Discovery of (S)-2-cyclopentyl-N-((1-isopropylpyrrolidin2-yl)-9-methyl-1-oxo-2,9-dihydro-1H-pyrrido[3,4-b]indole-4-carboxamide (VU0453379): a novel, CNS penetrant Glucagon-Like Peptide 1 (GLP-1) positive allosteric modulator (PAM)

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General Experimental

All reactions were carried out employing standard chemical techniques under inert atmosphere. Solvents used for extraction, washing, and chromatography were HPLC grade. Unless otherwise noted, all reagents were purchased from Aldrich Chemical Co. and were used without further purification. Analytical thin
layer chromatography was performed on 250 µm silica gel plates from Sorbent Technologies. Analytical HPLC was performed on an Agilent 1200 LCMS with UV detection at 215 nm and 254 nm along with ELSD detection and electrospray ionization, with all final compounds showing > 95% purity and a parent mass ion consistent with the desired structure. All NMR spectra were recorded on a 400 MHz Brüker AV-400 instrument. $^1$H chemical shifts are reported as δ values in ppm relative to the residual solvent peak (MeOD = 3.31, CDCl$_3$ = 7.26). Data are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet), coupling constant (Hz), and integration. $^{13}$C chemical shifts are reported as δ values in ppm relative to the residual solvent peak (MeOD = 49.0, CDCl$_3$ = 77.16). Low resolution mass spectra were obtained on an Agilent 1200 LCMS with electrospray ionization. High resolution mass spectra were recorded on a Waters QToF-API-US plus Acquity system with electrospray ionization. Automated flash column chromatography was performed on a Teledyne ISCO Combiflash Rf system. Preparative purification of library compounds was performed on a Gilson 215 preparative LC system. Optical rotations were acquired on a Jasco P-2000 polarimeter at 23 °C and 589 nm. The specific rotations were calculated according to the equation $[\alpha]_D^{22} = \frac{100x}{l \times c}$ where $l$ is path length in decimeters and $c$ is the concentration in g/100 mL.

**In vitro pharmacology methods**

**Human GLP-1R Cell Line**

Human GLP-1R 9-3-H cells were obtained by clonal selection of a GLP-1R-overexpressing cell line derived from ChemiSCREEN™ human recombinant GLP-1 receptor cells that also overexpress a promiscuous G-protein (Millipore #HTS163C; Millipore, Billerica, MA, USA). Cell cultures were maintained in Dulbecco’s Modified Eagles Medium (DMEM) with 10% fetal calf serum (FCS), 4.0 mM L-glutamine, 1X non-essential amino acids (NEAAs), and 10.0 mM hydroxyethyl piperazineethanesulfonic acid (HEPES), and they were retained under selection using 500 µg/mL geneticin and 500 µg/mL hygromycin B.

**Calcium mobilization assays:**

The fluorescence-based calcium assays were performed as previously reported with a few changes. Briefly, human GLP-1R 9-3-H cells and glucagon R cells were plated in a 20 µL mixed suspension (65% and 35%, respectively) of cells at a density of 15,000 total cells per well in a 384-well plate (#781091; Greiner Bio-one, Monroe, NC) in plating medium (DMEM supplemented with 4.0 mM L-glutamine, 10% FCS, 1X NEAA, and 10.0 mM HEPES) using a Multidrop Combi (Thermofisher Scientific, Waltham, MA) and incubated overnight at 37 °C and 5% CO$_2$. Individual 384-well plates of cells were transferred to a Cytomat Automated Incubator (Thermo Fisher Scientific) on the day of screening and were processed through the primary calcium-screening assay with an integrated suite of automated instrumentation. Each screening day was monitored and systematically staggered on a time-locked scheduler using Polara Scheduling Software (Thermofisher Scientific) integrated with an ELx405CW cell washer (Bio-Tek, Winooski, VT), a Multidrop Combi, and a Functional Drug Screening System (FDSS) 6000 (Hamamatsu, Bridgewater, NJ). Plates were handled and transferred using an F-3 robotic arm (Thermofisher Scientific).
Each plate was first washed on the ELx405CW and then loaded with the calcium-sensitive dye fluo-4 AM (Invitrogen, Grand Island, NY) at a final concentration of 2.0 µM. Plates were then incubated at ambient temperature for 40 min, and the dye was then removed by washing, leaving 20 µL per well of assay buffer. The cell plate was then introduced alongside a 384-well. Data were obtained as max-min fluorescent ratios and then normalized to percentage of maximal acetylcholine response. The single point values represent mean values obtained from at least three independent determinations performed in triplicate or greater (error bars represent +/- SEM) unless otherwise specified.

For Ca$^{2+}$ mobilization assays measuring GLP-1 CRC fold shift, human GLP-1R 9-3-H cells were plated in the manner described above. Test compounds were serially diluted into assay buffer to a 2X (20 µM) stock concentration in 0.6% dimethylsulfoxide (DMSO); stock compounds were added to the assay for a final stock concentration of 10 µM and a final DMSO concentration of 0.3%. An eight-point concentration range of GLP-1 (or 1 or 2) was serially diluted in assay buffer to 10X final concentration. FLEXstation II protocols were carried out as described above; data were obtained as max-min fluorescent ratios and then normalized to percentage of maximum GLP-1 response. Calculation of GLP-1 EC$_{50}$ was performed using the curve-fitting software of GraphPad Prism (version 5.01). GLP-1 fold shift was calculated as a ratio of GLP-1 EC$_{50}$ in the presence of vehicle to the GLP-1 EC$_{50}$ in the presence of test compound. Data shown represent mean values obtained from at least three independent determinations performed in triplicate or greater (error bars represent ± SEM) unless otherwise specified.

**β-Arrestin Recruitment and GPCR Internalization Assays:**

The recruitment of β-arrestin was measured using PathHunter™ Express hGLP-1R CHO cells (DiscoverX; Fremont, CA) and the internalization of GLP-1R was measured using PathHunter™ Express hGLP-1R U2OS cells (DiscoverX; Fremont, CA). For each assay, single use cell vials were reconstituted in 12 mL of CP reagent and plated at 20 µL per well in 384-well plates (Greiner Bio-one, Austria). The following day, cells were treated with 2.5 µL of compound and incubated at 37 °C for 15-30 minutes, followed by treatment with 2.5 µL of 10$^{-10}$ to 10$^{-6}$ M liraglutide (Novo Nordisk, Denmark) and incubated at 37 °C for 90-180 minutes. All incubation times used were according to manufacturer’s specific instructions. Substrate was added to each well and after a 60 minute room temperature incubation, luminescence values were obtained using a Perkin Elmer Enspire (Perkin Elmer, Foster City, CA).

**DMPK Methods**

**Plasma protein and brain homogenate binding:**

The degree of plasma protein binding of each test compound was determined essentially as previously described (Gentry et al., 2013). The protein binding of each compound was determined in plasma via equilibrium dialysis employing RED Plates (ThermoFisher Scientific, Rochester, NY). Plasma was added to the 96 well plate containing test compound and mixed thoroughly for a final compound concentration of 5 µM. Subsequently, an aliquot of the plasma-compound mixture was transferred to the cis chamber (red) of the RED plate, with a phosphate buffer (25 mM, pH 7.4) in the trans chamber. The RED plate was sealed and incubated for 4 hours at 37°C with shaking. At completion, aliquots from each chamber were diluted 1:1 with either plasma (cis) or buffer (trans) and transferred to a new 96 well plate,
at which time ice-cold acetonitrile containing internal standard (50 ng/mL carbamazepine) (2 volumes) was added to extract the matrices. The plate was centrifuged (3000 rcf, 10 min) and supernatants transferred and diluted 1:1 (supernatant: water) into a new 96 well plate, which was then sealed in preparation for LC/MS/MS analysis. Each compound was assayed in triplicate within the same 96-well plate. Fraction unbound was determined using the following equation:

\[
F_u = \frac{\text{Conc}_{\text{buffer}}}{\text{Conc}_{\text{plasma}}}
\]

A similar approach was used to determine the degree of brain homogenate binding, which employed the same methodology and procedure with the following modifications: 1) a final compound concentration of 1 µM was used, 2) naïve rat brains were homogenized in DPBS (1:3 composition of brain:DPBS, w/w) using a Mini-Bead Beater machine in order to obtain brain homogenate, which was then utilized in the same manner as plasma in the previously described plasma protein binding assay. Fraction unbound was determined using the following equation:

\[
\text{Diluted}: f_{u,d} = \frac{\text{Conc}_{\text{buffer}}}{\text{Conc}_{\text{plasma}}}
\]

\[
\text{Undiluted}: f_u = \left(\frac{1}{f_{u,d}}-1\right)^{1/D}
\]

where D = dilution factor

**Intrinsic clearance:**

The intrinsic clearance of each test compound was determined essentially as previously described (Gentry et al., 2013). Human or rat hepatic microsomes (0.5 mg/mL) and 1 µM test compound were incubated in 100 mM potassium phosphate pH 7.4 buffer with 3 mM MgCl\(_2\) at 37 °C with constant shaking. After a 5 min preincubation, the reaction was initiated by addition of NADPH (1 mM). At selected time intervals (0, 3, 7, 15, 25, and 45 min), aliquots were taken and subsequently placed into a 96-well plate containing cold acetonitrile with internal standard (50 ng/mL carbamazepine). Plates were then centrifuged at 3000 rcf (4° C) for 10 min, and the supernatant was transferred to a separate 96-well plate and diluted 1:1 with water for LC/MS/MS analysis. The in vitro half-life (\(T_{1/2}\), min, Eq. 1), intrinsic clearance (\(CL_{\text{int}}\), mL/min/kg, Eq. 2) and subsequent predicted hepatic clearance (\(CL_{\text{hep}}\), mL/min/kg, Eq. 3) was determined employing the following equations:

\[
(1) \quad T_{1/2} = \frac{\ln(2)}{k}
\]

where k represents the slope from linear regression analysis of the natural log percent remaining of test compound as a function of incubation time

\[
(2) \quad CL_{\text{int}} = \frac{0.693}{\text{in vitro}T_{1/2}} \times \frac{mL \text{ incubation}}{x} \times \frac{mg \text{ microsomes}}{x} \times \frac{45mg \text{ microsomes}}{gram \text{ liver}} \times \frac{20^o \text{ gram liver}}{kg \text{ body wt}}
\]
scale-up factors: of 20 (human) or 45 (rat)

\[
(3) \quad CL_{\text{hep}} = \frac{\frac{Q_h}{h} \cdot CL \text{ int}}{\frac{Q_h}{h} + CL \text{ int}}
\]

where \( Q_h \) (hepatic blood flow, mL/min/kg) is 21 (human) or 70 (rat).

**LC/MS/MS Bioanalysis of Samples from Plasma Protein Binding and Intrinsic Clearance Assays:**

Samples were analyzed essentially as previously described (Gentry et al., 2013) on a Thermo Electron TSQ Quantum Ultra triple quad mass spectrometer (San Jose, CA) via electrospray ionization (ESI) with two Thermo Electron Accella pumps (San Jose, CA), and a Leap Technologies CTC PAL autosampler (Carrboro, NC). Analytes were separated by gradient elution on a dual column system with two Thermo Hypersil Gold (2.1 x 30 mm, 1.9 µm) columns (San Jose, CA) thermostated at 40°C. HPLC mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. The gradient started at 10% B after a 0.2 min hold and was linearly increased to 95% B over 0.8 min; hold at 95% B for 0.2 min; returned to 10% B in 0.1 min. The total run time was 1.3 min and the HPLC flow rate was 0.8 mL/min. While pump 1 ran the gradient method, pump 2 equilibrated the alternate column isocratically at 10% B. Compound optimization, data collection and processing was performed using Thermo Electron's QuickQuan software (v2.3) and Xcalibur (v2.0.7 SP1).

**In vivo DMPK experimental:**

Plasma pharmacokinetics (PK) of compound (S)-9b (VU0453379) rat were obtained via contract with Frontage Laboratories (Exton, PA). Serial sampling of plasma (10 time points) following a single intravenous administration (1 mg/kg; formulated at 1 mg/mL in 10% EtOH 50% PEG400 40% saline) to male Sprague-Dawley rats (n = 3) was performed in order to obtain PK parameters (non-compartmental analysis). After 24 hr, re-administration of the same dose (and formulation) was performed, and single time point (0.25 hr post-re-administration) samples of plasma and brain were collected in order to assess brain distribution (brain:plasma partition coefficient, \( K_p \)). *In vivo* samples were analyzed via LC/MS/MS utilizing electrospray ionization (ESI) and MRM transition(s) specific to the analyte.

**Animal care and use:**

All animal study procedures were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health regulations of animal care covered in Principles of Laboratory Animal Care (National Institutes of Health).

**Static Islet Experiments**

Murine islets were isolated from 9- to 10-week-old C57BL/6 J male mice as previously described (Brissova et al., 2002). Purified islets were cultured overnight at 37 °C in RPMI-1640 with 5.6 mM glucose. After overnight culture, glucose-stimulated insulin secretion (GSIS) of islets was assessed using a static incubation protocol. Static incubation studies were performed in 12-well plates for 60 minutes at
37°C and 5% CO₂ in RPMI-1640 containing 5.6 mM or 16.7 mM glucose in the presence of 10 nM exendin-4 alone or with 30 μM compound. Islets were matched for size and number. Insulin concentration was determined by radioimmunoassay (Millipore, St. Charles, MO, USA). Insulin secretion was normalized to islet equivalents (IEQ), representing islet volume.

**Behavioral Pharmacology**

**Animals.** Male Sprague-Dawley rats weighing between 275 and 300 grams (Harlan Laboratories, Inc Indianapolis, IN) were used for the behavioral studies and were housed maintained under a 12-h light/dark cycle (lights on at 6 AM, lights off at 6 PM) with free access to food and water. The experimental protocols, which were performed during the light cycle, were approved by the Institutional Animal Care and Use Committee of Vanderbilt University and conformed to the guidelines established by the National Research Council Guide for the Care and Use of Laboratory Animals.

**Catalepsy**
Rats were administered haloperidol (1.5mg/kg, i.p., dissolved in 8.5% lactic acid) 60 minutes prior to vehicle (10% Tween 80), (S)-9b, VU0453379, (10 and 30mg/kg, i.p.) or Neurocrine A2A antagonist (56.6mg/kg, i.p.). After an additional 30 minutes pretreatment interval, all rats were assessed in the catalepsy model. Catalepsy was measured by placing the forepaws of each rat gently onto a horizontal bar placed 6 cm from the testing surface and with the body positioned at an angle of ~45° to the testing surface. The latency in seconds required for the rat to remove one or both forepaws from the bar was measured with a testing cutoff of 30 seconds.

**Spontaneous Locomotor Activity**
Locomotor activity studies were conducted by using a SmartFrame Open Field System (Kinder Scientific, San Diego, CA) equipped with 16 × 16 infrared photobeams located 1 in above the floor of the chamber. Rats were pretreated with vehicle or VU0453379 (10–30 mg/kg i.p.) for 30 min and then placed in the open-field chambers for a 60-min test session. Ambulation was measured as the total number of photobeam breaks per 30-min interval and recorded with a Pentium I computer equipped with Motor Monitor System software (Kinder Scientific). Data were analyzed by one-way ANOVA followed by Dunnett's test using JMP version 11 statistical software (SAS Institute, Cary, NC).

**Compound Experimental and Characterization**

**Scheme 1:**

![Chemical Diagram]

Reaction Conditions: a) HATU, DIEA, DMF; b) 4M HCl in dioxane, DCM; c) NaBH(OAc)₃, DCM
tert-butyl (R,S)-2-((2-cyclopentyl-9-methyl-1-oxo-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamido)methyl)pyrrolidine-1-carboxylate (10). To a vial was added 2-cyclopentyl-9-methyl-1-oxo-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxylic acid 9 (100 mg, 0.32 mmol; ChemDiv), 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (135 mg, 0.35 mmol; Oakwood Products, Inc.; Lot No.: E03J), and N,N-diisopropylethylamine (168 µL, 0.97 mmol), which was then dissolved in N,N-Dimethylformamide (2 mL). This mixture was stirred 30 minutes at room temperature to allow activation of the acid before adding 2-Aminomethyl-1-Boc-pyrrolidine (68 mg, 0.34 mmol). The resultant mixture was stirred an additional 15 hours at room temperature. Upon completion by LC/MS, the reaction mixture was dissolved in 1:1 water/CH₂Cl₂ (10 mL), and extracted with CH₂Cl₂ (10 mL). The combined organic layers were washed once with a 5% LiCl solution (15 mL), passed through a phase separator, and concentrated in vacuo. The brown residue was then purified by automated flash chromatography using 40-70% ethyl acetate in hexanes to elute. Desired product was afforded in 89% yield. ¹H NMR (400 MHz, CDCl₃) δ ppm): 8.46 (s, 1H), 7.99 (s, 1H), 7.47–7.41 (m, 2H), 7.23–7.19 (m, 1H), 5.51–5.47 (m, 1H), 4.34 (s, 3H), 4.15–4.07 (m, 1H), 3.82–3.77 (m, 3H), 3.47–3.34 (m, 3H) 2.25–2.16 (m, 2H), 2.12–2.05 (m, 1H), 1.98-1.74 (m, 10H), 1.36 (s, 9H); ¹³C NMR (100.6 MHz, CDCl₃) δ (ppm): 166.93, 156.73, 141.51, 127.01, 126.79, 125.05, 120.76, 120.31, 113.50 109.73, 80.23, 57.09, 55.71, 47.53, 32.52, 32.48, 31.46, 30.13, 28.38, 24.49, 24.10; HRMS (TOF, ES+) calc’d for C₂₈H₃₇N₄O₄ (M+1), 493.2809; found 493.2810.

(R,S)-2-cyclopentyl-9-methyl-1-oxo-N-(pyrrolidin-2-ylmethyl)-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamide (11). To a round-bottom flask was added at room temperature tert-butyl 2-((2-cyclopentyl-9-methyl-1-oxo-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamido)methyl)pyrrolidine-1-carboxylate (140 mg, 0.28 mmol) dissolved in DCM (8mL). 4M HCl in dioxane (4 mL) was added to this solution while stirring, and the resultant solution was stirred 10 hours at room temperature. At this point a light brown precipitate had formed, and reaction completion was confirmed via LC/MS. Solvent was removed in vacuo to afford the desired product in quantitative yield as the HCl salt.
(R,S)-2-cyclopentyl-N-((1-ethylpyrrolidin-2-yl)methyl)-9-methyl-1-oxo-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamide (8). To a round-bottom flask was added at room temperature (S)-2-cyclopentyl-9-methyl-1-oxo-N-(pyrrolidin-2-ylmethyl)-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamide 11 (100 mg, 0.26 mmol) dissolved in CH$_2$Cl$_2$ (2 mL) and dry acetaldehyde (100 µL). The resultant mixture was stirred at room temperature for 5 minutes before adding sodium triacetoxyborohydride (83 mg, 0.39 mmol), at which point the mixture was stirred an additional 4 hours. Upon completion by LC/MS, the reaction was quenched with sodium bicarbonate (10 mL), and extracted with CH$_2$Cl$_2$. The combined organic layers were dried by passage through a phase separator and concentrated in vacuo. The orange residue was taken up in dimethyl sulfoxide and purified via reverse-phase preparative HPLC using acetonitrile in water with 0.5% NH$_4$OH added to elute. Pure fractions were pooled and concentrated to dryness in vacuo to afford desired product as a foamy yellow solid in 74% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ (ppm): 8.35 (d, $J$= 8 Hz, 1H), 7.54-7.44 (m, 3H), 7.29-7.21 (m, 1H), 6.92 (br, 1H), 5.51-5.47 (m, 1H), 4.34 (s, 3H), 3.86-3.80 (m, 1H), 3.47-3.42 (m, 1H), 3.20-3.16 (m, 1H) 2.79 (br, 1H), 2.33-2.19 (m, 4H), 2.02-1.87 (m, 3H), 1.82-1.69 (m, 7H) 1.09 (t, $J$= 7.2, 3H); $^{13}$C NMR (100.6 MHz, CDCl$_3$) δ (ppm): 167.25, 156.63, 141.41, 126.91, 126.84, 125.30, 124.31, 120.39, 120.16, 113.38, 109.97, 62.85, 55.78, 53.54, 48.43, 40.99, 32.62, 32.59, 31.44, 28.33, 24.66, 22.95, 13.81; HRMS (TOF, ES+) calc’d for C$_{25}$H$_{33}$N$_3$O$_2$ (M+1), 421.2598; found 421.2596.

Scheme 2:

Reaction Conditions: a) HATU, DIEA, DMF; b) 4M HCl in dioxane, DCM; c) NaBH(OAc)$_3$, DCM
tert-butyl \((S)-2-\text{cyclopentyl-9-methyl-1-oxo-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamido)methyl}\)pyrrolidine-1-carboxylate (4). To a vial was added \(2-\text{cyclopentyl-9-methyl-1-oxo-2,9-dihydro-1H-pyrido}[3,4-b]\)indole-4-carboxylic acid \(9\) (300 mg, 0.97 mmol; ChemDiv), \(1-[\text{Bis(dimethylamino)methylene-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate}\) (404 mg, 1.06 mmol; Oakwood Products, Inc.; Lot No.: E03J), and \(N,N\)-diisopropylethylamine (505 µL, 2.90 mmol), which was then dissolved in \(N,N\)-Dimethylformamide (3 mL). This mixture was stirred 30 minutes at room temperature to allow activation of the acid before adding \((S)-2-\text{Aminomethyl-1-Boc-pyrrolidine}\) (203 mg, 1.02 mmol). The resultant mixture was then stirred an additional 15 hours at room temperature. Upon completion by LC/MS, the reaction mixture was dissolved in 1:1 water/\(\text{CH}_2\text{Cl}_2\) (15 mL), and extracted with \(\text{CH}_2\text{Cl}_2\) (15 mL). The combined organic layers were washed once with a 5% LiCl solution (20 mL), passed through a phase separator, and concentrated \textit{in vacuo}. The brown residue was then purified by automated flash chromatography using 40-70% ethyl acetate in hexanes to elute. Desired product was afforded in 82% yield. \(^1\text{H NMR} (400 \text{ MHz, CDCl}_3) \delta \text{ ppm): 8.46} (s, 1H), 7.99 (s, 1H), 7.49-7.41 (m, 2H), 7.23-7.19 (m, 1H), 5.51-5.47 (m, 1H), 4.34 (s, 3H), 4.13-4.07 (m, 1H), 3.81-3.78 (m, 3H), 3.47-3.34 (m, 3H) 2.25-2.16 (m, 2H), 2.12-2.05 (m, 1H), 1.98-1.74 (m, 10H), 1.37 (s, 9H); \(^{13}\text{C NMR} (100.6 \text{ MHz, CDCl}_3) \delta \text{ ppm): 166.94, 156.74, 141.52, 127.02, 126.80, 125.07, 120.76, 120.76, 113.51, 109.75, 80.24, 57.10, 55.72, 47.53, 32.53, 32.49, 31.46, 30.13, 28.39, 24.49, 24.07; \text{HRMS (TOF, ES+)}\) calc’d for \(C_{28}H_{37}N_4O_4\) (M+1), 493.2809; found 493.2811.

\((S)-2-\text{cyclopentyl-9-methyl-1-oxo-N-(pyrrolidin-2-ylmethyl)-2,9-dihydro-1H-pyrido[3,4-b]indole-carboxamide}\) (11). To a round-bottom flask was added at room temperature \(tert\)-butyl \((S)-2-\text{cyclopentyl-9-methyl-1-oxo-2,9-dihydro-1H-pyrido}[3,4-b]\)indole-4-carboxamido)methyl\)pyrrolidine-1-carboxylate \(10\) (390 mg, 0.80 mmol) dissolved in \(\text{DCM}\) (10 mL). 4M HCl in dioxane (5 mL) was added to this solution while stirring, and the resultant solution was stirred 10 hours at room temperature. At this point a light brown precipitate had formed, and reaction completion was confirmed via LC/MS. Solvent was removed \textit{in vacuo} to afford the desired product in quantitative yield as the HCl salt.
(S)-2-cyclopentyl-N-((1-ethylpyrrolidin-2-yl)methyl)-9-methyl-1-oxo-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamide (8). To a round-bottom flask was added at room temperature (S)-2-cyclopentyl-9-methyl-1-oxo-N-(pyrrolidin-2-ylmethyl)-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamide 11 (100 mg, 0.26 mmol) dissolved in CH$_2$Cl$_2$ (2 mL) and dry acetaldehyde (100 µL). The resultant mixture was stirred at room temperature for 5 minutes before adding sodium triacetoxyborohydride (222 mg, 0.39 mmol), at which point the mixture was stirred an additional 4 hours. Upon completion by LC/MS, the reaction was quenched with sodium bicarbonate (10 mL), and extracted with CH$_2$Cl$_2$. The combined organic layers were dried by passage through a phase separator and concentrated in vacuo. The orange residue was taken up in dimethyl sulfoxide and purified via reverse-phase preparative HPLC using acetonitrile in water with 0.5% NH$_4$OH added to elute. Pure fractions were pooled and concentrated to dryness in vacuo to afford desired product as a foamy yellow solid in 76% yield. Specific rotation $[\alpha]_{D}^{23} = -29.178^\circ$ (c = 1.0, MeOH); $^1$H NMR (400 MHz, CDCl$_3$) δ (ppm): 8.34 (d, $J = 8$ Hz, 1H), 7.54-7.43 (m, 3H), 7.28-7.21 (m, 1H), 6.90 (br, 1H), 5.51-5.47 (m, 1H), 4.33 (s, 3H), 3.85-3.79 (m, 1H), 3.46-3.41 (m, 1H), 3.19-3.15 (m, 1H), 2.92-2.88 (m, 1H), 2.79 (br, 1H), 2.32-2.21 (m, 4H), 2.02-1.86 (m, 3H), 1.82-1.68 (m, 7H) 1.08 (t, $J = 7.2$, 3H); $^{13}$C NMR (100.6 MHz, CDCl$_3$) δ (ppm): 167.25, 156.63, 141.42, 126.91, 126.85, 125.3, 124.32, 120.40, 120.16, 113.39, 109.98, 62.84, 55.78, 53.54, 48.42, 41.00, 32.63, 32.60, 31.45, 28.34, 24.66, 22.96, 13.83; HRMS (TOF, ES+) calc’d for C$_{25}$H$_{33}$N$_4$O$_2$ (M+1), 421.2598; found 421.2597.

Scheme 3:

Reaction Conditions: a) HATU, DIEA, DMF; b) 4M HCl in dioxane, DCM; c) NaBH(OAc)$_3$, DCM
(R)-2-cyclopentyl-9-methyl-1-oxo-N-((1-ethylpyrrolidin-2-yl)methyl)-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamide (11). To a round-bottom flask was added at room temperature tert-butyl (R)-2-((2-cyclopentyl-9-methyl-1-oxo-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamido)methyl)pyrrolidine-1-carboxylate 10 (130 mg, 0.26 mmol) dissolved in DCM (8 mL). 4M HCl in dioxane (4 mL) was added to this solution while stirring, and the resultant solution was stirred 10 hours at room temperature. At this point a light brown precipitate had formed, and reaction completion was confirmed via LC/MS. Solvent was removed in vacuo to afford the desired product in quantitative yield as the HCl salt.

(R)-2-cyclopentyl-9-methyl-1-oxo-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamide (11). To a round-bottom flask was added at room temperature tert-butyl (R)-2-((2-cyclopentyl-9-methyl-1-oxo-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamido)methyl)pyrrolidine-1-carboxylate 10 (130 mg, 0.26 mmol) dissolved in DCM (8 mL). 4M HCl in dioxane (4 mL) was added to this solution while stirring, and the resultant solution was stirred 10 hours at room temperature. At this point a light brown precipitate had formed, and reaction completion was confirmed via LC/MS. Solvent was removed in vacuo to afford the desired product in quantitative yield as the HCl salt.
9-methyl-1-oxo-N-(pyrrolidin-2-ylmethyl)-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamide 11 (100 mg, 0.26 mmol) dissolved in CH₂Cl₂ (2 mL) and dry acetaldehyde (100 µL). The resultant mixture was stirred at room temperature for 5 minutes before adding sodium triacetoxyborohydride (83 mg, 0.39 mmol), at which point the mixture was stirred an additional 4 hours. Upon completion by LC/MS, the reaction was quenched with sodium bicarbonate (10 mL), and extracted with CH₂Cl₂. The combined organic layers were dried by passage through a phase separator and concentrated in vacuo. The orange residue was taken up in dimethyl sulfoxide and purified via reverse-phase preparative HPLC using acetonitrile in water with 0.5% NH₄OH added to elute. Pure fractions were pooled and concentrated to dryness in vacuo to afford desired product as a foamy yellow solid in 81% yield. Specific rotation [α]²³ёт = +28.77° (c = 1.0, MeOH); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.33 (d, J= 8 Hz, 1H), 7.52-7.43 (m, 3H), 7.24-7.20 (m, 1H), 6.89 (br, 1H), 5.50-5.46 (m, 1H), 4.32 (s, 3H), 3.83-3.79 (m, 1H), 3.43-3.39 (m, 1H), 3.15-3.13 (m, 1H), 2.92-2.87 (m, 1H), 2.76 (br, 1H), 2.31-2.19 (m, 4H), 2.98-1.91 (m, 3H), 1.82-1.69 (m, 7H) 1.07 (t, J= 7.2, 3H); ¹³C NMR (100.6 MHz, CDCl₃) δ (ppm) 167.22, 156.58, 141.37, 126.85, 126.81, 125.22, 124.26, 120.37, 120.12, 113.39, 109.94, 62.72, 55.75, 53.51, 48.33, 41.03, 32.60, 32.57, 31.40, 28.33, 24.63, 22.91, 13.87; HRMS (TOF, ES+) calc’d for C₂₅H₃₃N₄O₂ (M+1), 421.2598; found 421.2599.

(S)-2-cyclopentyl-N-((1-isopropylpyrrolidin-2-yl)methyl)-9-methyl-1-oxo-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamide (9b). To a round-bottom flask was added at room temperature (S)-2-cyclopentyl-9-methyl-1-oxo-N-(pyrrolidin-2-ylmethyl)-2,9-dihydro-1H-pyrido[3,4-b]indole-4-carboxamide 11 (200 mg, 0.51 mmol) dissolved in CH₂Cl₂ (4 mL) and dry acetone (100 µL). The resultant mixture was stirred at room temperature for 5 minutes before adding sodium triacetoxyborohydride (150 mg, 0.71 mmol), at which point the mixture was stirred an additional 4 hours. Upon completion by LC/MS, the reaction was quenched with sodium bicarbonate (5 mL), and extracted with CH₂Cl₂. The combined organic layers were dried by passage through a phase separator and concentrated in vacuo. The orange residue was taken up in dimethyl sulfoxide and purified via reverse-phase preparative HPLC using acetonitrile in water with 0.5% NH₄OH added to elute. Pure fractions were pooled and concentrated to dryness in vacuo to afford desired product as a foamy yellow solid in 73% yield. Specific rotation [α]²³ёт = -20.6° (c = 1.0, MeOH); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.32 (d, J= 8.2 Hz, 1H), 7.49-7.45 (m, 2H), 7.39-7.37 (m, 1H), 7.24-7.20 (m, 1H), 7.08 (m, 1H), 5.47-5.43 (m, 1H), 4.24 (s, 3H), 3.74-3.68 (m, 1H), 3.38-3.32 (m, 1H), 3.12-3.07 (m, 1H), 3.02-2.95 (m, 1H), 2.94-2.89 (m, 1H), 2.57-2.51 (m, 1H), 2.25-2.22 (m, 2H), 1.96-1.81 (m, 3H) 1.81-1.67 (m, 7H), 1.12 (d, J= 6.6 Hz, 3H), 1.02 (d, J= 6.4 Hz, 3H); ¹³C NMR (100.6 MHz, CDCl₃) δ (ppm): 166.93, 156.23, 141.05, 126.54, 126.47, 124.77, 124.06, 120.09, 119.92, 113.34, 109.64, 58.17, 55.52, 49.70, 47.36, 42.47, 32.35, 32.32, 31.05,
29.06, 24.41, 23.48, 22.23, 16.24; HRMS (TOF, ES+) calc’d for C_{26}H_{35}N_{4}O_{2} (M+1), 435.5915; found 435.5917.

**Eurofins Lead Profiling Data**

A radioligand binding panel of 68 targets (GPCRs, ion channels, transporters, and nuclear hormones) with data reported as % inhibition of radioligand binding at a 10 µM concentration of (S)-9b (VU0453379) from two independent determinations.

**Supplemental Table 1: Eurofins Profiling of (S)-9b (VU0453379).**

| Target/Protein                  | Species | % Inhibition |
|--------------------------------|---------|--------------|
| Adenosine A<sub>1</sub>        | Human   | -1           |
| Adenosine A<sub>2A</sub>       | Human   | 6            |
| Adenosine A<sub>3</sub>        | Human   | 14           |
| Adrenergic α<sub>1A</sub>      | Rat     | 16           |
| Adrenergic α<sub>1B</sub>      | Rat     | -10          |
| Adrenergic α<sub>1D</sub>      | Human   | 9            |
| Adrenergic α<sub>2A</sub>      | Human   | 37           |
| Adrenergic β<sub>1</sub>       | Human   | 24           |
| Adrenergic β<sub>2</sub>       | Human   | -8           |
| Androgen (Testosterone) AR     | Rat     | 11           |
| Bradykinin B<sub>1</sub>       | Human   | 12           |
| Bradykinin B<sub>2</sub>       | Human   | 11           |
| Calcium Channel L-Type, Benzothiazepine | Rat | 41       |
| Calcium Channel L-Type, Dihydropyridine | Rat | 22       |
| Calcium Channel N-Type         | Rat     | 2            |
| Cannabinoid CB<sub>1</sub>     | Human   | 48           |
| Dopamine D<sub>1</sub>         | Human   | 5            |
| Dopamine D<sub>2S</sub>        | Human   | 8            |
| Dopamine D<sub>3</sub>         | Human   | 26           |
| Dopamine D<sub>4,2</sub>       | Human   | -2           |
| Endothelin ET<sub>A</sub>      | Human   | 11           |
| Endothelin ET<sub>B</sub>      | Human   | 3            |
| Epidermal Growth Factor (EGF)  | Human   | 11           |
| Estrogen ERα                   | Human   | 15           |
| GABA<sub>A</sub>, Flunitrazepam, Central | Rat | 12       |
| GABA<sub>A</sub>, Muscimol, Central | Rat | -1       |
| GABA<sub>B1A</sub>            | Human   | -2           |
| Glucocorticoid                 | Human   | 1            |
| Glutamate, Kainate             | Rat     | -6           |
| Glutamate, NMDA, Agonism       | Rat     | 1            |
| Glutamate, NMDA, Glycine       | Rat     | -2           |
| Substance                                | Species | Value |
|-----------------------------------------|---------|-------|
| Glutamate, NMDA, Phencyclidine          | Rat     | 18    |
| Histamine H₁                             | Human   | 22    |
| Histamine H₂                             | Human   | 25    |
| Histamine H₃                             | Human   | 26    |
| Imidazoline I₂, Central                  | Rat     | 15    |
| Interleukin IL-1                         | Mouse   | -1    |
| Leukotriene, Cysteiny CysLT₁            | Human   | 22    |
| Melatonin MT₁                           | Human   | 37    |
| Muscarinic M₁                           | Human   | -1    |
| Muscarinic M₂                           | Human   | 1     |
| Muscarinic M₃                           | Human   | 12    |
| Neuropeptide Y Y₁                       | Human   | 5     |
| Neuropeptide Y Y₂                       | Human   | 3     |
| Nicotinic Acetylcholine                  | Human   | 8     |
| Nicotinic Acetylcholine α₁, Bungarotoxin| Human   | 1     |
| Opiate δ₁ (OP1, DOP)                     | Human   | 6     |
| Opiate κ (OP2, KOP)                      | Human   | 5     |
| Opiate μ (OP3, MOP)                      | Human   | 5     |
| Phorbol Ester                           | Mouse   | 2     |
| Platelet Activating Factor (PAF)        | Human   | 7     |
| Potassium Channel [K_ATP]               | Human   | 5     |
| Potassium Channel hERG                  | Human   | 39    |
| Prostanoid EP₄                           | Human   | 30    |
| Purinergic P2X                           | Rabbit  | 28    |
| Purinergic P2Y                           | Rat     | 4     |
| Rolipram                                 | Rat     | 4     |
| Serotonin (5-HT₁ₐ)                      | Human   | 3     |
| Serotonin (5-HT₂ₙ)                      | Human   | 41    |
| Serotonin (5-HT₃₁)                      | Human   | -4    |
| Sigma σ₁                                | Human   | 47    |
| Sodium Channel, Site 2                  | Rat     | 49    |
| Tachykinin NK₁                          | Human   | 23    |
| Thyroid Hormone                         | Rat     | 7     |
| Transporter, Dopamine (DAT)             | Human   | 42    |
| Transporter, GABA                        | Rat     | -2    |
| Transporter, Norepinephrine (NET)       | Human   | 28    |
| Transporter, Serotonin (SERT)           | Human   | 22    |
Supplemental Figure 1: Effect of (S)-9b, VU0453379, on spontaneous locomotor activity in male Sprague Dawley rats. No sedation was observed, supporting the efficacy noted in the HIC assay. Vehicles was 10% Tween 80.