

## Supplementary Material

### “Discovery of an Isothiazole-Based Phenylpropanoic Acid GPR120 Agonist as a Development Candidate for Type 2 Diabetes”

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#### 1. General Procedures

<sup>1</sup>H NMR spectra were determined with a Bruker Biospin International AG-300 or AG-400 spectrometers at 300 or 400 MHz. Chemical shifts ( $\delta$ ) are reported in parts per million (ppm) relative to residual chloroform (7.26 ppm), TMS (0 ppm), or CD<sub>3</sub>OD (4.87 ppm) as an internal reference with coupling constants (J) reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Electrospray (ES)

mass spectra were recorded in positive or negative mode on a Micromass Platform spectrometer. The purity of all compounds described here was determined by analytical LC/MS using a Shimadzu LCMS-2110EV with analytical LC using Shiseido Capcell Pak C18 MGS3 column (3.0X50 mm, 3.5  $\mu$ ), 0.05% TFA in water as mobile phase A and 0.05% TFA in acetonitrile as mobile phase B at 1 mL/min flow, gradient from 10% to 100% B in 1.7 minutes, 100% B for 1.5 minutes, 100 to 10% B in 0.13 minutes, monitored by UV absorption at 215 nm using PDA and ELSD. The purity of all compounds was found to be > 95%. Thin-layer chromatography (TLC) was performed on Merck PLC pre-scored plates 60F<sub>254</sub>. Prep-HPLC conditions: (1#waters2767-5) column, SunFire Prep C18, 19\*150mm H Prep C-001(T)18600256819513816414 04; Mobile Phase, phase A: water with 0.05% TFA, phase B: CH<sub>3</sub>CN (20% CH<sub>3</sub>CN up to 50% in 8 min, up to 100 % in 0.1 min, hold 100% in 1.9 min, down to 20% in 0.1 min, hold 20% in 1.9 min); Detector, UV 220 & 254 nm. Unless otherwise noted, reagents were obtained from commercial sources and were used without further purification.

## 2. Synthetic procedures and data

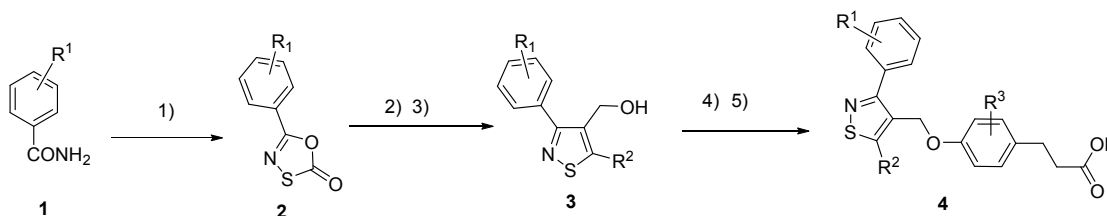
The general synthetic routes to prepare isothiazoles **4a** to **4y** and **9a** to **9c** are outlined in Schemes 1-3. In all cases, a convergent approach was employed that involved either a SN<sub>2</sub> reaction between a substituted isothiazolyl methyl methanesulfonate (methanesulfonates of **3** or **7**) and a substituted ethyl 3-(4-hydroxyphenyl)propanoate or a Mitsunobu coupling of a substituted isothiazolylmethanol (**12**) with a substituted ethyl 3-(4-hydroxyphenyl)propanoate, followed by hydrolysis of the esters into the corresponding phenyl propanoic acids under basic conditions.<sup>1</sup> The synthesis of isothiazolylmethanols **3** started with the conversion of commercially available benzamides **1** into the corresponding phenyloxathiazolone by treatment with chlorocarbonylsulfonyl chloride and potassium carbonate,<sup>2</sup> followed by cycloadditions with various ethyl alkynoates and reduction of the resulting isothiazoyl esters by DIBAL. (3-Chloro-5-(4-ethylphenyl)isothiazol-4-yl)methanol **7** was prepared in four steps, involving reaction of 4-ethylbenzaldehyde **5** with propanedinitrile in the presence of piperidine, reaction of the resulting phenylmethylenemalononitrile with S<sub>2</sub>Cl<sub>2</sub> to form 3-chloro-5-(4-ethylphenyl)isothiazole-4-carbonitrile **6**, acid catalyzed hydrolysis of the nitrile to the acid, and LAH reduction. Commercially available 5-amino-3-(trifluoromethyl)isothiazole-4-carbonitrile **10** was reacted

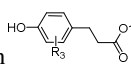
with isopentyl nitrite and bromine in 3-methyl-1-nitrobutane under modified Sandmeyer conditions followed by hydrolysis to give the corresponding acid **11**, which was then reduced to the corresponding alcohol and then coupled with 4-chloro-phenylboronic acid under Suzuki–Miyaura conditions to afford isothiazolymethanol **12**.

### References:

- 1) Tsunoda, T.; Yamamiya, Y.; Ito, S. 1,1'-(azodicarbonyl) dipiperidin -tributylphosphine, a new reagent system for Mitsunobu reaction *Tetrahedron Lett.* **1993**, *34*, 1639.
- 2) McKie, M. C.; Paton, R.M. Nitrile sulfides. Part 9. Synthesis of 3-arylisothiazoles. *J. Chem. Res., Synopses*, **1987**, *8*, 245.

### Scheme 1. Synthesis of isothiazoles **4**

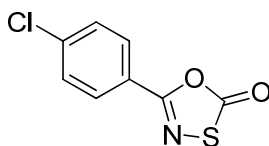


Reagents and conditions: 1) chlorocarbonylsulfonyl chloride,  $K_2CO_3$  reflux overnight in toluene, 75~92%; 2)  $R^2 \equiv CO_2Et$ , 1,3-di-Cl-benzene,  $180^\circ C$  for 18 h, 80~88%; 3) DIBAL in toluene,  $-78^\circ C$  1 h, 72~85%; 4)  $MsCl$ , TEA,  $0^\circ C$  1h and then ,  $K_2CO_3$  in DMF r.t. to  $80^\circ C$  for 4 h-overnight, 62~81%; 5)  $LiOH \cdot H_2O$ , THF/MeOH/water (1:1:1), r,t-overnight for 2 h, 85~98%

The target compounds **4** were synthesized in accordance with an analogous synthetic method described in Scheme 1.

### 3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-methylphenyl)propanoic acid (**4f**)

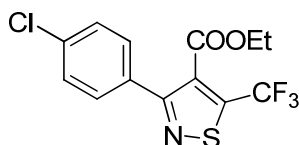
#### Step 1: 5-(4-chlorophenyl)-2H-1,3,4-oxathiazol-2-one



Into a 100-mL round-bottom flask, was placed 4-chlorobenzamide (3.0 g, 19.28 mmol, 1.00 equiv), chloro(chlorosulfanyl)methanone (5.03 g, 38.40 mmol, 1.99 equiv), toluene (30 mL). The resulting solution was stirred overnight at  $100^\circ C$  in an oil bath. The reaction progress was

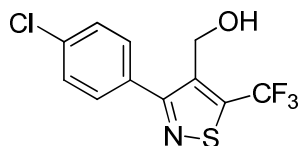
monitored by GCMS/TLC/LCMS (ethyl acetate/petroleum ether = 1:20). The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column with petroleum ether. This resulted in 3.8 g (92%) of 5-(4-chlorophenyl)-2H-1,3,4-oxathiazol-2-one as a white solid. Mass spectrum (ESI, m/z): Calcd. for C<sub>8</sub>H<sub>4</sub>ClNO<sub>2</sub>S, 212.97, found 214.2 (M+H).

Step 2: Ethyl 3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazole-4-carboxylate



Into a 50-mL sealed tube, was placed 5-(4-chlorophenyl)-2H-1,3,4-oxathiazol-2-one (2.2 g, 10.30 mmol, 1.00 equiv), ethyl 4,4,4-trifluorobut-2-ynoate (2.5 g, 15.05 mmol, 1.46 equiv), 1,3-dichlorobenzene (20 mL). The resulting solution was stirred for 18 h at 150°C in an oil bath. The reaction progress was monitored by LCMS/GCMS/TLC (ethyl acetate/petroleum ether = 1:20). The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column with petroleum ether. This resulted in 2.8 g (81%) of ethyl 3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazole-4-carboxylate as a yellow solid. Mass spectrum (ESI, m/z): Calcd. for C<sub>13</sub>H<sub>9</sub>ClF<sub>3</sub>NO<sub>2</sub>S, 335.00, found 336.0 (M+H).

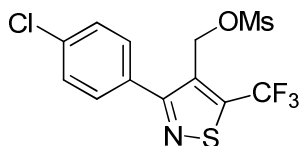
Step 3: [3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methanol



Into a 50-mL round-bottom flask (1 atm) purged and maintained with an inert atmosphere of nitrogen, was placed ethyl 3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazole-4-carboxylate (1.0 g, 2.98 mmol, 1.00 equiv), toluene (5.0 mL). This was followed by the addition of DIBAL-H (20% in toluene) (4.23 g, 29.79 mmol, 2.00 equiv) dropwise with stirring at -78°C. The resulting solution was warmed to room temperature and stirred for 1 h at 30°C. The reaction progress was monitored by LCMS. The reaction was then quenched by the addition of 10 mL of water and 10 mL NH<sub>4</sub>Cl. The resulting solution was extracted with 3x20 mL of dichloromethane and the organic layers combined and dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:10). This resulted in 0.72 g (82%) of [3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-

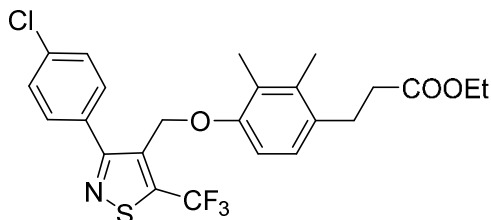
thiazol-4-yl]methanol as a yellow solid. Mass spectrum (ESI, m/z): Calcd. for  $C_{11}H_7ClF_3NOS$ , 292.99, found 294.0 (M+H).

Step 4: [3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methyl methanesulfonate



Into a 50-mL round-bottom flask, was placed [3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methanol (80 mg, 0.27 mmol, 1.00 equiv), dichloromethane (2.0 mL), triethylamine (83 mg, 0.82 mmol, 3.01 equiv). This was followed by the addition of MsCl (62 mg, 0.54 mmol, 2.00 equiv) dropwise with stirring at 0°C. The resulting solution was stirred for 1 h at 30°C. The reaction progress was monitored by LCMS. The reaction was then quenched by the addition of 10 mL of water. The resulting solution was extracted with 3x10 mL of dichloromethane and the organic layers combined. The resulting mixture was washed with 2x20 mL of sodium chloride. The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 0.12 g of [3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methyl methanesulfonate as a yellow solid. This crude could be used for the next step directly. Mass spectrum (ESI, m/z): Calcd. for  $C_{12}H_9ClF_3NO_3S_2$ , 370.97, found 372.0 (M+H).

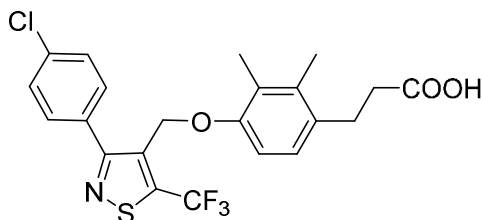
Step 5: Ethyl 3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate



Into a 50-mL round-bottom flask, was placed [3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methyl methanesulfonate (101 mg, 0.27 mmol, 1.00 equiv), ethyl 3-(2,3-difluoro-4-hydroxyphenyl)propanoate (prepared according to PCT Application WO2010/048207A2) (81 mg, 0.35 mmol, 1.30 equiv), N,N-dimethyl formamide (3.0 mL), potassium carbonate (113 mg, 0.82 mmol, 3.01 equiv). The resulting solution was stirred overnight at 30°C. The reaction progress was monitored by LCMS. The reaction was then quenched by the addition of 10 mL of water. The resulting solution was extracted with 3x10 mL

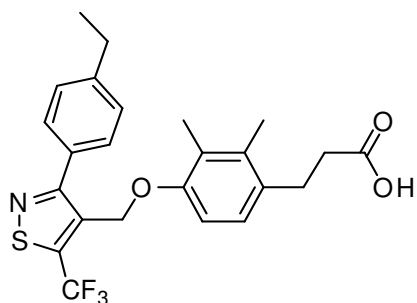
of ethyl acetate and the organic layers combined and dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by TLC-Plate with Petroleum ether/EtOAc = 6:1. This resulted in 0.10 g (73%) of ethyl 3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-methylphenyl)propanoate as a light yellow solid. Mass spectrum (ESI, m/z): Calcd. for C<sub>24</sub>H<sub>23</sub>ClF<sub>3</sub>NO<sub>3</sub>S, 497.10, found 498.4 (M+H).

Step 6: 3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-methylphenyl)propanoic acid



Into a 50-mL round-bottom flask, was placed ethyl 3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate (101 mg, 0.20 mmol, 1.00 equiv), tetrahydrofuran (2.0 mL), a solution of LiOH (100 mg, 4.18 mmol, 20.91 equiv) in water (2.0 mL). The resulting solution was stirred overnight at 30°C. The reaction progress was monitored by LCMS. The pH value of the solution was adjusted to 1 with aqueous HCl (2 N). The resulting mixture was concentrated under vacuum. The solids were collected by filtration and washed by hexane and EtOAc. Then the solid was dried under vacuum. This resulted in 85 mg (89%) of 3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.65 (d, *J* = 7.5 Hz, 2H), 7.42 (d, *J* = 7.8 Hz, 2H), 6.95 (d, *J* = 5.5 Hz, 1H), 6.62 (d, *J* = 6.0 Hz, 1H), 5.01 (s, 2H), 2.98 (t, *J* = 7.5 Hz, 2H), 2.66 (t, *J* = 7.8 Hz, 2H), 2.25 (s, 3H), 2.12 (s, 3H). Mass spectrum (ESI, m/z): Calcd. for C<sub>22</sub>H<sub>19</sub>ClF<sub>3</sub>NO<sub>3</sub>S, 469.07, found 470.1 (M+H).

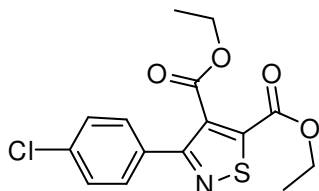
**3-(4-((3-(4-ethylphenyl)-5-(trifluoromethyl)isothiazol-4-yl)methoxy)-2,3-dimethylphenyl)propanoic acid (4e)**



The title compound was prepared according to the procedure described in **4f** starting from 4-ethylbenzamide using ethyl 3-(2,3-dimethyl-4-hydroxyphenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.63 (d, *J* = 7.0 Hz, 2H), 7.28 (d, *J* = 7.0 Hz, 2H), 6.98 (d, *J* = 6.5 Hz, 1H), 6.70 (d, *J* = 6.5 Hz, 1H), 5.03 (s, 2H), 2.98 (t, *J* = 5.5 Hz, 2H), 2.68 (q, *J* = 7.5 Hz, 2H), 2.60 (t, *J* = 6.0 Hz, 2H), 2.25 (s, 3H), 2.12 (s, 3H), 1.28 (t, *J* = 7.0 Hz, 3H). Mass spectrum (ESI, *m/z*): Calcd. for C<sub>24</sub>H<sub>24</sub>F<sub>4</sub>NO<sub>3</sub>S, 463.14, found 464.5 (M+H).

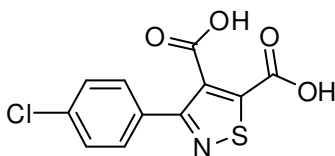
### **3-(4-((3-(4-chlorophenyl)isothiazol-4-yl)methoxy)-2,3-dimethylphenyl)propanoic acid (4a)**

#### Step 1: 4,5-diethyl 3-(4-chlorophenyl)-1,2-thiazole-4,5-dicarboxylate



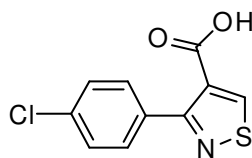
Into a 150-mL sealed tube, was placed 5-(4-chlorophenyl)-2H-1,3,4-oxathiazol-2-one (2 g, 9.65 mmol, 1.00 equiv), 1,4-diethyl but-2-ynedioate (3.29 g, 19.33 mmol, 2.00 equiv), 1,3-dichlorobenzene (30 mL). The resulting solution was stirred for 16 h at 148°C. The resulting mixture was concentrated under vacuum. This resulted in 1 g (31%) of 4,5-diethyl 3-(4-chlorophenyl)-1,2-thiazole-4,5-dicarboxylate as brown oil. Mass spectrum (ESI, *m/z*): Calcd. for C<sub>15</sub>H<sub>14</sub>ClNO<sub>4</sub>S, 339.03, found 340.3 (M+H).

#### Step 2: 3-(4-chlorophenyl)-1,2-thiazole-4,5-dicarboxylic acid



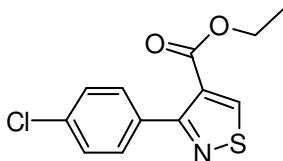
Into a 100-mL round-bottom flask, was placed a solution of 4,5-diethyl 3-(4-chlorophenyl)-1,2-thiazole-4,5-dicarboxylate (3.28 g, 9.84 mmol, 1.00 equiv) in ethanol (20 mL) and a solution of sodium hydroxide (2.36 g, 59.00 mmol, 6.00 equiv) in water(20 mL). The resulting solution was stirred for 2.5 h at 90°C in an oil bath. The pH value of the solution was adjusted to 3 with hydrogen chloride (1 mol/L). The resulting solution was extracted with 3x150 mL of dichloromethane. The organic layers were combined, washed with 1x10 mL of brine, dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 1.86 g (69%) of 3-(4-chlororphenyl)-1,2-thiazole-4,5-dicarboxylic acid as a yellow solid. Mass spectrum (ESI, m/z): Calcd. for C<sub>11</sub>H<sub>6</sub>ClNO<sub>4</sub>S, 282.97, found 284.3 (M+H).

Step 3: 3-(4-chlorophenyl)-1,2-thiazole-4-carboxylic acid



Into a 50-mL round-bottom flask, was placed 3-(4-chlorophenyl)-1, 2-thiazole-4,5-dicarboxylic acid (1.8697 g, 6.74 mmol, 1.00 equiv), 1,2-dichlorobenzene (20 mL). The resulting solution was stirred for 20 min at 200°C. The resulting mixture was concentrated, and then added 30 mL of petroleum ether slowly when it is hot. The solids were collected by filtration, washed with petroleum ether. This resulted in 1.35 g (86%) of 3-(4-chlorophenyl)-1, 2-thiazole-4-carboxylic acid as a white solid. Mass spectrum (ESI, m/z): Calcd. for C<sub>10</sub>H<sub>6</sub>ClNO<sub>2</sub>S, 238.98, found 240.2 (M+H).

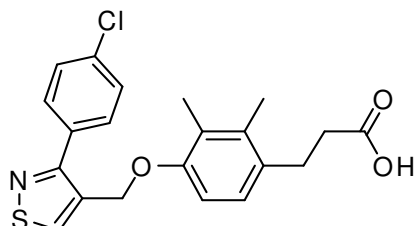
Step 4: Ethyl 3-(4-chlorophenyl)-1,2-thiazole-4-carboxylate



Into a 50-mL round-bottom flask, was placed ethanol (10 mL), thionyl chloride (440 mg, 3.70 mmol, 1.00 equiv), 3-(4-chlorophenyl)-1, 2-thiazole-4-carboxylic acid (860 mg, 3.69 mmol, 1.00 equiv). The resulting solution was stirred for 6 h at 90°C in an oil bath. The resulting mixture was concentrated under vacuum and taken up in 150 mL of dichloromethane, washed with 1x30 mL of brine. The solution was dried over anhydrous sodium sulfate and concentrated

under vacuum. This resulted in 0.72 g (75%) of ethyl 3-(4-chlorophenyl)-1, 2-thiazole-4-carboxylate as brown oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>12</sub>H<sub>10</sub>ClNO<sub>2</sub>S, 267.01, found 268.3 (M+H).

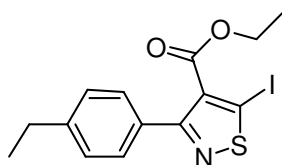
Step 5: 3-(4-((3-(4-chlorophenyl)isothiazol-4-yl)methoxy)-2,3-dimethylphenyl)propanoic acid



The title compound was prepared according to the procedure described in Example **4f** by coupling (3-(4-chlorophenyl)isothiazol-4-yl)methyl methanesulfonate and ethyl 3-(4-hydroxy-2,3-dimethylphenyl)propanoate followed by hydrolysis to afford the desired product as an off-white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.78 (s, 1H), 7.70 (d, *J* = 7.5 Hz, 2H), 7.42 (d, *J* = 7.5 Hz, 2H), 6.98 (d, *J* = 7.6 Hz, 1H), 6.68 (d, *J* = 7.8 Hz, 1H), 5.05 (s, 2H), 2.96 (t, *J* = 7.5 Hz, 2H), 2.62 (t, *J* = 7.6 Hz, 2H), 2.24 (s, 3H), 2.15 (s, 3H). Mass spectrum (ESI, m/z): Calcd. for C<sub>21</sub>H<sub>20</sub>ClNO<sub>3</sub>S, 401.09, found 402.1 (M+H).

**3-(4-[[3-(4-ethylphenyl)-5-(propan-2-yl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid (4d)**

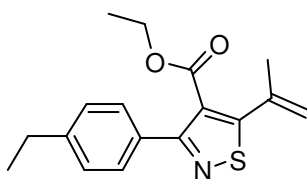
Step 1: Ethyl 3-(4-ethylphenyl)-5-iodo-1,2-thiazole-4-carboxylate



Into a 100-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed tetrahydrofuran (15 mL). This was followed by the addition of bis(propan-2-yl)amine (1.34 g, 13.24 mmol, 1.50 equiv). The mixture was cooled to -78°C. To this was added n-BuLi (2.5M) (5.28 mL). The mixture was stirred at -78°C for 40 minutes. To the mixture was added a solution of ethyl 3-(4-ethylphenyl)-1,2-thiazole-4-carboxylate (2.3 g, 8.80 mmol, 1.00 equiv) in tetrahydrofuran (5 mL). The mixture was stirred at -78°C for 40 minutes. To the mixture was added a solution of I<sub>2</sub> (3.35 g, 13.19 mmol, 1.50 equiv) in tetrahydrofuran (5 mL).

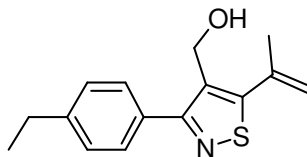
The resulting solution was stirred for 40 min at  $-78^{\circ}\text{C}$ . The reaction was then quenched by the addition of 80 mL of water. The resulting solution was extracted with 3x80 mL of ethyl acetate and the organic layers combined and concentrated under vacuum. The residue was applied onto a silica gel column and eluted with ethyl acetate/petroleum ether (1:20). The collected fractions were combined and concentrated under vacuum. This resulted in 2.1 g (62%) of ethyl 3-(4-ethylphenyl)-5-iodo-1,2-thiazole-4-carboxylate as red oil. Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{14}\text{H}_{14}\text{INO}_2\text{S}$ , 386.98, found 388.3 (M+H).

Step 2: Ethyl 3-(4-ethylphenyl)-5-(prop-1-en-2-yl)-1,2-thiazole-4-carboxylate



Into a 25-mL round-bottom flask, was placed ethyl 3-(4-ethylphenyl)-5-iodo-1,2-thiazole-4-carboxylate (200 mg, 0.52 mmol, 1.00 equiv), 4,4,5,5-tetramethyl-2-(prop-1-en-2-yl)-1,3,2-dioxaborolane (130.24 mg, 0.78 mmol, 1.50 equiv), sodium carbonate (164.34 mg, 1.55 mmol, 3.00 equiv),  $\text{Pd}(\text{dppf})\text{Cl}_2$  (37.82 mg, 0.05 mmol, 0.10 equiv), DME/ $\text{H}_2\text{O}$  (6 mL). The resulting solution was stirred for 16 h at  $95^{\circ}\text{C}$  in an oil bath under nitrogen. The resulting solution was extracted with 150 mL of dichloromethane. The organic layer was washed with 1x30 mL of brine, dried over anhydrous sodium sulfate and concentrated. The residue was applied onto a silica gel column and eluted with ethyl acetate/petroleum ether (1:20). This resulted in 287.4 mg of ethyl 3-(4-ethylphenyl)-5-(prop-1-en-2-yl)-1,2-thiazole-4-carboxylate as brown oil. Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{17}\text{H}_{19}\text{NO}_2\text{S}$ , 301.11, found 302.4 (M+H).

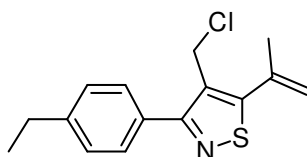
Step 3: [3-(4-ethylphenyl)-5-(prop-1-en-2-yl)-1,2-thiazol-4-yl]methanol



Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed a solution of ethyl 3-(4-ethylphenyl)-5-(prop-1-en-2-yl)-1,2-thiazole-4-

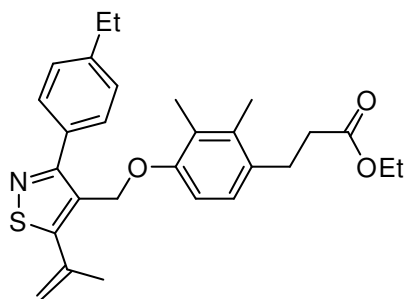
carboxylate (280 mg, 0.93 mmol, 1.00 equiv) in toluene (6 mL). DIBAL-H (1.32 g, 2.50 equiv) was added at  $-78^{\circ}\text{C}$ . The resulting solution was stirred for 4 h at  $-78^{\circ}\text{C}$ . The reaction was then quenched by the addition of 5 mL of water. The resulting solution was extracted with 200 mL of ethyl acetate. The organic layer was washed with 1x30 mL of brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by TLC method with petroleum ether/ethyl acetate (3:1). This resulted in 305.3 mg (crude) of [3-(4-ethylphenyl)-5-(prop-1-en-2-yl)-1,2-thiazol-4-yl]methanol as yellow oil. Mass spectrum (ESI, m/z): Calcd. for  $\text{C}_{15}\text{H}_{17}\text{NOS}$ , 259.10, found 260.4 (M+H).

Step 4: 4-(chloromethyl)-3-(4-ethylphenyl)-5-(prop-1-en-2-yl)isothiazole



Into a 50-mL round-bottom flask, was placed a solution of [3-(4-ethylphenyl)-5-(prop-1-en-2-yl)-1,2-thiazol-4-yl]methanol (305.3 mg, 1.18 mmol, 1.00 equiv) in dichloromethane (15 mL),  $\text{MsCl}$  (268.8 mg, 2.00 equiv), triethylamine (297.6 mg, 2.94 mmol, 2.50 equiv). The resulting solution was stirred for 40 min at  $25^{\circ}\text{C}$ . 10.0 mL water was added to the mixture. The organic phase was separated and washed by 10.0 mL brine, dried over anhydrous sodium sulfate. The resulting mixture was concentrated under vacuum and residue used in next step directly without further purification. Mass spectrum (ESI, m/z): Calcd. for  $\text{C}_{15}\text{H}_{16}\text{ClNS}$ , 277.07, found 278.4 (M+H).

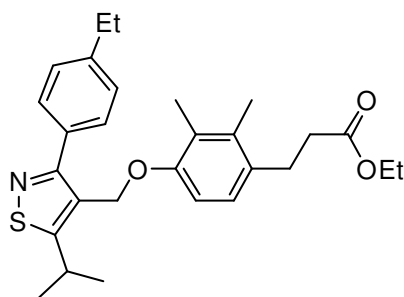
Step 5: Ethyl 3-(4-[[3-(4-ethylphenyl)-5-(prop-1-en-2-yl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate



Into a 50-mL round-bottom flask, was placed a solution of 4-(chloromethyl)-3-(4-ethylphenyl)-5-(prop-1-en-2-yl)-1,2-thiazole (326.5 mg, 1.18 mmol, 1.00 equiv) in N,N-

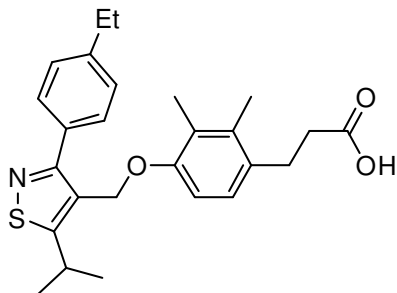
dimethylformamide (6 mL), potassium carbonate (488.0 mg, 3.53 mmol, 3.00 equiv), ethyl 3-(4-hydroxy-2,3-dimethylphenyl) propanoate (392.5 mg, 1.77 mmol, 1.50 equiv). The resulting solution was stirred for 12 h at 25°C and 30 mL ethyl acetate and 20 mL water were added to the mixture. The organic layer was separated and washed with 3x10 mL of brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by TLC method with petroleum ether/ethyl acetate (6:1). This resulted in 111.3 mg (20%) of ethyl 3-(4-[[3-(4-ethylphenyl)-5-(prop-1-en-2-yl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethyl phenyl) propanoate as yellow oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>28</sub>H<sub>33</sub>NO<sub>3</sub>S, 463.22, found 464.6 (M+H).

Step 6: Ethyl 3-(4-[[3-(4-ethylphenyl)-5-(propan-2-yl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate



Into a 25-mL round-bottom flask, was placed a solution of ethyl 3-(4-[[3-(4-ethylphenyl)-5-(prop-1-en-2-yl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate (76.3 mg, 0.16 mmol, 1.00 equiv) in ethanol (5 mL) and palladium on carbon (10%, 76 mg). The mixture was then subject to an atmosphere of hydrogen and stirred for 12 h at 25°C. The solids were filtered out. The resulting mixture was concentrated under vacuum. This resulted in 73.1 mg (95%) of ethyl 3-(4-[[3-(4-ethylphenyl)-5-(propan-2-yl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl) propanoate as white oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>28</sub>H<sub>35</sub>NO<sub>3</sub>S, 465.23, found 466.6 (M+H).

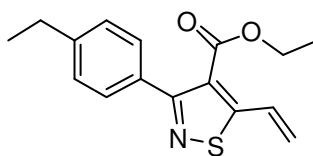
Step 7: 3-(4-[[3-(4-ethylphenyl)-5-(propan-2-yl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl) propanoic acid



Into a 25-mL round-bottom flask, was placed ethyl 3-(4-[[3-(4-ethylphenyl)-5-(propan-2-yl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate (73.1 mg, 0.16 mmol, 1.00 equiv), LiOH (73 mg, 3.05 mmol, 19.42 equiv), tetrahydrofuran/H<sub>2</sub>O (5 mL). The resulting solution was stirred for 12 h at 25°C. The resulting solution was concentrated. The pH value of the residue was adjusted to 3 with 1M hydrogen chloride. The resulting solution was extracted with 3x40 mL of dichloromethane and the organic layers combined and concentrated. The crude product was purified by Prep-HPLC with the following conditions (1#-Waters 2767-5): Column, SunFire Prep C<sub>18</sub>, 5µm, 19\*100mm; mobile phase, Water and CH<sub>3</sub>CN (30% CH<sub>3</sub>CN up to 90% in 10 min, up to 100% in 2 min, down to 30% in 2 min; Detector, UV 220&254nm. This resulted in 60 mg (85%) of 3-(4-[[3-(4-ethylphenyl)-5-(propan-2-yl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl) propanoic acid as a white solid. <sup>1</sup>H NMR (300MHz,CD<sub>3</sub>OD) δ 7.58 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 6.98 (d, *J* = 8.7 Hz, 1H), 6.75 (d, *J* = 8.4 Hz, 1H), 4.94 (s, 2H), 3.44-3.53 (m, 1H), 2.92 (t, *J* = 8.1 Hz, 2H), 2.68 (m, 2H), 2.52 (t, *J* = 7.2 Hz, 2H), 2.24 (s, 3H), 2.10 (s, 3H), 2.14 (s, 6H), 1.25 (t, *J* = 7.5 Hz, 3H). Mass spectrum (ESI, m/z): Calcd. for C<sub>26</sub>H<sub>31</sub>NO<sub>3</sub>S, 437.20, found 438.2 (M+H).

### 3-(4-[[5-ethyl-3-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid (4c)

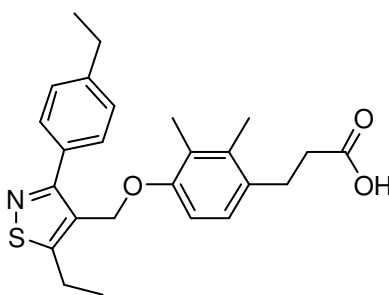
Step 1: Ethyl 5-ethenyl-3-(4-ethylphenyl)-1,2-thiazole-4-carboxylate



Into a 50-mL round-bottom flask (1 atm) purged and maintained with an inert atmosphere of nitrogen, was placed toluene (5 mL), ethyl 3-(4-ethylphenyl)-5-iodo-1,2-thiazole-4-carboxylate (300 mg, 0.77 mmol, 1.00 equiv), tributyl(ethenyl)stannane (370 mg, 1.17 mmol,

1.51 equiv), Pd(PPh<sub>3</sub>)<sub>4</sub> (89.4 mg, 0.08 mmol, 0.10 equiv). The resulting solution was stirred for 16 h at 95°C. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:20). The collected fractions were combined and concentrated under vacuum. This resulted in 200 mg (90%) of ethyl 5-ethenyl-3-(4-ethylphenyl)-1,2-thiazole-4-carboxylate as yellow oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>16</sub>H<sub>17</sub>NO<sub>2</sub>S, 287.10, found 288.2 (M+H).

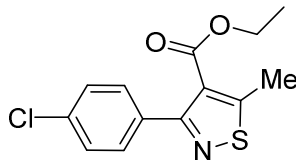
Step 2: 3-(4-[[5-ethyl-3-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid



The title compound was prepared according to the procedure described in Example **4d** by transformation of ethyl 5-ethenyl-3-(4-ethylphenyl)-1,2-thiazole-4-carboxylate into 4-(chloromethyl)-3-(4-ethylphenyl)-5-vinylisothiazole and then coupled with ethyl 3-(4-hydroxy-2,3-dimethylphenyl) propanoate and hydrogenation with 10% Pd/C followed by hydrolysis of ethyl 3-(4-[[5-ethyl-3-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate to afford the desired product as an off-white solid. <sup>1</sup>H NMR (300MHz, CD<sub>3</sub>OD) δ 7.58 (d, *J* = 8.1 Hz, 2H), 7.27 (d, *J* = 8.1 Hz, 2H), 6.97 (d, *J* = 8.7 Hz, 1H), 6.74 (d, *J* = 9.0 Hz, 1H), 5.04 (s, 2H), 3.05 (m, 2H), 2.92 (t, *J* = 8.1 Hz, 2H), 2.69 (m, 2H), 2.52 (t, *J* = 7.8 Hz, 2H), 2.24 (s, 3H), 2.09 (s, 3H), 1.39 (t, *J* = 7.5 Hz, 3H), 1.28 (t, *J* = 7.8 Hz, 3H). Mass spectrum (ESI, m/z): Calcd. for C<sub>25</sub>H<sub>29</sub>NO<sub>3</sub>S, 423.19, found 424.2 (M+H).

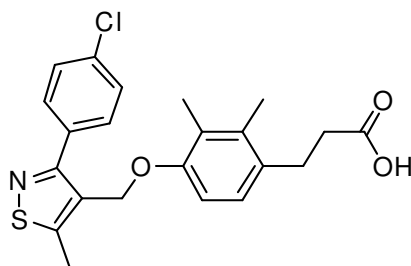
**3-(4-((3-(4-chlorophenyl)-5-methylisothiazol-4-yl)methoxy)-2,3-methylphenyl)propanoic acid (4b)**

Step 1: ethyl 3-(4-chlorophenyl)-5-methylisothiazole-4-carboxylate



Into a 25-mL round-bottom flask, was placed ethyl 3-(4-chlorophenyl)-5-iodoisothiazole-4-carboxylate prepared according to the same procedure described in Example **4d** (200 mg, 0.51 mmol, 1.00 equiv),  $\text{Me}_4\text{Sn}$  (106 mg, 0.77 mmol, 1.5 equiv),  $\text{Pd}(\text{PPh}_3)_4$  (58 mg, 0.05 mmol, 0.10 equiv) in toluene (10 mL). The resulting solution was stirred for 16 h at  $110^\circ\text{C}$  in an oil bath under nitrogen. The resulting solution was extracted with 100 mL of ethyl acetate. The organic layer was washed with 1x30 mL of brine, dried over anhydrous sodium sulfate and concentrated. The residue was applied onto a silica gel column and eluted with ethyl acetate/heptanes (5%). This resulted in 115 mg of ethyl 3-(4-chlorophenyl)-5-methylisothiazole-4-carboxylate as colorless oil. Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{13}\text{H}_{12}\text{ClNO}_2\text{S}$ , 281.03, found 282.5 (M+H).

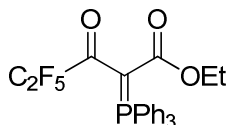
Step 2: 3-(4-((3-(4-chlorophenyl)-5-methylisothiazol-4-yl)methoxy)-2,3-methylphenyl)propanoic acid



The title compound was prepared according to the procedure described in Example **4d** by transformation of ethyl 3-(4-chlorophenyl)-5-methylisothiazole-4-carboxylate into (3-(4-chlorophenyl)-5-methylisothiazol-4-yl)methanol and then coupled with ethyl 3-(4-hydroxy-2,3-dimethylphenyl) propanoate followed by hydrolysis of ethyl 3-(4-((3-(4-chlorophenyl)-5-methylisothiazol-4-yl)methoxy)-2,3-methylphenyl)propanoate to afford the desired product as an off-white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.60 (d,  $J = 7.1$  Hz, 2H), 7.38 (d,  $J = 7.0$  Hz, 2H), 6.88 (m,  $J = 5.6$  Hz, 1H), 6.70 (m,  $J = 5.6$  Hz, 1H), 4.95 (s, 2H), 2.90 (m, 2H), 2.65 (m, 2H), 2.55 (s, 3H), 2.25 (s, 3H), 2.10 (s, 3H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{22}\text{H}_{22}\text{ClNO}_3\text{S}$ , 415.10, found 416.5 (M+H).

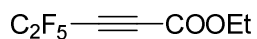
**3-(4-[[3-(4-ethylphenyl)-5-(pentafluoroethyl)-1, 2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid (4g)**

Step 1: Ethyl 4, 4, 5, 5, 5-pentafluoro-3-oxo-2-(triphenyl-[5]-phosphanylidene)pentanoate



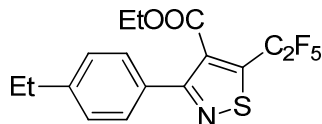
Into a 250-mL 3-necked round-bottom flask (1 atm) purged and maintained with an inert atmosphere of nitrogen, was placed (2-ethoxy-2-oxoethyl)triphenylphosphonium bromide (17.16 g, 39.97 mmol, 1.00 equiv), tetrahydrofuran (100 mL). The solution was cooled to 0°C in the ice/water bath. Triethylamine (8.888 g, 87.83 mmol, 2.20 equiv) was added dropwise and stirred at this temperature, stirred for 15 min. Then pentafluoropropanoyl 2,2,3,3,3-pentafluoropropanoate (13.64 g, 43.99 mmol, 1.10 equiv) was added dropwise and stirred for another 2h. The solids were collected by filtration, washed by the cool THF, collected the organic phase and concentrated. 100 mL distilled water was added to the residue, and stirred violently. The white solid appeared. Filtered and dried under the vacuum. This resulted in 14.4 g (73%) of ethyl 4,4,5,5,5-pentafluoro-3-oxo-2-(triphenyl-[5]-phosphanylidene) pentanoate as a white solid. Mass spectrum (ESI, m/z): Calcd. for C<sub>25</sub>H<sub>20</sub>F<sub>5</sub>O<sub>3</sub>P, 494.11, found 495.4 (M+H).

Step 2: Ethyl 4,4,5,5,5-pentafluoropent-2-ynoate



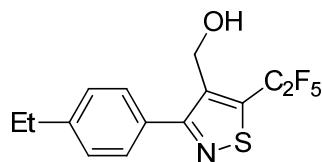
Into a 10-mL round-bottom flask, was placed ethyl 4,4,5,5,5-pentafluoro-3-oxo-2-(triphenyl-[5]-phosphanylidene)pentanoate (7.4 g, 14.97 mmol, 1.00 equiv). It was thermolyzed under reduced pressure (0.1~10 torr). Once the distillation pot reached to 140 degree C, the solid phosphorane began to melt and evolution of acetylene. The mixture was heated to 220~240 degree C and the acetylene was collected in the Ethanol-Liquid nitrogen bath. This resulted in 0.6 g (19%) of ethyl 4,4,5,5,5-pentafluoropent-2-ynoate as orange oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>7</sub>H<sub>5</sub>F<sub>5</sub>O<sub>2</sub>, 216.02, found 217.4 (M+H).

Step 3: Ethyl 3-(4-ethylphenyl)-5-(perfluoroethyl)isothiazole-4-carboxylate



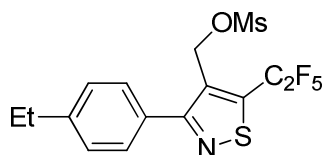
Into a 10-mL sealed tube, was placed 5-(4-ethylphenyl)-2H-1,3,4-oxathiazol-2-one (155 mg, 0.75 mmol, 1.00 equiv), ethyl 4,4,5,5,5-pentafluoropent-2-ynoate (250 mg, 1.16 mmol, 1.55 equiv), 1,3-dichlorobenzene (3 mL). The resulting solution was stirred for 16 h at 150°C in an oil bath. The reaction progress was monitored by LCMS. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:19). The resulted in a mixture of the product 0.192 g as the yellow oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>16</sub>H<sub>14</sub>F<sub>5</sub>NO<sub>2</sub>S, 379.07, found 380.3 (M+H).

Step 4: 3-(4-ethylphenyl)-5-(pentafluoroethyl)-1, 2-thiazol-4-yl]methanol



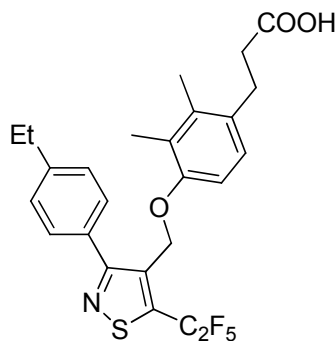
Into a 50-mL 3-necked round-bottom flask (1 atm) purged and maintained with an inert atmosphere of nitrogen, was placed ethyl 3-(4-ethylphenyl)-5-(pentafluoroethyl)-1,2-thiazole-4-carboxylate (180 mg, 0.47 mmol, 1.00 equiv), toluene (10 mL). This was followed by the addition of DIBAL-H (25% in toluene) (1.35 g, 5.00 equiv) dropwise with stirring at -78°C. The resulting solution was warmed to 30°C naturally and stirred for 2 h. The reaction progress was monitored by LCMS. The reaction was then quenched by the addition of 10 mL of water at 0°C, the organic phase was separated and the aqueous phase was extracted with 3x20 mL of ethyl acetate and the organic layers combined. The resulting mixture was washed with 2x30 mL of sodium chloride. The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by TLC. This resulted in 0.108 g (67%) of [3-(4-ethylphenyl)-5-(pentafluoroethyl)-1, 2-thiazol-4-yl]methanol as light yellow oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>14</sub>H<sub>12</sub>F<sub>5</sub>NOS, 337.06, found 338.3 (M+H).

Step 5: [3-(4-ethylphenyl)-5-(pentafluoroethyl)-1,2-thiazol-4-yl]methyl methanesulfonate



Into a 100-mL round-bottom flask, was placed [3-(4-ethylphenyl)-5-(pentafluoroethyl)-1,2-thiazol-4-yl]methanol (108 mg, 0.32 mmol, 1.00 equiv), dichloromethane (10 mL). The solution was cooled to 0 degree C. MsCl (73 mg, 0.64 mmol, 2.00 equiv) and triethylamine (0.097 g, 0.96 mmol, 3.00 equiv) were added dropwise. The resulting solution was stirred for 2 h at 25°C. The reaction progress was monitored by LCMS. The reaction was then quenched by the addition of 5 mL of water. Organic phase was separated and the aqueous phase was extracted with 3x10 mL of dichloromethane and the organic layers combined. The resulting mixture was washed with 2x20 mL of sodium chloride. The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 0.150 g (crude) of [3-(4-ethylphenyl)-5-(pentafluoroethyl)-1,2-thiazol-4-yl]methyl methanesulfonate as a white solid. And it could be used for the next step directly. Mass spectrum (ESI, m/z): Calcd. for C<sub>15</sub>H<sub>14</sub>F<sub>5</sub>NO<sub>3</sub>S<sub>2</sub>, 415.03, found 416.4 (M+H).

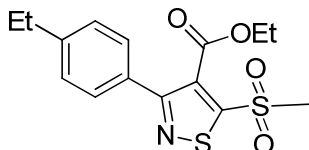
Step 6: 3-(4-[[3-(4-ethylphenyl)-5-(pentafluoroethyl)-1, 2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid



The title compound was prepared according to the procedure described in Example **4f** followed by coupling [3-(4-ethylphenyl)-5-(pentafluoroethyl)-1,2-thiazol-4-yl]methyl methanesulfonate and ethyl 3-(4-hydroxy-2,3-dimethylphenyl)propanoate to afford the desired product as an off-white solid. <sup>1</sup>H-NMR (300MHz, CD<sub>3</sub>OD) δ 7.60 (d, *J* = 8.1Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 1H), 5.03 (s, 2H), 2.91 (t, *J* = 8.1 Hz, 2H), 2.68 (q, *J*<sub>1</sub> = 15.3 Hz, *J*<sub>2</sub> = 7.5 Hz, 2H), 2.51 (t, *J* = 8.4 Hz, 2H), 2.23 (s, 3H), 2.03 (s, 3H), 1.02 (t, *J* = 7.8 Hz, 3H). Mass spectrum (ESI, m/z): Calcd. for C<sub>25</sub>H<sub>24</sub>F<sub>5</sub>NO<sub>3</sub>S, 513.14, found 514.1 (M+H).

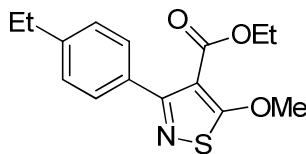
**3-(4-[[3-(4-ethylphenyl)-5-methoxy-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid (4h)**

Step 1: Ethyl 3-(4-ethylphenyl)-5-methanesulfonyl-1,2-thiazole-4-carboxylate



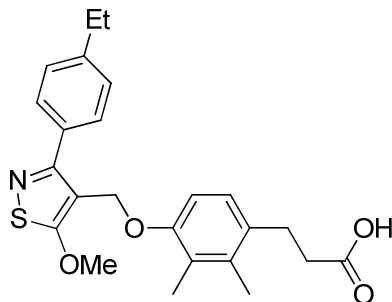
Into a 50-mL round-bottom flask, was placed N,N-dimethylformamide (8 mL), CuI (391 mg, 2.05 mmol, 1.99 equiv), ethyl 3-(4-ethylphenyl)-5-iodo-1,2-thiazole-4-carboxylate (400 mg, 1.03 mmol, 1.00 equiv), sodium methanesulfinate (421.7 mg, 4.13 mmol, 4.00 equiv). The resulting solution was stirred for 16 h at 80°C. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:20). The collected fractions were combined and concentrated under vacuum. This resulted in 170 mg (48%) of ethyl 3-(4-ethylphenyl)-5-methanesulfonyl-1,2-thiazole-4-carboxylate as a yellow solid. Mass spectrum (ESI, m/z): Calcd. for C<sub>15</sub>H<sub>17</sub>NO<sub>4</sub>S<sub>2</sub>, 339.06, found 340.1 (M+H).

Step 2: Methyl 3-(4-ethylphenyl)-5-methoxy-1,2-thiazole-4-carboxylate



Into a 100-mL round-bottom flask, was placed a solution of ethyl 3-(4-ethylphenyl)-5-methanesulfonyl-1,2-thiazole-4-carboxylate (150 mg, 0.44 mmol, 1.00 equiv), methanol (15 mL), MeONa (119.47 mg, 5.00 equiv). The resulting solution was stirred for 2 h at 70°C in an oil bath. The resulting solution was extracted with 2x15 mL of dichloromethane and the organic layers combined. The resulting mixture was washed with 2x30 mL of brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solids were filtered out. The resulting mixture was concentrated under vacuum. This resulted in 141.4 mg (crude) of methyl 3-(4-ethylphenyl)-5-methoxy-1,2-thiazole-4-carboxylate as white oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>15</sub>H<sub>17</sub>NO<sub>3</sub>S, 291.09, found 292.1 (M+H).

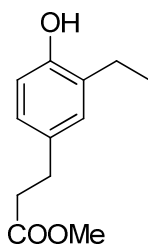
Step 3: 3-(4-[[3-(4-ethylphenyl)-5-methoxy-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid



The title compound was prepared according to the procedure described in Example **4f** by coupling of (3-(4-ethylphenyl)-5-methoxyisothiazol-4-yl)methyl methanesulfonate and ethyl 3-(4-hydroxy-2,3-dimethylphenyl)propanoate followed by hydrolysis to afford the desired product as an off-white solid.  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.63 (d,  $J = 8.1$  Hz, 2H), 7.25 (d,  $J = 7.8$  Hz, 1H), 6.95 (d,  $J = 8.1$  Hz, 1H), 6.75 (d,  $J = 8.1$  Hz, 1H), 4.86 (s, 2H), 4.14 (s, 3H), 2.91 (t,  $J = 7.8$  Hz, 2H), 2.68 (q,  $J_1 = 15.2$  Hz,  $J_2 = 7.5$  Hz, 2H), 2.51 (t,  $J = 7.8$  Hz, 2H), 2.23 (s, 3H), 2.06 (s, 3H), 1.25 (t,  $J = 7.8$  Hz, 3H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{24}\text{H}_{27}\text{NO}_4\text{S}$ , 425.17, found 426.2 (M+H).

### 3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-3-ethylphenyl)propanoic acid (**4i**)

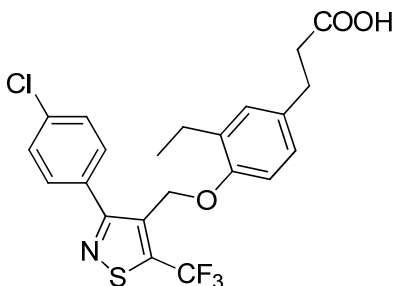
Step 1: Methyl 3-(3-ethyl-4-hydroxyphenyl)propanoate



Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed methyl 3-(3-bromo-4-hydroxyphenyl)propanoate (220 mg, 0.85 mmol, 1.00 equiv),  $\text{Pd}(\text{dppf})\text{Cl}_2$  (25.76 mg, 0.04 mmol, 0.04 equiv),  $\text{Cs}_2\text{CO}_3$  (616.4 mg), tetrahydrofuran (5 mL), triethylborane (1.26 mL). The resulting solution was stirred overnight at  $65^\circ\text{C}$  in an oil bath. The reaction was then quenched by the addition of 5 mL of water. The resulting mixture was concentrated under vacuum. The resulting solution was diluted with 20 mL of EA. The resulting mixture was washed with 3x10 mL of  $\text{H}_2\text{O}$ . The mixture was dried over anhydrous sodium sulfate. The residue was applied onto a silica gel column with ethylacetate/petroleum

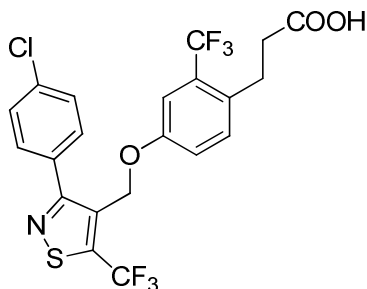
ether (10:90). This resulted in 300 mg (170%) of methyl 3-(3-ethyl-4-hydroxyphenyl)propanoate as colorless oil. Mass spectrum (ESI, m/z): Calcd. for  $C_{12}H_{16}O_3$ , 208.11, found 209.2 (M+H).

Step 2: 3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-3-ethylphenyl)propanoic acid



The title compound was prepared according to the procedure described in Example 4f starting from 4-chlorobenzamide using ethyl 3-(4-hydroxy-3-ethylphenyl) propanoate as coupling agent to afford the desired product as an off-white solid.  $^1H$  NMR (400MHz,  $CD_3OD$ )  $\delta$ : 7.70 (d,  $J = 9.2$  Hz, 2H), 7.46 (d,  $J = 8.8$  Hz, 2H), 7.02 (t,  $J = 9.6$  Hz, 2H), 6.86 (d,  $J = 8.0$  Hz, 1H), 5.11 (s, 2H), 2.86 (t,  $J = 7.6$  Hz, 2H), 2.45-2.60 (m, 4H), 1.07 (t,  $J = 7.6$  Hz, 3H). Mass spectrum (ESI, m/z): Calcd. for  $C_{22}H_{19}ClF_3NO_3S$ , 469.07, found 468.1 (M-H).

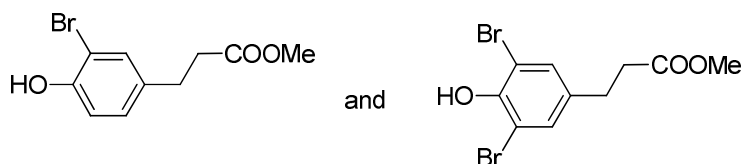
**3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-4-yl]methoxy]-2-(trifluoromethyl)phenyl)propanoic acid (4j)**



The title compound was prepared according to the procedure described in Example 4f starting from 4-chlorobenzamide using ethyl 3-(4-hydroxy-2-trifluoromethylphenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid.  $^1H$  NMR (300MHz,  $CD_3OD$ )  $\delta$  7.66-7.78 (m, 2H), 7.41-7.51 (m, 3H), 7.12-7.16 (m, 2H), 5.20 (s, 2H), 3.05 (t,  $J = 7.8$  Hz, 2H), 2.58 (t,  $J = 8.4$  Hz, 2H). Mass spectrum (ESI, m/z): Calcd. for  $C_{21}H_{14}ClF_6NO_3S$ , 509.03, found 508.1 (M-H).

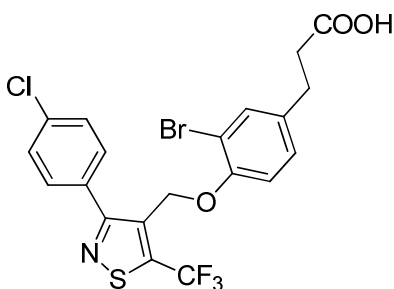
**3-(3-bromo-4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]phenyl)propanoic acid (4k)**

Step 1: Methyl 3-(3-bromo-4-hydroxyphenyl)propanoate and Methyl 3-(3,5-dibromo-4-hydroxyphenyl)propanoate



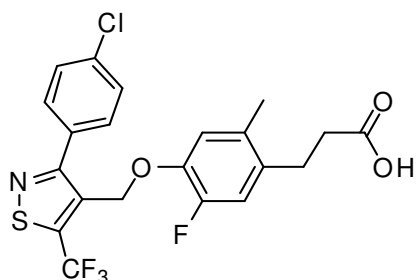
Into a 50-mL round-bottom flask, was placed methyl 3-(4-hydroxyphenyl)propanoate (1 g, 5.55 mmol, 1.00 equiv). This was followed by the addition of Br<sub>2</sub> (880 mg, 5.51 mmol, 1.00 equiv) dropwise with stirring at 0°C. To this was added methanol (25 mL). The resulting solution was stirred overnight at 0°C in a water/ice bath. The reaction was then quenched by the addition of 5 of sodium carbonate. The resulting mixture was concentrated under vacuum. The residue was diluted with 10 mL of water, extracted with 1x20 mL of ethyl acetate and the organic layers were combined. The mixture was dried over anhydrous magnesium sulfate and concentrated. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (0-15.4%). This resulted in 1.1 g (83%) of methyl 3-(3-bromo-4-hydroxyphenyl)propanoate as a white solid and 0.3 g of methyl 3-(3,5-dibromo-4-hydroxyphenyl)propanoate as a white solid. Methyl 3-(3-bromo-4-hydroxyphenyl)propanoate : Mass spectrum (ESI, m/z): Calcd. for C<sub>10</sub>H<sub>11</sub>BrO<sub>3</sub>, 257.99, found 259.1 (M+H). Methyl 3-(3,5-dibromo-4-hydroxyphenyl)propanoate: Mass spectrum (ESI, m/z): Calcd. for C<sub>10</sub>H<sub>10</sub>Br<sub>2</sub>O<sub>3</sub>, 335.90, found 337.1 (M+H).

Step 2: 3-(3-bromo-4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]phenyl)propanoic acid



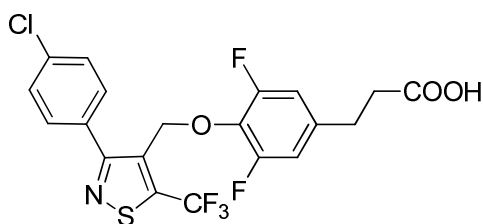
The title compound was prepared according to the procedure described in Example **4f** starting from 4-chlorobenzamide using ethyl 3-(4-hydroxy-3-bromophenyl) propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 7.73 (d,  $J = 8.8$  Hz, 2H), 7.47 (d,  $J = 8.8$  Hz, 3H), 7.19 (d,  $J = 8.4$  Hz, 1H), 7.99 (d,  $J = 8.4$  Hz, 1H), 5.14 (s, 2H), 2.88 (t,  $J = 7.6$  Hz, 2H), 2.60 (t,  $J = 7.6$  Hz, 2H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{20}\text{H}_{14}\text{BrClF}_3\text{NO}_3\text{S}$ , 518.95, found 520.0 (M+H).

**3-(4-((3-(4-chlorophenyl)-5-(trifluoromethyl)isothiazol-4-yl)methoxy)-5-fluoro-2-methylphenyl)propanoic acid (4l)**



The title compound was prepared according to the procedure described in Example **4f** starting from 4-chlorobenzamide using ethyl 3-(5-fluoro-4-hydroxy-2-methylphenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.73 (d,  $J = 8.0$  Hz, 2H), 7.47 (d,  $J = 8.1$  Hz, 2H), 6.77 (d,  $J = 8.5$  Hz, 1H), 6.93 (d,  $J = 8.5$  Hz, 2H), 5.10 (s, 2H), 2.88 (t,  $J = 8.5$  Hz, 2H), 2.63 (d,  $J = 8.2$  Hz, 2H), 2.24 (s, 3H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{21}\text{H}_{16}\text{ClF}_4\text{NO}_3\text{S}$ , 473.05, found 474.5 (M+H).

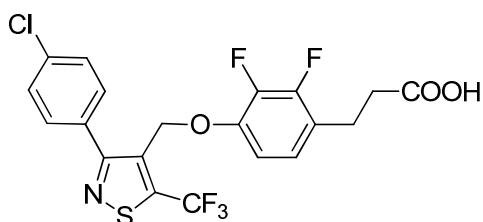
**3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-3,5-difluorophenyl)propanoic acid (4m)**



The title compound was prepared according to the procedure described in Example **4f** starting from 4-chlorobenzamide using tert-butyl 3-(3,5-difluoro-4-hydroxyphenyl)propanoate as

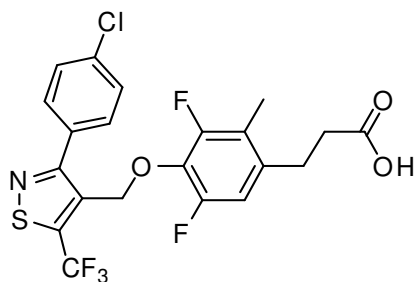
coupling agent followed by hydrolysis to afford the desired product as an off-white solid.  $^1\text{H}$  NMR (300MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.77 (d,  $J = 8.7$  Hz, 2H), 7.51 (d,  $J = 8.4$  Hz, 2H), 6.86 (d,  $J = 9.6$  Hz, 2H), 5.20 (s, 2H), 2.87 (t,  $J = 7.5$  Hz, 2H), 2.59 (t,  $J = 7.5$  Hz, 2H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{20}\text{H}_{13}\text{ClF}_5\text{NO}_3\text{S}$ , 477.02, found 478.0 (M+H).

**3-(4-[[3-(4-chlorophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-difluorophenyl)propanoic acid (4n)**



The title compound was prepared according to the procedure described in Example 4f starting from 4-chlorobenzamide using tert-butyl 3-(2,3-difluoro-4-hydroxyphenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as a white solid.  $^1\text{H}$ -NMR (300MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.69 (d,  $J = 8.4$  Hz, 2H), 7.51 (d,  $J = 8.4$  Hz, 2H), 7.01 (t,  $J = 7.5$  Hz, 1H), 6.85 (t,  $J = 7.5$  Hz, 1H), 5.19 (s, 2H), 2.94 (t,  $J = 7.8$  Hz, 2H), 2.61 (t,  $J = 7.8$  Hz, 2H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{20}\text{H}_{13}\text{ClF}_5\text{NO}_3\text{S}$ , 477.02, found 478.1 (M+H).

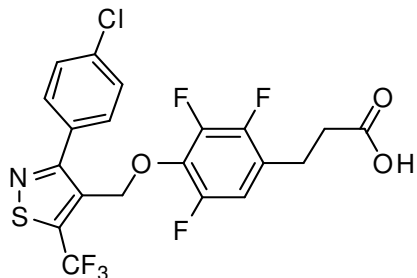
**3-(4-((3-(4-chlorophenyl)-5-(trifluoromethyl)isothiazol-4-yl)methoxy)-3,5-difluoro-2-methylphenyl)propanoic acid (4o)**



The title compound was prepared according to the procedure described in Example 4f starting from 4-chlorobenzamide using ethyl 3-(2-methyl-3,5-difluoro-4-hydroxyphenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.85 (d,  $J = 7.1$  Hz, 2H), 7.48 (d,  $J = 7.0$  Hz, 2H),

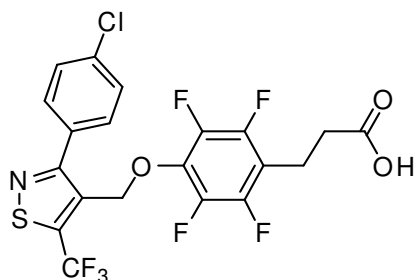
6.75 (d,  $J = 8.3$  Hz, 1H), 5.11 (s, 2H), 2.92 (t,  $J = 8.2$  Hz, 2H), 2.63 (t,  $J = 8.1$  Hz, 2H), 2.20 (s, 3H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $C_{21}H_{15}ClF_5NO_3S$ , 491.04, found 492.1 (M+H).

**3-(4-((3-(4-chlorophenyl)-5-(trifluoromethyl)isothiazol-4-yl)methoxy)-2,3,5-trifluorophenyl)propanoic acid (4p)**



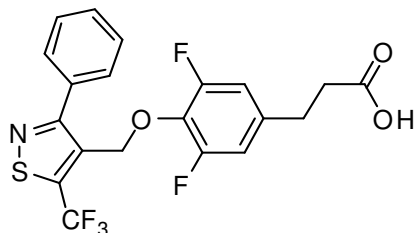
The title compound was prepared according to the procedure described in Example **4f** starting from 4-chlorobenzamide following using ethyl 3-(2,3,5-trifluoro-4-hydroxyphenyl) propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.82 (d,  $J = 8.1$  Hz, 2H), 7.53 (d,  $J = 8.0$  Hz, 2H), 6.81 (m, 1H), 5.18 (s, 2H), 2.97 (t,  $J = 7.5$  Hz, 2H), 2.71 (t,  $J = 7.5$  Hz, 2H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $C_{20}H_{12}ClF_6NO_3S$ , 495.01, found 496.1 (M+H).

**3-(4-((3-(4-chlorophenyl)-5-(trifluoromethyl)isothiazol-4-yl)methoxy)-2,3,5,6-tetrafluorophenyl)propanoic acid (4q)**



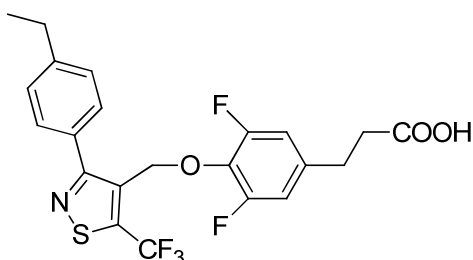
The title compound was prepared according to the procedure described in Example **4f** starting from 4-chlorobenzamide using ethyl 3-(2,3,4,5-tetrafluoro-4-hydroxyphenyl) propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.81 (d,  $J = 8.5$  Hz, 2H), 7.52 (d,  $J = 8.0$  Hz, 2H), 5.21 (s, 2H), 3.05 (m,  $J = 6.5$  Hz, 2H), 2.72 (m,  $J = 6.5$  Hz, 2H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $C_{20}H_{11}ClF_7NO_3S$ , 513.00, found 514.1 (M+H).

**3-(3,5-difluoro-4-((3-phenyl-5-(trifluoromethyl)isothiazol-4-yl)methoxy)phenyl)propanoic acid (4r)**



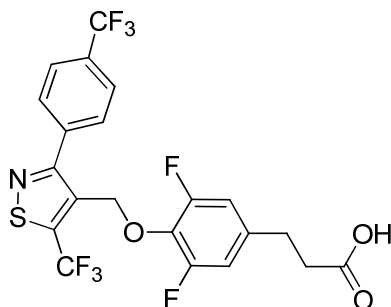
The title compound was prepared according to the procedure described in Example **4f** starting from benzamide using ethyl 3-(3,5-difluoro-4-hydroxyphenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.78 (m, 2H), 7.48 (m, 3H), 6.75 (d, *J* = 8.2 Hz, 2H), 5.12 (s, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 2.67 (t, *J* = 7.5 Hz, 2H). Mass spectrum (ESI, *m/z*): Calcd. for C<sub>20</sub>H<sub>14</sub>F<sub>5</sub>NO<sub>3</sub>S, 443.06, found 444.5 (M+H).

**3-(4-[[3-(4-ethylphenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-3,5-difluoro phenyl)propanoic acid (4s)**



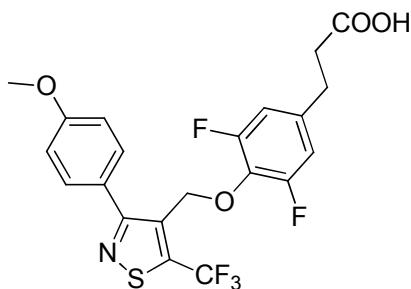
The title compound was prepared according to the procedure described in Example **4f** starting from 4-Et-benzamide using ethyl 3-(3,5-difluoro-4-hydroxyphenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as a white solid. <sup>1</sup>H-NMR (300Hz, CD<sub>3</sub>OD): δ 7.67 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 9.6 Hz, 2H), 5.21 (s, 2H), 2.87 (t, *J* = 7.5 Hz, 2H), 2.74 (q, *J*<sub>1</sub> = 7.5Hz, *J*<sub>2</sub> = 15.2 Hz, 2H), 2.61 (t, *J* = 7.8 Hz, 2H), 1.30 (t, *J* = 7.5 Hz, 3H). Mass spectrum (ESI, *m/z*): Calcd. for C<sub>22</sub>H<sub>18</sub>F<sub>5</sub>NO<sub>3</sub>S, 471.09, found 472.1 (M+H).

**3-(3, 5-difluoro-4-[[5-(trifluoromethyl)-3-[4-(trifluoromethyl)phenyl]-1,2-thiazol-4-yl]methoxy] phenyl)propanoic acid (4t)**



The title compound was prepared according to the procedure described in Example 4f starting 4-CF<sub>3</sub>-benzamide using ethyl 3-(4-hydroxy-3,5-difluorophenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid. <sup>1</sup>H NMR (300MHz, CD<sub>3</sub>OD) δ 7.99 (d, *J* = 8.1 Hz, 2H), 7.82 (d, *J* = 8.1 Hz, 2H), 6.86 (d, *J* = 13.5Hz, 2H), 5.23 (s, 2H), 2.87 (t, *J* = 7.2 Hz, 2H), 2.60 (t, *J* = 7.5 Hz, 2H). Mass spectrum (ESI, m/z): Calcd. for C<sub>21</sub>H<sub>13</sub>F<sub>8</sub>NO<sub>3</sub>S, 511.05, found 509.9 (M-H).

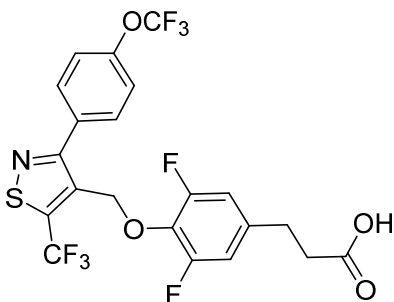
**3-(3,5-difluoro-4-[[3-(4-methoxyphenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy] phenyl)propanoic acid (4u)**



The title compound was prepared according to the procedure described in Example 4f starting from 4-OMe-benzamide using ethyl 3-(4-hydroxy-3,5-difluoro) phenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid. <sup>1</sup>H NMR (300MHz, CD<sub>3</sub>OD) δ 7.69 (d, *J* = 6.6 Hz, 2H), 6.99 (d, *J* = 9.0 Hz, 2H), 6.83 (d, *J* = 9.6 Hz, 2H), 5.15 (s, 2H), 3.84 (s, 3H), 2.83 (t, *J* = 7.5 Hz, 2H), 2.50 (t, *J* = 7.8 Hz, 2H). Mass spectrum (ESI, m/z): Calcd. for C<sub>21</sub>H<sub>16</sub>F<sub>5</sub>NO<sub>4</sub>S, 473.07, found 472.1 (M-H).

**3-[3, 5-difluoro-4-([3-[4-(trifluoromethoxy)phenyl]-5-(trifluoromethyl)-1,2-thiazol-4-**

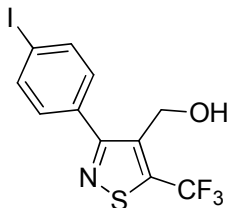
**yl]methoxy)phenyl]propanoic acid (4v)**



The title compound was prepared according to the procedure described in Example **4f** starting from 4-OCF<sub>3</sub>-benzamide using ethyl 3-(4-hydroxy-3,5-difluoro) phenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as a white solid. <sup>1</sup>HNMR (300MHz, CD<sub>3</sub>OD) δ 7.89 (d, *J* = 9.0 Hz, 2H), 7.42 (d, *J* = 7.8 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 5.21 (s, 1H), 2.87 (t, *J* = 7.8 Hz, 2H), 2.60 (t, *J* = 7.8 Hz, 2H). Mass spectrum (ESI, *m/z*): Calcd. for C<sub>21</sub>H<sub>13</sub>F<sub>8</sub>NO<sub>4</sub>S, 527.04, found 525.9 (M-H).

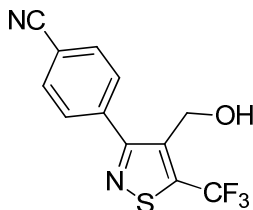
**3-(4-((3-(4-cyanophenyl)-5-(trifluoromethyl)isothiazol-4-yl)methoxy)-3,5-difluorophenyl)propanoic acid (4w)**

Step 1: 3-(4-iodophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methanol



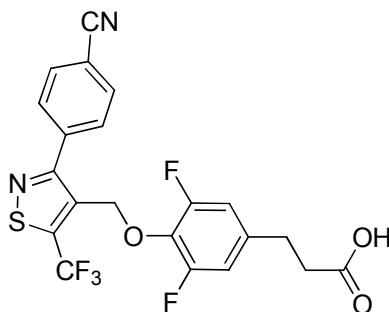
The title compound was prepared according to the procedure described in Example **4f** starting from 4-iodobenzamide to afford [3-(4-iodophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methanol as a white solid. Mass spectrum (ESI, *m/z*): Calcd. for C<sub>11</sub>H<sub>7</sub>F<sub>3</sub>INOS, 384.92, found 386.1 (M+H).

Step 2: 4-[4-(hydroxymethyl)-5-(trifluoromethyl)-1,2-thiazol-3-yl]benzonitrile



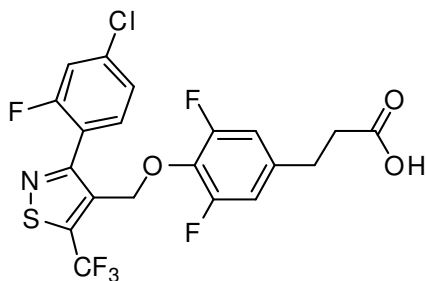
Into a 50-mL round-bottom flask was placed [3-(4-iodophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methanol (148 mg, 0.38 mmol, 1.00 equiv), N,N-dimethylformamide (5 mL), CuCN (52 mg). The resulting solution was stirred overnight at 130°C in an oil bath. The resulting solution was diluted with 15 mL of ethyl acetate. The resulting mixture was washed with 5x10 mL of saturated aqueous sodium chloride solution. The mixture was dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The residue was applied to a silica gel column with ethyl acetate/petroleum ether (17:83), to afford 68 mg (62%) of 4-[4-(hydroxymethyl)-5-(trifluoromethyl)-1,2-thiazol-3-yl]benzotrile as a white solid. Mass spectrum (ESI, m/z): Calcd. for C<sub>12</sub>H<sub>7</sub>F<sub>3</sub>N<sub>2</sub>OS, 284.02, found 285.1 (M+H).

Step 3: 3-(4-[[3-(4-cyanophenyl)-5-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-3,5-difluorophenyl) propanoic acid



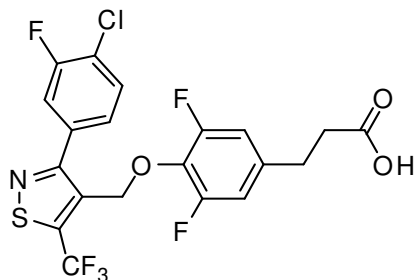
The title compound was prepared according to the procedure described in Example 4f by coupling 4-[4-(hydroxymethyl)-5-(trifluoromethyl)-1,2-thiazol-3-yl]benzotrile, with ethyl 3-(3,5-difluoro-4-hydroxyphenyl)propanoate followed by hydrolysis to afford the desired product as an off-white solid. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ: 7.98 (d, *J* = 8.4 Hz, 2H), 7.87 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 9.6 Hz, 2H), 5.23 (s, 2H), 2.87 (t, *J* = 7.8 Hz, 2H), 2.60 (t, *J* = 7.8 Hz, 2H). Mass spectrum (ESI, m/z): Calcd. for C<sub>21</sub>H<sub>13</sub>F<sub>5</sub>N<sub>2</sub>O<sub>3</sub>S, 468.06, found 466.9 (M-H).

**3-(4-((3-(4-chloro-2-fluorophenyl)-5-(trifluoromethyl)isothiazol-4-yl)methoxy)-3,5-difluorophenyl)propanoic acid (4x)**



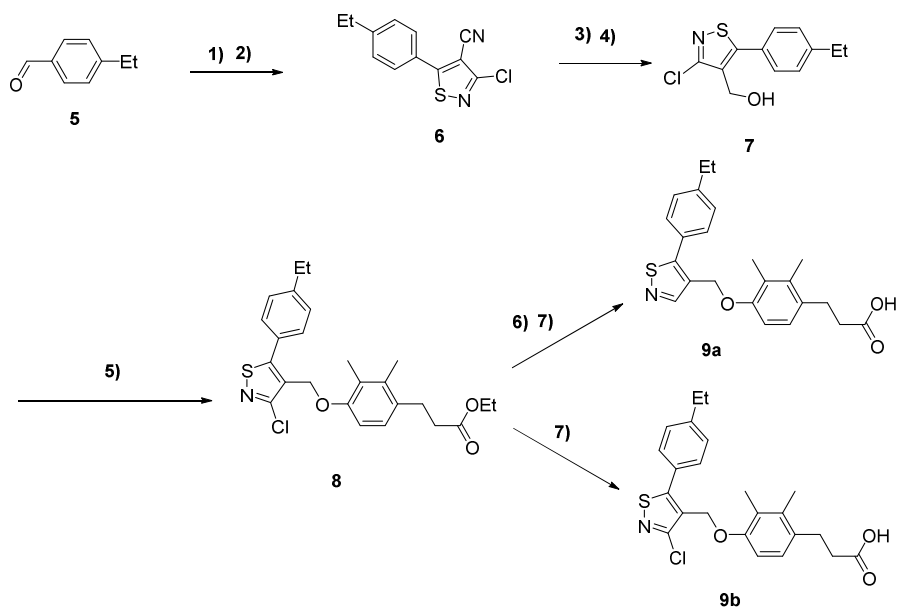
The title compound was prepared according to the procedure described in Example **4f** starting from 2-fluoro-4-chlorobenzamide using ethyl 3-(3,5-difluoro-4-hydroxyphenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.25 (s, 1H), 7.06 (d,  $J = 6.0$  Hz, 2H), 6.68 (d,  $J = 7.8$  Hz, 2H), 5.11 (s, 2H), 2.88 (t,  $J = 5.8$  Hz, 2H), 2.65 (t,  $J = 6.2$  Hz, 2H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{20}\text{H}_{12}\text{ClF}_6\text{NO}_3\text{S}$ , 495.01, found 496.0 (M+H).

**3-(4-((3-(4-chloro-3-fluorophenyl)-5-(trifluoromethyl)isothiazol-4-yl)methoxy)-3,5-difluorophenyl)propanoic acid (4y)**



The title compound was prepared according to the procedure described in Example **4f** starting from 3-fluoro-4-chlorobenzamide using ethyl 3-(3,5-difluoro-4-hydroxyphenyl)propanoate as coupling agent followed by hydrolysis to afford the desired product as an off-white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.76 (dd,  $J = 8.5, 5.5$  Hz, 1H), 7.70 (d,  $J = 7.2$  Hz, 1H), 7.55 (dd,  $J = 8.5, 7.2$  Hz, 1H), 6.79 (d,  $J = 8.8$  Hz, 2H), 5.11 (s, 2H), 2.98 (t,  $J = 8.0$  Hz, 2H), 2.70 (t,  $J = 8.0$  Hz, 2H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{20}\text{H}_{12}\text{ClF}_6\text{NO}_3\text{S}$ , 495.01, found 496.2 (M+H).

**Scheme 2.** Synthesis of isothiazoles **9a** and **9b**

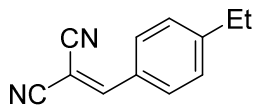


**Reagents and conditions:** 1) propanedinitrile, piperidine in *n*-butanol, r.t. 16 h, 56%; 2) S<sub>2</sub>Cl<sub>2</sub>, pyridine, 145°C for 5h, 57%; 3) H<sub>2</sub>SO<sub>4</sub>, 135°C for 3 h followed by NaNO<sub>2</sub> in water, 0-50°C for 0.5 h, 23%; 4) LAH in THF, 0°C to r.t for 12 h, 42%; 5) MsCl, TEA; ethyl 3-(4-hydroxy-2,3-dimethylphenyl)propanoate, K<sub>2</sub>CO<sub>3</sub> in DMF 25°C for overnight, 72%; 6) H<sub>2</sub> (1 atm), 5% Pd/C in EtOH, r.t. overnight, 36%; 7) Li-OH.H<sub>2</sub>O, THF/MeOH/water (1:1:1), r,t for 2 h, 90~95%

The target compounds **9a** and **9b** were synthesized in accordance with the synthetic method described in Scheme 2.

### 3-(4-[[5-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid (**9a**)

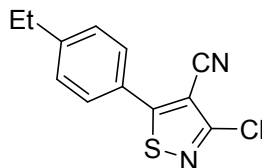
#### Step 1: 2-[(4-ethylphenyl)methylidene]propanedinitrile



Into a 500-mL round-bottom flask, was placed 4-ethylbenzaldehyde (10 g, 70.80 mmol, 1.00 equiv, 95%), propanedinitrile (6.4 g, 96.88 mmol, 1.30 equiv), *n*-butanol (200 mL). This was followed by the addition of piperidine (1 mL) dropwise with stirring. The resulting solution was stirred for 16 h at 20°C. The resulting mixture was concentrated under vacuum. The resulting solution was extracted with 3x25 mL of ethyl acetate and the organic layers combined. The resulting mixture was washed with 3x10 mL of sodium chloride (aq). The mixture was dried over anhydrous sodium sulfate. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:8). This resulted in 8.5 g (56%) of 2-[(4-

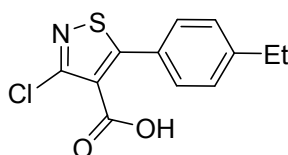
ethylphenyl)methylidene]propanedinitrile as a white crystal. Mass spectrum (ESI, m/z): Calcd. for  $C_{12}H_{10}N_2$ , 182.08, found 183.2 (M+H).

Step 2: 3-chloro-5-(4-ethylphenyl)-1, 2-thiazole-4-carbonitrile



Into a 100-mL round-bottom flask, was placed 2-[(4-ethylphenyl)methylidene]propanedinitrile (5.0 g, 24.70 mmol, 1.00 equiv, 90%),  $S_2Cl_2$  (7.4 g, 2.00 equiv), pyridine (217 mg, 2.74 mmol, 0.10 equiv). The resulting solution was stirred for 5 h at 140-150°C in an oil bath. The reaction mixture was cooled with a water/ice bath. The resulting solution was extracted with 3x20 mL of ethyl acetate and the organic layers combined. The resulting mixture was washed with 1x15 mL of sodium chloride (aq). The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 3.5 g (crude) of 3-chloro-5-(4-ethylphenyl)-1, 2-thiazole-4-carbonitrile as a brown solid. Mass spectrum (ESI, m/z): Calcd. for  $C_{12}H_9ClN_2S$ , 248.02, found 249.2 (M+H).

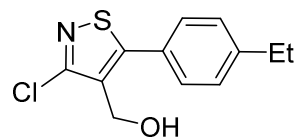
Step 3: 3-chloro-5-(4-ethylphenyl)-1, 2-thiazole-4-carboxylic acid



Into a 250-mL round-bottom flask, was placed 3-chloro-5-(4-ethylphenyl)-1,2-thiazole-4-carbonitrile (3.1 g, 11.22 mmol, 1.00 equiv, 90%) and sulfuric acid (15 mL), The resulting solution was stirred for 3 h at 130-140°C in an oil bath, and then cooled 0°C. A solution of  $NaNO_2$  (1.25 g, 18.12 mmol, 1.45 equiv) was added dropwise. Adjusted so that the temperature did not exceed 30°C, and then added into water (150 mL), and the mixture was heated at 50°C for 30 min and cooled. The resulting solution was extracted with 3x20 ml of ether and the organic layers combined. The ether extract was extracted with (3x10ml) of sodium hydroxide (200 mL, 10%), the extract was made acidic with concentrated HCl to pH=5-6, and was extracted with 3x15 ml of ether, the ether solution was dried by  $MgSO_4$  and concentrated. This

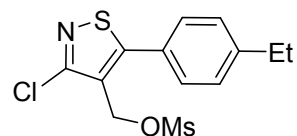
resulted in 800 mg (23%) of 3-chloro-5-(4-ethylphenyl)-1,2-thiazole-4-carboxylic acid as a brown solid. Mass spectrum (ESI, m/z): Calcd. for  $C_{12}H_{10}ClNO_2S$ , 267.01, found 268.2 (M+H).

Step 4: [3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methanol



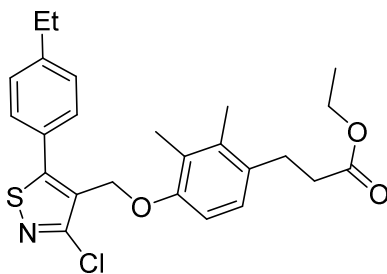
Into a 100-mL 3-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed LAH (57 mg, 1.50 mmol, 2.00 equiv), tetrahydrofuran (5 mL), 3-chloro-5-(4-ethylphenyl)-1,2-thiazole-4-carboxylic acid (200 mg, 0.67 mmol, 1.00 equiv, 90%). The resulting solution was stirred for 4 h at 60°C in an oil bath. The reaction mixture was cooled to 0 degree C. The reaction was then quenched by the addition of 5 mL of sodium hydroxide (3%) at 0°C for 1h. The solids were filtered out. The filter was concentrated and applied onto the TLC-Plate with ethyl acetate/petroleum ether (1:5). This resulted in 80 mg (42%) of [3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methanol as a white solid. Mass spectrum (ESI, m/z): Calcd. for  $C_{12}H_{12}ClNOS$ , 253.03, found 254.2 (M+H).

Step 5: [3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methyl methanesulfonate



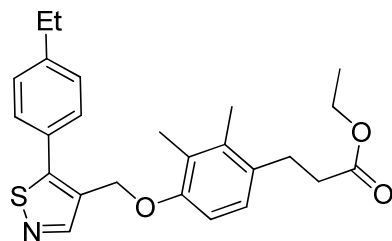
Into a 100-mL round-bottom flask, was placed [3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methanol (140 mg, 0.50 mmol, 1.00 equiv, 90%), dichloromethane (5 mL), triethylamine (168 mg, 1.66 mmol, 3.00 equiv), methanesulfonyl chloride (126 mg, 1.10 mmol, 2.00 equiv). The resulting solution was stirred for 30 min at 25°C. The reaction was then quenched by the addition of 10 mL of water. The resulting solution was extracted with 3x10 mL of ethyl acetate and the organic layers combined. The resulting mixture was washed with 1x10 mL of brine. The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 145 mg of [3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methyl methanesulfonate as light yellow oil. Mass spectrum (ESI, m/z): Calcd. for  $C_{13}H_{14}ClNO_3S_2$ , 331.01, found 332.2 (M+H).

Step 6: ethyl 3-(4-[[3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate



Into a 100-mL round-bottom flask, was placed [3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methyl methanesulfonate (100 mg, 0.27 mmol, 1.00 equiv, 90%), ethyl 3-(4-hydroxy-2,3-dimethylphenyl)propanoate (80 mg, 0.36 mmol, 1.20 equiv), potassium carbonate (125 mg, 0.90 mmol, 3.00 equiv), N,N-dimethylformamide (4 mL). The resulting solution was stirred overnight at 25°C. The resulting solution was diluted with 5 mL of H<sub>2</sub>O. The resulting solution was extracted with 3x10 mL of ethyl acetate and the organic layers combined. The resulting mixture was washed with 1x10 mL of brine. The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 101 mg (crude) of ethyl 3-(4-[[3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate as brown oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>25</sub>H<sub>28</sub>ClNO<sub>3</sub>S, 457.15, found 458.2 (M+H).

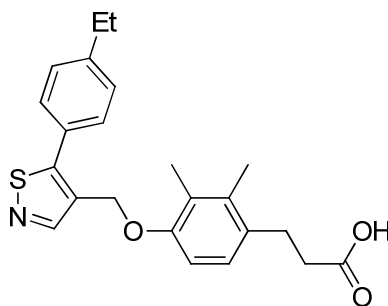
Step 7: Ethyl 3-(4-[[5-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate



Into a 100-mL round-bottom flask purged and maintained with an inert atmosphere of H<sub>2</sub>, was placed ethyl 3-(4-[[3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate (60 mg, 0.12 mmol, 1.00 equiv, 90%), Palladium carbon (60 mg), ethanol (2 mL). The resulting solution was stirred overnight at 25°C. The solids were filtered out. The filter was concentrated. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:5). This resulted in 20 mg (36%) of ethyl 3-(4-[[5-(4-ethylphenyl)-1,2-thiazol-4-

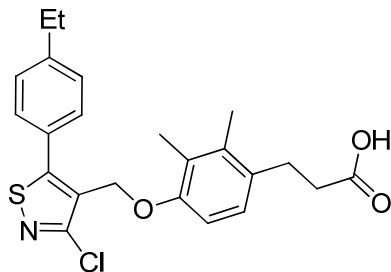
yl]methoxy]-2,3-dimethylphenyl)propanoate as colorless oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>25</sub>H<sub>29</sub>NO<sub>3</sub>S, 423.19, found 424.5 (M+H).

Step 8: 3-(4-[[5-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid



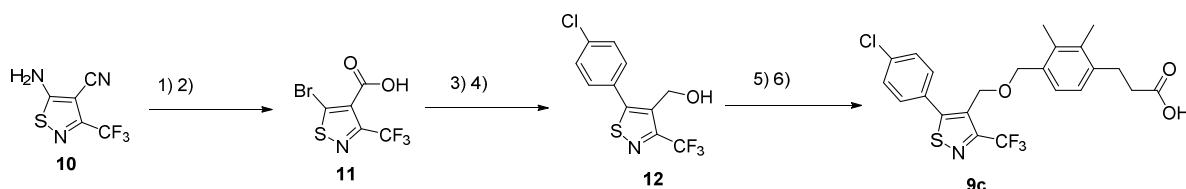
Into a 50-mL round-bottom flask, was placed ethyl 3-(4-[[5-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate (20 mg, 0.04 mmol, 1.00 equiv, 90%), LiOH (20 mg, 0.84 mmol, 21.00 equiv), tetrahydrofuran (1 mL), water (1 mL). The resulting solution was stirred overnight at 25°C. The resulting mixture was concentrated under vacuum. The resulting solution was diluted with 1 mL of H<sub>2</sub>O. The pH value of the solution was adjusted to 2-3 with hydrogen chloride (2 mol/L). The solids were collected by filtration. The crude product (2 mL) was purified by Prep-HPLC with the following conditions (1#-Waters 2767-5): Column, SunFire Prep C18, 5µm, 19\*100mm; mobile phase, Water of 0.05% TFA and CH<sub>3</sub>CN (40% CH<sub>3</sub>CN up to 90% in 5 min, up to 100% in 0.1 min, hold 100% in 1.9 min, down to 40% in 0.1 min, hold 40% in 1.9 min); Detector, UV 220&254nm. This resulted in 15 mg (95%) of 3-(4-[[5-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid as a white solid. <sup>1</sup>H NMR (300MHz, CD<sub>3</sub>OD) δ 8.57 (s, 1H), 7.48 (d, *J* = 8.1 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 6.95 (d, *J* = 8.1 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 5.09 (s, 2H), 2.90 (t, *J* = 6.9 Hz, 2H), 2.71 (dd, *J* = 7.8, 15.3 Hz, 2H), 2.48-2.50 (m, 2H), 2.23 (s, 3H), 2.10 (s, 3H), 1.27 (t, *J* = 7.5 Hz, 3H). Mass spectrum (ESI, m/z): Calcd. for C<sub>23</sub>H<sub>25</sub>NO<sub>3</sub>S, 395.16, found 396.2 (M+H).

**3-(4-[[3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoic acid (9b)**



The title compound was prepared according to the procedure described in Example **4f** by hydrolysis of ethyl 3-(4-[[3-chloro-5-(4-ethylphenyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate to afford the desired product as an off-white solid.  $^1\text{H NMR}$  (400MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.47 (d,  $J = 8.4$  Hz, 2H), 7.35 (d,  $J = 8.0$  Hz, 2H), 6.96(d,  $J = 8.4$  Hz, 1H), 6.74 (d,  $J = 8.4\text{Hz}$ , 1H), 4.98 (s, 2H), 2.92 (t,  $J = 7.2$  Hz, 2H), 2.72 (q,  $J = 7.6, 15.2$  Hz, 2H), 2.51 (t,  $J = 8.4$  Hz, 2H), 2.23 (s, 3H), 2.09 (s, 3H), 1.27 (t,  $J = 8.0$  Hz, 3H). Mass spectrum (ESI,  $m/z$ ): Calcd. for  $\text{C}_{23}\text{H}_{24}\text{ClNO}_3\text{S}$ , 429.12, found 430. 1 (M+H).

### Scheme 3. Synthesis of isothiazole **9c**

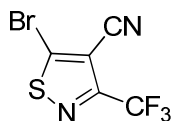


**Reagents and conditions:** 1) isopentyl nitrite,  $\text{Br}_2$  in 3-methyl-1-nitrobutane  $0^\circ\text{C}$  for 0.5 h, 85%; 2)  $\text{H}_2\text{SO}_4$ ,  $135^\circ\text{C}$  for 3 h followed by  $\text{NaNO}_2$  in water,  $0-50^\circ\text{C}$  for 0.5 h, 75%; 3)  $\text{BH}_3$  in THF,  $0^\circ\text{C}$  to r.t. for 12 h, 25%; 4) (4-chloro-phenyl)boronic acid,  $\text{Pd}(\text{PPh}_3)_4$  (cat),  $\text{K}_3\text{PO}_4$  in dioxane/water (1:1)  $90^\circ\text{C}$  for 3 h, 78%; 5) ethyl 3-(4-hydroxy-2,3-dimethylphenyl)propanoate,  $n\text{-Bu}_3\text{P}$ , ADDP in toluene  $80^\circ\text{C}$  for 4 h, 70%; 6)  $\text{LiOH}\cdot\text{H}_2\text{O}$ , THF/MeOH/water (1:1:1), r.t. for 2 h, 88%

The target compounds **9c** was synthesized in accordance with the synthetic method described in Scheme 3.

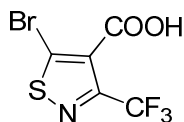
### 3-(4-[[5-(4-chlorophenyl)-3-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl) propanoic acid (**9c**)

#### Step 1: 5-bromo-3-(trifluoromethyl)-1,2-thiazole-4-carbonitrile



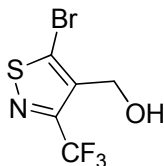
Into a 100-mL round-bottom flask, was placed 3-methyl-1-nitrobutane (14.4 g, 122.92 mmol, 6.00 equiv), isopentyl nitrite (30 mL), Br<sub>2</sub> (32.4 g, 202.74 mmol, 10.00 equiv). This was followed by the addition of 5-amino-3-(trifluoromethyl)-1,2-thiazole-4-carbonitrile (4.0 g, 20.71 mmol, 1.00 equiv), in portions at 0°C. The resulting solution was stirred for 30 min at 0°C in a water/ice bath. The reaction progress was monitored by TLC (ethyl acetate/petroleum ether = 1:4). The resulting solution was diluted with 30 mL of DCM. The resulting mixture was washed with 2x50 mL of Na<sub>2</sub>SO<sub>3</sub>/H<sub>2</sub>O. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:10). This resulted in 4.5 g (85%) of 5-bromo-3-(trifluoromethyl)-1,2-thiazole-4-carbonitrile as yellow crude oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>5</sub>BrF<sub>3</sub>N<sub>2</sub>S, 255.89, found 256.9 (M+H).

Step 2: 5-bromo-3-(trifluoromethyl)-1,2-thiazole-4-carboxylic acid



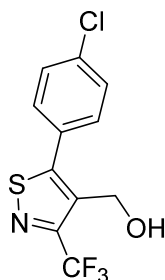
Into a 100-mL round-bottom flask, was placed 5-bromo-3-(trifluoromethyl)-1,2-thiazole-4-carbonitrile (4.2 g, 16.34 mmol, 1.00 equiv), con.H<sub>2</sub>SO<sub>4</sub> (10 mL). This was followed by the addition of a solution of NaNO<sub>2</sub> (1.69 g, 24.49 mmol, 1.50 equiv) in water (5 mL) dropwise with stirring at 0°C. To this was added water (25 mL) dropwise with stirring at 0°C. The resulting solution was stirred for 2 days at 20°C. The resulting solution was allowed to react, with stirring, for an additional 5 h while the temperature was maintained at 50°C in an oil bath. The reaction progress was monitored by LCMS. The resulting solution was extracted with 3x50 mL of ethyl acetate and the organic layers combined and dried over anhydrous sodium sulfate. The solids were filtered out. The resulting mixture was concentrated under vacuum. This resulted in 3.4 g of 5-bromo-3-(trifluoromethyl)-1,2-thiazole-4-carboxylic acid as yellow crude oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>5</sub>HBrF<sub>3</sub>NO<sub>2</sub>S, 274.89, found 273.9 (M-H).

Step 3: [5-bromo-3-(trifluoromethyl)-1, 2-thiazol-4-yl]methanol



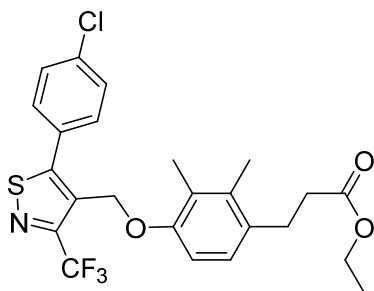
Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed 5-bromo-3-(trifluoromethyl)-1,2-thiazole-4-carboxylic acid (800 mg, 2.90 mmol, 1.00 equiv), tetrahydrofuran (3 mL). This was followed by the addition of  $\text{BH}_3$  (14.5 mL, 5.00 equiv) dropwise with stirring at  $0^\circ\text{C}$ . The resulting solution was stirred overnight at  $25^\circ\text{C}$ . The reaction progress was monitored by TLC (ethyl acetate/petroleum ether = 1:5). The reaction was then quenched by the addition of 20 mL of methanol. The reaction mixture was heated to reflux for 1 h. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:5). This resulted in 200 mg (25%) of [5-bromo-3-(trifluoromethyl)-1,2-thiazol-4-yl]methanol as yellow oil. Mass spectrum (ESI, m/z): Calcd. for  $\text{C}_5\text{H}_3\text{BrF}_3\text{NOS}$ , 260.91, found 261.9 (M+H).

Step 4:[5-(4-cholorophenyl)-3-(trifluoromethyl)-1,2-thiazol-4-yl]methanol



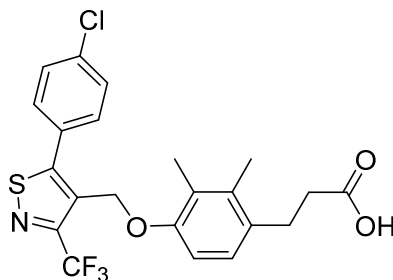
Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed [5-bromo-3-(trifluoromethyl)-1,2-thiazol-4-yl]methanol (100 mg, 0.38 mmol, 1.00 equiv), dioxane (3 mL), (4-chlorophenyl)boronic acid (113 mg, 0.75 mmol, 1.97 equiv),  $\text{Pd}(\text{PPh}_3)_4$  (44 mg, 0.04 mmol, 0.10 equiv),  $\text{K}_3\text{PO}_4$  (402 mg, 1.89 mmol, 4.96 equiv). The resulting solution was stirred for 3 h at  $90^\circ\text{C}$  in an oil bath. The solvent was removed and the residue was applied onto a TLC plate with ethyl acetate/petroleum ether (1:3). This resulted in 84 mg (crude) of [5-(4-chlorophenyl)-3-(trifluoromethyl)-1,2-thiazol-4-yl]methanol as yellow oil. Mass spectrum (ESI, m/z): Calcd. for  $\text{C}_{11}\text{H}_7\text{ClF}_3\text{NOS}$ , 292.99, found 294.1 (M+H).

Step 5: Ethyl 3-(4-[[5-(4-cholorophenyl)-3-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl)propanoate



Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed [5-(4-chlorophenyl)-3-(trifluoromethyl)-1,2-thiazol-4-yl]methanol (100 mg, 0.35 mmol, 1.00 equiv), tol (3 mL), ethyl 3-(4-hydroxy-2,3-dimethylphenyl)propanoate (154 mg, 0.69 mmol, 1.99 equiv), ADDP (174 mg, 0.70 mmol, 2.00 equiv), n-Bu<sub>3</sub>P (140 mg, 0.69 mmol, 1.99 equiv). The resulting solution was stirred overnight at 60°C. The resulting mixture was concentrated under vacuum. The residue was applied onto a TLC plate with ethyl acetate/petroleum ether (1/5). This resulted in 123 mg (crude) of ethyl 3-(4-[[5-(4-chlorophenyl)-3-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl) propanoate as colorless oil. Mass spectrum (ESI, m/z): Calcd. for C<sub>24</sub>H<sub>23</sub>ClF<sub>3</sub>NO<sub>3</sub>S, 497.10, found 498.5 (M+H).

Step 6: 3-(4-[[5-(4-chlorophenyl)-3-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl) propanoic acid



The title compound was prepared according to the procedure described in Example **4f** by hydrolysis of ethyl 3-(4-[[5-(2,4-dimethylphenyl)-3-(trifluoromethyl)-1,2-thiazol-4-yl]methoxy]-2,3-dimethylphenyl) propanoate to afford the desired product as an off-white solid. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ: 7.60 (d, *J* = 7.0 Hz, 2H), 7.38 (d, *J* = 7.5 Hz, 2H), 6.92 (d, *J* = 6.5 Hz, 1H), 6.60 (d, *J* = 6.5 Hz, 1H), 4.85 (s, 2H), 2.90 (t, *J* = 7.2 Hz, 2H), 2.51 (t, *J* = 7.2 Hz, 2H), 2.37 (s, 3H), 2.15 (s, 3H). Mass spectrum (ESI, m/z): Calcd. for C<sub>22</sub>H<sub>19</sub>ClF<sub>3</sub>NO<sub>3</sub>S, 469.07, found 468.1 (M-H).

## 2. **In vitro biological assays**

### 1. Human GPR120 in Calcium Flux Assay

#### a) Assay Principle:

This in vitro assay serves to assess test compound agonist activity against the short splice variant (SVS with Accession number NM\_001195755.1 confirmed by sequencing data) of the GPR120 receptor. The Human Short splice variant #2 (NM\_001195755.1) is missing an in-frame coding exon compared to variant 1 (the Human Long splice variant NM\_181745.3), resulting in a shorter isoform (GPR120-S) lacking a 16 aa protein segment compared to isoform GPR120-L. The assay platform utilizes HEK-293 cells stably transfected to express the Human GPR120 short form. These cells are first loaded with the Ca<sup>2+</sup> sensitive dye, Fluo-4 NW. Upon stimulation, intracellular released Ca<sup>2+</sup> can bind to the dye and alter its fluorescence intensity. This increase in fluorescence signal, and thus the flux in intracellular [Ca<sup>2+</sup>], is detected and quantitated by fluorescence imaging using a FLIPR reader. The effect of the agonist is measured as a function of concentration and used to calculate an EC<sub>50</sub> based upon a response curve.

#### b) Assay Procedure:

A Human GPR120 clone (Genbank accession number NM\_001195755.1) was placed into the pcDNA3.1 mammalian expression vector carrying the neomycin resistance gene. A stable mammalian cell was generated by placing the above clone into a HEK293 background. Clonal cells responding to long chain fatty acids had expression levels of GPR120 confirmed by RT-qPCR. In this procedure 2500 cells / well were employed. Human HEK-GPR120 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)/F12 medium supplemented with 10% fetal bovine serum (FBS), 1% L-Glutamine and 1% penicillin/streptomycin and 0.5 mg/ml G-418. Cells were split 2 times a week to keep the cells in the log-phase growth.

In preparation for the assay, HEK cells stably transfected with Human GPR120 (2.5K cells per well in 25uL growth medium) were seeded into 384-well plates and then incubated overnight (37°C, 5% CO<sub>2</sub>). The next day, the media was changed to 20 µL assay buffer and the cell starved for 1h at 37°C. The dye loading solution (2X dye) was prepared using 10 mL assay buffer, 100 µL of 250 mM probenecid, 1 bottle of

Component A, and 20 µl of dye in DMSO. Twenty µL of the 2X dye loading buffer was then added to each well. The plates were incubated at 37°C for 30 min, then at room temperature for an additional 15 minutes, before performing the assay on FLIPR.

Test compounds were prepared in assay buffer (2 uL of cpd + 198 uL assay buffer, final DMSO in assay plate is 0.2%) at the desired concentration, more particularly at 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.391, 0.195, 0.098, 0.049, 0.024 and 0.012 µM.

The assay was performed on a FLIPR plate reader using the following parameters. Baseline was read for 10 seconds at 1 sec intervals. The program was set to transfer 10 µL of ligand from compound plate to cell plate after baseline reading. Aspiration was executed at: 10 µL/sec speed, 4.6 µL height; Dispensing was executed at: 30 µL/sec speed, 45 µL height. After compound addition, each well was read for 300 sec, with measurements collected at 1 sec intervals.

The kinetic data from the FLIPR was based upon a 5 minute window for data collection. The fluorescence of each sample well was used for individual calculations of a normalized RFU value, which was defined as maximum response minus the minimum response. The normalized fluorescence reading (RFU) was calculated as follows:

$$\text{RFU} = F_{\text{max}} - F_{\text{min}}$$

The data were fitted to a sigmoidal dose-response curve with a variable Hill slope (< 2) using nonlinear regression with GraphPad Prism 5.0 to calculate the EC<sub>50</sub> values. The Fitting Equation was:  $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope})})$ , where X is the log of the concentration and Y is the response.

## 2. Human GPR120 DiscoverX PathHunter Beta-Arrestin assay

### a) Assay Principle:

The binding of an agonist (medium/long chain fatty acids or small molecule agonists) to the G-protein-coupled receptor GPR120 activates phospholipase C, leading to release of intracellular Ca<sup>+2</sup> through the generation of inositol 1,4,5-trisphosphate (InsP3 or IP3). GPR120 activation can also trigger intracellular signaling via recruitment of Beta-Arrestin. In the present method, agonist-induced activation of the human GPR120 receptor is monitored through the use of PathHunter CHO-K1 GPR120 Beta-Arrestin Cell Line engineered by DiscoverX, as detailed below. The cell lines are designed to co-

express both the ProLink/Enzyme Donor (PK)-tagged GPCR and the Enzyme Activator (EA)-tagged Beta-Arrestin fusion proteins. Upon GPR120 receptor stimulation / activation, the EA-tagged Beta-Arrestin portion is translocated to the tagged receptor, where the two enzyme fragments are brought within close proximity. Under these conditions, these fragments can interact and form an active Beta-gal enzyme complex through Enzyme Fragment Complementation (EFC). This active Beta-gal complex can enzymatically hydrolyse the substrate to produce a detectable light signal; therefore, activation as a function of agonist concentration can be expressed as an EC<sub>50</sub> value to determine relative compound activities. This in vitro assay therefore serves to assess compound agonist activity of the GPR120.

b) Assay Procedure:

In Procedure  $\beta$ -arrestin, the cell used were PathHunter CHO-K1 GPR120  $\beta$ -Arrestin Cell Line, expressing the long form of human GPR120 (Genbank accession number NM\_181745), with 3000 cells per well. The selected CHO-K1 GPR120  $\beta$ -Arrestin cells were cultured in Ham's F12 media supplemented with 10% fetal bovine serum (FBS), 1% Glutamine, 1x p/s, 800  $\mu$ g/mL G418 and 300  $\mu$ g/mL Hygromycin B (for selection). Cell stocks were maintained and grown in a sub-confluent state using standard cell culture procedures. The day before the experiment, the cells were harvested with non-enzymatic cell dissociation buffer and re-suspended in complete growth media at the desired concentration. A Corning 384-plate was then seeded with the proper number of cells in a volume of 25  $\mu$ L, per well. The seeded plates were incubated overnight at 37°C.

On the day of the experiment, the Assay Buffer containing (a) HBSS with Ca<sup>++</sup> and Mg<sup>++</sup>, (b) 20 mM HEPES, and (c) 0.1% BSA stabilizer (pH 7.4) was prepared. The growth medium was gently removed from the cell plates and 20  $\mu$ L of Assay Buffer added to each well. The plate was then incubated at 37°C for 60 min. Test compounds were serially diluted in Assay Buffer to desired concentrations (more particularly to one or more of the following  $\mu$ M concentrations: 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625, 0.1953125, 0.09765625, 0.048828125, 0.024414063, 0.012207031. Five  $\mu$ L of compound dilution was then added to each well and the plate incubated at 37°C for 90 min. The detection reagents were prepared according to the manufacture's instruction.

Twelve  $\mu\text{L}$  of the detection reagents were added to each well and the plate incubated at room temperature for 60 min.

The plates were read on an EnVision instrument, using Protocol name: Luminescence, Plate type: 384 Costar, Measurement height: 3 mm, Measurement time: 1 s, Aperture: 384 Plate aperture. The % activity relative to the positive control was calculated using the following equation:

The % Activity values were plotted versus the concentration of test compound and fitted to a sigmoidal dose-response curve with a Hill slope = 1 (fixed value) using nonlinear regression with GraphPad Prism 5.0 to calculate the  $\text{EC}_{50}$  values. The Fitting Equation was:  $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$ , where X is the log of the concentration and Y is the response.

### 3. Early ADME, PK and PD Profiles of 4x and 4y

Cpd	Kinetic solubility		Microsomal Stability			MDCK		PPB			P450 inhibition
	PBS	pH=2	human	rat	mouse	A to B, $P_{app}$	B to A/ A to B	human	rat	mouse	
	$\mu\text{M}$	$\mu\text{M}$	% remaining at 10 min			$\times 10^6 \text{ cm/s}$		%	%	%	
<b>4x</b>	38.9	7.21	95.5	86.7	92.2	17.5	1.05	99.87	99.81	99.68	all > 10 $\mu\text{M}$
<b>4y</b>	41.1	1.66	93.1	91.1	104.6	21.2	1.85	99.97	99.94	99.85	all > 10 $\mu\text{M}$

PK in DIO C57/Bl Mice				DIO Dose Response OGTT (glucose lowering)				
PO 20 mg/kg in 0.5% Methocel				PO in 0.5% methocel				
Cpd	$C_{max}$	$\text{AUC}_{last}$	$t_{1/2}$	10 mg/kg	3 mg/kg	1 mg/kg	0.3 mg/kg	0.1 mg/kg
	ng/mL	h*ng/mL	hour	Delta AUC	Delta AUC	Delta AUC	Delta AUC	Delta AUC
<b>4x</b>	9268	46822	5.23	not tested	-83%	-61%	-34%	-3%
<b>4y</b>	6590	38765	4.28	-74%	-40%	-33%	2%	not tested

### 4. Cerep panel screening of 4x

Assays	Concentration (M)	% of inhibition	Reference compounds		
			Reference Compound	IC50 (nM)	(nH)
A1 (h) (antagonist radioligand)	1.00E-05	-	DPCPX	1.6	(1.2)
A2A (h) (agonist radioligand)	1.00E-05	-	NECA	16	(0.7)
A3 (h) (agonist radioligand)	1.00E-05	32	IB-MECA	0.22	(0.5)
alpha 1 (non-selective) (antagonist radioligand)	1.00E-05	-	prazosin	0.26	(1.4)
alpha 2 (non-selective) (antagonist radioligand)	1.00E-05	-	yohimbine	92	(0.9)
beta 1 (h) (agonist radioligand)	1.00E-05	-	atenolol	320	(0.9)
AT1 (h) (antagonist radioligand)	1.00E-05	-	saralasin	0.50	(0.6)
BZD (central) (agonist radioligand)	1.00E-05	-	diazepam	14	(0.8)
B2 (h) (agonist radioligand)	1.00E-05	-	NPC 567	35	(1.1)

CCK1 (CCKA) (h) (agonist radioligand)	1.00E-05	-	CCK-8s	0.17	(1.0)
D1 (h) (antagonist radioligand)	1.00E-05	-	SCH 23390	0.30	(0.9)
D2S (h) (antagonist radioligand)	1.00E-05	14	(+)butaclamol	2.1	(1.2)
ETA (h) (agonist radioligand)	1.00E-05	-	endothelin-1	0.054	(1.2)
GABA (non-selective) (agonist radioligand)	1.00E-05	-	GABA	92	(0.9)
GAL2 (h) (agonist radioligand)	1.00E-05	-	galanin	0.39	(1.0)
CXCR2 (IL-8B) (h) (agonist radioligand)	1.00E-05	22	IL-8	0.32	(1.9)
CCR1 (h) (agonist radioligand)	1.00E-05	-	MIP-1a	0.082	(2.0)
H1 (h) (antagonist radioligand)	1.00E-05	-	pyrilamine	1.4	(1.1)
H2 (h) (antagonist radioligand)	1.00E-05	-	cimetidine	328	(1.0)
MC4 (h) (agonist radioligand)	1.00E-05	10	NDP-a-MSH	0.20	(1.0)
MT1 (ML1A) (h) (agonist radioligand)	1.00E-05	-	melatonin	0.37	(0.6)
M1 (h) (antagonist radioligand)	1.00E-05	-	pirenzepine	19	(1.2)
M2 (h) (antagonist radioligand)	1.00E-05	-	methoctramine	35	(1.0)
M3 (h) (antagonist radioligand)	1.00E-05	12	4-DAMP	0.76	(1.2)
NK2 (h) (agonist radioligand)	1.00E-05	18	[Nleu10]-NKA (4-10)	5.4	(0.8)
NK3 (h) (antagonist radioligand)	1.00E-05	11	SB 222200	5.7	(0.9)
Y1 (h) (agonist radioligand)	1.00E-05	-	NPY	0.11	(1.5)
Y2 (h) (agonist radioligand)	1.00E-05	-	NPY	0.072	(0.9)
NTS1 (NT1) (h) (agonist radioligand)	1.00E-05	-	neurotensin	0.41	(0.9)
delta 2 (DOP) (h) (agonist radioligand)	1.00E-05	14	DPDPE	4.6	(0.9)
kappa (KOP) (agonist radioligand)	1.00E-05	60	U 50488	0.82	(1.0)
mu (MOP) (h) (agonist radioligand)	1.00E-05	-	DAMGO	0.55	(0.7)
NOP (ORL1) (h) (agonist radioligand)	1.00E-05	-	nociceptin	1.5	(0.9)
5-HT1A (h) (agonist radioligand)	1.00E-05	15	8-OH-DPAT	0.76	(1.3)
5-HT1B (antagonist radioligand)	1.00E-05	-	serotonin	15	(0.9)
5-HT2A (h) (antagonist radioligand)	1.00E-05	-	ketanserin	0.81	(1.0)
5-HT2B (h) (agonist radioligand)	1.00E-05	26	(±)DOI	8.4	(0.8)
5-HT3 (h) (antagonist radioligand)	1.00E-05	-	MDL 72222	5.1	(1.0)
5-HT5a (h) (agonist radioligand)	1.00E-05	48	serotonin	165	(1.0)
5-HT6 (h) (agonist radioligand)	1.00E-05	-	serotonin	241	(0.8)
5-HT7 (h) (agonist radioligand)	1.00E-05	-	serotonin	0.57	(1.4)
sst (non-selective) (agonist radioligand)	1.00E-05	-	somatostatin-14	0.32	(0.9)
VPAC1 (VIP1) (h) (agonist radioligand)	1.00E-05	-	VIP	0.27	(1.0)
V1a (h) (agonist radioligand)	1.00E-05	-	[d(CH2)51,Tyr(Me)2]-AVP	0.80	(1.5)
Ca2+ channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)	1.00E-05	-	D 600	52	(0.5)
KV channel (antagonist radioligand)	1.00E-05	-	a-dendrotoxin	0.24	(0.9)
SKCa channel (antagonist radioligand)	1.00E-05	-	apamin	0.021	(1.5)
Na+ channel (site 2) (antagonist radioligand)	1.00E-05	-	veratridine	7900	(0.8)
Cl- channel (GABA-gated) (antagonist radioligand)	1.00E-05	40	picrotoxinin	151	(1.0)
norepinephrine transporter (h) (antagonist radioligand)	1.00E-05	45	protriptyline	3.5	(1.2)
dopamine transporter (h) (antagonist radioligand)	1.00E-05	28	BTCP	8.6	(1.8)
5-HT transporter (h) (antagonist radioligand)	1.00E-05	-	imipramine	1.8	(0.9)

For the test compounds, the results are expressed as a percent inhibition of control specific binding (mean values; n=2 )

The symbol + indicates an increase in the binding higher than 50%

The symbol - indicates an inhibition of less than 10%

## 5. In vivo assays

### a) GPR120 DIO Mice OGTT Screening

18-22 week old, C57Bl6 mice on a high fat diet (60% HFD) for 12-16 weeks (ave. body weight ~ 37-41g) were fasted for 6 hr, with removal of food occurring at 7 am on the morning of the study. The animals were sorted into treatment groups the day before the study by body weight. Animals outside the bounds of ~30-50g were left out of the study. The animals had been handled and shammed a total of 5-8 days (1-3 days immediately prior to the study). Glucose (in 1mL syringes) was drawn up the morning of the study. Test compounds were kept spinning and were only drawn into 1ml syringes prior to study commencement. Animals were bled via tail snip to determine basal glucose levels prior to dosing of treatments. An Ascensia BREEZE Blood Glucose Monitoring System by Bayer was used for determining glucose levels.

Animals were moved into the testing room at ~9-11 am, to yield them time to acclimate. The bleeds and dosing started at approximately 1 pm in 30-second intervals per animal. All groups were dosed 30 minutes prior to glucose administration at a dose volume of 10 ml/kg (the dose volume was calculated separately for each individual animal). Test compounds were administered at one or more of the following dosages in 0.5% methocel: 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg and 10 mg/kg.

Thirty minutes after the first dose (with test compound) animals were bled again for a second baseline, or T = 0, and immediately dosed with glucose (20% solution; TEKNOVA, 250 ml sterile bottle w/ catalogue number G0525) via a PO injection. The exact dose volume for glucose was also calculated separately for each individual animal. Blood glucose was measured at 15, 30, 45, 60, and 90 minutes post-glucose administration via the snipped tail. If an animal reached a value of “HI”, the upper limit of the glucometer (600 mg/dl) was substituted as the blood glucose value and the study was analyzed as normal with no exclusions. If 50% or more of any treatment group reaches a “HI” value at least once, the study was considered invalid and repeated. Glucose values were typed into an EXCEL spreadsheet where they were used to calculate glucose AUC and delta AUC post-compound and post-glucose. The glucose excursion curves and the different versions of the AUC’s were graphed in GraphPad Prism 5.

#### Statistical Methods:

Note: All statistics completed in this study were completed using the statistical software package GraphPad Prism 5. Standard procedures for analyzing data sets from screening GPR120 compounds in DIO mouse OGTT's were as listed here below. In addition to the statistics that were run using GraphPad Prism 5, Microsoft Excel was used to calculate the percent changes in AUC from vehicle groups as detailed below.

Change from -30 to 0 BSLN Glucose, Raw Glucose AUC -30 to 90 min, Delta Glucose AUC -30 to 90 min, Raw Glucose AUC 0 to 90 min, Delta Glucose AUC 0 to 90 min were analyzed using Column Statistics Analysis, with mean values used to calculate % change from the vehicle mean group, as well as mean, SEM and / or % change from vehicle, where appropriate; and using One-Way ANOVA w/ a Tukey Post-Test (Comparing All Pairs of Columns) with each treatment group examined to see if it was statistically significant compared to vehicle (\*\*\*= P<0.001).

#### b) PK studies:

Male SPF Sprague–Dawley rats, each weighing ~250 g, were used. The tested compound was dissolved in 0.5% methocel for total dissolution. Before dosing, the formulations were stored at room temperature, protected from light, and analyzed quantitatively; the stability of the formulations was checked at the day of dosing. Oral dose (10 mg/kg) administration was by gastric intubation, and blood samples were collected at 0.5, 1, 2, 4, 8, and 24 h post administration. Intravenous dose administration (2.0 mg/kg) was in the jugular vein, and blood samples were collected at 7 and 20 min, and 1, 2, 4, 8, and 24 h post administration. From four different animals the complete plasma profiles were taken. A limited PK analysis was performed using WinNonlin Professional (Version 3.3, Pharsight, Mountain View, CA, USA). Analysis was carried out on the plasma concentration time profiles obtained from each animal (n = 4). Oral PK parameters observed were maximum plasma concentration ( $C_{max}$ ), time to reach the maximum plasma concentration ( $T_{max}$ ), plasma half-life ( $t_{1/2}$ ), and exposure of the compound calculated by the area under the curve ( $AUC_{last}$  and  $AUC_{inf}$ ). PK parameters after i.v. administration were volume of distribution ( $V_d$ ), total plasma clearance (Cl) and area under the curve ( $AUC_{last}$  and  $AUC_{inf}$ ).

C57 bl/6J Mice are normally fasted overnight, then dosed intravenously (IV) at levels of 5 mg/kg and by oral gavage (PO) at a levels of 20 mg/kg with drug candidate. Food is returned at 4 hr post dose. Blood samples (0.25–2.0 ml depending on the species) are collected post dose into tubes containing an appropriate anticoagulant. Blood samples are centrifuged for cell removal, and 100 mL plasma is transferred to a clean vial, placed on dry ice, and subsequently stored in a -70°C freezer prior to analysis. Plasma samples are normally prepared as follows. Two volumes of acetonitrile containing internal standard is added to one volume of plasma to precipitate proteins. Samples are centrifuged (3000 g for 5 min) and supernatant removed for analysis by LC-MS-MS. Calibration standards are prepared by adding appropriate volumes of stock solution directly into plasma and treated identically to collected plasma samples. Calibration standards are typically prepared in the range of 2 ng/ml to 10 µg/mL for quantitation. QC samples are prepared in parallel at high, medium and low concentrations in an identical manner and they are used to ensure the quality of the assay results. No more than 2 of the 6 QC standards are allowed to differ by more than 20% of their nominal value. LC-MS-MS analysis is performed utilizing multiple reaction monitoring for detection of characteristic ions for each drug candidate, additional related analytes and internal standard. Detection below 2 ng/mL may require alternate sample preparation procedures and will increase analysis turnaround times. Plasma concentrations are measured as described above to determine a concentration vs time profile. The area under the plasma concentration vs time curve (AUC) is calculated using the linear trapezoidal method. Fitting of the data to obtain pharmacokinetic parameters is generally carried out using non-compartmental analysis. Subsequent fitting with compartmental models can be carried out to aid in simulation of different dosing regimens. Compartmental analysis requires a data set with well defined phases to be useful. Parameters are expressed for individual animals as well as mean  $\pm$  standard deviations. The CV% is a measure of the variability of the individual parameters. The exception is the mouse where individual time points are obtained from individual animals so only a mean curve is obtained.