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Syntheses and Binding Affinities of 6-Nitroquipazine Analogues for Serotonin Transporter. Part 2:† 4-Substituted 6-Nitroquipazines

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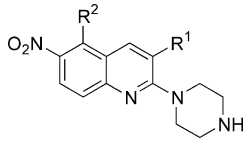
Abstract—Eleven 4-substituted derivatives of 6-nitroquipazine were synthesized and evaluated for their abilities to displace [^{3}H]citalopram binding to the rat cortical synaptic membranes. Among them, 4-chloro-6-nitroquipazine was shown to possess the highest binding affinity ($K_i = 0.03$ nM) which was approximately 6 times higher than that of 6-nitroquipazine ($K_i = 0.17$ nM) itself. In this paper, we describe the syntheses of 4-substituted 6-nitroquipazine derivatives, the results of corresponding biological evaluation and the SAR study.

Introduction

Serotonin (5-hydroxytryptamine or 5-HT) has been known as a neurotransmitter associated with many psychiatric diseases including depression, anxiety, obsessive-compulsive disorder, psychosis, eating disorder, and dependence in human brain.[2,3] It could not only bind to seven receptor subclasses but also be reuptaken into presynaptic neurons through its transporter, which plays an important role in modulation of serotonin concentrations in neuronal synapses. Therefore, inhibition of serotonin transporter results in increased concentration of serotonin in the synaptic cleft. Serotonin transporter (SERT) is an integral protein consisted of 12 transmembrane domains with the NH_2- and COOH- terminal domains in the cytoplasm.[4,5]

It has been reported that 6-nitroquipazine (**1**, 6-NQ) has higher binding affinity for SERT than other selective serotonin reuptake inhibitors (SSRIs, i.e., citalopram, fluoxetine, fluvoxamine, paroxetine and sertraline) approved by the Food and Drug Administration as antidepressants.[6–10] Although a few number of 6-NQ derivatives such as 5-iodo-6-nitroquipazine (**2**) have been reported,[11–15] the detailed SAR studies on them have not been performed. Previously, we reported not only a novel synthetic route of 6-NQ itself[16] but also syntheses and in vitro biological results of several 6-NQ derivatives toward SERT.[1] Moreover, during efforts for the syntheses of several analogues, 3-(3-[^{18}F]fluoropropyl)-6-nitroquipazine (**3**, $K_i = 0.32$ nM) was prepared as a positron emission tomography (PET) imaging agent.[17] The biodistribution and the metabolic decomposition rate of **3** were investigated in mice brain. Through these previous studies, we found that the substitution of several alkyl groups at C3 position would be tolerated, while a nitro group at C6 position and a piperazine group at C2 position play a critically important role in retaining the strong binding affinity for SERT. In this paper, 4-substituted 6-NQ derivatives were synthesized and tested for their potential abilities to displace [^{3}H]citalopram binding to the rat cortical synaptic membranes.



1, R^1=H, R^2=H,
2, R^1=H, R^2=I,
3, R^1=$CH_2CH_2CH_2[^{18}F]F$, R^2=H,

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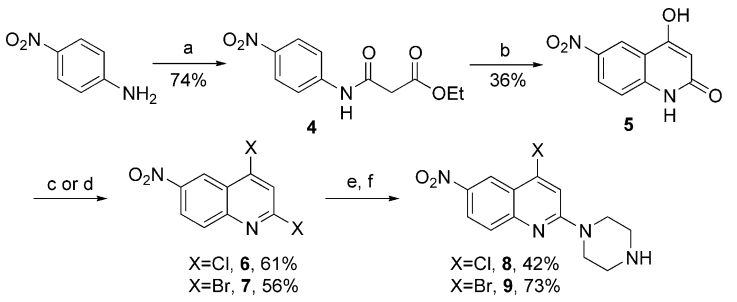


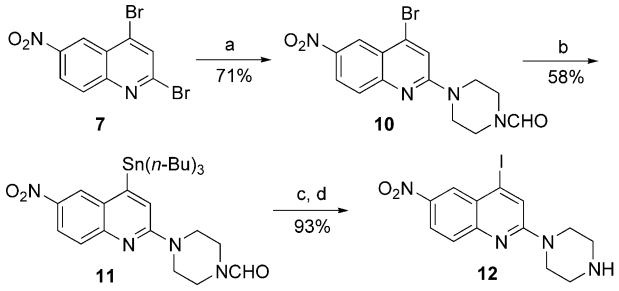
Scheme 1. Reagents and conditions: (a) diethylmalonate, 150 °C, 12 h; (b) PPA, 120 °C, 3 h; (c) $POCl_3$, reflux, 6 h; (d) $POBr_3$, CH_3CN, reflux, 3 h; (e) 1-piperazinecarboxaldehyde, DMF, 60 °C, 3 h; (f) 4 M H_2SO_4, THF, 80 °C, 3 h.

Chemistry

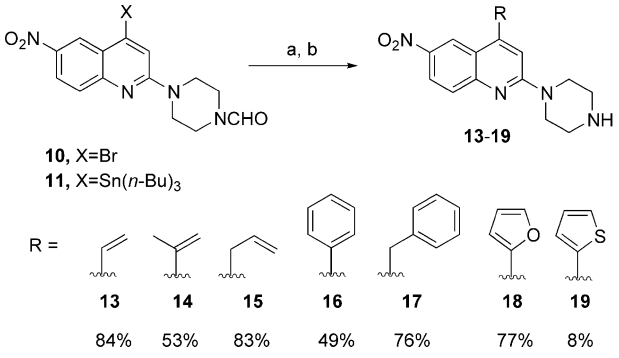
The key intermediate required to synthesize 4-substituted 6-NQ is either 2,4-dichloro- (**6**) or 2,4-dibromo-6-nitroquinoline (**7**), which was prepared in three steps from 4-nitroaniline as shown in Scheme 1. *N*-(4-Nitrophenyl)malonamic acid ethyl ester (**4**) was synthesized by the reaction of 4-nitroaniline and 10 equiv of neat diethylmalonate at 150 °C for 12 h, isolated by column chromatography after removal of excess malonate by distillation. The ring formation of **4** by heating with polyphosphoric acid gave 4-hydroxy-6-nitro-2(1*H*)quinolinone (**5**) in 36% yield. This polar and insoluble compound was purified by dissolving in aqueous NaOH (1 N) solution and supernatant substance was filtered out. The filtrate was acidified by addition of aqueous H_2SO_4 (4 M) solution until slightly acidic to give pale brown precipitate **5**. 2,4-Dichloro-6-nitroquinoline (**6**) was obtained by chlorination of **5** with phosphorus oxychloride in 61% yield. Likewise, refluxing of **5** with phosphorus oxybromide in acetonitrile gave 2,4-dibromo-6-nitroquinoline (**7**) in 56% yield. 4-Chloro- (**8**) and 4-bromo-6-nitroquipazine (**9**) were synthesized by the reaction of corresponding 2,4-dihalo-6-nitroquinolines and 1.5 equiv of 1-piperazinecarboxaldehyde in DMF at 60 °C for 3 h, followed by deformylation of *N*-formyl moiety in 4 M H_2SO_4 at 80 °C for 3 h.

N-Formyl-4-bromo-6-nitroquipazine (**10**) obtained by the reaction of 2,4-dibromoquinoline and 1-piperazinecarboxaldehyde was used for the introduction of several substituents including iodine at C4 position. As shown in Scheme 2, the palladium catalyzed coupling reaction of heteroaryl bromide (**10**) and bis(tri-*n*-butyltin) gave *N*-formyl-4-tributylstannanyl-6-nitroquipazine (**11**) in 58% yield. The tributyltin moiety was substituted with iodine under oxidative condition to give *N*-formyl-4-iodo-6-nitroquipazine, which was hydrolyzed using aqueous H_2SO_4 (4 M) to form 4-iodo-6-nitroquipazine (**12**).

Various 4-substituted 6-NQs were synthesized by Stille coupling reaction under the similar condition (Scheme 3). Compounds **14**, **16**, **17**, and **19** were prepared by the reaction of *N*-formyl-4-tributylstannanyl-6-nitroquipazine with 2-bromopropene, iodobenzene, benzylbromide and 2-bromothiophene, respectively. Compounds **13**, **15**, and **18** were obtained by the reaction of *N*-formyl-4-



Scheme 2. Reagents and conditions: (a) 1-piperazinecarboxaldehyde, DMF, 120 °C, 3 h; (b) bis(tri-*n*-butyltin), $Pd(PPh_3)_4$, dioxane, reflux, 12 h; (c) NaI, H_3PO_4, dichloramine T, EtOH, rt, 30 min; (d) 4 M H_2SO_4, THF, 80 °C, 3 h.

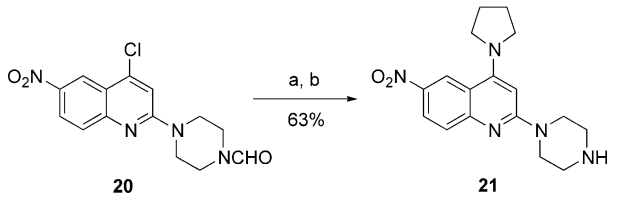


Scheme 3. Reagents and conditions: (a) $Pd(PPh_3)_4$, R-Y (Y = Sn(*n*-Bu)$_3$, Br or I), dioxane, reflux, 2–32 h; (d) 4 M H_2SO_4, THF, 80 °C, 3

bromo-6-nitroquipazine (**10**) and tributyl(vinyl)tin, allyltributyltin and 2-tributylstannanylfuran, respectively.

After Stille coupling reaction, the toxic by-product, tributyltin halide was quenched by addition of 10% aqueous KF solution into reaction mixture, which was stirred for 3 h. The resulting insoluble substances, white tributyltin polymer and palladium metal were removed by filtration with Celite.

The nucleophilic aromatic substitution reaction of **20** with pyrrolidine at C4 position was carried out by heating at 120 °C for 3 h. Deprotection of *N*-formyl moiety under acidic condition with 4 M H_2SO_4 afforded 6-nitro-4-(pyrrolidin-1-yl)quipazine (**21**) (Scheme 4).



20 21

Scheme 4. Reagents and conditions: (a) pyrrolidine, DMF, 120 °C, 3 h; (b) 4 M H_2SO_4, THF, 80 °C, 3 h.

Binding Studies

According to the method of our previous study,[1] using crude synaptic membranes prepared from the cerebral cortex of male Sprague–Dawley rats,[18] 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 unlabelled 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.[19] Table 1 illustrates the structures and the in vitro binding affinities of eleven 4-substituted derivatives of 6-NQ for the 5-HT transporter, including 6-NQ, fluoxetine, and paroxetine used as reference compounds.

Discussion

N-Formylated 4-halo-6-NQs **10** and **20** were synthesized from 4-nitroaniline and diethylmalonate in four steps as shown in Scheme 1. We synthesized new eleven 6-NQ derivatives including 4-chloro-6-NQ (**8**). Eight 6-NQ derivatives except **8**, **9** and **21** were prepared from a key tri-*n*-butylstannane intermediate **11** by Stille coupling. Compound **21** was obtained by nucleophilic aromatic substitution of **20** with pyrrolidine.

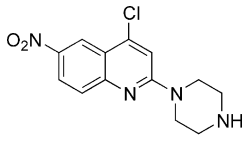
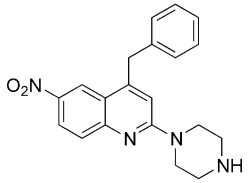
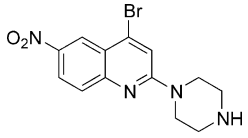
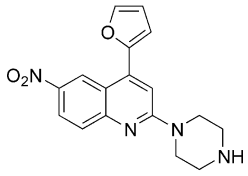
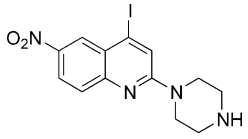
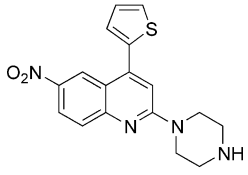
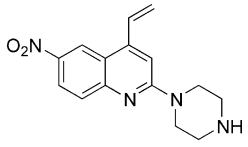
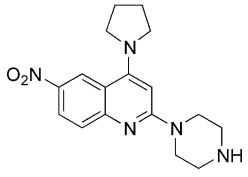
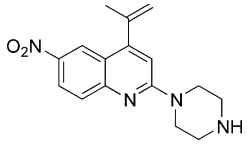
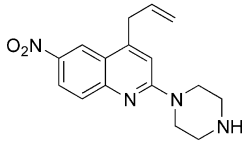
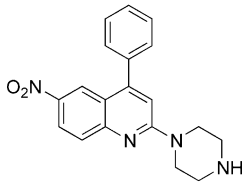
The results of binding affinities of 10 compounds are shown in Table 1. The binding affinities of the 10 6-NQ derivatives are affected by steric hindrance, electronic inductive effect and electronic resonance effect. First, we found a propensity that the bulkier substituent was, the lower binding affinity was. In other words, binding site of SERT is sensitive to steric hindrance with C4 position of 6-NQ. According to this propensity, 4-chloro-6-NQ (**8**) substituted with the smallest group, that is, chlorine atom showed the highest binding affinity among 11 6-NQ derivatives. Surprisingly, the K_i value of 4-chloro-6-NQ (**8**) was 0.03 nM for the rat cortical SERT. This is approximately 6-fold higher than that of 6-NQ itself and the best binding affinity among the SSRIs reported until now, except for ADAM (2-((2-((dimethylamino)-methyl)phenyl)thio)-5-iodoaniline) ($K_i = 0.013$ nM) synthesized by Kung et al.[20] Other halogen derivatives (K_i value of 4-bromo-6-NQ (**9**) = 0.37 nM, K_i value of 4-iodo-6-NQ (**10**) = 1.73 nM) for the rat cortical SERT. As mentioned above, the bulkiness of substituents is responsible for decreasing the binding affinities of the rest 10 6-NQs. In contrast to 4-chloro-6-NQ (**8**), for example, 4-benzyl-6-NQ (**17**) substituted with the bulkiest group has the worst inhibition constant ($K_i = 126.86$ nM) and much lower binding affinity (approximately more than 4000-fold) than **8**. The sensitivity of SERT to the size of substituents indicates that C4 position of 6-NQs is very close to the residue of SERT in the binding site, so there exists steric repulsion between the substituent on C4 position and the binding pocket of SERT. The fact that chlorine is larger than hydrogen but result in better binding affinity could be explained by an electronic factor. By changing from chlorine to other bulkier halogens, the binding affinities for the rat cortical SERT drops by the factor of one order: K_i value of 4-bromo-6-NQ (**9**) = 0.37 nM, K_i value of 4-iodo-6-NQ (**10**) = 1.73 nM. The binding affinity of 4-bromo-6-NQ (**9**) for 5-HT uptake site was obtained by Hashimoto et al. and showed similar trend.[21]

Second, in addition to the aspect of steric hindrance, the electronic environments of the binding pocket should also be taken into account. When compound **18** was compared with compound **19**, we found that there was a significant difference between them in spite of their similar bulkiness. Based on the fact that the furanyl group having oxygen atom with ability to induce hydrogen bond interaction resulted in higher binding affinity than compound **19**, it could be expected that hydrogen bond donor to ligand might be existed in the binding pocket. Although the steric effect of substituents is superior to electronic effect, the hydrogen bond interaction with the residue of SERT would compensate, to some extent, for the drawback resulted from steric repulsion.

The last substituent effect is the electronic resonance effect that influences electron density of quinoline ring. The chemical shifts of H3 of quinoline in ppm are: **1**, 7.01; **8**, 7.14; **9**, 7.81; **12**, 7.96; **13**, 7.06; **16**, 6.95; **17**, 6.77; **18**, 7.25; **19**, 7.05 and **21**, 5.90. With regard to compounds **16**, **19** and **21** having similar size of the substituents and no direct electronic effect to the binding site, ability of amino group (**21**) to donate nonpair electrons to quinoline ring is likely to be superior to the other two ligands **16**, **19**. There is, however, little difference in binding affinities among them.

In summary, we synthesized 11 6-NQ derivatives and performed in vitro test and SAR study. The substitution on C4 position of 6-NQ is largely restricted due to steric repulsion for the binding site. However, a group with hydrogen bond acceptor would reduce disadvantage provided by its bulkiness. We also found that the electron density of quinoline was not an important factor. The binding affinity of 4-chloro-6-NQ (**8**) for SERT is so potent in picomolar level ($K_i = 30$ pM) that this compound would be likely to serve as a new lead compound in the development of potent SSRI. Therefore, more modifications based on 4-chloro-6-NQ (**8**) are currently being pursued.

Table 1. Structures and binding data on the 5-HT transporter of the quipazine derivatives[a]

	Compd	*K*i (nM)		Compd	*K*i (nM)
8		0.03±0.01	**17**		126.86±14.53
9		0.37±0.03	**18**		5.23±0.53
12		1.73±0.02	**19**		67.24±9.49
13		1.82±0.09	**21**		61.12±17.53
14		—[b]		Fluoxetine (Prozac)	22.13±1.77
15		1.67±0.08		Paroxetine	0.53±0.08
16		60.03±17.17	**1**	6-Nitroquipazine	0.17±0.03

[a]The values represent mean SEM of 3–4 separate experiments done in duplicate.
[b]Not measured.

Acknowledgements

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