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Serotonin Transporter Inhibitors: Synthesis and Binding Potency of 2′-Methyl- and 3′-Methyl-6-nitroquipazine

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Abstract—Racemic 2′-methyl- and 3′-methyl-6-nitroquipazine ligands were selected as targets, synthesized and evaluated at the serotonin transporter employing an in vitro competitive inhibition assay with [^{3}H]paroxetine and rat cortical membrane. The 2′-methyl-6-nitroquipazine was found to be 50 times more potent than the 3′-methyl-substituted counterpart and of comparable potency to the known high affinity agent 5-iodo-6-nitroquipazine.

Introduction

The serotonin transporter (SERT) is an integral membrane protein that is responsible for the reuptake (transport) of the central nervous system biogenic amine 5-hydroxy-tryptamine (5-HT) from the synaptic cleft.[1] Transport of 5-HT is sensitive to the presence of nanomolar concentrations of SERT inhibitor ligands,[2,3] including serotonin selective reuptake inhibitor drugs (SSRIs). The SSRIs are thought to bind to the SERT[4] at the same site as 5-HT or a closely overlapping site(s).[5] The structural features of the SSRI agents are diverse, encompassing agents with either one or two aromatic structural components.[2,6] These drugs along with other SERT ligands have been utilized to develop a limited number of SERT inhibitor pharmacophore models.[7–9] The models describe regions in space for two distinct aromatic moieties that are connected to a carbon backbone containing an amine atom terminus. In some cases, one of the aromatic moieties may be joined to the chain through a heteroatom linkage. The models[7] may be utilized as initial design constructs for the development of new SERT inhibitors.

Investigations to map the SERT in living brain have employed positron emission tomography (PET) imaging techniques.[10,11] These studies have led to the discovery of a limited number of PET imaging agents that are appended with either fluorine-18 or carbon-11 positron-emitting nuclides. The location on the ligands where the short-lived, radiolabels are placed often influences the radiotracer syntheses and stability and also receptor affinity and selectivity.[11,12] Thus, many variables must be considered in the development of a PET ligand. Based on these criteria, the approach in our laboratory towards developing new SERT PET agents has encompassed the use of the SERT pharmacophore models and examination of select structure–affinity relationship (SAR) studies of potent SERT ligands.

Of particular interest are SERT ligands that are composed with only one aromatic structural component, a heteroatom linkage and a minimum of other functional groups. For example, analogue studies[13–16] of the established SERT inhibitor 6-nitroquipazine (6-nitro-2-piperazinylquinoline, DU-24565),[13,17] shown in Figure 1 as ligand **1**, have provided the SERT selective 5-iodo-6-nitroquipazine, ligand **2** (INQUIP). When evaluated in vitro with rat cortical membrane SERT and competitive inhibition binding conditions ([^{3}H]paroxetine), agent **2** has been found to be a more potent ligand (inhibition binding constant of **2**, $K_i = 0.19 \pm 0.09$ nM) than the parent drug **1** ($K_i = 0.23 \pm 0.06$ nM).[16]

Target Ligands

Our interest in the quipazine drug series has been to identify locations on the parent drug **1** that would be suitable for bearing an alkyl group ultimately containing a positron atom label. Since existing SERT pharmacophore models of well known high affinity SSRI ligands describe regions for two distinct aromatic

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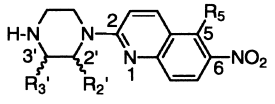


Ligand	R_2'	R_3'	R_5
1	H	H	H
2	H	H	I
3	CH_3	H	H
4	H	CH_3	H

Figure 1. Established serotonin transporter inhibitors (**1** and **2**) and the target ligands (**3** and **4**).

moieties[7,8] and inhibitor 6-nitroquipazine **1** contains only one aromatic member (quinoline ring), then our ligand design approach encompassed the placement of an alkyl group at prospective locations where a second aromatic moiety could be accommodated according to the models. The 2′- or 3′-positions of the piperazine ring of **1** were thought to be these structural locations. Therefore, we sought to initially probe the SAR of racemic 2′- and 3′-substituted variants of 6-nitroquipazine, **1**. The 2′-methyl- and 3′-methyl-6-nitroquipazines, **3** and **4**, respectively, were chosen as target ligands. Finally, analogues of **1** with substituents placed at these positions are unknown.[18]

Methyl groups were favored as initial test substituents based on their synthetic accessibility and value in the past to effectively probe SAR trends in the SSRI paroxetine drug family.[19] Furthermore, preliminary data acquired with methyl-substituted analogues could guide the subsequent study and development of related fluorine atom or methoxyl group appended derivatives (2′-fluoromethyl and 2′-methoxymethyl, respectively; structures not shown) of **3** and **4** as potential PET ligands. Other investigations have revealed that decreased SERT binding potency can be observed by placing unconstrained alkyl groups at or near the terminal amine end of SERT inhibitors.[16,19] Therefore, it was thought that target ligand **4** would serve to further define the sensitivity of this pharmacophore region to simple substituents. Described below are the racemic syntheses of ligands **3** and **4** and the assessment of their in vitro binding potency utilizing rat cortical SERT and [^{3}H]paroxetine.

Syntheses

Preparation of the target ligands **3** and **4** followed the synthetic routes shown in Scheme 1.[20–22] The pathways were patterned after related quipazine syntheses[23,24] and due to the preliminary nature of the investigation they were effected in a racemic fashion. To produce ligand **3**, the more accessible 4-position of (*rac*)-2-methylpiperazine **5** (Lancaster Synthesis) was protected with triphenylmethyl chloride[25] to afford the amine **6**. The coupling of **6** with 2-chloroquinoline (Aldrich Chemical Co.) at reflux temperature[23] for an extended period (125 h) in the presence of carbonate buffer[26] yielded the adduct **7**. Vigorous conditions were required for the coupling, which were considered a consequence of the steric demands imposed by the methyl group adjacent to the 1-position amine reacting center of **6**. Deprotection[27] of intermediate **7** provided the amine **8**. Regiospecific nitration[24] of **8** at 0 °C afforded the target ligand **3**,[28] in an overall 10% yield from starting material **5**. Formation of ligand **4** involved the coupling of **5** with 2-chloroquinoline to yield the amine **6**. Nitration of **6** afforded the target agent **4**,[29] in an overall yield of 55%.

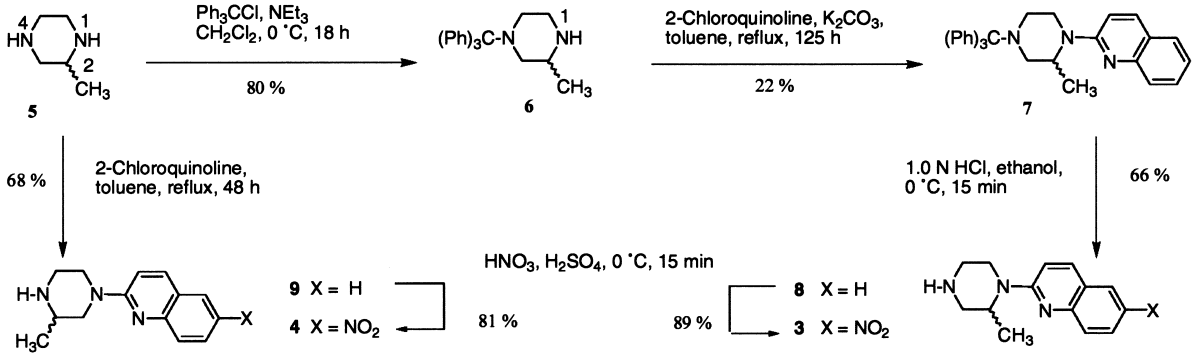
Binding Assessments

The in vitro inhibition binding constants (K_i) of ligands **1**, **3** and **4** were determined using an established competition assay (rat cortical SERT, [^{3}H]paroxetine)[30] which is a modified method of Habert.[31] The measured K_i data (mean±S.E.M., $n=3$) are summarized in Table 1. The in vitro assays were performed with aliquots of

Table 1. The in vitro inhibition binding constants (K_i) and equipotent molar ratios (EPMR) of the standard 6-nitroquipazine (**1**) and the target analogues (**3**–**4**)

Ligand	Inhibition constant, K_i (nM)[a]	EPMR[b]
1	0.163±0.053	1.0
3	0.081±0.061	0.49
4	4.56±2.4	28

[a]Data from competition assays using rat cortical membranes labeled with [^{3}H]paroxetine. The K_i data calculated from IC_{50} values represent the mean±S.E.M. of three or more individual determinations.
[b]The equipotent molar ratio (EMPR) is the ratio of K_i values; K_i analogue (**3** or **4**): K_i 6-nitroquipazine (**1**, standard).



Scheme 1. Synthetic routes to the target ligands **3** and **4**.

partially purified rat cortex membrane tissue (Pel-Freeze Biologicals, Inc.). Suspensions were incubated with 0.25 nM [^{3}H]paroxetine (DuPont-NEN, sp. act. 2.5 Ci mmol^{-1}) and decreasing concentrations (10^{-6} to 10^{-11} M) of **1** (standard; Research Biochemicals, Inc.), **3**, **4** or unlabeled paroxetine (SmithKline-Beecham). Incubations were terminated by dilution with ice-cold buffer and then filtered and washed rapidly on a Brandell cell harvester, air dried and then counted with a Packard scintillation counter.

Specific binding was defined as the binding difference in the presence and absence of unlabeled paroxetine. Nonspecific binding was defined by 1 μM paroxetine to the incubation media. Binding data were analyzed by conventional Hill plots (data not shown) where percent of total specific binding versus −log molar concentration of inhibitor **1**, **3**, **4** or unlabeled paroxetine (six or more data points per curve) were plotted and the inhibitor concentrations (IC_{50}) required to obtain 50% inhibition of [^{3}H]paroxetine binding were determined. Assay data points were accomplished in triplicate and the experiments were repeated three times on different days. The inhibition binding constants (K_i) were calculated from the established relationship[32] $K_i = IC_{50}/(1 + [L]/K_D)$ where [L] = is the concentration of free (unbound) [^{3}H]paroxetine and $K_D = 0.15$ nM.[31]

As shown in Table 1, the measured K_i values (nM) indicate that ligand **3** is more potent than the parent agent **1**, whereas ligand **4** is less potent than **1** for displacing [^{3}H]paroxetine from rat cortical SERT. Ligand **3** was 50 times more potent than **4** in the competition assay. The equipotent molar ratios (EPMR) calculated for **3** and **4** (K_i of **3** or **4**:K_i of **1**) are 0.49 and 28, respectively. A similar assay carried out earlier by others[16,33] evaluating 5-iodo-6-nitroquipazine **2** and the parent ligand **1** provided an EMPR value of 0.83 (K_i of **2**:K_i of **1** = 0.19 nM:0.23 nM). A comparison of the EPMR values for ligands **2** versus **3** reveals that the 2′-methyl-6-nitroquipazine **3** is of similar potency to the 5-iodo variant **2**.

Conclusions

The 6-nitroquipazine analogues **3** and **4** were selected as target ligands based on the methyl substituent locations corresponding to possible points of attachment of a second aromatic ring found in established pharmacophore models of well known SSRI ligands. Methyl groups were employed as simple structure–affinity probes in order to define which regiochemical analogue might serve as a lead agent for subsequent elaboration into fluorine atom or methoxyl group containing derivatives to serve as potential PET ligands. Agents **3** and **4** were synthesized by direct routes and were provided in adequate amounts for in vitro binding evaluations. Using competitive inhibition assays with partially purified rat cortical SERT, the 2′-methyl-substituted ligand **3** was found to be more potent than the parent agent **1**, and 50-fold more potent than the 3′-methyl-substituted agent **4** for displacing [^{3}H]paroxetine. Ligand **3** was found to be of comparable potency relative to the known high affinity SERT selective agent 5-iodo-6-nitroquipazine, ligand **2**.

Since placement of a methyl group at the 2′-position of **1** serves to enhance SERT affinity, then it is plausible that certain substituents at this location may be interacting with a portion of the SERT binding domain that normally accommodates the second aromatic ring moiety found in most SSRIs. The reduced binding potency of ligand **4** suggests that the 3′-position of the parent drug **1** is a location that is sensitive to substitution. This observation parallels an earlier ligand trend, where placement of simple alkyl groups at or near the terminal amine end of some SERT inhibitors results in decreased SERT affinity.

The racemic 2′-methyl-6-nitroquipazine, ligand **3**, is a potent SERT inhibitor that serves as a new lead agent for further study. Investigations to improve the synthetic yield of **3** and to assess the eudesmic ratio of the enantiomers of **3** are in progress. Efforts to prepare the 2′-fluoromethyl- and 2′-methoxymethyl-analogues of **1** as non-radioactive congeners of prospective PET agents and also experiments to demonstrate SERT selectivity of ligand **3** and variants are underway. The results from these SARs and related studies shall be reported in the near future.

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28. Ligand **3** was isolated in pure form as an orange colored glass; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (3H, d, J = 7.1 Hz), 2.84 (1H, dt, J = 12.3 Hz, 4.8 Hz), 2.98 (1H, d, J = 11.5 Hz), 3.07 (1H, dd, J = 12.5 Hz, 4.5 Hz), 3.14–3.28 (2H, m), 4.0–4.15 (1H, m), 4.46 (1H, br d, J = 12.5 Hz), 4.68 (3H, br s), 7.03 (1H, d, J = 9.5 Hz), 7.64 (1H, d, J = 9.1 Hz), 7.95 (1H, d, J = 9.1 Hz), 8.29 (1H, dd, J = 9.5 Hz, 2.5 Hz), 8.54 (1H, d, J = 2.5 Hz). Anal. ($C_{14}H_{16}N_4O_2$) C, H.
29. Agent **4** was obtained in pure form as an orange-yellow semi-solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (3H, d, J = 6.2 Hz), 1.75 (1H, br s), 2.70 (1H, t J = 12.5 Hz), 2.6–3.00 (2H, m), 3.09 (1H, t, J = 12.2 Hz), 3.18 (1H, d, J = 12.1 Hz), 4.52 (2H, d, J = 12.5 Hz), 7.07 (1H, d, J = 9.0 Hz), 7.66 (1H, d, J = 9.5 Hz), 7.96 (1H, d, J = 9.5 Hz), 8.30 (1H, dd, J = 9.0 Hz, 2.5 Hz), 8.53 (1H, d, J = 2.5 Hz). Anal. ($C_{14}H_{16}N_4O_2$) C, H.
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