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SYNTHESIS OF 12,12a-DIHYDRO-7,12a-DIAZACYCLOHEPTA[*cd*]-BENZ[*g*]AZULEN-12-ONE (CYCLOHEPTA[*mn*]PYRROLO[2,1-*c*][1,4]-BENZODIAZEPIN-12-ONE) AND EVALUATION OF CYTOTOXIC ACTIVITY AGAINST HeLa S3 CELLS

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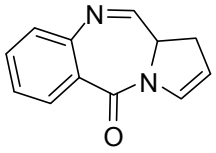
Abstract – 12,12a-dihydro-7,12a-diazacyclohepta[*cd*]benz[*g*]azulen-12-one (cyclohepta[*mn*]pyrrolo[2,1-*c*][1,4]benzodiazepin-12-one) derivative (**7**) was synthesized by the intramolecular cyclization of methyl 2-(3-phenyl-1-azaazulen-8-yl)aminobenzoate. Compound (**7**) showed extremely strong cytotoxic activity against HeLa S3 cells (IC_{50}=0.543±0.063 μM).

The 3a,9-diazabenz[*f*]azulene (pyrrolo[2,1-*c*][1,4]benzodiazepine: PBD) ring system (**1**), a skeleton of a group of potent naturally occurring antitumor antibiotics from *Streptomyces* species, are of considerable interest for their potentiality as antitumor agents, gene regulators, and DNA probs. Many synthetically and pharmacological investigations related to PBD and its analogs were reported.[1,2]

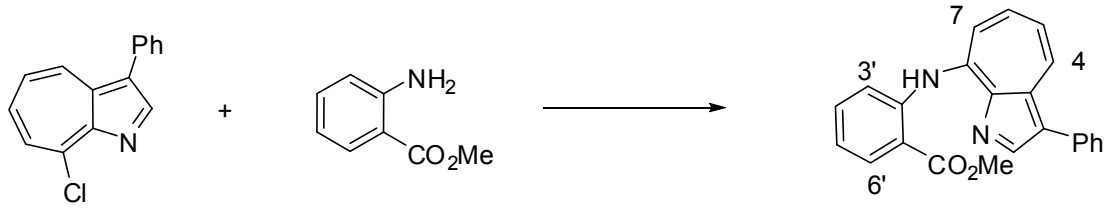


1

1-Azaazulenes[3] are attracted attention for their pharmaceutical and biological interests[4,5] as well as their characteristic physical and chemical properties. We also reported that the cytotoxic activities against

HeLa S3 cells of 1-zaazulene derivatives.[6-8] In the line of our interests for the synthesis of antitumoral 1-azaazulene derivatives, we proceeded to synthesize of fused 1-azaazulenes containing PBD skeleton.

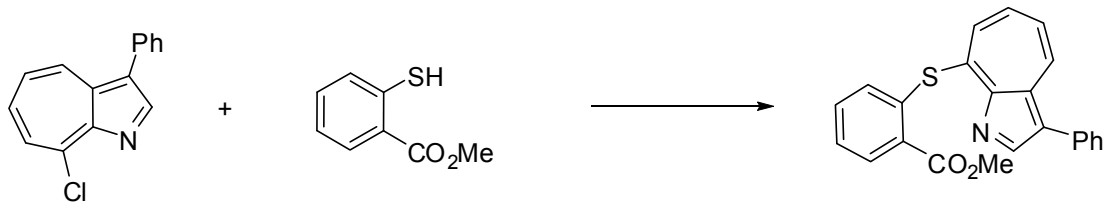
It is considered that 12,12a-dihydro-7,12a-diazacyclohepta[*cd*]benz[*g*]azulene (cyclohepta[*mn*]pyrrolo-[2,1-*c*][1,4]benzodiazepine) system would be derived from 2-(1-azaazulen-8-yl)aminobenzoic acid derivative by intramolecular cyclization. It is known that 8-(arylamino)-1-azaazulene derivatives were easily obtained by nucleophilic substitution of 8-halo-1-azaazulenes with arylamines.[9,10] Therefore, we synthesized methyl 2-(3-phenyl-1-azaazulen-8-yl)aminobenzoate (**2**) by the treatment of 8-chloro-3-phenyl-1-azaazulene (**3**) with methyl 2-aminobenzoate (**4**) in dioxane for 30min at rt in 89% yield. In the IR spectrum of **2**, an ester carbonyl peak was seen at 1725 cm^{-1} and an NH peak was seen at 3433 cm^{-1}. In its ^1H NMR spectrum, an ester methyl singlet was seen at δ 3.93 (s) and an NH signal was seen at δ 8.24 (br s). In the aromatic protons resonated region, protons owing to 1-azaazulene ring were seen at δ 7.03 (like t, J = 9.2, H-5), 7.46 (like t, J = 9.5, H-6), 7.56 (s, H-2), 7.56 (d, J = 9.6, H-7), and 8.21 (d, J = 9.6, H-4) together with phenyl protons (9H). From these results as well as HRMS (*m/z* 355.1418 $[M+H]^+$), we assigned the structure.



3 **4** **2**

Scheme 1

The reaction can expand to other substituted benzoic acid, and the reaction of **3** with methyl 2-mercaptobenzoate (**5**) gave **6** in 66% yield (Scheme 2).



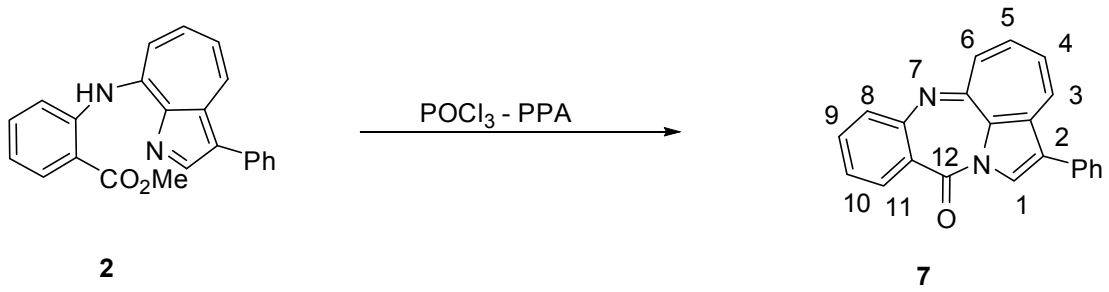
3 **5** **6**

Scheme 2

Previously, we reported the successful cyclization reaction of ethyl heteroarylamino-1-azaazulene-3-carboxylate with $POCl_3$-polyphosphoric acid (PPA).[6] Therefore, we adequate the condition to the

annulation of **2**. Thus, we treated **2** with $POCl_3$-PPA for 1 h at 150 ºC under argon atmosphere and obtained 2-phenyl-12,12a-dihydro-7,12a-diazacyclohepta[*cd*]benz[*f*]azulen-12-one (**7**) in 43% yield as red needles (Scheme 3). In the IR spectrum of **7**, a carbonyl peak was seen at 1631 cm^{-1}, which is equitable to an amide carbonyl. In its ^1H NMR spectrum, signals owing to azaazulene ring moiety at δ 7.56 (ddd, J = 10.0, 9.6, and 1.6, H-4), 7.59 (ddd, 10.8, 10.0, and 0.8, H-5), 8.34 (s, H-1), 8.70 (dd, J = 9.6 and 0.8, H-3), and 9.30 (d, J = 10.8, H-6) and signals owing to benzene moiety 7.40 (ddd, J = 8.0, 7.2, and 1.2, H-9), 7.57 (dd, J = 8.0 and 1.6, H-8), 7.78 (ddd, J = 8.0, 7.2, and 1.6, H-10), and 8.47 (dd, J = 8.0 and 1.2, H-11) were seen together with phenyl protons (7.43 (tt, J = 7.6 and 1.6, H-*p*-Ph), 7.54 (dd, J = 8.4 and 7.6, H-*m*-Ph), and 7.61(dd, J = 8.4 and 1.6, H-*o*-Ph)). From these results as well as HRMS (*m/z* 345.0971 $[M+Na]^+$), we assigned the structure.

Similar treatment of **6** with $POCl_3$-PPA resulted in complex results and did not give distinct products.



2 **7**

Scheme 3

The cytotoxic activity against HeLa S3 cells of obtained compounds (**2**, **6**, and **7**) were evaluated by MTT assay. Compounds (**2** and **6**) showed medium cytotoxic activity and **7** showed extremely strong cytotoxic activity as expected.

Table 1. Cytotoxic evaluation of compounds (**2a**, **6**, and **7a**) expressed in μM

	2	**6**	**7**
IC_{50}	31.1 ± 2.3[a]	12.9 ± 2.3[a]	0.543 ± 0.063

[a] The precipitates were appeared at the concentration of 1% DMSO of the culture fluid.

In summary, we presented a facile synthetic method for new annulated pyrrolo[2,1-*c*][1,4]benzodiazepine system having a strong cytotoxic activity against HeLa S3 cells, and the system is expected as a leading compound for new anticancer drugs.

EXPERIMENTAL

Melting points were determined with a Yanagimoto micro-melting point MP JP-3 apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker AVANCE 400S (400 MHz) using $CDCl_3$ as a solvent with tetramethylsilane as an internal standard; *J* values are recorded in Hz. IR spectra were recorded for KBr pellets on a Nicolet FT-IR AVTAR 370DTGS. Mass specra (ESI-MS) were taken with JEOL JMS-T100CS. Merck Kieselgel 60 was used for column chromatography.

Reaction of 8-chloro-3-phenyl-1-azaazulene (3) with methyl 2-aminobenzoate (4)

A mixture of **3** (0.106 g, 0.44 mmol) and **4** (0.117 g, 0.77 mmol) in dry dioxane (5 mL) was stirred for 30 min at rt. To the mixture hexane (30 mL) was added. The precipitates were corrected by filtration and HCl salt of **2** was obtained. The salt was dissolved in water, then the solution was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. Evaporation of the extract to give **2** (0.140 g, 89%).

2: Orange scales (from hexane-$CHCl_3$), mp 147.0-147.5 ºC; ^{1}H NMR δ 3.93 (3H, s, OMe), 7.03 (1H, like t, *J* = 9.2, H-5), 7.20 (1H, dd, *J* = 8.0 and 7.8, H-5'), 7.37 (1H, t, *J* = 7.3, H-*p*-Ph), 7.46 (1H, like t, *J* = 9.5, H-6), 7.50 (2H, t, *J* = 7.3, H-*m*-Ph), 7.55 (1H, dd, *J* = 8.0 and 7.8, H-4'), 7.56 (1H, d, *J* = 9.6, H-7), 7.56 (1H, s, H-2), 7.56 (1H, d, *J* = 7.8, H-3'), 7.57 (2H, d, *J* = 7.3, H-*o*-Ph), 8.10 (1H, d, *J* = 7.8, H-6'), 8.21 (1H, d, *J* = 9.6, H-4), and 8.24 (1H, br s, NH); ν_{max}/cm^{-1} 3214 (OH), 1633 (C=O). MS *m/z* 355 ($[M+H]^+$, 100%). HRMS: Calcd. for $C_{23}H_{19}N_2O_2$: 355.1447. Found: *m/z* 355.1418. HCl salt of **2**: Yellow needles (from hexane-CH_2Cl_2), mp 170 ºC (decomp); ^{1}H NMR δ 3.78 (3H, s, OMe), 7.24 (1H, d, *J* = 11.6, H-7), 7.28 (1H, dd, *J* = 10.4 and 9.2, H-5), 7.46 (1H, td, *J* = 7.6 and 1.6, H-*p*-Ph), 7.48 (2H, dd, *J* = 7.2 and 1.6, H-*o*-Ph), 7.53 (1H, d, *J* = 8.0, H-3'), 7.54 (1H, ddd, *J* = 8.0, 7.3 and 1.4, H-4'), 7.55 (2H, dd, *J* = 7.6 and 7.2, H-*m*-Ph), 7.66 (1H, dd, *J* = 11.6 and 9.2, H-6), 7.72 (1H, dd, *J* = 8.0 and 7.3, H-5'), 8.02 (1H, d, *J* = 2.8, H-2), 8.17 (1H, dd, *J* = 8.0 and 1.4, H-6'), 8.28 (1H, d, *J* = 10.4, H-4), 12.56 (1H, s, NH), and 15.25 (1H, br s, NH).

Reaction of 8-chloro-3-phenyl-1-azaazulene (3) with methyl 2-mercaptobenzoate (5)

A mixture of **3** (0.100 g, 0.42 mmol) and **5** (0.122 g, 0.73 mmol) in dry dioxane (5 mL) was stirred for 30 min at rt. To the mixture hexane (30 mL) was added. The precipitates were corrected by filtration and HCl salt of **6** was obtained as yellow powders. The salt was dissolved in water, then the solution was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. Evaporation of the extract to give **6** (0.099 g, 66%) was obtained as orange needles.

6 : Red powders (from hexane-$CHCl_3$), mp 52.0-53.9 ºC; ^{1}H NMR δ 3.78 (3H, s, OMe), 7.23 (1H, d, *J* = 8.8, H-7), 7.40 (1H, dd, *J* = 8.0 and 6.8, H-5'), 7.43 (1H, d, *J* = 9.0, H-4), 7.52 (2H, like t, *J* = 6.4, H-*m*-Ph), 7.56 (1H, like t, *J* = 8.0, H-*p*-Ph), 7.59-7.62 (4H, m, H-3', 4', and H-*o*-Ph), 7.75-7.79 (1H, m,

H-5), 7.95-7.99 (1H, m, H-6), 8.61 (1H, d, *J* = 8.0, H-6'), and 8.73 (1H, s, H-2); ν_{max}/cm^{-1} 1719 (C=O). MS *m/z* 372 (([M+H]$^+$, 100%), HRMS: Calcd. for $C_{23}H_{18}N_2O_2S$: 372.1058. Found: *m/z* 372.1044. HCl salt of **6**: Orange-yellow prisms (from hexane-CH_2Cl_2), mp 154 ºC (decomp); ^{1}H NMR δ 3.82 (3H, s, OMe), 7.48-7.61 (5H, m, Ph), 7.57 (1H, d, *J* = 9.6, H-7), 7.72 (1H, ddd, *J* = 7.6, 7.2, and 2.4, H-5') , 7.75 (1H, ddd, *J* = 7.6, 6.4, and 2.4, H-4'), 7.81 (1H, like t, *J* = 9.6, H-5), 7.86 (1H, dd, *J* = 6.4 and 2.4, H-3'), 7.89 (1H, dd, *J* = 10.0 and 9.6, H-6), 8.09 (1H, dd, *J* = 7.2 and 2.4, H-6'), 8.78 (1H, s, H-2), 8.81 (1H, dd, *J* = 10.0 and 1.2, H-4), and 14.50 (1H, br s, NH).

Cyclization of methyl 2-(3-phenyl-1-azaazulen-8-yl)aminobenzoate (2)

Under argon atmosphere, a mixture of **2** (0.034 g, 0.10 mmol), PPA (5 mL), and $POCl_3$ (5 mL) was heated for 1 h at 150 ºC, then ice-water (20 mL) was added. The mixture was neutralized with aq Na_2CO_3, and extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and evaporated. Chromatography of the residue with hexane-AcOEt (1:1) and recrystallization from hexane-CH_2Cl_2 gave **7** (0.014 g, 43%).

7: Red needles (from hexane-CH_2Cl_2), mp 230 ºC (decomp); ^{1}H NMR δ 7.40 (1H, ddd, *J* = 8.0, 7.2, and 1.2, H-9), 7.43 (2H, tt, *J* = 7.6 and 1.6, H-*p*-Ph), 7.54 (2H, dd, *J* = 8.4 and 7.6, H-*m*-Ph), 7.56 (1H, ddd, *J* = 10.0, 9.6, and 1.6, H-4), 7.57 (1H, dd, *J* = 8.0 and 1.6, H-8), 7.61(1H, dd, *J* = 8.4 and 1.6, H-*o*-Ph), 7.59 (1H, ddd, *J* = 10.8, 10.0, and 0.8, H-5), 7.78 (1H, ddd, *J* = 8.0, 7.2, and 1.6, H-10), 8.34 (1H, s, H-1), 8.47 (1H, dd, *J* = 8.0 and 1.2, H-11), 8.70 (1H, dd, *J* = 9.6 and 0.8, H-3), and 9.30 (1H, d, *J* = 10.8, H-6); ν_{max}/cm^{-1} 1631 (C=O). HRMS: Calcd. for $C_{22}H_{14}N_2NaO$: 345.1004. Found: *m/z* 345.0971 ([M+Na]$^+$).

Biological assay

HeLa S3 cells were obtained from AIST and used after cultivation. The cultivated HeLa S3 cells were cell counted and the culture fluid was prepared to the cell consistency of 2×10^4 cell/mL. The compounds were added to the medium in DMSO solutions. To the aliquot of the culture fluid, which was incubated for 3 h at 37 ºC, the test sample was added and then the culture fluid was incubated for 72 h. To the culture fluid, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) solution was added, and incubated for 4 h. Then the sample was centrifuged at 3000 rpm for 10 min at 4 ºC, and the solvent was evaporated. Then DMSO was added to obtained mixture. The MTT-formazan was dissolved by plate-mixing and OD540 was measured. The rate of outlive determined to refer with un-dosed control. Dose-response curve was drawn up and IC_{50} was pursued. Every experiment in the cytotoxic assay was replicated four times in order to define the IC values.

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