640 μL) was added dropwise to a solution of 4-chloro-3-phenylquipazine

(**13a**, 1.0 g, 3.08 mmol) in concentrated H_2SO_4 (15 mL) at 0 °C and stirred for 30 min. The reaction mixture was poured in ice water (100 mL) and basified with 4 N NaOH and then extracted with dichloromethane. The organic layer was dried with sodium sulfate and evaporated. The product **14a** was obtained pale as a yellow solid (867 mg, 68%) by flash column chromatography (10% MeOH/CH_2Cl_2): ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.07 (d, J = 2.4 Hz, 1H), 8.38–8.47 (m, 3H), 7.89 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 3.22–3.26 (m, 4H), 2.70–2.75 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 158.2, 147.5, 145.1, 141.3, 140.8, 140.4, 128.7, 126.3, 123.5, 121.9, 121.5, 119.4, 119.1, 47.7, 42.9; MS (ESI) m/z: 414 (MH^+). HRMS (CI): Calcd for $C_{19}H_{17}ClN_5O_4$ (MH^+) 414.0969. Found: 414.0962.

5.40. 4-Chloro-6-nitro-3-(4-nitrobenzyl)quipazine (14b)

This compound was prepared by the same method as for **14a**. **14b**: 72%, yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.04 (d, J = 2.6 Hz, 1H), 8.44 (dd, J = 9.2 and 2.6 Hz, 1H), 8.17 (d, J = 8.6 Hz, 2H), 7.96 (d, J = 9.2 Hz, 1H), 7.33 (d, J = 8.4 Hz, 2H), 4.62 (s, 2H), 3.28–3.33 (m, 4H), 2.98–3.03 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 163.7, 149.3, 146.9, 145.6, 145.4, 144.5, 129.4, 128.6, 126.1, 123.9, 123.9, 122.8, 121.3, 51.7, 45.8, 36.0; MS (ESI) m/z: 428 (MH^+). HRMS (CI): Calcd for $C_{20}H_{19}ClN_5O_4$ (MH^+) 428.1126. Found: 428.1129.

5.41. 4-Chloro-2-(4-methylpiperazin-1-yl)-6-nitroquinoline (15)

4-Chloro-6-nitroquipazine (**2**, 450 mg, 1.54 mmol), KOH (230 mg, 4.1 mmol) and methyl iodide (170 μL, 2.8 mmol) were added to DMF (15 mL) and stirred for 1 h at rt. The reaction mixture was quenched by water and extracted with ethyl acetate and then dried. The product **15** was obtained yellow solid (339 mg, 72%) by flash column chromatography (3% MeOH/CH_2Cl_2): ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.92 (d, J = 2.6 Hz, 1H), 8.34 (dd, J = 9.2 and 2.6 Hz, 1H), 7.67 (d, J = 9.2 Hz, 1H), 7.17 (s, 1H), 3.80–3.85 (m, 4H), 2.52–2.57 (m, 4H), 2.37 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 158.0, 151.8, 144.4, 142.4, 127.6, 124.3, 121.2, 119.7, 110.5, 54.7, 46.0, 44.8; MS (ESI) m/z: 307 (MH^+). HRMS (CI): Calcd for $C_{14}H_{16}ClN_4O_2$ (MH^+) 307.0962. Found: 307.0965.

5.42. In vitro serotonin transporter binding studies

As described in Section 3, we have measured the binding affinities according to the method published in our previous studies[1,4,6] using crude synaptic membranes prepared from the cerebral cortex of male Sprague–Dawley rats. See the detailed procedure in our previous study.[6]

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