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SYNTHESIS, PHOTOCHEMICAL PROPERTIES, AND CYTOTOXICITY OF 10-ALKYLPHENAZIN-2(10*H*)-ONES

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Abstract – In the present study, the synthesis of a variety of 10-alkylphenazin-2(10*H*)-ones by oxidative coupling between *N*-alkylbenzene-1,2-diamine and 1,2,4-benzenetriol under an oxygen atmosphere was realized, and their photochemical and biological properties were investigated. 10-Methylphenazin-2(10*H*)-one, a reddish orange pigment, exhibited fluorescence in the visible light region upon exposure to ultraviolet light. Interestingly, the fluorescence from 10-methylphenazin-2(10*H*)-one consisted of two components. In addition, the cytotoxic evaluation of various synthetic compounds was performed. Among the compounds tested in this study, 10-methylphenazin-2(10*H*)-one exhibited the most selective cytotoxic activity against human cancer A549 cells compared to normal MRC-5 cells.

INTRODUCTION

Phenazine is an azaarene derived from anthracene by replacing its carbon atoms at the positions 9 and 10 with nitrogen atoms (Figure 1). Several naturally occurring phenazines have been isolated from microorganisms such as *Pseudomonas* and *Streptomyces*.[1,2] These compounds exhibited broad and diverse biological activities such as antimicrobial, antiviral, and antitumor. Particularly, 10-alkylphenazin-2(10*H*)-ones (**1**) are a minor subgroup of phenazines, while endophenazine B[3,4] and chromophenazine C[5] are natural products containing this scaffold.

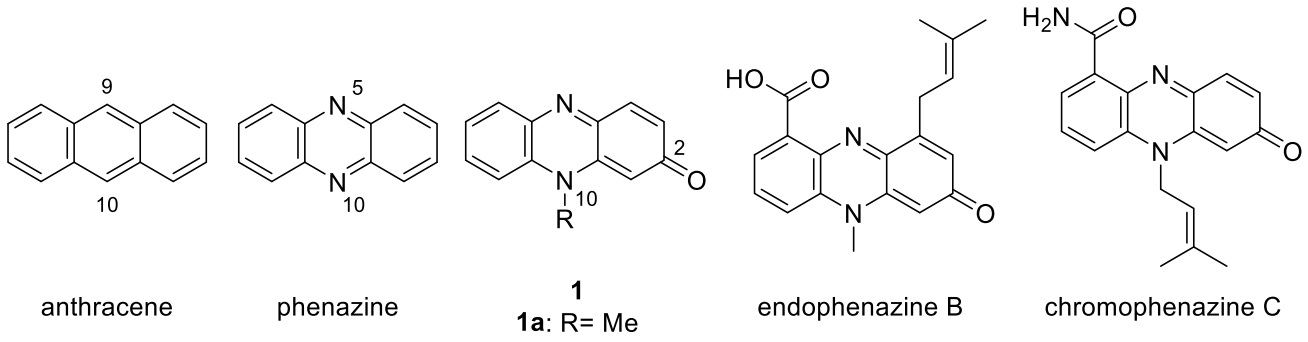
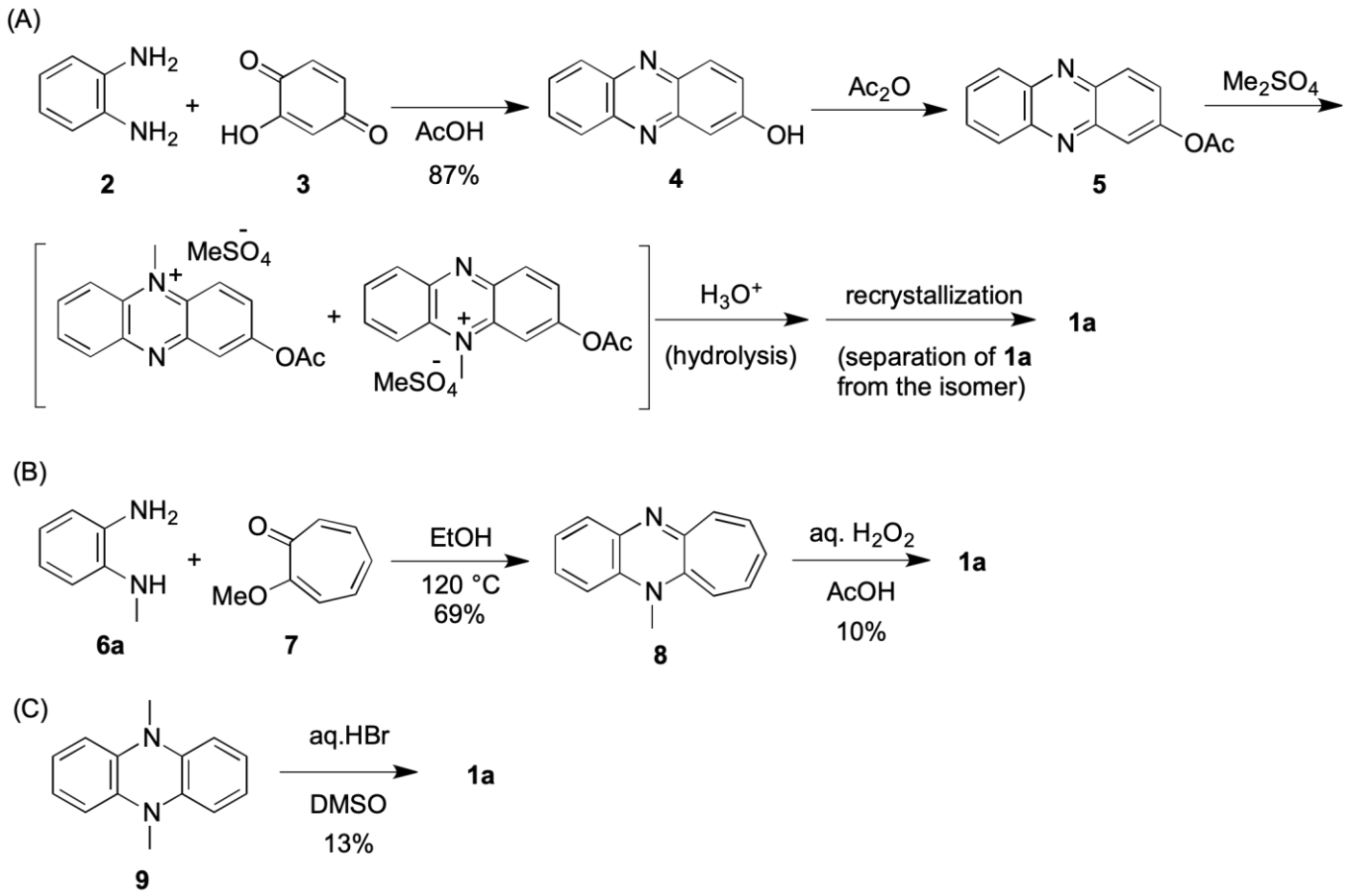


Figure 1. Structures of anthracene, phenazine, 10-alkylphenazin-2(10*H*)-ones (**1**), 10-methylphenazin-2(10*H*)-one (**1a**), endophenazine B, and chromophenazine C

Although several reports on the synthesis of phenazine and its derivatives are present in the literatures,[1,2] the methods to access **1** are rather limited. The previous syntheses of 10-methylphenazin-2(10*H*)-one (**1a**), one of the simplest phenazine derivatives, are summarized in Scheme 1. Kerhrmann and coworkers described the coupling of benzene-1,2-diamine (**2**) with 2-hydroxy-1,4-benzoquinone (**3**) to afford phenazin-2-ol (**4**), which was subsequently acetylated to yield **5** (Scheme 1A).[6,7] Methylation of **5** with methyl sulfate gave two methylphenazinium ions. Acidic hydrolysis of the methylphenazinium ions, and separation of **1a** from its isomer by recrystallization yielded pure **1a**. However, this method suffered from a lack of selectivity at the methylation step as well as cumbersome and time-consuming separation of **1a** from its isomer by recrystallization. Shindo and coworkers reported another synthetic route based on the coupling between *N*-methylbenzene-1,2-diamine (**6a**) and 2-methoxytropone (**7**), followed by oxidation of the resultant compound **8** with H_2O_2 (Scheme 1B).[8] It was reported that compound **1a** could be produced as a byproduct of various reactions.[9-13] For example, Sugimoto and coworkers reported that treatment of 5,10-dimethyl-5,10-dihydrophenazine (**9)** with aqueous HBr solution in dimethyl sulfoxide (DMSO), which led to the formation of **1a** as byproduct in 13% yield along with that of phenazine as main product in 77% yield (Scheme 1C).[9] For the physical properties of **1a**, the ionization constant[14] and

oxidation-reduction constant[7] were reported. However, to the best of our knowledge, the photochemical properties and biological activities of **1a** have not been reported.

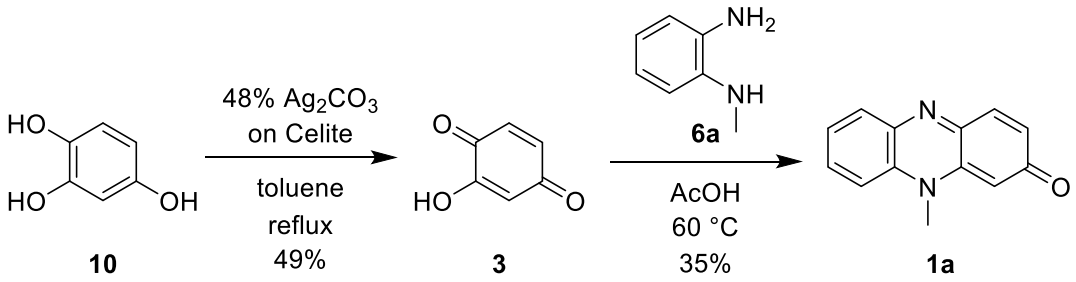
The limited synthetic accessibility of 10-alkylphenazin-2(10*H*)-ones has prevented further investigations of their photochemical properties and biological activities. Therefore, the development of more effective and straightforward methodology for the synthesis of **1a** and its derivatives is desirable. Previously, our group established the synthesis of *N*-alkylphenazin-1-ones by the oxidative condensation of pyrogallols with *N*-substituted benzene-1,2-diamines under an oxygen atmosphere.[15,16] We envisioned that our method will be also applicable to the synthesis of 10-alkylphenazin-2(10*H*)-ones and lead to the discovery of their novel properties. In this paper, we developed a synthetic route of 10-alkylphenazin-2(10*H*)-ones. The photochemical properties of **1a** were investigated by ultraviolet–visible (UV-Vis) absorption, steady-state fluorescence, and time-resolved fluorescence spectroscopy analyses. Furthermore, the cytotoxic activity of the synthetic 10-alkylphenazin-2(10*H*)-ones against human cancer A549 and normal MRC-5 cells was evaluated.



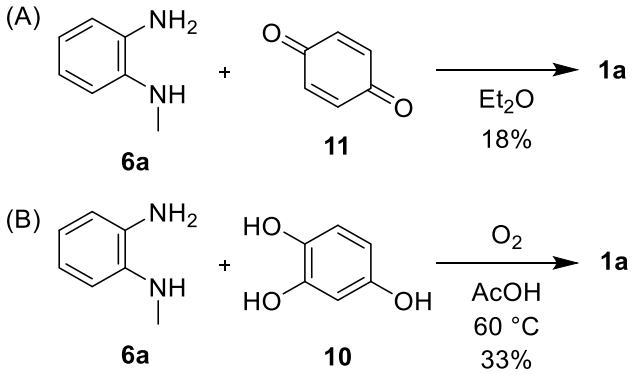
Scheme 1. Selected syntheses of 10-methylphenazin-2(10*H*)-one

RESULTS AND DISCUSSION

First, the direct coupling between 2-hydroxy-1,4-benzoquinone (**3**) and *N*-methylbenzene-1,2-diamine (**6a**) according to the procedure reported by Kerhrmann and coworkers[6,7] was examined (Scheme 2). Oxidation of 1,2,4-benzenetriol (**10**) with 48% Ag_2CO_3 on Celite gave **3** in 49% yield.[17] Coupling between **3** and **6a** with AcOH furnished **1a** in moderate yield of 35%. Thus, **1a** was obtained in 17% overall yield from **10**. Next, the direct oxidative coupling between **6a** and 1,4-benzoquinone (**11**) or 1,2,4-benzenetriol (**10**) was examined (Scheme 3).[15,16] Compound **6a** was treated with **11** (2.0 equiv), which can act both as a coupling partner and as an oxidant, in Et_2O to give **1a** in 18% yield (Scheme 3A). Reacting **6a** with **10** under an oxygen atmosphere in AcOH afforded **1a** in 33% yield (Scheme 3B). Thin layer chromatography (TLC) analysis of the reaction mixture indicates the consumption of **6a** and the formation of unidentified byproducts. Because these byproducts are more polar than **6a**, the oxidative decomposition of **6a** will be accompanied by formation of **1a**. Other reaction conditions including solvent and temperature were also investigated; however, the yield of **1a** was not improved. Therefore, the reaction shown in Scheme 3B provided the optimal conditions.



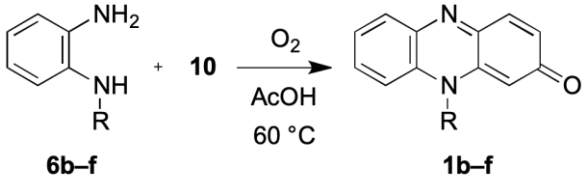
Scheme 2. Two-step synthesis of **1a** from **10**



Scheme 3. Synthesis of **1a** by oxidative coupling between **6a** and **11** or **10**

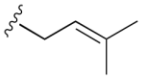
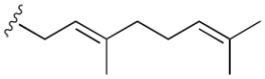
The conditions depicted in Scheme 3B were found to be effective for the preparation of 10-alkylphenazin-2(10*H*)-ones (Table 1). The reaction of *N*-ethylbenzene-1,2-diamine (**6b**) with 1,2,4-benzenetriol (**10**) under an oxygen atmosphere afforded **1b** in 28% yield (entry 1). 10-Decylphenazin-2(10*H*)-one (**1c**) and 10-benzylphenazin-2(10*H*)-one (**1d**) were synthesized in 21% and 32% yields, respectively (entries 2 and 3). Furthermore, this method was employed for the synthesis of 10-prenylphenazin-2(10*H*)-one (**1e**) and 10-geranylphenazin-2(10*H*)-one (**1f**) (entries 4 and 5). It is worth noting that compound **1e** is the core skeleton of chromophenazine C.

Table 1. Synthesis of **1b–f** by oxidative coupling between **6b–f** and **10**



6b–f **1b–f**

b: R = CH_2Me, **c**: R = $(CH_2)_9Me$, **d**: R = Bn

e: R =  **f**: R = 

entry	R	substrate	product	yield (%)[a]
1	Et	**6b**	**1b**	28
2	*n*-decyl	**6c**	**1c**	21
3	Bn	**6d**	**1d**	32
4	prenyl	**6e**	**1e**	29
5	geranyl	**6f**	**1f**	21

[a] Isolated yield.

Next, the photochemical properties of **1a** were investigated. UV-Vis and fluorescence spectra of **1a** in MeCN and MeOH were measured (Figure 2). The solutions of **1a** were reddish-orange due to the broad absorption ranging from 400 to 600 nm. Compound **1a** showed three emission features at 504, 543, and 601 nm in MeCN upon excitation at 410 nm. The absolute quantum yield (Φ) of **1a** in MeCN was determined to be 1.5% upon excitation at 410 nm. The degassing of oxygen gas from the solution did not change the quantum yield, validating the emission to be fluorescence. It is likely that the low quantum yield may be caused by the self-absorption (overlap of absorption and fluorescence spectra). On the other hand, **1a** exhibited a broad emission band at 543 nm in MeOH. These results indicate that the solvent greatly influenced the emission properties of **1a**. Interestingly, the time-resolved fluorescence spectrum of **1a** in MeCN indicates that **1a** has two fluorescence components at the maximum wavelengths of 610 and

500 nm (see Figure S1 in Supporting Information). Their fluorescence lifetimes were determined to be 2.4 and 7.1 ns, respectively, from the theoretical curve fitting of the fluorescence decay curves of **1a** in Figure 3. The excitation spectrum for the emission wavelength of 610 nm is different from that for the emission wavelength of 500 nm, suggesting the presence of two different components (Figure 4). The excitation spectrum for the emission wavelength of 610 nm is similar to the UV-Vis spectrum of **1a**. This result indicates that the fluorescence at 610 nm should be derived from **1a**. Thus, the short lifetime component observed at the maximum wavelength of 610 nm is attributed to the relaxation from S_1 to S_0 in **1a**. The origin of the long lifetime component observed at the maximum wavelength of 500 nm remains unclear. One possibility is that this component might be attributed to zwitterionic species **1a′**, one of the resonance structures of **1a** (Figure 5), because 5-methylphenazinium methyl sulfate displayed a weak broad emission band at 525 nm upon excitation at 360 nm.[18]

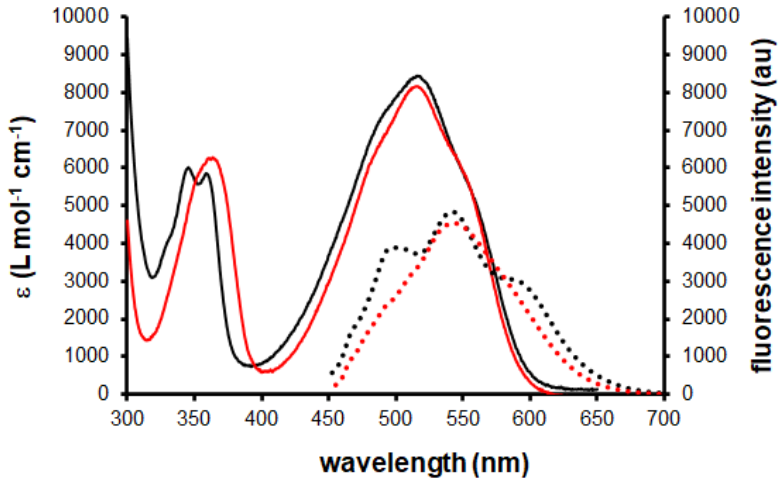


Figure 2. UV−Vis (solid lines) and fluorescence spectra (dashed lines) of **1a** upon excitation at 410 nm in MeCN (c = 1 × 10^{-4} M, black) and MeOH (c = 1 × 10^{-4} M, red) at 25 °C

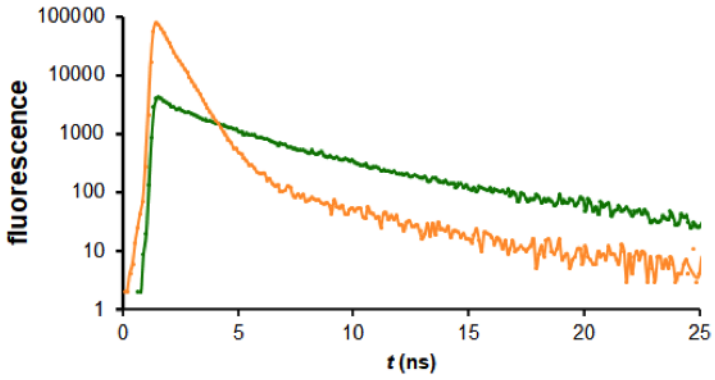


Figure 3. Fluorescence decay curves of **1a** at 610 nm (orange) and 500 nm (green) upon excitation at 410 nm in MeCN (1 × 10^{-4} M) at 25 °C

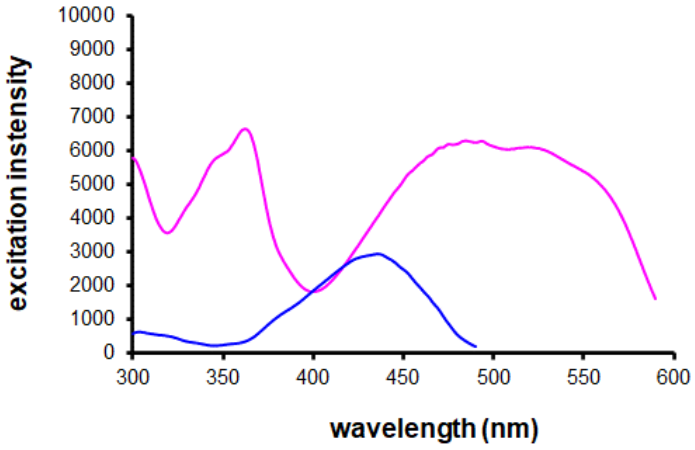


Figure 4. Excitation spectra of **1a** for the emission wavelengths of 610 nm (pink) and 500 nm (blue) in MeCN (1×10^{-4} M)

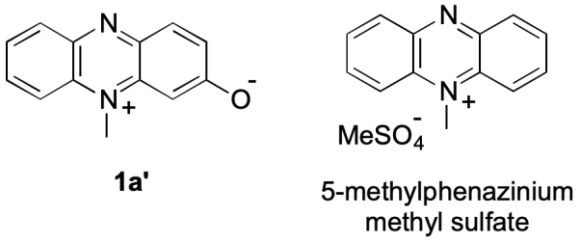


Figure 5. Structures for zwitterionic species **1a′** and 5-methylphenazinium methyl sulfate

The cytotoxic activity of **1a–f** against human A549 cells and normal MRC-5 cells was evaluated. The fifty percent inhibitory concentrations (IC_{50}) of cell viability and selectivity indexes (SI) are summarized in Table 2. The IC_{50} values were determined using the WST-8 assay.[19] The selectivity index is defined as the ratio of the IC_{50} value against normal cells to the corresponding IC_{50} value against cancer cells of similar tissue.[20,21] Generally, compounds with a SI value higher than 2 are considered to be selectively cytotoxic against cancer cells.[21] Compounds **1a**, **1b**, **1d**, and **1e** displayed cytotoxicity against cancer A549 cells at a concentration below 1.0 μM. Compounds **1c** and **1f** were comparably less active. Compound **1c** did not show cytotoxicity against A549 cells even at 10 μM. Compounds **1a**, **1d**, **1e**, and **1f** showed cytotoxicity against normal MRC-5 cells at a concentration below 1.0 μM. Among the compounds tested in this study, compound **1a** exhibited the most selective cytotoxicity against A549 cells with an SI value of 2.2.

Table 2. Cytotoxic activity of **1a**–**f** against cancer and normal cells (IC_{50}, μM)[a] along with their selectivity indexes

compound	IC_{50} (μM)		selectivity index[b]
	A549	MRC-5	
1a	0.40 ± 0.006	0.88 ± 0.033	2.2
1b	0.83 ± 0.025	1.53 ± 0.164	1.8
1c	>10	3.08 ± 0.404	–[d]
1d	0.26 ± 0.004	0.31 ± 0.034	1.2
1e	0.38 ± 0.020	0.66 ± 0.040	1.7
1f	1.50 ± 0.220	0.40 ± 0.044	0.27
SN-38[c]	0.071 ± 0.0004	0.046 ± 0.016	0.65

[a]The fifty percent inhibitory concentrations (IC_{50}) of cell viability were determined using the WST-8 assay. The IC_{50} values are expressed as the mean ± SD of triplicate experiments. [b]The selectivity index (SI) is defined as the IC_{50} value against normal MRC-5 cells divided by the IC_{50} value against A549 cells. [c]SN-38 was used as a positive control. [d]Not determined.

CONCLUSION

In our previous studies, we developed the synthesis of *N*-alkylphenazin-1-ones by the oxidative condensation of pyrogallols with *N*-substituted benzene-1,2-diamines under an oxygen atmosphere.[15,16] In this study, this method was applied to the synthesis of 10-alkylphenazin-2(10*H*)-ones. To our delight, 10-alkylphenazin-2(10*H*)-ones were prepared by simply treating *N*-alkylbenzene-1,2-diamines with 1,2,4-benzenetriol in AcOH under an oxygen atmosphere. Using the products obtained by this route, the photochemical properties of 10-methylphenazin-2(10*H*)-one were described. 10-Methylphenazin-2(10*H*)-one is a reddish-orange pigment and displayed orange-red fluorescence upon exposure to UV light. Interestingly, the analysis of the time-resolved fluorescence spectra uncovered the presence of two emission features for the compound. The biological evaluation of various synthetic analogues against human cancer A549 cells and normal MRC-5 cells revealed that 10-methylphenazin-2(10*H*)-one exerted selective cytotoxic activity towards A549 cells. We believe that these results will contribute to the design and synthesis of novel 10-alkylphenazin-2(10*H*)-ones with interesting physicochemical and biological properties. Further synthetic, physicochemical, and biological studies of 10-alkylphenazin-2(10*H*)-ones are currently ongoing in our laboratory and will be reported in due course.

EXPERIMENTAL

Solvents and reagents were used without further purification unless otherwise noted. Analytical TLC was performed using Silica gel 60 F_{254} plates (0.25 mm, normal phase, Merck) or Silica gel 60 RP-18 F_{254}s plates (0.25 mm, reversed phase, Merck). Preparative TLC (PTLC) was performed using Silica gel 60 F_{254} plates (0.5 mm, normal phase, Merck). Flash column chromatography was performed using silica gel (particle size 40–63 μm; 230–400 mesh ASTM; SiliaFlash F60, SiliCycle Inc.). Reversed phase flash chromatography was performed using octadecylsilyl (ODS) silica gel (particle size 15–30 μm, FUJIFILM Wako Pure Chemical Co.), unless otherwise noted. Melting point (Mp) data were determined using a Yanaco MP-J3 instrument and were uncorrected. IR spectra were recorded on a Horiba FT-720 spectrometer, using NaCl (neat) or KBr pellets (solid). ^{1}H and proton-decoupled ^{13}C (^{13}C{1H}) NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 and 100 MHz, respectively), using chloroform-*d* ($CDCl_3$) as solvent. Chemical shift values are expressed in δ (ppm) relative to tetramethylsilane (TMS, δ 0.00 ppm) or the residual solvent resonance ($CDCl_3$, δ 77.0 ppm for ^{13}C NMR). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (*J*; Hz), and integration. Mass spectra were obtained by a Sciex X500R quadrupole time-of-flight (QTOF) high resolution mass spectrometer using electrospray ionization (ESI). UV–Vis and fluorescence spectra were measured on a Hitachi U-3010 UV–Vis spectrometer and JASCO FP-8300 spectrometer, respectively. Fluorescence lifetime was counted on a time-correlated single-photon-counting spectrometer (HORIBA, Japan). Analytical HPLC was performed with a Shimadzu HPLC system (system control: SCL-10A, UV detector: SPD-M10A, pump: LC-M10A) equipped with a TOSOH TSK-gel reversed phase chromatography column (ODS-100Z, 4.6 × 150 mm) column, by eluting with a gradient solvent system of MeOH : H_2O (40:60 to 100:0 over 30 min). A549 cells were purchased from the Riken Cell Bank. MRC-5 cells were purchased from National Institute of Biomedical Innovation, Health and Nutrition. *N*-Methylbenzene-1,2-diamine (**6a**) and *N*-(3-methylbut-2-en-1-yl)benzene-1,2-diamine (**6e**) were prepared according to a reported procesure.[15]

***N*-Ethylbenzene-1,2-diamine (6b).** Bromoethane (120.0 μL, 1.87 mmol) was added to a suspension of *o*-phenylenediamine (**2**) (308.3 mg, 2.85 mmol) and K_2CO_3 (385.6 mg, 2.79 mmol) in DMF (10 mL). The mixture was stirred at rt for 1 day. The reaction was quenched by the addition of water. The mixture was diluted with EtOAc. After the layers were separated, the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to give a residue. The residue was purified by silica gel column chromatography (hexane/EtOAc = 2:1) to give **6b** (134.5 mg, 53%) as a brown oil. IR (neat) ν_{max} = 3388, 3330, 3043, 2970, 2929, 2870, 1711, 1622, 1599, 1510 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84–6.79 (m, 1H), 6.70–6.63 (m, 3H), 3.23 (brs, 3H), 3.12 (q, *J* = 7.1 Hz, 2H), 1.27 (td, *J* = 7.1, 0.8 Hz, 3H); ^{13}C{1H}

NMR (100 MHz, $CDCl_3$) δ 137.9, 133.9, 120.6, 118.3, 116.3, 111.5, 38.6, 14.9; HRMS (ESI/ QTOF) *m*/*z*: $[M+H]^+$ Calcd for $C_8H_{13}N_2$ 137.1073; Found 137.1072.

***N*-Decylbenzene-1,2-diamine (6c).** 1-Bromodecane (385.0 μL, 1.86 mmol) was added to a suspension of *o*-phenylenediamine (**2**) (300.0 mg, 2.77 mmol) and K_2CO_3 (384.0 mg, 2.78 mmol) in DMF (15 mL). The mixture was stirred at rt for 1 day. The reaction was quenched by the addition of water. The mixture was diluted with EtOAc. After the layers were separated, the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to give a residue. The residue was purified by silica gel column chromatography (hexane/EtOAc = 2:1) to give **6c** (261.7 mg, 57%) as a brown oil. IR (neat) ν_{max} = 3415, 3344, 3008, 2956, 2927, 2854, 1711, 1622, 1597, 1510 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.83–6.79 (m, 1H), 6.69–6.62 (m, 3H), 3.23 (brs, 3H), 3.06 (t, *J* = 7.2 Hz, 2H), 1.67–1.60 (m, 2H), 1.43–1.27 (brm, 14H), 0.88 (t, *J* = 6.7 Hz, 3H); $^{13}C\{1H\}$ NMR (100 MHz, $CDCl_3$) δ 138.1, 133.9, 120.6, 118.2, 116.3, 111.4, 44.2, 31.8, 29.63, 29.55, 29.52, 29.4, 29.3, 27.2, 22.6, 14.0; HRMS (ESI/ QTOF) *m*/*z*: $[M+H]^+$ Calcd for $C_{16}H_{29}N_2$ 249.2325; Found 249.2324.

***N*-Benzylbenzene-1,2-diamine (6d).** Benzyl bromide (220.0 μL, 1.85 mmol) was added to a suspension of *o*-phenylenediamine (**2**) (300.2 mg, 2.78 mmol) and K_2CO_3 (338.9 mg, 2.45 mmol) in DMF (10 mL). The mixture was stirred at rt for 2 h. The reaction was quenched by the addition of water. The mixture was diluted with EtOAc. After the layers were separated, the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to give a residue. The residue was purified by silica gel column chromatography (hexane/EtOAc = 4:1) to give **6d** (255.0 mg, 70%) as a brown oil. The structure of **6d** was confirmed by comparison of its ^1H NMR spectrum with that reported.[22]

(*E*)-*N*-(3,7-Dimethylocta-2,6-dien-1-yl)benzene-1,2-diamine (6f). Geranyl bromide (370.0 μL, 1.86 mmol) was added to a suspension of *o*-phenylenediamine (**2**) (300.3 mg, 2.78 mmol) and K_2CO_3 (384.6 mg, 2.78 mmol) in DMF (10 mL). The mixture was stirred at rt for 2 h. The reaction was quenched by the addition of water. The mixture was diluted with EtOAc. After the layers were separated, the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to give a residue. The residue was purified by silica gel column chromatography (hexane/EtOAc = 4:1) to give **6f** (261.0 mg, 57%) as a brown oil. IR (neat) ν_{max} = 3406, 3334, 3005, 2968, 2927, 2856, 1720, 1666, 1620, 1599 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.83 (td, *J* = 8.0, 1.6 Hz, 1H), 6.73–6.66 (m, 3H), 5.38 (t, *J* = 6.4 Hz, 1H), 5.10 (t, *J* = 6.8 Hz, 1H), 3.71 (d, *J* = 6.4 Hz, 2H), 3.31 (brs, 3H), 2.13–2.03 (m, 4H), 1.71 (s, 3H), 1.69 (s, 3H), 1.61 (s, 3H); $^{13}C\{1H\}$ NMR (100 MHz, $CDCl_3$) δ 138.9, 137.9, 134.2, 131.7, 123.9, 121.6, 120.6, 118.5, 116.3, 111.8, 42.2, 39.5, 26.4, 25.7, 17.7, 16.4; HRMS (ESI/ QTOF) *m*/*z*: $[M+H]^+$ Calcd for $C_{16}H_{25}N_2$ 245.2012 ; Found 245.2021.

2-Hydroxycyclohexa-2,5-diene-1,4-dione (3). Compound **10** (100.0 mg, 0.79 mmol) was added to a suspension of 48% Ag_2CO_3 on Celite (438.0 mg, 0.83 mmol) in toluene (60 mL). The mixture was refluxed under argon atmosphere for 15 min. The mixture was filtered through Celite and washed with toluene. The resultant mixture was concentrated to give **3** (47.8 mg, 49%) as a yellow solid.[17] Mp 115–116 °C; IR (KBr) ν_{max} = 3222, 3072, 1682, 1655, 1637, 1593 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.98 (brs, 1H), 6.83 (d, *J* = 10.0 Hz, 1H), 6.75 (dd, *J* = 10.0, 2.0 Hz, 1H), 6.15 (d, *J* = 2.0 Hz, 1H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 187.7, 183.2, 154.4, 139.3, 132.1, 108.4; HRMS (ESI/ QTOF) *m*/*z*: $[M+H]^+$ Calcd for $C_6H_5O_3$ 125.0233 ; Found 125.0236.

10-Methylphenazin-2(10*H*)-one (1a). A solution of **6a** (30.0 mg, 0.25 mmol) and **3** (30.5 mg, 0.25 mmol) in AcOH (2.5 mL) was stirred under an O_2 atmosphere at 60 °C in the dark for 1 h. The reaction was quenched by the addition of water. The mixture was diluted with $CHCl_3$. After the layers were separated, the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to give a residue. The residue was purified by reversed phase flash chromatography ($MeOH/H_2O$ = 4/1) to give **1a** (18.1 mg, 35%) as a dark red solid. Mp 186–189 °C (lit.[9] 202–204 °C); IR (KBr) ν_{max} = 2954, 2920, 2850, 2362, 1720, 1630, 1601, 1537 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.72–7.68 (m, 1H), 7.62 (d, *J* = 9.6 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 1H), 7.47–7.43 (m, 1H), 7.09 (dd, *J* = 9.6, 2.0 Hz, 1H), 6.21 (d, *J* = 1.6 Hz, 1H), 3.79 (s, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 184.3, 147.9, 138.4, 136.1, 135.6, 134.5, 132.7, 131.8, 131.6, 124.2, 113.5, 99.7, 33.5; HRMS (ESI/ QTOF) *m*/*z*: $[M+H]^+$ Calcd for $C_{13}H_{11}N_2O$ 211.0866 ; Found 211.0862. The purity of compounds **1a** was determined to be >99% by analytical HPLC (Figure S22 in Supporting Information).

Preparation of 1a (Scheme 3A). A solution of **6a** (30.0 mg, 0.25 mmol) and **11** (53.6 mg, 0.50 mmol) in Et_2O (2.0 mL) was stirred under an argon (Ar) atmosphere at rt in the dark for 1 day. The reaction was quenched by the addition of water. The mixture was diluted with $CHCl_3$. After the layers were separated, the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to give a residue. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1), reversed phase flash chromatography ($MeOH/H_2O$ = 4/1) and PTLC ($CHCl_3$/MeOH = 9/1) to give **1a** (9.4 mg, 18%).

General Procedure for the Oxidative Condensation between *N*-Alkylbenzene-1,2-diamine (6a–f) with 1,2,3-Benzenetriol (10) (Scheme 3B and Table 1). A solution of **6** (0.25 mmol) and **10** (0.25 mmol) in AcOH (2.0 mL) was stirred under an O_2 atmosphere at 60 °C in the dark until no further TLC changes were observed. The reaction was quenched by the addition of water. The mixture was diluted with $CHCl_3$. After the layers were separated, the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to give a residue. The residue was purified by silica gel column chromatography and reversed phase flash chromatography.

10-Methylphenazin-2(10*H*)-one (1a). Following the general procedure, the reaction was performed using **6a** (30.0 mg, 0.25 mmol) and **10** (31.0 mg, 0.25 mmol) in AcOH (2.0 mL) under an O_2 atmosphere for 6.5 h. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1) and reversed phase flash chromatography (MeOH/H_2O = 4/1) to give **1a** (17.1 mg, 33%).

10-Ethylphenazin-2(10*H*)-one (1b). Following the general procedure, the reaction was performed using **6b** (33.4 mg, 0.25 mmol) and **10** (31.0 mg, 0.25 mmol) in AcOH (2.0 mL) under an O_2 atmosphere for 3.5 h. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1) and reversed phase flash chromatography (MeOH/H_2O = 4/1) to give **1b** (15.2 mg, 28%) as a dark red solid. Mp 169–171 °C; IR (KBr) ν_{max} = 2958, 2927, 2873, 2858, 1730, 1628, 1601, 1581 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.73–7.69 (brm, 1H), 7.63 (d, *J* = 9.6 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.09 (dd, *J* = 9.6, 2.0 Hz, 1H), 6.24 (d, *J* = 2.0 Hz, 1H), 4.33 (q, *J* = 7.2 Hz, 2H), 1.52 (t, *J* = 7.2 Hz, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 184.4, 147.9, 137.1, 136.0, 135.7, 134.6, 132.8, 131.7, 130.9, 124.2, 113.2, 99.1, 41.4, 10.9; HRMS (ESI/ QTOF) *m*/*z*: $[M+H]^+$ Calcd for $C_{14}H_{13}N_2O$ 225.1022; Found 225.1017.

10-Decylphenazin-2(10*H*)-one (1c). Following the general procedure, the reaction was performed using **6c** (61.0 mg, 0.25 mmol) and **10** (31.0 mg, 0.25 mmol) in AcOH (2.0 mL) under an O_2 atmosphere for 4.5 h. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1) and reversed phase flash chromatography (MeOH/H_2O = 4/1) to give **1c** (17.3 mg, 21%) as a dark red solid. Mp 115–116 °C; IR (KBr) ν_{max} = 2954, 2922, 2850, 1734, 1626, 1601, 1531 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.72–7.68 (m, 1H), 7.63 (d, *J* = 9.6 Hz, 1H), 7.50–7.43 (m, 2H), 7.09 (dd, *J* = 9.6, 2.0 Hz, 1H), 6.20 (d, *J* = 2.0 Hz, 1H), 4.20 (t, *J* = 8.4 Hz, 2H), 1.92–1.84 (brm, 2H), 1.57–1.50 (brm, 2H), 1.44–1.30 (brm, 12H), 0.89 (t, *J* = 7.2 Hz, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 184.4, 147.9, 137.3, 136.0, 135.7, 134.6, 132.7, 131.7, 131.1, 124.1, 113.4, 99.3, 46.6, 31.8, 29.52, 29.45, 29.3, 29.2, 27.0, 25.7, 22.7, 14.1; HRMS (ESI/ QTOF) *m*/*z*: $[M+H]^+$ Calcd for $C_{22}H_{29}N_2O$ 337.2274; Found 337.2273.

10-Benzylphenazin-2(10*H*)-one (1d). Following the general procedure, the reaction was performed using **6d** (48.7 mg, 0.25 mmol) and **10** (31.0 mg, 0.25 mmol) in AcOH (2.0 mL) under an O_2 atmosphere for 4 h. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1) and reversed phase flash chromatography (MeOH/H_2O = 4/1) to give **1d** (22.3 mg, 32%) as a dark red solid. Mp 205–207 °C; IR (KBr) ν_{max} = 2954, 2920, 2850, 1720, 1630, 1601, 1543, 1529 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.65–7.59 (m, 2H), 7.47–7.40 (m, 2H), 7.37–7.31 (m, 3H), 7.16–7.14 (m, 2H), 7.04 (dd, *J* = 10.0, 2.0 Hz, 1H), 6.10 (d, *J* = 2.0 Hz, 1H), 5.45 (s, 2H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 184.5, 148.4, 138.1, 135.9, 135.6, 134.6, 132.9, 132.7, 131.9, 131.5, 129.3 (2C),

128.2, 125.9 (2C), 124.4, 113.9, 100.4, 50.7; HRMS (ESI/ QTOF) *m*/*z*: $[M+H]^+$ Calcd for $C_{19}H_{15}N_2O$ 287.1179; Found 287.1179.

10-(3-Methylbut-2-en-1-yl)phenazin-2(10*H*)-one (1e). Following the general procedure, the reaction was performed using **6e** (43.3 mg, 0.25 mmol) and **10** (31.0 mg, 0.25 mmol) in AcOH (2.0 mL) under an O_2 atmosphere for 2.2 h. The residue was purified by reversed phase flash chromatography ($MeOH/H_2O$ = 4/1) and silica gel column chromatography ($CHCl_3$/MeOH = 9/1) to give **1e** (19.0 mg, 29%) as a dark red solid. Mp 189–191 °C; IR (KBr) ν_{max} = 2958, 2918, 2850, 1730, 1628, 1597, 1531, 1462 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.69–7.65 (m, 1H), 7.62 (d, *J* = 10.0 Hz, 1H), 7.46–7.43 (m, 2H), 7.08 (dd, *J* = 9.6, 2.0 Hz, 1H), 6.15 (d, *J* = 2.0 Hz, 1H), 5.15–5.12 (brm, 1H), 4.84 (d, *J* = 5.6 Hz, 2H), 1.94 (s, 3H), 1.79 (s, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 184.3, 148.0, 138.7, 137.7, 136.0, 135.7, 134.5, 132.6, 131.5, 131.4, 124.1, 116.1, 113.7, 99.7, 45.5, 25.5, 18.6; HRMS (ESI/ QTOF) *m*/*z*: $[M+H]^+$ Calcd for $C_{17}H_{17}N_2O$ 265.1335; Found 265.1337.

(*E*)-10-(3,7-Dimethylocta-2,6-dien-1-yl)phenazin-2(10*H*)-one (1f). Following the general procedure, the reaction was performed using **6f** (60.0 mg, 0.25 mmol) and **10** (31.0 mg, 0.25 mmol) in AcOH (2.0 mL) under an O_2 atmosphere for 5 h. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1) and reversed phase flash chromatography ($MeOH/H_2O$ = 4/1) to give **1f** (16.8 mg, 21%) as a dark red solid. Mp 110–112 °C; IR (KBr) ν_{max} = 2916, 2846, 1718, 1630, 1601, 1587, 1543, 1529 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.69–7.62 (m, 2H), 7.46–7.41 (m, 2H), 7.09 (dd, *J* = 9.6, 2.0 Hz, 1H), 6.16 (d, *J* = 1.6 Hz, 1H), 5.15–5.09 (brm, 1H), 5.04–4.99 (brm, 1H), 4.86–4.85 (m, 2H), 2.08 (brs, 4H), 1.93 (s, 3H), 1.63 (s, 3H), 1.57 (s, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 184.3, 148.1, 142.1, 137.7, 136.1, 135.7, 134.5, 132.6, 132.2, 131.5, 131.4, 124.1, 123.3, 115.9, 113.8, 99.7, 45.6, 39.2, 26.2, 25.7, 17.7, 17.1; HRMS (ESI/ QTOF) *m*/*z*: $[M+H]^+$ Calcd for $C_{22}H_{25}N_2O$ 333.1961; Found 333.1967.

Cytotoxicity Evaluation. Cell growth was evaluated using Cell Counting Kit-8 according to the manufacturer's instructions based on the WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt] assay.[19] For the assay, the adherent A549 and MRC-5 cells were cultured in a 96-well plate with each well containing 2000 cells in a total volume of 100 μL. The concentration of DMSO in the cell cultures was 0.1–0.2% (v/v). The plates also included blank wells (0 cells) and control wells (2000 cells/100 μL for the adherent cells). A549 and MRC-5 cells were preincubated for 24 h before exposure to the test compounds. The plates were incubated with various concentrations of each compound for 72 h. At the end of incubation, 10 μL of the solution provided with the cell counting kit was then added, and the resulting mixture incubated for 2 h at 37 °C. The absorbance values were then measured at 450 nm with a

96-well plate reader. Cell growth inhibition was evaluated as the ratio of the absorbance of the sample to that of the control.

Measurement of UV−vis, Fluorescence Spectra, and Quantum Yields. UV−Vis spectrum of **1a** was measured in MeCN ($c = 1 \times 10^{-4}$ M) and MeOH ($c = 1 \times 10^{-4}$ M) at 25 ℃ using a quartz cell with a 10 mm path length. Fluorescence spectrum was measured in MeCN ($c = 1 \times 10^{-4}$ M) and MeOH ($c = 1 \times 10^{-4}$ M) at 25 ℃ at an excitation wavelength of 410 nm and 490 nm. Fluorescence lifetime of **1a** was measured in MeCN ($c = 1 \times 10^{-4}$ M). Fluorescence lifetime was counted on a time-correlated single-photon-counting spectrometer with an excitation light source of a pulsed laser diode (NanoLED-405L, HORIBA; wavelength: 410 nm, pulse width: <200 ps, pulse energy: 11 pJ) and a longpass filter (cut-on wavelength 370 nm). The emission spectra at different times after excitation were obtained by averaging the integrated fluorescence intensity for different time windows at every wavelength after the excitation pulse. The photoluminescence (PL) quantum yields were determined using absolute PL quantum yield spectrometer (C9920-02, Hamamatsu).

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