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An efficient synthesis of the optically active isomers of 2*H*-1,4-benzoxazine derivatives, novel KATP channel modulators

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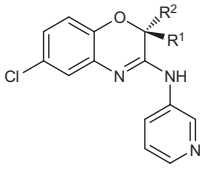
ABSTRACT

2*H*-1,4-Benzoxazine amidine derivatives are drugs acting as modulators of the skeletal muscle and pancreatic beta cell ATP-sensitive-K^+ (KATP) channels. With the aim of evaluating the influence of absolute configuration on the biological activity of these drugs, we herein report the optimization of a synthetic route to obtain both enantiomers of some of these compounds with improved chemical yield and high enantiomeric excess.

1. Introduction

Potassium channel openers (KCO) are chemically diverse compounds that belong to a number of structural classes. Many studies report the activities of these derivatives against the ATP-sensitive K^+ (KATP) channel subunits expressed in cell lines, primary targets for KCO action. These compounds show a broad range of therapeutic applications, including hypertension, angina, hypoglycemia, neuromuscular disorders, and epilepsy and exert their effects on pancreatic beta cells, neurons, and cardiac muscle by modulating KATP channels, changing the cellular electrical activity.[1,2]

Recently we reported on some 2*H*-1,4-benzoxazine amidine derivatives, which have emerged as novel skeletal muscle KATP channel modulators. In the presence of ATP, these molecules display KCO activity, whereas in the absence of the nucleotide they display a blocking action. Moreover, in vitro and in vivo, they show a combined activating/blocking action toward pancreatic beta cell KATP channels that confers a significant glycemic control, which supports their use as novel antidiabetic drugs.[3–5] With the aim of investigating the influence of the absolute configuration on the biological activity of these drugs, we decided to synthesize the stereoisomers of a series of chiral compounds (Fig. 1). For this reason, a preliminary study was conducted on compound **1** in order to identify the best pathway to follow.



Cpd	R^1	R^2	Cpd	R^1	R^2
(*R*)-**1**	H	CH_3	(*R*)-**5**	H	Cy
(*S*)-**1**	CH_3	H	(*S*)-**5**	Cy	H
(*R*)-**2**	H	CH_2CH_3	(*R*)-**6**	H	CH_2Cy
(*S*)-**2**	CH_2CH_3	H	(*S*)-**6**	CH_2Cy	H
(*R*)-**3**	H	$(CH_2)_2CH_3$	(*R*)-**7**	H	CH_2Ph
(*S*)-**3**	$(CH_2)_2CH_3$	H	(*S*)-**7**	CH_2Ph	H
(*R*)-**4**	H	$CH(CH_3)_2$	(*R*)-**8**	H	$(CH_2)_2Ph$
(*S*)-**4**	$CH(CH_3)_2$	H	(*S*)-**8**	$(CH_2)_2Ph$	H

Figure 1.

2. Results and discussion

Different attempts were carried out in order to obtain both enantiomers of this derivative. All synthetic routes included the involvement of key intermediates 6-chloro-2-methyl-2*H*-1,4-benzoxazine-3-ones **9a**, **9b**, obtained by the nitration of commercially available (*S*)- or (*R*)-2-(4-chloro-phenoxy)propanoic acid, respectively, followed by a one-pot reduction-cyclization with iron in hydrochloric acid as shown in Scheme 1.

Thereafter, different reaction conditions were investigated for the optimization of the pathway leading to (*R*)- or (*S*)-**1**. At first, we chose the same synthetic method used for the preparation of the racemate reacting **9a** or **9b** with 3-amino-pyridine in the presence of $TiCl_4$ and anisole (Scheme 2, method A).[4,6] This procedure gave reasonably good chemical yields but led to racemization.

For our next attempt, a good leaving group at the 3-position of the benzoxazinones **9a**–**b** was introduced in order to facilitate a nucleophilic substitution reaction from 3-amino-pyridine. Thus we turned these key intermediates into the corresponding (*S*)- or (*R*)-6-chloro-2-methyl-2*H*-1,4-benzoxazine-3-thiones using

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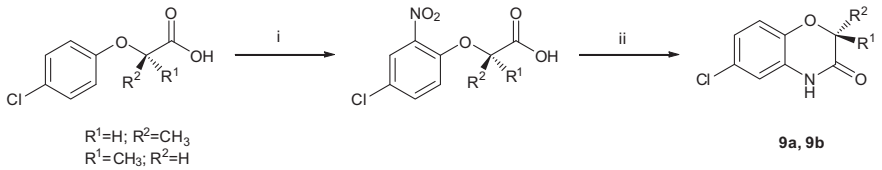
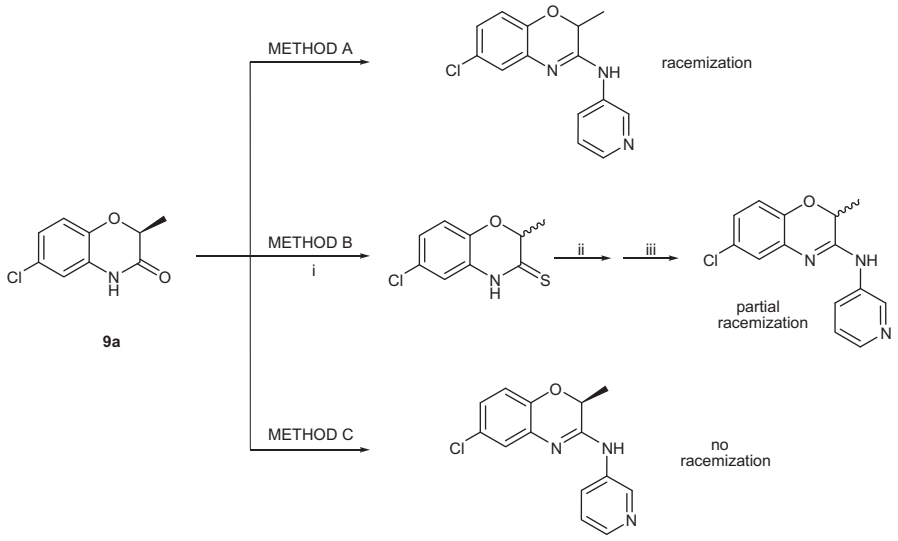


Scheme 1. Reagents and conditions: (i) 90% HNO_3, 0 °C, 4 h; (ii) Fe, 6 M HCl, 1,4-dioxane, reflux, 4 h.



Scheme 2. Reagents and conditions: Method A: 3-amino-pyridine, $TiCl_4$, anisole, anhydrous toluene, reflux, 24 h. Method B: (i) P_2S_5, toluene, reflux, 9 h or Lawesson's reagent, anhydrous toluene, reflux, 1 h; (ii) 1 M KOH, CH_3I, rt, 2 h; (iii) 3-amino-pyridine, 120 °C, 3 h. Method C: 3-amino-pyridine, Et_3N, $POCl_3$, anhydrous acetonitrile, rt, 4 h.

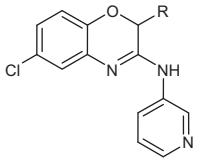
Lawesson's reagent or P_2S_5; sulfur was then methylated to give the corresponding iminothioethers, which were finally reacted with 3-amino-pyridine (Scheme 2, method B). In this way, the target compounds were obtained in optically active forms, but unfortunately their enantiomeric excess was not reproducible. We also found that the preparation of the thiones occurred with partial racemization which was dependent on the reaction time, concentration of the reaction solution, and crystallization conditions.

On the basis of these results, we attempted to design a synthetic process, which involved milder conditions, short reaction times and, at the same time, avoided the problems due to the malodorous properties of sulfurated compounds. We decided to follow a different synthetic route (Scheme 2, method C) which in a single step, transformed **9a**–**b** into the corresponding imido chloride intermediates with $POCl_3$ and easily converted them into (*S*)- or (*R*)-**1** by smooth condensation with 3-amino-pyridine. This procedure was previously reported in the literature but not applied to the preparation of optically active chiral amidines.[7,8]

Under these conditions, the reaction led to the isolation of the target compounds in moderate to good yields with an easy purification procedure consisting of a filtration of the solid residue, which was washed out and crystallized from chloroform/*n*-hexane. This method did not show any racemization and also allowed us to retrieve the unreacted starting products **9a**–**b** by column chromatography of the mother liquors. The amount of unreacted benzoxazinones was higher if the purification of (*R*)- or (*S*)-**1** was carried out by column chromatography allowing us to hypothesize that silica gel catalyzes the conversion of (*R*)- or (*S*)-**1** into the starting benzoxazinones.

The stereoisomers of analogues **2**–**8** were also prepared by following this procedure and in Table 1 the chemical yields and enantiomeric excesses obtained in the last step of the synthesis of these compounds are reported.

Table 1



Compound	R	Yield	ee[a] (%)
(*R*)-**1**	CH_3	19[b]	95
(*S*)-**1**	CH_3	45	97
(*R*)-**2**	CH_2CH_3	76	91
(*S*)-**2**	CH_2CH_3	87	94
(*R*)-**3**	$(CH_2)_2CH_3$	83	96
(*S*)-**3**	$(CH_2)_2CH_3$	37[b]	97
(*R*)-**4**	$CH(CH_3)_2$	25[b]	99
(*S*)-**4**	$CH(CH_3)_2$	37	98
(*R*)-**5**	Cy	62	99
(*S*)-**5**	Cy	39[b]	99
(*R*)-**6**	CH_2Cy	71	99
(*S*)-**6**	CH_2Cy	85	98
(*R*)-**7**	CH_2Ph	59	99
(*S*)-**7**	CH_2Ph	64	99
(*R*)-**8**	$(CH_2)_2Ph$	65	99
(*S*)-**8**	$(CH_2)_2Ph$	52	99

[a] Determined by HPLC analysis on Chiralcel OD or Chiralcel OD-R column (see Supplementary data).

[b] In these cases, the purification process was carried out by column chromatography.

3. Conclusion

In conclusion, we have reported on an alternative and efficient method to synthesize highly pure optically active isomers of chiral 2*H*-1,4-benzoxazine amidine derivatives overcoming problems due to the lability of the stereogenic center under the conditions used in previous attempts. This will allow us to study how the absolute configuration can affect the biological activity of these promising modulators of the pancreatic and skeletal muscle ATP-sensitive-K^+ channels.

4. Experimental

4.1. General

Column chromatography was performed on ICN Silica Gel 60 Å (63–200 μm) as a stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus and are uncorrected. Mass spectra were recorded on an HP GC/MS 6890-5973 MSD spectrometer, electron impact 70 eV, equipped with HP chemstation. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian-Mercury 300 (300 MHz) spectrometer at room temperature. Chemical shifts are expressed as parts per million (δ). The purity of the final compounds was >95%, as confirmed by combustion analysis carried out with a Eurovector Euro EA 3000 model analyzer.

Optical rotations were measured with a Perkin–Elmer 341 polarimeter at room temperature (20 °C): Concentrations are expressed as g (100 mL)$^{-1}$. The enantiomeric excesses of the final compounds were determined by HPLC analysis on Chiralcel OD or Chiralcel OD-R column (4.6 mm i.d. × 250 mm, Daicel Chemical Industries, Ltd, Tokyo, Japan). Analytical liquid chromatography was performed on a PE chromatograph equipped with a Rheodyne 7725i model injector, a 785A model UV/vis detector, a series 200 model pump and an NCI 900 model interface. Chemicals were from Aldrich (Milan, Italy), AlfaAesar (Karlsruhe, Germany) or Acros (Milan, Italy) and were used without any further purification.

4.2. Synthesis of (*S*)- or (*R*)-6-chloro-2-methyl-2*H*-1,4-benzoxazine-3-one 9a, 9b

An ice-cooled solution of (*S*)- or (*R*)-2-(4-chloro-phenoxy)propanoic acid (6 g) in 90% HNO_3 (10 mL) was stirred at 0 °C for 4 h. After the reaction was complete, the mixture was poured into ice and then extracted by diethyl ether (3 × 50 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give a yellow oil (7.18 g), which was dissolved in 1,4-dioxane (140 mL), after which were added with Fe (15.1 g) and 6 M HCl (140 mL) and refluxed. The reaction progress was checked by thin layer chromatography (TLC). After 4 h, the resulting mixture was poured into ice and extracted with diethyl ether (3 × 70 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated giving a red-brown solid that was purified by column chromatography over silica gel using petroleum ether/ethyl acetate (8:2) and then crystallized from *n*-hexane to afford the title compound as a white powder (5.2 g). 90% Yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.5 (1H, s, NH, exchange with D_2O), 6.91–7.17 (3H, m, aromatics), 4.88 (1H, q, J = 7.5 Hz, CH), 1.54 (3H, d, J = 7.5 Hz, CH_3). GC–MS *m*/*z*: 199 [34, $(M+2)^+$], 197 (100, M^+), 156 (30), 154 (90). (*R*)-6-Chloro-2-methyl-2*H*-1,4-benzoxazine-3-one, $[\alpha]_D^{20} = +39$ (*c* 1.0, MeOH); ee = 99% (Chiralcel OD-R column, methanol/water 90:10 as a mobile phase, flow rate: 0.4 mL min^{-1}, detection: 254 nm); (*S*)-6-chloro-2-methyl-2*H*-1,4-benzoxazine-3-one, $[\alpha]_D^{20} = -39$ (*c* 1.0, MeOH); ee = 99% (Chiralcel OD-R column, methanol/water 90:10 as a mobile phase, flow rate: 0.4 mL min^{-1}, detection: 254 nm).

4.3. Synthesis of (*R*)-6-chloro-2-methyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-1: Method A

To an ice-cooled suspension of $TiCl_4$ (6 mmol) in anhydrous toluene (35 mL) and anisole (1 mL) was added 3-amino-pyridine (50 mmol) and (*R*)-6-chloro-2-methyl-2*H*-1,4-benzoxazine-3-one (5 mmol) at 0 °C and then refluxed for 24 h. The reaction was cooled at room temperature, quenched with 96% ethanol and ammonium hydroxide (30%), filtered on a Celite pad, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give a dark brown oil, which was purified by column chromatography over silica gel using petroleum ether/ethyl acetate/methanol (8:1.5:0.5) and then crystallized from chloroform/*n*-hexane to afford the title compound as a white powder. 28% Yield. $[\alpha]_D^{20} = 0$ (*c* 1.0, MeOH), ee = 0% (Chiralcel OD-R column, methanol/water 90:10 as a mobile phase, flow rate: 0.4 mL min^{-1}, detection: 254 nm).

4.4. Synthesis of (*R*)-6-chloro-2-methyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-1: Method B

(*R*)-6-Chloro-2-methyl-2*H*-1,4-benzoxazine-3-one (2.4 g) and P_2S_5 (12 g) were dissolved in toluene and refluxed for 9 h. The reaction was then cooled to room temperature and filtered. The solution was concentrated in vacuo, dissolved in chloroform, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to give a yellow solid, which was purified by column chromatography over silica gel using petroleum ether/ethyl acetate (95:5) and then crystallized from chloroform/*n*-hexane to afford (*R*)-6-chloro-2-methyl-2*H*-1,4-benzoxazine-3-thione as a yellow powder (1.69 g; yield 69%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.50 (3H, d, J = 7.5 Hz, CH_3), 4.97 (1H, q, J = 7.5 Hz, CH), 6.91–7.17 (3H, m, aromatics), 11.1 (1H, s, NH, exchange with D_2O). The thio-derivative so obtained was dissolved in 1 M KOH (50 mL) after which was added CH_3I (1.76 g). The resulting mixture was stirred for 2 h at room temperature, then diluted with H_2O, and extracted (3 × 75 mL) with diethyl ether. The organic portion was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give (*R*)-6-chloro-2-methyl-3-methylsulfanyl-2*H*-1,4-benzoxazine as a yellow oil, in quantitative yield. GC–MS *m*/*z* 229 [28, $(M+2)^+$], 227 (76, M^+), 182 (36), 180 (100).

This product was mixed with 3-amino-pyridine at 120 °C. The reaction progress was checked by thin layer chromatography (TLC) and after 3 h the crude was purified by column chromatography over silica gel using petroleum ether/ethyl acetate/methanol (8:1,5:0,5) and crystallized from chloroform/*n*-hexane to afford the title compound as a white powder. 72% Yield. mp = 199–200 °C. (*R*)-**1**, $[\alpha]_D^{20} = +46$ (*c* 1.0, MeOH), ee = 20%. (Chiralcel OD-R column, methanol/water 90:10 as a mobile phase, flow rate: 0.4 mL min^{-1}, detection: 254 nm).

Alternatively, the intermediate (*R*)-6-chloro-2-methyl-2*H*-1,4-benzoxazine-3-thione could be synthesized by adding Lawesson's reagent (2.5 mmol) to a stirred solution of (*R*)-6-chloro-2-methyl-2*H*-1,4-benzoxazine-3-one (5 mmol) in anhydrous toluene (50 mL) and heating at reflux for 1 h. Next the solvent was concentrated and the crude solid purified by column chromatography over silica gel using petroleum ether/ethyl acetate (85:15) to give a yellow solid (90% yield). In both cases, the conversion of benzoxazine-3-one into benzoxazine-3-thione was associated with partial racemization (Chiralcel OD-R column, methanol/water 90:10 as a mobile phase, flow rate: 0.4 mL min^{-1}, detection: 254 nm) whose extension depended on the reaction time, concentration of the reaction solution, and crystallization conditions.

4.5. Synthesis of (*R*)- or (*S*)-6-chloro-2-methyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-1 or (*S*)-1: Method C

At first, Et_3N (2 mmol) and $POCl_3$ (2 mmol) were added under a nitrogen atmosphere to a solution of (*R*)- or (*S*)-6-chloro-2-methyl-2*H*-1,4-benzoxazine-3-one (1 mmol) in anhydrous acetonitrile (5 mL) at room temperature. After 20 min, 3-amino-pyridine (2 mmol) was added and the mixture was stirred for 4 h. Then the resulting precipitate was filtered off, dissolved in ethyl acetate, washed with cooled 1.5 M NaOH and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give a pale yellow solid. Compound (*R*)-**1** was purified by column chromatography over silica gel using petroleum ether/ethyl acetate/methanol (8:1.5:0.5) and crystallized from chloroform/*n*-hexane to give a white powder; compound (*S*)-**1** was directly purified by crystallization from chloroform/*n*-hexane.

4.6. (*S*)-6-Chloro-2-methyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*S*)-1

45% Yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.30 (3H, d, J = 6.7 Hz, CH_3), 4.91 (1H, q, J = 6.7 Hz, CH), 6.84–7.09 (3H, m, aromatics), 7.32–7.37, 8.21–8.26, 8.36–8.39 and 8.91 (4H, m, aromatics), 9.63 (1H, s, NH, exchange with D_2O); GC–MS m/z 275 [32, $(M+2)^+$], 273 (100, M^+), 131 (39), $[\alpha]_D^{20} = -256$ (c 1.0, MeOH), ee = 97% (Chiralcel OD-R column, methanol/water 90:10 as a mobile phase, flow rate: 0.4 mL min^{-1}, detection: 254 nm); mp = 200–202 °C. Anal. Calcd for $C_{14}H_{12}N_3ClO$: C, 61.43; H, 4.42; N, 15.35. Found: C, 61.21; H, 4.48; N, 15.37.

4.7. (*R*)-6-Chloro-2-methyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-1

19% Yield. $[\alpha]_D^{20} = +250$ (c 1.0, MeOH), ee = 95%; mp = 202–204 °C. Anal. Calcd for $C_{14}H_{12}N_3ClO$: C, 61.43; H, 4.42; N, 15.35. Found: C, 61.13; H, 4.44; N, 15.52.

4.8. Synthesis of (*R*)- or (*S*)-6-chloro-2-substituted-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazines 2–8

All compounds were synthesized using Method C.

4.9. (*R*)-6-Chloro-2-ethyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-2

76% Yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.94 (3H, t, J = 7.1 Hz, $CHCH_2CH_3$), 1.60 (2H, q, J = 7.1 Hz, $CHCH_2CH_3$), 4.67 (1H, t, J = 7.1 Hz, $CHCH_2CH_3$), 6.86–6.98 and 7.07–7.09 (3H, m, aromatics), 7.27–7.37, 8.21–8.23, 8.37–8.40 and 8.91 (4H, m, aromatics), 9.67 (1H, s, NH, exchange with D_2O); GC–MS m/z 289 [36, $(M+2)^+$], 287 (100, M^+), 259 (65); $[\alpha]_D^{20} = +273$ (c 1.0, MeOH), ee = 91% (Chiralcel OD-R column, methanol/0.02N $HClO_4$/$NaClO_4$ 70:30 pH 3.5 as a mobile phase, flow rate: 0.4 mL min^{-1}, T = 35 °C, detection: 254 nm); mp = 169–170 °C. Anal. Calcd for $C_{15}H_{14}N_3ClO$: C, 62.61; H, 4.90; N, 14.60. Found: C, 62.18; H, 4.97; N, 14.67.

4.10. (*S*)-6-Chloro-2-ethyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*S*)-2

87% Yield. $[\alpha]_D^{20} = -280$ (c 1.0, MeOH), ee = 94%; mp = 165–167 °C. Anal. Calcd for $C_{15}H_{14}N_3ClO$: C, 62.61; H, 4.90; N, 14.60. Found: C, 62.24; H, 4.92; N, 14.54.

4.11. (*R*)-6-Chloro-2-propyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-3

83% Yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.87 (3H, t, J = 7.1 Hz, CH_2CH_3), 1.30–1.66 (4H, m, $CH_2CH_2CH_3$), 4.77 [1H, dd, J = 9.6, 6.6 Hz, $CH(CH_2)_2$], 6.85–7.08 (3H, m, aromatics), 7.32–7.36, 8.29–8.39 and 8.91 (4H, m, aromatics), 9.64 (1H, s, NH, exchange with D_2O); GC–MS m/z 303 [20, $(M+2)^+$], 301 (60, M^+), 259 (100), $[\alpha]_D^{20} = +272$ (c 1.0, MeOH), ee = 96% (Chiralcel OD column, *n*-hexane/isopropanol/DEA 95:5:0.1 as a mobile phase, flow rate: 1 mL min^{-1}, detection: 254 nm); mp = 168–169 °C. Anal. Calcd for $C_{16}H_{16}N_3ClO$: C, 63.68; H, 5.34; N, 13.92. Found: C, 63.18; H, 5.62; N, 13.50.

4.12. (*S*)-6-Chloro-2-propyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*S*)-3

37% Yield. $[\alpha]_D^{20} = -274$ (c 1.0, MeOH), ee = 97%; mp = 164–165 °C. Anal. Calcd for $C_{16}H_{16}N_3ClO$: C, 63.68; H, 5.34; N, 13.92. Found: C, 62.66; H, 5.51; N, 13.57.

4.13. (*R*)-6-Chloro-2-isopropyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-4

25% Yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 and 1.09 [6H, 2 d, J = 6.6 Hz, $CH(CH_3)_2$], 2.15 [1H, m, J = 6.6 Hz, $CH(CH_3)_2$], 4.31 (1H, d, J = 8.0 Hz, CHCH), 6.82–7.00 (3H, m, aromatics), 7.26–7.31, 8.10–8.23 (4H, m, aromatics), 8.49 (1H, s, NH, exchange with D_2O); GC–MS m/z 303 [37, $(M+2)^+$], 301 (100, M^+), 258 (94), $[\alpha]_D^{20} = +373$ (c 1.0, MeOH), ee = 99% (Chiralcel OD column, *n*-hexane/isopropanol/DEA 95:5:0.1 as a mobile phase, flow rate: 1 mL min^{-1}, detection: 254 nm); mp = 108–109 °C. Anal. Calcd for $C_{16}H_{16}N_3ClO$: C, 63.68; H, 5.34; N, 13.92. Found: C, 63.18; H, 5.32; N, 13.77.

4.14. (*S*)-6-Chloro-2-isopropyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*S*)-4

37% Yield. $[\alpha]_D^{20} = -373$ (c 1.0, MeOH), ee = 98%; mp = 106–109 °C. Anal. Calcd for $C_{16}H_{16}N_3ClO$: C, 63.68; H, 5.34; N, 13.92. Found: C, 63.598; H, 5.37; N, 13.46.

4.15. (*R*)-6-Chloro-2-cyclohexyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-5

62% Yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.09–1.99 (11H, m, Cy), 4.33 (1H, d, J = 7.7 Hz, CHCH), 6.82–7.03 (3H, m, aromatics), 7.29–7.33, 8.24–8.25 (4H, m, aromatics), 8.55 (1H, s, NH, exchange with D_2O); GC–MS m/z 343 [37, $(M+2)^+$], 341 (100, M^+), 259 (100), $[\alpha]_D = +353$ (c 1.0 in MeOH), ee = 99% (Chiralcel OD column, *n*-hexane/isopropanol/DEA 95:5:0.1 as a mobile phase, flow rate: 1 mL min^{-1}, detection: 254 nm); mp = 168–169 °C. Anal. Calcd for $C_{19}H_{20}N_3ClO$: C, 66.76; H, 5.90; N, 12.29. Found: C, 66.28; H, 5.93; N, 11.74.

4.16. (*S*)-6-Chloro-2-cyclohexyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*S*)-5

39% Yield. $[\alpha]_D^{20} = -353$ (c 1.0, MeOH), ee = 99%; mp = 170–172 °C. Anal. Calcd for $C_{19}H_{20}N_3ClO$: C, 66.76; H, 5.90; N, 12.29. Found: C, 66.34; H, 5.88; N, 12.16.

4.17. (*R*)-6-Chloro-2-cyclohexylmethyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-6

71% Yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.81–1.87 (13H, m, CH_2Cy), 4.85–4.89 (1H, m, C*H*CH_2), 6.84–6.96 and 7.07–7.09 (3H, m, aromatics), 7.32–7.37, 8.20–8.23, 8.36–8.39 and 8.91 (4H, m, aromatics), 9.68 (1H, s, NH, exchange with D_2O); GC–MS *m*/*z* 357 [10, $(M+2)^+$], 355 (29, M^+), 259 (100), $[\alpha]_D^{20} = +256$ (*c* 1.0, MeOH), ee = 98% (Chiralcel OD column, *n*-hexane/isopropanol/DEA 98:2:0.01 as a mobile phase, flow rate: 0.5 mL min^{-1}, detection: 254 nm); mp = 129–131 °C. Anal. Calcd for $C_{20}H_{22}N_3ClO$: C, 67.50; H, 6.23; N, 11.81. Found: C, 67.39; H, 6.31; N, 11.95.

4.18. (*S*)-6-Chloro-2-cyclohexylmethyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*S*)-6

85% Yield. $[\alpha]_D^{20} = -256$ (*c* 1.0, MeOH), ee = 99%; mp = 132–134 °C. Anal. Calcd for $C_{20}H_{22}N_3ClO$: C, 67.50; H, 6.23; N, 11.81. Found: C, 67.37; H, 6.33; N, 11.22.

4.19. (*R*)-2-Benzyl-6-chloro-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-7

59% Yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.84–2.87 (2H, m, CH_2CH), 4.95–4.99 (1H, m, CH_2C*H*), 6.78–6.81, 6.98–7.02 and 7.13–7.40 (8H, m, aromatics), 8.24–8.26, 8.38–8.41 and 8.92 (4H, m, aromatics), 9.76 (1H, s, NH, exchange with D_2O); GC–MS *m*/*z* 351 [15, $(M+2)^+$], 349 (44, M^+), 258 (100); $[\alpha]_D^{20} = +273$ (*c* 1.0, MeOH), ee = 99% (Chiralcel OD column, *n*-hexane/isopropanol/DEA 80:20:0.1 as a mobile phase, flow rate: 1 mL min^{-1}, detection: 320 nm); mp = 207–208 °C dec. Anal. Calcd for $C_{20}H_{16}N_3ClO$: C, 68.67; H, 4.61; N, 12.01. Found: C, 68.29; H, 4.68; N, 11.76.

4.20. (*S*)-2-Benzyl-6-chloro-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*S*)-7

64% Yield. $[\alpha]_D^{20} = -273$ (*c* 1.0, MeOH), ee = 99%; mp = 204–206 °C. Anal. Calcd for $C_{20}H_{16}N_3ClO$: C, 68.67; H, 4.61; N, 12.01. Found: C, 68.17; H, 4.70; N, 11.72.

4.21. (*R*)-6-Chloro-2-phenethyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*R*)-8

65% Yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.84–1.91 (2H, m, CH_2CH), 2.62–2.83 (2H, m, PhCH_2), 4.77 (1H, CH_2C*H*), 6.86–7.36 (8H, m, aromatics), 8.21–8.23, 8.36–8.39 and 8.88–8.90 (4H, m, aromatics), 9.70 (1H, s, NH, exchange with D_2O); GC–MS *m*/*z* 365 [15, $(M+2)^+$], 363 (43, M^+), 259 (100); $[\alpha]_D^{20} = +244$ (*c* 1.0, MeOH), ee = 99% (Chiralcel OD column, *n*-hexane/isopropanol/DEA 80:20:0.1 as a mobile phase, flow rate: 1 mL min^{-1}, detection: 320 nm); mp = 179–181 °C. Anal. Calcd for $C_{21}H_{18}N_3ClO$: C, 69.32; H, 4.99; N, 11.55. Found: C, 69.36; H, 4.98; N, 11.35.

4.22. (*S*)-6-Chloro-2-phenethyl-3-(pyridin-3-yl-amino)-2*H*-1,4-benzoxazine (*S*)-8

52% Yield. $[\alpha]_D^{20} = -244$ (*c* 1.0, MeOH), ee = 99%; mp = 183–185 °C. Anal. Calcd for $C_{21}H_{18}N_3ClO$: C, 69.32; H, 4.99; N, 11.55. Found: C, 69.18; H, 5.16; N, 11.20.

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