Supporting Information

A Photodeactivatable Antagonist for Controlling CREB Dependent Gene Expression

Takuma Imoto,† Masafumi Minoshima,† Tatsushi Yokoyama,‡ Ben P. Emery,§ Steven D. Bull,§ Haruhiko Bito† and Kazuya Kikuchi*†§‖

†Division of Advanced Science and Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan.
‡Department of Neurochemistry, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.
§Department of Chemistry, University of Bath, Bath, BA2 7AY, U.K.
§Immunology Frontier Research Center, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan.
‖Quantum Information and Quantum Biology Division, Institute for Open and Transdisciplinary Research Initiatives, Osaka University, 2-1, Yamadaoka, Suita, Osaka 565-0871, Japan.
Materials and instruments

Unless otherwise specified, all reagents were purchased from chemical suppliers, including Tokyo Chemical Industries, Wako Pure Chemical, or Sigma-Aldrich Chemical Co., and used without further purification. Analytical thin-layer chromatography was performed using 60F254 silica plates (Merck & Co., Inc., Kenilworth, NJ, USA) and spots were visualized under UV light. Silica gel column chromatography was performed using BW-300 (Fuji Silycia Chemical Ltd.), with flash autopurification carried out using an Isolera Spectra (Biotage) employing SNAP Ultra and SNAP C18 cartridges. Reverse-phase high-performance liquid chromatography (RP-HPLC) analyses were performed using an Inertsil ODS-3 column (4.6 × 250 mm; GL Sciences) and a HPLC system comprised of a pump (PU-2080; JASCO, or Chromaster® 5110, HITACHI) and detector (MD-2010 plus and FP-2020; JASCO, or Chromaster® 5430 and Chromaster® 5440, HITACHI). In the case of the Chromaster® HPLC system, sample injections were performed using an auto sampler (Chromaster® 5210, HITACHI). Preparative HPLC was performed with an Inertsil ODS-3 column (10.0 × 250 mm; GL Sciences, Inc.) using a HPLC system with a PU-2087 pump (Jasco) and UV-2075 detector (Jasco). Buffer A was comprised of 0.1% TFA in H2O, while buffer B was comprised of 0.1% TFA in acetonitrile. Nuclear magnetic resonance (NMR) spectra were recorded on an AVANCE500HD instrument (Bruker, Billerica, MA, USA) at 500 MHz for 1H NMR spectra and 125 MHz for 13C NMR spectra using tetramethylsilane as internal standard. UV-Vis absorption spectra were obtained using a V-650 spectrometer (Jasco). Fluorescence microscopic analyses were carried out using a confocal laser scanning microscope (Olympus, FLUOVIEW FV10i) equipped with a 60× lens. Laser illumination was performed with a Xe lamp (Asahi, MAX-302), with laser illumination of cells performed with a fluorescence microscope (KEYENCE, BZ-X710) equipped with a 10× lens. Real-time PCR experiments were performed using a Thermal Cycler Dice® Real Time System Lite (Takara Bio Inc). The dual-luciferase® reporter assay system was purchased from Promega Co., Ltd., and cytotoxicity LDH Assay Kit-WST purchased from DOJINDO Laboratories (Kumamoto, Japan). Luminescence and absorbance levels were measured using an ARVO™ MX microplate reader (PerkinElmer). CRE (pGL4.29) and pRL-TK were obtained from Promega Co., Ltd.

Photolysis measurement

Samples were prepared in 0.1 M HEPES buffer (pH 7.4) contained 50 μM compound (1% DMSO). The sample solutions were added to a quartz cell 10 × 10 mm and irradiated by a Xe light source (MAX-303, Asahi Spectra) equipped with a bandpass filter (365 ± 5 nm, 405 ± 5 nm or 430 ± 5 nm).

Kinetic analysis of the photolysis of PCI-1 and PCI-2

Rate constants for photolysis were obtained from time-course experiments. PCI-1 or PCI-2 (50 μM) and benzoic acid (100 μM, internal standard) were irradiated using a Xe lamp (light intensity: 4
mW cm$^{-2}, \lambda_{ex} = 365 \pm 5$ nm, or 10 mW cm$^{-2}, \lambda_{ex} = 405 \pm 5$ nm) in a 200 µL mixture of 20 mM HEPES buffer (pH 7.4) containing 10% DMSO at room temperature. HPLC analyses were performed using a Chromaster® system after injection of each sample (100 µL) using an auto sampler. Relative peak areas were obtained by dividing the PCI or photodegraded PCI peak areas by the benzoic acid peak area. The rate constants for the decomposition of PCI-1-2 ($k$) were determined by fitting the kinetic data to the following equation (Eq. 1):

$$[\text{PCI}] = [\text{PCI}]_0 e^{-kt} \quad \text{(Eq. 1)}$$

$[\text{PCI}]$ and $[\text{PCI}]_0$ are the measured and initial concentrations of the starting material, respectively.

Quantum yields for photolysis were determined using the following equation (Eq 2):

$$\Phi = (I\sigma_{90\%})^{-1} \quad \text{(Eq. 2)}$$

$I$ is the radiant power in einstein·cm$^{-2}$·s$^{-1}$, $\sigma$ is the decadic extinction coefficient (10$^3$ times the molar extinction coefficient, $\varepsilon$) in cm$^{-1}$·mol$^{-1}$, and $t_{90\%}$ is the irradiation time in seconds for 90% photoconversion to product. The UV intensity of the lamp $I$ was measured by potassium ferrioxalate actinometry.

Cell culture

HEK293T cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) and two antibiotics (100 units/mL penicillin and 0.1 mg/mL streptomycin). For the reporter assays, the cells were maintained in 96-well plates at 37 °C throughout the experiments.

Inhibition of CREB-mediated gene transcription

The reporter assay was performed by transfecting HEK293T cells with CRE and pRL-TK using Lipofectamine 3000 (Invitrogen) according to the manufacturer’s protocol. After incubation of the transfected cells in 5% CO$_2$ at 37 °C for 24 h, the cells were incubated with the photodegraded CBP inhibitor (CI-1) PCI-1 or PCI-2 in DMEM containing 10% FBS for 30 min. The cells were then treated with forskolin (10 µM final concentration) and incubated in 5% CO$_2$ at 37 °C for 6 h and the media then removed. After addition of a lysis buffer, Luciferase assay reagent II was added and the firefly luminescence levels measured (exposure time: 2 sec). Subsequently, the Stop and Glo® reagent was added and the renilla luminescence levels measured (exposure time: 2 sec). Relative intensities were obtained by dividing the luminescence intensity of the firefly luciferase by the renilla luciferase intensity.
**Dual-luciferase reporter assay with photodeactivation of PCIs**

HEK293T cells in a 96-well plate were treated with 6 μM of a test compound and then each well irradiated by light ($\lambda_{ex} = 365$ nm, 10 mW cm$^{-2}$) for 5 min. Forskolin (final concentration of 10 μM) was added 30 min after the light irradiation and the cells then incubated at 37 °C for 6 h before their inhibitory activities were measured.

**Photolysis measurement of PCIs after light exposure to cells**

HEK293T cells ($3 \times 10^4$ cells/well) in 96-well plate were treated with 6 μM PCIs and irradiated with light ($\lambda_{ex} = 365$ nm, 10 mW cm$^{-2}$ for 5 min). After irradiation, the cells and the supernatants were harvested by centrifugation. The cells were lysed by cell lysis buffer and compounds extracted by adding acetonitrile to the cell lysate, with the resultant supernatants then centrifuged (13,200 rpm for 5 min) to remove proteins and nucleic acids as precipitates. The supernatants and organic extracts were analyzed by HPLC.

**Cytotoxicity LDH assay**

HEK293T cells were collected and replated into 96-well plates ($3 \times 10^4$ cells/well) in DMEM containing 10% FBS and the cells incubated for overnight. The cells were then treated with the indicated concentration of CBP inhibitor in DMEM containing 10% FBS, with the cytotoxicity LDH assay then performed after 4 h according to the manufacture’s guidelines.

**Real-time PCR Experiment**

HEK293T cells were replated into 24-well plates in DMEM containing 10% FBS overnight. 6 μM of each compound were added to each well which were then irradiated with light ($\lambda_{ex} = 365$ nm, 10 mW cm$^{-2}$ for 5 min). All experimental conditions were conducted in duplicate. Forskolin (final concentration of 10 μM) was added 1 h after light irradiation. The cells (~1 x 10$^6$ cells from two wells) were incubated at 37 °C for 45 min and the total RNA then extracted using an Epiquik total RNA isolation kit (EpiGentek). The isolated RNA was treated with 1 unit of DNase I (Promega Co. Ltd) to degrade the residual DNA. 25 ng RNA samples were used for reverse transcription and subsequent real-time PCR using a One Step TB Green® PrimeScript™ RT-PCR Kit II (Takara Bio Inc). The following primer sets were used: target NR4A2 gene (forward: 5’-CTTGGCACAGTTGAGATGAAATG-3’, reverse: 5’-CGAGGAAATTTAAGTGGACAG-3’) and reference HPRT1 (Hypoxanthine-guanine phosphoribosyltransferase 1) gene (forward: 5’-CCTGGCGTCGTGATTAGTGATG-3’, reverse: 5’-CGAGCAAGACGTTCCAGTCCCTG-3’). Reverse transcription was performed at 42 °C for 5 min and then terminated through heating at 95 °C for 10 sec. Each of the 40 PCR cycles consisted of a 5 sec denaturation step at 95 °C and a 30 sec
hybridization and DNA synthesis step at 60 °C. Relative gene expression levels were analyzed by a $2^{-\Delta\Delta CT}$ method using HPRT1 as a reference gene.
Scheme S1. Synthesis of PCI-1

1. Synthesis of PCI-1
Scheme S2. Synthesis of PCI-2
Scheme S3. Synthesis of CI-1

1. **2** to **14**
   - **2** (4-hydroxyphenyl)propionic acid
   - **14** (4-carboxyphenyl)propionic acid
   - Reaction: TFA in DCM
   - Yield: 96%

2. **8** to **15**
   - **8** (4-carboxyphenyl)propionic acid
   - **15** (4-carboxyphenyl)propionic acid
   - Reaction: PyBOP, DIPEA in DCM
   - Yield: 9%

3. **15** to **16**
   - **15** (4-carboxyphenyl)propionic acid
   - **16** (4-carboxyphenyl)propionic acid
   - Reaction: 2 M NaOH aq. in MeOH/THF
   - Yield: 86%

4. **16** to **17 (CI-1)**
   - **16** (4-carboxyphenyl)propionic acid
   - **17 (CI-1)** (4-carboxyphenyl)propionic acid
   - Reaction: TFA in DCM
   - Yield: 36%
Figure S1. Photolysis of photodeactivatable CBP inhibitors. HPLC traces of photolysis of 50 µM PCI-1 (a-c) and PCI-2 (d-f) at different wavelengths in 20 mM HEPES buffer (pH 7.4). (a and d) $\lambda = 365$ nm ± 5 nm (4 mW cm$^{-2}$), (b and e) $\lambda = 405$ nm ± 5 nm (10 mW cm$^{-2}$), (c and f) $\lambda = 430$ nm ± 5 nm (10 mW cm$^{-2}$). Asterisks indicate benzoic acid used as an internal standard.

Figure S2. Absorption spectra of PCIs. Absorption spectra of 10 µM PCI-1 (a) and PCI-2 (b) in 20 mM HEPES buffer (pH 7.4).
Figure S3. Time course of photolysis of PCIs at different wavelengths. 50 µM PCI-1 (blue) or PCI-2 (red) was illuminated at different wavelengths in 20 mM HEPES buffer (pH 7.4). The amount of remaining PCI was analyzed by HPLC. (a) $\lambda = 365$ nm ± 5 nm (4 mW cm$^{-2}$), (b) $\lambda = 405$ nm ± 5 nm (10 mW cm$^{-2}$). Error bars denote ± SD ($N = 3$).

Figure S4. Photostability of photolyzed product (CI-1). 50 µM CI-1 was illuminated in 20 mM HEPES buffer (pH 7.4). The remaining CI-1 was analyzed by HPLC. Light intensity; 4 mW cm$^{-2}$, $\lambda = 365$ nm ± 5 nm. Error bars denote ± SD ($N = 3$).
Figure S5. Photolysis of PCIs for cellular experiment. HPLC analyses of photolysis products by illumination of 100 µM PCI-1 (left) and PCI-2 (right) in HBSS. \( \lambda = 365 \) nm (10 mW cm\(^{-2}\)) after 10 min.

Figure S6. Confirmation of photolysis of PCIs in optical regulation of cellular CREB function. HPLC analyses of supernatant (top) and extracts (bottom) of cells incubated with PCIs in HBSS (1% DMSO), before and after illumination for 5 min (\( \lambda = 365 \) nm, 10 mW cm\(^{-2}\)).
Figure S7. Cytotoxicity of PCIs. Cytotoxicity LDH assay results for (a) PCI-1 and (b) PCI-2. Cells were treated with different concentrations of CBP inhibitor in DMEM (1% DMSO). Error bars denote ± SD (N = 3).

Figure S8. Cytotoxicity of PCIs with light illumination. Cytotoxicity LDH assays carried out on PCI-1 and PCI-2. Cells treated with 6 µM CBP inhibitor in HBSS (1% DMSO), with and without light illumination (λ = 365 nm (10 mW cm⁻²)) for 5 min. Error bars denote ± SD (N = 3).
Figure S9. Luciferase activity in the presence of different concentrations of (a) PCI-1 and (b) PCI-2. Error bars denote ± SD (N = 3).
Synthesis of compounds

Compounds 4, 7, and 8 were prepared using variants of previously reported procedures.\textsuperscript{1,2}

\begin{center}
\begin{tikzpicture}
\node[anchor=west] at (0,0) {\Huge O};
\node at (0,-0.5) {\Huge OH};
\end{tikzpicture}
\end{center}

Synthesis of compound 1

Acetic anhydride (945 mL, 10 mmol) was added dropwise to a stirred solution of 2,3-dihydroxynaphthalene (1.6 g, 10 mmol) and TEA (1 mL) in DMF (40 mL) at room temperature, with the reaction mixture then stirred at room temperature overnight. The solvent was then removed under reduced pressure, the crude residue diluted with water (100 mL) and then extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with brine (50 mL), dried over Na\textsubscript{2}SO\textsubscript{4} and the solvent removed under reduced pressure to afford a crude residue that was washed with DCM to afford compound 1 as a white solid (497 mg, 2.46 mmol, 25%). \textsuperscript{1}H NMR (500 MHz, DMSO-\textsubscript{d6}): \(\delta\) 10.11 (br, 1 H), 7.73 (m, 2H), 7.56 (m, 1H), 7.34 (m, 3H), 2.31 (s, 3H). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}): \(\delta\) 169.2, 145.9, 139.2, 132.5, 128.6, 127.5, 126.3, 126.2, 124.4, 120.0, 112.3, 21.1. HRMS (FAB\textsuperscript{+}) \textit{m/z}: Calcd for C\textsubscript{12}H\textsubscript{10}O\textsubscript{3} [M\textsuperscript{+}] 202.0630, found 202.0628.

\begin{center}
\begin{tikzpicture}
\node[anchor=west] at (0,0) {\Huge O};
\node at (0,-0.5) {\Huge NHBoc};
\end{tikzpicture}
\end{center}

Synthesis of compound 2

A solution of diethylazodicarboxylate (2.2 mol/L DEAD in toluene, 7.85 mL, 17.3 mmol) was added dropwise to a stirred solution of compound 1 (2.43 g, 12.0 mmol), Ph\textsubscript{3}P (4.72 g, 18.0 mmol) and 2-(\textit{tert}-butoxycarbonylamino)-1-ethanol (2.21 g, 13.7 mmol) in THF (40 mL) at 0 °C. The reaction mixture was then warmed to room temperature and stirred overnight. Solvent was removed under reduced pressure and the crude residue purified by flash silica chromatography [hexanes and ethyl acetate (9/1, v/v)] to give compound 2 as a white solid (1.59 g, 4.60 mmol, 38%). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) 7.73 (m, 1H), 7.71 (m, 1H), 7.49 (s, 1H), 7.43 (m, 1H), 7.37 (m, 1H), 7.22 (s, 1H), 5.01 (br, 1H), 4.20 (m, 2H), 3.56 (m, 2H), 2.37 (s, 3H), 1.45 (s, 9H). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}): \(\delta\) 169.5, 156.0, 149.0, 140.5, 132.5, 128.6, 127.3, 126.6, 126.3, 124.5, 120.3, 108.6, 79.5, 67.9, 39.9, 28.4, 20.7. HRMS (FAB\textsuperscript{+}) \textit{m/z}: Calcd for C\textsubscript{19}H\textsubscript{23}NO\textsubscript{5} [M\textsuperscript{+}] 345.1576, found 345.1576.
Synthesis of compound 3

Compound 2 (380 mg, 1.12 mmol) was dissolved in MeOH–THF (1:1, v/v, 10 mL) and 2 M NaOH aq. (10 mL) then added, with the reaction mixture then stirred at room temperature overnight. Solvent was removed under reduced pressure and the crude residue acidified with 2 M HCl (12 mL) at 0 °C, before being diluted with water (100 mL) and extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with brine (20 mL), dried over Na₂SO₄ and solvent removed under reduced pressure to obtain compound 3 as a white solid (203.9 mg, 0.672 mmol, 60%). ¹H NMR (500 MHz, DMSO-d₆): δ 9.07 (s, 1H), 7.67 (d 1H), 7.61 (m, 1H), 7.25 (m, 3H), 7.15 (m, 1H), 4.05 (m, 2H), 3.32 (m, 2H), 1.40 (s, 9H). ¹³C NMR (125 MHz, CDCl₃): δ 156.6, 146.7, 146.0, 123.0, 128.8, 126.4, 126.3, 124.4, 123.8, 109.7, 106.7, 80.0, 68.9, 40.1, 28.4. HRMS (FAB⁺) m/z: Calcd for C₁₄H₂₁O₄ [M]+ 303.1471, found 303.1465.

Synthesis of compound 4

4-Chloro-2-nitrobenzyl alcohol (1.88 g, 10.0 mmol), CBr₄ (4.64 g, 14.0 mmol) and Ph₃P (4.47 g, 17.0 mmol) in DCM (40 mL) were stirred at room temperature overnight. Solvent was removed under reduced pressure and the crude residue purified by flash silica chromatography [hexanes and ethyl acetate (9/1, v/v)] to give compound 4 as a yellow oil (1.95 g, 7.78 mmol, 78%). ¹H NMR (500 MHz, CDCl₃): δ 8.05 (d, J = 2.0 Hz, 1H), 7.59 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 7.53 (d, J = 8.0 Hz, 1H), 4.79 (s, 2H). ¹³C NMR (125 MHz, CDCl₃): δ 148.2, 135.4, 133.8, 133.6, 131.4, 125.7, 28.0. HRMS (EI⁺) m/z: Calcd for C₇H₅BrClNO₂ [M]+ 248.9192, found 248.9194.

Synthesis of compound 5

Compound 3 (151 mg, 0.500 mmol), compound 4 (150 mg, 0.598 mmol) and K₂CO₃ (83.6 mg, 0.605 mmol) were refluxed in THF (30 mL) overnight, with solvent then removed under reduced
pressure to afford a crude residue that was purified by flash silica chromatography [DCM and ethyl acetate (99/1, v/v)] to give compound 5 as a yellow solid (104 mg, 0.220 mmol, 44%). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.19 (d, \(J = 2.0\) Hz, 1H), 7.98 (d, \(J = 8.0\) Hz, 1H), 7.68 (m, 3H), 7.36 (m, 2H), 7.19 (d, \(J = 5.0\) Hz, 2H), 5.60 (s, 2H), 5.10 (s, 1H), 4.22 (m, 2H), 3.66 (m, 2H), 1.25 (s, 9H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 156.0, 148.6, 147.9, 147.3, 134.3, 134.1, 132.3, 130.0, 129.5, 129.1, 126.5, 126.4, 125.1, 124.8, 124.6, 109.2, 108.6, 79.6, 68.2, 67.3, 40.1, 28.4. HRMS (FAB\(^+\)) m/z: Calcd for C\(_{24}\)H\(_{26}\)ClN\(_2\)O\(_6\) [M+H]\(^+\) 473.1401, found 473.1487.

**Synthesis of compound 6**

TFA (4 mL) was added dropwise to a stirred solution of compound 5 (48.9 mg, 0.103 mmol) in DCM (4 mL) at room temperature and the reaction mixture stirred at room temperature overnight. Solvent was then removed under reduced pressure to obtain compound 6 as a yellow solid (49.3 mg, 0.101 mmol, 98%). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 9.82 (br, 3H), 7.94 (s, 1H), 7.66 (m, 2H), 7.59 (m, 2H), 7.38 (m, 2H), 7.18 (s, 1H), 7.15 (s, 1H), 5.46 (s, 2H), 4.36 (t, \(J = 4.5\) Hz, 2H), 3.61 (m 2H). \(^{13}\)C NMR (125 MHz, MeOD-d\(_4\)): \(\delta\) 148.5, 147.8, 134.3, 133.3, 131.2, 131.0, 129.8, 129.6, 126.3, 126.2, 124.7, 124.5, 109.2, 109.1, 67.3, 65.2, 39.0. HRMS (FAB\(^+\)) m/z: Calcd for C\(_{19}\)H\(_{18}\)ClN\(_2\)O\(_4\) [M+H]\(^+\) 373.0950, found 373.0961.

**Synthesis of Compound 7**

Diisopropyl azodicarboxylate (88 mL, 72 mmol) was added dropwise to a stirred solution of methyl 3-hydroxy-2-naphthoate (15.5 g, 76.7 mmol), 3-(tert-butoxycarbonylamino)-1-propanol (15.9 g, 90.7 mmol) and Ph\(_3\)P (22.7 g, 86.5 mmol) in dry THF (45 mL) at 0 °C under a N\(_2\) atmosphere and the resulting mixture stirred at room temperature overnight. Solvent was then removed under reduced pressure and the crude residue purified by flash silica chromatography [hexane-AcOEt (4/1, v/v)] to afford a solid that was washed with diethyl ether to afford compound 7 as a white solid (7.6 g, 21 mmol, 30%). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.40 (s, 1H), 7.83 (d, \(J = 8.0\) Hz, 1H), 7.72 (d, \(J = 8.0\) Hz, 1H), 7.52 (t, \(J = 8.0\) Hz, 1H), 7.38 (t, \(J = 8.0\) Hz, 1H), 7.20 (s, 1H), 5.93 (s, 1H), 4.22 (t, \(J = 5.5\) Hz, 2H), 3.97 (s, 3H), 3.44 (m, 2H), 2.10 (m, 2H), 1.46 (s, 9H).\(^{13}\)C NMR (125 MHz, DMSO-d\(_6\)): \(\delta\) 166.7, 156.1, 154.6, 136.1, 131.9, 128.9, 128.8, 127.5, 126.9, 124.9, 122.6, 108.2, 77.9, 66.7, 52.5, 37.6, 29.5, 28.7. HRMS (FAB\(^+\)) m/z: Calcd for C\(_{20}\)H\(_{26}\)NO\(_3\) [M+H]\(^+\) 360.1805, found 360.1806.
Synthesis of compound 8

2 M aq. NaOH (20 mL) was added to a solution of compound 7 (3.34 g, 9.29 mmol) in MeOH-THF (1/1, v/v, 40 mL) and the resulting mixture stirred at room temperature overnight. Solvent was removed under reduced pressure and the crude residue acidified with 2 M aq. HCl, extracted with DCM and the organic layer dried over Na₂SO₄. The organic layer was then evaporated under reduced pressure to afford a crude solid that was washed with diethyl ether to afford compound 8 as a white solid (2.69 g, 7.79 mmol, 84%). ¹H NMR (500 MHz, DMSO-d₆): δ 12.79 (s, 1H), 8.21 (s, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.54 (m, 1H), 7.41 (m, 2H), 6.91 (m, 1H), 4.14 (t, J = 6.0 Hz, 2H), 3.15 (m, 2H), 1.90 (m, 2H), 1.37 (s, 9H).¹³C NMR (125 MHz, DMSO-d₆): δ 167.9, 156.1, 154.6, 135.8, 131.9, 128.9, 128.8, 127.5, 126.9, 124.9, 122.6, 108.2, 77.9, 66.7, 52.5, 37.6, 29.5, 28.7.

Synthesis of PCI-1 (9)

PyBOP (18.3 mg, 0.0351 mmol) and DIPEA (10 µL) were added to a solution of compound 6 (15.8 mg, 0.0324 mmol) in DCM (6 mL) and the reaction mixture stirred at 0 °C for 5 min. DIPEA (10 µL) and compound 8 (14.0 mg, 0.0388 mmol) were then added at 0 °C and the stirred reaction mixture warmed to room temperature overnight. Solvent was removed under reduced pressure, then the crude residue diluted with water (100 mL) and extracted with DCM (2 × 100 mL). The combined organic layers were washed with brine (20 mL), dried over Na₂SO₄ and the organic solvent removed under reduced pressure to afford a crude residue. TFA (4 mL) was added dropwise to a stirred solution of this crude residue in DCM (4 mL) at room temperature and the reaction mixture then stirred at room temperature overnight. Solvent was then removed under reduced pressure and the crude residue purified by HPLC to obtain PCI-1 as a yellow solid (19.3 mg, 0.0270 mmol, 83%). ¹H NMR (500 MHz, CDCl₃): δ 10.64 (s, 1H), 8.85 (s, 1H), 8.53 (d, J = 9.0 Hz, 1H), 8.49 (br, 1H), 7.90 (br, 1H), 7.88
(d, J = 8.5 Hz, 1H), 7.81 (d, J = 8.5 Hz, 2H), 7.50 (m, 2H), 7.38 (m, 2H), 7.17 (s, 1H), 6.84 (s, 2H), 4.50 (t, J = 5.0 Hz, 2H), 4.33 (t, J = 5.0 Hz, 2H), 4.24 (t, J = 5.0 Hz, 2H), 3.23 (t, J = 5.0 Hz, 2H), 3.12 (q, J = 5.0 Hz, 2H), 2.18 (m, 2H), 1.32 (s, 2H).

13C NMR (125 MHz, MeOD-d4): δ 170.9, 155.3, 150.8, 150.2, 149.5, 137.5, 135.7, 133.7, 132.3, 131.9, 131.6, 130.0, 129.9, 128.4, 128.3, 128.1, 127.1, 126.6, 126.4, 126.3, 126.2, 118.8, 111.4, 111.0, 109.2, 69.2, 69.1, 69.1, 41.1, 40.4, 28.2, 1.4.

HRMS (FAB+) m/z: Calcd for C33H31ClN3O6 [M]⁺ 600.1896, found 600.1908.

Synthesis of compound 10

Compound 3 (90 mg, 0.30 mmol), 4,5-dimethoxy-2-nitrobenzyl bromide (99 mg, 0.36 mmol) and K2CO3 (83.6 mg, 0.605 mmol) were dissolved in THF (20 mL) and the reaction mixture refluxed overnight. Solvent was removed under reduced pressure and the crude residue purified by flash silica chromatography [hexanes and ethyl acetate (9/1, v/v)] to give compound 10 as a yellow solid (107 mg, 0.215 mmol, 72%). 1H NMR (500 MHz, CDCl3): δ 7.77 (s, 1H), 7.68 (m, 2H), 7.56 (s, 1H), 7.35 (m, 2H), 7.25 (s, 1H), 7.17 (s, 1H), 5.60 (s, 2H), 4.11 (s, 1H), 4.22 (m, 2H), 4.00 (s, 3H), 3.65 (br, 2H) 1.44 (s, 9H). 13C NMR (125 MHz, CDCl3): δ 155.8, 154.0, 148.5, 147.8, 138.9, 129.4, 129.3, 126.6, 126.4, 124.7, 124.6, 109.3, 108.9, 108.4, 108.0, 106.6, 79.8, 68.1, 67.5, 56.4, 56.3, 40.2, 28.4. HRMS (FAB+) m/z: Calcd for C26H31N2O8 [M+H]⁺ 499.2002, found 499.2072.

Synthesis of compound 11

TFA (5 mL) was added dropwise to a solution of compound 10 (107 mg, 0.215 mmol) in DCM (5 mL) at room temperature, with the reaction mixture then stirred at room temperature overnight, with solvent then removed under reduced pressure to afford compound 11 as a yellow solid (95.8 mg, 0.187 mmol, 87%). 1H NMR (500 MHz, CDCl3): δ 8.12 (m, 3H), 7.77 (m, 3H), 7.54 (s, 2H), 7.50 (s, 1H), 7.47 (s, 1H), 7.37 (m, 2H), 5.59 (s, 2H), 4.35 (m, 2H), 3.92 (s, 3H), 3.90 (s, 3H), 3.35 (m, 2H). 13C NMR (125 MHz, CDCl3): δ 154.0, 148.6, 147.9, 139.0, 129.4, 129.3, 126.6, 126.4, 125.1, 124.8, 124.7,
124.6, 109.3, 109.0, 108.5, 108.0, 67.6, 56.4, 56.3, 28.4, 28.3 HRMS (FAB\(^+\)) m/z: Calcd for C\(_{21}\)H\(_{23}\)N\(_2\)O\(_6\) [M+H]\(^+\) 399.1551 found 399.1550.

Synthesis of compound 12

DIPEA (10 µL) and PyBOP (63.7 mg, 0.122 mmol) were added to a solution of compound 11 (51.4 mg, 0.100 mmol) in DCM (25 mL) and the reaction mixture stirred at 0 °C for 5 min. DIPEA (10 µL) and compound 8 (41.2 mg, 0.120 mmol) was then added at 0 °C and the stirred reaction mixture then warmed to room temperature overnight. Solvent was removed under reduced pressure, the crude residue diluted with water (100 mL) and extracted with DCM (2 × 100 mL). The combined organic layers were washed with brine (20 mL), dried over Na\(_2\)SO\(_4\), the organic solvent removed under reduced pressure to afford a crude residue purified by flash silica chromatography [hexanes and ethyl acetate (9/1, v/v)] to give compound 12 as a yellow solid (44.2 mg, 0.0609 mmol, 61%).\(^1\)H NMR (500 MHz, DMSO-d\(_6\)): \(\delta\) 8.57 (m, 1H), 8.33 (m, 1H), 7.92 (d, \(J = 8.0\) Hz, 1H), 7.82 (m, 2H), 7.69 (s, 1H), 7.55 (m, 2H), 7.47 (m, 2H), 7.35 (m 3H), 6.83 (m, 1 H), 5.56 (s, 2H), 4.35 (t, \(J = 6.0\) Hz, 2H), 4.11 (t, \(J = 6.0\) Hz, 2H), 3.85 (m, 6H), 3.03 (m, 2H), 1.84 (m, 2H), 1.32 (m, 2H), 1.27 (s, 9H). \(^{13}\)C NMR (125 MHz, DMSO-d\(_6\)): \(\delta\) 165.7, 156.2, 154.0, 153.8, 148.9, 148.2, 148.0, 139.4, 135.6, 131.6, 129.7, 129.4, 128.9, 128.6, 128.3, 127.9, 126.9, 126.8, 125.0, 124.8, 124.6, 111.5, 109.0, 108.8, 107.8, 78.0, 67.4, 67.3, 66.3, 56.4, 56.3, 37.0, 29.3, 28.6. HRMS (FAB\(^+\)) m/z: Calcd for C\(_{40}\)H\(_{43}\)N\(_3\)O\(_{10}\)Na [M+Na]\(^+\) 748.2846, found 748.2857.
Synthesis of PCI-2 (13)

TFA (5 mL) was added dropwise to a stirred solution of compound 12 (44.2 mg, 0.0609 mmol) in DCM (5 mL) at room temperature with the reaction mixture then stirred at room temperature overnight. Solvent was removed under reduced pressure and the crude residue then purified by HPLC to obtain PCI-2 as a yellow solid (34.2 mg, 0.0463 mmol, 76%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.70 (m, 1H), 8.11 (s, 1H), 7.82 (m, 8H), 7.70 (s, 1H), 7.54 (m, 2H), 7.49 (m, 1H), 7.43 (s, 1H), 7.39 (m, 1H), 7.36 (m, 2H), 5.76 (s, 2H), 4.34 (m, 2H), 4.25 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.01 (s, 2H). $^{13}$C NMR (125 MHz, MeOD-$d_4$): $\delta$ 167.0, 153.8, 153.6, 148.9, 148.3, 148.1, 139.6, 135.3, 130.5, 129.7, 129.4, 128.6, 128.3, 127.9, 126.9, 126.8, 126.1, 124.9, 124.8, 124.7, 117.1, 110.8, 109.2, 109.0, 108.5, 107.8, 67.4, 67.1, 66.5, 56.4, 55.9, 37.4, 26.7. HRMS (FAB$^+$) $m/z$: Calcd for C$_{35}$H$_{36}$N$_3$O$_8$ [M+H]$^+$ 626.2497, found 626.2504.

Synthesis of compound 14

TFA (4 mL) added dropwise to a stirred solution of compound 2 (1.49 g, 4.31 mmol) in DCM (4 mL) at room temperature and the reaction mixture then stirred at room temperature overnight. Solvent was then removed under reduced pressure to obtain compound 14 as a white solid (1.49 g, 4.14 mmol, 96%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 9.67 (br, 2H), 7.68 (m, 2H), 7.44 (m, 2H), 7.37 (m, 1H), 7.12 (s, 1H), 4.32 (m, 2H), 3.34 (m, 2H), 2.29 (s, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 171.4, 147.2, 139.7, 132.2, 128.3, 127.3, 126.8, 126.7, 125.3, 120.6, 108.9, 64.1, 39.1, 20.6. HRMS (FAB$^+$) $m/z$: Calcd for C$_{14}$H$_{16}$NO$_3$ [M+H]$^+$ 246.1125, found 246.1133.
Synthesis of compound 15

PyBOP (2.02 g, 3.96 mmol) and DIPEA (680 µL) were added to a stirred solution of compound 14 (1.42 g, 3.96 mmol) in DCM (40 mL), with the reaction mixture then stirred at 0 °C for 5 min. 3-(3-((tert-butoxycarbonyl)amino)propoxy)-2-naphthoic acid (1.37 g, 3.96 mmol) was then added at 0 °C and the stirred reaction mixture warmed to room temperature overnight. Solvent was removed under reduced pressure, the crude residue diluted with water (100 mL) and extracted with DCM (2 × 100 mL) and the combined organic layers washed with brine (20 mL) and dried over Na₂SO₄. The organic solvent was removed under reduced pressure and the crude residue purified by HPLC to obtain compound 15 as a white solid (20.6 mg, 0.356 mmol, 9%). ¹H NMR (500 MHz, CDCl₃): δ 8.69 (s, 1H), 8.33 (m, 1H), 7.88 (m, 1H), 7.72 (m, 1H), 7.71 (m, 1H), 7.69 (m, 1H), 7.57 (m, 1H), 7.50 (m, 1H), 7.49 (m, 1H), 7.38 (m, 1H), 7.36 (m, 1H), 7.30 (m, 1H), 7.18 (s, 1H), 4.38 (t, J = 6.0 Hz 2H), 4.18 (t, J = 6.0 Hz, 2H), 4.00 (m, 2H), 3.18 (m, 2H), 2.24 (m, 3H), 1.93 (m, 2H), 1.35 (s, 9H) ¹³C NMR (125 MHz, CDCl₃): δ 169.4, 165.8, 156.1, 153.9, 149.2, 140.5, 135.7, 133.5, 132.5, 129.1, 128.2, 128.1, 127.2, 126.7, 126.3, 126.2, 124.6, 124.5, 122.7, 120.4, 129.0, 107.3, 67.9, 66.5, 53.4, 39.5, 37.6, 29.5, 28.3, 28.2, 20.5. HRMS (FAB⁺) m/z: Calcd for C₃₃H₃₆N₂O₇Na [M+Na]+ 595.2420, found 595.2434.
Synthesis of compound 16

2 M NaOH aq. (2 mL) was added to a stirred solution of compound 15 (57.3 mg, 0.10 mmol) in MeOH–THF (1:1, 2 mL, v/v) and the reaction mixture then stirred at room temperature overnight. The solvent was removed under reduced pressure to afford a crude residue that was acidified with 2 M HCl (3 mL) at 0 °C, diluted with water (10 mL) and then extracted with DCM (2 × 10 mL). The combined organic layers were washed with brine (20 mL), dried over Na₂SO₄ and concentrated to obtain compound 16 as a white solid (29.6 mg, 0.086 mmol, 86%). ¹H NMR (500 MHz, CDCl₃): δ 8.76 (s, 1H), 8.70 (m, 1H), 7.88 (m, 1H), 7.72 (m, 1H), 7.63 (m, 2H), 7.49 (m, 1H), 7.38 (m, 1H), 7.29 (m, 3H), 7.24 (s, 1H), 7.17 (s, 1H), 7.05 (s, 1H), 4.38 (t, J = 6.0 Hz 2H), 4.18 (t, J = 6.0 Hz, 2H), 4.00 (m, 2H), 3.18 (m, 2H), 2.24 (m, 3H), 1.93 (m, 2H), 1.35 (s, 9H). ¹³C NMR (125 MHz, CDCl₃): δ 166.2, 156.8, 153.8, 147.1, 146.2, 136.5, 135.7, 134.9, 134.0, 130.9, 129.8, 129.5, 129.3, 129.2, 128.9, 128.3, 128.2, 126.5, 126.3, 126.2, 125.3, 124.6, 124.3, 123.7, 122.5, 109.7, 107.8, 107.1, 106.8, 79.9, 68.6, 68.0, 67.3, 65.7, 39.7, 37.4, 30.9, 29.7, 28.4, 28.3, 25.6. HRMS (FAB⁺) m/z: Calcd for C₃₁H₃₄N₂O₆Na [M+Na]⁺ 553.2315, found 553.2325.

Synthesis of CI-1 (17, major product after photolysis of PCIs)

TFA (2 mL) was added dropwise to a stirred solution of compound 16 (53.1 mg, 0.10 mmol) in DCM (2 mL) at room temperature and the reaction mixture stirred overnight. Solvent was then removed under reduced pressure to obtain CI-1 as a white solid (19.9 mg, 0.036 mmol, 36%). ¹H NMR (500 MHz, MeOD-d₄): δ 8.09 (s, 1H), 7.82 (m, 2H), 7.67 (m, 1H), 7.60 (m, 1H), 7.53 (m, 1H), 7.42 (m, 1H), 7.39 (s, 1H), 7.29 (s, 1H), 7.26 (m, 2H), 7.18 (s, 1H), 4.34 (m, 4H), 3.95 (t, J = 5.0 Hz, 2H), 3.31 (m, 2), 3.18 (t, J = 6.0 Hz, 2H), 2.20 (m, 2H). ¹³C NMR (125 MHz, MeOD-d₄): δ 169.0, 153.2, 147.7, 146.4, 135.5, 129.9, 129.7, 129.1, 128.1, 127.8, 126.4, 126.2, 125.5, 125.4, 124.6, 123.9, 123.3, 109.7, 107.4, 107.1, 67.3, 66.9, 39.0, 38.3, 26.2. HRMS (FAB⁺) m/z: Calcd for C₂₆H₂₇N₂O₄ [M+H]⁺ 431.1965, found 431.1976.
References
1. McAllister, L. A.; Bechle, B. M.; Dounay, A. B.; Evrard, E.; Gan, X.; Ghosh, S.; Kim, J.-Y.; Parikh, V. D.; Tuttle, J. B.; Verhoest, P. R. A General Strategy for the Synthesis of Cyclic N-Aryl Hydroxamic Acids via Partial Nitro Group Reduction. *J. Org. Chem.* **2011**, *76*, 3484-3497.
2. Xie, F. C.; Li, B. X. B.; Kassenbrock, A.; Xue, C. H.; Wang, X. Y.; Qian, D. Z.; Sears, R. C.; Xiao, X. S. Identification of a Potent Inhibitor of CREB-Mediated Gene Transcription with Efficacious in Vivo Anticancer Activity. *J. Med. Chem.* **2015**, *58*, 5075-5087.
3 Pfaffl, M. W. A New Mathematical Model for Relative Quantification in Real-Time RTPCR. *Nucleic Acids Res.* **2001**, *29*, e45.
$^{1}H$ and $^{13}C$ NMR spectra
| Parameter          | Value          |
|--------------------|----------------|
| n                  | 3              |
| D1                 | 2.0000000000   |
| D1                  | 0.0000000000   |
| T0                  | 1              |
| CHANNEL 1         | 125.768300 MHz |
| P1                  | 100            |
| P1                  | 0.00 ppm       |
| CHANNEL 2         | 125.768300 MHz |
| P2                  | 100            |
| CHANNEL 3         | 125.768300 MHz |
| P3                  | 100            |
| CHANNEL 4         | 125.768300 MHz |
| P4                  | 100            |
| CHANNEL 5         | 125.768300 MHz |
| P5                  | 100            |
| CHANNEL 6         | 125.768300 MHz |
| P6                  | 100            |
| CHANNEL 7         | 125.768300 MHz |
| P7                  | 100            |
| CHANNEL 8         | 125.768300 MHz |
| P8                  | 100            |

Additionally, there are various other parameters related to the acquisition and processing of the data, such as solvent, acquisition time, and processing settings.
