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SYNTHESIS OF BENZOTROPONE-ANNULATED 1-AZAAZULENES AND RELATED COMPOUNDS BY SUZUKI-MIYAURA COUPLING / ALDOL CONDENSATION CASCADE REACTION AND EVALUATIONS OF THEIR CYTOTIXIC ACTIVITY AGAINST HELA S3 CELLS†

Makoto Nakatani,[a] Hiroyuki Fujii,[b]* Toshihiro Murafuji,[a] Takahiro Gunji,[c] Reiko Ikeda,[c] Takeo Konakahara,[c] and Noritaka Abe[c]*

[a]Graduate School of Medicine, Yamaguchi University, Yoshida, Yamaguchi 753-8512, Japan; [b]Science Research Center, Yamaguchi University, Yoshida, Yamaguchi 753-8512, Japan; [c]Department of Pure and Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, Noda, Chiba 278-8510, Japan: E-mail noriabe@rs.noda.tus.ac.jp

Abstract – One-pot Suzuki-Miyaura coupling/aldol condensation cascade reactions of 3-acetyl-2-bromo-1-azaazulene (**1b**) with 2-formylphenylboronic acid and the reaction of 2-bromo-3-formyl-1-azaazulene (**6b**) with 2-acetylphenylboronic acid gave 5-azacyclohepta[*b*]benz[*h*]azulen-11(11*H*)-one (**5**) and 5-azacyclohepta[*b*]benz[*h*]azulen-13(13*H*)-one (**8**), respectively, in good yields. The 13-hydroxy-12,13-dihydro-derivative (**4**) of **5** was isolated as an intermediate in the former reaction. Similar reaction of **1b** with 2-formylthiophene-3-boronic acid under similar conditions gave 4-aza-1-thia-1,10-dihydroazuleno[2,1-*e*]azulen-10-one (**10**) in high yield. Similar reaction of **1b** with 3-formythiophene-2-boronic acid did not give a corresponding cyclization product, instead 3-acetyl-1-azaazulen-2(1*H*)-one was obtained. Compounds (**4**, **5**, and **10**) showed strong cytotoxic activity against HeLa S3 cells, whereas compound (**8**) did not show the cytotoxic activity.

INTRODUCTION

The chemistry of troponoid compounds began as natural product chemistry at first, and are attracted

† Dedicated to Professor Dr. Albert Padwa on the occasion of his 75th birthday.

attention from peculiarities of their structures and reactions, as well as their numerical pharmaceutical utilities.[1] Recently, troponoid compounds were investigated from a viewpoint of functionality, such as liquid crystals.[2]

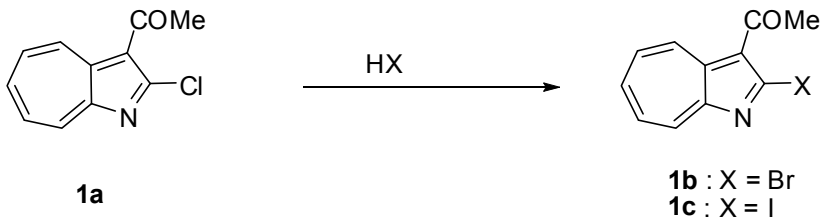
Arenotropones (arene-annulated cycloheptatrieneones), such as benzotropones, benzotropolones, and pyridotropones, etc., are formally considered as a special case of tropones, and recently some researches were made from the interests of their physical properties[3] and biological activities.[4-6] As a view point of functionality of benzotropones, conjugated polymers containing tropones and benzotropones were synthesized.[7]

In connection with arenotropones, we recently reported the synthesis of 1-azaazulene fused tropones and their cytotoxicity toward HeLa S3 cells.[8] It is known that benzotropones have some different character compared with tropones. So, in the line of the synthesis of bioactive azaazulenes,[9] we have now advanced the investigation to the synthesis of azaazulenobenzotropones.

RESULTS AND DISCUSSION

Numerous synthetic methods for synthesis of benzotropones are known,[10] and recently new and efficient approaches were reported.[4,11] In the methods, it seems that the intramolecular aldol condensation which is adopted for the synthesis of dibenzotropone from 2-acetyl-2'-formylbiphenyl.[6,12] Recently, one-pot Suzuki-Miyaura coupling/aldol condensation cascade reaction was reported as an efficient synthetic method for dibenzotropones.[13] We previously reported the synthesis of 2-(aryl substituted)-1-azaazulenes by Suzuki-Miyaura coupling.[14] Therefore we examined the Suzuki-Miyaura coupling of 3-acethyl-2-halo-1-azaazulenes with 2-formylphenylbronic acid and the reaction of 3-formyl-2-halo-1-azaazulenes with 2-acetylphenylboronic acid.

3-Acetyl-2-bromo-1-azaazulene (**1b**) and 3-acetyl-2-iodo-1-azaazulene (**1c**) were synthesized by the treatment of 3-acetyl-2-chloro-1-azaazulene (**1a**)[15] with 48% HBr or 55% HI at 100 ℃ in 96% and 72% yields, respectively (Scheme 1).



1a

1b : X = Br
1c : X = I

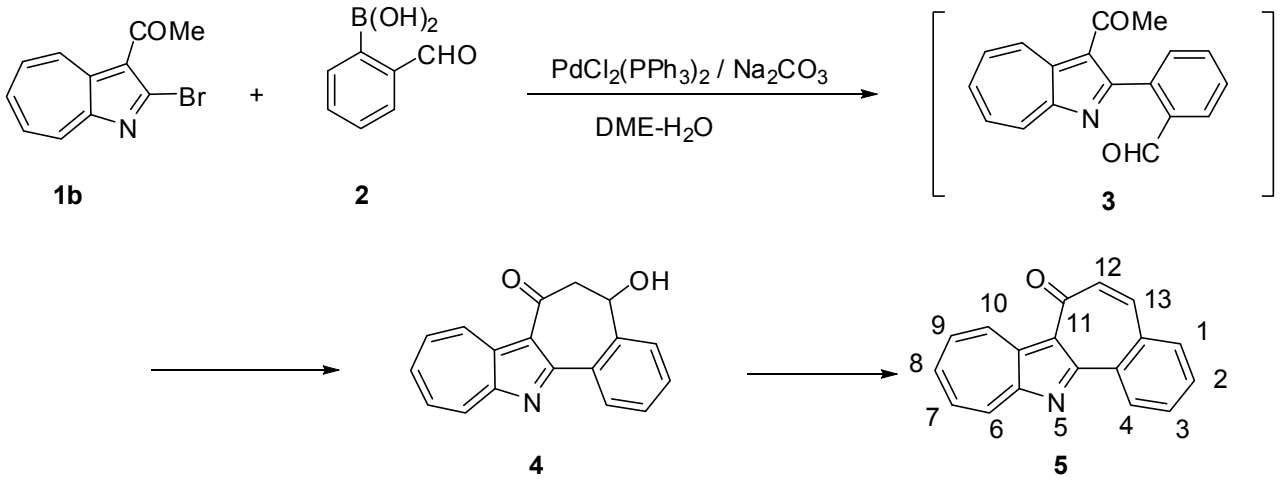
Scheme 1

The reactions of **1a** with 2-formylphenylbronic acid (**2**) under some conditions using $Pd(OAc)_2$, $PdCl_2(PPh_3)_2$, $PdCl_2(dppf) \cdot CH_2Cl_2$, or $Pd_2(dba)_3$ as Pd-catalyst, KF or K_2CO_3 as base,

(2-biphenyl)di-*t*-butylphosphine or xantphos as ligand, and THF, toluene, or dioxane as solvent, but the reactions underwent only scarcely, and no distinct product was obtained. Reaction of **1b** or **1c** with **2** gave unstable yellow product, being considered to be **3** from the ^{1}H NMR spectrum. Compound **3** easily decomposed in $CDCl_3$ in NMR tube for 24 h at rt, and an attempt for isolation of **3** was failed.

Treatment of **1b** with **2** in the presence of $PdCl_2(PPh_3)_2$ and Na_2CO_3 in DME-H_2O for 6 h at 60 ℃ gave yellow needles (**4**) in 57% yield. The ^{1}H NMR spectrum of **4** showed a proton assignable to OH at δ 5.73 (1H, d, *J* 4.1, disappeared by addition of D_2O), a methine proton assignable to H-13 at δ 5.03 (1H, td (*J* 5.8 and 4.1), changed to t (*J* 5.8) by addition of D_2O) and methylene protons assignable to H-12 at δ 3.16 (2H, d, *J* 5.8, not changed by addition of D_2O) together with aromatic protons. The ^{13}C NMR spectrum showed a carbonyl carbon at δ 195.1, a methine carbon at δ 67.2 and a methylene carbon at δ 53.2 together with 15 aromatic carbons. In its IR spectrum, a carbonyl peak was seen at 1633 cm^{-1} and an OH peak at 3214 cm^{-1}. From these results as well as elemental analysis, we assigned the structure of **4** as 13-hydroxy-5-azacyclohepta[*b*]benz[*h*]azulen-11(11*H*)-one.

When the reaction was carried out for 48 h at 70 ℃, **5** was obtained as orange needles in 65% yield at a stroke. In the ^{1}H NMR spectrum of **5**, a couple of AB doublets were seen at δ 6.97 and 7.54 (*J* 12.8) together with aromatic protons. The ^{13}C NMR spectrum showed a carbonyl carbon at δ 185.0 together with 17 aromatic carbons. In its IR spectrum, a carbonyl peak was seen at 1575 cm^{-1}. From these results as well as elemental analysis, we assigned the structure of **5** as 5-azacyclohepta[*b*]benz[*h*]azulen-11(11*H*)-one.

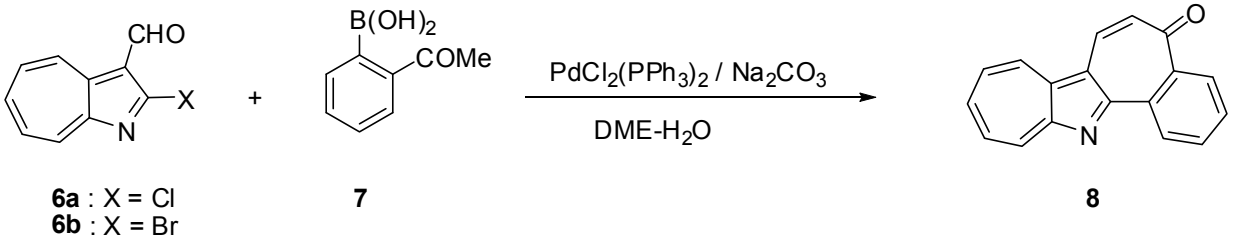


1b **2** **3** **4** **5**

Scheme 2

Next we examined the synthesis of **8**, an isomer of **5**, by the reaction of 3-formyl-2-haro-1-azaazulene (**6**) with 2-acetylphenylboronic acid (**7**). The reaction of 2-chloro-3-formyl-1-azaazulene (**6a**) with **7** did not proceed under the conditions as mentioned for the reaction of **1b** with **2**. The reaction of

2-bromo-3-formyl-1-azaazulene (**6b**) with **7** under similar conditions for 22 h at 70 ℃ gave **8** as purple needles in 35% yield. In the ^{1}H NMR spectrum of **8**, a couple of AB doublets were seen at δ 6.94 and 8.02 (J 12.1) together with aromatic protons. The ^{13}C NMR spectrum showed a carbonyl carbon at δ 189.7 together with 15 aromatic carbons. In its IR spectrum, a carbonyl peak was seen at 1579 cm^{-1}. From these results as well as elemental analysis, we assigned the structure of **8** as 5-azacyclohepta[*b*]benz[*h*]azulen-13(13*H*)-one.



6a : X = Cl
6b : X = Br

7

8

Scheme 3

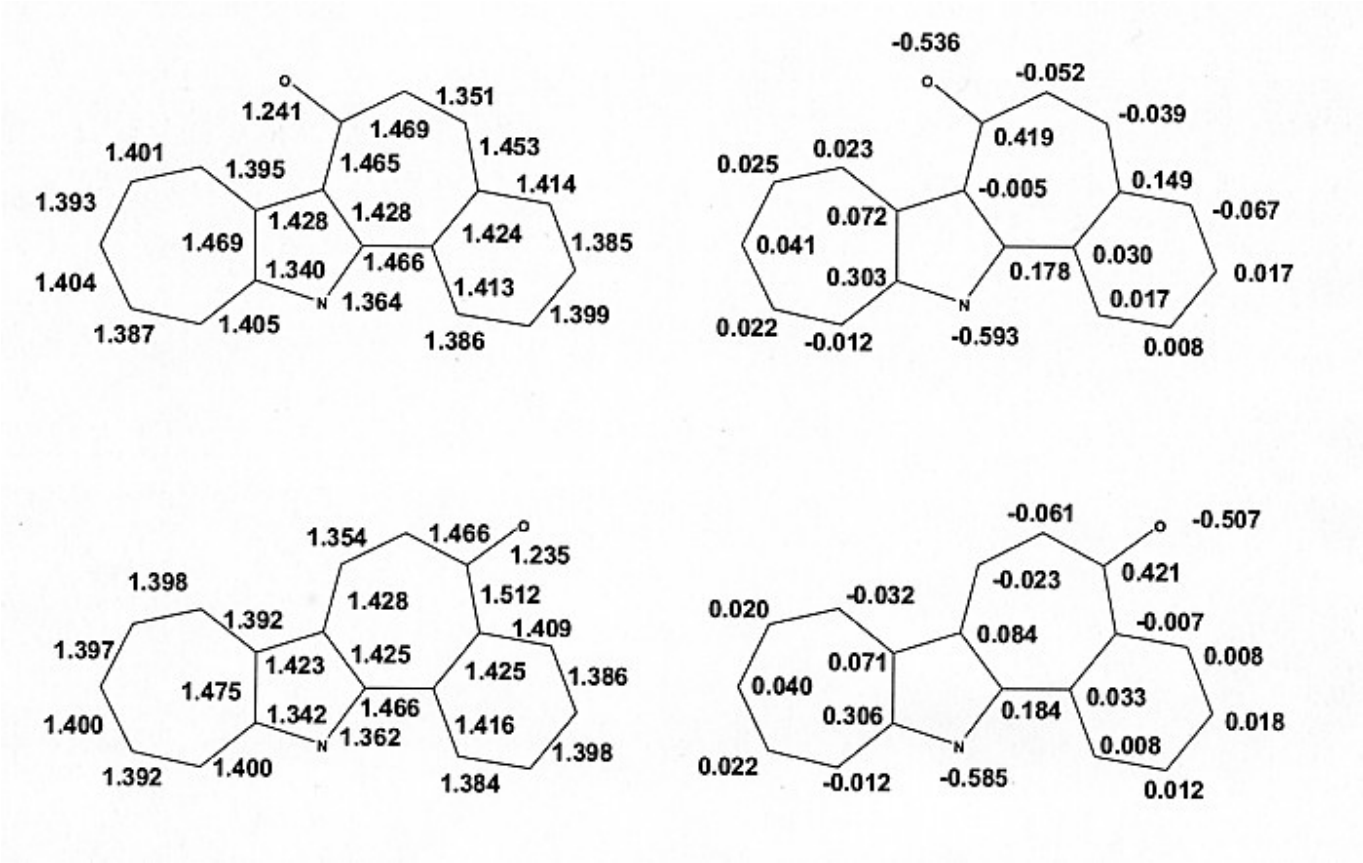


Figure 1. The calculated bond lengths and Mulliken atomic charges of **5** (upper) and **8** (lower). The structures were optimized by Gaussian '03: B3LYP/6-31G*. Bond lengths (left) are given in Å. Atomic charges (right) with hydrogens summed into heavy atoms.

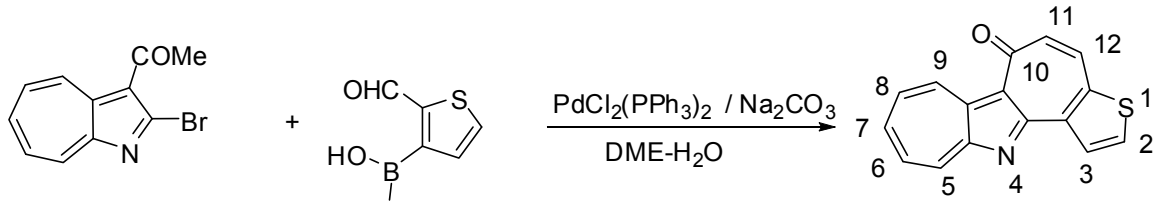
Unfortunately, we could not obtain favorable crystals of **5** and **8** for the X-ray analysis. Therefore, to obtain information about the structures of **5** and **8**, we performed DFT calculations at the B3LYP/6-31G*

level performed the use of the Gaussian 03 package (Figure 1). It seems that bond alternations scarcely existed in **5** and **8**, in spite of the observation of some contributions of conjugated enone form from the information about bond lengths. Interestingly, it seems cationic charges distribute on the ring carbons and anionic charges mainly exist on carbonyl oxygen and N-13 in **5**. On the other hand, it seems charges distribute over the ring in **8**. From these considerations, we advanced that **5** has a character of **5A** and **8** has a character of **8A** (Figure 2). The facts that the color of **5** is orange and the color of **8** is purple would consist with the consideration.

5A **8A**

Figure 2

As expansion of the reaction, we next examined the reaction of **1b** with formylthiopheneboronic acids. Treatment of **1b** with 2-formylthiophene-3-boronic acid (**9**) under the conditions for 20 h at 70 ℃ gave 4-aza-1-thia-1,10-dihydroazuleno[2,1-*e*]azulen-10-one (**10**) in 81% yield as orange needles. In the ^{1}H NMR spectrum of **10**, a couple of AB doublets were seen at δ 7.03 and 7.65 (*J* 12.5) assignable to H-11 and H-12 and a couple of AB doublets were seen at δ 7.69 and 8.58 (*J* 5.4) assignable to H-2 and H-3 together with 5 seven-membered ring protons. The ^{13}C NMR spectrum showed a carbonyl carbon at δ 185.3 together with 15 aromatic carbons. In its IR spectrum, a carbonyl peak was seen at 1568 cm^{-1}. From these results as well as elemental analysis, we assigned the structure.

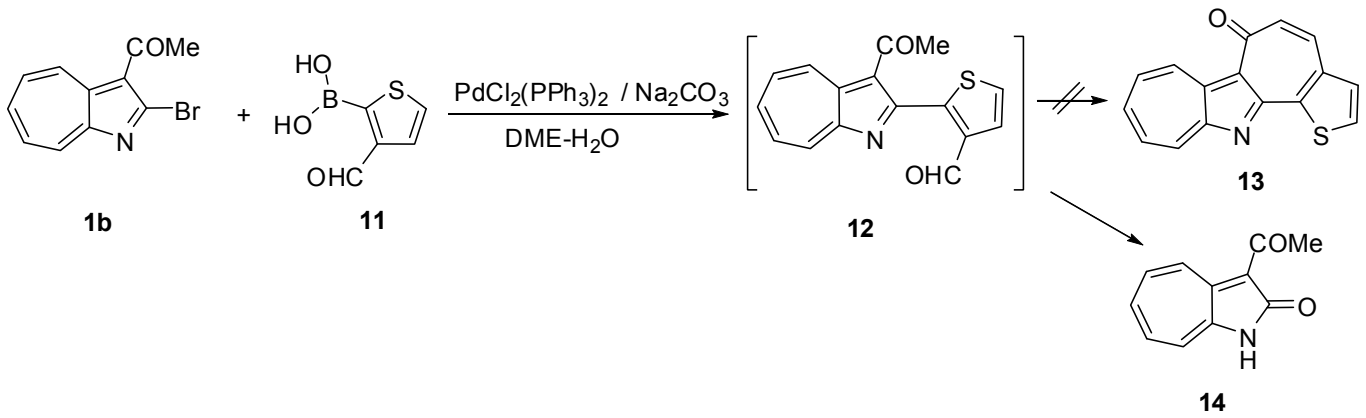


1b **9** **10**

Scheme 4

On the contrary, the reaction of the reaction of **1b** with 3-formylthiophene-2-boronic acid (**11**) under similar conditions did not give 4-aza-3-thia-3,10-dihydroazuleno[2,1-*e*]azulen-10-one (**13**), instead the formation of 3-acetyl-1-azaazulen-2(1*H*)-one[15-17] (**14**) was confirmed. In the reaction, colorless spot being

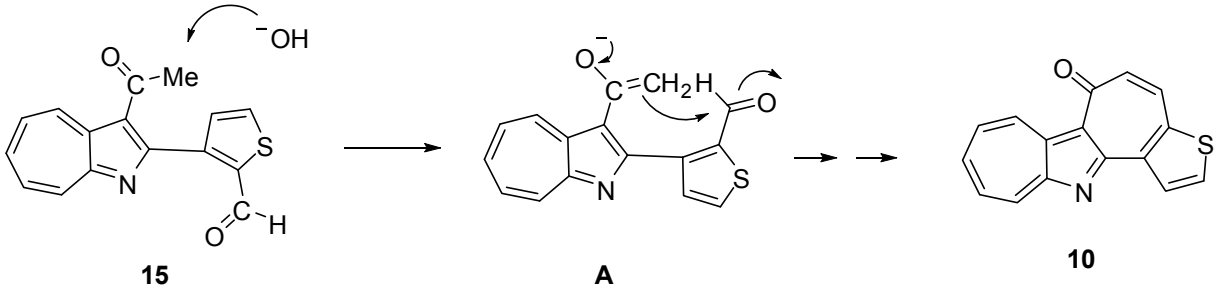
considered as 3-formylthiophene was seen on TLC, but 3-formylthiophene was not isolated. When the reaction was carried out in the absence of **11**, **1b** did not react at all at and **14** was not produced at the conditions, and the result showed **14** was not produced by the hydrolysis of **1b**. In addition, when above reaction of **1b** with **11** was carried out in the presence of Pd-catalyst under the water-free conditions, the reaction did not proceed. Therefore, it is considered that **14** would be produced from an unstable intermediate (**12**).



1b **11** **12** **13** **14**

Scheme 5

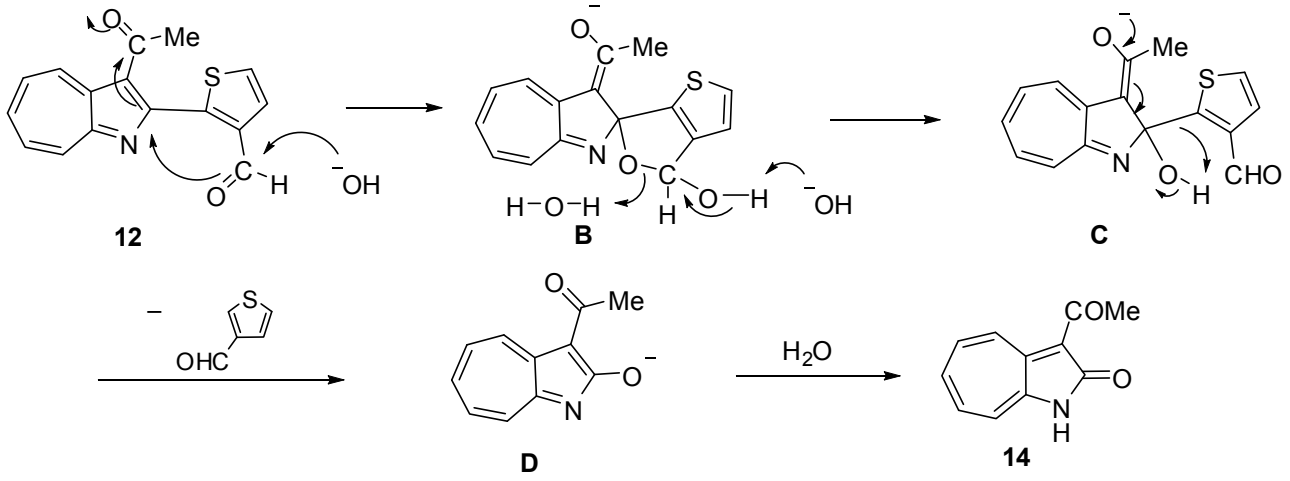
Plausible mechanisms for the formation of **10** and **14** are shown in Scheme 6 and Scheme 7. It is considered that a deference of the reactivity of the formyl groups on the thiophene ring affects the reaction. When the reactivity of the formyl group of 3-acetyl-2-(2-formylthiophen-3-yl)-1-azaazulene (**15**) is lower than that of the acetyl group, the enolate (**A**) is produced, and the aldol condensation undergoes to give cyclization product (**10**) (Scheme 6).



15 **A** **10**

Scheme 6

When the nucleophilic attack of hydroxide ion to the carbonyl carbon of the formyl group on 3-acetyl-2-(3-formylthiophen-2-yl)-1-azaazulene (**12**) take preference over the formation of the enolate by abstraction of a proton from the acetyl group on **12** by hydroxide ion, **B** is produced. Deprotonation from **A** by hydroxide ion and a successive reaction as shown with the curved arrows affords **C**. Elimination of 3-formylthiphene from **C** furnishes **14** *via* **D** (Scheme 7).



Scheme 7

Evaluation of cytotoxic activity

Compounds (**4**, **5**, **8** and **10**) were evaluated for their cytotoxic activity against HeLa S3 cells by MTT assay. The IC_{50} values [μM] are summarized in Table 1. Except for **8**, the compounds showed substantially potent cycotoxic activity, and **5** showed most strong. On the other hand, the compound (**8**) did not show the cytotoxic activity. It seems that the position of the carbonyl group affected the cytotoxic activity.

Table 1. Cytotoxic evaluation of compounds (**4**, **5**, **8**, and **10**) expressed in μM

	4	**5**	**8**	**10**
IC_{50}	10.5 ± 0.5	≧ 3.2 ± 0.6 [a]	> 100	4.6 ± 0.2 [b]

[a] The precipitates appeared at the concentration. [b] The precipitates appeared at 1%DMSO-culture fluid.

CONCLUSION

We successfully synthesized 1-azaazulene-annulated benzotropones and thienotropones in one-pot Suzuki-Miyaura coupling/Aldol condensation cascade reaction. Obtained compounds (**4**, **5**, and **10**) have strong cytotoxic activity against HeLa S3 cells, except for 5-azacyclohepta[*b*]benz[*h*]azulen-13(13*H*)-one (**8**).

EXPERIMENTAL

Melting points were determined with a Yanagimoto micro-melting point MP JP-3 apparatus and were uncorrected. ^{1}H NMR spectra and ^{13}C NMR spectra (including HH-COSY and CH-COSY NMR) were recorded on a Bruker AVANCE 400S (400 MHz for ^{1}H and 100.6 MHz for ^{13}C) 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 on a JASCO V-670 using $CHCl_3$ as a solvent. Mass spectra (ESI-MS) were taken with JEOL JMS-T100CS. Merck Kieselgel 60 was used for column chromatography.

Synthesis of 3-acetyl-2-bromo-1-azaazulene (1b) and 3-acetyl-3-iodo-1-azaazulene (1c)

A mixture of **1a** (0.1058 g, 0.5145 mmol) and 48% HBr (6 mL) was heated at 100 ℃ for 70 min, then poured into H_2O (50 mL). The mixture was neutralized with $NaHCO_3$, and extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and evaporated. Chromatography of the residue with hexane-AcOEt (1 : 1) gave **1b** (0.1242 g, 96.5%).

Similar treatment of **1a** with 55% HI at 100 ℃ for 90 min gave **1c** (72%).

1b : Yellow needles (from hexane-CH_2Cl_2), mp 165-166 ℃ (decomp); ^{1}H NMR δ 2.89 (3H, s, Me), 7.98-8.03 (2H, m, H-5 and 7), 8.12 (1H, dddd, *J* 11.0, 9.8, 1.2 and 1.0, H-6), 8.75 (1H, dd, *J* 10.2 and 1.2, H-8), and 9.79 (1H, dd, *J* 10.4 and 1.0, H-4); ^{13}C NMR δ 31.9, 123.1, 133.3, 134.3, 137.9, 138.5, 140.6, 146.7, 148.6, 158.0, and 195.3; ν_{max} / cm^{-1} 1633 (C=O); λ_{max} nm (log ε) 254 (4.00), 270 (3.99), 296 (4.56), 331 (3.94), 361 (3.31), and 455 (2.97). *Anal.* Calcd for $C_{11}H_8NBrO$: C, 52.83; H, 3.22; N, 5.60. Found: C, 52.70; H, 3.36; N, 5.58.

1c : Yellow needles (from hexane-CH_2Cl_2), mp 154-156 ℃ (decomp); ^{1}H NMR δ 2.94 (3H, s, Me), 7.94-8.00 (2H, m, H-5 and 7), 8.12 (1H, dddd, *J* 9.80, 9.8, 1.1 and 0.9, H-6), 8.76 (1H, dd, *J* 10.1 and 1.1 H-8), and 9.71 (1H, dd, *J* 10.4 and 0.9, H-4); ^{13}C NMR δ 32.0, 125.3, 128.8, 132.9, 133.8, 137.65, 137.8, 140.8, 145.9, 159.7, and 195.6; ν_{max} / cm^{-1} 1622 (C=O). *Anal.* Calcd for $C_{11}H_8NIO$: C, 44.47; H, 2.71; N, 4.71. Found: C, 44.32; H, 2.74; N, 4.59.

Reaction of 3-acetyl-2-bromo -1-azaazulene with 2-formylphenylboronic acid

a) A mixture of **1b** (0.0815 g, 0.3259 mmol), **2** (0.0742 g, 0.4947 mmol), $PdCl_2(PPh_3)_2$ (0.0226 g, 0.0322 mmol), Na_2CO_3 (0.150 g, 1.415 mmol) in DME (3 mL)-H_2O (1.5 mL) was heated at 60 ℃ for 6 h, then water was added. The mixture was extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and evaporated. Chromatography of the residue with $CHCl_3$–AcOEt (2 : 1) gave **4** (0.0512 g, 57%).

4 : Yellow needles (from hexane-CH_2Cl_2), mp 193-195 ℃; ^{1}H NMR (DMSO-d_6) δ 3.15 (2H, d, *J* 5.6, H-12), 5.03 (1H, td, *J* 5.6 and 4.2, H-13), 5.77 (1H, d, *J* 4.2, OH, disappeared by addition of D_2O), 7.49 (1H, ddd, *J* 7.5, 7.4, and 1.6, H-3), 7.54 (1H, ddd, *J* 7.4, 7.3, and 1.5, H-2), 7.65 (1H, br d, *J* 7.3, H-1), 8.08-8.20 (3H, m, H-7, 8, and 9), 8.52 (1H, dd, *J* 7.5 and 1.5, H-4), 8.81 (1H, dd, *J* 10.2 and 1.3, H-6), and 9.76 (1H, d, *J* 9.8, H-10); ^{13}C NMR (DMSO-d_6) δ 53.2, 67.2, 120.2, 126.3, 127.7, 130.7, 131.4, 131.8, 134.0, 134.6, 138.4, 140.6, 145.1, 147.3, 159.3, 165.3, 195.1; ν_{max} / cm^{-1} 3214 (OH), 1633 (C=O); λ_{max}

nm (log ε) 282 (4.28, sh), 316 (4.61), 365 (4.16), 466 (3.07), 496 (2.93, sh), and 540 (2.00, sh). *Anal*. Calcd for $C_{18}H_{13}NO_2$: C, 78.53; H, 4.76; N, 5.09. Found: C, 78.37; H, 4.73; N, 5.00.

b) A mixture of **1b** (0.237 g, 0.948 mmol), **2** (0.185 g, 1.234 mmol), $PdCl_2(PPh_3)_2$ (0.035 g, 0.0499 mmol), Na_2CO_3 (0.501 g, 4.723 mmol) in DME (10 mL)-H_2O (5 mL) was heated at 70 ℃ for 48 h, then water was added. The mixture was extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and evaporated. Chromatography of the residue with $CHCl_3$–AcOEt (3 : 1) gave **5** (0.1585 g, 65%).

5 : Orange needles (from cyclohexane-THF), mp 159-162 ℃; ^{1}H NMR δ 6.97 (1H, d, *J* 12.8, H-12), 7.54 (1H, d, *J* 12.8, H-13), 7.69 (1H, ddd, *J* 8.0, 6.6, and 1.6, H-2), 7.75-7.79 (2H, m, H-1 and 3), 8.05-8.16 (3H, m, H-7, 8, and 9), 8.93 (1H, dd, *J* 10.5 and 1.4, H-6), 9.55 (1H, dd, *J* 7.8 and 1.6, H-4), and 10.45 (1H, dd, *J* 9.5 and 1.6, H-10); ^{13}C NMR (DMSO-d_6) δ 122.5, 130.6, 131.2, 131.3, 133.6, 133.9, 134.0, 134.6, 134.7, 134.9, 138.9, 139.4, 139.8, 141.7, 147.6, 158.9, 161.9, and 185.0; ν_{max} / cm^{-1} 1575 (C=O); λ_{max} nm (log ε) 286 (4.37), 326 (4.62), 365 (4.11), 395 (3.87), 480 (2.88), and 515 (2.56, sh). *Anal*. Calcd for $C_{18}H_{11}NO$: C, 84.03; H, 4.31; N, 5.44. Found: C, 82.92; H, 4.48; N, 5.47.

Reaction of 2-bromo-3-formyl-1-azaazulene (6b) with 2-acetylphenylboronic acid (7)

A mixture of **6b** (0.0080 g, 0.03389 mmol), **7** (0.0102 g, 0.0622 mmol), $PdCl_2(PPh_3)_2$ (0.0060 g, 0.00855 mmol), Na_2CO_3 (0.0289 g, 0.2727 mmol) in DME (0.5 mL)-H_2O (0.25 mL) was heated at 70 ℃ for 22 h, then water was added. The mixture was extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and evaporated. Chromatography of the residue with $CHCl_3$–AcOEt (3 : 1) gave **8** (g, 35%).

8 : Purple needles (from hexane-CH_2Cl_2), mp 213-215 ℃; ^{1}H NMR δ 6.94 (1H, d, *J* 12.1, H-12), 7.77 (1H, ddd, *J* 8.3, 8.1, and 1.3, H-2), 7.87 (1H, ddd, *J* 8.3, 8.1, and 1.5, H-3), 7.86-7.91 (1H, m, H-7), 7.96 (1H, ddd, *J* 9.9, 9.7, and 1.0, H-9), 8.02 (1H, d, *J* 12.1, H-11), 8.01-8.07 (1H, m, H-8), 8.58 (1H, dd, *J* 8.1 and 1.5, H-1), 8.83 (1H, d, *J* 9.6, H-6), 8.87 (1H, dd, *J* 9.7 and 1.0, H-10), and 9.46 (1H, dd, *J* 8.1 and 1.3, H-4); ^{13}C NMR δ 121.1, 128.8, 128.9, 130.1, 130.9, 131.3, 131.6, 131.9, 132.3, 133.0, 133.4, 137.8, 138.7, 139.0, 145.5, 158.1, 163.4, and 189.7; ν_{max} / cm^{-1} 1579 (C=O); λ_{max} nm (log ε) 272 (4.21), 320 (4.64), 364 (4.51), 381 (4.32), 440 (2.33), 537 (3.21), 543 (3.22), 552 (3.22), 555 (3.22), 598 (2.96, sh). *Anal*. Calcd for $C_{18}H_{11}NO$: C, 84.03; H, 4.31; N, 5.44. Found: C, 83.94; H, 4.36; N, 5.35.

Reaction of 1b with 2-formylthiophene-3-boronic acid (9)

A mixture of **1b** (0.0281 g, 0.1124 mmol), **9** (0.0215 g, 0.1378 mmol), $PdCl_2(PPh_3)_2$ (0.0083 g, 0.01183 mmol), Na_2CO_3 (0.0603 g, 0.5689 mmol) in DME (1 mL)-H_2O (0.5 mL) was heated at 70 ℃ for 20 h, then water was added. The mixture was extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and evaporated. Chromatography of the residue with CH_2Cl_2 gave **10** (0.0240 g, 81%).

10 : Orange needles (from EtOAc), mp 213-215 ℃; ^{1}H NMR δ 7.03 (1H, d, *J* 12.5, H-11), 7.65 (1H, d, *J* 12.5, H-12), 7.69 (1H, d, *J* 5.4, H-2), 8.06-8.17 (3H, m, H-6, 7, and 8), 8.58 (1H, d, *J* 5.4, H-3), 8.92 (1H, d, *J* 9.5, H-5), and 10.58 (1H, dd, *J* 9.2 and 1.8, H-9); ^{13}C NMR δ 123.6, 128.2, 129.0, 130.6, 132.8, 133.4, 134.0, 138.0, 139.0, 139.9, 140.2, 141.5, 148.3, 159.6, 161.0, and 185.3; ν_{max} / cm^{-1} 1568 (C=O). MS (ESI) *m/z* 549 ([2M+Na]$^+$, 100%), 288 ([M+Na]$^+$, 71%), 264 ([M+H]$^+$, 6%). HRMS (ESI): Calcd for $C_{16}H_{10}NOS$: 264.04831. Found: *m/z* 264.04548.

Reaction of 1b with 2-formylthiophene-3-boronic acid (9)

A mixture of **1b** (0.0754 g, 0.301 mmol), **11** (0.0660 g, 0.423 mmol), $PdCl_2(PPh_3)_2$ (0.0122 g, 0.0174 mmol), Na_2CO_3 (0.1779 g, 0.1678 mmol) in DME (3 mL)-H_2O (1.5 mL) was heated at 70 ℃ for 22 h, then water was added. The mixture was extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and evaporated. Chromatography of the residue with $CHCl_3$–AcOEt (1 : 1) gave 2-acetyl-1-azaazulen-2(1*H*)-one[15-17] (**14**) (0.0202 g, 36%).

14 : Yellow needles (from EtOAc), mp 293-295 ℃ (decomp), (lit.[15], mp 265-270 ℃; lit.[16], mp 297-299 ℃; lit.[17], mp 293.5-294.5 ℃ (decomp)); ^{1}H NMR δ 2.75 (3H, s, Me), 7.44 (1H, like tm, *J* 8.7, H-6), 7.61 (1H, dd, *J* 9.4 and 8.6, H-7), 7.61 (1H, d, *J* 8.6, H-8), 7.69 (1H, like tm, *J* 10.0, H-5), 9.45 (1H, d, *J* 11.0, H-4), and 11.12 (1H, br, NH); ν_{max} / cm^{-1} 1640 and 1625 (C=O); *Anal*. Calcd for $C_{11}H_9NO_2$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.45; H, 4.78; N, 7.51.

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 ℃, 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 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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