Conformational Analysis of the NMDA Receptor Antagonist (1*S*,2*R*)-1-Phenyl-2-[(*S*)-1-aminopropyl]-*N*,*N*-diethylcyclopropanecarboxamide (PPDC) Designed by a Novel Conformational Restriction Method Based on the Structural Feature of Cyclopropane Ring

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Received November 26, 2001; accepted April 16, 2002

(1*S*,2*R*)-1-Phenyl-2-[(*S*)-1-aminopropyl]-*N*,*N*-diethylcyclopropanecarboxamide (2b, PPDC), a new class of potent *N*-methyl-D-aspartic acid (NMDA) receptor antagonist, was designed based on a new method for restricting the conformation of compounds having a cyclopropane ring. The three-dimensional structures of PPDC obtained by the three different methods of X-ray crystallographic analysis, usual MM2-calculations in vacuum, and MM2 calculations based on the nuclear Overhauser effect (NOE) data in D_2O are similar, which are in accord with that hypothesized. These results suggest that this conformational restriction method is particularly effective in designing novel biologically active molecules.

Key words NMDA receptor; cyclopropane; conformational restriction; NMR; calculation

The synthesis of conformationally restricted analogs of a lead compound often results in an improvement of the specific binding affinity for the target molecule.[1—3] Restricting the conformation of a biologically active compound is also effective in investigating the bioactive conformation,[3] which is the conformation the compound assumes in binding to its target molecule.[1—3] In the design of conformationally restricted analogs, it is essential that the conformationally restricted analog should be as similar as possible to the parent compound in size, shape, and molecular weight.[1] Conformationally restricted analogs have usually been designed and synthesized by introducing often bulky cyclic moieties into the lead compounds. Consequently, the chemical and physical properties of these analogs can be quite different from those of the original leads. Because of its structure, a cyclopropane ring is likely to be effective in restricting the conformation of a molecule without changing the chemical and physical properties of the lead compound.[4—8] In fact, cyclopropane rings have already been successfully used to restrict the bioactive conformations of neurotransmitters,[4,5] amino acids,[6] peptides[7] and nucleosides.[8]

We devised a new method for restricting the conformation of compounds having a cyclopropane ring,[9—11] and applied it to the design of the conformationally restricted analogs of (±)-(*Z*)-2-aminomethyl-1-phenyl-*N*,*N*-diethylcyclopropanecarboxamide [milnacipran, (±)-**1**],[12—16] a clinically efficient antidepressant having a cyclopropane structure, to develop efficient NMDA (*N*-methyl-D-aspartic acid) receptor antagonists.[17—25] The structures of the conformationally restricted analogs are shown in Fig. 1.

Various antagonists of NMDA receptors have been developed since the receptors may be involved in both chronic and acute neurodegenerative disorders.[26—28] Some have been shown to be effective in experimental models of epilepsy and stroke.[26—28] Unfortunately, the non-competitive inhibitors currently available frequently have serious behavioral effects[29—31] and cause neuronal vacuolization,[32] while competitive inhibitors are often inactive *in vivo* because of their poor permeability through the blood-brain barrier.[33,34] Therefore, another efficient NMDA receptor antagonist is eagerly desired.

We previously synthesized the above mentioned series of conformationally restricted analogs of milnacipran, and the pharmacological studies showed that (1*S*,2*R*)-1-phenyl-2-[(*S*)-1-aminopropyl]-*N*,*N*-diethylcyclopropanecarboxamide (**2b**, PPDC) is a new class of potent NMDA receptor antagonists.[19,21,23] In this report, we describe detailed three-dimensional structures of PPDC to clarify the receptor-binding conformation.

Design and Pharmacological Effect of PPDC Milnacipran, which shows a potent antidepressant effect due to competitive inhibition of the re-uptake of serotonin (5-HT) in the central nervous system (CNS),[12—16] is also recognized as a non-competitive NMDA receptor antagonist.[35] Although the binding affinity of (±)-**1** for the NMDA receptor is not high, the compound has the advantage of sufficiently pene-

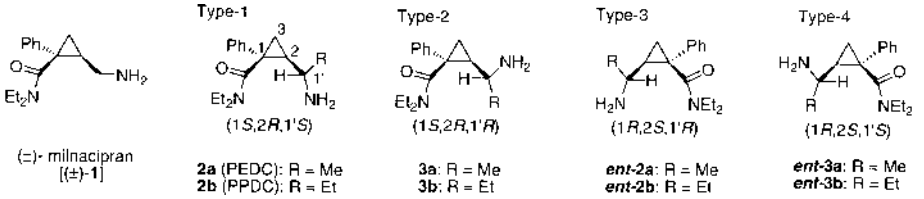

Fig. 1. Milnacipran and Its Conformationally Restricted Analogs

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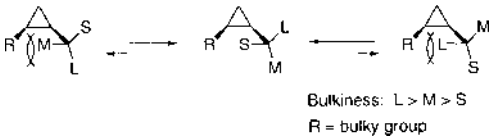

Fig. 2. Conformational Restriction by the Repulsion between Adjacent Substituents on a Cyclopropane Ring

trating into the brain without serious side effects,[15,16] making it a clinically useful antidepressant and therefore a good lead for an efficient NMDA receptor antagonist. This may be because the structure of milnacipran is clearly different from that of previous NMDA receptor antagonists. Thus, we designed and synthesized conformationally restricted analogs of milnacipran using the characteristic cyclopropane structure to increase their specific affinity for the NMDA receptor and thereby to develop potent NMDA receptor antagonists.[17—25]

Adjacent substituents on a cyclopropane ring mutually exert significant steric repulsion because they are fixed in an eclipsed conformation to each other. Consequently, conformations of the substituents on a cyclopropane ring can be restricted by the steric effect of adjacent substituents, especially when they are bulky, as indicated in Fig. 2. We hypothesized that this structural feature of the cyclopropane ring system could be used as a conformational restriction method, and therefore designed the conformationally restricted analogs of milnacipran (±)-**1**. Because the primary amino function of (±)-**1** is essential for the binding affinity for the NMDA receptor,[35] we assumed that the conformation of the aminomethyl moiety would significantly affect the activity of the compound. While the aminomethyl moiety is not so bulky and may freely rotate at least to some extent, the conformers A and B may be preferable to conformer C because of the serious steric repulsion with the bulky diethylcarbamoyl group in conformer C, as shown in Fig. 3. Based on these considerations, we designed four types of conformationally restricted analogs of (±)-**1** with different stereochemistries, *i.e.*, Type-1 and Type-2, and their enantiomers Type-3 and Type-4, as shown in Fig. 1.[9] In these analogs, an alkyl group introduced at the α-position of the amino function restricts the location of the amino group in space due to steric repulsion with the diethylcarbamoyl group. Therefore, the conformation of these compounds can be limited depending on the configuration of the alkyl group introduced; conformer B would be predominant in Type-1 and its enantiomer Type-3, while conformer A would be predominant in Type-2 and its enantiomer Type-4, as shown in Fig. 3.

The conformationally restricted analogs were successfully synthesized and their biological evaluations showed that the analogs with a Type-1 configuration, *i.e.*, **2a** (PEDC) and **2b** (PPDC), are potent NMDA receptor antagonists with IC_{50} values approximately 30-fold stronger than that of (±)-**1**.[17] PPDC (**2b**), in particular, was the most likely candidate since it was a potent NMDA receptor antagonist virtually devoid of the inhibitory effect on 5-HT-uptake, while **2a** (PEDC) was a strong 5-HT-uptake inhibitor like the parent compound milnacipran.[17] Pharmacological studies on PPDC, together with the structural features of PPDC, which are markedly different from those of the previous antagonists, suggest that PPDC represents a new class of NMDA receptor antagonists.[19,21,22]

Conformational Analysis of PPDC (2b) As reported

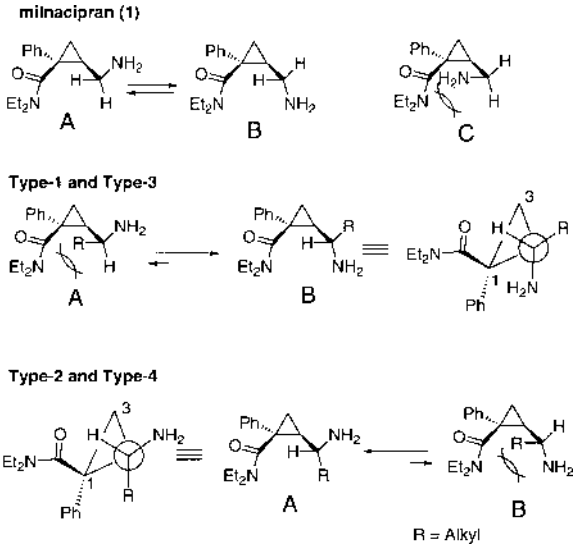

Fig. 3. Conformation Restriction of Milnacipran by Introducing an Alkyl Group

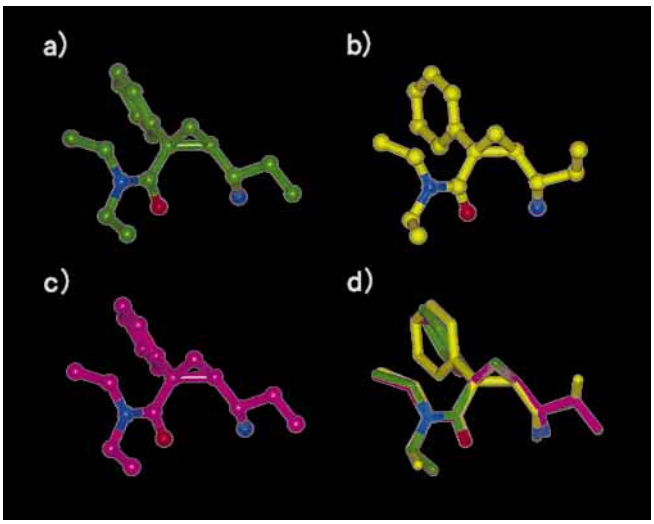

Fig. 4. Conformational Analyses of PPDC (**2b**)

a) Usual MM2 calculation structure with simulated annealing method; b) X-ray crystallographic structure (the enantiomeric structure of the X-ray-analyzed structure of *ent*-**2b**); c) MM2 calculation structure by simulated annealing method based on the NOE data; d) the superimposition of the three structures shown in a, b, and c.

previously, our hypothesis regarding conformational restriction based on the structural feature of the cyclopropane ring described above has been supported by X-ray crystallographic analyses of *ent*-**2b**, the enantiomer of PPDC, and its 1′-diastereomer *ent*-**3b**.[9] In the crystals, *ent*-**2b** and its 1′-diastereomer *ent*-**3b** assume the expected conformations as shown in Fig. 3: the biologically essential amino group of *ent*-**2b** is positioned anti to C3 (Type-3, conformer B) whereas the amino group of *ent*-**3b** is positioned *anti* to C1 (Type-4, conformer A). In the solid state structure of *ent*-**2b**, any intermolecular interaction was not observed, it is possible that the crystal structure is different from that in solution. Thus, we further investigated the conformation of PPDC in solution as well as in vacuum.

The stable conformation of PPDC was calculated by the usual MM2 method in vacuum. As shown in Fig. 4a, the calculated conformation is similar to the structure by X-ray

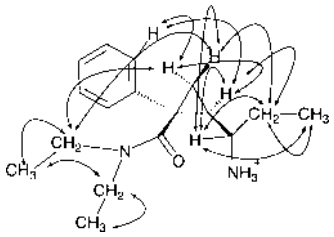

Fig. 5. The NOE Observed in NOESY Spectra of PPDC (**2b**)

crystallography, shown in Fig. 4b[36]; the amino group is positioned *anti* to C3 and the bulky diethylcarbamoyl group is placed outside of the molecule probably due to the steric repulsion with the adjacent substituent at the 2-position.

The three-dimensional structure in aqueous solution, which should be very important from the viewpoint of the bioactive conformation, was next investigated. The structure was constructed according to the previously reported method[37] by MM2 calculations with a simulated annealing method based on the NOE constraints of the intramolecular proton pairs measured in D_2O, and the observed NOE in the nuclear Overhauser effect spectroscopy (NOESY) spectra were shown in Fig. 5. As shown in Fig. 4c, the NOE-based structure is almost the same as that obtained by the calculations in vacuum (Fig. 4a). Figure 4d is the superimposition of these three structures, and shows that the conformations of PPDC obtained by the three different methods are analogous and in agreement with the speculated conformer B, shown in Fig. 3.

These results suggest that the conformation of the substituents on a cyclopropane ring of PPDC would be significantly restricted by the steric effect of adjacent substituents even in solution, as we hypothesized, and that it would bind to the NMDA receptor in a conformation similar to conformer B (Fig. 2). As described, the developed conformational restriction method based on the structural feature of the cyclopropane ring is very effective and can be applied to design other novel biologically active molecules.

Experimental

PPDC was synthesized according to the previously reported method.[9] All of the ^{1}H-NMR spectra were measured on a JEOL JNM LA400 spectrometer (400 MHz). The NMR spectra of PPDC (hydrochloride, 15 mM) were measured in D_2O at 37 °C. NOESY spectra were recorded in the phase-sensitive mode using the methods of States and coworkers.[36] The intensities of the NOE cross peaks in the NOESY spectrum recorded with the mixing time of 200 ms were used to obtained inter-proton distances. NOE cross peaks were separated into three distance categories depending on cross peak intensity. Strong NOE were given an upper distance constraint of 2.5 Å while medium and weak NOEs were given values of 3.0 and 3.5 Å, respectively. For distance constraints that involved nonequivalent methylene, methyl and aromatic ring protons, which could not be stereospecifically, assigned, the pseudoatom treatment was used. These provided the distance constraints, which were used for subsequent structure calculations. Three-dimensional structures, which satisfy the NOE constraints of the intramolecular proton pairs were constructed by simulated annealing calculations[37] using Discover-Insight (MSI) as the program. All calculations of other potential functions were performed following the protocol in the program Discover using the standard parameters on IRIS Indogo2 Solid Impact R10000. The structure of PPDC in vacuum was also constructed by simulated annealing calculations using Discover-Insight (MSI) on IRIS Indogo2 Solid Impact R10000. The X-ray crystallographic data of *ent*-**2b** were previously reported.[9]

Acknowledgments This investigation was supported by a Grant-in-Aid for Creative Scientific Research (13NP0401) from the Japan Society for Promotion of Science. We are grateful to Daiso Co., Ltd. for gift of chiral epichlorohydrins.

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