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Syntheses and binding affinities of 6-nitroquipazine analogues for serotonin transporter. Part 5: 2′-Substituted 6-nitroquipazines☆

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Abstract—Five C2′-substituted 6-nitroquipazine (6-NQ) derivatives were prepared and evaluated in terms of their biological abilities (K_i) to displace [^{3}H]citalopram binding to serotonin transporter. The relationship between their structure and biological activities revealed that shorter alkyl groups tend to possess higher binding affinity. Both compounds **12a** and **12c** were found to have the equally highest binding affinity ($K_i = 0.43 \pm 0.02$ nM).

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is well known as a monoamine neurotransmitter that plays an important role in the modulation of mood and sleep. After interaction with serotonin receptors at post-synapse, some 5-HTs are reuptaken to pre-synaptic neuron cell through the serotonin transporter (SERT) that is an integral membrane protein consisting of 12 transmembrane domains. Alterations of serotonin concentration in the neuronal synaptic cleft could cause various psychiatric diseases such as schizophrenia, anxiety, neurodegenerative, emesis, and depression.[2,3] In most cases, reduction of serotonin concentration in synapse region is responsible for such diseases. For the treatment of these diseases, therefore, it is desirable to increase the concentration of 5-HT is desirable and it can be achieved by inhibition of 5-HT reuptake with several chemical drugs known as selective specific reuptake inhibitors (SSRIs), that is, paroxetine, fluoxetine, ADAM, (+)-McN-5652, citalopram, sertraline, and 6-nitroquipazine, which are believed to bound to the similar region of SERT.[4–7] Intriguingly, although other inhibition compounds usually have less structural similarity, most SSRI compounds commonly have one or two aromatic groups and one amine group maintaining constant distance and position with each other. Like many successful SSRIs, 6-nitroquipazine (6-NQ, **1**, $K_i = 0.17$ nM) also has high binding affinity,[4,5] and selectivity against other serotonin binding proteins in spite of its structural simplicity, expecting its therapeutic and diagnostic potency.[6,7] We already developed the efficient synthesis of a member of 6-NQ derivatives[1,8] as well as 6-NQ itself[9] for our continuous interest in the development of radioisotope-labeled compounds for PET (positron emission tomography)[8c] and SPECT (single photon emission computed tomography)[10] image. While we have made an effort to introduce substitutions to quinoline ring, Gerdes et al. tried to modify piperazine ring.[11] They reported the synthesis and biological evaluation of 2′- and 3′-methyl-6-NQs (**2** and **3**) as racemic mixtures. Between them, **2** has better binding affinity than **3**. In this paper, we report the synthesis and biological study of additional 6-NQ derivatives substituted with several alkyl groups through ether linkage at C2′ position of piperazine (Fig. 1).

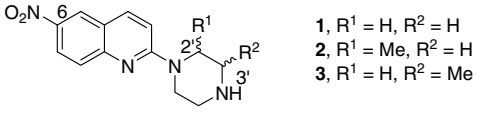

Figure 1.

Keywords: Serotonin transporter; 6-Nitroquipazine; SAR; 5-Hydroxytryptamine.
☆ See Ref. 1.
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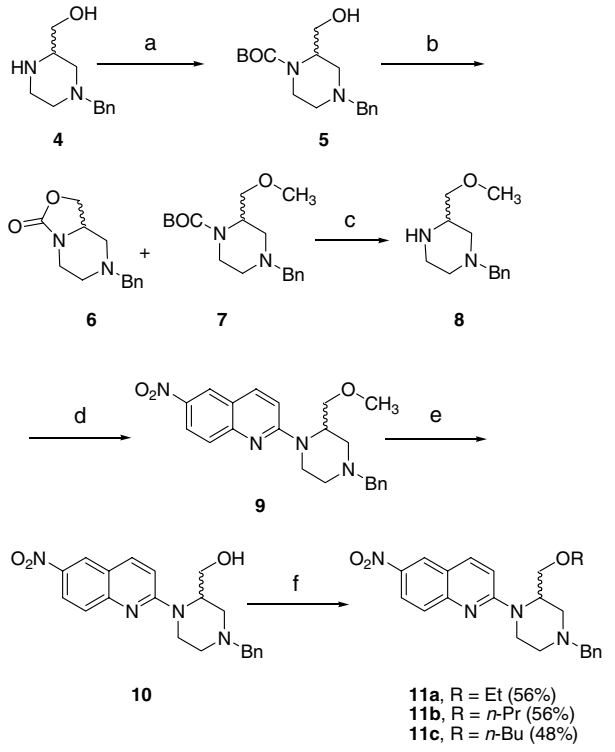
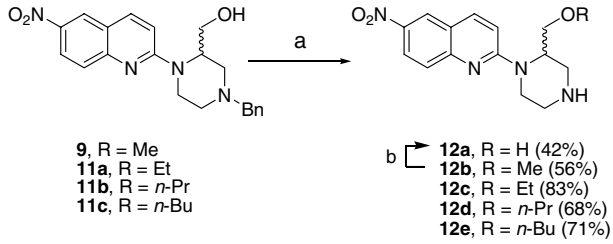
2. Chemistry

Mono *N*-benzylpiperazine compound **4** was prepared as a racemic mixture by following a literature procedure in three steps starting from *N*,*N*′-dibenzylethylenediamine and diethyl bromomalonate.[12] *N*-Boc protection of compound **4** was performed by the reaction of **4** with $(Boc)_2O$ in the presence of triethylamine to give 85% of **5**. *O*-Alkylation of hydroxyl group of compound **5** with several alkyl halides was first tried using NaH in anhydrous THF (Scheme 1).

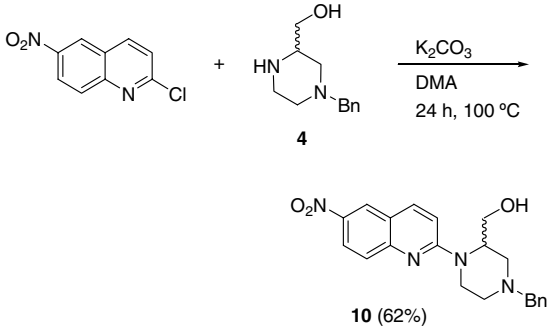
However, only *O*-methylated compound **7** was yielded moderately when iodomethane was used, also including 28% of **6**. When other alkyl halides were used in this reaction, lactam compound **6** was mainly obtained by internal transacylation reaction as an undesired product. Therefore, alkyl derivatization through etherification was postponed until the preparation of 6-nitroquipazine derivative **10**. *N*-Boc group of **7** was selectively deprotected under acidic condition to give compound **8**. This mono free amine piperazine was incorporated into 6-nitroquinoline using 2-chloro-6-nitroquinoline, which was prepared by previous method.[9,13] That reaction was performed in the presence of potassium carbonate in DMA at 130 °C for 10 h, affording 6-NQ derivative **9** in 92% yield. Demethylation of 2′-methoxy group of **9** was carried out by the treatment of boron tribromide at 0 °C to give 2′-hydroxymethyl-6-NQ (**10**) in 36% yield. 2′-Alkylated 6-NQs (**11a**–**11c**) were obtained by



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, CH_2Cl_2, 0 °C, 4 h, 85%; (b) NaH, CH_3I, THF, 0 °C, N_2, 3 h, 69%; (c) 4 M H_2SO_4, THF, rt, 4 h, 99%; (d) 2-chloro-6-nitroquinoline, K_2CO_3, DMA, 130 °C, N_2, 10 h, 92%; (e) BBr_3, CH_2Cl_2, 0 °C, N_2, 3 h, 36%; (f) RX, NaH, DMF, N_2, 0–60 °C, 4 h.



Scheme 2. Reagents and conditions: (a) i—1-chloroethyl chloroformate, dichloroethane, reflux, N_2, 3 h; ii—MeOH, reflux, N_2, 1 h; (b) BBr_3, CH_2Cl_2, 0 °C, N_2, 3 h.



Scheme 3.

alkylation of 2′-hydroxyl-6-NQ (**10**) with three alkyl halides and NaH in moderate yields 48–56%, where anhydrous DMF solvent was used instead of THF that was used for *O*-methylation of compound **5**.

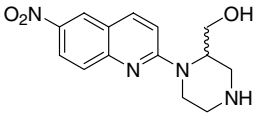
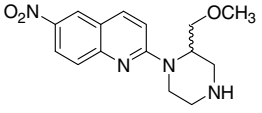
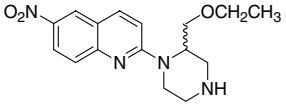
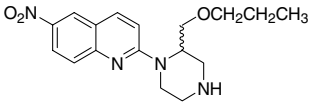
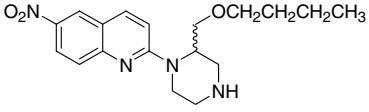
Finally, 2′-alkylated 6-NQs (**12b**–**12e**) were synthesized from corresponding *N*-benzyl-2′-alkylated 6-NQs (**9**, **11a**–**11c**) by treating with 1-chloroethyl chloroformate, followed by heating in MeOH in 56–83% yield (Scheme 2). However, since debenzylation of *N*-benzyl-2′-hydroxymethyl-6-NQ (**10**) to make 2′-hydroxymethyl-6-NQ (**12a**) under the same condition was not successful, compound **12a** was prepared by demethylation of **12b** with boron tribromide.

It was interesting to note that in the middle of modification of hydroxyl functional group, we found that *N*-benzyl-2′-hydroxymethyl-6-NQ (**10**) could be synthesized as well by direct S_NAr reaction of 2-chloro-6-nitroquinoline and mono *N*-benzylated 2-hydroxymethylpiperazine **4** in DMA solvent under heating condition (Scheme 3).

3. Binding studies

For in vitro biological study, crude synaptic membranes prepared from the cerebral cortex of male Sprague–Dawley rats[14] were used according to the method of our previous study.[1,8] Five 6-NQ derivatives **12a**–**12e** were tested in the form of racemic mixtures and their K_i values are summarized in Table 1. The K_d value of [^{3}H]citalopram measured by Scatchard analysis of the equilibrium-saturation experiment was 1.12 nM. Competition binding assays were performed by measuring the concentrations of displaced [^{3}H]citalopram by test compounds which inhibited the specific binding by

Table 1. Structure and biology data of 6-nitroquipazine derivatives on SERT[a]

Compound		K_i (nM)
12a		0.43 ± 0.02
12b		0.68 ± 0.02
12c		0.43 ± 0.02
12d		5.67 ± 0.57
12e		15.36 ± 1.74
1	6-NQ	0.24 ± 0.00
	Fluoxetine	8.70 ± 1.31

[a] The values represent means ± SEM of 3–4 separate experiments.

50% (IC_{50} values), using 1 nM [^{3}H]citalopram and 11 different concentrations of **12a**–**12e** compounds ranging from 10^{-11} to 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 software. Inhibition binding constant (K_i) values were subsequently calculated from IC_{50} values using the Cheng–Prusoff equation.[15] Table 1 illustrates the in vitro binding affinities of **12a**–**12e** targeting SERT, including 6-NQ, fluoxetine.

4. Discussion

As shown in Table 1, 2′-substituted 6-NQs have slightly lower binding affinities than mother molecule 6-NQ. However, it should be noted that with regard to that all compounds are racemic mixtures, purified single enantiomers must have better activities than mirror image compounds as well as racemic mixtures. By comparison of structure to inhibition constant (K_i) for each compound, obviously, it was found that shorter alkyl groups tend to have better binding affinities. Such trend is quietly consistent with our previous studies on 6-NQ derivatives. Therefore, it could be explained in the same manner as previous papers by sterically restricted surroundings. Ethoxy group of compound (**12c**) seems to be in a middle place. Three compounds, for example, hydroxymethyl (**12a**), methoxymethyl (**12b**), and ethoxymethyl (**12c**) derivatives, maintain similarly high binding affinities (K_i = 0.43–0.68 nM) regardless of their polarity. Little difference of binding affinity among **12a**–**12c** is probably because the oxygen in 2′ position might interact with amino acid residue of SERT through weak hydrogen bond. However, the considerable decrease of binding affinity after ethoxymethyl group was found and it is strongly due to increase of alkyl bulkiness. As mentioned above, 6-NQ is a quietly optimized compound by proper interaction through hydrophobic nitroquinoline moiety and hydrophilic amine moiety, maintaining reasonable distance from SERT. Two longer alkyl groups, *n*-propoxymethyl (**12d**) and *n*-butoxymethyl (**12e**), may brake the optimal interaction between 6-NQ and binding pocket of SERT, giving rise to relatively high K_i value.

In summary, we have prepared five 6-NQ derivatives substituted with a series of alkoxymethyl groups on 2′ position and their in vitro assay was performed for SAR study. Compounds **12a** and **12c** have shown an equally high binding affinity even though these analogous are racemic mixtures. Subsequent SAR study revealed that binding pocket region of SERT is sterically restricted so that shorter alkyl groups trend to possess higher binding affinities.

5. Experimental

5.1. General method

Flash column chromatography was done using silica gel (EM Science, 230–400 mesh ASTM). Solvents and reagents were purchased form the following commercial sources: Aldrich, Sigma, Lancaster, Acros, and TCI. Analytical thin layer chromatography (TLC) was performed with Merck silica gel F-254 glass-backed plates. ^{1}H and ^{13}C NMR spectra were obtained on Varian Gemini-2000 (200 and 400 MHz) spectrometers and are reported in parts per million downfield from internal tetramethylsilane. Mass spectra were obtained on HP590 GC/MS 5972 MSD spectrometer.

5.1.1. 4-Benzyl-2-(hydroxymethyl)piperazine (4). 100 mg (36%) as a pale brown oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.20 (m, 5H), 3.57 (dd, 1H, J = 9.6, 2.8 Hz), 3.53–3.45 (m, 3H), 3.03–2.98 (m, 1H), 2.95–2.86 (m, 2H), 2.71 (d, 2H, J = 11.6 Hz), 2.42 (br s, 2H), 2.11 (td, 1H, J = 10.8, 3.0 Hz), 1.90 (t, 1H, J = 10.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.9, 128.3, 127.4, 126.2, 62.6, 62.5, 55.6, 55.0, 52.8, 52.7, 48.4, 44.1; MS (EI) *m*/*z* (relative intensity) 206 [M]$^+$, 189, 176, 134 (100), 120.

5.1.2. 1-Benzyl-3-(hydroxymethyl)-1-*tert*-butyloxycarbonyl piperazine (5). To a solution of compound **4** (1.55 g, 7.51 mmol) in dichloromethane (40 mL) was added a solution of $(Boc)_2O$ (1.959 g, 8.26 mmol) in dichloromethane (10 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was added into a brine solution (100 mL) and extracted with dichloromethane

(3× 25 mL). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash column chromatography (40% EtOAc/hexane) gave the desired product **5** (1.97 g, 85%) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.24 (m, 5H), 4.07 (br s, 1H), 3.94–3.90 (m, 2H), 3.87–3.83 (m, 2H), 3.48 (t, 2H, J = 14.0 Hz), 3.37 (br s, 1H), 2.97 (d, 1H, J = 14.0 Hz), 2.81 (d, 1H, J = 11.8 Hz), 2.28 (dd, 1H, J = 9.8, 2.8 Hz), 2.08 (t, 1H, J = 13.6 Hz), 1.45 (t, 9H, J = 14.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.1, 137.1, 128.9, 128.4, 127.4, 79.9, 66.2, 62.9, 54.9, 52.5, 51.2, 41.5, 28.3; MS (FAB) *m/z* 307 $[M+H]^+$, 251. HRMS (FAB) *m/z* $C_{17}H_{26}O_3N_2$ $[M+H]^+$ Calcd: 307.2022. Found: 307.2016.

5.1.3. 7-Benzylhexahydrooxazolo[3,4-*a*]pyrazin-3-one (6). MS (EI) *m/z* 232 $[M]^+$, 187, 159, 146, 133.

5.1.4. 4-Benzyl-2-(methoxymethyl)-1-*tert*-butyloxycarbonyl piperazine (7). To a solution of compound **5** (4.63 g, 15.11 mmol) in anhydrous tetrahydrofuran (100 mL) was added portionwise NaH (785 mg, 19.64 mmol) at 0 °C under N_2 atmosphere. After 5 min, iodomethane (2.60 mg, 18.13 mmol) was added into the reaction mixture and stirred at room temperature for 3 h. The reaction solution was added into a brine solution (300 mL) and extracted with EtOAc (3× 100 mL). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The desired product **7** (3.34 g, 69%) was obtained by flash column chromatography (40% EtOAc/hexane) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.19 (m, 5H), 4.19 (br s, 1H), 3.85 (d, 1H, J = 12.2 Hz), 3.51 (t, 1H, J = 8.2 Hz), 3.45 (d, 2H, J = 4.2 Hz), 3.31 (s, 3H), 3.00 (t, 1H, J = 12.4 Hz), 2.87 (d, 1H, J = 11.8 Hz), 2.71 (d, 1H, J = 12.2 Hz), 2.07–1.96 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.7, 138.0, 128.5, 128.1, 128.0, 126.8, 79.3, 69.7, 62.5, 60.1, 58.5, 52.5, 52.4, 50.0, 40.0, 28.2, 20.7, 14.0; MS (FAB) *m/z* (relative intensity) 321 $[M+H]^+$, 265 (100), 263, 231. HRMS (FAB) *m/z* $C_{18}H_{28}O_3N_2$ $[M+H]^+$ Calcd: 321.2178. Found: 321.2181.

5.1.5. 1-Benzyl-3-(methoxymethyl)piperazine (8). To a solution of compound **7** (1.78 g, 5.55 mmol) in tetrahydrofuran (100 mL) was added 4 M aqueous sulfuric acid (10 mL) and stirred at room temperature for 4 h. The reaction mixture was added slowly into water (300 mL) and 4 N aqueous NaOH was added until slightly basic to litmus. The organic compounds were extracted with dichloromethane (2× 50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash column chromatography (5% MeOH/dichloromethane) gave the desired product **8** (1.22 g, 99%) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.23 (m, 5H), 3.54–3.47 (m, 2H), 3.78–3.31 (m, 4H), 3.28 (t, 1H, J = 8.2 Hz), 3.04–2.96 (m, 2H), 2.90 (t, 1H, J = 12.6 Hz), 2.74 (t, 2H, J = 12.2 Hz), 2.25 (s, 1H), 2.10 (t, 1H, J = 12.6 Hz), 1.84 (t, 1H, J = 12.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.8, 128.9, 128.0, 126.8, 74.7, 63.2, 58.9, 55.6, 54.3, 53.4, 44.9; MS (FAB) *m/z* (relative intensity) 221 $[M+H]^+$, 219 (100), 175. HRMS (FAB) *m/z* $C_{13}H_{20}ON_2$ $[M+H]^+$ Calcd: 221.1654. Found: 221.1661.

5.1.6. 2-[4-Benzyl-2-(methoxymethyl)piperazin-1-yl]-6-nitroquinoline (9). A suspension of compound **8** (475 mg, 2.16 mmol) and K_2CO_3 (895 mg, 6.47 mmol) in anhydrous DMA (20 mL) was stirred at room temperature under N_2 atmosphere. After 5 min, 2-chloro-6-nitroquionoline (455 mg, 2.16 mmol) was added into the reaction mixture and stirred at 130 °C for 10 h. The reaction mixture was cooled to room temperature, filtered with Celite, and washed with EtOAc. Water (200 mL) was added, and organic compounds were extracted with EtOAc (3× 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash column chromatography (20% EtOAc/hexane) gave the desired product **9** (780 mg, 92%) as a yellow amorphous solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (d, 1H, J = 2.4 Hz), 8.39 (dd, 1H, J = 9.2, 2.6 Hz), 8.01 (d, 1H, J = 12.2 Hz), 7.38–7.26 (m, 6H), 6.93 (s, 1H), 3.85 (t, 1H, J = 8.4 Hz), 3.71–3.55 (m, 5H), 3.29 (d, 1H, J = 12.0 Hz), 3.18 (s, 3H), 2.94 (q, 2H, J = 13.6 Hz), 2.65 (d, 1H, J = 12.0 Hz), 2.52 (t, 1H, J = 12.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 151.5, 141.7, 138.4, 138.0, 128.8, 128.3, 127.2, 127.0, 124.2, 123.5, 121.0, 111.1, 70.2, 62.6, 59.1, 53.1, 52.9, 51.7, 40.6; MS (FAB) *m/z* 392 $[M+H]^+$. HRMS (FAB) *m/z* $C_{22}H_{24}O_3N_4$ $[M+H]^+$ Calcd: 392.1848. Found: 392.1847.

5.1.7. 2-[4-Benzyl-2-(hydroxymethyl)piperazin-1-yl]-6-nitroquinoline (10). To a solution of compound **9** (0.62 g, 1.59 mmol) in anhydrous dichloromethane (20 mL) was added slowly boron tribromide (3.18 mL, 3.18 mmol) at 0 °C, and stirred for 3 h. Water (100 mL) was added into the reaction mixture and organic compounds were extracted with EtOAc (3× 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash column chromatography (40% EtOAc/hexane) gave the desired product **10** (220 mg, 36%) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, 1H, J = 2.4 Hz), 8.29 (dd, 1H, J = 9.4, 2.7 Hz), 7.97 (d, 1H, J = 9.2 Hz), 7.62 (d, 1H, J = 9.2 Hz), 7.39–7.29 (m, 5H), 7.07 (d, 1H, J = 9.2 Hz), 4.90 (t, 2H, J = 13.0 Hz), 4.25 (br s, 1H), 4.16–4.09 (m, 1H), 3.99 (dd, 1H, J = 9.4, 2.8 Hz), 3.69 (t, 1H, J = 14.0 Hz), 3.59 (d, 1H, J = 12.0 Hz), 3.55 (d, 1H, J = 12.0 Hz), 3.17 (d, 1H, J = 11.6 Hz), 3.05 (d, 1H, J = 13.2 Hz), 2.45 (dd, 1H, J = 9.6, 2.8 Hz), 2.29 (t, 1H, J = 13.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 151.2, 141.9, 138.8, 137.0, 129.0, 128.6, 127.6, 127.0, 124.3, 123.7, 121.1, 111.1, 66.0, 62.9, 55.5, 52.7, 51.8, 42.5; MS (FAB) *m/z* (relative intensity) 379 $[M+H]^+$, 154 (100). HRMS (FAB) *m/z* $C_{21}H_{23}O_3N_4$ $[M+H]^+$ Calcd: 379.1770. Found: 379.1772.

5.2. General procedure for the preparation of 2-[4-benzyl-2-(alkoxymethyl)piperazin-1-yl]-6-nitroquinoline (11a–11c)

A solution of NaH (20 mg, 0.40 mmol) in anhydrous DMA (15 mL) was added to a solution of compound **10** (100 mg, 0.26 mmol) in anhydrous DMA (5 mL) at 0 °C under N_2 atmosphere. After 5 min, iodomethane

(162 μL, 0.26 mmol) was added into the reaction mixture and stirred at 60 °C for 4 h. The reaction mixture was cooled to room temperature and the reaction was quenched by adding water (200 mL). Organic compounds were extracted with dichloromethane (2× 50 mL), dried over Na_2SO_4, and concentrated in vacuo. Flash column chromatography (5% MeOH/dichloromethane) gave the desired product **11a**.

5.2.1. 2-[4-Benzyl-2-(ethoxymethyl)piperazin-1-yl]-6-nitroquinoline (11a). 60 mg (56%) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, 1H, J = 2.4 Hz), 8.28 (dd, 1H, J = 9.3, 2.7 Hz), 7.93 (d, 1H, J = 9.3 Hz), 7.64 (d, 1H, J = 9.2 Hz), 7.37–7.26 (m, 5H), 7.09 (d, 1H, J = 9.3 Hz), 4.62 (t, 2H, J = 13.0 Hz), 3.81 (t, 1H, J = 8.6 Hz), 3.65 (dd, 1H, J = 9.2, 2.6 Hz), 3.60 (d, 1H, J = 13.2 Hz), 3.52 (d, 1H, J = 13.0 Hz), 3.33 (s, 3H), 3.30–3.23 (m, 1H), 3.09 (d, 1H, J = 14.0 Hz), 2.97 (d, 1H, J = 14.1 Hz), 2.25–2.05 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 151.4, 141.6, 138.3, 138.0, 128.8, 128.2, 127.0, 124.2, 123.4, 121.0, 111.2, 67.7, 66.7, 62.6, 53.0, 52.8, 52.0, 40.7, 15.1; MS (FAB) *m/z* (relative intensity) 407 $[M+H]^+$, 154, 136 (100). HRMS (FAB) *m/z* $C_{23}H_{27}O_3N_4$ $[M+H]^+$ Calcd: 407.2083. Found: 407.2071.

5.2.2. 2-[4-Benzyl-2-(propoxymethyl)piperazin-1-yl]-6-nitroquinoline (11b). 62 mg (56%) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, 1H, J = 2.4 Hz), 8.27 (dd, 1H, J = 9.3, 2.7 Hz), 7.92 (d, 1H, J = 9.2 Hz), 7.63 (d, 1H, J = 9.3 Hz), 7.41–7.24 (m, 5H), 7.09 (d, 1H, J = 9.3 Hz), 4.60 (br s, 2H), 3.83 (t, 1H, J = 9.2 Hz), 3.67–3.59 (m, 2H), 3.49 (d, 1H, J = 13.2 Hz) 3.41–3.33 (m, 2H), 3.26 (t, 1H, J = 14.0 Hz), 3.12 (d, 1H, J = 12.1 Hz), 2.98 (d, 1H, J = 14.0 Hz), 2.32–2.16 (m, 2H), 1.52–1.36 (m, 2H), 0.77 (t, 3H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 151.4, 141.7, 138.2, 138.0, 128.8, 128.2, 127.1, 127.0, 124.2, 123.5, 121.0, 111.3, 73.1, 68.0, 62.7, 53.0, 52.1, 40.6, 22.8, 10.4; MS (FAB) *m/z* 421 $[M+H]^+$, 154. HRMS (FAB) *m/z* $C_{24}H_{29}O_3N_4$ $[M+H]^+$ Calcd: 421.2240. Found: 421.2248.

5.2.3. 2-[4-Benzyl-2-(butoxymethyl)piperazin-1-yl]-6-nitroquinoline (11c). 55 mg (48%) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, 1H, J = 2.4 Hz), 8.27 (dd, 1H, J = 9.3, 2.7 Hz), 7.91 (d, 1H, J = 9.1 Hz), 7.63 (d, 1H, J = 9.3 Hz), 7.39–7.26 (m, 5H), 7.09 (d, 1H, J = 9.2 Hz), 4.60 (br s, 2H), 3.83 (t, 1H, J = 9.0 Hz), 3.68–3.58 (m, 2H), 3.49 (d, 1H, J = 13.0 Hz) 3.44–3.37 (m, 2H), 3.25 (t, 1H, J = 14.0 Hz), 3.11 (d, 1H, J = 13.2 Hz), 2.98 (d, 1H, J = 14.1 Hz), 2.25–2.08 (m, 2H), 1.44–1.34 (m, 2H), 1.26–1.14 (m, 2H), 0.80 (t, 3H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 151.4, 141.7, 138.2, 138.0, 128.8, 128.3, 127.1, 127.0, 124.2, 123.5, 121.0, 111.3, 71.2, 68.1, 62.7, 53.0, 52.1, 40.6, 31.6, 19.1, 13.8; MS (FAB) *m/z* 435 $[M+H]^+$, 154. HRMS (FAB) *m/z* $C_{25}H_{31}O_3N_4$ $[M+H]^+$ Calcd: 435.2396. Found: 435.2386.

5.3. General procedure for the preparation of 2-[2-(alkoxymethyl)piperazin-1-yl]-6-nitroquinoline (12b–12e)

To a solution of compound **9** (60 mg, 0.15 mmol) in anhydrous dichloromethane (5 mL) was added slowly 1-chloroethyl chloroformate (0.02 mL, 0.22 mmol) at room temperature under N_2 atmosphere and stirred under reflux for 2 h. The reaction mixture was cooled to room temperature and solvent was removed by rotary evaporator. The residue was dissolved in methanol (20 mL) at room temperature under N_2 atmosphere and stirred under reflux for 1 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was dissolved in dichloromethane (20 mL) and water (20 mL), and then organic layer was separated. Organic compounds were extracted with dichloromethane (2× 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated by rotary evaporator. Flash column chromatography (10% MeOH/dichloromethane) gave the desired product **12b**.

5.3.1. 2-[(2-Methoxymethyl)piperazin-1-yl]-6-nitroquinoline (12b). 87 mg (56%) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (d, 1H, J = 2.4 Hz), 8.39 (dd, 1H, J = 9.2, 2.6 Hz), 8.01 (d, 1H, J = 12.2 Hz), 7.38–7.26 (m, 6H), 6.93 (s, 1H), 3.85 (t, 1H, J = 8.4 Hz), 3.71–3.55 (m, 5H), 3.29 (d, 1H, J = 12.0 Hz), 3.18 (s, 3H), 2.94 (q, 2H, J = 13.6 Hz), 2.65 (d, 1H, J = 12.0 Hz), 2.52 (t, 1H, J = 12.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 151.5, 141.7, 138.4, 138.0, 128.8, 128.3, 127.2, 127.0, 124.2, 123.5, 121.0, 111.1, 70.6, 59.1, 50.3, 45.8, 45.3, 40.9; MS (FAB) *m/z* (relative intensity) 303 $[M+H]^+$ (100), 287, 257. HRMS (FAB) *m/z* $C_{15}H_{18}O_3N_4$ $[M+H]^+$ Calcd: 303.1457. Found: 303.1465.

5.3.2. 2-[2-(Ethoxymethyl)piperazin-1-yl]-6-nitroquinoline (12c). 39 mg (83%) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, 1H, J = 2.4 Hz), 8.29 (dd, 1H, J = 9.3, 2.7 Hz), 7.97 (d, 1H, J = 9.3 Hz), 7.65 (d, 1H, J = 9.3 Hz), 7.09 (d, 1H, J = 9.4 Hz), 4.64 (br s, 1H), 4.52 (d, 1H, J = 13.0 Hz), 3.88 (dd, 1H, J = 9.3, 2.7 Hz), 3.63 (dd, 1H, J = 9.2, 2.6 Hz), 3.53 (dd, 1H, J = 9.2, 2.6 Hz), 3.50 (d, 2H, J = 7.0 Hz), 3.36 (d, 1H, J = 12.2 Hz), 3.26 (dd, 1H, J = 9.2, 2.3 Hz), 3.20 (d, 1H, J = 11.5 Hz), 3.00 (dd, 1H, J = 9.3, 2.7 Hz), 2.88 (q, 1H, J = 8.4 Hz), 1.15 (t, 3H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 151.5, 141.8, 138.4, 127.1, 124.2, 123.6, 121.1, 111.1, 68.2, 66.8, 51.0, 46.3, 45.8, 41.3, 15.3; MS (FAB) *m/z* 317 $[M+H]^+$. HRMS (FAB) *m/z* $C_{16}H_{21}O_3N_4$ $[M+H]^+$ Calcd: 317.1614. Found: 317.1615.

5.3.3. 6-Nitro-2-(2-propoxymethylpiperazin-1-yl)quinoline (12d). 31 mg (68%) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, 1H, J = 2.4 Hz), 8.28 (dd, 1H, J = 9.3, 2.7 Hz), 7.94 (d, 1H, J = 9.4 Hz), 7.63 (d, 1H, J = 9.4 Hz), 7.09 (d, 1H, J = 9.4 Hz), 4.61 (br s, 1H), 4.51 (dd, 1H, J = 13.0 Hz), 3.86 (dd, 1H, J = 9.3, 2.7 Hz), 3.63 (dd, 1H, J = 9.2, 2.6 Hz), 3.40 (t, 2H, J = 7.0 Hz), 3.36 (d, 1H, J = 14.0 Hz), 3.23 (dd, 1H, J = 9.2, 2.3 Hz), 3.17 (d, 1H, J = 11.5 Hz), 2.98 (dd, 1H, J = 9.3, 2.7 Hz), 2.86 (q, 1H, J = 8.3 Hz), 2.07 (br s, 1H), 1.45–1.58 (m, 2H), 0.84 (t, 3H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 158.7, 151.5, 141.7, 138.4, 127.0, 124.2, 123.5, 121.1, 111.1, 73.1, 68.5, 51.1, 46.5, 46.0, 41.4, 22.9, 10.5; MS (FAB) *m/z* 331 $[M+H]^+$. HRMS (FAB) *m/z* $C_{17}H_{23}O_3N_4$ $[M+H]^+$ Calcd: 331.1770. Found: 331.1778.

5.3.4. 2-[(2-Butoxymethyl)piperazin-1-yl]-6-nitroquinoline (12e). 31 mg (71%) as a bright yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, 1H, J = 2.4 Hz), 8.27 (dd, 1H, J = 9.3, 2.7 Hz), 7.93 (d, 1H, J = 9.4 Hz), 7.62 (d, 1H, J = 9.4 Hz), 7.08 (d, 1H, J = 9.4 Hz), 4.60 (br s, 1H), 4.50 (dd, 1H, J = 12.4 Hz), 3.85 (dd, 1H, J = 9.3, 2.7 Hz), 3.62 (dd, 1H, J = 9.2, 2.6 Hz), 3.44 (t, 2H, J = 7.0 Hz), 3.31 (d, 1H, J = 12.0 Hz), 3.22 (dd, 1H, J = 9.2, 2.3 Hz), 3.16 (d, 1H, J = 11.0 Hz), 2.98 (dd, 1H, J = 9.3, 2.7 Hz), 2.86 (t, 1H, J = 14.0 Hz), 1.90 (br s, 1H), 1.43–1.51 (m, 2H), 1.22–1.32 (m, 2H), 0.84 (t, 3H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 151.4, 141.7, 138.3, 127.0, 124.2, 123.5, 121.0, 111.1, 71.3, 68.4, 51.2, 46.5, 46.0, 41.4, 31.7, 19.2, 13.8; MS (FAB) m/z 345 $[M+H]^+$. HRMS (FAB) m/z $C_{18}H_{25}O_3N_4$ $[M+H]^+$ Calcd: 345.1927. Found: 345.1926.

5.3.5. 2-[2-(Hydroxymethyl)piperazin-1-yl]-6-nitroquinoline (12a). The desired compound was prepared from methoxy compound **8b** (20 mg, 0.066 mmol) and boron tribromide (0.33 mL, 0.33 mmol) using a method analogue to the preparation of compound **6** and purified by flash chromatography (10% MeOH/dichloromethane), giving **12a** (8 mg, 42%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, 1H, J = 2.6 Hz), 8.30 (dd, 1H, J = 9.4, 2.8 Hz), 7.98 (d, 1H, J = 9.6 Hz), 7.63 (d, 1H, J = 9.2 Hz), 7.09 (d, 1H, J = 9.0 Hz), 4.86 (br s, 1H), 4.28–4.14 (m, 2H), 4.03 (dd, 1H, J = 10.8, 3.8 Hz), 3.60 (td, 1H, J = 12.6, 3.5 Hz), 3.38 (d, 1H, J = 12.2 Hz), 3.26–3.19 (m, 1H), 3.08 (dd, 1H, J = 12.2, 4.4 Hz), 2.93 (td, 1H, J = 12.0, 4.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.2, 151.3, 142.3, 138.3, 127.2, 124.3, 123.7, 121.4, 111.3, 64.8, 52.4, 42.9, 30.0, 18.9; MS (CI) m/z 289 $[M+H]^+$, 259, 190. HRMS (FAB) m/z $C_{14}H_{16}O_3N_4$ $[M+H]^+$ Calcd: 289.1301. Found: 289.1297.

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