Copyrine Alkaloids: Synthesis, Spectroscopic Characterization, and Antimycotic/Antimycobacterial Activity of A- and B-Ring-Functionalized Sampangines

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Several A- and B-ring-substituted sampangines were synthesized and evaluated for antifungal and antimycobacterial activity against AIDS-related opportunistic infection pathogens. Electrophilic halogenation provided a channel for structural elaboration of the sampangine B-ring at position 4, while the synthesis of A-ring 3-substituted sampangines and benzo[4,5]sampangine (24) were achieved from the corresponding functionalized cleistopholines. Two-dimensional NMR spectroscopy was used to rigorously characterize the A- and B-ring substituent patterns. Structure–activity relationship studies revealed the activity of the sampangines was enhanced by the presence of a substituent at position 3 or by a 4,5-benzo group.

Introduction

The characteristic breakdown in the immune system that is associated with acquired immunodeficiency syndrome (AIDS) is often manifested in the form of serious opportunistic infections (OI).[1] Prior to the AIDS epidemic, the management of disseminated OI in immunosuppressed patients involved the reduction of immunosuppressive therapy coupled with chemotherapy. Since the course of immunosuppression in AIDS patients cannot be currently halted or reversed, the only recourse is treatment of the OI in these individuals, even though the infection is a reflection of a more complicated underlying immune disorder. Unfortunately, immunosuppressed patients appear to be more resistant to conventional antibiotic therapy than normal individuals.

The most common systemic fungal infections in AIDS patients are cryptococcosis, which occurs in 7–10% of AIDS patients and is due to *Cryptococcus neoformans*, and candidiasis, which occurs in ~5–7% of AIDS patients and is due primarily to *Candida albicans*.[2-8] Other important, but less frequent opportunistic fungal pathogens that cause life-threatening disseminated mycoses include *Aspergillus*, *Fusarium*, and *Histoplasma*. Atypical mycobacteria is also now recognized as a common bacterial AIDS-related OI, occurring in greater than 50% of AIDS patients.[9-14] Precise statistics regarding the occurrence of this disease are difficult to obtain since it is often not recognized until post-mortem examination. The most common cause of atypical mycobacteriosis is *Mycobacterium avium-intracellulare*, which is actually a complex of the two closely related strains *M. avium* and *M. intracellulare*.

The significant shortcomings of the drugs currently available for the treatment of OI has intensified the search for new, more effective agents. As part of an extensive program aimed at the discovery and development of prototype antibiotics from higher plants, we reported recently the isolation and structure elucidation of the antifungal copyrine alkaloid, 3-methoxysampangine.[15] Herein we describe the first total synthesis of this natural product and several other A- and B-ring sampangine analogs that were developed as part of a structure–activity relationship study, as well as the in vitro activity of

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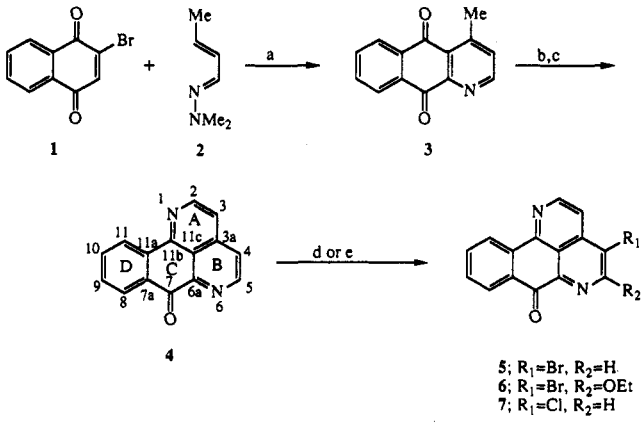
Scheme I [a]



[a] (a) Xylene, reflux; (b) $(MeO)_2CHNMe_2$, DMF, 120 °C; (c) NH_4Cl, HOAc, 120 °C; (d) $C_5H_5NHBr_3$, $CHCl_3$, reflux; (e) NCS, DMF, 100 °C.

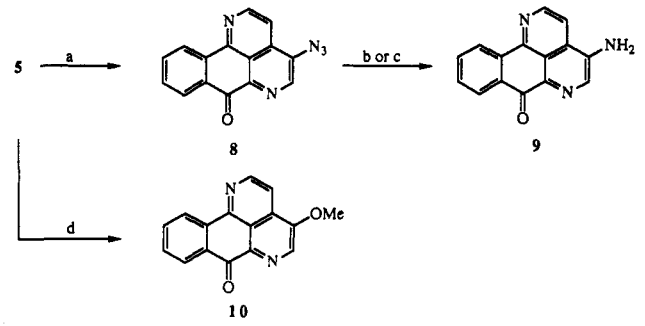
these compounds against fungal and atypical mycobacterial OI pathogens.

Results and Discussion

Chemistry. Cleistopholine (3) was obtained through the hetero Diels–Alder reaction of quinone 1 with hydrazone 2, followed by an in situ elimination of dimethylammonium bromide from the cycloadduct.[16] The condensation of 3 with dimethylformamide dimethyl acetal provided sampangine (4)[17] in 27% overall yield on a scale suitable for analog development (Scheme I).

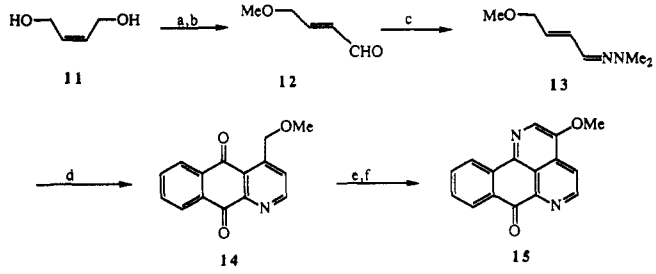
Mental dissection of the sampangine molecular skeleton revealed the presence of an unsymmetrically substituted 2,7-naphthyridine nucleus as the AB-ring segment. The known electrophilic halogenation and nucleophilic aromatic substitution chemistry of 2,7-naphthyridines[18–21] suggested such chemistry would provide a channel for regioselective functionalization of the B-ring of 4. To this end, the electrophilic halogenation of 4 with bromine in pyridine delivered only 4-bromosampangine (5), the yield of which was enhanced to 64% by the employment of pyridinium bromide perbromide in chloroform. The latter set of conditions also yielded small amounts (1%) of 6 that presumably arose through a reaction of the ethanol present in the commercial chloroform with trace amounts of 4,5-dibromosampangine produced in the halogenation reaction. The electrophilic halogenation of 4 with *N*-chlorosuccinimide in hot DMF[22] likewise provided 4-chlorosampangine (7) in 53% yield. The direct fluorination of 4 with several fluorinating agents[23] (*N*-fluoropyridinium triflate or tetrabutylammonium fluoride) under a variety of conditions has thus far proven unsuccessful, however.

Scheme II [a]



[a] (a) NaN_3, Me_2CO, H_2O, reflux; (b) MeOH, reflux; (c) H_2S, piperidine, MeOH, 10 °C; (d) NaOMe, MeOH, reflux.

Scheme III [a]



[a] (a) Me_2SO_4, NaOH, H_2O; (b) PCC, CH_2Cl_2; (c) NH_2NMe_2, $CaCl_2$; (d) 1, xylene, reflux; (e) $(MeO)_2CHNMe_2$, DMF, 120 °C; (f) NH_4Cl, HOAc, 120 °C.

Nucleophilic aromatic substitution reactions of 5 have culminated in the syntheses of three additional sampangines, (Scheme II). Subjection of 5 to a refluxing methanolic solution of sodium methoxide afforded the known 4-methoxysampangine (10) in high yield. The facility with which this substitution reaction occurred led to the use of other nucleophiles such as azide and amide anion. Indeed, the photolabile 4-azidosampangine (8) was isolated in 80% yield from the reaction of 5 with sodium azide in refluxing acetone–water. Thermal decomposition[24] of 8 provided the highly fluorescent 4-amino analog 9. An alternate route to 9 employed hydrogen sulfide[25] as a reductant of 8, a process which was used in the single-pot generation of 8 in 91% yield by addition of the reductant to the vessel in which 8 was formed. The reaction of 5 with potassium amide also provided 8, but in substantially diminished yields relative to the above procedures.

These results indicated that a different approach would be needed in the synthesis of A-ring sampangine derivatives. In this regard, a modification of the hetero Diels–Alder procedure[16] was utilized in the first total synthesis of 3-methoxysampangine (15) (Scheme III) and 3-methylsampangine (18) (Scheme IV). The cycloaddition reaction of 1 with (*E*)-4-methoxy-2-butenal *N*,*N*-dimethylhydrazone (13), the latter in turn prepared from the previously unreported aldehyde 12, gave 4′-methoxycleistopholine (14) in 12% yield. The modest yield of this reaction, in comparison to that of Scheme I, was a

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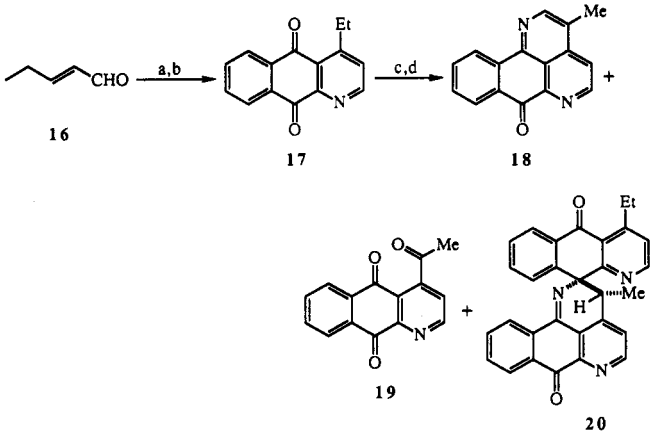
Scheme IV [a]



[a] (a) NH_2NMe_2; (b) 1, xylene, reflux; (c) $(MeO)_2CHNMe_2$, DMF, 120 °C; (d) NH_4Cl, HOAc, 120 °C.

characteristic with the homocleistopholine (17) synthesis as well. Generation of the A-rings in 15 and 18 unfortunately also proceeded in diminished yield (6%) relative to that of sampangine (52%). The TLC, IR, and ^1H and ^{13}C NMR data of 15 were identical in all respects to that of the authentic natural product. In the synthesis of 18, at least two additional compounds, 4'-oxohomocleistopholine (19) and dimer 20, were produced in comparable quantities. The exact yields associated with the formation of 19 and 20 remain undetermined due to difficulty in isolation. Chromatography of the reaction product gave fractions consisting of recovered 17 (11%), 18 (6%), and a fraction comprised of 18–20. Recrystallization of the latter fraction from ethyl acetate led to an unusual manual separation of the crystalline mixture under a low power stereoscopic microscope; 18 crystallized as long yellow needles, 19 as nearly perfect golden octahedra, and dimer 20 as rectangular yellow plates. The structure of 20 was established firmly through X-ray crystallographic analysis (Figure 1).

Finally, the ascididemin[26,27] analog, benzo[4,5]sampangine (24), was prepared by the procedure outlined in Scheme V. Conjugate addition of 2'-aminoacetophenone (22) onto 1,4-naphthoquinone (21) delivered an intermediate quinone that was cyclized to benzo[2,3]cleistopholine (23).[28] Condensation of 23 with dimethylformamide dimethyl acetal gave 24 in 34% overall yield.

Spectroscopic Characterization. The attainment of 10 and 15 by total synthesis permitted rigorous structural establishment of A- and B-ring substituent assignments in the sampangines. The ^1H and ^{13}C NMR spectral assignments of 10 and 15 are compared with that for 4 in Tables I and II, respectively. The structural assignments for each compound reside largely on two-dimensional ^1H–^{13}C long-range heterocorrelated (HETCOR) connectivity experiments.[15] The ^1H NMR spectra of all three compounds contain an ABMX system characteristic of the 1,2-disubstituted benzene D-ring nucleus. The aromatic A- and B-ring protons of 10 and 15 resonate as a downfield doublet (H_2 or H_5, respectively), a singlet (H_5 or H_2,

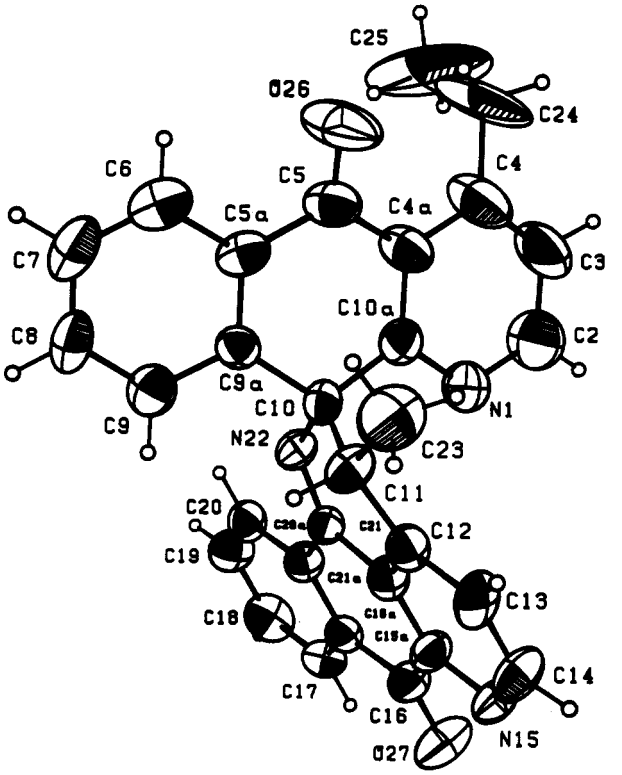
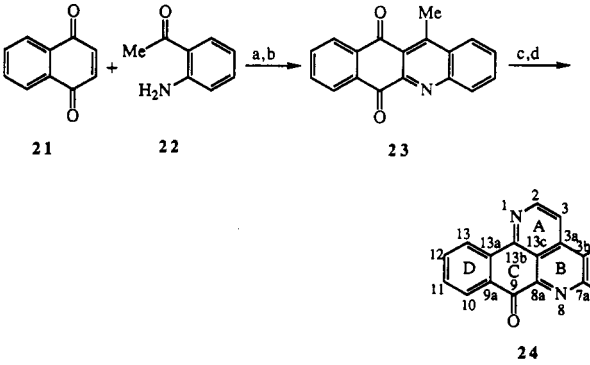


Figure 1. Thermal-ellipsoid plot of compound 20 illustrating the atom-numbering scheme. The hydrogen atom radii are arbitrarily reduced.

Scheme V [a]



[a] (a) $CeCl_3 \cdot 7H_2O$, EtOH, air; (b) AcOH, H_2SO_4, reflux; (c) $(MeO)_2CHNMe_2$, DMF, 120 °C; (d) NH_4Cl, HOAc, 120 °C.

respectively), and an upfield doublet (H_3 or H_4, respectively), chemical shifts of these resonances being the defining feature distinguishing the molecules. In contrast, the A- and B-ring protons of 4 resonate as two downfield sets of doublets (H_2 and H_5) and two upfield sets of doublets (H_3 and H_4). Long-range HETCOR connectivity experiments unambiguously defined H_8 in all three compounds through a three-bond correlation with the carbonyl carbon atom. With this knowledge, the D-ring protons H_9, H_{10}, and H_{11} were identified from the correlated spectroscopy (COSY) and long-range HETCOR experiments. A three-bond HETCOR connectivity of the H_{11} doublet of doublets with C_{11b}, and thence C_{11b} with the H_2 singlet of 15 or the H_2 doublet of 10, established firmly the location of the methoxy group. Similarly, the three-bond HETCOR connectivity of C_{11b} with one of the downfield doublet resonances of 4 likewise led to a clean assignment of H_2 in this molecule and hence the remaining AB-ring protons. Further interpretation of the ^1H–^{13}C one- and three-bond

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Table I. ^1H NMR Data for 4, 10, and 15

position	chemical shift, ppm ($CDCl_3$) sampangine (4)	4-methoxysampangine (10)	3-methoxysampangine (15)[a]
2	8.88 (d, J = 5.8 Hz, 1 H)	8.89 (d, J = 5.8 Hz, 1 H)	8.36 (s, 1 H)
3	7.71 (d, J = 5.8 Hz, 1 H)	8.00 (d, J = 5.8 Hz, 1 H)	–
3a	–	–	–
4	7.92 (d, J = 5.5 Hz, 1 H)	–	8.21 (d, J = 5.4 Hz, 1 H)
5	9.13 (d, J = 5.5 Hz, 1 H)	8.66 (s, 1 H)	9.13 (d, J = 5.4 Hz, 1 H)
6a	–	–	–
7	–	–	–
7a	–	–	–
8	8.46 (dd, J = 7.8, 1.2 Hz, 1 H)	8.49 (dd, J = 7.9, 1.2 Hz, 1 H)	8.43 (dd, J = 7.8, 1.2 Hz, 1 H)
9	7.69 (ddd, J = 7.8, 7.8, 1.2 Hz, 1 H)	7.69 (ddd, J = 7.9, 7.9, 1.2 Hz, 1 H)	7.61 (ddd, J = 7.8, 7.8, 1.2 Hz, 1 H)
10	7.83 (ddd, J = 7.8, 7.8, 1.2 Hz, 1 H)	7.82 (ddd, J = 7.9, 7.9, 1.2 Hz, 1 H)	7.78 (ddd, J = 7.8, 7.8, 1.2 Hz, 1 H)
11	8.82 (dd, J = 7.8, 1.2, 1 H)	8.85 (dd, J = 7.9, 1.2 Hz, 1 H)	8.65 (dd, J = 7.8, 1.2 Hz, 1 H)
11a	–	–	–
11b	–	–	–
11c	–	–	–
OCH_3	–	4.25 (s, 3 H)	4.18 (s, 3 H)

[a] See ref 15.

Table II. ^{13}C NMR Data for 4, 10, and 15

position	chemical shift, ppm ($CDCl_3$)[a] sampangine (4)	4-methoxy-sampangine (10)	3-methoxy-sampangine (15)[b]
2	147.1 (1)	146.6 (1)	126.8 (1)
3	118.9 (1)	114.3 (1)	149.9 (0)
3a	138.3 (0)	130.3 (0)	131.8 (0)
4	123.2 (1)	152.7 (0)	118.8 (1)
5	148.2 (1)	128.9 (1)	148.0 (1)
6a	147.5 (0)	141.0 (0)	147.2 (0)
7	181.5 (0)	181.1 (0)	182.0 (0)
7a	132.0 (0)	132.8 (0)	131.5 (0)
8	128.1 (1)	128.4 (1)	128.5 (1)
9	131.1 (1)	131.2 (1)	130.2 (1)
10	134.4 (1)	134.2 (1)	134.6 (1)
11	125.1 (1)	125.3 (1)	124.6 (1)
11a	135.0 (0)	135.6 (0)	135.7 (0)
11b	150.7 (0)	150.4 (0)	143.2 (0)
11c	119.3 (0)	120.0 (0)	119.7 (0)
OCH_3	–	56.9 (3)	56.6 (3)

[a] Numbers in parentheses refer to the number of attached protons. [b] See ref 15.

Table III. ^1H and ^{13}C NMR Data for Benzo[4,5]Sampangine (24)

position	chemical shift, ppm ($CDCl_3$) ^1H NMR	^{13}C NMR[a]
2	8.97 (d, J = 5.7 Hz, 1 H)	148.9 (1)
3	8.30 (d, J = 5.7 Hz, 1 H)	115.5 (1)
3a	–	137.8 (0)
3b	–	123.5 (0)
4	8.55 (dd, J = 7.1, 1.4 Hz, 1 H)	122.9 (1)
5	7.84 (ddd, J = 7.1, 7.0, 1.4 Hz, 1 H)	130.3 (1)
6	7.93 (ddd, J = 7.1, 7.0, 1.4 Hz, 1 H)	131.6 (1)
7	8.55 (dd, J = 7.1, 1.4 Hz, 1 H)	133.1 (1)
7a	–	145.8 (0)
8a	–	146.0 (0)
9	–	182.2 (0)
9a	–	132.5 (0)
10	8.44 (dd, J = 7.8, 1.0 Hz, 1 H)	128.7 (1)
11	7.66 (ddd, J = 7.8, 7.4, 1.0 Hz, 1 H)	131.2 (1)
12	7.80 (ddd, J = 7.8, 7.4, 1.0 Hz, 1 H)	134.9 (1)
13	8.79 (dd, J = 7.8, 1.0 Hz, 1 H)	125.8 (1)
13a	–	136.1 (0)
13b	–	150.5 (0)
13c	–	117.0 (0)

[a] Numbers in parentheses refer to the number of attached protons.

correlations culminated in the other NMR assignments illustrated in Tables I and II for 4, 10, and 15.

The ^1H and ^{13}C NMR assignments for 24 are listed in Table III. The proton NMR spectrum of 24 consisted of eight resonances for the two ABMX systems and a downfield and upfield set of doublets for H_2 and H_3, respectively. Assignment of the 8.45 ppm doublet of doublets to H_{10} followed from the long-range HETCOR connectivity of this proton with carbonyl atom C_9. The remaining protons of this ABMX system and carbon atoms C_{9a} through C_{13b} were identified from the COSY, ^1H–^{13}C HETCOR, and long-range HETCOR experiments. A three-bond correlation of the downfield proton doublet with C_{13b} led to the assignment of this resonance to H_2. The other ^1H NMR assignments relied upon the long-range HETCOR connectivity of the H_3 doublet with carbon atom C_{3b} and thence the C_{3b} resonance with the H_5 doublet of doublet of doublets. One and three-bond correlations in the HETCOR experiments were used to assign the B- and E-ring carbon resonances with the exception of C_{8a}, which was defined by default. The A-, B-, and E-ring ^{13}C NMR assignments for 24 closely parallel that reported for ascididemin.[26,27]

Biological Evaluation. The sampangines and cleistopholines were evaluated in vitro for activity versus the yeasts *Candida albicans* NIH B311 and *Cryptococcus neoformans* ATCC 32264, the filamentous fungus *Aspergillus fumigatus* ATCC 26934, and the atypical mycobacterium *Mycobacterium intracellulare* ATCC 23068 using a qualitative agar-well diffusion assay.[15] Compounds that exhibited significant activity in the qualitative screen were subjected to a 2-fold serial broth dilution assay to quantitate the activity. Table IV summarizes the minimum inhibitory concentrations (MIC) of these compounds, in comparison with the positive controls amphotericin B and rifampin,[1,10] against the above pathogens.

Examination of the in vitro data revealed the broad spectrum antifungal/antimycobacterial activity of the sampangines was enhanced by the presence of a 3-methoxy, 3-methyl, or 4,5-benzo group relative to that of 4. Indeed, compounds 18 and 24 exhibited activities in vitro that were comparable to that of the positive controls amphotericin B and rifampin. The addition of a 4-halo, 4-alkoxy, or 4-amino substituent to the sampangine nucleus, however, resulted in compounds with reduced antifungal/antimycobacterial activities relative to that of 4. The cleistopholines were similarly less effective than 4, 15, 18, or 24 against the same microorganisms, reflecting the

Table IV. In Vitro Antifungal and Antimycobacterial Activities

compound	organism[a] Ca[b]	Cn[c]	Af[d]	Mi[e]
cleistopholines				
3	12.5	1.56	100	12.5
17	100	3.12	NT	12.5
23	25	25	NT	1.56
sampangines				
4	1.56	0.78	1.56	0.78
5	3.12	0.10	50	3.12
6	NT	25	NT	25
7	0.39	0.10	NT	3.12
8	25	6.25	NT	NT
9	100	25	NT	NT
10	3.12	1.56	NT	3.12
15	1.56	0.78	0.78	1.56
18	0.39	0.39	0.78	0.39
20	NT	NT	NT	NT
24	0.39	1.56[f]	0.39	0.39
controls				
amphotericin B	0.78	0.39	0.39	NT
rifampin	NT	NT	NT	0.78

[a] Activity expressed as minimum inhibitory concentration in μg/mL; NT = not tested due to negative qualitative assay. [b] Ca = *Candida albicans* B311 in yeast nitrogen broth. [c] Cn = *Cryptococcus neoformans* ATCC 32264 in Mycophil broth. [d] Af = *Aspergillus fumigatus* ATCC 26934 in Sabouraud dextrose broth. [e] Mi = *Mycobacterium intracellulare* ATCC 23068 in Mueller–Hinton broth. [f] Tested in yeast nitrogen broth.

potential biological importance of the azaquinone nucleus in the sampangines.

Conclusions. The syntheses, spectroscopic characterization, and antifungal/antimycobacterial evaluation of several A- and B-ring-substituted sampangines were achieved. Electrophilic halogenation reactions of sampangine provided the 4-bromo and 4-chloro derivatives **5** and **7**, the former of which was converted to the 4-azido-, 4-amido-, and 4-methoxysampangines (**8–10**). The A-ring 3-substituted sampangines **15** and **18** and benzo[4,5]-sampangine (**24**) were obtained from the corresponding functionalized cleistopholines. Two-dimensional NMR spectroscopic techniques led to an unambiguous assignment of A- and B-ring substituent patterns in the sampangines. Evaluation of the sampangines against AIDS-related fungal and mycobacterial opportunistic infection pathogens revealed the activity of these compounds to be enhanced by the presence of a 3-substituent (e.g., **15** and **18**) or a 4,5-benzo ring (e.g., **24**) and decreased by the presence of a 4-substituent.

Experimental Section

General. Tetrahydrofuran (THF) was dried over sodium and benzophenone. 2-Bromo-1,4-naphthoquinone (**1**)[29] and (*E*)-2-butenal *N,N*-dimethylhydrazone (**2**)[30] were prepared by literature methods. Other reagents and solvents were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received.

Melting points (uncorrected) were determined in open capillary tubes with a Thomas-Hoover capillary melting point apparatus. Infrared (IR) spectra were recorded on a Perkin-Elmer 281B spectrophotometer. The NMR spectra were obtained on a Varian VXR-300 FT spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C NMR. Chemical shifts are reported as ppm downfield relative to tetramethylsilane. The numbers in parentheses for the ^{13}C NMR data refer to the number of attached protons on that carbon atom as determined by the attached-proton test (APT). Two-dimensional NMR spectra were obtained using standard Varian pulse sequences for COSY and HETCOR. The long-range HETCOR experiments were optimized for $^3J_{C-H}$ = 10 Hz and $^3J_{C-H}$ = 5 Hz. High resolution mass spectra were obtained at the Mass Spectrometry Laboratory, Department of Chemistry, University of Kansas, Lawrence, Kansas. Elemental analyses were performed by Ms. Paulanne Rider using a Perkin-Elmer Model 240 analyzer at the Chemical Instrumentation Laboratory, Northern Illinois University, DeKalb, Illinois.

Analytical thin-layer chromatography (TLC) was performed on Merck silica gel F-254 aluminum or polyester-backed plates. Visualization was achieved with shortwave ultraviolet light and/or Dragendorff reagent spray. Column chromatography was performed on Merck 230–400 mesh silica gel.

General Procedure for the Hetero Diels–Alder Reaction. 4-Methylbenzo[*g*]quinoline-5,10-dione (Cleistopholine, 3). (*E*)-2-Butenal *N,N*-dimethylhydrazone (**2**, 37.0 g, 0.330 mol) in xylene (100 mL) was added rapidly to a xylene solution (500 mL) of 2-bromo-1,4-naphthoquinone (**1**, 60.0 g, 0.253 mol) and the dark mixture heated at reflux for 6 h under a nitrogen atmosphere. The mixture was then transferred to a large separatory funnel, and the solids coating the wall of the reaction vessel were washed thoroughly with EtOAc (6 × 250 mL). The combined organic phase and washings were extracted with 2 N H_2SO_4 solution (1 × 1000 mL followed by 2 × 750 mL). The acid extracts were combined, chilled in ice, and made basic (~pH 10, test paper) with 6 N NaOH solution before extracting with EtOAc (4 × 1000 mL). The latter organic layers were dried over K_2CO_3 and concentrated to dryness. Flash silica gel chromatography while eluting with EtOAc provided pure **3** (29.3 g, 52%) as a yellow-brown solid. An analytical sample was obtained by crystallization of the chromatographed product from EtOAc: mp 202–203 °C (lit.[16] mp 198–201 °C); IR (KBr) 1680, 1660, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.28 (br s, 3 H), 7.47 (dd, 1 H, J = 4.9, 0.7 Hz), 7.76–7.82 (m, 2 H), 8.19–8.24 (m, 1 H), 8.30–8.34 (m, 1 H), 8.86 (d, 1 H, J = 4.9 Hz); ^{13}C NMR ($CDCl_3$) 22.8 (3), 127.1 (1), 127.3 (1), 129.1 (0), 131.2 (1), 132.5 (0), 133.8 (0), 134.1 (1), 134.5 (1), 150.0 (0), 151.5 (0), 153.4 (1), 181.9 (0), 184.7 (0) ppm). Anal. (exact mass, HREIMS) calcd for $C_{14}H_{19}NO_2$ *m/e* 223.0633, found 223.0633. Anal. ($C_{14}H_9NO_2$) C, H, N.

7*H*-Naphtho[1,2,3-*ij*][2,7]naphthyridin-7-one (Sampangine, 4). Dimethylformamide dimethyl acetal (8.63 mL, 0.065 mol) was added to a solution of **3** (11.16 g, 0.050 mol) in DMF (40 mL). The mixture was heated under a nitrogen atmosphere for 30 min by submerging the reaction vessel into an oil bath preheated to 120 °C. Ammonium chloride (25.75 g, 0.48 mol) and glacial acetic acid (85 mL) were then added to the hot reaction followed by continued heating at 120 °C for an additional 30 min. After allowing to cool, the mixture was poured onto H_2O (1500 mL) and extracted with CH_2Cl_2 (4 × 500 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution (3 × 500 mL), H_2O (3 × 500 mL), dried over K_2CO_3, and concentrated to dryness. Flash silica gel chromatography of this material using EtOAc as eluant provided pure **4** (6.00 g, 52%). An analytical sample was obtained by crystallization from EtOAc: mp 220–222 °C (lit.[16] mp 216–218 °C); IR (KBr) 1670, 1615, 1590, 1400, 1380 cm^{-1}; ^1H and ^{13}C NMR (see Tables I and II). Anal. (exact mass, HREIMS) calcd for $C_{15}H_8N_2O$ *m/e* 232.0637 found 232.0636. Anal. ($C_{15}H_8N_2O$) C, H, N.

Electrophilic Bromination of Sampangine. 4-Bromo-7*H*-naphtho[1,2,3-*ij*][2,7]naphthyridin-7-one (4-Bromosampangine, 5) and 4-Bromo-5-ethoxy-7*H*-naphtho[1,2,3-*ij*][2,7]-naphthyridin-7-one (4-Bromo-5-ethoxysampangine, 6). A mixture of pyridinium bromide perbromide[31] (4.80 g, 15.0 mmol) and **4** (2.32 g, 10.0 mmol) in $CHCl_3$ (100 mL) was heated at reflux for 24 h. After cooling, the mixture was poured into a separatory funnel and washed with saturated aqueous $NaHCO_3$ solution (2 × 250 mL). The organic layer was dried (K_2CO_3) and concentrated to dryness. The residual solids were subjected to flash

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silica gel chromatography while eluting with $CHCl_3$ to give pure **5** (2.00 g, 64%) and **6** (0.05 g, 1%). An analytical sample of **6** was obtained by crystallization from $CHCl_3$. Compound **5**: mp 244–246 °C; IR (KBr) 1670, 1590, 1400, 1320 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.72 (ddd, 1 H, J = 7.9, 7.9, 1.4 Hz), 7.86 (ddd, 1 H, J = 7.9, 7.9, 1.4 Hz), 7.96 (d, 1 H, J = 5.9 Hz), 8.46 (dd, 1 H, J = 7.9, 1.4 Hz), 8.85 (dd, 1 H, J = 7.9, 1.4 Hz), 8.99 (d, 1 H, J = 5.9 Hz), 9.28 (s, 1 H); ^{13}C NMR ($CDCl_3$) 118.3 (1), 120.5 (0), 123.7 (0), 125.8 (1), 128.7 (1), 131.8 (1), 132.3 (0), 135.0 (1), 135.1 (0), 138.6 (0), 146.7 (0), 148.6 (1), 150.2 (1), 151.7 (0), 181.6 (0) ppm. Anal. (exact mass, HREIMS) calcd for $C_{15}H_7BrN_2O$ m/e 309.9742, found 309.9741. Anal. ($C_{15}H_7BrN_2O$) C, H, N. Compound **6**: mp 200–201 °C; IR (KBr) 1670, 1592, 1570, 1430, 1382, 1365, 1330 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.56 (t, 3 H, J = 7.1 Hz), 4.79 (q, 2 H, J = 7.1 Hz), 7.67 (ddd, 1 H, J = 7.3, 7.3, 1.4 Hz), 7.79 (ddd, 1 H, J = 7.3, 7.3, 1.4, Hz), 7.82 (d, 1 H, J = 6.1 Hz), 8.36 (dd, 1 H, J = 7.3, 1.4 Hz), 8.72 (d, 1 H, J = 6.1 Hz), 8.75 (dd, 1 H, J = 7.3, 1.4 Hz); ^{13}C NMR ($CDCl_3$) 14.6 (3), 64.7 (2), 107.4 (0), 117.5 (0), 117.8 (1), 125.5 (1), 128.2 (1), 131.4 (1), 132.2 (0), 134.5 (1), 135.0 (0), 140.9 (0), 144.6 (0), 147.2 (1), 151.7 (0), 159.9 (0), 181.4 (0) ppm. Anal. (exact mass, HREIMS) calcd for $C_{17}H_{11}BrN_2O$ m/e 354.0004, found 354.0000. Anal. ($C_{17}H_{11}BrN_2O$) C, H, N.

4-Chloro-7*H*-naphtho[1,2,3-*ij*][2,7]naphthyridin-7-one (4-Chlorosampangine, 7). *N*-Chlorosuccinimide (200 mg, 1.5 mmol) was added to a suspension of **4** (232 mg, 1.0 mmol) in DMF (10 mL) and the mixture stirred at 100 °C for 24 h.[22] The mixture was then poured onto H_2O (100 mL), and the solids were isolated by filtration. The crude product was purified by column chromatography eluting with $CHCl_3$/EtOAc (9:1) to give **7** (142 mg, 53%). Crystallization from EtOAc provided an analytical sample: mp 262–263 °C; IR (KBr) 1670, 1590, 1410, 1315 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.68 (ddd, 1 H, J = 7.6, 7.6, 1.4 Hz), 7.81 (ddd, 1 H, J = 7.6, 7.6, 1.4 Hz), 7.95 (d, 1 H, J = 5.9 Hz), 8.41 (dd, 1 H, J = 7.6, 1.4 Hz), 8.76 (dd, 1 H, J = 7.6, 1.4 Hz), 8.93 (d, 1 H, J = 5.9 Hz), 9.09 (s, 1 H); ^{13}C NMR ($CDCl_3$) 115.7 (1), 120.0 (0), 125.6 (1), 128.5 (1), 131.7 (1), 132.0 (0), 132.2 (0), 134.8 (1), 135.0 (0), 136.9 (0), 146.0 (0), 147.2 (1), 148.2 (1), 151.5 (0), 181.2 (0) ppm. Anal. (exact mass, HREIMS) calcd for $C_{15}H_7ClN_2O$ m/e 266.0247, found 262.0245. Anal. ($C_{15}H_7ClN_2O$) C, H, N.

4-Azido-7*H*-naphtho[1,2,3-*ij*][2,7]naphthyridin-7-one (4-Azidosampangine, 8). A solution of sodium azide (650 mg, 10.0 mmol) in H_2O (5 mL) was added to a suspension of **5** (312 mg, 1.0 mmol) in acetone (20 mL). The mixture was stirred at reflux for 1 h, the acetone evaporated, and H_2O (50 mL) added. The product was extracted into $CHCl_3$ (4 × 25 mL) and the organic layer dried (Na_2SO_4) and concentrated. Flash silica gel chromatography while eluting with $CHCl_3$/EtOAc (95:5) in a dark room gave pure **8** (220 mg, 80%). An analytical sample was obtained by crystallization from $CHCl_3$: mp 271–272 °C; UV (MeOH) λ_{max} 207 (log ϵ 4.59), 226 (log ϵ 4.60), 250 (log ϵ 4.55), 260 (log ϵ 4.56), 267 (sh, log ϵ 4.50), 295 (log ϵ 3.93), 307 (log ϵ 3.89), 400 (log ϵ 4.10), 416 (log ϵ 4.08); IR (KBr) 2120, 1670, 1590, 1485, 1405, 1375, 1335, 1310 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.71 (ddd, 1 H, J = 7.7, 7.7, 1.4 Hz), 7.84 (ddd, 1 H, J = 7.7, 7.7, 1.4 Hz), 7.87 (d, 1 H, J = 5.8 Hz), 8.48 (dd, 1 H, J = 7.7, 1.4 Hz), 8.84 (dd, 1 H, J = 7.7, 1.4 Hz), 8.90 (d, 1 H, J = 5.8 Hz), 8.94 (s, 1 H); ^{13}C NMR ($CDCl_3$) 114.3 (1), 119.7 (0), 125.5 (1), 128.6 (1), 131.4 (0), 131.5 (1), 132.5 (0), 134.5 (1), 135.3 (0), 136.0 (0), 137.3 (1), 143.7 (0), 147.4 (1), 150.9 (0), 181.1 (0). Anal. (exact mass, HREIMS) calcd for $C_{15}H_7N_5O$ m/e 273.0650, found 273.0621. Anal. ($C_{15}H_7N_5O \cdot {}^1/_2H_2O$) C, H, N.

4-Amino-7*H*-naphtho[1,2,3-*ij*][2,7]naphthyridin-7-one (4-Aminosampangine, 9). Method A. Hydrogen sulfide[25] was bubbled through a solution containing **8** (160 mg, 0.58 mmol) and piperidine (2 drops) in MeOH (20 mL) that was precooled to 10 °C. After 30 min, the temperature of the reaction was allowed to rise to ambient temperature, and after an additional 30 min the reaction was stopped. The solvent was evaporated, and the residual solids were chromatographed over silica gel using $CHCl_3$/MeOH (9:1) as eluant to give **9** (136 mg, 95%). The chromatography of **9** is easily monitored through its fluorescent characteristics. An analytical sample was obtained by crystallization from DMSO: mp >325 °C; UV (MeOH) 207 (log ϵ 4.34), 252 (log ϵ 4.16), 269 (log ϵ 4.15), 348 (log ϵ 3.56), 461 (log ϵ 4.11); IR (KBr) 3300 (br) 1725, 1625, 1585, 1560, 1505, 1460, 1385, 1335, 1300 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.72 (ddd, 1 H, J = 7.5, 7.5, 1.3 Hz), 7.83 (ddd, 1 H, J = 7.5, 7.5, 1.3 Hz), 7.95 (br s, 2 H), 8.21 (d, 1 H, J = 5.9 Hz), 8.26 (dd, 1 H, J = 7.5, 1.3 Hz), 8.38 (s, 1 H), 8.75 (dd, 1 H, J = 7.5, 1.3 Hz), 8.81 (d, 1 H, J = 5.9 Hz); ^{13}C NMR (DMSO-d_6) 115.7 (1), 119.8 (0), 124.1 (0), 124.7 (1), 127.0 (1), 130.9 (1), 132.0 (1), 132.8 (0), 132.9 (0), 133.1 (1), 134.7 (0), 144.3 (1), 145.1 (0), 148.5 (0), 178.4 (0) ppm. Anal. (exact mass, HREIMS) calcd for $C_{15}H_9N_3O$ m/e 247.0746, found 247.0744. Anal. ($C_{15}H_9N_3O \cdot H_2O$) C, H; N: calcd 24.81; found, 24.38.

Method B. A mixture of **5** (622 mg, 2.0 mmol) in acetone (40 mL) and sodium azide (1.30 g, 20.0 mmol) in H_2O (10 mL) was heated at reflux for 1 h. The acetone was then removed by evaporation, MeOH (40 mL) added, and the mixture cooled to 10 °C. Piperidine (2 drops) was added and a stream of hydrogen sulfide bubbled through the reaction. After 30 min, the temperature was allowed to rise to 23 °C and the reaction continued for an additional 30 min. The solvent was then evaporated and the residue chromatographed as above to give **9** (450 mg, 91%).

Method C. A solution of **8** (273 mg, 1.0 mmol) in MeOH (50 mL) was heated at reflux for 7 days.[24] Following evaporation of the solvent, the residue was chromatographed as above to give **9** (119 mg, 48%).

4-Methoxy-7*H*-naphtho[1,2,3-*ij*][2,7]naphthyridin-7-one (4-Methoxysampangine, 10). A solution of **5** (934 mg, 3.0 mmol) and sodium methoxide (1.62 g, 30.0 mmol) in dry MeOH (100 mL) was refluxed for 12 h. The MeOH was then evaporated and $CHCl_3$ (500 mL) added. The organic solution was washed with H_2O (4 × 100 mL), dried (K_2CO_3), and concentrated. Pure **10** (660 mg, 84%), by TLC analysis, was obtained by trituration with EtOAc. An analytical sample was obtained by crystallization from $CHCl_3$: mp 279–280 dec; IR (KBr) 1670, 1595, 1570, 1500, 1405, 1375, 1320 cm^{-1}; ^1H and ^{13}C NMR (see Tables I and II). Anal. (exact mass, HREIMS) calcd for $C_{16}H_{10}N_2O_2$ m/e 262.0742, found 262.0740. Anal. ($C_{16}H_{10}N_2O_2$) C, H, N. The mp, IR, and ^1H and ^{13}C NMR data reported are consistent with those reported for eupomatidine-2, an alkaloid recently isolated and assigned structure **10**.[32]

(*E*)-4-Methoxy-2-butenal (12). A solution of (*Z*)-2-buten-1,4-diol (88.11 g, 1.00 mol), sodium hydroxide (55.99 g, 1.40 mol), and H_2O (230 mL) was heated to 70 °C before adding dimethyl sulfate (53.9 mL, 0.57 mol) dropwise. The mixture was then stirred for 2 h at 80 °C before continuously extracting the product with Et_2O in a 1-L extraction apparatus for 26 h. The ether extract was dried ($MgSO_4$) and concentrated by rotary evaporation. Distillation of the product through a 10-cm Vigreaux column gave two fractions; the first fraction (bp 28–34 °C, 25 mmHg) was identified as (*Z*)-1,4-dimethoxy-2-butene (13.70 g, 11%) and the higher boiling fraction (bp 92–100 °C, 25 mmHg) as (*Z*)-4-methoxy-2-buten-1-ol (38.42 g, 66%).[33]

To a suspension of pyridinium chlorochromate[34] (63.3 g, 0.29 mol) in CH_2Cl_2 (500 mL) was added a CH_2Cl_2 (80 mL) solution of (*Z*)-4-methoxy-2-buten-1-ol (28.0 g, 0.27 mol). The mixture immediately darkened and evolved heat. After stirring for 2.5 h at ambient temperature, the mixture was diluted with Et_2O (2000 mL) and filtered through a bed of Florisil. The residual solids in the flask were washed well with Et_2O and the washes passed through the Florisil bed. The organic filtrate was concentrated to an oil and this oil distilled (bp 66–68 °C, 20 mmHg) with a short-path still to give **12** (14.30 g, 52%). The colorless product turns light yellow shortly after distillation but can be stored overnight in a −20 °C freezer before use: IR (neat) 2990, 2920, 2820, 2720, 1690, 1640, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.36 (s, 3 H), 4.15 (dd, 2 H, J = 4.2, 2.0 Hz), 6.27 (ddt, 1 H, J = 15.8, 8.0, 2.0 Hz), 6.78 (dt, 1 H, J = 15.8, 4.2 Hz), 9.52 (d, 1 H, J = 8.0 Hz). Anal. (exact mass, HREIMS) calcd for $C_5H_8O_2$ m/e 100.0524; found 100.0524. Anal. ($C_5H_8O_2$) C, H.

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(*E*)-4-Methoxy-2-butenal *N,N*-Dimethylhydrazone (13). *N,N*-Dimethylhydrazine (10.90 mL, 0.14 mol) was added dropwise over 15 min to **12** (13.06 g, 0.13 mol) while cooling the mixture with an ice bath.[30] The bath was then removed and the mixture stirred for 1.5 h at ambient temperature. Calcium chloride (20 g) was added to the reaction and allowed to stand for 15 min, and the product was decanted. Distillation (bp 102–110 °C, 25 mmHg) of the oil through a 10-cm Vigreaux column provided pure **13** (14.45 g, 78%): IR (neat) 2850, 2820, 1560, 1465, 1445, 1375 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.80 (s, 6 H), 3.26 (s, 3 H), 3.93 (dd, 2 H, J = 6.2, 1.2 Hz), 5.74 (dt, 1 H, J = 15.8, 6.2 Hz), 6.31 (ddt, 1 H, J = 15.8, 8.9, 1.2 Hz), 6.92 (d, 1 H, J = 8.9 Hz). Anal. (exact mass, HREIMS) calcd for $C_7H_{14}N_2O$ m/e 142.1106, found 142.1105. Anal. ($C_7H_{14}N_2O$) C, H, N.

4-(Methoxymethyl)benzo[*g*]quinoline-5,10-dione (4′-Methoxycleistopholine, 14). The general procedure described above for cleistopholine was followed beginning with **13** (12.25 g, 86.0 mmol) and **1** (15.71 g, 66.0 mmol) in xylene (160 mL). Flash silica gel chromatography of the brown product obtained upon workup and elution with EtOAc/petroleum ether (7:3) provided **14** (1.96 g, 12%). An analytical sample was prepared by crystallization from EtOAc: mp 172–173 °C; IR (KBr) 1680, 1665, 1590, 1300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.59 (s, 3 H), 5.12 (s, 2 H), 7.80–7.84 (m, 2 H), 8.07 (dt, 1 H, J = 5.0, 1.0 Hz), 8.21–8.24 (m, 1 H), 8.34–8.37 (m, 1 H), 9.04 (d, 1 H, J = 5.0 Hz); ^{13}C NMR ($CDCl_3$) 59.1 (3), 72.1 (2), 125.3 (1), 127.1 (0), 127.2 (1), 127.6 (1), 132.6 (0), 133.4 (0), 134.4 (1), 134.6 (1), 149.7 (0), 152.7 (0), 154.3 (1), 181.7 (0), 184.9 (0). Anal. (exact mass, HREIMS) calcd for $C_{15}H_{11}NO_3$ m/e 253.0739; found 253.0744. Anal. ($C_{15}H_{11}NO_3 \cdot {}^1/_4H_2O$) C, H, N.

3-Methoxy-7*H*-naphtho[1,2,3-*ij*][2,7]naphthyridin-7-one (3-Methoxysampangine, 15). The general procedure described above for sampangine was followed beginning with **14** (1.00 g, 3.95 mmol). Chromatography of the crude product on flash silica gel while eluting with $CHCl_3$/EtOAc (9:1) gave a yellow fraction containing the desired product plus impurities. This fraction was rechromatographed as above to give pure **15** (0.06 g, 6%). An analytical sample was obtained by crystallization from $CHCl_3$: mp 225–227 °C (lit. mp 213–215 °C); IR (KBr) 1673, 1598, 1570, 1380, 1300 cm^{-1}; ^1H and ^{13}C NMR (see Tables I and II). Anal. (exact mass, HREIMS) calcd for $C_{16}H_{10}N_2O_2$ m/e 262.0742, found 262.0747. Anal. ($C_{16}H_{10}N_2O_2 \cdot {}^1/_2H_2O$) C, H, N: calcd, 71.27; found 70.82. The TLC, IR, and ^1H and ^{13}C NMR data for this compound were identical in all respects to that of the authentic natural product.

4-Ethylbenzo[*g*]quinoline-5,10-dione (Homocleistopholine, 17). *N,N*-Dimethylhydrazine (42.0 mL 0.55 mol) was added dropwise to (*E*)-2-pentenal (42.06 g, 0.50 mol) at such a rate that the reaction temperature could be maintained at about 0 °C. The mixture was then stirred for 1 h at ambient temperature and the organic phase separated and dried (K_2CO_3). Distillation (bp 84–86 °C, 25 mmHg; lit.[35] bp 60 °C, 15 mmHg) through a 10-cm Vigreaux column gave (*E*)-2-pentenal *N,N*-dimethylhydrazone (51.3 g, 81%): n^{20} 1.5104; IR (neat) 2960, 2870, 2850, 2820, 2780, 1565, 1470, 1460, 1445 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (t, 3 H, J = 7.4 Hz), 2.16–2.05 (m, 2 H), 2.76 (s, 6 H), 5.82 (dt, 1 H, J = 15.6, 6.3 Hz), 6.14 (dd, 1 H, J = 15.6, 8.8 Hz), 6.97 (d, 1 H, J = 8.8 Hz).

A solution of (*E*)-2-pentenal *N,N*-dimethylhydrazone (49.15 g, 0.39 mol) in xylene (100 mL) was quickly added to a xylene (600 mL) solution of **1** (71.12 g, 0.30 mol) and the dark colored mixture heated at reflux for 6 h under a nitrogen atmosphere. Workup followed the procedure described above for cleistopholine. Chromatography provided pure **17** (10.90 g, 14%). An analytical sample was obtained by crystallization from EtOAc: mp 157–158 °C; IR (KBr) 1680, 1665, 1590, 1575, 1450, 1340, 1300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (t, 3 H, J = 7.4 Hz), 3.28 (q, 2 H, J = 7.4 Hz), 7.48 (d, 1 H, J = 5.0 Hz), 7.71–7.78 (m, 2 H), 8.14–8.18 (m, 1 H), 8.25–8.28 (m, 1 H), 8.87 (d, 1 H, J = 5.0 Hz); ^{13}C NMR ($CDCl_3$) 14.1 (3), 28.0 (2), 127.1 (1), 127.2 (1), 128.5 (0), 129.3 (1), 132.4 (0), 133.9 (0), 134.0 (1), 134.5 (1), 150.2 (0), 153.6 (1), 157.2 (0), 181.8 (0), 184.5 (0) ppm. Anal. (exact mass, HREIMS) calcd for $C_{15}H_{11}NO_2$ m/e 237.0790, found 237.0788. Anal. ($C_{15}H_{11}NO_2$) C, H, N.

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Synthesis of 3-Methyl-7*H*-naphtho[1,2,3-*ij*][2,7]-naphthyridin-7-one (3-Methylsampangine, 18), 4-(Methylcarbonyl)benzo[*g*]quinoline-5,10-dione (4′-Oxohomocleistopholine, 19), and 2,3-Dihydro-4′-ethyl-3α-methylspiro[7*H*-naphtho[1,2,3-*ij*][2,7]naphthyridine-2α,10′-benzo[*g*]quinoline]-5′,7-dione, 20. The general procedure outlined above for sampangine was followed beginning with **17** (7.12 g, 30.0 mmol). Evaporation of the CH_2Cl_2 extract provided a product that was a complex mixture by TLC analysis. Flash silica gel chromatography of this material while eluting with $CHCl_2$–EtOAc (9:1) gave fractions consisting of recovered **17** (0.75 g, 11%), **18** (0.45 g, 6%), and a mixture of **18–20**. The latter mixture was separated by crystallization from EtOAc and manual sorting of the crystal types; **18** crystallized as long yellow needles, **19** as nearly perfect golden octahedra (0.45 g, 6%), and **20** as rectangular yellow plates (0.41 g, 6%). Yields for **19** and **20** represent minimal quantities present as actually isolated by this procedure. Compound **18**: 219–220 °C; IR (KBr) 1665, 1590, 1570, 1370, 1310 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.65 (s, 3 H), 7.62 (ddd, 1 H, J = 7.8, 7.8, 1.3 Hz), 7.76 (ddd, 1 H, J = 7.8, 7.8, 1.3 Hz) 7.93 (d, 1 H, J = 5.6 Hz) 8.38 (dd, 1 H, J = 7.8, 1.3 Hz), 8.56 (br s, 1 H), 8.65 (dd, 1 H, J = 7.8, 1.3 Hz), 9.08 (d, 1 H, J = 5.6 Hz); ^{13}C NMR ($CDCl_3$) 15.3 (3), 119.0 (0), 120.5 (1), 124.9 (1), 127.2 (0), 128.3 (1), 130.8 (1), 131.9 (0), 134.5 (1), 135.6 (0), 138.4 (0), 146.8 (1), 148.0 (0), 148.2 (1), 149.1 (0), 182.0 (0) ppm. Anal. (exact mass, HREIMS) calcd for $C_{16}H_{10}N_2O$ m/e 246.0793, found 246.0784. Anal. ($C_{16}H_{10}N_2O$) C, H, N. Compound **19**: mp 208–210 °C; IR (KBr) 1700, 1675, 1665, 1580, 1465, 1450, 1350, 1335, 1305 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.61 (s, 3 H), 7.45 (d, 1 H, J = 4.7 Hz), 7.81–7.91 (m, 3 H), 8.22–8.26 (m, 1 H), 8.38–8.42 (m, 1 H), 9.14 (d, 1 H, J = 4.7 Hz); ^{13}C NMR ($CDCl_3$) 30.4 (3), 123.7 (1), 126.9 (0), 127.5 (1), 128.1 (1), 132.3 (0), 132.9 (0), 134.9 (1), 135.2 (1), 149.3 (0), 151.5 (0), 155.3 (1), 180.7 (0), 182.7 (0), 202.0 (0) ppm. Anal. (exact mass, HREIMS) calcd for $C_{15}H_9NO_3$ m/e 251.0582, found 251.0576. Anal. ($C_{15}H_9NO_3$) C, H, N. Compound **20**: mp 273–274 °C; IR (KBr) 2980, 1665, 1620, 1590, 1570, 1550, 1455, 1310, 1280, 1240, 1200, 1160, 1030, 965, 930, 855, 790, 780, 760, 722, 710, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (d, 3 H, J = 7.0 Hz), 1.26 (t, 3 H, J = 7.4 Hz), 3.18–3.32 (m, 2 H), 3.61 (dq, 1 H, J = 1.3, 7.0 Hz), 7.08 (d, 1 H, J = 4.9 Hz), 7.22 (dd, 1 H, J = 4.8, 1.3 Hz), 7.60 (ddd, 1 H, J = 7.8, 5.8, 3.0 Hz), 7.68–7.78 (m, 4 H), 8.07 (d, 1 H, J = 4.9 Hz), 8.29 (dd, 1 H, J = 7.8, 1.0 Hz), 8.46–8.50 (m, 2 H), 8.87 (d, 1 H, J = 4.8 Hz); ^{13}C NMR ($CDCl_3$) 11.9 (3), 14.5 (3), 28.1 (2), 42.8 (1), 68.0 (0), 123.1 (1), 124.1 (0), 125.3 (1), 125.5 (1), 125.7 (0), 126.4 (1), 127.6 (1), 128.2 (1), 128.3 (1), 131.6 (1), 132.6 (0), 132.8 (0), 133.7 (1), 133.8 (1), 134.9 (0), 143.3 (0), 146.3 (0), 147.8 (0), 151.4 (1), 153.5 (1), 156.3 (0), 156.9 (0), 157.0 (0), 182.8 (0), 186.0 (0) ppm. Anal. (exact mass, HREIMS) calcd for $C_{30}H_{21}N_3O_2$ m/e 455.1634, found 455.1635. Anal. ($C_{30}H_{21}N_3O_2 \cdot {}^1/_2C_4H_8O_2$) C, H, N.

12-Methylbenz[*b*]acridine-6,11-dione (Benzo[2,3]-cleistopholine, 23). A mixture of absolute EtOH (600 mL), 1,4-naphthoquinone (4.74 g, 30.0 mmol), 2′-aminoacetophenone (3.37 g, 30 mmol), and cerium trichloride heptahydrate (1.66 g, 3.0 mmol) was warmed to effect solution and then allowed to stand at ambient temperature while a steady stream of air was continuously blown into the reaction mixture for 24 h.[27] The red precipitate was collected by filtration and washed with a small amount of absolute EtOH. The filtrate was subjected to the above procedure twice, and a total of 5.26 g (60%) of 2-(2′-acetylanilino)-1,4-naphthoquinone was obtained as red needles: mp 177–179 °C; ^1H NMR ($CDCl_3$) δ 2.66 (s, 3 H), 6.99 (s, 1 H), 7.06 (d, 1 H, J = 9.0 Hz), 7.14 (ddd, 1 H, J = 6.0, 6.0, 1.0 Hz), 7.55 (ddd, 1 H, J = 9.0, 6.0, 1.0 Hz), 7.65 (ddd, 1 H, J = 8.0, 8.0, 1.5 Hz), 7.73 (ddd, 1 H, J = 8.0, 8.0, 1.5 Hz), 7.93 (dd, 1 H, J = 6.0, 1.0 Hz), 8.05 (dd, 1 H, J = 9.0, 1.0 Hz), 8.13 (dd, 1 H, J = 9.0, 1.0 Hz).

To a cold, stirred suspension of 2-(2′-acetylanilino)-1,4-naphthoquinone (4.00 g, 15.7 mmol) in glacial acetic acid (132 mL) was slowly added concentrated H_2SO_4 (13.2 mL). The reaction mixture was then gently refluxed for 15 min, cooled, and poured onto ice–H_2O (2000 mL). The yellow precipitate was collected and washed with a small amount of ice–H_2O to give **23** (3.23 g, 100%) as fine greenish-yellow needles: mp 237–239 °C dec (lit.[28] mp 236 °C); IR (KBr) 1680, 1655, 1590, 1495, 1375 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.22 (s, 3 H), 7.69 (ddd, 1 H, J = 6.7,

Table V. Crystal Data and Summary of Intensity Data Collection and Structure Refinement for Compound 20

compound	$C_{30}H_{21}N_3O_2$
color/shape	yellow/parallelepiped
formula weight	455.52
space group	$P2_1/n$
temperature, °C	18
cell constants[a]	
a, Å	8.426 (8)
b, Å	21.535 (9)
c, Å	12.644 (9)
β, deg	101.23 (6)
cell volume, Å^3	2250
formula units/unit cell	4
D_{calc}, g cm^{-3}	1.34
μ_{calcd}, cm^{-1}	0.93
diffractometer/scan	Enraf-Nonius CAD-4/ω-2θ
radiation, graphite monochromator	Mo Kα (λ = 0.71073)
maximum crystal dimensions, mm	0.30 × 0.40 × 0.45
scan width	0.80 + 0.35 tan θ
standard reflections	$\bar{4}81$, 067, 166, 156, $\bar{3}83$, $37\bar{4}$, 056
decay of standards	±4%
reflections measured	4361
2θ range, deg	$2 \le 2\theta \le 50$
range of h, k, l	+10, +25, ±15
reflections obsd [$F_o \ge 5\sigma(F_o)$][b]	1351
computer programs[c]	SHELX[d]
structure solution	SHELXS[e]
no. of parameters varied	322
weights	$[\sigma(F_o)^2 + 0.002F_o^2]^{-1}$
GOF	0.80
$R = \Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$	0.064
R_w	0.079
largest feature final difference map	0.2 e Å^{-3}

[a] Least-squares refinement of $((\sin\theta)/\lambda)^2$ values for 25 reflections $\theta > 11°$. [b] Corrections: Lorentz-polarization. [c] Neutral scattering factors and anomalous dispersion corrections from ref 35. [d] See ref 37. [e] See ref 36.

6.7, 1.3 Hz), 7.76–7.78 (m, 1 H), 7.78–7.84 (m, 1 H), 7.84 (ddd, 1 H, J = 6.7, 6.7, 1.3 Hz), 8.25 (dd, 1 H, J = 6.0, 2.5 Hz), 8.29 (br d, 1 H, J = 6.7 Hz), 8.34 (dd, 1 H, J = 6.0, 2.5 Hz), 8.39 (br d, 1 H, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$) 16.9 (3), 125.3 (0), 125.3 (1), 127.4 (1), 127.4 (1), 129.4 (1), 129.7 (0), 132.2 (1), 132.3 (1), 133.3 (0), 133.9 (1), 134.6 (1), 135.3 (0), 148.2 (0), 148.5 (0), 151.8 (0), 182.0 (0), 184.9 (0) ppm. Anal. (exact mass, HREIMS) calcd for $C_{18}H_{11}N_2O$ m/e 273.0789, found 273.0779. Anal. ($C_{18}H_{11}N_2$) C, H, N; calcd 79.11; found, 78.54.

9*H*-Benzo[*f*]naphtho[1,2,3-*ij*][2,7]naphthyridin-9-one (Benzo[4,5]sampangine, 24). The general sampangine procedure described above was followed beginning with **23** (2.38 g, 8.7 mmol). The procedure provided **24** (1.82 g, 56%) as yellow needles: mp 260–262 °C, IR (KBr) 1680, 1590, 1442, 1390, 1300 cm^{-1}; ^{1}H and ^{13}C NMR (see Table III). Anal. (exact mass, HREIMS) calcd for $C_{19}H_{10}N_2O$ m/e 282.0792, found 282.0783. Anal. ($C_{19}H_{10}N_2O$) C, H; N: calcd, 80.84; found, 80.33.

X-ray Crystallography. Crystals of compound **20** were grown from EtOAc. A yellow, parallelepiped single crystal of this substance was mounted on a pin. The space group was determined to be the centric $P2_1/c$ from the systematic absences. Diffraction experiments were performed at 18 °C on an Enraf-Nonius CAD-4 diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Cell constants from setting angles of 25 reflections ($\theta > 11°$). Intensity data were corrected for Lorentz-polarization effects. θ_{max} = 50°; h 0 to 10, k 0 to 25, l −15 to 15. Standard reflections $\bar{4}81$, 067, 166, 156, $\bar{3}83$, $37\bar{4}$, 056 observed every 3600 s of data collection time; variation = ±4%; 4361 reflections measured, 1351 independent observed reflections [$F_o \ge 5\sigma(F_o)$]. The data collection parameters are summarized in Table V.

The structure was solved utilizing the SHELXS direct-methods program.[36] Least-squares refinement with isotropic thermal parameters led to R = 0.128. The geometrically constrained hydrogen atoms were placed in calculated positions 0.95 Å from the bonded carbon atom and allowed to ride on that atom with B fixed at 5.5 Å^2. The methyl hydrogen atoms were included as a rigid group with rotational freedom at the bonded carbon atom (C–H = 0.95 Å, B = 5.5 Å^2). Scattering factors and anomalous-dispersion corrections were from the *International Tables for X-ray Crystallography*[37] and the structure was refined with SHELX 76.[38] $\sum w(|F_o| - |F_c|)^2$ minimized, weights = $[\sigma(F_o)^2 + 0.002(F_o)^2]^{-1}$, 322 parameters varied. Refinement of non-hydrogen atoms with anisotropic temperature factors led to the final values of R = 0.064 and R_w = 0.079. Δ/σ in final least squares refinement cycle <0.01, $\Delta\rho$ <0.2 e Å^{-3} in final difference map.

Biological Methods. In vitro evaluation of activity versus *Candida albicans* NIH B311, *Cryptococcus neoformans* ATCC 32264, *Aspergillus fumigatus* ATCC 26934, and *Mycobacterium intracellulare* ATCC 23068 was accomplished by using the agar-well diffusion assay previously described,[15] with the following modifications. *C. albicans* NIH B311, which served to induce experimental disseminated candidiasis, was used for the initial qualitative evaluation of anticandidal activity. The organism was grown in Sabouraud dextrose broth (SDB) for 24 h at 37 °C, after which the cells were harvested by centrifugation (4 °C, 2000 rpm, 3 min). After centrifugation, the cells were washed and suspended in sterile 0.9% saline to give a final concentration of 10^6 CFU/mL (adjusted with a hemacytometer). Inocula of *C. neoformans* and *A. fumigatus* were prepared by suspension of the surface growth of stock agar slants in sterile H_2O as previously described.[2,5] Culture plates (15 × 100 mm) for the qualitative assay were prepared from 25 mL of Sabouraud dextrose agar. With sterile cotton swabs, the plates were streaked with the suspension of the appropriate test organism. Cylindrical plugs were removed from the agar plates with a sterile cork borer to produce wells with diameters of approximately 11 mm. To the wells was added 100 µL of a solution or suspension of an extract, fraction, or pure compound. Pure compounds were tested at 1 mg/mL. When solvents other than H_2O, EtOH, MeOH, dimethyl sulfoxide, dimethylformamide, or acetone were required to dissolve the compounds, solvent blanks were included. Antifungal activity was recorded as the width (in mm) of the zone of inhibition, measured from the edge of the agar well to the edge of the zone, following incubation of the plates for 24 h for *C. albicans* (37 °C) or 48 h for *A. fumigatus* (30 °C) and *C. neoformans* (26 °C). The antifungal agent amphotericin B was included as a positive control in each assay. For qualitative in vitro antimycobacterial evaluation, *M. intracellulare* ATCC 23068 was grown in Lowenstein–Jensen (L–J) medium for 48 h at 37 °C. The remainder of the assay was conducted as described above except the organism was cultured in Mueller–Hinton broth. Rifampin was used as a positive control in the antimycobacterial assay.

Compounds that exhibited significant activity in the qualitative screen (>4-mm zone) were subjected to a quantitative assay to determine the minimum inhibitory concentration (MIC). The method used to determine the MIC was the 2-fold serial broth dilution assay[15] in yeast nitrogen both (Difco Laboratories) for *C. albicans*, Mycophil broth for *C. neoformans*, Sabouraud dextrose broth for *A. fumigatus*, and Mueller–Hinton broth for *M. intracellulare*. The inoculum for the MIC determination was prepared as described for the qualitative evaluation. With a calibrated sterile wire loop, each tube was inoculated with 10 µL of the suspension. The MIC was taken as the lowest concentration of a compound that inhibited the growth of the test organisms after an appropriate incubation period (37 °C for 24 and 48 h for *C. albicans*; 30 °C for 48 and 72 h for *A. fumigatus*; 26 °C for 48 and 72 h for *C. neoformans*; 37 °C for 72 h for *M. intracellulare*). The antifungal agent amphotericin B was included as a positive control in each antifungal assay and rifampin in the antimycobacterial evaluation. MIC values were confirmed by replicate evaluations.

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Supplementary Material Available: Tables of final fractional coordinates, bond distances and angles, least-squares planes, and thermal parameters (7 pages); observed and calculated structure factors for compound **20** (3 pages). Ordering information is given on any current masthead page.