Preventing Morphine-Seeking Behavior through the Re-Engineering of Vincamine’s Biological Activity

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ABSTRACT: Innovative discovery strategies are essential to address the ongoing opioid epidemic in the United States. Misuse of prescription and illegal opioids (e.g., morphine, heroin) has led to major problems with addiction and overdose. We used vincamine, an indole alkaloid, as a synthetic starting point for dramatic structural alterations of its complex, fused ring system to synthesize 80 diverse compounds with intricate molecular architectures. A select series of vincamine-derived compounds were screened for both agonistic and antagonistic activities against a panel of 168 G protein-coupled receptor (GPCR) drug targets. Although vincamine was without an effect, the novel compound 4 (V2a) demonstrated antagonistic activities against hypocretin (orexin) receptor 2. When advanced to animal studies, 4 (V2a) significantly prevented acute morphine-conditioned place preference (CPP) and stress-induced reinstatement of extinguished morphine-CPP in mouse models of opioid reward and relapse. These results demonstrate that the ring distortion of vincamine offers a promising way to explore new chemical space of relevance to opioid addiction.

INTRODUCTION

An invigorated effort to improve pain management was implemented across the United States (U.S.) in the mid-1990s.1 Hospitals and inpatient settings began to include “pain scores” as a quality measure for patients. Many clinicians responded to patients’ elevated pain scores by prescribing opioid medications more frequently. Initially, opioid therapies were not believed to be highly addictive; however, we now know that there are significant ramifications associated with opioid misuse as the U.S. is amidst a health-care crisis resulting from opioid addiction and death-related overdoses.1−4

The opioid epidemic has produced devastating public health, social, and economic consequences.1−3 Several legal and illegal opioids have played a critical role in the growing crisis and can be categorized as either prescription pain relievers (morphine, codeine, oxycodone), synthetic opioids (fentanyl), or heroin. In 2017, more than 130 people died every day in the U.S. from overdosing on opioids.5 According to the National Survey on Drug Use and Health, nearly 12 million individuals in the U.S. misused opioids in 2015.6 It is also reported that nearly 80% of heroin users initially misused prescription opioids.7 The Center for Disease Control and Prevention estimates the economic burden of prescription misuse alone to be $78.5 billion each year.2 In response to the unprecedented need to treat opioid addiction, there is an increased emphasis to discover new and innovative therapeutic agents for opioid use disorders.

Several natural products have been shown to be excellent starting points for generating diverse and complex compound libraries, which are of importance to those involved in drug discovery.8−18 Our group is interested in diverse indole-based compounds,14,19 as many are known to bind and modulate an array of therapeutically relevant biological targets, from complex natural products (e.g., vincristine binds microtubules for cancer treatments) to structurally simple synthetic indoles (e.g., ondansetron binds serotonin receptors for nausea therapies).19,20 Therefore, we hypothesized that a unique library composed of architecturally diverse indole-based compounds could lead to promising discoveries of relevance to opioid addiction.

Vincamine (1) is an indole alkaloid found in the leaves of Vinca minor and is available on an affordable decagram scale (∼$36/g). This indole alkaloid is a prescription medicine (Oxybral SR) used to improve cerebral blood flow and cognitive function. Despite vincamine’s interesting biological activity profile, it has no known activities against targets.
relevant to opioid addiction; however, we aimed to use vincamine as a starting point to rapidly generate complex and diverse indole-based scaffolds to explore new chemical space through biological screens against a diverse panel of drug targets. The overarching goal of this work is to re-engineer the biological activity of vincamine through dramatic alterations of its inherently complex molecular architecture and identify promising new lead compounds for opioid abuse.

A diverse library of stereochemically complex compounds, with scaffolds related to 2–9 (Figure 1), was rapidly generated in one to six synthetic steps from vincamine using an orchestrated series of chemoselective ring cleavage, ring rearrangement, and ring fusion reactions. Using this ring distortion approach, we synthesized an intricate and diverse library of 80 indole-based small molecules featuring unique and complex molecular architectures (Supporting Information Figures 1 and 2). We evaluated a subset of the library against a panel of 168 G protein-coupled receptor (GPCR) drug targets, several of which demonstrated activity profiles distinct from vincamine and other complex, indole-based small molecules synthesized during these investigations.

RESULTS AND DISCUSSION

Ring Distortion of Vincamine To Rapidly Generate Diverse, Stereochemically Complex Indole-Based Compounds. Following an initial query of the literature, we identified two ring cleavage transformations from vincamine that were useful during our ring distortion efforts.21,22 We developed a modified version of the first ring cleavage21 to afford 12, which is a three-step synthesis from vincamine using (1) lithium aluminum hydride, (2) sodium periodate, and then (3) potassium hydroxide (91% yield/three steps; ≥3.5 g for each step; Scheme 1). The second ring cleavage reaction from vincamine utilizes methyl propiolate in methanol to afford structures similar to 17 as a single diastereomer.22 In this reaction, the tertiary amine of vincamine undergoes a conjugate addition into methyl propiolate, in turn, activating the C–N bond adjacent to the indole heterocycle for ring cleavage. We used various alcohol solvents as nucleophiles to attack the carbon adjacent to the 2-position of the indole heterocycle, which occurs with complete inversion of stereochemistry. We found sterically encumbered alcohol nucleophiles to induce a stereoinversion of the α-hydroxyester center in vincamine during this reaction. Presumably, stereoinversion occurs through a ring-opening and -closing pathway to furnish 18 and related analogues. An interesting feature of 18 is the hydrogen-bonding interaction between the inverted hydroxyl and resultant isopropyl ether oxygen atom that can be seen in the X-ray structure of 18 (Scheme 1). We believe that this stereoinversion is favored and occurs to avoid steric

Figure 1. Rapid syntheses of diverse and stereochemically complex molecules from the indole alkaloid vincamine (1).
interactions between bulky ether groups and the methyl ester that would be on the same face in the resulting products. In addition, this ring cleavage reaction is applicable to several vincamine-derived substrates.

In addition, cyanogen bromide (CNBr) was utilized for regioselective C–N ring cleavage reactions, similar to our previous studies with yohimbine. Compound 16 was treated with cyanogen bromide and 2-iodobenzyl alcohol to afford indole-promoted C–N ring cleavage product 2 in a 40% yield as a single diastereomer. Alternatively, a von Braun reaction that occurs through a second-order nucleophilic substitution (SN2) pathway can be employed utilizing cyanogen bromide in N,N-dimethylformamide (DMF) under microwave conditions to yield alternative ring cleavage scaffold 10 (45% yield, two steps), which was elaborated into a series of amide analogues 11.
During this study, we found 12 to be an excellent starting point for innovative and provocative ring distortion reactions. One of our synthetic goals was to carry out the selective oxidation of the indole nucleus upon treatment with \textit{meta}-chloroperbenzoic acid (\textit{m}-CPBA) to afford C-3 hydroxylated products (e.g., 6). The hydroxylation of indoles at C-3 provides a synthetic opportunity to access interesting ring rearrangement scaffolds from vincamine following a base-promoted 1,2-alkyl shift to a spiro-3-oxindoles. During these studies, 16 was stereoselectively hydroxylated with \textit{m}-CPBA and trifluoroacetic acid (TFA; Scheme 1) in methylene chloride to afford 6 as a single diastereomer in a 34\% yield (axially protonated amine of 16 hydrogen bond directs \textit{m}-CPBA; \textit{β}-hydroxylation affords 6; see X-ray). Subsequent exposure of 6 to sodium hydroxide induced a semipinacol rearrangement to furnish spiroindoxyl 9 in an 82\% yield.

The carboxylic acid of 12 readily undergoes amidation reactions using ethyl chloroformate, or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) with catalytic 4-dimethylaminopyridine (DMAP), followed by the addition of diverse amines to afford 13 (18 amide derivatives, 65\% average yield; Scheme 1). In contrast to the hydroxylation of the indole nucleus (e.g., conversion of 16 to 6), we discovered that amide analogues (13) undergo an intriguing oxidative ring fusion reaction upon treatment with \textit{m}-CPBA and TFA (Schemes 1 and 2). Surprisingly, no C-3 hydroxylated products were observed upon treatment of amides 13 with \textit{m}-CPBA and TFA; however, this transformation was robust and afforded 13 unique ring-fused analogues of 15 in a 31–56\% yield (42\% average yield). Rationalizing the reaction mechanism for the oxidative ring fusion was an initial challenge. However, we hypothesized that \textit{m}-CPBA initially reacts with the indole of 13 to give the expected 3-hydroxyindolenine intermediate. Then, we proposed that TFA (17 equiv) under refluxing conditions leads to a subsequent elimination (dehydration), resulting in intermediate 14, which undergoes a final 5-exo-trig cyclization between the amide nitrogen (nucleophile) and the resonance-stabilized carbocation adjacent to the C-2 position of the indole heterocycle, rationally leading to the observed ring-fused scaffolds (15).

To test our hypothesis regarding the mechanism of the oxidative ring fusion reaction (conversion of 13 to 15), we utilized the hypervalent iodine reagent \textit{[bis(trifluoroacetoxy)-iodo]benzene} (PIFA) and water as a milder oxidizing system to isolate desired C-3 hydroxylated 19 (Scheme 2) in a 26\% yield. 19 is an important intermediate regarding our proposed...
reaction mechanism that leads to the formation of ring fusion analogues (15). Upon exposure of 19 to TFA (without m-
CPBA) in refluxing methylene chloride, we were delighted to
see the rapid formation of ring fusion compound 24 in a 74%
yield in 2.5 h (Scheme 2; the X-ray of analogue 25 confirms C–
N bond formation upon ring closure). These findings support
our proposed mechanism regarding the conversion of amides
(13) to novel ring fusion analogues (15).

Biological Evaluation against a Diverse Panel of
GPCR Drug Targets. Following synthesis, seven unique
vincamine-derived compounds were selected to represent a
maximal chemical diversity from the synthesized library
(analogues 3–9; one from each of the distinct scaffold types
from vincamine; chemoinformatic analyses of vincamine-
derived analogues can be seen in the Supporting Information
Figure 6) and screened against a panel of 168 G protein-
coupled receptor (GPCR) drug targets in cell-based assays
(Figure 2; Supporting Information Figures 3 and 4).23 Each
compound was screened at 20 μM for agonist and antagonist
activities against the GPCR panel, which contained targets
relevant to opioid addiction (e.g., opioid receptors OPRM1,
OPRD1, OPRK1, OPRL1; cannabinoid receptor 1 CNR126
relevant to opioid addiction (e.g., opioid receptors OPRM1,
activities against the GPCR panel, which contained targets
occurring by 7 weeks (left bars). Forced swimming (right bars) reinstated place preference in vehicle or vincamine-treated mice.

Figure 3. Results of 4 (V2a) and vincamine in morphine-CPP studies in mouse models of opioid reward and relapse. (A) 4 pretreatment prevents morphine-conditioned place preference (CPP) in mice. In place conditioning, mice exhibited significant morphine-CPP (left bars), but 4 (60 nmol, i.c.v.) did not significantly differ from initial preferences (center bars). 4, but not vincamine, pretreatment 20 min prior to morphine significantly prevented morphine-CPP. n = 16–30 mice/group. * p < 0.05 vs preconditioning response; † p < 0.05 vs morphine-CPP, Tukey post hoc test. (B) Stress-induced reinstatement of morphine-CPP in mice prevented by 4 pretreatment. Mice exhibited significant morphine-CPP, with extinction occurring by 7 weeks (left bars). Forced swimming (right bars) reinstated place preference in vehicle or vincamine-treated mice. 4 pretreatment prevented stress-induced reinstatement. n = 16–21 mice/group; 58 mice total. * p < 0.05 vs preconditioning response (leftmost bar); † p < 0.05 vs post CPP (second bar on left); ‡ p < 0.05 vs stress-induced reinstatement (striped red bar, center), Tukey’s post hoc test.

rate), and 8 (one hit, 0.6% hit rate). As a comparator in this
screen, yohimbine (26; indole alkaloid) was shown to
antagonize seven GPCRs (4.2% hit rate) that include
adrenergic, dopamine, and serotonin receptors, which has
previously been reported in the literature.23

The “gain-of-function” GPCR antagonistic activities regarding
3–5, 7, and 8 compared to vincamine were the initial proof-
of-concept result that our ring distortion approach could lead
to molecules with re-engineered biological activities (based on
a >70% differential antagonistic activity in screen comparing
vincamine to ring distortion analogues). Concentration-
response experiments were subsequently performed with select
compounds (4, 5, 7) for antagonistic activities against specific
GPCRs. These experiments allowed for the determination of
potency for analogues 4, 5, and 7 against relevant GPCRs,
resulting in several IC₅₀ values in the low micromolar range.

Following initial screens, we turned our attention to
analogue 4 (V2a), which demonstrates antagonistic activity
against hypocretin (orexin) receptor 2 (HCRT2); IC₅₀ = 2.26
μM; Figure 2). We were encouraged by initial results, as
hypocretin receptor 2 antagonism recently demonstrated a
dose-dependent reduction of heroin self-administration in
rats.27 In addition, analogue 4 demonstrated a good selectivity
profile for targeting HCRT2 in the GPCR panel, including no
significant modulatory activities (<20%) of opioid receptors at
20 μM.

Stability concerns regarding the potential acid-labile N,N’-
carbon (sp²) center adjacent to the 2-position of the indole
nucleus of 4 were probed experimentally. Encouragingly, we
found that 4 could be readily interconverted to the
respective HCl salt and free base of 4, including NMR
spectra). In addition, analogue 4 proved to have robust acid
stability following 24 h exposure to acidic media at 37 °C
(92.1% recovery in pH 2.0; 99.2% recovery in pH 4.88;
Supporting Information). With the selective HCRT2
antagonistic activity and chemical stability results in hand, we
were curious to know if 4 could attenuate opioid-seeking in mouse models using behavioral pharmacological approaches.

**Animal Studies To Determine the Efficacy of 4 (V2a) in Morphine-Seeking Mouse Models.** We evaluated 4 (V2a) to determine the potential for this novel compound to prevent acute morphine-conditioned place preference (CPP) and stress-induced reinstatement of extinguished morphine-CPP in mice. A major problem in treating individuals with opioid addiction is the occurrence of relapse, which is often caused by a stressful event. For humans, examples of stressful life events that could lead to relapse (reinstatement) include the loss of employment or a relationship; however, in reinstatement experiments in the lab, mice are subjected to a forced swim test (FST) that triggers a stress response that leads to subsequent morphine-seeking behaviors.

In counterbalanced conditioned place preference experiments in mice, intracerebroventricular (i.c.v.) treatment of 4 was without a significant effect alone, but a 20 min pretreatment prior to conditioning prevented acute morphine-CPP [two-way analysis of variance (ANOVA), $F_{(3,170)} = 4.65, p = 0.004$; Figure 3A]. A similar pretreatment with the parent vincamine was without a significant effect on morphine-CPP (final preference, $219 \pm 74 s, p = 0.998$ vs morphine-CPP, Tukey’s post hoc test), further suggesting that the effects of 4’s in vivo efficacy are due to HCRTR2 antagonism. Pretreatment with 4 also significantly prevented stress-induced reinstatement of extinguished morphine-seeking behavior (one-way ANOVA, $F_{(6,24)} = 9.76, p < 0.0001$; Figure 3B) compared to that of a vehicle control in mice subjected to a forced swim test, whereas vincamine did not. Combined, this data clearly indicates that ring distortion can be used to re-engineer vincamine’s biological activity and 4 holds promise for further exploration as a potential treatment for opioid abuse.

**Molecular Modeling of 4 (V2a) in HCRTR2.** We used molecular modeling to determine a binding mode of 4 (V2a) in the antagonistic binding site of HCRTR2. Our goal was to establish a rational design pathway for future development efforts. Initially, we docked the HCRTR2 antagonists suvorexant and EMPA to their respective binding conformations (suvorexant to PDB ID 4S0V, EMPA to PDB ID 5WQC). The redocking of these antagonists using Glide XP and AutoDock4 generated binding poses close to crystallographic binding modes with both root-mean-square deviation (RMSD) less than 1.0 Å, whereas redocking using Glide SP could not find the crystallographic binding modes with both RMSD larger than 7.0 Å. Therefore, we found our customized docking protocols with Glide XP and AutoDock4 to be better than Glide SP scoring, which was reasonable as the binding site of HCRTR2 is relatively closed with a deep binding cleft. When compound 4 was docked to both modeled conformations, we found the 4S0V conformation to be significantly better than 5WQC, since using a 1.5 Å RMSD threshold, 4S0V, yields an 11% conformational clustering with $-10.2 \text{ kcal/mol}$ binding energy whereas 5WQC yields a 4% conformational clustering with $-9.1 \text{ kcal/mol}$. This result made sense, as suvorexant has structural similarities to 4.

The binding mode of 4 (V2a) to HCRTR2 matches that of suvorexant at several corresponding functional groups/fragments, which include (1) the indole group of 4 to the benzoxazole group of suvorexant, (2) azacyclohexyl to diazepam, and (3) triazole to triazole (see the Supporting Information Figure S). A major difference is that the terminal benzyl group of 4 binds deeply into an extra subpocket of HCRTR2 (binding model seen in Figure 4). The indole of 4 binds at a major hydrophobic region (major residues of Pro131, Cys107, and Val138, not shown for clarity). The triazole of 4 has an aromatic interaction with Tyr317, the same for the terminal benzene with Phe227. Collectively, our data suggests that 4 is an ideal template to develop improved HCRTR2 antagonists using molecular modeling to rationally design new agents to treat opioid abuse.

**CONCLUSIONS**

In conclusion, we have synthesized a diverse collection of complex compounds from vincamine bearing distinct molecular architectures. During these investigations, we discovered a novel oxidative ring fusion reaction that led to the discovery of 4 (V2a), which demonstrates HCRTR2 antagonistic activities. As HCRTR2 was recently reported to be relevant in heroin addiction, 4 was advanced to animal studies and demonstrated in vivo efficacy in acute and reinstatement mouse models of morphine reward. In addition, we established a model for 4 binding to HCRTR2 using molecular docking, which will be utilized to direct future analogue design and...
synthesis. These findings further support the therapeutic potential of HCRTR2 antagonists to treat opioid abuse. Finally, this work demonstrates the significant potential ring distortion has in re-engineering the biological activity of natural products, such as vincamine, for new applications of critical importance to human health.

**EXPERIMENTAL SECTION**

**General Information.** The purities of all compounds evaluated in biological assays were confirmed to be ≥95% by liquid chromatography–mass spectrometry (LC–MS) using a Shimadzu Prominence high-performance liquid chromatography (HPLC) system, an AB Sciex 3200 QTRAP spectrometer, and a Kinetics EVO C18 column (50 mm × 2.1 mm × 2.6 μm) with a 30 min linear gradient from 30 to 95% acetonitrile in 5 mM ammonium bicarbonate at a flow rate of 0.25 mL/min. All GPCR screens and concentration-dependent experiments were conducted at DiscoverX (https://www.discoverx.com/arrestin). All animal experiments were carried out at the University of Florida and authorized by the Institutional Animal Care and Use Committee (IACUC) under protocol 201910772. The University of Florida’s accreditation number for animal welfare work is A3377-01. The University of Florida also holds continuous accreditation from AAALAC since 1960. Anhydrous solvents were transferred via syringe to flame-dried glassware, which was cooled under a stream of dry argon. All microwave reactions were carried out in sealed tubes in an Anton Paar Monowave 300 microwave synthesis reactor. Constant power was applied to ensure reproducibility. Temperature control was automated via an IR sensor, and all indicated temperatures correspond to the maximal temperature reached during each experiment. General Information. Crude products, such as vincamine, for new applications of critical importance to human health.

**Synthesis Procedure for the Generation of 2-(1S,12bS)-1-Ethyl-2H,2,3H,4H,6H,7H,12H,12bH-indolo[2,3-alquinolinizin-1-Yl]-N-cyclopentylacetamide.** Compound 12 (138 mg, 0.441 mmol) was added to a flame-dried round-bottom flask and dissolved in anhydrous dichloromethane (4.5 mL). The reaction was cooled to 0 °C, and triethylamine (89 μL, 0.582 mmol) was added dropwise. After stirring for 5 min, ethyl chloroformate (47 μL, 0.485 mmol) was added, and the reaction continued to stir for an additional 30 min. Cyclpentylamine (0.435 mL, 4.41 mmol; 0.3 M in dichloromethane) and 4-dimethylaminopyridine (DMAP, one crystal) in anhydrous dichloromethane were then added to the reaction mixture, which was allowed to slowly warm to room temperature. The reaction was continued to stir for 3 h and, upon completion (monitored by thin-layer chromatography, TLC), quenched with brine, extracted with dichloromethane, and the organic layer was dried with sodium sulfate. The organic layer was then filtered, concentrated in vacuo, and the crude material was purified via column chromatography 100% hexanes to 3:2 hexanes/ethyl acetate to afford 2 (130 mg, 40%) as a yellow-brown residue. 1H NMR: (500 MHz, CDCl₃, δ 10.07 (s, 1H), 7.80 (d, δ J = 7.9 Hz, 1H), 7.51 (d, δ J = 7.9 Hz, 1H), 7.48 (d, δ J = 8.2 Hz, 1H), 7.30 (d, δ J = 8.2, 7.5 Hz, 1H), 7.26–7.18 (m, 2H), 7.13 (d, δ J = 8.3, 7.5 Hz, 1H), 6.96 (d, δ J = 7.6, 1.8 Hz, 1H), 4.54 (s, 1H), 4.24 (d, δ J = 11.6 Hz, 1H), 4.13 (d, δ J = 11.6 Hz, 1H), 3.70 (m, 1H), 3.58 (s, 3H), 3.21 (d, δ J = 15.4, 3.3 Hz, 1H), 3.14 (d, δ J = 14.2, 4.9 Hz, 1H), 3.01–2.92 (m, 2H), 2.45 (d, δ J = 13.3 Hz, 1H), 2.37 (m, 1H), 2.31 (d, δ J = 13.4 Hz, 1H), 1.65–1.40 (m, 4H), 1.31 (m, 1H), 0.99 (t, δ J = 7.5 Hz, 3H). 13C NMR: (100 MHz, CDCl₃, δ 174.7, 140.3, 139.6, 136.4, 132.9, 130.4, 129.7, 128.2, 127.4, 122.8, 119.7, 119.6, 118.4, 112.2, 111.9, 99.3, 78.8, 75.2, 53.6, 53.0, 51.8, 46.0, 38.3, 30.7, 26.9, 26.2, 23.7, 8.2. High-resolution mass spectrometry (HRMS) [electrospray ionization (ESI)]: calc'd for C₂₈H₃₂IN₃O₃Na [M + Na]⁺: 432.2258, found: 432.2261. [α]_D^20 = −25° (c 0.10 g/100 mL, MeOH). Note: the stereochecmy of 2 was determined using one-dimensional (1-D) nuclear Overhauser enhancement spectroscopy (NOESY) (see NMR spectrum; Supporting Information).
additional 14 h. Upon completion (by TLC), the reaction was quenched with brine, extracted with dichloromethane, and the organic layer was dried with sodium sulfate. The organic layer was filtered, concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 24:5:74:5:1 hexanes/dichloromethane/triethylamine. Following the column, the compound was dissolved in chloroform and washed with deionized water to afford 4 (210 mg, 49%) as a yellow solid. H NMR: (500 MHz, CDCl3) δ 9.13 (s, 1H), 7.40 (d, J = 8.9 Hz, 2H), 7.21–7.14 (m, 2H), 7.10–7.04 (m, 3H), 6.78 (s, 1H), 6.73 (d, J = 6.7 Hz, 2H), 4.68 (d, J = 14.7 Hz, 1H), 4.55 (d, J = 15.1 Hz, 1H), 4.09 (d, J = 15.1 Hz, 1H), 4.05 (d, J = 14.9 Hz, 1H), 3.36 (dt, J = 12.0, 6.3 Hz, 1H), 3.27 (dt, J = 11.8, 4.8 Hz, 1H), 3.02–2.88 (m, 3H), 2.70–2.58 (m, 2H), 2.24 (d, J = 16.9 Hz, 1H), 2.03 (dt, J = 13.8, 4.0 Hz, 1H), 1.77 (m, 1H), 1.57 (sextet, J = 7.4 Hz, 1H), 1.44 (td, J = 13.1, 3.7 Hz, 1H), 1.37–1.20 (m, 2H), 0.80 (t, J = 7.4 Hz, 3H). 13C NMR: (100 MHz, CDCl3) δ 173.7, 143.1, 137.2, 134.7, 133.0, 128.0, 128.6, 127.9, 127.6, 122.4, 121.6, 119.4, 118.7, 114.0, 111.9, 82.7, 52.9, 50.2, 49.8, 44.7, 43.6, 35.3, 32.8, 25.9, 21.8, 17.8, 9.6. HRMS (ESI): calcd for C21H18NO4 [M + H]+: 341.1257, found: 341.1253.

Synthesis Procedure for the Generation of 5, (115,19R)-15-(3-Bromobenzoyl)-11-ethyl-19-methoxy-8,15-diazatetracyclo[9.6.2.015.18]nonadeca-1(18),2,4,6-tetraen-9-one. The resulting organic layer was then concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 82:16:5:1 hexanes/ethyl acetate/triethylamine to afford 5 (294 mg, 34%) as a white crystalline solid. 1H NMR: (400 MHz, CDCl3) δ 7.49 (d, J = 7.6 Hz, 1H), 7.41 (d, J = 7.0 Hz, 1H), 7.31 (dd, J = 8.2, 7.3 Hz, 1H), 7.18 (dd, J = 8.2, 7.3 Hz, 1H), 4.48 (m, 1H), 3.55 (s, 3H), 3.12 (s, 1H), 3.06–2.97 (m, 2H), 2.88 (d, J = 13.5 Hz, 1H), 2.62 (dd, J = 14.9, 9.8, 6.4 Hz, 1H), 2.39 (d, J = 13.5 Hz, 1H), 2.27 (dd, J = 12.2, 7.8, 6.4 Hz, 1H), 2.10–1.95 (m, 1H), 1.92 (m, 1H), 1.74 (sextet, J = 7.5 Hz, 1H), 1.63 (m, 1H), 1.60–1.42 (m, 2H), 1.05 (t, J = 7.5 Hz, 3H). Note: 1H spectrum referenced TMS at 0.00 ppm. 13C NMR: (100 MHz, CDCl3) δ 183.8, 173.6, 154.1, 139.9, 129.3, 126.2, 121.0, 82.5, 71.5, 55.6, 51.5, 50.8, 40.7, 38.9, 32.2, 31.3, 29.7, 21.4, 8.1. HRMS (ESI): calcd for C18H16N2O2 [M + H]+: 334.0846, found: 334.0846.

General Amidation Procedure for the Synthesis of 7, (Methyl (4aS,12bS)-12-[2-[(4-Bromophenyl)formamido]ethyl]-1-cyano-4a-ethyl-1H,2H,3H,4H,4aH,12H-indolo[1,2,3-cd]naphthalidine-6-carboxylate. Compound 8 (33.5 mg, 0.089 mmol) was added to a flame-dried round-bottom flask and dissolved in dichloromethane (2.0 mL). The solution was then cooled to 0°C before triethylamine (25.0 μL, 0.177 mmol) was added dropwise. 4-Bromobenzoyl chloride (21.4 mg, 0.097 mmol) and 4-dimethylaminopyridine (DMAP, one crystal) were added as a solution in dichloromethane. The reaction was allowed to stir for 3 h as it slowly warmed to room temperature. Upon completion (by TLC), the reaction was quenched with brine, extracted with dichloromethane, and dried with sodium sulfate. The resulting organic layer was then filtered, concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 100% hexanes to 3:1 hexanes/ethyl acetate to afford 7 (17 mg, 59%) as a white-brown solid. H NMR: (400 MHz, CDCl3) at 65 °C) δ 8.38 (tt, J = 8.2, 0.8 Hz, 1H), 7.52–7.44 (m, 3H), 7.39 (td, J = 7.7, 1.1 Hz, 1H), 7.27 (td, J = 7.5, 1.0 Hz, 1H), 7.13 (m, 1H), 7.10 (dd, J = 8.0, 7.8 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 4.54 (s, 1H), 3.64 (m, 1H), 3.52–3.39 (m, 2H), 3.29 (m, 3H), 3.16 (m, 1H), 2.94 (d, J = 17.6 Hz, 1H), 2.71 (dt, J = 14.0, 4.9 Hz, 1H), 2.36 (dd, J = 17.6, 1.3 Hz, 1H), 1.90 (m, 1H), 1.68–1.57 (m, 4H), 0.89 (t, J = 7.6 Hz, 3H), 0.78 (m, 1H). 13C NMR: (100 MHz, CDCl3 at 65 °C) δ 172.7, 170.4, 141.1, 134.6, 134.9, 133.0, 131.4, 130.6, 130.4, 126.6, 126.1, 125.0, 123.0, 120.6, 119.5, 117.2, 75.8, 56.8, 51.6, 50.2, 44.4, 41.8, 31.6, 27.3, 23.5, 22.5, 8.2. HRMS (ESI): calcd for C15H16BrN2O2 [M + H]+: 309.1434, found: 309.1447. MP: 68–70 °C. [α]20 D: +52° (c 0.12 g /100 mL, MeOH).

General Procedure for the Synthesis of 8, 54, and 55. Compound 8 (416 mg, 1.37 mmol) was added to a flame-dried round-bottom flask and dissolved in chloroform (137 mL). Then, phenol (5.10 g, 55.0 mmol) was added to the reaction mixture after methyl propiolate (204 μL, 2.06 mmol) was added dropwise. The reaction was heated to 62 °C for 7 h. Upon completion (as monitored by TLC), the reaction was cooled down to room temperature, quenched with saturated aqueous sodium bicarbonate, and extracted with dichloromethane. The organic layer was collected and dried with sodium sulfate, filtered,
and concentrated in vacuo. The resulting crude material was then purified via column chromatography using a gradient of 100% hexanes to 3:2 hexanes/ethyl acetate to afford 8 (247 mg, 35%), 54 (96.2 mg, 14%), and 55 (191 mg, 27%) as white solids. Note: this reaction gave various product distributions. For example, this reaction was scaled to 3.4 grams of 81 (103 mmol), and, following the procedure (methyl propiolate: 1.4 mL, 14.4 mmol; phenol: 19.4 g, 206 mmol; chloroform, 206 mL), we isolated 2.5 g of 8 (47% yield), 267 mg of 54 (5%), and 637 mg of 55 (12%).

**Synthesis Procedure for the Generation of 9, (1R,12S,19S)-12-Ethyl-9,16-diazapatentacyclo[10.6.10.9.8.6.10.9.18]nonadeca-3,5,7-triene-2,10-dione.** Compound 6 (12.9 mg, 0.038 mmol) was added to a round-bottom flask and dissolved in methanol (1.5 mL). Sodium hydroxide (8.0 mg, 0.19 mmol) was added, and the reaction was heated to 64 °C for 3 h after being equipped with a reflux condenser. Upon completion, the reaction was quenched with brine, extracted with chloroform, and the organic layer was dried with sodium sulfate. The organic layer was filtered, concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 74:5:2.5 hexanes/ethyl acetate/triethylamine to afford 9 (9.7 mg, 82%) as a white-brown solid. 1H NMR: (400 MHz, CDCl3) δ 8.29 (d, J = 8.2 Hz, 1H), 7.75 (d, J = 7.7 Hz, 1H), 7.66 (td, J = 7.8, 1.4 Hz, 1H), 7.24 (m, 1H), 3.44 (d, J = 15.6 Hz, 1H), 3.11 (m, 1H), 3.03 (d, δ = 8.8, 7.4 Hz, 1H), 2.53 (s, 1H), 2.49 (m, 1H), 2.30 (td, J = 11.4, 3.1 Hz, 1H), 2.17 (td, J = 12.4, 5.5 Hz, 1H), 2.11 (dd, J = 15.6, 2.1 Hz, 1H), 1.95 (td, J = 11.3, 7.8 Hz, 1H), 1.57−1.67 (m, 2H), 1.61 (m, 1H), 1.21 (m, 1H), 0.91 (m, 1H), 0.78−0.69 (m, 4H). 13C NMR (100 MHz, CDCl3) δ 2009, 170.9, 152.3, 136.9, 125.0, 124.5, 122.7, 119.9, 75.5, 68.8, 51.6, 51.0, 40.9, 38.4, 38.3, 32.1, 20.3, 7.1. HRMS (ESI): calcld for C23H18N2O4 [M + H+] 311.1754, found: 311.1756. MP: 115−117 °C. [α]20D +63° (0.27 g/100 mL, MeOH).

**Synthesis Procedure for the Generation of 10, Methyl (4A,12S,19S)-12-(2-Bromoethyl)-1-cyano-4-ethyl-1H,2H,3H,4H,4aH,12bH-indolo[2,1-h],7-naphthyridine-6-carboxylate.** Compound 81 (503 mg, 1.50 mmol) was added to a flame-dried microwave vial and dissolved in N,N-dimethylformamide (15 mL). Then, a 3.0 M solution of cyanogen bromide in dichloromethane (1.5 mL, 4.50 mmol) was added dropwise to the reaction, which was subsequently subjected to microwave irradiation for 6 min at 100 °C. Upon completion of the reaction (monitored by TLC), the reaction was cooled down to room temperature, diluted with ethyl acetate, and quenched with brine (3 x 100 mL). The organic layer was collected and dried with sodium sulfate, then filtered, and finally concentrated in vacuo. The crude material was purified via column chromatography using a gradient of 100% chloroform to 99:1 chloroform/acetone to yield 10 (310 mg, 47%) as an orange-white solid. 1H NMR: (400 MHz, CDCl3) δ 7.59 (d, J = 7.6, 1H), 7.28−7.14 (m, 3H), 6.15 (d, J = 1.5 Hz, 1H), 4.11 (d, J = 1.5 Hz, 1H), 3.93 (s, 3H), 3.76 (td, J = 9.5, 7.1 Hz, 1H), 3.63 (td, J = 9.5, 7.1 Hz, 1H), 3.48−3.29 (m, 3H), 3.07 (td, J = 12.1, 3.0 Hz, 1H), 2.01 (d, J = 13.4 Hz, 1H), 1.79−1.56 (m, 2H), 1.30 (td, J = 13.1, 3.9 Hz, 1H), 1.11−0.96 (m, 2H), 0.69 (t, J = 7.5 Hz, 3H). Note: 1H spectrum referenced TMS at 0.0 ppm. 13C NMR (100 MHz, CDCl3) δ 163.3, 134.6, 130.0, 128.0, 127.9, 123.9, 121.2, 119.2, 117.5, 116.6, 112.7, 56.5, 52.8, 49.9, 39.4, 32.2, 31.5, 31.4, 28.6, 21.7, 8.1. HRMS (ESI): calcld for C26H18BrN2O4Na [M + Na]+: 464.0944, found: 464.0959. MP: 115−117 °C. [α]20D −76° (0.24 g/100 mL, CH2Cl2).

**HRMS (ESI):** calcld for C41H28N4O6 [M + H]+: 515.2540, found: 515.2549. MP: 157−159 °C. [α]20D ≈ −134° (0.15 g/100 mL, MeOH).

**HRMS (ESI):** calcld for C41H28N4O6 [M + H]+: 515.2540, found: 515.2549. MP: 240−242 °C. decomposed. [α]20D −74° (0.10 g/100 mL, CH2Cl2).

**HRMS (ESI):** calcld for C41H28N4O6 [M + H]+: 515.2540, found: 515.2549. MP: 240−242 °C. decomposed. [α]20D −74° (0.10 g/100 mL, CH2Cl2).

**HRMS (ESI):** calcld for C41H28N4O6 [M + H]+: 515.2540, found: 515.2549. MP: 240−242 °C. decomposed. [α]20D −74° (0.10 g/100 mL, CH2Cl2).
Synthesis Procedure for the Generation of 12, 2-((1S,12bS)-1-Ethyl-2H,3H,4H,6H,7H,12H,12bH-indolo[2,3-a]quinolin-1-yl)acetic Acid. Lactam 80 (3.53 g, 12.0 mmol) was reacted with potassium hydroxide in seven equal portions in a flame-dried microwave vial. Each portion of 80 was dissolved in ethanol (8.5 mL), and a 0.5 M aqueous potassium hydroxide solution (5 mL) was added. The resulting reaction mixture was then subjected to microwave irradiation for 20 min at 180 °C. The seven individually reacted portions were then combined in an Erlenmeyer flask and acidified to pH 5−6. The resulting solution was then extracted with dichloromethane. The organic layer was collected and then dried with sodium sulfate, filtered, and concentrated in vacuo.

The crude material was purified via column chromatography using a 99:1 dichloromethane/triethylamine to a 94:5:1 dichloromethane/methanol/triethylamine mixture and reacted for 3 h. Upon completion (by TLC), the reaction was heated to reflux and added with aqueous ethyl acetate and ethyl acetate.

The organic layer was recovered and concentrated in vacuo. The crude material was collected, dissolved in methanol (13 mL), and methyl propiolate (18 μL, 0.198 mmol) was then added dropwise, and the reaction was stirred at room temperature for 5 min before heating to reflux for 2 h. Upon completion (by TLC), the reaction was cooled with aqueous brine and extracted with ethyl acetate. The organic layer was collected, dried with sodium sulfate, filtered, and concentrated in vacuo.

The crude material was purified via column chromatography using a gradient of 100% hexanes to 2:1 hexanes/ethyl acetate to afford 17 (44.9 mg, 83%) as a white solid. 1H NMR: (400 MHz, CDCl$_3$ at 50 °C) δ 8.46 (d, J = 7.4 Hz, 1H), 7.55 (d, J = 13.3 Hz, 1H), 7.48 (dd, J = 7.4, 0.7 Hz, 1H), 7.39 (td, J = 7.4, 0.7 Hz, 1H), 7.32 (td, J = 7.4, 0.7 Hz, 1H), 4.80 (d, J = 13.4 Hz, 1H), 4.14 (s, 1H), 3.72 (m, 1H), 3.71 (s, 3H), 3.55 (dd, J = 14.2, 6.8 Hz, 1H), 3.21 (s, 3H), 3.14 (td, J = 14.1, 2.6 Hz, 1H), 3.01 (d, J = 17.3 Hz, 1H), 2.73 (m, 3H), 2.09 (ddd, J = 17.3 Hz, 1H), 2.33 (m, 1H), 1.94 (sextet, J = 7.6 Hz, 1H), 1.88−1.69 (m, 3H), 1.62−1.52 (m, 2H), 0.87 (t, J = 7.7 Hz, 3H), 0.84 (m, 1H). 13C NMR: (100 MHz, CDCl$_3$ at 50 °C) δ 169.6, 169.4, 150.4, 135.5, 133.7, 128.9, 126.0, 124.1, 118.9, 118.2, 117.2, 87.9, 75.5, 58.9, 56.5, 55.8, 50.9, 43.6, 40.9, 34.1, 26.6, 22.4, 20.0, 7.9. HRMS (ESI): calc for C$_{25}$H$_{30}$N$_{2}$O$_{2}$ [M + H]: 411.2278; found: 411.2269. MP: 116−118 °C. [α]$_D^{20}$ +37° (c 0.13 g/100 mL, CH$_2$Cl$_2$).

Synthesis Procedure for the Generation of 18 and 63. Vincamine (100 mg, 0.282 mmol) was added to a flame-dried round-bottom flask and dissolved in isopropanol (28 mL). Methyl propiolate (41 μL, 0.462 mmol) was then added dropwise to the mixture, and the reaction was heated to reflux for 2 h. Upon completion, the reaction was cooled and then concentrated in vacuo. Finally, the crude material was purified via column chromatography using a gradient of 100% hexanes to 3:1 hexanes/ethyl acetate to afford 18 (84.4 mg, 60%) as a colorless solid and V3m (35.7 mg, 25%) as a clear-brown residue.

Synthesis Procedure for the Generation of 16, Methyl 2- ((1S,12bS)-1-Ethyl-2H,3H,4H,6H,7H,12H,12bH-indolo[2,3-a]quinolin-1-yl)acetate. Compound 12 (1.51 g, 4.84 mmol) was added to a round-bottom flask and dissolved in anhydrous N,N-dimethylformamide (48 mL). The solution was cooled to 0 °C, and then anhydrous potassium carbonate (1.34 g, 9.69 mmol) and iodomethane (0.362 mL, 5.81 mmol) were added sequentially. The resulting reaction mixture was warmed slowly to room temperature and reacted for 3 h. Upon completion by TLC, the reaction was quenched with brine, extracted with ethyl acetate, and the organic layer was washed with deionized water three times (~1 L). The organic layer was dried with sodium sulfate, filtered, concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 82:16:5:1 hexanes/ethyl acetate/triethylamine to afford 16 (1.18 g, 75%) as a brown solid. 1H NMR: (400 MHz, CDCl$_3$) δ 7.83 (s, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.15 (td, J = 8.0, 1.3 Hz, 1H), 7.09 (td, J = 8.0, 1.1 Hz, 1H), 3.49 (s, 3H), 3.38 (m, 1H), 3.09−2.97 (m, 3H), 2.89 (m, 1H), 2.68−2.53 (m, 2H), 2.40 (td, J = 12.5, 2.8 Hz, 1H), 2.06−1.86 (m, 3H), 1.82 (m, 1H), 1.67−1.55 (m, 3H), 1.18 (t, J = 7.7 Hz, 3H). 13C NMR: (100 MHz, CDCl$_3$) δ 173.7, 136.1, 132.6, 126.8, 121.7, 119.4, 117.9, 112.4, 110.9, 66.3, 56.9, 54.0, 51.2, 40.4, 38.1, 32.3, 31.4, 22.2, 22.1, 6.2. HRMS (ESI): calc for C$_{25}$H$_{28}$N$_{2}$O: [M + H]: 327.2067; found: 327.2074. MP: 127−129 °C. [α]$_D^{20}$ +52° (c 0.74 g/100 mL, CH$_2$Cl$_2$).
3H), 0.92 (d, J = 5.9 Hz, 3H), 0.86 (t, J = 7.5 Hz, 3H). 13C NMR: (100 MHz, CDCl3 at 50 °C) δ 172.0, 169.6, 151.3, 135.4, 134.6, 127.6, 123.0, 120.3, 118.5, 111.9, 111.0, 86.7, 82.3, 71.8, 68.9, 58.0, 56.1, 53.4, 50.6, 46.5, 41.8, 34.1, 29.4, 23.4, 22.3 (2), 21.2, 8.1. Note: the presence of two carbon signals at 22.3 ppm was elucidated by a HSQC experiment. HRMS (ESI): calcd for C28H38N2O6Na [M + Na]+: 521.2622, found: 521.2628. MP: 158−160 °C. [α]D 20: +62° (c 0.27 g/100 mL, CH2Cl2).

Analogue 63, methyl (9S,11S,19R)-11-ethyl-9-hydroxy-15-[1(E)-3-methoxy-3-oxoprop-1-en-1-yl]-19-(propan-2-yloxy)-8,15-diazatetracyclo[9.6.2.02,7.08,18]nonadeca-1(18),2,4,6-tetraene-9-carboxylate: 1H NMR: (400 MHz, CDCl3 at 50 °C) δ 7.61 (d, J = 13.3 Hz, 1H), 7.51 (m, 1H), 7.22−7.12 (m, 3H), 4.82 (d, J = 13.3 Hz, 1H), 4.55 (s, 1H), 4.40 (s, 1H), 3.80 (s, 3H), 3.72 (m, 1H), 3.59−3.47 (m, 2H), 3.16 (dd, J = 15.7, 13.8 Hz, 1H), 3.00−2.81 (m, 2H), 2.85 (d, J = 13.7 Hz, 1H), 2.30 (dd, J = 13.8, 9.2 Hz, 1H), 1.93−1.70 (m, 4H), 1.60−1.49 (m, 2H), 1.22 (dd, J = 15.9, 7.9 Hz, 1H), 1.14 (d, J = 6.0 Hz, 3H), 0.90 (d, J = 6.1 Hz, 3H), 0.86 (s, J = 7.6 Hz, 3H). 13C NMR: (100 MHz, CDCl3 at 50 °C) δ 175.0, 169.7, 150.6, 135.6, 135.2, 128.3, 122.9, 120.5, 118.5, 112.6, 112.3, 87.3, 83.2, 70.3, 67.5, 59.4, 56.7, 54.0, 50.7, 41.0, 40.9, 33.2, 27.3, 23.7, 22.0 (2), 21.3, 8.1. Note: justification for the overlapping carbon signals at 22.0 ppm is based on HSQC studies of 18. HRMS (ESI): calcd for C28H38N2O6Na [M + Na]+: 521.2622, found: 521.2628. [α]D 20: +58° (c 0.10 g/100 mL, CH2Cl2).

Synthesis Procedure for the Generation of 19, 2-[(1S,7aR,12bS)-1-Ethyl-7a-hydroxy-1H,2H,3H,4H,6H,7H,7aH,12bH-indolo[2,3-a]quinolizin-1-yl]-N-benzylacetamide. Compound 28 (403 mg, 1.00 mmol) was added to a round-bottom flask and dissolved in acetonitrile/water (2:1, 30 mL). The resulting solution was cooled to 0 °C, and a 0.3 M solution of [bis(trifluoroacetoxy)iodo]benzene (PIFA; 860 mg, 2.00 mmol) in acetonitrile was added slowly via syringe over 5 min. The reaction was stirred for 3 h at 0 °C and then quenched with cold saturated aqueous sodium bicarbonate. The mixture was then extracted with dichloromethane, washed with brine, and the organic layer was dried with sodium sulfate. The organic layer was filtered, concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 49.5:49.5:1 hexanes/ethyl acetate/triethylamine to afford 19 (109 mg, 26%) as a brown residue. 1H NMR: (500 MHz, CDCl3) δ 7.49 (d, J = 7.5 Hz, 1H), 7.41 (d, J = 7.2 Hz, 1H), 7.32 (dd, J = 8.2, 7.7 Hz, 1H), 7.29−7.16 (m, 4H), 7.12 (d, J = 7.4 Hz, 2H), 6.09 (t, J = 5.3 Hz, 1H), 4.36 (dd, J = 14.6, 5.9 Hz, 1H), 3.87 (dd, J = 14.4, 5.0 Hz, 1H), 3.12 (s, 1H), 3.08−2.99 (m, 2H), 2.65 (m, 1H), 2.60 (d, J = 14.5 Hz, 1H), 2.40 (d, J = 13.6 Hz, 1H), 2.30 (dd, J = 12.2, 7.1, 1.8 Hz, 1H), 2.13−1.82 (m, 5H), 1.65 (m, 1H), 1.54 (dd, J = 13.9, 9.9, 3.3 Hz, 1H), 1.47 (td, J = 13.1, 3.9 Hz, 1H), 1.09 (t, J = 7.4 Hz, 3H). 13C NMR: (100 MHz, CDCl3) δ 184.1, 171.9, 151.9, 139.9, 138.3, 129.3, 128.7, 128.0, 128.7, 126.2, 121.0, 82.6, 72.0, 55.7, 50.9, 43.6, 41.0, 40.8, 32.9, 32.2, 29.9, 21.4, 8.3. HRMS (ESI): calcd for C26H32N3O2 [M + H]+: 418.2489, found: 418.2484. [α]D 20: +16° (c 0.37 g/100 mL, CH2Cl2). Note: correlated spectroscopy (COSY) and 1-D rotating frame overhauser enhancement spectroscopy (ROESY) were used to determine the relative stereochemistry of 19 (see the Supporting Information).
General Procedure for the Oxidative Ring Fusion Synthesis of 20−25 and 46−52 from Corresponding Aminide Precursors (e.g., 30): Amide 30 (44.6 mg, 0.127 mmol) was added to a flame-dried round-bottom flask and dissolved in anhydrous dichloromethane (5.1 mL). The reaction was cooled to 0 °C, and trifluoroacetic acid (0.175 mL, 2.16 mmol) was added. The resultant solution was stirred for 5 min, and then m-chloroperbenzoic acid (21.9 mg, 0.127 mmol) was added as a 0.3 M solution in anhydrous dichloromethane. The reaction was then refluxed for 24 h. Upon completion, the reaction was quenched with 3 M aqueous ammonium hydroxide. The resulting mixture was then extracted with dichloromethane, washed with brine, and the organic layer was dried with sodium sulfate. The organic layer was filtered, concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 79.5:19.5:1 hexanes/ethyl acetate/triethylamine to afford ring fusion product 20 (18.5 mg, 42%) as a green-yellow residue.

Analogue 20, (15S,17S)-20-cyclopropyl-17-ethyl-3,13,20-triazapentacyclo[11.7.0.1^7,1^7.0^9,1^9]icos-2(10),4,6,8-tetraen-19-one. Yield: 42%; 18.5 mg 20; green-yellow residue. \[^1H\text{NMR}: (400 MHz, CDCl_3) \delta 9.47 (s, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.51 (d, J = 7.8 Hz, 1H), 7.17 (td, J = 7.4, 1.0 Hz, 1H), 7.09 (dd, J = 8.2, 7.5 Hz, 1H), 3.53 (dd, J = 11.6, 9.1, 4.5 Hz, 1H), 3.21−3.10 (m, 2H), 3.04 (m, 1H), 3.03 (d, J = 16.7 Hz, 1H), 2.89−2.72 (m, 2H), 2.17 (d, J = 17.1 Hz, 1H), 2.14 (m, 1H), 1.94 (m, 1H), 1.81 (m, 1H), 1.59−1.35 (m, 1H), 1.13 (sextet, J = 7.5 Hz, 1H), 0.80 (m, 1H; buried under triplet), 0.78 (t, J = 7.5 Hz, 3H), 0.55 (m, 1H), 0.39−0.22 (m, 2H). \[^13C\text{NMR}: (100 MHz, CDCl_3) \delta 175.5, 136.9, 133.3, 126.2, 122.1, 119.2, 118.3, 113.5, 112.2, 84.6, 50.4, 49.9, 44.5, 43.7, 33.1, 26.7, 24.7, 22.6, 20.0, 9.4, 6.8, 5.4\)], HRMS (ESI): [M + H\(^+\)] calculated for C\(_{24}\)H\(_{32}\)N\(_3\)O\(_2\): 400.2383, found: 400.2393. MP: 170−172 °C. \([\alpha]\)\(_D\)\(^{22}\): +48° (c 0.17 g/100 mL, CH\(_2\)Cl\(_2\)). Analogue 21, (15S,17S)-17-ethyl-20-(prop-2-yn-1-yl)-3,13,20-triazapentacyclo[11.7.0.1^7,1^7.0^9,1^9]icos-2(10),4,6,8-tetraen-19-one. Yield: 56%; 27.5 mg 21; off-white residue. \[^1H\text{NMR}: (400 MHz, CDCl_3) \delta 9.65 (s, 1H), 7.66 (dt, J = 8.1, 0.8 Hz, 1H), 7.53 (s, d, J = 7.9 Hz, 1H), 7.20 (ddd, J = 7.9, 6.9, 1.2 Hz, 1H), 7.11 (ddd, J = 8.1, 6.9, 0.8 Hz, 1H), 3.94 (ddd, J = 17.3, 2.6 Hz, 1H), 3.61 (dd, J = 17.3, 2.6 Hz, 1H), 3.47−3.34 (m, 2H), 3.25 (m, 1H), 3.16 (d, J = 17.0 Hz, 1H), 3.01 (dt, J = 12.8, 4.2 Hz, 1H), 2.94−2.79 (m, 2H), 2.31 (d, J = 17.0 Hz, 1H), 2.05 (m, 1H), 1.98 (t, J = 2.6 Hz, 1H), 1.88 (m, 1H), 1.68−1.52 (m, 2H), 1.47 (m, 1H), 1.33 (sextet, J = 7.6 Hz, 1H), 0.86 (t, J = 7.6 Hz, 3H). \[^13C\text{NMR}: (100 MHz, CDCl_3) \delta 174.4, 137.5, 131.6, 126.0, 122.4, 119.2, 118.2, 114.4, 112.4, 83.6, 78.9, 78.0, 50.1, 49.6, 43.9, 43.7, 32.8, 29.8, 27.1, 22.2, 18.0, 9.5\)]. HRMS (ESI): calculated for C\(_{22}\)H\(_{28}\)N\(_2\)O\(_2\) [M + H\(^+\): 348.2070, found: 348.2075. \([\alpha]\)\(_D\)\(^{22}\): +23° (c 0.23 g/100 mL, CH\(_2\)Cl\(_2\)).
Synthesis Procedure for the Generation of 24 from 19. Compound 19 (260 mg, 0.062 mmol) was added to a flame-dried round-bottom flask and dissolved in anhydrous dichloromethane (2.5 mL). The reaction was cooled to 0 °C, and triluoroacetic acid (0.085 mL, 1.05 mmol) was added. The reaction was slowly warmed to room temperature for 4 h. Then, the reaction temperature was adjusted to 80 °C for an additional 3 h. Upon completion by TLC, the reaction was quenched with deionized water. The crude mixture was filtered, concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 79.5:19.5:1 hexanes/ethyl acetate/triethylamine to afford 24 (18.3 mg, 74%) as a light green solid. Note: this experiment was critical to understanding the reaction mechanism for the oxidative ring fusion to related structures.

Procedure for the Synthesis of 38, 2-[[15,12bS]-1-Ethyl-1H,2H,3H,4H,6H,7H,12H,12bH-indolo[2,3-a]quinolin-1-yl-N-(3,5-dimethoxypyridin-2-yl)acetamide. 12 (38.2 mg, 0.105 mmol) was added to a flame-dried round-bottom flask and dissolved in anhydrous dichloromethane (2.6 mL). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (24.0 μL, 0.136 mmol) was added dropwise, and the reaction was stirred for 5 min. After a period of 20 min, 3,5-dimethoxyaniline (21.0 mg, 0.136 mmol) as a 0.3 M solution in anhydrous dichloromethane was added, and the reaction proceeded for 72 h. Upon completion, the reaction was quenched with deionized water, extracted with ethyl acetate, and the organic layer was dried with sodium sulfate, filtered, and concentrated in vacuo. The crude product was then purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 79.5:19.5:1 hexanes/ethyl acetate/triethylamine to afford 38 (10.1 mg, 21%) as a white-brown residue. 1H NMR: (500 MHz, CDCl3) δ 8.14 (s, 1H), 7.89 (s, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.13 (dd, J = 8.0, 7.5 Hz, 1H), 7.06 (d, J = 8.3, 7.5 Hz, 1H), 6.50 (d, J = 2.2 Hz, 2H), 6.12 (s, J = 2.2 Hz, 1H), 3.69 (s, 6H), 3.45 (s, 1H), 3.15–3.05 (m, 2H), 2.98 (s, 1H), 2.76–2.67 (m, 2H), 2.64 (td, J = 11.4, 3.5 Hz, 1H), 2.47 (td, J = 12.8, 2.3 Hz, 1H), 2.27 (d, J = 14.3 Hz, 1H), 2.10–1.93 (m, 4H), 1.70–1.58 (m, 2H), 1.20 (t, J = 7.6 Hz, 3H). 13C NMR: (100 MHz, CDCl3) δ 170.0, 161.0, 140.1, 136.3, 132.7, 126.9, 122.1, 119.8, 118.1, 112.3, 111.1, 97.6, 96.5, 67.2, 56.9, 55.5, 54.2, 43.1, 41.0, 33.2, 32.6, 22.3, 22.2, 8.4. HRMS (ESI): calcd for C22H29N3O2 [M + H]+: 368.2333, found: 368.2333. 

Procedure for the Chemical Synthesis of 44, (15,17S)-17-Ethyl-20-(3-phenylprop-2-yn-1-yl)-3,13,20-triazapentacyclo[11.7.0.01,17.02,10.04,9]icosa-2(10),4,6,8-tetraen-19-one. To a flame-dried round-bottom flask was added tetrakis(triphenylphosphine)-palladium(0) (10.3 mg, 0.0089 mmol), copper(I) iodide (3.4 mg, 0.018 mmol), and iodo benzene (20.0 μL, 0.177 mmol). These reagents were then dissolved in anhydrous N,N-dimethylformamide (2.0 mL), and triethylamine (22.0 μL, 0.160 mmol) was finally added dropwise. 21 (30.8 mg, 0.089 mmol) as a 0.6 M solution in anhydrous N,N-dimethylformamide was added, and the reaction proceeded at room temperature for 4 h. Then, the reaction temperature was adjusted to 80 °C for an additional 3 h. Upon completion by TLC, the reaction was quenched with deionized water. The crude reaction mixture was extracted with ethyl acetate, washed with brine, and the organic layer was dried with sodium sulfate. The organic layer was filtered, concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 79.5:19.5:1 hexanes/ethyl acetate/triethylamine to afford 44 (17.0 mg, 45%) as a brown residue. 1H NMR: (500 MHz, CDCl3) δ 8.33 (s, 1H), 7.50 (d, J = 7.6 Hz, 1H), 7.38 (d, J = 7.7 Hz, 1H), 7.19–7.04 (m, 5H), 6.78 (d, J = 7.6 Hz, 2H), 4.09 (d, J = 17.4 Hz, 1H), 3.99 (d, J = 17.4 Hz, 1H), 3.38–3.43 (m, 2H), 2.99 (d, J = 11.0, 3.7 Hz, 1H), 2.92–2.76 (m, 3H), 2.28 (d, J = 17.1 Hz, 1H), 2.00 (d, J = 13.1 Hz, 1H), 1.82 (q, J = 12.3 Hz, 1H), 1.68–1.46 (m, 2H), 1.42 (m, 1H), 1.23 (m, 1H), 0.80 (t, J = 7.5 Hz, 3H). 13C NMR: (100 MHz, CDCl3) δ 174.1, 137.6, 132.1, 131.8, 128.1, 128.0, 126.9, 122.6, 119.4, 118.4, 114.6, 112.4, 84.3, 83.4, 82.5, 50.1, 49.9, 44.2, 43.9, 32.8, 30.4, 26.9, 22.3, 18.4, 9.5. HRMS (ESI): calcd for C28H30N3O [M + H]+: 424.2383, found: 424.2380. [α]D20 = +3° (c 0.10 g/100 mL, CH2Cl2).

For C23H23NO3 [M + H]+: 448.2595, found: 448.2595. [α]D20 = −40° (c 0.10 g/100 mL, CH2Cl2).
General Procedure for the Synthesis of 69–72. Compound 79 (30.1 mg, 0.107 mmol) was added to a flame-dried round-bottom flask and dissolved in chloroform (30.8 mL). Phenol (402 mg, 4.28 mmol) and 4-nitrobenzyl bromide (48.0 mg, 0.220 mmol) were added as one portion, and then methyl propiolate (14.0 mmol) was added dropwise at room temperature. The reaction was stirred for 2 h. Upon completion, the reaction was then cooled down to room temperature, quenched with saturated aqueous brine and extracted with dichloromethane. The organic layer was then dried with sodium sulfate, filtered, concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 100% hexanes to 3:1 hexanes/ethyl acetate to afford 69 (28.0 mg, 57%) as a clear solid.

Procedure for the Synthesis of 73. A 10 mL N,N-dimethylformamide (2.8 mL) with 0.1 M sodium carbonate was added to stir at room temperature for 21 h. Upon completion by TLC, the reaction mixture was then diluted with brine and extracted with dichloromethane. The organic layer was dried with sodium sulfate, filtered, concentrated in vacuo, and the crude material was purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 74.5:24.5:1 hexanes/ethyl acetate/triethylamine to 99:1 ethyl acetate/triethylamine to afford 74 (503 mg, 99%) as a clear-yellow residue.

Synthesis Procedure for the Generation of 74, (11S,19R)-11-Ethyl-19-methoxy-8,15-diazatetracyclo[9.6.2.03,8.16]nonadeca-1(18),2,4,6-tetraen-9-one. Compound 17 (640 mg, 1.56 mmol) was added to a stirring solution of 1.0 M hydrogen chloride in methanol (15.6 mL). The reaction was allowed to stir at room temperature for 1.5 h until complete. The reaction mixture was then diluted with brine and extracted with ethyl acetate. The organic layer was collected via a separatory funnel, dried with sodium sulfate, filtered, and concentrated in vacuo. The crude material was then purified via column chromatography using a gradient of 99:1 hexanes/triethylamine to 74.5:24.5:1 hexanes/ethyl acetate/triethylamine to 99:1 ethyl acetate/triethylamine to afford 74 (503 mg, 99%) as a clear-yellow residue.

HRMS (ESI): calcd for C33H36N3O3 [M + H]+: 490.2853, found: 490.2856. [\(\alpha\)_D]74°: +20 (c 0.2 g/100 mL, CH\(_2\)Cl\(_2\)).
Procedure for the Synthesis of 76. Methyl (9S,11S,19R)-15-Ethyl-1,11-diazapentacyclo[9.6.2.07,19.015,19.022,22]nonadeca-2,4,6,8(18)-tetraen-17-ol. Lithium aluminum hydride (142 mg, 3.94 mmol) was then added portionwise at 0 °C before allowing to warm to room temperature. The reaction mixture was then heated to 66 °C and allowed to stir for 1 h. After that time, the reaction was cooled and then quenched with 1 M sodium hydroxide (aqueous solution, 2 mL), followed by the addition of distilled water (10 mL). The resulting mixture was then filtered through a frit funnel containing celite. The filtrate was then rinsed with warm ethyl acetate. The organic solution containing the product was dried with sodium sulfate and concentrated in vacuo to afford 74 (81.7 g, 97%) as a yellow-white solid. Note: 74 is a known compound (CAS No. 3282-95-4).

1H NMR: (400 MHz, CDCl3) δ 7.63 (m, 1H), 7.48 (m, 1H), 7.17–7.09 (m, 2H), 4.10 (d, J = 11.4 Hz, 1H), 3.87 (d, J = 11.4 Hz, 1H), 3.71 (s, 1H), 3.28–3.29 (m, 4H), 2.55–2.47 (m, 2H), 2.33 (td, J = 12.3, 3.0 Hz, 1H), 2.26–2.13 (m, 2H), 2.04 (d, J = 15.1 Hz, 1H), 1.69 (m, 1H), 1.34 (m, 1H), 1.32–1.30 (m, 2H, 0.91 (t, J = 7.5 Hz, 1H). 13C NMR: (100 MHz, CDCl3) δ 174.8, 173.5, 133.7, 127.9, 123.1, 120.6, 118.4, 117.9, 112.1, 111.9, 83.0, 75.5, 56.8, 56.4, 54.5, 41.0, 40.6, 36.2, 32.6, 26.8, 23.5, 21.4, 8.1. HRMS (ESI): calcd for C21H25NO2Na [M + Na]+: 287.1686; found: 287.1691. 

Procedure for the Synthesis of 77. (15S,19S)-15-Ethyl-1,11-diazapentacyclo[9.6.2.07,19.015,19.022,22]nonadeca-2,4,6,8(18)-tetraen-17-ol. Lithium aluminum hydride (142 mg, 3.94 mmol) was then added portionwise at 0 °C before allowing to warm to room temperature. The reaction mixture was then heated to 66 °C and allowed to stir for 1 h. After that time, the reaction was cooled and then quenched with 1 M sodium hydroxide (aqueous solution, 2 mL), followed by the addition of distilled water (10 mL). The resulting mixture was then filtered through a frit funnel containing celite. The filtrate was then rinsed with warm ethyl acetate. The organic solution containing the product was dried with sodium sulfate and concentrated in vacuo to afford 74 (81.7 g, 97%) as a yellow-white solid. Note: 74 is a known compound (CAS No. 3282-95-4).

1H NMR: (400 MHz, CDCl3) δ 7.63 (m, 1H), 7.48 (m, 1H), 7.17–7.09 (m, 2H), 4.10 (d, J = 11.4 Hz, 1H), 3.87 (d, J = 11.4 Hz, 1H), 3.71 (s, 1H), 3.28–3.29 (m, 4H), 2.55–2.47 (m, 2H), 2.33 (td, J = 12.3, 3.0 Hz, 1H), 2.26–2.13 (m, 2H), 2.04 (d, J = 15.1 Hz, 1H), 1.69 (m, 1H), 1.34 (m, 1H), 1.32–1.30 (m, 2H, 0.91 (t, J = 7.5 Hz, 1H). 13C NMR: (100 MHz, CDCl3) δ 134.0, 132.4, 129.3, 121.3, 120.1, 118.6, 105.7, 84.8, 67.6, 59.5, 50.8, 44.3, 44.2, 34.5, 28.9, 25.9, 20.8, 16.9, 7.8. MP: 180–182 °C. [α]D20 +10.0 (c 0.17 g/100 mL, CH3Cl2), lit. +10.6 (c 0.17 g/100 mL, CH3Cl2). Note: Compound 78 is an intermediate en route to 12 and 17.
Procedure for the Synthesis of 80, (15S,19S)-15-Ethyl-11-diaza-diazapentacyclo[9.6.2.0^2,7.0^8,18.0^15,19]nonadec-2,4,6,8(18)-tetraen-17-one. Compound 78 (4.17 g, 12.8 mmol) was added to a round-bottom flask and dissolved in 3:1 solution of tetrahydrofuran/water (128 mL). Sodium periodate (13.7 g, 64.0 mmol) was added portionwise, and the reaction was stirred at room temperature for 4 h. The reaction was quenched with saturated aqueous sodium hydroxide solution and then extracted with ethyl acetate/methanol:triethylamine to yield 80 (4.5 g, 95%) as a light yellow solid. Note: Compound 81 is a known compound (apovincamine, CAS No. 4880-92-6), and this procedure converting vincamine to 81 was adapted from the literature.\(^4\)\(^1\)\(^H\) NMR: (400 MHz, CDCl\(_3\)) \(\delta 7.47\) (d, \(J = 7.7\) Hz, 1H), 7.23 (d, \(J = 8.3\) Hz, 1H), 7.17 (td, \(J = 7.1, 1.1\) Hz, 1H), 7.13 (td, \(J = 7.2, 0.9\) Hz, 1H), 6.14 (s, 1H), 4.17 (s, 1H), 3.95 (s, 3H), 3.38 (dd, \(J = 13.9, 6.2\) Hz, 1H), 3.27 (td, \(J = 11.7, 5.5\) Hz, 1H), 3.03 (m, 1H), 2.70–2.60 (m, 2H), 2.54 (dd, \(J = 16.4, 3.4\) Hz, 1H), 2.00–1.86 (m, 2H), 1.75 (m, 1H), 1.52 (d, \(J = 13.7\) Hz, 1H), 1.42 (dt, \(J = 13.2, 2.9\) Hz, 1H), 1.06–0.98 (m, 4H).\(^13\)C NMR: (150 MHz, CDCl\(_3\)) \(\delta 164.0, 153.4, 130.9, 129.2, 128.4, 128.3, 122.0, 120.5, 118.5, 112.6, 108.9, 80.0, 52.7, 51.7, 45.1, 38.0, 28.7, 27.5, 20.4, 16.5, 8.9. MP: 159°–161 °C. [\(\alpha\)_\text{D}]\(^20\) +112\(^\circ\) (c 0.6 g/100 mL, CHCl\(_3\)), lit. +98 (c 0.5 g/100 mL, MeOH).\(^45\) Note: Compound 81 is an intermediate en route to 10.

Synthesis Procedure for the Generation of 81, Methyl (15S,19S)-15-Ethyl-11-diaza-diazapentacyclo[9.6.2.0^2,7.0^8,18.0^15,19]nonadec-2,4,6,8(18)-16-pentaene-17-carboxylate. Vincamine 1 (5.1 g, 14.4 mmol) was added to a round-bottom flask and dissolved in toluene (48 mL). p-Toluenesulfonic acid monohydrate (5.5 g, 28.8 mmol) was added to the solution, and the flask was equipped with a Dean–Stark trap and heated at reflux for 2 h. Upon completion of the reaction (monitored by TLC), the mixture was basified to pH 6–7 with 1 M aqueous sodium hydroxide solution and then extracted with ethyl acetate. The organic layer was collected and dried with sodium sulfate, filtered, and concentrated in vacuo. The crude material was then purified via column chromatography using a 99:1 hexanes/triethylamine to 49.5:49.5:1 hexanes/ethyl acetate/triethylamine to afford 81 (4.5 g, 95%) as a light yellow solid. Note: 81 is a known compound (apovincamine, CAS No. 4880-92-6), and this procedure converting vincamine to 81 was adapted from the literature.\(^4\)\(^1\)\(^H\) NMR: (400 MHz, CDCl\(_3\)) \(\delta 7.47\) (d, \(J = 7.7\) Hz, 1H), 7.23 (d, \(J = 8.3\) Hz, 1H), 7.17 (td, \(J = 7.1, 1.1\) Hz, 1H), 7.13 (td, \(J = 7.2, 0.9\) Hz, 1H), 6.14 (s, 1H), 4.17 (s, 1H), 3.95 (s, 3H), 3.38 (dd, \(J = 13.9, 6.2\) Hz, 1H), 3.27 (td, \(J = 11.7, 5.5\) Hz, 1H), 3.03 (m, 1H), 2.70–2.60 (m, 2H), 2.54 (dd, \(J = 16.4, 3.4\) Hz, 1H), 2.00–1.86 (m, 2H), 1.75 (m, 1H), 1.52 (d, \(J = 13.7\) Hz, 1H), 1.42 (dt, \(J = 13.2, 2.9\) Hz, 1H), 1.06–0.98 (m, 4H).\(^13\)C NMR: (150 MHz, CDCl\(_3\)) \(\delta 164.0, 153.4, 130.9, 129.2, 128.4, 128.3, 122.0, 120.5, 118.5, 112.6, 108.9, 80.0, 52.7, 51.7, 45.1, 38.0, 28.7, 27.5, 20.4, 16.5, 8.9. MP: 159°–161 °C. [\(\alpha\)_\text{D}]\(^20\) +112\(^\circ\) (c 0.6 g/100 mL, CHCl\(_3\)), lit. +98 (c 0.5 g/100 mL, MeOH).\(^45\) Note: Compound 81 is an intermediate en route to 10.

Procedure for the Chemical Synthesis of 84, Methyl (4αS,12bS)-12-(2-Aminoethyl)-1-cyano-4α-ethyl-1H,2H,3H,4H,4aH,12bH-indolol[1,2-h]-1,7-naphthyridine-6-carboxylate. Compound 85 (157 mg, 0.388 mmol) was added to a flame-dried round-bottom flask and dissolved in methanol (6.50 mL). Triphenylphosphine (153 mg, 0.582 mmol) was then added to the resulting solution, and the reaction mixture was heated to reflux. The reaction proceeded for 1 h before being cooled down to room temperature and concentrated in vacuo. The crude material was purified by column chromatography using a gradient of 99:1 ethyl acetate/triethylamine to 89:5:9:5 ethyl acetate/methanol:triethylamine to yield 84 (122 mg, 83%) as a clear residue.\(^1\)\(^H\) NMR: (400 MHz, CDCl\(_3\)) \(\delta 7.59\) (d, \(J = 7.5\) Hz, 3H), 4.13 (d, \(J = 1.7\) Hz, 1H), 3.92 (s, 3H), 3.38 (d, \(J = 12.2\) Hz, 1H), 3.17–2.86 (m, 5H), 2.18 (s, 2H), 2.03 (d, \(J = 13.2\) Hz, 1H), 1.81–1.61 (m, 2H), 1.35 (td, \(J = 14.0, 4.2\) Hz, 1H), 0.99 (q, \(J = 7.5\) Hz, 2H), 0.67 (t, \(J = 7.5\) Hz, 3H).\(^13\)C NMR: (100 MHz, CDCl\(_3\)) \(\delta 163.5, 134.8, 130.2, 128.7, 127.8, 127.2, 123.8, 121.0, 119.7, 118.2, 116.7, 112.5, 56.6, 52.7, 49.9, 42.4, 39.5, 32.3, 31.4, 28.1, 21.8, 8.0. HRMS (ESI): calcld for C\(_{22}\)H\(_{26}\)N\(_4\)O\(_2\)Na [M + Na]+: 513.8408, found: 513.8404.
Procedure for the Chemical Synthesis of 85, Methyl (4aS,12bS)-12-(2-Azidoethyl)-1-cyano-4a-ethyl-1H,2H,3H,4H,4aH,12bH-indolo[1,2-h],7-naphthylidine-6-carboxylate. Compound 10 (256 mg, 0.379 mmol) was added to a flame-dried microwave flask and dissolved in N,N-dimethylformamide (6.00 mL). Sodium azide (301 mg, 4.63 mmol) was then added to the resulting solution, and the reaction mixture was subjected to microwave irradiation at 100 °C for 6 min. Upon completion of this reaction (monitored by TLC), the reaction was cooled down to room temperature, diluted with ethyl acetate, and quenched with brine. The organic layer was collected and dried with sodium sulfate, filtered, and concentrated in vacuo. The crude material was finally purified via column chromatography using a gradient of 100% chloroform to 99:1 chloroform/acetone to yield 85 (174 mg, 73%) as a white solid. 

1H NMR (400 MHz, CDCl3) δ 7.58 (m, 1H), 7.26 (m, 1H), 7.23–7.15 (m, 2H), 6.15 (d, J = 1.7 Hz, 1H), 4.11 (d, J = 1.7 Hz, 1H), 3.96 (s, 3H), 3.72 (ddd, J = 12.2, 8.2, 5.7 Hz, 1H), 3.61 (ddd, J = 12.2, 7.9, 4.1 Hz, 1H), 3.48 (dp, J = 10.3, 2.1 Hz, 1H), 3.21–3.03 (m, 3H), 2.13 (dt, J = 13.8, 3.5 Hz, 1H), 1.89–1.70 (m, 2H), 1.42 (td, J = 13.2, 4.2 Hz, 1H), 1.17–1.10 (m, 2H), 0.75 (t, J = 7.5 Hz, 3H). Note: 1H spectrum referenced TMS at 0.00 ppm.

13C NMR: δ 163.4, 163.4, 163.1, 162.8, 128.1, 127.4, 124.0, 121.2, 119.3, 116.7, 116.6, 112.7, 56.8, 52.8, 51.5, 50.1, 39.4, 32.3, 31.4, 24.4, 21.8, 8.1. HRMS (ESI): calcd for C22H24N6O2Na [M + Na]+ 434.1, 429.1, 419.1, 416.1, 414.1, 310.3, 256.3, 243.2, 229.2, 205.2, 192.2, 168.2, 56.8, 52.8, 51.5, 50.1, 39.4, 32.3, 31.4, 24.4, 21.8, 8.1.

Synthesis of 36. Methyl (4aS,12bS)-12-(2-Azidoethyl)-1-cyano-4a-ethyl-1H,2H,3H,4H,4aH,12bH-indolo[1,2-h],7-naphthylidine-6-carboxylate. Compound 10 (256 mg, 0.379 mmol) was added to a flame-dried microwave flask and dissolved in N,N-dimethylformamide (6.00 mL). Sodium azide (301 mg, 4.63 mmol) was then added to the resulting solution, and the reaction mixture was subjected to microwave irradiation at 100 °C for 6 min. Upon completion of this reaction (monitored by TLC), the reaction was cooled down to room temperature, diluted with ethyl acetate, and quenched with brine. The organic layer was collected and dried with sodium sulfate, filtered, and concentrated in vacuo. The crude material was then purified using silica gel column chromatography to yield 36 (174 mg, 73%) as a white solid.

1H NMR: δ 7.26 (m, 1H), 7.23–7.15 (m, 2H), 6.15 (d, J = 1.7 Hz, 1H), 4.11 (d, J = 1.7 Hz, 1H), 3.96 (s, 3H), 3.72 (ddd, J = 12.2, 8.2, 5.7 Hz, 1H), 3.61 (ddd, J = 12.2, 7.9, 4.1 Hz, 1H), 3.48 (dp, J = 10.3, 2.1 Hz, 1H), 3.21–3.03 (m, 3H), 2.13 (dt, J = 13.8, 3.5 Hz, 1H), 1.89–1.70 (m, 2H), 1.42 (td, J = 13.2, 4.2 Hz, 1H), 1.17–1.10 (m, 2H), 0.75 (t, J = 7.5 Hz, 3H). Note: 1H spectrum referenced TMS at 0.00 ppm.

13C NMR: δ 163.4, 163.4, 163.1, 162.8, 128.1, 127.4, 124.0, 121.2, 119.3, 116.7, 116.6, 112.7, 56.8, 52.8, 51.5, 50.1, 39.4, 32.3, 31.4, 24.4, 21.8, 8.1. HRMS (ESI): calcd for C22H24N6O2Na [M + Na]+ 434.1, 429.1, 419.1, 416.1, 414.1, 310.3, 256.3, 243.2, 229.2, 205.2, 192.2, 168.2, 56.8, 52.8, 51.5, 50.1, 39.4, 32.3, 31.4, 24.4, 21.8, 8.1.

Chemical structures of 85 and 36 are shown in Figure 1. The chemical structures of all other compounds are shown in the Supporting Information.

Biology. In Vitro Screening against GPCR Drug Targets and Dose–Response Experiments (Performed at DiscoverX). Path-Hunter β-Agonist Assays. PathHunter cell lines were expanded by agonist challenge at the EC80 concentration. The intermediate determination, cells were preincubated with an antagonist followed by agonist challenge at the EC80 concentration. The final assay determination, cells were incubated with a sample to induce a response.

Intermediate dilution of sample stocks was performed to generate a 5× sample in assay buffer. 5 μL of 5× sample was added to cells and incubated at 37 °C prior to testing. For agonist determination, cells were incubated with a sample to induce a response. Intermediate dilution of sample stocks was performed to generate a 5× sample in assay buffer. 5 μL of 5× sample was added to cells and incubated at 37 °C prior to testing. For agonist determination, cells were incubated with a sample to induce a response.

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Data Analysis. CPP data are presented as the difference in the time spent in drug- and vehicle-associated chambers. All data were analyzed with Student’s t tests or by repeated-measures two-way ANOVA as appropriate. Significant results demonstrated by ANOVA were further analyzed for significance with Tukey’s honestly significant difference (HSD) post hoc test to assess group differences.34 For all repeated measures ANOVA, simple main effects and simple main effect contrasts are presented following significant interactions, with the difference in the time spent on the treatment- vs vehicle-associated side as the dependent measure and conditioning status as the between-groups factor. Effects were considered significant when P < 0.05. All effects are expressed as mean ± standard error of the mean (SEM). Molecular Modeling. Structure Preparation. The three-dimensional (3-D) structure of 4 (V2a) was prepared by LigPrep (Schrodinger, LLC). The human OX2 orexin receptor (hypocretin receptor 2) structures were obtained from Protein Data Bank under the codes 4S0V and 5WQC. The protein structures were pretreated with Protein Preparation Wizard (Schrodinger, LLC) to assign proper protonation states for residues. The cocrystallized ligands, water molecules, and additives were removed. Docking with AutoDock4.2.30 Gasteiger charges were added onto both receptor and ligand atoms. A grid box was centered at where the cocrystallized ligand is (1) Suvorexant in 4S0V structure and (2) EMPA in 5WQC structure, respectively. Dimensions of the box were set to 76 × 76 × 70 grid points with a spacing of 0.375 Å. Lamarckian genetic algorithm (LGA) was used as the search method. All docked conformations were clustered at an RMSD of 1.5 Å. Docking with Glide SP51 and Glide XP52 (Schrodinger, LLC). The grid box center was set as the same as the one used in AutoDock4.2. The outer box dimensions were set to 30 Å × 30 Å × 30 Å, and the inner box dimensions were set to 10 Å × 10 Å × 10 Å. The precision mode was set to either SP or XP. At most, three conformations were required to report. All dockings were performed using the Maestro suite (release of 2016-04).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/10.1021/acs.jmedchem.9b01924.

Supporting figures, supporting (synthetic) schemes, full characterization data [1H, 13C NMR, and two-dimensional (2-D) NMR spectra, high-resolution mass spectra, melting points for solids, observed color and state of compounds] for new compounds, X-ray analyses for select compounds, X-ray analyses for select compounds, X-ray analyses for select compounds, X-ray analyses for select compounds, X-ray analyses for select compounds, X-ray analyses for select compounds, X-ray analyses for select compounds, X-ray analyses for select compounds, X-ray analyses for select compounds, X-ray analyses for select compounds, X-ray analyses for select compounds, and submitted to the Cambridge Crystallographic Data Center (CCDC) website (CCDC codes: 6 (V1p), 158422; 17 (V3r), 1584221; 18 (V3l), 1584220; and 25 (V2i), 1848065).

Accession Codes

The authors will release the atomic coordinates and experimental data upon article publication. X-ray structures for 6 (V1p), 17 (V3r), 18 (V3l), and 25 (V2i) were obtained and submitted to the Cambridge Crystallographic Data Center (CCDC) website (CCDC codes: 6 (V1p), 1584222; 17 (V3r), 1584221; 18 (V3l), 1584220; and 25 (V2i), 1848065).

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Notes

The authors declare no competing financial interest.

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**ABBREVIATIONS**

Avg, average; calcd, calculated; CPP, conditioned place preference; CNBr, cyanogen bromide; Cys, cysteine; DMF, dimethylformamide; GPCR, G protein-coupled receptor; HCRT2, hypocretin receptor 2; i.c.v., intracerebroventricular; μM, micromolar; nmol, nanomoles; Pro, proline; Val, valine

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