Discovery of α-Obscurine Derivatives as Novel $Ca_v3.1$ Calcium Channel Blockers

Lei Wang+,[a, b] Wen-Yan Li+,[a, b] Zai-Feng Yuan,*[a] and Qin-Shi Zhao*[a]

Voltage-gated calcium channels (VGCCs), particularly T-type calcium channels (TTCCs), are crucial for various physiological processes and have been implicated in pain, epilepsy, and cancer. Despite the clinical trials of TTCC blockers like Z944 and MK8998, none are currently available on the market. This study investigates the efficacy of *Lycopodium* alkaloids, particularly as natural product-based TTCC blockers. We synthesized eighteen derivatives from α-obscurine, a lycodine-type alkaloid, and identified five derivatives with significant $Ca_v3.1$ blockade activity. The most potent derivative, compound **7**, exhibited an IC_{50} value of 0.19 ± 0.03 μM and was further analyzed through molecular docking, revealing key interactions with $Ca_v3.1$. These findings provide a foundation for the structural optimization of $Ca_v3.1$ calcium channel blockers and present compound **7** as a promising lead compound for drug development and a tool for chemical biology research.

1. Introduction

Ion channels are pivotal targets for drug development, with Voltage-gated calcium channels (VGCCs) being a primary focus.[1] VGCCs are categorized into two families, one is high-voltage calcium channels (HVAs), and the other is low-voltage calcium channels (LVAs). T-type calcium channels (TTCCs), comprising $Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$, are classified as LVAs.[2] Recent studies have highlighted the significance of TTCCs in pain,[3] epilepsy,[4] and cancer[5] pathophysiology, prompting the development of numerous TTCC blockers.[6] Notably, Z944[7] and MK8998[8] have progressed to clinical trials, distinguishing themselves from the first-generation TTCC blocker mibefradil by their selectivity (Figure 1).[9] Despite these advancements, no TTCC blockers are currently available on the market, underscoring the need for continued discovery efforts. Natural products and their derivatives, including lycoplanine A,[10] cyclovirobuxine D,[11] and hyphenrone K,[12] have emerged as valuable sources for TTCC blockers (Figure 1). Among these, *Lycopodium* alkaloids from Lycopodiaceae plants are classified into Lycopodine, Fawcettimine, Lycodine, and Phlegmarine types[13] (Figure 2). The structural complexity of *Lycopodium* alkaloids often translates into a high degree of specificity and affinity for biological targets like T-type calcium channels, Acetylcholinesterase (AChE), etc.[14]

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α-Obscurine (**1**), a lycodine-type alkaloid isolated from *Lycopodium japonicum*, was firstly reported in 1953.[15] However, reports detailing its bioactivity are exceedingly scarce. Previously, our research group discovered that Casuattimines A, B and D, which belong to the Lycodine-type *Lycopodium* alkaloids, exhibited inhibitory effects on $Ca_v3.1$.[14b] We hypothesized that Lycodine-type alkaloids possessed potential as blockers of $Ca_v3.1$. Given the ease of obtaining Lycodine-type α-obscurine from *Lycopodium japonicum*, we conducted structural modifications on α-obscurine with the aim of discovering novel $Ca_v3.1$ calcium channel blockers. Consequently, this study found that α-obscurine derivatives could serve as novel $Ca_v3.1$ calcium channel blockers, providing lead compounds or chemical tools for mechanistic exploration. We synthesized eighteen derivatives from α-obscurine and identified five of them with significant $Ca_v3.1$ blockade activity.

2. Results and Discussion

2.1. Chemistry

The synthesis of eighteen derivatives from α-obscurine was achieved (Scheme 1 and 2). Initial attempts to oxidize the A ring of α-obscurine with $Pb(OAc)_4$ effectively yielded compound **2**, featuring oxidation of the A ring and demethylation of the C ring.[16] Subsequent reaction products of compound **2** with Tf_2O, TsCl, MsCl, and BnBr were identified. Notably, the chemical shifts of C-9 and C-13 in compound **3** remained consistent, while notable shifts were observed for C-1, C-4, C-5, C-6, H-2, and H-3, indicating Tf_2O's reaction with the pyridone oxygen of the A ring (Figure S1).[17]

This pattern was corroborated by the conversion of compound **3** to **4** using $Pd(OAc)_2$, indirectly confirming the structure of compound **3**. Similar NMR variations in compounds **5** and **6** suggested that TsCl and MsCl also targeted the pyridone oxygen. HSQC and HMBC analyses revealed BnBr's reaction with the A ring's pyridone, with the chemical shift of C-

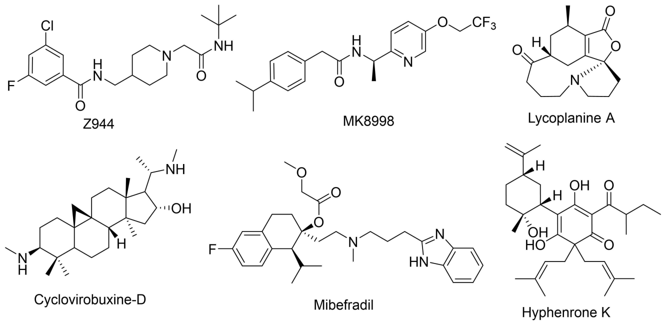


Figure 1. Representative T-type calcium channel blockers.

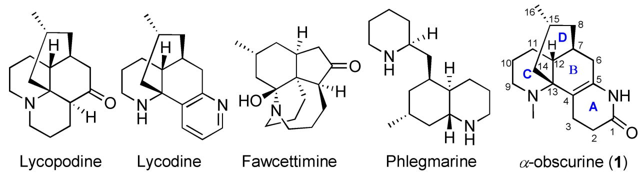
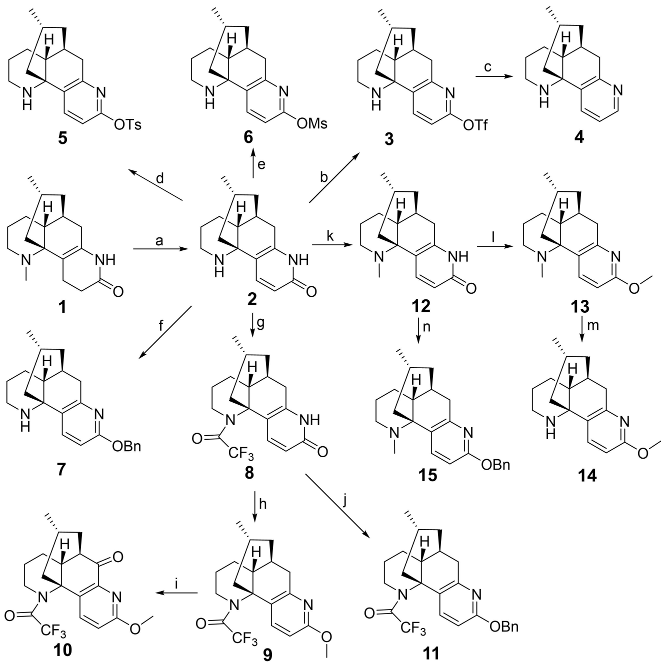
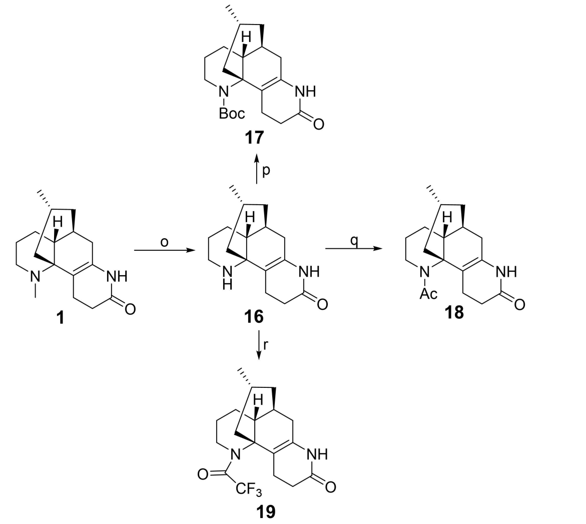


Figure 2. Representative types of *Lycopodium* alkaloids and α-obscurine (**1**).



Scheme 1. Synthesis of α-obscurine derivatives **2–15**. [a] $Pb(OAc)_4$, DCM, r.t, 4h, 54%; [b] Tf_2O, pyridine, DCM, −50 °C, 1 h, 73%; [c] $Pd(OAc)_2$, dppf, HCO_2NH_4, Et_3N, DMF, 60 °C, 2 h, 73%; [d] TsCl, Et_3N, DMAP, THF, 50 °C, 5 h, 58%; [e] MsCl, Et_3N, DMAP, THF, 50 °C, 3 h, 77%; [f] BnBr, Ag_2CO_3, Toluene, 60 °C, 2 h, 45%; [g] TFAA, Et_3N, DCM, r.t, 2 h, 62%; [h] CH_3I, Ag_2CO_3, DCM, r.t, overnight, dark, 70%; [i] AIBN, NBS, CCl_4, 80 °C, 3 h, 34%; [j] BnBr, Ag_2CO_3, Toluene, 60 °C, 2 h, 68%; [k] CH_3I, Ag_2CO_3, DCM, 2 h, dark, 72%; [l] CH_3I, Ag_2CO_3, DCM, overnight, dark, 66%; [m] $KMnO_4$, *t*-BuOH/H_2O(1 : 1), 80 °C, 2 h, 54%; [n] BnBr, Ag_2CO_3, Toluene, 60 °C, 2 h, 78%.

17 at 68.9 ppm confirming the reaction site (Figure S3).[18] Compound **8** was synthesized from compound **2** and TFAA, with NMR data revealing the notable change of chemical shifts of C-9 and C-13 and indicating TFAA's reaction with the C ring (Figure S2). Derivatives **9** and **11** were obtained by treating



Scheme 2. Synthesis of α-obscurine derivatives **16–19**. [o] DDQ, 1,4-dioxane, 110 °C, 2 h, 57%; [p] Boc_2O, Et_3N, THF, 60 °C, 2 h, 57%; [q] AcCl, Et_3N, DCM, r.t, 2 h, 46%; [r] TFAA, Et_3N, DCM, r.t, 1 h, 77%.

compound **8** with CH_3I and BnBr, respectively. Compound **9** underwent further transformation with AIBN and NBS to yield compound **10**,[19] while compounds **12** and **13** were synthesized from reactions involving CH_3I. Compound **14** was derived from compound **13** using $KMnO_4$. Finally, compound **15** was obtained by treating compound **12** with BnBr.

Compound **16** was obtained from **1** by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), resulting demethylation of the C ring. The reactions of compound **16** with Boc_2O, AcCl, TFAA synthesized compounds **17**, **18**, **19**.

2.2. Biological activity

We investigated the effect of compounds **1–19** on the $Ca_v3.1$ voltage-gated calcium channel (Compounds **2** and **12** were insoluble in DMSO). Our results revealed that compounds **3**, **5**, **7**, **9** and **17** greatly inhibited the currents of $Ca_v3.1$ channels, Compared to the parent compound (**1**), these five derivatives demonstrated markedly enhanced inhibitory effects, with compounds **7** achieving nearly 100% inhibition(Figure 3).

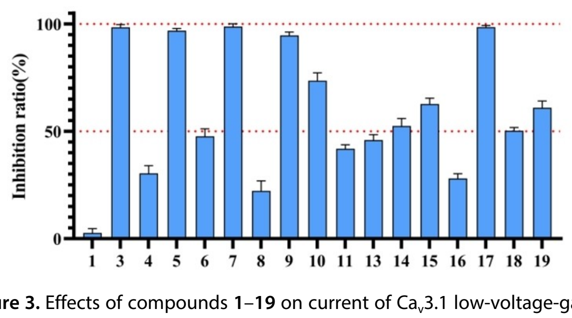


Figure 3. Effects of compounds **1–19** on current of $Ca_v3.1$ low-voltage-gated calcium channel at 50 μM. All the data were represented as mean ± SD (n ≥ 3).

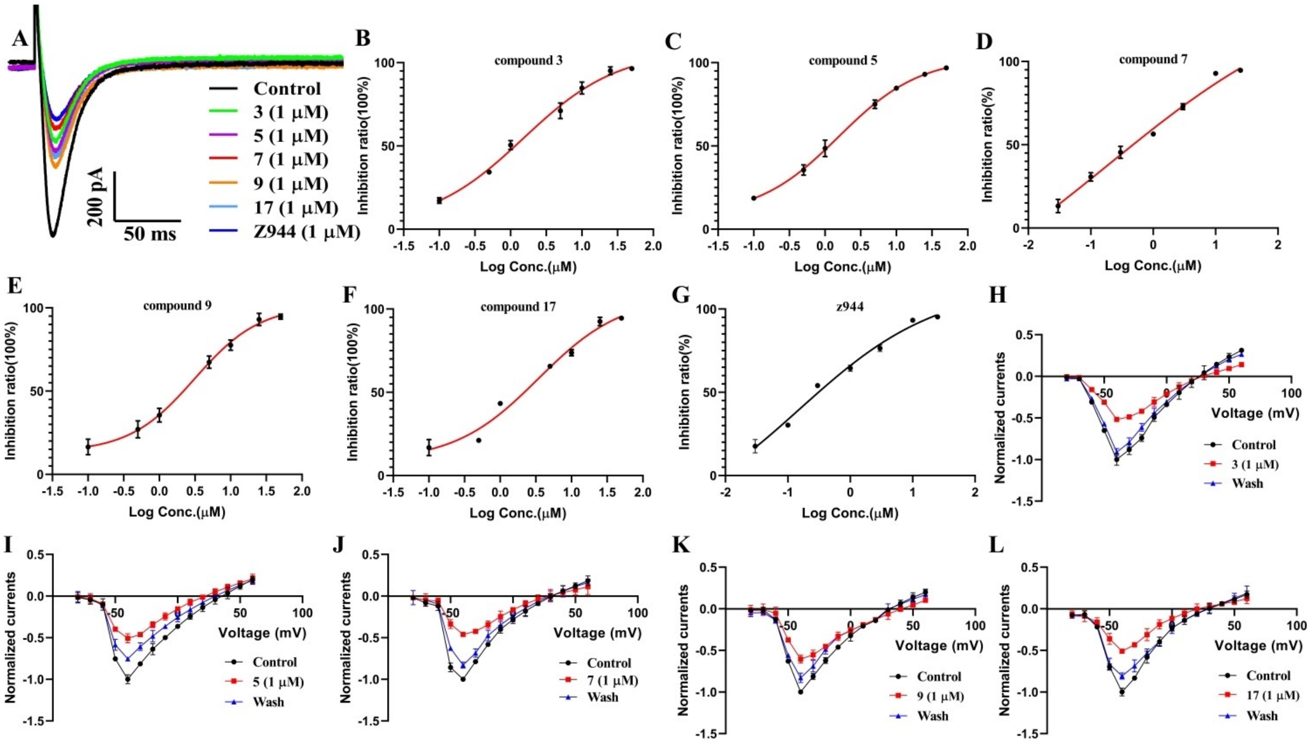


Figure 4. Inhibitory effect of compounds **3**, **5**, **7**, **9**, **17** and Z944 on $Ca_v3.1$ calcium channel. (A) Representative whole-cell $Ca_v3.1$ currents elicited by a 150 ms depolarization of −40 mV in the absence (control) and presence of 1 μM of **3**, **5**, **7**, **9**, **17** and Z944 (positive control). (B–G) Dose-responsive curves of **3**, **5**, **7**, **9**, **17** and Z944 on peak current of $Ca_v3.1$. Data points represent mean ± SD ($n \geq 3$). Solid curve represents fit to the Hill equation. (H–L) Normalized current-voltage curves of $Ca_v3.1$ control (black), $Ca_v3.1$ with 1 μM **3**, **5**, **7**, **9**, **17**, respectively (red). All the data were represented as mean ± SD ($n \geq 3$).

And compounds **3**, **5**, **7**, **9** and **17** inhibited the currents of $Ca_v3.1$ in a dose-dependent manner with IC_{50} values of 1.55 ± 0.25 μM, 1.58 ± 0.46 μM, 0.19 ± 0.03 μM, 3.10 ± 0.37 μM, and 3.60 ± 0.88 μM, respectively. (Figure 4B–F). And as a positive control, Z944 also significantly inhibited the current of $Ca_v3.1$ channels with IC_{50} values of 0.14 ± 0.06 μM (Figure 4G). The normalized current-voltage curve showed that all the five compounds had significant blockade effects on $Ca_v3.1$ and might be reversible blockers (Figure 4H–L).

These findings facilitated a preliminary structure-activity relationship analysis. The comparison of compound **4** with another ten pyridine-type compounds highlighted the critical role of the oxygen atom at C1 for $Ca_v3.1$ blockade activity. The inhibition ratios of compounds **9** and **10** suggested that carbonyl groups at C-6 diminish $Ca_v3.1$ blockade activity. Conversely, benzene and trifluoromethyl groups were found to enhance $Ca_v3.1$ blockade activity, as evidenced by the inhibition ratios of compounds **3**, **5**, **6**, **7**, and **14**. Notably, the trifluoroacetyl group's impact on blockade activity was dual-sided, as inferred from the inhibition ratios of compounds **1**, **7**, **9**, **11**, **14**, **19**. The inhibition ratios of compounds **1**, **7**, **13**, **14**, **16** showed that the C ring' s *N*-methyl was adverse to $Ca_v3.1$ blockade activity. The enhancement of $Ca_v3.1$ blockade activity was significant by the Boc groups of C ring.

2.3. Moleculardocking

Given the strong blockade activity of **7**, its interaction with $Ca_v3.1$ was investigated through molecular docking. Molecular docking results revealed hydrogen bonding between the oxygen atom of compound **7** and the amino acid residue Lys1462. The pyridine and phenyl groups of compound **7** and Phe956 participate in π-π interactions, while π-alkyl interactions were observed between the methyl group of compound **7** and Phe868. Alkyl-alkyl interactions were also identified between compound **7** and Leu872, as well as Leu920 (Figure 5). The molecular docking results suggest that compound **7** exhibits

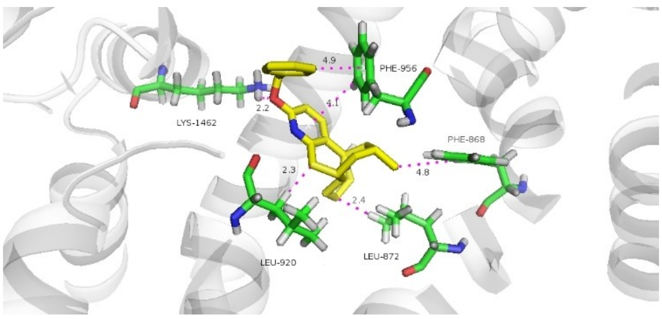


Figure 5. The proposed interaction model of compound **7** with $Ca_v3.1$. The ligand is depicted as sticks color-coded by element type (C, yellow; O, red; N, blue; polar H, gray), and key amino acid residues are represented as sticks color-coded by element type (C, green; O, red; N, blue; H, gray). Key interactions are highlighted with purple dotted lines.

strong interactions with key residues in $Ca_v3.1$, indicating its potential as a promising TTCC blocker. These findings not only enhance our understanding of the mechanisms of TTCC blockers but also provide valuable insights for the future design of more effective drugs targeting this channel. Also, molecular docking between Z944 and $Ca_v3.1$ was carried out. The molecular docking results of compound **7** and Z944 was summarized in Table S1.

3. Conclusions

This study synthesized eighteen derivatives from α-obscurine, a compound from plants traditionally used for treating arthritic pain. The $Ca_v3.1$ blockade activity of compounds **3**, **5**, **7**, **9** and **17** was significantly enhanced, with IC_{50} values of 1.55 ± 0.25 μM, 1.58 ± 0.46 μM, 0.19 ± 0.03 μM, 3.10 ± 0.37 μM, and 3.60 ± 0.88 μM, respectively. The robust blockade activity of compound **7** warranted further exploration of its interaction with $Ca_v3.1$ through molecular docking. These insights lay the groundwork for the structural refinement of compound **7**, positioning it as a promising lead for drug development. Additionally, compound **7** will serve as a chemical biology tool for future investigations into TTCC-related mechanisms, contributing to advancements in the treatment of conditions related to TTCC dysregulation.

Experimental Section

Chemistry

The preparation of α-obscurine

Lycopodium japonicum was collected from GuiZhou, China in June 2015. The sample was identified by Prof. Xiao Cheng (Kunming Institute of Botany). A voucher specimen (201507110P1) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

The dried whole plant of *Lycopodium japonicum* (29 kg) underwent methanol extraction at room temperature using 60 L of methanol for three 24-hour periods. Following this, the methanol extract was partitioned between ethyl acetate and a 1‰ hydrochloric acid aqueous solution. The aqueous layer was then adjusted to pH 10 with ammonia water and extracted with chloroform, yielding 30 g of crude alkaloids. These crude alkaloids were further separated using alkaline alumina column chromatography, transitioning from a chloroform/methanol solvent system of 20:1 to 1:1, and finally eluted with methanol to produce fractions Fr. 1–Fr. 5. From fraction Fr. 2 (3.5 g), α-obscurine (980 mg) was isolated through silica gel column chromatography, employing a chloroform/methanol gradient of 70:1 to 5:1.

General procedures

Unless otherwise specified, all reactions were carried out in dry container under the protection of Argon, and monitored by TLC. The crude products were purified by column chromatography on silica gel (200–300 mesh). All reagents were commercially available and used without further purification. THF was distilled from Na, DCM, DMF and toluene was distilled from CaH_2 before use. All ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz or 600 MHz spectrometer in $CDCl_3$, CD_3OD and acetone-d_6. The solvent signals were used as references. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are reported in hertz (Hz). HR-MS were recorded on Agilent 6540 Q-TOF spectrometer.

Synthesis of Compounds 2–19

(4aR,5S,10bR,12R)-12-methyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthrolin-8(7H)-one(2)

To a solution of **1** (200 mg, 0.73 mmol) in DCM (10 mL) was added $Pb(OAc)_4$ (646.3 mg, 1.46 mmol) at rt. After 3 h, additional $Pb(OAc)_4$ (323.2 mg, 0.73 mmol) was added. The reaction mixture was stirred at rt for one hour, followed by the addition of K_2CO_3. The mixture was then filtered and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol/diethylamine = 30:1:0.05) to give compound **2** (102 mg, 54% yield) as a yellow solid. ^1H-NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 9.3 Hz, 1H), 6.45 (d, *J* = 9.3 Hz, 1H), 2.94 (dd, *J* = 18.8, 7.0 Hz, 1H), 2.80–2.72 (m, 1H), 2.48–2.40 (m, 2H), 2.01 (dq, *J* = 6.8, 3.3 Hz, 1H), 1.74–1.67 (m, 1H), 1.60–1.42 (m, 6H), 1.25 (ddt, *J* = 13.2, 10.0, 4.3 Hz, 3H), 1.03 (t, *J* = 11.6 Hz, 1H), 0.80 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0, 144.9, 139.9, 118.0, 117.3, 54.5, 49.7, 44.6, 43.1, 41.4, 33.2, 29.8, 27.8, 25.9, 25.8, 21.9. HRMS (ESI) calculated for $C_{16}H_{22}N_2O$ $[M+H]^+$ 259.1805, found 259.1805.

(4aR,5S,10bR,12R)-12-methyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthrolin-8-yl trifluoromethanesulfonate(3)

To a solution of **2** (25 mg, 0.10 mmol) in DCM (1 mL) was added Tf_2O (33 μL, 0.19 mmol) and pyridine (78 μL, 0.96 mmol) at −50 °C. After 1h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol = 25:1) to give compound **3** (27 mg, 73% yield) as a yellow solid. ^1H-NMR (600 MHz, $CDCl_3$) δ 8.06 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 8.3 Hz, 1H), 3.09 (dd, *J* = 19.2, 7.2 Hz, 1H), 2.81 (ddt, *J* = 13.7, 3.9, 2.0 Hz, 1H), 2.67 (d, *J* = 19.2 Hz, 1H), 2.40–2.34 (m, 1H), 2.09 (dq, *J* = 6.8, 3.3 Hz, 1H), 1.77 (ddt, *J* = 13.2, 4.1, 2.5 Hz, 1H), 1.62–1.43 (m, 6H), 1.37–1.30 (m, 1H), 1.18 (dtt, *J* = 25.2, 12.5, 3.0 Hz, 3H), 0.79 (d, *J* = 6.1 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.9, 153.5, 138.9, 137.1, 118.6 (q, *J*(C–F) = 324.9 Hz), 112.4, 56.1, 50.9, 44.5, 43.4, 41.3, 34.8, 33.6, 27.8, 26.0, 25.8, 21.9. HRMS (ESI) calculated for $C_{17}H_{21}F_3N_2O_3$ S $[M+H]^+$ 391.1298, found 391.1299.

(4aR,5S,10bR,12R)-12-methyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthroline(4)

To a solution of **3** (25 mg, 0.06 mmol) in DMF (1 mL) was added $Pd(OAc)_2$ (1 mg, 0.01 mmol), Dppf (4 mg, 0.01 mmol), HCO_2NH_4 (20 mg, 0.32 mmol) and Et_3N (18 μL, 0.13 mmol) at rt. After removing oxygen from the reaction vessel, the resulting mixture was heated to 60 °C for 2 h under an argon atmosphere, and then the reaction mixture was poured into saturated aqueous NaCl (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol = 4:1) to give compound **4** (11 mg, 73% yield) as white solid. ^{1}H-NMR (600 MHz, CD_3OD) δ 8.31 (d, J = 4.9 Hz, 1H), 7.90 (d, J = 7.9 Hz, 1H), 7.33–7.24 (m, 1H), 3.16 (dd, J = 18.8, 7.3 Hz, 1H), 2.76 (d, J = 12.7 Hz, 1H), 2.69 (d, J = 18.7 Hz, 1H), 2.46–2.41 (m, 1H), 2.16–2.10 (m, 1H), 1.81 (d, J = 13.2 Hz, 1H), 1.73 (d, J = 12.7 Hz, 1H), 1.67–1.62 (m, 1H), 1.57 (d, J = 13.7 Hz, 2H), 1.50–1.46 (m, 1H), 1.41–1.37 (m, 1H), 1.34–1.26 (m, 2H), 1.19–1.13 (m, 2H), 0.81 (d, J = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 159.6, 147.5, 137.5, 135.4, 123.3, 57.8, 51.5, 44.7, 44.5, 42.0, 35.7, 34.7, 27.5, 27.1, 27.0, 22.4. HRMS (ESI) calculated for $C_{16}H_{22}N_2$ $[M+H]^+$ 243.1856, found 243.1857.

(4aR,5S,10bR,12R)-12-methyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthrolin-8-yl 4-methylbenzenesulfonate(5)

To a solution of **2** (23 mg, 0.09 mmol) in THF (1 mL) was added TsCl (34 mg, 0.18 mmol), Et_3N (37 μL, 0.27 mmol) and DMAP (1 mg, 0.01 mmol) at rt. The resulting mixture was heated to 50 °C for 5 h, and then the reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol = 9:1) to give compound **5** (21 mg, 58% yield) as a yellow oil. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.93 (dd, J = 15.0, 8.2 Hz, 3H), 7.33 (d, J = 8.0 Hz, 2H), 6.94 (d, J = 8.3 Hz, 1H), 3.06–2.91 (m, 2H), 2.82 (d, J = 13.3 Hz, 1H), 2.50 (d, J = 19.1 Hz, 1H), 2.45 (s, 3H), 2.43–2.38 (m, 1H), 2.05 (dq, J = 6.8, 3.2 Hz, 1H), 1.71 (d, J = 13.9 Hz, 1H), 1.53 (s, 3H), 1.49 (d, J = 8.5 Hz, 2H), 1.40 (t, J = 7.2 Hz, 1H), 1.34 (dd, J = 12.1, 4.0 Hz, 1H), 1.21–1.13 (m, 2H), 0.78 (d, J = 5.8 Hz, 3H). ^{13}C NMR (150 MHz, CD_3Cl) δ 158.0, 154.4, 145.0, 137.8, 135.1, 133.9, 129.7, 128.8, 113.1, 55.9, 51.0, 44.5, 43.6, 41.3, 34.8, 33.6, 27.9, 26.0, 25.8, 22.0, 21.7. HRMS (ESI) calculated for $C_{23}H_{28}N_2O_3S$ $[M+H]^+$ 413.1893, found 413.1897.

(4aR,5S,10bR,12R)-12-methyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthrolin-8-yl methanesulfonate(6)

To a solution of **2** (20 mg, 0.08 mmol) in THF (1 mL) was added MsCl (12 μL, 0.15 mmol), Et_3N (32 μL, 0.23 mmol) and DMAP (2 mg, 0.02 mmol) at rt. The resulting mixture was heated to 50 °C for 3 h, and then the reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol = 9:1) to give compound **6** (20 mg, 77% yield) as yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.3 Hz, 1H), 6.96 (d, J = 8.3 Hz, 1H), 3.48 (s, 3H), 3.08 (dd, J = 19.1, 7.1 Hz, 1H), 2.79 (ddt, J = 13.4, 4.3, 1.9 Hz, 1H), 2.63 (d, J = 19.1 Hz, 1H), 2.45–2.35 (m, 1H), 2.07 (dt, J = 6.7, 3.5 Hz, 1H), 1.78–1.73 (m, 1H), 1.62–1.45 (m, 6H), 1.25–1.14 (m, 4H), 0.78 (d, J = 5.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.9, 154.8, 138.3, 135.3, 113.2, 56.0, 50.9, 44.5, 43.5, 41.2, 40.6, 34.9, 33.7, 27.8, 26.0, 25.8, 21.9. HRMS (ESI) calculated for $C_{17}H_{24}N_2O_3$ S $[M+H]^+$ 337.1580, found 337.1577.

(4aR,5S,10bR,12R)-8-(benzyloxy)-12-methyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthroline(7)

To a solution of **2** (30 mg, 0.12 mmol) in Toluene (1.5 mL) was added BnBr (28 μL, 0.23 mmol) and Ag_2CO_3 (96 mg, 0.35 mmol) at rt. The resulting mixture was heated to 60 °C for 2 h, and then the reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol = 20:1) to give compound **7** (18 mg, 45% yield) as a yellow solid. ^{1}H-NMR (600 MHz, CD_3OD) δ 7.71 (d, J = 8.5 Hz, 1H), 7.45–7.43 (m, 2H), 7.37–7.33 (m, 2H), 7.30–7.27 (m, 1H), 6.69 (d, J = 8.5 Hz, 1H), 5.29 (s, 2H), 3.04 (dd, J = 18.8, 7.2 Hz, 1H), 2.71 (ddt, J = 12.5, 4.1, 2.0 Hz, 1H), 2.55 (d, J = 18.8 Hz, 1H), 2.44 (td, J = 12.5, 3.2 Hz, 1H), 2.05 (dq, J = 6.8, 3.3 Hz, 1H), 1.79–1.74 (m, 1H), 1.66–1.60 (m, 2H), 1.57–1.48 (m, 2H), 1.45 (dd, J = 8.0, 1.9 Hz, 1H), 1.38–1.32 (m, 1H), 1.25–1.18 (m, 3H), 0.80 (d, J = 5.9 Hz, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 163.0, 157.4, 138.9, 137.7, 129.4, 129.2, 129.0, 128.8, 109.3, 68.9, 57.3, 51.5, 44.9, 44.8, 42.0, 36.1, 35.0, 27.6, 27.1, 27.1, 22.4. HRMS (ESI) calculated for $C_{23}H_{28}N_2O$ $[M+H]^+$ 349.2274, found 349.2275.

(4aR,5S,10bR,12R)-12-methyl-1-(2,2,2-trifluoroacetyl)-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthrolin-8(7H)-one(8)

To a solution of **2** (21.5 mg, 0.08 mmol) in DCM (1 mL) was added TFAA (11 μL, 0.17 mmol) and Et_3N (35 μL, 0.25 mmol) at rt. After 2 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol/diethylamine = 20:1:0.05) to give compound **8** (18 mg, 62% yield) as brown powder. ^{1}H-NMR (600 MHz, CD_3OD) δ 7.31 (d, J = 9.4 Hz, 1H), 6.45 (d, J = 9.3 Hz, 1H), 3.74 (dtd, J = 14.9, 3.4, 1.7 Hz, 1H), 3.03–2.94 (m, 2H), 2.86–2.79 (m, 1H), 2.36 (d, J = 19.0 Hz, 1H), 2.15 (dq, J = 5.9, 2.9 Hz, 1H), 1.96 (ddd, J = 12.7, 4.1, 2.6 Hz, 1H), 1.70 (qt, J = 11.5, 3.0 Hz, 3H), 1.63 (ddd, J = 17.2, 8.4, 6.3 Hz, 2H), 1.48–1.41 (m, 1H), 1.39–1.32 (m, 2H), 0.90 (d, J = 5.7 Hz, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 165.4, 159.4(q, J(C–F) = 35.0 Hz), 146.3, 141.4, 119.3, 118.2(q, J(C–F) = 290.6 Hz), 117.0, 67.0, 46.8, 45.3, 45.0, 43.2, 35.1, 30.3, 27.9, 27.4, 26.5, 22.4. HRMS (ESI) calculated for $C_{18}H_{21}F_3N_2O_2$ $[M+H]^+$ 355.1628, found 355.1631.

2,2,2-trifluoro-1-((4aR,5S,10bR,12R)-8-methoxy-12-methyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthrolin-1-yl)ethan-1-one(9)

To a solution of **8** (32 mg, 0.09 mmol) in DCM (1 mL) was added CH_3I (281 μL, 4.52 mmol) and Ag_2CO_3(75 mg, 0.27 mmol) at rt. After the resulting mixture was stirred overnight, the reaction mixture was filtered, poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 30:1) to give compound **9** (23 mg, 70% yield) as a yellow solid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.37 (d, J = 8.6 Hz, 1H), 6.61 (d, J = 8.5 Hz, 1H), 3.91 (s, 3H), 3.70–3.63 (m, 1H), 3.12 (dd, J = 19.1, 7.4 Hz, 1H), 2.99 (ddd, J = 13.0, 3.8, 1.8 Hz, 1H), 2.72 (ddd, J = 14.8, 12.4, 2.5 Hz, 1H), 2.60 (d, J = 19.1 Hz, 1H), 2.12 (dq, J = 6.7, 3.2 Hz, 1H), 1.92 (dt, J = 12.7, 3.3 Hz, 1H), 1.71–1.63 (m, 3H), 1.60 (ddd, J = 11.9, 5.1, 3.0 Hz, 2H), 1.37 (qd, J = 12.8, 4.5 Hz, 1H), 1.30–1.27 (m, 1H), 1.27–1.22 (m, 1H), 0.81 (d, J = 6.1 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ

162.3, 158.2(q, J(C–F) = 31.1 Hz), 154.9, 137.5, 125.5, 116.8(q, J(C–F) = 330.1 Hz), 109.2, 67.0, 53.8, 46.5, 44.0, 43.9(q, J(C–F) = 3.8 Hz), 42.6, 34.4, 34.3, 26.6, 26.6, 25.6, 22.1. HRMS (ESI) calculated for $C_{19}H_{23}F_3N_2O_2$ $[M+H]^+$ 369.1784, found 369.1786.

(4aR,5R,10bR,12R)-8-methoxy-12-methyl-1-(2,2,2-trifluoroacetyl)-1,2,3,4,4a,5-hexahydro-6H-5,10b-propano-1,7-phenanthrolin-6-one(10)

Into a 5 mL sealed tube, **9** (20 mg, 0.05 mmol), CCl_4 (1 mL), AIBN (5 mg, 0.03 mmol), and NBS (39 mg, 0.22 mmol) were added at room temperature. After the resulting mixture was stirred at 80 °C for 3 h under an argon atmosphere, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 60:1) to give compound **10** (7 mg, 34% yield) as a brown solid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.57 (d, J = 8.6 Hz, 1H), 7.05 (d, J = 8.6 Hz, 1H), 4.06 (s, 3H), 3.81–3.76 (m, 1H), 3.20 (ddd, J = 13.4, 4.2, 1.6 Hz, 1H), 2.83 (dt, J = 5.0, 2.7 Hz, 1H), 2.76 (ddd, J = 14.9, 12.3, 2.6 Hz, 1H), 2.26 (ddd, J = 13.1, 4.3, 2.3 Hz, 1H), 1.93 (dq, J = 13.1, 2.4, 1.7 Hz, 1H), 1.84 (dd, J = 13.4, 11.8 Hz, 1H), 1.76–1.71 (m, 1H), 1.65 (ddd, J = 10.5, 5.1, 2.7 Hz, 2H), 1.44 (tdd, J = 13.5, 5.0, 3.3 Hz, 2H), 1.33 (tt, J = 12.7, 5.4 Hz, 1H), 0.89 (d, J = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.9, 163.6, 158.5(q, J(C–F) = 35.6 Hz), 146.3, 137.9, 133.0, 118.4, 116.6(q, J(C–F) = 292.7 Hz), 66.6, 53.9, 51.8, 47.4, 44.0(q, J(C–F) = 3.9 Hz), 43.9, 37.4, 27.1, 26.9, 25.1, 21.8. HRMS (ESI) calculated for $C_{19}H_{21}F_3N_2O_3$ $[M+H]^+$ 383.1577, found 383.1580.

1-((4aR,5S,10bR,12R)-8-(benzyloxy)-12-methyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthrolin-1-yl)-2,2,2-trifluoroethan-1-one(11)

To a solution of **8** (35 mg, 0.10 mmol) in Toluene (2 mL) was added BnBr (24 μL, 0.20 mmol) and Ag_2CO_3 (68 mg, 0.25 mmol) at rt. After the resulting mixture was heated to 60 °C for 2 h, the reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 50:1) to give compound **11** (30 mg, 68% yield) as yellow oil. ^{1}H-NMR (600 MHz, CD_3OD) δ 7.45–7.42 (m, 2H), 7.39 (d, J = 8.5 Hz, 1H), 7.36–7.32 (m, 2H), 7.31–7.26 (m, 1H), 6.72 (d, J = 8.5 Hz, 1H), 5.32 (s, 2H), 3.73–3.67 (m, 1H), 3.14 (dd, J = 19.1, 7.4 Hz, 1H), 2.99 (ddd, J = 13.0, 4.0, 1.7 Hz, 1H), 2.78 (ddd, J = 14.8, 12.0, 2.9 Hz, 1H), 2.57 (d, J = 19.0 Hz, 1H), 2.15 (dq, J = 6.9, 3.1 Hz, 1H), 1.97 (dt, J = 12.7, 3.3 Hz, 1H), 1.73 (ddt, J = 12.9, 4.2, 2.0 Hz, 1H), 1.69–1.62 (m, 4H), 1.42–1.33 (m, 2H), 1.27–1.21 (m, 1H), 0.84 (d, J = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 163.6, 159.3(q, J(C–F) = 33.5 Hz), 156.8, 138.8, 138.3, 129.4, 129.0, 128.8, 126.9, 118.3(q, J(C–F) = 286.8 Hz), 110.3, 68.9, 68.5, 48.1, 45.4, 45.3(q, J(C–F) = 3.7 Hz), 43.8, 35.8, 35.7, 27.9, 27.8, 26.7, 22.6. HRMS (ESI) calculated for $C_{25}H_{27}F_3N_2O_2$ $[M+H]^+$ 445.2097, found 445.2098.

(4aR,5S,10bR,12R)-1,12-dimethyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthrolin-8(7H)-one(12)

To a solution of **2** (31 mg, 0.12 mmol) in DCM (1.5 mL) was added CH_3I (15 μL, 0.24 mmol) and Ag_2CO_3 (99 mg, 0.36 mmol) at rt. After the resulting mixture was stirred for 2 h in the dark, the reaction mixture was filtered, poured into saturated aqueous $NaHCO_3$ (10 mL), and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol/diethylamine = 20:1:0.05) to give compound **12** (24 mg, 72% yield) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 13.33 (br s, 1H), 7.80 (d, J = 9.3 Hz, 1H), 6.42 (d, J = 9.3 Hz, 1H), 2.95 (dd, J = 18.7, 6.9 Hz, 1H), 2.78 (d, J = 7.7 Hz, 1H), 2.68 (td, J = 13.5, 2.8 Hz, 1H), 2.55 (s, 4H), 2.40 (d, J = 18.6 Hz, 1H), 1.99 (dq, J = 6.8, 3.2 Hz, 1H), 1.83 (dt, J = 12.5, 3.6 Hz, 1H), 1.76 (dt, J = 13.1, 4.0 Hz, 1H), 1.67 (dt, J = 12.8, 3.0 Hz, 1H), 1.44 (ddt, J = 11.5, 4.7, 2.4 Hz, 1H), 1.34 (d, J = 4.4 Hz, 2H), 1.26 (dd, J = 12.9, 3.9 Hz, 1H), 1.18–1.08 (m, 2H), 0.80 (d, J = 5.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0, 144.3, 141.0, 119.9, 117.1, 57.9, 50.4, 46.4, 43.1, 36.1, 34.4, 33.3, 29.6, 26.5, 26.5, 22.2, 19.3. HRMS (ESI) calculated for $C_{17}H_{24}N_2O$ $[M+H]^+$ 273.1961, found 273.1960.

(4aR,5S,10bR,12R)-8-methoxy-1,12-dimethyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthroline(13)

To a solution of **12** (30 mg, 0.12 mmol) in DCM (1.5 mL) was added CH_3I (361 μL, 5.81 mmol) and Ag2CO3 (96 mg, 0.35 mmol) at rt. After the resulting mixture was stirred overnight in the dark, the reaction mixture was filtered, poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 10:1) to give compound **13** (21 mg, 66% yield) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 8.6 Hz, 1H), 6.54 (d, J = 8.5 Hz, 1H), 3.89 (s, 3H), 3.05 (dd, J = 18.7, 7.0 Hz, 1H), 2.70 (td, J = 13.6, 2.8 Hz, 1H), 2.60 (s, 3H), 2.56–2.46 (m, 2H), 2.02 (dq, J = 6.6, 3.1 Hz, 1H), 1.88 (dt, J = 12.7, 3.6 Hz, 1H), 1.79 (dt, J = 13.2, 4.0 Hz, 1H), 1.72–1.68 (m, 1H), 1.47 (ddq, J = 10.4, 4.3, 2.1 Hz, 1H), 1.37 (d, J = 10.4 Hz, 2H), 1.28–1.18 (m, 3H), 1.12–1.06 (m, 1H), 0.77 (d, J = 5.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.8, 155.3, 137.3, 129.9, 108.0, 59.1, 53.2, 50.4, 47.8, 43.9, 36.3, 35.2, 34.5, 34.0, 26.8, 26.5, 22.4, 19.6. HRMS (ESI) calculated for $C_{18}H_{26}N_2O$ $[M+H]^+$ 287.2118, found 287.2117.

4aR,5S,10bR,12R)-8-methoxy-12-methyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthroline(14)

A solution of **13** (30 mg, 0.12 mmol) and $KMnO_4$ in t-BuOH/H_2O (1:1, 1.5 mL) was stirred at 80 °C. After 2 h, the mixture was cooled to rt and then was poured into saturated aqueous $Na_2S_2O_3$ (10 mL), filtered, and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 9:1) to give compound **14** (15 mg, 54% yield) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 8.5 Hz, 1H), 6.57 (d, J = 8.4 Hz, 1H), 3.90 (s, 3H), 3.03 (dd, J = 18.8, 7.2 Hz, 1H), 2.76 (ddt, J = 12.9, 4.0, 2.1 Hz, 1H), 2.55 (d, J = 18.8 Hz, 1H), 2.45 (td, J = 13.2, 12.5, 4.0 Hz, 1H), 2.04 (dq, J = 6.5, 3.0 Hz, 1H), 1.75 (dt, J = 8.5, 2.4 Hz, 1H), 1.59–1.41 (m, 6H), 1.33–1.21 (m, 3H), 1.11 (t, J = 11.4 Hz, 1H), 0.78 (d, J = 5.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.0, 155.9, 136.2, 127.9, 108.1, 55.7, 53.2, 51.1, 44.8, 43.8, 41.4, 35.3, 33.9, 27.9, 26.1, 25.9, 22.0. HRMS (ESI) calculated for $C_{17}H_{24}N_2O$ $[M+H]^+$ 273.1961, found 273.1961.

(4aR,5S,10bR,12R)-8-(benzyloxy)-1,12-dimethyl-2,3,4,4a,5,6-hexahydro-1H-5,10b-propano-1,7-phenanthroline(15)

To a solution of **12** (20 mg, 0.07 mmol) in Toluene (1 mL) was added BnBr (17 μL, 0.15 mmol) and Ag_2CO_3 (60 mg, 0.22 mmol) at rt. After the resulting mixture was heated to 60 °C for 2 h, the reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol = 30:1) to give compound **15** (20.8 mg, 78% yield) as yellow oil. ^{1}H-NMR (600 MHz, Acetone-d_6) δ 7.94 (d, J = 8.5 Hz, 1H), 7.49 (dd, J = 7.4, 1.6 Hz, 2H), 7.38 (dd, J = 8.4, 6.8 Hz, 2H), 7.33–7.29 (m, 1H), 6.66–6.56 (m, 1H), 5.33 (d, J = 1.3 Hz, 2H), 3.05 (dd, J = 18.7, 7.0 Hz, 1H), 2.67 (td, J = 13.6, 2.8 Hz, 1H), 2.61 (s, 3H), 2.56–2.53 (m, 1H), 2.50 (d, J = 18.8 Hz, 1H), 1.94 (dt, J = 12.9, 3.6 Hz, 1H), 1.82 (dddd, J = 17.1, 13.2, 8.6, 3.9 Hz, 1H), 1.73 (ddt, J = 12.7, 5.1, 2.4 Hz, 1H), 1.53–1.42 (m, 3H), 1.33 (ddd, J = 12.0, 3.8, 2.0 Hz, 1H), 1.30–1.24 (m, 3H), 1.08 (ddt, J = 13.1, 4.4, 2.5 Hz, 1H), 0.78 (d, J = 6.3 Hz, 3H). ^{13}C NMR (150 MHz, Acetone-d_6) δ 162.1, 156.0, 139.0, 138.2, 131.0, 129.1, 128.9, 128.4, 109.5, 67.7, 59.7, 51.1, 48.6, 44.6, 36.5, 35.9, 35.1, 34.9, 27.7, 27.4, 22.6, 20.2. HRMS (ESI) calculated for $C_{24}H_{30}N_2O$ $[M+H]^+$ 363.2431, found 363.2424.

(4aR,5S,10bR,12R)-12-methyl-2,3,4,4a,5,6,9,10-octahydro-1H-5,10b-propano-1,7-phenanthrolin-8(7H)-one(16)

To a solution of **1** (21 mg, 0.08 mmol) in 1,4-dioxane (1 mL) was added DDQ (69 mg, 0.31 mmol) at rt. After the resulting mixture was heated to 110 °C for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol/diethylamine = 25:1:0.05) to give compound **16** (11.4 mg, 57% yield) as a yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 2.79 (dd, J = 12.6, 3.9 Hz, 1H), 2.41 (ddt, J = 39.1, 17.9, 8.1 Hz, 4H), 2.28 (dd, J = 16.6, 7.9 Hz, 1H), 2.17 (dtd, J = 14.2, 7.1, 3.7 Hz, 1H), 1.88–1.83 (m, 1H), 1.72–1.56 (m, 5H), 1.43 (ddq, J = 35.2, 14.0, 6.9 Hz, 5H), 1.19 (td, J = 13.6, 4.0 Hz, 1H), 0.84 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 130.9, 112.2, 55.4, 46.1, 44.6, 43.5, 42.7, 33.5, 31.2, 30.1, 27.3, 26.5, 26.0, 22.0, 18.8. HRMS (ESI) calculated for $C_{16}H_{24}N_2O$ $[M+H]^+$ 261.1961, found 261.1963.

tert-butyl(4aR,5S,10bR,12R)-12-methyl-8-oxo-2,3,4,4a,5,6,7,8,9,10-decahydro-1H-5,10b-propano-1,7-phenanthroline-1-carboxylate (17)

To a solution of **16** (20 mg, 0.07 mmol) in THF (1 mL) was added Boc_2O (33 mg, 0.15 mmol) and Et_3N (32 μL, 0.23 mmol) at rt. After the resulting mixture was heated to 60 °C for 2 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/isopropanol/diethylamine = 30:1:0.05) to give compound **17** (15.8 mg, 57% yield) as white solid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.69 (s, 1H), 4.15–4.11 (m, 1H), 2.78–2.73 (m, 1H), 2.48–2.38 (m, 4H), 2.29 (dt, J = 16.8, 8.3 Hz, 1H), 2.10–2.04 (m, 1H), 1.90 (dd, J = 7.1, 3.5 Hz, 1H), 1.66 (d, J = 18.4 Hz, 1H), 1.63–1.59 (m, 2H), 1.58 (t, J = 3.6 Hz, 1H), 1.55 (d, J = 2.9 Hz, 1H), 1.52 (d, J = 11.9 Hz, 1H), 1.44 (d, J = 1.3 Hz, 12H), 1.20 (td, J = 12.7, 4.1 Hz, 1H), 0.91 (d, J = 6.1 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 155.7, 130.8, 111.7, 79.3, 63.4, 44.4, 43.9, 43.8, 42.7, 33.9, 31.0, 29.8, 28.5, 27.4, 27.2, 25.4, 22.3, 19.9. HRMS (ESI) calculated for $C_{21}H_{32}N_2O_3$ $[M+H]^+$ 361.2486, found 361.2485.

(4aR,5S,10bR,12R)-1-acetyl-12-methyl-2,3,4,4a,5,6,9,10-octahydro-1H-5,10b-propano-1,7-phenanthrolin-8(7H)-one(18)

To a solution of **16** (20 mg, 0.07 mmol) in DCM (1 mL) was added AcCl (11 uL, 0.15 mmol) and Et_3N (32 μL, 0.23 mmol) at rt. After 2 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc/diethylamine = 4:1:0.05) to give compound **18** (10.7 mg, 46% yield) as colorless oil. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.22 (s, 1H), 3.64 (d, J = 13.9 Hz, 1H), 3.12 (d, J = 11.8 Hz, 1H), 2.72 (t, J = 12.8 Hz, 1H), 2.49 (dq, J = 16.2, 7.7 Hz, 2H), 2.44–2.37 (m, 2H), 2.15 (d, J = 1.7 Hz, 3H), 2.09–2.05 (m, 1H), 1.94–1.92 (m, 1H), 1.69–1.51 (m, 8H), 1.28–1.16 (m, 2H), 0.91 (dd, J = 6.1, 1.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.0, 171.2, 131.0, 111.2, 65.5, 46.1, 44.0, 43.6, 42.7, 33.9, 31.0, 29.8, 27.1, 27.1, 26.1, 25.6, 22.2, 20.3. HRMS (ESI) calculated for $C_{18}H_{26}N_2O_2$ $[M+H]^+$ 303.2067, found 303.2066.

(4aR,5S,10bR,12R)-12-methyl-1-(2,2,2-trifluoroacetyl)-2,3,4,4a,5,6,9,10-octahydro-1H-5,10b-propano-1,7-phenanthrolin-8(7H)-one(19)

To a solution of **16** (18 mg, 0.07 mmol) in DCM (1 mL) was added TFAA (10 ul, 0.14 mmol) and Et_3N (30 μL, 0.21 mmol) at rt. After 1 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with DCM (5 mL×3). The combined organic phases were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc/diethylamine = 7:3:0.05) to give compound **19** (19 mg, 77% yield) as colorless oil. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.91 (s, 1H), 3.79–3.72 (m, 1H), 3.04 (ddd, J = 13.3, 4.2, 1.7 Hz, 1H), 2.78 (ddd, J = 14.6, 12.4, 2.3 Hz, 1H), 2.57–2.42 (m, 3H), 2.37 (dtt, J = 16.5, 8.0, 2.4 Hz, 1H), 2.09–2.03 (m, 1H), 1.98 (dq, J = 6.3, 2.9 Hz, 1H), 1.79 (ddd, J = 11.3, 5.5, 2.5 Hz, 1H), 1.77–1.60 (m, 5H), 1.55 (h, J = 7.9, 7.3 Hz, 2H), 1.49 (dd, J = 13.4, 11.6 Hz, 1H), 1.26–1.20 (m, 1H), 0.92 (d, J = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.6, 157.8 (q, J(C–F) = 33.9 Hz), 132.5, 116.9 (q, J(C–F) = 281.4 Hz), 109.6, 67.4, 44.6 (q, J(C–F) = 4.1 Hz), 44.3, 42.9, 42.6, 34.1, 31.0, 29.8, 27.3, 26.7, 25.6, 22.2, 20.3. HRMS (ESI) calculated for $C_{18}H_{23}F_3N_2O_2$ $[M+H]^+$ 357.1784, found 357.1783.

Biological Activity

HEK293T cells (purchased from ATCC) were cultured at 37 °C with 5% CO_2 in Dulbecco's Modified Eagle Medium (VivaCell, Shanghai, China) with glucose, L-glutamine, pyruvate, 10% FBS (VivaCell, Shanghai, China) and 1% Pen-Strep (VivaCell, Shanghai, China). Cells were seeded at low density onto 24-well plates 24 hours before transfection. Adherent cells were transfected using Lipofectamine 3000 reagent (Invitrogen) with 300 ng $Ca_v3.1$ cDNA and recorded after 48 h. Whole-cell voltage-clamp recordings were performed at rt (24 °C). The peak currents of $Ca_v3.1$ were elicited by 150 ms depolarization from a holding potential of −100 mV to −40 mV at 4 s intervals. Borosilicate glass micropipettes were pulled to produce a resistance of 4–6 MΩ and filled with intracellular recording solution containing 130 mM CsCl, 2 mM $MgCl_2$, 10 mM EGTA, 5 mM Na-ATP, 10 mM HEPES (pH 7.2 with CsOH). The extracellular recording solution was composed of 145 mM CsCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Glucose, 10 mM HEPES (pH 7.4 with CsOH).[10] The current trace of $Ca_v3.1$ in different states was analyzed by the Clampfit 10.6. Data were processed using the software Graphpad Prism 8.0.

Molecular docking

The crystallographic structure of $Ca_v3.1$ (PDB: 6KZP) was downloaded from Protein Data Bank. Protein and ligand preparation was carried out using Pymol and Autodock Tools v1.5.6. Autodock Vina were used to carry out the molecular docking.[20] The conformation of the lowest affinity was chosen to analyze the protein-ligand interaction by Protein-Ligand Interaction Profiler (http://plip-tool.-biotec.tu-dresden.de/plip-web/plip/index). The result was visualized by Pymol.

Supplementary Material

The data that support this study are available in the supplementary material of this article.

Author Contributions

L. Wang performed the synthesis of compounds **2**–**19**. *W.-Y. Li* performed the evaluation of biological activity. The research design and supervision were carried out by *Z.-F. Yuan* and *Q.-S. Zhao*. All authors revised and approved the final manuscript.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: α-obscurine · $Ca_v3.1$ *Lycopodium* alkaloids · T-type calcium channels · Voltage-gated calcium channels

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