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SYNTHESIS AND SOME REACTIONS OF 11-AZACYCLOHEPT[*a*]AZULEN-3(3*H*)-ONES AND EVALUATION OF THEIR CYTOTOXIC ACTIVITY AGAINST HELA S3 CELLS

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Abstract – Aldol condensation of 2,3-diformyl-1-azaazulene with acetone, methoxyacetone, and benzylacetone in aq. NaOH solution gave corresponding 11-azacyclohept[*a*]azulen-3(3*H*)-one (**2a**), 2-methoxy-derivative (**2b**) and 2-benzyl-derivative (**2d**) in good yields. Hydrolysis of **2b** using hot HBr gave 2-hydroxy-11-azacyclohept[*a*]azulen-3(3*H*)-one (**2f**). Acetylation of **2f** gave 2-acetoxy-11-azacyclohept[*a*]azulen-3(3*H*)-one (**2g**). Reaction of the compounds (**2a**, **2b**, **2f**) with diethyl malonate in Ac_2O under heating gave 1,9(11b*H*)-11b-azaazuleno[1,2,3-*cd*]azulenedione derivatives (**4a**, **4b**, **4f**). Compounds (**2b**, **2f**) showed weak cytotoxic activity against HeLa S3 cells.

INTRODUCTION

Tricyclic and tetracyclic systems of containing azulene nucleus such as azulenotropylium ions, azulenotropones and azulenoazulenes are attracted attentions form the view of aromaticity and bioactivity.[1-3] Several azulenotropones and azulenoazulenes have been synthesized notably by K. Takase,[4-6] C. Jutz,[7] T. Toda,[8] Z. Yoshida,[9] and S. Kuroda,[10] and M. Nitta.[11] Although many researches

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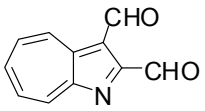
Dedicated to Professor Emeritus Akira Suzuki on occasion of his 80th birthday.

of fused azaazulene chemistry were proceeding,[12] researches about aza-analogs of azulenotropylium ions, azulenotropones, and azulenoazulenes were few,[13-16] and the report was not observed about the reaction of azaazulenotropones except the *N*-protonation[13,14] and the *N*-methylation[14]. Recently, we reported the synthesis of 2,3-diformyl-1-azaazulene,[17] which would be a key product for construction of fused 1-azaazulene systems. We now report the facile synthesis 11-azacyclohept[*a*]azulen-3(3*H*)-ones (11-azaazuleno[1,2-*d*]tropones) (**2**) and the addition-cyclization reaction of **2** with diethyl malonate.

RESULTS AND DISCUSSION

Treatment of 2,3-diformyl-1-azaazulene (**1**) with acetone in aq. NaOH solution for 10 min at rt gave 11-azacyclohept[*a*]azulen-3(3*H*)-one (**2a**) in 48% yield. It is reported that **2a** was prepared by the oxidation of dicyclohepta[*b*,*d*]pyrrole with MnO_2 by Nitta, but the yield low (9%) and the reaction of **2a** was scarcely investigated.[14] In the ^{1}H NMR spectrum of **2a**, seven-membered ring protons in the azaazulene moiety were seen at δ 7.96 (ddd, *J* 9.8, 9.6, and 0.4, H-7), 8.03 (dd, *J* 10.0 and 9.8, H-9), 8.15 (t, *J* 9.8, H-8), 8.88 (dd, *J* 10.0 and 0.4, H-10), and 8.93 (d, *J* 9.6, H-6), where bond-alternation was not observed. On the other hand, seven-membered ring protons of tropone moiety were seen at δ 6.95 (dd, *J* 12.1 and 2.4, H-4), 7.25 (d, *J* 12.4 and 2.4, H-2), 8.07 (d, *J* 12.1, H-5), and 8.08 (d, *J* 12.4, H-1). The results suggest that **2a** would have a 1,4-pentadien-3-one fused with 1-azaazulene structure. Unfortunately, we could not obtain a favorable crystal of **2a** for the X-ray analysis. Therefore, to clarify the consideration, we performed the molecular orbital calculation by Gaussian '03 using RHF/6-31G*[18]. The calculated bond lengths for **2a**, shown in Figure 1, supported our consideration.



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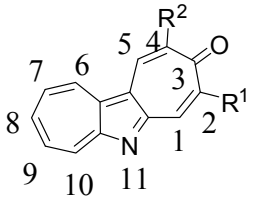
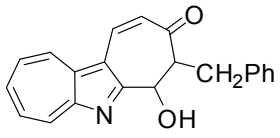


2a : $R^1 = R^2 = H$
2b : R^1 = OMe, $R^2 = H$
2c : $R^1 = H$, R^2 = OMe
2d : $R^1 = CH_2Ph$, $R^2 = H$
2e : $R^1 = R^2 = CO_2Et$
2f : R^1 = OH, $R^2 = H$
2g : R^1 = OAc, $R^2 = H$



3

The condensation of **1** with acetone underwent in good yield. Therefore, our method is superior to Nitta's method. The method could be extended to the reaction with other acetone derivatives. Thus **1** was treated with methoxyacetone in aq. NaOH solution for 10 min at rt to give only

2-methoxy-11-azacyclohept[*a*]azulen-3(3*H*)-one (**2b**) in 63% yield, and 4-methoxy-11-azacyclohept[*a*]-azulen-3(3*H*)-one[13] (**2c**) was not obtained. Melting point of obtained compound (mp 214-215 ℃) differed from that of the reported **2c** (mp 209.5-211.5 ℃)[13]. The electronic spectra of **2b** and **2c**[13] were distinctly different as shown in Figure 2. In the ^{1}H NMR spectrum of **2b**, methoxy proton was seen at δ 4.11, and a singlet signal assigned to H-1 proton was seen at δ 7.68 and AB-doublet protons were seen at δ 7.13 (1H, d, *J* 12.2, H-4) 8.17 (1H, d, *J* 12.2, H-5), except for the seven-membered ring protons of the azaazulene moiety. From the results, we assigned the structure.

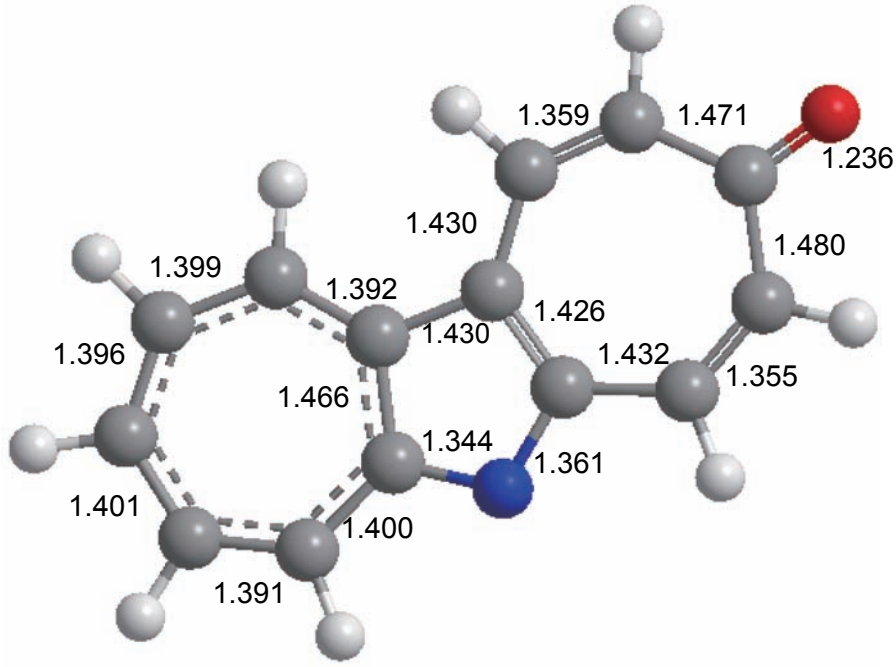


Figure 1. Calculated bond lengths and the optimized structure of **2a**.

When **1** was treated with benzylacetone for 10 min at rt, **2d** (34%) and **3** (22%) were obtained. In the ^{1}H NMR spectrum of **3**, two methine protons were observed at δ 3.56 (1H, dddd, *J* 8.1, 7.7, 6.0, and 1.2, H-2), 5.21 (1H, d, *J* 6.0, H-1) and benzyl protons were seen at δ 2.60 (1H, dd, *J* 13.8 and 7.7) and 2.77 (1H, dd, *J* 13.8 and 8.1), except for the seven-membered ring protons of the azaazulene moiety. In the ^{13}C NMR spectrum of **3**, two methine carbons were observed at δ 59.1 and 68.8 together with benzyl carbon at δ 34.3. In the IR spectrum of **3**, characteristic peaks were observed at 3444 (OH), 1623 (C=O), and 1603 cm^{-1} (C=C). From the results, we assigned the structure. Prolonged treatment of **1** with benzylacetone (for 2h at rt) gave **2d** in 74% yield and none of **3**. It is considered that intermediate (**3**) was obtained at first, then successive dehydration afforded **2d**.

Similar treatment of **1** with diethyl 1,3-acetonedicarboxylate gave **2e** in 13% yield. For improvement of the yield, **1** was treated under azeotropic conditions in the presence of $NHEt_2$ in benzene under reflux for 4 h, but **2e** was obtained only in 5% yield.

Hydrolysis of **2b** was achieved by treatment with 48% HBr under reflux for 3 h and 2-hydroxy-11-azacyclohept[*a*]azulen-3(3*H*)-one (**2f**) was obtained in 88% yield. Compound **2f** was acetylated with Ac_2O in the presence of pyridine for 1 h at rt, and 2-acetoxy-11-azacyclohept[*a*]azulen-3(3*H*)-one (**2g**) was obtained in 61% yield, where the isomeric acetate could not be isolated.

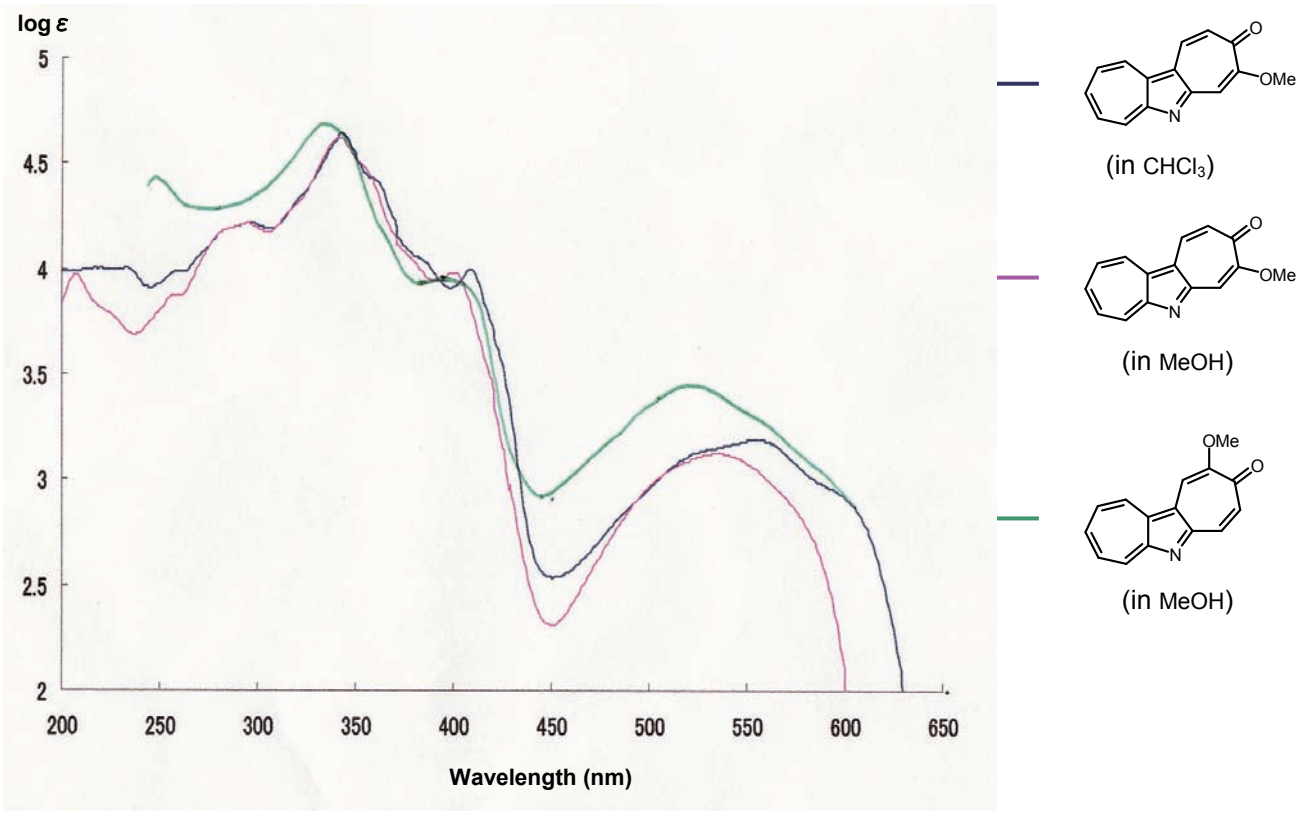


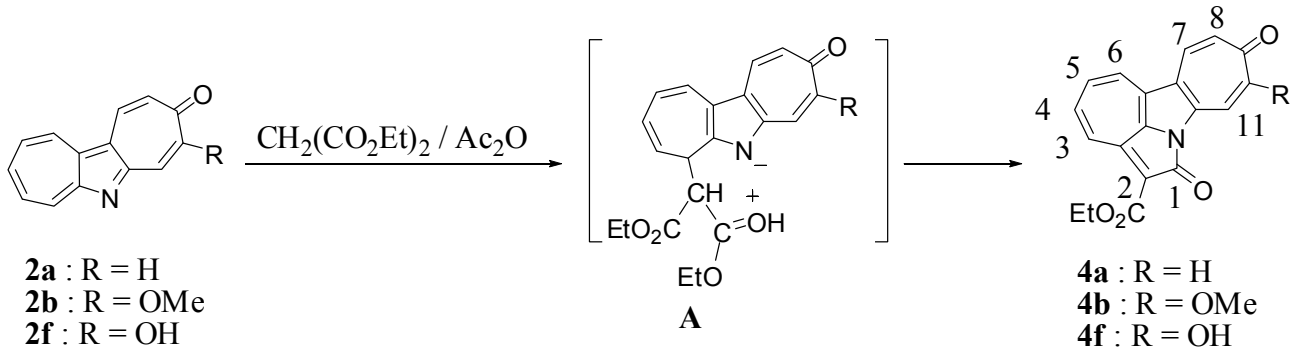
Figure 2. Electronic spectra of **2b** and **2c**.

It is well known that 8,8-dicyanoheptafulvene was obtained by the condensation of tropone with malononitrile in refluxing Ac_2O.[19] Therefore we examined the reaction of **2a** with malononitrile, but distinct product was not isolated. When **2a** was treated with diethyl malonate in refluxing Ac_2O for 1.5 h gave 11b-azaazuleno[1,2,3-*cd*]azulene-1,9(11b*H*)-dione derivative (**4a**) in 24 % yield. In the ^{1}H NMR spectrum of **4a**, one ethyl ester signals were observed at δ 1.45 (*J* 7.1, Me) and 4.43 (*J* 7.1, OCH_2) and four proton signals owing to seven-membered ring of azaazulene moiety were seen at δ 7.51 (dd, *J* 10.4 and 10.3, H-5), 7.81 (dd, *J* 11.0 and 10.3, H-4), 8.04 (d, *J* 10.4, H-6), and 8.71 (d, *J* 11.0, H-3), and tropone moiety at δ 7.07 (dd, *J* 12.2 and 2.5, H-8), 7.22 (dd, *J* 12.4 and 2.5, H-10), 7.73 (dd, *J* 12.2, H-7), and 8.48 (d, *J* 12.4, H-11). In the IR spectrum of **4a**, three carbonyl signals were seen at 1738, 1674, and 1619 cm^{-1}, owing to ester, γ-lactim, and tropone carbonyls, respectively. From the results, we

assigned the structure. Michael addition of the enol form of diethyl malonate to the C-10 of **2a** would generate intermediate **A**, and successive cyclization and dehydrogenation furnished to **4a**.

Similar treatment of **2b** and **2d** with diethyl malonate in refluxing Ac_2O gave **4b** (39%) and **4f** (25%), respectively.

As **2a** was not reacted at the carbonyl of tropone moiety as above, we examined the reaction of **2a** with 2,4-dinitrophenyl hydrazine to substantiate the reactivity. But we could not obtain distinct products, and failed to elicit the reactivity of the carbonyl group.



2a : R = H
2b : R = OMe
2f : R = OH

A

4a : R = H
4b : R = OMe
4f : R = OH

Evaluation of cytotoxic activity

Compounds (**2b** and **2f**) were evaluated for their cytotoxic activity against HeLa S3 cells. The IC_{50} values [μM] were 41 and 19 for **2b** and **2f**, respectively. The results revealed that the compounds (**2b** and **2f**) showed weak activity against HeLa S3 cells.

EXPERIMENTAL

Mps are measured using a Yanagimoto micro-melting apparatus and uncorrected. ^{1}H NMR spectra (including HH-COSY and CH-COSY NMR) were recorded on a Bruker AVANCE 400S (400 MHz) and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400S (100.6 MHz) using $CDCl_3$ as a solvent with tetramethylsilane as an internal standard unless otherwise stated; *J* values are recorded in Hz. IR spectra were recorded for KBr pellets on a Nicolet FT-IR AVTAR 370DTGS unless otherwise stated. Electronic spectra were recorded with JASCO V-570 spectrophotometer. Elemental analyses were taken with a Perkin Elmer 2400II. Kieselgel 60 and activated alumina C300 were used for column chromatography and Kieselgel 60G was used for thin-layer chromatography.

Synthesis of 11-azacyclohept[*a*]azulen-3(3*H*)-one (2a)

A solution of 2,3-diformyl-1-azaazulene (**1**) (0.0988 g, 0.534 mmol) and Me_2CO (0.06 mL, 0.82 mmol) in 0.11 M NaOH solution (100 mL, 1.1 mmol) was stirred for 10 min at room temperature. The mixture

was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, and evaporated. The residue was chromatographed with EtOAc to give **2a** (0.0531 g, 48%) as purple powders.

2a: Purple micro needles (from EtOH), mp 195 ℃ (decomp.); δ_H 6.95 (1H, dd, *J* 12.1 and 2.4, H-4), 7.25 (1H, d, *J* 12.4 and 2.4, H-2), 7.96 (1H, ddd, *J* 9.8, 9.6, and 0.4, H-7), 8.03 (1H, dd, *J* 10.0 and 9.8, H-9), 8.07 (1H, d, *J* 12.1, H-5), 8.08 (1H, d, *J* 12.4, H-1), 8.15 (1H, t, *J* 9.8, H-8), 8.88 (1H, dd, *J* 10.0 and 0.4, H-10), and 8.93 (1H, d, *J* 9.6, H-6); δ_H(TFA-*d*) 7.71 (1H, dd, *J* 12.0 and 2.0, H-4), 7.92 (1H, d, *J* 12.1 and 2.0, H-2), 8.47 (1H, d, 12.1, H-1), 8.83 (1H, dd, *J* 10.0 and 9.9, H-7), 8.86 (1H, dd, *J* 10.1 and 9.8, H-9), 8.88 (1H, d, *J* 12.0, H-5), 8.98 (1H, dd, *J* 10.0 and 9.8, H-8), 9.39 (1H, d, *J* 10.1, H-10), and 9.87 (1H, d, *J* 9.9, H-6); δ_C 107.5, 120.0, 130.4, 131.9, 132.5, 135.8, 138.7, 139. 9, 134.0, 141.0, 141.4, 143.6, 149.7, and 184.9; ν_{max} / cm^{-1} 1592 (C=O); λ_{max}($CHCl_3$) nm (log ε) 286 (4.11), 328 (4.52), 342 (4.66), 360 (3.97, sh), 385 (3.86), 406 (3.42), 500 (2.82, sh), 541 (3.03, sh), 568 (3.07), and 616 (2.84, sh); λ_{max}(MeOH) nm (log ε) 286 (4.16), 328 (4.60), 342 (4.72), 358 (4.06, sh), 384 (3.93), 406 (3.49), 541 (3.15, sh), 566 (3.17), and 607 (2.97, sh). *Anal*. Calcd for $C_{14}H_9NO \cdot H_2O$: C, 74.65; H, 4.92; N, 6.22. Found: C, 74.44; H, 4.98; N, 6.19.

Synthesis of 2-methoxy-11-azacyclohept[*a*]azulen-3(3*H*)-one (2b)

A solution of **1** (0.0981 g, 0.530 mmol) and methoxyacetone (0.07 mL, 0.76 mmol) in 0.055 M NaOH solution (200 mL, 1.1 mmol) was stirred for 10 min at rt. The mixture was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, and evaporated. The residue was chromatographed with EtOAc to give **2b** (0.0795 g, 63%) as reddish purple leaflets.

2b: Reddish purple leaflets (from hexane-CH_2Cl_2), mp 214-215 ℃; δ_H 4.11 (3H, s, OMe), 7.13 (1H, d, *J* 12.2, H-4), 7.68 (1H, s, H-1), 7.95 (1H, td, *J* 9.4 and 1.2, H-7), 7.99 (1H, ddd, *J* 9.6, 9.4, and 1.2, H-9), 8.10 (1H, ddd, *J* 9.6, 9.4, and 1.4, H-8), 8.17 (1H, d, *J* 12.2, H-5), 8.77 (1H, dd, *J* 9.6 and 1.4, H-10), and 8.86 (1H, d, *J* 9.4, H-6); δ_H(TFA-*d*) 4.35 (3H, s, OMe), 7.92 (1H, d, *J* 11.9, H-4), 8.13 (1H, s, H-1), 8.81-8.96 (3H, m, H-7, 8, and 9), 9.06 (1H, d, *J* 11.9, H-5), 9.36 (1H, d, *J* 9.9, H-10), and 9.82 (1H, d, *J* 9.8, H-6); δ_C 56.7, 110.1, 121.9, 128.2, 129.4, 130.9, 132.9, 133.1, 137.1, 139.0, 144.9, 160.0, 161.3, 162.4, and 181.7; ν_{max} / cm^{-1} 1614 (C=O); λ_{max}($CHCl_3$) nm (log ε) 259 (3.99), 284 (4.19, sh), 296 (4.22), 322 (4.34, sh), 343 (4.64), 358 (4.43, sh), 382 (4.05, sh), 409 (4.00), 528 (3.14, sh), 555 (3.19), and 592 (2.96, sh); λ_{max} (MeOH) nm (log ε) 258 (3.88), 285 (4.19, sh), 294 (4.22), 318 (4.29, sh), 341 (4.63), 376 (4.07, sh), 400 (3.98), and 535 (3.12). *Anal*. Calcd for $C_{15}H_{11}NO_2 \cdot 2H_2O$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.12; H, 5.88; N, 5.34.

Reaction of 1 with benzylacetone

a) A solution of **1** (0.093 g, 0.502 mmol) and benzylacetone (0.113 g, 0.762 mmol) in 0.048 M NaOH solution (100 mL, 4.80 mmol) was stirred for 10 min at rt. The mixture was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, and evaporated. The residue was chromatographed with EtOAc to give **2d** (0.0471 g, 34%) and **3** (0.0334 g, 22%), successively.

b) A solution of **1** (0.0370 g, 0.200 mmol) and benzylacetone (0.113 g, 0.762 mmol) in 0.0445 M NaOH solution (100 mL, 4.45 mmol) was stirred for 2 h at rt. The mixture was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, and evaporated. The residue was chromatographed with EtOAc to give **2d** (0.0442 g, 74%).

2d: violet powders (from hexane-CH_2Cl_2), mp 208-210 ℃; δ_H 4.14 (2H, s, CH_2), 7.01 (1H, d, *J* 12.2, H-4), 7.23 (1H, tt, *J* 7.2 and 2.2, H-*p*-Ph), 7.31 (2H, dd, *J* 7.7 and 7.2, H-*m*-Ph), 7.37 (2H, dd, *J* 7.7 and 2.2), H-*o*-Ph), 7.91 (1H, dd, *J* 9.8 and 9.5, H-7), 7.97 (1H, dd, *J* 9.9 and 9.6, H-9), 8.01 (1H, d, *J* 12.2, H-5), 8.08 (1H, dd, *J* 9.9 and 9.8, H-8), 8.12 (1H, s, H-1), 8.79 (1H, d, *J* 9.6, H-10), and 8.86 (1H, d, *J* 9.5, H-6); δ_C 40.9, 124.0, 126.5, 128.3, 128.6, 129.6, 130.5, 131.1, 132.3, 124.5, 134.6, 138.6, 139.2, 140.1, 144.5, 152.1, 159.1, 161.8, and 187.3; ν_{max} / cm^{-1} 1597 (C=O). *Anal*. Calcd for $C_{21}H_{15}NO \cdot H_2O$: C, 79.98; H, 5.43; N, 4.44. Found: C, 79.66; H, 5.32; N, 4.64.

3: Red powders (from hexane-CH_2Cl_2), mp 202 ℃ (decomp.); δ_H 2.60 (1H, dd, *J* 13.8 and 7.7, C<u>H</u>$_2$), 2.77 (1H, dd, *J* 13.8 and 8.1, C<u>H</u>$_2$), 3.56 (1H, dddd, *J* 8.1, 7.7, 6.0, and 1.2, H-2), 5.21 (1H, d, *J* 6.0, H-1), 6.24 (1H, d, *J* 12.3 and 1.2, H-4), 6.94 (2H, dt, *J* 7.5 and 1.5, H-*o*-Ph), 7.11 (1H, tt, *J* 7.0 and 1.5, H-*p*-Ph), 7.16 (2H, dd, *J* 7.5 and 7.0, H-*m*-Ph), 7.56 (1H, d, *J* 12.3, H-5), 7.76 (1H, dd, *J* 10.0 and 9.7, H-7), 7.80 (1H, dd, *J* 9.8 and 9.7, H-9), 7.97 (1H, ddd, *J* 9.8, 9.7, and 0.8, H-8), 8.57 (1H, d, *J* 10.0, H-6), and 8.63 (1H, dd, *J* 9.5 and 0.8, H-10), (OH was not observed); δ_C 34.3, 59.1, 68.8, 119.5, 125.9, 126.3, 128.3, 128.9, 130.5, 131.6, 133.8, 137.6, 138.3, 139.7, 144.5, 157.0, 169.9, and 196.9; ν_{max} / cm^{-1} 3444 (OH), 1623 (C=O), and 1603 (C=C). *Anal*. Calcd for $C_{21}H_{17}NO_2$: C, 79.98; H, 5.43; N, 4.44. Found: C, 80.06; H, 5.52; N, 4.28.

Reaction of 1 with diethyl acetone-1,3-dicarboxylate

a) A solution of **1** (0.0951 g, 0.514 mmol) and diethyl acetone-1,3-dicarboxylate (0.20 mL, 1.20 mmol) in 0.25 M NaOH solution (50 mL, 1.25 mmol) was stirred for 10 min at rt. The mixture was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, and evaporated. The residue was chromatographed with EtOAc to give **2e** (0.0215 g, 13%) as violet powders.

b) To the solution of diethyl acetone-1,3-dicarboxylate (0.20 mL, 1.20 mmol) and $NHEt_2$ (0.15 mL) in dry benzene (50 mL) was added benzene solution (20 mL) of **1** (0.1149 g, 0.625 mmol), and the mixture was refluxed for 4 h using Dean-Stark trap. The mixture was evaporated and the residue was

chromatographed with EtOAc to give **2e** (0.0015 g, 5%).

2e: Violet powders (from AcOEt), mp 148-149 ℃; δ_H 1.41 (3H, t, *J* 7.0, Me), 1.42 (3H, t, *J* 7, Me), 4.43 (4H, q, *J* 7.0, CH_2), 8.05 (1H, dd, *J* 10.1 and 9.9, H-9), 8.10 (1H, dd, *J* 9.8 and 9.5, H-7), 8.23 (1H, dd, *J* 9.9 and 9.5, H-8), 8.55 (1H, s, H-1), 8.77 (1H, s, H-5), 8.93 (1H, d, *J* 10.1, H-10), and 9.05 (1H, d, *J* 9.5, H-6); δ_C 14.1, 14.2, 61.9, 62.1, 122.9, 130.9, 131.5, 131.6, 133.3, 134.2, 136.3, 140.3, 141.1, 141.8, 145.9, 159.8, 160.8, 166.2, 167.1, and 184.2; ν_{max} / cm^{-1} 1736, 1695, and 1626 (C=O); λ_{max}($CHCl_3$) nm (log ε) 296 (4.32, sh), 334 (4.59), 341 (4.58), 376 (4.12, sh), 523 (3.00, sh), 553 (3.04), and 592 (2.84, sh). *Anal*. Calcd for $C_{20}H_{17}NO_5$: C, 68.37; H, 4.88; N, 3.99. Found: C, 68.57; H, 4.93; N, 3.75.

Synthesis of 2-hydroxy-11-azacyclohept[*a*]azulen-3(3*H*)-one (2f)

A mixture of **2b** (0.0317 g, 0.134 mmol) and 48% HBr (10 mL) was stirred for 3 h under heating at 160 ℃. The mixture was neutralized with Na_2CO_3 and extracted with $CHCl_3$. The extract was dried over Na_2SO_4, and evaporated. The compound **2f** (0.0263 g, 88%) was obtained as purple powders.

2f: Purple scales (from EtOH-EtOAc), mp 178-180 ℃; δ_H 5.11 (1H, br, OH), 7.31 (1H, d, *J* 12.0, H-4), 7.97 (1H, ddd, *J* 9.6, 9.5 and 1.4, H-7), 8.04 (1H, ddd, *J* 9.6, 9.3, and 1.4, H-9), 8.07 (1H, s, H-1), 8.08 (1H, dd, *J* 9.6 and 9.5, H-8), 8.43 (1H, d, *J* 12.0, H-5), 8.82 (1H, dd, *J* 9.3 and 1.8, H-10), and 8.88 (1H, d, *J* 9.5, H-6); δ_C 11.9, 122.8, 123.8, 125.0, 131.9, 132.7, 133.4, 135.2, 137.4, 139.0, 145.5, 160.4, 161.0, 162.6, and 180.3; ν_{max} / cm^{-1} 3249 (OH) and 1625 (C=O); λ_{max}($CHCl_3$) nm (log ε) 276 (4.04), 292 (4.06), 300 (4.09), 321 (4.16), 339 (4.32, sh), 350 (4.38), 391 (3.80, sh), 396 (3.78, sh), 410 (3.68, sh), 428 (3.44, sh), 510 (3.20), 533 (3.18), and 581 (2.91, sh); λ_{max}(MeOH) nm (log ε) 259 (3.77), 265 (3.82), 285 (3.95, sh), 297 (4.05, sh), 320 (4.23), 360 (4.29), 409 (3.65), 506 (3.79), and 531 (3.78); λ_{max}(MeOH + 1 drop of aq. NaOH) nm (log ε) 259 (3.77), 265 (3.82), 285 (3.94, sh), 298 (4.05, sh), 321 (4.25), 359 (4.28), 424 (3.65), 508 (3.79), and 532 (3.78). *Anal*. Calcd for $C_{14}H_9NO_2$·EtOAc: C, 69.44; H, 5.51; N, 4.50. Found: C, 69.41; H, 5.35; N, 4.24.

Acetylation of 2f

A mixture of **2f** (0.0185 g, 0.083 mmol), Ac_2O (1 mL), and a drop of pyridine was stirred for 1 h at rt. To the mixture water was added, and the mixture was extracted with $CHCl_3$. The extract was dried over Na_2SO_4, and evaporated. The residue was chromatographed with EtOAc to give **2g** (0.0134 g, 61%) as violet powders.

2g: Violet needles (from MeOH-CH_2Cl_2), mp 188 ℃; δ_H 2.44 (1H,s, Me), 7.10 (1H, d, *J* 12.2, H-4), 7.99 (1H, td, *J* 10.0 and 9.8, H-9), 8.05 (1H, dd, *J* 9.8 and 9.6, H-7), 8.10 (1H, s, H-1), 8.14 (1H, d, *J* 12.2, H-5), 8.17 (1H, t, *J* 9.8, H-8), 8.82 (1H, d, *J* 10.0, H-10), and 8.88 (1H, d, *J* 9.6, H-6); δ_C 20.8, 124.0,

126.3, 129.4, 139.5, 131.0, 132.9, 135.0, 139.2, 140.8, 144.6, 155.6, 159.2, 159.5, 168.8, and 180.0; ν_{max} / cm^{-1} 1754 and 1603 (C=O); $\lambda_{max}(CHCl_3)$ nm (log ε) 259 (3.95), 288 (4.23, sh), 312 (4.36, sh), 327 (4.45), 340 (4.53), 360 (4.43), 384 (3.94, sh), 494 (3.82, sh), 503 (3.82), and 525 3.81, sh). *Anal*. Calcd for $C_{16}H_{11}NO_3$: C, 72.45; H, 4.18; N, 5.28. Found: C, 72.41; H, 4.33; N, 5.24.

Reaction of 11-azacyclohept[*a*]azulen-3(3*H*)-ones (2a, 2b, 2f) with diethyl malonate

A mixture of **2a** (0.0872 g, 0.42 mmol) and diethyl malonate (0.35 mL, 2.3 mmol) in Ac_2O (10 mL) was refluxed for 1.5 h. To the mixture was added water, then the mixture was extracted with $CHCl_3$. The extract was dried over Na_2SO_4, and evaporated. The residue was chromatographed with EtOAc to give **4a** (0.318g, 24%) as red powders.

4a: Red powders (from CH_2Cl_2), mp 137-138 ℃; δ_H 1.45 (3H, t, *J* 7.1, Me), 4.43 (2H, q, *J* 7.1, OCH_2), 7.07 (1H, dd, *J* 12.2 and 2.5, H-8), 7.22 (1H, dd, *J* 12.4 and 2.5, H-10), 7.51 (1H, dd, *J* 10.4 and 10.3, H-5), 7.73 (1H, dd, *J* 12.2, H-7), 7.81 (1H, dd, *J* 11.0 and 10.3, H-4), 8.04 (1H, d, *J* 10.4, H-6), 8.48 (1H, d, *J* 12.4, H-11), and 8.71 (1H, d, *J* 11.0, H-3); δ_C 14.5, 60.4, 125.0, 126.5, 128.6, 129.4, 130.3, 130.9, 131.4, 135.2, 136.7, 136.8, 139.0, 140.7, 141.8, 149.6, 162.3, 163.4 and 187.3; ν_{max} / cm^{-1} 1738, 1674, 1619 (C=O), and 1588 (C=C); $\lambda_{max}(CHCl_3)$ nm (log ε) 296 (4.32, sh), 334 (4.59), 341 (4.58), 376 (4.12, sh), 418 (3.23, sh), 518 (2.99, sh), 553 (3.04), and 604 (2.77, sh). *Anal*. Calcd for $C_{19}H_{13}NO_4$: C, 71.47; H, 4.10; N, 4.39. Found: C, 71.53; H, 3.85; N, 4.21.

Similar treatment of **2b** and **2f** gave **4b** (39%) and **4f** (21%), respectively.

4b: Reddish brown powders (from cyclohexane), mp 223-224 ℃; δ_H 1.46 (3H, t, *J* 7.1, Me), 4.12 (3H, s, OMe), 4.45 (2H, q, *J* 7.1, OCH_2), 7.24 (1H, d, *J* 12.4, H-8), 7.58 (1H, dd, *J* 10.3 and 9.8, H-5), 7.83 (1H, d, *J* 12.4, H-7), 7.85 (1H, dd, *J* 11.0 and 9.8, H-4), 8.11 (1H, d, *J* 10.3, H-6), 8.27 (1H, s, H-11), and 8.79 (1H, d, *J* 11.0, H-3); ν_{max} / cm^{-1} 1721, 1690, 1613 (C=O), and 1585 (C=C). *Anal*. Calcd for $C_{20}H_{15}NO_5$: C, 68.76; H, 4.33; N, 4.01. Found: C, 69.02; H, 4.55; N, 3.86.

4f: Red powders (from CH_2Cl_2), mp 132-133 ℃; δ_H 1.45 (3H, t, *J* 7.1, Me), 4.43 (2H, q, *J* 7.1, OCH_2), 7.25 (1H, d, *J* 12.4, H-8), 7.52 (1H, dd, *J* 10.4 and 9.6, H-5), 7.82 (1H, d, *J* 12.4, H-7), 7.83 (1H, dd, *J* 11.0 and 9.6, H-4), 8.05 (1H, d, *J* 10.4, H-6), 8.56 (1H, s, H-11), and 8.71 (1H, d, *J* 11.0, H-3) (OH was not observed); ν_{max} / cm^{-1} 3294 (OH), 1720, 1690, 1613 (C=O), and 1585 (C=C). *Anal*. Calcd for $C_{19}H_{13}NO_5$: C, 68.06; H, 3.91; N, 4.18. Found: C, 67.72; H, 3.86; N, 4.28.

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 cells/ml. The compounds added to the medium in DMSO solutions. To the aliquot of the culture fluid, which was incubated for 3 h at 37 ℃, 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 ℃, and the solvent was evaporated. Then DMSO was added to the 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 cycotoxic assay was replicated twice in order to define the IC values.

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