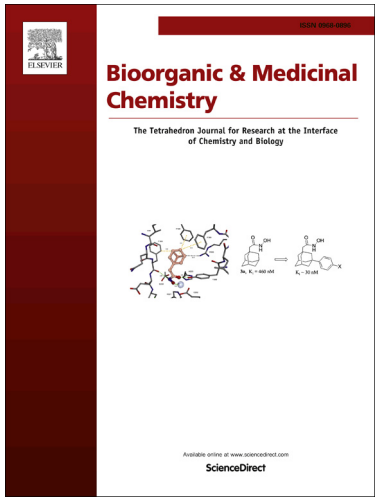

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Design, Synthesis and Biological Evaluation of a Novel Series of Peripheral-Selective Noradrenaline Reuptake Inhibitor

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Abstract

Centrally acting noradrenaline reuptake inhibitor (NRI) is reportedly effective for patients with stress urinary incontinence (SUI) by increasing urethral closure in the clinical Phase IIa study with esreboxetine. Noradrenaline transporters are expressed in both central and peripheral nervous systems and the contribution of each site to efficacy has not been clarified. This report describes the development of a series of peripheral-selective 7-phenyl-1,4-oxazepane NRIs to investigate the contribution of the peripheral site to increasing urethral resistance in rats. (6*S*,7*R*)-1,4-Oxazepane derivative **7** exhibited noradrenaline transporter inhibition with high selectivity against inhibitions of serotonin and dopamine transporters. A replacement of hydroxyl with acetamide group contributed to enhancement of peripheral selectivity by increasing molecular polarity. Compound **12**, *N*-{[(6*S*,7*R*)-7-(3,4-dichlorophenyl)-1,4-oxazepan-6-yl]methyl}acetamide 0.5 fumarate, which showed effectively no brain penetration in rats, increased urethral resistance in a dose-dependent manner and exhibited a maximal effect on par with esreboxetine. These results demonstrate that the urethral resistance-increasing effects of NRI in rats are mainly caused by the inhibition of noradrenaline transporters in the peripheral sites.

KEYWORDS: Peripheral-selective noradrenaline reuptake inhibitor, Stress urinary incontinence, 7-Aryl-1,4-oxazepane derivatives.

ABBREVIATIONS: SUI, Stress urinary incontinence; DAT, dopamine transporter; SERT, serotonin transporter; NET, norepinephrine transporter; hERG, human ether-a-go-go-related gene K+ channel; CNS, central nervous system; Boc, *tert*-butoxycarbonyl.

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1. Introduction

Stress urinary incontinence (SUI) is a disorder of lower urinary tract functions, and is defined as involuntary urine leakage during events increasing abdominal pressure such as laughing, sneezing, coughing and lifting. Prevalence of SUI in women increases over 20 years old, and it is reported that the symptom is found in 33.9% of women aged 40 years and older.[1] Current treatment options of SUI are very limited; physical therapy and surgery are standard treatment.[2] In 2004, duloxetine, an antidepressant based on serotonin and noradrenaline reuptake inhibition, was approved for treatment of SUI in only a few countries in EU.[3] Esreboxetine, which is a centrally acting selective noradrenaline reuptake inhibitor (NRI), also showed a significant reduction in frequency of incontinence episodes in SUI patients compared with placebo in the US study.[4] However, all the antidepressants, including duloxetine are obliged to be labeled with a "black box warning" about increased risks of suicidality in children, adolescent and young adults.[5] Noradrenaline transporter (norepinephrine transporter, NET), which is responsible for the reuptake of extracellular noradrenaline, is known to express in both central and peripheral nervous systems. Central and peripheral enhancement of the noradrenaline system are each recognized to have the potential to increase urethral resistance; the former acts at the presynaptic

cells of motor neurons innervating the external urethral sphincter in the sacral spinal cord, and the latter acts at the terminus of the autonomic hypogastric nerve innervating the urethral smooth muscle.[6] However, contribution of each site to the efficacy was not clarified so far. It was considered that if peripheral-selective NRIs, which inhibit the peripheral NET dominantly, showed anti-SUI effects, the compounds would be useful drugs for the treatment of SUI without causing central adverse effects. Therefore, we attempted to synthesize novel peripheral and monoamine selective NRIs, and assessed their biological potential as a novel class of therapeutic agent for SUI.

The design of peripheral and monoamine selective NRIs is outlined in Figure 1. Most reported monoamine reuptake inhibitors have a lipophilic aromatic ring and basic amino group as the core motif.[7] Among them, some structurally rigid inhibitors such as amitifadine or milnacipran possess two or three carbon atoms between the aromatic ring and the amino group. We supposed these structures are essential for the monoamine reuptake inhibitory activities, and generated a primary scaffold I, which consists of an aromatic ring, a linker with three carbon atoms, and an amine moiety. Independent study about two carbon atoms linker series will be disclosed in due course. On the other hand, 7-membered 1,4-diazepan-2-one derivative II, a serotonin, noradrenaline and dopamine reuptake inhibitor (triple reuptake inhibitor) was reported by our laboratory. This compound showed relatively noradrenaline selective reuptake inhibition.[8] As a design strategy for selective NRI, we applied the "7-membered ring" of II as a noradrenaline selective core ring to "three carbon atoms" in a primary scaffold I, and designed the core scaffold of 4-phenylazepine and 7-phenyl-1,4-oxazepane III. To achieve peripheral selectivity, hydrophilic functional group (FG) was additionally located on the core ring to increase polarity of the molecule. Indeed, similar approach was reported in peripherally restricted Cannabinoid 1 (CB1) antagonist study, which is succeeded in reducing the psychiatric side effects.[9] In a series of the studies, the most common strategy is addition of polar groups to known brain penetrant CB1 antagonists to provide peripherally-selective compounds.[10] In addition, these classes of monoamine reuptake inhibitors might have a drug-drug interaction liability through CYP2D6 inhibition and hERG inhibition due to the

cationic amphiphilic drug (CAD) structure.[11] Aware of these risks, we evaluated and prioritized compounds based on CYP2D6 inhibition.

We set out to explore novel peripheral-selective NRIs based on scaffold III, and found that 4-phenylazepane derivative **1** and 7-phenyl-1,4-oxazepane derivative **2** demonstrated potent NET inhibitory activity with moderate selectivity against serotonin transporter (SERT) and dopamine transporter (DAT) inhibitions. Thus, we selected the compounds as leads, and studied the substituents R on the benzene ring and the hydrophilic substituents FG to obtain the novel compounds with superior NRI activity and peripheral selectivity.

[Figure 1]

2. Chemistry

The synthesis of the 4-phenylazepane derivative **1** is shown in Scheme 1. Addition of dichlorophenyl magnesium bromide to commercially available cyclohexenyl ketone **18** and subsequent cyano group addition afforded cyclohexyl ketone **20**. After protection of the ketone **20** as the 1,3-dioxolane, the nitrile of compound **21** was reduced to hydroxymethyl group in two steps. Deprotection of the 1,3-dioxolane moiety of compound **22** and silylation of the hydroxyl group gave cyclohexanone **23**. Ring expansion by Beckmann rearrangement provided azepan-2-one derivative **24**. The amide motif of compound **24** was reduced to amine using borane-tetrahydrofuran complex. After treatment in acidic condition, the amine group was protected by Boc group and deprotected by treating with hydrogen chloride to afford the 4-phenylazepane derivative **1** as the HCl salt.

[Scheme 1]

The synthesis of the 7-phenyl-1,4-oxazepane derivatives **2**–**4** is shown in Scheme 2. Commercially available nitrile derivative **25** was transformed to phenylketone **26** using the corresponding Grignard

reagent. After addition of acetonitrile anion to **26**, the cyano group was reduced to give the corresponding amine **28**. Reductive alkylation of the amine with benzaldehyde and subsequent acylation with chloroacetyl chloride furnished amide **29**. Intramolecular cyclization of **29** under basic condition afforded 7-phenyl-1,4-oxazepan-3-one **30**. After reduction of amide **30** to amine derivative **31**, replacement of the *N*-benzyl with Boc protection gave compound **32**, followed by removal of the *p*-methoxy benzyl group of **32** using ceric ammonium nitrate afford hydroxymethyl derivative **33**. Finally, optical resolution of **33** was carried out using preparative chiral HPLC and typical deprotection gave 7-phenyl-1,4-oxazepane derivatives **2**–**4** as the HCl salt.

[Scheme 2]

The preparation of 6-hydroxymethyl 7-phenyl-1,4-oxazepane derivatives **5**–**8** is illustrated in Scheme 3. Morita-Baylis-Hillman reaction of dichlorobenzaldehyde **34** with methyl acrylate afforded ester **35**. Michael addition of benzyl amine gave a mixture of two diastereomers (*rac*-**36**, *rac*-**37**), which were separated by silica gel column chromatography. After transformation of the methyl ester *rac*-**36** to the silyl ether *rac*-**38** via a hydroxymethyl intermediate, *N*-selective acylation with chloroacetyl chloride gave amide *rac*-**39**. Cyclization of *rac*-**39** under basic conditions gave 6-substituted 1,4-oxazepan-3-one *rac*-**40**. After reduction of the amide group, *N*-benzyl group was replaced with Boc along with de-protection of hydroxyl group afforded hydroxymethyl derivative *rac*-**42**. Optical resolution of *rac*-**42** using preparative chiral HPLC, followed by typical deprotection of Boc group afforded (6*S*,7*R*)-**7** and (6*R*,7*S*)-**8**. *Erythro* isomer *rac*-**37** was converted to hydroxymethyl derivative (6*S*,7*S*)-**5** and (6*R*,7*R*)-**6** in the similar manner as *threo* isomer. The absolute stereochemistry of **5** and **7** were confirmed by single-crystal X-ray analysis (Figure 2).

[Scheme 3]

[Figure 2]

The preparation of (6*S*,7*R*)-7-phenyl-1,4-oxazepane derivatives **9**–**17** is shown in Scheme 4. Carboxylic acid **45** was prepared by Pinnick oxidation via aldehyde intermediate **44** and subsequent condensation with the desired amines and typical deprotection of Boc gave corresponding carboxamides **9**–**11**. The hydroxyl group of compound **43a**–**e** was converted into an amine **48a**–**e** via the mesylate **46a**–**e** and azide **47a**–**e**, and was then treated with acetyl chloride and hydrogen chloride furnish acetamide **12** and **14**–**17** as the HCl or fumaric acid salt. Extended acetamide **13** was synthesized via a cyano intermediate followed by reduction of the cyano group, acetylation of the amine with acetyl chloride and treatment in hydrochloric acid.

[Scheme 4]

3. Results and discussion

The synthesized compounds were evaluated for their *in vitro* reuptake inhibitory activity against human NET, SERT and DAT, expressed as IC_{50} values. Brain penetration was evaluated by investigating NET occupancy in rat brain after intravenous injection at 3 mg/kg.

First, we evaluated configuration of the substituents on the 7-membered ring and position of the hydrophilic substituents FG in III (Table 1). In an initial SAR study, the 3,4-dichlorophenyl group was adopted as a NET selective Ar moiety on this oxazepane/azepane system.[11] The 4-Phenylazepane derivative **1** and 7-phenyl-1,4-oxazepane derivative **2** showed potent NET inhibitory activity with moderate selectivity against SERT and DAT. The oxazepane derivative **2** demonstrated weaker CYP2D6 inhibition than the corresponding azepane **1** (19% vs 44%) suggesting that a lower ClogP value is favorable to avoid this liability (ClogP; **2**: 1.9, **1**: 2.9).[12] The (7*S*)-isomer **4** showed approximately 100-fold potent NET inhibition compared to the (7*R*)-isomer **3**. In contrast, neither SERT nor DAT inhibitory activity was affected by the absolute configuration. These results indicated that the

absolute configuration of 7-position on 1,4-oxazepane ring was critical for the NET inhibitory activity in the 7-hydroxymethyl series. Interestingly, potent NET inhibition was conserved among four stereoisomers [**5** (6*S*,7*S*), **6** (6*R*,7*R*), **7** (6*S*,7*R*) and **8** (6*R*,7*S*)] in the 6-hydroxymethyl series. DAT inhibitory activities were weak in all the stereo isomers. On the other hand, SERT inhibitory activity was dependent on the configuration of phenyl groups. The (7*S*)-isomers **5** and **8** showed stronger SERT inhibition than the (7*R*)-isomers **6** and **7**. Between the (7*R*)-isomers, the trans isomer **7** exhibited higher selectivity against SERT and DAT than the cis isomer **6**. Regarding CYP2D6 inhibition, the trans isomers showed lower potential than cis isomers (**5** and **6** vs **7** and **8**). Among the evaluated compounds, the (6*S*,7*R*)-compound **7** showed the most potent NET inhibitory activity, the highest selectivity against SERT and DAT inhibitions, and relatively low CYP2D6 liability. Thus we selected this stereoisomer as the core template.

[Table 1]

Compound **7** showed highly selective NRI profiles. However, peripheral selectivity, which was estimated by rat brain NET occupancy, was insufficient, as rat brain NET occupancy was 68%, indicating that the compound penetrated into the brain as shown in Table 2. To achieve superior peripheral selectivity, introduction of amide motif at the 6-position was conducted (Table 2). Replacement of hydroxymethyl group with carbamoyl group (**9**) gave a reduction in NET inhibitory activity. Methyl capping of the carbamoyl nitrogen atom (**10** and **11**) increased NET inhibitory activity along with lipophilicity (CLogP; **9**: 1.8, **10**: 2.1, **11**: 2.6), resulting in poor peripheral selectivity (rat brain NET occupancy = 68%). Enhancement of DAT inhibition was observed in compound **11**. Inversion of the amide linker (**12**) enhanced NET inhibition. In addition, compound **12** showed excellent peripheral selectivity. The profiles appear to be correlated with the ClogP values. Linker extension (**13**) decreased the inhibitory activity, suggesting that the length of the linker is critical, especially for the NET inhibition. Next, substituents on benzene ring were evaluated to investigate the effects on

selectivity against SERT and DAT.[13] Replacement of the chlorine atom at the 3-position on benzene ring with fluorine (**14**) retained high selectivity. Meanwhile, similar exchange at the 4-position (**15**) decreased the selectivity. These replacements maintained the peripheral selective profiles. Replacement of the 3 or 4-chlorine with a methyl group (**16** and **17**) also did not improve selectivity against SERT and DAT. Among the evaluated compounds, compound **12** demonstrated the most potent and selective NET inhibition as well as the greatest peripheral selectivity and a reduced potential for the liabilities often seen with CAD structures (CYP2D6 at 10 μM: 26%, hERG inhibition at 10 μM: 15%).[14] Thus, compound **12** was selected for further *in vivo* evaluation.

[Table 2]

The *in vivo* activities of compound **12** were evaluated by measuring effects on leak point pressure, which indicates urethral resistance, in rats. The leak point pressure was measured at 0.5 h after i.v. administration of compound **12** and 0.5 h after s.c. administration of esreboxetine. As shown in Figure 3, compound **12** significantly increased urethral resistance in a dose-dependent manner and the maximum leak point pressure elevation was comparable to that by esreboxetine. Considering the negative CNS-penetrating property of compound **12** (NET occupancy = -14%), the urethral resistance-increasing effects of compound **12** in rats were suggested to be mainly mediated by the inhibition of peripheral NET. Esreboxetine treatment increases urethral closure in the rat model, as well as in a human clinical trial, and this increased urethral closure appears to decrease the frequency of incontinence episodes in SUI patients.[16] Taken together, these findings suggest that peripheral-selective NRI have the potential to show anti-SUI efficacy in humans.

[Figure 3]

4. Conclusion

Novel peripheral-selective NRIs were designed, and a compound with low CNS-penetration property was evaluated in a model of SUI. Optimization of (6*S*,7*R*)-1,4-oxazepane derivatives led to the potent and highly NET selective compound **12** which exhibited excellent peripheral selective effects. Compound **12** showed significant increase in urethral resistance in rats with the comparable maximum effects to the central acting, and clinically effective esreboxetine. These results suggest that urethral resistance-increasing effects of NRI are mainly caused by inhibition of NETs in the peripheral sites and this series of peripheral-selective NRIs would be a novel class of therapeutic agent for SUI.

5. Experimental Section

5.1. Chemistry

Melting points were determined with a Yanagimoto melting point apparatus or a Büchi melting point apparatus B-545 and are uncorrected. ^{1}H NMR spectra were obtained at 300 MHz on a Varian Ultra-300, or a Bruker DPX-300 spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. Peak multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, doublet of doublet of doublets; dt, double triplet; td, triple doublet; quin., quintet; brs, broad singlet; m, multiplet. Elemental analyses were carried out by Takeda Analytical Laboratories Ltd. Reactions were followed by TLC on Silica gel 60 F 254 precoated TLC

plates (E. Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.). Chromatographic separations were carried out on silica gel 60 (0.063–0.200 or 0.040–0.063 mm, E. Merck) or basic silica gel (Chromatorex® NH, 100–200 mesh, Fuji Silysia Chemical Ltd.) using the indicated eluents. Yields are unoptimized. The HPLC analyses were performed using a Shimadzu UFLC instrument. Elution was done with a gradient of 5–90% solvent B in solvent A (solvent A was 0.1% TFA in water, and solvent B was 0.1% TFA in MeCN) through a L-column 2 ODS (3.0 × 50 mm, 2 μm) column at 1.2 mL min^{-1}. Area % purity was measured at 254 nm. Abbreviations for solvents are the following: Et_2O, diethyl ether; MeOH, methanol; EtOH, ethanol; BuOH, butanol; EtOAc, ethyl acetate; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; MeCN, acetonitrile; $CDCl_3$, chroloform-d; Et_3N, triethylamine; Boc_2O, di-*tert*-butyl dicarbonate; TBDMSCl, *tert*-butyldimethylsilyl chloride; WSC•HCl, water soluble carbodiimide hydrochloride (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride); HOBt•NH_3, 1-hydroxybenzotriazole ammonium salt; DIBAL-H, diisobutylaluminium hydride; DMAP, *N*,*N*-dimethyl-4-aminopyridine.

5.1.1. Typical procedure of obtaining compounds 1–17 by deprotection of Boc.

Typical procedure A: To a solution of Boc intermediate in EtOH (0.2 M) was added 14.7 M HCl in EtOH (10–100 eq.) at room temperature. The mixture was stirred at room temperature for 1–24 h and then concentrated in vacuo. The residue was crystallized or triturated with EtOAc-hexane to give target compound.

Typical procedure B: To a solution of Boc intermediate was added 4 M HCl in EtOAc (10–100 eq.) at room temperature. The mixture was stirred at room temperature for 1–24 h and then concentrated in vacuo. The residue was crystallized or triturated with EtOAc-hexane to give target compound.

5.1.2. 3-(3,4-Dichlorophenyl)cyclohex-2-enone (19). A suspension of Mg (1.04 g, 42.8 mmol) in THF (10 mL) was added 4-bromo-1,2-dichlorobenzene (9.67 g, 42.8 mmol) in THF (20 mL) and I_2 (1 spatura) at room temperature. The mixture was stirred at 45°C under N_2 for 1 h and then 3-ethoxycyclohex-2-en-1-one **18** (4.76 mL, 35.7 mmol) in THF (10 mL) was added at 0°C. The mixture was stirred at room temperature under N_2 for 1 h. The mixture was added with 1 M HCl aq. at 0°C,

extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **19** (5.51 g, 64%) as a colorless crystal. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16 (2H, quin, *J* = 6.3 Hz), 2.43−2.54 (2H, m), 2.72 (2H, td, *J* = 6.0, 1.5 Hz), 6.37 (1H, t, *J* = 1.5 Hz), 7.33−7.41 (1H, m), 7.45−7.51 (1H, m), 7.61 (1H, d, *J* = 2.3 Hz).

5.1.3. 1-(3,4-Dichlorophenyl)-3-oxocyclohexanecarbonitrile (20). To a solution of **19** (5.0 g, 20.7 mmol) in DMF (60 mL) and water (15 mL) was added Et_3N hydrochloride (4.28 g, 31.1 mmol) and potassium cyanide (2.70 g, 41.5 mmol) at room temperature. The mixture was stirred at 90°C for 3 h. The mixture was poured into sat. $NaHCO_3$ aq. at room temperature, extracted with EtOAc, washed with sat. NH_4Cl aq. and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **20** (1.50 g, 27%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10−2.27 (3H, m), 2.33−2.48 (2H, m), 2.55−2.65 (1H, m), 2.75−2.83 (1H, m), 2.86−2.94 (1H, m), 7.33 (1H, dd, *J* = 8.5, 2.5 Hz), 7.52 (1H, d, *J* = 8.3 Hz), 7.57 (1H, d, *J* = 2.3 Hz).

5.1.4. 7-(3,4-Dichlorophenyl)-1,4-dioxaspiro[4.5]decane-7-carbonitrile (21). To a solution of **20** (1.5 g, 5.59 mmol) in toluene (15 mL) was added ethylene glycol (620 μL, 11.2 mmol) and pyridinium *p*-toluenesulfonate (70.0 mg, 0.28 mmol) at room temperature. The mixture was stirred 125°C for 3 h. The mixture was poured into water, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **21** (1.30 g, 74%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48–1.64 (1H, m), 1.65–1.78 (1H, m), 1.82–1.98 (3H, m), 2.08–2.39 (3H, m), 3.88–4.01 (2H, m), 4.05–4.17 (2H, m), 7.32–7.39 (1H, m), 7.42–7.51 (1H, m), 7.60 (1H, d, *J* = 2.3 Hz).

5.1.5. (7-(3,4-Dichlorophenyl)-1,4-dioxaspiro[4.5]decan-7-yl)methanol (22). To a solution of **21** (1.3 g, 4.16 mmol) in toluene (15 mL) was added 1.6 M DIBAL-H in toluene (3.61 mL, 5.41 mmol) under N_2 at -78°C. The mixture was stirred at -78°C to room temperature under N_2 for 1 h and then 1 M HCl aq. (1.6 mL) was added. After stirring for 1 h, the mixture was poured into water, extracted with

EtOAc, washed with and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give 7-(3,4-dichlorophenyl)-1,4-dioxaspiro[4.5]decane-7-carbaldehyde (920 mg, 70%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (2H, d, *J* = 5.3 Hz), 1.70–1.88 (3H, m), 2.00 (1H, s), 2.50–2.65 (2H, m), 3.90–4.08 (4H, m), 7.11 (1H, dd, *J* = 8.7, 2.3 Hz), 7.33–7.46 (2H, m), 9.35 (1H, d, *J* = 1.9 Hz). To a solution of 7-(3,4-dichlorophenyl)-1,4-dioxaspiro[4.5]decane-7-carbaldehyde (920 mg, 2.92 mmol) in MeOH (10 mL) was added $NaBH_4$ (120 mg, 3.17 mmol) at 0°C. The mixture was stirred at 0°C to room temperature overnight. The mixture was neutralized with 0.1 M HCl aq. at 0°C, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **22** (910 mg, 98%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45–1.56 (1H, m), 1.59–1.99 (7H, m), 2.16 (1H, d, *J* = 14.0 Hz), 3.60–3.71 (1H, m), 3.75–3.85 (1H, m), 3.86–4.07 (4H, m), 7.22 (1H, dd, *J* = 8.3, 2.3 Hz), 7.39 (1H, d, *J* = 8.7 Hz), 7.46 (1H, d, *J* = 2.3 Hz).

5.1.6. 3-((*tert*-Butyldimethylsilyloxy)methyl)-3-(3,4-dichlorophenyl)cyclohexanone (23). To a solution of **22** (910 mg, 2.87 mmol) in acetone (5 mL) was added 1 M HCl aq. (5 mL, 5.00 mmol) at room temperature. The mixture was stirred 65°C for 3 h. The mixture was poured into water at room temperature, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give a colorless oil. To a solution of the oil in THF (5 mL) was added TBDMSCl (452 mg, 3.00 mmol), Et_3N (569 μL, 4.09 mmol) and DMAP (10 mg, 0.08 mmol) at room temperature. The mixture was stirred 65°C overnight. The mixture was poured into water at room temperature and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **23** (880 mg, 79%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.01–0.04 (6H, m), 0.80–0.95 (9H, m), 1.52–1.64 (1H, m), 1.87–

2.04 (1H, m), 2.13–2.45 (4H, m), 2.71–2.90 (2H, m), 3.37–3.50 (1H, m), 3.54–3.67 (1H, m), 7.21 (1H, dd, *J* = 8.7, 2.3 Hz), 7.44 (1H, d, *J* = 8.3 Hz), 7.47 (1H, d, *J* = 2.3 Hz).

5.1.7. 5-((*tert*-Butyldimethylsilyloxy)methyl)-5-(3,4-dichlorophenyl)azepan-2-one (24). To a solution of **23** (880 mg, 2.27 mmol) in MeOH (2 mL) and pyridine (2 mL) was added hydroxylamine hydrochloride (316 mg, 4.54 mmol). The mixture was stirred at 65°C for 1 h. The mixture was poured into water at room temperature, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue in pyridine (4 mL) was added *p*-toluenesulfonyl chloride (866 mg, 4.54 mmol) and DMAP (10 mg, 0.08 mmol). The mixture was stirred 80°C overnight. The mixture was poured into water at room temperature, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **24** (66 mg, 7%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.05–0.05 (6H, m), 0.89–0.98 (9H, m), 1.65–1.80 (1H, m), 1.87–2.09 (2H, m), 2.48–2.62 (1H, m), 2.88–2.98 (1H, m), 3.03–3.12 (1H, m), 3.17–3.43 (2H, m), 3.48 (2H, s), 6.01 (1H, brs), 7.41–7.46 (1H, m), 7.48–7.53 (1H, m), 7.61 (1H, d, *J* = 2.3 Hz). MS m/z: 402 $[M+H]^+$.

5.1.8. *tert*-Butyl 4-(3,4-dichlorophenyl)-4-(hydroxymethyl)azepane-1-carboxylate. To a solution of **24** (66 mg, 0.16 mmol) in THF (2 mL) was added 1.2 M borane-tetrahydrofuran complex THF solution (2 mL, 2.40 mmol) at room temperature. The mixture was stirred 65°C for 2 h and then 1 M HCl aq. (1 mL, 1.00 mmol) was added. The mixture was stirred at 60°C for 1 h. After cooling to room temperature, Et_3N (500 μL, 3.60 mmol) and Boc_2O (57 μL, 0.25 mmol) were added. The mixture was stirred at room temperature for 3 h. The mixture was poured into water, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give *tert*-butyl 4-(3,4-dichlorophenyl)-4-(hydroxymethyl)azepane-1-carboxylate (53.0 mg, 86%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (1H, brs), 1.33–1.44 (9H, m), 1.57–1.69 (2H, m), 1.73–1.93 (2H, m), 2.18–2.42 (2H, m), 3.02–3.39 (3H, m), 3.46 (2H, s), 3.51–3.79 (1H, m), 7.15 (1H, dd, *J* = 8.5, 1.7 Hz), 7.38 (1H, d, *J* = 1.9 Hz), 7.44 (1H, d, *J* = 8.7 Hz).

5.1.9. (4-(3,4-Dichlorophenyl)azepan-4-yl)methanol monohydrochloride (1). Typical procedure A, 57%, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.45–1.66 (1H, m), 1.86 (2H, dd, J = 14.6, 10.0 Hz), 2.01–2.22 (2H, m), 2.33 (1H, dd, J = 15.9, 8.0 Hz), 2.88 (1H, dd, J = 13.6, 9.5 Hz), 3.01 (2H, t, J = 5.1 Hz), 3.16–3.28 (1H, m), 3.35 (2H, d, J = 5.3 Hz), 4.89 (1H, t, J = 5.3 Hz), 7.32–7.41 (1H, m), 7.54–7.66 (2H, m), 8.95 (2H, brs). MS m/z: 274.1 $[M+H]^+$.

5.1.10. 1-(3,4-Dichlorophenyl)-2-(4-methoxyphenoxy)ethanone (26). To a suspension of Mg (2.23 g, 91.7 mmol) and I_2 (5 mg) in Et_2O (20 mL) was added a solution of 1-bromo-3,4-dichlorobenzene (20.7 g, 91.7 mmol) in Et_2O (20 mL), and the mixture was stirred at 35°C for 1 h. The mixture was cooled to -10°C, and Et_2O (100 mL) was added. A solution of [(4-methoxybenzyl)oxy]acetonitrile **25** (10 g, 61.2 mmol) in Et_2O (20 mL) was added dropwise at -10°C, and the mixture was stirred for 1 h to room temperature. To the mixture was added 1 M HCl aq. (200 mL), and the mixture was stirred for 1 h, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc) to give **26** (15.6 g, 81%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.72–3.86 (3H, m), 5.12 (2H, s), 6.76–6.98 (4H, m), 7.57 (1H, d, J = 8.3 Hz), 7.84 (1H, dd, J = 8.5, 2.1 Hz), 8.09 (1H, d, J = 1.9 Hz).

5.1.11. 3-(3,4-Dichlorophenyl)-3-hydroxy-4-(4-methoxyphenoxy)butanenitrile (27). To a mixed solution of MeCN (241 μL, 4.5 mmol) and THF (2 mL) was added dropwise a solution of *n*-butyllithium in hexane (1.6 M, 3 mL, 4.81 mmol), and the mixture was stirred at -78°C for 30 min. To the mixture was added dropwise a solution of **26** (1.0 g, 3.21 mmol) in THF (3 mL), and the mixture was stirred at -78°C for 10 min, and then stirred for 1 h to room temperature. To the mixture was added water, and the mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **27** (932 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.86–3.14 (2H, m), 3.29 (1H, s), 3.77 (3H, s), 4.06–4.24 (2H, m), 6.84 (4H, s), 7.33–7.42 (1H, m), 7.50 (1H, d, J = 8.7 Hz), 7.70 (1H, d, J = 2.3 Hz).

5.1.12. 4-Amino-2-(3,4-dichlorophenyl)-1-(4-methoxyphenoxy)butan-2-ol (28). To a suspension of $LiAlH_4$ (2.53 g, 66.6 mmol) in Et_2O (50 mL) was added $AlCl_3$ (2.96 g, 22.2 mmol), and the mixture was stirred at 0°C for 30 min. To the mixture was added dropwise a solution of **27** (11.7 g, 33.3 mmol) in THF (50 mL), and the mixture was stirred at 0°C for 15 min, and then stirred to room temperature for 2 h. Ice was added and the precipitate was filtered off through Celite. The filtrate was diluted with water, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give **28** (10.8 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.94–2.10 (1H, m), 2.25 (1H, ddd, J = 14.4, 10.2, 3.8 Hz), 2.63–2.83 (1H, m), 2.99–3.14 (1H, m), 3.17–3.47 (3H, m), 3.68–3.78 (3H, m), 3.85 (1H, d, J = 9.1 Hz), 3.94–4.05 (1H, m), 6.67–6.89 (4H, m), 7.41 (2H, s), 7.75 (1H, s).

5.1.13. *N*-Benzyl-2-chloro-*N*-[3-(3,4-dichlorophenyl)-3-hydroxy-4-(4-methoxyphenoxy)butyl]acetamide (29). To a solution of **28** (500 mg, 1.40 mmol) in MeOH (2 mL) was added $MgSO_4$ (253 mg, 2.1 mmol), Et_3N (78 μL, 0.56 mmol) and benzaldehyde (148 μL, 1.47 mmol), and the mixture was stirred at room temperature for 1.5 h. To the mixture was added $NaBH_4$ (264 mg, 7.0 mmol), and the mixture was stirred at 0°C for 10 min, and then stirred to room temperature for 1.5 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue was diluted with water, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give an oil (440 mg). To a solution of the oil in THF (3 mL) was added Et_3N (149 μL, 1.08 mmol) and chloroacetyl chloride (86 μL, 1.08 mmol), and the mixture was stirred at 0°C for 10 min, and then stirred to room temperature for 2 h. The mixture was diluted with water, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **29** (395 mg, 69%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.13–2.43 (2H, m), 2.99 (1H, ddd, J = 15.4, 11.0, 4.9 Hz), 3.26–3.60 (2H, m), 3.68–4.22 (7H, m), 4.40–4.66 (2H, m), 6.67–6.97 (4H, m), 7.07–7.72 (8H, m).

5.1.14. 4-Benzyl-7-(3,4-dichlorophenyl)-7-[(4-methoxyphenoxy)methyl]-1,4-oxazepan-3-one (30). To a solution of **29** (390 mg, 0.75 mmol) in THF (40 mL) was added NaO*t*Bu (72 mg, 0.75 mmol), and

the mixture was stirred at 0°C for 2 h and then stirred to room temperature for 14 h. After adding water, the mixture was concentrated. The residue was diluted with water, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **30** (256 mg, 70%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.36 (1H, dd, *J* = 15.3, 10.0 Hz), 2.80 (1H, dd, *J* = 15.6, 8.1 Hz), 3.22 (1H, dd, *J* = 13.8, 7.7 Hz), 3.56–3.88 (5H, m), 4.00 (1H, d, *J* = 9.4 Hz), 4.20 (1H, d, *J* = 17.0 Hz), 4.41–4.62 (2H, m), 4.63–4.76 (1H, m), 6.60–6.85 (4H, m), 7.15–7.40 (6H, m), 7.45 (1H, d, *J* = 8.7 Hz), 7.53 (1H, d, *J* = 1.9 Hz). MS m/z: 486.1 $[M+H]^+$.

5.1.15. 4-Benzyl-7-(3,4-dichlorophenyl)-7-[(4-methoxyphenoxy)methyl]-1,4-oxazepane (31). To a solution of $LiAlH_4$ (351 mg, 9.24 mmol) in Et_2O (10 mL) was added $AlCl_3$ (412 mg, 3.08 mmol), and the mixture was stirred at 0°C for 10 min. To the mixture was added dropwise a solution of **30** (2.25 g, 4.62 mmol) in THF (10 mL) and the mixture was stirred at 0°C for 15 min and to room temperature for 2 h. After adding ice, the precipitate was filtered off through Celite. The filtrate was diluted with water, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give **31** (1.18 g, 49%) as a oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18–2.45 (1H, m), 2.52–2.88 (5H, m), 3.46–3.63 (2H, m), 3.63–3.84 (5H, m), 3.85–4.00 (2H, m), 6.57–6.83 (4H, m), 7.16–7.44 (7H, m), 7.60 (1H, d, *J* = 2.3 Hz). MS m/z: 472.4 $[M+H]^+$

5.1.16. *tert*-Butyl 7-(3,4-dichlorophenyl)-7-[(4-methoxyphenoxy)methyl]-1,4-oxazepane-4-carboxylate (32). To a solution of **31** (1.18 g, 2.5 mmol) in MeCN (5 mL) was added Et_3N (415 μL, 3.00 mmol) and 1-chloroethyl chloroformate (327 μL, 3.00 mmol), and the mixture was stirred at 90°C for 1.5 h. The solvent was evaporated under reduced pressure and MeOH (20 mL) was added to the residue. The mixture was stirred at 80°C for 1 h and then solvent was evaporated under reduced pressure. To a solution of the residue in THF (5 mL) was added Et_3N (415 μL, 3.00 mmol) and Boc_2O (689 μL, 3.00 mmol), and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was added water, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in

vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **32** (793 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (9H, s), 2.12–2.36 (1H, m), 2.71 (1H, dd, *J* = 15.3, 7.3 Hz), 3.25–3.63 (3H, m), 3.67–4.00 (8H, m), 6.64–6.89 (4H, m), 7.18–7.33 (1H, m), 7.43 (1H, d, *J* = 8.3 Hz), 7.57 (1H, d, *J* = 2.3 Hz). MS m/z: 382.1 $[M+H\text{-}Boc]^+$

5.1.17. *tert*-Butyl 7-(3,4-dichlorophenyl)-7-(hydroxymethyl)-1,4-oxazepane-4-carboxylate (33). To a solution of **32** (639 mg, 1.32 mmol) in MeCN (8 mL) and water (2 mL) was added ceric ammonium nitrate (2.18 g, 4.00 mmol) at 0°C, and the mixture was stirred at 0°C for 30 min. The mixture was added water, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **33** (440 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (9H, s), 1.87–2.08 (1H, m), 2.17 (1H, d, *J* = 9.1 Hz), 2.45 (1H, dd, *J* = 14.9, 7.4 Hz), 3.20–3.97 (8H, m), 7.19 (1H, dd, *J* = 8.5, 2.1 Hz), 7.39–7.52 (2H, m). MS m/z: 320.3 $[M+H\text{-}t\text{Bu}]^+$.

5.1.18. *tert*-Butyl (7*S*)-7-(3,4-dichlorophenyl)-7-(hydroxymethyl)-1,4-oxazepane-4-carboxylate, *tert*-Butyl (7*R*)-7-(3,4-dichlorophenyl)-7-(hydroxymethyl)-1,4-oxazepane-4-carboxylate. A racemate (50.4 g) of **33** was separated by SFC (column: CHIRALPAK AD-H, 30 mmID×250 mmL, manufactured by DAICEL CHEMICAL INDUSTRIES, LTD., mobile phase: carbon dioxide/2-propanol = 65/35) to give (7*S*)-compound having a longer retention time and (7*R*)-compound having a shorter retention time.

5.1.19. [7-(3,4-Dichlorophenyl)-1,4-oxazepan-7-yl]methanol monohydrochloride (2). Typical procedure B, 99%, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.36 (1H, dd, *J* = 16.3, 9.8 Hz), 2.76 (1H, dd, *J* = 16.3, 8.0 Hz), 3.13 (3H, brs), 3.24–3.52 (3H, m), 3.62 (1H, dd, *J* = 13.8, 7.8 Hz), 3.93–4.08 (1H, m), 4.97–5.21 (1H, m), 7.35 (1H, dd, *J* = 8.3, 1.9 Hz), 7.57 (1H, d, *J* = 1.9 Hz), 7.63 (1H, d, *J* = 8.7 Hz), 9.18 (2H, brs). MS m/z: 276.2 $[M+H]^+$.

5.1.20. [(7*R*)-7-(3,4-Dichlorophenyl)-1,4-oxazepan-7-yl]methanol monohydrochloride (3). Typical procedure B, 82%, Colorless crystal. ^{1}H NMR (300 MHz, DMSO–d_6) δ 2.36 (1H, dd, *J* = 16.4, 9.6 Hz), 2.76 (1H, dd, *J* = 16.4, 7.7 Hz), 3.00–3.20 (3H, m), 3.24–3.37 (2H, m), 3.40–3.49 (1H, m), 3.56–3.69 (1H, m), 3.94–4.07 (1H, m), 5.09 (1H, t, *J* = 5.9 Hz), 7.35 (1H, dd, *J* = 8.5, 2.1 Hz), 7.57 (1H, d, *J* = 2.3 Hz), 7.60–7.67 (1H, m), 9.00–9.27 (2H, m). MS m/z: 276.1 $[M+H]^+$. Mp: 235–238 °C. Anal. Calcd for $C_{12}H_{16}NO_2Cl_3$: C,46.10; H,5.16; N,4.48. Found: C,46.17; H,5.12; N,4.53.

5.1.21. [(7*S*)-7-(3,4-Dichlorophenyl)-1,4-oxazepan-7-yl]methanol monohydrochloride (4). Typical procedure B, 79%, Colorless crystal. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.34 (1H, dd, *J* = 16.2, 9.8 Hz), 2.76 (1H, dd, *J* = 16.6, 7.6 Hz), 3.01–3.21 (3H, m), 3.24–3.37 (2H, m), 3.40–3.50 (1H, m), 3.55–3.67 (1H, m), 3.95–4.07 (1H, m), 5.08 (1H, t, *J* = 5.9 Hz), 7.35 (1H, dd, *J* = 8.7, 2.3 Hz), 7.57 (1H, d, *J* = 1.9 Hz), 7.61–7.67 (1H, m), 8.83–9.24 (2H, m). MS m/z: 276.1 $[M+H]^+$. Mp: 235–238 °C. Anal. Calcd for $C_{12}H_{16}NO_2Cl_3$: C,46.10; H,5.16; N,4.48. Found: C,46.01; H,5.17; N,4.59. $[\alpha]^{25}_D$ +46.5 (c 0.25, MeOH).

5.1.22. Methyl 2-[(3,4-dichlorophenyl)(hydroxy)methyl]prop-2-enoate (35). Methyl acrylate (96 mL, 1.1 mol) was added to a mixture of **34** (125 g, 714 mmol) and 1,4-Diazabicyclo[2.2.2]octane (24.0 g, 214 mmol) in MeCN (100 mL) at room temperature. The mixture was stirred at room temperature for 3 days. The mixture was concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **35** (155 g, 83%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.17 (1H, d, *J* = 6.0 Hz), 3.75 (3H, s), 5.50 (1H, d, *J* = 5.7 Hz), 5.85 (1H, s), 6.37 (1H, s), 7.22 (1H, dd, *J* = 8.3, 1.9 Hz), 7.41 (1H, d, *J* = 8.3 Hz), 7.48 (1H, d, *J* = 1.9 Hz).

5.1.23. Methyl (2*RS*,3*RS*)-2-[(benzylamino)methyl]-3-(3,4-dichlorophenyl)-3-hydroxypropanoate (*rac*-36), Methyl (2*SR*,3*RS*)-2-[(benzylamino)methyl]-3-(3,4-dichlorophenyl)-3-hydroxypropanoate (*rac*-37). To a mixture of **35** (13.6 g, 52.1 mmol) and Et_3N (8.74 mL, 62.6 mmol) in MeOH (100 mL) was added benzylamine (6.84 mL, 62.6 mmol), and the mixture was stirred at room temperature for 3 days. The mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (silica gel, hexane/EtOAc) to give the less polar ***rac*-36** (12.7 g, 63%) and more polar

***rac*-37** (4.17 g, 21%). ***rac*-36**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.66–2.76 (2H, m), 3.11–3.23 (1H, m), 3.66–3.79 (5H, m), 5.27 (1H, d, *J* = 4.9 Hz), 7.10 (1H, dd, *J* = 8.3, 1.5 Hz), 7.26–7.40 (6H, m), 7.45 (1H, d, *J* = 1.9 Hz). ***rac*-37**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.85–2.98 (2H, m), 3.01–3.12 (1H, m), 3.58 (3H, s), 3.71–3.86 (2H, m), 5.12 (1H, d, *J* = 3.8 Hz), 7.09 (1H, dd, *J* = 8.3, 1.9 Hz), 7.26–7.45 (7H, m).

5.1.24. (1*RS*,2*SR*)-3-(Benzylamino)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-1-(3,4-dichlorophenyl)propan-1-ol (*rac*-38). To a suspension of $CaCl_2$ (67.8 g, 611 mmol) in THF (450 mL) and EtOH (200 mL) was added $NaBH_4$ (30.8 g, 815 mmol) at room temperature. To the mixture was added dropwise ***rac*-36** (150 g, 407 mmol) in THF (200 mL) and EtOH (200 mL) at 0°C. The mixture was stirred at room temperature overnight. The mixture was neutralized with 6M HCl aq. (160 mL) at 0°C and stirred at room temperature overnight. The mixture was neutralized with 8M NaOH aq. (40 mL), and then filtered through Celite. The filtrate was concentrated in vacuo, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give an colorless oil. To a solution of the oil in THF (1 L) was added Et_3N (62.6 mL, 449 mmol) at 0°C and then TBDMSCl (61.6 g, 409 mmol) in THF (350 mL) was added at room temperature. The mixture was stirred overnight, and then imidazole (33.4 g, 490 mmol) was added. The mixture was stirred at room temperature for 5h, and then DMAP (21 g, 172 mmol) was added. After stirring overnight, the mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give ***rac*-38** (113 g, 61%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (6H, s), 0.88 (9H, s), 1.68–1.81 (1H, m), 2.84–3.06 (2H, m), 3.40–3.49 (1H, m), 3.49–3.59 (1H, m), 3.77 (2H, s), 4.82–4.90 (1H, m), 7.12–7.17 (1H, m), 7.25–7.39 (6H, m), 7.46 (1H, d, *J* = 1.9 Hz). MS m/z: 454.1 $[M+H]^+$.

5.1.25. *N*-Benzyl-*N*-[(2*RS*,3*SR*)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-3-(3,4-dichlorophenyl)-3-hydroxypropyl]-2-chloroacetamide (*rac*-39). A solution of 2-chloroacetyl chloride (28.1 mL, 351 mmol) in THF (200 mL) was added to a mixture of ***rac*-38** (145 g, 319 mmol) and Et_3N (53.4 mL, 383 mmol) in THF (1 L) at 0°C dropwise. The mixture was stirred at ambient temperature over weekend and

then concentrated in vacuo. The residue was diluted with water, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo to give ***rac*-39** (169 g, quant.). MS m/z: 514.1 $[M+H-OH]^+$.

5.1.26. (6*RS*,7*SR*)-4-Benzyl-6-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-7-(3,4-dichlorophenyl)-1,4-oxazepan-3-one (*rac*-40). 1 M NaOH aq. (479 mL, 479 mmol) was added to a solution of ***rac*-39** (169 g, 319 mmol) in THF (1.2 L). The mixture was stirred at room temperature overnight and then concentrated in vacuo. The residue was diluted with water, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give ***rac*-40** (100 g, 64%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.06 (6H, d, *J* = 1.5 Hz), 0.85 (9H, s), 1.90–2.03 (1H, m), 3.20 (1H, dd, *J* = 10.4, 6.6 Hz), 3.32 (1H, dd, *J* = 10.4, 4.3 Hz), 3.53–3.63 (1H, m), 3.63–3.71 (1H, m), 4.31 (1H, d, *J* = 1.5 Hz), 4.33–4.38 (1H, m), 4.47 (1H, d, *J* = 14.4 Hz), 4.51–4.58 (1H, m), 4.85 (1H, d, *J* = 14.4 Hz), 7.13 (1H, dd, *J* = 8.3, 2.3 Hz), 7.27–7.45 (7H, m). MS m/z: 494.0 $[M+H]^+$.

5.1.27. (6*RS*,7*SR*)-4-Benzyl-6-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-7-(3,4-dichlorophenyl)-1,4-oxazepane (*rac*-41). To a suspension of $AlCl_3$ (16.2 g, 122 mmol) in THF (800 mL) was added $LiAlH_4$ (13.9 g, 365 mmol) at 0°C and the mixture was stirred at 0°C for 20 min. A solution of ***rac*-40** (100 g, 203 mmol) in THF (500 mL) was added to the mixture at 0°C for 2 h. After being stirred at 0°C for 3.5 h, a solution of potassium sodium (+)-tartrate tetrahydrate (286 g, 1.01 mol) in water (500 mL) was added to the reaction mixture at 0°C. The mixture was stirred overnight. The precipitate was removed by filtration, and the filtrate was concentrated in vacuo. The residue was diluted with water, extracted with EtOAc, washed with brine, dried over Na_2SO_4 and concentrated in vacuo to give ***rac*-41** (94 g, 96%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.05 (6H, s), 0.83 (9H, s), 2.07–2.23 (1H, m), 2.57–2.77 (2H, m), 2.88 (2H, d, *J* = 3.8 Hz), 3.39–3.49 (1H, m), 3.51–3.64 (2H, m), 3.65 (2H, s), 3.93–4.04 (1H, m), 4.42 (1H, d, *J* = 8.3 Hz), 7.22 (1H, dd, *J* = 8.3, 2.3 Hz), 7.24–7.42 (6H, m), 7.49 (1H, d, *J* = 2.3 Hz).

5.1.28. *tert*-Butyl (6*RS*,7*SR*)-7-(3,4-dichlorophenyl)-6-(hydroxymethyl)-1,4-oxazepane-4-carboxylate (*rac*-42). Chloroformic acid 1-chloroethyl ester (25.5 mL, 234 mmol) was added to a solution of ***rac*-41** (93.7 g, 195 mmol) in MeCN (1 L). The mixture was stirred at room temperature for 30 min and then concentrated in vacuo. 1 M HCl aq. (10 mL) was added to the residue in MeOH (1 L). The mixture was stirred at 80°C for 2 h. After cooling, the mixture was added Boc_2O (47.0 mL, 205 mmol) and Et_3N (32.6 mL, 234 mmol) at 0°C. The mixture was stirred at room temperature overnight and then concentrated in vacuo. The residue was diluted with water, extracted with EtOAc, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give ***rac*-42** (67.3 g, 92%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (9H, s), 2.06–2.19 (1H, m), 3.05–3.30 (2H, m), 3.43 (1H, dd, *J* = 14.7, 6.4 Hz), 3.49–3.67 (2H, m), 4.04–4.12 (3H, m), 4.21 (1H, dd, *J* = 10.0, 4.7 Hz), 4.33 (1H, d, *J* = 9.8 Hz), 7.19 (1H, dd, *J* = 8.3, 1.9 Hz), 7.40 (1H, d, *J* = 8.3 Hz), 7.48 (1H, d, *J* = 1.9 Hz). MS m/z: 275.9 $[M+H]^+$.

5.1.29. *tert*-Butyl (6*S*,7*R*)-7-(3,4-dichlorophenyl)-6-(hydroxymethyl)-1,4-oxazepane-4-carboxylate, *tert*-Butyl (6*R*,7*S*)-7-(3,4-dichlorophenyl)-6-(hydroxymethyl)-1,4-oxazepane-4-carboxylate. *Rac*-42 (67.3 g) was separated by HPLC (CHIRALPAK AD, 50 mmID×500 mmL, manufactured by DAICEL CHEMICAL INDUSTRIES, LTD., mobile phase: hexane/ethanol = 900/100) to give **(6*S*,7*R*)-42** (31.3 g, >99.9% ee., recovery rate 100%) having a shorter retention time and **(6*R*,7*S*)-42** (29.9 g, >99.6% ee., recovery rate 98%) having a longer retention time.

5.1.30. ((6*S*,7*R*)-7-(3,4-Dichlorophenyl)-1,4-oxazepan-6-yl)methanol monohydrochloride (7). Typical procedure A, 95%, Colorless crystal. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.36–2.46 (1H, m), 3.05–3.31 (5H, m), 3.42–3.52 (1H, m), 3.74–3.86 (1H, m), 3.99–4.11 (1H, m), 4.44 (1H, d, *J* = 10.2 Hz), 4.93–5.03 (1H, m), 7.38 (1H, dd, *J* = 8.3, 1.9 Hz), 7.64–7.70 (2H, m), 9.07–9.51 (2H, m). MS m/z: 276.1 $[M+H]^+$. Anal. Calcd for $C_{12}H_{16}NO_2Cl_3$•0.5 H_2O: C,44.81;H,5.33;N,4.35. Found: C,44.81;H,5.17;N,4.28. $[\alpha]^{25}_D$ +13.5 (c 0.25, MeOH).

5.1.31. ((6*R*,7*S*)-7-(3,4-Dichlorophenyl)-1,4-oxazepan-6-yl)methanol monohydrochloride (8). Typical procedure A, 90%, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.43–2.48 (1H, m), 3.07–3.17 (2H, m), 3.20–3.30 (3H, m), 3.41–3.53 (1H, m), 3.75–3.90 (1H, m), 4.06 (1H, dt, *J* = 13.9, 4.4 Hz), 4.45 (1H, d, *J* = 10.2 Hz), 4.97 (1H, brs), 7.39 (1H, dd, *J* = 8.3, 2.3 Hz), 7.63–7.71 (2H, m), 9.49 (2H, brs). MS m/z: 276.1 $[M+H]^+$. $[\alpha]^{25}_D$ –16.8 (c 0.25, MeOH).

Cis derivatives **5** and **6** were prepared in a similar manner as trans isomer.

5.1.32. (1*RS*,2*RS*)-3-(Benzylamino)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-1-(3,4-dichlorophenyl)propan-1-ol. 64%. ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.02–0.02 (6H, m), 0.82–0.91 (9H, m), 2.01–2.13 (1H, m), 2.65–2.74 (1H, m), 2.80–2.91 (1H, m), 3.36 (1H, dd, *J* = 10.5, 6.4 Hz), 3.55 (1H, dd, *J* = 10.5, 6.0 Hz), 3.71–3.84 (2H, m), 5.01 (1H, d, *J* = 3.8 Hz), 7.09 (1H, dt, *J* = 8.3, 1.1 Hz), 7.26–7.38 (6H, m), 7.43 (1H, d, *J* = 1.9 Hz). MS m/z: 454.1 $[M+H]^+$.

5.1.33. *N*-Benzyl-*N*-[(2*RS*,3*RS*)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-3-(3,4-dichlorophenyl)-3-hydroxypropyl]-2-chloroacetamide. Quant. MS m/z: 514.1 $[M+H\text{-}OH]^+$.

5.1.34. (6*RS*,7*RS*)-4-Benzyl-6-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-7-(3,4-dichlorophenyl)-1,4-oxazepan-3-one. 78%. ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.10 (3H, s), -0.07 (3H, s), 0.83 (9H, s), 2.16–2.30 (1H, m), 3.18 (1H, dd, *J* = 10.4, 4.7 Hz), 3.42–3.55 (2H, m), 3.72 (1H, dd, *J* = 14.7, 6.8 Hz), 4.12 (1H, d, *J* = 14.4 Hz), 4.34 (1H, d, *J* = 15.1 Hz), 4.58–4.72 (2H, m), 5.28 (1H, d, *J* = 14.7 Hz), 7.09 (1H, dd, *J* = 8.5, 2.5 Hz), 7.27–7.45 (7H, m). MS m/z: 494.2 $[M+H]^+$.

5.1.35. (6*RS*,7*RS*)-4-Benzyl-6-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-7-(3,4-dichlorophenyl)-1,4-oxazepane. Quant. ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.11 (3H, s), -0.09 (3H, s), 0.74–0.83 (9H, m), 2.35–2.49 (1H, m), 2.56–2.86 (4H, m), 3.18–3.27 (1H, m), 3.31–3.41 (1H, m), 3.62–3.79 (3H, m), 3.85–3.95 (1H, m), 4.98 (1H, d, *J* = 4.5 Hz), 7.18 (1H, dd, *J* = 8.3, 1.9 Hz), 7.24–7.41 (6H, m), 7.48 (1H, d, *J* = 1.9 Hz).

5.1.36. *tert*-Butyl (6*RS*,7*RS*)-7-(3,4-dichlorophenyl)-6-(hydroxymethyl)-1,4-oxazepane-4-carboxylate. 88%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43–1.55 (9H, m), 2.24–2.39 (1H, m), 3.08–3.96 (7H, m), 4.14–4.33 (2H, m), 4.64 (1H, d, *J* = 2.3 Hz), 7.10 (1H, dd, *J* = 8.3, 1.5 Hz), 7.30–7.45 (2H, m).

5.1.37. *tert*-Butyl (6*S*,7*S*)-7-(3,4-dichlorophenyl)-6-(hydroxymethyl)-1,4-oxazepane-4-carboxylate, ***tert*-Butyl (6*R*,7*R*)-7-(3,4-dichlorophenyl)-6-(hydroxymethyl)-1,4-oxazepane-4-carboxylate.** *tert*-Butyl (6*RS*,7*RS*)-7-(3,4-dichlorophenyl)-6-(hydroxymethyl)-1,4-oxazepane-4-carboxylate (15.8 g) was separated by HPLC (CHIRALPAK AD, 50 mmID×500 mmL, manufactured by DAICEL CHEMICAL INDUSTRIES, LTD., mobile phase: hexane/2-propanol = 800/200) to give (6*S*,7*S*)-compound (7.9 g, >99.9% ee., recovery rate 100%) having a shorter retention time and (6*R*,7*R*)-compound (7.4 g, >99.9% ee., recovery rate 93%) having a longer retention time.

5.1.38. [(6*S*,7*S*)-7-(3,4-Dichlorophenyl)-1,4-oxazepan-6-yl]methanol monohydrochloride (5). Typical procedure A, 35%, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.65 (1H, brs), 2.96–3.16 (2H, m), 3.19–3.41 (4H, m), 3.85–3.98 (1H, m), 3.99–4.10 (1H, m), 4.82 (1H, brs), 5.02 (1H, d, *J* = 4.1 Hz), 7.35 (1H, dd, *J* = 8.7, 1.5 Hz), 7.58–7.65 (2H, m), 9.35 (2H, brs). MS m/z: 276.1 $[M+H]^+$. $[\alpha]^{25}_D$ -62.7 (c 0.25, MeOH).

5.1.39. [(6*R*,7*R*)-7-(3,4-Dichlorophenyl)-1,4-oxazepan-6-yl]methanol monohydrochloride (6). Typical procedure A, 81%, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.59–2.72 (1H, m), 2.97–3.17 (2H, m), 3.17–3.34 (4H, m), 3.85–3.99 (1H, m), 3.99–4.11 (1H, m), 4.82 (1H, brs), 5.02 (1H, d, *J* = 4.5 Hz), 7.35 (1H, dd, *J* = 8.3, 1.9 Hz), 7.57–7.66 (2H, m), 9.24 (1H, brs), 9.61 (1H, brs). MS m/z: 276.1 $[M+H]^+$. $[\alpha]^{25}_D$ +64.6 (c 0.25, MeOH).

5.1.40. *tert*-Butyl (6*R*,7*R*)-7-(3,4-dichlorophenyl)-6-formyl-1,4-oxazepane-4-carboxylate (44). To a solution of **43a** (8.3 g, 22.1 mmol) in MeCN (110 mL) was added Dess-Martin periodinane (11.2 g, 26.5 mmol) at 0°C. The mixture was stirred at 0°C for 4 h, and then $NaHCO_3$ aq. and $Na_2S_2O_3$ aq. were added. After 30 min, the mixture was extracted with EtOAc, washed with water and brine, dried over

Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **44** (5.41 g, 66%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43–1.56 (9H, m), 2.87–3.37 (2H, m), 3.48–3.77 (2H, m), 3.80–4.20 (2H, m), 4.34 (1H, d, *J* = 15.1 Hz), 4.69–5.13 (1H, m), 7.18 (1H, d, *J* = 8.3 Hz), 7.33–7.40 (1H, m), 7.40–7.56 (1H, m), 9.46–9.85 (1H, m). MS m/z: 274.0 $[M+H\text{-}Boc]^+$.

5.1.41. (6*R*,7*R*)-4-(*tert*-Butoxycarbonyl)-7-(3,4-dichlorophenyl)-1,4-oxazepane-6-carboxylic acid (45). To a mixture of **44** (5.4 g, 14.4 mmol), 2-methyl-2-butene (34 mL, 32 mmol) and *t*-BuOH (75 mL) in THF (75 mL) was added sodium chlorite (7.83 g, 86.6 mmol) and sodium dihydrogenphosphate (12.1 g, 101 mmol) in H_2O (120 mL) at 0°C. The mixture was stirred at 0°C for 2 h. The mixture was poured into water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **45** (4.49 g, 80%) as a yellow amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34–1.56 (9H, m), 2.73–3.08 (1H, m), 3.14–3.91 (3H, m), 3.94–4.25 (3H, m), 4.73 (1H, d, *J* = 10.2 Hz), 7.16 (1H, dd, *J* = 8.3, 1.9 Hz), 7.37 (1H, d, *J* = 8.3 Hz), 7.45 (1H, d, *J* = 1.9 Hz).

5.1.42. (6*R*,7*R*)-*tert*-Butyl 6-carbamoyl-7-(3,4-dichlorophenyl)-1,4-oxazepane-4-carboxylate. To a solution of **45** (150 mg, 0.38 mmol) in DMF (1.92 mL) was added WSC•HCl (88 mg, 0.46 mmol) and HOBt•NH_3 (70.2 mg, 0.46 mmol). The mixture was stirred at room temperature overnight. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc) to give (6*R*,7*R*)-*tert*-butyl 6-carbamoyl-7-(3,4-dichlorophenyl)-1,4-oxazepane-4-carboxylate. (183 mg, quant.) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (9H, s), 2.49–2.86 (1H, m), 3.46–4.05 (6H, m), 4.75 (1H, d, *J* = 9.1 Hz), 5.05–6.38 (2H, m), 7.17 (1H, dd, *J* = 8.3, 1.9 Hz), 7.38 (1H, d, *J* = 8.0 Hz), 7.46 (1H, d, *J* = 1.9 Hz). MS m/z: 289.0 $[M+H\text{-}Boc]^+$.

5.1.43. (6*R*,7*R*)-7-(3,4-Dichlorophenyl)-1,4-oxazepane-6-carboxamide monohydrochloride (9). Typical procedure A, 91%, Colorless crystal. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.06–3.18 (1H, m), 3.21–3.48 (4H, m), 3.86–3.99 (1H, m), 4.03–4.14 (1H, m), 4.77 (1H, d, *J* = 9.8 Hz), 7.09 (1H, s), 7.29 (1H, dd, *J* = 8.3, 1.9 Hz), 7.53–7.60 (2H, m), 7.63 (1H, d, *J* = 8.3 Hz), 9.27–9.69 (2H, m). MS m/z: 289.0 $[M+H]^+$. Anal. Calcd for $C_{12}H_{15}N_2O_2Cl_3$•0.2 H_2O: C,43.78;H,4.71;N,8.51. Found: C,43.81;H,4.68;N,8.59. $[\alpha]^{25}_D$ +8.7 (c 0.25, MeOH).

Compounds **10** and **11** were prepared in same manner using corresponding amine, followed by typical deprotection.

5.1.44. (6*R*,7*R*)-7-(3,4-Dichlorophenyl)-*N*-methyl-1,4-oxazepane-6-carboxamide monohydrochloride (10). Typical procedure B, 29% in two steps, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (3H, d, *J* = 4.5 Hz), 3.05–3.15 (1H, m), 3.22–3.46 (4H, m), 3.88–4.02 (1H, m), 4.05–4.16 (1H, m), 4.78 (1H, d, *J* = 9.8 Hz), 7.20–7.27 (1H, m), 7.55 (1H, d, *J* = 1.5 Hz), 7.61 (1H, d, *J* = 8.3 Hz), 7.99–8.09 (1H, m), 9.44–9.71 (2H, m). MS m/z: 303.0 $[M+H]^+$. $[\alpha]^{25}_D$ -19.2 (c 0.25, MeOH).

5.1.45. (6*R*,7*R*)-*tert*-Butyl 7-(3,4-dichlorophenyl)-6-(dimethylcarbamoyl)-1,4-oxazepane-4-carboxylate. 69%, Colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45–1.53 (9H, m), 2.67–2.84 (6H, m), 2.99–3.57 (2H, m), 3.75–4.24 (3H, m), 4.72–4.87 (1H, m), 7.14 (1H, dd, *J* =8.3, 1.9 Hz), 7.36 (1H, d, *J* =8.3 Hz), 7.44 (1H, d, *J* =1.9 Hz). MS m/z: 417.1 $[M+H]^+$.

5.1.46. (6*R*,7*R*)-7-(3,4-Dichlorophenyl)-*N*,*N*-dimethyl-1,4-oxazepane-6-carboxamide monohydrochloride (11). Typical procedure B, 60%, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.65 (6H, d, *J* =1.5 Hz), 3.15–3.28 (1H, m), 3.36–3.52 (3H, m), 3.83–4.10 (2H, m), 4.11–4.24 (1H, m), 4.75 (1H, d, *J* =9.8 Hz), 7.25 (1H, dd, *J* =8.5, 2.1 Hz), 7.55 (1H, d, *J* =2.3 Hz), 7.64 (1H, d, *J* =8.3 Hz), 9.58 (2H, brs). MS m/z: 317.3 $[M+H]^+$. $[\alpha]^{25}_D$ -12.0 (c 0.25, MeOH).

5.1.47. *tert*-Butyl (6*S*,7*R*)-7-(3,4-Dichlorophenyl)-6-{[(methylsulfonyl)oxy]methyl}-1,4-oxazepane-4-carboxylate (46a). To a solution of **43a** (4.66 g, 12.4 mmol) in THF (50 mL) was added Et_3N (3.45

mL, 24.8 mmol) and methanesulfonyl chloride (1.44 mL, 18.6 mmol) at 0°C. The mixture was stirred at 0°C for 2 h and at room temperature for 3 h. The mixture was neutralized with sat. $NaHCO_3$ aq., extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo to give **46a** (5.73 g, quant.) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (9H, s), 2.33–2.46 (1H, m), 2.89 (1H, brs), 3.02 (2H, brs), 3.41–3.55 (1H, m), 3.57–3.90 (4H, m), 3.97–4.16 (3H, m), 4.20 (1H, d, *J* = 9.4 Hz), 7.16 (1H, dd, *J* = 8.3, 1.9 Hz), 7.41–7.47 (2H, m).

5.1.48. *tert*-Butyl (6*S*,7*R*)-6-(azidomethyl)-7-(3,4-dichlorophenyl)-1,4-oxazepane-4-carboxylate (47a). To a solution of **46a** (10 g, 22.0 mmol) in DMF (100 mL) was added sodium azide (2.15 g, 33.0 mmol) at room temperature. The mixture was stirred at 80°C for 3 h. The mixture was poured into water at room temperature, extracted with EtOAc, washed with sat. NH_4Cl aq. and water, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give **47a** (8.89 g, quant.) as a colorless amorphous solid. MS m/z: 301.0 $[M+H\text{-}Boc]^+$.

5.1.49. *tert*-Butyl (6*R*,7*R*)-6-(aminomethyl)-7-(3,4-dichlorophenyl)-1,4-oxazepane-4-carboxylate (48a). To a solution of **47a** (8.89 g, 22.2 mmol) in THF (85 mL) and water (17 mL) was added triphenylphosphine (6.97 g, 26.6 mmol). The mixture was stirred at room temperature overnight. The mixture was poured into water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, EtOAc–MeOH) to give **48a** (7.62 g, 92%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38–1.60 (9H, m), 1.84–2.04 (1H, m), 2.36–2.75 (2H, m), 3.23–3.39 (1H, m), 3.45–3.70 (2H, m), 3.76–4.25 (4H, m), 7.16 (1H, d, *J* =7.9 Hz), 7.34–7.47 (2H, m). MS m/z: 375.4 $[M+H]^+$.

5.1.50. *tert*-Butyl (6*R*,7*R*)-6-[(acetylamino)methyl]-7-(3,4-dichlorophenyl)-1,4-oxazepane-4-carboxylate. Acetyl chloride (86 μL, 1.20 mmol) was added to a solution of **48a** (300 mg, 0.80 mmol) and Et_3N (167 μL, 1.20 mmol) in THF (4 mL) at room temperature. The mixture was poured into water, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give *tert*-butyl (6*R*,7*R*)-6-

[(acetylamino)methyl]-7-(3,4-dichlorophenyl)-1,4-oxazepane-4-carboxylate (272 mg, 82%) as a colorless amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (9H, s), 1.98 (3H, s), 2.26 (1H, brs), 2.93–3.62 (5H, m), 3.89–4.20 (4H, m), 7.17–7.25 (2H, m), 7.42 (1H, d, *J* =8.3 Hz), 7.52 (1H, s). MS m/z: 317.0 $[M+H\text{-}Boc]^+$.

5.1.51. *N*-(((6*S*,7*R*)-7-(3,4-Dichlorophenyl)-1,4-oxazepan-6-yl)methyl)acetamide 0.5 fumarate (12). To a solution of *tert*-butyl (6*R*,7*R*)-6-[(acetylamino)methyl]-7-(3,4-dichlorophenyl)-1,4-oxazepane-4-carboxylate (310 mg, 0.74 mmol) in EtOH (1 mL) was added 14.7 M HCl in EtOH (2 mL). The mixture was stirred at room temperature for 3 h and concentrated in vacuo. The residue was neutralized with 1 M NaOH aq., diluted with water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc). The residue in EtOAc (3 mL) was added a solution of fumaric acid (83.9 mg, 0.72 mmol) in EtOH (2 mL) and concentrated in vacuo. The residue was crystallized from EtOAc–EtOH to give **12** (187 mg, 67%) as a colorless crystal. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.73 (3H, s), 2.21 (1H, td, *J* = 8.8, 4.3 Hz), 2.78–3.10 (6H, m), 3.60 (1H, ddd, *J* = 12.8, 7.9, 4.9 Hz), 3.92 (1H, dt, *J* = 12.8, 3.6 Hz), 4.28 (1H, d, *J* = 9.4 Hz), 6.49 (1H, s), 7.39 (1H, dd, *J* = 8.3, 1.9 Hz), 7.53–7.65 (1H, m), 7.67 (1H, d, *J* = 1.9 Hz), 7.90 (1H, t, *J* = 5.5 Hz). MS m/z: 317.3 $[M+H]^+$. Mp: 167–170 °C. Anal. Calcd for $C_{14}H_{18}Cl_2N_2O_2 \bullet 0.5C_4H_4O_4 \bullet 0.2H_2O$: C,50.72; H,5.43; N,7.39. Found: C,50.62; H,5.24; N,7.43. $[\alpha]^{25}_D$ –7.4 (c 0.25, MeOH).

Compounds **14**–**17** were prepared in same manner using **43b**–**43e**.

5.1.52. (6*S*,7*R*)-*tert*-Butyl 7-(4-chloro-3-fluorophenyl)-6-((methylsulfonyloxy)methyl)-1,4-oxazepane-4-carboxylate (46b). Quant., Pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (9H, s), 2.30–2.46 (1H, m), 3.41–4.38 (12H, m), 7.05 (1H, d, *J* = 7.9 Hz), 7.15 (1H, dd, *J* = 9.6, 1.7 Hz), 7.34–7.46 (1H, m).

5.1.53. (6*S*,7*R*)-*tert*-Butyl 6-(azidomethyl)-7-(4-chloro-3-fluorophenyl)-1,4-oxazepane-4-carboxylate (47b). 64%, Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (9H, s), 2.09–2.36 (1H, m), 3.13–4.43 (9H, m), 7.00–7.23 (2H, m), 7.34–7.50 (1H, m). MS m/z: 285.2 $[M+H\text{-}Boc]^+$.

5.1.54. (6*R*,7*R*)-*tert*-Butyl 6-(aminomethyl)-7-(4-chloro-3-fluorophenyl)-1,4-oxazepane-4-carboxylate (48b). 82%, Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (9H, s), 1.83–2.12 (1H, m), 2.44–2.68 (2H, m), 3.24–3.46 (1H, m), 3.47–4.24 (6H, m), 7.04 (1H, d, *J* = 7.5 Hz), 7.15 (1H, d, *J* = 9.8 Hz), 7.31–7.43 (1H, m). MS m/z: 359.2 $[M+H]^+$.

5.1.55. (6*R*,7*R*)-*tert*-Butyl 6-(acetamidomethyl)-7-(4-chloro-3-fluorophenyl)-1,4-oxazepane-4-carboxylate. 49%, Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (9H, s), 1.98 (3H, s), 2.17–2.34 (1H, m), 2.96–3.43 (4H, m), 3.47–3.65 (1H, m), 3.93–4.19 (4H, m), 7.13 (1H, d, *J* = 8.3 Hz), 7.19–7.25 (1H, m), 7.36 (1H, t, *J* = 7.9 Hz).

5.1.56. *N*-(((6*S*,7*R*)-7-(4-Chloro-3-fluorophenyl)-1,4-oxazepan-6-yl)methyl)acetamide monohydrochloride (14). Typical procedure A, 98%, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.79 (3H, s), 2.56–2.72 (1H, m), 2.74–3.01 (2H, m), 3.01–3.40 (4H, m), 3.74–3.90 (1H, m), 3.92–4.09 (1H, m), 4.43 (1H, d, *J* = 9.8 Hz), 7.35 (1H, d, *J* = 7.9 Hz), 7.53–7.68 (2H, m), 8.12–8.28 (1H, m), 9.44 (1H, brs), 9.93 (1H, brs). MS m/z: 301.3 $[M+H]^+$.

5.1.57. (6*S*,7*R*)-*tert*-Butyl 7-(3-chloro-4-fluorophenyl)-6-((methylsulfonyloxy)methyl)-1,4-oxazepane-4-carboxylate (46c). 98%, Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (9H, brs), 2.40 (1H, brs), 2.79–3.12 (3H, m), 3.39–3.56 (1H, m), 3.57–3.92 (4H, m), 3.95–4.17 (3H, m), 4.20 (1H, d, *J* = 9.8 Hz), 7.09–7.24 (2H, m), 7.40 (1H, dd, *J* = 7.0, 2.1 Hz).

5.1.58. (6*S*,7*R*)-*tert*-Butyl 6-(azidomethyl)-7-(3-chloro-4-fluorophenyl)-1,4-oxazepane-4-carboxylate (47c). Quant., Colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (9H, brs), 2.14 (1H, brs), 3.18 (2H, d, *J* = 8.3 Hz), 3.46–3.91 (5H, m), 4.02–4.24 (2H, m), 7.08–7.23 (2H, m), 7.38 (1H, dd, *J* = 7.2, 1.9 Hz). MS m/z: 285.4 $[M+H\text{-}Boc]^+$.

5.1.59. (6*R*,7*R*)-*tert*-Butyl 6-(aminomethyl)-7-(3-chloro-4-fluorophenyl)-1,4-oxazepane-4-carboxylate (48c). 92%, Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (9H, s), 1.83–2.04 (1H, m), 2.39–2.66 (2H, m), 3.32 (1H, d, *J* = 4.2 Hz), 3.48–3.70 (2H, m), 3.76–4.14 (4H, m), 7.04–7.14 (1H, m), 7.17 (1H, d, *J* = 7.2 Hz), 7.39 (1H, d, *J* = 6.8 Hz). MS m/z: 359.2 $[M+H]^+$.

5.1.60. (6*R*,7*R*)-*tert*-Butyl 6-(acetamidomethyl)-7-(3-chloro-4-fluorophenyl)-1,4-oxazepane-4-carboxylate. Quant., Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (9H, s), 1.98 (3H, s), 2.27 (1H, brs), 2.92–3.31 (3H, m), 3.37 (1H, dd, *J* = 14.9, 5.1 Hz), 3.48–3.62 (1H, m), 3.94–4.08 (3H, m), 4.08–4.17 (1H, m), 7.06–7.15 (1H, m), 7.19 (1H, brs), 7.24–7.30 (1H, m), 7.47 (1H, d, *J* = 7.2 Hz). MS m/z: 301.3 $[M+H\text{-Boc}]^+$.

5.1.61. *N*-(((6*S*,7*R*)-7-(3-Chloro-4-fluorophenyl)-1,4-oxazepan-6-yl)methyl)acetamide 0.5 fumarate (15). The compound was prepared in the similar manner with compound **12**. 43%, Colorless crystal. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.74 (3H, s), 2.20 (1H, td, *J* = 9.0, 4.0 Hz), 2.78–3.06 (6H, m), 3.60 (1H, ddd, *J* = 12.9, 8.0, 4.9 Hz), 3.91 (1H, dt, *J* = 12.8, 3.5 Hz), 4.27 (1H, d, *J* = 9.6 Hz), 6.52 (1H, s), 7.38–7.44 (2H, m), 7.62 (1H, d, *J* = 7.7 Hz), 7.81–7.96 (1H, m). MS m/z: 301.1 $[M+H]^+$. $[\alpha]^{25}_D$ -9.9 (c 0.25, MeOH).

5.1.62. (6*S*,7*R*)-*tert*-Butyl 6-(azidomethyl)-7-(4-chloro-3-methylphenyl)-1,4-oxazepane-4-carboxylate (47d). 25% in two steps from **43d**, ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45–1.56 (9H, m), 2.12–2.27 (1H, m), 2.38 (3H, s), 3.09–3.26 (2H, m), 3.41–3.91 (5H, m), 4.01–4.19 (2H, m), 7.06 (1H, d, *J* = 7.9 Hz), 7.17 (1H, s), 7.32 (1H, d, *J* = 8.3 Hz). MS m/z: 281.0 $[M+H\text{-Boc}]^+$.

5.1.63. (6*R*,7*R*)-*tert*-Butyl 6-(aminomethyl)-7-(4-chloro-3-methylphenyl)-1,4-oxazepane-4-carboxylate (48d). 94%, Colorless oil. MS m/z: 355.3 $[M+H]^+$.

5.1.64. (6*R*,7*R*)-*tert*-Butyl 6-(acetamidomethyl)-7-(4-chloro-3-methylphenyl)-1,4-oxazepane-4-carboxylate. 79%, Colorless oil. MS m/z: 397.3 $[M+H]^+$.

5.1.65. *N*-(((6*S*,7*R*)-7-(4-Chloro-3-methylphenyl)-1,4-oxazepan-6-yl)methyl)acetamide monohydrochloride (16). Typical procedure B, 74%, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78 (3H, s), 2.34 (3H, s), 2.55–2.67 (1H, m), 2.86 (2H, t, *J* = 5.9 Hz), 3.04–3.45 (4H, m), 3.70–3.85 (1H, m), 3.91–4.04 (1H, m), 4.34 (1H, d, *J* = 10.2 Hz), 7.29 (1H, dd, *J* = 8.3, 1.9 Hz), 7.36–7.50 (2H, m), 8.09 (1H, t, *J* = 5.7 Hz), 9.24 (1H, brs), 9.75 (1H, brs). MS m/z: 297.2 $[M+H]^+$. $[\alpha]^{25}_D$ -3.6 (c 0.25, MeOH).

5.1.66. (6*R*,7*R*)-*tert*-Butyl 6-(aminomethyl)-7-(3-chloro-4-methylphenyl)-1,4-oxazepane-4-carboxylate (48e). 76% in three steps from **43e**, Colorless oil. MS m/z: 355.1 $[M+H]^+$.

5.1.67. (6*R*,7*R*)-*tert*-Butyl 6-(acetamidomethyl)-7-(3-chloro-4-methylphenyl)-1,4-oxazepane-4-carboxylate. 79%, Colorless oil. MS m/z: 397.3 $[M+H]^+$.

5.1.68. *N*-(((6*S*,7*R*)-7-(3-Chloro-4-methylphenyl)-1,4-oxazepan-6-yl)methyl)acetamide monohydrochloride (17). Typical procedure B, 75%, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78 (3H, s), 2.33 (3H, s), 2.54–2.67 (1H, m), 2.85 (2H, t, *J* = 6.0 Hz), 3.01–3.43 (4H, m), 3.70–3.85 (1H, m), 3.91–4.06 (1H, m), 4.34 (1H, d, *J* = 10.2 Hz), 7.22–7.42 (2H, m), 7.52 (1H, d, *J* = 1.1 Hz), 7.99–8.16 (1H, m), 9.14 (1H, brs), 9.69 (1H, brs). MS m/z: 297.2 $[M+H]^+$. $[\alpha]^{25}_D$ -7.9 (c 0.25, MeOH).

5.1.69. (6*R*,7*R*)-*tert*-Butyl 6-(2-acetamidoethyl)-7-(3,4-dichlorophenyl)-1,4-oxazepane-4-carboxylate. Potassium cyanide (149 mg, 2.29 mmol) was added to a solution of **46a** (260 mg, 0.57 mmol) in DMF (2 mL). The mixture was stirred at 80°C overnight. The mixture was quenched with sat. $NaHCO_3$ aq. and extracted with EtOAc. The organic layer was separated, washed with water, 0.1 M HCl aq. and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give cyano intermediate as a colorless oil. To the $AlCl_3$ (50.4 mg, 0.38 mmol) in THF (2.0 mL) was added $LiAlH_4$ (43.4 mg, 1.14 mmol) at 0°C. The mixture was stirred at 0°C for 1 h. To the mixture was added dropwise the cyano intermediate in THF (2 mL) at 0°C. The mixture was stirred at 0°C for 1 h. The mixture was poured into a solution of potassium

sodium (+)-tartrate tetrahydrate in water at room temperature and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. To the residue in THF (2 mL) was added acetylchloride (15 μL, 0.21 mmol) and Et_3N (29 μL, 0.21 mmol) at 0°C. The mixture was stirred at room temperature for 3 h. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc) to give (6*R*,7*R*)-*tert*-butyl 6-(2-acetamidoethyl)-7-(3,4-dichlorophenyl)-1,4-oxazepane-4-carboxylate (45.0 mg, 18%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (2H, d, *J* = 4.5 Hz), 1.51 (9H, s), 1.94 (3H, s), 3.11–3.39 (2H, m), 3.43–3.70 (5H, m), 3.77 (1H, d, *J* = 14.7 Hz), 3.96 (1H, d, *J* = 10.2 Hz), 4.05–4.13 (1H, m), 6.91 (1H, brs), 7.06–7.15 (1H, m), 7.36–7.44 (2H, m). MS m/z: 331 [M+H-Boc]$^+$.

5.1.70. *N*-(2-((6*R*,7*R*)-7-(3,4-Dichlorophenyl)-1,4-oxazepan-6-yl)ethyl)acetamide monohydrochloride (13). Typical procedure B, 34%, Colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09–1.31 (2H, m), 2.88 (1H, brs), 3.03–3.45 (6H, m), 3.72–4.05 (5H, m), 4.34 (1H, d, *J* = 10.2 Hz), 7.36 (1H, d, *J* = 8.3 Hz), 7.61–7.71 (2H, m), 7.81–7.91 (1H, m), 9.04–9.35 (1H, m), 9.46–9.80 (1H, m). MS m/z: 331.1 $[M+H]^+$. $[\alpha]^{25}_D$ +11.8 (c 0.16, MeOH).

5.1.71. X-ray structure analysis

Crystal data for compound **5**: $C_{12}H_{16}Cl_2NO_2^+ \cdot Cl^- \cdot 0.16H_2O$, MW = 315.51; crystal size, 0.26 x 0.12 x 0.06 mm; colorless, platelet; monoclinic, space group $P2_1$, a = 20.4010(10) Å, b = 6.8708(4) Å, c = 20.4513(11) Å, $\alpha = \gamma = 90°$, β = 93.862(7)°, V = 2860.2(3) Å^3, Z = 8, Dx = 1.465 g/cm^3, T = 100 K, μ = 5.774 mm^{-1}, λ = 1.54187 Å, R_1 = 0.099, wR_2 = 0.254, Flack Parameter[17] = 0.07(4).

Crystal data for compound **7**: $C_{12}H_{16}Cl_2NO_2^+ \cdot Cl^- \cdot 0.25H_2O$, MW = 317.13; crystal size, 0.20 x 0.19 x 0.05 mm; colorless, platelet; monoclinic, space group $C2$, a = 14.2479(3) Å, b = 6.52995(12) Å, c =

30.7562(6) Å, $\alpha = \gamma = 90°$, $\beta = 102.022(8)°$, $V = 2798.74(12)$ Å^3, $Z = 8$, $Dx = 1.505$ g/cm^3, $T = 100$ K, $\mu = 5.908$ mm^{-1}, $\lambda = 1.54187$ Å, $R_1 = 0.025$, $wR_2 = 0.061$, Flack Parameter[17] = 0.017(13).

All measurements were made on a Rigaku R-AXIS RAPID-191R diffractometer using graphite monochromated Cu-Kα radiation. The structure was solved by direct methods with SIR2008[18] and was refined using full-matrix least-squares on F^2 with SHELXL-2013.[19] All non-H atoms were refined with anisotropic displacement parameters.

CCDC 1044796 for compounds **5** and CCDC 1044795 for compounds **7** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/Community/Requestastructure/Pages/DataRequest.aspx

5.2. Monoamine reuptake inhibition

5.2.1. Preparation of human monoamine expressing cell

Human serotonin transporter cDNA was amplified from human brain cDNA library by PCR, and inserted into pCRII-TOPO vector (manufactured by Invitrogen). The base sequence was confirmed and modified, and subcloned to pcDNA3.1 vector (manufactured by Invitrogen), whereby a human serotonin transporter expression plasmid was constructed.

Human norepinephrine transporter cDNA was purchased from Invitrogen, and the base sequence was confirm and modified, and subcloned to pcDNA3.1 vector, whereby a human norepinephrine transporter expression plasmid was constructed.

Human dopamine transporter expression plasmid was prepared by following procedure. SRα promoter contained in pTB1411 described in JP-A-H5-076385 was cleaved with restriction enzyme HindIII (manufactured by TAKARA BIO INC.), blunt-ended, further cleaved with restriction enzyme EcoRI (manufactured by TAKARA BIO INC.), and fragmented. On the other hand, pCI vector was cleaved with restriction enzyme BglII (manufactured by TAKARA BIO INC.), blunt-ended with T4DNA polymerase, and further cleaved with restriction enzyme EcoRI (manufactured by TAKARA BIO INC.).

Into this site was inserted a SRα promoter fragment to give pCI-SRa. Then, pCI-SRa was cleaved with restriction enzyme ClaI (manufactured by TAKARA BIO INC.) and blunt–ended. Into this site was inserted a 1.63Kb fragment obtained by cleaving pGFP-C1 (manufactured by TOYOBO) with restriction enzyme Bsu36I (manufactured by Daiichi Pure Chemicals Co., Ltd.) followed by blunt-ending, whereby pMSRα neo was prepared. Human dopamine transporter cDNA was amplified from human substantia nigra cDNA library by PCR, and inserted into the pCRII vector (manufactured by Invitrogen). The base sequence was confirmed, modified and subcloned to pMSRα neo, whereby a human dopamine transporter expression plasmid was constructed.

The monoamine transporter expression plasmids thus prepared were introduced into CHO-K1 cells using FuGENE6 (manufactured by Roche Diagnostics) and according to the attached protocol, whereby each expressing cell was established.

5.2.2. Human serotonin transporter inhibitory activity

CHO cells stably expressing a human serotonin transporter were used for the measurement of human serotonin transporter inhibitory activity. Unless particularly indicated, these CHO cells were cultured in a Ham/F12 medium (Invitrogen) containing 10% fetal bovine serum (MOREGATE). The cultured cells that reached almost confluent were rinsed with PBS (Invitrogen), detached with Trypsin/EDTA (Invitrogen), and collected by a centrifugal operation. The obtained cells were counted, and diluted to 3×10^5 cells per 1 mL medium, the mixture was dispensed to a 96 well white plate (Corning) at 100 μL per well, and cultured overnight in a CO_2 incubator. Then, an assay buffer (126 mM NaCl, 4.95 mM KCl, 1.26 mM KH_2PO_4, 1.26 mM $MgSO_4$, 10 mM HEPES, 2.32 mM $CaCl_2$, 5.52 mM Glucose, 0.5% BSA) was prepared, the medium in the cell plate was removed and the assay buffer was added by 80 μL. A test compound was diluted with the assay buffer to a 10-fold concentration of the final concentration, and the mixture was dispensed to a 96 well polypropylene plate. The diluted test compound was dispensed to the cell plate by 10 μL. [^{3}H]-5-Hydroxytryptamine (GE Healthcare) was diluted with the assay buffer to 200 nM, and the mixture was dispensed to the cell plate by 10 μL. At 20 min from [^{3}H]-5-hydroxytryptamine addition, the assay buffer was removed by suction, and the plate was washed twice

with 150 μL of PBS (Invitrogen) per well. Microscinti 20 (PerkinElmer) was dispensed to each well by 100 μL, and the mixture was stirred for about 30 min. The radioactivity was measured by TopCount (PerkinElmer). The inhibitory activity of each compound (10 μM) was calculated as a relative activity value based on the inhibitory activity of 10 μM Paroxetine (serotonin transporter inhibitor) as 100%. IC_{50} and 95% confidence interval were calculated by XLfit (n=2).

5.2.3. Human norepinephrine transporter inhibitory activity

CHO cells stably expressing human norepinephrine transporter were used for the measurement of human norepinephrine transporter inhibitory activity. Unless otherwise indicated, these CHO cells were cultured in Ham/F12 medium (Invitrogen) containing 10% fetal bovine serum (MOREGATE). The cultured cells that reached almost confluent were rinsed with PBS (Invitrogen), detached with Trypsin/EDTA (Invitrogen), and collected by a centrifugal operation. The obtained cells were counted, and diluted to 3×10^5 cells per 1 mL medium, and the mixture was dispensed to a 96 well white plate (Corning) at 100 μL per well, and cultured overnight in a CO_2 incubator. Then, an assay buffer (126 mM NaCl, 4.95 mM KCl, 1.26 mM KH_2PO_4, 1.26 mM $MgSO_4$, 10 mM HEPES, 2.32 mM $CaCl_2$, 5.52 mM Glucose, 0.5% BSA) was prepared, the medium in the cell plate was removed and the assay buffer was added by 80 μL. A test compound was diluted with the assay buffer to a 10-fold concentration of the final concentration, and the mixture was dispensed to a 96 well polypropylene plate. The diluted test compound was dispensed to the cell plate by 10 μL. [^{3}H]-Norepinephrine (GE Healthcare) was diluted with the assay buffer to 200 nM, and the mixture was dispensed to the cell plate by 10 μL. At 45 min from [^{3}H]-norepinephrine addition, the assay buffer was removed by suction, and the plate was washed twice with 150 μL of PBS (Invitrogen) per well. Microscinti 20 (PerkinElmer) was dispensed to each well by 100 μL, and the mixture was stirred for about 30 min. The radioactivity was measured by TopCount (PerkinElmer). The inhibitory activity of each compound (10 μM) was calculated as a relative activity value based on the inhibitory activity of 10 μM DMI (norepinephrine transporter inhibitor) as 100%. IC_{50} and 95% confidence interval were calculated by XLfit (n=2).

5.2.4. Human dopamine transporter inhibitory activity

CHO cells stably expressing human dopamine transporter were used for the measurement of human dopamine transporter inhibitory activity. Unless otherwise indicated, these CHO cells were cultured in Ham/F12 medium (Invitrogen) containing 10% fetal bovine serum (MOREGATE). One day before the assay, the cultured cells that reached almost confluent were rinsed with PBS (Invitrogen), detached with Trypsin/EDTA (Invitrogen), and collected by a centrifugal operation. The obtained cells were counted, and diluted to 3×10^5 cells per 1 mL medium, and the mixture was dispensed to a 96 well white plate (Corning) at 100 μL per well, and cultured overnight in a CO_2 incubator. On the day of the test, an assay buffer (126 mM NaCl, 4.95 mM KCl, 1.26 mM KH_2PO_4, 1.26 mM $MgSO_4$, 10 mM HEPES, 2.32 mM $CaCl_2$, 5.52 mM Glucose, 0.5% BSA) was prepared, the medium in the cell plate was removed and the assay buffer was added by 80 μL. A test compound was diluted with the assay buffer to a 10-fold concentration of the final concentration, and the mixture was dispensed to a 96 well polypropylene plate. The diluted test compound was dispensed to the cell plate by 10 μL. [^{3}H]-Dopamine (GE Healthcare) was diluted with the assay buffer to 200 nM, cold dopamine was diluted to 10 μM, and the mixture was dispensed to the cell plate by 10 μL. At 60 min from [^{3}H]-dopamine addition, the assay buffer was removed by suction, and the plate was washed twice with 150 μL of PBS (Invitrogen) per well. Microscinti 20 (PerkinElmer) was dispensed to each well by 100 μL, and the mixture was stirred for about 30 min. The radioactivity was measured by TopCount (PerkinElmer). The inhibitory activity of each compound (10 μM) was calculated as a relative activity value based on the inhibitory activity of 100 μM Nomifensine (dopamine transporter inhibitor) as 100%. IC_{50} and 95% confidence interval were calculated by XLfit (n=2).

5.3. NET ex vivo binding assay in rat brain cortex

Adults female rats of Sprague-Dawley strain (CLEA Japan) were studied using experimental protocols approved by Takeda's Experimental Animal Care and Use Committee. CNS penetrating property was measured by investigating occupation rate of NET in the brain cortex after drug administration. After rats were anesthetized with intraperitoneal injection of urethane (Wako Pure Chemical Industries, Ltd.), the test compound was intravenously injected. Twenty minutes after the injection, the cortex in the left

brain was taken off and used for *ex vivo* binding study with [^{3}H]-nisoxetine (NEN). Fifteen times volume of assay buffer [50 mmol/L Tris Cl (pH 7.5), 125 mmol/L NaCl, 5 mmol/L KCl] as the taken cortex weight was added, homogenized, and plasma membrane fraction was prepared so that protein concentration of the fraction was 4 mg/mL. Six hundred μL of the membrane fraction, 200 μL of the assay buffer and 100 μL of [^{3}H]-nisoxetine solution (25 nmol/L) were mixed, and 100 μL of desipramine (10 μmol/L) or assay buffer was added, and the mixture was incubated at room temperature for 1 hour. The mixture was suctioned through GF/B glass filter (Whatman), and the glass filter was washed with assay buffer. Radio activity with the glass filter was measured using scintillation counter (Aloka, LSC6100). The NET occupation rate was calculated with specific binding of the vehicle group as 100%.

5.4. Evaluation of urethral resistance-increasing effects in rat

After rats were anesthetized with isoflurane (Abbott Japan) inhalation, the spinal cord was transected at the T8-T9 level after laminectomy. After closing the wound site on the back, the urinary bladder was exposed through an abdominal incision. Two polyethylene catheters (PE-100; Intramedic, Becton Dickson and Company) with a fire-flared tip were inserted into the bladder from the dome and secured with a ligature for bladder filling and pressure recording, and then, the abdomen was closed. Rats were placed in Bollman restraint cage (KN-326/3, Natsume Seisakusho). A pressure transducer (DX-100; Nihon Koden) connected to an amplifier (1257; NEC), an analog-to-digital converter (MP-100 BIOPAK systems) and a computer equipped with a data converting software (AcqKnowledge; BIOPAK systems) was used to record the intravesical pressure. Data was acquired at the rate of 100 Hz.

The bladder was then filled continuously with saline (0.1 mL/sec) containing Evans blue dye (Wako Pure Chemical) via the catheter using infusion pump-. The intravesical pressure was raised by infusion until leakage of the Evans blue solution was observed from the urethral orifice. The peak of recorded intravesical pressure was recognized as the lowest intravesical pressure inducing urinary leakage, and the value was referred as the leak point pressure (LPP). As urine leakage theoretically occurs when intravesical pressure exceeds total urethral resistance, LPP is considered to show the total urethral

resistance. Measurements were repeated, and the mean of last three LPPs was calculated. The vehicle and the test compound were intravenously or subcutaneously administered, and LPP was again measured 30 minutes after the administration. Data were analyzed with paired ***t***-test, and P values <0.05 was considered to be significant.

5.5. CYP2D6 inhibition

Inhibition activity of test compounds of CYP2D6 was evaluated by incubating 5 μmol/L bufuralol with 2 nmol/L CYP2D6 derived from CYP2D6-expressing insect cells (BD Biosciences) in the presence of 10 μmol/L test compound. The incubation mixture was allowed to stand for 60 min at 27°C. The concentration of 1'-hydroxybufuralol was measured by LC/MS/MS.

Acknowledgment. We thank Mr. Mitsuyoshi Nishitani for X-ray structure analysis.

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Figure. 1. Design strategy of peripheral-selective noradrenaline reuptake inhibitor

Figure. 2. ORTEP drawing of compound **5** (left) and **7** (right), thermal ellipsoids are drawn at 50% probability.

Figure. 3. *In vivo* profile of compound **12**. Data are expressed as the means ± SE in 6-10 rats. **P<0.01 compared with pre-value (paired ***t***-test).

Scheme 1. Synthesis of Compounds **1** [a]

[a] Reagents and conditions: (a) 4-bromo-1,2-dichlorobenzene, Mg, I_2, THF, 0°C to rt; (b) KCN, Et_3N·HCl, DMF, H_2O, 90°C; (c) $(CH_2OH)_2$, PPTS, toluene, 125°C; (d) DIBAL in toluene and then 1 M HCl aq., -78°C to rt; (e) $NaBH_4$, MeOH, 0°C to rt; (f) 1 M HCl aq., acetone, 65°C; (g) TBDMSCl, Et_3N, DMAP, THF, 65°C; (h) NH_2OH·HCl, MeOH, pyridine, 65°C, and then *p*-TsCl, DMAP, pyridine, 80°C; (i) 1) BH_3-THF, THF, 65°C, 2) 1 M HCl aq., 60°C and then, Boc_2O and Et_3N, rt. (j) 11.7 M HCl in EtOH, EtOH, rt.

Scheme 2. Synthesis of Compounds **2, 3** and **4**[a]

[a] Reagents and conditions: (a) 4-bromo-1,2-dichlorobenzene, Mg, I_2, Et_2O, -10°C to rt; (b) CH_3CN, *n*BuLi, THF, -78°C to rt; (c) $LiAlH_4$, $AlCl_3$, Et_2O, THF, 0°C to rt; (d) 1) PhCHO, $MgSO_4$, Et_3N, MeOH and then $NaBH_4$, 0°C to rt, 2) chloroacetyl chloride, THF, Et_3N, 0°C to rt; (e) NaO*t*Bu, THF, 0°C to rt; (f) $LiAlH_4$, $AlCl_3$, Et_2O, THF, 0°C to rt; (g) 1) 1-chloroethyl chloroformate, Et_3N, CH_3CN, 90°C, 2) MeOH, 80°C, 3) Boc_2O, Et_3N, THF, rt; (h) CAN, CH_3CN, water, 0°C; (i) optical resolution by HPLC; (j) 4 M HCl in EtOAc, rt.

Scheme 3. Synthesis of 6-hydroxymethyl 7-phenyl-1,4-oxazepane derivatives **5-8**[a]

[a] Reagents and conditions: (a) methyl acrylate, DABCO, DBU, CH_3CN, rt; (b) $BnNH_2$, Et_3N, MeOH, rt; (c) 1) $CaCl_2$, $NaBH_4$, THF, EtOH, 0°C to rt; 2) TBDMSCl, Et_3N, imidazole, DMAP, THF, 0°C to rt; (d) chloroacetyl chloride, Et_3N, THF, 0°C to rt; (e) 1 M NaOH aq., THF, 0°C to rt; (f) $LiAlH_4$, $AlCl_3$, THF, 0°C to rt; (g) 1) 1-chloroethyl chloroformate, CH_3CN, rt, 2) 1 M HCl aq., MeOH, 80°C, 3) Boc_2O, Et_3N, 0°C to rt; (h) optical resolution by HPLC; (i) 4 M HCl in EtOAc or 11.7 M HCl in EtOH, EtOH, rt.

Scheme 4. Synthesis of (6*S*,7*R*)-7-phenyl-1,4-oxazepane derivatives **9-17**[a]

[a] Reagents and conditions: (a) Dess-Martin periodinane, CH_3CN, 0°C; (b) 2-methyl-2-butene, $NaClO_2$, NaH_2PO_4, *t*BuOH, THF, water, 0°C; (c) WSC, $R^3R^4NH_2$, Et_3N, DMF, rt; (d) 4 M HCl in EtOAc or 11.7 M HCl in EtOH, EtOH, rt; (e) MsCl, Et_3N, THF, 0°C to rt; (f) NaN_3, DMF, 80°C; (g) PPh_3, THF, H_2O, rt; (h) AcCl, Et_3N, THF, rt; (i) 11.7 M HCl in EtOH, rt. and then fumaric acid; (j) 1) KCN, DMF, 80°C, 2) $LiAlH_4$, $AlCl_3$, THF, 0°C.

Table 1. Monoamine reuptake inh., selectivity and CYP2D6 inh. of 7-membered ring compounds[a]

[a] All compound were isolated as HCl salt. [b] These values were calculated from the results of two experiments. [c] 95% confidience interval for each IC_{50} value. [d] CYP2D6 inhibition expressed as its % inhibition value at the drug concentration of 10 μM.

Table 2. Monoamine reuptake inh., selectivity and NET occupancy of (6*S*,7*R*)-1,4-oxazepane derivatives compounds.

[a,b] See corresponding footnotes of Table 1. [c] 3 mg/kg, i.v. [d] isolated as fumaric acid salt.

Figure1

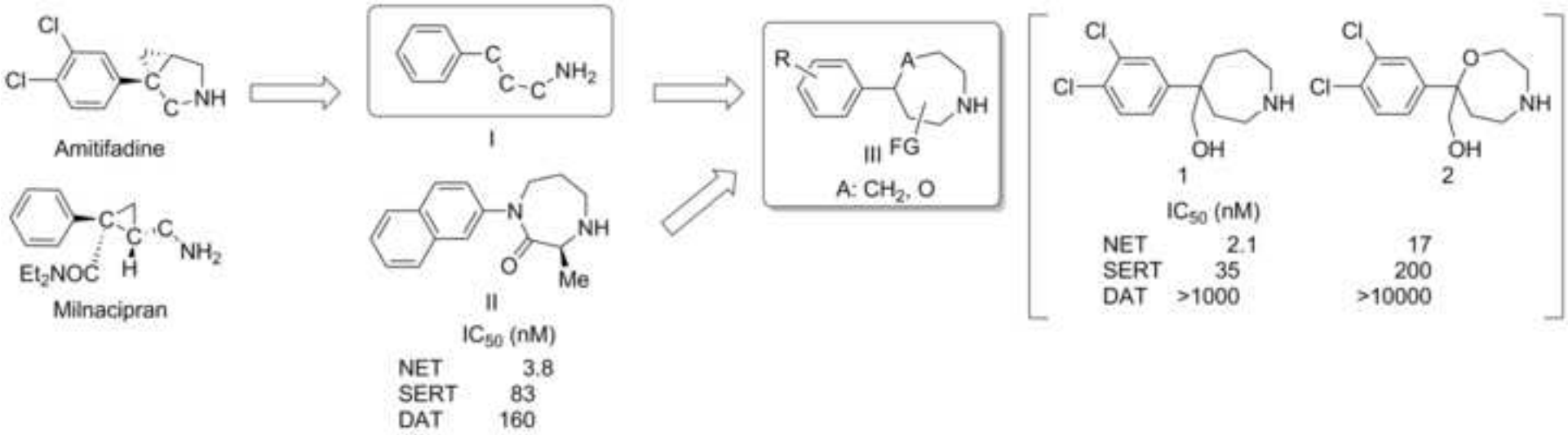


Figure2

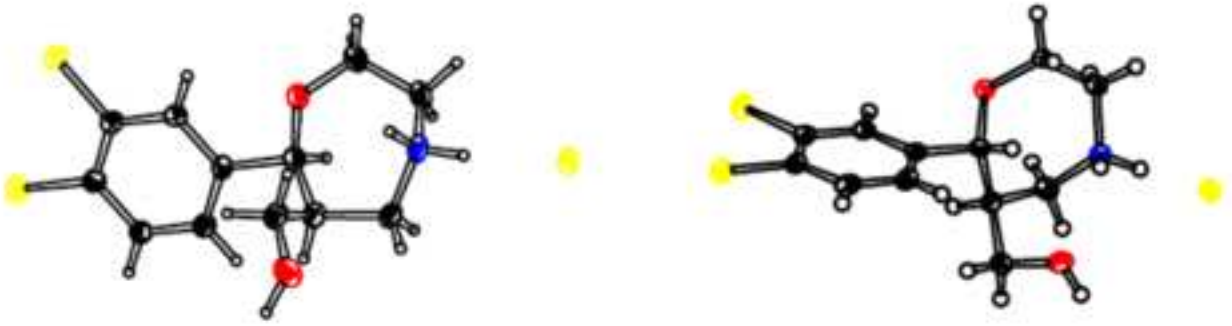
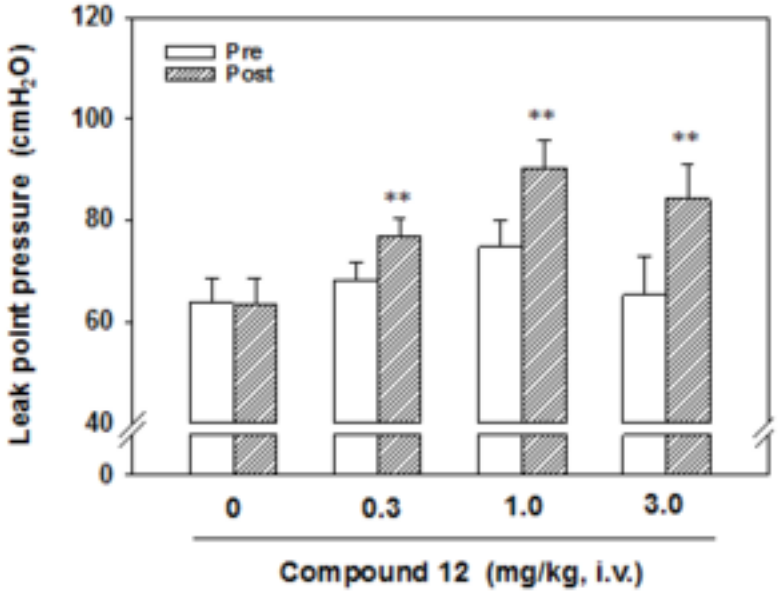
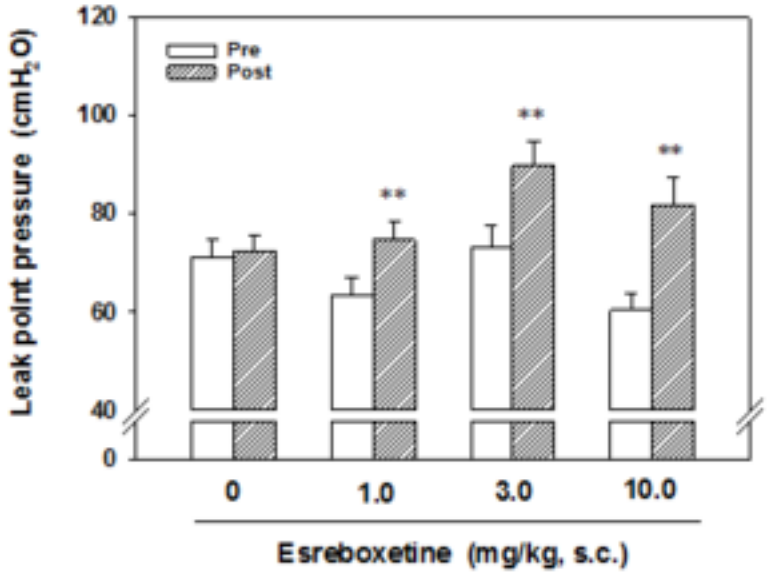
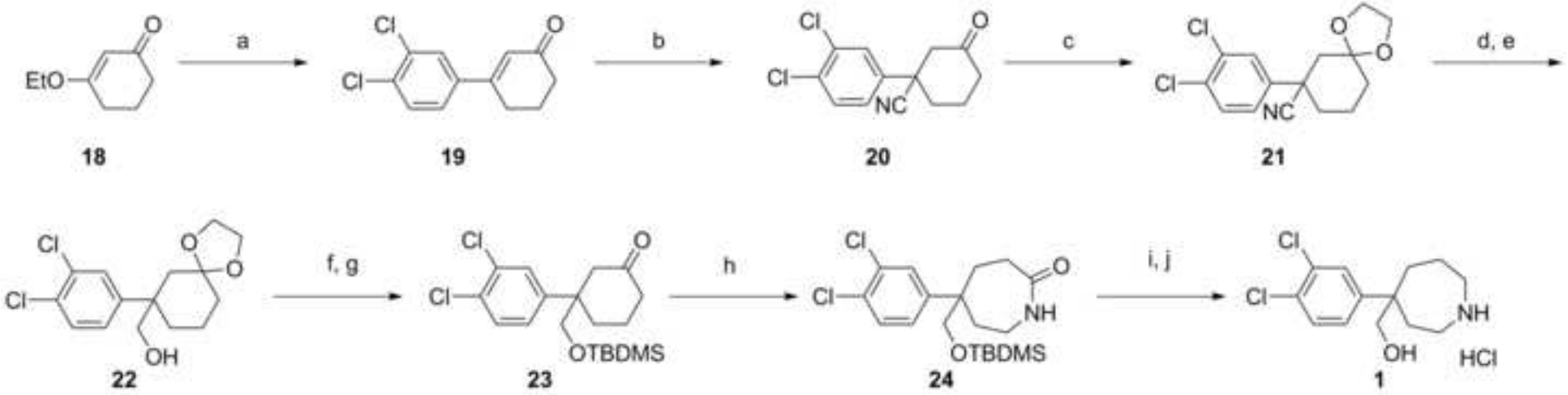


Figure3

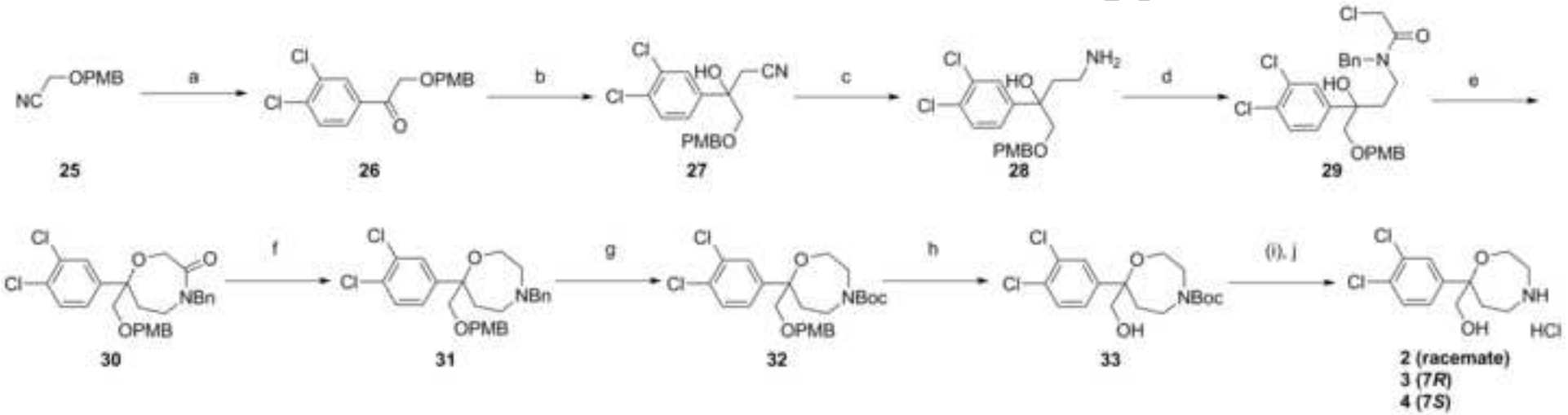




Scheme 1



Scheme 2

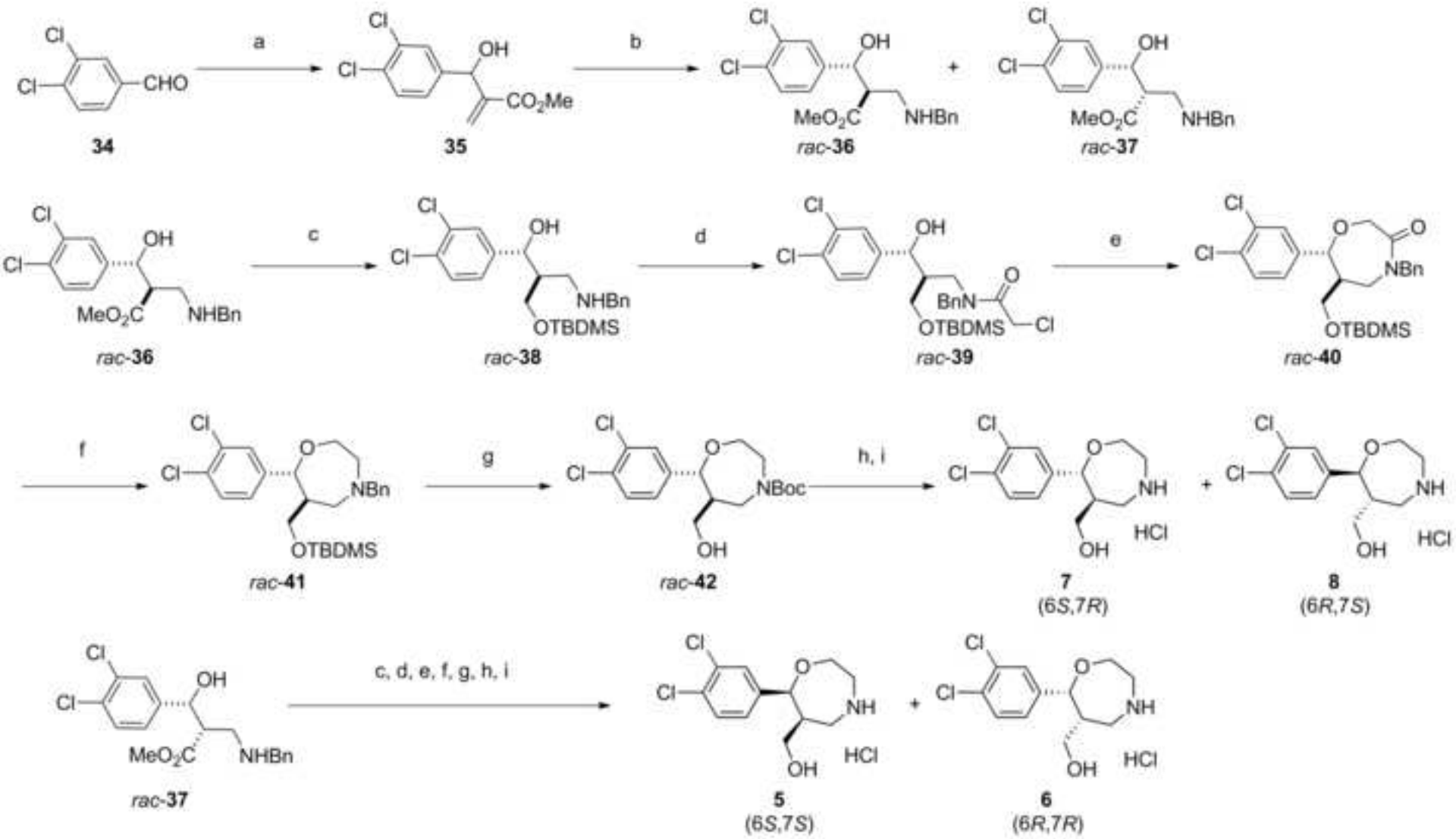


25 **26** **27** **28** **29**

30 **31** **32** **33**

2 (racemate)
3 (7*R*)
4 (7*S*)

Scheme 3



Scheme 4

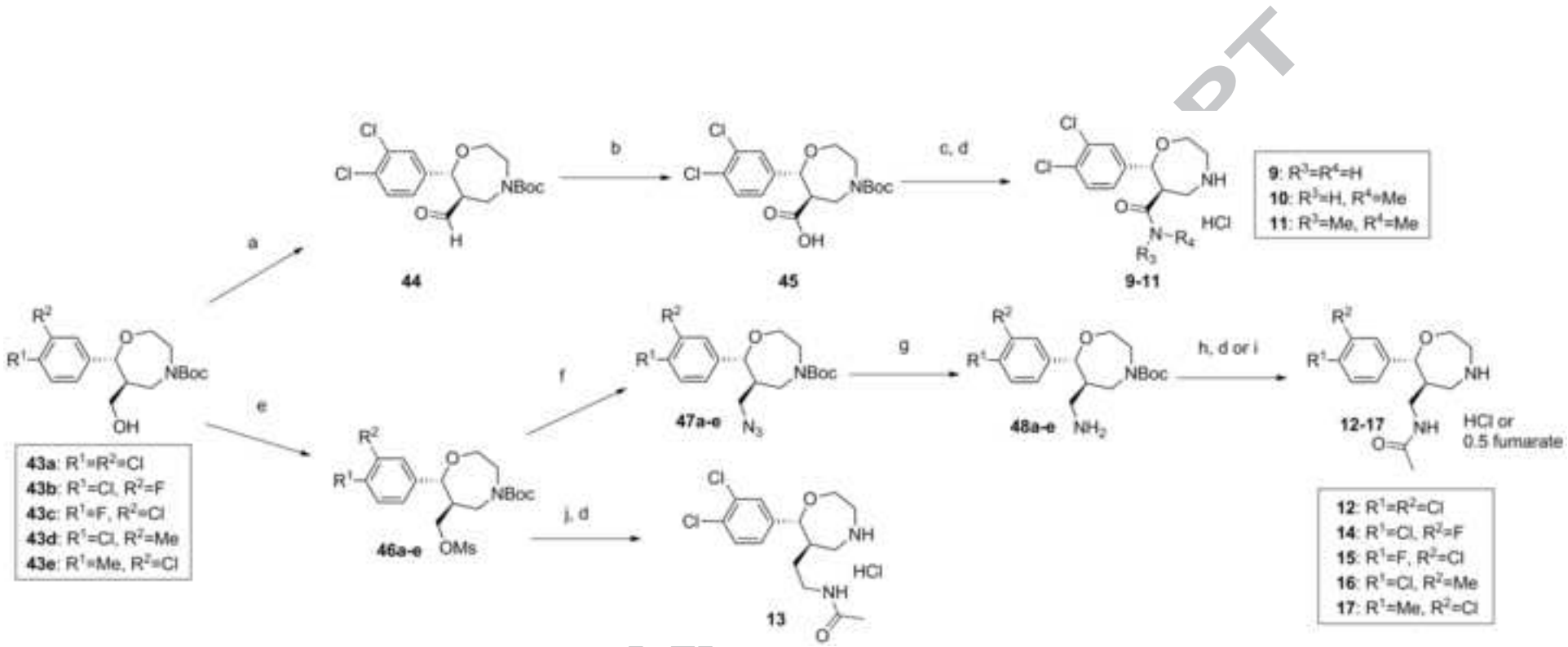
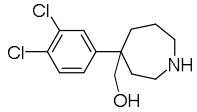
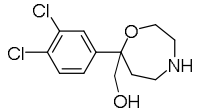
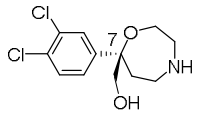
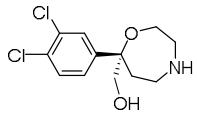
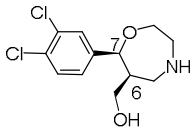
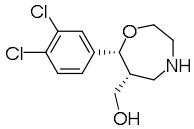
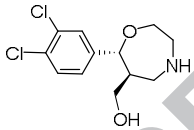
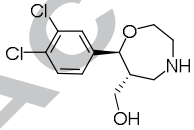
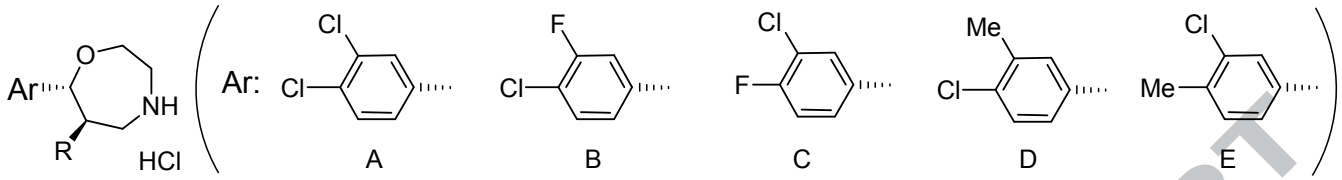


Table 1. Monoamine reuptake inh., selectivity and CYP2D6 inh. of 7-membered ring compounds[a]

Cmpd.	Structure	Stereo	Reuptake inh. IC50[b] (nM), 95% CI[c]			Selectivity		CYP2D6 inhibition (%)[d]
			NET	SERT	DAT	SERT/NET	DAT/NET	
1		racemate	2.1 (1.4-3.2)	35 (29-42)	>1000	17	>480	44
2		racemate	17 (15-19)	200 (150-270)	>10000	12	>590	19
3		7*R*	700 (530-910)	330 (240-450)	>10000	0.47	>14	21
4		7*S*	5.6 (4.4-7.1)	200 (150-250)	>10000	35	>1800	23
5		6*S*, 7*S*	8.5 (7.4-9.8)	11 (10-13)	>1000	1.3	>120	58
6		6*R*, 7*R*	6.0 (5.1-7.1)	120 (77-180)	>1000	20	>170	47
7		6*S*, 7*R*	2.0 (1.3-2.9)	97 (71-130)	3100 (1600-6000)	50	1800	34
8		6*R*, 7*S*	5.8 (4.7-7.2)	36 (31-41)	1400 (950-1900)	6.1	230	45

[a] All compound were isolated as HCl salt. [b] These values were calculated from the results of two experiments. [c] 95% confidience interval for each IC50 value. [d] CYP2D6 inhibition expressed as its % inhibition value at the drug concentration of 10 uM.

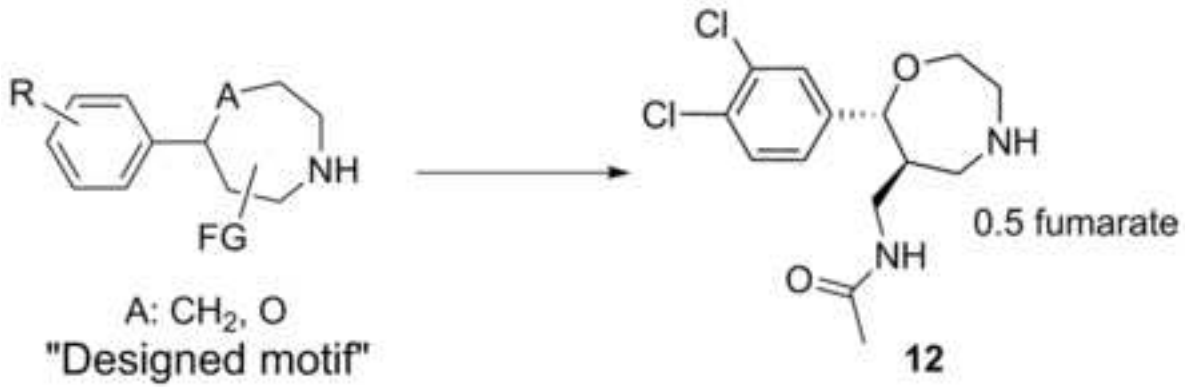
Table 2. Monoamine reuptake inh., selectivity and NET occupancy of (6*S*,7*R*)-1,4-oxazepane derivatives compounds



Cmpd.	Ar	R	Reuptake inh. IC50[a] (nM), 95% CI[b]			Selectivity		NET occupancy in rat brain(%)[c]	ClogP
			NET	SERT	DAT	SERT/NET	DAT/NET		
7	A	CH_2OH	2.0 (1.3-2.9)	97 (71-130)	3100 (1600-6000)	50	1800	68	1.0
9	A	$CONH_2$	52 (45-61)	780 (630-960)	>10000	15	>190	-	1.8
10	A	CONHMe	12 (8.2-18)	>1000	>1000	>83	>83	-	2.1
11	A	$CONMe_2$	0.77 (0.66-0.89)	350 (270-450)	70 (46-100)	460	91	68	2.6
12[d]	A	CH_2NHAc	0.33 (0.16-0.67)	150 (120-180)	1500 (1000-2100)	450	4400	-14	0.71
13	A	$(CH_2)_2NHAc$	5.8 (4.2-8.0)	200 (160-250)	>1000	35	>170	-	1.2
14	B	CH_2NHAc	2.3 (1.9-2.7)	>1000	>1000	>440	>440	-2.8	0.26
15[d]	C	CH_2NHAc	13 (11-16)	>1000	>1000	>76	>76	1.7	0.26
16	D	CH_2NHAc	3.3 (2.3-4.8)	480 (280-820)	>1000	150	>300	-	0.61

17	E	CH_2NHAc	3.4 (2.3-5.0)	680 (500-930)	>1000	200	>300	-	0.61

[a.b] See corresponding footnotes of Table 1. [c] 3 mg/kg, i.v. [d] isolated as fumaric acid salt.



Reuptake inhibitions (NET/SERT/DAT):
IC_{50}= 0.33 / 150 / 1500 nM
NET Occupancy in rat brain: -14%
Urethral resistance-increasing effect in rats