SUPPORTING INFORMATION

Defining the mechanism of action of S1QELs, specific suppressors of superoxide production in quinone-reaction site in mitochondrial complex I

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Figure S1
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Table S1
General synthetic methods

All moisture- and air-sensitive reactions were performed in oven-dried glassware under nitrogen or argon atmosphere with dry solvents under anhydrous conditions using standard syringe septum techniques. $^1$H-NMR spectra were recorded at 400 or 500 MHz with Bruker AVANCE III 400 or 500 spectrometers, respectively, using tetramethylsilane (TMS) as the internal standard. $^{13}$C-NMR spectra were recorded at 100 or 125 MHz, respectively. Chemical shifts (δ) were given in ppm relative to TMS with coupling constants (J) in Hz. The mass spectra were recorded on a Shimadzu LCMS-8040 with ESI source. Thin-layer chromatography (TLC) was performed on Merk TLC plate Silica-gel 60F254, and the spot was detected by iodine, anis, phosphomolybdic acid, or UV absorbance. Dry solvents were either used as purchased or freshly distilled using common practices where appropriate. HPLC purification was carried out with a Shimadzu LC-10 AS. Elution profiles were monitored at 254 nm with a Shimadzu SPD-10A.

Abbreviations

Ac$_2$O, acetic acid anhydride; AcOH, acetic acid; Boc, tert-butoxycarbonyl; chloramine T, sodium p-toluenesulfonchloramide trihydrate; DIBAL-H, diisobutylaluminium hydride; DMAP, 4-dimethylaminopyridine; DMF, N,N-dimethylformamide; Et$_3$N, triethylamine; HATU, N-[(dimethylamino)-1H-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide; LAH, lithium aluminium hydride; Lawesson’s reagent, 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-dithione; MS, mass spectrometry; NBS, N-bromosuccinimide; rt, rt; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography.
Scheme 1

Reagents and conditions: (a) Lawesson’s reagent, THF, reflux, 4 h, 81% and 92% (for 2a and 2b); (b) ethyl 2-chloroacetoacetate, EtOH, reflux, 4 h, 91% and 78% (for 3a and 3b); (c) LiAlH4, THF, 0 °C to rt, 1 h, 88% and 85% (for 4a and 4b); (d) SOCl2, DMF, CH2Cl2, 40 °C, 4 h; (e) NaCN, DMF, rt, 16 h, 87% and 50% (for 6a and 6b, 2 steps); (f) BH3·Me2S, THF, reflux, 4 h; (g) ethyl chloroglyoxylate, Et3N, THF, reflux, 1 h, 91% and 88% (for 9a and 9b); (h) 6.0 M NaOH aq., MeOH/THF, rt, overnight, 88% and 83% (for 10a and 10b); (i) HATU, N-methylmorpholine, DMF, rt, 16 h, 30%, 48% and 43% (for S1QEL1.1, S1QEL1.5, and S1QEL1.5_D1, 2 steps).

Synthesis of S1QEL1.1, S1QEL1.5, and S1QEL1.5_D1.
**p-Methylbenzothioamide (2a)**

To a solution of *p*-toluamide (1a) (1.7 g, 13 mmol) in anhydrous THF (40 mL) was added Lawesson’s reagent (5.1 g, 13 mmol) under an argon atmosphere, and the mixture was refluxed for 4 h (ref. 1). The reaction mixture was concentrated in vacuo, diluted with ethyl acetate, washed with saturated aqueous NaHCO₃ and brine, and the organic layer was dried over anhydrous MgSO₄. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 2% MeOH/CHCl₃) to afford 2a (1.5 g, 10 mmol, 81%): ¹H-NMR (400 MHz, CDCl₃) δ 7.79 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 7.9 Hz, 2H), 2.39 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃) δ 142.9, 136.2, 129.1 (2C), 127.0 (2C), 21.4; ESI-MS (*m/z*) 125.0 [M+H]⁺.

**p-Fluorobenzothioamide (2b)**

2b was prepared from *p*-fluorobenzamide (1b) according to the procedure described for 2a. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 2% MeOH/CHCl₃) to afford 2a (1.7 g, 11 mmol, 92%): ¹H-NMR (400 MHz, CDCl₃) δ 7.93-7.90 (m, 2H), 7.07 (t, *J* = 8.6 Hz, 2H), 2.30 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃) δ 166.5, 164.0, 135.5, 129.7, 129.6, 115.6, 115.4; ESI-MS (*m/z*) 156.0 [M+H]⁺.

**Ethyl 4-methyl-2-(*p*-tolyl)thiazole-5-carboxylate (3a)**

To a solution of 2a (2.0 g, 13 mmol) in ethanol (20 mL) was added ethyl 2-chloroacetoacetate (2.6 g, 16 mmol), and the mixture was refluxed for 4 h (ref. 1). Then, the reaction mixture was cooled 0 °C and stirred for 16 h to form white precipitate. The precipitate was collected by filtration, washed with ice-cooled ethanol, and dried *in vacuo* to afford pure 3a (3.2 g, 12.1 mmol, 91%): ¹H-NMR (400 MHz, DMSO-*d₆*) δ 7.81 (d, *J* = 8.2 Hz, 2H), 7.26 (d, *J* = 7.9 Hz, 2H), 4.23 (q, *J* = 7.1 Hz, 2H), 2.60 (s, 3H), 2.29 (s, 3H), 1.23 (t, *J* = 7.1, 3H); ¹³C-NMR (100 MHz, DMSO-*d₆*) δ 169.7, 161.9, 160.7, 142.0, 130.4, 130.0 (2C), 127.0 (2C), 121.2, 61.63, 21.5, 17.7, 14.6; ESI-MS (*m/z*) 262.1 [M+H]⁺.
Ethyl 2-(p-fluorophenyl)-4-methylthiazole-5-carboxylate (3b)

3b was prepared from 2b according to the procedure described for 3a to afford 3b (1.3 g, 5.0 mmol, 78%): $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 7.97-7.94 (m, 2H), 7.14 (t, $J$ = 8.7 Hz, 2H), 4.36(q, $J$ = 7.2 Hz, 2H), 2.77 (s, 3H), 1.39 (t, $J$ = 7.1, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ 168.8, 165.9, 162.4, 161.2, 129.6, 129.1, 129.0, 122.1, 116.5, 116.3, 61.5, 26.4, 17.7, 14.5; ESI-MS (m/z) 266.1 [M+H]$^+$. 

(4-Methyl-2-(p-tolyl)thiazol-5-yl)methanol (4a)

To an ice-cooled suspension of LiAlH$_4$ (1.7 g, 46 mmol) in anhydrous THF (60 mL) was added 3a (3.0 g, 12 mmol) in THF (20 mL) under a nitrogen atmosphere, and the mixture was warmed up to the room temperature and stirred for further 1 h. The reaction was quenched with water, extracted with ethyl acetate, washed with brine and the organic layer was dried over anhydrous MgSO$_4$. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 5% MeOH/CHCl$_3$) to afford 4a (2.2 g, 10 mmol, 88%): $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 7.77 (d, $J$ = 8.0 Hz, 2H), 7.22 (d, $J$ = 8.1 Hz, 2H), 4.80 (s, 2H), 2.42 (s, 3H), 2.38 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ 166.5, 150.3, 140.2, 131.0, 130.5, 130.0 (2C), 126.3 (2C), 56.9, 21.4, 21.5, 15.1; ESI-MS (m/z) 220.1 [M+H]$^+$. 

(2-(p-Fluorophenyl)-4-methylthiazol-5-yl)methanol (4b)

4b was prepared from 3b according to the procedure described for 4a. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 5% MeOH/CHCl$_3$) to afford 4b (932 mg, 4.17 mmol, 85%): $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 7.87-7.84 (m, 2H), 7.10 (t, $J$ = 8.5 Hz, 2H), 4.80 (s, 2H), 2.42 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ 165.2, 162.8, 150.5, 131.4, 130.2, 128.5, 128.4, 116.3, 116.1, 57.0, 26.4, 15.3; ESI-MS (m/z) 224.1 [M+H]$^+$. 

S4
5-(Chloromethyl)-4-methyl-2-(p-tolyl)thiazole (5a)

To a solution of 4a (2.0 g, 9.1 mmol) in anhydrous dichloromethane (50 mL) were added thionyl chloride (6.5 g, 55 mmol) and DMF (1 drop) at room temperature under nitrogen atmosphere. After stirring at 40 °C for 3 h, the solvent was removed in vacuo to afford crude product of 5a, which was subjected to the next reaction without further purification: ESI-MS (m/z) 238.1 [M+H]+.

5-(Chloromethyl)-2-(p-fluorophenyl)-4-methylthiazole (5b)

5b was prepared from 4b according to the procedure described for 5a. The crude 5b was subjected to the next reaction without further separation: ESI-MS (m/z) 242.1 [M+H]+.

2-(4-Methyl-2-(p-tolyl)thiazol-5-yl)acetonitrile (6a)

To a solution of 5a (2.0 g) in anhydrous DMF (60 mL) was added sodium cyanide (1.3 g, 27 mmol) at room temperature under nitrogen atmosphere, and the mixture was stirred for 16 h. The reaction was quenched with water, extracted with ethyl acetate, washed with brine and dried over MgSO4. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, CHCl3) to afford 6a (1.8 g, 7.9 mmol, 81%): 1H-NMR (400 MHz, CDCl3) δ 7.79 (d, J = 9.9 Hz, 2H), 7.23 (d, J = 7.9 Hz, 2H), 3.84 (s, 2H), 2.45 (s, 3H), 2.39 (s, 3H); 13C-NMR (100 MHz, CDCl3) δ 166.5, 151.7, 140.7, 130.5, 129.7 (2C), 126.4, 126.3 (2C), 117.9, 21.4, 15.3, 15.1; ESI-MS (m/z) 229.1 [M+H]+.

2-(2-(p-Fluorophenyl)-4-methylthiazol-5-yl)acetonitrile (6b)

6b was prepared from 5b according to the procedure described for 6a. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, CHCl3) to afford 6a (469 mg, 2.02 mmol, 50%): 1H-NMR (400 MHz, CDCl3) δ 7.89-7.85 (m, 2H), 7.12 (t, J = 8.6 Hz, 2H), 3.85 (s, 2H), 2.45 (s, 3H); 13C-NMR (100 MHz, CDCl3) δ 166.5, 151.7, 140.7, 130.5, 129.7 (2C), 126.4, 126.3 (2C), 117.9, 21.4, 15.3, 15.1; ESI-MS (m/z) 233.1 [M+H]+.
2-(4-Methyl-2-((p-tolyl)thiazol-5-yl)ethan-1-amine (7a)

To a solution of 6a (200 mg, 0.88 mmol) in anhydrous THF (20 mL) was added borane-dimethyl sulfide complex (2.0 M in THF, 1.76 mL, 3.52 mmol) at room temperature under nitrogen atmosphere, and the mixture was refluxed for 4 h. The reaction was carefully quenched by the addition of methanol, followed by the extraction of the mixture with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na$_2$SO$_4$ to give 7a as a crude product, which was subjected to the next step without further purification: $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 7.76 (d, $J$ = 8.1 Hz, 2H), 7.20 (d, $J$ = 7.9 Hz, 2H), 2.97 (t, $J$ = 6.3 Hz, 2H), 2.89(t, $J$ = 6.4 Hz, 2H), 2.41 (s, 3H), 2.37 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ 164.5, 149.5, 139.7, 131.2, 129.5 (2C), 128.8, 126.0 (2C), 43.3, 30.7, 21.4, 15.2; ESI-MS (m/z) 233.1 [M+H]$^+$.

2-(2-((p-Fluorophenyl)-4-methylthiazol-5-yl)ethan-1-amine (7b)

7b was prepared from 6b according to the procedure described for 7a. The crude product 7b was subjected to the next reaction without further purification: ESI-MS (m/z) 237.1 [M+H]$^+$.

Ethyl N-(m-acetamidophenyl)-oxamate (9a)

To an ice-cooled solution of m-aminoacetanilide (8a) (500 mg, 3.33 mmol) in anhydrous THF (14 mL) were added triethylamine (741 mg, 7.32 mmol) and ethyl chloroglyoxylate (500 mg, 3.66 mmol) at room temperature under nitrogen atmosphere, and the mixture was refluxed for 1 h. The reaction was quenched with water, extracted with ethyl acetate, washed with brine, and dried over anhydrous MgSO$_4$ to afford pure 9a (758 mg, 3.03 mmol, 91%): $^1$H-NMR (400 MHz, MeOD) $\delta$ 8.00 (d, $J$ = 2.0 Hz, 1H), 7.43-7.39 (m, 2H), 7.30 (t, $J$ = 6.4 Hz, 2H), 4.39 (q, $J$ = 7.1 Hz, 2H), 2.13 (s, 3H), 1.40 (t, $J$ = 7.1 Hz, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ 171.9, 161.9, 157.4, 140.6, 138.9, 130.3, 118.3, 117.7, 113.9, 64.3, 24.0, 14.4; ESI-MS (m/z) 251.1 [M+H]$^+$. 

S6
**Ethyl N-(3,4-dimethylphenyl)oxamate (9b)**

9b was prepared from 3,4-dimethylaniline (8b) according to the procedure described for 8a (640 mg, 2.9 mmol, 91%): \(^1\)H-NMR (400 MHz, MeOD) \(\delta\) 7.46 (d, \(J = 1.8\) Hz, 1H), 7.43 (dd, \(J = 8.1, 2.3\) Hz, 1H), 7.12 (d, \(J = 8.1\) Hz, 1H), 4.39 (q, \(J = 7.1\) Hz, 2H), 2.27 (s, 3H), 2.25 (s, 3H), 1.41 (t, \(J = 7.1\) Hz, 3H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta\) 162.0, 157.2, 138.4, 136.2, 135.1, 131.0, 123.1, 120.0, 64.2, 20.1, 19.4, 14.4; ESI-MS (\(m/z\)) 222.1 [M+H]\(^+\).

**N-(m-Acetamidophenyl)oxamic acid (10a)**

To a solution of 9a (400 mg, 1.60 mmol) in a mixture of methanol/THF (12 mL/4 mL) was added 6.0 M aqueous NaOH (0.8 mL). After stirring at room temperature for 16 h, the mixture was acidified with 1.0 M HCl to pH 4 ~ 5, followed by the removal of organic solvent in vacuo. The residue was extracted with ethyl acetate, washed with brine, and dried over MgSO\(_4\). The removal of the organic solvent gave 10a (313 mg, 1.41 mmol, 88%): \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 10.15 (s, 1H), 10.12 (s, 1H), 7.99 (s, 1H), 7.33 (t, \(J = 10.0\) Hz, 2H), 7.18 (t, \(J = 8.0\) Hz, 1H), 4.39 (q, \(J = 7.1\) Hz, 2H), 2.13 (s, 3H); \(^{13}\)C-NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 168.4, 164.2, 162.7, 139.6, 138.5, 128.7, 114.6, 110.7, 24.0; ESI-MS (\(m/z\)) 221.1 [M-H]\(^-\).

**N-(3,4-Dimethylphenyl)oxamic acid (10b)**

10b was prepared from 3,4-dimethylaniline (9b) according to the procedure described for 10a (530 mg, 2.75 mmol, 83%): \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 10.5 (s, 1H), 7.52 (d, \(J = 1.9\) Hz, 1H), 7.45 (dd, \(J = 8.2, 2.2\) Hz, 1H), 7.08 (d, \(J = 8.2\) Hz, 1H), 2.19 (s, 3H), 2.17 (s, 3H); \(^{13}\)C-NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 162.3, 156.7, 136.4, 135.4, 132.5, 129.6, 121.5, 117.9, 19.7, 18.9; ESI-MS (\(m/z\)) 192.1 [M-H]\(^-\).
**N-(m-Acetamidophenyl)-N’-(2-(4-methyl-2-(p-tolyl)thiazol-5-yl)ethyl)oxamide (S1QEL1.1)**

To a solution of 7a (200 mg) and 10a (250 mg, 1.08 mmol) in DMF (2.0 mL) were added N-methylmorpholine (455 mg, 4.50 mmol) and HATU (513 mg, 1.35 mmol) sequentially under nitrogen atmosphere. After stirring at room temperature for 16 h, the reaction was quenched with brine, extracted with ethyl acetate, and dried over anhydrous MgSO₄. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 10% MeOH/CHCl₃), which was further purified by recrystallization from CHCl₃ to afford pure S1QEL1.1 (118 mg, 0.27 mmol, 30%): ¹H-NMR (400 MHz, DMSO-δ₆) δ 10.55 (s, 1H), 9.97 (s, 1H), 9.13 (t, J = 6.1 Hz, 1H), 8.07 (s, 1H), 7.73 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 14.3 Hz, 1H), 7.37 (d, J = 15.2 Hz, 1H), 7.27-7.21 (m, 3H), 3.43 (q, J = 6.5 Hz, 2H), 3.03 (t, J = 6.9 Hz, 2H), 2.33(s, 3H), 2.03 (s, 3H); ¹³C-NMR (100 MHz, DMSO-δ₆) δ 168.4, 163.2, 160.1, 158.4, 149.6, 139.5, 137.8, 130.7, 129.7, 128.8, 128.4, 125.6, 115.5, 111.4, 25.5, 24.0, 20.9, 14.8; ESI-MS (m/z) 437.2 [M+H]+.

**N-(3,4-Dimethylphenyl)-N’-(2-(2-(p-fluorophenyl)-4-methylthiazol-5-yl)ethyl)oxamide (S1QEL1.5)**

S1QEL1.5 was prepared from 7b and 10b according to the procedure described for S1QEL1.1. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 10% MeOH/CHCl₃), which was further purified by recrystallization from ethanol to afford S1QEL1.5 (138 mg, 0.34 mmol, 48%, 2 steps from 6b): ¹H-NMR (400 MHz/ CDCl₃) δ 9.13 (s, 1H), 7.86-7.84 (m, 2H), 7.77 (d, J = 1.3, 1H), 7.40 (d, J = 1.9, 1H), 7.36 (dd, J = 8.1, 2.2 Hz, 1H), 7.13-7.07 (m, 3H), 3.63 (q, J = 6.7 Hz, 2H), 3.07 (t, J = 6.9 Hz, 2H), 2.41 (s, 3H), 2.26 (s, 3H), 2.24 (s, 3H), 2.03 (s, 3H); ¹³C-NMR (100 MHz/ CDCl₃) δ 163.9, 160.5 (2C), 157.1, 150.6, 137.8, 134.2 (2C), 130.4 (2C), 128.4, 128.3, 127.7, 121.2, 117.5, 116.2, 116.0, 41.1, 26.6, 20.1, 19.5, 15.3; ESI-MS (m/z) 412.3 [M+H]+.
**N-(3,4-Dimethylphenyl)-N’-(2-(4-methyl-2-(p-tolyl)thiazol-5-yl)ethyl)oxamide (S1QEL1.5_D1)**

S1QEL1.5_D1 was prepared from 7a and 10b according to the procedure described for S1QEL1.1. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 10% MeOH/CHCl₃), which was further purified by recrystallization from ethanol to afford S1QEL1.5_D1 (255 mg, 0.62 mmol, 43%, 2 steps from 6a): ¹H-NMR (400 MHz, CDCl₃) δ 9.15 (s, 1H), 7.83 (t, J = 6.1 Hz, 1H), 7.76 (d, J = 6.4 Hz, 2H), 7.40 (d, J = 2.0 Hz, 1H), 7.36 (dd, J = 8.2, 2.4 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 8.1 Hz, 1H) 3.62 (q, J = 6.8 Hz, 2H), 3.06 (t, J = 6.9 Hz, 2H), 2.41 (s, 3H), 2.38 (s, 3H), 2.26 (s, 3H), 2.24 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃) δ 165.3, 160.5, 157.2, 150.4, 140.1, 137.8, 134.2, 130.4, 129.7 (2C), 127.1, 126.4 (2C), 121.2, 117.4, 41.1, 26.6, 21.6, 20.1, 19.5, 15.3; ESI-MS (m/z) 408.3 [M+H]+.
Scheme 2

Reagents and conditions: (a) phthalic anhydride, AcOK, AcOH, reflux, 3 h, 82%; (b) PCl₅, toluene, reflux, 3 h, 87%; (c) bis(2-chloroethyl)amine hydrochloride, 2-(2-methoxyethoxy)ethanol, 150 °C, 16 h, 52%; (d) Et₃N, CH₂Cl₂, rt, 5 h, 62% and 86% (for 16a and 16b); (e) H₂NN₂·H₂O, EtOH, reflux, 7 h, 73% and 92% (for 17a and 17b); (f) SOCl₂, toluene, 75 °C, 5 h; (g) Et₃N, DMAP, CH₂Cl₂, rt, 3 h, 63% and 41% (for S1QEL.2.1 and S1QEL.2.3, 2 steps).

Synthesis of S1QEL.2.1 and S1QEL.2.3.

Reagents and conditions: (a) phthalic anhydride, AcOK, AcOH, reflux, 3 h, 82%; (b) PCl₅, toluene, reflux, 3 h, 87%; (c) bis(2-chloroethyl)amine hydrochloride, 2-(2-methoxyethoxy)ethanol, 150 °C, 16 h, 52%; (d) Et₃N, CH₂Cl₂, rt, 5 h, 62% and 86% (for 16a and 16b); (e) H₂NN₂·H₂O, EtOH, reflux, 7 h, 73% and 92% (for 17a and 17b); (f) SOCl₂, toluene, 75 °C, 5 h; (g) Et₃N, DMAP, CH₂Cl₂, rt, 3 h, 63% and 41% (for S1QEL.2.1 and S1QEL.2.3, 2 steps).
Potassium 2-(1,3-dioxoisoindolin-2-yl)ethane-1-sulfonate (12)

To a solution of 2-aminoethanesulfonic acid (11) (2.0 g, 16 mmol) in acetic acid (8.0 mL) was added potassium acetate (1.4 g, 14 mmol) at room temperature. The mixture was refluxed for 10 min, followed by the addition of phthalic anhydride (2.6 g, 18 mmol). After stirring the mixture under reflux for further 3 h, the reaction mixture was cooled to 0 °C to form white precipitate. The precipitate was collected by filtration, washed with ice-cooled ethanol, and dried in vacuo to afford pure 12 (3.9 g, 13 mmol, 82%): 1H-NMR (400 MHz, DMSO-d6) δ 7.86-7.81 (m, 4H), 3.86-3.82 (m, 2H), 2.78-2.74 (m, 2H); 13C-NMR (100 MHz, DMSO-d6) δ 167.7, 134.3, 131.8, 122.9, 48.7, 34.5; ESI-MS (m/z) 294.2 [M+H]+.

2-(1,3-Dioxoisoindolin-2-yl)ethane-1-sulfonyl chloride (13)

To a solution of 12 (1.6 g, 5.5 mmol) in anhydrous toluene (8.0 mL) was added phosphorus pentachloride (1.0 g, 4.9 mmol) in one portion. After stirring the mixture under reflux for 1 h, additional phosphorous pentachloride (1.0 g, 4.9 mmol) was added, and the resulting mixture was refluxed for further 1 h. The mixture was concentrated in vacuo, then the residue was washed with ice-cooled water to afford 13 (1.3 g, 4.8 mmol, 87%): 1H-NMR (400 MHz, CDCl3) δ 7.91-7.89 (m, 2H), 7.79-7.76 (m, 2H), 4.36 (t, J = 6.5 Hz, 2H), 4.09 (t, J = 6.5 Hz, 2H); 13C-NMR (100 MHz, CDCl3) δ 167.5 (2C), 134.7 (2C), 131.8 (2C), 124.0 (2C), 61.6, 33.0; ESI-MS (m/z) 274.5 [M+H]+.

1-(p-Methoxyphenyl)piperazine (15a)

To a solution of p-anisidine (14) (500 mg, 4.06 mmol) in 2-(2-methoxyethoxy)ethanol (1.5 mL) was added bis(2-chloroethyl)amine hydrochloride (725 mg, 4.06 mmol), and the mixture was stirred at 150 °C for 16 h. The mixture was cooled to room temperature, and it was diluted with diethyl ether (150 mL) to form brown solid. The solid was collected by filtration and dried in vacuo to afford 15a (402 mg, 2.09 mmol, 52%): 1H-NMR (400 MHz, CDCl3) δ 7.02 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 9.0 Hz, 2H), 3.77 (s, 3H), 3.48 (d, J = 6.7 Hz, 8H); 13C-NMR (100 MHz, CDCl3) δ 124.9, 120.3, 115.0 (4C), 55.8, 49.0 (2C), 43.6 (2C); ESI-MS (m/z) 193.2 [M-H].
2-(2-((4-\text{-}Methoxyphenyl)piperazin-1-yl)sulfonyl)ethyl)isoindoline-1,3-dione (16a)

To a solution of (15a) (400 mg, 2.08 mmol) and triethylamine (442 mg, 4.38 mmol) in dichloromethane (10 mL) was added 13 (627 mg, 2.30 mmol) in small portions, and the mixture was stirred at room temperature for 5 h. The reaction was quenched with 1.0 M aqueous HCl, extracted with dichloromethane, and dried over anhydrous MgSO₄. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 10% MeOH/CHCl₃) to afford 16a (555 mg, 1.3 mmol, 62%): ¹H-NMR (400 MHz, CDCl₃) δ 7.88-7.86 (m, 2H), 7.75-7.73 (m, 2H), 6.90-6.83 (m, 4H), 4.17 (t, \(J = 7.0\) Hz, 2H), 3.77 (s, 3H) 3.47 (t, \(J = 4.9\) Hz, 4H), 3.38 (t, \(J = 7.0\) Hz, 2H), 3.11 (t, \(J = 4.9\) Hz, 4H); ¹³C-NMR (100 MHz, CDCl₃) δ 167.9 (2C), 154.8, 134.5 (2C), 132.1 (2C), 124.0, 123.8 (2C), 119.5 (2C), 114.8 (2C), 55.8, 51.3 (2C), 46.9 46.1 (2C), 32.6; ESI-MS (m/z) 430.2 [M+H]⁺.

2-((4-\text{-}Methoxyphenyl)piperazin-1-yl)sulfonyl)ethan-1-amine (17a)

To a solution of 16a (550 mg, 1.28 mmol) in ethanol (10 mL) was added hydrazine monohydrate (78 mg, 1.54 mmol), and the mixture was heated under reflux for 1 h. The reaction was quenched by the addition of 1.0 M aqueous HCl, followed by the removal of insoluble material by filtration. The filtrate was basified with 2.0 M aqueous NaOH, then extracted with ethyl acetate. The organic layer was dried over anhydrous Na₂SO₄ to afford 17a (353 mg, 1.18 mmol, 92%): ¹H-NMR (400 MHz, DMSO-\(d₆\)) δ 6.92 (d, \(J = 9.1\) Hz, 2H), 6.83(t, \(J = 9.1\) Hz, 2H), 3.77 (s, 3H) 3.31-3.26 (m, 6H), 3.06 (t, \(J = 4.7\) Hz, 4H), 2.98 (t, \(J = 7.1\) Hz, 2H); ¹³C-NMR (100 MHz, DMSO-\(d₆\)) δ 153.6, 144.9, 118.3 (2C), 114.4 (2C), 55.3, 49.9 (2C), 49.6 45.3 (2C), 35.5; ESI-MS (m/z) 360.2 [M+H]⁺.
To a solution of 18 (300 mg, 1.6 mmol) in toluene (3.0 mL) was added thionyl chloride (213 mg, 1.89 mmol) and DMF (1 drop), and the mixture was stirred at 75 °C for 5 h. The mixture was concentrated in vacuo to provide a crude acyl chloride 19.

To a solution of 17a (200 mg, 0.67 mmol), triethylamine (135 mg, 1.34 mmol), and N,N-dimethyl-4-aminopyridine (4 mg, 0.03 mmol) in anhydrous dichloromethane (5.0 mL) was added 19 in dichloromethane (1.0 mL), and the mixture was stirred at room temperature for 3 h. The reaction was quenched with water, extracted with dichloromethane, dried over anhydrous MgSO₄. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, /CHCl₃) to afford 16a (129 mg, 0.28 mmol, 41%): ¹H-NMR (400 MHz, CDCl₃) δ 6.92-6.84 (m, 4H), 6.25 (t, J = 6.8 Hz, 1H), 3.78-3.74 (m, 5H), 3.42 (t, J = 4.9 Hz, 4H), 3.15-3.09 (m, 6H), 2.05 (tt, J = 12.2, 3.5 Hz, 1H), 1.90 (d, J = 13.8 Hz, 2H), 1.82 (d, J = 12.6 Hz, 2H), 1.82 (m, 2H), 1.28-1.18 (m, 7H), 0.92-0.87 (m, 5H); ¹³C-NMR (100 MHz, CDCl₃) δ 176.7, 154.9, 145.2, 119.5 (2C), 114.8 (2C), 55.8, 51.2 (2C), 46.1 (2C), 45.6, 37.1 (2C), 33.8, 32.7 (2C), 29.7 (2C), 29.3 (2C), 23.2, 14.3; ESI-MS (m/z) 466.4 [M+H]+.

2-(2-((4-Phenylpiperazin-1-yl)sulfonyl)ethyl)isoindoline-1,3-dione (16b)

16b was prepared from 15b according to the procedure described for 16a. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 2% MeOH/CHCl₃) to afford 16b (456 mg, 1.14 mmol, 86%): ¹H-NMR (400 MHz, CDCl₃) δ 7.88-7.86 (m, 2H), 7.75-7.72(m, 2H), 7.28 (t, J = 8.1 Hz, 2H), 6.94-6.91 (m, 3H), 4.18 (t, J = 7.1 Hz, 2H), 3.47 (t, J = 5.0 Hz, 4H), 3.38 (t, J = 7.0 Hz, 2H), 3.24 (t, J = 5.0 Hz, 4H); ¹³C-NMR (100 MHz, CDCl₃) δ 167.7 (2C), 150.6, 134.6 (2C), 132.1 (2C), 129.5 (2C), 123.8 (2C), 117.2 (2C), 121.0, 49.6 (2C), 45.8, 45.6 (2C), 32.4; ESI-MS (m/z) 400.1 [M+H]+.
2-((4-phenylpiperazin-1-yl)sulfonyl)ethan-1-amine (17b)

17b was prepared from 16b according to the procedure described for 17a (147 mg, 0.55 mmol, 73%): \(^1\)H-NMR (400 MHz, MeOD) \(\delta\) 7.74 (d, \(J = 8.1\) Hz, 2H), 7.52-7.43(m, 3H), 3.84 (t, \(J = 4.7\) Hz, 4H), 3.75 (t, \(J = 4.8\) Hz, 4H), 3.57 (t, \(J = 6.8\) Hz, 2H), 3.37 (t, \(J = 6.7\) Hz, 2H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta\) 153.6, 130.3 (2C), 121.8, 118.3 (2C), 51.6, 51.0 (2C), 47.0 (2C), 37.1; ESI-MS (\(m/z\)) 270.1 [M+H]\(^+\).

(1S,4R)-4-butyl-N-(2-((4-phenylpiperazin-1-yl)sulfonyl)ethyl)cyclohexane-1-carboxamide (S1QEL2.3)

S1QEL2.3 was prepared from 17b and 19 according to the procedure described for S1QEL2.1. The crude product was purified by silica-gel column chromatography (Wako gel\(^\circ\) C-200, CHCl\(_3\)), which was further purified by recrystallized from CHCl\(_3\) to afford S1QEL2.3 (203 mg, 0.47 mmol, 63%): \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.29 (t, \(J = 6.3\) Hz, 4H), 6.24 (t, \(J = 5.7\) Hz, 1H), 3.76 (q, \(J = 5.8\) Hz, 2H),3.43 (t, \(J = 5.0\) Hz, 4H), 3.26 (t, \(J = 5.0\) Hz, 4H), 3.10 (t, \(J = 5.8\) Hz, 2H), 2.05 (tt, \(J = 12.2, 3.5\) Hz, 1H), 1.90 (d, \(J = 13.8\) Hz, 2H), 1.82 (d, \(J = 12.5\) Hz, 2H), 1.82 (m, 2H), 1.28-1.18 (m, 7H), 0.96-0.88 (m, 5H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta\) 176.5, 150.7, 129.3 (2C), 121.0, 117.1 (2C), 49.6 (2C), 45.7 (2C), 45.5, 36.9 (2C), 33.6, 32.4 (2C), 29.5 (2C), 29.1 (2C), 23.0, 14.1; ESI-MS (\(m/z\)) 436.3 [M+H]\(^+\).
Scheme 3

**Synthesis of S1QEL1.1_D1, S1QEL1.1_D2, and S1QEL1.1_D3.**

Reagents and conditions: (a) HCOOH, reflux, 16 h, 71%; (b) H₂, Pd/C, EtOH, rt, 2 h, quant.; (c) Boc₂O, Et₃N, THF, rt, 16 h, 84%; (d) NaH, MeI, DMF, rt, 2 h, 45%; (e) H₂, Pd/C, EtOH, rt, 2 h, 90%; (f) Boc₂O, Et₃N, THF, rt, 16 h, 68%; (g) ethyl chloroglyoxylate, Et₃N, THF, reflux, 1 h, 92%, 81%, and quant. (for 27a, 27b, and 27c); (h) 6.0 M aq. NaOH, MeOH/THF, rt, 16 h, 46%, 97%, and quant. (for 28a, 28b, and 28c); (i) 7a, HATU, N-methylmorpholine, DMF, rt, 4 h, 1.8% (for S1QEL1.1_D4); (j) TFA, CH₂Cl₂, rt, 3 h, 9.1% and 8.3% (for S1QEL1.1_D3 and S1QEL1.1_D2, 2 steps).
**N-(m-Nitrophenyl)formamide (21)**

A mixture of \( m \)-nitroaniline (20) (1.38 g, 10.0 mmol) and formic acid (15 mL) was heated under reflux for 16 h. The mixture was concentrated *in vacuo*. The crude product was purified by recrystallization from methanol to afford 21 (1.18 g, 7.08 mmol, 71%) as a mixture of rotamers (84:16 on \(^1\)H-NMR): \(^1\)H-NMR (400 MHz, DMSO-\(d_6\), major rotamer) \( \delta \) 10.68 (br, 1H), 8.61 (t, \( J = 2.1 \) Hz, 1H), 8.37 (s, 1H), 7.95-7.86 (m, 2H), 7.69-7.58 (m, 1H); \(^1\)H-NMR (400 MHz, DMSO-\(d_6\), minor rotamer) \( \delta \) 10.68 (br, 1H), 8.94 (s, 1H), 8.02 (t, \( J = 1.9 \) Hz, 1H), 7.95-7.86 (m, 1H), 7.69-7.58 (m, 2H); \(^13\)C-NMR (100 MHz, DMSO-\(d_6\)) \( \delta \) 160.5, 148.1, 139.3, 130.5, 125.2, 118.3, 113.5.

**N-(m-Aminophenyl)formamide (22a)**

A solution of 21 (332 mg, 2.01 mmol) in anhydrous ethanol (16 mL) was stirred with 10% palladium on carbon (56 mg) under hydrogen atmosphere. After stirring at room temperature for 2 h, the mixture was filtered through a pad of Celite\(^\text{\circledR}\). The filtrate was concentrated *in vacuo* to afford 22a (272 mg, 2.01 mmol, quant.) as a mixture of rotamers (68:32 on \(^1\)H-NMR): \(^1\)H-NMR (400 MHz, DMSO-\(d_6\), major rotamer,) \( \delta \) 9.85 (br s, 1H), 8.15 (d, \( J = 1.9 \) Hz, 1H), 6.92 (t, \( J = 8.0 \) Hz, 1H), 6.88 (t, \( J = 2.0 \) Hz, 1H), 6.65 (dd, \( J = 7.9, 0.9 \) Hz, 1H), 6.29 (dd, \( J = 8.0, 1.4 \) Hz, 1H), 5.04 (br s, 1H); \(^1\)H-NMR (400 MHz, DMSO-\(d_6\), minor rotamer) \( \delta \) 9.90 (br s, 1H), 8.62 (d, \( J = 11.1 \) Hz, 1H), 6.93 (t, \( J = 7.9 \) Hz, 1H), 6.34-6.29 (m, 3H), 5.10 (br s, 1H); \(^13\)C-NMR (100 MHz, DMSO-\(d_6\), major rotamer) \( \delta \) 159.7, 149.3, 138.98, 129.5, 110.2, 107.5, 105.2; \(^13\)C-NMR (100 MHz, DMSO-\(d_6\), minor rotamer) \( \delta \) 162.7, 149.9, 139.02, 130.2, 110.3, 105.7, 103.4; ESI-MS (\( m/z \)) 136.2 [M+H]\(^+\).
**tert-Butyl (m-nitrophenyl)carbamate (23)**

To an ice-cooled solution of m-nitroaniline (20) (1.4 g, 10 mmol) and di-tert-butyl dicarbonate (2.2 g, 10 mmol) in anhydrous THF (50 mL) was added N,N-dimethyl-4-aminopyridine (1.3 g, 11 mmol) under a nitrogen atmosphere. After stirring at room temperature for 16 h, the reaction mixture was diluted with water, followed by the removal of organic solvent under reduced pressure. The residue was extracted with ethyl acetate, washed with brine, and the organic layer was dried over MgSO4. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 0-10% methanol/chloroform) to afford 24 (2.0 g, 8.4 mmol, 84%): 1H-NMR (400 MHz, CDCl3) δ 8.31 (t, J = 2.2 Hz, 1H), 7.88 (ddd, J = 8.2, 2.2, 0.8 Hz, 1H), 7.69 (d, J = 7.9 Hz, 1H), 7.43 (t, J = 4.1 Hz, 1H), 6.83 (br s, 1H), 1.54 (s, 9H); 13C-NMR (100 MHz, CDCl3) δ 152.5, 148.9, 139.9, 129.9, 124.1, 117.8, 113.3, 81.8, 28.4 (3C); ESI-MS (m/z) 239.1 [M+H]+.

**tert-Butyl methyl(m-nitrophenyl)carbamate (24)**

To an ice-cooled solution of 23 (1.7 g, 7.0 mmol) in anhydrous DMF (14 mL) was added NaH (336 mg, 8.40 mmol, 60% in mineral oil), and the mixture was stirred at room temperature for 1 h. The reaction mixture was cooled with ice-bath, then iodomethane (1.5 g, 11 mmol) was added, followed by the stirring of the mixture for further 2h. The reaction was quenched with water, extracted with ethyl acetate, washed with brine, and dried over anhydrous MgSO4. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 0-10% methanol/chloroform) to afford 25 (790 mg, 3.13 mmol, 45%): 1H-NMR (400 MHz, CDCl3) δ 8.16 (t, J = 2.2 Hz, 1H), 8.01 (ddd, J = 8.2, 2.2, 1.0 Hz, 1H), 7.63 (dd, J = 8.1, 1.2 Hz, 1H), 7.49 (t, J = 8.1 Hz, 1H), 3.33 (s, 3H), 1.49 (s, 9H); 13C-NMR (100 MHz, CDCl3) δ 154.2, 148.5, 145.0, 131.1, 129.3, 120.0, 119.9, 119.7, 81.7, 37.1, 28.5 (3C); ESI-MS (m/z) 253.1 [M+H]+.

**tert-Butyl (m-aminophenyl)(methyl)carbamate (22b)**

22b was prepared from 24 according to the procedure described for 22a (624 mg, 2.81 mmol, 90%): 1H-NMR (400 MHz, CDCl3) δ 7.10 (t, J = 7.9 Hz, 1H), 6.66-6.62 (m, 2H), 6.55-6.52 (m, 1H), 3.52 (br s, 2H), 3.21 (s, 3H), 1.45 (s, 9H); 13C-NMR (100 MHz, CDCl3) δ 155.0, 146.2, 145.0, 129.5, 116.4, 113.0, 112.9, 80.3, 37.5, 28.5 (3C); ESI-MS (m/z) 223.1 [M+H]+.
**tert-butyl (m-aminophenyl)carbamate (22c)**

To an ice-cooled solution of m-phenylenediamine (26) (400 mg, 3.71 mmol) and triethylamine (374 mg, 3.71 mmol) in anhydrous THF (20 mL) was added di-tert-butyl dicarbonate (808 mg, 3.71 mmol) under nitrogen atmosphere. After stirring at room temperature for 16 h, the mixture was concentrated in vacuo. The residue was resuspended in toluene, washed with brine, and the organic layer was dried over anhydrous MgSO$_4$. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 30% ethyl acetate/n-hexane) to afford 22c (523 mg, 2.51 mmol, 68%): 1H-NMR (400 MHz, CDCl$_3$) $\delta$ 7.03 (t, $J = 8.0$ Hz, 1H), 6.97 (br s, 1H), 6.55-6.52 (m, 1H), 6.40 (br s, 1H), 6.37-6.34 (m, 1H), 3.66 (br s, 2H), 1.51 (s, 9H); 13C-NMR (100 MHz, CDCl$_3$) $\delta$ 147.3, 139.5, 129.8, 110.0, 108.7, 105.2, 80.5, 28.5 (3C); ESI-MS ($m/z$) 209.1 [M+H]$^+$.  

**Ethyl N-(m-formamidophenyl)oxamate (26a)**

To an ice-cooled solution of 22a (272 mg, 2.00 mmol) in anhydrous THF (7 mL) were added triethylamine (405 mg, 4.00 mmol) and ethyl chloroglyoxylate (300 mg, 2.20 mmol) under a nitrogen atmosphere. After refluxed for 1 h, the mixture was quenched with water, extracted with ethyl acetate, washed with brine, and dried over MgSO$_4$ to afford 26a (287 mg, 1.21 mmol, 61%) as a mixture of rotamers (77:23 on 1H-NMR): 1H-NMR (500 MHz, MeOD, major rotamer) $\delta$ 8.27 (s, 1H), 8.03 (t, $J = 2.0$ Hz, 1H), 7.47-7.42 (m, 2H), 7.33 (t, $J = 8.1$ Hz, 1H), 4.38 (q, $J = 7.2$ Hz, 2H), 1.39 (t, $J = 7.1$ Hz, 3H); 1H-NMR (500 MHz, MeOD, minor rotamer) $\delta$ 8.72 (s, 1H), 7.65 (s, 1H), 7.47-7.42 (1H, m), 7.34 (t, $J = 8.1$ Hz, 1H), 7.02-6.99 (m, 1H), 4.38 (q, $J = 7.1$ Hz, 2H), 1.39 (t, $J = 7.1$ Hz, 3H); 13C-NMR (125 MHz, MeOD, including rotamic pairs) $\delta$ 163.2, 160.3, 160.2, 155.9, 138.1, 137.5, 129.7, 129.0, 116.6, 116.5, 112.1, 62.8, 12.8; ESI-MS ($m/z$) 237.1 [M+H]$^+$. 
ethyl N-((tert-butoxycarbonyl)(methyl)amino)phenyl)oxamate (26b)

26b was prepared from 22b according to the procedure described for 26a (587 mg, 1.82 mmol, 81%): 1H-NMR (400 MHz, CDCl3) δ 1H-NMR (400 MHz, CDCl3) δ 8.87 (br s, 1H), 7.65 (t, J = 2.1 Hz, 1H), 7.40 (dd, J = 8.2, 0.9 Hz, 1H), 7.32 (t, J = 8.1 Hz, 1H), 7.08 (dd, J = 8.0, 1.9, 0.9 Hz, 1H), 4.43 (q, J = 7.1 Hz, 2H), 3.27 (s, 3H), 1.47 (s, 9H), 1.43 (t, J = 7.1 Hz, 3H); 13C-NMR (100 MHz, CDCl3) δ 161.1, 154.8, 154.1, 144.9, 136.7, 129.4, 122.6, 117.2, 116.8, 80.9, 64.0, 37.4, 28.5 (3C), 14.2; ESI-MS (m/z) 323.4 [M+H]+.

ethyl N-((tert-Butoxycarbonyl)amino)phenyl)oxamate (26c)

26c was prepared from 22c according to the procedure described for 26a (677 mg, 2.20 mmol, quant.): 1H-NMR (400 MHz, CDCl3) δ 8.85 (br s, 1H), 7.76 (t, J = 2.1 Hz, 1H), 7.42-7.39 (m, 1H), 7.28 (t, J = 8.2 Hz, 1H), 7.14-7.11 (m, 1H), 6.54 (s, 1H), 4.42 (q, J = 7.1 Hz, 2H), 1.52 (s, 9H), 1.43 (t, J = 7.1 Hz, 3H); 13C-NMR (100 MHz, CDCl3) δ 160.9, 154.0, 152.7, 139.4, 137.1, 129.9, 115.4, 114.4, 109.8, 81.0, 63.9, 28.5 (3C), 14.1; ESI-MS (m/z) 309.1 [M+H]+.

N-(m-Formamidophenyl)oxamic acid (27a)

To a solution of 26a (278 mg, 1.18 mmol) in methanol (7.5 mL) and THF (2.5 mL) was added 6.0 M aqueous NaOH (0.6 mL). After stirring at room temperature for 16 h, the reaction mixture was acidified with aqueous 6.0 M HCl to pH 1~2. The removal of organic solvent in vacuo gave 27a as a white precipitate, which was collected by filtration (116 mg, 0.56 mmol, 46%). 27a was immediately subjected to the next reaction without further purification: ESI-MS (m/z) 207.1 [M−H]−.
N-(m-((tert-Butoxycarbonyl)(methyl)amino)phenyl)oxamic acid (27b)

27b was prepared from 26b according to the procedure described for 27a. (514 mg, 1.82 mmol, 97%): $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.78 (br s, 1H), 7.73 (s, 1H), 7.56 (d, $J = 8.2$ Hz, 1H), 7.27 (t, $J = 8.1$ Hz, 1H), 7.03 (dd, $J = 8.1$, 1.1 Hz, 1H), 3.11 (s, 3H), 1.33 (s, 9H); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$ 162.3, 154.1, 153.1, 144.1, 137.8, 129.1, 122.0, 117.9, 117.8, 80.2, 37.2, 28.2 (3C); ESI-MS ($m/z$) 293.1 [M$-$H]$^-$.

N-(m-((tert-Butoxycarbonyl)amino)phenyl)oxamic acid (27c)

27c was prepared from 26c according to the procedure described for 27a (561 mg, 2.00 mmol, quantitatively): $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.63 (s, 1H), 9.32 (s, 1H), 7.86 (s, 1H), 7.30 (dt, $J = 8.2$, 1.5 Hz, 1H), 7.20 (t, $J = 8.0$ Hz, 1H), 7.14 (dt, $J = 8.2$, 1.5 Hz, 1H), 1.43 (s, 3H); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$ 164.9, 162.1, 153.1, 140.1, 137.6, 129.1, 115.6, 115.3, 111.5, 79.5, 28.4 (3C); ESI-MS ($m/z$) 279.2 [M$-$H]$^-$.

N-(m-Formamidophenyl)-N'-((2-(4-methyl-2-(p-tolyl)thiazol-5-yl)ethyl)oxamide (S1QEL1.1_D1)

S1QEL1.1_D4 was prepared from 7a and 27a according to the procedure described for S1QEL1.1. The crude product was purified by recrystallization from ethanol to afford S1QEL1.1_D1 (3.1 mg, 0.0073 mmol, 1.8%) as a mixture of rotamers (75:25 on $^1$H-NMR): $^1$H-NMR (400 MHz, MeOD/CDCl$_3$ = 1:1, major rotamer) $\delta$ 8.24 (s, 1H), 7.98 (s, 1H), 7.70 (d, $J = 8.1$ Hz, 2H), 7.44-7.37 (m, 2H), 7.29 (t, $J = 8.1$ Hz, 1H), 7.22 (d, $J = 8.2$ Hz, 1H), 3.57 (t, $J = 7.0$ Hz, 2H), 3.07 (t, $J = 7.0$ Hz, 2H), 2.39 (s, 3H), 2.36 (s, 3H); $^1$H-NMR (400 MHz, MeOD/CDCl$_3$ = 1:1, minor rotamer) $\delta$ 8.68 (s, 1H) 7.70 (d, $J = 8.1$ Hz, 2H) 7.67-7.64 (m, 2H), 7.44-7.37 (m, 1H), 7.31 (t, $J = 8.2$ Hz, 1H), 7.22 (d, $J = 8.2$ Hz, 1H), 3.57 (t, $J = 7.0$ Hz, 2H), 3.07 (t, $J = 7.0$ Hz, 2H), 2.39 (s, 3H), 2.36 (s, 3H); $^{13}$C-NMR (100 MHz, MeOD/CDCl$_3$ (1:1), including rotamic pairs) $\delta$ 166.9, 164.4, 161.6, 161.4, 159.0, 150.6, 141.3, 139.2, 138.4, 131.7, 131.0, 130.5 (2C), 130.3, 129.1, 127.1 (2C), 117.6, 117.5, 117.3, 115.9, 112.9, 111.2, 41.9, 29.9, 21.6, 14.9; ESI-MS ($m/z$) 423.1 [M+H]$^+$. 

S20
**tert-Butyl methyl(m-(2-((2-(4-methyl-2-(p-tolyl)thiazol-5-yl)ethyl)amino)-2-oxoacetamido)phenyl) carbamate (28b)**

28b was prepared from 7a and 27b according to the procedure described for S1QEL1.1. The crude 29b was subjected to the next reaction without further purification: ESI-MS (m/z) 509.2 [M+H]+.

**tert-Butyl (m-(2-((2-(4-methyl-2-(p-tolyl)thiazol-5-yl)ethyl)amino)-2-oxoacetamido)phenyl) carbamate (28c)**

28b was prepared from 7a and 27b according to the procedure described for S1QEL1.1. The crude 29c was used in next reaction without further purification: ESI-MS (m/z) 495.2 [M+H]+.

**N-(2-(4-Methyl-2-(p-tolyl)thiazol-5-yl)ethyl)-N’-(m-(methylamino)phenyl)oxamide (S1QEL1.1_D3)**

To a solution of crude 28b in anhydrous dichloromethane (2.0 mL) was added trifluoroacetic acid (0.2 mL), and the mixture was stirred at room temperature for 16 h. The reaction was quenched with water, extracted with dichloromethane, and dried over anhydrous Na2SO4. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 30% ethyl acetate/n-hexane) to afford S1QEL1.1_D3 (23 mg, 0.056 mmol, 9.1%, 2 steps from 7a): 1H-NMR (400 MHz, CDCl3) δ 9.11 (s, 1H), 7.77 (dt, J = 8.2, 1.8 Hz, 2H), 7.72 (m, 1H), 7.21 (dd, J = 8.5, 0.5 Hz, 2H), 7.16 (t, J = 8.0 Hz, 1H), 7.03 (t, J = 2.1 Hz, 1H), 6.84 (ddd, J = 7.9, 2.0, 0.8 Hz, 1H), 6.43 (ddd, J = 8.1, 2.3, 0.8 Hz, 1H), 3.83 (br s, 1H), 3.63 (q, J = 6.8 Hz, 2H), 3.07 (t, J = 6.9 Hz, 2H), 2.84 (s, 2H), 2.42 (s, 3H), 2.38 (s, 3H); 13C-NMR (100 MHz, CDCl3) δ 165.3, 160.5, 157.2, 150.4, 150.3, 140.2, 137.5, 131.2, 130.1, 129.8 (2C), 127.1, 126.4 (2C), 109.8, 108.8, 103.7, 41.2, 30.8, 26.6, 21.6, 15.3; ESI-MS (m/z) 409.2 [M+H]+.
N-(m-aminophenyl)-N’-(2-(4-methyl-2-(p-tolyl)thiazol-5-yl)ethyl)oxamide (S1QEL1.1_D2)

S1QEL1.1_D2 was prepared from 28c according to the procedure described for S1QEL1.1_D3. The crude product was purified by reverse-phase HPLC using acetonitrile/water system as an eluent to afford pure S1QEL1.1_D2 (12 mg, 0.031 mmol, 8.3% for 2 steps): ¹H-NMR (400 MHz, MeOD) δ 8.07 (t, J = 2.0 Hz, 1H), 7.74 (dt, J = 8.2, 1.8 Hz, 2H), 7.67 (ddd, J = 8.3, 2.0, 0.9 Hz, 1H), 7.49 (t, J = 8.1 Hz, 1H), 7.28 (d, J = 8.0 Hz, J 2H), 7.15 (ddd, J = 7.9, 2.1, 0.9 Hz, 1H), 3.59 (dt, J = 8.7, 3.4 Hz, 2H), 3.11 (t, J = 6.8 Hz, 2H), 2.41 (s, 3H), 2.38 (s, 3H); ¹³C-NMR (100 MHz, MeOD) δ 167.6, 161.7, 150.1, 142.5, 140.5, 133.3, 131.8, 131.2, 131.0 (2C), 130.3, 127.5 (2C), 121.4, 119.9, 115.6, 41.7, 27.0, 21.5, 14.5; ESI-MS (m/z) 395.1 [M+H]⁺.
Scheme 4<sup>d</sup>

Reagents and conditions: (a) Lawesson’s reagent, 1,4-dioxane, reflux, 0.5 h, quant.; (b) ethyl 2-chloroacetoacetate, EtOH, reflux, 3 h, 75% (2 steps); (c) DIBAL-H, THF, -78 to 0 °C, 7 h, 82%; (d) SOCl₂, DMF, CH₂Cl₂, 40 °C, 4 h, crude; (e) NaCN, DMF, rt, 16 h, 95% (2 steps); (f) BH₃·Me₂S, THF, reflux, 4 h; (g) Boc₂O, DMAP, THF, rt, 16 h, 57% (2 steps); (h) sodium L-ascorbate, Cul, trans-N,N'-dimethylcyclohexane-1,2-diamine, NaN₃, rt to reflux, 15 min; (i) LiAlH₄, THF, -40 °C, 1 h, 90% (2 steps); (j) ICl, NaHCO₃, MeOH / CH₂Cl₂, rt, 4 h, 52%; (k) i) NaNO₂, 6.0 M HCl, 0 °C, 0.5 h, ii) NaN₃, rt, 2 h, 27%; (l) 10a, HATU, N-methylmorpholine, DMF, rt, 16 h, 20%; (m) bis(tributyltin), Pd(PPh₃)₄, 1,4-dioxane, 50 °C, 16 h, 17%; (n) <sup>[125]I</sup>Nal, chloramine T, 0.2 M Aq. KH₂PO₄, rt, 10 min, 13%.

<sup>d</sup>Synthesis of S1QEL1.1_PD1 and <sup>[125]I</sup>S1QEL1.1_PD1.
**p-Iodobenzothioamide (30)**

To a solution of *p*-iodobenzamide (29) (4.0 g, 16 mmol) in 1,4-dioxane (40 mL) was added Lawesson's reagent (3.9 g, 9.7 mmol), and the mixture was heated under reflux for 30 min. The reaction mixture was concentrated in vacuo. The residue was purified by silica-gel column chromatography (Wako gel® C-200, 2% MeOH/CHCl₃) to afford 30 (4.2 g, 16 mmol): ¹H-NMR (400MHz, CDCl₃) δ 7.76 (dt, *J* = 8.6, 2.1 Hz, 2H), 7.59 (dt, *J* = 8.6, 2.1 Hz, 2H); ¹³C-NMR (100 MHz, CDCl₃) δ 201.1, 138.7, 137.9 (2C), 128.6 (2C), 99.4; ESI-MS (m/z) 263.9 [M+H]+.

**Ethyl 2-(*p*-iodophenyl)-4-methylthiazole-5-carboxylate (31)**

To a solution of 30 (4.9 g, 16 mmol) in ethanol (20 mL) was added ethyl 2-chloroacetooacetate (3.2 g, 19.4 mmol), and the mixture was refluxed for 4 h. Then, the reaction mixture was cooled to 0°C and the stirring was continued for 16 h to provide white precipitate. The precipitate was collected by filtration, washed with ice-cooled ethanol, and dried *in vacuo* to afford 31 (4.5 g, 12 mmol, 75%): ¹H-NMR (400MHz, CDCl₃) δ 7.79 (dt, *J* = 8.6, 2.0 Hz, 2H), 7.69 (dt, *J* = 8.6, 2.0 Hz, 2H), 4.36(q, *J* = 7.1 Hz, 2H), 2.77 (s, 3H), 1.39 (t, *J* = 7.1, 3H); ¹³C-NMR (100MHz, DMSO-d₆) δ 168.7, 162.3, 161.2, 138.3 (2C), 132.6, 128.3 (2C), 122.3, 97.6, 61.5, 17.6, 14.5; ESI-MS (m/z) 373.9 [M+H]+.

**(2-(*p*-Iodophenyl)-4-methylthiazol-5-yl)methanol (32)**

To a solution of 31 (4.5 g, 12 mmol) in THF was added DIBAL-H (1.0 M in THF, 30 mL, 30 mmol) at -78 °C under nitrogen atmosphere, and the reaction mixture was allowed to warm up to -50 °C for 7 h with stirring. The reaction was quenched with saturated aqueous Rochelle salt, followed by vigorous stirring until the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous MgSO₄ and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 5% MeOH/CHCl₃) to afford 32 (3.2 g, 9.8 mmol, 82%): ¹H-NMR (400MHz, MeOH/CDCl₃ = 1:1) δ 7.79 (dt, *J* = 8.6, 2.1 Hz, 2H), 7.62 (d, *J* = 8.6, 2.1 Hz,
2H), 4.76 (s, 2H), 2.44 (s, 3H); $^{13}$C-NMR (100MHz, MeOH/CDCl$_3$ = 1:1) $\delta$ 165.9, 150.3, 138.8 (2C), 133.6, 128.4 (2C), 108.3, 96.4, 56.4, 14.8; ESI-MS (m/z) 331.9 [M+H]$^+$.  

![Chemical structure of 5-(Chloromethyl)-2-(p-iodophenyl)-4-methylthiazole (33)](image)

**5-(Chloromethyl)-2-(p-iodophenyl)-4-methylthiazole (33)**

To a solution of 32 (3.2 g, 9.7 mmol) in anhydrous dichloromethane (19 mL) were added thionyl chloride (6.9 g, 58 mmol) and DMF (1 drop) under nitrogen atmosphere, and the mixture was stirred at 40 °C for 3 h. The mixture was concentrated under reduced pressure to afford crude 33, which was subjected to the next reaction without further purification; ESI-MS (m/z) 349.9 [M+H]$^+$.  

![Chemical structure of 2-(2-(p-Iodophenyl)-4-methylthiazol-5-yl)acetonitrile (34)](image)

**2-(2-(p-Iodophenyl)-4-methylthiazol-5-yl)acetonitrile (34)**

To a solution of 33 (3.2 g) in DMF (22 mL) was added sodium cyanide (1.4 g, 29 mmol) under nitrogen atmosphere, and the reaction mixture was stirred at room temperature for 16 h. The reaction was quenched with water, extracted with ethyl acetate, washed with brine, and the organic layer was dried over anhydrous MgSO$_4$. The crude product was purified by silica-gel column chromatography (Wako gel C-200, CHCl$_3$) to afford 34 (3.1 g, 9.1 mmol, 95%, 2 steps): $^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 7.78 (dt, $J$ = 8.6, 2.0 Hz, 2H), 7.61 (dt, $J$ = 8.6, 2.1 Hz, 2H), 3.85 (s, 2H), 2.46 (s, 3H); $^{13}$C-NMR (100MHz, CDCl$_3$) $\delta$ 165.1, 152.1, 138.1, 132.6, 127.8, 118.9 (2C), 116.1 (2C), 96.5, 15.1, 14.2; ESI-MS (m/z) 340.9 [M+H]$^+$.  

![Chemical structure of 2-(2-(p-Iodophenyl)-4-methylthiazol-5-yl)ethan-1-amine (35)](image)

**2-(2-(p-Iodophenyl)-4-methylthiazol-5-yl)ethan-1-amine (35)**

To a solution of 34 (1.5 g, 4.3 mmol) in anhydrous THF (34 mL) was added borane-dimethyl sulfide complex (2.0 M in THF, 8.7 mL, 17 mmol) under nitrogen atmosphere, and the mixture was heated under reflux for 30 min. The reaction was quenched by the addition of methanol and water, extracted with ethyl acetate, washed with brine, dried over anhydrous Na$_2$SO$_4$. The crude amine 35 was subjected to the next reaction without further purification; ESI-MS (m/z) 345.1 [M+H]$^+$.  

S25
tert-Butyl (2-(2-([p]-iodophenyl)-4-methylthiazol-5-yl)ethyl)carbamate (36)

To a solution of crude amine 35 (1.4 g) in anhydrous THF (25 mL) were added di-tert-butyldicarbonate (1.9 mg, 8.7 mmol) and N,N-dimethyl-4-aminopyridine (53 mg, 0.43 mmol), and the reaction mixture was stirred at room temperature for 16 h. The reaction was quenched with water, extracted with ethyl acetate, and the organic layer was dried over anhydrous MgSO4. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, CHCl3) to afford 36 (1.1 g, 2.49 mmol, 57%, 2 steps): 1H-NMR (400MHz, CDCl3) δ 7.74 (dt, J = 8.6, 2.1 Hz, 2H), 7.61 (dt, J = 8.6, 2.1 Hz, 2H), 3.36 (q, J = 6.2 Hz, 2H), 2.97 (t, J = 6.5 Hz, 2H), 2.40 (s, 3H), 1.44 (s, 9H); 13C-NMR (100MHz, CDCl3) δ 163.5, 155.9, 150.8, 138.2 (2C), 133.5, 129.4, 127.9 (2C), 95.8, 63.1, 41.9, 28.6 (3C), 27.3, 15.2; ESI-MS (m/z) 445.1 [M+H]+.

tert-Butyl (2-(2-([p]-azidophenyl)-4-methylthiazol-5-yl)ethyl)carbamate (37)

To a solution 36 (1.1 g, 2.5 mmol) in ethanol (21 mL) and water (9.0 mL) were added sodium ascorbate (50 mg, 0.25 mmol), copper(I) iodide (95 mg, 0.50 mmol), trans-,N,N'-dimethylcyclohexane-1,2-diamine (105 mg, 0.74 mmol), and sodium azide (483 mg, 7.43 mmol), and the mixture was heated under reflux for 15 min. Then, the reaction was quenched with water, extracted with ethyl acetate, washed with brine, and the organic layer was dried over MgSO4. The azide 37 was immediately subjected to the next reaction without further purification: ESI-MS (m/z) 360.2 [M+H]+.

tert-Butyl (2-(2-([p]-aminophenyl)-4-methylthiazol-5-yl)ethyl)carbamate (38)

To a suspension of LiAlH4 (94 mg, 2.5 mmol) in anhydrous THF (15 mL) was added dropwise a solution of 37 (900 mg) in THF (10 mL) at -40 °C under nitrogen atmosphere. After stirring the mixture at -40 °C for 1 h, the reaction was quenched with the addition of saturated aqueous Rochelle salt. The resulting mixture was extracted with ethyl acetate, washed with brine, and the organic layer was dried over anhydrous MgSO4. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 5% MeOH, CHCl3) to afford 38 (741 mg, 2.2 mmol, 90%, 2 steps): 1H-NMR (400MHz, CDCl3) δ 7.68 (dt, J = 8.6, 2.1 Hz, 2H), 6.68 (dt, J = 8.6, 2.3 Hz, 2H), 4.67 (br s, 1H), 3.86 (br s, 2H), 3.35(q, J = 5.8 Hz, 2H), 2.93 (t, J = 6.5 Hz, 2H), 2.37 (s, 3H), 1.44 (s, 9H); 13C-NMR (100MHz, CDCl3) δ 165.4, 156.0, 149.7, 148.2, 133.3, 127.8 (2C), 124.7, 115.1 (2C), 79.7, 42.0, 28.6 (3C), 27.2, 15.2; ESI-MS (m/z) 334.2 [M+H]+.
**tert-Butyl (2-(2-(4-amino-3-iodophenyl)-4-methylthiazol-5-yl)ethyl)carbamate (39)**

To a solution of **38** (1.8 g, 5.5 mmol) and sodium bicarbonate (898 mg, 5.53 mmol) in methanol (20 mL) was added iodine monochloride (929 mg, 11.1 mmol) in dichloromethane. After stirring at room temperature for 4 h, the reaction was quenched with saturated aqueous Na$_2$S$_2$O$_3$, extracted with ethyl acetate and washed with brine, and the organic layer was dried over anhydrous MgSO$_4$. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 2% MeOH/CHCl$_3$) to afford **39** (1.3 g, 2.9 mmol, 52%): ESI-MS (m/z) 460.1 [M+H]$^+$. 

**2-(2-(4-Azido-3-iodophenyl)-4-methylthiazol-5-yl)ethan-1-amine (40)**

To a solution of **39** (285 mg, 0.62 mmol) in 6.0 M aqueous HCl (6.0 mL) was added sodium nitrite (103 mg, 1.49 mmol) in water at 0 °C, and the mixture was stirred at 0 °C for 30 min. Then, sodium azide (121 mg, 1.86 mmol) was added to the mixture at 0°C, followed by the stirring at room temperature for further 2 h. The reaction mixture was diluted with ethyl acetate, basified with 6.0 M aqueous NaOH. The aqueous layer was again extracted with ethyl acetate, and the combined organic layer was dried over anhydrous Na$_2$SO$_4$. The crude product was purified by preparative reverse-phase HPLC using MeOH/water system as an eluent to afford **40** (65 mg, 0.17 mmol, 27%): $^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 8.32 (t, $J$ = 1.8 Hz, 1H), 7.88 (dt, $J$ = 8.4, 2.2 Hz, 1H), 7.14 (dd, $J$ = 8.4, 2.2 Hz, 1H), 3.00 (q, $J$ = 6.6 Hz, 2H), 2.91 (t, $J$ = 6.6 Hz, 2H), 2.41 (s, 3H); $^{13}$C-NMR (100MHz, CDCl$_3$) $\delta$ 161.6, 150.3, 142.7, 137.5, 132.3, 130.4, 118.6, 88.3, 43.5, 30.9, 15.3; ESI-MS (m/z) 386.0 [M+H]$^+$. 

S27
**$N$-(3-Acetamidophenyl)-$N'$-(2-(2-(4-azido-3-iodophenyl)-4-methylthiazol-5-yl)ethyl)oxamide**  

(S1QEL1.1_PD1)

S1QEL1.1_PD1 was prepared from 40 and 10a according to the procedure described for S1QEL1.1. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 5% MeOH/CHCl₃), followed by the recrystallization from ethanol to afford S1QEL1.1_PD1 (20 mg, 0.03 mmol, 20%): ¹H-NMR (400MHz, CDCl₃) δ 9.22 (s, 1H), 8.32 (d, $J = 1.9$ Hz, 1H), 7.92 (s, 1H), 7.88 (dd, $J = 8.4, 2.0$ Hz, 1H), 7.69 (t, $J = 6.4$ Hz, 1H), 7.38-7.29 (m, 3H), 7.20 (s, 1H), 7.15 (d, $J = 8.3$ Hz, 1H), 3.64 (q, $J = 6.7$ Hz, 2H), 3.09 (t, $J = 6.9$, 2H), 2.42 (s, 3H), 2.18 (s, 3H); ¹³C-NMR (100MHz, MeOD) δ 170.2 (2C), 163.2, 160.5, 157.5, 139.1, 137.6 (2C), 136.8, 129.7, 129.5, 129.3, 127.6 (2C), 118.7 (2C), 111.7, 88.0, 40.4, 29.5, 23.4, 13.7; ESI-MS (m/z) 590.1 [M+H⁺].

To a solution S1QEL1.1_PD1 (18 mg, 0.031 mmol) in anhydrous 1,4-dioxane (1 mL) were added bis(tributyltin) (89 mg, 0.15 mmol) and tetrakis(triphenylphosphine)palladium (0) (3.5 mg, 0.0030 mmol) under argon atmosphere. After stirred at room temperature for 16 h, the mixture was concentrated under reduced pressure. The residue was purified by silica-gel column chromatography (Wako gel® C-200, 2% MeOH/CHCl₃), followed by further purification by preparative reverse-phase HPLC using MeOH/water system as an eluent to afford 41 (3.9 mg, 0.0051 mmol, 17%): ¹H-NMR (400MHz, CDCl₃) δ 9.22 (s, 1H), 8.90-7.85 (m, 3H), 7.69 (t, $J = 6.1$ Hz, 1H), 7.37-7.29 (m, 3H), 7.20 (s, 1H), 7.16 (d, $J = 8.1$ Hz, 1H), 3.64 (q, $J = 6.7$ Hz, 2H), 3.08 (t, $J = 7.0$, 2H), 2.42 (s, 3H), 2.18 (s, 3H), 1.57-1.50 (m, 6H), 1.38-1.29 (m, 6H), 1.15-1.11 (m, 6H), 0.89 (t, $J = 7.3$ Hz, 9H); ¹³C-NMR (125MHz, CDCl₃) δ 168.5 (2C), 164.8, 160.2, 157.4, 150.7, 138.9, 135.6 (2C), 130.3, 130.1, 128.1, 127.3, 117.4 (2C), 116.7, 115.6, 111.1, 40.4, 29.5, 23.4, 13.7; ESI-MS (m/z) 754.3 [M+H⁺] (maximum peak for ¹²⁰Sn-isotope).
To a solution of the tin-precursor 41 (1.0 mM, in ethanol, 20 μL) in screw-capped 1.5mL plastic-tube was added [125I]NaI (PerkinElmer, NEZ033A, 1 mCi, 2000 Ci/mmol, 10 μL). The radio-iodination was initiated by adding freshly prepared aqueous chloramine T (3.0 mM in KPi buffer, pH 7.4, 20 μL), and the mixture was incubated at room temperature for 10 min (Ref. 2). The reaction was quenched with 5% aqueous NaHSO₃ (100 μL), and the resulting mixture was extracted with chloroform (100 μL x 3). The combined organic layer was concentrated using vacuum-centrifugal evaporator. The crude product was dissolved in methanol (50 μL), and subjected to HPLC (Shimazu LC-10 AS) purification using a C18 column (COSMOSIL 5C18-MS-II, 4.6 x 150 mm, Nacalai Tesque) at a flow rate of 0.8 mL/min with methanol/water system as an eluent.

The column was eluted with isocratic 10% methanol in 5 min, then isocratic 90% methanol in 15 min. The fraction was collected every 30 s (~400 μL) and the radioactivity was measured by γ-counting system (COBRATMII, Packard). The strong radioactive fractions corresponding to the retention time of [125I]S1QEL1.1_PDI (14.5 min) were combined and the solvent was evaporated by vacuum-centrifugal concentrator. [125I]S1QEL1.1_PDI was stored as an ethanolic solution (1 mCi/mL) at -18 °C. The radiochemical yield of [125I]S1QEL1.1_PDI from the initial [125I]NaI was 13%. The radiochemical purity and the specific activity were >99% and 2000 Ci/mmol, respectively (judged from HPLC and TLC analyses).
Scheme 5

Synthesis of S1QEL1.1_PD2 and [125I]S1QEL1.1_PD2.

Reagents and conditions: (a) \(N\)-bromosuccinimide, conc. \(H_2SO_4\), 60 °C, 16 h, 96%; (b) Fe, \(NH_4Cl\), MeOH/water, 60 °C, 4 h, 82%; (c) sodium \(L\)-ascorbate, Cul, \(N,N\)'-dimethylethylenediamine, \(NaN_3\), EtOH/H\(_2\)O, reflux, 1 h, 87%; (d) \(Ac_2O\), \(Et_3N\), CH\(_2\)Cl\(_2\), rt, 5 h, 25%; (e) ethyl chloroglyoxylate, \(Et_3N\), THF, reflux, 1 h, 88%; (f) 6.0 M aq. NaOH, MeOH/THF, rt, 15 min, quant.; (g) 36, HATU, \(N\)-methylmorphorine, DMF, rt, 3 h, 15%; (h) bis(tributyltin), \(Pd(PPh_3)_4\), 1,4-dioxane, 50 °C, 1 d, 12%; (i) \(^{[125I]}\)NaI, chloramine T, 0.2 M aq. KH\(_2\)PO\(_4\), rt, 10 min, 34%.
1-Bromo-3,5-dinitrobenzene (43)

A solution of \(m\)-dinitrobenzene 42 (3.03 g, 18.0 mmol) in concentrated sulfuric acid (50 mL) was heated at 60 °C, followed by the addition of \(N\)-bromosuccinimide (3.84 g, 21.6 mmol) in several portions. After stirring at 60 °C for 16 h, the reaction mixture was cooled to room temperature and poured into crushed ice. The mixture was extracted with dichloromethane and dried over anhydrous MgSO\(_4\). The crude product was purified by silica-gel column chromatography (Wako gel\textsuperscript{®} C-200, 5% ethyl acetate / \(n\)-hexane) to afford 43 (4.26 g, 17.2 mmol, 96%): \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.01 (t, \(J = 2.0\) Hz, 1H), 8.71 (d, \(J = 2.0\) Hz, 2H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta\) 149.02, 132.21 (2C), 124.02, 117.56 (2C).

5-Bromobenzene-1,3-diamine (44)

To a solution of 43 (4.26 g, 17.2 mmol) in methanol (20 mL) and water (20 mL) were added ammonium chloride (7.38 g, 138 mmol) and iron powder (4.82 g, 86 mmol). After stirring at 60 °C for 4 h, the mixture was filtered through a pad of Celite\textsuperscript{®}, followed by the removal of methanol under reduced pressure. The resulting aqueous solution was extracted with dichloromethane, and the organic layer was dried over anhydrous Na\(_2\)SO\(_4\). The crude product was purified by silica-gel column chromatography (Wako gel\textsuperscript{®} C-200, 30% ethyl acetate/\(n\)-hexane) to afford 44 (2.64 g, 14.1 mmol, 82%): \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 6.25 (d, \(J = 2.0\) Hz, 2H), 5.90 (t, \(J = 2.0\) Hz, 1H), 3.60 (br s, 4H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta\) 148.7 (2C), 123.7, 108.9 (2C), 100.2; ESI-MS (m/z) 187.0 [M+H]+ (for 79Br-isotope), 189.0 [M+H]+ (for 81Br-isotope).

5-Azidobenzene-1,3-diamine (45)

To a solution of 44 (1.87 g, 10.0 mmol) in ethanol (25 mL) and water (25 mL) were added sodium ascorbate (200 mg, 1.01 mmol), copper(I) iodide (381 mg, 2.0 mmol), \(N,N\)’-dimethylethlenediamine (264 mg, 3.02 mmol) and sodium azide (1.30 g, 20.4 mmol), and the mixture was heated under reflux for 15 min. Then, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous MgSO\(_4\). The crude product was purified by silica-gel column chromatography (Wako
gel® C-200, 2% methanol/chloroform) to afford 45 (1.30 g, 8.72 mmol, 87%): \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.79 (s, 3H), 3.64 (br s, 4H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta\) 148.8 (2C), 142.1, 98.5, 96.6 (2C); ESI-MS (\(m/z\)) 150.1 [M+H]\(^+\).

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\text{\begin{figure}[h]
\centering
\includegraphics[width=0.1\textwidth]{n-3-amino-5-azidophenylacetamide.png}
\caption{N-(3-Amino-5-azidophenyl)acetamide (46)}
\end{figure}}
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\(N\)-(3-Amino-5-azidophenyl)acetamide (46)

To an ice-cooled solution of 45 (1.30 g, 8.72 mmol) in anhydrous dichloromethane (50 mL) were added triethylamine (1.06 g, 10.5 mmol) and acetic anhydride (0.89 g, 8.72 mmol) under nitrogen atmosphere. After stirring the mixture at room temperature for 5 h, it was concentrated \textit{in vacuo}. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 5% ethyl acetate/n-hexane) to afford 46 (416 mg, 2.18 mmol, 25%): \(^1\)H-NMR (400 MHz, MeOD) \(\delta\) 6.73 (t, \(J = 1.7\) Hz, 1H), 6.65 (t, \(J = 1.8\) Hz, 1H), 6.14 (t, \(J = 1.9\) Hz, 1H), 2.08 (s, 3H); \(^{13}\)C-NMR (100 MHz, MeOD) \(\delta\) 171.7, 151.1, 142.4, 141.9, 104.3, 102.1, 101.0, 23.9; ESI-MS (\(m/z\)) 192.1 [M+H]\(^+\).

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\text{\begin{figure}[h]
\centering
\includegraphics[width=0.1\textwidth]{ethyl-n-3-acetamido-5-azidophenyloxamate.png}
\caption{Ethyl N-(3-acetamido-5-azidophenyl)oxamate (47)}
\end{figure}}
\]

Ethyl \(N\)-(3-acetamido-5-azidophenyl)oxamate (47)

To an ice-cooled solution of 46 (416 mg, 2.18 mmol) in anhydrous THF (10 mL) were added triethylamine (440 mg, 4.35 mmol) and ethyl chloroglyoxylate (327 mg, 2.39 mmol) under nitrogen atmosphere, and the mixture was refluxed for 1h. The reaction was quenched with water, extracted with ethyl acetate, washed with brine, and dried over anhydrous MgSO\(_4\) to afford 46 (560 mg, 1.92 mmol, 88%): \(^1\)H-NMR (400 MHz, MeOD) \(\delta\) 7.72 (t, \(J = 1.8\) Hz, 1H), 7.30 (t, \(J = 1.9\) Hz, 1H), 7.23 (t, \(J = 1.9\) Hz, 1H), 4.38 (q, \(J = 7.1\) Hz, 2H), 2.12 (s, 3H), 1.39 (t, \(J = 7.1\) Hz, 3H); \(^{13}\)C-NMR (100 MHz, MeOD) \(\delta\) 172.9, 161.5, 157.3, 142.6, 141.9, 140.2, 109.5, 64.2, 23.9, 14.2; ESI-MS (\(m/z\)) 292.1 [M+H]\(^+\).

\[
\text{\begin{figure}[h]
\centering
\includegraphics[width=0.1\textwidth]{n-3-acetamido-5-azidophenyloxamic acid.png}
\caption{N-(3-Acetamido-5-azidophenyl)oxamic acid (48)}
\end{figure}}
\]

\(N\)-(3-Acetamido-5-azidophenyl)oxamic acid (48)
To a solution of 47 (58.3 mg, 0.20 mmol) in methanol (1.5 mL) and THF (0.5 mL) was added 6.0 M aqueous NaOH (0.1 mL), and the mixture was stirred at room temperature for 16 h. The mixture was acidified with 6.0 M HCl to pH 1~2, followed by the removal of organic solvent in vacuo. The resulting aqueous solution was extracted with ethyl acetate, washed with brine, and dried over anhydrous MgSO₄ to afford 48 (52.6 mg, 0.20 mmol, quant): ¹H-NMR (400 MHz, DMSO-dB₆) δ 10.76 (s, 1H), 10.16 (s, 1H), 7.79 (t, J = 1.5 Hz, 1H), 7.27 (t, J = 1.7 Hz, 1H), 7.20 (t, J = 1.8 Hz, 1H), 2.03 (s, 3H); ¹³C-NMR (100 MHz, DMSO-d₆) δ 169.4, 162.2, 157.6, 141.1, 140.4, 139.5, 107.8, 105.9, 102.0, 24.3; ESI-MS (m/z) 262.1 [M−H]⁻.

\[ \text{N-(3-Acetamido-5-azidophenyl)-N'-(2-(2-(4-iodophenyl)-4-methylthiazol-5-yl)ethyl)oxamide} \]

(S1QEL1.1_PD2) was prepared from 35 and 48 according to the procedure described for S1QEL1.1. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 30-50% ethyl acetate/n-hexane) to afford S1QEL1.1_PD2 (15.4 mg, 0.026 mmol, 15%): ¹H-NMR (400 MHz, MeOD/CDCl₃ =1/1) δ 7.78 (dt, J = 8.6, 2.1 Hz, 2H), 7.62-7.58 (m, 3H), 7.30 (t, J = 2.0 Hz, 1H), 7.24 (t, J = 1.9 Hz, 1H), 3.60 (t, J = 7.0 Hz, 2H), 3.11 (t, J = 7.0 Hz, 2H), 2.43 (s, 3H), 2.14 (s, 3H); ¹³C-NMR (100 MHz, MeOD/CDCl₃ =1/1) δ 171.2, 165.0, 161.0, 158.5, 150.9, 142.1, 141.1, 139.0, 138.7 (2C), 133.4, 129.6, 128.3 (2C), 108.4, 107.6, 106.8, 96.3, 41.2, 24.0, 14.8; ESI-MS (m/z) 590.1 [M+H]+.
N-(3-Acetamido-5-azidophenyl)-N’-(2-(4-methyl-2-(4-(tributylstannyl)phenyl)thiazol-5-yl)ethyl)oxamide (49)

49 was prepared from S1QEL1.1_PD2 according to the procedure described for 41. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 30-50% ethyl acetate/n-hexane) to afford 50 (1.2 mg, 0.0016 mmol, 12%): $^1$H-NMR (400 MHz, MeOD/CDCl$_3$ = 1/1) δ 7.78 (d, $J = 8.1$ Hz, 2H), 7.62 (s, 1H), 7.54 (d, $J = 8.2$ Hz, 2H), 7.30 (t, $J = 1.9$ Hz, 1H), 7.26 (t, $J = 1.9$ Hz, 1H), 3.60 (t, $J = 7.0$ Hz, 2H), 3.11 (t, $J = 7.0$ Hz, 2H), 2.43 (s, 3H), 2.14 (s, 3H), 1.61-1.53 (m, 6H), 1.40-1.30 (m, 6H), 1.13-1.08 (m, 6H), 0.90 (t, $J = 7.3$ Hz, 9H); $^{13}$C-NMR (400 MHz, MeOD/CDCl$_3$ = 1/1) δ 171.1, 166.8, 161.0, 158.6, 150.5, 146.0, 142.1, 141.1, 139.1, 137.6 (2C), 133.4, 128.9, 126.0 (2C), 108.5, 107.6, 106.8, 41.3, 29.7 (3C), 27.9 (3C), 26.6, 24.0, 14.8, 13.9 (3C), 10.1 (3C); ESI-MS (m/z) 754.3 [M+H]$^+$ (maximum peak for $^{120}$Sn isotope).

N-(3-Acetamido-5-azidophenyl)-N’-(2-(4-$^{125}$I]iodophenyl)-4-methylthiazol-5-yl)ethyl)oxamide ([$^{125}$I]S1QEL1.1_PD2)

[$^{125}$I]S1QEL1.1_PD2 was prepared from 49 according to the same procedure described for [$^{125}$I]S1QEL1.1_PD1. The radiochemical yield from the initial [$^{125}$I]NaI was 34%. The radiochemical purity and the specific activity were >99% and 2,000 Ci/mmol, respectively (judged from HPLC and radio-TLC). [$^{125}$I]S1QEL1.1_PD2 was stored as an ethanoic solution (1 mCi/mL) at 4 °C.

References (for synthetic procedures)
1. Li, Z., Qiu, Q., Xu, X., Wang, X., Jiao, L., Su, X., Pan, M., Huang, W., and Qian, H. (2016) Design, synthesis and structure-activity relationship studies of new thiazole-based free fatty acid receptor 1 agonists for the treatment of type 2 diabetes. Eur. J. Med. Chem. 113, 246-257.
2. Uno, S., Kimura, H., Murai, M., and Miyoshi, H. (2019) Exploring the quinone/inhibitor-binding pocket in mitochondrial respiratory complex I by chemical biology approaches. J. Biol. Chem. 294, 679-696.
Figure S1
Structures of AL1 and $[^{125}\text{I}]\text{AzQ}$ used in the present study.
Figure S2
Schematic diagram of the pinpoint chemical modification of the 49 kDa Asp160. The 49 kDa Asp160 can be modified by an externally added TAMRA-N3 through the two-step conjugation procedure, LDT and click chemistry (see refs. 31, 32).
Table S1. Proteins identified by MALDI-TOF MS

| Protein name (Swiss Prot accession No.) | Matched peptides | MOWSE score $^a$ (Sequence coverage) | Observed m/z (MH$^+$) | Mr | Matched in-gel triptic digests | Peptide sequence | Residues |
|------------------------------------------|------------------|---------------------------------------|----------------------|----|--------------------------------|-----------------|---------|
| Complex I-ND1 subunit (P03887)           | 7                | 51 (15%)                              | 979.544              | 978.5367 | 978.5321 | VLGYMQLLR | 27-34 |
|                                          |                  |                                       | 1107.639             | 1106.6320 | 1106.6270 | VLGYMQLRK | 27-35 |
|                                          |                  |                                       | 2050.167             | 2049.1601 | 2049.1622 | KGPNVVGPYGLLQPIADAIK | 35-54 |
|                                          |                  |                                       | 1922.077             | 1921.0701 | 1921.0673 | GPNVVGPYGLLQPIADAIK | 36-54 |
|                                          |                  |                                       | 876.526              | 875.5187  | 875.5229  | YALIGALR | 127-134 |
|                                          |                  |                                       | 1649.860             | 1648.8535 | 1648.8548 | FRYDQMLHLLWK | 280-291 |
|                                          |                  |                                       | 1346.698             | 1345.6908 | 1345.6853 | YDQLMHLLWK | 282-291 |
| ADP/ATP carrier (P02722)                 | 12               | 72 (48%)                              | 1219.737             | 1218.7297 | 1218.6608 | DFLAGGVAAAISK | 11-23 |
|                                          |                  |                                       | 1169.605             | 1168.5977 | 1168.5655 | EQGFLSFWR | 64-72 |
|                                          |                  |                                       | 856.507              | 855.4997  | 855.4926  | GNLANVIR | 73-89 |
|                                          |                  |                                       | 1446.790             | 1445.7827 | 1445.7343 | YFPTQALNFAFK | 81-92 |
|                                          |                  |                                       | 1004.563             | 1003.5557 | 1003.5451 | QIFLGGVDR | 97-105 |
|                                          |                  |                                       | 2796.347             | 2795.3397 | 2795.3377 | Y FAGNLASGGAAGATSLCFVYPLDFAR + carbamidomethyl (C) | 112-138 |
|                                          |                  |                                       | 1927.115             | 1926.1077 | 1926.0363 | GLYQGFNVSQGHIIYR | 172-188 |
|                                          |                  |                                       | 1205.615             | 1204.6077 | 1204.5764 | AAYFGVYDTAK | 189-199 |
|                                          |                  |                                       | 1644.763             | 1643.7557 | 1643.7072 | GADIMYTGTVDCWR + carbamidomethyl (C) | 246-259 |
|                                          |                  |                                       | 1660.796             | 1659.7617 | 1659.7021 | GADIMYTGTVDCWR + carbamidomethyl (C) + oxidation (M) | 246-259 |
|                                          |                  |                                       | 902.496              | 901.4887  | 901.4770  | GAWSNVLR | 273-280 |
|                                          |                  |                                       | 1739.973             | 1738.9657 | 1738.9328 | GMGGAFV LV LVEIKK | 281-296 |

$^a$MOWSE score is $-10\log P$, where $P$ is the probability that the observed match is a random event. Scores greater than 54 are significant ($p<0.05$).