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Photoinduced Nitric Oxide Release from Nitrobenzene Derivatives

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Abstract: A new type of photoinduced nitric oxide (NO) donors was designed from nitrobenzene derivatives. Visible-light irradiation of 2,6-dimethylnitrobenzenes bearing extended π-electron systems at the 4-position revealed efficient NO release using ESR analysis and the Griess assay. Computational study and ultraviolet spectrum analysis suggested that the NO-releasing activity was closely related to the conformation of the nitro group, the absorption intensity, and the length of the conjugated π-electron system. Employing the photodependent cytotoxicity of compound **14** against HCT116 human colon cancer cells, it was demonstrated that 4-substituted-2,6-dimethylnitrobenzene analogues are useful NO donors for the time- and site-controlled NO treatment.

Introduction

Nitric oxide (NO), a simple diatomic free radical, has proven in recent years to be involved in the maintenance and regulation of vital functions[1] and is one of the most fascinating and studied compounds in biological chemistry, although for decades it was merely viewed as an environmental pollutant. The development and use of NO donors have played important roles in research on NO physiology,[2] and the significance of these donors has recently been reaffirmed not only from the perspective of reagents for biological studies but also with a view to their application as pharmaceuticals.[3] To date, a number of NO donors have been developed[2,4] and utilized. However, many of the currently used NO donors such as 1-hydroxy-2-oxo-3-(aminoalkyl)-1-triazenes (NOCs)[5] and 4-alkyl-2-hydroxyimino-5-nitro-3-hexenes (NORs)[6] are reagents that release NO by spontaneous autolysis. For further research on NO physiology and potential therapeutic application, it appeared desirable to liberate NO in living systems in a time- and site-controlled manner. This concept led to the identification of several photochemically triggered NO donors such as metal nitrosyl compounds[7] and some caged nitric oxides.[8] The duration and site of NO release from these compounds can be controlled by changing the interval and position of light exposure. However, some of these photoinducible NO donors have problems associated with stability and toxicity. For example, the NO-releasing rate of metal nitrosyl compounds such as dipotassium pentachloronitrosylruthenate and sodium nitroprusside (SNP) varies with changes in pH, and SNP has toxic consequences attributable to the cyanide ligand.[9]

We previously found 6-nitrobenzo[*a*]pyrene (6-nitroBaP) (Figure 1) to be a photoinducible NO-releasing agent[10] whose NO-releasing mechanism is completely different from those of well-known photochemically triggered NO donors. 6-NitroBaP releases NO with the concomitant formation of 6-oxylBaP radical under visible-light irradiation (Figure 1). Based on the fact that 1-nitroBaP and 3-nitroBaP with sterically less hindered nitro

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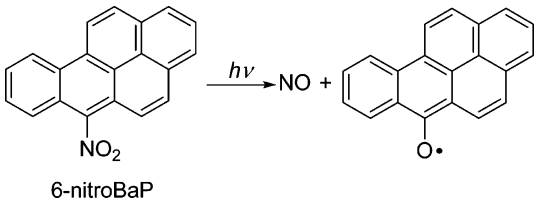

Figure 1. 6-NitroBaP as a photoinducible NO-releasing agent.

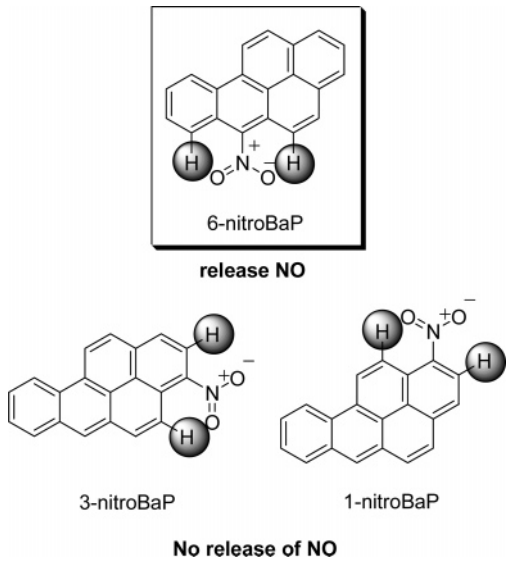

Figure 2. Structures of 1-, 3-, and 6-nitroBaP.

groups did not release NO (Figure 2) and the results of computational studies by our group[10] as well as another,[11] it was suggested that the nonplanar torsional conformation of the nitro substituent in relation to the aromatic ring is important for the light-induced release of NO. In view of this finding, regulation of the nitro group conformation could give simpler nitrobenzene derivatives the ability to release NO. Contributing to this line of thought, we report that some nitrobenzene derivatives with extended π-electron conjugations have potent NO-releasing activity in response to visible-light irradiation. A simulation study and ultraviolet spectrum analysis of these compounds suggested that the NO-releasing activity was closely related to the conformation of the nitro group, the absorption wavelength, and the length of the conjugated π-electron system. Employing the photodependent cytotoxicity of compound **14** against HCT116 human colon cancer cells, it was demonstrated that nitrobenzene derivatives are useful as time- and site-controlled NO donors.

Results

Molecular Design. As NO donor candidates, we focused on 2,6-dimethylnitrobenzene derivatives, which are expected to have nonplanar conformations of the nitro group in their simple structures (Figure 3). Electron-donating groups (**2** and **3**) and olefins (**4**−**11**, and **14**) were chosen as substituents at the 4-position so that the derivatives would have maximum absorption at a longer wavelength, because it is desirable that compounds release NO under visible-light irradiation without affecting the living organisms. In addition, the extension of conjugated π-electron systems by the introduction of olefins at the 4-position may increase the NO-releasing ability by stabilizing the oxylradical which is speculated to be formed concomitantly with the generation of NO.[10] Compounds with one methyl group (**12**) and without a methyl group (**13**) were also prepared as reference compounds.

ESR Analysis. Detection of NO released from nitrobenzene derivatives was carried out by an ESR spin-trapping method with *N*-methyl-D-glucamine dithiocarbamate (MGD)−Fe^{2+} complex, which reacts with NO to give a [$(MGD)_2$−Fe^{2+}−NO] stable paramagnetic complex.[12] On ESR spectroscopy, the complex is observed as a broadened three-line spectrum consisting of $a^N = 1.25$ mT and $g^{iso} = 2.04$. The prepared 2, 6-dimethylnitrobenzene derivatives were photoirradiated in aqueous DMSO and subsequently subjected to ESR spectroscopy.

No ESR signal was detected with compounds **1**, **2**, and **3** bearing an OH and the NMe_2 group at the 4-position of 2,6-dimethylnitrobenzene, respectively (data not shown), whereas a 5-min photoirradiation[13] of compound **4** bearing a styrenyl group at the 4-position exhibited a characteristic three-line spectrum on ESR (Figure 4A), which indicates that NO was released from compound **4** by photoirradiation. NO-releasing activity was distinctly dependent on the two methyl groups introduced at the ortho position to the nitro group on the phenyl ring. The lack of the methyl groups (**12** and **13**) led to a much less potent NO donor and a compound devoid of the ability to release NO, respectively (Figure 4). We next evaluated the ESR signal intensity of compound **4** and its derivatives **5**−**11** after 1-, 3-, and 5-min photoirradiation (Figure 5). All of the tested compounds were shown to release NO during these photoirradiation periods. Time-dependent increases in the ESR signal intensity indicated the photolytic generation of NO from these compounds. With the extension of the conjugated π-electron system (see **5**−**9**) the NO-releasing activity increased except for that of compound **7**, which was less potent than **4**. Compounds with longer conjugated π-electron systems showed greater activity when comparing **4** or **8** with **9**. Next, we studied the effect of substituents at the other phenyl ring of compound **4**. It was shown that 3′,5′-dimethoxy compound **11** was more potent, while 2′,6′-dimethyl analogue **10** significantly reduced the activity.

Griess Assay. For further confirmation of NO production via the photolysis of nitrobenzene derivatives, the Griess method[14] was used to evaluate production by detecting NO_2^- resulting from NO autoxidation in aqueous solution. The visible absorption at 546 nm of the red color formed upon diazo coupling of the Griess reagents allowed for evaluation of the NO formed after photolysis of the nitrobenzene derivatives. After photoirradiation for 1 and 2 h, the absorbance at 546 nm was measured.

The Griess assay was used to investigate the NO-releasing efficiency of **4**, **5**, **8**, and **9**, positive compounds in the ESR assay, and **12** and **13**, negative compounds in the ESR assay (Figure 6). Compounds **4**, **5**, **8**, and **9** bearing two methyl groups at the ortho positions of the nitro group released NO in a time-

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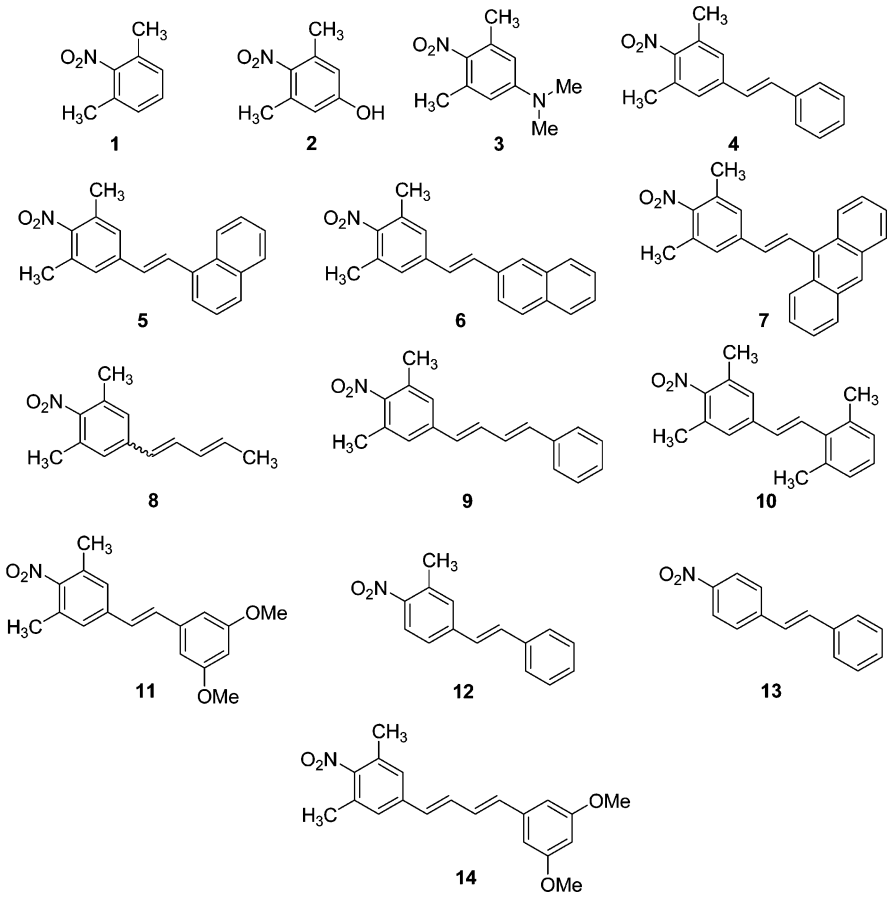

Figure 3. Structures of nitrobenzene derivatives.

Figure 4. ESR spectra of [$(MGD)_2$−Fe−NO] complex after photoirradiation in the presence of compounds **4**, **12**, and **13**. Samples contained 1 mM **4** (A), **12** (B), or **13** (C), 75 mM MGD, and 20 mM $FeSO_4$ in distilled water (containing 25% DMSO); ESR spectra were recorded after photoirradiation for 5 min with a modulation amplitude of 2.0 G and a microwave power of 16 mW.

dependent manner under visible light, while an extreme reduction in the NO-releasing activity was observed with compounds having a single methyl group (**12**) and lacking a methyl group (**13**). On comparing the four positive compounds (**4**, **5**, **8**, and **9**), the longer their conjugated π-electron systems, the better NO donors they were. The results from the Griess assay were consistent with those obtained by ESR analysis.

To determine the chemical yield of the NO-releasing reaction, compound **9**, the most active compound in both the ESR and Griess assays was subjected to a longer period of photoirradiation and the NO-release rate was measured by the Griess method (Figure 7). The generation of NO from compound **9** increased linearly until 6 h of photoirradiation and then reached a plateau. The yield of NO formation for the photochemical reaction determined by the Griess method was 55%.

Cytotoxicity Against Cancer Cells. It has been reported that NO is a mediator of the cytotoxic action of macrophages toward tumor cells through inhibition of mitochondrial enzyme activity and DNA synthesis.[15] Therefore, we determined whether NO

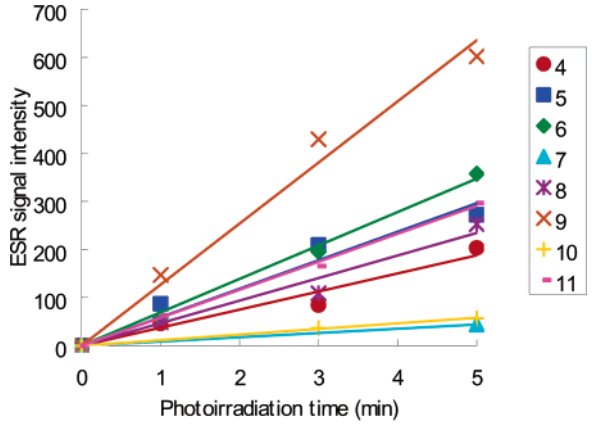

Figure 5. ESR signal intensities of [$(MGD)_2$−Fe−NO] complex after photoirradiation in the presence of compounds **4**−**11**. Samples contained 1 mM **4**−**11**, 75 mM MGD, and 20 mM $FeSO_4$ in water (containing 25% DMSO); ESR spectra were recorded after photoirradiation for 1, 3, or 5 min with a modulation amplitude of 2.0 G and a microwave power of 16 mW.

induced from nitrobenzene derivatives by photoirradiation can function as a cytotoxic agent. For the application of our NO donors in a biological study, we prepared compound **14**. In the ESR and the Griess assays, the NO-releasing activity of compound **14** was comparable to that of compound **9** (see Supporting Information), the most potent compound among all those prepared for this study. Compound **14** has the advantage of solubility in aqueous media compared with compound **9**. It

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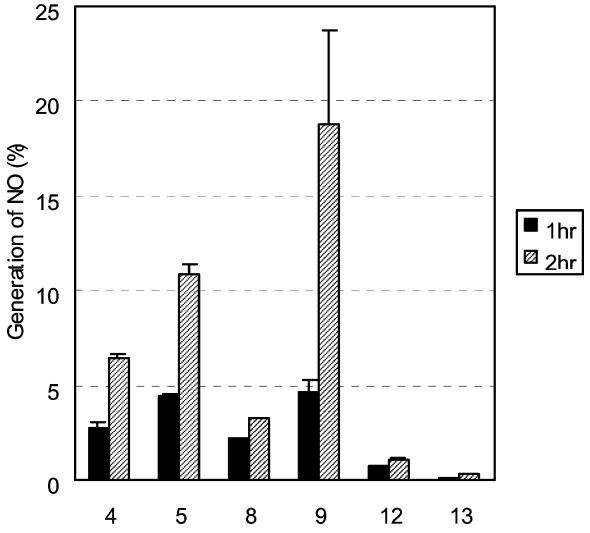


Figure 6. Griess assay results for compounds **4**, **5**, **8**, **9**, **12**, and **13** (n = 3, mean ±SD).

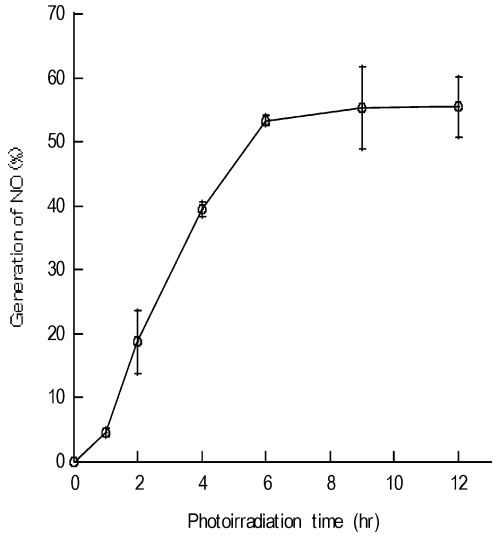


Figure 7. NO-release rate of compound **9** (n = 3, mean ±SD).

was more easily available for the cellular experiments as a DMSO solution. In the presence of a 2.5 μM concentration of compound **14**, light of 330−380 nm wavelength was shed for 1 min on a fixed area of a culture plate containing human colon cancer HCT116 cells.[16] The cells were observed after a 24-h incubation at 37 °C under dark conditions. As shown in Figure 8, the cells had detached and appeared dead within the area of irradiation (Figure 8a), which indicates that the cytotoxicity was quite dependent on the photoirradiation. Treatment with compound **14** without photoirradiation had no effect on the cells in 24 h. The photoirradiation alone without compound **14** also had no effect. This cytotoxic effect was diminished in the presence of NADH and P450 nor, an NO reductase (Figure 8c), which confirmed that NO was mainly responsible for the cytotoxicity against the HCT 116 cells. There were no changes at a position 3.5 mm from the center of irradiation, i.e., outside the photoirradiation area, regardless of the absence (Figure 8b) or the presence of P450 nor (Figure 8d). Preirradiation to the solution of compound **14** for 30 min abolished its cytotoxic effects,

(16) NO release from compound **14** under assay conditions was confirmed by the Griess Method (see Supporting Information).

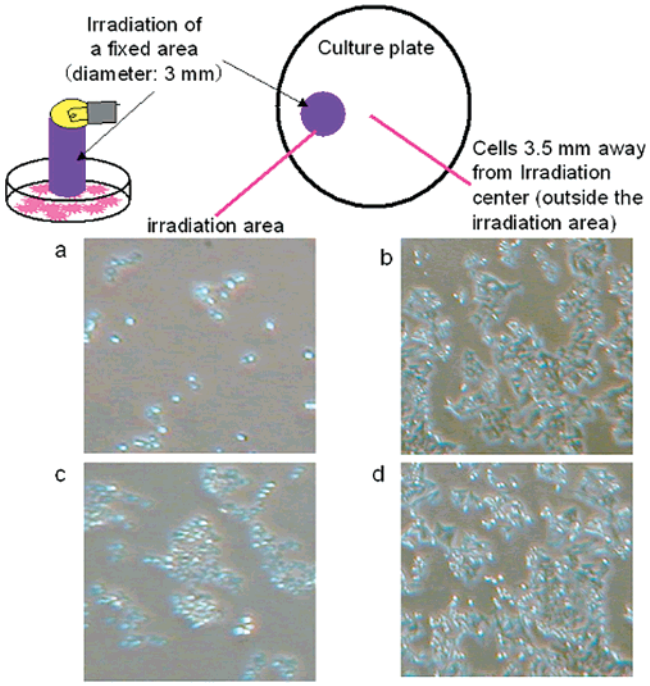


Figure 8. Cytotoxic activity of NO released from compound **14** under photoirradiation. Representative phase-contrast images of cells within the irradiation area in the absence (a) and the presence (c) of P450 nor are shown. Cells at 3.5 mm away from the irradiation center in the absence (b) and the presence (d) of P450 nor are also presented. The experiments were duplicated, and at least 4 areas were observed in each culture plate. In all experiments, the same results were obtained.

indicating that the stable products after liberation of NO or photoinduced decomposition are nontoxic.

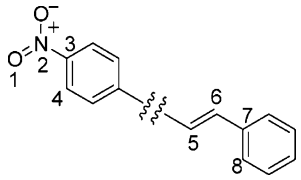
Discussion

MM2 calculations and the ultraviolet−visible-light absorption spectra measurements were carried out to determine which characteristic is necessary for NO generation under visible-light irradiation (Table 1).

It is assumed that a nitroarene gives rise to NO via nitritearene, which is produced from the nitroarene by an intramolecular rearrangement mechanism (Figure 9), and the nonplanar torsional conformation of the nitro group in relation to the aromatic ring gives an advantage in the nitro-to-nitrite rearrangement.[10,11] Therefore, the dihedral angle between the plane of the nitro group and the phenyl ring ($\Delta O^1N^2C^3C^4$) for compounds **1**−**13** was initially calculated to clarify the effect of the two methyl groups on the conformation of the nitro group. The dihedral angles for dimethyl compounds **1**−**11** were around 45°, while those of monomethyl **12** and no-methyl **13** were 0°. Considering this calculation result and the fact that compounds **12** and **13** did not generate NO efficiently, it was suggested that two methyl groups at the ortho positions of the nitro group are indispensable for inclining the nitro group to the plane of the phenyl ring and for the subsequent intramolecular rearrangement from nitro to nitrite.

Another important factor is the intensity of light energy absorption, which is considered to be essential for the excitation of compounds or further torsional conformation change of the nitro group.[11] Indeed, compounds with a longer conjugated π-electron system, which were expected to have maximum absorption at a longer wavelength, were inclined to enhance

Table 1. Results of MM2 Calculations and Ultraviolet Absorption Measurements



compds	$O^1N^2C^3C^4$ [a] (deg)	$C^5C^6C^7C^8$ [b] (deg)	λ max (nm)	ϵ max ($\times 10^4\ M^{-1}\ cm^{-1}$)	ESR signal[c] ($\times 10^2$ au)
1	44.8		255	0.14	nd
2	43.6		354	0.14	nd
3	44.4		396	0.65	nd
4	44.6	30.3	307	2.4	2.0
5	44.6	50.3	329	2.0	2.8
6	44.7	30.3	320	2.7	3.6
7	44.6	79.8	389	1.1	0.4
8	44.6		289	1.4	2.5
9	44.6	28.5	332	4.3	6.0
10	44.7	66.7	287	1.6	0.6
11	44.7	32.1	308	2.6	3.0
12	0.l	30.0	343	2.2	0.4
13	0.0	30.0	349	2.6	nd

[a] Dihedral angle (deg) between the planes of the nitro group and the phenyl moiety. [b] Dihedral angle (deg) between the ethylene moiety and the aromatic ring. [c] ESR signal intensity of [$(MGD)_2$−Fe−NO] complex after 5-min irradiation. nd = not detected.

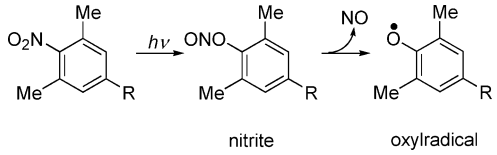


Figure 9. Predicted mechanism for NO generation from 4-substituted-2,6-dimethylnitrobenzene derivatives.

the NO-releasing activity. The results of the ultraviolet−visible-light spectroscopic investigation of nitrobenzene derivatives were in good agreement with those obtained by the ESR assay. Specifically, all of the compounds with an adequate ability to provide NO (**4**, **5**, **6**, **8**, **9**, and **11**) had strong absorption in the range of irradiating light (wavelength of >300 nm), while compounds having weak absorption (**1** and **3**) under these conditions did not generate NO although they had two methyl groups at the ortho positions of the nitro group. These results support the idea that NO is released from nitrobenzene derivatives by light energy absorption and not by any other factor.

However, compounds **7** and **10**, which have two methyl groups, had adequate absorption at wavelengths of >300 nm and seemed to have long conjugated π-electron systems, hardly having any ability to release NO. The calculated dihedral angle between the ethylene moiety and the aromatic ring ($\Delta C^5C^6C^7C^8$) of compounds **7** and **10** was 79.8° and 66.7°, respectively. These values were greater than those of other compounds. This suggested that compounds **7** and **10** have difficulty in creating a planar structure, and therefore there is little conjugation between the two aromatic rings. It can be concluded that the lack of a conjugated π-electron system made it difficult for compounds **7** and **10** to absorb the light energy necessary for generating NO.[17]

(17) In the case of compound **7**, the absorption at 386 nm, which is due to the anthracene ring, has little to do with NO generation since the nitrobenzene moiety and the anthracene ring are not conjugated.

Longer conjugated π-electron systems can also contribute to the resonance stabilization of the oxylradicals generated by N−O bond fission of the nitrite. The destabilization of the oxylradical caused by the lack of a π-conjugated system may be the reason that compounds **3**, **7**, and **10** did not have a significant ability to release NO.

Unfortunately, the oxylradical, which is considered to be formed concomitantly with the generation of NO, could not be detected because of its instability. However, the result of the cellular assay using compound **14** indicated that the degradation products derived from the oxylradical did not appear to interfere with the biological NO experiment. Therefore, nitrobenzene derivatives may be useful NO donors, with the advantage that the time of light exposure, the duration of NO release, and the site of NO action can be controlled.

All the synthesized compounds were very stable in crystal forms under a dark condition. It was also confirmed that the photoinduced cytotoxic effect of compound **14** was maintained at least for 4 weeks when stored at −20 °C as a concentrated solution in DMSO. This stability of the compounds in storage appears to give usefulness in the biological applications as NO-releasing reagents.

Conclusion

In the present study, we characterized several 4-substituted-2,6-dimethylnitrobenzenes as a new type of NO donor. The computational study and ultraviolet−visible-light spectrum analysis also highlighted the significance of the conformation of the nitro group, the intensity of light energy absorption, and the length of the conjugated π-electron system. More importantly, one of our compounds demonstrated its activity as an NO donor in a biological study.

Experimental Section

General Methods. Melting points were determined using a Yanagimoto micromelting point apparatus or a Büchi 545 melting point apparatus and were left uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a JEOL JNM-LA400 spectrometer with $CDCl_3$ as the solvent. Chemical shifts (δ) were reported in parts per million relative to the internal standard tetramethylsilane. Elemental analysis was performed with a Yanaco CHN CORDER NT-5 analyzer, and all values were within ±0.4% of the calculated values. High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-SX102A mass spectrometer. GC−MS analyses were performed on a Shimadzu GCMS-QP2010. Ultraviolet−visible-light spectra were recorded on a HITACHI U-3000 spectrophotometer or an Agilent 8453 spectrophotometer. Compound **1** was purchased from Wako Pure Chemical Industries, and MGD was obtained from DOJINDO. All other reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku and used without purification. Flash column chromatography was performed using Silica Gel 60 (particle size 0.046−0.063 mm) supplied by Merck. In ESR analysis and the Griess assay, irradiation was performed through a Pyrex filter with a 300-W photoreflector lamp.

3,5-Dimethyl-4-nitrophenol (2). Compound **2** was prepared by a previously reported method:[18] mp 109−111 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 6.56 (2H, d, J = 0.49 Hz), 5.19 (1H, s), 2.30 (6H, s). MS (EI) m/z: 167 (M^+). Anal. Calcd for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.33; H, 5.55; N, 8.61.

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(3,5-Dimethyl-4-nitrophenyl)dimethylamine (3). Compound **3** was prepared by a previously reported method:[19] mp 115−116 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 6.31 (3H, s), 3.01 (6H, s), 2.37 (6H, s). MS (EI) *m*/*z*: 194 (M^+). Anal. Calcd for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N,14.42. Found: C, 61.71; H, 7.30; N, 14.58.

1,3-Dimethyl-2-nitro-5-[(1*E*,3*E*)-4-(3,5-dimethoxyphenyl)-1,3-butadienyl]benzene (14). Step 1: Preparation of 3,5-Dimethyl-4-nitrobenzylbromide. To phosphorus tribromide (1.16 g, 4.29 mmol) was added 3,5-dimethyl-4-nitrobenzyl alcohol[20] (489 mg, 2.70 mmol) under argon with cooling by an ice bath, and the solution was stirred for 5 h at room temperature. The reaction mixture was poured into water and extracted with $CHCl_3$. The $CHCl_3$ layer was separated, washed with brine, and dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 9/1) gave 610 mg (93%) of 3,5-dimethyl-4-nitrobenzylbromide as a pale yellow solid: mp 49−50 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.15 (2H, s), 4.40 (2H, s), 2.31 (6H, s). MS (EI) *m*/*z*: 243 (M^+, ^{79}Br), 245 (M^+, ^{81}Br).

Step 2: Preparation of Diethyl 3,5-Dimethyl-4-nitrobenzylphosphonate. A mixture of 3,5-dimethyl-4-nitrobenzylbromide (694 mg, 2.84 mmol) obtained above, tetrabutylammonium iodide (77 mg, 0.21 mmol), and triethyl phosphite (596 mg, 3.59 mmol) was stirred for 6 h at 120 °C. The reaction mixture was subjected to silica gel flash chromatography ($CHCl_3$/MeOH = 100/1) to give 873 mg (q.y.) of 3,5-dimethyl-4-nitrobenzylphosphonate as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.06 (2H, d, J = 2.4 Hz), 4.10−4.02 (4H, m), 3.09 (2H, d, J = 22.2 Hz), 2.30 (6H, s), 1.28 (6H, t, J = 7.1 Hz). MS (EI) *m*/*z*: 301 (M^+). HRMS: calcd for $C_{13}H_{20}NO_5P$, 301.108; found, 301.108.

Step 1′: Preparation of 3-(3,5-Dimethoxyphenyl)acrylonitrile. To a suspension of sodium hydride (60%, 1.27 g, 31.8 mmol) in THF (30 mL) was added a solution of cyanomethylphosphonic acid diethyl ester (500 mg, 2.82 mmol) in THF (10 mL) under argon with cooling by an ice bath, and the solution was stirred for 15 min at room temperature. To the mixture was added to 3,5-dimethoxybenzaldehyde (656 mg, 3.95 mmol), and the reaction mixture was stirred for 17 h at room temperature. The mixture was poured into water and extracted with $CHCl_3$. The $CHCl_3$ layer was separated, washed with brine, and dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 4/1) gave 833 mg (q.y.) of 3-(3,5-dimethoxyphenyl)acrylonitrile as a white solid; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.32 (1H, d, J = 16.5 Hz), 6.57 (2H, d, J = 2.2 Hz), 6.53 (1H, t, J = 2.2 Hz), 5.85 (1H, d, J = 16.6 Hz), 3.81 (6H, s).

Step 2′: Preparation of 3-(3,5-Dimethoxyphenyl)propenal. To a solution of 3-(3,5-dimethoxyphenyl)acrylonitrile (1.31 g, 7.56 mmol) obtained above in toluene (30 mL) was added diisobutyl aluminum hydride (1.0 M, 15.1 mL, 15.1 mmol) under argon at −50 °C, and the solution was stirred for 22 h at room temperature. The mixture was poured into 1 N aqueous HCl and extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 4/1) gave 378 mg (26%.) of 3-(3,5-dimethoxyphenyl)propenal as a pale yellow crystal; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 9.70 (1H, d, J = 10.9 Hz), 7.41 (1H, d, J = 15.9 Hz), 6.71−6.65 (3H, m), 6.55 (1H, t, J = 2.3 Hz), 3.83 (6H, s).

Step 3: Preparation of 1,3-Dimethyl-2-nitro-5-[(1*E*,3*E*)-4-(3,5-dimethoxyphenyl)-1,3-butadienyl]benzene (14). Compound **14** was prepared from diethyl 3,5-dimethyl-4-nitrobenzylphosphonate obtained in step 2 and 3-(3,5-dimethoxyphenyl)propenal obtained above using the same procedure described in step 1′ in a 55% yield: mp 132−133 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.16 (2H, s), 6.93 (2H, m), 6.62 (4H, m), 6.40 (1H, s), 3.83 (6H, s), 2.33 (6H, s). MS (EI) *m*/*z*: 339 (M^+). Anal. Calcd for $C_{19}H_{19}NO_3$: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.63; H, 6.23; N, 4.55.

Compounds **4**−**13** were prepared from the corresponding diethylphosphonates and aldehydes using the procedure described for **14** (step 1′).

(*E*)-3,5-Dimethyl-4-nitrostilbene (4): mp 101−103 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.52 (2H, d, J = 7.3 Hz), 7.38 (2H, dd, J = 7.1, 7.8 Hz), 7.32−7.28 (1H, m), 7.25 (2H, s), 7.14 (1H, d, J = 16.3 Hz), 7.02 (1H, d, J = 16.3 Hz), 2.35 (6H, s). MS (EI) *m*/*z*: 253 (M^+). Anal. Calcd for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.83; H, 6.12; N, 5.62.

1-[(*E*)-2-(3,5-Dimethyl-4-nitrophenyl)ethenyl]naphthalene (5): mp 164−165 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.20 (1H, d, J = 8 Hz), 7.94−7.83 (3H, m), 7.74 (1H, d, J = 7.3 Hz), 7.57−7.49 (3H, m), 7.34 (2H, s), 7.07 (1H, d, J = 16.1 Hz), 2.39 (6H, s). MS (EI) *m*/*z*: 303 (M^+). Anal. Calcd for $C_{20}H_{17}NO_2$: C, 79.19; H, 5.65; N, 4.62. Found: C, 79.30; H, 5.67; N, 4.66.

2-[(*E*)-2-(3,5-Dimethyl-4-nitrophenyl)ethenyl]naphthalene (6): mp 147−149 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.88−7.82 (4H, m), 7.72 (1H, dd, J = 1.7, 8.8 Hz), 7.52−7.46 (2H, m), 7.31 (1H, d, J = 16.3 Hz), 7.30 (2H, s), 7.14 (1H, d, J = 16.34), 2.37 (6H, s). MS (EI) *m*/*z*: 303 (M^+). Anal. Calcd for $C_{20}H_{17}NO_2$: C, 79.19; H, 5.65; N, 4.62. Found: C, 78.96; H, 5.70; N, 4.62.

9-[(*E*)-2-(3,5-Dimethyl-4-nitrophenyl)ethenyl]anthracene (7): mp 219−221 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.44 (1H, s), 8.31−8.28 (2H, m), 8.05−8.02 (2H, m), 7.98 (1H, d, J = 16.6 Hz), 7.52−7.46 (4H, m), 7.42 (2H, s), 6.90 (1H, d, J = 16.6 Hz), 2.41 (6H, s). MS (EI) *m*/*z*: 353 (M^+). Anal. Calcd for $C_{24}H_{19}NO_2$: C, 81.56; H, 5.42; N, 3.96. Found: C, 81.70; H, 5.75; N, 3.86.

1,3-Dimethyl-2-nitro-5-(1,3-petadienyl)benzene (8): yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.09 (2H, s), 6.80−6.73 (1H, m), 6.33 (1H, d, J = 15.8 Hz), 6.25−6.18 (1H, m), 5.94−5.88 (1H, m), 2.31 (6H, s), 1.83 (3H, d, J = 6.8 Hz). MS (EI) *m*/*z*: 217 (M^+). HRMS: Calcd for $C_{13}H_{15}NO_2$, 217.110; found, 217.110.

1,3-Dimethyl-2-nitro-5-[(1*E*,3*E*)-4-phenyl-1,3-butadienyl]benzene (9): mp 126−130 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.45 (2H, d, J = 7.1 Hz), 7.35 (2H, dd, J = 7.3, 7.8 Hz), 7.26 (1H, m), 7.17 (2H, s), 7.104−6.903 (2H, m), 6.73 (1H, d, J = 14.6 Hz), 6.57 (1H, d, J = 14.9 Hz), 2.33 (6H, s). MS (EI) *m*/*z*: 279 (M^+). Anal. Calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.01. Found: C, 77.17; H, 6.29; N, 5.03.

5-[(*E*)-2-(2,6-Dimethylphenyl)ethenyl]-1,3-dimethyl-2-nitrobenzene (10): mp 147−149 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.88−7.82 (4H, m), 7.72 (1H, dd, J = 1.7, 8.8 Hz), 7.52−7.46 (2H, m), 7.31 (1H, d, J = 16.3 Hz), 7.30 (2H, s), 7.14 (1H, d, J = 16.3 Hz), 2.37 (6H, s). MS (EI) *m*/*z*: 303 (M^+). Anal. Calcd for $C_{20}H_{17}NO_2$: C, 79.19; H, 5.65; N, 4.62. Found: C, 78.96; H, 5.70; N, 4.62.

5-[(*E*)-2-(3,5-Dimethoxyphenyl)ethenyl]-1,3-dimethyl-2-nitrobenzene (11): mp 104−105 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.24 (2H, s), 7.07 (1H, d, J = 16.4 Hz), 6.99 (1H, d, J = 16.4 Hz), 6.67 (2H, d, J = 2.2 Hz), 6.43 (1H, t, J = 2.2 Hz), 3.85 (6H, s), 2.35 (6H, s); MS (EI) *m*/*z*: 313 (M^+); Anal. Calcd for $C_{18}H_{19}NO_4$: C, 68.99; H, 6.11; N, 4.47. Found: C, 68.79; H, 6.11; N, 4.57.

(*E*)-3-Methyl-4-nitrostilbene (12): mp 85−87 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.03 (1H, d, J = 8.5 Hz), 7.54 (2H, dd, J = 1.5, 7.3 Hz), 7.48−7.38 (4H, m), 7.35−7.31 (1H, m), 7.23 (1H, d, J = 16.6 Hz), 7.09 (1H, d, J = 16.6 Hz), 2.66 (3H, s). MS (EI) *m*/*z*: 239 (M^+). Anal. Calcd for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85. Found: C, 75.14; H, 5.44; N, 6.03.

(*E*)-4-Nitrostilbene (13): mp 161−162 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.22 (2H, ddd, J = 2, 2.4, 6.8 Hz), 7.64 (2H, ddd, J = 2, 2.4, 8.8 Hz), 7.56 (2H, d, J = 7.1 Hz), 7.42−7.39 (2H, m), 7.36−7.31 (1H, m), 7.28 (1H, d, J = 16.3 Hz), 7.15 (1H, d, J = 16.3 Hz). MS (EI) *m*/*z*: 225 (M^+). Anal. Calcd for $C_{14}H_{11}NO_2$: C, 74.65; H, 4.92; N, 6.22. Found: C, 74.65; H, 4.92; N, 6.22.

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ESR Analysis. The Fe^{2+} complex of MGD [$Fe^{2+}-MGD_2$, (Fe−MGD)] was used to trap NO. Fresh stock solutions of Fe−MGD (1:4) were prepared by adding ferrous ammonium sulfate to an aqueous solution of MGD. A sample containing 1 mM of compounds **1**−**14** and 20 mM Fe−MGD in distilled water (25% DMSO) was introduced to a quartz flat curette cell. ESR spectra were recorded after light irradiation at a distance of 10 cm with a JES-RE 2X spectrometer (JEOL Co. Ltd., Tokyo, Japan). The spectrometer settings were modulation frequency, 100 kHz; modulation amplitude, 2.0 G; scan time, 4 min; microwave power, 16 mW; and microwave frequency, 9.42 GHz.

Griess Assay. The Griess reagents were prepared by mixing acetic acid (5 mL), sulfanilic acid (500 mg), *N*-1-naphthylethylenediamine dihydrochloride (50 mg), and distilled water (95 mL). A sample containing 200 μM compounds **4**, **5**, **8**, **9**, **12**, or **13** in *o*-dichlorobenzene (2 mL) and distilled water (1 mL) was photoirradiated for the specified times at a distance of 12 cm. The mixture was treated with the Griess reagents (1 mL), and it was agitated for 15 min. The aqueous layer was separated with a fixed angle centrifuge for 5 min at 5000 rpm, and the absorbance at 546 nm was measured. The results were expressed as the percentile of the conversion ratio of the testing compounds.

Cytotoxicity Against HCT116 Cells. HCT116 human colon cancer cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in McCoy5A culture medium containing penicillin and streptomycin, supplemented with fetal bovine serum as described in the ATCC instructions. The cells were maintained at 37 °C in a humidified 5% (v/v) CO_2 incubator under sub-confluent conditions. For the experiments, the cells were plated at 2.5×10^5 cells per 6-cm culture dish 2 days before treatments and incubated at 37 °C. On the day of the experiment, the culture medium was refreshed and reduced to 2 mL per 6-cm culture dish. The cells were then treated with compound **14** in 2 μL of DMSO giving a final concentration of 2.5 μM, which resulted in a final DMSO concentration in the culture media of less than 0.1% (v/v), at which concentration there were no apparent effects from the DMSO. The cells were also treated with compound **14** in the same manner described above in the presence of 40 nM P450 nor (Wako Pure Chemical Industry Co. Ltd, Osaka, Japan) and 2 μM NADH. The treated cells were subsequently subjected to the photoirradiation for 1 min by using the light-source (100 W mercury lamp) of a fluorescence microscope (Olympus BX60/BX-FLA with UplanFl 10X objective lens) with a WU filter (330−380 nm band-pass filter). The irradiation area was set as a circular area with a diameter of around 3 mm. After 24 h incubation, the cells were observed under an inverted phase-contrast microscope, and the images were taken with a digital camera and processed with a personal computer.

MM2 Calculations. Force-field (MM2) minimizations of compounds **1**−**13** were performed using Macromodel 8.0 software.[21] All structures were fully optimized with each parameter set as follows: force field, MM2*; method, LBFGS; max no. iterations, 10 000; converge on, gradient; convergence threshold, 0.05.

Supporting Information Available: Ultraviolet−visible-light spectra of compounds **1**−**14** and the ESR and Griess assay results of compound **14** are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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