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Syntheses and Binding Affinities of 6-Nitroquipazine Analogues for Serotonin Transporter. Part 1

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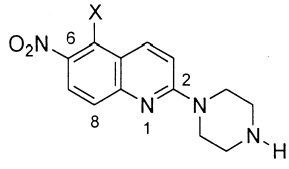
Abstract—6-Nitroquipazine has been known as one of the most potent and selective inhibitors of serotonin transporter in vitro and in vivo. Nine derivatives of 6-nitroquipazine were synthesized and tested for their potential abilities to displace [^{3}H]citalopram binding to the rat cortical membranes.

Serotonin (5-hydoxytryptamine or 5-HT) is a neurotransmitter considered to be related to depression and other psychiatric diseases. 5-HT reuptake sites in the mammalian brain have been studied extensively with radiotracers such as [^{3}H]imipramine, [^{3}H]paroxetine, and [^{3}H]citalopram. 6-Nitroquipazine (**1**, 6-NQ) has been known as one of the most potent and selective antagonists for serotonin transporter (SERT) in vitro[1,2] and in vivo,[3,4] showing higher potency ($K_i = 0.17$ nM) than paroxetine ($K_i = 0.58$ nM) or citalopram ($K_i = 1.50$ nM) for 5-HT reuptake site. Imaging of brain using radiopharmaceuticals is, therefore, expected to be a useful tool for understanding of dysfunction of psychiatric diseases as well as pathological studies of neurotransmission of 5-HT.



1, X=H
1a, X=^{123}I
1b, X=^{76}Br

Although Mathis et al. reported syntheses of 5-[^{123}I]iodo- (**1a**) and 5-[^{76}Br]bromo-6-nitroquipazine (**1b**) as a SPECT and a PET imaging agent, respectively,[5–7] their synthetic methods of quipazine analogues were too inefficient and tricky to give target molecules in good yield. Until we recently reported the regioselective synthesis of 6-nitroquipazine in three steps, in overall 82% yield,[8] only few structure modification of 6-nitroquipazine has been reported.[9] This regioselective synthesis enabled us to modify the structure of 6-NQ and to synthesize the compounds we designed. In this paper, we reported the synthesis of several 6-NQ derivatives and the measurement of their biological activities for 5-HT transporter to find out structure–activity relationship of 6-NQ derivatives.

Chemistry

Nine new quipazine derivatives were prepared according to our synthetic route recently reported.[8] In Scheme 1, 2-chloro-6-nitroquinoline (**11**) was obtained from hydrocarbostyril[9] (**12**). Compounds **2**–**5** were prepared by simple displacement reactions with various corresponding amines.

The first step of our regioseletive synthetic route of 6-NQ resulted in addition of a nitro group. Dinitro compound **13** was also obtained in harsher nitration condition (Scheme 2). Rest steps are the same as the synthesis of 6-NQ.

To check the electronic effects of a nitro group on C6 postion, three 6-haloquipazines were prepared according to Scheme 3. Halogenation of hydrocarbostyril underwent only on C6 position and the rest steps—chlorination followed by aromatization and aromatic substitution reaction—are the same as the synthesis of 6-NQ. As an

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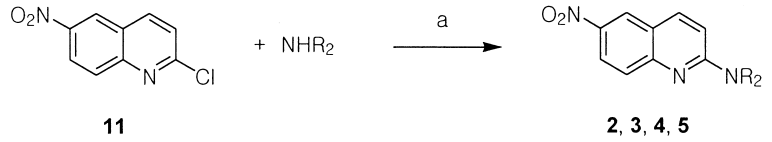
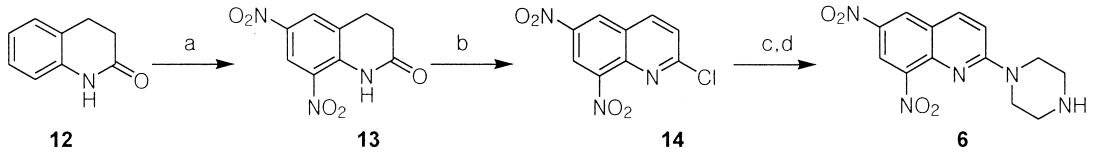
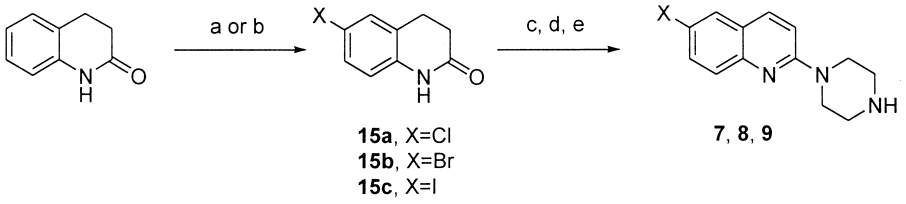


Scheme 1. Reagents and conditions: (a) 2-methylpiperazine (yield, 71%), or *N,N'*-dimethylethylenediamine (56%), or ethylenediamine (95%), or homopiperazine (70%), dioxane, reflux, 4 h.



Scheme 2. Reagents and conditions: (a) concd H_2SO_4, concd HNO_3, 100 °C, 30 min, 47%; (b) $POCl_3$, DMF, 60 °C, 4 h, 98%; (c) 1-piperazinecarboxaldehyde, DMF, rt, 5 min, 70%; (d) 4 M H_2SO_4, 60 °C, 3 h 30 min, 80%.



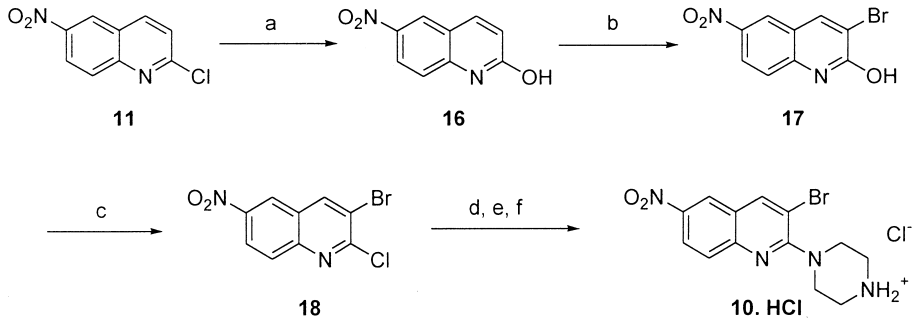
Scheme 3. Reagents and conditions: (a) $NaXO_3/H_2O$, HX, AcOH, rt, X = Cl, Br, 20 min, 55%, 95%, respectively; (b) ICl, AcOH, rt, 12 h, 91%; (c) $POCl_3$, DDQ, DMF, rt, 6 h, 87–93%; (d) 1-piperazinecarboxaldehyde, DMF, 80 °C, 3–10 h, 42–57%; (e) 4 M H_2SO_4, 80 °C, 3–5 h, 57–96%.

alternative route, Sandmeyer reactions from 6-aminoquipazine were examined. Although the reduction of 6-NQ using 10% palladium on charcoal provided 6-aminoquipazine in high yield, several undesired and inseparable products were formed in Sandmeyer reaction.

3-Bromo-6-NQ was prepared by reaction of 6-nitro-2-quinolone (**16**) with sodium bromate in acidic condition, followed by aromatic substitution reaction with piperazine. Compound **16** was obtained from **11** by refluxing in acetic acid (Scheme 4).

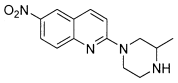
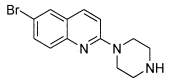
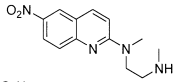
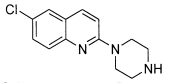
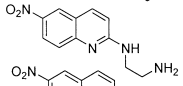
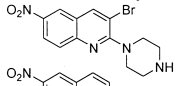
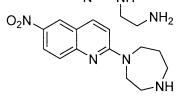
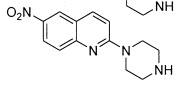
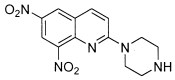
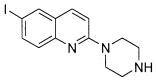
Binding studies

Binding of [^{3}H]citalopram to the 5-HT transporter was measured according to the method of Pähkla et al.[10] with slight modifications using crude synaptic membranes prepared from the rat cerebral cortex.[11] 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 unlabelled compounds between 10^{-11} and 10^{-5} M. IC_{50} values were determined from the competition binding data using computer-assisted curve fitting with GraphPad Prism 3.0 program. Inhibition binding



Scheme 4. Reagents and conditions: (a) AcOH, 100 °C, 10 h, 99%; (b) $NaBrO_3/H_2O$, HBr, 100 °C, 4 h, 76%; (c) $POCl_3$, DMF, 100 °C, 30 min, 93%; (d) 1-piperazinecarboxaldehyde, DMF, 120 °C, 6 h, 97%; (e) 4 M H_2SO_4, 100 °C, 5 h, 50%; (f) HCl(g), MeOH-ether.

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

Compounds		K_i (nM)	Compounds		K_i (nM)
2		22.19 ± 1.70	**8**		0.91 ± 0.07
3		8.43 ± 0.45	**9**		1.68 ± 0.13
4		164.30 ± 4.22	**10**		12.62 ± 1.44
5		1.90 ± 0.15	**1**		0.17 ± 0.03
6		312.85 ± 2.85		Fluoxetine (Prozac)	22.13 ± 1.77
7		6.60 ± 0.52		Paroxetine	0.53 ± 0.08

[a]The values represent mean ± S.E.M of 3–4 separate experiments done in duplicate.

constant (K_i) values were subsequently calculated from IC_{50} values using the Cheng-Prusoff equation.[12] Table 1 illustrates the in vitro binding affinities of 2–10 for the 5-HT transporter including 6-NQ, fluoxetine, and paroxetine.

Discussion

6-NQ has high binding affinity ($K_i = 0.17$ nM) for SERT, while quipazine itself has much lower affinity ($K_i = 63$ nM).[13] Quipazine and its derivatives have been used for the study of serotonin receptor. A nitro group at C6 position plays an important role in retaining the strong binding affinity for SERT. To obtain information on the possible roles of the different substituents on 6-NQ, the structure of 6-NQ was modified. Cappelli[14] and Morreale[15] groups recently reported a comparative molecular field analysis (CoMFA) and pharmacological evaluation of quipazine with subtypes of serotonin receptor. Due to the difficulty of the structural modifications of 6-NQ with remaining 6-nitro group, only few reports of structural modifications of 6-NQ are published. The purpose of this research is to synthesize and evaluate enough number of derivatives of 6-NQ to do CoMFA study.

Introduction of a methyl group to piperazine ring dramatically decreases the affinity by about 2 orders of magnitude (compound **2** versus **1**). Ring-opened compounds **3** and **4** also show reduction in affinities. The fact that primary amine **4** has much lower affinity implies the importance of secondary amine for binding. Compound **5** having homopiperazine shows an affinity slightly higher than nanomolar affinity. As same as the proposed interaction of quipazine in the $5\text{-}HT_3$ receptor by Cappelli et al., secondary amine is important for the charge-assisted interaction between the protonated terminal piperazine nitrogen atom and a negatively charged carboxylic amino acid residue in the SERT.[14]

Addition of a nitro group to C8 (compound **6**) and a bromo group to C3 (compound **10**), and replacement of a nitro group on C6 with an iodo, a bromo or a chloro group (compound **7**–**9**) lead to the wide range of affinities. If the hydrogen-bonding between the heterocyclic nitrogen atom and a suitable hydrogen bond donor in the SERT is important, the electronic density of the nitrogen atom in quinoline ring would greatly be affected by variable substituents.

More syntheses of 6-NQ derivatives are being currently pursued. In addition, more biological studies with some 6-NQ derivatives showing good binding affinities are in progress.

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