Supporting Information

Inhibiting Ferroptosis through Disrupting the NCOA4-FTH1 Interaction: A New Mechanism of Action

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Table of Contents

1 Supporting Figures  S3
2 Experimental Section  S8
   Synthesis and Characterization  S8
   Cell Culture  S27
   Antibodies and Reagents  S27
   Cell Viability Assay  S28
   SiRNA-Mediated Knockdown of GPX4  S28
   Measurement/Imaging of Lipid ROS  S28
   Glutathione Measurements  S29
   DPPH Assay  S29
   Iron Chelating Studies  S29
   High-Content Imaging Studies  S29
   Localization of Intracellular Fe$^{2+}$  S30
   Western Blotting Analysis and Coimmunoprecipitation Assay  S30
   Pull-Down Assay  S31
   Chemical Labeling of 14d and Fluorescence Microscopy  S31
   qPCR  S32
   Protein Expression and Purification  S32
   Surface Plasmon Resonance Analysis  S32
   ELISA for Protein-Protein Interaction Study  S32
   Sprague-Dawley Rats  S33
   Transient Cerebral Ischemia Model in SD Rats  S33
   Post Surgery Neurological Evaluation  S34
   TTC Staining  S34
   H&E and Nissl Staining  S34
   MDA Assay  S34
   4-HNE Assay  S34
3 $^1$H and $^{13}$C NMR Spectra  S36
4 References  S63
1 Supporting Figures

**Figure S1.** Inhibitory activities of the indicated compounds against Erastin-induced ferroptosis in HT22 cells. Cells were treated with 1 μM Erastin in the absence or presence of the indicated concentrations of compounds for 24 h. Cell viability was assayed using a CCK-8 kit. Data shown represent the mean ± SEM from three independent experiments.

**Figure S2.** (A) Viability of HT22 cells cultured for 24 h in cystine-free media alone or in combination with 0.5 μM 9a, 0.5 μM Fer-1 or 20 μM DFO. (B) Effect of 9a on BSO-induced ferroptosis in HT22 cells. Cells were treated with 100 μM BSO in the absence
or presence of the indicated concentrations of 9a for 12 h. (C) Dose-response relationship for inhibition of ML210 (10 μM, 24 h)-induced death in HT22 cells by 9a, Fer-1 and DFO. (D) Morphology of HT22 cells treated with 1 μM RSL3 in the absence or presence of 0.5 μM Fer-1 or the indicated concentrations of 9a for 24 h. Scale bar, 100 μm. (E) Dose-response relationship for inhibition of Erastin (1 μM, 24 h), RSL3 (1 μM, 24 h) or ML210 (1 μM, 24 h)-induced death in HT-1080 cells by 9a. (F) Flow cytometry analyses of lipid ROS levels in HT-1080 cells. Cells were transfected with si-NC or si-GPX4 for 12 h, then treated with or without 0.5 μM 9a, 0.5 μM Fer-1 or 20 μM DFO for 36 h. (G) Effect of 9a on the lethality of various compounds in HT22 cells. Cells were pre-treated with the indicated concentrations of 9a for 0.5 h and then treated with 0.5 μM staurosporine (STS) for 24 h, 2 mM H₂O₂ for 12 h, or 20 ng/mL TNF-α (T), 20 nM SM-164 (S), 20 μM z-VAD-fmk (Z) for 12 h. Data shown represent the mean ± SEM from three independent experiments.

Figure S3. (A) Effect of 9a on the levels of GSH in HT22 cells. After treatment as indicated for 12 h, the contents of GSH were measured using the commercial assay kit. (B) Immunoblot analysis of HT22 cells treated as indicated for 12 h. (C) Quantification of the immunoblot analysis in B. Data shown represent the mean ± SEM from three independent experiments; **p < 0.01.
Figure S4. (A) Confocal imaging of LysoTracker Red (red) in HT22 cells treated with 0.5 μM 9a for 6 h. LysoTracker Red stains the lysosomes. (B) Quantification of the number of LysoTracker foci normalized to cell number in A. Approximately 1000 cells were measured per condition. (C) Confocal imaging of FerroOrange (red) in HT-1080 cells treated with 0.5 μM 9a or 50 μM DFO for 6 h. Nuclei were stained with Hoechst 33342 (blue). (D) Quantification of the fluorescence intensity of FerroOrange. (E) Confocal imaging of FerroOrange (red) and LysoTracker Green (green) in HT-1080 cells treated with 0.5 μM 9a for 6 h. The colocalized foci are indicated by white arrows and shown in an enlarged image of the yellow box. (F) Quantification of the fluorescence intensity of FerroOrange colocalized with LysoTracker Green per cell in E. Approximately 150 cells were measured per condition. (G) Confocal imaging of LysoTracker Red (red) in HT-1080 cells treated with 0.5 μM 9a for 6 h. (H) Quantification of the number of LysoTracker foci normalized to cell number in G. Approximately 1000 cells were measured per condition. (B, D and H) Data shown represent the mean ± SEM from three independent experiments; ***p < 0.001.
Figure S5. (A) Excitation and emission spectra of 9a. (B) Confocal microscopy analysis of the distribution of 9a in live cells. HT22 or HT-1080 cells were incubated with 10 μM 9a in the absence or presence of 1 μM RSL3 for 2 h. (C) Quantification of 9a-positive vesicles colocalizing with GFP-LC3. At least 400 cells were measured per condition. Data shown represent the mean ± SEM from three independent experiments. (D) Structures of compounds 9a and 14d. (E) Chemical strategy to label small molecules in cells. (F) Confocal images showing the subcellular localization of labeled 14d (red). HT22 cells were treated with 10 μM 14d for 2 h. The autophagosomes were detected using antibody against LC3. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, blue). 14d was labeled by means of click chemistry as described in experimental section.

Figure S6. 9a does not colocalize with mitochondria or ER in live cells. Confocal imaging of 9a (magenta) in (A) HT22 or (B) HT-1080 cells. Cells were incubated with 10 μM 9a for 2 h. Mito-Tracker Green or Mito-Tracker Deep Red stains the
mitochondria. ER-Tracker Red stains the ER.

Figure S7. (A) Confocal imaging of A549 cells stably expressing GFP-LC3 upon different treatments. Cells were treated with 25 μM CQ or the indicated concentrations of 9a for 6 h. (B) Quantification of the number of GFP-LC3 puncta per cell. Approximately 200 cells were measured per condition. Data shown represent the mean ± SEM from three independent experiments; ***p < 0.001. (C) Immunoblot analysis of HT22 cells treated as indicated for 6 h. (D) Quantification of the immunoblot analysis in C. Data shown represent the mean ± SEM from three independent experiments; **p < 0.01. (E) Dose-response relationship for inhibition of RSL3 (1 μM, 24 h)-induced death in HT22 cells by Biotin-14d or 14d.
2 Experimental Section

2.1 Synthesis and Characterization

$^1$H and $^{13}$C NMR spectra were recorded on a Bruker Ascend\textsuperscript{TM} 400 or Ascend\textsuperscript{TM} 500 spectrometer. The coupling constant values ($J$) are given in Hz. The abbreviations used in NMR data are mentioned as, singlet = s, doublet = d, triplet = t, multiplet = m, double doublet = dd, and broad singlet = brs. Mass spectra (MS) were recorded on a Shimadzu LCMS-2010A instrument with an ESI detector. High-resolution mass spectra (HRMS) were recorded on a Shimadzu LCMS-IT-TOF mass spectrometer. Flash column chromatography was performed using silica gel (200-300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. All reaction progress was monitored by thin layer chromatography (TLC) on precoated silica gel GF254 (Qingdao Haiyang Chemical Co. Ltd) and the spots were detected under UV light (254 nm). All products had purification values of > 95%. All reagents and solvents were of analytical reagent grade and used without further purification unless specified. No unexpected or unusually high safety hazards were encountered.

Scheme S1. Synthesis of 4a-4h

Reagents and conditions: (a) $\text{K}_2\text{CO}_3$, DMF, 110 °C, 16 h; (b) Ni, $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$, MeOH, 60 °C, 2 h; (c) 1. TBTU, DIPEA, DMF, r.t., 6 h; 2. AcOH, reflux, overnight.

General procedure for the synthesis of 1a-1d: 5-chloro-2-nitroaniline (997.6 mg, 5.8 mmol), cyclic amines (6.9 mmol), and $\text{K}_2\text{CO}_3$ (1.28 g, 9.3 mmol) were added into dry DMF (10 mL). The mixture was stirred at 110 °C for 16 h. After cooling to room temperature, water was added and the mixture was extracted with EtOAc. The organic layer was dried over anhydrous Na$_2$SO$_4$ and concentrated. The crude solid was purified through flash column chromatography with EtOAc (0-30%) in CH$_2$Cl$_2$ to afford 1a-1d as a bright yellow solid.

5-(4-Methylpiperazin-1-yl)-2-nitroaniline (1a): Following the general procedure, 1a was obtained from the reaction of 1-methylpiperazine with 5-chloro-2-nitroaniline in
71% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.01 (d, J = 9.7 Hz, 1H), 6.28 (dd, J = 9.7, 2.8 Hz, 1H), 6.13 (s, 2H), 5.95 (d, J = 2.8 Hz, 1H), 3.37 (t, J = 5.2 Hz, 4H), 2.52 (t, J = 5.2 Hz, 4H), 2.34 (s, 3H). ESI-MS m/z: 237.1 [M+H]+.

5-(4-Methylpiperidin-1-yl)-2-nitroaniline (1b): Following the general procedure, 1b was obtained from the reaction of 4-methylpiperidine with 5-chloro-2-nitroaniline in 76% yield. ¹H NMR (500 MHz, CDCl₃) δ 7.91 (d, J = 9.5 Hz, 1H), 6.36 (d, J = 9.5 Hz, 1H), 6.16 (s, 1H), 3.96 (d, J = 13.0 Hz, 2H), 2.92 (t, J = 12.6 Hz, 2H), 1.75 (d, J = 13.2 Hz, 2H), 1.71 - 1.63 (m, 1H), 1.28 - 1.18 (m, 2H), 0.99 (d, J = 6.4 Hz, 3H). ESI-MS m/z: 222.1 [M+H]+.

2-Nitro-5-(piperidin-1-yl)aniline (1c): Following the general procedure, 1c was obtained from the reaction of piperidine with 5-chloro-2-nitroaniline in 83% yield. ¹H NMR (500 MHz, CDCl₃) δ 7.91 (d, J = 9.7 Hz, 1H), 6.19 (dd, J = 9.7, 1.2 Hz, 1H), 6.08 (s, 2H), 5.85 (d, J = 2.5 Hz, 1H), 3.32 - 3.27 (m, 4H), 1.61 - 1.54 (m, 6H). ESI-MS m/z: 224.1 [M+H]+.

5-Morpholino-2-nitroaniline (1d): Following the general procedure, 1d was obtained from the reaction of morpholine with 5-chloro-2-nitroaniline in 79% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.03 (d, J = 9.7 Hz, 1H), 6.27 (dd, J = 9.7, 2.6 Hz, 1H), 6.13 (s, 2H), 5.95 (d, J = 2.6 Hz, 1H), 3.85 - 3.80 (m, 4H), 3.33 - 3.28 (m, 4H). ESI-MS m/z: 224.1 [M+H]+.

General procedure for the synthesis of 4a-4h: The mixture of intermediates 1a-1d (5.0 mmol), hydrazine hydrate (5 mL) and a catalytic amount of Ni in MeOH (30 mL) was stirred at 60 °C for 2 h. After cooling to room temperature, the solution was filtrated and the filtrate was evaporated to dryness under reduced pressure to give intermediates 2a-2d as black solids (90-95%). The intermediates 3a-3b were synthesized according to our previous report.¹ The reactions of intermediates 3a-3b and 2a-2d were performed following the previously reported methods with slight modification.¹ Briefly, to a solution of 3a-3b (1.0 mmol) in DMF (5 mL), DIPEA (0.21mL, 1.2 mmol) and TBTU (385 mg, 1.2 mmol) was added. The resulting mixture was stirred at 0 °C for 0.5 h. Then the desired diaminobenzene derivatives 2a-2d (1.1 mmol) was added. The mixture was stirred at room temperature for 6 h and then quenched with ice water. The
precipitated solid was filtered, washed with water, and dried in vacuo to afford the appropriate amide, which was used in the next step without further purification. Next, AcOH (10 mL) was added and the mixture was refluxed overnight. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was purified through flash column chromatography.

2-(3-(6-(4-Methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (4a): Following the general procedure above, 4a was obtained from the reaction of 2a with 3a as a white solid in 43% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.74 (s, 1H), 7.41 - 7.28 (m, 3H), 7.21 - 7.10 (m, 4H), 7.07 - 6.88 (m, 3H), 4.47 (s, 2H), 3.62 (t, $J$ = 5.4 Hz, 2H), 3.23 (s, 4H), 2.98 (t, $J$ = 5.2 Hz, 2H), 2.65 (s, 4H), 2.39 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 150.6, 150.4, 148.0, 137.9, 135.8, 134.7, 134.3, 131.2, 129.5, 128.4, 126.6, 126.3, 125.9, 118.8, 115.7, 115.5, 113.5, 111.7, 97.1, 54.9 (2C), 50.2, 49.7, 49.7, 45.8, 45.6, 28.2. HRMS (ESI): calcd for C$_{27}$H$_{29}$N$_5$ [M+H]$^+$ 424.2496, found 424.2521.

2-(3-(6-(4-Methylpiperidin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (4b): Following the general procedure above, 4b was obtained from the reaction of 2b with 3a as a white solid in 50% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 7.72 (s, 1H), 7.53 - 7.43 (m, 2H), 7.35 (t, $J$ = 7.3 Hz, 1H), 7.27 - 7.16 (m, 4H), 7.06 (d, $J$ = 5.8 Hz, 1H), 6.99 - 6.85 (m, 2H), 4.47 (s, 2H), 3.66 - 3.52 (m, 4H), 2.96 (s, 2H), 2.69 - 2.56 (m, 2H), 1.75 - 1.66 (m, 2H), 1.52 - 1.42 (m, 1H), 1.35 - 1.24 (m, 2H), 0.94 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 150.4, 148.5, 143.7, 139.1, 137.8, 134.7, 134.4, 131.1, 129.6, 128.4, 126.6, 126.3, 126.0, 123.4, 123.1, 115.7, 115.6, 111.8, 99.6, 50.9, 49.7 (2C), 45.6, 34.0 (2C), 30.2, 28.2, 21.9. HRMS (ESI): calcd for C$_{28}$H$_{30}$N$_4$ [M+H]$^+$ 423.2543, found 423.2548.

2-(3-(6-(Piperidin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (4c): Following the general procedure above, 4c was obtained from the reaction of 2c with 3a as a white solid in 48% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 7.60 (s, 1H), 7.35 (d, $J$ = 6.2 Hz, 2H), 7.23 (t, $J$ = 6.4 Hz, 1H), 7.17 - 6.92 (m, 6H), 6.80 (s, 1H), 4.36 (s, 2H), 3.50 (s, 6H), 2.84 (s, 2H), 1.59 - 1.51 (m, 4H), 1.44 - 1.40 (m, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 150.6, 150.5, 149.1, 137.8, 135.8,
134.8, 134.4, 131.2, 129.7, 128.5, 126.4, 126.1, 118.9, 115.9, 115.6, 114.5, 111.8, 97.7, 52.1, 51.6, 49.8, 45.7, 28.3, 25.9, 25.7, 24.0. HRMS (ESI): calcd for C_{27}H_{32}N_{4} [M+H]^+ 409.2387, found 409.2390.

4-(2-(3-(3,4-Dihydroisoquinolin-2(1H)-yl)phenyl)-1H-benzo[d]imidazol-6-yl)morpholine (4d): Following the general procedure above, 4d was obtained from the reaction of 2d with 3a as a white solid in 43% yield. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.71 (s, 1H), 7.48 (t, \(J = 8.1\) Hz, 1H), 7.34 (t, \(J = 8.0\) Hz, 1H), 7.27 - 7.23 (m, 1H), 7.21 - 7.11 (m, 5H), 6.99 - 6.88 (m, 2H), 4.47 (s, 2H), 3.79 - 3.71 (m, 4H), 3.61 (t, \(J = 5.8\) Hz, 2H), 3.12 - 3.04 (m, 4H), 2.95 (t, \(J = 5.7\) Hz, 2H). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 150.9, 150.1, 147.5, 138.7, 134.4, 134.1, 131.7, 131.0, 129.3, 129.1, 128.7, 127.9, 127.0, 126.1, 125.9, 125.5, 115.6, 115.2, 111.8, 66.0 (2C), 50.1 (2C), 49.5, 45.3, 27.9. HRMS (ESI): calcd for C_{26}H_{28}N_{4} [M+H]^+ 411.2179, found 411.2196.

2-(6-(6-(4-Methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline (4e): Following the general procedure above, 4e was obtained from the reaction of 2a with 3b as a white solid in 45% yield. \(^1\)H NMR (400 MHz, CD_{3}OD) \(\delta\) 7.63 - 7.56 (m, 2H), 7.36 - 7.31 (m, 2H), 7.26 - 7.21 (m, 4H), 7.13 (d, \(J = 8.9\) Hz, 1H), 6.93 (d, \(J = 8.5\) Hz, 1H), 4.88 (s, 2H), 4.03 (t, \(J = 5.2\) Hz, 2H), 3.44 - 3.37 (m, 4H), 3.09 - 3.02 (m, 6H), 2.70 (s, 3H). \(^{13}\)C NMR (100 MHz, CD_{3}OD) \(\delta\) 159.7, 153.0, 149.2, 147.1, 139.5, 136.6, 135.7, 129.4, 127.6, 127.4, 127.2, 125.3, 125.2, 118.7, 117.0, 112.4, 110.8, 108.8, 55.6 (2C), 50.8 (2C), 48.0, 45.0, 43.6, 29.9. HRMS (ESI): calcd for C_{26}H_{28}N_{6} [M+H]^+ 425.2448, found 425.2450.

2-(6-(6-(4-Methylpiperidin-1-yl)-1H-benzo[d]imidazol-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline (4f): Following the general procedure above, 4f was obtained from the reaction of 2b with 3b as a white solid in 50% yield. \(^1\)H NMR (400 MHz, CDCl_{3}) \(\delta\) 7.75 (d, \(J = 7.3\) Hz, 1H), 7.60 - 7.52 (m, 2H), 7.24 - 7.13 (m, 5H), 7.05 (d, \(J = 8.3\) Hz, 1H), 6.69 (d, \(J = 8.5\) Hz, 1H), 4.76 (s, 2H), 3.89 (t, \(J = 5.8\) Hz, 2H), 3.60 (d, \(J = 12.0\) Hz, 2H), 2.95 (t, \(J = 5.7\) Hz, 2H), 2.73 (t, \(J = 11.2\) Hz, 2H), 1.75 (d, \(J = 11.2\) Hz, 2H), 1.55 - 1.37 (m, 3H), 0.98 (d, \(J = 5.8\) Hz, 3H). \(^{13}\)C NMR (100 MHz, CDCl_{3}) \(\delta\) 158.0, 150.0, 149.3, 144.4, 138.5, 135.3, 134.2, 128.4, 126.7, 126.5, 126.3, 116.7, 116.4, 116.3, 110.8, 108.2, 101.1, 101.1, 51.8, 47.2, 42.6, 34.1 (2C), 30.6, 29.8, 29.0, 21.9.
HRMS (ESI): calcd for C_{27}H_{29}N_{5} [M+H]^+ 424.2496, found 424.2516.

2-(6-(6-(Piperidin-1-yl)-1H-benzo[d]imidazol-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline (4g): Following the general procedure above, 4g was obtained from the reaction of 2c with 3b as a white solid in 48% yield. \(^1\)H NMR (500 MHz, CD_{3}OD) \(\delta\) 7.64 (t, 1H), 7.54 - 7.47 (m, 2H), 7.28 - 7.10 (m, 5H), 7.09 - 7.02 (m, 1H), 6.86 - 6.80 (m, 1H), 4.80 (s, 2H), 3.95 (t, \(J = 5.8\) Hz, 2H), 3.11 (s, 4H), 2.96 (t, \(J = 5.5\) Hz, 2H), 1.80 - 1.69 (m, 4H), 1.62 - 1.53 (m, 2H). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 157.8, 150.0, 149.1, 146.6, 146.6, 138.4, 138.0, 135.5, 135.1, 134.5, 128.4, 126.5, 126.2, 125.9, 119.2, 114.7, 109.2, 106.9, 97.8, 51.3 (2C), 46.6, 41.9, 28.1, 25.6 (2C), 23.9.

HRMS (ESI): calcd for C_{26}H_{27}N_{5} [M+H]^+ 410.2339, found 410.2349.

4-(2-(6-(3,4-Dihydroisoquinolin-2(1H)-yl)pyridin-2-yl)-1H-benzo[d]imidazol-6-yl)morpholine (4h): Following the general procedure above, 4h was obtained from the reaction of 2d with 3b as a white solid in 56% yield. \(^1\)H NMR (400 MHz, CDCl_{3}) \(\delta\) 7.74 - 7.61 (m, 3H), 7.25 - 7.18 (m, 4H), 7.01 (d, \(J = 7.9\) Hz, 2H), 6.75 (d, \(J = 8.4\) Hz, 1H), 4.79 (s, 2H), 3.95 (t, \(J = 5.9\) Hz, 2H), 3.91 (t, \(J = 4.6\) Hz, 4H), 3.19 (t, \(J = 4.4\) Hz, 4H), 3.02 (t, \(J = 5.8\) Hz, 2H). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 157.9, 150.3, 148.3, 146.6, 138.5, 138.4, 135.5, 135.1, 134.6, 128.5, 126.6, 126.3, 126.0, 119.3, 113.5, 109.3, 107.1, 97.3, 66.3 (2C), 50.5, 50.0 (2C), 46.5, 28.1. HRMS (ESI): calcd for C_{25}H_{25}N_{3}O [M+H]^+ 412.2151, found 412.2151.

**Scheme S2. Synthesis of 9a-9k**

**Reagents and conditions:** (a) MeSO_{3}H, NaN_{3}, CH_{2}Cl_{2}, 0 °C to r.t., overnight; (b) AlLiH_{4}, THF, 0 °C to reflux, 4 h; (c) K_{2}CO_{3}, Cul, L-proline, DMSO, 80 °C, 24 h, N_{2};
(d) LiOH, THF, 90 °C, 3 h; (e) 1. TBTU, DIPEA, DMF, r.t., 6 h; 2. AcOH, reflux, overnight. 9j-9k were obtained via the demethylation of 9h-9i.

The intermediates 5a-5b, 6a-6b, 7a and 7c were synthesized according to our previous report. 1

**General procedure for the synthesis of 5a-5d:** 7-methoxy-2,3-dihydroinden-1-one (4.31 g, 26.6 mmol) or 5,7-dimethoxy-2,3-dihydroinden-1-one (5.11 g, 26.6 mmol) was dissolved in the mixed solvent of CH₂Cl₂ (40 mL) and MeSO₃H (methanesulfonic acid, 40 mL). NaN₃ (3.46 g, 53.2 mmol) was added slowly in an ice-water bath. Then the mixture was stirred overnight at room temperature. In an ice-water bath, 20% NaOH (aq) was added to make the solution neutral. The aqueous solution was extracted with CH₂Cl₂ and the obtained organic layer was combined, washed with brine, dried over Na₂SO₄ and concentrated. The residue was purified by flash column chromatography with EtOAc/hexane (2:1) elution.

**8-Methoxy-3,4-dihydroisoquinolin-1(2H)-one (5c):** Following the general procedure above, 5c was obtained as a white solid in 61% yield. ¹H NMR (400 MHz, CD₃OD) δ 7.41 (dd, J = 8.4, 7.6 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 6.86 (dd, J = 7.6, 0.8 Hz, 1H), 3.85 (s, 3H), 3.37 (t, J = 6.4 Hz, 2H), 2.90 (t, J = 6.4 Hz, 2H). ESI-MS m/z: 178.0 [M+H]+.

**6,8-Dimethoxy-3,4-dihydroisoquinolin-1(2H)-one (5d):** Following the general procedure above, 5d was obtained as a white solid in 63% yield. ¹H NMR (400 MHz, CD₃OD) δ 6.49 (s, 1H), 6.44 (s, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.36 (t, J = 6.4 Hz, 2H), 2.87 (t, J = 6.4 Hz, 2H). ESI-MS m/z: 208.0 [M+H]+.

**General procedure for the synthesis of 6a-6d:** To the mixture of 5c (1.77 g, 10 mmol) or 5d (2.07 g, 10 mmol) in dry THF (50 mL), a solution of LiAlH₄ in dry THF (1 mol/L, 20 mL) was added dropwise in an ice-water bath. The reaction mixture was stirred at reflux for 4 h, and then quenched by the sequential addition of THF and 30% NaOH solution in an ice-water bath. The mixture was filtered through celite and washed with MeOH. The residue was concentrated under reduced pressure and purified by flash column chromatography with EtOAc/MeOH (10:1) elution.

**8-Methoxy-1,2,3,4-tetrahydroisoquinoline (6c):** Following the general procedure
above, *6c* was obtained as a white oil in 51% yield. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.10 (t, $J = 8.5$ Hz, 1H), 6.73-6.61 (m, 2H), 3.94 (s, 2H), 3.79 (s, 3H), 3.35 (t, $J = 6.4$ Hz, 2H), 3.01 (t, $J = 6.4$ Hz, 2H). ESI-MS m/z: 164.1 [M+H]$^+$.  

**6,8-Dimethoxy-1,2,3,4-tetrahydroisoquinoline (6d):** Following the general procedure above, *6d* was obtained as a white oil in 54% yield. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 6.46 (d, $J = 1.8$ Hz, 1H), 6.41 (s, 1H), 4.10 (s, 2H), 3.85 (s, 3H), 3.81 (s, 3H), 3.37 (t, $J = 6.4$ Hz, 2H), 3.02 (t, $J = 6.4$ Hz, 2H). ESI-MS m/z: 194.1 [M+H]$^+$.  

**General procedure for the synthesis of 7a-7i:** To a dried sealed vessel were added 1,2,3,4-tetrahydroisoquinoline derivatives (20 mmol), anhydrous K$_2$CO$_3$ (8.34 g, 60 mmol), CuI (0.8 g, 4 mmol), L-proline (0.92 g, 8 mmol), ethyl 3-iodobenzoate (5.52 g, 20 mmol), and DMSO (25 mL). The mixture was stirred at 80°C for 24 h under N$_2$ environment. Upon consumption of the starting material, the reaction mixture was cooled down to room temperature, poured into ice cold water, and extracted with EtOAc. The combined organic layer was dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography with hexane/EtOAc (5:1) to provide the intermediates *7a-7i* as a white oil.  

**Ethyl 3-(6-chloro-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (7b):** Following the general procedure, *7b* was obtained from the reaction of 6-chloro-1,2,3,4-tetrahydroisoquinoline with ethyl 3-iodobenzoate in 59% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.63 (s, 1H), 7.50 (d, $J = 7.6$ Hz, 1H), 7.33 (t, $J = 7.9$ Hz, 1H), 7.19 - 7.16 (m, 2H), 7.15 - 7.09 (m, 2H), 4.42 - 4.35 (m, 4H), 3.59 (t, $J = 5.9$ Hz, 2H), 2.97 (t, $J = 5.7$ Hz, 2H), 1.40 (t, $J = 7.1$ Hz, 3H). ESI-MS m/z: 316.1 [M+H]$^+$.  

**Ethyl 3-(6-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (7d):** Following the general procedure, *7d* was obtained from the reaction of 1,2,3,4-tetrahydroisoquinolin-6-ol with ethyl 3-iodobenzoate in 56% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.62 (s, 1H), 7.48 (d, $J = 7.6$ Hz, 1H), 7.32 (t, $J = 7.9$ Hz, 1H), 7.12 (dd, $J = 8.2$, 2.0 Hz, 1H), 7.04 (d, $J = 8.2$ Hz, 1H), 6.69 (dd, $J = 8.2$, 2.0 Hz, 1H), 6.65 (s, 1H), 4.41 - 4.36 (m, 4H), 3.57 (t, $J = 5.8$ Hz, 2H), 2.94 (t, $J = 5.7$ Hz, 2H), 1.40 (t, $J = 7.1$ Hz, 3H). ESI-MS m/z: 298.1 [M+H]$^+$.  

**Ethyl 3-(7-methyl-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (7e):** Following the
general procedure, 7e was obtained from the reaction of 7-methyl-1,2,3,4-tetrahydroisoquinoline with ethyl 3-iodobenzoate in 55% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.65 (s, 1H), 7.50 (d, $J = 7.5$ Hz, 1H), 7.35 (t, $J = 7.9$ Hz, 1H), 7.17 - 7.12 (m, 2H), 6.89 - 6.83 (m, 1H), 6.81 (s, 1H), 4.44 - 4.37 (m, 4H), 3.61 (t, $J = 5.8$ Hz, 2H), 3.00 (t, $J = 5.7$ Hz, 2H), 1.61 (s, 3H), 1.42 (t, $J = 7.2$ Hz, 3H). ESI-MS m/z: 296.1 [M+H]$^+$.  

**Ethyl 3-(8-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (7f):** Following the general procedure, 7f was obtained from the reaction of 6c with ethyl 3-iodobenzoate in 62% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.23 (s, 1H), 8.12 (d, $J = 7.8$ Hz, 1H), 7.84 (dd, $J = 8.2$, 2.2 Hz, 1H), 7.67 (t, $J = 8.0$ Hz, 1H), 7.06 (t, $J = 7.9$ Hz, 1H), 6.71 (d, $J = 8.1$ Hz, 1H), 6.63 (d, $J = 7.7$ Hz, 1H), 4.58 (s, 2H), 4.31 (q, $J = 7.1$ Hz, 2H), 3.90 (t, $J = 5.8$ Hz, 2H), 1.40 (t, $J = 7.1$ Hz, 3H). ESI-MS m/z: 312.1 [M+H]$^+$.  

**Ethyl 3-(8-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (7g):** Following the general procedure, 7g was obtained from the reaction of 1,2,3,4-tetrahydroisoquinolin-8-ol with ethyl 3-iodobenzoate in 67% yield. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.23 (s, 1H), 8.12 (d, $J = 7.8$ Hz, 1H), 7.84 (dd, $J = 8.2$, 2.2 Hz, 1H), 7.67 (t, $J = 8.0$ Hz, 1H), 7.06 (t, $J = 7.9$ Hz, 1H), 6.71 (d, $J = 8.1$ Hz, 1H), 6.63 (d, $J = 7.7$ Hz, 1H), 4.42 (q, $J = 7.1$ Hz, 2H), 3.99 (t, $J = 6.4$ Hz, 2H), 3.25 (s, 2H), 3.13 (t, $J = 6.3$ Hz, 2H), 1.40 (t, $J = 7.1$ Hz, 3H). ESI-MS m/z: 298.1 [M+H]$^+$.  

**Ethyl 3-(6,8-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (7h):** Following the general procedure, 7h was obtained from the reaction of 6d with ethyl 3-iodobenzoate in 54% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.68 (s, 1H), 7.47 (d, $J = 7.5$ Hz, 1H), 7.32 (t, $J = 7.9$ Hz, 1H), 7.21 - 7.14 (m, 1H), 6.34 (s, 1H), 6.30 (s, 1H), 4.38 (q, $J = 7.1$ Hz, 2H), 4.29 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.57 (t, $J = 5.7$ Hz, 2H), 2.94 (t, $J = 5.5$ Hz, 2H), 1.40 (t, $J = 7.1$ Hz, 3H). ESI-MS m/z: 342.1 [M+H]$^+$.  

**Ethyl 3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (7i):** Following the general procedure, 7i was obtained from the reaction of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline with ethyl 3-iodobenzoate in 60% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.66 - 7.62 (m, 1H), 7.50 - 7.46 (m, 1H), 7.32 (t, $J = 7.9$ Hz, 1H), 7.16 - 7.10
General procedure for the synthesis of 9a-9k: To the mixture of 7a-7i (10 mmol) in THF (50 mL), LiOH (1.19 g, 50 mmol) dissolved in the solution of EtOH/H₂O (5:1, 5 mL) was added. After stirred at 90 °C for 3 h, the solvent was evaporated in vacuo. A small amount of ice-water was added to dissolve the residue. 1N HCl aqueous solution was added slowly to make the solution neutral. The resulting precipitate was isolated by filtration and dried under vacuum to afford the desired intermediates 8a-8i, which were used in the next step without further purification. The reaction of 8a-8i and 2a was performed to afford the desired products 9a-9i according to the procedure for the synthesis of 4a-4h. 9j and 9k was obtained via the demethylation of 9h and 9i, respectively. Briefly, to a solution of 9h or 9i (0.12 mmol) in dry CH₂Cl₂ (15 mL), BBr₃ (0.11 mL, 1.2 mmol) was added dropwise at -20 °C under N₂. The reaction mixture was then allowed to warm to room temperature and stirred for another 4 h until complete, as indicated by TLC. The reaction was carefully mixed with saturated aqueous NaHCO₃ at 0 °C to make the solution neutral. The mixture was extracted with EtOAc twice, and the organic layer was combined and dried over anhydrous Na₂SO₄. The residue was filtered, concentrated, and purified by flash column chromatography with CH₂Cl₂/MeOH (6:1) elution to provide 9j or 9k.

6-Fluoro-2-(3-(6-(4-methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (9a): 9a was obtained from the reaction of 8a with 2a as a yellow solid in 48% yield. ¹H NMR (400 MHz, CD₃OD) δ 7.65 (t, J = 1.8 Hz, 1H), 7.42 (d, J = 8.8 Hz, 1H), 7.37 (m, 1H), 7.31 (t, J = 7.9 Hz, 1H), 7.15 (dd, J = 9.4, 5.6 Hz, 1H), 7.07 - 7.03 (m, 2H), 6.98 (dd, J = 8.8, 2.2 Hz, 1H), 6.87 - 6.80 (m, 2H), 4.39 (s, 2H), 3.57 (t, J = 6.0 Hz, 2H), 2.94 (m, 6H), 2.85 (s, 4H), 2.48 (s, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 162.8 (d, J = 242.8 Hz), 153.7, 152.3, 149.1, 138.4 (d, J = 7.7 Hz), 131.7, 131.4 (d, J = 2.6 Hz), 130.9 (2C), 130.6, 129.3 (d, J = 8.1 Hz), 117.9, 117.7, 116.7, 115.6 (d, J = 21.1 Hz), 114.1 (2C), 114.0 (d, J = 21.8 Hz), 102.7, 55.7 (2C), 51.0, 50.9, 47.3, 45.1, 43.5, 30.0. HRMS (ESI): calcd for C₂₇H₂₈N₅F [M+H]+ 442.2402,
6-Chloro-2-(3-(6-(4-methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (9b): 9b was obtained from the reaction of 8b with 2a as a yellow solid in 50% yield. $^1$H NMR (400 MHz, CD$_3$OD) δ 7.71 (s, 1H), 7.52 (d, $J = 8.8$ Hz, 1H), 7.46 (d, $J = 7.6$ Hz, 1H), 7.38 (t, $J = 8.0$ Hz, 1H), 7.32 - 7.26 (m, 1H), 7.18 - 7.14 (m, 3H), 7.11 (dd, $J = 8.2$, 2.2 Hz, 1H), 7.05 (dd, $J = 8.8$, 1.7 Hz, 1H), 4.42 (s, 2H), 3.61 (t, $J = 5.8$ Hz, 2H), 3.34 (s, 4H), 3.14 (s, 4H), 2.97 (t, $J = 5.7$ Hz, 2H), 2.73 (s, 3H). $^{13}$C NMR (125 MHz, CD$_3$OD) δ 159.7, 156.5, 152.4, 146.6, 142.9, 140.6, 140.1, 139.1, 138.0, 137.5, 135.4, 134.9, 133.1, 128.2, 125.6, 125.3, 121.6, 119.8, 109.0, 63.2 (2C), 58.7, 58.2 (2C), 54.7, 53.5, 37.4. HRMS (ESI): calcd for C$_{27}$H$_{28}$N$_5$Cl $[M+H]^+$ 458.2106, found 458.2098.

6-Methoxy-2-(3-(6-(4-methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (9c): 9c was obtained from the reaction of 8c with 2a as a yellow solid in 52% yield. $^1$H NMR (400 MHz, CD$_3$OD) δ 7.75 (s, 1H), 7.55 (d, $J = 8.8$ Hz, 1H), 7.49 - 7.39 (m, 2H), 7.21 (d, $J = 2.0$ Hz, 1H), 7.16 (d, $J = 8.2$ Hz, 2H), 7.11 (dd, $J = 8.8$, 2.2 Hz, 1H), 6.82 - 6.75 (m, 2H), 4.46 (s, 2H), 3.80 (s, 3H), 3.67 (s, 3H), 3.14 (s, 4H), 2.97 (t, $J = 5.7$ Hz, 2H), 2.84 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 157.9, 156.5, 152.4, 146.6, 142.9, 140.6, 140.1, 139.1, 138.0, 137.5, 135.4, 134.9, 133.1, 125.6, 125.3, 121.6, 119.8, 109.0, 63.2 (2C), 58.7, 58.2 (2C), 54.7, 53.5, 37.4. HRMS (ESI): calcd for C$_{28}$H$_{31}$N$_5$O $[M+H]^+$ 454.2601, found 454.2604.

2-(3-(6-(4-Methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (9d): 9d was obtained from the reaction of 8d with 2a as a yellow solid in 47% yield. $^1$H NMR (500 MHz, CD$_3$OD) δ 7.70 (s, 1H), 7.49 (d, $J = 8.8$ Hz, 1H), 7.43 (d, $J = 7.6$ Hz, 1H), 7.37 (t, $J = 7.9$ Hz, 1H), 7.14 - 7.08 (m, 2H), 7.06 - 7.01 (m, 2H), 6.63 (dd, $J = 8.3$, 2.1 Hz, 1H), 6.60 (s, 1H), 4.38 (s, 2H), 3.60 (t, $J = 5.9$ Hz, 2H), 3.25 (t, $J = 3.9$ Hz, 4H), 2.93 (t, $J = 5.7$ Hz, 2H), 2.79 (t, $J = 4.1$ Hz, 4H), 2.45 (s, 3H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 155.8, 151.1, 150.4, 147.8, 135.7, 131.1, 129.5, 127.5, 124.5, 123.2, 122.7, 118.2, 115.6, 115.4, 114.6, 113.9, 113.4, 111.7, 111.2, 54.8 (2C), 49.8 (2C), 49.2, 45.7, 45.5, 28.4. HRMS (ESI): calcd for C$_{27}$H$_{29}$N$_5$O $[M+H]^+$
7-Methyl-2-(3-(6-(4-methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (9e): 9e was obtained from the reaction of 8e with 2a as a yellow solid in 58% yield. $^1$H NMR (400 MHz, CD$_3$OD) δ 7.74 (s, 1H), 7.53 (d, $J$ = 8.8 Hz, 1H), 7.47 (d, $J$ = 7.6 Hz, 1H), 7.40 (t, $J$ = 7.9 Hz, 1H), 7.32 - 7.26 (m, 1H), 7.17 (d, $J$ = 1.5 Hz, 1H), 7.13 (dd, $J$ = 8.2, 1.9 Hz, 1H), 7.09 - 7.05 (m, 1H), 7.04 - 6.97 (m, 2H), 4.44 (s, 2H), 3.63 (t, $J$ = 5.8 Hz, 2H), 3.38 - 3.34 (m, 4H), 3.11 (t, $J$ = 4.4 Hz, 4H), 2.95 (t, $J$ = 5.8 Hz, 2H), 2.71 (s, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 152.2, 150.8, 147.7, 135.6, 133.9, 131.7, 130.1, 129.9, 128.2, 127.2, 127.1, 125.1, 124.4, 118.6, 116.3, 116.2, 115.2, 112.6, 111.1, 54.5 (2C), 50.0, 49.5 (2C), 46.0, 45.0, 28.6, 21.1. HRMS (ESI): calcd for C$_{28}$H$_{31}$N$_5$ [M+H]$^+$ 438.2652, found 438.2662.

8-Methoxy-2-(3-(6-(4-methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (9f): 9f was obtained from the reaction of 8f with 2a as a yellow solid in 53% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.84 (s, 1H), 7.49 (d, $J$ = 7.7 Hz, 2H), 7.29 (t, $J$ = 8.0 Hz, 1H), 7.09 (t, $J$ = 7.9 Hz, 1H), 7.04 - 6.87 (m, 2H), 6.90 (d, $J$ = 8.7 Hz, 1H), 6.71 - 6.63 (m, 2H), 4.31 (s, 2H), 3.77 (s, 3H), 3.40 (t, $J$ = 5.5 Hz, 2H), 3.12 (t, $J$ = 4.0 Hz, 4H), 2.78 (t, $J$ = 5.2 Hz, 2H), 2.58 (t, $J$ = 4.0 Hz, 4H), 2.33 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 156.1, 152.3, 151.2, 147.9, 136.1, 133.7, 132.5, 130.8, 129.9, 129.9, 126.9, 122.9, 120.8, 116.5, 116.4, 115.2, 113.1, 107.3, 101.9, 56.0, 55.3 (2C), 54.9 (2C), 50.2, 45.9, 45.7, 29.2. HRMS (ESI): calcd for C$_{28}$H$_{31}$N$_5$O [M+H]$^+$ 454.2601, found 454.2602.

2-(3-(6-(4-Methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinolin-8-ol (9g): 9g was obtained from the reaction of 8g with 2a as a yellow solid in 49% yield. $^1$H NMR (500 MHz, CD$_3$OD) δ 8.22 (s, 1H), 7.97 (d, $J$ = 8.8 Hz, 1H), 7.93 (d, $J$ = 7.7 Hz, 1H), 7.84 (t, $J$ = 7.7 Hz, 1H), 7.63 - 7.57 (m, 2H), 7.51 - 7.44(m, 2H), 7.19 (d, $J$ = 7.7 Hz, 1H), 7.13 (d, $J$ = 8.0 Hz, 1H), 4.91 (s, 2H), 4.11 (t, $J$ = 6.0 Hz, 2H), 3.69 (t, $J$ = 4.5 Hz, 4H), 3.38 (t, $J$ = 6.0 Hz, 2H), 3.12 (t, $J$ = 4.5 Hz, 4H), 2.83 (s, 3H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 155.7, 151.0, 150.4, 147.6, 135.6, 131.0, 129.4, 127.3, 124.4, 123.2, 122.7, 118.1, 115.7, 115.5, 114.7, 113.9, 113.3, 111.8, 111.1, 54.4 (2C), 50.0 (2C), 49.4, 45.7, 45.6, 28.6. HRMS (ESI): calcd for C$_{27}$H$_{29}$N$_5$O [M+H]$^+$
440.2445, found 440.2473.

6.8-Dimethoxy-2-(3-(6-(4-methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (9h): 9h was obtained from the reaction of 8h with 2a as a yellow solid in 50% yield. $^1$H NMR (400 MHz, DMSO-$_d$6) $\delta$ 7.78 (s, 1H), 7.55 (d, $J = 6.0$ Hz, 1H), 7.46 (d, $J = 8.5$ Hz, 1H), 7.35 (t, $J = 7.7$ Hz, 1H), 7.30 - 7.18 (m, 1H), 7.07 - 6.92 (m, 2H), 6.44 (s, 1H), 6.38 (s, 1H), 4.25 (s, 2H), 3.83 (s, 3H), 3.74 (s, 3H), 3.57 (s, 2H), 3.19 (s, 4H), 2.90 (s, 2H), 2.71 (s, 4H), 2.38 (s, 3H). $^{13}$C NMR (125 MHz, DMSO-$_d$6) $\delta$ 158.7, 156.7, 150.7, 147.4, 142.9, 136.4, 131.1, 129.6, 127.6, 124.4, 123.3, 118.5, 116.0, 115.7, 114.6, 112.0, 110.8, 104.3, 96.2, 55.4 (2C), 55.2 (2C), 54.3 (2C), 49.3, 45.2, 44.8, 28.9. HRMS (ESI): calcd for C$_{29}$H$_{33}$N$_3$O$_2$ [M+H]$^+$ 484.2707, found 484.2706.

6.7-Dimethoxy-2-(3-(6-(4-methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (9i): 9i was obtained from the reaction of 8i with 2a as a yellow solid in 51% yield. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.65 (s, 1H), 7.43 (d, $J = 8.8$ Hz, 1H), 7.37 - 7.33 (m, 1H), 7.30 (t, $J = 7.9$ Hz, 1H), 7.08 - 7.03 (m, 2H), 6.98 (dd, $J = 8.8$, 2.2 Hz, 1H), 6.73 (s, 1H), 6.67 (s, 1H), 4.33 (s, 2H), 3.74 (s, 3H), 3.71 (s, 3H), 3.56 (t, $J = 5.8$ Hz, 2H), 3.27 - 3.22 (m, 4H), 2.95 - 2.90 (m, 4H), 2.85 (t, $J = 5.8$ Hz, 2H), 2.54 (s, 3H). $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 153.8, 152.5, 149.2, 149.1, 149.1, 131.7, 130.9, 130.9, 128.2, 127.8, 127.7, 118.1, 117.7, 116.7, 114.3, 113.1, 111.3, 103.0, 95.3, 56.6, 56.5, 55.7 (2C), 51.3, 51.0 (2C), 47.9, 43.5, 29.4. HRMS (ESI): calcd for C$_{29}$H$_{33}$N$_3$O$_2$ [M+H]$^+$ 484.2707, found 484.2727.

2-(3-(6-(4-Methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline-6,8-diol (9j): Yellow solid, 73%. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.75 (s, 1H), 7.53 (d, $J = 8.8$ Hz, 1H), 7.47 (d, $J = 7.7$ Hz, 1H), 7.41 (t, $J = 7.9$ Hz, 1H), 7.18 (d, $J = 2.0$ Hz, 1H), 7.14 (dd, $J = 8.2$, 1.8 Hz, 1H), 7.07 (dd, $J = 8.8$, 2.2 Hz, 2H), 6.23 (d, $J = 2.2$ Hz, 1H), 6.18 (d, $J = 2.1$ Hz, 1H), 4.32 (s, 2H), 3.60 (t, $J = 5.7$ Hz, 2H), 3.37 (t, $J = 4.4$ Hz, 4H), 3.14 (t, $J = 4.8$ Hz, 4H), 2.90 (t, $J = 5.6$ Hz, 2H), 2.73 (s, 3H). $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$ 157.3, 155.9, 153.8, 152.8, 148.7, 140.1, 137.8, 136.0, 131.5, 130.8, 117.8, 117.6, 116.6, 114.1, 113.9, 107.2, 103.0, 101.3, 55.5 (2C), 50.4 (2C), 47.4, 46.9, 44.6, 30.3. HRMS (ESI): calcd for C$_{27}$H$_{29}$N$_3$O$_2$ [M+H]$^+$
2-(3-(6-(4-Methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol (9k): Yellow solid, 75%. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.72 (s, 1H), 7.50 (d, $J = 7.4$ Hz, 2H), 7.36 (t, $J = 7.9$ Hz, 1H), 7.06 (d, $J = 7.5$ Hz, 1H), 7.01 - 6.89 (m, 2H), 6.64 (s, 1H), 6.56 (s, 1H), 4.32 (s, 2H), 3.58 (t, $J = 5.8$ Hz, 2H), 3.15 (brs, 4H), 2.79 (t, $J = 5.7$ Hz, 2H), 2.27 (s, 4H), 1.92 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 151.1, 150.5, 147.8, 143.9, 143.7, 131.1, 129.7, 129.5, 124.8, 124.5, 115.6, 115.5, 115.2, 114.0, 113.9, 113.5, 111.8, 114.8, 114.0, 113.9, 113.5, 111.8, 54.8 (2C), 49.9, 49.3 (2C), 45.9, 45.7, 27.5. HRMS (ESI): calcd for C$_{27}$H$_{29}$N$_5$O$_2$ [M+H]$^+$ 456.2394, found 456.2421.

Scheme S3. Synthesis of 14a-14d

Reagents and conditions: (a) (Boc)$_2$O, DMAP, THF, 0.5 h; (b) NaH, MeCN, 60 °C, 24 h; (c) 1. HCl, 1,4-dioxane, r.t., 3 h; 2. K$_2$CO$_3$, CuI, L-proline, DMSO, 80 °C, 24 h, N$_2$; (d) LiOH, THF, 90 °C, 3 h; (e) 1. TBTU, DIPEA, DMF, r.t., 6 h; 2. AcOH, reflux, overnight.

tert-Butyl 6-hydroxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (10a): To the mixture of 1,2,3,4-tetrahydroisoquinolin-6-ol (2.0 g, 13.4 mmol) and DMAP (27 mg) in THF (60 mL), di-tert-butyl dicarbonate (3.07 mL, 13.4 mmol) was added dropwise at 0 °C. The mixture was then stirred at room temperature for 0.5 h. After the solvent was removed under reduced pressure, the residue was dissolved in CH$_2$Cl$_2$, washed with saturated NaHCO$_3$, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated in vacuo. The residue was purified through flash column chromatography with hexane/EtOAc (5:1) elution to afford 10a as a white oil in 70% yield. $^1$H NMR (400
MHz, CDCl₃) δ 6.94 (d, J = 8.2 Hz, 1H), 6.68 (d, J = 7.4 Hz, 1H), 6.63 (s, 1H), 5.59 (s, 1H), 4.48 (s, 2H), 3.61 (t, J = 5.6 Hz, 2H), 2.76 (t, J = 5.8 Hz, 2H), 1.49 (s, 9H). ESI-MS m/z: 149.0 [M+H-Boc]+.

**General procedure for the synthesis of 11a-11d:** The intermediate 10a (373.5 mg, 1.5 mmol) was dissolved in acetonitrile (10 mL), and NaH (60% dispersion in mineral oil, 72.9 mg, 1.8 mmol) was added at 0 °C. After stirring at room temperature for 1 h, the desired alkyl bromide (1.8 mmol) was added and the reaction mixture was stirred at 60 °C for 24 h. After completion of the reaction, the mixture was diluted with water and extracted with EtOAc and brine. The organic fraction was dried over anhydrous Na₂SO₄ and the solvent was evaporated *in vacuo*. The residue was purified through flash column chromatography with hexane/EtOAc (10:1) elution to afford 11a-11d.

**tert-Butyl 6-ethoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (11a):** Following the general procedure, 11a was obtained from the reaction of bromoethane with 10a as a colorless oil in 79% yield. ¹H NMR (400 MHz, CDCl₃) δ 6.93 (d, J = 8.3 Hz, 1H), 6.67 (dd, J = 8.4, 2.4 Hz, 1H), 6.59 (d, J = 2.4 Hz, 1H), 4.43 (s, 2H), 3.94 (q, J = 7.0 Hz, 2H), 3.55 (t, J = 5.6 Hz, 2H), 2.72 (t, J = 5.6 Hz, 2H), 1.42 (s, 9H), 1.33 (t, J = 7.0 Hz, 3H). ESI-MS m/z: 177.1 [M+H-Boc]+.

**tert-Butyl 6-propoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (11b):** Following the general procedure, 11b was obtained from the reaction of 1-bromopropane with 10a as a colorless oil in 72% yield. ¹H NMR (400 MHz, CDCl₃) δ 7.00 (d, J = 8.4 Hz, 1H), 6.74 (dd, J = 8.4, 2.5 Hz, 1H), 6.67 (d, J = 2.3 Hz, 1H), 4.50 (s, 2H), 3.90 (t, J = 6.6 Hz, 2H), 3.59 (t, J = 5.6 Hz, 2H), 2.79 (t, J = 5.6 Hz, 2H), 1.84 - 1.74 (m, 2H), 1.49 (s, 9H), 1.03 (t, J = 7.4 Hz, 3H). ESI-MS m/z: 191.1 [M+H-Boc]+.

**tert-Butyl 6-isopropoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (11c):** Following the general procedure, 11c was obtained from the reaction of 2-bromopropane with 10a as a colorless oil in 65% yield. ¹H NMR (400 MHz, CDCl₃) δ 7.00 (d, J = 8.4 Hz, 1H), 6.73 (dd, J = 8.4, 2.5 Hz, 1H), 6.66 (d, J = 2.3 Hz, 1H), 4.54 - 4.46 (m, 3H), 3.62 (t, J = 5.6 Hz, 2H), 2.78 (t, J = 5.6 Hz, 2H), 1.48 (s, 9H), 1.32 (d, J = 6.1 Hz, 6H). ESI-MS m/z: 191.1 [M+H-Boc]+.

**tert-Butyl 6-(prop-2-yn-1-yl oxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate (11d):**
Following the general procedure, 11d was obtained from the reaction of 3-bromoprop-1-yne with 10a as a colorless oil in 71% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.05 (d, $J$ = 8.3 Hz, 1H), 6.84 (dd, $J$ = 8.3, 1.6 Hz, 1H), 6.77 (s, 1H), 4.69 (d, $J$ = 1.2 Hz, 2H), 4.53 (s, 2H), 3.64 (t, $J$ = 5.4 Hz, 2H), 2.83 (t, $J$ = 5.4 Hz, 2H), 2.53 (s, 1H), 1.51 (s, 9H). ESI-MS m/z: 187.1 [M+H-Boc]$^+$. 

**General procedure for the synthesis of 12a-12d:** The Boc derivative 11a-11d (1.0 mmol) was dissolved in 1,4-dioxane (5 mL), and HCl (1.25 mL, 4 M in 1,4-dioxane) was added. The reaction mixture was stirred at room temperature for 3 h, and then was concentrated in vacuo to afford the crude. To a dried sealed vessel were added the crude, anhydrous K$_2$CO$_3$ (553 mg, 4.0 mmol), CuI (38 mg, 0.2 mmol), L-proline (46 mg, 0.4 mmol), ethyl 3-iodobenzoate (276 mg, 1.0 mmol), and DMSO (10 mL). Then the mixture was stirred at 80 °C for 24 h under N$_2$ environment. After cooling to room temperature, water was added and the mixture was extracted with EtOAc. Then, the combined organic layer was dried over anhydrous Na$_2$SO$_4$ and concentrated. The residue was purified through flash column chromatography with hexane/EtOAc (10:1) elution to afford compounds 12a-12d.

**Ethyl 3-(6-ethoxy-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (12a):** Following the general procedure, 12a was obtained as a yellow oil in 65% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.64 - 7.61 (m, 1H), 7.47 (d, $J$ = 7.6 Hz, 1H), 7.32 (t, $J$ = 7.9 Hz, 1H), 7.12 (dd, $J$ = 8.1, 2.3 Hz, 1H), 7.08 (d, $J$ = 8.4 Hz, 1H), 6.76 (dd, $J$ = 8.4, 2.6 Hz, 1H), 6.70 (d, $J$ = 2.4 Hz, 1H), 4.41 - 4.35 (m, 4H), 4.02 (q, $J$ = 7.0 Hz, 2H), 3.58 (t, $J$ = 5.9 Hz, 2H), 2.96 (t, $J$ = 5.8 Hz, 2H), 1.43 - 1.37 (m, 6H). ESI-MS m/z: 326.1 [M+H]$^+$. 

**Ethyl 3-(6-propoxy-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (12b):** Following the general procedure, 12b was obtained as a yellow oil in 67% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.63 (s, 1H), 7.47 (d, $J$ = 7.5 Hz, 1H), 7.32 (t, $J$ = 7.9 Hz, 1H), 7.12 (dd, $J$ = 8.3, 2.5 Hz, 1H), 7.08 (d, $J$ = 8.4 Hz, 1H), 6.77 (dd, $J$ = 8.4, 2.0 Hz, 1H), 6.71 (s, 1H), 4.42 - 4.34 (m, 4H), 3.91 (t, $J$ = 6.6 Hz, 2H), 3.58 (t, $J$ = 5.8 Hz, 2H), 2.96 (t, $J$ = 5.8 Hz, 2H), 1.85 - 1.75 (m, 2H), 1.40 (t, $J$ = 7.2 Hz, 3H), 1.04 (t, $J$ = 7.4 Hz, 3H). ESI-MS m/z: 340.1 [M+H]$^+$. 

**Ethyl 3-(6-isopropoxy-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (12c):** Following
the general procedure, **12c** was obtained as a yellow oil in 55% yield. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.64 - 7.61 (m, 1H), 7.47 (d, \(J = 7.6\) Hz, 1H), 7.32 (t, \(J = 7.9\) Hz, 1H), 7.12 (dd, \(J = 8.2, 2.5\) Hz, 1H), 7.07 (d, \(J = 8.4\) Hz, 1H), 6.75 (dd, \(J = 8.4, 2.5\) Hz, 1H), 6.70 (d, \(J = 2.3\) Hz, 1H), 4.59 - 4.47 (m, 1H), 4.41 - 4.35 (m, 4H), 3.58 (t, \(J = 5.8\) Hz, 2H), 2.96 (t, \(J = 5.8\) Hz, 2H), 1.40 (t, \(J = 7.1\) Hz, 3H), 1.33 (d, \(J = 6.1\) Hz, 6H). ESI-MS m/z: 340.1 [M+H]^+.

**Ethyl 3-(6-(prop-2-yn-1-yloxy)-3,4-dihydroisoquinolin-2(1H)-yl)benzoate (12d):** Following the general procedure, **12d** was obtained as a yellow oil in 61% yield. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.64 - 7.61 (m, 1H), 7.47 (d, \(J = 7.6\) Hz, 1H), 7.32 (t, \(J = 7.9\) Hz, 1H), 7.12 (dd, \(J = 8.2, 2.5\) Hz, 1H), 7.07 (d, \(J = 8.4\) Hz, 1H), 6.75 (dd, \(J = 8.4, 2.5\) Hz, 1H), 6.70 (d, \(J = 2.3\) Hz, 1H), 4.59 - 4.47 (m, 1H), 4.41 - 4.35 (m, 4H), 3.58 (t, \(J = 5.8\) Hz, 2H), 2.96 (t, \(J = 5.8\) Hz, 2H), 1.40 (t, \(J = 7.1\) Hz, 3H), 1.33 (d, \(J = 6.1\) Hz, 6H). ESI-MS m/z: 336.1 [M+H]^+.

**General procedure for the synthesis of 14a-14d:** The synthesis of **14a-14d** was conducted according to the procedure for the synthesis of **9a-9i**.

**6-Ethoxy-2-(3-(6-(4-methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (14a):** Yellow solid, 40%. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.80 (s, 1H), 7.53 (d, \(J = 7.3\) Hz, 1H), 7.46 (d, \(J = 8.6\) Hz, 1H), 7.32 (t, \(J = 7.8\) Hz, 1H), 7.15 (d, \(J = 7.9\) Hz, 1H), 7.08 - 7.01 (m, 2H), 6.80 - 6.69 (m, 2H), 4.61 (s, 2H), 4.34 - 4.27 (m, 4H), 3.52 (t, \(J = 5.4\) Hz, 2H), 2.91 (t, \(J = 5.4\) Hz, 2H), 2.44 (s, 1H), 1.33 (t, \(J = 7.1\) Hz, 3H). ESI-MS m/z: 468.2758, found 468.2767.

**2-(3-(6-(4-Methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-6-propoxy-1,2,3,4-tetrahydroisoquinoline (14b):** Yellow solid, 41%. \(^1\)H NMR (400 MHz, CD\(_3\)OD) \(\delta\) 7.74 (s, 1H), 7.54 (d, \(J = 8.7\) Hz, 1H), 7.47 (d, \(J = 7.4\) Hz, 1H), 7.41 (t, \(J = 7.9\) Hz, 1H), 7.18 (s, 1H), 7.14 (dd, \(J = 8.3, 3.6\) Hz, 2H), 7.08 (dd, \(J = 8.8, 2.0\) Hz, 1H), 6.79 - 6.73 (m, 2H), 4.44 (s, 2H), 3.92 (t, \(J = 6.5\) Hz, 2H), 3.64 (t, \(J = 5.8\) Hz, 2H), 3.38 - 3.33 (m, 4H), 3.07 - 2.97 (m, 6H), 2.66 (s, 3H), 1.82 - 1.74 (m, 2H), 1.05 (t, \(J = 7.4\) Hz, 3H). \(^{13}\)C NMR (125 MHz, CD\(_3\)OD) \(\delta\) 163.7, 162.1, 157.6, 150.8, 136.3, 131.5,
130.5, 130.1, 130.0, 128.0, 126.7, 122.2, 116.2, 115.9, 114.1, 113.8, 113.2, 112.9, 112.3, 69.3, 54.9 (2C), 49.9, 49.9, 49.6, 45.9, 45.6, 28.9, 22.5, 10.9. HRMS (ESI): calcd for \( \text{C}_{30}\text{H}_{35}\text{N}_{5}\text{O} [\text{M+H}]^+ 482.2914 \), found 482.2921.

6-Isopropoxy-2-(3-(6-(4-methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-1,2,3,4-tetrahydroisoquinoline (14c): Yellow solid, 37%. \(^1\)H NMR (400 MHz, CD\( _3 \)OD) \( \delta \) 7.71 (s, 1H), 7.51 (d, \( J = 8.7 \) Hz, 1H), 7.45 (d, \( J = 7.6 \) Hz, 1H), 7.37 (t, \( J = 7.9 \) Hz, 1H), 7.29 - 7.25 (m, 1H), 7.13 (s, 1H), 7.10 - 7.06 (m, 1H), 7.03 (s, 1H), 2.65 - 2.61 (s, 6H), 1.28 (t, \( J = 6.0 \) Hz, 3H). \(^13\)C NMR (125 MHz, CD\( _3 \)OD) \( \delta \) 156.4, 152.3, 151.0, 147.5, 135.8, 130.2, 129.5, 127.5, 127.2, 126.2, 124.0, 117.4, 116.4, 116.1, 115.1, 114.0, 112.5, 111.0, 101.4, 69.6, 54.1 (2C), 49.5, 49.2 (2C), 46.1, 43.4, 28.8, 21.0 (2C). HRMS (ESI): calcd for \( \text{C}_{30}\text{H}_{35}\text{N}_{5}\text{O} [\text{M+H}]^+ 482.2914 \), found 482.2926.

2-(3-(6-(4-Methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)-6-(prop-2-yn-1-yloxy)-1,2,3,4-tetrahydroisoquinoline (14d): Yellow solid, 39%. \(^1\)H NMR (400 MHz, DMSO-d\( _6 \)) \( \delta \) 7.74 (s, 1H), 7.51 (d, \( J = 7.6 \) Hz, 1H), 7.46 (d, \( J = 8.8 \) Hz, 1H), 7.41 - 7.35 (m, 1H), 7.20 (d, \( J = 8.1 \) Hz, 1H), 7.07 (dd, \( J = 8.2, 2.0 \) Hz, 1H), 7.02 (s, 1H), 6.96 (dd, \( J = 8.8, 2.2 \) Hz, 1H), 6.88 - 6.80 (m, 2H), 4.77 (d, \( J = 2.3 \) Hz, 2H), 4.42 (s, 2H), 3.61 (t, \( J = 5.6 \) Hz, 2H), 3.54 (t, \( J = 2.3 \) Hz, 1H), 3.23 - 3.15 (m, 4H), 2.94 (t, \( J = 5.6 \) Hz, 2H), 2.81 - 2.71 (m, 4H), 2.44 (s, 3H). \(^13\)C NMR (125 MHz, DMSO-d\( _6 \)) \( \delta \) 156.1, 151.7, 150.8, 147.7, 136.4, 131.5, 130.0, 128.1, 127.6, 125.8, 124.1, 119.1, 116.2, 116.1, 114.6, 114.6, 113.5, 112.2, 110.7, 79.9, 78.6, 55.8, 54.5 (2C), 49.6, 49.5 (2C), 45.9, 45.0, 28.9. HRMS (ESI): calcd for \( \text{C}_{30}\text{H}_{31}\text{N}_{5}\text{O} [\text{M+H}]^+ 478.2601 \), found 478.2577.

**Scheme S4. Synthesis of 17a-17c**

**Reagents and conditions:** (a) K\(_2\)CO\(_3\), CuI, L-proline, DMSO, 80 °C, 24 h, N\(_2\); or
Na₂CO₃, Pd(PPh₃)₄, 1,4-dioxane/H₂O, 80 °C, 16 h, N₂; (b) LiOH, THF, 90 °C, 3 h; (c) 1. TBTU, DIPEA, DMF, r.t., 6 h; 2. AcOH, reflux, overnight.

**General procedure for the synthesis of 15a-15c:** The reaction of 2-(piperazin-1-yl)pyrimidine or morpholine with ethyl 3-iodobenzoate was performed to afford the desired compounds 15a-15b according to the procedure for the synthesis of 7a-7i. For the synthesis of 15c, to the mixture of 5-bromobenzo[d][1,3]dioxole (400 mg, 2 mmol) and (3-ethoxycarbonyl)phenyl)boronic acid (388 mg, 2 mmol) in the solvent of 1,4-dioxane/H₂O (4:1, 15 mL), Na₂CO₃ (424 mg, 4 mmol) and Pd(PPh₃)₄ (115.5 mg, 0.1 mmol) were added. The reaction mixture was stirred at 80 °C for 16 h under N₂ environment, cooled to room temperature, and filtered through a pad of celite. The filtrate was partitioned between 1.0 N NaOH and CH₂Cl₂ and extracted with CH₂Cl₂. The combined organic layer was dried over Na₂SO₄ and concentrated in vacuo. The residue was purified through flash column chromatography with hexane/EtOAc (20:1) elution to afford compound 15c.

**Ethyl 3-(4-(pyrimidin-2-yl)piperazin-1-yl)benzoate (15a):** White oil, 73%. ¹H NMR (400 MHz, CDCl₃) δ 8.34 (d, J = 4.7 Hz, 2H), 7.66 - 7.62 (m, 1H), 7.55 (d, J = 7.7 Hz, 1H), 7.33 (t, J = 7.9 Hz, 1H), 7.15 (dd, J = 8.2, 2.1 Hz, 1H), 6.52 (t, J = 4.7 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 4.00 (t, J = 5.2 Hz, 4H), 3.31 (t, J = 5.2 Hz, 4H), 1.40 (t, J = 7.1 Hz, 3H). ESI-MS m/z: 313.1 [M+H]⁺.

**Ethyl 3-morpholinobenzoate (15b):** White oil, 78%. ¹H NMR (400 MHz, CDCl₃) δ 7.60 - 7.57 (m, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.33 (t, J = 7.9 Hz, 1H), 7.09 (dd, J = 8.0, 2.3 Hz, 1H), 4.37 (q, J = 7.1 Hz, 2H), 3.90 - 3.82 (m, 4H), 3.23 - 3.15 (m, 4H), 1.39 (t, J = 7.1 Hz, 3H). ESI-MS m/z: 236.1 [M+H]⁺.

**Ethyl 3-(benzo[d][1,3]dioxol-5-yl)benzoate (15c):** White oil, 76%. ¹H NMR (500 MHz, CDCl₃) δ 8.20 (t, J = 1.7 Hz, 1H), 7.99 (dd, J = 7.7, 1.1 Hz, 1H), 7.70 (dd, J = 7.7, 1.2 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.11 - 7.08 (m, 2H), 6.92 - 6.88 (m, 1H), 6.01 (s, 2H), 4.41 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H). ESI-MS m/z: 271.0 [M+H]⁺.

**General procedure for the synthesis of 17a-17c:** The synthesis of 17a-17c was conducted according to the procedure for the synthesis of 9a-9i.

6-(4-Methylpiperazin-1-yl)-2-(3-(4-(pyrimidin-2-yl)piperazin-1-yl)phenyl)-1H-
benzo[d]imidazole (17a): White solid, 45%. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.33 (d, $J$ = 4.8 Hz, 2H), 7.69 (s, 1H), 7.53 - 7.47 (m, 2H), 7.37 (t, $J$ = 7.8 Hz, 1H), 7.13 - 7.08 (m, 2H), 7.03 (dd, $J$ = 8.8, 2.1 Hz, 1H), 6.59 (t, $J$ = 4.8 Hz, 1H), 3.95 (t, $J$ = 5.2 Hz, 4H), 3.34 - 3.30 (m, 4H), 3.24 (t, $J$ = 4.8 Hz, 4H), 2.77 (t, $J$ = 4.8 Hz, 4H), 2.44 (s, 3H).

$^{13}$C NMR (100 MHz, DMSO-$_d_6$) $\delta$ 161.7, 158.5 (3C), 151.8, 148.2, 131.6 (2C), 130.0 (2C), 117.5, 117.2, 113.6 (2C), 110.9 (3C), 55.1 (2C), 50.1, 48.6 (2C), 45.9, 43.7 (2C), 41.8. HRMS (ESI): calcd for C$_{26}$H$_{30}$N$_8$ [M+H]$^+$ 455.2666, found 455.2701.

4-(3-(6-(4-Methylpiperazin-1-yl)-1H-benzo[d]imidazol-2-yl)phenyl)morpholine (17b): White solid, 40%. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.64 (s, 1H), 7.53 - 7.43 (m, 2H), 7.34 (t, $J$ = 8.0 Hz, 1H), 7.09 (s, 1H), 7.00 (d, $J$ = 8.7 Hz, 2H), 3.79 (t, $J$ = 4.8 Hz, 4H), 3.20 - 3.13 (m, 8H), 2.64 (t, $J$ = 4.8 Hz, 4H), 2.33 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$_d_6$) $\delta$ 151.4, 150.8, 147.8, 131.1, 129.5, 129.1, 118.0, 117.0, 116.1, 113.9, 113.7, 112.4, 110.7, 66.1 (2C), 54.8 (2C), 49.9 (2C), 48.4 (2C), 45.7. HRMS (ESI): calcd for C$_{22}$H$_{22}$N$_5$O [M+H]$^+$ 378.2288, found 378.2289.

2-(3-(Benzo[d][1,3]dioxol-5-yl)phenyl)-6-(4-methylpiperazin-1-yl)-1H-benzo[d]imidazole (17c): Brown solid, 44%. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.24 (s, 1H), 7.95 (d, $J$ = 7.7 Hz, 1H), 7.62 (d, $J$ = 7.8 Hz, 1H), 7.54 - 7.49 (m, 2H), 7.19 (s, 1H), 7.18 - 7.16 (m, 1H), 7.11 (s, 1H), 7.04 (dd, $J$ = 8.8, 2.0 Hz, 1H), 6.90 (d, $J$ = 7.8 Hz, 1H), 5.99 (s, 2H), 3.21 (t, $J$ = 4.8 Hz, 4H), 2.67 (t, $J$ = 4.8 Hz, 4H), 2.37 (s, 3H). $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 152.7, 149.7, 149.7, 148.9, 142.9, 135.8, 131.5, 130.5, 129.1, 125.8, 125.7, 121.7, 116.6, 109.5, 108.5, 102.6, 56.1 (2C), 51.6 (2C), 46.0. HRMS (ESI): calcd for C$_{23}$H$_{24}$N$_4$O$_2$ [M+H]$^+$ 413.1972, found 413.1998.

Scheme S5. Synthesis of Biotin-14d

Reagents and conditions: CuSO$_4$5H$_2$O, sodium ascorbate, MeOH/H$_2$O, overnight, N$_2$. Biotin-PEG$_2$-N$_3$ (22 mg, 0.05 mmol) and compound 14d (24 mg, 0.05 mmol) were dissolved in MeOH (2 mL) and the solution was slowly diluted with water (0.5 mL).
After addition of aqueous CuSO$_4$·5H$_2$O solution (20 μL, 1 M) and freshly prepared aqueous sodium ascorbate solution (20 μL, 1 M), the reaction mixture was stirred overnight at room temperature under N$_2$. The solvent was removed under reduced pressure. The residue was purified by preparative HPLC to afford Biotin-14d as a yellow solid (16 mg, 35% yield) (10% to 75% MeCN in H$_2$O containing 0.1% HCOOH over 30 min; flow rate 3 mL/min). Purity: 98.6% by HPLC. HRMS (ESI): calcd for C$_{48}$H$_{63}$N$_{11}$O$_6$S [M+2H]$^{2+}$ 461.7415, found 461.7418.

2.2 Cell Culture. All cell lines were cultured at 37°C with 5% CO$_2$ in a humidified incubator. HT22, HEK-293T and A549 cells stably expressing GFP-LC3 were maintained in DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin/streptomycin (Gibco). HT-1080 cells (Male) were maintained in MEM (Gibco) supplemented with 10% FBS and 1% penicillin/streptomycin. All cell lines used for experiments were passaged no more than 15 times.

2.3 Antibodies and Reagents. The following primary antibodies were used in this study: FTH1 (Cell Signaling Technology 4393, Western blotting (WB) 1:1000), ferritin (Rockland Immunochemicals 200-401-090-0100, immunofluorescence (IF) 1:100), NCOA4 (Bethyl Laboratories A302-272A, WB 1:1000, IP 8 μg/mg protein), LC3 (Cell Signaling Technology 12741, WB 1:1000, IF 1:100), GPX4 (Abcam ab125066, WB 1:5000), LAMP2 (Proteintech 66301-1-lg, IF 1:100), HRP-conjugated His-tag (Proteintech HRP-66005, WB 1:5000), Myc-tag (Yeasen 30601ES20, WB 1:5000), Flag-tag (Yeasen 30503ES60, WB 1:5000, IP 1:100), GAPDH (Cell Signaling Technology 2118, WB 1:1000), β-actin (Bioworld AP0060, WB 1:1000), α-tubulin (Sigma-Aldrich T6199, WB 1:1000). The following secondary antibodies were used: HRP-conjugated anti-rabbit IgG (Cell Signaling Technology 7074, WB 1:3000), HRP-conjugated anti-mouse IgG (Cell Signaling Technology 7076, WB 1:3000), Alexa Fluor 594 anti-rabbit IgG (Abcam ab150080, IF 1:1000), Alexa Fluor Plus 488 anti-mouse IgG (Invitrogen A32723, IF 1:1000), Alexa Fluor Plus 488 anti-rabbit IgG (Invitrogen A32731, IF 1:1000). The following reagents were used: BODIPY-C11 (Invitrogen, D3861), carboxy-H$_2$DCFDA (Sigma-Aldrich, 287810), FerroOrange (Dojindo, F374),...
LysoTracker Green (Beyotime, C1047S), LysoTracker Red (Beyotime, C1046), ER-Tracker Red (Beyotime, C1041), MitoTracker Deep Red (Yeasen, 40743ES50), Erastin (Targetmol, T1765), RSL3 (Targetmol, T3646), ML210 (Targetmol, T8375), Ferrostatin-1 (Targetmol, T6500), Deferoxamine mesylate (DFO, Targetmol, T1637), Vitamin C (MedChemExpress, HY-B0166), BSO (Aladdin, B113387), staurosporine (Targetmol, T6680), chloroquine (A506569, Sangon Biotech), DPPH (Sigma-Aldrich, D9132).

2.4 Cell Viability Assay. Cells were plated in 96-well microplates (Corning) at a density of 3,000 cells/well. To evaluate the inhibitory activities of compounds against ferroptosis, cells were incubated with 1 μM Erastin or RSL3 ± compounds, ± Fer-1, or ± DFO for 24 h. Cell viability was assayed using a Cell Counting Kit-8 (CCK-8) kit. Briefly, after indicated treatment, 10 μL CCK-8 solution was added to each well and incubated for 4 h at 37 °C. Absorbance was measured at 450 nm. DMSO controls were taken as having 100% viability. Three independent biological replicates were performed for the experiments.

2.5 SiRNA-Mediated Knockdown of GPX4. HT-1080 cells were plated in 96-well microplates at a density of 5,000 cells/well and then transfected with a GPX4-targeting siRNA (synthesized by Sangon) or scrambled siRNA using Lipofectamine 3000 Transfection Reagent (Invitrogen) in Opti-MEM Reduced Serum Medium (Invitrogen) for 12 h. The transfected cells were then treated with or without compounds for 36 h. Cell viability was assayed using a CCK-8 kit. Three independent biological replicates were performed for the experiments.

2.6 Measurement/Imaging of Lipid ROS. Lipid ROS was analyzed by flow cytometry: HT22 cells were plated in 6-well plates (Corning) at a density of 3×10^4 cells/well. After treatment, cells were washed twice with 1×PBS and then stained with 5 μM BODIPY-C11 dye (diluted in serum-containing DMEM) for 30 min at 37 °C in an incubator. Following staining, cells were washed with PBS, trypsinized, and collected in 1 ml PBS. Flow cytometry data were collected on guava easyCyte™ (Merck) with an excitation wavelength of 488 nm. A minimum of 10,000 cells were analyzed for each condition. Data analysis was performed using the FlowJo 10 software. For cytosolic ROS, cells
were stained with 10 μM carboxy-H₂DCFDA (diluted in DMEM).

Lipid ROS imaging: HT22 cells were seeded in glass bottom 96-well plates (ThermoFisher Scientific) at a density of 3,000 cells/well and grew overnight. After treatment, cells were co-stained with BODIPY-C11 dye and Hoechst 33342 (1μg/mL). After wash, digital images were directly recorded using a FV3000 laser scanning confocal microscope (Olympus) with a 60× objective lens.

2.7 Glutathione Measurements. HT22 cells were seeded into 6-well plates at a density of 5×10⁴ cells/well. After treated as indicated for 12 h, cells were collected and prepared for measurement of total intracellular glutathione (GSH+GSSG) using the assay kit (Beyotime) according to the manufacturer's instructions. Three independent biological replicates were performed for the experiments.

2.8 DPPH Assay. Reagents (9a, Fer-1, vitamin C, and DMSO) were diluted in ethanol and pipetted into a 96-well plate. DPPH solution (final working concentration 100 μM) was added to start the reaction. The mixture was incubated in the dark for 30 min at room temperature. Absorbance at 517 nm was measured. All values were normalized to DMSO (= 1.0). Three independent biological replicates were performed for the experiments.

2.9 Iron Chelating Studies. Iron chelating properties of 9a were investigated with a UV-2600 spectrophotometer (Shimadzu, Japan) using 1 cm path length quartz cuvette. FeSO₄ or FeCl₃ (50 μM) solution was added into the solution containing DFO or compound 9a at the concentration of 50 μM in HEPES buffer (20 mM, pH = 7.4) with 150 mM NaCl. The UV-Vis spectra in the range of 200-600 nm for DFO and 290-500 nm for 9a were recorded.

2.10 High-Content Imaging Studies. HT22 and HT-1080 cells were seeded in 96-well plates and grew overnight. After treated with 0.5 μM 9a or 50 μM DFO for 6 h, cells were co-stained with FerroOrange (1 μM) and Hoechst 33342 (1μg/mL) in serum-free medium for 30 min at 37 °C in an incubator. Following staining, cells were washed with PBS. The Cellomics ArrayScan Vti (ThermoFisher Scientific) high-content imaging platform was used for the quantification of the fluorescence intensity of FerroOrange in live cells. The high-content analysis automatically focused in the fluorescence
channel of Hoechst 33342 and captured the channel of FerroOrange with exposed time for 0.5 s. The data were acquired from approximately 3000 cells per sample. Three independent biological replicates were performed for the experiments.

2.11 Localization of Intracellular Fe^{2+}. HT22 and HT-1080 cells were seeded in glass bottom 96-well plates and grew overnight. After treated as indicated in the main figure, cells were co-stained with FerroOrange (1 μM) and LysoTracker Green (50 nM) in serum-free medium for 30 min at 37 °C in an incubator, and washed with PBS. Digital images were recorded using a FV3000 laser scanning confocal microscope (Olympus) with a 60× objective lens. The images were analyzed with Imaris software (Bitplane Corp.).

2.12 Western Blotting Analysis and Coimmunoprecipitation Assay. For Western blotting, after washing with cold PBS, cells were lysed in RIPA buffer (Beyotime) with protease inhibitor cocktail. Lysates were collected, briefly sonicated, and centrifuged at 13,000 rpm for 15 min at 4 °C. The supernatant was transferred into a new tube. The protein concentrations were determined by Pierce™ BCA Protein Assay kit (ThermoFisher Scientific). Samples were diluted in SDS-PAGE loading buffer (5×, reduced, Fdbio science) and denatured at 100 °C for 5 min before loading in 12% SDS-PAGE gels. Proteins were then transferred to PVDF membranes (Millipore). After blocking with 5% non-fat milk, the membrane was incubated with primary antibodies overnight at 4 °C. Washed by Tris-buffered saline with 0.1% Tween-20 (TBST) for three times, the membranes were incubated with corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for 1.5 h. Chemiluminescent detection was performed using ECL Western blotting solution (Tanon) and Chemiluminescence imager (Tanon 5200, China). Quantification of the bands was performed using ImageJ software (National Institutes of Health).

For the endogenous interaction assay, HT-1080 cells were treated and lysed with NP-40 lysis buffer (Beyotime) containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40 and protease inhibitors. The cell lysates were incubated with NCOA4 antibody overnight at 4 °C. Immunocomplexes were captured using protein A/G magnetic beads (ThermoFisher Scientific) for 1 h at room temperature. Eluted immunocomplexes were
subjected to SDS-PAGE and immunoblotted with specific antibodies to the proteins of interest. For the exogenous interaction assay, HEK-293T cells were plated on 10 cm culture dishes and grown to ~60 % confluence, then transfected with FTH1-Myc-His and NCOA4-Flag plasmids. After 24 h, cells were collected and lysed with NP-40 lysis buffer as described above. Extracts were immunoprecipitated with anti-Flag antibody and beads (MedChemExpress), and then assessed by immunoblot analysis with the indicated antibodies.

2.13 Pull-Down Assay. Biotin-PEG₃-N₃ (20 μM) and the varying concentrations of probe (Biotin-14d) were preincubated with streptavidin magnetic beads (MedChemExpress) overnight at 4 °C. The beads were then washed three times in the wash buffer (PBS supplemented with 0.05% Tween-20 and 0.1% BSA). HEK-293T cells expressing FTH1-Myc-His and NCOA4-Flag were harvested and lysed in RIPA buffer with protease inhibitor cocktail. After centrifugation at 13,000 rpm for 15 min at 4 °C, the supernatant (1 mg/mL) was collected and equally divided into five parts, one of them preincubated with 9a (20 μM) for 6 h at 4 °C, then incubated with the beads in PBS overnight at 4 °C, respectively. After incubation, beads were washed three times with the wash buffer. The bead-bound proteins were eluted, separated by SDS-PAGE, and visualized by Western blotting.

2.14 Chemical Labeling of 14d and Fluorescence Microscopy. HT22 cells were seeded in glass bottom 96-well plates, cultured at ~80% confluence, then treated for 2 h with 10 µM 14d. Cells were fixed with 4% paraformaldehyde for 15 min, prior to permeabilization (Triton X-100, 0.5% in PBS, 20 min) and washed three times with 1% BSA/PBS. The click reaction cocktail was prepared from BeyoClick™ EdU-647 kit (Beyotime) according to the manufacturer’s protocol. Briefly, mixing 430 µL of click reaction buffer with 20 µL of CuSO₄ solution, 1 µL azide 647, 50 µL click additive solution (sodium ascorbate) to reach a final volume of ~500 µL. Cells were incubated with the click reaction cocktail in the dark at room temperature for 30 min, then washed three times with 3% BSA/PBS. For immunofluorescence, cells were blocked with 5% BSA for 30 min at 37 °C, then incubated with LC3 antibody in blocking buffer overnight at 4 °C. After rinsing in PBS, cells were incubated with Alexa Fluor Plus 488
goat anti-rabbit IgG for 30 min at 37 °C. Before imaging, cells were treated with 4',6-diamidino-2-phenylindole (DAPI, Invitrogen) for 10 min at room temperature. After washing, digital images were recorded using a FV3000 laser scanning confocal microscope (Olympus) with a 60× objective lens.

2.15 qPCR. Total RNA was isolated using RNAsiso plus (TaKaRa) according to the manufacturer’s protocol. Total RNA (1 μg) was used for cDNA synthesis using the ReverTra Ace qPCR RT Master Mix (TOYOBO) according to the manufacturer’s protocol. cDNA was used to amplify specific target genes by SYBR Green Real-time PCR Master Mix (TOYOBO). The data were normalized to the internal control β-actin. Relative gene expression levels were calculated using the delta Ct (2−ΔΔCt) method. Sequences of the primers used for qPCR are: PTGS2, forward 5'-CTGCGCCTTTTCAAGGATGG-3' and reverse 5'-GGGGATACACCTCTCCACCA-3'; FTH1, forward 5'-GCCGAGAAACTGATGAAGCTGC-3' and reverse 5'-GCACACTCCATTGCATTCAAGCC-3'; FTL, forward 5'-CTCCTGGGTTTTACCCCATTC-3' and reverse 5'-ACGCAGCTCAGTAACAGTCC-3'; β-actin, forward 5'-AGATCAAGATCATTGCTCCTC-3' and reverse 5'-ACGCAGCTCAGTAACAGTCC-3'.

2.16 Protein Expression and Purification. The NCOA4383-522 cDNA-coding region was synthesized and cloned into the BamHI and XhoI sites of pET28a(+) (expression of 8xHis-tagged proteins). Expression was achieved in Escherichia coli BL21 (DE3) induced with 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG) at 16 °C overnight. The bacteria were then pelleted, resuspended in lysis buffer (50 mM Tris-HCl pH 7.4, 400 mM NaCl, 10% glycerol, 2 mM β-mercaptoethanol), and sonicated. The proteins were purified using Ni-NTA resin (Qiagen) and a Superdex 75 column (GE Healthcare). The protein purity was above 95% as assessed by SDS-PAGE.

2.17 Surface Plasmon Resonance Analysis. The binding affinity of compounds to NCOA4383-522 was measured using Biacore 8K (GE Healthcare) with a CM5 sensor chip (GE Healthcare). Solutions of compounds were prepared with running buffer by serial dilutions from stock solutions. The samples were then injected at a flow rate of 30 μL/min for 120 s of association phase, followed with 100 s of disassociation phase at 25 °C. Data were analyzed with Biacore Insight Evaluation software.
2.18 ELISA for Protein-Protein Interaction Study. Ferritin antibody (0.5 μg/mL, 100 μL in PBS per cell) was coated on a 96-well Nunc Maxisorp plate (ThermoFisher Scientific) overnight at 4 °C. After washing with PBS supplemented with 0.05% Tween-20 (PBST), 1% BSA/PBST was used for blocking for 1 h at room temperature. Plates were washed twice in PBST. Recombinant human FTH1 protein (10 μg/mL, 100 μL in 1% BSA/PBST per cell) was added to the wells and incubated for 1 h at room temperature. The wells were washed three times with PBST and incubated with purified NCOA4383-522 (10 μg/mL, 100 μL in 1% BSA/PBST per cell) with varying concentrations of compound for 1 h. NCOA4383-522 was preincubated with compound for 0.5 h prior to its addition to the plate. After washing with PBST, HRP-conjugated His-tag antibody (diluted 1:5000 in 1% BSA/PBST, 100 μL per cell) was bound for 1 h. The wells were washed three times with PBST and HRP substrate 3,3′,5,5′-tetramethylbenzidine (TMB) solution was added and incubated for 20 min. The reaction was then stopped by addition of 1 M H2SO4 and absorbance was measured at 450 nm.

2.19 Sprague-Dawley Rats. Male Sprague-Dawley (SD) rats weighing 180-220 g were obtained from the Laboratory Animal Center of Sun Yat-sen University (Guangzhou, China). SD rats were housed individually under standard conditions of temperature and humidity, and a 12 h light-dark cycle, with free access to food and water before use. Adequate measures were taken to minimize pain or discomfort during surgeries. All experimental procedures were conducted based on the protocols approved by the Institution Animal Care and Use Committee of Sun Yat-sen University.

2.20 Transient Cerebral Ischemia Model in SD Rats. Transient cerebral ischemia was induced by intraluminal middle cerebral artery occlusion (MCAO). Briefly, rats weighing 220-250 g were anesthetized with intraperitoneal injection of phenobarbital (60 mg/kg). The left common carotid artery and external carotid artery were separated and ligated through a median cervical incision, and the pterygofrontal artery was separated as well. An arterial clamp was placed in the proximal and distal carotid arteries, and a 4-0 monofilament nylon suture was inserted through the incision, to a depth of 17-20 mm. The filament was withdrawn to restore blood flow (reperfusion) after 1.5 h of MCAO. All the SD rats subjected to tMCAO were randomly divided into
four groups (n = 18 per group). Vehicle, Fer-1 (10 mg/kg), or compound (10 mg/kg) was administered by intraperitoneal injection 0.5 h before and 2 h after MCAO occlusion. All the sham-operated control rats were performed the same surgical procedure without insertion of a filament. The animal’s body temperature was maintained at 37.0 ± 0.5 °C during the whole surgical procedure using a heat pad. We excluded rats from further studies if excessive bleeding occurred during surgery or the rat failed to recover from anesthesia.

2.21 Post Surgery Neurological Evaluation. The neurological assessment post-surgery was performed by an investigator blinded to the experimental groups. After 24 h of MCAO/reperfusion, the neurological deficit of each rat was evaluated by a five-point scale as described previously: 0, no observable deficit; 1, unable to extend the left forepaw; 2, circling to the left side; 3, reclination to the left side at rest; 4, failure to move spontaneously.²

2.22 TTC Staining. After 24 h of MCAO/reperfusion, rats were sacrificed. The brain was removed rapidly and placed in -20 °C for 20 min. Coronal slices were made at 2 mm intervals from the frontal poles, and sections were immersed in 1% 2,3,5-triprynyltetrazolium chloride (TTC) in phosphate-buffered saline at 37 °C for 20 min. The brain slices were fixed in 4 % paraformaldehyde at 4 °C until imaging. The red staining represented normal and healthy brain tissue and the white represented infarct tissue. The infarct volumes were calculated as follows: (area of contralateral hemisphere - area of ipsilateral noninfarcted region)/ area of contralateral hemisphere × 100 %.

2.23 H&E and Nissl Staining. Brains were fixed overnight in 4 % paraformaldehyde, embedded in paraffin, and serially sectioned, deparaffinized, and hydrated. The sections were stained with Hematoxylin and Eosin (H&E) or 0.1 % cresyl violet for routine histological examination with a light microscope.

2.24 MDA Assay. The level of MDA in lesioned hemisphere was measured using a kit (#S0131, Beyotime) in accordance with the manufacturer’s instructions. The values were normalized by protein concentration.

2.25 4-HNE Assay. For a ~30 mg piece of brain tissue, add ~270 μL lysis buffer to the
tube and homogenize with an electric homogenizer. The extract was then centrifuged for 30 min at 13,000 g at 4 °C to get supernatant. The 4-HNE assay was carried out using an ELISA kit following the manufacturer’s instructions (#ab238538, Abcam). The values were normalized by protein concentration.

**Statistical Analysis.** Graphs were drawn and statistics were analyzed by using GraphPad Prism 8 Software. Data were analyzed using two-tailed Student’s t test or one-way ANOVA and expressed as mean ± SEM. A p value less than 0.05 was considered statistically significant.
$^1$H and $^{13}$C NMR of 4a
\[ ^1H \text{ and } ^{13}C \text{ NMR of } 4b \]
$^1$H and $^{13}$C NMR of 4c
$^1$H and $^{13}$C NMR of 4d
$^1$H and $^{13}$C NMR of 4e
$^1$H and $^{13}$C NMR of 4f
$^1$H and $^{13}$C NMR of 4g
$^{1}H$ and $^{13}C$ NMR of 4h
$^1$H and $^{13}$C NMR of 9a
$^1\text{H}$ and $^{13}\text{C}$ NMR of 9b
$^1$H and $^{13}$C NMR of 9c
$^1$H and $^{13}$C NMR of 9d
$^1$H and $^{13}$C NMR of 9e
$^1$H and $^{13}$C NMR of 9f
$^1$H and $^{13}$C NMR of 9g
$^{1}H$ and $^{13}C$ NMR of 9h
$^1$H and $^{13}$C NMR of 9i
$^1$H and $^{13}$C NMR of 9j
$^{1}H$ and $^{13}C$ NMR of 9k
$^{1}$H and $^{13}$C NMR of 14a
$^1$H and $^{13}$C NMR of $^{14b}$
$^{1}H$ and $^{13}C$ NMR of 14c
$^{1}H$ and $^{13}C$ NMR of 14d
$^1$H and $^{13}$C NMR of 17a
\(^1\)H and \(^{13}\)C NMR of 17b
$^1$H and $^{13}$C NMR of 17c
HPLC analysis of Biotin-14d

HRMS spectrum of Biotin-14d
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