Electronic Supplementary Information

Rhodamine Cyclic Hydrazide as Fluorescent Probe for the Detection of Hydroxyl Radical

Minjeong Kim, Sung-Kyun Ko, Hyemi Kim, Injae Shin* and Jinsung Tae*

Department of Chemistry, Yonsei University, Seoul, 120-749 Korea

CONTENTS

1. General Methods S2
2. Synthesis of 1 from Rhodamine 6G S3
3. Synthesis of 2 from Rhodamine 6G S4
4. Synthesis of 1, 2-diallylhydrazine dihydrochloride S5
5. Synthesis of 3 from the reaction of 2 and •OH S5
6. Proposed mechanism of reaction between 2 and •OH S6
7. HPLC analysis after reaction of 2 with •OH S7
8. Time dependent fluorescence intensity changes of 1, 2 with •OH in various buffers. S8
9. Relative fluorescence intensity changes of 1 in the presence of ROS&RNS S9
10. Color changes of 1 with •OH and other ROS & RNS S12
11. UV-Vis absorption changes of 1 S13
12. Time dependent fluorescence intensity changes of 1 S14
13. Fluorescence titration of 1 with •OH S15
14. H₂O₂ concentration dependent fluorescence intensity changes S16
15. FeSO₄ concentration dependent fluorescence intensity changes S17
16. Fluorescence spectra of 1 at various pH values S18
17. Effect of scavengers S19
18. Fluorescence imaging of cells with probe 1. S20
19. Reference S21
20. ¹H NMR and ¹³C NMR copies of 1 S22
21. ¹H NMR and ¹³C NMR copies of 2 S23
22. ¹H NMR and ¹³C NMR copies of 3 S24
23. ¹H NMR and ¹³C NMR copies of 1, 2-diallylhydrazine dihydrochloride S25

S1
1. General Methods

**General Synthetic Materials and Methods:** Silica gel 60 (230-400 mesh, Merck) was used for column chromatography. Analytical thin layer chromatography was performed using Merck 60 F254 silica gel (precoated sheets, 0.25 mm thick). All reagents and solvents for reactions were used as received with the following exceptions. 1, 2-Dichloromethane (CH$_2$Cl$_2$) and triethylamine (Et$_3$N) were distilled from calcium hydride (CaH$_2$). All other chemicals used were purchased from Sigma-Aldrich and were used as received.

**Spectroscopic Materials and Methods:** Nuclear magnetic resonance (NMR) spectra were recorded in CDCl$_3$ unless otherwise stated, with tetramethylsilane (TMS) as internal reference at ambient temperature, mainly on a Bruker Avance II-400 Fourier Transform Spectrometer operating at 400 MHz for $^1$H and at 100.6 MHz for $^{13}$C. Mass spectra were recorded on a ZQ-4000 LC-MS and QUATTRO LC Triple Quadrupole Tandem mass spectrometer for both low resolution and high resolution mass spectra. The pH was recorded by HI-8014 instrument (HANNA). Melting points were measured on a Z289078 (Sigma-Aldrich) microscope. Infrared absorption spectra were recorded as a solution in CH$_2$Cl$_2$ on a Avatar 360 FT-IR spectrophotometer. HPLC spectra were recorded using an Agilent Technology 1260 infinity. Fluorescence emission spectra were obtained using a Hitachi F-4500 spectrofluorimeter linked to a Pentium PC running SpectraCalc software package. The slit width was 5.0 nm for both excitation and emission. The photon multiplier voltage was 400 V. A circulating H$_2$O/DMF bath was used during all experiments to regulate the temperature at 25±1 °C. Samples were contained in 10.0 nm path length quartz cuvettes (3.5 mL volume). Upon excitation at 500 nm, the emission spectra were integrated over the range 510-630 nm. All measurements were conducted at least in triplicate.
2. Synthesis of 1 from Rhodamine 6G.

Rhodamine 19 was prepared according to the known procedure.\(^1\)

1, 2-Pyrazolidine dihydrochloride was prepared according to the known procedure.\(^2\)

To a rhodamine 6G (12.2 g, 25.5 mmol) in EtOH (300 mL) was added sodium hydroxide (3.04 g, 76.4 mmol) in H\(_2\)O (45 mL) and the reaction mixture was stirred for 5 h under reflux conditions. After the solution was cooled to room temperature, distilled H\(_2\)O (50 mL) was added to the solution. The solution was cooled to 0 °C. The resulting precipitates were filtered and dried at 70 °C for 30 min to give the 7.0 g (66%) of rhodamine 19.

To a solution of rhodamine 19 (973 mg, 2.35 mmol) in dichloromethane (20 mL) was added phosphorus oxychloride (0.65 mL, 7.04 mmol) over 2 min. The solution was refluxed for 4 h. The reaction mixture was cooled and evaporated in vacuo to give rhodamine acid chloride, which was used for next step without purification.

To 1,2-pyrazolidine dihydrochloride (270 mg, 1.86 mmol) in dichloromethane (8 mL) were added slowly triethylamine (1.10 mL, 7.80 mmol) and then the crude rhodamine acid chloride in dichloromethane (10 mL). And the reaction mixture was stirred for 12 h at room temperature. The mixture was concentrated under vacuum and the crude product was purified by column chromatography (hexanes/EtOAc = 2/1 to 1/1) to give 330 mg of compound \(\mathbf{1}\) as a pale red solid (46% yield).
from rhodamine 19).

**Compound 1**: $R_f = 0.25$ (silica gel, hexanes/EtOAc = 1/2); mp 253-255 °C; $^1$H NMR (400 MHz, CDCl$_3$) = 8.21-8.19 (m, 1H), 7.40-7.26 (m, 2H), 6.97-6.95 (m, 1H), 6.69 (s, 2H), 6.33 (s, 2H), 3.74 (t, $J = 4.5$ Hz, 2H), 3.53 (bs, 2H), 3.21 (q, $J = 4.5$ Hz, 4H), 2.60 (t, $J = 4.0$ Hz, 2H), 1.97 (s, 6H), 1.86-1.80 (m, 2H), 1.32 (t, $J = 4.5$ Hz, 6H); $^{13}$C NMR (100.6 MHz, CDCl$_3$) = 160.8, 151.9, 147.4, 144.8, 132.3, 131.2, 129.0, 128.3, 127.2, 126.7, 117.4, 108.2, 96.0, 63.8, 48.1, 44.8, 38.5, 22.5, 17.1, 15.0; IR (film, cm$^{-1}$) 3432, 3367, 2973, 2927, 2869, 1634, 1571, 1516, 1468, 1417, 1345, 1263, 1215, 1154, 1094, 1014, 914, 875, 812, 747; HRMS (FAB) $m/z$ calcld for C$_{29}$H$_{33}$N$_4$O$_2$ [(M+H)$^+$] 469.2525; found 469.2522.

### 3. Synthesis of 2 from Rhodamine 6G.

![Chemical reaction diagram]

To the 1, 2-diallylhydrazine dihydrochloride (380 mg, 2.10 mmol) dissolved in dichloromethane (10 mL) were added triethylamine (1.2 mL, 8.8 mmol) slowly and then crude rhodamine acid chloride in dichloromethane (10 mL). And the reaction mixture was stirred for 12 h at room temperature. The mixture was concentrated under vacuum and the crude product was purified by column chromatography (hexanes/EtOAc = 5:1 to 3:1) to give 430 mg of compound 2 as pink-red solid (36% from Rhodamine 19).

**Compound 2**: $R_f = 0.20$ (silica gel, hexanes/EtOAc = 2/1); mp 185-187 °C; $^1$H NMR (400 MHz, CDCl$_3$) = 8.28-8.26 (m, 1H), 7.58-7.50 (m, 2H), 7.32-7.30 (m, 1H), 6.49 (s, 4H), 5.53-5.43 (m, 1H), 4.93-4.66 (m, 5H), 3.81 (bs, 2H), 3.49 (s, 2H), 3.22 (q, $J = 4.3$ Hz, 6H), 1.96 (s, 6H), 1.33-1.30 (t, $J =$...
4.4 Hz, 6H); $^{13}$C NMR (100.6 MHz, CDCl$_3$) = 164.2, 153.3, 147.0, 138.6, 134.8, 132.3, 132.1, 130.2, 128.7, 127.8, 127.7, 117.8, 116.9, 115.8, 97.8, 64.3, 56.5, 52.7, 38.5, 17.0, 14.8; IR (film, cm$^{-1}$) 3434, 3384, 3074, 2973, 2869, 1635, 1574, 1512, 1417, 1342, 1281, 1213, 1155, 1001, 920, 816, 753; HRMS (FAB) m/z calcd for C$_{32}$H$_{37}$N$_4$O$_2$ [(M+H)$^+$] 509.2838; found 509.2851.

4. Synthesis of 1,2-diallylhydrazine dihydrochloride.

![Reaction scheme]

Di-tert-butyl 1,2-diallylhydrazine-1, 2-dicarboxylate was prepared according to the known procedure.$^3$

Di-tert-butyl 1,2-diallylhydrazine-1, 2-dicarboxylate (5.3 g, 16.9 mmol) was dissolved in 4 M hydrogen chloride in 1,4-dioxane (20 mL). And the reaction mixture was stirred for 3 h at room temperature. Diethyl ether (10 mL) was added to the mixture and the mixture was stirred for 10 min. The resulting precipitates were filtered and dried at room temperature for 1 h to give the 2.5 g (89%) of 1, 2-diallylhydrazine dihydrochloride.

1, 2-Diallylhydrazine dihydrochloride: $R_f = 0.15$ (silica gel, DCM/MeOH = 20/1); mp 163-165 °C; $^1$H NMR (400 MHz, MeOD) = 9.62 (bs, 2H), 5.91-5.86 (m, 2H), 5.34-5.29 (m, 2H), 5.23 (dd, $J = 1.0, 6.5$ Hz, 2H), 3.59-3.57 (m, 4H); $^{13}$C NMR (100.6 MHz, MeOD) = 131.1, 120.9, 50.5; IR (film, cm$^{-1}$) 3414, 2259, 2199; HRMS (FAB) m/z calcd for C$_{6}$H$_{13}$N$_2$ [(M+H)$^+$] 113.0000; found 113.1004.

5. Synthesis of 3 from the reaction of 2 and -OH.

![Reaction scheme]

To a solution of 2 (100 mg, 0.19 mmol) in dichloromethane (10 mL) was added hydroxyl
radical (20 eq). The reaction mixture was stirred at room temperature for 4 h. The organic layer was separated and washed with water (20 mL×3) and saturated NaCl solution (20 mL×3) and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel (DCM/MeOH = 20/1 to 10/1) to give the 10 mg of 3 (12%) as a red solid.

**Compound 3:** $R_f = 0.25$ (silica gel, DCM/MeOH = 8/1); mp 190-192 °C; $^1$H NMR (400 MHz, CDCl$_3$) = 7.64-7.57 (m, 3H), 7.56-7.53 (bs, 2H), 7.32-7.29 (m, 1H), 7.26 (d, $J = 5.5$ Hz, 1H), 7.01 (s, 2H), 6.52 (s, 2H), 6.30-6.20 (m, 1H), 5.93-5.50 (m, 2H), 5.38-5.28 (m, 1H), 4.91 (dd, $J = 2.5$, 6.5 Hz, 1H), 4.61 (d, $J = 10.8$ Hz, 1H), 4.34 (d, $J = 3.0$ Hz, 2H), 3.55-3.48 (m, 5H), 2.24 (s, 6H), 1.35 (t, $J = 4.5$ Hz, 6H); $^{13}$C NMR (100.6 MHz, CDCl$_3$) = 169.5, 157.1, 156.0, 154.5, 143.6, 136.8, 133.6, 131.2, 129.5, 129.4, 128.8, 126.0, 125.3, 117.3, 113.5, 93.4, 43.3, 38.4, 18.5, 13.9; IR (film, cm$^{-1}$) 3214, 2975, 2926, 1648, 1606, 1499, 1443, 1315, 1185, 1024; LRMS (FAB) $m/z$ calcd for C$_{32}$H$_{35}$N$_4$O$_2$ [(M+H)$^+$] 506; found 506.

**6. Proposed mechanism of reaction between 2 and •OH.**
7. HPLC analysis after reaction of 2 with •OH

HPLC analysis: at 520 nm, ACN/H₂O= 60 : 40, flow rate 1.0 mL/min, retention time: 9.2 min (2) and 10.8 min (3)

a) Hydroxyl radical (•OH, 1.0 equiv) was added to a solution of 2 (50 μM) in Tris-HCl buffer (DMF 1% v/v) at pH 7.4 (25 °C). After given time periods, the solutions were quenched with excess Tempol to remove remaining •OH and extract with CH₂Cl₂. The CH₂Cl₂ layers were analyzed by HPLC.

b) Changes of HPLC peaks of 2 and 3 versus time after reaction of 2 with •OH.

(Note: The HPLC intensities of 3 are decreasing after initial formation as shown in Figures a) and b). The reaction of 2 and hydroxyl radical generates several spots on TLC analysis, which implies that the product(s) and reactive radical intermediates further react with hydroxyl radical to generate complex mixture.)
8. Time dependent fluorescence intensity changes of 1, 2 (10 μM) with •OH (1.0 equiv) in various buffer.

Hydroxyl radical (•OH, 1.0 equiv) was added to a solution of 1 (10 μM) in Tris-HCl buffer, HEPES buffer, PBS buffer, H2O (DMF 1% v/v) at pH 7.4 (25 °C) (Ex. 500 nm; Em. 550 nm).

Hydroxyl radical (•OH, 1.0 equiv) was added to a solution of 2 (10 μM) in Tris-HCl buffer, HEPES buffer, PBS buffer, H2O (DMF 1% v/v) at pH 7.4 (25 °C) (Ex. 500 nm; Em. 551 nm). To make hydroxyl radical (•OH), Fenton reagents [CuCl2 and H2O2] was respectively added in various buffers containing 1, 2 (10 μM).

(Note: It is not clear why probe 1 displays quite different responses to hydroxyl radical in different buffer solutions.)
9. Relative fluorescence intensity of 1 (10 μM) in the presence of various reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Hydroxyl radical (•OH, 1.0 equiv), hydrogen peroxide (H₂O₂), alkylperoxyl radical (ROO•), nitric oxide (NO•), superoxide (•O₂⁻), hypochlorous acid (HOCl), singlet oxygen (¹O₂), peroxy nitrite (ONOO⁻) (5.0 equiv respectively) was added to a solution of 1 (10 μM) in Tris-HCl buffer (DMF 1% v/v) at pH 7.4 (25 °C).

Relative fluorescence intensities (at 550 nm) of 1 (10 μM) in the presence of ROS/RNS.

To make hydroxyl radical (•OH), Fenton reagents [FeSO₄ and H₂O₂] was respectively added in Tris-HCl buffer containing 1 (10 μM) and mixture was incubated for 30 min. (Ex. 500 nm; Em. 550 nm)
Generation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

Various ROS and RNS including \( \bullet \text{OH}, \text{H}_2\text{O}_2, \text{ROO}^\bullet, \text{NO}^\bullet, \text{O}_2^-, \text{HOCl}, \text{I}_2^-, \text{ONOO}^- \) were prepared according to the following methods.

1) Preparation of \( \bullet \text{OH} \) (hydroxyl radical) (final 10 \( \mu \text{M} \))\(^{4,5,6}\)

(a) CuCl\(_2\) (copper(II) chloride) was dissolved in H\(_2\)O and H\(_2\)O\(_2\) was diluted in H\(_2\)O respectively. CuCl\(_2\) solution (2 mM) and H\(_2\)O\(_2\) solution (20 mM) was added to make 10 \( \mu \text{M} \) \( \bullet \text{OH} \) in Tris-HCl buffer (DMF 1% v/v) containing 1 at room temperature and the spectrum was measured 30 min later.

(b) FeSO\(_4\)-7H\(_2\)O (ferrous sulfate hydrate) was dissolved in H\(_2\)O and H\(_2\)O\(_2\) was diluted in H\(_2\)O respectively. FeSO\(_4\)-7H\(_2\)O solution (2 mM) and H\(_2\)O\(_2\) solution (20 mM) was added to make 10 \( \mu \text{M} \) \( \bullet \text{OH} \) in Tris-HCl buffer (DMF 1% v/v) containing 1 at room temperature and the spectrum was measured 30 min later. The molar ratio of FeSO\(_4\) to H\(_2\)O\(_2\) is 1:10

2) Preparation of H\(_2\)O\(_2\) (hydrogen peroxide) (10 mM)\(^7\)

H\(_2\)O\(_2\) was diluted in H\(_2\)O and stirring for 20 min. Then H\(_2\)O\(_2\) solution was added in Tris-HCl buffer (DMF 1% v/v) containing 1 at room temperature and the spectrum was measured 30 min later.

3) Preparation of ROO\(^\bullet\) (alkyl peroxide) (10 mM)\(^8\)

2, 2-azobis (2-aminodipropyl) dihydrochloride was diluted in H\(_2\)O and stirring for 20 min. Then ROO\(^\bullet\) solution was added in Tris-HCl buffer (DMF 1% v/v) containing 1 at room temperature and the spectrum was measured 30 min later.

4) Preparation of NO\(^\bullet\) (Nitric oxide) (10 mM)\(^8\)

SNP (sodium nitroferricyanide(III) dihydrate) was diluted in H\(_2\)O and stirring for 20 min. Then NO\(^\bullet\) solution was added in Tris-HCl buffer (DMF 1% v/v) containing 1 at room temperature and the spectrum was measured 30 min later.

5) Preparation of \( \text{O}_2^\bullet^- \) (superoxide radical) (10 mM)\(^9\)

KO\(_2\) (potassium superoxide) was dissolved in DMSO and stirring for 20 min. Then \( \text{O}_2^\bullet^- \) solution was added in Tris-HCl buffer (DMF 1% v/v) containing 1 at room temperature and the spectrum was measured 30 min later.

6) Preparation of HOCl (hypochlorous acid) (10 mM)\(^7\)

NaOCl was dissolved in 0.1 M NaOH (aq) and stirring for 20 min. Then HOCl solution was added in
Tris-HCl buffer (DMF 1% v/v) containing I at room temperature and the spectrum was measured 30 min later.

7) Preparation of $^1$O$_2$ (singlet oxygen) (10 mM)$^{10}$
NaOCl was dissolved in 1 mM of H$_2$O$_2$ to make 10 mM solution and stirring for 20 min. Then $^1$O$_2$ solution was added in Tris-HCl buffer (DMF 1% v/v) containing I at room temperature and the spectrum was measured 30 min later.

8) Preparation of ONOO$^-$ (peroxynitrite)$^8$
NaOH (aq) was added in a mixture of NaNO$_2$ (sodium nitrite) 0.6 M, H$_2$O$_2$ (hydrogen peroxide) 0.7 M and hydrochloric acid 0.6 M to make 1.5 M alkaline. The excess H$_2$O$_2$ was removed by passing the solution through a short column of manganese dioxide. The resulting solution was stored at lower than -18 °C. The solution was thawed immediately before use. The concentration of peroxynitrite was determined by measuring the absorption of the solution at 302 nm. The extinction coefficient of peroxynitrite solution in 0.1 M NaOH (aq) is 1670 M$^{-1}$cm$^{-1}$ at 302 nm.
10. Color changes of 1 (20 μM) with •OH and other ROS & RNS.

\[ \text{•OH, } H_2O_2, \text{ ROO•, NO•, } \cdot O_2^- \text{, HOCl, } ^1O_2, \text{ ONOO}^- \text{ (5.0 equiv respectively)} \] was added to a solution of 1 (20 μM) in Tris-HCl buffer (DMF 1% v/v) at pH 7.4 (25 °C).

Color changes were taken pictures after incubation for 30 min.
11. UV-Vis absorption of 1 (10 μM) in the presence of various reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Hydroxyl radical (•OH), hydrogen peroxide (H$_2$O$_2$), alkylperoxy radical (ROO•), nitric oxide (NO•), superoxide (•O$_2^-$), hypochlorous acid (HOCl), singlet oxygen (¹O$_2$), peroxynitrite (ONOO•) (5.0 equiv respectively) was added to a solution of 1 (10 μM) in Tris-HCl buffer (DMF 1% v/v) at pH 7.4.

Relative UV-Vis absorptions of 1 (10 μM) in presence of ROS/RNS.
12. Time dependent fluorescence intensity changes of 1 (10 μM) with •OH (1 equiv) in Tris-HCl buffer (DMF 1% v/v) at pH 7.4 (25 °C). (Ex. 500 nm; Em. 550 nm)
13. Fluorescence titration of 1 (10 μM) with •OH (various concentration) in Tris-HCl buffer (DMF 1% v/v) at pH 7.4 (25 °C). (Ex. 500 nm; Em. 550 nm)\(^d\)

Fluorescence titration (Ex. 550 nm) of 1 (10 μM) in presence of •OH (micro molar concentration = 4, 8, 12, 16, 20, 40, 60, 80, 100 μM).

A solution of 1 (2.0 mL) was placed in a quartz cell and the fluorescence spectrum was recorded. Fluorescence intensity changes (Ex. 550 nm) were recorded after incubation for 30 min at pH 7.4 (25 °C).

Fluorescence intensity changes (Ex. 550 nm) v.s. micro molar concentration of •OH.
14. H$_2$O$_2$ concentration dependent fluorescence intensity changes.

H$_2$O$_2$ was added to a solution of 1 (10 μM) in Tris-HCl buffer containing 10 μM FeSO$_4$ (DMF 1% v/v) and mixture was incubated for 30 min at pH 7.4 (25 °C). (Ex. 500 nm; Em. 550 nm)

Fluorescence intensity changes (Ex. 550 nm) v.s. micro molar concentration of H$_2$O$_2$. 

---

Electronic Supplementary Material (ESI) for Chemical Communications
This journal is © The Royal Society of Chemistry 2013
15. FeSO₄ concentration dependent fluorescence intensity changes.¹¹,¹²,¹³

FeSO₄ was added to a solution of 1 (10 μM) in Tris-HCl buffer containing 10 μM H₂O₂ (DMF 1% v/v) and mixture was incubated for 30 min at pH 7.4 (25 °C). (Ex. 500 nm; Em. 550 nm)

Fluorescence intensity changes (Ex. 550 nm) v.s. micro molar concentration of FeSO₄.
16. Fluorescence spectra of 1 (10 μM) with •OH (1 equiv) in H₂O (DMF 1% v/v) at various pH values. (Ex. 500 nm; Em. 550 nm)
17. Effect of scavengers (DMSO\textsuperscript{6}, t-butanol, hydroquinone\textsuperscript{14}, TEMPOL\textsuperscript{15} (0.1 mM)) on fluorescence intensity of 1 (10 μM) containing •OH (10 μM) in Tris-HCl buffer (DMF 1% v/v, 25 °C) (Ex. 500 nm; Em. 550 nm).

Scavengers (0.1 mM)* and hydroxyl radical (10 μM) was simultaneously added to a solution of 1 (10 μM) in Tris-HCl buffer (DMF 1% v/v) and mixture was incubated for 30 min at pH 7.4 (25 °C). (Ex. 500 nm; Em. 550 nm)

* Scavengers : DMSO, t-butanol, hydroquinone, TEMPOL (0.1 mM)

It found that hydroquinone and TEMPOL can be used for scavenger of •OH.
18. Fluorescence imaging of cells with probe 1.16

Fluorescent detection of intracellular hydroxyl radicals generated by Fenton’s reagent.
A549 (human lung adenocarcinoma epithelial cells) and RAW264.7 cells (murine
macrophage-like cells) obtained from American Type Culture Collection (Manassas, VA)
were maintained in DMEM (Dulbecco’s Modified Eagle Medium) supplemented with 10%
fetal bovine serum (FBS), 50 units/mL of penicillin and 50 μg/mL of streptomycin. A549 and
RAW264.7 cells were seeded in a 24-well plate at a density of 1 × 10^4 cells per well in
culture media. After 24 h, the cells were treated with 20 μM probe in culture media
containing 0.1% (v/v) DMF for 1 h at 37 °C. After washing with PBS to remove the
remaining probe, the cells were incubated with 20 μM of Fenton’s reagent (FeSO₄ : H₂O₂,
1:10) for 1 h at 37 °C. Separately, the probe-treated cells were incubated with 5 mM
TEMPOL in 0.1% (v/v) DMSO for 1 h and then treated with 20 μM of Fenton’s reagent for 1
h. After being washed with PBS, the treated cells were analyzed by using confocal
microscopy (LSM 510 META, Carl Zeiss, Germany) (λ_{ex} = 543 nm).

Fluorescent detection of intracellular hydroxyl radicals produced by PMA stimulation.
A549 and RAW264.7 cells were treated with 20 μM probe for 1 h at 37 °C. After being
washed with PBS to remove the remaining probe, the cells were incubated with 10 ng/mL
PMA for 1 h to generate hydroxyl radicals. Separately, the probe-treated cells were incubated
with 5 mM TEMPO in 0.1% (v/v) DMSO for 1 h and then stimulated by treatment with 10
ng/mL PMA for 1 h. After being washed with PBS, the treated cells were imaged by using
confocal microscopy (λ_{ex} = 543 nm).
19. Reference

20. $^1$H NMR and $^{13}$C NMR copies of 1.
21. $^1$H NMR and $^{13}$C NMR copies of 2.
22. $^1$H NMR and $^{13}$C NMR copies of 3.
23. $^1$H NMR and $^{13}$C NMR copies of 1,2-diallyhydrazine dihydrochloride.