Regular Article

Chemical Synthetic Platform for Chlorpromazine Oligomers That Were Reported as Photo-degradation Products of Chlorpromazine

Taiki Kohiki, Yusuke Nishikawa, Tsubasa Inokuma, Akira Shigenaga,* and Akira Otaka*

Institute of Biomedical Sciences and Graduate School of Pharmaceutical Sciences, Tokushima University; Tokushima 770–8505, Japan.

Received August 30, 2017; accepted September 12, 2017

A synthetic platform for chlorpromazine (CPZ) oligomers, which could be generated via photo-reaction of CPZ, is essential to promote their biological and structural studies. In this paper, the first synthetic platform for CPZ oligomers is described. A photo-irradiation experiment of CPZ to confirm whether the structure of the CPZ dimer generated by the photo-irradiation was identical to that prepared by our synthetic method is also reported.

Key words chlorpromazine (CPZ); chlorpromazine dimer; chlorpromazine trimer; chlorpromazine oligomer; photo-reaction; photosensitization

Chlorpromazine (CPZ) (n=1) is the first synthetic antipsychotic drug employed for treatment of mental disorders25) (Fig. 1). It was first developed by Charpentier et al. as an antihistaminic agent,3) and its potency in psychiatric treatment was then reported by Laborit et al. in 1952.7) Schizophrenia is one of the major diseases treatable by CPZ, and its mechanism of action is thought to be antagonistic effect on the dopamine D2 receptor.5) As CPZ was widely used, its photo-toxic and photo-allergic adverse effects were also reported6); therefore, its photo-degradation reaction has been intensely studied.7–11) In the previous studies, the main focus was on structures and bioactivities of monomeric derivatives that were generated via photo-irradiation of CPZ. However, bioactivities of several photo-generated CPZ oligomers have also been shown so far. The oligomers were first reported as potential causative agents of unfavorable effects such as hemolysis and inflammation.12–14) A beneficial bioactivity, which could be an alternative action of mechanism, was recently clarified by Fukui and colleagues.15) They have been studying on human D-amino acid oxidase (hDAO) that is a potential risk factor for schizophrenia and is involved in glutamate-mediated neurotransmission. Because antipsychotic drugs can affect not only dopaminergic but also glutamatergic neurotransmission,16) they focused on hDAO-inhibitory activity of CPZ and its photo-generated oligomers. In their paper, photo-generation of CPZ dimer, trimer and tetramer was confirmed and the high therapeutic effect of CPZ. A proposed structure of the oligomer is shown in Fig. 1.8,15) Huang and Sands carried out several experiments to determine the structure of the oligomer, but a position of C–C bond formation was proposed not based on experimental results, but based on speculation of a reaction mechanism.17) Fukui’s group attempted to clarify the structure using NMR, but the effort was hampered by broadening of the peaks.15) Therefore, there is no experimental evidence that supports the position of the C–C bond formation. To promote biological and structural studies on the oligomers, we considered that constant supply of the structurally defined oligomers is essential. In this paper, development of a synthetic platform for the CPZ oligomers 1, the structure had been proposed,8,15) is first reported. Whether dimer 1 (n=2) could be generated by UV-induced photo-degradation of CPZ monomer 1 (n=1) is also described.

Results and Discussion

A synthetic strategy for the preparation of CPZ oligomer 1 is shown in Chart 1. In this study, not dimethylamino derivatives, but tert-butoxycarbonyl (Boc)-protected building blocks 2 and 3 were employed to facilitate purification of synthetic intermediates by standard normal phase column chromatography. The synthetic platform is as follows: Step 1) replacement of chlorine with boron; Step 2) coupling of the obtained boron derivative with aryl bromide; Step 3) removal of Boc groups; Step 4) methylation of the generated secondary amines. This platform should enable synthesis of CPZ oligomer 1 by repetition of Steps 1 and 2.

We first attempted to synthesize building blocks 2 (Chart 2). Starting from chlorophenothiazine 4, a chloropropyl unit was introduced in accordance with a report, with slight modification.17) Treatment of chloride 5 with NaI followed by substitution by methylamine and subsequent Boc protection generated building block 2. Preparation of brominated building block 3 was next attempted (Chart 3). Regioselective bromination of 4 with N-bromosuccinimide (NBS)18) afforded a brominated phenothiazine, and it was converted to 3 similarly as conversion of 4 to 2 in Chart 2. Dimer 1 (n=2) was then synthesized as follows (Chart 4): Chlorine of 2 was replaced by a pinacol borane in the presence of palladium catalyst and 2-dicyclohexylphosphino-2’4’,6’-trisopropylbiphényl (XPhos) to generate 7.19) Suzuki–Miyaura coupling20,21) of boronate 7

Fig. 1. Structure of CPZ (n=1) and Its Oligomers (n>1)

* To whom correspondence should be addressed. e-mail: shigenaga.akira@tokushima-u.ac.jp; aotaka@tokushima-u.ac.jp

© 2017 The Pharmaceutical Society of Japan
and bromide 322) followed by removal of the Boc groups under acidic conditions and subsequent reductive methylation of the generated secondary amines then successfully afforded CPZ dimer 1 (n=2). To demonstrate practicality of our synthetic platform, the synthetic protocol was applied to preparation of CPZ trimer 1 (n=3) (Chart 5). Following the substitution of chlorine of dimer 8 to pinacol borane, product 9 was coupled with bromide 3 and generated trimer 10 was converted to desired 1 (n=3) employing a procedure similar to that for the dimer.23) These results clearly demonstrate that our synthetic platform enables facile access to the CPZ oligomers.

Finally, we examined whether photo-degradation of CPZ monomer generates dimer 1 (n=2). CPZ monomer 1 (n=1) dissolved in water was irradiated with UV for 2.5 h at room tem-
temperature. During the reaction, color of the reaction mixture was changed from colorless to brown. The obtained mixture was then analyzed using LC/MS (Fig. 2). Whereas substrate \( \text{I} (n=1) \) remained as the major component (Fig. 2A), generation of dimers was observed by detection using MS \( (m/z = 601, \text{corresponding to } \text{I} (n=2) + \text{H})^+ \) (Fig. 2B). Unexpectedly, the masses of seven peaks identical to that of \( \text{I} (n=2) \) were detected (peaks a to g in Fig. 2B). Because CPZ has seven possible reactive points to react with photo-generated dechlorinated radical \( \text{II}^{10,24} \) to generate the monochlorinated dimers (Chart 6), we speculated that radical \( \text{II} \) reacted with CPZ with low regioselectivity to generate all seven isomers. Finally, co-injection of the photo-degradation products with chemically synthesized dimer \( \text{I} (n=2) \) clarified that \( \text{I} (n=2) \) is one of the non-major photo-products corresponding to the peak g (Fig. 2C).

Conclusion

A chemical synthetic platform for preparation of CPZ oligomers \( \text{I} \), which were reported as photo-degradation products of CPZ, was established. It was then unexpectedly clarified that the photo-generated monochlorinated CPZ dimer is not only \( \text{I} (n=2) \), but also a mixture of its isomers. This suggests that biological studies of the CPZ oligomers examined so far employed mixtures of the isomers; therefore, synthetic platforms, including ours reported in this paper, for preparation of each isomer should contribute to further biological and pharmacological study of photo-generated CPZ oligomers to elucidate which isomers are really responsible for the bioactivity and photo-toxicity.

Experimental

General Methods All reactions were carried out under a positive pressure of argon at room temperature unless otherwise noted. For column chromatography, silica Gel 60N (spherical, neutral, Kanto Chemical Co., Inc., Japan) was employed. TLC was performed on precoated plates (0.25 mm, silica gel Merck Kiesegel 60F245). NMR spectra were recorded using a Bruker AV400N at 400 MHz frequency for \( ^1\text{H} \) and a JEOL JNM-AL300 at 300 or 75 MHz frequency for \( ^1\text{H} \) or \( ^{13}\text{C} \), respectively. Chemical shifts are calibrated to the solvent signal. A Waters MICROMASS® LCT PRIME TM (electro-
spray ionization-time-of-flight (ESI-TOF)) was employed for measurement of high resolution (HR) mass spectra.25 IR spectra were measured using a JASCO FT-IR 6200. Melting point was obtained on MICRO MELTING POINT APPARATUS (YANAGIMOTO, Japan) and was uncorrected. Elemental analysis was performed by CHN-CORDER (YANAGIMOTO).

VL-30L (VILBER LOURMAT, 2 × 15 W, power = 60 W, 365 nm tube) was employed for UV-irradiation experiment because UVA was widely used for photo-degradation of CPZ.7–14 For the obtained suspension was stirred at room temperature overnight. After addition of water, the reaction mixture was obtained by TLC (hexane–EtOAc = 4:1 (v/v)).

2-Chloro-10-(3-chloropropyl)-10H-phenothiazine (5) To a suspension of sodium hydride (NaH) (55% (w/w)), 542 mg, 12.4 mmol) in MeCN (solvent B) was used for elution (flow rate: 1 mL/min; gradient of solvent A in solvent B: 30 to 65% over 30 min).

2-Chloro-10H-phenothiazin-10-yl(propyl)(methyl)carbamate (2) Chloride 5 (1.23 g, 3.96 mmol) and NaI (5.94 g, 39.6 mmol) in acetone (15 mL) were refluxed overnight. In addition of water, the reaction mixture was extracted with EtOAc. The combined organic layer was dried over Na2SO4, filtered and concentrated in vacuo. The residue was purified by column chromatography (hexane–EtOAc = 100:1 (v/v)), and alkylated product 5 (1.23 g, 3.96 mmol, 93%) was obtained as pale yellow oil: 1H-NMR (CDCl3, 400 MHz) δ: 2.20 (2H, quint, J = 6.3 Hz), 3.63 (2H, t, J = 6.3 Hz), 4.02 (2H, t, J = 6.3 Hz), 6.84 (1H, d, J = 1.8 Hz), 6.88–6.91 (2H, m), 6.94 (1H, td, J = 7.5 and 1.2 Hz), 7.02 (1H, d, J = 8.0 Hz), 7.12–7.19 (2H, m); 13C-NMR (CDCl3, 100 MHz) δ: 29.4, 42.2, 44.0, 115.8, 115.9, 122.5, 123.4, 124.1, 125.3, 127.5, 127.6, 128.0, 133.3, 144.2, 146.3; HR-MS ESI-TOF m/z: Calcd for C15H13Cl2N2O2S: [M + Na]+ 351.0389. Found 351.0400.

tert-Butyl [3-(2-Chloro-10H-phenothiazin-10-yl)propyl](methyl)carbamate (2) Chloride 5 (1.23 g, 3.96 mmol) and NaI (5.94 g, 39.6 mmol) in acetone (15 mL) were refluxed overnight. In addition of water, the reaction mixture was extracted with EtOAc. The combined organic layer was dried over Na2SO4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (hexane–EtOAc = 70:1 then 10:1 (v/v)) and product 2 (1.43 g, 3.53 mmol, 89% over 3 steps) was obtained as white solid: mp 88°C; 1H-NMR (CDCl3, 300 MHz) δ: 1.41 (9H, brs), 2.01 (2H, quint, J = 6.8 Hz), 2.79 (3H, s), 3.33 (2H, brs), 3.85 (2H, t, J = 6.8 Hz), 6.82 (1H, d, J = 2.0 Hz), 6.86 (1H, d, J = 8.0 Hz), 6.89 (1H, d, J = 10.0, 1.8 Hz), 6.94 (1H, t, J = 7.5 Hz), 7.03 (1H, d, J = 8.0 Hz), 7.12–7.19 (2H, m); 13C-NMR (CDCl3, 75 MHz, 50°C) δ: 25.5, 28.4, 34.6, 44.8, 46.5 (br), 79.4, 115.8, 115.9, 122.4, 123.0, 124.0 (br), 125.3 (br), 127.4, 127.6, 127.9, 133.3, 144.5, 146.5, 155.8 (br); Anal. Calcd for C14H12Cl2N2O2S: C, 58.0; H, 4.6; N, 11.1; Found: C, 58.0; H, 4.3; N, 11.2.

7-Bromo-2-chloro-10-(3-chloropropyl)-10H-phenothiazine (6) NBS (4.12 g, 23.1 mmol) in THF (43.0 mL) was added to chlorophenothiazine 4 (5.00 g, 21.4 mmol) in THF (50.0 mL) at 0°C slowly, and the resulting mixture was stirred at room temperature for 3.5 h. To the mixture was added Na2SO4 until color of the mixture changes from green to yellow, and the resulting suspension was extracted with EtOAc after addition of water. The combined organic layer was washed with water (3 times) followed by brine, dried over Na2SO4, filtered, and concentrated in vacuo. The obtained solid was washed with water and then dissolved in EtOAc. The solution was dried over Na2SO4, filtered and concentrated in vacuo to give 6.38 g of a crude brominated product. The obtained crude material (6.38 g, 20.4 mmol) was alkylated as similar to 4 in Chart 2, and product 6 (4.93 g, 12.7 mmol, 59% over 2 steps) was obtained as colorless oil: 1H-NMR (CDCl3, 400 MHz) δ: 2.18 (2H, quint, J = 6.3 Hz), 3.63 (2H, t, J = 6.3 Hz), 3.99 (2H, t, J = 6.3 Hz), 6.72 (1H, d, J = 8.3 Hz), 6.84 (1H, d, J = 2.0 Hz), 6.90 (1H, dd, J = 8.3, 2.0 Hz), 7.01 (1H, d, J = 8.0 Hz), 7.22–7.27 (2H, m); 13C-NMR (CDCl3, 75 MHz) δ: 29.2, 42.0, 44.0, 115.3, 116.0, 116.9, 122.9, 123.3, 127.6, 128.1, 129.9, 130.1, 133.5, 145.3, 145.9; HR-MS (ESI-TOF) m/z: Calcd for C16H11BrClN2O2S (M+Na+) 386.9251. Found 386.9257.

tert-Butyl [3-(7-Bromo-2-chloro-10H-phenothiazin-10-yl)propyl](methyl)carbamate (3) Substrates 6 (816 mg, 2.10 mmol) was converted to 3 as similar to conversion of 2, and product 3 (782 mg, 1.62 mmol, 77% over 3 steps) was obtained as white amorphous: 1H-NMR (CDCl3, 300 MHz, 50°C) δ: 1.39 (9H, s), 1.93 (2H, quint, J = 6.8 Hz), 2.75 (3H, s), 3.27 (2H, t, J = 6.8 Hz), 3.74 (2H, t, J = 6.8 Hz), 6.61 (1H, d, J = 8.4 Hz), 6.75 (1H, d, J = 2.0 Hz), 6.82 (1H, d, J = 8.0, 1.8 Hz), 6.92 (1H, d, J = 8.0 Hz), 7.13–7.21 (2H, m); 13C-NMR (CDCl3, 75 MHz, 50°C) δ: 25.1, 28.2, 34.4, 44.7, 46.2 (br), 79.2, 115.0, 115.7, 116.6, 122.5, 122.9 (br), 127.2, 127.8, 129.5, 129.8, 133.3, 143.4, 145.8, 155.4; HR-MS (ESI-TOF) m/z: Calcd for C17H13BrClN2O2S (M+Na+) 505.0328. Found 505.0319; IR (neat) 754, 807, 852, 915, 1108, 1123, 1245, 1280, 1412, 1455, 1553, 1587, 2867, 2958, 3062 cm–1.

tert-Butyl [3-(7-Bromo-2-chloro-10H-phenothiazin-10-yl)propyl](methyl)carbamate (3) Chloride 2 (1.43 g, 3.53 mmol, bis(pinacolato) diboron (1.79 g, 7.05 mmol), potassium acetate (416 mg, 4.24 mmol), Pd(OAc)2 (15.9 mg, 71 µmol) and XPhos (67.3 mg, 134 µmol) were added to 1,4-dioxane (35.3 mL), and the obtained mixture was refluxed overnight. When remaining of the substrate had been observed by TLC (hexane–EtOAc = 4:1 (v/v)), XPhos...
(33.7 mg, 70.7 µmol) was added to the reaction mixture and it was refluxed for additional 8.5h. After addition of water and EtOAc, the reaction mixture was filtered through cotton and the mixture was extracted with EtOAc. The combined organic layer was washed with sat. aq. NaHCO₃ followed by brine, filtered and concentrated in vacuo. The resulting residue was purified by column chromatography (hexane–EtOAc=20:1 then 5:1 (v/v)) and product 7 (1.57 g, 3.16 mmol, 90%) was obtained as pale yellow amorphous: ¹H-NMR (CDCl₃, 300 MHz, 50°C) δ: 1.32 (12H, s), 1.42 (9H, s), 2.01 (2H, quint, J=6.7 Hz), 2.78 (3H, s), 3.33 (2H, t, J=7.7 Hz), 3.92 (2H, t, J=6.7 Hz), 6.79–6.91 (2H, m), 7.06–7.17 (3H, m), 7.29 (1H, s), 7.37 (1H, d, J=7.7 Hz); ¹³C-NMR (CDCl₃, 75 MHz, 50°C) δ: 24.7, 25.4, 28.3, 34.3, 44.5, 46.4, 79.1, 83.6, 115.5, 121.1, 122.3, 125.2, 126.8, 127.1, 127.3, 129.0, 129.5, 144.6, 145.0, 155.7; HR-MS (ESI-TOF) m/z: Calcd for C₃₆H₃₄ClN₂O₆S₂ [M+H⁺] 795.2781. Found 795.2762; 24.9, 25.6, 25.7, 28.5, 34.5, 34.6, 44.8, 46.6, 79.4, 83.9, 113.9, 115.8, 120.9, 121.2, 122.7, 125.7, 125.8, 127.0, 127.2, 127.6, 127.9, 129.3, 135.9, 139.6, 144.5, 145.2, 145.8, 155.8; HR-MS (ESI-TOF) m/z: Calcd for C₃₂H₃₀ClN₂O₆S₂ [(M+Na⁺)] 878.4073. Found 878.4009; IR (KBr) 751, 806, 931, 1039, 1105, 1132, 1169, 1219, 1444, 1573, 1765, 2940 cm⁻¹.

**Di-tet-buty1 [(8'-8-Chloro-10H,10'H-2,3'-biphenothiazine)-10,10'-diyl]bis(propane-3,1-diyl)]bis(methylcarbamate) (9)** Chloride 8 (200 mg, 259 µmol) was converted to 9 as similar to conversion of 2 to 7, and product 9 (195 mg, 225 µmol, 87%) was obtained as yellow amorphous: ¹H-NMR (CDCl₃, 300 MHz, 50°C) δ: 1.34 (12H, s), 1.41 (9H, s), 1.43 (9H, s), 2.06 (4H, quint, J=6.8 Hz), 2.78 (3H, s), 2.81 (3H, s), 3.25–3.45 (4H, m), 3.90–4.10 (4H, m), 6.82–7.45 (13H, m); ¹³C-NMR (CDCl₃, 75 MHz, 50°C) δ: 24.9, 25.6, 25.7, 28.5, 34.5, 34.6, 44.8, 46.6, 79.4, 83.9, 113.9, 115.8, 120.9, 121.2, 122.7, 125.7, 125.8, 127.0, 127.2, 127.6, 127.9, 129.3, 135.9, 139.6, 144.5, 145.2, 145.8, 155.8; HR-MS (ESI-TOF) m/z: Calcd for C₃₀H₂₈ClN₂O₆S₂ [(M+Na⁺)] 878.4073. Found 878.4009; IR (KBr) 751, 809, 1147, 1249, 1356, 1393, 1417, 1458, 1578, 1693, 2929, 2977 cm⁻¹.

**Di-tet-buty1 [(10'-3-[3-(3-t-Butoxy carbonyl(methyl)-amino)-a-pyrrole]-8'-chloro-10H,10'H-2,3',3'-terphenothiazine)-10,10'-diyl]bis(propane-3,1-diyl)]bis(methylcarbamate) (10)** Dimer 9 (100 mg, 116 µmol) was converted with bromide 3 (46.8 mg, 96.7 µmol) to generate trimer 10 as similar to coupling of 7 and 3. In this case NaN₃ was employed instead of K₂CO₃. Product 10 (90.6 mg, 79.3 µmol, 82%) was obtained as yellow amorphous: ¹H-NMR (CDCl₃, 300 MHz, 50°C) δ: 1.41 (18H, s), 1.44 (9H, s), 1.95–2.18 (6H, m), 2.79 (3H, s), 2.81 (3H, s), 2.83 (3H, s), 3.22–3.45 (6H, m), 3.90 (2H, t, J=6.8 Hz), 3.90–4.25 (4H, m), 6.82–7.39 (19H, m); ¹³C-NMR (CDCl₃, 75 MHz, 50°C) δ: 24.9, 25.4, 25.6, 25.7, 28.5, 34.5, 34.7, 44.8, 44.9, 46.6, 79.4, 79.5, 114.0, 115.8, 115.9, 120.1, 121.1, 122.6, 122.7, 125.8, 125.9, 126.0, 127.3, 127.6, 127.7, 128.1, 133.5, 135.6, 139.5, 143.9, 144.5, 144.8, 145.2, 145.6, 154.5, 163.5; HR-MS (ESI-TOF) m/z: Calcd for C₃₅H₂₅ClN₂O₆S₂ [(M+Na⁺)] 1179.4079. Found 1179.4088; IR (KBr) 749, 808, 1150, 1247, 1394, 1457, 1569, 1692, 2930, 2975 cm⁻¹.

**3',3'-(8'-Chloro-10H,10'H-2,3',3'-terphenothiazine)-10,10'-triyl]tris(N,N-dimethylpropan-1-amine) (1 (n=3))** Boc derivative 10 (25.0 mg, 21.9 µmol) was converted to CPZ trimer 1 (n=3) as similar to conversion of 8 to 1 (n=2), and product 1 (n=3) (16.8 mg, 19.0 µmol, 87% over 2 steps) was obtained as yellow amorphous: ¹H-NMR (CDCl₃, 400 MHz) δ: 1.91–2.02 (4H, m), 2.21 (6H, s), 2.23 (6H, s), 2.40 (2H, t, J=7.0 Hz), 2.41 (2H, t, J=7.0 Hz), 3.90 (2H, t, J=7.0 Hz), 3.97 (2H, t, J=7.0 Hz); ¹³C-NMR (CDCl₃, 75 MHz) δ: 25.1, 25.3, 25.7, 29.5, 45.6, 57.0, 57.2, 113.8, 115.7, 115.9, 120.7, 120.8, 122.3, 122.5, 123.0, 123.6, 124.0, 125.1, 125.2, 125.4, 125.6, 125.9, 127.2, 127.4, 127.6, 127.7, 127.9, 133.3, 133.7, 139.2, 143.7, 145.1, 145.7, 146.3; HR-MS (ESI-TOF) m/z: Calcd for C₆₃H₅₃ClN₆O₆S₃ [(M+H⁺)] 1799.4079. Found 1799.4088; IR (KBr) 749, 808, 1150, 1247, 1394, 1457, 1569, 1692, 2930, 2975 cm⁻¹.
for C$_5$H$_7$ClN$_6$S$_3$ ([M$+2$H]$^+$) 442.1748. Found 442.1777; IR (KBr) 1039, 1457, 1569, 2768, 2819, 2856, 2926 cm$^{-1}$.

**UV-Irradiation Experiment of CPZ Monomer 1 (n=1)**

A solution of CPZ monomer 1 (n=1) (7.0 mg, 22 µmol) in water (200 µL) was irradiated by UV (distance between the UV lamp and the reaction mixture: 3 cm) for 2.5 h at room temperature. The resulting mixture was analyzed with or without synthetic dimer 1 (n=2) using LC/MS. LC conditions were shown in the General methods section.

**Acknowledgments**

The authors express our appreciation to Professors Kiyoshi Fukui and Yusuke Kato (Tokushima University) for involving us in a research on hDAO. This research was supported in part by PRESTO, Japan Science and Technology Agency (JST), and Grant-in-Aid for Scientific Research (KAKENHI, Grant Number 15K07858) from the Japan Society for the Promotion of Science (JSPS). T.K. acknowledges a financial support from Faculty of Pharmaceutical Sciences, Tokushima University. Measurement of high resolution mass spectra was performed at the Tokushima Regional Base for Industry–Academia–Government Joint Research.

**Conflict of Interest**

The authors declare no conflict of interest.

**References**