A Novel Synthesis of Benzo[*c*]phenanthridine Skeleton and Biological Evaluation of Isoquinoline Derivatives

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Benzo[*c*]phenanthridine skeleton was synthesized from easily available starting *N*-methyl-*o*-toluamide 2 and *o*-methylbenzonitrile 5 in 7 steps. Radical cyclization of styrene 10 afforded 6,11-dimethyl-6,11-dihydro-5*H*-indeno[1,2-*c*]isoquinolin-5-one 13. Most 3-arylisoquinolines have displayed strong activities against human tumor cell lines. Especially, indenoisoquinolinone 13 exhibited excellent cytotoxicity (IC_{50}=0.002 μg/ml; HCT 15).

Key words benzo[*c*]phenanthridine; indenoisoquinoline; antitumor activity; radical cyclization

Naturally occurring benzo[*c*]phenanthridine alkaloid which has more than 80 members is characterized by the basic skeleton **1**. These alkaloids have been attractive to synthetic organic chemists and biochemists over the last 2 decades since such compounds have shown interesting biological properties.[1)] Several total syntheses of benzo[*c*]phenanthridine alkaloids have been reported.[2)] As part of our endeavor to develop potential antitumor agents, we have studied the benzo[*c*]phenanthridines and 3-arylisoquinolines.[3)] In this paper we describe an efficient synthesis of benzo[*c*]phenanthridine skeleton with the biological study of 3-arylisoquinolines, benzo[*c*]phenanthridinone and indeno[1,2-*c*]isoquinolinone.

Retrosynthetic consideration of benzo[*c*]phenanthridine indicates that the coupling of *o*-methylbenzonitrile with *o*-toluamide might afford 3-arylisoquinoline which could be transferred to the aldehyde. Benzo[*c*]phenanthridine can be formed by an intramolecular ring cyclization method as outlined below.

Our strategy is based on the synthesis of 3-arylisoquinoline **12** which is a crucial intermediate for the formation of C ring of benzo[*c*]phenanthridine. *N*-Methyl-*o*-toluamide **2** was basified with two equivalent *n*-butyl lithium to give the dianion which was treated with *o*-methylbenzonitrile **5** to afford the 3-(2-methyl)phenylisoquinolin-1(2*H*)-one **6** in 55% yield.[4)] When we performed the reaction with *N*-methyl-*o*-toluamide **2** and *o*-vinylbenzonitrile **3**, the desired product **4** was not obtained.[5)] Methylation of **6** with MeI/60%NaH in tetrahydrofuran (THF) provided the *N*-methylated product **7** without yielding *o*-methylated compound in good yield. Bromination of **7** afforded the tribrominated one **8** in 64% yield. In this reaction, we could also detect the 4-bromo and dibrominated compounds by ^{1}H-NMR when we quenched it while doing the reaction. Tribrominated product **8** was then treated with $CaCO_3$ in dimethyl formamide (DMF) to provide the aldehyde **9** and the consecutive Wittig reaction with methyltriphenylphosphonium bromide afforded the styrene **11** in 87% and 90% yield, respectively. Oxyfunctionalization of olefin **10** with thallium trinitrate in MeOH gave the acetal **11** in 60% yield which was then hydrolyzed with 10% HCl to provide the desired 5-methylbenzo[*c*]phenanthridin-6-one **1** in 34% yield.[6)] In this reaction we assumed that the hydrolysis produced the aldehyde **12** and the consecutive intramolecular enamide-aldehyde cyclization occurred under an acidic condition. After the ring formation, dehydration and debromination would easily occur thus producing a fully aromatized ring system of benzo[*c*]phenanthridine. When the styrene **10** was reacted with tributyl tin hydride in the presence of azobiscyclohexanecarbonitrile (ACCN), 6,11-dimethyl-6,11-dihydro-5*H*-indeno[1,2-*c*]isoquinolin-5-one **13** was obtained exclusively instead of benzo[*c*]phenanthridine. This result could be explained by Baldwin's rule that 5-exo-trig pathway is favored than 6-endo-trig in a general way of ring closure reaction.[7)] The substituted starting *N*-methyl-*o*-tolunitrile and *o*-methylbenzonitrile seem to be easily prepared. So, the substituted natural benzo[*c*]phenanthidine alkaloids as well as indeno[1,2-*c*]isoquinolines could be synthesized using this method.

All experimental procedures for testing cytotoxicity of synthesized compounds followed the NCI protocol based on the Sulforhodamine B (SRB) method with four human tumor cell lines.[8)] Most synthetic compounds exhibited relatively strong cytotoxicities. The activities of 3-arylisoquinolines could be rationalized by our pharmacophore model studies.[3)] Interestingly, indeno[1,2-*c*]isoquinolinone **13** showed stronger *in vitro* antitumor activity than doxorubicin in our evaluation system. The results obtained from this biological study are listed in Table 1. We believe that indenoisoquinolines provide interesting clues to further explore the antitumor potential of this class of compound.

In conclusion, we succeeded in the convenient synthesis of benzo[*c*]phenanthridine skeleton in 7 steps from the starting

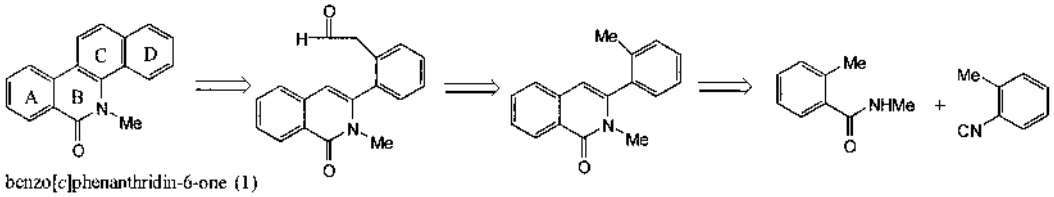

Chart 1

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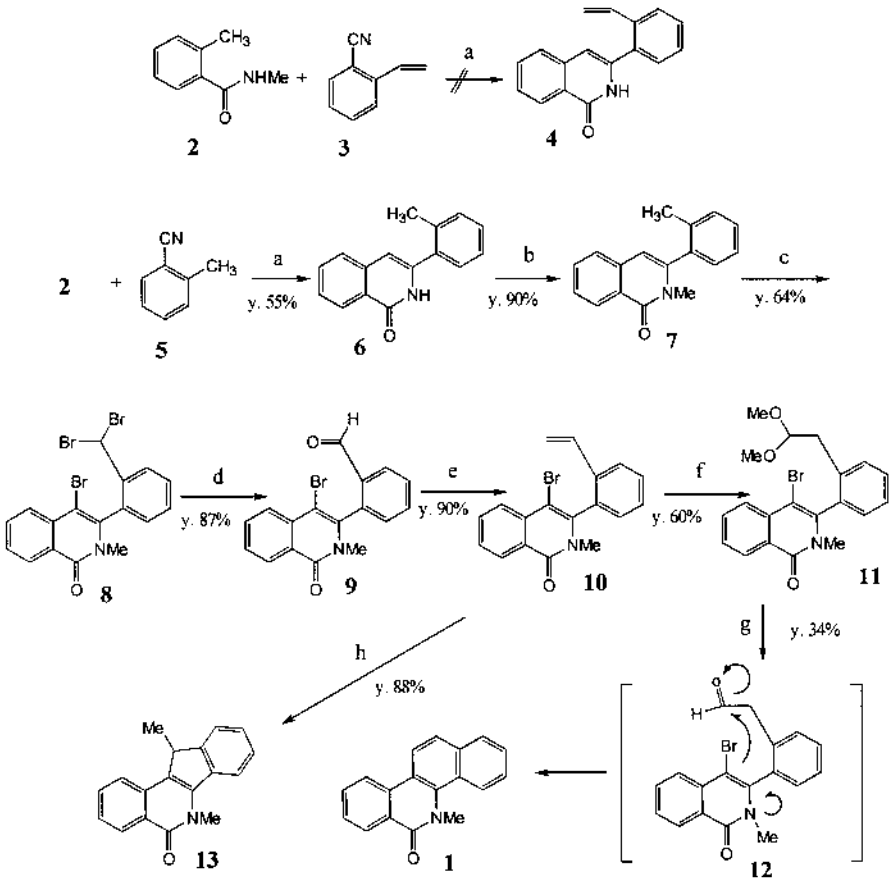

a, *n*-BuLi, −50 °C; b, 60% NaH, MeI; c, Br_2, CCl_4, *hv*; d, $CaCO_3$, H_2O, DMF; e, $Ph_3P^+CH_3\ Br^-$, *n*-BuLi, THF; f, $Tl(NO_3)_3 \cdot 3H_2O$, MeOH; g, 10% HCl, MeOH; h, ACCN, *n*-Bu_3SnH.

Chart 2

Table 1. *In Vitro* Cytotoxicities of Compounds (μg/ml)

Compd.	A549	SK-OV-3	SK-MEL-2	HCT 15
1	8.322	5.158	2.285	0.651
6	2.941	2.362	1.405	1.043
7	7.916	11.930	3.346	8.636
8	4.575	5.757	0.447	0.975
9	5.705	9.278	0.813	0.477
10	7.684	6.112	0.642	5.732
11	>100	>100	98.68	86.92
13	0.003	0.009	<0.001	0.002
Doxorubicin	0.011	0.068	0.071	0.113

Tumor cell lines: A 549 (human lung), SKOV-3 (human ovarian), SK-MEL-2 (human melanoma), HCT 15 (human colon).

material. In addition, we discovered very strong antitumor indenoisoquinoline which might be an important compound for the development of antitumor agents. The synthesis of natural benzo[*c*]phenanthridines using our method is under investigation.

Experimental

Melting points were determined on a Electrothermal IA9200 melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H-NMR) were recorded on a Bruker AC 80 and a Varian 300 spectrometer, using TMS as the internal standard; chemical shifts are reported in parts per million and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). IR spectra were recorded on a Perkin-Elmer 783 spectrometer and a Nicolet instrument using KBr pellets. Elemental analyses were performed on a CaHo Erba elemental analyzer. Solvents were routinely distilled prior to use. Anhydrous THF was distilled from sodium-benzophenon ketyl. Column chromatography was performed on Merck silica gel 60 (70—230 mesh). TLC was carried out using plates coated with silicagel 60F 254 purchased from Merck Co. Reagents were obtained from commercial suppliers and were used without purification.

2-Methyl-3-(2-methylphenyl)-1(2*H*)-isoquinolinone (7) 60% NaH (410 mg, 17.1 mmole) was added portionwise to a solution of **6**[4] (2 g, 8.54 mmole) in THF (50 ml) at 0 °C under nitrogen. The mixture was stirred for 1 h at 0 °C and then CH_3I (2.4 g, 17 mmole) was added. The reaction mixture was warmed to 50—60 °C and stirred for 2 h. The reaction mixture was quenched with water and extracted with ethyl acetate. The organic phase was washed with water, brine, dried over Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography on silica gel with hexane : ethyl acetate=10 : 1 to give **7** (1.9 g, 90%) as a colorless oil. IR neat cm^{-1}: 1650 (amide carbonyl). ^{1}H-NMR ($CDCl_3$) δ: 8.47 (1H, d, J=7.8 Hz, C_8-H), 7.66—7.25 (7H, m, Ar-H), 6.41 (1H, s, C_4-H), 3.92 (3H, s, NMe), 2.19 (3H, s, Me). MS *m/z* (%): 249 (M^+, 100), 248 (79), 234 (46). *Anal.* Calcd for $C_{17}H_{15}NO$: C, 81.90; H, 6.06; N, 5.62. Found: C, 81.65; H, 6.32; N, 5.72.

4-Bromo-3-[2-(dibromomethyl)phenyl]-2-methyl-1(2*H*)-isoquinolinone (8) Bromine (1.93 g, 12.08 mmole) in CCl_4 (10 ml) was slowly added to a solution of **7** (1 g, 4.03 mmole) and $NaHCO_3$ (2.8 g) in CCl_4 (40 ml). The reaction mixture was warmed to 50 °C and stirred for 1 h under irradiation and then was diluted with water and extracted with methylene chloride. The combined organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated to dryness which was purified by column chromatography on silica gel with hexane : ethyl acetate=20 : 1 to give **8** (1.24 g, 64%) as a pale orange solid. mp 206—207 °C. IR (KBr) cm^{-1}: 1660 (amide carbonyl). ^{1}H-NMR ($CDCl_3$) δ: 8.53 (1H, d, J=7.0 Hz, Ar-H), 8.18 (1H, d, J=7.0 Hz, Ar-H), 7.99 (4H, m, Ar-H), 6.70 (1H, s, $CHBr_2$), 3.31 (3H, s, NMe). MS *m/z* (%): 485 (M^++2 , 2), 487 (M^++4, 100), 407 (76). *Anal.* Calcd for $C_{17}H_{12}Br_3NO$: C, 42.01; H, 2.49; N, 2.88. Found: C, 42.25; H, 2.79; N, 2.86.

2-(4-Bromo-2-methyl-1-oxo-1,2-dihydro-3-isoquinolinyl)benzaldehyde (9) The mixture of **8** (100 mg, 0.21 mmole), calcium carbonate (72 mg), water (30 ml) and DMSO (15 ml) was heated to reflux overnight. The reaction mixture was cooled to room temperature and taken up with methylene chloride. The organic phase was washed with water, brine, dried over Na_2SO_4 and concentrated to dryness to give a solid. The residue was purified by column chromatography on silica gel with hexane : ethyl acetate=4 : 1 to give **10** (63 mg, 87%) as a pale yellow solid. mp 153—154 °C. IR (KBr) cm^{-1}: 1645 (amide carbonyl), 1710 (aldehyde carbonyl). ^{1}H-NMR ($CDCl_3$) δ: 10.01 (1H, s, –CHO), 8.52 (1H, d, J=7.8 Hz, C_5-H), 8.08 (1H, d, J=7.5 Hz, C_8-H), 7.94 (1H, d, J=7.5 Hz, Ar-H), 7.84—7.56 (5H, m, Ar-H), 3.28 (3H, s, NMe). MS *m/z* (%): 342 (M^++2, 8), 341 (M^+, 7), 263 (21), 262 (100), 247 (14), 232 (9). *Anal*. Calcd for $C_{17}H_{12}BrNO_2$: C, 59.67; H, 3.53; N, 4.09. Found: C, 59.91; H, 3.43; N, 4.29.

4-Bromo-2-methyl-3-(2-vinylphenyl)-1(2*H*)-isoquinolinone (10) *n*-BuLi (1.5 M soln. in hexane, 0.67 ml, 1.0 mmol) was added to a solution of methyltriphenylphosphonium bromide (314 mg, 0.9 mmol) in THF (40 ml) at room temperature under nitrogen. After 1 h stirring, a solution of **9** (200 mg, 0.6 mmol) in THF (10 ml) was added to the above reaction mixture. After 30 min stirring, the reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography on silica gel with hexane : ethyl acetate=6 : 1 to afford **10** (180 mg, 90%) as a colorless oil. IR neat cm^{-1}: 1650 (amide carbonyl). ^{1}H-NMR ($CDCl_3$) δ: 8.52 (1H, d, J=7.2 Hz, Ar-H), 7.98 (1H, d, J=7.8 Hz, Ar-H), 7.80—7.40 (6H, m, Ar-H), 6.49 (1H, dd, J=17.4, 11.1 Hz, $\underline{CH}$=CH_2), 5.77 (1H, dd, J=17.4, 0.9 Hz, CH=$\underline{CH_2}$), 5.22 (1H, dd, J=11.1, 0.9 Hz, CH=$\underline{CH_2}$), 3.27 (3H, s, NMe). MS *m/z* (%): 341 (M^++2, 8), 261 (38), 260 (100), 259 (12), 258 (18), 246 (22), 245 (41), 217 (9), 216 (9), 202 (12). *Anal*. Calcd for $C_{18}H_{14}BrNO$: C, 63.55; H, 4.15; N, 4.12. Found: C, 63.85; H, 4.12; N, 4.29.

4-Bromo-3-[2-(2,2-dimethoxyethyl)phenyl]-2-methyl-1(2*H*)-isoquinolinone (11) A solution of thallium (III) nitrate trihydrate (566 mg, 1.27 mmol) in methanol (10 ml) was added to a solution of **10** (175 mg, 0.73 mmol) in methanol (10 ml) at room temperature, and then the reaction mixture was warmed to 80—90 °C. After stirring for 12 h, the saturated $NaHCO_3$ solution (10 ml) was added to the reaction mixture and extracted with methylene chloride. The combined organic phase was washed with water, brine, dried over Na_2SO_4 and concentrated to dryness to yield the residue which was purified by column chromatography on silica gel with hexane : ethyl acetate=6 : 1 to give **11** (177 mg, 60%) as a colorless oil. IR neat cm^{-1}: 1662 (amide carbonyl). ^{1}H-NMR ($CDCl_3$) δ: 8.47 (1H, d, J=8.0 Hz, Ar-H), 7.93—7.25 (7H, m, Ar-H), 4.53 (1H, dd, J=5.0, 6.1 Hz, –$CH(OMe)_2$), 3.37, 3.29 (each 3H, each s, –OMe), 2.84 (1H, dd, J=6.1, 15 Hz, –CH_2–), 2.69 (1H, dd, J=5.0, 15 Hz, –CH_2–). MS *m/z* (%): 401 (M^+, 26), 370 (9), 322 (100), 306 (12). *Anal*. Calcd for $C_{20}H_{20}BrNO_3$: C, 59.71; H, 5.01; N, 3.48. Found: C, 59.61; H, 5.22; N, 3.47.

5-Methylbenzo[c]phenanthridin-6 (5*H*)-one (1) A solution of **11** (130 mg, 0.32 mmol) and 10% hydrochloric acid (10 ml) in MeOH (30 ml) was heated to reflux overnight. Methanol of the reaction mixture was evaporated off and the residue was taken up in methylene chlororide. The solution was washed with water, brine, dried over Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography on silica gel with hexane : ethyl acetate=5 : 1 to give **1** (28 mg, 34%) as a colorless solid. mp 135—136 °C. IR (KBr) (cm^{-1}): 1650 (amide carbonyl). ^{1}H-NMR ($CDCl_3$) δ: 8.59 (1H, dd, J=8.1, 1.5 Hz, Ar-H), 8.40—7.53 (9H, m, Ar-H), 4.06 (3H, s, NMe). MS *m/z* (%): 259 (M^+, 8), 258 (100). *Anal*. Calcd for $C_{18}H_{13}NO$: C, 83.38; H, 5.05; N, 5.40. Found: C, 83.45; H, 5.25; N, 5.48.

6,11-Dimethyl-6,11-dihydro-5*H*-indeno[1,2-*c*]isoquinolin-5-one (13) The mixture of **10** (200 mg, 0.59 mmol) and azobiscyclohexanecarbonitrile (100 mg) in toluene (15 ml) was stirred at 0 °C for 30 min. and then the reaction mixture was warmed to room temperature. To this reaction mixture *n*-tributyltin hydride (430 mg, 1.48 mmol) was added and stirred for 2 h at 60 °C. The reaction was quenched with water and extracted with ethyl acetate. The organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography on silica gel with hexane : ethyl acetate=10 : 1 to yield **13** (135 mg, 88%) as a pale yellow oil. IR neat cm^{-1}: 1650 (amide carbonyl). ^{1}H-NMR ($CDCl_3$) δ: 8.55 (1H, d, J=8.0 Hz, Ar-H), 8.04—7.10 (7H, m, Ar-H), 4.14 (3H, s, –NMe), 4.02 (1H, q, J=7.2 Hz, CH), 1.59 (3H, d, J=7.5 Hz, –Me). MS *m/z* (%): 261 (M^+, 100), 245 (48). *Anal*. Calcd for $C_{18}H_{15}NO$: C, 82.73; H, 5.79; N, 5.36. Found: C, 82.53; H, 5.55; N, 5.38.

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