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Synthesis and Cytotoxicity of Sempervirine and Analogues

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Abstract

Sempervirine and analogues were synthesized using a route featuring Sonogashira and Larock Pd-catalyzed reactions. Structure–activity relationships were investigated using three human cancer cell lines. 10-Fluorosempervirine is the most potently cytotoxic member of the family yet described.

Graphical abstract

Many natural products and their derivatives are important therapeutic agents, particularly in the treatment of infections and cancers.¹ Modern synthetic methods, especially those not relying upon the intrinsic reactivity of groups present in the natural material, have lowered the barrier to conducting SAR studies in natural product scaffolds to improve efficacy and therapeutic index. We recently reported a general approach to the synthesis of tetracyclic and pentacyclic alkaloids² featuring highly substrate-tolerant Sonogashira³ and Larock⁴ reactions. Here we report using the route for an SAR study in the sempervirine series.

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Notes

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Supporting Information

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¹H and ¹³C NMR spectra for new compounds (PDF)

Sempervirine has attracted significant interest since the pioneering structure elucidation⁵ and synthetic work⁶ of Woodward, who proposed its canonical forms (Figure 1). Several elegant syntheses have been reported, notably by Swan,⁷ Westphal,⁸ Potts,⁹ and Gribble.¹⁰ It has been targeted due to its antitumor activity, arising from topoisomerase 1 inhibition, DNA intercalation, and other mechanisms.¹¹ Routes to sempervirine generally involve moderately long synthetic sequences, proceed with a modest overall yield, and have limitations in scope for preparing the natural material or a small set of analogues.

Previous sempervirine analogues prepared include Woodward's indole *N*-methyl derivative⁶ and Lipi ska's three analogues, wherein the E-ring (Figure 1) was replaced by fused cyclopentyl, cycloheptyl, and cyclooctyl rings, with each product obtained in six steps in about 4% overall yield, using a route featuring an inverse electron demand Diels–Alder reaction between a 1,2,4-triazine diene and a cyclic enamine.¹² Lipi ska later reported four C10 methoxy analogues having five-, six-, seven-, and eight-membered E-rings, in 2–10% yield by a route using a Fischer indole synthesis¹² and Gribble's method¹⁰ for C-ring construction. Malhotra et al. used a microwave-assisted version of Westphal's method⁸ to prepare the C11 methoxy analogue in 32% yield,¹³ a compound previously made by Huebner et al.¹⁴ Malhotra et al. also prepared a compound in 42% yield, wherein the E-ring was replaced with two phenyl groups.¹³ To our knowledge, the biological activity for these compounds is unreported.

Our route uses an initial regioselective semireduction of 3-isoquinolone (**2**, Scheme 1). Literature reduction methods required excesses of expensive and harsh reagents (SbF₃ or SbF₅ in CF₃SO₃H).¹⁵ In a solvent survey using 1 mol % of PtO₂, acetic acid gave unwanted pyridone ring saturation. Adding TFA favored the desired phenyl ring reduction, with 10:1 TFA/triflic acid giving full regioselectivity. The triflate **3** was then prepared (94% yield, two steps) and used in a Sonogashira reaction (92%) followed by a Larock indole synthesis reaction that proceeded with high regioselectivity¹⁶ and then a triflate-promoted cyclization (91%, two steps).² DDQ oxidation provided sempervirine triflate **1** (96%). The route is six steps and 76% yield, with intermediate **8** and the natural product purified by precipitation. Given the efficiency, ease of purification, and expected substrate tolerances, we felt that an SAR study for sempervirine was feasible.

Altering ring size within the core is a drastic change not generally possible by natural product modification. A seven-membered ring analogue of dihydrosempervirine was prepared in three steps and 60% yield from triflate **3** (Scheme 2). In the Larock cyclization, catalyst loading was increased 2-fold relative to the Scheme 1 protocol. The lower yield seen for a longer alkyl chain (compound **10** vs **5**) suggests hydroxyl group chelation in the alkyne palladation step. The Larock reaction to access the five-membered ring analogue **16** (Scheme 2) was unsuccessful, perhaps due to poor coordination by the propargylic alcohol group and/or gramine-like instability of product **15**.

Structural diversity in the central ring is possible: a C7 substituent can be installed by using hydroxyester **17**¹⁷ to give tetracyclic esters **20** and **21** (Scheme 3). This core ring system and pattern of substitution are similar to that of javacarboline (**22**).

Using electron-rich bromoanilines permits access to electronrich sempervirine analogues (**29** and **30**, Scheme 4). Here, intermediates **25** and **26** were not purified, and salts **27–30** were isolated by precipitation. Compound **30** in another salt form has been described.¹³

Electron-deficient derivatives are accessible (**37** and **38**, Scheme 5). For **38**, the DDQ aromatization reaction required 7 days and excess oxidant. As another variation, isoquinolinone **2** semireduction can be omitted, providing the aromatic E-ring analogue **43** (Scheme 6).

Sempervirine analogues with substituents in the A-, C-, and/ or E-ring are thus accessible. Presumably, the use of C1- or C4-substituted analogues of isoquinoline **2** would also allow access to D-ring analogues (our previous paper described D-ring aza analogues).² The B-ring, with only one site for substitution, can likely also be modified by N-alkylation of an intermediate or the final product. *The ability to modify rings A–E of sempervirine by one general method illustrates the versatility and utility of the Larock and Sonogashira reactions.*

Because sempervirine has multiple mechanisms of antitumor activity,¹¹ a single biochemical assay is inadequate to assess potency. We conducted standard whole-cell antitumor viability MTT assays¹⁸ in three sempervirine-sensitive human tumor lines: Raji Burkitt lymphoma, MDA-MB-231 breast cancer, and HeLa cervical cancer cells. Compounds **44–50** (Figure 2)² were also evaluated. Growth inhibition EC₅₀ estimates were determined using a 6-point dilution protocol.¹⁸ Active compounds (estimated EC₅₀ < 3 μ M) were re-evaluated using a 12-point protocol.

Growth inhibition curves for sempervirine and two more potent analogues are shown in Figure 3. A 2.3–3.4-fold enhancement of potency for **38** relative to that for sempervirine **1** was found. *Compound 38 is, to our knowledge, the most cytotoxic sempervirine analogue known.* Enhanced potency and improved pharmacokinetic properties are likely required for animal use of **38**.¹⁹ Growth inhibition results for all other less active compounds are shown in Table 1, based upon the 6-point assay.¹⁸ Compounds with a nonaromatic central ring were inactive at 10 μ M (**8**, **12**, **20**, **27**, **28**, **35**, **36**, **42**, and **44–49**), thus extended planarity is essential for activity.

In conclusion, these methods allow extensive modifications to sempervirine, with 10-fluorosempervirine (**38**) being the most potently cytotoxic analogue yet described.

EXPERIMENTAL SECTION

General Methods

Infrared (IR) spectra were collected on an FT-IR spectrometer. ¹H NMR spectra were recorded at 400 MHz in parts per million (ppm) downfield from an internal standard, CHCl₃ (δ 7.26) or DMSO (δ 2.54). ¹³C NMR spectra were recorded at 100 MHz in parts per million (ppm) downfield from an internal standard, CHCl₃ (δ 77.36) or DMSO (δ 40.45). High-resolution mass spectra (HRMS) were recorded on a TOF LC/MS spectrometer. Reagents and anhydrous solvents used were obtained from commercial vendors. Flash

column chromatography was performed to purify compounds as indicated, using 60 Å mesh silica columns and automated instruments.

General Procedure for DDQ Oxidation Reactions

Preparation of Sempervirine Triflate (1)—A solution of **8** (106 mg, 0.25 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (85 mg, 0.375 mmol) in acetic acid (1 mL) was stirred at 110 °C for 4 h. After being cooled to room temperature, the resulting precipitate was filtered and recrystallized with acetic acid, providing **1** (101 mg, 96%) as a yellow solid: IR (neat cm^{-1}) 3186, 1651, 1222, 1028, 745; ^1H NMR (400 MHz, DMSO) δ 13.29 (s, 1H), 9.27 (s, 1H), 8.89 (d, J = 6.8 Hz, 1H), 8.72 (d, J = 6.8 Hz, 1H), 8.69 (s, 1H), 8.43 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.75 (t, J = 7.6 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 3.20 (br, 2H), 3.04 (br, 2H), 1.95 (br, 4H); ^{13}C NMR (100 MHz, DMSO) δ 149.9, 141.2, 135.3, 133.6, 130.8, 130.0, 129.6, 126.6, 122.4, 122.3, 121.6, 121.3, 119.9, 116.6, 113.3, 29.6, 26.6, 22.1, 22.0; HRMS (ES) m/e calcd for $\text{C}_{19}\text{H}_{17}\text{N}_2$ ($\text{M} - \text{OTf}$) $^+$ 273.1392, found 273.1399.

5,6,7,8-Tetrahydroisoquinolin-3-yl Trifluoromethanesulfonate (3)—A solution of isoquinolin-3-ol (**2**) (2 g, 13.79 mmol) and PtO_2 (30 mg, 0.132 mmol) in trifluoroacetic acid (10 mL) and triflic acid (1 mL) was stirred under 50 psi H_2 for 1 h. The mixture was filtered, and to the solution was added ice water. The resulting mixture was made basic (pH 8) with saturated aqueous Na_2CO_3 and extracted with ethyl acetate. The extracts were combined and evaporated to provide 5,6,7,8-tetrahydroisoquinolin-3-ol (2 g, 98%) as a solid. To a solution of 5,6,7,8-tetrahydroisoquinolin-3-ol (200 mg, 1.343 mmol) and Et_3N (163 mg, 1.612 mmol) in CH_2Cl_2 (5 mL) at 0 °C was then added trifluoromethanesulfonic anhydride (0.245 mL, 1.447 mmol) slowly, followed by stirring for 10 min. Water was added, and the CH_2Cl_2 layer was separated and then filtered through a pad of silica gel. Evaporation of the CH_2Cl_2 provided compound **3** (360 mg, 96%) as an oil: IR (neat cm^{-1}) 2942, 1416, 1201, 927, 834; ^1H NMR (400 MHz, CDCl_3) δ 8.05 (s, 1H), 6.87 (s, 1H), 2.83–2.80 (m, 2H), 2.78–2.75 (m, 2H), 1.87–1.78 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ 154.2, 152.3, 148.9, 134.6, 120.5 (CF_3), 117.4 (CF_3), 115.0, 29.4, 26.1, 22.5, 22.1; HRMS (ES) m/e calcd for $\text{C}_{10}\text{H}_{11}\text{F}_3\text{NO}_3\text{S}$ ($\text{M} + \text{H}$) $^+$ 282.0406, found 282.0408.

General Procedure for Sonogashira Coupling Reactions

4-(5,6,7,8-Tetrahydroisoquinolin-3-yl)but-3-yn-1-ol (5)—A solution of 5,6,7,8-tetrahydroisoquinolin-3-yl triflate (**3**) (1.69 g, 6.0 mmol), 3-butyne-1-ol (**4**) (504 mg, 7.2 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (127 mg, 0.18 mmol), and CuI (69 mg, 0.36 mmol) in Et_3N (6 mL) was stirred at 80 °C for 1 h. Silica gel was added, and the resulting mixture was evaporated and separated by flash chromatography (SiO_2 , EtOAc), providing compound **5** (1100 mg, 92%) as a solid: IR (neat cm^{-1}) 3183, 2937, 1597, 1053, 695; ^1H NMR (400 MHz, CDCl_3) δ 8.15 (s, 1H), 7.04 (s, 1H), 3.85 (t, J = 6.4 Hz, 2H), 2.68–2.65 (m, 6H), 1.77–1.75 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ 150.3, 146.9, 140.0, 132.8, 127.1, 87.1, 81.8, 61.0, 28.7, 26.4, 24.1, 22.6, 22.4; HRMS (ES) m/e calcd for $\text{C}_{13}\text{H}_{16}\text{NO}$ ($\text{M} + \text{H}$) $^+$ 202.1226, found 202.1229.

General Procedure for Larock Indole Synthesis Reactions

2-(2-(5,6,7,8-Tetrahydroisoquinolin-3-yl)-1H-indol-3-yl)ethanol (**7**)—2-

Bromoaniline (**6**) (52 mg, 0.3 mmol), **5** (62 mg, 0.3 mmol), Pd(OAc)₂ (1.8 mg, 0.0075 mmol), 1,1'-bis(diphenylphosphino)-ferrocene (8.4 mg, 0.015 mmol), and KHCO₃ (90 mg, 0.9 mmol) were added to dry and degassed DMF (2 mL). The solution was heated for 4 h at 110 °C, after which time the reaction was complete, as determined by LCMS analysis. Water was added, and the mixture was extracted with ethyl acetate. The organic extracts were combined, dried, and purified by flash chromatography (SiO₂, hexanes/EtOAc = 1:1), providing compound **7** (84 mg, 96%) as a solid: IR (neat cm⁻¹) 3182, 2925, 1603, 1042, 737; ¹H NMR (400 MHz, CDCl₃) δ 9.40 (br, 1H), 8.13 (s, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 7.44 (s, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.21 (t, *J* = 7.2 Hz, 1H), 7.11 (t, *J* = 7.2 Hz, 1H), 4.05 (t, *J* = 6.0 Hz, 2H), 3.26 (t, *J* = 6.0 Hz, 2H), 2.80 (br, 2H), 2.67 (br, 2H), 1.80 (br, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 148.8, 147.7, 147.4, 136.5, 134.1, 131.7, 129.3, 123.0, 121.3, 119.7, 118.9, 112.6, 111.8, 64.0, 29.2, 27.5, 26.3, 22.7, 22.5; HRMS (ES) *m/e* calcd for C₁₉H₂₁N₂O (M + H)⁺ 293.1648, found 293.1656.

General Procedure for Triflate Cyclization Reactions

7,8-Dihydrosempervirine (8)—To a solution of compound **7** (44 mg, 0.15 mmol) and Et₃N (24 mg, 0.225 mmol) in dry CHCl₃ (1.4 mL) at 0 °C was slowly added trifluoromethanesulfonic anhydride (34 μL, 0.18 mmol). After being stirred for an additional 5 min, the resulting yellow precipitate was collected by filtration, and the solid was washed with CHCl₃ and then identified as compound **8** as a yellow solid (60.2 mg, 95%): IR (neat cm⁻¹) 3297, 1558, 1149, 1031, 748; ¹H NMR (400 MHz, DMSO) δ 12.21 (s, 1H), 8.81 (s, 1H), 8.01 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.40 (t, *J* = 7.2 Hz, 1H), 7.22 (t, *J* = 7.2 Hz, 1H), 4.83 (br, 2H), 3.38 (br, 2H), 3.05 (br, 2H), 2.88 (br, 2H), 1.86 (br, 4H); ¹³C NMR (100 MHz, DMSO) δ 158.2, 145.4, 140.2, 139.8, 134.4, 126.6, 125.9, 125.7, 121.5, 121.2, 121.0, 116.9, 113.4, 55.9, 29.8, 26.4, 21.9, 21.8, 19.8; HRMS (ES) *m/e* calcd for C₁₉H₁₉N₂ (M – OTf)⁺ 275.1548, found 275.1553.

5-(5,6,7,8-Tetrahydroisoquinolin-3-yl)pent-4-yn-1-ol (10)—By the general Sonogashira coupling procedure, 5,6,7,8-tetrahydroisoquinolin-3-yl triflate (**3**) (141 mg, 0.5 mmol), 4-pentyn-1-ol (**9**) (51 mg, 0.6 mmol), PdCl₂(PPh₃)₂ (11 mg, 0.015 mmol), and CuI (6 mg, 0.03 mmol) in Et₃N (1 mL) at 80 °C for 1 h provided compound **10** (92 mg, 86%) as an oil after flash chromatography (SiO₂, EtOAc): IR (neat cm⁻¹) 3280, 2931, 1595, 1473, 1060; ¹H NMR (400 MHz, CDCl₃) δ 8.18 (s, 1H), 7.05 (s, 1H), 3.81 (t, *J* = 6.0 Hz, 2H), 2.78 (br, 1H), 2.71–2.66 (m, 4H), 2.55 (t, *J* = 7.2 Hz, 2H), 1.89–1.82 (m, 2H), 1.80–1.73 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 150.6, 146.7, 140.4, 132.6, 127.2, 89.1, 81.0, 61.7, 31.5, 28.7, 26.4, 22.7, 22.5, 16.20; HRMS (ES) *m/e* calcd for C₁₄H₁₈NO (M + H)⁺ 216.1383, found 216.1385.

3-(2-(5,6,7,8-Tetrahydroisoquinolin-3-yl)-1H-indol-3-yl)propan-1-ol (11)—By the general Larock indole synthesis procedure, 2-bromoaniline (**6**) (48 mg, 0.28 mmol), alkyne **10** (60 mg, 0.28 mmol), Pd(OAc)₂ (3.2 mg, 0.014 mmol), 1,1'-bis-(diphenylphosphino)ferrocene (15.5 mg, 0.028 mmol), and KHCO₃ (84 mg, 0.84 mmol) in DMF (1.4 mL) for 2 h provided **11** (65 mg, 76%) as an oil after flash chromatography (SiO₂,

hexanes/EtOAc = 1:1): IR (neat cm^{-1}) 2931, 1604, 1436, 955, 734; ^1H NMR (400 MHz, CDCl_3) δ 9.18 (s, 1H), 8.20 (s, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.38 (d, J = 7.6 Hz, 1H), 7.34 (s, 1H), 7.24 (td, J = 6.8 and 0.8 Hz, 1H), 7.14 (td, J = 8.0 and 0.8 Hz, 1H), 5.58 (br, 1H), 3.62 (t, J = 5.6 Hz, 2H), 3.26 (t, J = 6.4 Hz, 2H), 2.72–2.65 (m, 4H), 2.10–2.04 (m, 2H), 1.81–1.73 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ 149.5, 148.5, 147.9, 136.6, 133.2, 131.9, 129.6, 123.2, 122.1, 119.8, 119.7, 114.4, 111.5, 60.1, 32.2, 29.2, 26.2, 22.7, 22.6, 20.6; HRMS (ES) m/e calcd for $\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}$ ($\text{M} + \text{H}$) $^+$ 307.1805, found 307.1804.

1,2,3,4,7,8,9,14-Octahydroindolo[2',3':3,4]azepino[1,2-b]-isoquinolin-6-ium

Triflate (12)—By the general triflate cyclization procedure, compound **11** (50 mg, 0.163 mmol), Et_3N (25 mg, 0.245 mmol), and trifluoromethanesulfonic anhydride (33 μL , 0.196 mmol) in dry CHCl_3 (1 mL) provided **12** (65 mg, 91%) as a yellow solid: IR (neat cm^{-1}) 3302, 1556, 1154, 1030, 745; ^1H NMR (400 MHz, DMSO) δ 11.85 (s, 1H), 8.85 (s, 1H), 8.17 (s, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.55 (d, J = 8.0 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 4.71–4.69 (m, 2H), 3.24 (t, J = 6.8 Hz, 2H), 3.07 (br, 2H), 2.90 (br, 2H), 2.42 (br, 2H), 1.88 (br, 4H); ^{13}C NMR (100 MHz, DMSO) δ 157.1, 145.6, 143.5, 138.7, 135.3, 128.8, 126.9, 126.7, 124.7, 122.1, 121.1, 121.0, 112.9, 60.3, 29.7, 26.6, 26.3, 24.9, 21.92, 21.89; HRMS (ES) m/e calcd for $\text{C}_{20}\text{H}_{21}\text{N}_2$ ($\text{M} - \text{OTf}$) $^+$ 289.1705, found 289.1709.

3-(5,6,7,8-Tetrahydroisoquinolin-3-yl)prop-2-yn-1-ol (14)—By the general Sonogashira coupling procedure, 5,6,7,8-tetrahydroisoquinolin-3-yl triflate **3** (140 mg, 0.5 mmol), 2-propyn-1-ol **13** (34 mg, 0.6 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (11 mg, 0.015 mmol), and CuI (6 mg, 0.03 mmol) in Et_3N (1 mL) at 80 $^\circ\text{C}$ for 1 h provided **14** (81 mg, 87%) as a solid after flash chromatography (SiO_2 , EtOAc): IR (neat cm^{-1}) 3143, 2934, 1041, 974, 868; ^1H NMR (400 MHz, CDCl_3) δ 8.21 (s, 1H), 7.13 (s, 1H), 4.51 (s, 2H), 3.84 (br, 1H), 2.73–2.68 (m, 4H), 1.83–1.75 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ 150.6, 147.1, 139.5, 133.4, 127.6, 87.6, 84.6, 51.4, 28.8, 26.5, 22.7, 22.4; HRMS (ES) m/e calcd for $\text{C}_{12}\text{H}_{14}\text{NO}$ ($\text{M} + \text{H}$) $^+$ 188.1070, found 188.1073.

Ethyl 2-Hydroxy-5-(5,6,7,8-tetrahydroisoquinolin-3-yl)pent-4-ynoate (18)—By the general Sonogashira coupling procedure, 5,6,7,8-tetrahydroisoquinolin-3-yl triflate (**3**) (160 mg, 0.569 mmol), ethyl 2-hydroxypent-4-ynoate (**17**) (97 mg, 0.683 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (12 mg, 0.0171 mmol), and CuI (6.5 mg, 0.0341 mmol) in Et_3N (1 mL) at 80 $^\circ\text{C}$ for 2 h provided **18** (145 mg, 94%) as an oil after flash chromatography (SiO_2 , hexanes/EtOAc = 1:1): IR (neat cm^{-1}) 2934, 1734, 1195, 1098, 1033; ^1H NMR (400 MHz, CDCl_3) δ 8.13 (s, 1H), 6.98 (s, 1H), 4.64 (br, 1H), 4.41 (t, J = 5.6 Hz, 1H), 4.23–4.15 (m, 2H), 2.92 (qd, J = 16.8 and 4.8 Hz, 2H), 2.63–2.58 (m, 4H), 1.74–1.68 (m, 4H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 173.1, 150.3, 146.7, 139.5, 132.8, 127.3, 84.5, 82.7, 69.3, 61.8, 28.5, 26.3, 26.1, 22.5, 22.3, 14.3; HRMS (ES) m/e calcd for $\text{C}_{16}\text{H}_{20}\text{NO}_3$ ($\text{M} + \text{H}$) $^+$ 274.1438, found 274.1439.

Ethyl 2-Hydroxy-3-(2-(5,6,7,8-tetrahydroisoquinolin-3-yl)-1H-indol-3-yl)propanoate (19)—By the general Larock indole synthesis procedure, 2-bromoaniline (**6**) (102 mg, 0.593 mmol), alkyne **18** (135 mg, 0.494 mmol), $\text{Pd}(\text{OAc})_2$ (5.6 mg, 0.0247 mmol), 1,1'-bis(diphenylphosphino)ferrocene (27.4 mg, 0.0494 mmol), and KHCO_3 (148

mg, 1.482 mmol) in DMF (2.5 mL) for 2 h provided **19** (120 mg, 67%) as an oil after flash chromatography (SiO₂, hexanes/EtOAc = 1:1): IR (neat cm⁻¹) 2929, 1729, 1603, 1205, 739; ¹H NMR (400 MHz, CDCl₃) δ 9.28 (s, 1H), 7.96 (s, 1H), 7.43–7.40 (m, 1H), 7.16 (s, 1H), 7.06–7.03 (m, 1H), 6.99–6.94 (m, 2H), 4.66 (t, *J* = 5.6 Hz, 1H), 4.36–4.28 (m, 1H), 4.21–4.13 (m, 1H), 3.45 (d, *J* = 5.6 Hz, 2H), 2.74–2.59 (m, 4H), 1.82–1.71 (m, 4H), 1.39 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 175.8, 148.2, 147.1, 146.6, 136.6, 134.6, 131.8, 128.9, 122.9, 121.7, 119.4, 118.5, 112.1, 110.1, 73.0, 61.3, 29.3, 29.1, 26.4, 22.8, 22.6, 14.6; HRMS (ES) *m/e* calcd for C₂₂H₂₅N₂O₃ (M + H)⁺ 365.1860, found 365.1861.

7-(Ethoxycarbonyl)-2,3,4,7,8,13-hexahydro-1H-indolo[2',3':3,4]-pyrido[1,2-b]isoquinolin-6-ium Triflate (20)—By the general triflate cyclization procedure, compound **19** (110 mg, 0.302 mmol), Et₃N (46 mg, 0.453 mmol), and trifluoromethanesulfonic anhydride (62 μL, 0.362 mmol) in dry CHCl₃ (2.0 mL) at room temperature for 1 h provided **20** (119 mg, 80%) as a yellow solid: IR (neat cm⁻¹) 3228, 1744, 1156, 1029, 768; ¹H NMR (400 MHz, DMSO) δ 12.32 (s, 1H), 8.82 (s, 1H), 8.13 (s, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.41 (t, *J* = 7.2 Hz, 1H), 7.22 (t, *J* = 7.6 Hz, 1H), 6.14 (d, *J* = 6.0 Hz, 1H), 4.17–.06 (m, 2H), 3.94 (d, *J* = 17.2 Hz, 1H), 3.74 (dd, *J* = 17.2 and 6.8 Hz, 1H), 3.19–3.06 (m, 2H), 2.98–2.83 (m, 2H), 1.97–1.83 (m, 4H), 1.10 (t, *J* = 6.8 Hz, 3H); ¹³C NMR (100 MHz, DMSO) δ 169.0, 160.3, 146.1, 140.1, 140.0, 134.7, 127.0, 125.7, 125.5, 121.8, 121.4, 121.3, 114.3, 113.6, 66.9, 63.7, 30.1, 26.5, 23.3, 21.8, 21.7, 14.7; HRMS (ES) *m/e* calcd for C₂₂H₂₃N₂O₂ (M – OTf)⁺ 347.1760, found 347.1763.

7-(Ethoxycarbonyl)-2,3,4,13-tetrahydro-1H-indolo[2',3':3,4]-pyrido[1,2-b]isoquinolin-6-ium Triflate (21)—By the general DDQ oxidation procedure, **20** (50 mg, 0.1 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (34 mg, 0.15 mmol) in acetic acid (1.0 mL) at 100 °C for 8 h provided **21** (40.2 mg, 82%) as a yellow solid: IR (neat cm⁻¹) 2938, 1722, 1220, 1158, 756; ¹H NMR (400 MHz, DMSO) δ 13.42 (s, 1H), 9.88 (s, 1H), 9.28 (s, 1H), 8.59 (s, 1H), 8.46 (d, *J* = 8.0 Hz, 1H), 7.76–7.69 (m, 2H), 7.51 (td, *J* = 8.0 and 1.2 Hz, 1H), 4.65 (q, *J* = 7.2 Hz, 2H), 3.16 (br, 2H), 3.05 (br, 2H), 2.00–1.93 (m, 4H), 1.59 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100 MHz, DMSO) δ 162.6, 151.4, 141.6, 134.8, 134.6, 132.6, 131.9, 130.1, 124.2, 123.4, 123.3, 122.9, 122.1, 121.2, 119.6, 113.8, 63.9, 29.6, 27.3, 22.1, 22.0, 14.9; HRMS (ES) *m/e* calcd for C₂₂H₂₁N₂O₂ (M – OTf)⁺ 345.1603, found 345.1601.

9-Methoxy-2,3,4,7,8,13-hexahydro-1H-indolo[2',3':3,4]pyrido-[1,2-b]isoquinolin-6-ium Triflate (23)—A solution of 2-bromo-3-methoxyaniline (**23**) (53 mg, 0.26 mmol), alkyne **5** (41 mg, 0.2 mmol), Pd(OAc)₂ (4.5 mg, 0.02 mmol), 1,1'-bis(diphenylphosphino)-ferrocene (22 mg, 0.04 mmol), and KHCO₃ (60 mg, 0.6 mmol) in DMF (1.0 mL) was degassed and stirred at 110 °C for 10 h. After being cooled to room temperature, water was added and the mixture was extracted with ethyl acetate. The organic extracts were combined, washed with water, filtered through a pad of silica gel, and dried to provide the crude intermediate **25**. Et₃N (31 mg, 0.3 mmol) and CHCl₃ (1.5 mL) were then added to this crude intermediate, and then trifluoromethanesulfonic anhydride (34 μL, 0.2 mmol) was slowly added to the mixture at 0 °C. After being stirred for 5 min, the resulting precipitate was filtered and washed with CHCl₃ to provide **27** (52 mg, 57%) as a yellow

solid: IR (neat cm^{-1}) 3246, 1513, 1247, 1032, 745; ^1H NMR (400 MHz, DMSO) δ 12.15 (s, 1H), 8.76 (s, 1H), 7.92 (s, 1H), 7.29 (t, J = 8.0 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 6.64 (d, J = 8.0 Hz, 1H), 4.79 (t, J = 6.8 Hz, 2H), 3.93 (s, 3H), 3.49 (t, J = 6.8 Hz, 2H), 3.01 (br, 2H), 2.84 (br, 2H), 1.85 (br, 4H); ^{13}C NMR (100 MHz, DMSO) δ 158.1, 155.8, 145.1, 141.2, 140.1, 134.1, 128.0, 124.6, 120.7, 116.8, 116.6, 106.3, 101.3, 56.3, 55.8, 29.8, 26.4, 22.0, 21.9, 21.5; HRMS (ES) m/e calcd for $\text{C}_{20}\text{H}_{21}\text{N}_2\text{O}$ ($\text{M} - \text{OTf}$) $^+$ 305.1654, found 305.1652.

11-Methoxy-2,3,4,7,8,13-hexahydro-1H-indolo[2',3':3,4]pyrido-[1,2-b]isoquinolin-6-ium Triflate (28)—A solution of 2-bromo-5-methoxyaniline (**24**) (51

mg, 0.25 mmol), **5** (51 mg, 0.25 mmol), $\text{Pd}(\text{OAc})_2$ (5.6 mg, 0.025 mmol), 1,1'-bis(diphenylphosphino)-ferrocene (28 mg, 0.05 mmol), and K_2CO_3 (87 mg, 0.625 mmol) in DMF (1.5 mL) was degassed and stirred at 110 $^\circ\text{C}$ for 4 h. After being cooled to room temperature, water was added and the mixture was extracted with ethyl acetate. The organic extracts were combined, washed with water, filtered through a pad of silica gel, and dried to provide the crude intermediate **26**. Et_3N (38 mg, 0.375 mmol) and CHCl_3 (2 mL) were then added to this crude intermediate, and then trifluoromethanesulfonic anhydride (42 μL , 0.25 mmol) was slowly added to the mixture at 0 $^\circ\text{C}$. After being stirred for 5 min, the resulting precipitate was filtered and washed with CHCl_3 to provide **28** (79 mg, 70%) as a yellow solid: IR (neat cm^{-1}) 3293, 1554, 1249, 1031, 810; ^1H NMR (400 MHz, DMSO) δ 11.98 (s, 1H), 8.71 (s, 1H), 7.87 (s, 1H), 7.62 (d, J = 8.8 Hz, 1H), 6.95 (d, J = 2.0 Hz, 1H), 6.86 (dd, J = 8.8 and 2.0 Hz, 1H), 4.79 (t, J = 7.2 Hz, 2H), 3.88 (s, 3H), 3.34 (t, J = 7.2 Hz, 2H), 3.01 (br, 2H), 2.84 (br, 2H), 1.85 (br, 4H); ^{13}C NMR (100 MHz, DMSO) δ 159.8, 157.9, 144.9, 141.2, 140.3, 133.5, 124.8, 122.2, 120.3, 120.2, 117.8, 112.9, 95.1, 56.3, 55.7, 29.8, 26.3, 22.0, 21.9, 19.9; HRMS (ES) m/e calcd for $\text{C}_{20}\text{H}_{21}\text{N}_2\text{O}$ ($\text{M} - \text{OTf}$) $^+$ 305.1654, found 305.1653.

9-Methoxy Sempervirine (29)—By the general DDQ oxidation procedure, **27** (46 mg, 0.1 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (30 mg, 0.13 mmol) in acetic acid (1.0 mL) at 100 $^\circ\text{C}$ for 30 min provided **29** (42 mg, 93%) as a yellow solid: IR (neat cm^{-1}) 3171, 1352, 1240, 1026, 733; ^1H NMR (400 MHz, DMSO) δ 13.17 (s, 1H), 9.21 (s, 1H), 8.78 (d, J = 6.8 Hz, 1H), 8.52 (s, 1H), 8.41 (d, J = 6.8 Hz, 1H), 7.66 (t, J = 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 4.12 (s, 3H), 3.17–3.12 (m, 2H), 3.02–2.98 (m, 2H), 1.97–1.89 (m, 4H); ^{13}C NMR (100 MHz, DMSO) δ 156.8, 149.7, 143.0, 135.5, 133.5, 131.4, 131.0, 129.8, 127.2, 121.3, 120.0, 117.9, 111.7, 106.1, 102.8, 56.8, 29.8, 26.8, 22.2, 22.2; HRMS (ES) m/e calcd for $\text{C}_{20}\text{H}_{19}\text{N}_2\text{O}$ ($\text{M} - \text{OTf}$) $^+$ 303.1497, found 303.1497.

11-Methoxy Sempervirine (30)—By the general DDQ oxidation procedure, **28** (68 mg, 0.15 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (52 mg, 0.225 mmol) in acetic acid (1.0 mL) at 100 $^\circ\text{C}$ for 2 h provided **30** (64 mg, 94%) as a yellow solid: IR (neat cm^{-1}) 1626, 1251, 1158, 1033, 799; ^1H NMR (400 MHz, DMSO) δ 13.06 (s, 1H), 9.15 (s, 1H), 8.81 (d, J = 5.2 Hz, 1H), 8.57 (d, J = 6.0 Hz, 1H), 8.52 (s, 1H), 8.24 (d, J = 8.8 Hz, 1H), 7.16 (s, 1H), 7.08 (d, J = 8.4 Hz, 1H), 3.98 (s, 3H), 3.15 (br, 2H), 3.00 (br, 2H), 1.93 (br, 4H); ^{13}C NMR (100 MHz, DMSO) δ 161.8, 149.4, 143.4, 135.2, 133.0, 130.9, 130.1, 127.1, 123.7,

122.7, 119.8, 116.2, 115.5, 113.2, 95.4, 56.6, 29.7, 26.7, 22.2, 22.2; HRMS (ES) m/e calcd for $C_{20}H_{19}N_2O$ ($M - OTf$)⁺ 303.1497, found 303.1500.

7,8-Dihydro-12-fluorosempervirine (35)—A solution of 2-bromo-6-fluoroaniline (**31**) (46 mg, 0.24 mmol), alkyne **5** (41 mg, 0.2 mmol), $Pd(OAc)_2$ (2.3 mg, 0.01 mmol), 1,1'-bis(diphenylphosphino)-ferrocene (11 mg, 0.02 mmol), and $KHCO_3$ (60 mg, 0.6 mmol) in DMF (1.0 mL) was degassed and stirred at 110 °C for 2 h. After being cooled to room temperature, water was added and the mixture was extracted with ethyl acetate. The organic extracts were combined, washed with water, filtered through a pad of silica gel, and dried to provide the crude intermediate **33**. Et_3N (31 mg, 0.3 mmol) and $CHCl_3$ (1.5 mL) were then added to this crude intermediate, and then trifluoromethanesulfonic anhydride (34 μL , 0.2 mmol) was slowly added to the mixture at 0 °C. After being stirred for 5 min, the resulting precipitate was filtered and washed with $CHCl_3$ to provide **35** (65 mg, 74%) as a yellow solid: IR (neat cm^{-1}) 3247, 1557, 1239, 1029, 787; 1H NMR (400 MHz, DMSO) δ 12.58 (s, 1H), 8.84 (s, 1H), 8.15 (s, 1H), 7.58 (d, $J = 8.0$ Hz, 1H), 7.25–7.14 (m, 2H), 4.86 (t, $J = 7.2$ Hz, 2H), 3.40 (t, $J = 7.2$ Hz, 2H), 3.03 (br, 2H), 2.88 (br, 2H), 1.86 (br, 4H); ^{13}C NMR (100 MHz, DMSO) δ 158.4, 151.4 (d, $^1J_{C-F} = 243.5$ Hz), 145.6, 139.8, 135.1, 129.4, 129.3, 127.9, 127.7, 127.1, 121.9, 121.9, 121.5, 117.5, 117.5, 117.5, 111.0, 110.9, 56.0, 29.9, 26.5, 21.9, 21.8, 19.8; HRMS (ES) m/e calcd for $C_{19}H_{18}FN_2$ ($M - OTf$)⁺ 293.1454, found 293.1450.

7,8-Dihydro-10-fluorosempervirine (36)—A solution of alkyne **5** (70 mg, 0.348 mmol), $Pd(OAc)_2$ (2 mg, 0.0087 mmol), 1,1'-bis(diphenylphosphino)ferrocene (10 mg, 0.0174 mmol), and $KHCO_3$ (104 mg, 1.04 mmol) in DMF (1.6 mL) was degassed and stirred at 110 °C. A solution of 2-bromo-4-fluoroaniline (**32**) (199 mg, 1.04 mmol) in DMF (0.4 mL) was then added slowly to this solution, and the mixture was maintained at 110 °C for 4 h. After being cooled to room temperature, the resulting mixture was quenched with water and extracted with ethyl acetate. The organic extracts were combined, washed with water, filtered through a pad of silica gel, and dried to provide the crude intermediate. Et_3N (53 mg, 0.522 mmol) and $CHCl_3$ (1 mL) were then added to this crude intermediate, and trifluoromethanesulfonic anhydride (60 μL , 0.348 mmol) was then added slowly to this mixture at 0 °C. After being stirred for 10 min, the resulting precipitate was filtered and washed with $CHCl_3$ to provide **36** (115 mg, 75%) as a yellow solid: IR (neat cm^{-1}) 3285, 1561, 1282, 1034, 813; 1H NMR (400 MHz, DMSO) δ 12.28 (s, 1H), 8.80 (s, 1H), 7.96 (s, 1H), 7.57–7.53 (m, 2H), 7.24 (td, $J = 9.2$ and 2.0 Hz, 1H), 4.84 (t, $J = 7.2$ Hz, 2H), 3.36 (t, $J = 7.2$ Hz, 2H), 3.03 (br, 2H), 2.87 (br, 2H), 1.86 (br, 4H); ^{13}C NMR (100 MHz, DMSO) δ 159.5 (d, $^1J_{C-F} = 233.4$ Hz), 158.3, 145.5, 139.9, 136.4, 134.9, 127.6, 125.9, 125.8, 121.2, 116.7, 116.7, 115.4, 115.1, 114.8, 114.7, 105.8, 105.6, 56.0, 29.9, 26.5, 21.9, 21.8, 19.8; HRMS (ES) m/e calcd for $C_{19}H_{18}FN_2$ ($M - OTf$)⁺ 293.1454, found 293.1450.

12-Fluorosempervirine (37)—By the general DDQ oxidation procedure, **35** (74 mg, 0.167 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (76 mg, 0.335 mmol) in acetic acid (1.8 mL) at 100 °C for 4 h provided **37** (66 mg, 90%) as a yellow solid: IR (neat cm^{-1}) 2948, 1406, 1222, 1026, 776; 1H NMR (400 MHz, DMSO) δ 13.40 (s, 1H), 9.17 (s, 1H), 8.79 (d, $J = 7.2$ Hz, 1H), 8.65 (s, 1H), 8.61 (d, $J = 7.2$ Hz, 1H), 8.15 (d, $J = 7.6$ Hz, 1H), 7.58

(dd, $J = 11.2$ and 8.0 Hz, 1H), 7.44–7.39 (m, 1H), 3.13 (br, 2H), 3.00 (br, 2H), 1.94 (br, 4H); ^{13}C NMR (100 MHz, DMSO) δ 151.0 (d, $^1J_{\text{C-F}} = 244.5$ Hz), 150.7, 136.0, 134.4, 131.7, 131.0, 129.6, 129.5, 127.5, 125.1, 125.1, 123.0, 122.9, 121.9, 121.8, 120.5, 118.8, 116.9, 114.3, 114.2, 29.8, 26.8, 22.13, 22.09; HRMS (ES) m/e calcd for $\text{C}_{19}\text{H}_{16}\text{FN}_2$ ($\text{M} - \text{OTf}$) $^+$ 291.1298, found 291.1299.

10-Fluorosempervirine (38)—By the general DDQ oxidation procedure, **36** (66 mg, 0.15 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (340 mg, 1.5 mmol) in acetic acid (1.0 mL) at 100 °C for 7 days provided **38** (34 mg, 52%) as a yellow solid: IR (neat cm^{-1}) 2943, 1651, 1248, 1153, 820; ^1H NMR (400 MHz, DMSO) δ 13.66 (br, 1H), 9.25 (s, 1H), 8.82 (d, $J = 6.8$ Hz, 1H), 8.76 (s, 1H), 8.62 (d, $J = 6.8$ Hz, 1H), 8.24 (dd, $J = 8.8$ and 2.0 Hz, 1H), 7.85 (dd, $J = 8.8$ and 4.0 Hz, 1H), 7.60 (td, $J = 9.2$ and 2.0 Hz, 1H), 3.17 (br, 2H), 3.03 (br, 2H), 1.94 (br, 4H); ^{13}C NMR (100 MHz, DMSO) δ 159.6 (d, $^1J_{\text{C-F}} = 234.9$ Hz), 150.5, 138.4, 136.0, 134.2, 131.9, 131.8, 126.7, 122.1, 122.0, 121.6, 121.5, 120.8, 118.3, 118.1, 117.0, 115.3, 115.2, 107.8, 107.6, 29.8, 26.8, 22.2, 22.1; HRMS (ES) m/e calcd for $\text{C}_{19}\text{H}_{16}\text{FN}_2$ ($\text{M} - \text{OTf}$) $^+$ 291.1298, found 291.1295.

13H-Indolo[2',3':3,4]pyrido[1,2-b]isoquinolin-6-ium Triflate (43)—By the general DDQ oxidation procedure, **42**² (42 mg, 0.1 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (34 mg, 0.15 mmol) in acetic acid (1.0 mL) at 100 °C for 30 min provided **43** (33 mg, 79%) as a yellow solid: IR (neat cm^{-1}) 3215, 1221, 1027, 885, 733; ^1H NMR (400 MHz, DMSO) δ 13.40 (s, 1H), 10.48 (s, 1H), 9.44 (s, 1H), 9.04 (d, $J = 6.0$ Hz, 1H), 8.70 (d, $J = 6.4$ Hz, 1H), 8.44 (d, $J = 8.0$ Hz, 1H), 8.34 (d, $J = 10.0$ Hz, 1H), 8.32 (d, $J = 8.4$ Hz, 1H), 8.15 (t, $J = 7.2$ Hz, 1H), 8.00 (t, $J = 7.2$ Hz, 1H), 7.78 (d, $J = 8.4$ Hz, 1H), 7.62 (t, $J = 7.2$ Hz, 1H), 7.42 (t, $J = 7.6$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO) δ 141.3, 140.7, 135.5, 135.4, 131.2, 130.9, 130.0, 129.3, 128.8, 127.9, 126.8, 125.8, 122.4, 122.2, 122.1, 119.4, 118.2, 117.7, 113.5; HRMS (ES) m/e calcd for $\text{C}_{19}\text{H}_{13}\text{N}_2$ ($\text{M} - \text{OTf}$) $^+$ 269.1079, found 269.1077.

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DEDICATION

This paper is dedicated to the memory of John Michael “Mike” Kane, an inspirational organic and medicinal chemist, mentor, and friend to T.D.B.

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17. Products of the Larock reactions reported in this study (compounds **7**, **11**, **19**, **25**, **26**, **33**, **34**, and **41**) were obtained with 95% regioselectivity, as no isomer was detected by ¹H and ¹³C NMR spectroscopy. In a few instances (**11**, **25**, **26**, **41**), there was analytical HPLC/LCMS evidence for the formation of trace amounts of a regioisomer (estimated yields ranging from 1 to 3%), but in no instances were these minor isomers isolated for full characterization.
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19. In comparable assays, the marketed topoisomerase 1 inhibitor topotecan has 2-5-fold higher potency, suggesting that additional rounds of optimization of compounds **29** and **38** may lead to compounds of significant activity. For the topotecan data, see: Mitsui I, Kumazawa E, Hirota Y, Aonuma M, Sugimori M, Ohsuki S, Uoto K, Ejima A, Terasawa H, Sato K. Jpn J Cancer Res. 1995; 86:776. [PubMed: 7559102]

Figure 1.
Sempervirine canonical forms and ring numbering (Woodward and Witkop, 1949).⁵

Figure 2.
Additional analogues tested.²

Figure 3.
Growth inhibition by the most potent compounds in the series.

Scheme 1.
Synthesis of Sempervirine Triflate

Scheme 2.
Approaches to Seven- and Five-Membered Ring Analogues

Scheme 3.
C-Ring Carboethoxy-Substituted Analogues

Scheme 4.
A-Ring Methoxy-Substituted Analogues

Scheme 5.
Fluorinated Analogues

Scheme 6.
Aromatic Analogues

Table 1

Estimated Growth Inhibition for All Other Compounds Tested, 6-Point MTT Assay

compound(s)	Raji EC ₅₀ (μ M)	MDA-MB-231 EC ₅₀ (μ M)	HeLa EC ₅₀ (μ M)
8, 12, 20, 27, 28, 35, 36, 42, 44, 45, 46, 47, 48, 49	>10	>10	>10
21, 30, 37, 43	3–10	3–10	3–10
50	3–10	3–10	>10