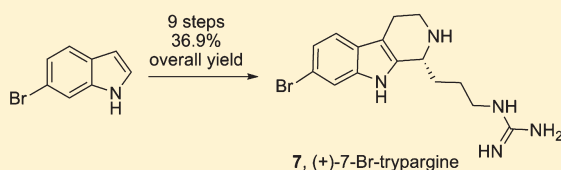


## Total Synthesis of (+)-7-Bromotrypargine and Unnatural Analogues: Biological Evaluation Uncovers Activity at CNS Targets of Therapeutic Relevance

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## Supporting Information

## ABSTRACT:



Target	% Inhibition (@10 $\mu$ M)	$K_i$ ( $\mu$ M)	$IC_{50}$ ( $\mu$ M)
H <sub>3</sub>	74	1.8	3.6
DAT	81	3.0	3.8
NET	82	1.9	1.9

The first total synthesis of (+)-7-bromotrypargine, a  $\beta$ -carboline alkaloid from *Ancornia* sp. is reported. The synthesis proceeds in nine steps, eight steps longest linear sequence, in 36.9% overall yield. Biological characterization found that (+)-7-bromotrypargine is a H<sub>3</sub> antagonist, and a selective inhibitor of the dopamine transporter (DAT) and norepinephrine transporter (NET), without inhibiting the serotonin transporter (SERT). Moreover, unlike electron rich congeners, (+)-7-bromotrypargine is not cytotoxic and thus represents an attractive starting point for chemical optimization; therefore, we piloted a number of chemistries for the synthesis of unnatural analogues.

Drug discovery based on natural products, and marine natural products in particular, has been a highly successful approach toward novel lead series and marketed therapeutics for both peripheral and central nervous system (CNS) indications.<sup>1,2</sup> A number of marine natural products, such as aplysamine **1**,<sup>3</sup> verogamine **2**,<sup>4</sup> conessine **3**,<sup>5</sup> and dispyrin **4**,<sup>6,7</sup> have displayed both affinity for and inhibition of the histamine subtype 3 (H<sub>3</sub>) receptor (Figure 1).<sup>8–10</sup> The H<sub>3</sub> receptor is a presynaptic autoreceptor within the Class A GPCR family, but it also functions as a heteroreceptor modulating levels of neurotransmitters such as dopamine, acetylcholine, norepinephrine, serotonin, GABA, and glutamate.<sup>8–10</sup> Thus, H<sub>3</sub>R has garnered a great deal of interest from the pharmaceutical industry for the possible treatment of obesity, epilepsy, sleep/wake, schizophrenia, Alzheimer's disease, neuropathic pain, and ADHD.<sup>8–10</sup> Importantly, conserved elements have been identified within small molecule H<sub>3</sub> ligand scaffolds that resulted in a highly predictive pharmacophore model, and many marine natural products conform to this model.<sup>8–10</sup>

$\beta$ -Carboline alkaloids are a prevalent class of biologically active natural products from marine organisms. They exhibit diverse structural features and distinct neuropharmacological profiles.<sup>11,12</sup> Our lab has worked extensively in this arena,<sup>13,14</sup> and we were recently attracted to a class of  $\beta$ -carboline alkaloids represented by the trypargines **5–7** (Figure 2), as these alkaloids mapped well onto the H<sub>3</sub> pharmacophore model and offered a synthetic

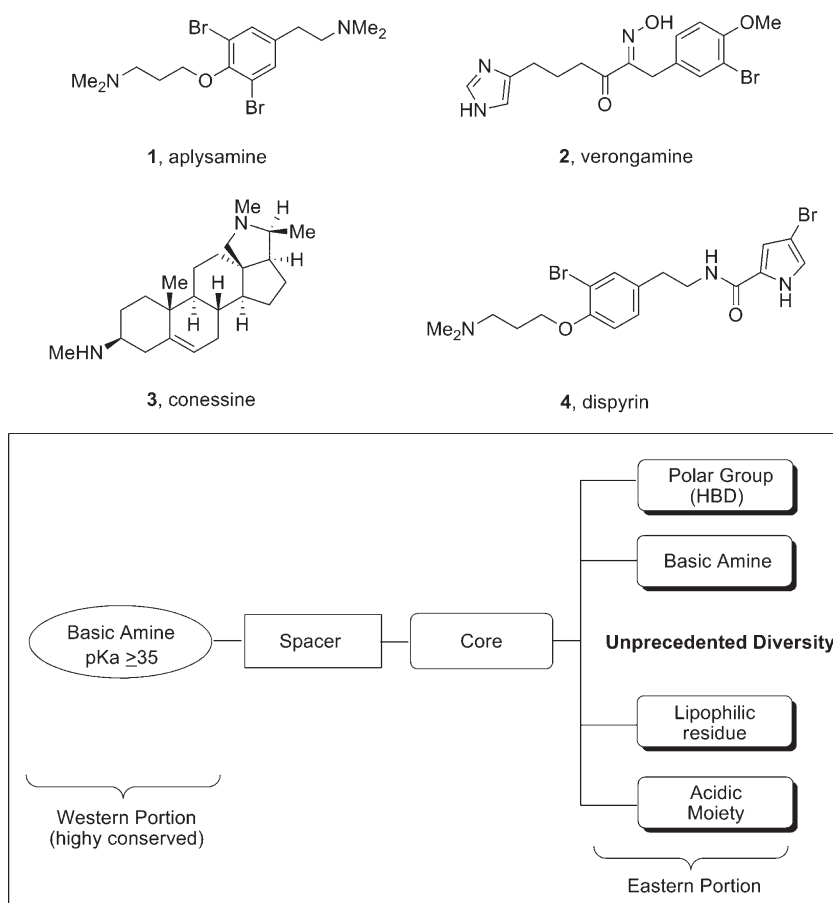
challenge.<sup>15,16</sup> Both trypargine **5**<sup>15</sup> and 6-hydroxytrypargine **6**<sup>16</sup> are highly toxic alkaloids. (+)-7-Bromotrypargine **7**, was only recently isolated by Quinn et al. from the Australian marine sponge *Ancornia* sp. and found to possess antimalarial activity.<sup>17</sup> 6-Bromotryptamine **8** was isolated along with **7** in similar quantities, and it is believed to be a key biosynthetic precursor.<sup>17</sup> Based on the neuropharmacological profiles of  $\beta$ -carboline alkaloids, and the electron-deficient nature of **7**, relative to the electron-rich congeners **5** and **6**, which might diminish the cytotoxicity, the total synthesis of **7** and biological evaluation seemed warranted.

Our retrosynthesis of **7** (Figure 3) afforded two approaches to close the tricyclic ring: either utilizing the recently reported Brønsted-acid-catalyzed, enantioselective Protio–Pictet–Spengler reaction<sup>18,19</sup> or a Bischler–Napieralski reaction,<sup>14</sup> followed by an asymmetric Noyori transfer hydrogenation protocol.<sup>20</sup> Both routes required the synthesis of 6-bromotryptamine **8**. As shown in Scheme 1, nitro-olefination of 6-bromo-1H-indole **9**, as prescribed by Büchi and Mak,<sup>21</sup> proceeded smoothly affording **10** in 98% yield. Exhaustive reduction then provided natural 6-bromotryptamine **8** in 76% yield.

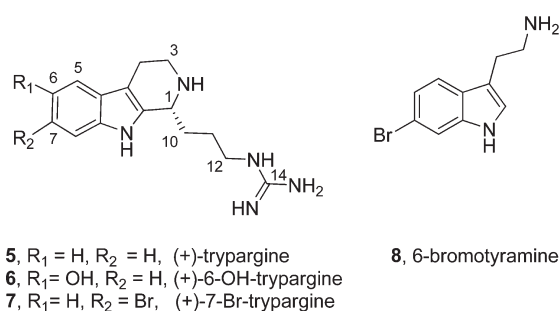
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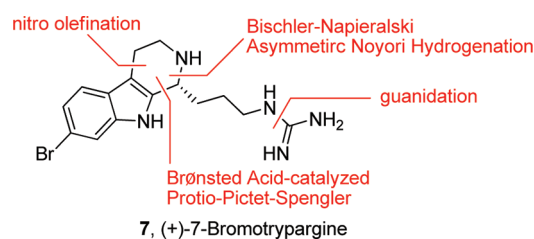


**Figure 1.** Marine natural products 1–4 with significant affinity for and inhibition of  $H_3$ , a target of interest for numerous CNS indications. Inset: refined  $H_3$  pharmacophore model, to which synthetic small molecule and natural product  $H_3$  antagonists conform.



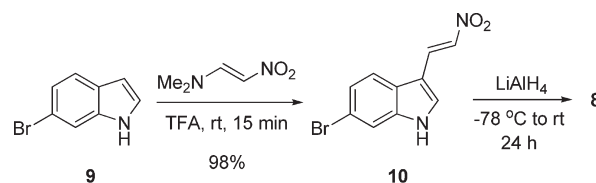
**Figure 2.** Structures of the (+)-tryptamines 5–7 and 6-bromotryptamine 8.

With large quantities of **8** in hand, we evaluated both the Jacobsen thiourea Brønsted-acid-catalyzed, enantioselective Protio–Pictet–Spengler reaction<sup>18</sup> as well as the Dixon chiral BINOL-derived phosphoric acid variation<sup>19</sup> with known aldehyde **11** to produce the key, chiral  $\beta$ -carboline core **13** (Scheme 2). Interestingly, neither protocol afforded **13**; however, electron rich congeners of **8**, such as **12**, proceeded smoothly, providing **14** in good % ee and conversion. Thus, the Brønsted-acid-catalyzed, enantioselective Protio–Pictet–Spengler approach with the more electron deficient **8** was not tractable. Indeed, the original reports by Jacobsen and Klausen, and Dixon et al. utilized only electron rich substrates.<sup>18,19</sup> Conversion to **13** could be forced under both protocols (~80% yield) at high temperatures, but enantioselectivity



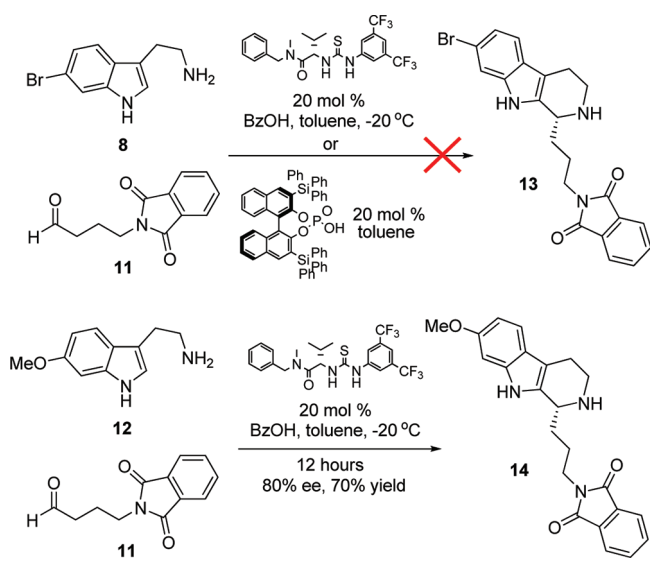
**Figure 3.** Retrosynthesis of (+)-7-bromotryptamine (**7**).

### Scheme 1. Synthesis of 6-Bromotryptamine (**8**)



eroded. Thus, focus shifted toward a Bischler–Napieralski/Noyori asymmetric transfer hydrogenation approach.<sup>14,20</sup>

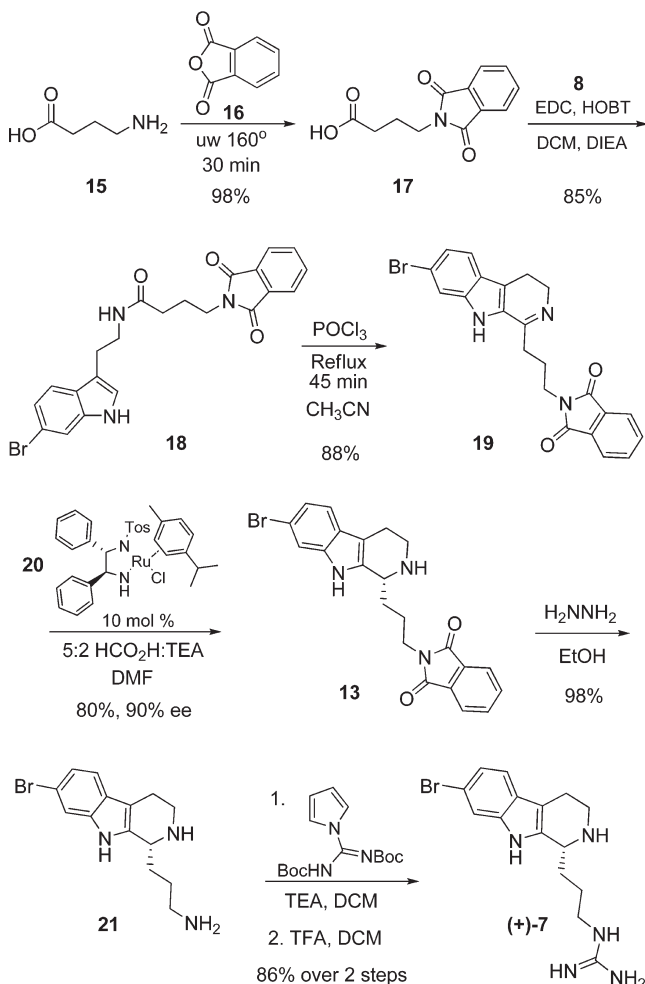
Key acid **17** was prepared in 98% yield by treating 4-amino-butyanoic acid **15** with anhydride **16** under microwave conditions (Scheme 3). Standard amide coupling conditions with **8** and **17**

Scheme 2. Brønsted-Acid-Catalyzed, Enantioselective Protio–Pictet–Spengler Approach to  $\beta$ -Carboline Core 13

provided amide **18**. The Bischler–Napieralski reaction<sup>14,20</sup> was facilitated by treatment with  $\text{POCl}_3$  to imine **19** in 88% yield. Noyori asymmetric transfer hydrogenation with (*S,S*)-ruthenium catalyst **20** delivered the  $\beta$ -carboline core **13** in 80% yield and 90% ee.<sup>20,22</sup> Removal of the phthalimide moiety with hydrazine to afford amine **21**, guanidation, and removal of the Boc protecting groups proceeded in 84% yield over the three steps, delivering, for the first time, (+)-7-bromotryptargine **7**. Synthetic **7** exhibited physical and spectroscopic data identical to that of natural **7**,<sup>17,22</sup> confirming the structure and absolute stereochemistry. Overall, the synthesis proceeds in nine steps, eight steps longest linear sequence, in 36.9% overall yield which provided significant material to enable detailed biological evaluation.

As **5** and **6** are highly toxic alkaloids, we first evaluated **7** in a standard cytotoxicity assay and found **7** to be nontoxic up to 20  $\mu\text{M}$ , suggesting the pharmacological profile might diverge from **5** and **6**.<sup>23</sup> This surprising result led us to study **7** in our standard HCT116 colon carcinoma cell viability assay.<sup>22–24</sup> Here, as well, **7** had no effect on HCT116 cell viability after 48 h. In contrast, advanced intermediate **21**, an unnatural analogue of **7**, displayed an  $\text{IC}_{50}$  of 3  $\mu\text{M}$  in this assay, completely killing the cells after 48 h. These data prompted examination of unnatural analogue **21** in additional colon carcinoma (SW620 and H520) cell lines.<sup>25</sup> Interestingly, **21** had dramatic effects inhibiting proliferation in BrdU assays as well as significant activity on cell viability.<sup>22</sup> In contrast, **7** was devoid of activity in all of these assays. Collectively, these data informed us of two important points: (1) the pharmacology of the more electron deficient (+)-7-bromotryptargine (**7**) is distinct from **5** and **6** and warrants further biological evaluation, as it lacks toxicity; and (2) unnatural analogue **21** possesses an intriguing pharmacological profile warranting the synthesis and characterization of additional unnatural analogues of **7**.

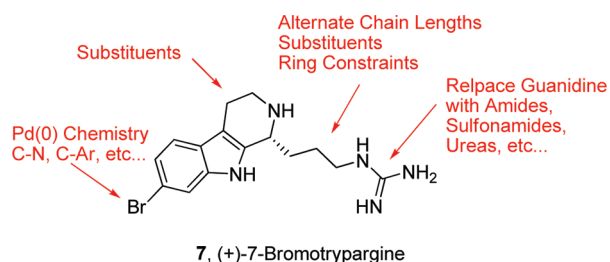
(+)-7-Bromotryptargine (**7**) was then evaluated in an external panel<sup>26</sup> of 68 GPCRs, ion channels, and transporters in radioligand binding assays in an attempt to identify a discrete molecular target with therapeutic relevance, a strategy that has been highly successful.<sup>6,7</sup> In this instance, **7** was found to have a very clean pharmacological profile, affording significant percent

Scheme 3. Synthesis of (+)-7-Bromotryptargine (**7**)Table 1. Pharmacological Profile of (+)-7-Bromotryptargine (**7**)

target	% inhibition (@10 $\mu\text{M}$ )	$K_i$ ( $\mu\text{M}$ )	$\text{IC}_{50}$ ( $\mu\text{M}$ )
H <sub>3</sub>	74	1.8	3.6
DAT	81	3.0	3.8
NET	82	1.9	1.9

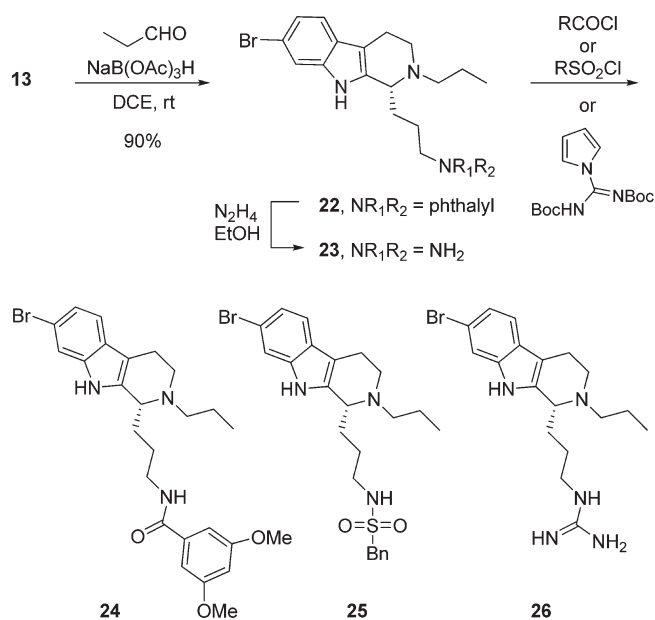
inhibition at only three targets: histamine H<sub>3</sub> (74% @ 10  $\mu\text{M}$ ), the dopamine transporter, DAT (81% @ 10  $\mu\text{M}$ ), and the norepinephrine transporter, NET (82% @ 10  $\mu\text{M}$ ). Interestingly, these are all targets for important pathologies of the CNS, and **7** did prove to possess activity at the H<sub>3</sub> receptor as envisioned. Indeed, **7** is now the fifth marine natural product with significant activity at H<sub>3</sub>, a target of interest for Alzheimer's disease, schizophrenia, ADHD, and other indications.<sup>3–6</sup> Full dose–response curves were generated for the three targets in both binding ( $K_i$ ) and functional ( $\text{IC}_{50}$ ) assays,<sup>22</sup> proving that **7** both bound to and inhibited the three targets with moderate potencies (Table 1).

(+)-7-Bromotryptargine (**7**) possessed a unique and rare pharmacological profile. There are three monoamine transporters (MATs) that control the extracellular levels of the monoamine neurotransmitters.<sup>27</sup> The three major MATs (SERT, DAT, and NET) control the reuptake of serotonin, dopamine, and



**Figure 4.** Diversity plan for unnatural analogues of (+)-7-bromotryptargine (7).

**Scheme 4. Synthesis of Unnatural Analogues 24–26 of (+)-7-Bromotryptargine (7)**



norepinephrine, respectively. Drugs targeting MATS are blockbuster and target either serotonin reuptake (SSRIs), serotonin and norepinephrine reuptake (SNRIs), or uptake of all three (triple reuptake inhibitors).<sup>27</sup> To date, only one drug, methylphenidate (Ritalin), targets only NET and DAT,<sup>28</sup> making 7 a novel chemotype, and a most attractive starting point for further optimization for dual NET/DAT activity and/or H<sub>3</sub> activity.

In order to optimize the dual NET/DAT activity, we would need to have reliable chemistry to prepare unnatural analogues of 7 (Figure 4) in short order, while also removing functionality, such as the guanidine, that would limit brain penetration. Thus, we piloted a number of “classical” reactions (reductive amination, acylation, sulfonylation, Suzuki coupling, etc.) that are mainstays in the pharmaceutical industry en route to unnatural analogues of 7 (Scheme 4). Prior to removal of the phthalimide moiety, the secondary amine 13 could undergo a reductive amination reaction to provide tertiary amine 22 in 90% yield. Removal of the phthalimide moiety liberated a primary amine 23 that was smoothly acylated 24, sulfonylated 25, or guanidated 26 to afford unnatural analogues of 7 in excellent isolated yields (>85%).

In summary, we have completed the first total synthesis of (+)-7-bromotryptargine (7) in nine steps (eight steps longest linear sequence) in 36.9% overall yield from commercial materials.

In the course of this work, we found that the Brønsted-acid-catalyzed, enantioselective Protio–Pictet–Spengler reaction works well for electron-rich substrates but fails for electron-deficient cores, and thus, it was ineffective in accessing 7. Biological evaluation of 7 and an advanced intermediate 21 proved very exciting, with 7 displaying divergent pharmacology from related β-carbolines 5 and 6, while 21 was extremely cytotoxic in multiple nontransformed and colon cancer cell lines. Importantly, receptor profiling efforts identified 7 as a moderately potent, dual NET/DAT inhibitor, and only the second known compound, and chemotype, to display such a pharmacological profile devoid of serotonin transporter (SERT) activity. This finding was in addition to the anticipated activity as a H<sub>3</sub> antagonist, based on the H<sub>3</sub> pharmacophore model. The intriguing pharmacological profile led us to then explore chemistry to access unnatural analogues. These reactions will serve as the groundwork for a larger effort aimed at unnatural analogue synthesis to develop structure–activity relationships (SARs) around 7 and to optimize dual NET/DAT activity. Efforts toward these aims are in progress and will be reported in due course.

## METHODS

**General.** The general chemistry, experimental information, and spectral data of all new compounds are supplied in the Supporting Information. Purity of all final compounds was determined by HPLC analysis and is >98%.

**Total Synthesis of (+)-Bromotryptargine (7).** 6-Bromo-3-(2-nitrovinyl)-1H-indole (10). 1-(Dimethylamino)-2-nitroethylene (1.6 g, 13.72 mmol) was dissolved in 20 mL of trifluoroacetic acid and stirred for 5 min. 6-Bromoindole (9) (3.23 g, 16.47 mmol) was dissolved in 15 mL of dichloromethane (DCM) and added to the stirring solution. The mixture was stirred for 15 min at room temperature and monitored for loss of starting indole by thin layer chromatography. After 15 min, the reaction was transferred to a 1 L separatory funnel, rinsing with DCM and then quenched with 200 mL water. The aqueous phase was extracted three times with DCM, and the combined organic phase was dried over MgSO<sub>4</sub>, filtered, and concentrated onto 10 g of silica. The product was purified on silica (EtOAc/hexanes 1:2) and yielded 3.61 g (98%) 6-bromo-3-(2-nitrovinyl)-1H-indole as a yellow solid. <sup>1</sup>H NMR (600.1 MHz, DMSO) δ (ppm): 8.39 (d, J = 13.5 Hz, 1H), 8.25 (s, 1H), 8.02 (d, J = 13.5 Hz, 1H), 7.95 (d, J = 8.51 Hz, 1H), 7.71 (d, J = 1.75 Hz, 1H), 7.35 (dd, J = 1.85, 8.51 Hz, 1H). <sup>13</sup>C NMR (150 MHz, DMSO) δ (ppm): 138.9, 137.1, 134.4, 132.3, 124.9, 124.1, 122.6, 116.3, 115.8, 108.6. HRMS (TOF, ES+) C<sub>10</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>Br [M+H]<sup>+</sup> calcd, 266.9769; found, 266.9769.

**6-Bromotryptamine (8).** A 250 mL Schlenk flask equipped with a stir bar and septa was flame-dried under vacuum. LiAlH<sub>4</sub> (2.03 g, 53.69 mmol) was added, and the flask then was sealed and vacuum purged three times, refilling with argon. Tetrahydrofuran (THF; 89 mL) was added, and the solution was stirred 5 min at room temperature before being cooled to −78 °C for 10 min. 6-Bromo-3-(2-nitrovinyl)-1H-indole 10 (2.39 g, 8.94 mmol) was dissolved in 89 mL of THF and added dropwise via syringe to the stirred solution. The reaction was allowed to warm to room temperature overnight. The reaction was monitored by thin layer chromatography over 24 h. After 24 h, the flask was placed in an ice bath and cooled for 5 min before the careful addition of 2 mL of water, followed by 2 mL of 15% aq. NaOH, followed by 6 mL of water. The flask was stirred for 30 min, and a small amount of MgSO<sub>4</sub> was added. The mixture was then filtered through a glass frit, washing the formed solid with dichloromethane. The resulting brown solution was then concentrated to afford a crude brown oil. The product was purified on silica (80:18:2 CHCl<sub>3</sub>/MeOH/NH<sub>4</sub>OH) and yielded 1.63 g (76%) 8

as a brown solid.  $^1\text{H}$  NMR (600.1 MHz, MeOD)  $\delta$  (ppm): 7.56 (d,  $J$  = 1.65 Hz, 1 H), 7.51 (d,  $J$  = 8.51 Hz, 1H), 7.21 (s, 1H), 7.18 (d,  $J$  = 7.51 Hz, 1H), 3.24 (t,  $J$  = 7.63 Hz, 2H), 3.12 (t,  $J$  = 7.63 Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, MeOD)  $\delta$  (ppm): 137.5, 126.2, 122.9, 121.2, 119.2, 114.3, 113.6, 112.4, 41.5, 27.8. HRMS (TOF, ES+)  $\text{C}_{10}\text{H}_{12}\text{N}_2\text{Br}$  [M+H]<sup>+</sup> calcd, 239.0184; found, 239.0182.

*N*-(2-(6-Bromo-1H-indol-3-yl)ethyl)-4-(1,3-dioxoisindolin-2-yl)butanamide (**18**). 6-Bromotryptamine (**8**) (1.0 g, 4.18 mmol), 4-(1,3-dioxoisindolin-2-yl)butanoic acid (**17**) (1.17 g, 5.01 mmol), EDC (1.60 g, 8.36 mmol), and HOBT (1.12 g, 8.36 mmol) were dissolved in 30 mL of 9:1 DCM/DIEA solution. The mixture was stirred for 12 h. Analysis by LCMS showed product conversion. The contents of the flask were transferred to a 500 mL separatory funnel, 150 mL of water was added, and the solution was extracted three times with DCM. The combined organic phase was dried over  $\text{MgSO}_4$  and concentrated under reduced pressure. The product was purified on silica (2% MeOH in DCM) and isolated **18** as a yellow solid 1.61 g (85%).  $^1\text{H}$  NMR (600.1 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 8.09 (s, 1H), 7.78 (dq,  $J$  = 8.45, 57.8 Hz, 4H), 7.52 (d,  $J$  = 1.65 Hz, 1H), 7.48 (d,  $J$  = 8.45 Hz, 1H), 7.22 (dd,  $J$  = 1.65, 8.45 Hz, 1H), 7.07 (d,  $J$  = 2.25 Hz, 1H), 5.93 (s, 1H), 3.69 (t,  $J$  = 6.36 Hz, 2H), 3.59 (q,  $J$  = 6.79, 6.10 Hz, 2H), 2.97 (t,  $J$  = 6.91 Hz, 2H), 2.17 (t,  $J$  = 7.13 Hz, 2H), 2.01 (p,  $J$  = 6.91 Hz, 2H), 1.58 (s, 1H).  $^{13}\text{C}$  NMR (150 MHz, MeOD)  $\delta$  (ppm): 171.7, 168.5, 137.0, 134.0, 131.9, 126.3, 123.2, 122.7, 122.5, 120.0, 115.7, 114.0, 113.3, 39.6, 37.1, 33.8, 25.1, 24.8. HRMS (TOF, ES+)  $\text{C}_{22}\text{H}_{21}\text{N}_3\text{O}_3\text{Br}$  [M+H]<sup>+</sup> calcd, 454.0766; found, 454.0764.

2-(3-(7-Bromo-4,9-dihydro-3H-pyrido[3,4-b]indol-1-yl)propyl)isindoline-1,3-dione (**19**). *N*-(2-(6-Bromo-1H-indol-3-yl)ethyl)-4-(1,3-dioxoisindolin-2-yl)butanamide (**18**) (1.23 g, 2.71 mmol) was placed in a 250 mL round-bottom flask. Acetonitrile (25 mL) was added, followed by  $\text{POCl}_3$  (2.08 g, 1.67 mmol). The flask was then brought to reflux for 1 h. Starting material loss and product conversion were monitored by TLC and LCMS. After 1 h, the flask was removed from the heating bath, and the excess  $\text{POCl}_3$  was evaporated under reduced pressure. The flask contents were then transferred to a 500 mL separatory funnel, washing with DCM, 100 mL of water was added, and the solution was extracted three times with DCM. The combined organic phase was concentrated and purified on silica (30:1  $\text{CHCl}_3/\text{MeOH}$ ) to afford 1.043 g (88%) **19**.  $^1\text{H}$  NMR (600.1 MHz, MeOD)  $\delta$  (ppm): 7.73 (s, 4H), 7.66 (d,  $J$  = 1.49 Hz, 1H), 7.54 (d,  $J$  = 8.74 Hz, 1H), 7.27 (dd,  $J$  = 1.51 Hz, 8.74, 1H), 3.94 (t,  $J$  = 8.97 Hz, 2H), 3.83 (t,  $J$  = 6.49 Hz, 2H), 3.15 (t,  $J$  = 9.08 Hz, 2H), 3.12 (t,  $J$  = 8.93 Hz, 1H), 2.28 (p,  $J$  = 7.03 Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz, MeOD)  $\delta$  (ppm): 169.5, 168.2, 141.8, 134.0, 131.6, 125.8, 135.1, 125.0, 122.9, 122.85, 122.81, 122.6, 115.5, 42.2, 36.5, 29.9, 25.5, 18.2. HRMS (TOF, ES+)  $\text{C}_{22}\text{H}_{19}\text{N}_3\text{O}_2\text{Br}$  [M+H]<sup>+</sup> calcd, 436.0661; found, 436.0663.

(*R*)-2-(3-(7-Bromo-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)propyl)isindoline-1,3-dione (**13**). A 100 mL round-bottom flask was charged with **18** (893 mg, 2.046 mmol) and **20** (118.4 mg, 0.204 mmol), and DMF (7.8 mL) was added. The flask was cooled to 0 °C, and a solution of 5:2 formic acid/triethylamine (1.75 mL) was added. The flask was sealed, stirred, and allowed to warm overnight. Reaction progress was monitored by LCMS. After 12 h, the flask contents were transferred to a 250 mL separatory funnel, washing with DCM. Water (50 mL) was added, and the solution was extracted three times with DCM. The combined organic phase was dried over  $\text{MgSO}_4$  and concentrated. The product was purified on silica (30:1  $\text{CHCl}_3/\text{MeOH}$ ) and yielded 717.4 mg (80%) **13** as a golden-brown solid.  $[\alpha]_{\text{D}}^{23}$  35.5° ( $c$  = 1.0, MeOH).  $^1\text{H}$  NMR (600.1 MHz, MeOD)  $\delta$  (ppm): 7.85 (m, 4H), 7.49 (d,  $J$  = 1.56 Hz, 1H), 7.38 (d,  $J$  = 8.50 Hz, 1H), 7.17 (dd,  $J$  = 1.56, 8.50 Hz, 1H), 4.75 (m, 1H), 3.84 (t,  $J$  = 6.51 Hz, 2H), 3.73 (m, 1H), 3.44 (m, 1H), 3.04 (m, 2H), 2.30 (m, 1H), 1.96 (m, 3H).  $^{13}\text{C}$  NMR (150 MHz, MeOD)  $\delta$  (ppm): 168.4, 137.5, 134.0, 131.8, 129.3, 124.8, 122.7, 122.3, 119.1, 115.4, 113.7, 106.2, 52.8, 41.3, 36.6, 29.0, 23.9, 17.8. HRMS

(TOF, ES+)  $\text{C}_{22}\text{H}_{21}\text{N}_3\text{O}_2\text{Br}$  [M+H]<sup>+</sup> calcd, 438.0817; found, 438.0814.

(*R*)-3-(7-Bromo-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)propan-1-amine (**21**). A 100 mL round-bottom flask was charged with **13** (514 mg, 1.17 mmol). EtOH was added (10 mL), followed by hydrazine hydrate (368  $\mu\text{L}$ , 11.72 mmol). The flask was brought to reflux for 45 min, monitoring by LCMS. After 45 min, the flask was cooled and the contents were concentrated to remove ethanol. The product was purified on silica (80:18:2  $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ ) and yielded **21** 317.3 mg (98%) as a brown solid.  $[\alpha]_{\text{D}}^{23}$  28.5° ( $c$  = 2.0, MeOH).  $^1\text{H}$  NMR (600.1 MHz, MeOD)  $\delta$  (ppm): 7.56 (d,  $J$  = 1.58 Hz, 1H), 7.39 (d,  $J$  = 8.43 Hz, 1H), 7.17 (dd,  $J$  = 1.58, 8.43 Hz, 1H), 4.77 (q,  $J$  = 4.41 Hz, 1H), 3.75 (m, 1H), 3.49 (m, 1H), 3.07 (m, 4H), 2.34 (m, 1H), 2.11 (m, 1H), 1.97 (m, 2H).  $^{13}\text{C}$  NMR (150 MHz, MeOD)  $\delta$  (ppm): 161.1, 160.9, 137.6, 129.1, 124.9, 122.4, 119.2, 115.4, 113.9, 106.5, 52.5, 41.2, 38.7, 28.6, 22.8, 17.8. HRMS (TOF, ES+)  $\text{C}_{14}\text{H}_{19}\text{N}_3\text{Br}$  [M+H]<sup>+</sup> calcd, 308.0862; found, 308.0759.

(+)-7-Bromotryptargine (**7**). A 50 mL round-bottom flask was charged with **21** (231 mg, 0.749 mmol) and DCM (3 mL).  $N,N'$ -Bis(Boc)-1H-pyrazole-1-carboxamide (255.8 mg, 0.824 mmol) and triethylamine (156  $\mu\text{L}$ , 1.12 mmol) were then added. The flask was stirred for 3 h and monitored by TLC and LCMS. After 3 h, 5 mL of trifluoroacetic acid was added and the reaction was stirred 4 h. Reaction progress was monitored by LCMS. After 4 h, the reaction was concentrated and the product was purified by reverse phase high performance liquid chromatography using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10) and yielded 225 mg (86% over two steps) **3** as a glassy yellow solid.  $[\alpha]_{\text{D}}^{23}$  40.0° ( $c$  = 1.0, MeOH).  $^1\text{H}$  NMR (600.1 MHz, DMSO)  $\delta$  (ppm): 11.3 (s, 1H), 9.49 (s, 1H), 9.00 (s, 1H), 7.73 (br s, 1H), 7.56 (d,  $J$  = 1.68 Hz, 1H), 7.46 (d,  $J$  = 8.50 Hz, 1H), 7.20 (br s, 2H), 7.18 (br s, 2H), 7.18 (dd,  $J$  = 1.68, 8.50 Hz, 1H), 4.69 (s, 1H), 3.58 (m, 1H), 3.34 (m, 1H), 3.18 (m, 2H), 2.93 (t,  $J$  = 5.5 Hz, 2H), 2.11 (m, 1H), 1.90 (m, 1H), 1.69 (m, 1H).  $^{13}\text{C}$  NMR (150 MHz, DMSO)  $\delta$  (ppm): 157.2, 137.5, 131.4, 125.2, 122.4, 120.3, 118.5, 114.9, 114.3, 106.6, 52.2, 40.9, 40.7, 28.9, 24.7, 18.4. HRMS (TOF, ES+)  $\text{C}_{15}\text{H}_{21}\text{N}_5\text{Br}$  [M+H]<sup>+</sup> calcd, 350.0980; found, 350.0982.

**Cell Culture and HCT116 Viability Assay.** HCT116 cells (ATCC CCL-247, passage 8–18, mycoplasma-negative on 07/14/09 by Lonza MycoAlert mycoplasma detection assay) were grown at 37 °C in a humidified incubator with 5%  $\text{CO}_2$  in DMEM + 10% FBS. Cells were plated in growth medium at 8000 cells per well (100  $\mu\text{L}$ ) in 96-well plates and allowed to attach for 24 h. No cells were plated in the first column. Fresh DMEM + 10% FBS + penicillin/streptomycin (100  $\mu\text{L}$ ) containing the final concentrations of dimethyl sulfoxide (DMSO) or epi-Lucentamycin A or podophyllotoxin dilutions in DMSO (DMSO concentration maintained at 0.1%) was replaced in each well. After 48 h of cell growth with respective treatments, WST-1 Cell Proliferation Reagent (Roche, 11644807001) was added (10  $\mu\text{L}$ ) to each well for 45–60 min at 37 °C. The plates were read (Molecular Devices) at 450 nm for the formazan product and at 690 nm as a reference. Background absorbance (medium only plus WST-1, first column) was averaged and subtracted from all values. Six replicates were used in the calculations per treatment concentration in duplicate experiments. Data was plotted as a percentage of metabolism of WST-1 in the DMSO-treated cells. A colorectal adenocarcinoma cell line, SW620, and a squamous cell lung carcinoma cell line, H520, were obtained from the American Type Culture Collection (Manassas, VA) and maintained in a humidified atmosphere of 5%  $\text{CO}_2$  in air at 37 °C. The cells were routinely cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Atlanta Biologicals, GA) and 100  $\mu\text{g}/\text{mL}$  penicillin-streptomycin.

**Proliferation Analysis.** SW620 and H520 cells ( $2.5 \times 10^4/100 \mu\text{L}$ ) were seeded in 96-well microtiter plates prior to treatment. Cells were treated with 10  $\mu\text{M}$  concentration of synthesized compound in triplicate for 48 h in RPMI 1640 supplemented medium and 100  $\mu\text{g}/\text{mL}$

penicillin-streptomycin. The CycLex Cellular BrdU ELISA Kit from MBL International (Woburn, MA) was used to measure proliferation. The RPMI medium was removed and replaced with 100  $\mu\text{L}$  of  $1 \times$  BrdU label mix in RPMI media for 2 h at 37  $^{\circ}\text{C}$  in 5%  $\text{CO}_2$  in the air. The BrdU label mix was removed, and 200  $\mu\text{L}$  of the fix/denature solution was added and incubated for 30 min at room temperature. The plate was drained, incubated with 50  $\mu\text{L}$  of primary antibody for 1 h at room temperature, rinsed with wash buffer, and incubated with 50  $\mu\text{L}$  of secondary antibody. The wells were rinsed with wash buffer followed by a single rinse with phosphate-buffered saline and drained. Fifty microliters of substrate solution was added and incubated for 6 min followed immediately by 50  $\mu\text{L}$  of stop solution. The change in proliferation was quantified by measuring the absorbance of the dye solution at 450 nm on a microtiter plate reader.

**Invasion Analysis.** SW620 cells ( $2.5 \times 10^5$ /mL) were seeded in 6-well plates prior to treatment. Cells were treated with 10  $\mu\text{M}$  concentration of synthesized compound for 24 h in RPMI 1640 supplemented medium and 100  $\mu\text{g}/\text{mL}$  penicillin-streptomycin. An amount of 40  $\mu\text{L}$  (2.5 mg/mL) of BD Matrigel Basement Membrane Matrix (BD Biosciences, Bedford, MA) was added to top of Fluoroblok invasion chambers (BD Biosciences). Then the cells were trypsinized and  $3 \times 10^5$ /250  $\mu\text{L}$  cells were added to the top of the chamber in serum-free RPMI medium, and 1 mL of RPMI medium with 10% FBS was added to the bottom of the well. Then the plates were incubated for 72 h at 37  $^{\circ}\text{C}$  in 5%  $\text{CO}_2$  in air. Invading cells were stained with Calcein AM as a fluorescent dye to count invading cells using a fluorometer (Molecular Devices Spectramax M5). All experiments were done in triplicate with a total of three independent replicates.

**Radioligand Binding and Functional Assays.** All biological assays were conducted at Ricerca Pharma according to published protocols.<sup>26</sup>

## ■ ASSOCIATED CONTENT

**S Supporting Information.** Experimental procedures and spectroscopic data for selected compounds and detailed pharmacology methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Author Contributions

C.W.L. conceived and directed the project and wrote the manuscript. J.T.B. performed all chemical synthesis. S.L.S. and B.C.C. performed cytotoxicity assays, and L.J.M. directed the efforts of S.L.S. and B.C.C.

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