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SYNTHESIS OF CRIBROSTATIN 6 AND ITS RELATED COMPOUNDS

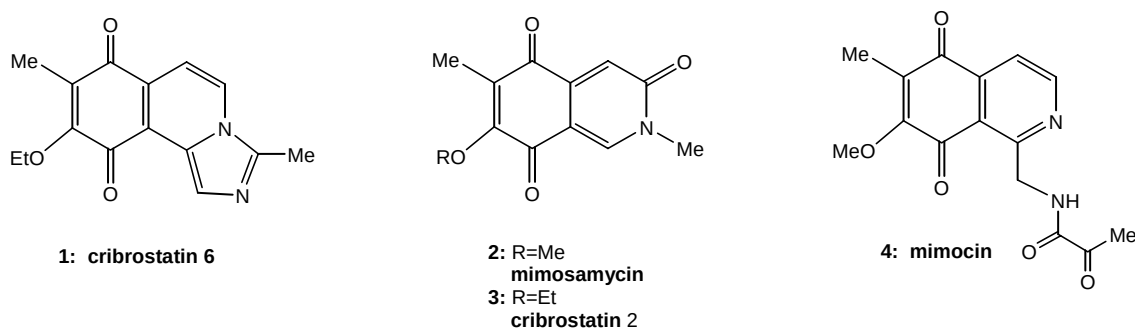
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Abstract – The synthesis of cribrostatin 6 (**1**), which shows good biological activity as a dark blue cancer cells growth inhibitor and a number of pathogenic bacteria and fungi, was achieved in two steps from 1-acetylaminoethyl-5,7-diethoxy-8-hydroxy-6-methylisoquinoline (**14**). The related compounds (**8~11**) were also synthesized, and the antimicrobial activities of **1** and its nine related compounds (**5~13**) were investigated.

Since mimosamycin (**2**) was isolated from a culture filtrate of *Streptomyces lavendulae*,^{1a} a series of structurally interesting and biologically active 5,8-isoquinolinedione alkaloids have been isolated from marine sources and from *Actinomycetes*.¹ Compound (**2**) shows antimicrobial activity, particularly against mycobacteria. Mimocin (**4**), isolated from a *Streptomyces lavendulae* metabolite,^{1b} contains a pyruvamide side chain at the C-1 position of 5,8-isoquinolinedione and exhibits strong antimicrobial activity, while cribrostatin 2 (**3**), isolated from the marine sponge *Cribochalina* sp.^{1c} contains an ethoxy group at the same position the ethoxy group in **1** and has been shown to exhibit activity against the P-388 lymphocytic leukemia cell line.

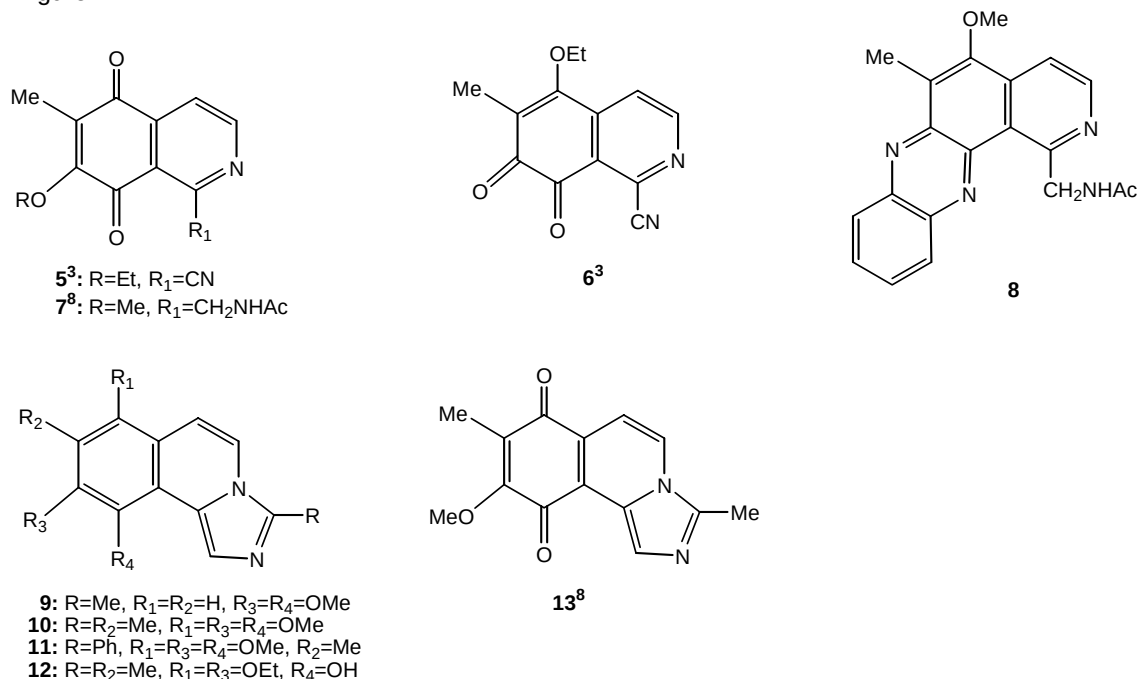
Figure 1



Recently Pettit and colleagues reported the isolation of cribrostatin 6 (**1**) from the marine sponge *Cribochalina* sp. The structure of **1**, based on spectral data and X-Ray crystal structure analysis,² was shown to consist of an isoquinolinedione skeleton, similar to that of mimosamycin (**2**), but containing a fused imidazole ring. Compound (**1**) shows good biological activity as a growth inhibitor of cancer cells and a number of pathogenic bacteria and fungi; its synthesis and bioactivity are therefore of interest. In

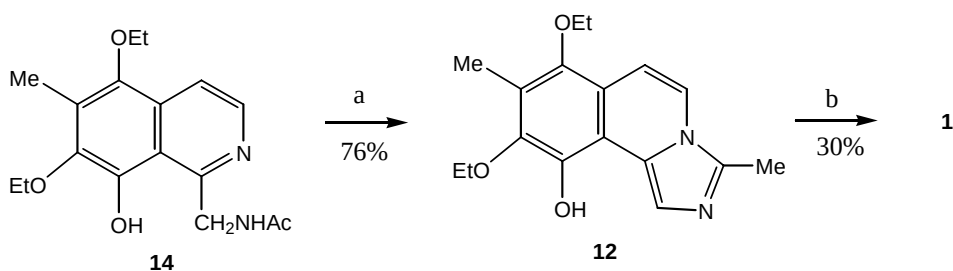
this study we report the synthesis of cribrastatin 6 (**1**) from 1-acetylaminoethyl-5,7-diethoxy-8-hydroxy-6-methylisoquinoline (**14**) in two steps, the preparation of related compounds (**8~11**), and an examination of the antimicrobial activities of **1** and its nine related compounds (**5~13**).

Figure 2



We have already reported the preparation of hydroxyisoquinoline (**14**),³ a starting material in the synthesis of cribrastatin 6 (**1**), from 2,4-diethoxy-3-methylphenol⁴ in eight steps according to a modified Pomeranz-Fritsch isoquinoline synthesis.⁵

Figure 3

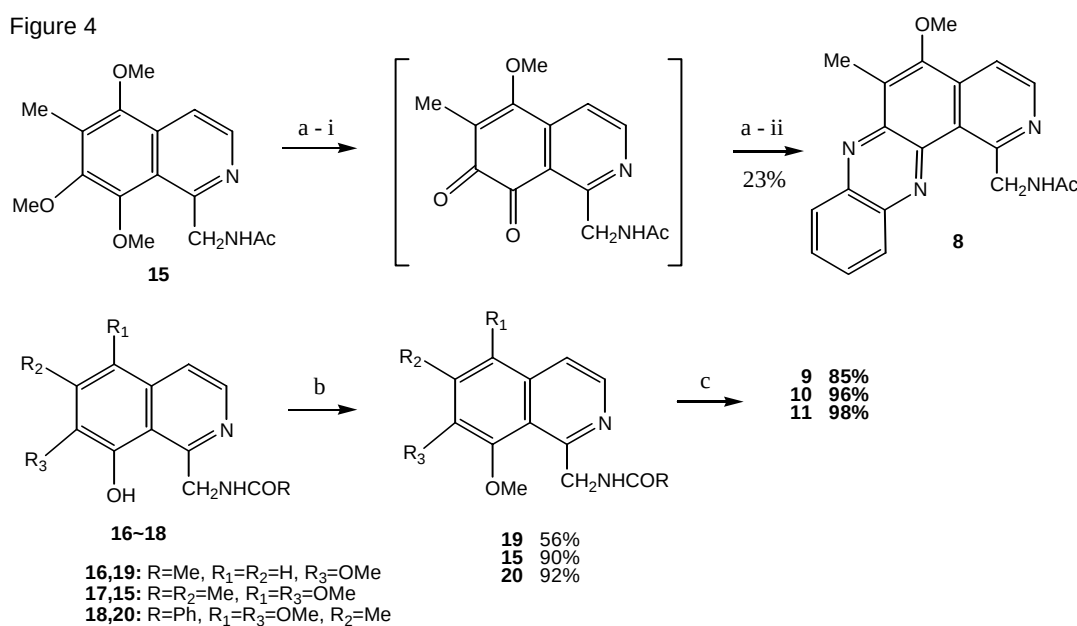


a) POCl_3 , toluene, 110-115°C, 15 min b) 60% HNO_3 , 10°C, 2h

Cyclization of **14** using POCl_3 ⁶ in toluene at 110-115°C for 15 min afforded imidazo[5,1-a]isoquinoline (**12**) in 76% yield. Treatment of **12** with 60% HNO_3 at 10°C for 2 h gave the desired product (**1**) in 30% yield. The spectroscopic data of synthetic **1** matched those of the authentic sample in all respects.

Pyrido[3,4-a]phenazine (**8**), a target molecule for studying antimicrobial activities, was prepared by condensation of 7,8-isoquinolinedione and *o*-phenylenediamine. Oxidation of trimethoxyisoquinoline (**15**) with ceric ammonium nitrate (CAN)⁷ in aqueous acetonitrile at 0-5°C for 0.5 h followed by condensation with *o*-phenylenediamine in EtOH at room temperature for 20 min afforded **8** in 23% yield.

Figure 4



a) i) CAN, MeCN, H₂O, 0–5°C, 0.5 h ii) *o*-C₆H₄(NH₂), EtOH, rt, 20 min
 b) CH₂N₂, Et₂O, rt, 3 h
 c) POCl₃, toluene, 110–115°C, 15 min

Hydroxyisoquinoline derivatives (**16**, **17** and **18**) have been prepared by catalytic hydrogenation of the corresponding 1-cyano-8-acyloxyisoquinolines over 10% Pd-C in MeOH containing HCl,⁸ which results in the intramolecular transfer of the acyl group from the oxygen atom to the nitrogen atom. O-Methylation of **16**, **17** and **18** with diazomethane in ether at room temperature for 3 h afforded the 8-methoxyisoquinolines (**19**, **15** and **20**) in 56%, 90% and 92% yields, respectively. Cyclization of **19**, **15** and **20** by the same procedure used for **12** furnished the corresponding imidazo[5,1-*a*]isoquinolines (**9**, **10** and **11**) in 85%, 96% and 98% yields, respectively.

The antimicrobial activities of cribrostatin 6 and nine related compounds against bacteria and fungi are studied. The results were shown in Table 1.

Table 1. Antimicrobial activities (MIC, µg/mL) of cribrostatin 6 (**1**) and its nine related compounds against bacteria and fungi.

Compound	1	5	6	7	8	9	10	11	12	13
Microorganism										
<i>B. subtilis</i>	10.4	5.2	-	83.3	-	-	-	20.8	-	2.6
<i>S. aureus</i>	10.4	2.6	-	-	-	-	-	-	-	10.4
<i>C. neoformans</i>	2.6	1.3	-	83.3	-	83.3	20.8	2.6	2.6	1.3
<i>T. menta-grophytes</i>	2.6	5.2	-	-	-	-	83.3	20.8	-	1.3

Antimicrobial activities of Cribrostatin 6 and nine related compounds against two bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and fungi (*Cryptococcus neoformans* and *Trichophyton mentagrophytes*) were determined by microbroth dilution method using brain heart infusion (Difco, USA) medium, and MIC values were determined at 24 to 72 h incubation at 37°C. – indicates the MIC values of above >100 µg/mL.

Cirobrostatin 6 and its ethyl derivative (**13**) showed higher antifungal activities, and their MIC values ranged from 1.3 to 2.6 $\mu\text{g/mL}$. Among them, compound (**13**) showed highest antimicrobial activity. Structure-activity relationship in these compounds suggests an importance of quinone structure in the molecule for the exhibition of their antimicrobial activity.

In summary, cribrostatin 6 (**1**) was synthesized in two steps from a known compound, and four other target molecules for the study of antimicrobial activities were also prepared. Cribrostatin 6 and its related compound such as **13** showed strong antimicrobial activities, and their antifungal activity are higher than that of their antibacterial activity.

EXPERIMENTAL

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ¹H-NMR spectra at 100 MHz and 270 MHz were measured in CDCl_3 or $\text{CDCl}_3+\text{CD}_3\text{OD}$ with tetramethylsilane as an internal standard. Anhydrous sodium sulfate was used for drying organic solvent extracts, and the solvent was removed with a rotary evaporator and finally under high vacuum. Column chromatography (flash chromatography) was performed with silica gel 60 (Merck, 230-400 mesh).

1-Acetylaminomethyl-7,8-dimethoxyisoquinoline (**19**)

1-Acetylaminomethyl-8-hydroxy-7-methoxyisoquinoline (**16**) (566 mg, 2.3 mmol) was added to an ether solution containing excess of CH_2N_2 and the mixture was stirred at rt for 3 h. The solvent was evaporated and the residue was chromatographed (eluting with ethyl acetate) to afford **19** (335 mg, 56%) as colorless prisms from benzene. mp 145-147°C. *Anal.* Calcd for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.35; H, 6.17; N, 10.72. IR(KBr) cm^{-1} : 3270, 1635. *Ms m/z* (%): 260(M^+ , 56), 217(100), 202(23). ¹H-NMR (CDCl_3) δ : 2.13(3H, s), 4.00(3H, s), 4.07(3H, s), 5.20(2H, d, $J=4$ Hz), 7.43(1H, d, $J=8$ Hz), 7.50(1H, d, $J=6$ Hz), 7.60(1H, d, $J=8$ Hz), 7.70-8.00(1H, br s), 8.25(1H, d, $J=6$ Hz).

O-Methylation of **17** and **18** were carried out by the same procedure as used for **19**

1-Acetylaminomethyl-5,7,8-trimethoxy-6-methylisoquinoline (**15**)

90% yield. mp 96-97°C (colorless prisms from CHCl_3 -MeOH). *Anal.* Calcd for $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_4$: C, 63.14; H, 6.62; N, 9.21. Found: C, 62.91; H, 6.78; N, 9.15. IR(KBr) cm^{-1} : 3320, 1640. *Ms m/z* (%): 304(M^+ , 65), 261(100), 231(56). ¹H-NMR (CDCl_3) δ : 2.14(3H, s), 2.37(3H, s), 3.84(3H, s), 3.92(3H, s), 4.00(3H, s), 5.15(2H, d, $J=5$ Hz), 7.71(1H, d, $J=6$ Hz), 7.60-8.10(1H, br s), 8.31(1H, d, $J=6$ Hz).

1-Benzoylaminomethyl-5,7,8-trimethoxy-6-methylisoquinoline (**20**)

92% yield. mp 133-135°C (colorless prisms from benzene). *Anal.* Calcd for $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_4$: C, 68.83; H, 6.05; N, 7.65. Found: C, 69.05; H, 6.06; N, 7.55. IR(KBr) cm^{-1} : 3340, 1655. *Ms m/z* (%): 366(M^+ , 48), 261(100), 231(31), 105(34). ¹H-NMR (CDCl_3) δ : 2.39(3H, s), 3.86(3H, s), 3.94(3H, s), 4.06(3H, s), 5.37(2H, d, $J=4$ Hz), 7.30-7.60(3H, m), 7.75(1H, d, $J=6$ Hz), 7.90-8.10(2H, m), 8.39(1H, d, $J=6$ Hz), 8.60-9.00(1H, br s).

7,9-Diethoxy-10-hydroxy-3,8-dimethylimidazo[5,1-*a*]isoquinoline (**12**)

1-Acetylaminomethyl-5,7-diethoxy-8-hydroxy-6-methylisoquinoline (**14**) (255 mg, 0.8 mmol) in toluene (4 mL) was treated with POCl_3 (675 mg, 4.4 mmol) under stirring at 110-115°C for 15 min, then poured into cold water (50 mL), adjusted to pH 7 with saturated aqueous NaHCO_3 solution and extracted with CHCl_3 (3 x 20 mL). The extract was washed with brine, dried and concentrated. The residue was

chromatographed (eluting with ethyl acetate) to afford **12** (182 mg, 76%) as light yellow prisms from CHCl_3 -hexane. mp 212-213°C. HRMS Calcd for $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_3$: 300.1474, Found: 300.1475. Ms m/z (%): 300(M^+ , 100), 271(40), 230(75), 202(26). IR(KBr) cm^{-1} : 3456. $^1\text{H-NMR}$ (CDCl_3) δ : 1.46(6H, t, $J=7.3$ Hz), 2.33(3H, s), 2.68(3H, s), 3.91(2H, q, $J=7.3$ Hz), 4.04(2H, q, $J=7.3$ Hz), 6.80-7.15(1H, br s), 7.01(1H, d, $J=7.6$ Hz), 7.45(1H, d, $J=7.6$ Hz), 7.97(1H, s).

Cyclization of **19**, **15** and **20** were carried out by the same procedure as used for **12**

9,10-Dimethoxy-3-methylimidazo[5,1-*a*]isoquinoline (9)

85% yield mp 144.5-146°C (colorless prisms from benzene-hexane). *Anal.* Calcd for $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_2$: C, 69.40; H, 5.83; N, 11.56. Found: C, 69.25; H, 5.77; N, 11.56. Ms m/z (%): 242(M^+ , 100), 186(57). $^1\text{H-NMR}$ (CDCl_3) δ : 2.65(3H, s), 3.97(3H, s), 4.02(3H, s), 6.67(1H, d, $J=8$ Hz), 7.02(1H, d, $J=8$ Hz), 7.27(1H, d, $J=8$ Hz), 7.40(1H, d, $J=8$ Hz), 7.97(1H, s).

7,9,10-Trimethoxy-3,8-dimethylimidazo[5,1-*a*]isoquinoline (10)

96% yield. mp 158-159°C (colorless prisms from CHCl_3 -hexane). *Anal.* Calcd for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_3$: C, 67.11; H, 6.34; N, 9.78. Found: C, 67.35; H, 6.37; N, 9.77. Ms m/z (%): 286(M^+ , 100), 271(35), 256(26), 230(28). $^1\text{H-NMR}$ (CDCl_3) δ : 2.30(3H, s), 2.65(3H, s), 3.80(3H, s), 3.95(3H, s), 3.98(3H, s), 6.97(2H, d, $J=8$ Hz), 7.47(1H, d, $J=8$ Hz), 7.89(1H, s).

7,9,10-Trimethoxy-8-methyl-3-phenylimidazo[5,1-*a*]isoquinoline (11)

98% yield. mp 86-88°C (colorless prisms from CHCl_3 -hexane). *Anal.* Calcd for $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_3$: C, 72.39; H, 5.79; N, 8.04. Found: C, 72.56; H, 5.89; N, 8.02. Ms m/z (%): 348(M^+ , 100), 333(21), 318(32). $^1\text{H-NMR}$ (CDCl_3) δ : 2.32(3H, s), 3.81(3H, s), 3.97(3H, s), 4.02(3H, s), 7.00(1H, d, $J=8$ Hz), 7.40-7.60(3H, m), 7.70-7.85(2H, m), 7.95(1H, d, $J=8$ Hz), 8.14(1H, s).

1-Acetylaminoethyl-5-methoxy-6-methylpyrido[3,4-*a*]phenazine (8)

A solution of CAN (1.37 g, 2.5 mmol) in water (1.5 mL) was added drop wise to **15** (152 mg, 0.5 mmol) suspended in acetonitrile-water (12 : 5, 8.5 mL) containing suspended pyridine-2,6-dicarboxylic acid *N*-oxide (0.46 g, 2.5 mmol) with stirring at 0-5°C. The mixture was stirred for an additional 0.5 h, diluted with water (40 mL), adjusted to pH 7 with saturated aqueous NaHCO_3 solution and extracted with CHCl_3 (3 x 20 mL). The extract was washed with brine, dried and concentrated. A mixture of the residue and *o*-phenylenediamine (40 mg, 0.37 mmol) in EtOH (3 mL) was stirred at rt for 20 min. The precipitated crystals were collected by filtration and recrystallized from CHCl_3 -ethyl acetate to give **8** (39 mg, 23%) as colorless powder. mp 262-263°C (decomp). *Anal.* Calcd for $\text{C}_{20}\text{H}_{18}\text{N}_4\text{O}_2$: C, 69.35; H, 5.24; N, 16.18. Found: C, 69.49; H, 5.09; N, 16.24. Ms m/z (%): 346(M^+ , 53), 303(100), 287(59), 272(29), 232(26). IR(KBr) cm^{-1} : 3300, 1635. $^1\text{H-NMR}$ ($\text{CDCl}_3+\text{CD}_3\text{OD}$) δ : 2.21(3H, s), 2.87(3H, s), 4.04(3H, s), 5.78(2H, s), 7.88-7.96(2H, m), 8.05(1H, d, $J=5.5$ Hz), 8.26-8.34(1H, m), 8.50-8.60(1H, m), 8.84(1H, d, $J=5.5$ Hz).

Cribrostatin 6 (1)

Imidazoisoquinoline (**12**) (30 mg, 0.1 mmol) in 60% HNO_3 (0.2 mL) was stirred at 10°C for 2 h, then basified with 5% aqueous NaHCO_3 solution and extracted with CHCl_3 (3 x 1 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 1 : 1) to afford **1** (8.0 mg, 30%). mp 171-172°C (dark blue needles from acetone). HRMS Calcd for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_3$: 270.1004, Found: 270.1003. Ms m/z (%): 270(M^+ , 100), 242(10), 214(25), 185(13),

172(7), 157(6), 145(10), 116(5). IR(KBr) cm^{-1} : 2924, 1662, 1630, 1614, 1528, 1318, 1174. ^1H -NMR (CDCl_3) δ : 1.43(3H, t, $J=7.3\text{ Hz}$), 2.08(3H, s), 2.77(3H, s), 4.42(2H, q, $J=7.3\text{ Hz}$), 7.27(1H, d, $J=7.3\text{ Hz}$), 7.91(1H, d, $J=7.3\text{ Hz}$), 8.30(1H, s). ^{13}C -NMR (100 MHz, CDCl_3) δ : 9.22, 12.36, 16.03, 69.77, 108.37, 123.64, 123.90, 124.63, 124.83, 125.48, 130.19, 137.50, 156.26, 180.48, 184.76.

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