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SYNTHESIS OF CRIBROSTATIN 6 AND ITS RELATED COMPOUNDS

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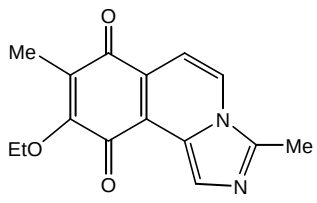
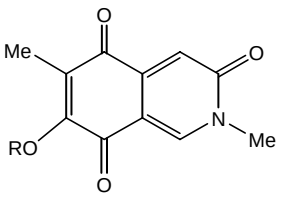
Abstract – The synthesis of cribrostatin 6 (**1**), which shows good biological activity as a dark blue cancer cells growth inhibitor and a number of pathogenic bacteria and fungi, was achieved in two steps from 1-acetylaminomethyl-5,7-diethoxy-8-hydroxy-6-methylisoquinoline (**14**). The related compounds (**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 *Cribrochalina* 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.

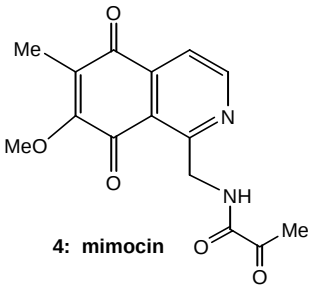
Figure 1



1: cribrostatin 6



2: R=Me **mimosamycin**
3: R=Et **cribrostatin** 2

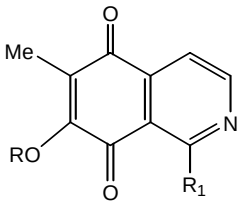


4: mimocin

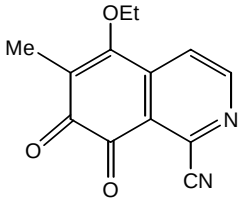
Recently Pettit and colleagues reported the isolation of cribrostatin 6 (**1**) from the marine sponge *Cribrochalina* sp. The structure of **1**, based on spectral data and X-Ray crystal structure analysis,[2] was shown to consist of an isoquinolinedione skeleton, similar to that of mimosamycin (**2**), 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 cribrostatin 6 (**1**) from 1-acetylaminomethyl-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**).

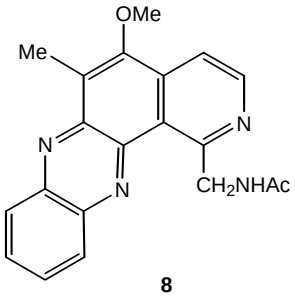
Figure 2



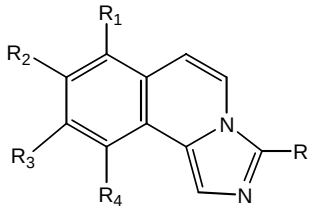
5[3]**:** R=Et, R_1=CN
7[8]**:** R=Me, R_1=CH_2NHAc



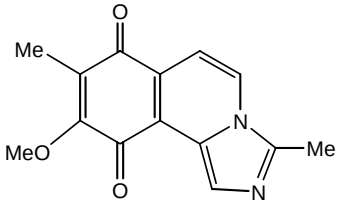
6[3]



8



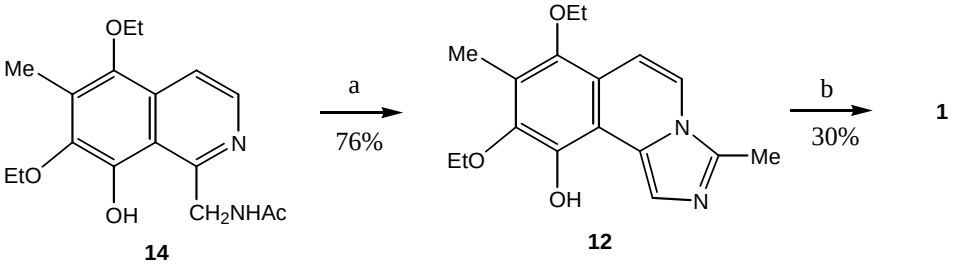
9: R=Me, R_1=R_2=H, R_3=R_4=OMe
10: R=R_2=Me, R_1=R_3=R_4=OMe
11: R=Ph, R_1=R_3=R_4=OMe, R_2=Me
12: R=R_2=Me, R_1=R_3=OEt, R_4=OH



13[8]

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

Figure 3



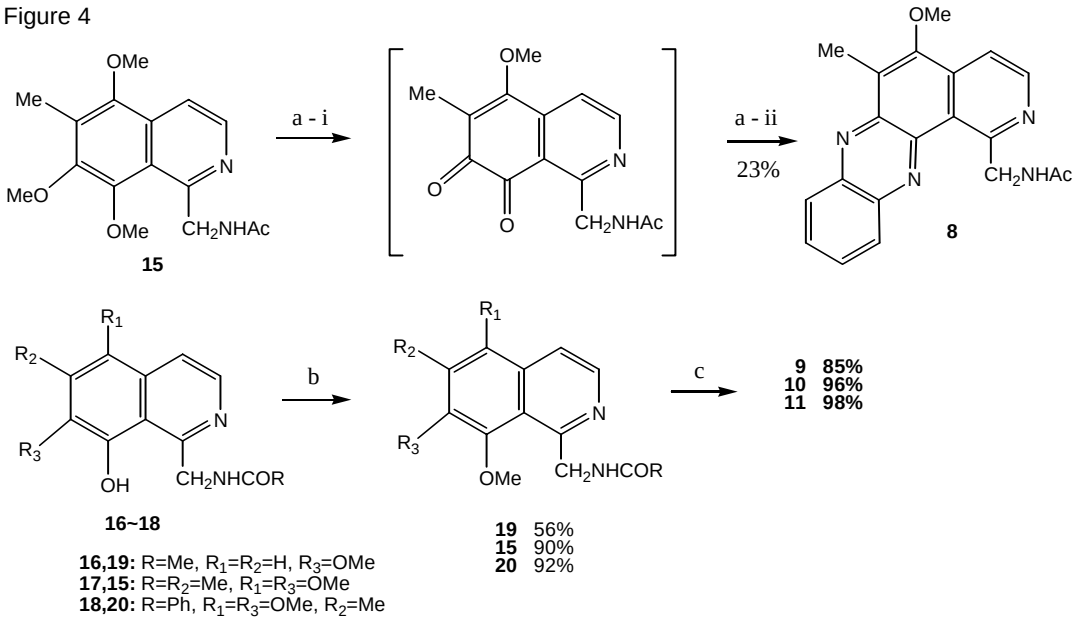
14 **12**

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℃ for 15 min afforded imidazo[5,1-*a*]isoquinoline (**12**) in 76% yield. Treatment of **12** with 60% HNO_3 at 10℃ 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℃ 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, H_2O, 0-5^oC, 0.5 h ii *o*-$C_6H_4(NH_2)$, EtOH, rt, 20 min b) CH_2N_2, Et_2O, rt, 3 h
c) $POCl_3$, toluene, 110~115^oC, 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										
B. subtilis	10.4	5.2	-	83.3	-	-	-	20.8	-	2.6
S. aureus	10.4	2.6	-	-	-	-	-	-	-	10.4
C. neoformans	2.6	1.3	-	83.3	-	83.3	20.8	2.6	2.6	1.3
T. menta-grophytes	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 μg/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. ^{1}H-NMR spectra at 100 MHz and 270 MHz were measured in $CDCl_3$ or $CDCl_3$+CD_3OD 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℃. *Anal.* Calcd for $C_{14}H_{16}N_2O_3$：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). ^{1}H-NMR（$CDCl_3$）δ：2.13(3H, s), 4.00(3H, s), 4.07(3H, s), 5.20(2H, d, *J*=4 H_Z), 7.43(1H, d, *J*=8 H_Z), 7.50(1H, d, *J*=6 H_Z), 7.60(1H, d, *J*=8 H_Z), 7.70-8.00(1H, br s), 8.25(1H, d, *J*=6 H_Z).

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℃ (colorless prisms from $CHCl_3$-MeOH). *Anal.* Calcd for $C_{16}H_{20}N_2O_4$：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). ^{1}H-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 H_Z), 7.71(1H, d, *J*=6 H_Z), 7.60-8.10(1H, br s), 8.31(1H, d, *J*=6 H_Z).

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

92% yield. mp 133-135℃ (colorless prisms from benzene). *Anal.* Calcd for $C_{21}H_{22}N_2O_4$：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). ^{1}H-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 H_Z), 7.30-7.60(3H, m), 7.75(1H, d, *J*=6 H_Z), 7.90-8.10(2H, m), 8.39(1H, d, *J*=6 H_Z), 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℃ 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 $C_{17}H_{20}N_2O_3$: 300.1474, Found: 300.1475. Ms *m/z* (%): 300(M^+, 100), 271(40), 230(75), 202(26). IR(KBr) cm^{-1}: 3456. ^{1}H-NMR（$CDCl_3$）δ：1.46(6H, t, *J*=7.3 H_Z), 2.33(3H, s), 2.68(3H, s), 3.91(2H, q, *J*=7.3 H_Z), 4.04(2H, q, *J*=7.3 H_Z), 6.80-7.15(1H, br s), 7.01(1H, d, *J*=7.6 H_Z), 7.45(1H, d, *J*=7.6 H_Z), 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 $C_{14}H_{14}N_2O_2$：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}H-NMR（$CDCl_3$）δ：2.65(3H, s), 3.97(3H, s), 4.02(3H, s), 6.67(1H, d, *J*=8 H_Z), 7.02(1H, d, *J*=8 H_Z), 7.27(1H, d, *J*=8 H_Z), 7.40(1H, d, *J*=8 H_Z), 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 $C_{16}H_{18}N_2O_3$：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}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 H_Z), 7.47(1H, d, *J*=8 H_Z), 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 $C_{21}H_{20}N_2O_3$：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}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 H_Z), 7.40-7.60(3H, m), 7.70-7.85(2H, m), 7.95(1H, d, *J*=8 H_Z), 8.14(1H, s).

1-Acetylaminomethyl-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) conteining 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 $C_{20}H_{18}N_4O_2$：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}H-NMR（$CDCl_3$+CD_3OD）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 H_Z), 8.26-8.34(1H, m), 8.50-8.60(1H, m), 8.84(1H, d, *J*=5.5 H_Z).

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 extruct 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 $C_{15}H_{14}N_2O_3$: 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. ^{1}H-NMR ($CDCl_3$) δ：1.43(3H, t, *J*=7.3 H_Z), 2.08(3H, s), 2.77(3H, s), 4.42(2H, q, *J*=7.3 H_Z), 7.27(1H, d, *J*=7.3 H_Z), 7.91(1H, d, *J*=7.3 H_Z), 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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