Supporting Information

Discovery of TUG-770: A Highly Potent Free Fatty Acid Receptor 1 (FFA1/GPR40) Agonist for Treatment of Type 2 Diabetes

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Synthetic Procedures and Compound Characterization

General

All commercial starting materials and solvents were used without further purification, unless otherwise stated. THF was freshly distilled from sodium/benzophenone. MeOH was freshly distilled from Mg. CH_2Cl_2 was distilled and stored over 4Å sieves. Pyridine was dried over 4Å sieves. DMF was purchased anhydrous from Sigma-Aldrich. Water used in the reactions was filtered (Milli-Q) and demineralized, but not degassed. Purification by flash chromatography was carried out using silica gel 60 (0.040-0.063 mm, Merck). ^{1}H and ^{13}C NMR spectra were calibrated relative to TMS internal standard or residual solvent peak. Purity was determined by HPLC and confirmed by inspection of NMR spectra. HPLC analysis was performed using a Dionex 120 C18 column (5µ, 4.6x150 mm); flow: 1 mL/min; 10% acetonitrile in water (0-1 min), 10-100% acetonitrile in water (1-10 min), 100% acetonitrile (11-15 min), with both solvents containing 0.05% TFA or 0.1% HCOOH as modifier; UV detection at 254 nm. High-resolution mass spectra (HRMS) were obtained on a Thermo Finnigan TSQ 700 using electrospray ionization (ESI) or a Bruker micrOTOF-Q II (ESI). All test compounds were of ≥95% purity unless otherwise stated.

General procedure I: Sonogashira coupling[1]

A Schlenk flask charged with Na_2PdCl_4 (1 mol%), 2-(di-*tert*-butylphosphino)-*N*-phenylindole (PIntB, 2 mol%), CuI (2 mol%), alkyne (if solid, 1 equiv), aryl halide (1.1 equiv), H_2O (0.2 mL/mmol) and TMEDA (1.8 mL/mmol) was evacuated and backfilled with argon three times. If liquid, the alkyne (1.1-2 equiv) was added at this point (procedure IB). The reaction mixture was heated to 80 ^{o}C. After consumption of the starting material monitored by TLC, the reaction mixture was cooled to room temperature, added water and extracted with EtOAc (x3). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated, and the residue was purified by flash chromatography.

General procedure II: ester hydrolysis

$LiOH{\cdot}H_2O$ (3 equiv) dissolved in H_2O (~2 mL/mmol ester) was added to a solution of the ester (1 equiv) in THF (~4 mL/mmol ester). The reaction was stirred at room temperature until complete consumption of the starting material as indicated by TLC, typically after 1-12 hours. The organic phases were combined, washed with brine and dried over $MgSO_4$ before concentration to give the corresponding carboxylic acid.

General procedure III: Wittig reaction

A round bottomed flask was added aryl aldehyde (1 equiv), ethyl bromoacetate (1.5 equiv), saturated aqueous $NaHCO_3$ (2 mL/mmol) and PPh_3 (1.4 equiv). Some reactions (procedure IIIB) were added EtOAc (1 mL/mmol). The reaction mixture was stirred vigorously at room temperature. After consumption of the starting materials the reaction was added water and extracted with EtOAc (x3). The organic phases were combined, washed with brine, dried over $MgSO_4$, concentrated under vacuum and purified by flash chromatography.

General procedure IV: reduction[2]

$CoCl_2{\cdot}6H_2O$ (0.1 equiv) and aryl acrylate (1 equiv) was dissolved in MeOH (2 mL/mmol) under argon atmosphere. $NaBH_4$ (1.25-2 equiv) was added in portions of 25-50 mg when the color faded from black-brown to pink. After consumption of the starting materials the reaction was added water and extracted with EtOAc (x3).

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[1] Christiansen, E.; Due-Hansen, M. E.; Ulven, T., A rapid and efficient Sonogashira protocol and improved synthesis of free fatty acid 1 (FFA1) receptor agonists. *J. Org. Chem.* **2010,** *75*, 1301-1304.

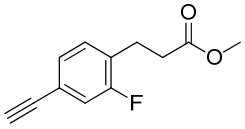
[2] Satoh, T.; Nanba, K.; Suzuki, S., Reduction of Organic Compounds with $NaBH_4$-Transition Metal Salt Systems .4. Selective Hydrogenation of Olefines in Unsaturated Esters. *Chem. Pharm. Bull.* **1971,** *19*, 817-820.

The organic phases were combined, washed with brine, dried over $MgSO_4$, concentrated under vacuum and purified by flash chromatography.

General procedure V: alkylation

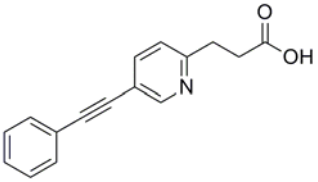
2-(Methylsulfonyl)ethanol or 3-(methylsulfonyl)propan-1-ol (1-1.1 equiv) was dissolved in DMSO (1 mL/mmol) and 2M NaOH solution (0.5-0.6 mL/mmol) was added drop-wise. The benzyl bromide (1 equiv) was added and the reaction stirred at room temperature and monitored by TLC. After consumption of the starting materials the reaction was added water and the mixture extracted with EtOAc (x3). The combined organic phase was washed with brine, dried over $MgSO_4$, concentrated under vacuum and the residue was purified by flash chromatography.

Methyl 3-(4-ethynyl-2-fluorophenyl)propanoate (2).



Step 1: Ethyl 3-(2-fluoro-4-((trimethylsilyl)ethynyl)phenyl)propanoate was prepared from ethyl 3-(4-bromo-2-fluorophenyl)propanoate (670 mg, 2.44 mmol) and trimethylsilylacetylene (0.64 mL, 4.93 mmol) according to the general procedure IB. After concentration the crude product was used directly in the next step. Step 2: The crude was dissolved in MeOH (25 mL), added potassium carbonate (683 mg, 4.94 mmol) and stirred vigorously for 3 hours at room temperature. The reaction was added water and extracted with EtOAc. The organic phases were combined, washed with brine, dried over $MgSO_4$, concentrated under vacuum and purified by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5) to give 375 mg (74%) of a yellow oil: R_f = 0.49 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.24–7.10 (m, 3H), 3.67 (s, 3H), 3.07 (s, 1H), 2.97 (t, J = 7.7 Hz, 2H), 2.63 (t, J = 7.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.9, 160.5 (d, J = 247.5 Hz), 130.7 (d, J = 6.1 Hz), 128.7 (d, J = 16.2 Hz), 128.1 (d, J = 4.0 Hz), 122.0 (d, J = 9.1 Hz), 118.9 (d, J = 24.2 Hz), 82.3 (d, J = 3.0 Hz), 77.8, 51.7, 33.9 (d, J = 1 Hz), 24.5 (d, J = 3.0 Hz); ESI-MS m/z 229.1 ($M+Na^+$).

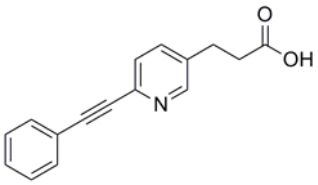
3-(5-(Phenylethynyl)pyridin-2-yl)propanoic acid (4)



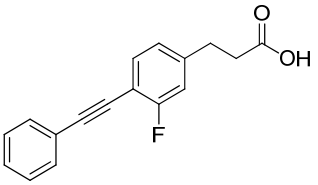
Step 1: (*E*)-Ethyl 3-(5-bromopyridin-2-yl)acrylate (**4a**) was prepared from 5-bromopicolinaldehyde (906 mg, 4.87 mmol) and ethyl 2-bromoacetate (0.80 mL, 7.21 mmol) according to the general procedure III to give 716 mg (91% yield based on recovered starting material) of a white solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:10): R_f = 0.47 (EtOAc:petroleum ether, 1:4); ^{1}H NMR ($CDCl_3$) δ 8.69 (d, J = 2.3 Hz, 1H), 7.84 (dd, J = 8.3 Hz, 2.4 Hz, 1H), 7.62 (d, J = 15.7 Hz, 1H), 7.31 (d, J = 8.3 Hz, 1H), 6.91 (d, J = 15.7 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.5, 151.5, 151.3, 142.0, 139.3, 125.0, 123.2, 121.4, 60.8, 14.3; ESI-MS m/z 278.0 ($M+Na^+$). Step 2: Ethyl 3-(5-bromopyridin-2-yl)propanoate (**4b**) was prepared from **4a** (700 mg, 2.73 mmol) and $NaBH_4$ (130 mg, 3.43 mmol) according to the general procedure IV to give 547 mg (77%) of a clear oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:10→1:5): t_R = 10.50 (HPLC); ^{1}H NMR ($CDCl_3$) δ 8.57 (d, J = 2.3 Hz, 1H), 7.71 (dd, J = 8.3 Hz, 2.4 Hz, 1H), 7.10 (d, J = 8.3 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.06 (t, J = 7.3 Hz, 2H), 2.78 (t, J = 7.3 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.8, 158.7, 150.3, 138.8, 124.4, 118.3, 60.5, 33.1, 32.2, 14.2; ESI-MS m/z 280.0 ($M+Na^+$). Step 3: Ethyl 3-(5-(phenylethynyl)pyridin-2-yl)propanoate (**4c**) was prepared from **4b** (133 mg, 0.51 mmol) and phenylacetylene (0.07 mL, 0.64 mmol) according to the general procedure IB to give 123 mg (86%) of pale yellow solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:3): R_f = 0.22 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 8.67 (d, J = 1.5 Hz, 1H), 7.71 (dd, J = 8.0 Hz, 2.2 Hz, 1H), 7.58–7.50 (m, 2H), 7.39–7.32 (m, 3H), 7.18 (d, J = 8.0 Hz, 1H), 4.13 (q, J = 7.1 Hz, 2H), 3.13 (t, J = 7.4 Hz, 2H), 2.80 (t, J = 7.4 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.9, 159.4, 151.8, 138.7, 131.7, 128.7, 128.4, 122.7, 122.5, 118.0, 92.1, 86.1, 60.5, 33.2, 32.9, 14.2; ESI-MS m/z 302.1 ($M+Na^+$). Step 4: **4** was prepared from **4c** (94 mg, 0.34 mmol) according to the general procedure II to give 79 mg (89%) of a pale yellow solid (t_R = 9.53, purity: 99.9% by HPLC): ^{1}H NMR (DMSO-d_6) δ 12.14 (s, 1H), 8.66

(dd, J = 2.2 Hz, 0.6 Hz, 1H), 7.88 (dd, J = 8.1 Hz, 2.2 Hz, 1H), 7.62–7.54 (m, 2H), 7.49–7.40 (m, 3H), 7.36 (d, J = 8.2 Hz, 1H), 3.02 (t, J = 7.3 Hz, 2H), 2.70 (t, J = 7.3 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 173.7, 159.9, 150.9, 138.6, 131.3, 129.0, 128.7, 122.6, 121.8, 116.7, 91.6, 86.5, 32.2, 32.1; ESI-HRMS calcd for $C_{16}H_{13}NO_2Na$ ($M+Na^+$) 252.1019, found 252.1018.

3-(6-(Phenylethynyl)pyridin-3-yl)propanoic acid (5)



Step 1: (*E*)-Ethyl 3-(6-bromopyridin-3-yl)acrylate (**5a**) was prepared from 6-bromonicotinaldehyde (909 mg, 4.89 mmol) and ethyl 2-bromoacetate (0.80 mL, 7.21 mmol) according to the general procedure III to give 1187 mg (95%) of a white solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:6): t_R = 11.55 (HPLC); ^{1}H NMR ($CDCl_3$) δ 8.49 (d, J = 2.5 Hz, 1H), 7.69 (dd, J = 8.3 Hz, 2.5 Hz, 1H), 7.61 (d, J = 16.1 Hz, 1H), 7.52 (d, J = 8.3 Hz, 1H), 6.50 (d, J = 16.1 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.0, 149.8, 143.5, 139.4, 136.3, 129.6, 128.4, 121.3, 60.9, 14.3; ESI-MS *m/z* 278.0 ($M+Na^+$). Step 2: Ethyl 3-(6-bromopyridin-3-yl)propanoate (**5b**) was prepared from **5a** (1152 mg, 4.50 mmol) and $NaBH_4$ (207 mg, 5.47 mmol) according to the general procedure IV to give 687 mg (59%) of a clear oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:12): t_R = 10.95 (HPLC); ^{1}H NMR (DMSO-d_6) δ 13.03 (d, J = 2.1 Hz, 1H), 12.41 (dd, J = 8.2 Hz, 2.6 Hz, 1H), 12.31 (d, J = 8.2 Hz, 1H), 8.79 (q, J = 7.1 Hz, 2H), 7.59 (t, J = 7.4 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 5.90 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 177.1, 155.6, 144.8, 144.2, 141.2, 132.8, 65.2, 39.4, 31.8, 19.3. Step 3: Ethyl 3-(6-(phenylethynyl)pyridin-3-yl)propanoate (**5c**) was prepared from **5b** (134 mg, 0.52 mmol) and phenylacetylene (0.07 mL, 0.64 mmol) according to the general procedure IB to give 101 mg (70%) of pale brownish solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:2): R_f = 0.16 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 8.49 (d, J = 1.8 Hz, 1H), 7.62–7.57 (m, 2H), 7.54 (dd, J = 8.0, 2.3 Hz, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.38–7.34 (m, 3H), 4.13 (q, J = 7.1 Hz, 2H), 2.98 (t, J = 7.5 Hz, 2H), 2.64 (t, J = 7.5 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.2, 150.2, 141.5, 136.1, 135.4, 132.0, 128.9, 128.4, 126.8, 122.4, 88.9, 88.6, 60.7, 35.2, 28.0, 14.2; ESI-MS *m/z* 302.1 ($M+Na^+$). Step 4: **5** was prepared from **5c** (75 mg, 0.27 mmol) according to the general procedure II to give 59 mg (82%) of a pale yellow solid (t_R = 9.20, purity: 99.1% by HPLC) after purification by flash chromatography (SiO_2, EtOAc[with 1.25% AcOH]:petroleum ether, 1:2): ^{1}H NMR (DMSO-d_6) δ 12.25 (s, 1H), 8.50 (d, J = 1.7 Hz, 1H), 7.73 (dd, J = 8.0 Hz, 2.3 Hz, 1H), 7.64–7.54 (m, 3H), 7.50–7.42 (m, 3H), 2.87 (t, J = 7.4 Hz, 2H), 2.61 (t, J = 7.5 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 173.4, 150.2, 139.9, 136.4, 136.3, 131.5, 129.2, 128.8, 126.7, 121.5, 88.9, 87.8, 34.3, 27.3; ESI-HRMS calcd for $C_{16}H_{13}NO_2Na$ ($M+Na^+$) 252.1019, found 252.1017.

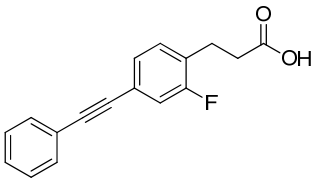


3-(3-Fluoro-4-(phenylethynyl)phenyl)propanoic acid (6).

Step 1: (*E*)-Ethyl 3-(4-bromo-3-fluorophenyl)acrylate (**6a**) was prepared from 4-bromo-3-fluorobenzaldehyde (500 mg, 2.46 mmol) and ethyl 2-bromoacetate (0.40 mL, 3.61 mmol) according to the general procedure IIIB to give 650 mg (97%) of a pale yellow oily product (purity ~90% by ^{1}H NMR) after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5): R_f = 0.50 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.57 (dd, J = 15.3 Hz, 5.3 Hz, 2H), 7.27 (d, J = 9.3 Hz, 1H), 7.17 (d, J = 8.3 Hz, 1H), 6.43 (d, J = 16.0 Hz, 1H), 4.27 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.4, 159.3 (d, J = 249.3 Hz), 142.1 (d, J = 3.0 Hz), 135.9 (d, J = 7.1 Hz), 134.0, 124.8 (d, J = 4.0 Hz), 120.3, 115.2 (d, J = 23.2 Hz), 111.0 (d, J = 21.2 Hz), 60.8, 14.3; ESI-MS *m/z* 273.0 ($M+H^+$). Step 2: Ethyl 3-(4-bromo-3-fluorophenyl)propanoate (**6b**) was prepared from **6a** (310 mg, 1.14 mmol) and $NaBH_4$ (77 mg, 2.04 mmol) at 0 °C according to the general procedure IV to give 270 mg (87%) of a clear oily product: t_R = 12.98 (HPLC); ^{1}H NMR ($CDCl_3$) δ 7.44 (t, J = 7.7 Hz, 1H), 6.98 (d, J = 9.5 Hz, 1H), 6.88 (d, J = 8.2 Hz, 1H), 4.13 (q, J = 7.1 Hz, 2H), 2.91 (t, J = 7.5 Hz, 2H),

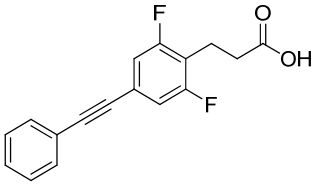
2.60 (t, J = 7.5 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.3, 159.0 (d, J = 248.5 Hz), 142.4 (d, J = 7.1 Hz), 133.4, 125.3 (d, J = 4.0 Hz), 116.5 (d, J = 22.2 Hz), 106.5 (d, J = 21.2 Hz), 60.6, 35.3, 30.2 (d, J = 1.2 Hz), 14.2. Step 3: Ethyl 3-(3-fluoro-4-(phenylethynyl)phenyl)propanoate (**6c**) was prepared from **6b** (137 mg, 0.50 mmol) and phenylacetylene (0.07 mL, 0.64 mmol) according to the general procedure IB to give 115 mg (78%) of a brown oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:8): R_f = 0.60 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.58–7.50 (m, 2H), 7.42 (t, J = 7.7 Hz, 1H), 7.38–7.30 (m, 3H), 7.00–6.92 (m, 2H), 4.13 (q, J = 7.1 Hz, 2H), 2.95 (t, J = 7.6 Hz, 2H), 2.62 (t, J = 7.6 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.4, 162.6 (d, J = 252.5 Hz), 143.5 (d, J = 7.4 Hz), 133.3 (d, J = 1.5 Hz), 131.7, 128.5, 128.4, 124.0 (d, J = 3.2 Hz), 123.0, 115.5 (d, J = 21.1 Hz), 109.7 (d, J = 15.9 Hz), 94.0 (d, J = 3.0 Hz), 82.7, 60.6, 35.3, 30.7 (d, J = 1.2 Hz), 14.2; ESI-MS *m/z* 261.1 ($M+H^+$). Step 4: **6** was prepared from **6c** (102 mg, 0.35 mmol) according to the general procedure II to give 85 mg (92%) of a pale yellow solid (t_R = 12.24, purity: 99.6% by HPLC): ^{1}H NMR (Acetone-d_6) δ 7.61–7.47 (m, 3H), 7.47–7.39 (m, 3H), 7.22–7.13 (m, 2H), 2.98 (t, J = 7.5 Hz, 2H), 2.68 (t, J = 7.5 Hz, 2H); ^{13}C NMR (Acetone-d_6) δ 173.6, 163.2 (d, J = 250.7 Hz), 145.9 (d, J = 7.6 Hz), 134.1, 132.2, 129.6, 129.5, 125.5 (d, J = 3.0 Hz), 123.7, 116.4 (d, J = 21.2 Hz), 110.0 (d, J = 15.2 Hz), 94.5 (d, J = 3.1 Hz), 83.3, 35.2, 31.2 (d, J = 1.3 Hz); ESI-HRMS calcd for $C_{17}H_{14}FO_2$ ($M+H^+$) 269.0972, found 269.0978.

3-(2-Fluoro-4-(phenylethynyl)phenyl)propanoic acid (7).



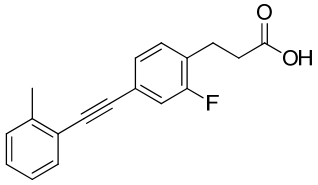
Step 1: (*E*)-Ethyl 3-(4-bromo-2-fluorophenyl)acrylate (**7a**) was prepared from 4-bromo-2-fluorobenzaldehyde (1005 mg, 4.93 mmol) and ethyl 2-bromoacetate (0.80 mL, 7.21 mmol) according to the general procedure III to give 1178 mg (87%) of a clear oil (white solid at 5 °C) after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5): t_R = 13.67 (HPLC); ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 16.2 Hz, 1H), 7.40 (t, J = 8.1 Hz, 1H), 7.34–7.27 (m, 2H), 6.52 (d, J = 16.2 Hz, 1H), 4.27 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.6, 160.9 (d, J = 258.3 Hz), 136.0 (d, J = 2.4 Hz), 129.9 (d, J = 3.7 Hz), 128.0 (d, J = 3.7 Hz), 124.4 (d, J = 9.9 Hz), 121.7 (d, J = 11.8 Hz), 121.5 (d, J = 6.7 Hz), 119.9 (d, J = 25.1 Hz), 60.8, 14.3. Step 2: Ethyl 3-(4-bromo-2-fluorophenyl)propanoate (**7b**) was prepared from **7a** (1124 mg, 4.12 mmol) and $NaBH_4$ (277 mg, 7.33 mmol) at 0 °C according to the general procedure IV to give 979 mg (86%) of a clear oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5): t_R = 13.24 (HPLC); ^{1}H NMR ($CDCl_3$) δ 7.22–7.17 (m, 2H), 7.10 (t, J = 8.1 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 2.93 (t, J = 7.6 Hz, 2H), 2.60 (t, J = 7.6 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.4, 161.0 (d, J = 251.5 Hz), 131.8 (d, J = 5.1 Hz), 127.3 (d, J = 3.0 Hz), 126.6 (d, J = 16.2 Hz), 120.2 (d, J = 9.1 Hz), 119.0 (d, J = 25.3 Hz), 60.6, 34.1 (d, J = 1.0 Hz), 24.2 (d, J = 2.0 Hz), 14.2; ESI-MS *m/z* 297.0 ($M+Na^+$). Step 3: Ethyl 3-(2-fluoro-4-(phenylethynyl)phenyl)propanoate (**7c**) was prepared from **7b** (139 mg, 0.51 mmol) and phenylacetylene (0.07 mL, 0.64 mmol) according to the general procedure IB to give 104 mg (69%) of an orange oily product after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5): R_f = 0.47 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.56–7.48 (m, 2H), 7.39–7.31 (m, 3H), 7.25–7.15 (m, 3H), 4.13 (q, J = 7.1 Hz, 2H), 2.98 (t, J = 7.6 Hz, 2H), 2.63 (t, J = 7.7 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.5, 160.7 (d, J = 246.4 Hz), 131.7, 130.6 (d, J = 6.1 Hz), 128.5, 128.4, 128.1 (d, J = 15.1 Hz), 127.5 (d, J = 3.0 Hz), 123.2 (d, J = 10.1 Hz), 122.9, 118.3 (d, J = 24.2 Hz), 89.9, 88.1 (d, J = 4.0 Hz), 60.6, 34.2, 24.6 (d, J = 2.0 Hz), 14.2; ESI-MS *m/z* 319.1 ($M+Na^+$). Step 4: **7** was prepared from **7c** (84 mg, 0.28 mmol) according to the general procedure II to give 63 mg (79%) of a pale yellow solid (t_R = 12.49, purity: 99.7% by HPLC) after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:2): ^{1}H NMR (DMSO-d_6) δ 12.24 (s, 1H), 7.59–7.53 (m, 2H), 7.49–7.41 (m, 3H), 7.40–7.30 (m, 3H), 2.87 (t, J = 7.6 Hz, 2H), 2.56 (t, J = 7.6 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 173.3, 160.0 (d, J = 245.4 Hz), 131.3, 130.9 (d, J = 5.1 Hz), 128.9, 128.7, 128.6, 127.5 (d, J = 3.0 Hz), 121.9, 121.8 (d, J = 10.1 Hz), 117.7 (d, J = 24.2 Hz), 89.7, 88.0 (d, J = 3.0 Hz), 33.3, 23.5; ESI-HRMS calcd for $C_{17}H_{13}FO_2Na$ ($M+Na^+$) 291.0793, found 291.0806.

3-(2,6-Difluoro-4-(phenylethynyl)phenyl)propanoic acid (8).



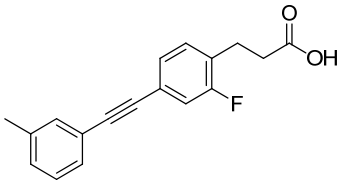
Step 1: (*E*)-Ethyl 3-(4-bromo-2,6-difluorophenyl)acrylate (**8a**) was prepared from 4-bromo-2,6-difluorobenzaldehyde (1006 mg, 4.55 mmol) and ethyl 2-bromoacetate (0.75 mL, 6.76 mmol) according to the general procedure IIIB to give 1274 mg (96%) of a yellow solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:4): R_f = 0.60 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.68 (d, *J* = 16.5 Hz, 1H), 7.18–7.11 (m, 2H), 6.72 (d, *J* = 16.5 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.6, 161.3 (dd, *J* = 260.1 Hz, 8.0 Hz), 129.7, 124.9 (dd, *J* = 8.7 Hz, 8.7 Hz), 123.4 (dd, *J* = 13.1 Hz, 13.1 Hz), 116.0 (dd, *J* = 27.1 Hz, 2.7 Hz), 111.8 (dd, *J* = 15.2 Hz, 15.2 Hz), 60.8, 14.3; ESI-MS *m/z* 291.0 (M+H$^+$). Step 2: Ethyl 3-(4-bromo-2,6-difluorophenyl)propanoate (**8b**) was prepared from **8a** (290 mg, 1.00 mmol) and $NaBH_4$ (102 mg, 2.70 mmol) at 0 ^{o}C according to the general procedure IV to give 202 mg (69%) of a clear oil (only ~80% pure by ^{1}H NMR) after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:2): t_R = 13.38 (HPLC); ^{1}H NMR ($CDCl_3$) δ 7.09–7.01 (m, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 2.95 (t, *J* = 7.8 Hz, 2H), 2.58 (t, *J* = 7.8 Hz, 2H), 1.24 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.2, 161.3 (dd, *J* = 251.5 Hz, 9.6 Hz), 127.9 (dd, *J* = 10.1 Hz, 10.1 Hz), 119.7 (dd, *J* = 12.1 Hz, 12.1 Hz), 115.2 (d, *J* = 29.8 Hz), 61.6, 33.4, 17.9 (dd, *J* = 2.5 Hz, 2.5 Hz), 14.1. Step 3: Ethyl 3-(2,6-difluoro-4-(phenylethynyl)phenyl)propanoate (**8c**) was prepared from **8b** (149 mg, 0.51 mmol) and phenylacetylene (0.07 mL, 0.64 mmol) according to the general procedure IB to give 90 mg (56%) of a pale yellow solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:10): R_f = 0.55 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.56–7.46 (m, 2H), 7.41–7.31 (m, 3H), 7.07–6.98 (m, 2H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.00 (t, *J* = 7.8 Hz, 2H), 2.60 (t, *J* = 7.8 Hz, 2H), 1.24 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.2, 161.1 (dd, *J* = 248.4 Hz, 11.1 Hz), 131.7, 128.8, 128.4, 123.3 (dd, *J* = 12.1 Hz, 12.1 Hz), 122.5, 116.9 (dd, *J* = 20.2 Hz, 20.2 Hz), 114.4 (dd, *J* = 19.9 Hz, 8.2 Hz), 90.9, 87.2 (dd, *J* = 4.0 Hz, 4.0 Hz), 61.6, 33.6, 18.1, 14.2; ESI-MS *m/z* 337.1 (M+Na$^+$). Step 4: **8** was prepared from **8c** (78 mg, 0.25 mmol) according to the general procedure II to give 69 mg (97%) of a white solid (t_R = 12.71, purity: 99.6% by HPLC): ^{1}H NMR (Acetone-d_6) δ 7.62–7.52 (m, 2H), 7.49–7.38 (m, 3H), 7.22–7.12 (m, 2H), 3.00 (t, *J* = 7.8 Hz, 2H), 2.63 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR (Acetone-d_6) δ 173.2, 162.1 (dd, *J* = 247.3 Hz, 10.1 Hz), 132.5, 129.9, 129.5, 124.1 (dd, *J* = 12.6 Hz, 12.6 Hz), 123.3, 118.3 (dd, *J* = 20.7 Hz, 20.7 Hz), 115.1 (dd, *J* = 20.0 Hz, 8.5 Hz), 91.6, 87.7 (dd, *J* = 3.7 Hz, 3.7 Hz), 33.5, 18.7 (dd, *J* = 2.5 Hz, 2.5 Hz); ESI-HRMS calcd for $C_{17}H_{13}F_2O_2$ (M+H$^+$) 287.0878, found 287.0881.

3-(2-Fluoro-4-(*o*-tolylethynyl)phenyl)propanoic acid (10).



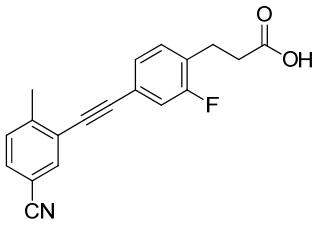
Step 1: Methyl 3-(2-fluoro-4-(*o*-tolylethynyl)phenyl)propanoate (**10a**) was prepared from **2** (110 mg, 0.53 mmol) and 1-bromo-2-methylbenzene (70 µL, 0.58 mmol) according to the general procedure I to give 82 mg (52%) of a pale yellow oily product after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:8): R_f = 0.35 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.47 (d, *J* = 7.5 Hz, 1H), 7.28–7.12 (m, 6H), 3.67 (s, 3H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.64 (t, *J* = 7.6 Hz, 2H), 2.49 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.9, 160.7 (d, *J* = 247.1 Hz), 140.3, 131.9, 130.6 (d, *J* = 6.1 Hz), 129.5, 128.6, 127.9 (d, *J* = 16.2 Hz), 127.4 (d, *J* = 3.0 Hz), 125.6, 123.5 (d, *J* = 10.1 Hz), 122.7, 118.2 (d, *J* = 23.2 Hz), 92.1 (d, *J* = 3.1 Hz), 88.9, 51.7, 34.0 (d, *J* = 1.2 Hz), 24.6 (d, *J* = 2.3 Hz), 20.7; ESI-MS *m/z* 297.1 (M+H$^+$). Step 2: **10** was prepared from **10a** (72 mg, 0.24 mmol) according to the general procedure II to give 65 mg (95%) of a white solid (t_R = 13.03, purity: 99.8% by HPLC): ^{1}H NMR ($CDCl_3$) δ 7.48 (d, *J* = 7.5 Hz, 1H), 7.28–7.12 (m, 6H), 3.00 (t, *J* = 7.5 Hz, 2H), 2.70 (t, *J* = 7.6 Hz, 2H), 2.50 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 178.5, 160.7 (d, *J* = 247.3 Hz), 140.3, 131.9, 130.6 (d, *J* = 6.1 Hz), 129.5, 128.6, 127.5 (d, *J* = 16.1 Hz), 127.5 (d, *J* = 3.2 Hz), 125.6, 123.6 (d, *J* = 10.1 Hz), 122.6, 118.2 (d, *J* = 24.2 Hz), 92.0 (d, *J* = 3.2 Hz), 89.0, 33.9, 24.3 (d, *J* = 2.1 Hz), 20.7; ESI-HRMS calcd for $C_{18}H_{16}FO_2$ (M+H$^+$) 283.1129, found 283.1139.

3-(2-Fluoro-4-(*m*-tolylethynyl)phenyl)propanoic acid (11).



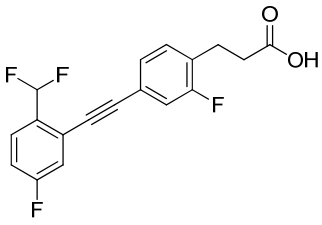
Step 1: Methyl 3-(2-fluoro-4-(*m*-tolylethynyl)phenyl)propanoate (**11a**) was prepared from **2** (104 mg, 0.51 mmol) and 1-iodo-3-methylbenzene (70 µL, 0.55 mmol) according to the general procedure IB to give 100 mg (67%) of a clear oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5): R_f = 0.58 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.37–7.30 (m, 2H), 7.26–7.20 (m, 2H), 7.20–7.13 (m, 2H), 3.67 (s, 3H), 2.98 (t, *J* = 7.7 Hz, 2H), 2.64 (t, *J* = 7.7 Hz, 2H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 173.0, 160.7 (d, *J* = 247.5 Hz), 138.1, 132.2, 130.6 (d, *J* = 5.1 Hz), 129.4, 128.7, 128.3, 127.9 (d, *J* = 16.2 Hz), 127.5 (d, *J* = 3.0 Hz), 123.3 (d, *J* = 10.1 Hz), 122.7, 118.3 (d, *J* = 24.2 Hz), 90.1, 87.8 (d, *J* = 3.0 Hz), 51.7, 34.0 (d, *J* = 1.0 Hz), 24.6 (d, *J* = 3.0 Hz), 21.2; ESI-MS *m/z* 319.1 (M+Na$^+$). Step 2: **11** was prepared from **11a** (51 mg, 0.17 mmol) according to the general procedure II to give 44 mg (90%) of a beige solid (t_R = 12.81, purity: 99.8% by HPLC): ^{1}H NMR ($CDCl_3$) δ 7.36–7.30 (m, 2H), 7.26–7.12 (m, 5H), 2.99 (t, *J* = 7.6 Hz, 2H), 2.70 (t, *J* = 7.7 Hz, 2H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 178.5, 160.7 (d, *J* = 246.2 Hz), 138.1, 132.2, 130.6 (d, *J* = 5.6 Hz), 129.4, 128.8, 128.3, 127.6 (d, *J* = 3.4 Hz), 127.4, 123.5 (d, *J* = 9.8 Hz), 122.7, 118.3 (d, *J* = 23.7 Hz), 90.2, 87.7 (d, *J* = 3.2 Hz), 33.9, 24.3 (d, *J* = 2.2 Hz), 21.2; ESI-HRMS calcd for $C_{18}H_{15}FO_2Na$ (M+Na$^+$) 305.0951, found 305.0948.

3-(4-((5-Cyano-2-methylphenyl)ethynyl)-2-fluorophenyl)propanoic acid (12).



Step 1: Methyl 3-(4-((5-cyano-2-methylphenyl)ethynyl)-2-fluorophenyl)-propanoate (**12a**) was prepared from **2** (110 mg, 0.53 mmol) and 3-iodo-4-methylbenzonitrile (140 mg, 0.58 mmol) according to the general procedure I to give 115 mg (68%) of pale yellow oily product after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5): R_f = 0.21 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.75 (s, 1H), 7.50 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 7.9 Hz, 1H), 7.25–7.17 (m, 3H), 3.68 (s, 3H), 3.00 (t, *J* = 7.6 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H), 2.56 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.8, 160.7 (d, *J* = 247.7 Hz), 145.6, 135.2, 131.5, 130.8 (d, *J* = 5.7 Hz), 130.4, 128.8 (d, *J* = 16.2 Hz), 127.6 (d, *J* = 4.0 Hz), 124.3, 122.4 (d, *J* = 9.1 Hz), 118.3 (d, *J* = 24.0 Hz), 118.3, 110.1, 94.2 (d, *J* = 3.1 Hz), 86.4, 51.7, 33.9 (d, *J* = 1.1 Hz), 24.6 (d, *J* = 2.3 Hz), 21.2; ESI-MS *m/z* 344.1 (M+Na$^+$). Step 2: **12** was prepared from **12a** (110 mg, 0.34 mmol) according to the general procedure II to give 71 mg (68%) of a white solid (t_R = 12.26, purity: 99.3% by HPLC) after purification by flash chromatography (SiO_2, EtOAc[with 1% AcOH]:petroleum ether, 2:3): ^{1}H NMR (Acetone-d_6) δ 7.88 (s, 1H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.47–7.29 (m, 3H), 2.99 (t, *J* = 7.6 Hz, 2H), 2.66 (dd, *J* = 14.8 Hz, 7.2 Hz, 2H), 2.59 (s, 3H); ^{13}C NMR (Acetone-d_6) δ 173.5, 161.6 (d, *J* = 245.4 Hz), 146.6, 135.8, 132.7, 132.1 (d, *J* = 5.7 Hz), 131.7, 130.3 (d, *J* = 15.9 Hz), 128.5 (d, *J* = 3.3 Hz), 125.0, 123.1 (d, *J* = 9.8 Hz), 118.9, 118.7, 111.0, 94.8 (d, *J* = 3.1 Hz), 87.1, 34.0, 24.8 (d, *J* = 2.6 Hz), 21.1; ESI-HRMS calcd for $C_{19}H_{14}FNO_2Na$ (M+Na$^+$) 330.0901, found 330.0892.

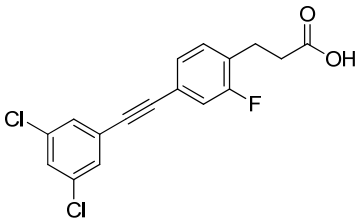
3-(4-((2-(Difluoromethyl)-5-fluorophenyl)ethynyl)-2-fluorophenyl)propanoic acid (13).



Step 1: Methyl 3-(4-((2-(difluoromethyl)-5-fluorophenyl)ethynyl)-2-fluoro-phenyl)propanoate (**13a**) was prepared from **2** (99 mg, 0.48 mmol) and 2-bromo-1-(difluoromethyl)-4-fluorobenzene (70 µL, 0.53 mmol) according to the general procedure I to give 118 mg (70%) of a pale yellow solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5): R_f = 0.47 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.66 (dd, *J* = 8.7 Hz, 5.6 Hz, 1H), 7.30–7.15 (m, 5H), 7.02 (t, *J* = 55.2 Hz, 1H), 3.68 (s, 3H), 3.00 (t, *J* = 7.6 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.8, 163.4 (dt, *J* = 252.2 Hz, 1.9 Hz), 160.7 (d, *J* = 247.9 Hz), 131.6 (dt, *J* = 22.8 Hz, 3.3 Hz), 130.9 (d, *J* = 5.7 Hz), 129.2 (d, *J* = 16.1 Hz), 127.7 (d, *J* = 3.3 Hz), 127.7 (dt, *J* = 10.9 Hz, 5.7 Hz), 123.8 (dt, *J* = 10.3 Hz, 6.1 Hz), 121.8 (d, *J* = 9.7 Hz), 119.2 (d, *J* = 23.6

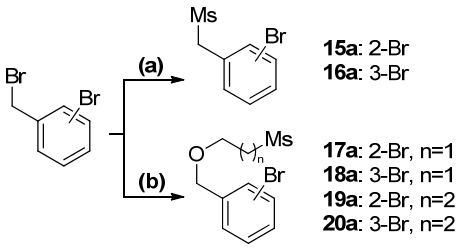
Hz), 118.4 (d, J = 24.1 Hz), 116.4 (d, J = 22.0 Hz), 112.8 (t, J = 238.8 Hz), 94.6 (d, J = 3.1 Hz), 84.3–84.2 (m), 51.7, 33.9 (d, J = 1.2 Hz), 24.6 (d, J = 2.3 Hz); ESI-MS *m/z* 373.1 ($M+Na^+$). Step 2: **13** was prepared from **13a** (75 mg, 0.21 mmol) according to the general procedure II to give 68 mg (95%) of a white solid (t_R = 12.53, purity: 98.8% by HPLC): ^{1}H NMR (Acetone-d_6) δ 7.78 (dd, J = 8.7 Hz, 5.6 Hz, 1H), 7.50–7.32 (m, 5H), 7.20 (t, J = 54.9 Hz, 1H), 3.00 (t, J = 7.6 Hz, 2H), 2.67 (t, J = 7.6 Hz, 2H); ^{13}C NMR (Acetone-d_6) δ 173.5, 164.4 (dt, J = 250.5 Hz, 1.9 Hz), 161.5 (d, J = 246.2 Hz), 132.7 (dt, J = 22.7 Hz, 3.2 Hz), 132.1 (d, J = 5.6 Hz), 130.8 (d, J = 16.0 Hz), 128.9 (dt, J = 9.7 Hz, 5.9 Hz), 128.7 (d, J = 3.3 Hz), 124.6 (dt, J = 10.4 Hz, 5.9 Hz), 122.6 (d, J = 9.8 Hz), 120.0 (d, J = 24.0 Hz), 119.0 (d, J = 24.4 Hz), 117.4 (d, J = 22.3 Hz), 114.1 (t, J = 237.0 Hz), 95.3 (d, J = 3.1 Hz), 94.9 (d, J = 2.8 Hz), 34.0 (d, J = 0.9 Hz), 24.8 (d, J = 2.6 Hz); ESI-HRMS calcd for $C_{18}H_{12}F_4O_2Na$ ($M+Na^+$) 359.0666, found 359.0680.

3-(4-((3,5-Dichlorophenyl)ethynyl)-2-fluorophenyl)propanoic acid (14).



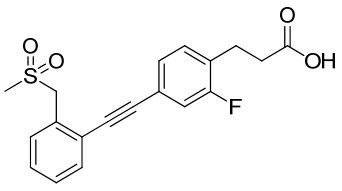
Step 1: Methyl 3-(4-((3,5-dichlorophenyl)ethynyl)-2-fluorophenyl)propanoate (**14a**) was prepared from **2** (103 mg, 0.50 mmol) and 1-bromo-3,5-dichlorobenzene (125 mg, 0.55 mmol) according to the general procedure I to give 128 mg (73%) of a pale yellow solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5): R_f = 0.49 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.39 (d, J = 1.9 Hz, 2H), 7.33 (t, J = 1.9 Hz, 1H), 7.24–7.20 (m, 2H), 7.19–7.14 (m, 1H), 3.68 (s, 3H), 2.99 (t, J = 7.6 Hz, 2H), 2.65 (t, J = 7.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.9, 160.6 (d, J = 247.5 Hz), 135.0, 130.8 (d, J = 5.7 Hz), 129.8, 129.0, 128.8, 127.7 (d, J = 3.3 Hz), 125.7, 122.1 (d, J = 9.8 Hz), 118.4 (d, J = 24.1 Hz), 90.4 (d, J = 3.0 Hz), 87.1, 51.7, 33.9 (d, J = 1.0 Hz), 24.6 (d, J = 2.0 Hz). Step 2: **14** was prepared from **14a** (100 mg, 0.29 mmol) according to the general procedure II to give 93 mg (96%) of a white solid (t_R = 14.38, purity: 99.9% by HPLC): ^{1}H NMR (DMSO-d_6) δ 12.25 (s, 1H), 7.73–7.68 (m, 1H), 7.67–7.60 (m, 2H), 7.45–7.30 (m, 3H), 2.88 (t, J = 7.5 Hz, 2H), 2.57 (t, J = 7.6 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 173.3, 160.0 (d, J = 245.4 Hz), 134.4, 131.1 (d, J = 5.1 Hz), 129.8, 129.5 (d, J = 16.0 Hz), 128.8, 127.8 (d, J = 3.1 Hz), 125.2, 121.0 (d, J = 9.8 Hz), 118.0 (d, J = 24.3 Hz), 90.6, 86.9, 33.3, 23.6 (d, J = 2.0 Hz); ESI-HRMS calcd for $C_{17}H_{11}Cl_2FO_2Na$ ($M+Na^+$) 359.0012, found 359.0007.

Scheme S1[a]



[a]Reagents and conditions: (a) sodium methanesulfinate, DMF, 60 ºC, 1 h, 94–97%; (b) 2-(methylsulfonyl)ethanol (**17a**+**18a**) or 3-(methylsulfonyl)propan-1-ol (**19a**+**20a**), 2M $NaOH_{(aq)}$, DMSO, 2–22 h, 7–54%.

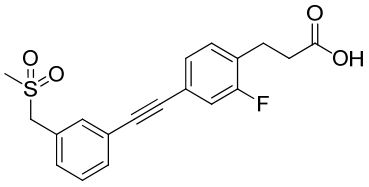
3-(2-Fluoro-4-((2-((methylsulfonyl)methyl)phenyl)ethynyl)phenyl)propanoic acid (15).



Step 1: 1-Bromo-2-((methylsulfonyl)methyl)benzene (**15a**): 2-Bromobenzyl-bromide (250 mg, 1.00 mmol), sodium methanesulfinate (303 mg, 2.97 mmol) and DMF (2.5 mL) was added to a 10 mL cone shaped flask and stirred at 60 °C for 1 hour. The reaction was cooled to room temperature, water was added and the mixture was extracted with EtOAc. The combined organic extracts was washed with water, washed with brine, dried over $MgSO_4$ and concentrated under vacuum to give 235 mg (94%) of a white solid: R_f = 0.34 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$)

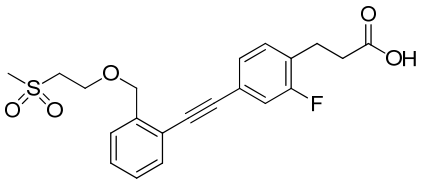
δ 7.67–7.63 (m, 1H), 7.61 (dd, *J* = 7.7 Hz, 1.5 Hz, 1H), 7.40 (td, *J* = 7.6 Hz, 0.9 Hz, 1H), 7.27 (td, *J* = 7.8 Hz, 1.6 Hz, 1H), 4.52 (s, 2H), 2.82 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 133.4, 133.1, 130.8, 128.5, 128.3, 125.0, 60.4, 39.9; ESI-MS *m/z* 270.9 ($M+Na^+$). Step 2: Methyl 3-(2-fluoro-4-((2-((methylsulfonyl)methyl)phenyl)ethynyl)phenyl)-propanoate (**15b**) was prepared from **2** (104 mg, 0.50 mmol) and **15a** (136 mg, 0.54 mmol) according to the general procedure I to give 136 mg (72%) of a pale yellow solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:2): R_f = 0.12 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.67–7.57 (m, 2H), 7.47–7.37 (m, 2H), 7.30–7.16 (m, 3H), 4.57 (s, 2H), 3.69 (s, 3H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.80 (s, 3H), 2.66 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.8, 160.7 (d, *J* = 248.1 Hz), 133.3 (d, *J* = 32.7 Hz), 132.8, 131.5, 131.0 (d, *J* = 5.7 Hz), 130.3, 129.5, 129.2, 127.5 (d, *J* = 3.3 Hz), 123.6, 122.0 (d, *J* = 9.7 Hz), 118.3 (d, *J* = 24.0 Hz), 93.4 (d, *J* = 3.2 Hz), 87.2, 59.5, 51.9, 39.4, 33.9 (d, *J* = 1.2 Hz), 24.6 (d, *J* = 2.3 Hz); ESI-MS *m/z* 375.1 ($M+H^+$). Step 3: **15** was prepared from **15b** (109 mg, 0.29 mmol) according to the general procedure II to give 100 mg (95%) of a pale yellow solid (t_R = 10.71, purity: 99.6% by HPLC): ^{1}H NMR (Acetone-d_6) δ 7.73–7.59 (m, 2H), 7.51–7.31 (m, 5H), 4.70 (s, 2H), 2.99 (t, *J* = 7.6 Hz, 2H), 2.93 (s, 3H), 2.67 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (Acetone-d_6) δ 173.5, 161.5 (d, *J* = 245.8 Hz), 134.1 (d, *J* = 6.2 Hz), 133.3, 132.7, 132.0, 130.1 (d, *J* = 16.0 Hz), 129.9, 129.7, 128.6 (d, *J* = 3.0 Hz), 124.9, 123.3 (d, *J* = 9.9 Hz), 118.8 (d, *J* = 24.3 Hz), 93.5 (d, *J* = 3.1 Hz), 88.4, 59.6, 40.5, 34.0, 24.8 (d, *J* = 2.5 Hz); ESI-HRMS calcd for $C_{19}H_{19}O_4S$ ($M+H^+$) 361.0467, found 361.0897.

3-(2-Fluoro-4-((3-((methylsulfonyl)methyl)phenyl)ethynyl)phenyl)propanoic acid (16).



Step 1: 1-Bromo-3-((methylsulfonyl)methyl)benzene (**16a**) was prepared as **16a** from 3-bromobenzylbromide (260 mg, 1.04 mmol) to give 252 mg (97%) of a white solid: R_f = 0.15 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.60–7.52 (m, 2H), 7.37 (d, *J* = 7.7 Hz, 1H), 7.30 (t, *J* = 7.8 Hz, 1H), 4.21 (s, 2H), 2.80 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 133.4, 132.4, 130.6, 130.4, 129.2, 123.1, 60.6, 39.4; ESI-MS *m/z* 270.9 ($M+Na^+$). Step 2: Methyl 3-(2-fluoro-4-((3-((methylsulfonyl)methyl)phenyl)ethynyl)phenyl)propanoate (**16b**) was prepared from **2** (102 mg, 0.50 mmol) and **16a** (138 mg, 0.55 mmol) according to the general procedure I to give 116 mg (62%) of a pale yellow solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:2): R_f = 0.12 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.59–7.52 (m, 2H), 7.41 (d, *J* = 4.9 Hz, 2H), 7.32–7.15 (m, 3H), 4.25 (s, 2H), 3.68 (s, 3H), 2.99 (t, *J* = 7.7 Hz, 2H), 2.80 (s, 3H), 2.65 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.9, 160.6 (d, *J* = 247.3 Hz), 133.5, 132.3, 130.7 (d, *J* = 5.7 Hz), 130.5, 129.3, 128.7, 128.4 (d, *J* = 16.0 Hz), 127.6 (d, *J* = 3.3 Hz), 124.0, 122.7 (d, *J* = 9.7 Hz), 118.3 (d, *J* = 23.9 Hz), 89.2 (d, *J* = 3.0 Hz), 88.8, 60.9, 51.7, 39.2, 33.9 (d, *J* = 1.2 Hz), 24.6 (d, *J* = 2.3 Hz); ESI-MS *m/z* 375.1 ($M+H^+$). Step 3: **16** was prepared from **16b** (90 mg, 0.24 mmol) according to the general procedure II to give 83 mg (96%) of a pale yellow solid (t_R = 10.56, purity: 99.7% by HPLC): ^{1}H NMR (Acetone-d_6) δ 7.68 (s, 1H), 7.62–7.45 (m, 3H), 7.40 (t, *J* = 7.9 Hz, 1H), 7.31 (dd, *J* = 16.8 Hz, 9.4 Hz, 2H), 4.47 (s, 2H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.90 (s, 3H), 2.66 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (Acetone-d_6) δ 173.5, 161.5 (d, *J* = 245.9 Hz), 134.7, 132.4, 132.2, 132.0 (d, *J* = 5.7 Hz), 131.1, 129.9 (d, *J* = 16.0 Hz), 129.8, 128.5 (d, *J* = 3.3 Hz), 124.0, 123.5 (d, *J* = 9.9 Hz), 118.7 (d, *J* = 24.2 Hz), 89.9, 89.2 (d, *J* = 3.2 Hz), 60.4, 39.9, 34.0, 24.8 (d, *J* = 2.5 Hz); ESI-HRMS calcd for $C_{19}H_{19}O_4S$ ($M+H^+$) 361.0467, found 361.0902.

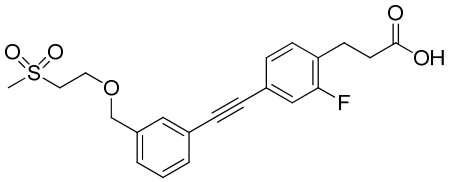
3-(2-Fluoro-4-((2-((2-(methylsulfonyl)ethoxy)methyl)phenyl)ethynyl)phenyl)propanoic acid (17).



Step 1: 1-Bromo-2-((2-(methylsulfonyl)ethoxy)methyl)benzene (**17a**) was prepared from 2-(methylsulfonyl)ethanol (275 mg, 2.22 mmol) and 2-bromobenzyl bromide (504 mg, 2.02 mmol) according to the general procedure V to give 144 mg (24%) of a white solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:3→1:0): R_f = 0.15 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.57 (dd, *J* = 8.0 Hz, 1.1 Hz, 1H), 7.40 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.33 (dt, *J* = 7.5 Hz, 1.2 Hz, 1H), 7.19 (dt, *J* = 7.7 Hz, 1.8 Hz,

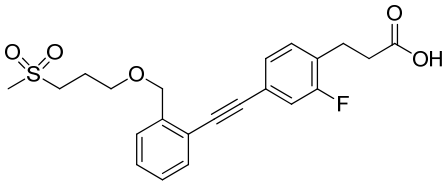
1H), 4.63 (s, 2H), 4.04–3.97 (m, 2H), 3.31–3.25 (m, 2H), 3.02 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 136.3, 132.9, 129.6, 129.5, 127.6, 123.2, 73.0, 64.5, 55.4, 43.1; ESI-MS *m/z* 315.0 ($M+Na^+$). Step 2: Methyl 3-(2-fluoro-4-((2-((2-(methylsulfonyl)ethoxy)methyl)phenyl)ethynyl)phenyl)propanoate (**17b**) was prepared from **2** (81 mg, 0.39 mmol) and **17a** (121 mg, 0.41 mmol) according to the general procedure I to give 88 mg (54%) of a yellow oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:2→1:1): R_f = 0.31 (EtOAc:petroleum ether, 1:1); ^{1}H NMR ($CDCl_3$) δ 7.54 (dd, *J* = 7.5 Hz, 1.3 Hz, 1H), 7.43 (dd, *J* = 7.5 Hz, 1.1 Hz, 1H), 7.37 (dt, *J* = 7.5 Hz, 1.5 Hz, 1H), 7.32 (dt, *J* = 7.4 Hz, 1.6 Hz, 1H), 7.28–7.17 (m, 3H), 4.77 (s, 2H), 4.04–3.98 (m, 2H), 3.68 (s, 3H), 3.26 (t, *J* = 5.0 Hz, 2H), 3.04–2.96 (m, 5H), 2.65 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.9, 160.7 (d, *J* = 246.6 Hz), 138.6, 132.5, 130.8 (d, *J* = 5.6 Hz), 128.9, 128.4 (d, *J* = 16.0 Hz), 128.2, 128.0, 127.5 (d, *J* = 3.3 Hz), 122.8 (d, *J* = 9.6 Hz), 122.0, 118.2 (d, *J* = 23.8 Hz), 92.8 (d, *J* = 3.1 Hz), 87.3, 71.8, 64.5, 55.4, 51.7, 43.1, 33.9 (d, *J* = 1.2 Hz), 24.6 (d, *J* = 2.3 Hz); ESI-MS *m/z* 441.1 ($M+Na^+$). Step 3: **17** was prepared from **17b** (72 mg, 0.17 mmol) according to the general procedure II to give 36 mg (52%) of a white solid (t_R = 11.03, purity: 99.3% by HPLC) after purification by flash chromatography (SiO_2, EtOAc[with 1% AcOH]:petroleum ether, 1:1): ^{1}H NMR (Acetone-d_6) δ 7.44 (dd, *J* = 7.6 Hz, 1.3 Hz, 2H), 7.30 (dt, *J* = 7.7 Hz, 1.5 Hz, 1H), 7.28–7.17 (m, 4H), 4.70 (s, 2H), 3.94–3.88 (m, 2H), 3.27–3.22 (m, 2H), 2.90–2.82 (m, 5H), 2.53 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (Acetone-d_6) δ 173.7, 161.6 (d, *J* = 244.8 Hz), 140.6, 133.0, 132.0 (d, *J* = 5.6 Hz), 129.9 (d, *J* = 15.8 Hz), 129.8, 129.1, 128.7, 128.5 (d, *J* = 3.3 Hz), 123.6 (d, *J* = 9.9 Hz), 122.5, 118.7 (d, *J* = 24.1 Hz), 93.4 (d, *J* = 3.2 Hz), 88.1, 71.9, 65.5, 55.5, 43.0, 34.1, 24.9 (d, *J* = 2.6 Hz); ESI-HRMS calcd for $C_{21}H_{21}FO_5SNa$ ($M+Na^+$) 427.0986, found 427.0966.

3-(2-Fluoro-4-((3-((2-(methylsulfonyl)ethoxy)methyl)phenyl)ethynyl)phenyl)propanoic acid (18).



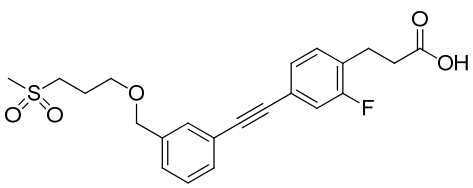
Step 1: 1-Bromo-3-((2-(methylsulfonyl)ethoxy)methyl)benzene (**18a**) was prepared from 2-(methylsulfonyl)ethanol (115 mg, 0.93 mmol) and 3-bromobenzyl bromide (251 mg, 1.00 mmol) according to the general procedure V to give 146 mg (54%) of a clear oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:3→1:0): R_f = 0.09 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.49–7.41 (m, 2H), 7.27–7.20 (m, 2H), 4.53 (s, 2H), 3.97–3.90 (m, 2H), 3.30–3.23 (m, 2H), 3.00 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 139.4, 131.2, 130.7, 130.2, 126.2, 122.7, 72.8, 64.3, 55.3, 43.2; ESI-HRMS calcd for $C_{10}H_{13}BrO_3SNa$ ($M+Na^+$) 314.9661, found 314.9654. Step 2: Methyl 3-(2-fluoro-4-((3-((2-(methylsulfonyl)ethoxy)methyl)phenyl)-ethynyl)phenyl)propanoate (**18b**) was prepared from **2** (83 mg, 0.40 mmol) and **18a** (134 mg, 0.46 mmol) according to the general procedure I to give 95 mg (57%) of a pale yellow solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:2→1:1): R_f = 0.21 (EtOAc:petroleum ether, 1:1); ^{1}H NMR ($CDCl_3$) δ 7.50–7.44 (m, 2H), 7.36 (t, *J* = 7.8 Hz, 1H), 7.32–7.28 (m, 1H), 7.26–7.15 (m, 3H), 4.56 (s, 2H), 3.97–3.92 (m, 2H), 3.68 (s, 3H), 3.26 (t, *J* = 5.1 Hz, 2H), 3.03–2.96 (m, 5H), 2.65 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.9, 160.7 (d, *J* = 246.3 Hz), 137.4, 131.3, 130.9, 130.7 (d, *J* = 5.6 Hz), 128.7, 128.2 (d, *J* = 16.0 Hz), 127.9, 127.6 (d, *J* = 3.3 Hz), 123.3, 123.0 (d, *J* = 9.8 Hz), 118.3 (d, *J* = 23.8 Hz), 89.5, 88.5 (d, *J* = 3.2 Hz), 73.2, 64.3, 55.3, 51.7, 43.2, 34.0 (d, *J* = 1.3 Hz), 24.6 (d, *J* = 2.3 Hz); ESI-MS *m/z* 441.1 ($M+Na^+$). Step 3: **18** was prepared from **18b** (87 mg, 0.21 mmol) according to the general procedure II to give 55 mg (65%) of a white solid (t_R = 10.88, purity: 99.4% by HPLC) after purification by flash chromatography (SiO_2, EtOAc[with 1% AcOH]:petroleum ether, 1:1): ^{1}H NMR (Acetone-d_6) δ 7.45–7.42 (m, 1H), 7.38–7.23 (m, 4H), 7.18 (dd, *J* = 7.8 Hz, 1.5 Hz, 1H), 7.13 (dd, *J* = 10.7 Hz, 1.4 Hz, 1H), 4.50 (s, 2H), 3.87–3.79 (m, 2H), 3.28–3.22 (m, 2H), 2.89–2.81 (m, 5H), 2.52 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (Acetone-d_6) δ 173.6, 161.6 (d, *J* = 244.8 Hz), 139.7, 132.0 (d, *J* = 5.7 Hz), 131.6, 131.5, 129.8 (d, *J* = 15.9 Hz), 129.6, 128.9, 128.5 (d, *J* = 3.3 Hz), 123.8, 123.7 (d, *J* = 9.8 Hz), 118.7 (d, *J* = 24.1 Hz), 90.4, 88.9 (d, *J* = 3.3 Hz), 73.0, 65.2, 55.4, 43.0, 34.1, 24.8 (d, *J* = 2.5 Hz); ESI-HRMS calcd for $C_{21}H_{21}FO_5SNa$ ($M+Na^+$) 427.0986, found 427.0972.

3-(2-Fluoro-4-((2-((3-(methylsulfonyl)propoxy)methyl)phenyl)ethynyl)phenyl)propanoic acid (19).



Step 1: 3-Bromopropan-1-ol (501 mg, 3.61 mmol) was dissolved in EtOH (4 mL) and sodium methanesulfinate (820 mg, 8.03 mmol) dissolved in water (4 mL) was added. The reaction was heated to 50 °C for 24 h. The reaction was cooled to room temperature and extracted with EtOAc. The organic phase was dried over $MgSO_4$ and concentrated under vacuum to give 330 mg (66%) of 3-(methylsulfonyl)propan-1-ol as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.82–3.77 (m, 2H), 3.24–3.15 (m, 2H), 2.95 (s, 3H), 2.15–2.05 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 60.3, 51.8, 40.8, 25.4. Step 2: 1-Bromo-2-((3-(methylsulfonyl)propoxy)methyl)benzene (**19a**) was prepared from 3-(methylsulfonyl)propan-1-ol (159 mg, 1.15 mmol) and 2-bromobenzyl bromide (250 mg, 1.00 mmol) according to the general procedure V to give 44 mg (7%) of a clear oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:3→1:0): R_f = 0.10 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.57–7.53 (m, 1H), 7.42 (dd, J = 7.6 Hz, 1.6 Hz, 1H), 7.32 (dt, J = 7.5 Hz, 1.1 Hz, 1H), 7.17 (dt, J = 7.8 Hz, 1.7 Hz, 1H), 4.57 (s, 2H), 3.68 (t, J = 5.8 Hz, 2H), 3.26–3.15 (m, 2H), 2.91 (s, 3H), 2.23–2.12 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 137.2, 132.7, 129.33, 129.27, 127.5, 123.0, 72.4, 68.2, 52.1, 40.7, 23.1; ESI-MS *m/z* 329.0 ($M+Na^+$). Step 3: Methyl 3-(2-fluoro-4-((2-((3-(methylsulfonyl)propoxy)methyl)phenyl)ethynyl)phenyl)propanoate (**19b**) was prepared from **2** (36 mg, 0.17 mmol) and **19a** (45 mg, 0.14 mmol) according to the general procedure I to give 29 mg (48%) of a sticky yellow oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:1): R_f = 0.22 (EtOAc:petroleum ether, 1:1); ^{1}H NMR ($CDCl_3$) δ 7.53 (dd, J = 7.6 Hz, 1.1 Hz, 1H), 7.44 (dd, J = 7.6 Hz, 0.6 Hz, 1H), 7.36 (dt, J = 7.5 Hz, 1.4 Hz, 1H), 7.30 (dt, J = 7.5 Hz, 1.4 Hz, 1H), 7.26–7.15 (m, 3H), 4.73 (s, 2H), 3.72–3.65 (m, 5H), 3.19–3.12 (m, 2H), 2.99 (t, J = 7.6 Hz, 2H), 2.85 (s, 3H), 2.65 (t, J = 7.6 Hz, 2H), 2.22–2.11 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 172.9, 160.7 (d, J = 246.5 Hz), 139.6, 132.3, 130.8 (d, J = 5.6 Hz), 128.8, 128.3 (d, J = 16.0 Hz), 127.8, 127.7, 127.5 (d, J = 3.3 Hz), 123.0 (d, J = 9.7 Hz), 121.7, 118.2 (d, J = 23.7 Hz), 92.6 (d, J = 3.1 Hz), 87.6, 71.2, 68.2, 52.1, 51.7, 40.6, 34.0 (d, J = 1.3 Hz), 24.6 (d, J = 2.3 Hz), 23.1; ESI-MS *m/z* 455.1 ($M+Na^+$). Step 4: **19** was prepared from **19b** (28 mg, 0.06 mmol) according to the general procedure II to give 24 mg (91%) of a white solid (t_R = 11.10, purity: 97.9% by HPLC): ^{1}H NMR (Acetone-d_6) δ 7.44–7.40 (m, 2H), 7.33–7.25 (m, 2H), 7.24–7.19 (m, 2H), 7.17 (dd, J = 10.7 Hz, 1.5 Hz, 1H), 4.64 (s, 2H), 3.60 (t, J = 6.1 Hz, 2H), 3.10–3.03 (m, 2H), 2.85 (t, J = 7.6 Hz, 2H), 2.77 (s, 3H), 2.53 (t, J = 7.6 Hz, 2H), 2.04–1.94 (m, 2H); ^{13}C NMR (Acetone-d_6) δ 173.5, 161.6 (d, J = 244.9 Hz), 141.4, 132.8, 132.0 (d, J = 5.6 Hz), 129.82 (d, J = 15.8 Hz), 129.78, 128.7, 128.44 (d, J = 3.3 Hz), 128.39, 123.7 (d, J = 9.8 Hz), 122.2, 118.7 (d, J = 24.1 Hz), 93.3 (d, J = 3.2 Hz), 88.2, 71.5, 69.3, 52.3, 40.7, 34.1, 24.8 (d, J = 2.6 Hz), 23.9; ESI-HRMS calcd for $C_{22}H_{23}FO_5SNa$ ($M+Na^+$) 441.1142, found 441.1140.

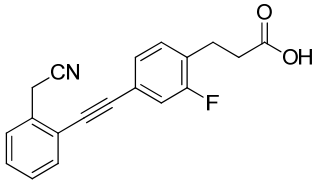
3-(2-Fluoro-4-((3-((3-(methylsulfonyl)propoxy)methyl)phenyl)ethynyl)phenyl)propanoic acid (20).



Step 1: 1-Bromo-3-((3-(methylsulfonyl)propoxy)methyl)benzene (**20a**) was prepared from 3-(methylsulfonyl)propan-1-ol (161 mg, 1.16 mmol) and 3-bromobenzyl bromide (250 mg, 1.00 mmol) according to the general procedure to give 131 mg (42%) of a clear oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:2→1:0): R_f = 0.13 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.48–7.45 (m, 1H), 7.45–7.41 (m, 1H), 7.25–7.21 (m, 2H), 4.48 (s, 2H), 3.61 (t, J = 5.9 Hz, 2H), 3.20–3.12 (m, 2H), 2.91 (s, 3H), 2.21–2.11 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 140.3, 130.9, 130.6, 130.1, 126.1, 122.6, 72.2, 68.0, 52.0, 40.7, 23.0; ESI-MS *m/z* 329.0 ($M+Na^+$). Step 2: Methyl 3-(2-fluoro-4-((3-((3-(methylsulfonyl)propoxy)methyl)phenyl)ethynyl)phenyl)propanoate (**20b**) was prepared from **2** (93 mg, 0.45 mmol) and **20a** (125 mg, 0.40 mmol) according to the general procedure I to give 87 mg (50%) of a sticky yellow oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:1): R_f = 0.13 (EtOAc:petroleum ether, 1:1); ^{1}H NMR ($CDCl_3$) δ 7.50–7.43 (m, 2H), 7.34 (t, J = 7.5 Hz, 1H), 7.31–7.27 (m, 1H), 7.26–7.22 (m, 1H), 7.21–7.16 (m, 2H), 4.51 (s, 2H), 3.67 (s, 3H), 3.62 (t, J = 5.8 Hz, 2H), 3.21–3.13 (m,

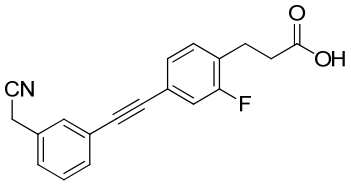
2H), 2.99 (t, J = 7.7 Hz, 2H), 2.91 (s, 3H), 2.64 (t, J = 7.7 Hz, 2H), 2.22–2.12 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 172.9, 160.7 (d, J = 246.2 Hz), 138.3, 131.1, 130.8, 130.7 (d, J = 5.6 Hz), 128.6, 128.1 (d, J = 15.9 Hz), 127.8, 127.6 (d, J = 3.3 Hz), 123.12, 123.05 (d, J = 9.8 Hz), 118.3 (d, J = 23.8 Hz), 89.7, 88.4 (d, J = 3.1 Hz), 72.6, 67.9, 52.1, 51.7, 40.7, 34.0 (d, J = 1.2 Hz), 24.6 (d, J = 2.4 Hz), 23.1; ESI-MS *m/z* 455.1 (M+Na$^+$). Step 3: **20** was prepared from **20b** (70 mg, 0.16 mmol) according to the general procedure II to give 58 mg (85%) of a white solid (t_R = 10.97, purity: 98.1% by HPLC): ^{1}H NMR (Acetone-d_6) δ 7.41 (d, J = 0.7 Hz, 1H), 7.36–7.32 (m, 1H), 7.29–7.23 (m, 3H), 7.18 (dd, J = 7.8 Hz, 1.5 Hz, 1H), 7.14 (dd, J = 10.7 Hz, 1.5 Hz, 1H), 4.43 (s, 2H), 3.52 (t, J = 6.1 Hz, 2H), 3.10–3.02 (m, 2H), 2.85 (t, J = 7.6 Hz, 2H), 2.81 (s, 3H), 2.53 (t, J = 7.6 Hz, 2H), 2.01–1.95 (m, 2H); ^{13}C NMR (Acetone-d_6) δ 173.5, 161.6 (d, J = 244.7 Hz), 140.2, 132.0 (d, J = 5.7 Hz), 131.4, 131.3, 129.7 (d, J = 15.9 Hz), 129.5, 128.8, 128.5 (d, J = 3.3 Hz), 123.8 (d, J = 9.9 Hz), 123.6, 118.7 (d, J = 24.1 Hz), 90.5, 88.8 (d, J = 3.1 Hz), 72.7, 69.0, 52.3, 40.7, 34.1, 24.8 (d, J = 2.6 Hz), 23.8; ESI-HRMS calcd for $C_{22}H_{23}FO_5SNa$ (M+Na$^+$) 441.1142, found 441.1135.

3-(4-((2-(Cyanomethyl)phenyl)ethynyl)-2-fluorophenyl)propanoic acid (22).



Step 1: Methyl 3-(4-((2-(cyanomethyl)phenyl)ethynyl)-2-fluorophenyl)propanoate (**22a**) was prepared from **2** (105 mg, 0.51 mmol) and 2-(2-iodophenyl)acetonitrile (149 mg, 0.61 mmol) according to the general procedure I to give 105 mg (64%) of pale yellow solid after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5): R_f = 0.29 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.53 (dd, J = 24.1 Hz, 7.4 Hz, 2H), 7.43–7.31 (m, 2H), 7.30–7.17 (m, 3H), 3.95 (s, 2H), 3.68 (s, 3H), 3.00 (t, J = 7.6 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.9, 160.7 (d, J = 248.5 Hz), 132.5, 131.8, 130.9 (d, J = 5.1 Hz), 129.3, 128.7 (d, J = 16.2 Hz), 128.3, 128.3, 127.6 (d, J = 3.0 Hz), 122.5, 122.4 (d, J = 9.1 Hz), 118.3 (d, J = 24.2 Hz), 117.3, 94.4 (d, J = 3.3 Hz), 86.5, 51.7, 33.9 (d, J = 1.0 Hz), 24.6 (d, J = 2.2 Hz), 22.8; ESI-MS *m/z* 344.1 (M+Na$^+$). Step 2: **22** was prepared from **22a** (73 mg, 0.23 mmol) according to the general procedure II to give 48 mg (69%) of a white solid (t_R = 11.65, purity: 98.7% by HPLC) after purification by flash chromatography (SiO_2, EtOAc[with 1% AcOH]:petroleum ether, 2:3): ^{1}H NMR (Acetone-d_6) δ 7.60 (dd, J = 17.5 Hz, 7.5 Hz, 2H), 7.53–7.33 (m, 5H), 4.16 (s, 2H), 2.99 (t, J = 7.6 Hz, 2H), 2.67 (t, J = 7.6 Hz, 2H); ^{13}C NMR (Acetone-d_6) δ 173.5, 161.5 (d, J = 245.8 Hz), 134.1, 133.2, 132.0 (d, J = 6.1 Hz), 130.4, 130.1 (d, J = 15.2 Hz), 129.6, 129.1, 128.5 (d, J = 3.0 Hz), 123.3 (d, J = 9.9 Hz), 123.3, 118.8 (d, J = 24.3 Hz), 118.4, 94.8 (d, J = 3.0 Hz), 87.4, 34.0, 24.8 (d, J = 2.5 Hz), 22.9; ESI-HRMS calcd for $C_{19}H_{14}FNO_2Na$ (M+Na$^+$) 330.0901, found 330.0907.

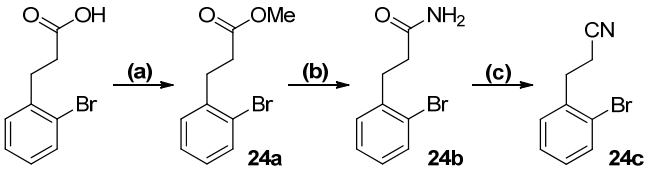
3-(4-((3-(Cyanomethyl)phenyl)ethynyl)-2-fluorophenyl)propanoic acid (23).



Step 1: Methyl 3-(4-((3-(cyanomethyl)phenyl)ethynyl)-2-fluorophenyl)-propanoate (**23a**) was prepared from **2** (104 mg, 0.50 mmol) and 2-(3-bromophenyl)acetonitrile (107 mg, 0.55 mmol) according to the general procedure IB to give 100 mg (62%) of a yellow oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:3): R_f = 0.39 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.52–7.46 (m, 2H), 7.37 (t, J = 7.6 Hz, 1H), 7.33–7.29 (m, 1H), 7.26–7.15 (m, 3H), 3.76 (s, 2H), 3.68 (s, 3H), 2.99 (t, J = 7.7 Hz, 2H), 2.65 (t, J = 7.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.9, 160.7 (d, J = 247.5 Hz), 131.3, 131.0, 130.7 (d, J = 6.1 Hz), 130.3, 129.3, 128.4 (d, J = 16.2 Hz), 127.9, 127.6 (d, J = 3.0 Hz), 124.0, 122.8 (d, J = 10.1 Hz), 118.4 (d, J = 24.2 Hz), 117.4, 89.1 (d, J = 3.0 Hz), 88.9, 51.7, 34.0 (d, J = 1.0 Hz), 24.6 (d, J = 2.0 Hz), 23.4; ESI-MS *m/z* 344.1 (M+Na$^+$). Step 2: **23** was prepared from **23a** (62 mg, 0.19 mmol) according to the general procedure II to give 53 mg (91%) of a pale yellow solid (t_R = 11.27, purity: 98.8% by HPLC): ^{1}H NMR ($CDCl_3$) δ 7.52–7.46 (m, 2H), 7.37 (t, J = 7.6 Hz, 1H), 7.33–7.28 (m, 1H), 7.27–7.16 (m, 3H), 3.76 (s, 2H), 3.00 (t, J = 7.6 Hz, 2H), 2.71 (t, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 178.3, 160.7 (d, J = 247.5 Hz), 131.3, 131.0, 130.7 (d, J = 6.1 Hz), 130.3, 129.3, 128.00 (d, J = 16.2 Hz), 127.96, 127.7 (d, J = 4.0 Hz), 124.0, 122.9 (d, J = 9.1 Hz), 118.4 (d, J = 24.2

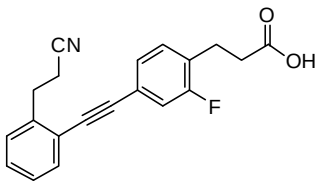
Hz), 117.4, 89.05 (d, J = 3.0 Hz), 88.98, 33.8 (d, J = 1.0 Hz), 24.3 (d, J = 2.0 Hz), 23.4; ESI-HRMS calcd for $C_{19}H_{14}FNO_2Na$ (M+Na^+) 330.0901, found 330.0901.

Scheme S2[a]



[a] Reagents and conditions: (a) AcCl, MeOH, 0 ºC → room temp, 2 d, 64%; (b) 30% $NH_{3(aq)}$, 1,4-dioxane, room temp, 24 h; (c) TsCl, pyridine, CH_2Cl_2, room temp, 1 d, then sat. $NaHCO_{3(aq)}$, room temp, 45 min, 67% over two steps.

3-(4-((2-(2-Cyanoethyl)phenyl)ethynyl)-2-fluorophenyl)propanoic acid (24).



Step 1: A dried round-bottom flask with dry MeOH (22 mL) under argon at 0 °C was added AcCl (1.9 mL, 26.2 mmol) dropwise over 5 min. The solution was stirred for 10 min before addition of 3-(2-bromophenyl)propanoic acid (2.01 g, 8.7 mmol). The reaction was allowed to reach room temperature and stirred for 2 days. The reaction was concentrated, redissolved in MeOH, concentrated and dried *in vacuo*. Purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:5) gave methyl 3-(2-bromophenyl)propanoate (**24a)** as a colorless oil (1.35 g, 64%): R_f = 0.46 (EtOAc:petroleum ether, 1:5); ^{1}H NMR ($CDCl_3$) δ 7.52 (d, J = 8.2 Hz, 1H), 7.27–7.18 (m, 2H), 7.06 (ddd, J = 8.1 Hz, 6.8 Hz, 2.5 Hz, 1H), 3.67 (s, 3H), 3.06 (t, J = 7.8 Hz, 2H), 2.65 (t, J = 7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 173.1, 139.8, 133.0, 130.5, 128.2, 127.6, 124.4, 51.7, 34.0, 31.5; ESI-MS *m/z* 265.0 (M+Na^+). Step 2: **24a** (601 mg, 2.48 mmol) was dissolved in 1,4-dioxane (15 mL) in a 100 mL round-bottom flask, to give a clear solution. Aqueous NH_3 (24%, 30 mL) was added, which turned the reaction mixture cloudy. The reaction was stirred at room temperature for 24 h, where after it was concentrated *in vacuo* to give crude 3-(2-bromophenyl)propanamide (**24b**) as a white solid (553 mg), which was used in the next step with no further purification. The crude product could be purified by flash chromatography (SiO_2, CH_2Cl_2:MeOH, 1:0.05) to give a white solid: R_f = 0.24 (CH_2Cl_2:MeOH, 1:0.05); ESI-MS *m/z* 228.0 (M+H^+). Step 3: To a solution of crude **24b** (301 mg, 1.32 mmol) and *p*-toluenesulfonyl chloride (502 mg, 2.6 mmol) in 5 mL CH_2Cl_2 in a 25 mL round bottom flask was added dry pyridine (0.53 mL, 6.6 mmol). The reaction was stirred at room temperature overnight, after which 6 mL sat. aq. $NaHCO_3$ was added and the mixture was stirred vigorously for 45 min. The layers were separated and the organic phase washed with 1M aq. HCl and sat. aq. $NaHCO_3$. The aqueous phases were extracted with CH_2Cl_2 and the combined organic phases dried over Na_2SO_4, concentrated *in vacuo* and purified by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:6) to give 3-(2-bromophenyl)propanenitrile (**24c**) as a clear oil (186 mg, 67% over two steps): R_f = 0.53 (SiO_2, CH_2Cl_2:MeOH, 10:1); ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 8.0 Hz, 1H), 7.34–7.27 (m, 2H), 7.19–7.12 (m, 1H), 3.09 (t, J = 7.4 Hz, 2H), 2.68 (t, J = 7.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 137.3, 133.3, 130.9, 129.3, 128.1, 124.2, 118.9, 32.2, 17.7; ESI-MS *m/z* 210.0 (M+H^+). Step 4: Methyl 3-(4-((2-(2-cyanoethyl)phenyl)ethynyl)-2-fluorophenyl)propanoate (**24d**) was prepared from **2** (108 mg, 0.52 mmol) and (**24c**) (120 mg, 0.57 mmol) according to the general procedure I to give 108 mg (62%) of a pale yellow oil after purification by flash chromatography (SiO_2, EtOAc:petroleum ether, 1:4): R_f = 0.23 (EtOAc:petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$) δ 7.54 (d, J = 7.6 Hz, 1H), 7.38–7.15 (m, 6H), 3.68 (s, 3H), 3.18 (t, J = 7.5 Hz, 2H), 3.00 (t, J = 7.6 Hz, 2H), 2.74 (t, J = 7.5 Hz, 2H), 2.65 (t, J = 7.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 173.0, 160.8 (d, J = 246.7 Hz), 139.9, 132.8, 130.9 (d, J = 5.6 Hz), 129.3, 129.2, 128.6 (d, J = 16.0 Hz), 127.6 (d, J = 3.3 Hz), 127.5, 122.8 (d, J = 9.6 Hz), 122.3, 119.2, 118.3 (d, J = 23.8 Hz), 92.9 (d, J = 3.2 Hz), 87.5, 51.8, 34.0 (d, J = 1.2 Hz), 30.8, 24.7 (d, J = 2.3 Hz), 18.2; ESI-Ms *m/z* 358.1 (M+Na^+). Step 5: **24** was prepared from **24d** (60 mg, 0.18 mmol) according to the general procedure II to give 57 mg (100%) of a white solid (t_R = 11.76, purity: 99% by HPLC): ^{1}H NMR (DMSO-d_6) δ 12.24 (s, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.45–7.31 (m, 6H), 3.12 (t, J = 7.1 Hz, 2H), 2.90 (t, J = 7.3 Hz, 2H), 2.88 (t, J = 7.7 Hz, 2H), 2.56 (t, J = 7.6 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 173.3,

160.0 (d, J = 244.7 Hz), 140.5, 132.0, 131.0 (d, J = 5.6 Hz), 129.2, 129.2, 128.8 (d, J = 16.0 Hz), 127.5, 127.2, 121.8 (d, J = 9.8 Hz), 121.4, 119.9, 117.7 (d, J = 23.8 Hz), 92.2 (d, J = 3.2 Hz), 87.5, 33.4, 29.3, 23.5 (d, J = 2.3 Hz), 17.2; ESI-HRMS calcd for $C_{20}H_{16}FNNaO_2$ ($M+Na^+$) 344.1063, found 344.1052.

Physicochemical properties

Aqueous solubility

10 µL of a 10 mM compound stock solution in DMSO was added to an Eppendorf tube containing 490 µL phosphate buffer (10 mM, pH = 7.4). The sample was shaken (25 °C, 800 rpm) for 24 h, centrifuged, and the supernatant transferred to an HPLC vial before analysis by HPLC. Each compound was analyzed in triplicate, each parallel with double injections. The solubility was calculated from the peak area relative to a reference sample (200 µM in MeOH/milliQ water, 60/40, v/v).

Chemical stability

5 µL of a 10 mM compound stock solution in DMSO was added to an Eppendorf tube containing 995 µL phosphate buffer (10 mM, pH = 7.4). The sample was shaken at 37 °C and 600 rpm for 12 days. 100 µL was withdrawn and analyzed by HPLC. The study was performed in duplicate, each parallel with double injections.

Biological Assays

Calcium Mobilization Assays

hFFA1 assay: 1321N1 cells stably transfected with human FFA1 were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 400 µg/mL G418. Calcium measurements were performed using a NOVOstar® microplate reader with a built-in pipetor (BMG LabTech, Offenburg, Germany). Cells were seeded in 96-well tissue-culture plates at a density of 30,000–40,000 cells per well. After 24 h, cells were washed twice in Krebs-HEPES buffer (KHB: 118.6 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 11.7 mM *D*-glucose, 10 mM HEPES (free acid), 1.3 mM $CaCl_2$ and 1.2 mM $MgSO_4$, pH 7.4) and loaded with 1.5 µM Oregon Green 488 BAPTA-1/AM (Molecular Probes, Eugene, OR) and 0.03% Pluronic F-127 (Invitrogen, Karlsruhe, Germany) for 1 h (37 °C, 5% CO_2). After addition of KHB, microplates were directly transferred to Novostar and kept at 37 °C under exclusion of light for 15 min until the measurement was started. Test compound solution was injected sequentially into separate wells and fluorescence was measured at 520 nm (bandwidth 25 nm) for 50 intervals of 0.4 seconds each. The excitation wavelength was 485 nm (bandwidth 25 nm).

mFFA1 assay: The mouse ortholog of FFA1 (mFFA1) was amplified from genomic mouse DNA without its stop codon by PCR and inserted into the pcDNA5 FRT/TO expression vector upstream of a sequence encoding enhanced yellow fluorescent protein. This plasmid was then used to generate a stable inducible Flp-In™ T-REx™ 293 cell line that express mFFA1 on demand following induction with the antibiotic doxycycline. Experiments to assess compound **22** at mFFA1 using a Ca^{2+} mobilization assay were then carried out on these cells by first plating 80000 cells per well in black with clear bottom 96 well plates, then inducing mFFA1 expression with doxycycline (100 ng/mL). Cells were then cultured overnight before labeling with the calcium sensitive dye Fura2-AM for 45 min. Cells were washed three times with Hank's Balanced Salt Solution (HBSS) and incubated 15 minutes in HBSS prior to the assay. Fura-2 fluorescent emission at 510 nm resulting from 340 or 380 nm excitation was then monitored using a Flexstation (Molecular Devices, Sunnyvale, CA, USA) plate reader set to incubate the assay plate at 37 °C. Basal fluorescence was measured for 16 s, test compounds were then added, and fluorescence was measured for an additional 74 s. The maximum difference in 340/380 ratios obtained before and after compound addition was then used to plot concentration-response data.

BRET β-Arrestin 2 Interaction Assay

Plasmids encoding human FFA4, human FFA1 or rat FFA1 fused at their C-terminal to enhanced yellow fluorescent protein were cotransfected into HEK 293 cells with a plasmid encoding β-arrestin 2 fused to Renilla luciferase. Cells were distributed into white 96-well plates 24 h post-transfection and then maintained in culture for another 24 h prior to their use. To conduct the assay, cells were first washed in Hank's Balanced Salt Solution and then the Renilla luciferase substrate coelenterazine h (2.5 µM), and the ligand of interest were added. Cells were incubated at 37 °C for 5 min before luminescence at 535 and 475 nm was measured using a Pherastar FS plate reader. The ratio of luminescence at 535/475 nm was then used to calculate the BRET response.

Label-Free Dynamic Mass Redistribution (DMR) Assay

Cell based dynamic mass redistribution assays were performed as described previously in detail,[8-9] using a beta version of the Corning® Epic® Biosensor (Corning, NY, USA) or the Enspire® benchtop optical label-free system in conjunction with the Mini Janus liquid handing station (Perkin Elmer, Hamburg, Germany). Briefly, refractive waveguide grating optical biosensors, integrated in 384-well microplates, allow extremely sensitive measurements of changes in local optical density in a detecting zone up to 150 nm above the surface of the sensor. Cellular mass movements induced upon GPCR activation can be detected by illuminating the underside of the biosensor with polychromatic light and measurement of changes in wavelength of the reflected

monochromatic light that is a sensitive function of the index of refraction. The magnitude of this wavelength shift (in picometers) is directly proportional to the amount of DMR.
Cells were seeded at a density of 15,000 cells/well (HEK-293 cells transfected with FFA1, FFA2 or FFA3) and 30,000 cells/well (INS-1E) on fibronectin-coated biosensor plates and were cultivated for 20-24 h (37 °C, 5% CO_2) to obtain confluent monolayers. Before the assay, cells were washed twice with Hank's buffered salt solution (HBSS) containing 20 mM HEPES and incubated for 1 h in the Epic® reader at 28 °C. The sensor plate was then scanned and a baseline optical signature was recorded. Hereafter, compound solutions were transferred into the biosensor plate and DMR was monitored for at least 5,000 s.

Counterscreens

Counterscreen on hFFA2 and hFFA3 were performed in a label-free dynamic mass redistribution (DMR) assay using transfected HEK-293 cells, see detailed procedure below.

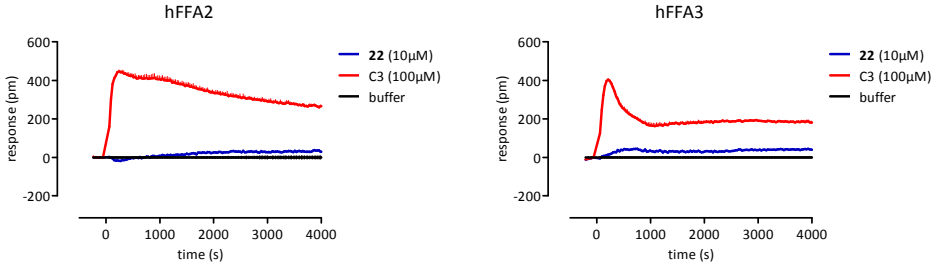


Figure S1. Counterscreen of **22** (10 µM) on hFFA2 and hFFA3-expressing HEK-293 cells. 15,000 cells/well were seeded into fibronectin-coated DMR plates and were cultivated overnight before stimulation with the test compound **22**, propionic acid (**C3**, 100 µM) as positive control or solvent (buffer).

Additional counterscreens were performed at Cerep Inc. **22** was dissolved in DMSO to a 10 mM stock solutions, which was diluted with water/HBSS to a final concentration of 10 µM. Results are shown in Table S1.

Table S1. Percent Inhibition of Radioligand Binding by **22** (10 µM)

Assay	% Inhibition
PPARγ (h) (agonist effect)	0
PPARγ (h) (antagonist effect)	-39
A_1 (h) (antagonist radioligand)	-11
A_{2A} (h) (agonist radioligand)	18
A_3 (h) (agonist radioligand)	-1
α_1 (non-selective) (antagonist radioligand)	-8
α_2 (non-selective) (antagonist radioligand)	-9
β_1 (h) (agonist radioligand)	9
β_2 (h) (agonist radioligand)	6
AT_1 (h) (antagonist radioligand)	-52
BZD (central) (agonist radioligand)	-15
B_2 (h) (agonist radioligand)	-1
CB_1 (h) (agonist radioligand)	-13
CCK_1 (CCK_A) (h) (agonist radioligand)	-39
D_1 (h) (antagonist radioligand)	-8
D_{2S} (h) (antagonist radioligand)	-12
ETA (h) (agonist radioligand)	-7

GABA (non-selective) (agonist radioligand)	-14
GAL_2 (h) (agonist radioligand)	-28
CXCR2 (IL-8B) (h) (agonist radioligand)	7
CCR1 (h) (agonist radioligand)	-1
H_1 (h) (antagonist radioligand)	1
H_2 (h) (antagonist radioligand)	-5
MC_4 (h) (agonist radioligand)	-23
MT_1 (ML_{1A}) (h) (agonist radioligand)	-10
M_1 (h) (antagonist radioligand)	-8
M_2 (h) (antagonist radioligand)	-22
M_3 (h) (antagonist radioligand)	4
NK_2 (h) (agonist radioligand)	-5
NK_3 (h) (antagonist radioligand)	1
Y_1 (h) (agonist radioligand)	-13
Y_2 (h) (agonist radioligand)	-22
NTS_1 (NT1) (h) (agonist radioligand)	-24
δ_2 (DOP) (h) (agonist radioligand)	8
κ (KOP) (agonist radioligand)	-13
μ (MOP) (h) (agonist radioligand)	13
NOP (ORL1) (h) (agonist radioligand)	-2
5-HT_{1A} (h) (agonist radioligand)	3
5-HT_{1B} (antagonist radioligand)	-6
5-HT_{2A} (h) (antagonist radioligand)	-3
5-HT_{2B} (h) (agonist radioligand)	7
5-HT_3 (h) (antagonist radioligand)	7
5-HT_{5a} (h) (agonist radioligand)	13
5-HT_6 (h) (agonist radioligand)	2
5-HT_7 (h) (agonist radioligand)	10
sst (non-selective) (agonist radioligand)	-7
$VPAC_1$ (VIP_1) (h) (agonist radioligand)	-6
V_{1a} (h) (agonist radioligand)	-1
Ca^{2+} channel (L, verapamil site, phenylalkylamine) (antagonist radioligand)	-5
K_V channel (antagonist radioligand)	-5
SK_{Ca} channel (antagonist radioligand)	-11
Na^+ channel (site 2) (antagonist radioligand)	-34
Cl^- channel (GABA-gated) (antagonist radioligand)	-12
norepinephrine transporter (h) (antagonist radioligand)	-20
dopamine transporter (h) (antagonist radioligand)	2
5-HT transporter (h) (antagonist radioligand)	12

In Vitro Toxicity

In vitro toxicity tests were performed at Cerep Inc. The results are given in Table S2. Compounds are considered to exert a toxic effect if there is a 40% effect on cell number, nuclear size and/or mitochondrial membrane potential, and a 20% effect on intracellular free calcium and membrane permeability.

Table S2. In vitro toxicity of **21** and **22** (1, 10 and 100 μM).

	21	**22**
Cell numbers	-9%, -7%, 12%	-19%, 12%, -5%
Free Ca^{2+}	0%, 1%, 8%	-2%, -1%, 3%
Nuclear size	3%, 6%, 13%	6%, 0%, 17%
Membrane permeability	1%, 3%, 3%	0%, 0%, 4%
Mitochondrial membrane potential	-5%, -2%, -14%	5%, 7%, 3%

Pharmacokinetic Analysis in Mice

The pharmacokinetic study in mice was performed at BioFocus, Inc. The pharmacokinetic properties of **22** were determined in mice (male, CD-1) following single intravenous (2.5 mg/kg) and oral (10 mg/kg) dosing. Intravenous doses were prepared at a concentration of 0.5 mg/mL in a mixture comprising 10% DMSO, 10% Cremophor EL and 80% mannitol solution (5% w/v in water) and administered in a volume of 5 mL/kg. Oral doses were prepared at a concentration of 1 mg/mL in a mixture comprising 10% DMSO, 10% Cremophor EL and 80% mannitol solution (5% w/v in water) and administered in a volume of 10 mL/kg. Blood samples obtained from three mice per time-point, at 8 time points: 5, 15, 30, 60 minutes, 2, 4, 6 and 8 hours after i.v. dosing, 15, 30, 60, 90 minutes, 2, 4, 6 and 8 hours after p.o. dosing. Blood was centrifuged to yield plasma which was analyzed for parent drug using LC-MS/MS (Waters Xevo TQ). Pharmacokinetic parameters were determined using the mean data from the mice (n=3) at each time-point. Non-compartmental analysis was performed using the software package PK Solutions 2.0 from Summit Research Services. AUC values were calculated by the trapezoidal method.

Glucose Tolerance Test in Normal Mice

Studies were conducted in accordance with UK Government Home Office regulations. 72 6-7 weeks old male C57Bl/6 mice (Charles River) were given chow diet (Bantin and Kingman, no 1 diet) and water *ad lib* and are given a few days rest to adapt to new environment and caging under controlled lighting conditions (lights on 8.00h, 12 h light/12 h dark) and at a room temperature of 21 ± 1 °C. Mice are randomized to achieve similar mean body weight in each cage. Each dose of test compound (2, 10, 50 or 250 mg/kg of **22**), vehicle or positive control (sitagliptin, 10 mg/kg) was given to 2 groups of 3 mice. Five hours prior to the start of the glucose tolerance test (07.00h), food was removed and animals were given clean cages. Mice were treated with vehicle or compound at 11.30h and glucose at 12.00h. Glucose was dissolved in water (2 g/10 mL) and given to the mice i.p. at a rate of 2 g/kg. Blood samples (20 μL) were taken for the analysis of glucose concentrations at -30, 0, 20, 40, 60, 90 and 120 minutes following glucose administration. 20 μL samples of blood were taken into disposable micro-pipettes (*Dade Diagnostics Inc., Aguada, Puerto Rico*) and glucose concentrations determined after mixing with 0.38 mL of haemolysis reagent. Duplicate 20 μL aliquots of this mixture was taken for each individual sample and placed in a 96-well assay plate. To each well was added 180 μL aliquots of glucose oxidase reagent (*ThermoTrace, Vicotria, Australia. Cat no TR5221*), the samples were mixed and then left for approximately 30 minutes. Samples were then analyzed automatically using a SpectraMax-250 and SoftMax Pro software (*Molecular Devices Corporation, 1311 Orleans Drive, Sunnyvale, California 94089, USA*). The results were converted into glucose concentration values using Prism software, version 3.0 (*GraphPad Software Inc., San Diego, California, USA*).

Glucose Tolerance Test in DIO Mice

C56Bl/6 male mice (Charles River, Manston, UK) were obtained at 5/6 weeks of age and fed on the 60% fat (by calories) diet D12492 (Research Diets Inc., New Brunswick, NJ 08901, USA) for 14 weeks. The mice were randomly assigned to either vehicle (10% w/v cremophor, 10% DMSO, 80% of 5% mannitol in water), **21** (20 mg/kg) or **22** (20 mg/kg). Each treatment was given to groups of 6 mice. The treatment was given daily for 4 weeks. A glucose tolerance test was undertaken on the first day of dosing as described above except the glucose load was given orally, at the rate of 3 g/kg, 30 min after giving the drug treatment. The glucose tolerance test was repeated after 22 days. In both cases **22** significantly improved glucose tolerance. After repeat dosing for 28 days, both the vehicle-treated mice and the mice given **22** were again subjected to a glucose tolerance test but on this occasion both sets of mice received compound **22** so that a comparison could be made between the efficacy of an acute treatment and chronic treatment in order to demonstrate any drug tolerance. Naive DIO mice acted as controls. During the study it was demonstrated that **22** had no effect on food intake, body weight, body composition (measured by dual energy X-ray absorptiometry, Piximus, Lunar Corp., Madison WI, USA) or plasma leptin concentration. **22** significantly improved the insulin sensitivity index (plasma glucose x plasma insulin) ($P < 0.05$ using Fisher's least-significant difference test).

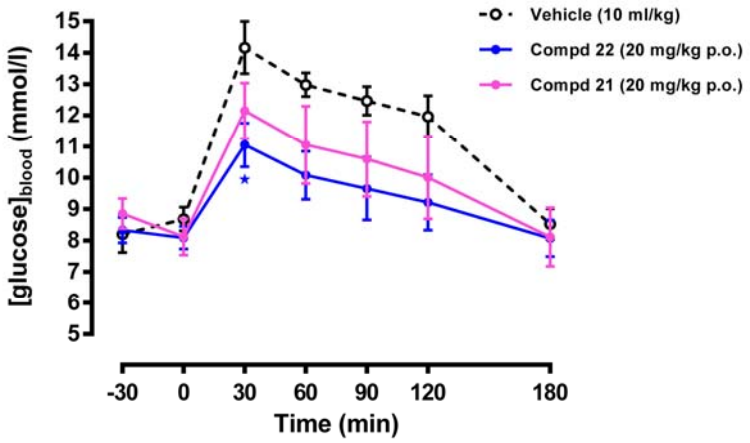


Figure S2. Oral glucose tolerance test (OGTT) in DIO mice after treatment with an acute single dose of **21** or **22** (20 mg/kg) or with vehicle.

Glucose Tolerance Test in Rats

Male Sprague-Dawley rats were obtained from Charles River (Manston, UK). The rats were fed on Bantin and Kingman No 1 chow diet (Hull, UK) ad-lib. Rats were randomly allocated to treatment groups of six and fasted overnight before being given the cremophor/DMSO/mannitol vehicle or **22** (10, 20 or 50 mg/kg) orally. Blood samples were obtained from the tail vein immediately prior to dosing with vehicle or **22** and 5 min later another blood sample taken followed by oral dosing of glucose (1 g/kg). Subsequent blood samples were taken at 5, 10, 15 and 30 min post-glucose. At each time point 20 µL of blood was used for the measurement of glucose as described above. For hormone assays, blood (200 µL) was collected into EDTA coated microvettes (Sarstedt, Numbrecht, Germany) and stored on ice for a maximum of 30 min followed by centrifugation at ~500g for 5 min. Plasma was stored at -80 °C until assayed for insulin. Plasma insulin was measured using plates with spots

in well pre-coated with capture antibodies to insulin (Mesoscale Discovery, Gaithersburg, MD, USA). 150 µL of blocker A solution was added to each well and the plate sealed and incubated for 1 h with shaking (300-1000 rpm) at room temperature. The plate is then washed three times with phosphate buffered saline containing 0.05% tween 20 (PBS-T). 20 µL of the metabolic assay working solution containing aprotinin is then dispensed into each well followed by 40 µL of sample and the plate sealed and incubated for 2 h at room temperature with vigorous shaking. The shaking accelerates the capture of the ligand. The plates are washed three times with PBS-T and then 25 µL of the detection antibody solution containing a Sulfo-Tag anti-mouse/rat insulin antibody. The plates are resealed and incubated for 1h with vigorous shaking. The plates are then washed three times with PBS-T and 150 µL read buffer added to provide the appropriate chemical environment for electrochemiluminescence. The plates are loaded into the MSD sector instrument where voltage application causes the labels bound to the electrode surface to emit light. The assay is calibrated against 8 samples giving a range of 0-50,000 pg/mL for insulin.