HETEROCYCLES, Vol. 71, No. 8, 2007, pp. 1801 – 1806. © The Japan Institute of Heterocyclic Chemistry
Received, 10th April, 2007, Accepted, 18th May, 2007, Published online, 22nd May, 2007, COM-07-11074

SYNTHESIS OF ARNOAMINE B AND RELATED COMPOUNDS

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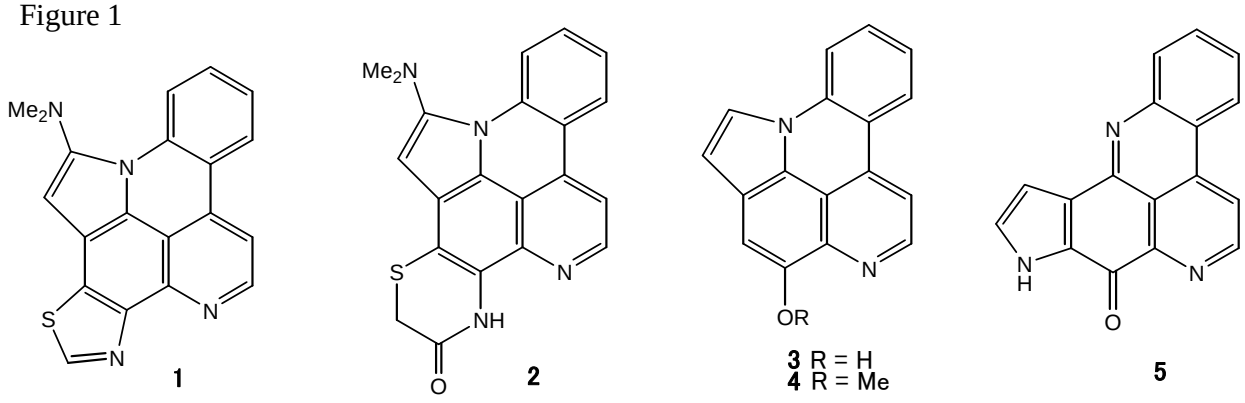
Abstract – The synthesis of arnoamine B (**4**), which inhibits the catalytic activity of topoisomerase II and exhibits selective cytotoxicity against human tumor cell lines, was achieved in four steps from 4-bromo-8-methoxy-6-methyl-5-nitroquinoline (**6**). The related compounds **11**~**14** were also synthesized, and the antimicrobial activities of **4** and its seven related compounds **6**, **8**, **9**, and **11**~**14** were investigated.

A series of structurally related polycyclic aromatic alkaloids containing pyridoacridine ring systems have been isolated from marine organisms.[1] The pyridoacridine system is of interest, as the skeleton contains an indole ring.[2~5]

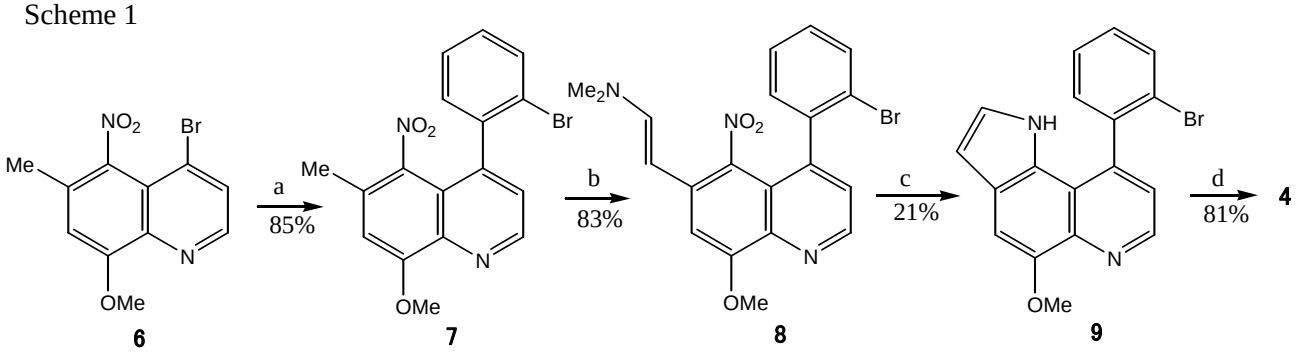
Figure 1



In 1992, Gunawardana *et al.* reported the isolation and structure elucidation of stellettamine (**1**),[2] which contains a thiazole ring fused to the pyridoacridine ring system, on the basis of NMR and single-crystal X-ray diffraction experiments. In 2000, cycloshermilamine D (**2**)[3] was isolated from the marine tunicate *Cystodytes violatinctus*, and its structure was established on the basis of NMR spectroscopic data. Arnoamine A (**3**) and B (**4**),[4] which have relatively simple structures, were obtained in 1998 from the brownish-purple ascidian *Cystodytes* sp., collected near Arno Atoll in the Republic of the Marshall Islands. The structures of **3** and **4** were proposed on the basis of interpretation of spectroscopic data, particularly those obtained from HMBC and NOE NMR experiments. Sebastianine A (**5**),[5] which contains the same pyridoacridine structure, was isolated from the ascidian *Cystodytes dellechiajei* by Torres *et al*.

Arnoamines have been found to inhibit the catalytic activity of topoisomerase II and exhibits selective cytotoxicity against human tumor cell lines; their syntheses and bioactivites are therefore of interest.

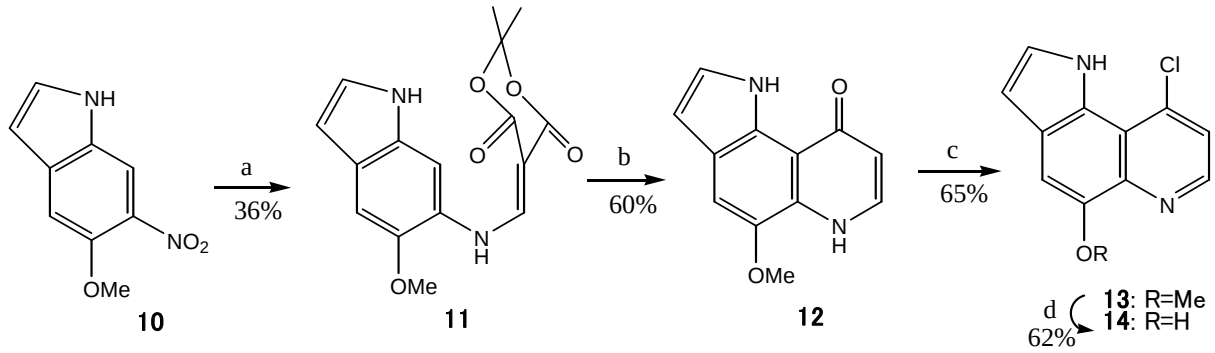
Scheme 1



a) $[(C_6H_5)_3P]_4Pd$, K_2CO_3/H_2O, Toluene, EtOH, reflux, 24h b) DMFDMA, 145°C, 96h c) Zn, AcOH/H_2O, 75°C, 30min
d) $Pd(Ac)_2$, $[(Me)_3C]_3P$, K_2CO_3, xylene, 110°C, 4h

In this study we report the synthesis of arnoamine B (**4**) from 4-bromo-8-methoxy-6-methyl-5-nitroquinoline (**6**) in four steps, the preparation of related compounds **11**~**14**, and an examination of the antimicrobial activities of **4** and its seven related compounds **6**, **8**, **9**, and **11**~**14**.

Scheme 2



a) i Pd-C, H_2, MeOH ii Meldrum's acid, $HC(OMe_3)_3$, reflux, 2 h b) $(C_6H_5)_2O$, reflux, 40min
c) $POCl_3$, 70°C, 1.5 h d) BBr_3, CH_2Cl_2, rt, 24 h

In 2000, Delfourne and co-workers accomplished the first synthesis of arnoamines A (**3**) and B (**4**) used thermolysis of arylaminomethylene Meldrum's acid derivative, biaryl cross coupling reaction, and the Fisher indole synthesis.[6] In 2006, Radchenko and co-workers reported a simple and effective approach to the synthesis of pyrido[4,3,2-*mn*]pyrrolo[3,2,1-*de*]acridine skeleton of arnoamines; the starting material was the known indole.[7]

We previously reported the preparation of 4-bromoquinoline (**6**),[8] a starting material in the synthesis of arnoamine B (**4**), from 6-methoxy-4-methyl-3-nitroaniline *via* thermolysis of arylaminomethylene Meldrum's acid derivative in three steps, and the indole moiety of **4** was introduced by the Leimgruber-Batcho indole synthesis.[9]

A palladium(0)-catalyzed cross coupling reaction of **6** with bromophenylboronic acid gave the

4-bromophenylquinoline (**7**) in excellent yield.[10] The 6-methyl group of **7** can be functionalized by condensation with *N*,*N*-dimethylformamide dimethyl acetal to provide the corresponding enamine (**8**) in 83% yield. Reductive cyclization of enamine (**8**) with zinc powder in 80% aqueous acetic acid afforded the indole (**9**) in 21% yield[11] and using iron powder in acetic acid-methanol at 110 ºC for 30 min resulted in 8% yield. Terminal cyclization of **9** with tri-*tert*-butylphosphine, palladium(III) acetate and K_2CO_3 in xylene furnished arnoamine B (**4**) in 81% yield.[12] The spectroscopic data of synthetic **4** matched those of authentic samples in all respects.

Enamine (**11**), a target molecule for examination of antimicrobial activities, was prepared by catalytic hydrogenation of 5-methyl-6-nitroindole (**10**)[13] over 10% Pd-C in methanol, followed by reaction with Meldrum's acid in trimethyl orthoformate in 36% yield.[14] Cyclization of **11** in refluxing diphenyl ether for 40 min afforded pyrrolo[2,3-*f*]quinolin-9-one (**12**) *via* unstable aminoketene in 60% yield.[14] Treatment of **12** with phosphorus oxychloride at 70 ºC for 1.5 h afforded methoxypyrrolo[2,3-*f*]quinoline (**13**) in 65% yield. Finally, demethylation of **13** with boron tribromide in methylene chloride at room temperature for 24 h gave hydroxypyrrolo[2,3-f]quinoline (**14**) in 62% yield.

The antimicrobial activities of arnoamine B and seven related compounds against bacteria and fungi were investigated, and the results are shown in Table 1.

Table 1. Antimicrobial activities (MIC, μg/mL) of arnoamine B (**4**) and seven related compounds against bacteria and fungi.

Compound	4	6	8	9	11	12	13	14
Microorganism								
B. subtilis	–	–	–	–	–	–	–	–
S. aureus	–	–	–	–	–	–	–	–
A. niger	–	–	–	–	–	–	–	8
C. albicans	–	–	–	–	–	–	–	8
Cr. neoformans	–	–	–	16	–	32[a]	32	4

Antimicrobial activities of arnoamine B and eight related compounds against two bacteria (*Bacillus subtilis* PCI 219 and *Staphylococcus aureus* 209P) and three fungi (*Aspergillus niger* IFM 5368, *Candida albicans* ATCC 90028and *Cryptococcus neoformans* ATCC 90112) 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 ℃. a indicates the MIC values of above 64 μg/ml.

In summary, arnoamine B (**4**) was synthesized in four steps from a known compound, and four other target molecules for examination of antimicrobial activity were also prepared. Among eight compounds tested, four compounds showed antifungal activities, but antibacterial activities were not observed. The compound **14** showed the highest antifungal activity, and the MIC value against *A. niger*, *C. albicans* and *Cr. neoformans* were 8.0, 8.0 and 4.0 μg/ml, respectively.

EXPERIMENTAL

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected.[1] H-NMR spectra at 270 MHz were measured in $CDCl_3$ 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).

4-(2'-Bromophenyl)-8-methoxy-6-methyl-5-nitroquinoline (7). 2 M Aqueous K_2CO_3 (1 mL, 2 mmol) was added to a mixture of **6** (297 mg, 1 mmol) and bromophenylboronic acid (301 mg, 1.5 mmol) in toluene (10 mL) and EtOH (0.52 mL) under argon. Tetrakis(triphenylphosphine)palladium(0) (46 mg, 0.04 mmol) was added to the vigorously stirred two-phase mixture and the resulting mixture was refluxed for 24 h. The reaction mixture was poured into water (50 mL) and extracted with EtOAc (3 x 20 mL). The extract was washed with brine, dried, and concentrated. The residue was chromatographed (eluting with hexane-EtOAc 1 : 2) to afford **7** (317 mg, 85%). mp 196-197 ℃ (light yellow prisms from $CHCl_3$-hexane). HRMS Calcd for $C_{17}H_{13}N_2O_3Br$: 372.0110. Found: 372.0108. Ms *m/z* (%): 374(M^++2, 99), 372(M^+, 100), 328(58), 326(65), 247(75), 218(60). IR(KBr) cm^{-1}: 1526, 1502, 1462, 1366, 1238. ^{1}H-NMR ($CDCl_3$) δ: 2.41(3H, s), 4.17(3H, s), 6.91(1H, s), 7.17-7.23(1H, m), 7.27-7.36(2H, m), 7.38(1H, d, *J*=4.3 Hz), 7.60-7.66(1H, m), 9.00(1H, d, *J*=4.3 Hz).

4-(2'-Bromophenyl)-8-methoxy-6-[β-*trans*-(*N*,*N*-dimethylamino)ethenyl]-5-nitroquinoline (8). A solution of **7** (373 mg, 1 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (3.8 mL) was heated at 145 ℃ in sealed tube for 4 days. After the reaction mixture was cooled, the precipitated crystals were collected by filtration and recrystallized from $CHCl_3$-hexane to give **8** (356 mg, 83%) as red needles. mp 239.5-240.5 ℃. *Anal.* Calcd for $C_{20}H_{18}N_3O_3Br$: C, 56.09; H, 4.24; N, 9.81. Found: C, 55.91; H, 4.25; N, 9.69. IR (KBr) cm^{-1}: 1634, 1604, 1496, 1390, 1240. ^{1}H-NMR ($CDCl_3$) δ: 2.88(6H, s), 4.16(3H, s), 5.07(1H, d, *J*=13.2 Hz), 6.98(1H, s), 7.00(1H, d, *J*=13.2 Hz), 7.20-7.35(4H, m), 7.63(1H, dd, *J*=7.6, 1.3 Hz), 8.80(1H, d, *J*=4.3 Hz). Ms *m/z* (%): 429(M^++2, 9), 427(M^+, 9), 412(46), 410(45), 320(42), 275(35), 247(33), 218(100).

9-(2'-Bromophenyl)-5-methoxypyrrolo[2,3-*f*]quinoline (9). The 5-nitroquinoline **(8)** (85.6 mg, 0.2 mmol) was suspended in 80% aqueous AcOH (0.3 mL) and heated at 75 ℃. Zinc dust (117 mg, 1.8 mmol) was added portionwise over 0.5 h and then the reaction was continued at 80 ℃ for 15 min. The reaction mixture was poured into water (30 mL), adjusted to pH 7 with saturated aqueous $NaHCO_3$ solution, and extracted with $CHCl_3$ (3 x 15 mL). The extract was washed with brine, dried, and concentrated. The residue was chromatographed (eluting with hexane-EtOAc 1 : 2) to afford **6** (15 mg, 21%). mp 178-179 ℃ (light yellow prisms from $CHCl_3$-hexane). HRMS Calcd for $C_{18}H_{13}N_2OBr$: 352.0211, Found: 352.0208. Ms *m/z* (%): 354(M^++2, 99), 352(M^+, 100), 351(64), 325(27), 323(27), 243(14). IR (KBr) cm^{-1}: 3476, 1478, 1362, 1316, 1152. ^{1}H-NMR ($CDCl_3$) δ: 4.14(3H, s), 6.57(1H, t, *J*=3.0 Hz), 6.97(1H, t, *J*=3.0 Hz), 7.29(1H, d, *J*=4.3 Hz), 7.34(1H, s), 7.43-7.57(3H, m), 7.87(1H, dd, *J*=7.9, 1.3 Hz), 8.96(1H, d, *J*=4.3 Hz).

Arnoamine B (**4**) To a solution of **9** (35 mg, 0.1mmol) in xylene (1.5 mL) were added

tri-*tert*-butylphosphine (12 mg, 0.06 mmol), palladium(III) acetate (4.5 mg, 0.02 mmol), and K_2CO_3 (41 mg, 0.3 mmol) and stirred at 110 ℃ for 4 h under N_2. The water (30 mL) was added to the reaction mixture and extracted with EtOAc (3 x 10 mL). The extract was washed with brine, dried, and concentrated. The residue was chromatographed (eluting with EtOAc) to afford **4** (22 mg, 81%). mp 235-236 ℃ (yellow prisms from $CHCl_3$-hexane). HRMS Calcd for $C_{18}H_{12}N_2O$: 272.0950, Found: 272.0946. Ms *m/z* (%): 272(M^+, 100), 271(89), 257(11), 242(36), 229(12). IR (KBr) cm^{-1}: 1610, 1470, 1388, 1360, 1282. ^{1}H-NMR ($CDCl_3$) δ: 4.21(3H, s), 7.16(1H, d, *J*=3.0 Hz), 7.49(1H, ddd, *J*=8.3, 7.3, 1.0 Hz), 7.56(1H, s), 7.73(1H, ddd, *J*=8.2, 7.3, 1.3 Hz), 8.00(1H, dd, *J*=8.2, 1.0 Hz), 8.03(1H, d, *J*=5.0 Hz), 8.12(1H, d, *J*=3.0 Hz), 8.48(1H, dd, *J*=8.3, 1.3 Hz), 9.13(1H, d, *J*=5.0 Hz). ^{1}H-NMR ($CDCl_3$+TFA-d) δ: 4.25(3H, s), 7.55(1H, d, *J*=3.3 Hz), 7.80(1H, dd, *J*=8.3, 7.3 Hz), 8.10(1H, s), 8.11(1H, dd, *J*=8.6, 7.3 Hz), 8.31(1H, d, *J*=8.6 Hz), 8.45(1H, d, *J*=6.6 Hz), 8.52(1H, d, *J*=3.3 Hz), 8.72(1H, d, *J*=8.3 Hz), 9.08(1H, d, *J*=6.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 56.37, 102.53, 107.33, 110.78, 114.16, 115.24, 116.80, 117.07, 120.29, 121.69, 124.04, 125.75, 131.07, 132.70, 134.88, 139.01, 147.51, 150.51. ^{13}C-NMR (100 MHz, $CDCl_3$+TFA-d) δ: 57.05, 109.68, 110.40, 110.56, 112.52, 113.53, 116.24, 116.75, 118.15, 119.26, 120.84, 126.02, 127.40, 135.76, 138.76, 141.35, 144.18.

5-[(5-Methoxyindol-6-ylamino)methylene]-2,2-dimethyl-4,6-dione-1,3-dioxane (11). 5-Methoxy-6-nitroindole (**10**) (38 mg, 0.2 mmol) in MeOH (8 mL) was hydrogenated for 1 h using 10% Pd-C (38 mg) as a catalyst under H_2 atomsphere. The catalyst was filtered off and the solvent was removed. A solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (26 mg, 0.18 mmol) in methyl orthoformate (2 mL) was refluxed for 2 h and the crude aminoindole was immediately added. The mixture was refluxed for another 2 h. After the reaction mixture was cooled, the precipitated crystals were collected by filtration and recrystallized from $CHCl_3$ to give **11** (23 mg, 36%) as yellow needles. mp 217-218 ℃. HRMS Calcd for $C_{16}H_{16}N_2O_5$: 316.1059, Found: 316.1062. Ms *m/z* (%): 316(M^+, 54), 258(12), 214(87), 199(20), 183(100), 171(23). IR (KBr) cm^{-1}: 3388, 1676, 1614, 1450, 1322, 1278. ^{1}H-NMR ($CDCl_3$) δ: 1.76(6H, s), 3.97(3H, s), 6.50(1H, t, *J*=2.3 Hz), 7.15(1H, s), 7.24(1H, t, *J*=3.0 Hz), 7.38(1H, s), 8.49(1H, br s), 8.69(1H, d, *J*=14.9 Hz), 11.68(1H, d, *J*=14.9 Hz).

5-Methoxypyrrolo[2,3-*f*]quinolin-9-one (12). A mixture of **11** (1.90 g, 6 mmol) and diphenyl ether (100 mL) was refluxed for 40 min. The reaction mixture was cooled and diluted with hexane (80 mL). The precipitated crystals were collected by filtration, washed with hexane (3 x 5 mL) and chromatographed (eluting with EtOAc) to afford **12** (771 mg, 60%). mp 209-210 ℃ (light yellow prisms from $CHCl_3$-hexane). HRMS Calcd for $C_{12}H_{10}N_2O_2$: 214.0742. Found: 214.0739. Ms *m/z* (%): 214(M^+, 66), 199(100), 143(17). IR (KBr) cm^{-1}: 3416, 1548, 1478, 1308, 1202, 1132. ^{1}H-NMR ($(CD_3)_2CO$) δ: 3.87(3H, s), 6.44(1H, d, *J*=7.3 Hz), 6.57(1H, t, *J*=3.0 Hz), 7.27(1H, t, *J*=3.0 Hz), 7.28(1H, s), 7.73(1H, d, *J*=7.3 Hz), 10.97(1H, br s).

9-Chloro-5-methoxypyrrolo[2,3-*f*]quinoline (13). A mixture of **12** (642 mg, 3 mmol) and $POCl_3$ (5 ml) was stirred at 70 ℃ for 1.5 h, poured into cold water (100 mL) and adjusted to pH 7 with saturated aqueous $NaHCO_3$ solution. The precipitated crystals were collected by filtration and chromatographed (eluting with hexane-EtOAc 1 : 1) to afford **13** (453 mg, 65%). mp 177-178 ℃ (light yellow crystals from $CHCl_3$-hexane). HRFABMS(glycerol, MH^+) calcd for $C_{12}H_{10}N_2OCl$ 233.0482. Found 233.0482. Ms

m/z (%): 234(M^++2, 32), 232(M^+, 100), 231(68), 203(37), 189(7). IR (KBr) cm^{-1}: 1612, 1474, 1358, 1312, 1232, 1196, 1146. ^{1}H-NMR ($CDCl_3$) δ: 4.11(3H, s), 6.69(1H, t, *J*=3.0 Hz), 7.35(1H, t, *J*=3.0 Hz), 7.36(1H, s), 7.51(1H, d, *J*=5.0 Hz), 8.73(1H, d, *J*=4.6 Hz), 9.93(1H, br s).

9-Chloro-5-hydroxypyrrolo[2,3-*f*]quinoline (14). To 9-chloroquinoline (**13**) (116 mg, 0.5 mmol) was added a solution of BBr_3 (1 M/CH_2Cl_2, 10 mL) under a dry nitrogen atmosphere. The solution was stirred at rt for 24 h, then poured into water (100 mL) and adjusted to pH 7 with saturated aqueous $NaHCO_3$ solution. The precipitated crystals were collected by filtration and chromatographed (eluting with hexane-EtOAc 5 : 1) to afford **14** (67 mg, 62%). mp 154-155 ℃ (light yellow powder from $CHCl_3$-hexane). HRMS Calcd for $C_{11}H_7N_2OCl$: 218.0247, Found: 218.0247. Ms *m/z* (%): 220(M^++2, 33), 218(M^+, 100), 190(24), 155(17). IR (KBr) cm^{-1}: 3476, 3384, 1416, 1370, 1282. ^{1}H-NMR ($CDCl_3$) δ: 6.70(1H, t, *J*=3.0 Hz), 7.39(1H, t, *J*=3.0 Hz), 7.49(1H, s), 7.55(1H, d, *J*=5.0 Hz), 8.07(1H, br s), 8.60(1H, d, *J*=4.6 Hz), 9.84(1H, br).

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