Discovery of SHR9352, a highly potent G protein biased mu-opioid receptor agonist

Xin Li,*† Wei He,† Yang Chen,† Guimei Yang,† Hong Wan,† Lei Zhang,† Qiyue Hu,† Jun Feng,† Zhigao Zhang,† Feng He,† Chang Bai,† Lianshan Zhang,‡ Li You,§ Weikang Tao†

†Shanghai Hengrui Pharmaceutical CO., LTD., 279 Wenjing Road, Shanghai 200245, China
‡Jiangsu Hengrui Medicine CO., LTD., Lianyungang, Jiangsu 222047, China
§Department of Anaesthesiology, Fudan University Shanghai Cancer center, 270 Dongan Road, Shanghai 200032, China

List of Contents

General Information -------------------------------------------------2
Synthetic procedure and characterization for new compounds--------3-16
Methods for in vitro, in vivo, and pharmacokinetic assays----------17-18
References---------------------------------------------------------20
General Information

$^1$H spectra were recorded on a Bruker Avance-400 NMR spectrometer using CDCl$_3$, CD$_3$OD or DMSO-d$_6$ as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shift (δ) are reported in parts per million (ppm) and coupling constant (J) are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet. Mass spectra were determined on a Finnigan LCQ Advantage (ESI) spectrometer (Finnigan LCQ Advantage MAX, Thermo). Products were purified by column chromatography (200-300 mesh, Yantai Huanghai), or prep-TLC (0.4-0.5 mm, GF-254, Liangchen Guiyuan).

Molecular modeling studies were done using a laptop computer with an Intel® i7-2720QM, 2.2 GHz CPU and 4GB RAM running the Microsoft Windows 7 professional operating system. The software package Molecular Operating Environment (MOE), Chemical Computing Group Inc., Montreal, H3A 2R7 Canada, http://www.chemcomp.com was used for the modeling study. The agonist bound MOR co-crystal complex (PDB: 5C1M) was downloaded and prepared using Structure Preparation within MOE, including adjust hydrogens and lone pairs using Protonate3D (Labute, P. Proteins. 2009, 75, 187.). Docking was conducted using the rigid receptor docking protocol and AMBER10: EHT force field with R-Field solvation. The docking site was defined by the original crystallographic ligand (BU72) in 5C1M. The generated poses are first placed using Triangle Matcher with London dG score. Up to 30 poses are passed to the refinement stage using GBVI/WDA dG score resulted in up to 10 poses kept.
Synthetic procedure and characterization for new compounds

(R)-N-(2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)chroman-4-amine (1)

(R)-2-(9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)acetaldehyde (23) (80 mg, 0.31 mmol) and (R)-chroman-4-amine hydrochloride 24 (115 mg, 0.62 mmol) were dissolved in CH$_2$Cl$_2$/MeOH (10 mL, v/v=5:1) followed by the addition of NaBH(OAc)$_3$ (197 mg, 0.93 mmol). The reaction was stopped after 12 h. After the reaction mixture was concentrated under rotavapor, the product 1 (30 mg, pale yellow oil) was isolated from Prep-TLC. Yield is 25%.

HRMS m/z (ESI): C$_{25}$H$_{32}$N$_2$O$_2$ [M+H]$^+$: 393.2537, found 393.2542.

$^1$H NMR (400 MHz, MeOH-d$_4$) δ 8.55(d, J= 5.0, 1H), 7.78(t, J= 7.5, 1H), 7.53(d, J= 8.3, 1H), 7.26(t, J= 5.2, 1H), 7.03-7.12(m, 2H), 6.79(t, J= 7.5, 1H), 6.71(d, J= 8.0, 1H), 4.05-4.23(m, 2H), 3.72-3.83(m, 1H), 3.58-3.64(m, 1H), 2.52-2.63(m, 2H), 2.41-2.48(m, 1H), 2.01-2.14(m, 2H), 1.85-1.99(m, 2H), 1.28-1.31(m, 10H), 1.06-1.16(m, 1H), 0.68-0.79(m, 1H).

$^{13}$C NMR (400 MHz, MeOH-d$_4$): δ 164.26, 154.60, 148.27, 148.27, 136.61, 129.04, 128.14, 123.48, 121.95, 121.20, 119.53, 116.37, 83.30, 61.94, 59.06, 50.55, 45.47, 44.62, 41.24, 40.84, 40.43, 33.49, 32.99, 26.79, 23.48, 21.84.

(S)-N-(2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)chroman-4-amine (2)

(S)-2-(9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)acetaldehyde (23) (80 mg, 0.31 mmol) and (S)-chroman-4-amine hydrochloride 25 (86 mg, 0.46 mmol) were dissolved in CH$_2$Cl$_2$/MeOH (10 mL, v/v=5:1) and stirred for 1 h before the addition of NaBH(OAc)$_3$ (263 mg, 1.24 mmol). The reaction was stopped after 12 h. After the reaction mixture was concentrated under rotavapor, the product 2 (30 mg, white solid) was isolated from Prep-TLC. Yield is 25%.

HRMS m/z (ESI): C$_{25}$H$_{32}$N$_2$O$_2$ [M+H]$^+$: 393.2537, found 393.2539.
$^1$H NMR (400 MHz, CDCl$_3$) δ 8.55 (d, J= 3.8, 1H), 7.80-7.76 (m, 1H), 7.53 (d, J= 8.0, 1H), 7.26-7.25 (m, 1H), 7.05-7.01 (m, 2H), 6.78-6.70 (m, 2H), 4.17-4.10 (m, 2H), 3.79-3.63 (m, 3H), 2.56-2.42 (m, 3H), 2.19-2.10 (m, 2H), 1.92-1.82 (m, 2H), 1.80-1.44 (m, 12H).

$^{13}$C NMR (400 MHz, MeOH-d$_4$): δ 158.95, 155.28, 145.49, 144.06, 131.09, 129.81, 125.73, 125.10, 120.51, 117.62, 115.37, 82.39, 60.97, 57.77, 51.82, 43.64, 40.88, 40.33, 39.51, 38.73, 34.31, 31.72, 24.14, 23.30, 22.03.

(5)-N-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)-1,2,3,4-tetrahydronaphthalen-1-amine (4)

![Chemical Structure](image)

(R)-2-(9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)acetaldehyde 23\(^1\) (20 mg, 0.14 mmol) and (S)-1,2,3,4-tetrahydronaphthalen-1-amine 26\(^4\) (86 mg, 0.46 mmol) were dissolved in CH$_2$Cl$_2$ (20 mL) and stirred for 1 h before the addition of NaBH(OAc)$_3$ (144 mg, 0.68 mmol). The reaction was stopped after 11 h. After the reaction mixture was concentrated under rotavapor, the product 4 (15 mg, yellow solid) was isolated from Prep-TLC. Yield is 28.3%.

HRMS m/z (ESI): C$_{26}$H$_{34}$N$_2$O [M+H]$^+$: 391.2744, found 391.2708.

(5)-4-((2-(R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethylamino)-1,2,3,4-tetrahydronaphthalen-1-ol (5)

(1S,4S)-4-((2-(R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethylamino)-1,2,3,4-tetrahydronaphthalen-1-ol (5)

(1R,4S)-4-((2-(R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethylamino)-1,2,3,4-tetrahydronaphthalen-1-ol (6)
(S)-4-((2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)amino)-3,4-dihydronaphthalen-1(2H)-one 9 (50 mg, 0.12 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL) at –78 °C followed by drop-wise addition of DIABL-H (1.0 M in Toluene, 0.29 mL). The reaction was quenched by MeOH (5 mL) after 2 h. The reaction mixture was concentrated under reduced pressure. The product was isolated separately as title compounds, 5 (18 mg, pale white solid, yield: 35%) and 6 (20 mg, pale white solid, yield, 39%).

5: HRMS m/z (ESI): C$_{26}$H$_{34}$N$_2$O$_2$ [M+H]$^+$: 407.2693, found 407.2682.

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.51 (d, J = 3.5, 1H), 7.50 (t, J = 7.3, 1H), 7.36 (d, J = 7.5, 1H), 7.33-7.30 (m, 3H), 7.21-7.18 (m, 2H), 4.83 (s, 1H), 4.25 (s, 1H), 3.81-3.75 (m, 2H), 2.85-2.83 (m, 1H), 2.36-2.30 (m, 5H), 1.98-1.80 (m, 2H), 1.78-1.60 (m, 9H), 1.48-1.25 (m, 5H).

$^{13}$C NMR (400 MHz, MeOH-d$_4$): δ 159.95, 145.78, 145.14, 135.44, 134.06, 131.09, 129.81, 124.10, 120.51, 117.75, 115.22, 91.06, 84.39, 66.07, 57.82, 45.33, 43.64, 42.14, 40.31, 39.55, 39.11, 33.14, 31.72, 27.14, 24.30, 23.03.

6: HRMS m/z (ESI): C$_{26}$H$_{34}$N$_2$O$_2$ [M+H]$^+$: 407.2693, found 407.2625.

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.51 (d, J = 3.8, 1H), 7.50 (t, J = 6.8, 1H), 7.36 (d, J = 7.8, 1H), 7.33-7.30 (m, 3H), 7.21-7.18 (m, 2H), 4.83 (s, 1H), 4.25 (s, 1H), 3.81-3.75 (m, 2H), 2.85-2.83 (m, 1H), 2.36-2.30 (m, 5H), 1.98-1.80 (m, 2H), 1.78-1.60 (m, 9H), 1.48-1.25 (m, 5H).

$^{13}$C NMR (400 MHz, MeOH-d$_4$): δ 161.24, 155.28, 144.89, 144.12, 134.09, 126.81, 125.55, 125.07, 121.51, 116.62, 114.37, 91.66, 83.39, 66.60, 57.77, 46.82, 43.64, 42.88, 40.33, 39.51, 38.73, 34.30, 31.72, 27.14, 23.64, 23.23.

(1S,4R)-4-methoxy-N-(2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)-1,2,3,4-tetrahydro naphthalen-1-amine (7)
i: \((1R,4S)-4-\{(2-(R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethylamino\}-1,2,3,4-tetrahydronaphthalen-1-ol\) 6 (46 mg, 0.11 mmol), di-\textit{t}-butyl dicarbonate (27 mg, 0.12 mmol) and triethyl amine (23 mg, 0.22 mmol) were dissolved in \(\text{CH}_2\text{Cl}_2\) (15 mL). The reaction was stopped after 16 h and concentrated under rotavapor. Product 27 (46 mg, white solid) was isolated through Prep-TLC. Yield: 82%.

MS m/z (ESI): [M+H]^+ : 507.3, found 507.3 [M+1].

\begin{align*}
ii: \text{\textit{t}-butyl} \\
((1S,4R)-4-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)\{(2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)carbamate\} 27 (46 mg, 0.091 mmol) was dissolved in \text{THF} (10 mL) followed by the addition of \text{NaH} (8 mg, 0.182 mmol). After 30 min, MeI (16 mg, 0.11 mmol) was added. The reaction was quenched by water (50 mL) and ethyl acetate (50 mL) after stirring 16 h. The organic phase was collected and concentrated. The crude product 28 (47 mg, brown solid) was used without any future purification.

MS m/z (ESI): [M+H]^+ : 521.4, found 521.3.

\begin{align*}
iii:\text{\textit{t}-butyl} \\
((1S,4R)-4-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)\{(2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)carbamate\} 28 (47 mg, 0.091 mmol) was dissolved in \text{CH}_2\text{Cl}_2 (10 mL) before the addition of \text{HCl} solution (4 M in dioxane, 0.1 mL). The reaction mixture was concentrated after 1h. The residue was added ethanol. Amonia was applied to adjust the pH to 8. The solution was concentrated under reduced pressure and the product 7 (36 mg, yellow solid) was isolated through Prep-TLC. Yield is 95%.

HRMS m/z (ESI): \text{C}_{27}\text{H}_{36}\text{N}_{2}\text{O}_{2} [M+H]^+ : 421.2850, found 421.2871.

\text{\textsuperscript{1}H NMR} (400 MHz, DMSO-\textit{d}6) \delta 8.55 (d, \textit{J} = 3.7, 1H), 7.75-7.72 (m, 1H), 7.46 (d, \textit{J} = 7.4, 1H), 7.37-7.32 (m, 2H), 7.28-7.15 (m, 3H), 4.67 (d, \textit{J} = 4.4, 1H), 4.30 (d, \textit{J} = 4.2, 1H), 3.97 (s, 1H), 3.64-3.50 (m, 2H), 3.35 (s, 3H), 2.41-2.26 (m, 2H), 2.16-2.06 (m, 2H), 2.04-1.87 (m, 2H), 1.86-1.72 (m, 4H), 1.62-1.21 (m, 8H), 1.04-0.94 (m, 1H), 0.68-0.61 (m, 1H).
$^{13}$C NMR (400 MHz, MeOH-d4): $\delta$ 162.01, 155.28, 145.49, 144.06, 130.52, 128.47, 125.73, 125.10, 120.51, 116.25, 113.42, 90.64, 83.45, 60.66, 59.54, 57.77, 51.82, 44.55, 41.02, 40.54, 39.01, 38.52, 34.13, 31.88, 26.44, 23.30, 22.03.

(1S,4R)-4-methoxy-N-(2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)-1,2,3,4-tetrahydronaphthalen-1-amine (8)

![Chemical structure of 8]

i: $(S)$-tert-butyl (4-oxo-1,2,3,4-tetrahydronaphthalen-1-yl)carbamate 21 (100 mg, 0.883 mmol) was dissolved in cooled (0 °C) toluene before the addition of $(R)$-2-methyl-CBS-oxazaborolidine (0.1 mL, 0.076 mmol). After 5 min, the borane dimethyl sulfide complex (0.88 mL, 0.76 mmol) was slowly added. The reaction was quenched after 2 h by brine (50 mL). The aqueous phase was extracted by ethyl acetate (30 mL) three times. The organic phase was combined, washed by brine (3 * 30 mL), dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The product 29 (60 mg, white solid, yield 60%) was isolated through prep-TLC.

MS m/z (ESI): [M$-$55]$^+$: 208.2, found 208.3.

ii: tert-butyl ((1S,4S)-4-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)carbamate 29 (30 mg, 0.11 mmol) was dissolved in CH$_2$Cl$_2$ (4 mL). Silver oxide (76 mg, 0.33 mmol) and methyl iodide (62 mg, 0.44 mmol) were then added into the reaction mixture. The reaction was stopped after 48 h. The crude product 30 (30 mg, yellow oil) was obtained after the reaction mixture filtration was concentrated and utilized for next step without any further purification.

MS m/z (ESI): [M+H]$^+$: 278.2, found 278.4.

iii: tert-butyl ((1S,4S)-4-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)carbamate 30 (30 mg, 0.11 mmol) was dissolved in CH$_2$Cl$_2$ (0.5 mL) and added HCl (4 M in dioxane, 1 mL). The mixture was allowed to stir for 2.5 h and concentrated to deliver crude product 31 (24 mg, white solid) without any further purification.

MS m/z (ESI): [M+H]$^+$: 178.2, found 178.4.
iv: (R)-2-(9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)acetaldehyde 23\(^1\) (29 mg, 0.11 mmol), crude product 31 (24 mg, 0.11 mmol) were dissolved in MeOH (4 mL) and stirred for 12 h before the addition of NaBH\(_4\) (8 mg, 0.22 mmol). The reaction was stopped after 15 min. After the reaction mixture was concentrated under rotavapor, the product 8 (4 mg, white solid) was isolated from Prep-TLC. Yield is 8.7%.

HRMS m/z (ESI): C\(_{27}\)H\(_{36}\)N\(_2\)O\(_2\) [M+H]\(^+\): 421.2850, found 421.2870.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.56 (d, J= 4.5, 1H), 7.66 (t, J= 6.0, 1H), 7.33 (d, J= 4.8, 1H), 7.15 (d, J= 8.2, 1H), 7.08-7.06 (m, 3H), 7.04 (d, J= 4.8, 1H), 3.76 (d, J= 4.6, 2H), 3.61-3.58 (br, 1H), 3.41 (s, 3H), 2.74-2.72 (m, 3H), 2.46 (d, J= 5.6, 1H), 2.32 (d, J= 5.4, 1H), 2.13-2.08 (m, 1H), 2.03-2.00 (m, 1H), 1.90 (d, J= 9.2, 1H), 1.75 -1.72 (m, 11H), 1.51-1.46 (m, 3H).

\(^{13}\)C NMR (400 MHz, MeOH-d4): \(\delta\) 159.21, 153.28, 146.40, 143.12, 130.18, 127.86, 126.37, 125.86, 122.55, 116.20, 114.44, 88.56; 84.36, 62.90, 58.12, 55.32; 52.20, 44.66, 41.86, 40.30, 39.87, 38.10, 33.25, 31.41, 25.35, 24.28, 21.96.

(S)-4-((2-(R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)amino)-3,4-dihydronaphthalen-1(2H)-one (9)

\[
\begin{align*}
\text{i: } (S)-1,2,3,4\text{-tetrahydronaphthalen-1-amine 19}^5 \text{ (3.0 g, 20.4 mmol), di-}\text{-}\text{tert-butyl dicarboxate (4.9 g, 44.5 mmol) and triethyl amine (5.7 mL, 40.8 mmol) were dissolved in CH}_2\text{Cl}_2\text{ (100 mL). The reaction was stopped after 12 h and washed by water (100 mL) and NaHCO}_3\text{ aqueous solution (100 mL). The organic phase was dried over Na}_2\text{SO}_4\text{ and concentrated to deliver product 20 (5.6 g, yellow oil) without further purification. MS m/z (ESI): } [M+H]^+ : 248.2, \text{ found 248.3.} \\
\text{ii: } (S)-\text{tert-butyl (1,2,3,4-tetrahydronaphthalen-1-yl)carbamate 20 (5.6 g, 20.4 mmol) was dissolved in acetone/water (90 mL, v/v=2:1) followed by addition of MgSO}_4\text{ (5.5 g, 45.66 mmol) and KMnO}_4\text{ (7.22 g, 45.66 mmol). The reaction was stopped after 12 h. The reaction was filtered and concentrated. The product 21 (3.1 g, pale white solid, yield: 52%) was isolated through flash chromatography. MS m/z (ESI): } [M+H]^+ : 262.3, \text{ found 262.3.}
\end{align*}
\]
iii: (S)-4-amino-3,4-dihyronaphthalen-1(2H)-one hydrochloride 21 (1.0 g, 3.83 mmol) was dissolved in CH$_2$Cl$_2$ (20 mL) followed by addition of HCl (4.0 M in dioxane, 8 mL). The reaction was stopped after 2 h and the mixture was concentrated. The residue was diluted with ethanol (10 mL) and followed by drop-wise addition of aqueous ammonia (30%) until the pH was adjusted to 8. The solution was then concentrated and product 22 (400 mg, green oil, yield: 64.8%) was isolated by Prep-TLC.

MS m/z (ESI): [M+H]$^+$: 162.3, found 162.3.

iv: (R)-2-((9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)acetaldehyde 23 (268 mg, 1.04 mmol) and (S)-4-amino-3,4-dihyronaphthalen-1(2H)-one hydrochloride 22 (200 mg, 1.24 mmol) were dissolved in CH$_2$Cl$_2$ (20 mL) and stirred for 1 h before the addition of NaBH(OAc)$_3$ (1.1 g, 5.18 mmol). The reaction was stopped after 2 h. After the reaction mixture was concentrated under rotavapor, the product 9 (136 mg, white solid) was isolated from Prep-TLC. Yield is 32%.

HRMS m/z (ESI): C$_{26}$H$_{32}$N$_2$O$_2$ [M+H]$^+$: 405.2537, found 405.2545.

1H NMR (400 MHz, CDCl$_3$) δ 8.73 (d, J=1.2, 1H), 8.15-8.09 (m, 2H), 7.83 (d, 1H), 7.81-7.69 (m, 3H), 7.47 (d, J = 7.6, 1H), 4.45 (t, J = 3.8, 1H), 3.77-3.74 (m, 2H), 3.03-2.98 (m, 1H), 2.75-2.68 (m, 3H), 2.51-2.44 (m, 5H), 2.05-2.01 (m, 2H), 1.57-1.48 (m, 7H), 1.20-1.05 (m, 1H), 0.80-0.77 (m, 1H).

13C NMR (400 MHz, MeOH-d$_4$): δ 198.83, 162.18, 148.36, 136.53, 133.71, 133.20, 127.44, 126.58, 126.12, 125.77, 122.43, 119.80, 87.06, 60.84, 57.75, 45.84, 42.65, 39.98, 38.58, 38.17, 34.42, 24.60, 24.21, 23.68, 23.20.

(E)-2-(((S)-4-((2-(R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)amino)-3,4-dihyronaphthalen-1(2H)-ylidene)acetonitrile (10)

|  |  |
|---|---|
| 21 | 22 |
|   | i | ii | iii |
| 24 | 10 |

i: diethyl cyanomethylphosphonate (200 mg, 0.76 mmol) was dissolved in cooled (0 °C) THF. NaH (61 mg, 1.52 mmol) was added slowly and the reaction was stirred for 30 min before the addition of 21 (200 mg, 0.76 mmol). The reaction was poured into ice water after 16 h and the mixture was extracted by ethyl acetate three times. Combined organic phase was dried over Na$_2$SO$_4$ and concentrated. The product 32 (150 mg, colorless oil) was isolated by prep-TLC. Yield is 69%.

MS m/z (ESI): [M+H]$^+$: 285.2, found 285.1.
ii: (S,E)-tert-butyl (4-(cyanomethylene)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamate 32 (150 mg, 0.52 mmol) was dissolved in CH$_2$Cl$_2$ (5 mL). HCl (1.0 M in dioxane, 2 mL) was added. The reaction mixture was concentrated after 3 h to afford the crude product 33 (110 mg, white solid) for the next step without further purification.

iii: (R)-2-(9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)acetaldehyde 23$^1$ (100 mg, 0.39 mmol), crude product 33 (85 mg, 0.39 mmol) were dissolved in CH$_2$Cl$_2$/MeOH (10 mL, v/v=10:1) followed by addition of NaBH(OAc)$_3$ (8 mg, 0.22 mmol). The reaction was stopped after 16 h. After the reaction mixture was concentrated under rotavapor, the product 10 (30 mg, light yellow oil) was isolated from Prep-TLC. Yield is 18%.

MS m/z (ESI): C$_{28}$H$_{33}$N$_3$O$^{[M+H]^+}$: 428.2696, found 428.2610.

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.57 (d, J= 4.0, 1H), 7.86-7.78 (m, 1H), 7.76-7.74 (m, 1H), 7.39-7.22 (m, 3H), 7.26-7.23 (m, 2H), 6.36-6.35 (m, 1H), 3.65-3.54 (m, 3H), 2.90-2.60 (m, 2H), 2.42-2.37 (m, 3H), 2.03-1.90 (m, 4H), 1.82-1.78 (m, 2H), 1.51-1.24 (m, 10H).

$^{13}$C NMR (400 MHz, MeOH-d$_4$): $\delta$ 164.26, 162.47, 148.21, 137.73, 136.11, 128.04, 125.78, 125.22, 125.13, 124.56, 124.45, 119.17, 117.01, 88.06, 86.39, 60.34, 57.61, 45.74, 45.71, 42.48, 39.78, 38.80, 37.49, 30.72, 25.76, 24.99, 24.01, 23.27.

(S)-N-(2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)-2,3-dihydro-1H-inden-1-amine (11)

(R)-2-(9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)acetaldehyde 23$^1$ (20 mg, 0.08 mmol) and (S)-2,3-dihydro-1H-inden-1-amine 34$^6$ (26 mg, 0.15 mmol) were dissolved in CH$_2$Cl$_2$ (10 mL) and stirred for 2 h before the addition of NaBH(OAc)$_3$ (49 mg, 0.23 mmol). The reaction was stopped after 12 h. After the reaction mixture was concentrated under rotavapor, the product 11 (5 mg) was isolated from Prep-TLC. Yield is 17%.

HRMS m/z (ESI): C$_{25}$H$_{32}$N$_2$O $[M+H]^+$: 377.2587, found 377.2544.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.63 (d, J= 5.0, 1H), 7.90 (t, J= 7.8, 1H), 7.60 (d, J= 8.3, 1H), 7.38 (s, 4H), 7.35 (d, J= 2.4, 1H), 4.65-4.70 (dd, J=7.2, 3.4, 1H), 3.76 (d, J= 5.5, 2H), 2.90-3.16 (m, 2H), 2.40-2.60 (m, 4H), 1.85-2.10 (m, 4H), 1.70-1.80 (m, 2H), 1.40-1.69 (m, 5H), 1.30-1.39 (m, 1H), 1.10-1.20 (m, 1H), 0.70-0.80 (m, 1H).
$^{13}$C NMR (400 MHz, MeOH-d4): δ 162.46, 148.68, 144.76, 137.23, 136.31, 129.84, 126.78, 125.22, 124.93, 121.86, 121.75, 83.06, 62.34, 58.65, 44.44, 40.98, 40.68, 40.57, 40.42, 33.42, 32.69, 29.51, 28.48, 23.46, 21.82.

$(1R,2R)$-2-methoxy-$N$-(2-((($R$)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)-2,3-dihydro-1H-inden-1-amine (12)

\[ \text{35} \xrightarrow{i} \text{36} \xrightarrow{ii} \text{37} \]

\[ \xrightarrow{iii} \text{12} \]

$i$: tert-butyl ((1R,2R)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)carbamate 35\(^1\) (350 mg, 1.34 mmol) was dissolved in CH$_2$Cl$_2$ (15 mL). Silver oxide (930 mg, 4.02 mmol), methyl iodide (0.25 mL, 4.02 mmol) and few activated 4 A MS were added. The reaction was stopped, filtered, and concentrated after 16 h. The residue was separated by prep-TLC to afford product 36 (200 mg, white solid). The yield is 57%.

MS m/z (ESI): [M-55]$^+$: 208.3, found 208.2.

$ii$: tert-butyl ((1R,2R)-2-methoxy-2,3-dihydro-1H-inden-1-yl)carbamate 36 (60 mg, 0.228 mmol) was dissolved in CH$_2$Cl$_2$ (5 mL) and TFA (0.5 mL) was added. After 2 h, the reaction mixture was concentrated to deliver crude product 37 (66 mg, yellow oil) without further purification.

MS m/z (ESI): [M+H]$^+$: 164.2, found 164.2.

$iii$: (R)-2-(9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)acetaldehyde 23\(^1\) (50 mg, 0.193 mmol) and crude product 37 (66 mg, 0.228 mmol) were dissolved in CH$_2$Cl$_2$ (15 mL) and stirred for 30 min before the addition of NaBH(OAc)$_3$ (200 mg, 0.965 mmol). The reaction was stopped after 12 h. After the reaction mixture was concentrated under rotavapor, the product 12 (25 mg, yellow oil) was isolated from Prep-TLC. Yield is 32%.

HRMS m/z (ESI): C$_{26}$H$_{34}$N$_2$O$_2$ [M+H]$^+$: 407.2693, found 407.2705.

$^1$H NMR (400 MHz, DMSO-d6) δ 8.55 (d, J= 3.8, 1H), 7.71 (t, J= 7.8, 1H), 7.58 (d, J= 7.5, 1H), 7.40 (d, J= 8.0, 1H), 7.28 (d, J=7.3, 1H), 7.25-7.10 (m, 3H), 4.39 (d, J= 4.4, 1H), 4.26 (d, J= 4.3, 1H), 3.82-3.70 (m, 5H), 3.30 (s, 3H), 2.88-2.30 (m, 2H), 2.40-2.26 (m, 2H), 1.96-1.91 (m, 2H), 1.85-1.62 (m, 4H), 1.61-1.24 (m, 6H).
\(^{13}\)C NMR (400 MHz, MeOH-d4): \(\delta\) 161.51, 147.14, 141.44, 140.23, 137.25, 127.15, 125.77, 125.15, 124.84, 122.55, 120.20, 89.04, 83.73, 68.34, 57.45, 45.17, 44.86, 42.48, 40.58, 39.51, 38.44, 34.43, 28.41, 27.18, 22.48, 20.81.

(S)-N-(2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)-3',4'-dihydro-2'H-spiro[[1,3]dithiolane-2,1'-naphthalen]-4'-amine (18)

\[
\begin{array}{c}
\text{9} \quad \text{+} \quad \text{HS}\text{-SH} \quad \rightarrow \quad \text{18}
\end{array}
\]

Compound 9 (35 mg, 0.0865 mmol), thiol (82 mg, 0.865 mmol), and PPTS (240 mg, 0.952 mmol) were dissolved in toluene (15 mL) at 110 °C. The reaction was stopped after 12 h and concentrated. The product 18 (40 mg, yellow solid) was isolated by prep-TLC. Yield is 96%. The ee is 98.3%.

HRMS m/z (ESI): C\(_{28}\)H\(_{36}\)N\(_2\)O\(_2\) [M+H]\(^{+}\): 481.2342, found 481.2354.

\(^1\)H NMR (400 MHz, DMSO-d6): \(\delta\) 8.55(dd, \(J= 4.5,1.0\), 1H), 7.83(d, \(J= 7.8\), 1H), 7.74(d, \(J= 7.8\), 1H), 7.48(d, \(J= 8.0\), 1H), 7.12-7.27(m, 4H), 3.80-3.86(m, 1H), 3.54-3.67(m, 5H), 3.44-3.51(m, 1H), 3.35-3.43(m, 1H), 2.39-2.49(m, 2H), 2.32-2.38(m, 1H), 2.10-2.19(m, 2H), 1.87-1.99(m, 2H), 1.71-1.84(m, 3H), 1.64-1.70(m, 1H), 1.27-1.63(m, 7H), 0.95-1.02(m, 1H), 0.60-0.71(m, 1H).

\(^{13}\)C NMR (400 MHz, CDCl3): \(\delta\) 164.32, 148.75, 139.93, 137.41, 136.19, 130.92, 128.26, 127.94, 127.75, 123.24, 121.89, 119.08, 82.90, 68.25, 54.68, 45.34, 41.54, 41.05, 40.76, 40.73, 40.60, 39.39, 34.35, 33.55, 29.69, 26.70, 23.99, 22.57.

Instrument: Agilent 1260 DA; chromatographic column: Chiralpak IF 150*4.6 mm, 5 um; Column Temperature: 35 °C; Speed: 1.0 ml/min. Mobile phase: 5% (ethanol, 0.1% DEA), 95% hexane.
(S)-6-fluoro-N-((2R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)chroman-4-amine (3)
The synthesis of 3 followed the similar procedure of 2.
HRMS m/z (ESI): C_{25}H_{31}FN_{2}O_{2} [M+H]^+: 411.2442, found 411.2442.

\[ \text{H NMR (400 MHz, CDCl3): } \delta 8.56 \text{ (d, J= 4.1, 1H), 7.67-7.64 (m, 1H), 7.34-7.31 (m, 1H), 7.16-7.14 (m, 1H), 6.84-6.74 (m, 2H), 6.73-6.7 (m, 1H), 4.02-4.08 (m, 2H), 3.78-3.75 (m, 3H), 2.66-2.12 (m, 6H), 2.1-1.59 (m, 9H), 1.35-1.18 (m, 4H).} \]

\[ \text{13C NMR (400 MHz, MeOH-d4): } \delta 164.04, 157.62, 155.27, 150.97, 150.95, 148.32, 136.66, 121.84, 121.29, 117.69, 117.61, 83.27, 62.29, 58.99, 50.68, 45.20, 44.01, 41.20, 40.45, 40.36, 33.60, 33.04, 26.32, 23.46, 21.88. \]

\[(S)-N-(2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-4-amine (13)\]

The synthesis of 13 followed the similar procedure of 4.
HRMS m/z (ESI): C_{24}H_{32}N_{2}OS [M+H]^+: 397.2308, found 397.2330.

\[ \text{H NMR (400 MHz, Methanol-d4): } \delta 8.64 \text{ (d, J= 4.5, 1H), 7.71-7.55 (m, 2H), 7.35 (d, J= 7.6, 1H), 7.20 (t, J= 7.4, 1H), 7.00 (d, J= 2.0, 1H), 3.93-3.73 (m, 3H), 3.28-3.05 (m, 2H), 2.78-2.46 (m, 5H), 2.43-1.95 (m, 5H), 1.91-1.67 (m, 8H), 1.22-1.15 (m, 1H), 0.79-0.71 (m, 1H).} \]

\[ \text{13C NMR (400 MHz, MeOH-d4): } \delta 162.64, 146.75, 138.30, 132.60, 127.55, 126.51, 125.60, 124.06, 121.51, 83.05, 55.04, 51.85, 43.22, 41.69, 40.42, 34.87, 33.42, 32.96, 29.49, 25.48, 24.61, 23.41, 22.60, 21.86. \]

\[(S)-4-((2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)amino)-5,6-dihydrobenzo[b]thiophen-7(4H)-one (14)\]
The synthesis of 14 followed the similar procedure of 4.
HRMS m/z (ESI): C_{24}H_{30}N_{2}O_{2}S [M+H]^+: 411.2101, found 411.2103.

$^1$H NMR (400 MHz, CDCl3) δ 8.54 (d, J= 4.0, 1H), 7.81-7.77 (m, 2H), 7.53 (d, J= 8.0, 1H), 7.26 (t, J= 2.1, 1H), 7.09 (d, J= 6.0, 1H), 3.88-3.75 (m, 3H), 2.62-2.46 (m, 5H), 2.43-1.95 (m, 5H), 1.91-1.55 (m, 8H), 1.10-1.02 (m, 1H), 0.75-0.71 (m, 1H).

$^{13}$C NMR (400 MHz, MeOH-d4): δ 200.64, 164.04, 156.75, 148.30, 136.60, 134.24, 127.55, 126.01, 125.66, 124.45, 83.30, 59.04, 52.85, 41.22, 41.09, 40.42, 34.87, 33.52, 32.97, 29.59, 25.44, 24.36, 23.47, 21.86.

(S)-7-(((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)amino)-6,7-dihydrobenzo[b]thiophene-4(5H)-one (15)

The synthesis of 15 followed the similar procedure of 4.
HRMS m/z (ESI): C_{24}H_{30}N_{2}O_{2}S [M+H]^+: 411.2101, found 411.2124.

$^1$H NMR (400 MHz, CDCl3) δ 8.56 (d, J= 4.0, 1H), 7.82-7.78 (m, 2H), 7.69(t, J= 8.0, 1H), 7.53 (d, J= 6.0, 1H), 7.11 (d, J= 5.6, 1H), 3.90-3.76 (m, 3H), 2.60-2.40 (m, 5H), 2.45-1.94 (m, 5H), 1.90-1.53 (m, 8H), 1.12-1.02 (m, 1H), 0.76-0.73 (m, 1H).

$^{13}$C NMR (400 MHz, MeOH-d4): δ 196.64, 167.04, 156.75, 148.30, 134.24, 129.64, 127.55, 126.01, 121.61, 120.21, 86.30, 59.04, 52.85, 43.22, 41.09, 40.42, 35.87, 33.72, 32.97, 29.59, 26.34, 24.98, 23.67, 21.96.

(S)-N-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)-6,7-dihydro-5H-spiro[benzo[b]thiophene-4,2'-[1,3]dioxolan]-7-amine (16)
The synthesis of 16 followed the similar procedure of 18.

HRMS m/z (ESI): C_{26}H_{34}N_{2}O_{3}S [M+H]^+: 455.2363, found 455.2350.

\(^1\)H NMR (400 MHz, CDCl3): \(\delta\) 8.57 (d, \(J= 4.0, 1H\)), 7.80 (t, \(J= 7.6, 1H\)), 7.51 (d, \(J= 8.0, 1H\)), 7.45 (d, \(J= 5.0, 1H\)), 7.30 (dd, \(J=7.2, 6 Hz\), \(1H\)), 6.92 (d, \(J= 5.2, 1H\)), 4.14-4.00 (m, 5H), 3.76 (dd, \(J=6.0, 3.0 Hz\), \(2H\)), 2.75-2.81 (m, \(1H\)), 2.41-2.60 (m, \(2H\)), 2.21-2.30 (m, \(2H\)), 1.86-2.13 (m, \(4H\)), 1.70-1.81 (m, \(2H\)), 1.41-1.69 (m, \(5H\)), 1.31-1.39 (m, \(3H\)), 1.10-1.20 (m, \(1 H\)), 0.71-0.80 (m, \(1H\)).

\(^{13}\)C NMR (400 MHz, MeOH-d4): \(\delta\) 166.64, 156.04, 152.85, 148.35, 135.64, 128.74, 126.55, 123.40, 121.80, 120.51, 86.30, 70.15, 69.65, 59.04, 52.85, 43.22, 41.09, 40.48, 35.87, 33.72, 32.97, 29.69, 26.14, 24.68, 23.67, 21.27.

\((S)-N-(2-((R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl)ethyl)-3',4'-dihydro-2'H-spiro[1,3]dioxolane-2,1'-naphthalen]-4'-amine (17)

The synthesis of 17 followed the similar procedure of 18.

HRMS m/z (ESI): C_{28}H_{36}N_{2}O_{3} [M+H]^+: 449.2799, found 449.2771.

\(^1\)H NMR (400 MHz, DMSO-d6): \(\delta\) 8.58 (d, \(J= 4.0, 1H\)), 7.81 (s, \(1H\)), 7.77-7.70 (m, \(1H\)), 7.51 (d, \(J= 8.0, 1H\)), 7.27-7.11 (m, \(4H\)), 3.85 (s, \(1H\)), 3.66-3.50 (m, \(5H\)), 3.51-3.42 (m, \(1H\)), 3.42-3.33 (m, \(1H\)), 2.48-2.35 (m, \(2H\)), 2.38-2.32 (m, \(1H\)), 2.20-2.08 (m, \(2H\)), 2.01-1.88 (m, \(2H\)), 1.85-1.75 (m, \(3H\)), 1.71-1.31 (m, \(8H\)), 1.00-0.96 (m, \(1H\)), 0.70-0.62 (m, \(1H\)).

\(^{13}\)C NMR (400 MHz, CDCl3): \(\delta\) 164.80, 149.75, 138.83, 136.59, 134.92, 128.00, 127.94, 127.50, 126.45, 123.50, 122.19, 121.58, 82.90, 70.75, 70.25, 68.25, 59.33, 54.68, 45.34, 41.50, 40.60, 39.19, 34.35, 33.40, 29.69, 26.50, 24.99, 21.67.
Methods for in vitro, in vivo, and pharmacokinetic assays

In vitro CYP inhibition assay

The inhibitory IC50 values of SHR9352 for five major P450 enzymes was determined in human liver microsomes (BD Gentest) by similar conditions as described in literatures.\(^8,9\)

In Vitro Cellular Assays.

Cell Culture and Cell Lines Development: The human OPRM1 gene (NM_000914.3, encoding human MOR), mouse OPRM1 gene (NM_001039652.1, mouse MOR), rat OPRM1 gene (NM_001038597.2, rat MOR), human OPRD1 gene (NM_000911.3, human DOR), and human OPRK1 gene (NM_000912.3, human KOR) were synthesized by GENEWIZ( Suzhou, China), and constructed into pcDNA3.1(+) vector (V79020, ThermoFisher Scientific). A Luciferase Reporter cell line was constructed by co-transfecting HEK293 cells with pcDNA3.1/ hOPRM1 (or pcDNA3.1/ hOPRD1, or pcDNA3.1/ hOPRK1, or pcDNA3.1/ mOPRM1, or pcDNA3.1/ rOPRM1) and pGL4.29[ luc2P/ CRE/Hygro]( 9PIE847, Promega). Cells were grown in MEM with 10% FBS, and 1mg/mL of neomycin and 200 µg/mL of hygromycin. CHO-K1 stably co-transfected to overexpress β-arrestin-2 fused to a β- galactosidase fragment and human OPRM1 gene fused to a complementary β-galactosidase fragment using the pCMV-ProLink plasmid purchased from DiscoveRx(PathHunter β-arrestin assay, DiscoveRx Corporation, Fremont California). The culture medium for CHO-K1 stable cells and reagents were also purchased from DiscoveRx. All compounds were dissolved in DMSO solution, and then diluted with DMSO.

cAMP Accumulation Assay

Receptor G protein mediated responses were determined by measuring changes in cAMP using the Luciferase Reporter cell lines and ONE-Glo™ Luciferase Assay System(Promega, USA). MOR, KOR, and DOR all couple to Gai, so G protein coupling was measured as inhibition of forskolin-stimulated cAMP accumulation in the presence of 1µM forskolin (Sigma catalog #F6886). cAMP accumulation assay was run using HEK293 stably transfected to opioid receptors and luciferase reporter genes. After 5 hours drug treatment, ONE-Glo™ Luciferase Assay Reagent was added to the cells for cell lysis and reaction signal reading by Luminometer (Victor 3, PerkinElmer, USA).

Detailed Procedure: The initial concentration was 1000 nM, 3-fold serial titration and 10 points were tested. On day 1, about 1 X 10^5 cells per well were seeded; On Day 2, compounds were titrated with DMSO and then transferred into each cell well on assay plate. Total assay volume was 100 µL per well. After compounds were added, the assay plate were incubated for 5 h\(^a\) at 37 °C and then 100 µL

---

\(^a\) The Luciferase Reporter cell line system contained an cAMP response element (CRE) that drove the transcription of the luciferase reporter gene luc2P. It was an indirect cAMP detection system which delivered a delayed signal. Only after reporter gene was transcripted, the signal could be detected. So more time was required for incubation. We have optimized the assay incubation time and it was found 5 h was a proper incubation time.
detection reagent were added into each well; Finally, after incubation for 5 min at room temperature, the assay plate were read by the microplate reader.

**β-Arrestin-2 Recruitment Assay**

The PathHunter enzyme complementation assay (DiscoveRx Corporation, Fremont, California) was performed according to the manufacturer’s description. Briefly, when β-arrestin-2 translocates to active receptor, the complementary β-galactosidase fragments fused to receptor and β-arrestin interact to form a functional enzyme, which is detected by chemiluminescence. β-arrestin-2 recruitment assay was run using CHO-K1 cells stably transfected to OPRM1 and overexpress β-arrestin-2 fused to a β-galactosidase fragment.

Detailed Procedure: compound initial concentration was 10000 nM, 3-fold titration and 10 points were tested. On day 1, about 1 X 10^4 cells per well were seeded. On day 2, compounds were titrated with DMSO and then transferred into each cell well on assay plate. Total assay volume was 100 µL per well. After compounds were added, the assay plate were incubated for 90 min at 37 °C and then add 50 µL detection reagent were added into each well, Finally, after incubation for 60 min at room temperature, the assay plate were read by the microplate reader.

**In vivo PK studies**

Animals utilized for preclinical studies include nude mice, rats and dogs. All animals were treated in accordance with Institutional Guide for the Care and Use of Laboratory Animals. ICR mice (around 20g, 9 males) were purchased from Sino-British Sippr/BK Lab Animal Co. Ltd (Shanghai) (SCXK 2013-0016), Sprague Dawley (SD) rats (200-250g, 2 males and 2 females) from Sino-British Sippr/BK Lab Animal Co. Ltd (Shanghai) (SCXK 2013-0016), and beagle dogs (9-13kg, 3 males) from Beijing Marshall Biotechnology Co., Ltd (SCXK 2016-0001). Plasma samples of nude mice, SD rats and dogs were collected at pre-dose and 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24 h after the i.v. or s.c. administration. The following figure shows the time-course PK for plasma:
**In vivo Rat Incisional Pain Study**

*In vivo* efficacy experiments were conducted in male wistar rats (200–250 g). Animals were housed in standard conditions with a 12-hour light/dark cycle for 72 hour prior to surgery.\textsuperscript{10,11} The measurement of tactile sensitivity for the injured hindpaw was obtained using von Frey filaments (UGO BASILE, #38450). Twenty-four hours after surgery, every 8 animals were randomly assigned to Model, TRV130, or SHR9352 treatment groups. Both compounds were administered i.v. as a bolus in the rat tail in a volume of 1 ml/kg. Allodynia was measured 30 minutes after drug administration. The percentage of pain reduction effect was calculated as follows: 

\[
\frac{\text{treatment group} - \text{model group}}{\text{model group}} \times 100.
\]

**In vivo Mouse Gastrointestinal Function Study- Small Intestinal Transit test**

*In vivo* gastrointestinal transit of the small intestine was measured using the charcoal meal test.\textsuperscript{12} After housed in standard conditions and fed with a standard laboratory food and water ad libitum for at least 72 hour, C57BL/6J mice (10 per drug treatment) were given an injection of saline (10 ul/g s.c.), TRV130 or SHR9352 20 min prior to an oral gavage of a charcoal meal containing a 5% aqueous suspension of charcoal (Sigma-Aldrich) in a 10% gum Arabic (Acros Organics, Fairlawn, NJ) solution at a volume of 10 ul/g. At 30 min, animals were sacrificed by cervical dislocation, and the small intestine, from the jejunum to the cecum, was dissected and the mysenry removed. The distance traveled by the leading edge of the charcoal meal was measured relative to the total length of the small intestine, and the percentage of gastrointestinal transit for each animal was calculated as follows:

\[
\text{percentage transit} = \frac{\text{charcoaldistance}}{\text{small intestine length}} \times 100.
\]
References

1. Compound 24 was reduced from corresponding chiral cyanide based on reported literature (Yamashita, D.; Gotchev, D.; Pitis, P.; Chen, X.; Liu, G.; Yuan, C. C. WO2012129495, 2012.). The ee of chiral cyanide is above 98%. The separation condition (for 5.4 g product mixtures) is as follows: Instrument: SFC-multigram (Berger Instruments Inc.); Column: Chiralpak AD-H (Daicel), 2cm I.D. * 25 cm Length, 5 um; Mobile phase: CO2/MeOH =(60/40); Flow Rate: 70 g/Min; Pressure: 120 bar; Temperature: 40 °C. Stack Inject cycling time: 6 min; loading weight each time: 200 mg. Under above condition, we got 2.5 g desired product with ee as 98.53%.

2. Schmidt, R. G.; Bayburt, E. K.; Latshaw, S. P.; Koenig, J. R.; Daanen, J. F.; McDonald, H. A.; Bianchi, B. R.; Zhong, C.; Joshi, S.; Honore, P.; Marsh, K. C.; Lee, C.; Faltynek, C. R.; Gomtsyan, A. Bioorg. Med. Chem. Lett. 2011, 21, 1338-1341.

3. Pressnitz, D. P.; Fuchs, C. S.; Sattler, J. H.; Knaus, T.; Macheroux, P.; Mutti, F. G.; Kroutil, W. ACS Catal. 2013, 3, 555-559.

4. Pable, O.; Guijarro, D.; Yus, M. Eur. J. Org. Chem. 2014, 31, 7034.

5. Liang, G.; Robert-Peillard, F.; Fruit, C.; Muller, P.; Dodd, R. H.; Dauban, P. Angew. Chem. Int. Ed. 2006, 45, 4641-4645.

6. Uiterweerd, P. G.; van der Sluis, M.; Kaptein, B.; de Lange, B.; Kellogg, R. M.; Broxterman, Q. B. Tetrahedron Asymmetry. 2003, 14, 3479-3485.

7. Probst, N.; Madarasz, A.; Valkonen, A.; Papai, I.; Rissansen, K.; Neuvonen, A.; Pihko, P. M. Angew. Chem. Int. Ed. 2012, 51, 8495-8499.

8. Li, G.; Huang,K., Nikolic, D., and B. van Breemen, R., High-Throughput Cytochrome P450 Cocktail Inhibition Assay for Assessing Drug-Drug and Drug-Botanical Interactions. Drug Metab Dispos 43:1670–1678, 2015.

9. FDA Guidance for Industry: Drug Interaction Studies — Study Design, Data Analysis, and Implications for Dosing and Labeling, 2006.

10. DeWire, S. M.; Yamashita, D. S; Rominger, D. H.; Liu, G; Cowan, C. L.; Graczyk, T. M.; Chen, X. T.; Pitis, P. M.; Gotchev, D.; Yuan, C.; Koblish, M.; Lark, M. W.; Violin, J. D. J. Pharmacol. Exp. Ther. 2013, 344, 708–717.

11. Brennan T. J.; Vandermeulen E. P.; Gebhart G. F. Characterization of a rat model of incisional pain. Pain 1996, 64 (3):493-501.

12. Raehal, K. M.; Walker, J. K.; Bohn, L. M. J. Pharmacol. Exp. Ther. 2005, 314, 1195–1201.