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Conformational Analysis of Tandospirone in Aqueous Solution: Lead Evolution of Potent Dopamine D_4 Receptor Ligands

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Abstract—The significant contribution of folded conformation (**2**) of the anxiolytic tandospirone (**1**) in aqueous solution was verified by dynamic ^{1}H NMR. A structurally rigid mimic of **2** was designed and synthesized to evaluate the implication of **2** towards neuroleptic receptor binding. The designed structures provided a new rigid scaffold for dopamine D_4 ligands.

Introduction

Tandospirone (**1**, Fig. 1),[1] developed as an anxiolytic drug, is an aryl-piperazine compound that binds to both 5-HT_{1A} and dopamine D_4 receptors.[2] Unlike buspirone, it has weak binding to the dopamine D_2 receptor[3] and binds to neither the benzodiazepine nor GABA receptor.[4]

The pharmacological profile of anxiolytic drugs is considered to be dependent on selectivity in receptor binding, for which molecular modeling studies have commonly described an aromatic ring binding site, a nitrogen binding site and a hydrophobic accessory binding site as primary binding sites.[5]

Arrangement of those parts is different among receptor types and it is difficult to identify those relative geometries for the aryl-piperazines because of structural flexibility.

In a previous paper,[6] we studied a conformational analysis of **1** in aqueous media using ^{1}H NMR together with molecular modeling and showed that there is a significant contribution of a folded conformer (**2**, Fig. 1) of **1** which is stabilized by hydrophobic interactions between the bicyclo ring moiety and the pyrimidine ring in aqueous media.

Folded conformations in aqueous solution have also been reported for Taxol[7] and substance P antagonists[8] and the importance of these conformations for bioactivity have been shown in both cases.

Conformational changes of **1** were further examined in the present study by means of dynamic ^{1}H NMR spectroscopy to estimate the activation energy of the conformational change. The result of the dynamic NMR prompted us to design structurally simulated rigid analogues of the folded conformation (**2**), to study the contribution of **2** to the anxiolytic activity of **1**.

Kinetics

Dynamic ^{1}H NMR studies of **1** were conducted in buffer solution.[9] For a kinetic run, a sample was placed in a pre-calibrated ^{1}H NMR probe that was preset to a specific temperature. After thermal equilibration of the sample, the ^{1}H NMR spectra were recorded at different temperatures ranging from 25 to 50 °C.

The resonances of the alkyl chain part of **1** should be affected by the temperature due to conformational

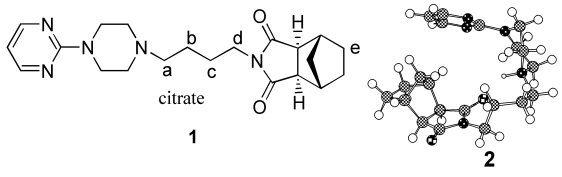

Figure 1. Chemical structure of tandospirone (**1**) (left) and its folded conformation (**2**) (right).

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changes if this rate is slow enough in the NMR time scale. The spectral lines of protons b are broad and complex at 25 °C as shown in Figure 2. When the temperature was increased gradually, the signal pattern of b became simpler. The chemical shift difference between the two protons at b were found to be different by more than 0.005 ppm at 25 °C; however, the resonances were essentially the same at 50 °C. This change of the pattern by temperature suggested two protons at the b position became nearly equivalent when conformation of **1** became flexible from **2** to an extended conformation. Protons at a, c, and d of **1** were hardly analyzed in a variable temperature because of multiplicity and overlapping. Because of the complex pattern of ^{1}H NMR resonances, we obtained only an approximate activation energy of the conformational change, 6–7 kcal/mol.[10] This observation confirmed the considerable contribution of **2** in the conformational space of **1** in aqueous solution.

Design of Conformational Mimics

As a practical method to study how a specific conformation of a compound contributes to its biological activities, we performed a structural search using a query which fills our hypothesis for designing conformational mimics. Our assumption consists of three critical points that are an aromatic ring, a basic nitrogen, and a hydrophobic region in relation to a pharmacophore of **1** as shown in Figure 3.

An aromatic ring and a basic nitrogen in the structure of **1** forming an aryl-piperazine moiety often regarded as a mimic structure of 5-hydroxy-tryptamine (5-HT), while a hydrophobic part is needed for higher 5-HT_{1A} affinity.[5] The acceptable distances in the query were acquired from the structure of the folded conformation (**2**).

The structural search was carried out using MDDR-3D[11] database and Cambridge Crystallographic Database[12] to find 234 and 32 compounds, respectively. Highly flexible compounds with more than three rotatable bonds were eliminated and remaining compounds were categorized to 18 groups based on similarity. Then, each of the groups were manually evaluated for both geometric and steric fitness to conformer **2**, and mezilamine (**3**), a known dopamine D_2 ligand[13] was selected as a preliminary lead structure.

The target compound to be synthesized (**4**) was designed by replacing the methyl group attached to the sulfur atom with a phenyl group because a molecular modeling study showed the methyl group is somewhat small to mimic the bulky bicyclo ring moiety of **2**. The X-ray crystallographic data[12] and modeling showed that the phenyl group of **4** attached to the pyrimidine ring via the sulfur atom is oriented perpendicular to the plane of a pyrimidine ring (Fig. 4).

Related analogues (**16**, **21a** and **21b**) were synthesized as shown in Scheme 2 varying the bulkiness of each hydrophobic moiety in **4** to examine further changes in receptor affinities.

Chemistry

The designed compound (**4**) was synthesized as shown in Scheme 1. Methylphenylsulfonium ylide (**5**) was prepared by the reaction of barbituric acid with methylphenyl sulfoxide in the presence of acetic anhydride. The methyl group was removed by treatment of **5** with $POCl_3$ to yield **6**. In the case of the related diphenyl sulfonium ylide compound, no reaction occurred and the starting ylide was recovered quantitatively. The methyl group at the sulfur atom of **5** was eliminated as

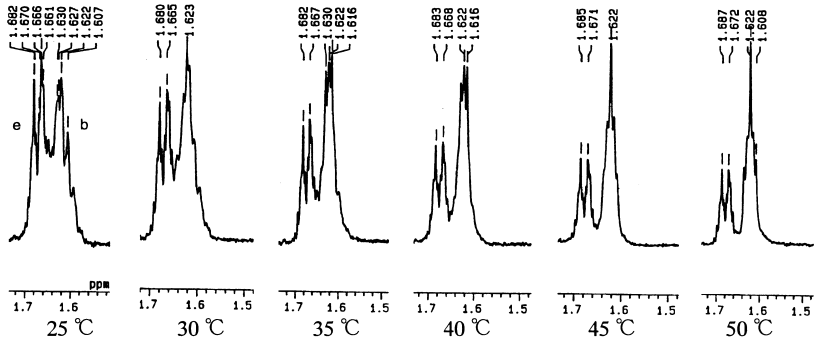


Figure 2. Expanded regions of ^{1}H NMR spectra of tandospirone (protons b and e) at variable temperatures.

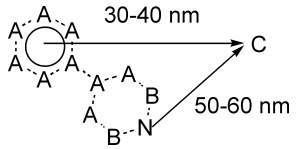


Figure 3. Query for 3D database search. A, B, and C denote carbon or nitrogen, carbon, and hydrophobic moiety, respectively.

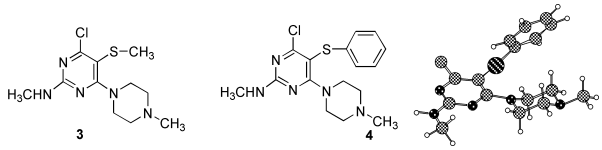
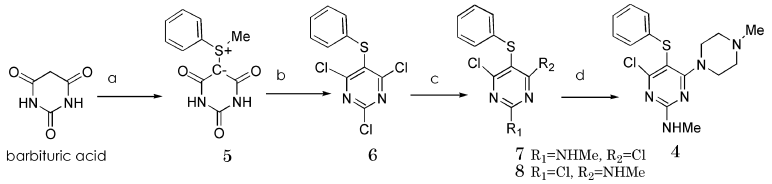
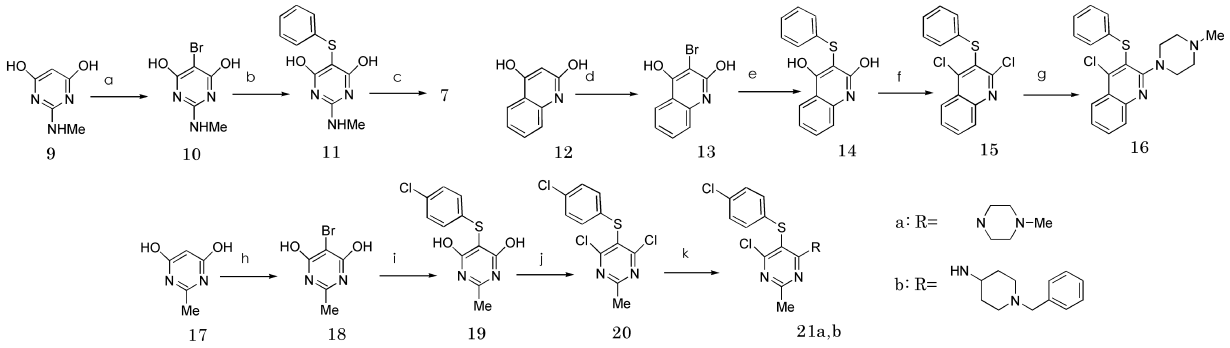


Figure 4. Structures of mezilamine (**3**) and designed compounds (**4**) with 3D structure from molecular modeling.



Scheme 1. Reagents, conditions, and yields: (a) methylphenylsulfoxide, Ac_2O/AcOH, 90 °C, 2 h, quantitative yield; (b) $POCl_3$/dimethyl aniline, reflux, 24 h, quantitative yield; (c) 40% $MeNH_2$ aq/methylethyl ketone, 5 °C, 2 h, then ambient temperature, 80% for **6** and 10% for **7**; (d) *N*-methylpiperazine, EtOH, reflux, 3 h, 60%.



Scheme 2. Reagents, conditions and yields: (a) Br_2/AcOH, rt, overnight, 40%; (b) thiophenol, NaH/DMF, reflux, 8 h, 95%; (c) $POCl_3$, reflux, 2 h, 40%; (d) Br_2/AcOH, rt, overnight, 84%; (e) thiophenol, NaH/DMF, rt, 4 h, 91%; (f) $POCl_3$, reflux, 2 h, 97%; (g) *N*-methylpiperazine, EtOH, reflux, 24 h, 22%; (h) Br_2/AcOH, rt, overnight, 40%; (i) *p*-chlorothiophenol, NaH/DMF, reflux, 8 h, 95%; (j) $POCl_3$, reflux, 2 h, 20%; (k) for **21a**: *N*-methylpiperazine, 2-butanone, K_2CO_3, H_2O, reflux, 1 h, 55%; for **21b**: 4-amino-1-benzylpiperidine, 2-butanone, K_2CO_3, H_2O, reflux, 1 h, 56%.

methyl chloride, whereas removal of phenyl group might be difficult by the same reaction.[14] The reaction of a primary amine with trichlorinated molecule (**6**) gave a mixture of isomers bearing the amino substituent in either position 2 (**7**) or 4 (**8**). These isomers can be separated easily by silica gel column chromatography. Major product was found to be the product substituted in position 2. 2-Methylamino-4,6-dichloro-5-phenylthio pyrimidine (**7**) was condensed with an *N*-methyl piperazine to yield **4**. The other isomer (**8**) was treated with *N*-methylpiperazine to give a condensed compound, which was confirmed to show only weak affinity ($K_i > 10^{-6}$) for the 5-HT_{1A} and dopamine receptors. In both cases, di-*N*-methylpiperazine substituted derivatives were not isolated under the reaction condition.

The structure of each isomer (**7** or **8**) was determined by ^{13}C NMR spectroscopy. Comparison of the ^{13}C chemical shifts in pyrimidines, **6**, **7**, and **8** permits their identification (Table 1).

Pyrimidine and trichlorinated compound (**6**) have two equivalent pyrimidine carbons. The isomer (**8**) has four different pyrimidine carbons, while isomer (**7**) has two equivalent pyrimidine carbons. Carbons 4 and 6 of the pyrimidine ring should be equivalent for **7** and non-equivalent for **8**. Thus, each isomer could be identified. Compound (**7**) was also synthesized as shown in Scheme 2. Pyrimidine (**9**) was prepared by the condensation of diethyl malonate and *N*-methyl guanidine. Bromination of **9** followed by substitution by thiophenol gave **11**, which was chlorinated with $POCl_3$ to give **7**. Related compounds (**16**, **21a**, and **21b**) were also synthesized in a similar manner as shown in Scheme 2.

Table 1. ^{13}C NMR chemical shifts of pyrimidines

Position of C	Pyrimidine	**6**	**7**	**8**
C(2)	158.8	153.5	167.2	161.7
C(4)	156.4	167.4	160.7	165.2
C(5)	121.4	128.3	112.1	125.5
C(6)	156.4	167.4	160.7	164.5

Results and Discussion

Designed compounds were evaluated in a receptor binding assay for D_2, D_4, and 5-HT_{1A} binding affinities as shown in Table 2.

Table 2. Receptor binding assay[15]

	K_i (nM)		
Compound	D_2	D_4	5-HT_{1A}
1	460[a]	7.2[a]	32[a]
3	5.4	>10,000	430
4	188	250	460
16	31	92	2000
21a	62	260	1500
21b	62	2.3	160

[a]See ref 2.

Compound **3**, originally reported as an anti-psychotic drug,[13] showed strong D_2 affinity and modest 5-HT_{1A} affinity. However, unlike tandospirone (**1**), **3** had no D_4 affinity.[16] Compound **4** designed from the folded conformation (**2**) showed D_2, D_4, and 5-HT_{1A} affinities. Compound **4** has a bulky and hydrophobic S-Ph group as an equivalent of the hydrophobic bicyclo group of **2**, and **3** has a S-Me group in the same position. When the binding profile of **4** was compared to that of **3**, significant enhancement of D_4 affinity was observed in **4**, whereas D_2 affinity of **4** was decreased. In contrast to this, no significant change was observed in 5-HT_{1A} affinity between **3** and **4**. That is, introduction of a bulky and hydrophobic moiety in **4** contributed to the dopamine D_4 affinity. These observations suggested that the folded conformation (**2**) of **1** was responsible for the dopamine D_4 affinity.

In addition to this, related compounds (**16**, **21a**, and **21b**) of **4** also had affinity to the D_4 receptor as well as the D_2 and 5-HT_{1A} receptors. Among them, compound **21b** showed the strongest D_4 affinity and the D_4/D_2 selectivity was also observed. Several dopamine D_4 selective ligands have been reported,[17] but most of them have flexible structures. Compound **4** was considered to be a new scaffold for structurally rigid dopamine D_4 selective ligands.

Summary

The rigid compound (**4**) designed from **2**, the folded conformation of **1** in aqueous solution, revealed that this conformation is mainly responsible for the dopamine D_4 affinity.

It was shown that conformational analysis of flexible drugs in aqueous solvent using NMR measurements and a three-dimensional database search could provide an additional way toward lead evolution processes in drug discovery.

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