Supporting information for:

**Structure-Based Design of of 3-(4-Aryl-1*H*-1,2,3-Triazol-1-yl)-Biphenyl Derivatives as $P2Y_{14}$ Receptor Antagonists**

Anna Junker, Ramachandran Balasubramanian, Antonella Ciancetta, Elisa Uliassi, Evgeny Kiselev, Chiara Martiriggiano, Kevin Trujillo, Giorgi Mtchedlidze, Leah Birdwell, Kyle A. Brown, T. Kendall Harden, and Kenneth A. Jacobson

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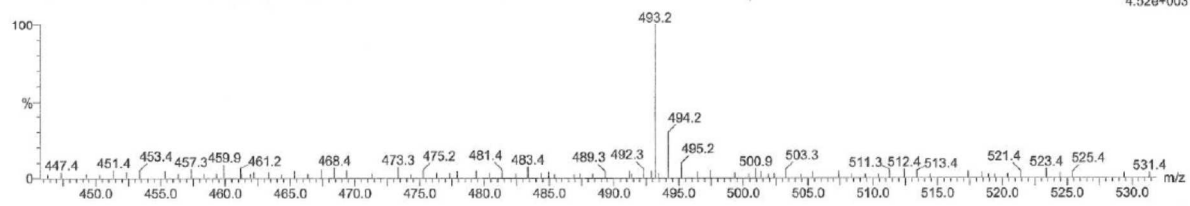
**5-(4-(4-(Trifluoromethyl)phenyl)-1H-1,2,3-triazol-1-yl)-4'-(Piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (65).**

Elemental Composition Report

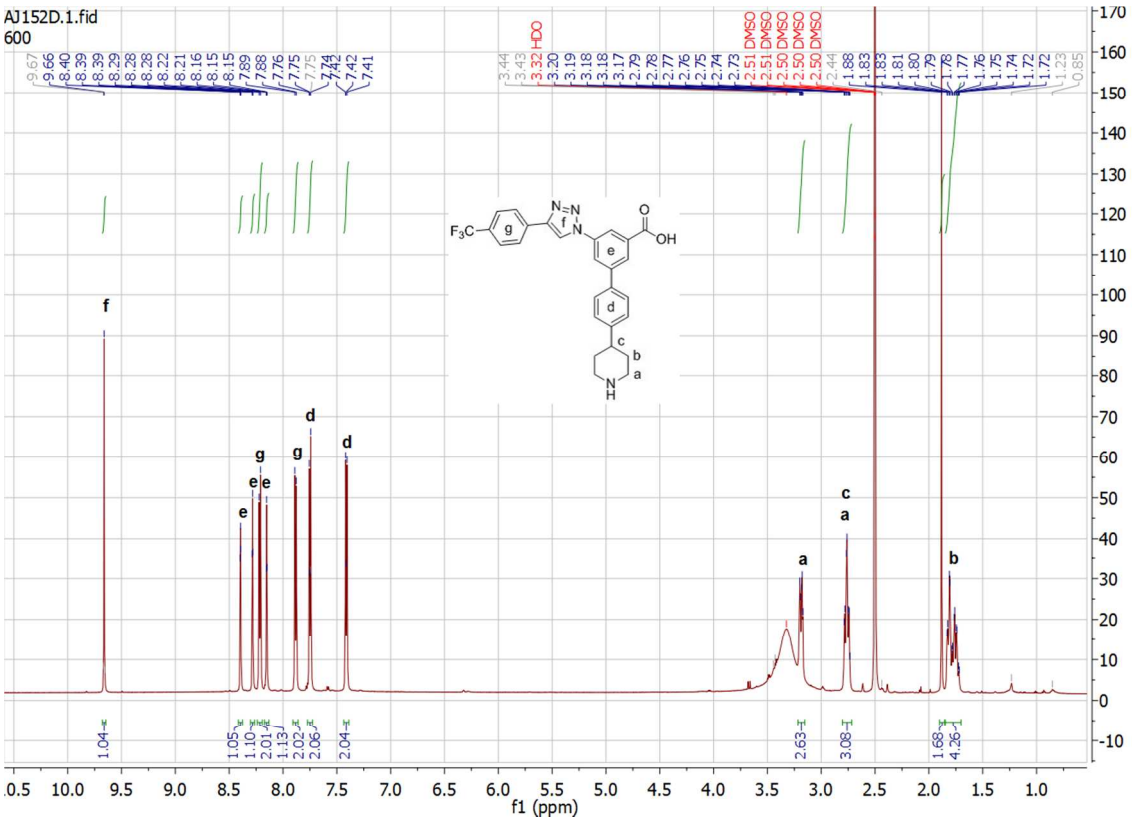
Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

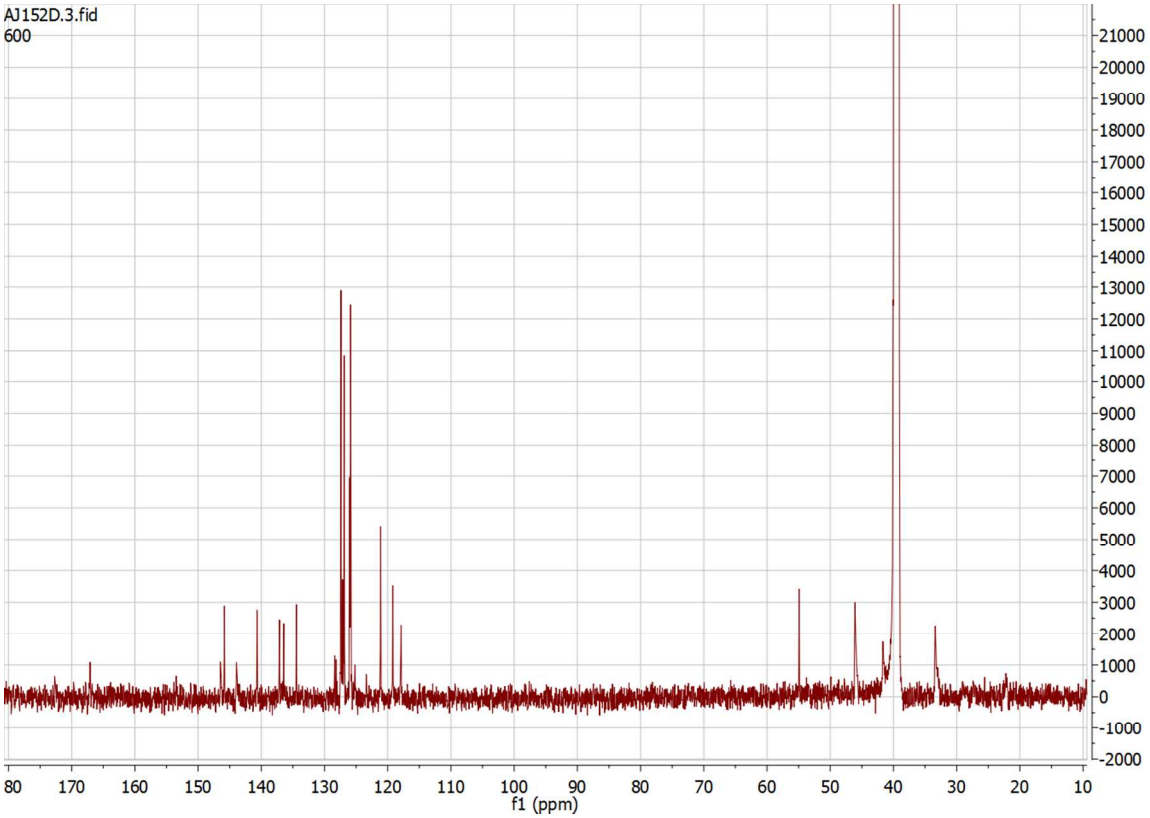
Monoisotopic Mass, Even Electron Ions
84 formula(e) evaluated with 3 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-120 H: 0-200 N: 4-4 O: 0-40 F: 3-3
15-Sep-2015
aj-15sep15-152d 162 (2.996) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00 )

TOF MS ES+
4.52e+003

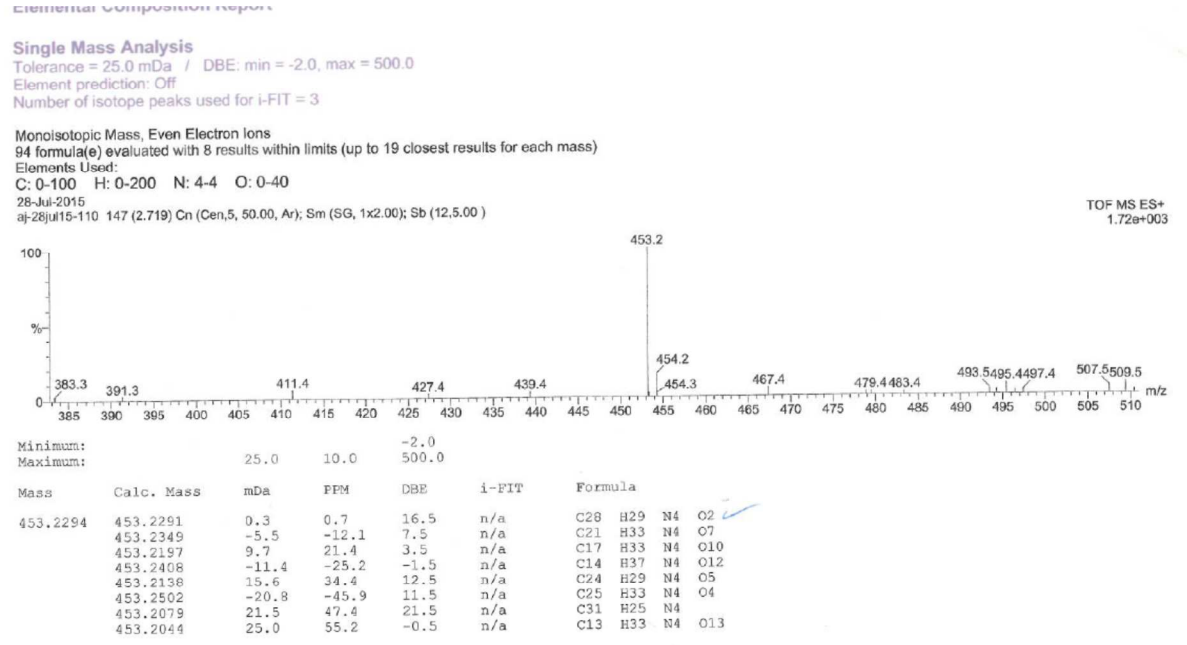



| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | -2.0 | | | |
| Maximum: | | 10.0 | 10.0 | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 493.1853 | 493.1851 | 0.2 | 0.4 | 16.5 | 47.4 | C27 H24 N4 O2 F3 |
| | 493.1910 | -5.7 | -11.6 | 7.5 | 76.3 | C20 H28 N4 O7 F3 |
| | 493.1758 | 9.5 | 19.3 | 3.5 | 136.0 | C16 H28 N4 O10 F3 |






**5-(4-(4-Ethylphenyl)-1H-1,2,3-triazol-1-yl)-4'-(piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (66).**



Single Mass Analysis
Tolerance = 25.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

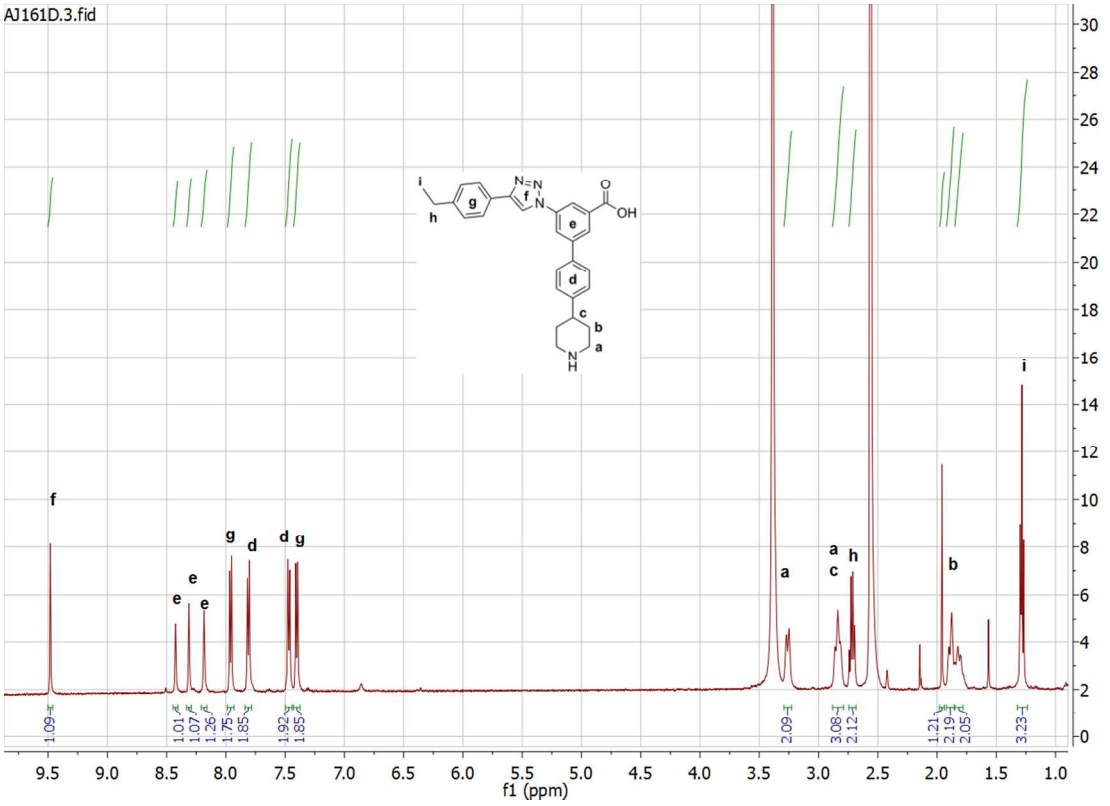
Monoisotopic Mass, Even Electron Ions
94 formula(e) evaluated with 8 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 4-4 O: 0-40

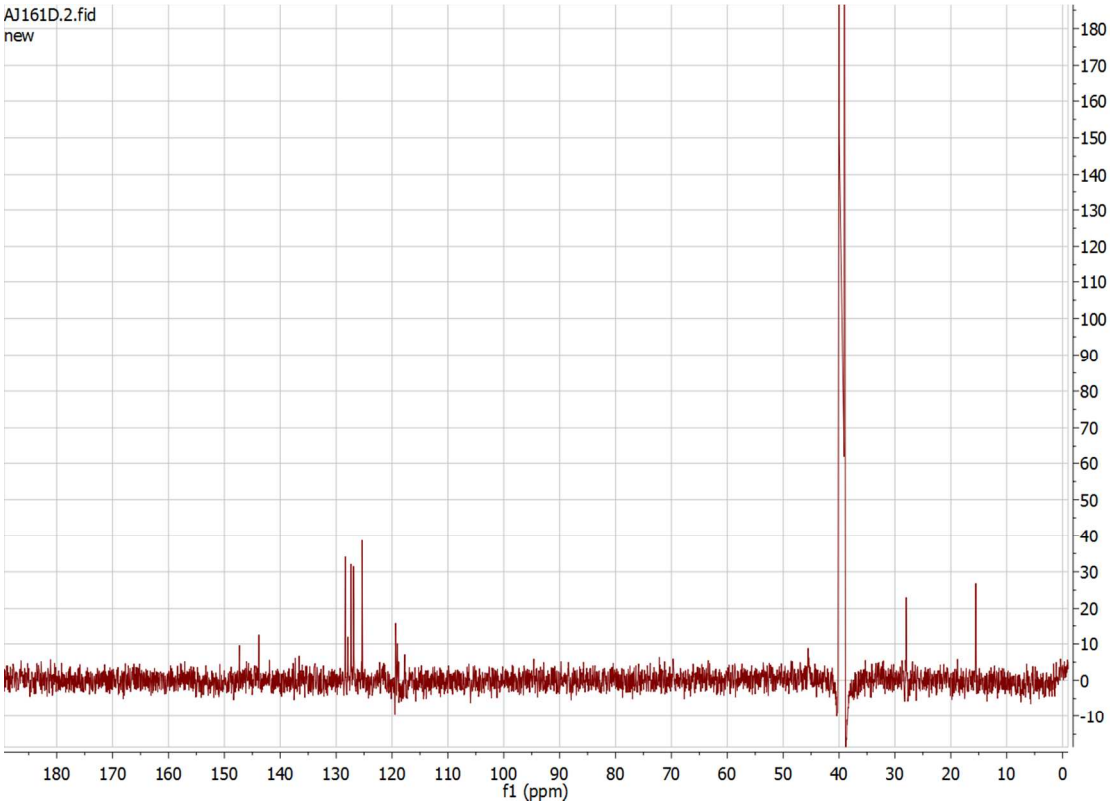
28-Jul-2015
aj-28jul15-110 147 (2.719) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00 )

TOF MS ES+
1.72e+003



| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 25.0 | 10.0 | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 453.2294 | 453.2291 | 0.3 | 0.7 | 16.5 | n/a | C28 H29 N4 O2 |
| | 453.2349 | -5.5 | -12.1 | 7.5 | n/a | C21 H33 N4 O7 |
| | 453.2197 | 9.7 | 21.4 | 3.5 | n/a | C17 H33 N4 O10 |
| | 453.2408 | -11.4 | -25.2 | -1.5 | n/a | C14 H37 N4 O12 |
| | 453.2138 | 15.6 | 34.4 | 12.5 | n/a | C24 H29 N4 O5 |
| | 453.2502 | -20.8 | -45.9 | 11.5 | n/a | C25 H33 N4 O4 |
| | 453.2079 | 21.5 | 47.4 | 21.5 | n/a | C31 H25 N4 |
| | 453.2044 | 25.0 | 55.2 | -0.5 | n/a | C13 H33 N4 O13 |


AJ161D.3.fid
f1 (ppm)


AJ161D.2.fid
new
f1 (ppm)

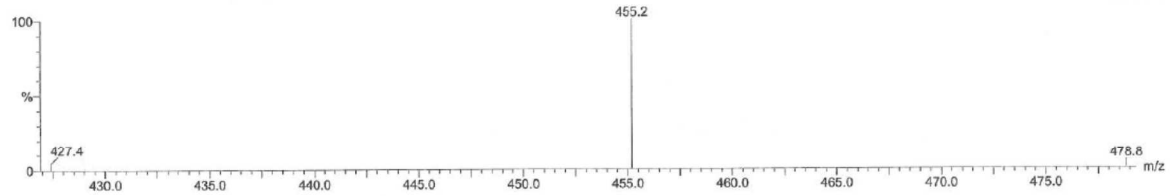
**5-(4-(4-(Hydroxymethyl)phenyl)-1H-1,2,3-triazol-1-yl)-4'-(piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (67).**

Elemental Composition Report

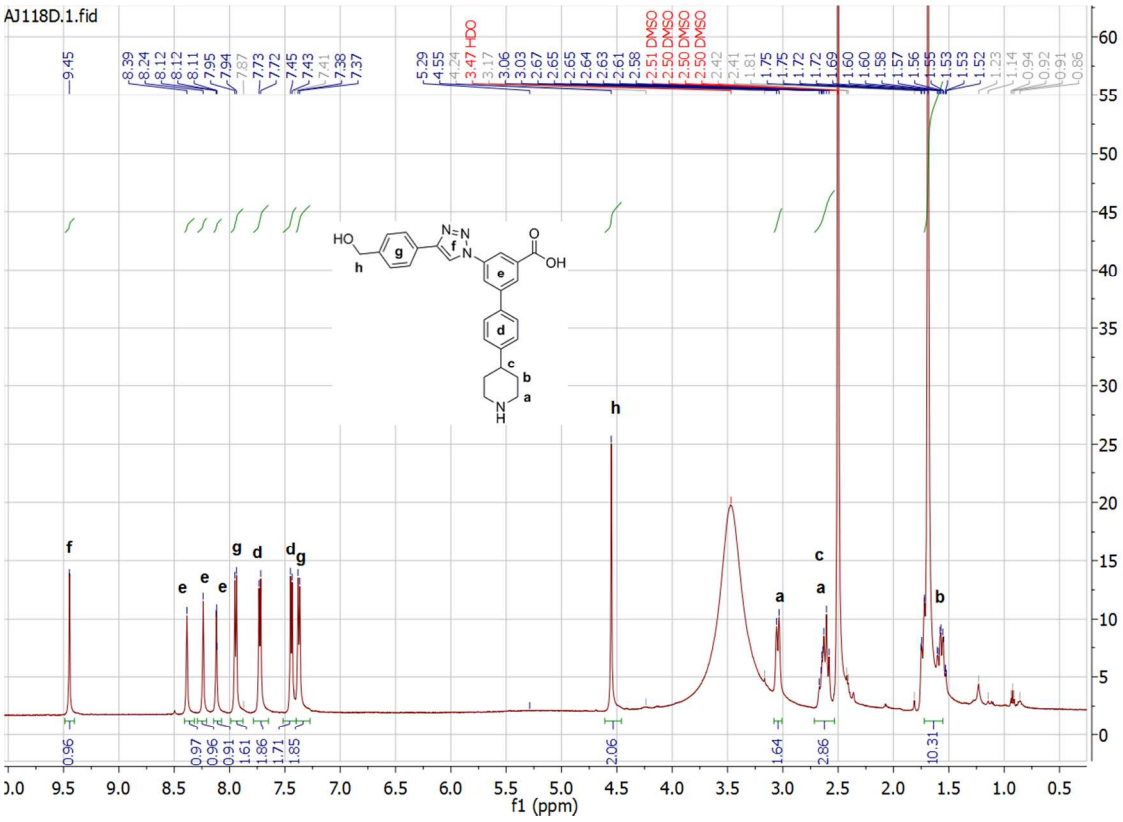
Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

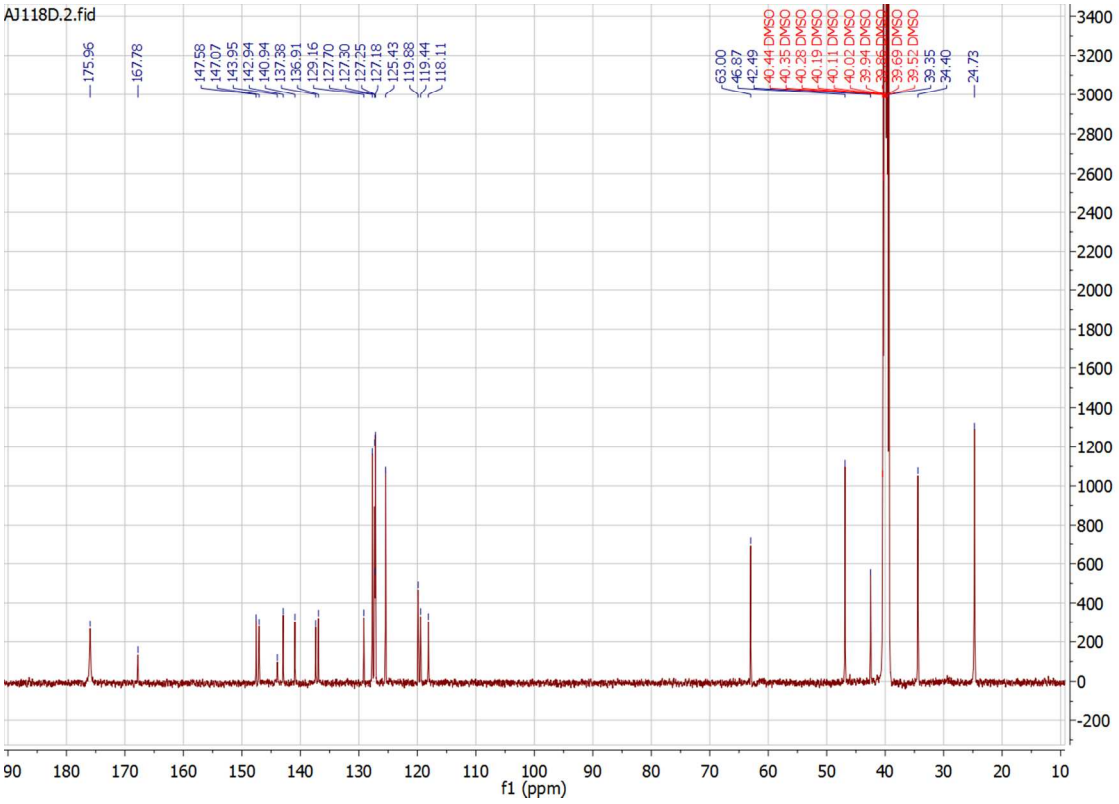
Monoisotopic Mass, Even Electron Ions
98 formula(e) evaluated with 3 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 4-4 O: 0-40
05-Aug-2015
aj-05aug15-118d 115 (2.127) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00 )

TOF MS ES+
3.38e+003

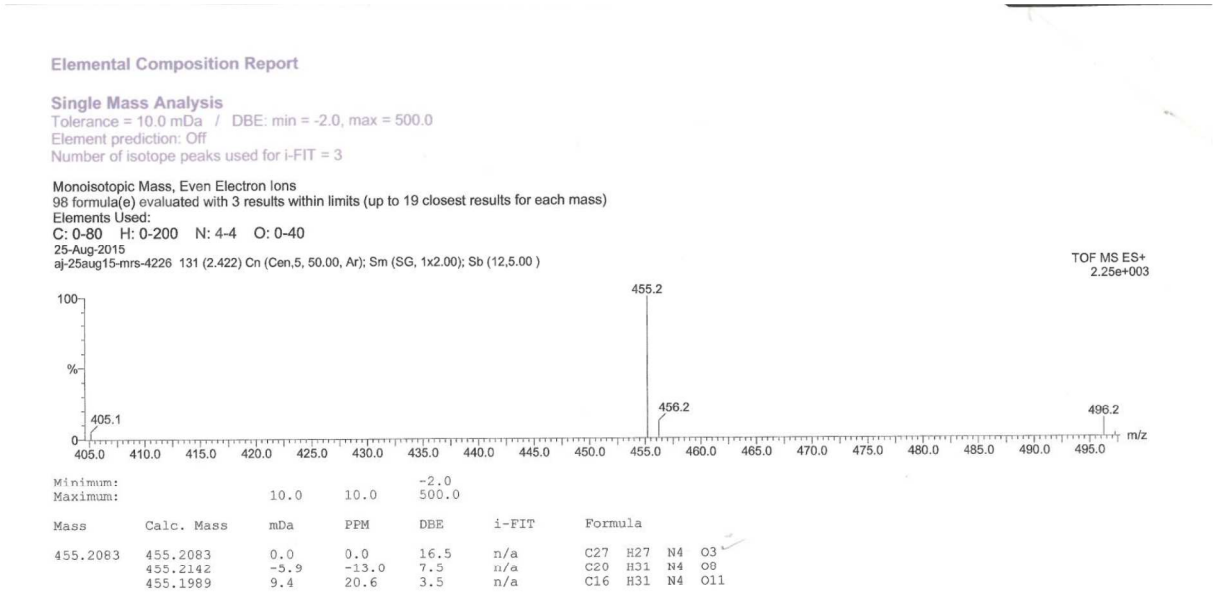



| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | 10.0 | 10.0 | -2.0 | | |
| Maximum: | | | | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 455.2081 | 455.2083 | -0.2 | -0.4 | 16.5 | n/a | C27 H27 N4 O3 |
| | 455.2142 | -6.1 | -13.4 | 7.5 | n/a | C20 H31 N4 O8 |
| | 455.1989 | 9.2 | 20.2 | 3.5 | n/a | C16 H31 N4 O11 |






**5-(4-(3-Methoxy-phenyl)-1H-1,2,3-triazol-1-yl)-4'-(piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (68).**



Elemental Composition Report

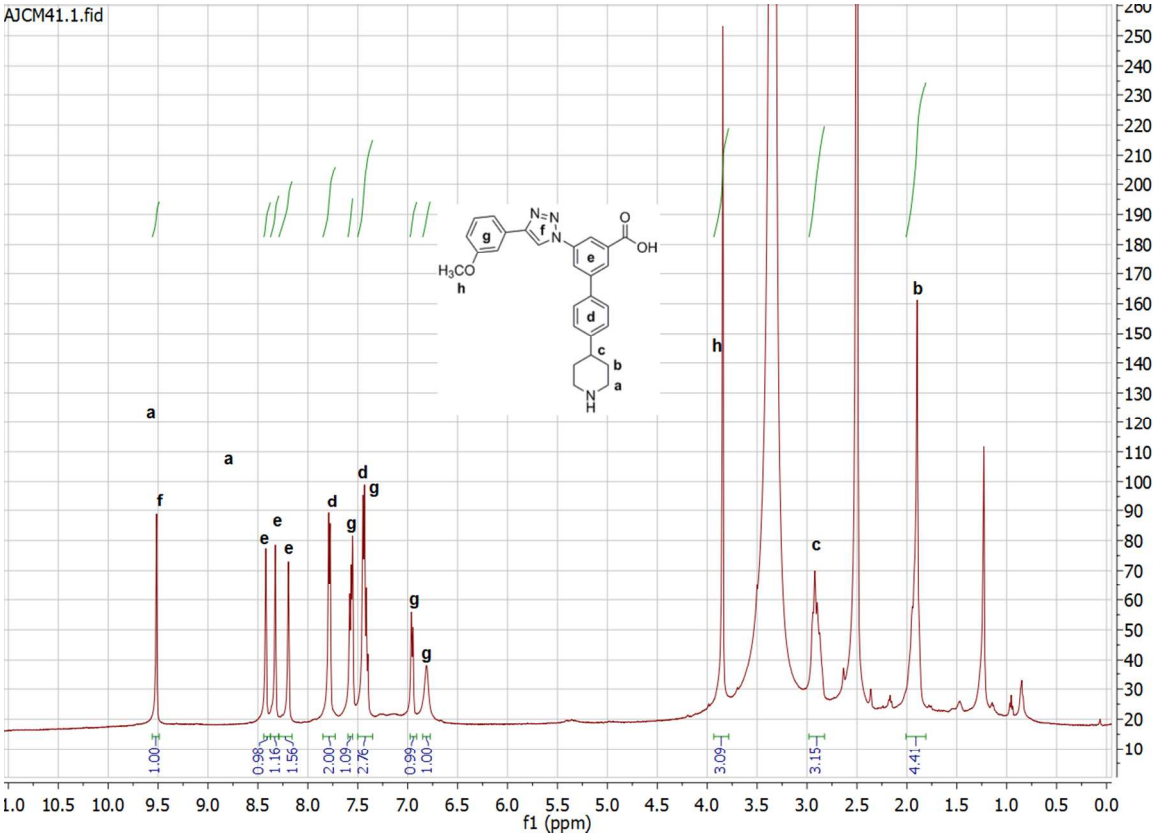
Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
98 formula(e) evaluated with 3 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-80 H: 0-200 N: 4-4 O: 0-40
25-Aug-2015
aj-25aug15-mrs-4226 131 (2.422) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00 )

TOF MS ES+
2.25e+003



| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 10.0 | 10.0 | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 455.2083 | 455.2083 | 0.0 | 0.0 | 16.5 | n/a | C27 H27 N4 O3 |
| | 455.2142 | -5.9 | -13.0 | 7.5 | n/a | C20 H31 N4 O8 |
| | 455.1989 | 9.4 | 20.6 | 3.5 | n/a | C16 H31 N4 O11 |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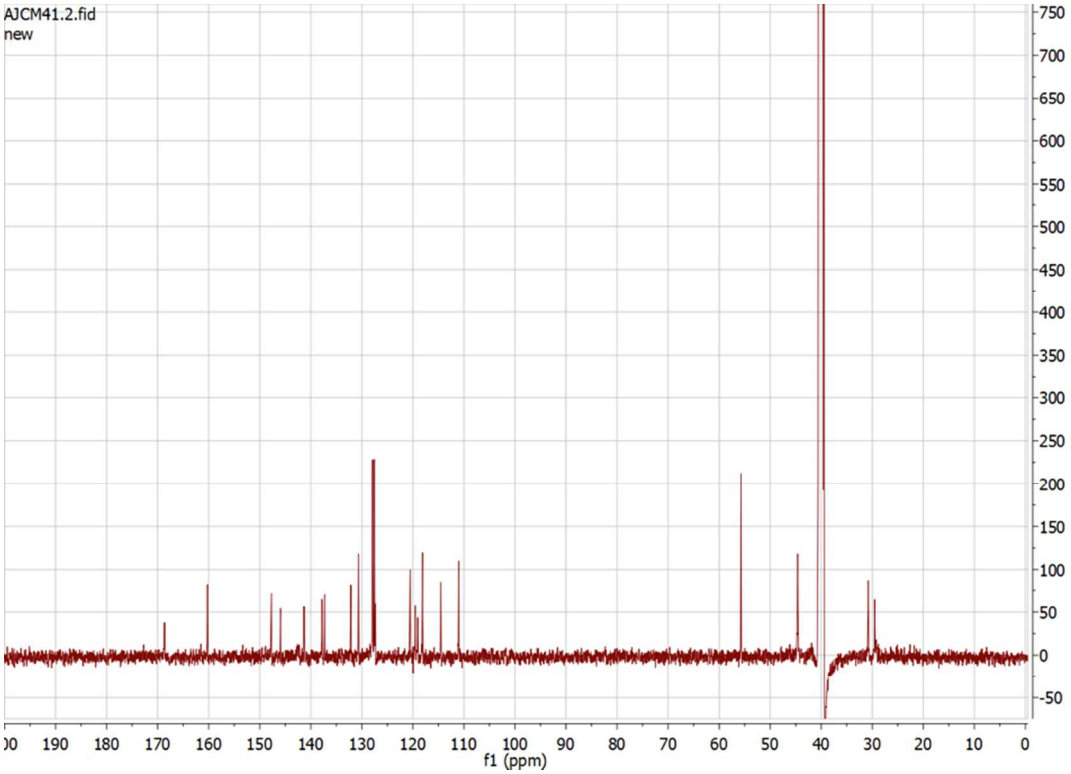
f1 (ppm)


AJCM41.2.fid
new
f1 (ppm)

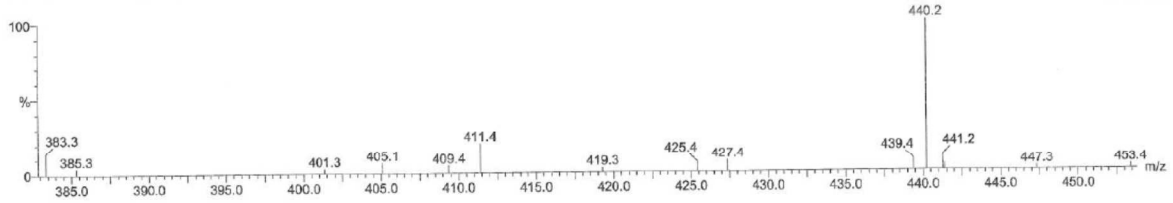
**5-(4-(4-Aminophenyl)-1H-1,2,3-triazol-1-yl)-4'-(piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (69)**

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

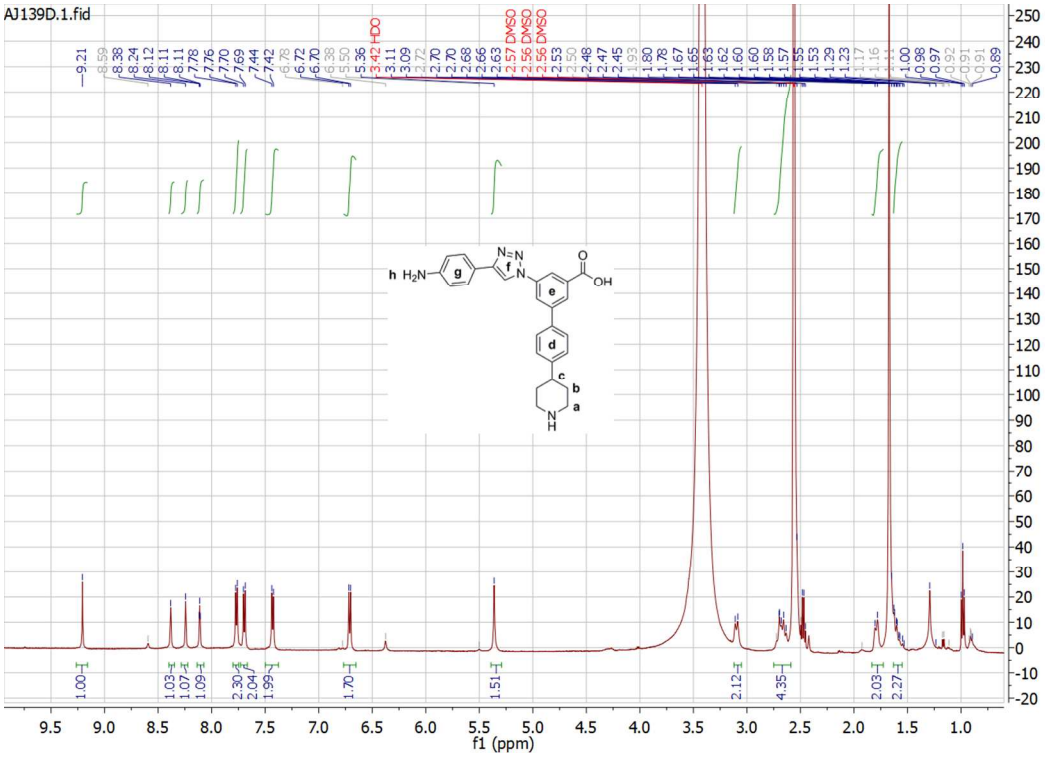
Monoisotopic Mass, Even Electron Ions
83 formula(e) evaluated with 2 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-120 H: 0-200 N: 5-5 O: 0-50

27-Aug-2015
aj-27aug15--139d 137 (2.534) Cn (Cen,5, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00 )

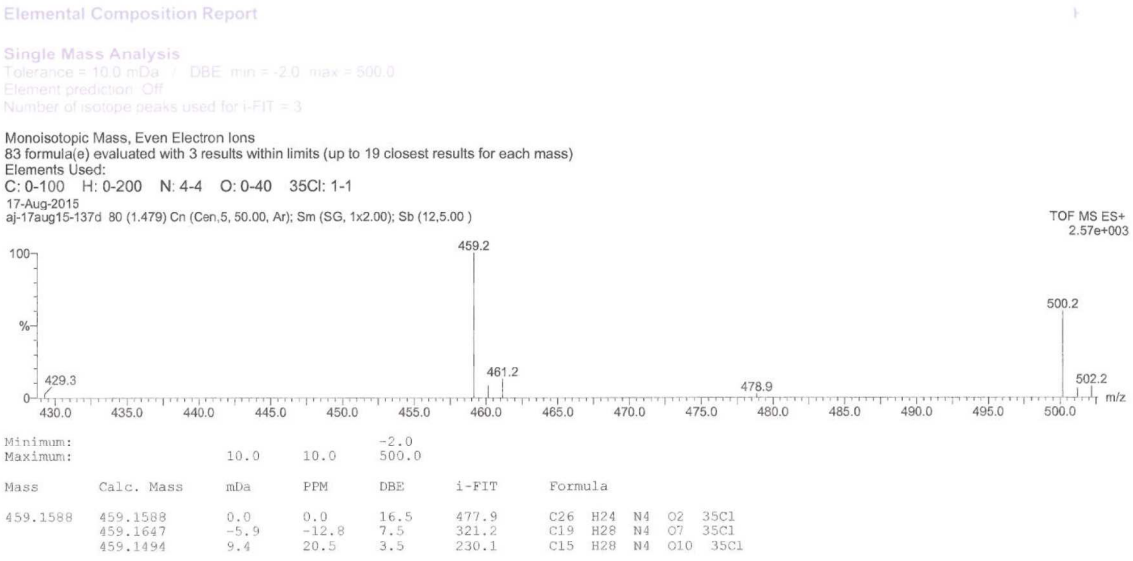
TOF MS ES+
4.50e+002



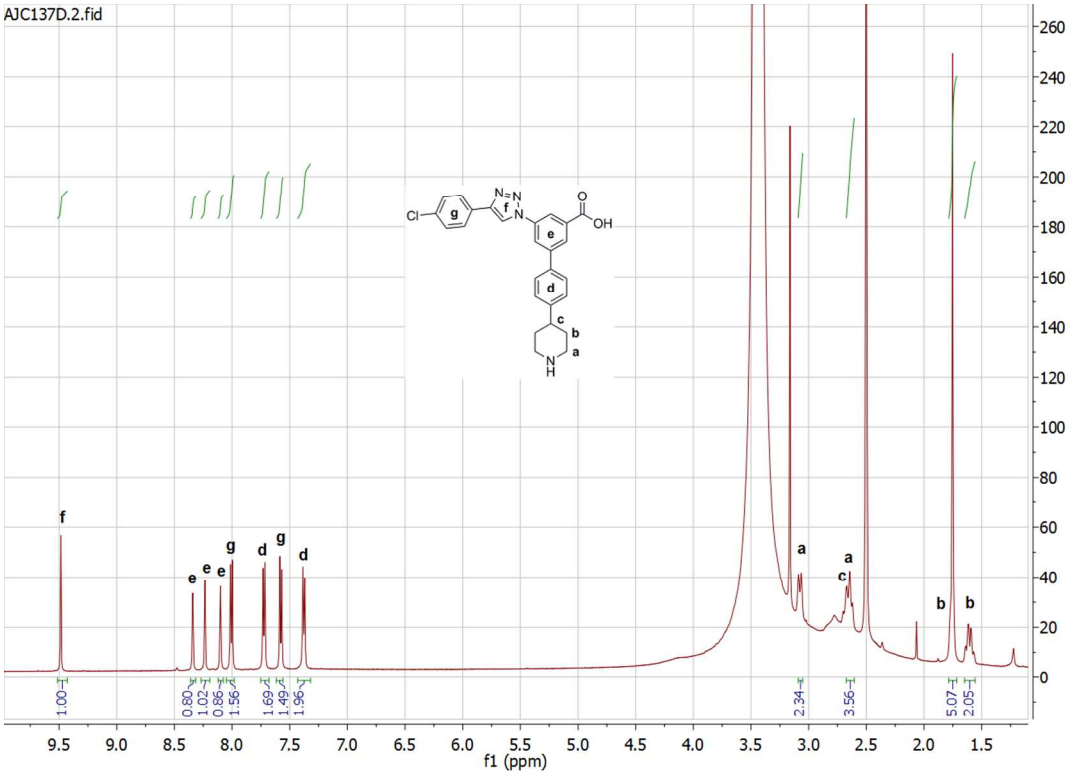

| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 10.0 | 10.0 | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 440.2094 | 440.2087 | 0.7 | 1.6 | 16.5 | n/a | C26 H26 N5 O2 |
| | 440.2145 | -5.1 | -11.6 | 7.5 | n/a | C19 H30 N5 O7 |

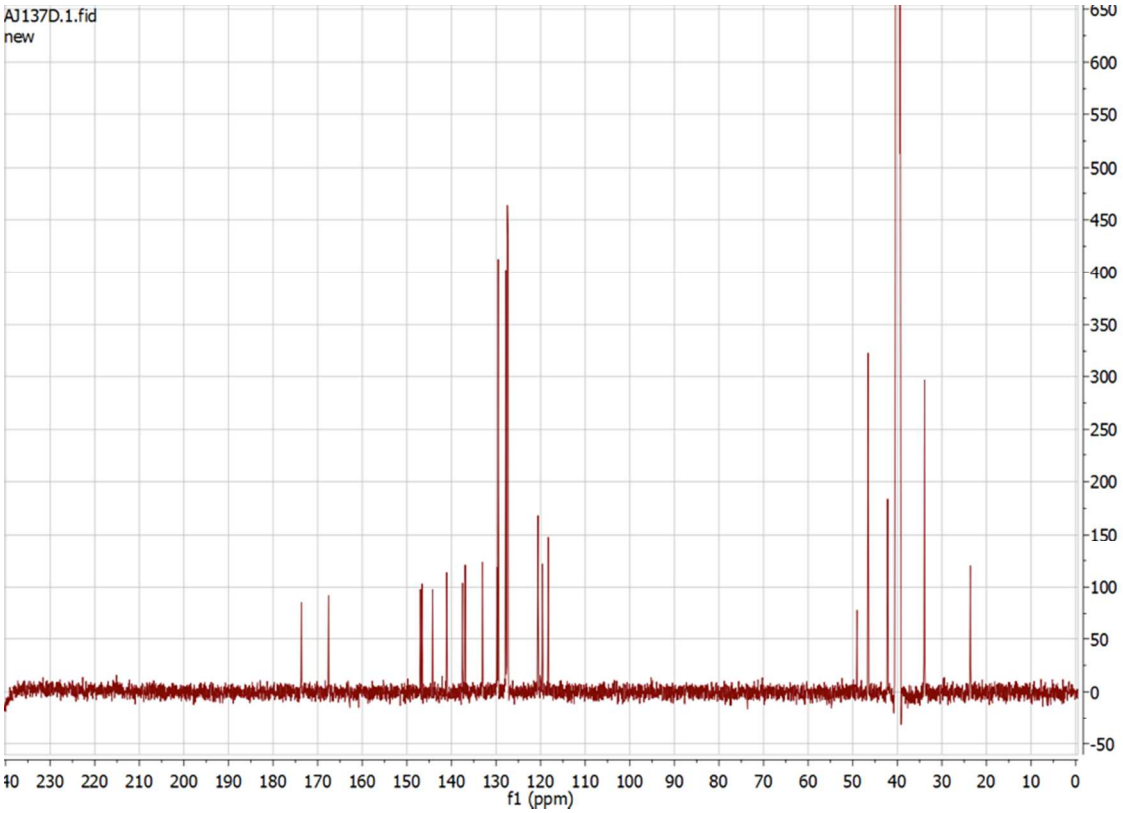



**5-(4-(4-Chlorophenyl)-1H-1,2,3-triazol-1-yl)-4'-(piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (70)**

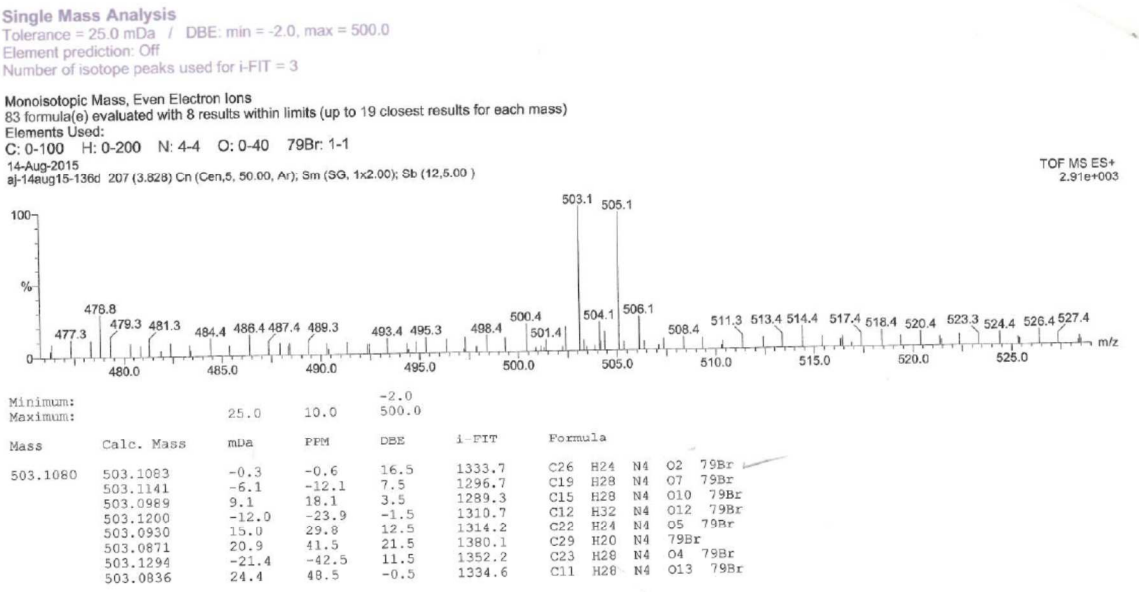



| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 10.0 | 10.0 | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 459.1588 | 459.1588 | 0.0 | 0.0 | 16.5 | 477.9 | C26 H24 N4 O2 35Cl |
| | 459.1647 | -5.9 | -12.8 | 7.5 | 321.2 | C19 H28 N4 O7 35Cl |
| | 459.1494 | 9.4 | 20.5 | 3.5 | 230.1 | C15 H28 N4 O10 35Cl |


AJC137D.2.fid
f1 (ppm)


AJ137D.1.fid
new
f1 (ppm)

**5-(4-(4-Bromophenyl)-1H-1,2,3-triazol-1-yl)-4'-(piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (71).**



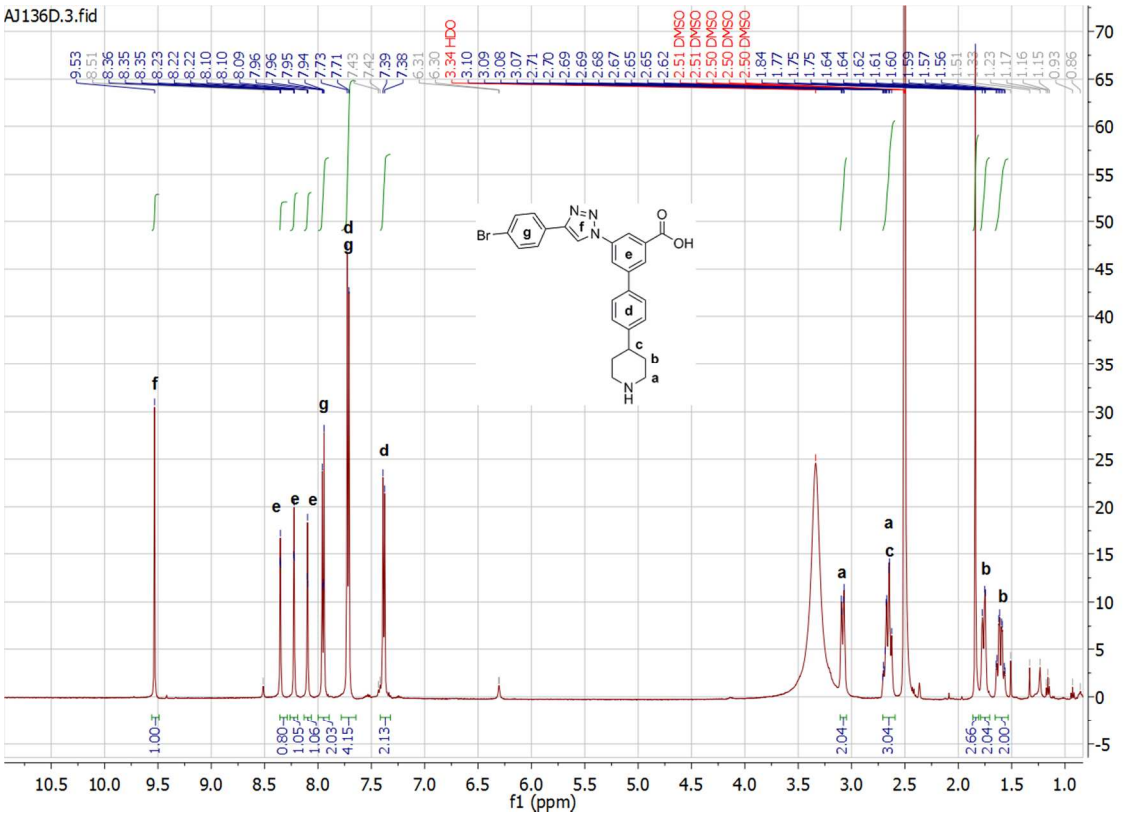
Single Mass Analysis
Tolerance = 25.0 mDa / DBE: min = -2.0, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

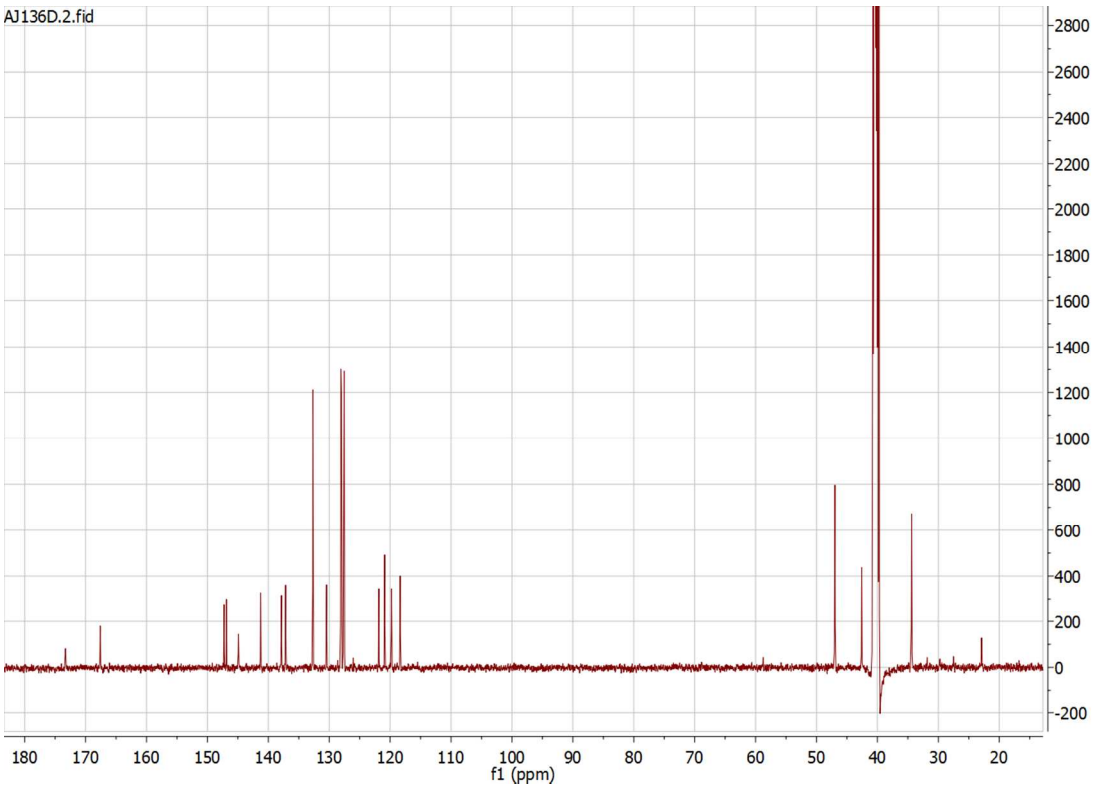
Monoisotopic Mass, Even Electron Ions
83 formula(e) evaluated with 8 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 4-4 O: 0-40 79Br: 1-1

14-Aug-2015
aj-14aug15-136d 207 (3.828) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00 )

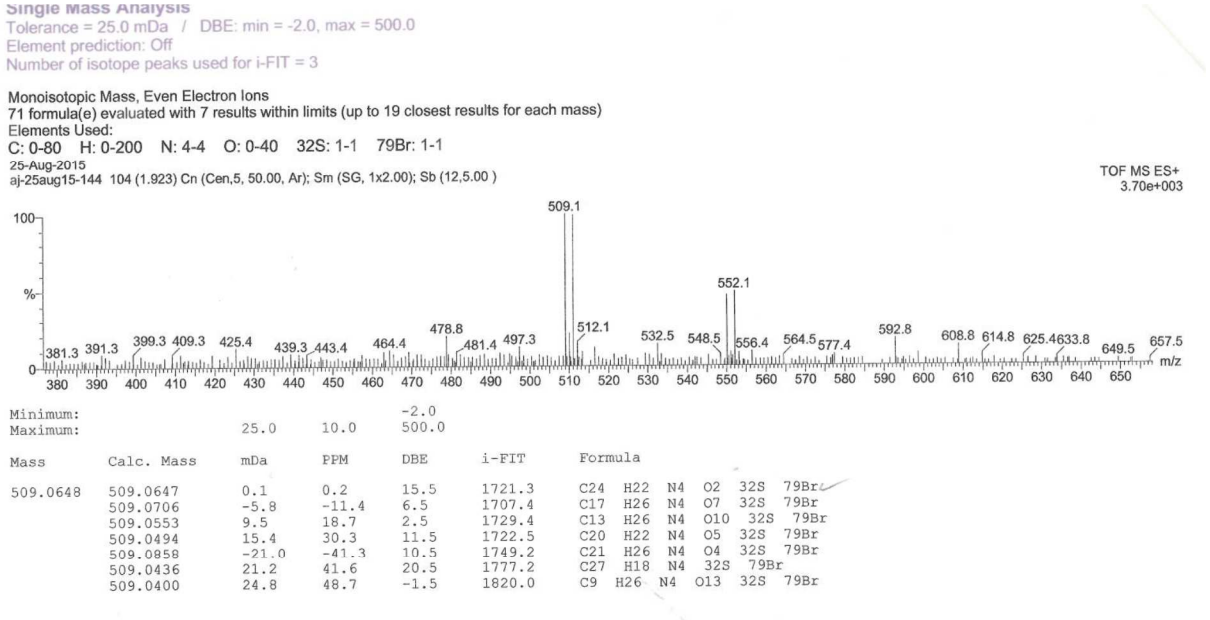
TOF MS ES+
2.91e+003

| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | 25.0 | 10.0 | -2.0 | | |
| Maximum: | | | | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 503.1080 | 503.1083 | -0.3 | -0.6 | 16.5 | 1333.7 | C26 H24 N4 O2 79Br |
| | 503.1141 | -6.1 | -12.1 | 7.5 | 1296.7 | C19 H28 N4 O7 79Br |
| | 503.0989 | 9.1 | 18.1 | 3.5 | 1289.3 | C15 H28 N4 O10 79Br |
| | 503.1200 | -12.0 | -23.9 | -1.5 | 1310.7 | C12 H32 N4 O12 79Br |
| | 503.0930 | 15.0 | 29.8 | 12.5 | 1314.2 | C22 H24 N4 O5 79Br |
| | 503.0871 | 20.9 | 41.5 | 21.5 | 1380.1 | C29 H20 N4 79Br |
| | 503.1294 | -21.4 | -42.5 | 11.5 | 1352.2 | C23 H28 N4 O4 79Br |
| | 503.0836 | 24.4 | 48.5 | -0.5 | 1334.6 | C11 H26 N4 O13 79Br |

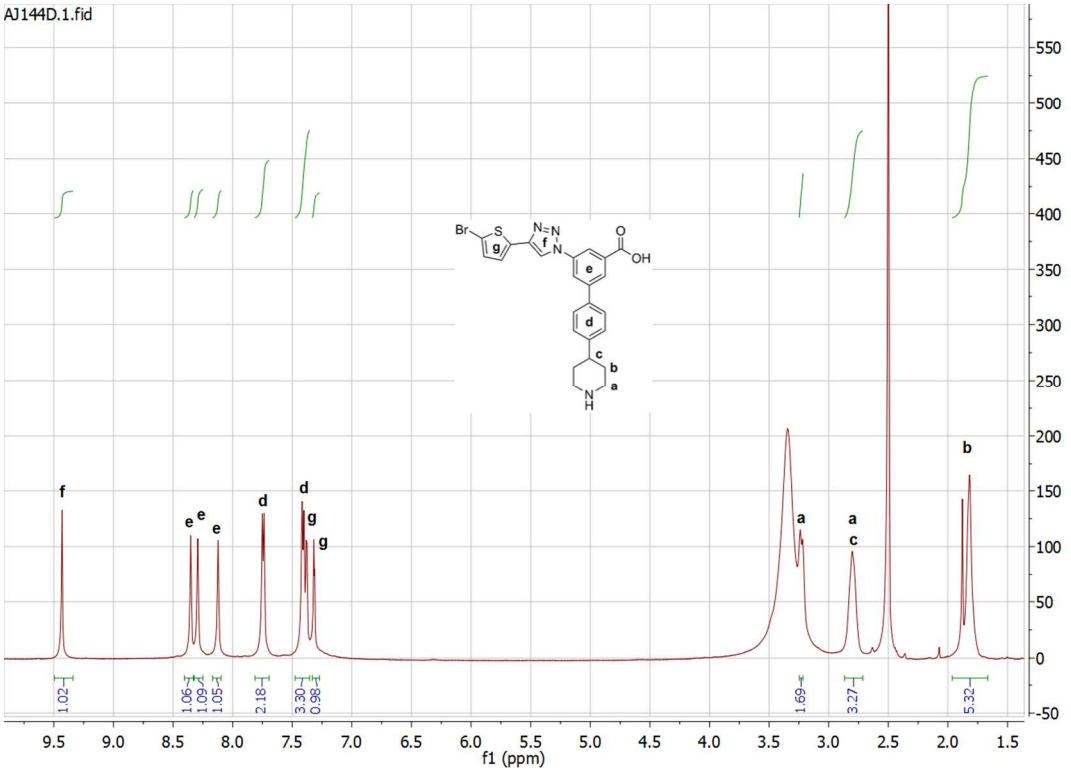





**5-(4-(5-Bromothiophen-2-yl)-1H-1,2,3-triazol-1-yl)-4'-(piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (75).**




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 25.0 | 10.0 | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 509.0648 | 509.0647 | 0.1 | 0.2 | 15.5 | 1721.3 | C24 H22 N4 O2 32S 79Br |
| | 509.0706 | -5.8 | -11.4 | 6.5 | 1707.4 | C17 H26 N4 O7 32S 79Br |
| | 509.0553 | 9.5 | 18.7 | 2.5 | 1729.4 | C13 H26 N4 O10 32S 79Br |
| | 509.0494 | 15.4 | 30.3 | 11.5 | 1722.5 | C20 H22 N4 O5 32S 79Br |
| | 509.0858 | -21.0 | -41.3 | 10.5 | 1749.2 | C21 H26 N4 O4 32S 79Br |
| | 509.0436 | 21.2 | 41.6 | 20.5 | 1777.2 | C27 H18 N4 32S 79Br |
| | 509.0400 | 24.8 | 48.7 | -1.5 | 1820.0 | C9 H26 N4 O13 32S 79Br |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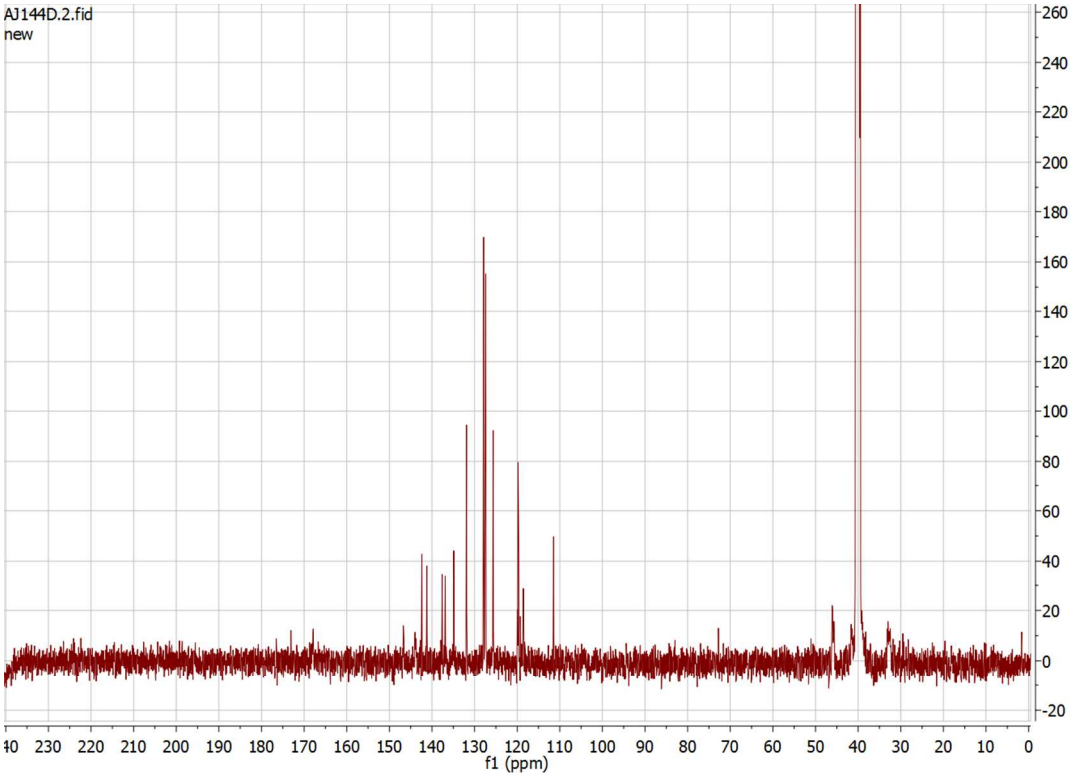
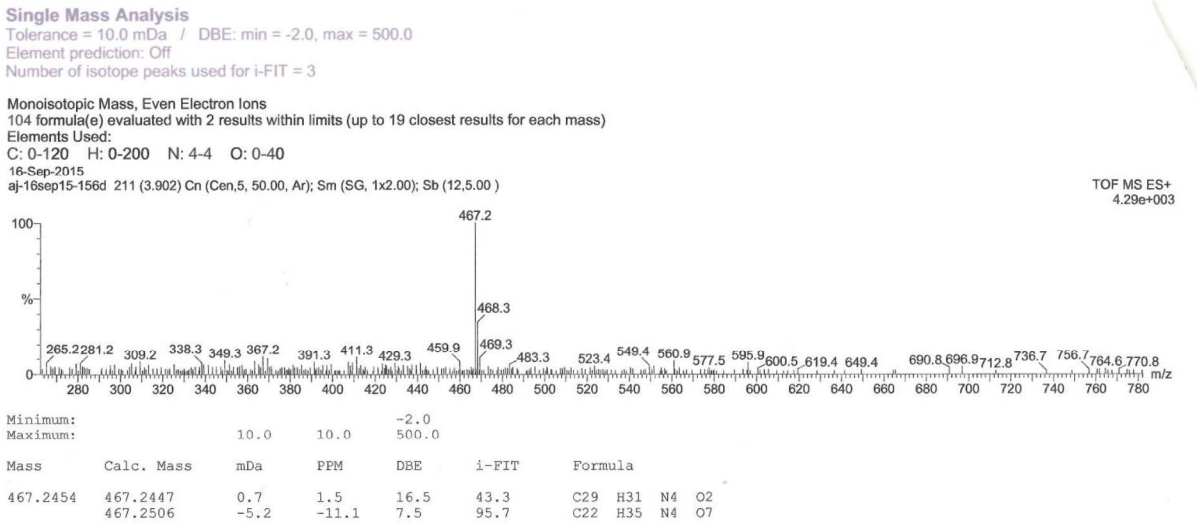
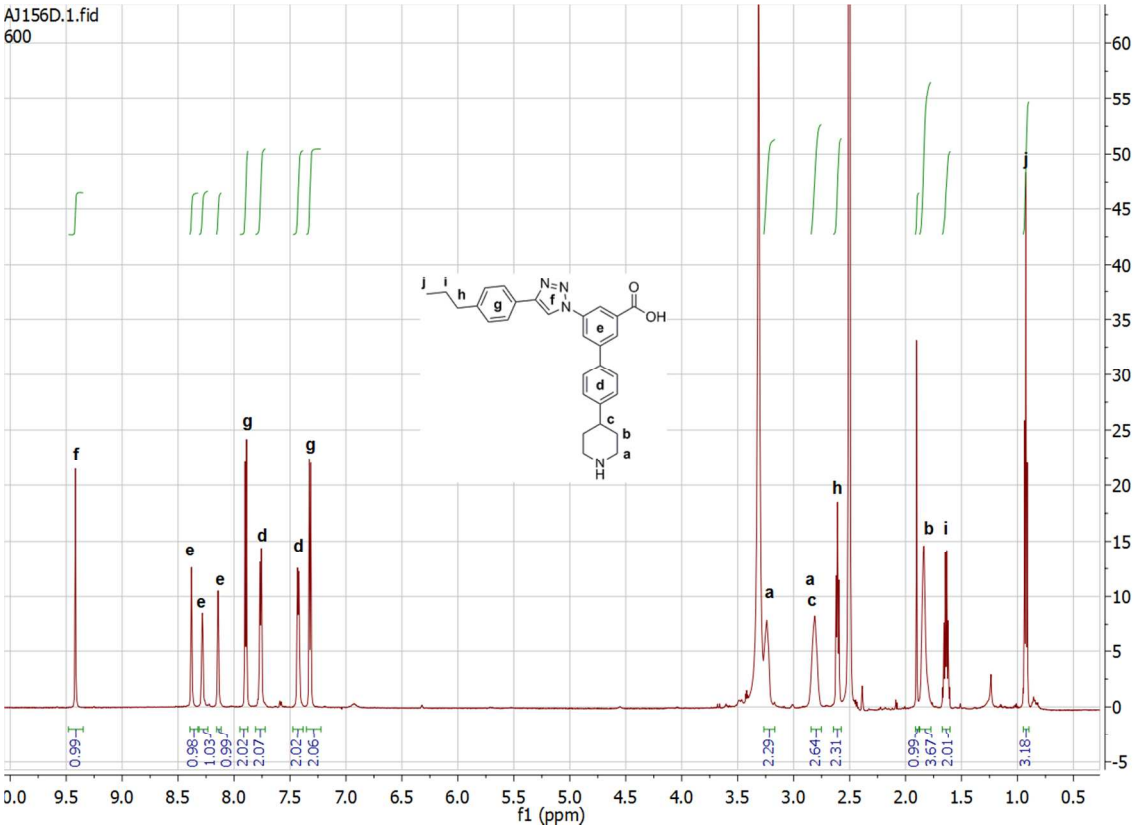
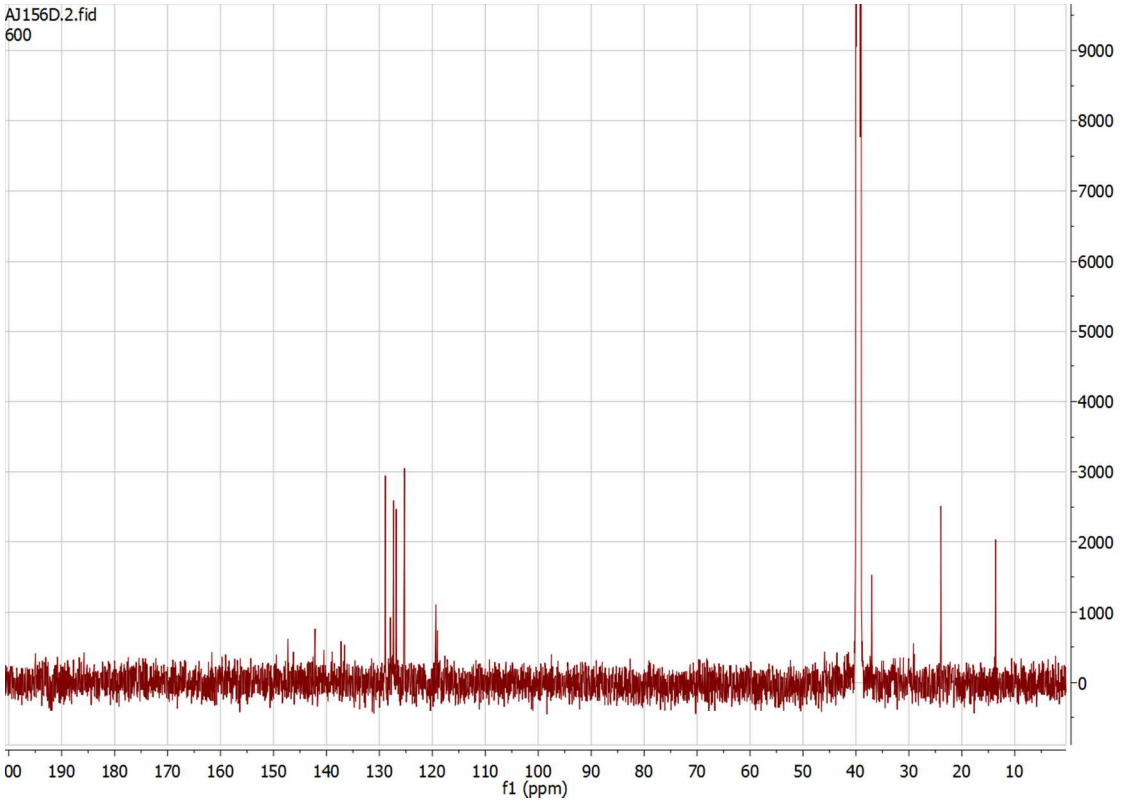
f1 (ppm)


AJ144D.2.fid
new
f1 (ppm)

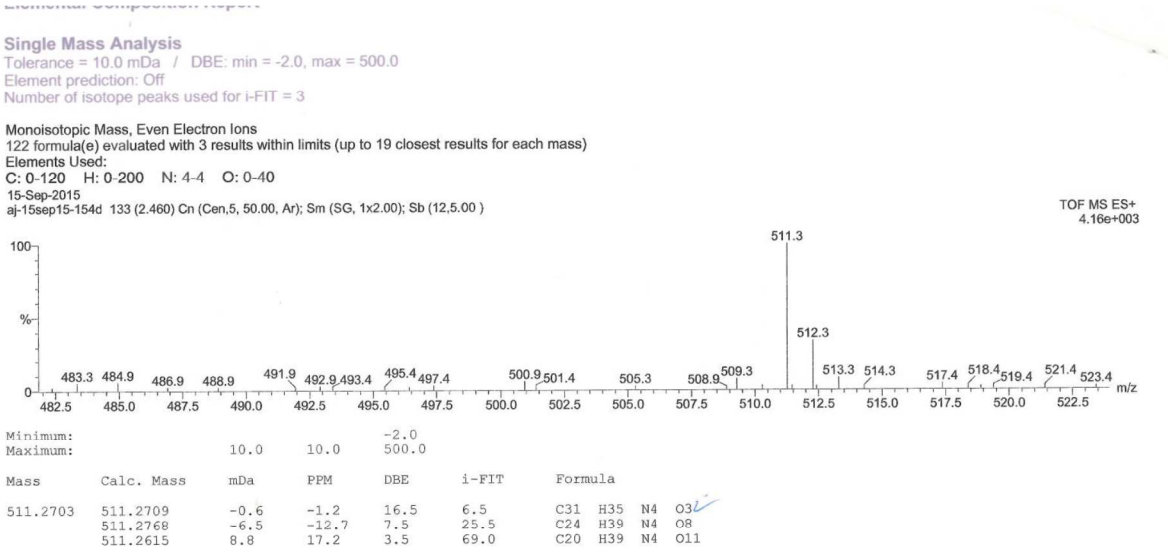
**5-(4-(4-Propylphenyl)-1H-1,2,3-triazol-1-yl)- 4'-(piperidin-4-yl)- [1,1'-biphenyl]-3-carboxylic acid (77)**



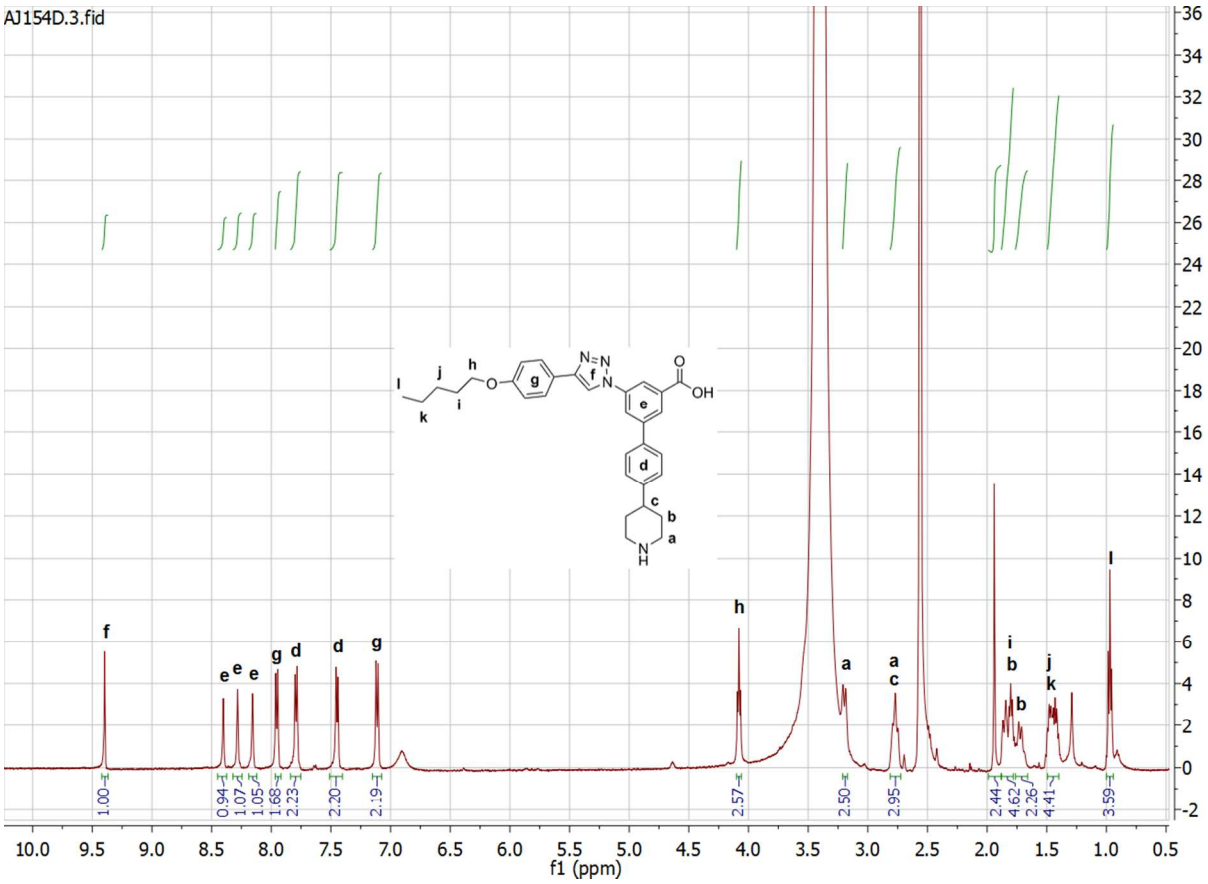



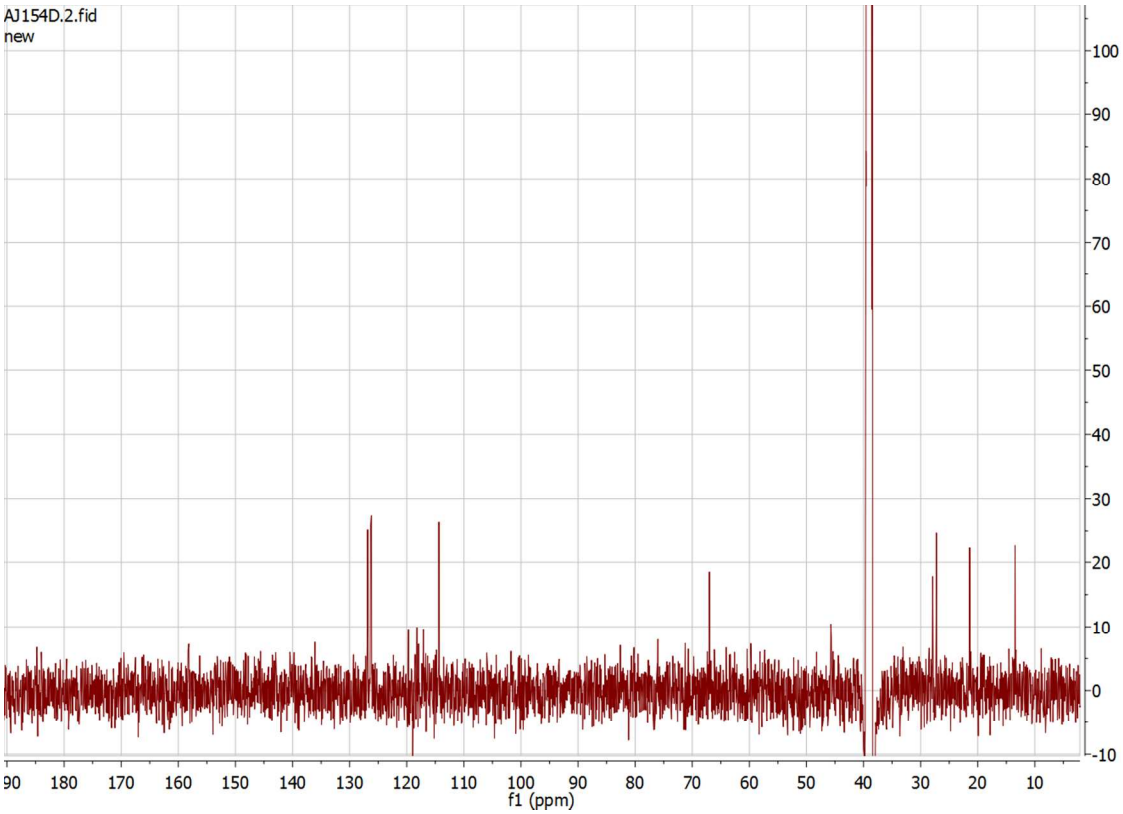



**5-(4-(4-(Pentyloxy)phenyl)-1H-1,2,3-triazol-1-yl)-4'-(piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (82)**




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -2.0 | | |
| Maximum: | | 10.0 | 10.0 | 500.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 511.2703 | 511.2709 | -0.6 | -1.2 | 16.5 | 6.5 | C31 H35 N4 O3 |
| | 511.2768 | -6.5 | -12.7 | 7.5 | 25.5 | C24 H39 N4 O8 |
| | 511.2615 | 8.8 | 17.2 | 3.5 | 69.0 | C20 H39 N4 O11 |


AJ154D.3.fid
f1 (ppm)


AJ154D.2.fid
new
f1 (ppm)

**Methyl 3-hydroxy-5-iodobenzoate (5b)**. 3-Hydroxy-5-iodobenzoic acid (**5a**, 264 mg, 1 mmol) was suspended in methanol (3 mL) and the solution was cooled to 0 °C in an ice bath. Thionyl chloride (0.5 mL, 7 mmol) was added to the mixture over the course of 30 min at 0 °C. The mixture was allowed to warm to room temperature and stirred for 16 h. The solvent was removed from the resulting light yellow solution under reduced pressure, and the residue was redissolved in dichloromethane (3 mL). The solution was washed with saturated aqueous sodium bicarbonate solution (1 mL) and water (1 mL). The organic layer was dried with $Na_2SO_4$ and filtered through a pad of silica gel, and concentrated *in vacuo* to provide **2** (92 mg, 33%). MS (ESI, m/z) 279 $[M+H]^+$. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.94 (s, 1H), 7.54 (s, 1H), 7.44 (s, 1H), 5.68 (s, 1H), 3.93 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ (ppm) = 165.9, 156.3, 132.6, 130.9, 129.2, 116.1, 93.9, 52.7.

**Methyl 3-hydroxy-5-((4-(trifluoromethyl)phenyl)ethynyl)benzoate (7)**. 1-Ethynyl-4-(trifluoromethyl)benzene (**6**, 102 mg, 0.6 mmol) was added to a degassed suspension of **5b** (110 mg, 0.4 mmol), bis(triphenylphosphine)palladium(II) dichloride (14 mg, 5 mol%) and copper(I) iodide (4 mg, 5 mol%) and triethylamine (0.3 mL, mmol) in anhydrous DMF (6 mL) at 0 °C. The reaction mixture was allowed to warm up to stirred until the complete consumption of **5b**. The reaction mixture was quenched with water (25 mL) and the organic products were extracted with ethyl acetate (3 × 5 mL). The combined extracts were washed with water (2 × 5 mL), brine (5 mL), dried with sodium sulfate and evaporated to dryness. The residue was subjected to flash column chromatography (silica gel), eluting with chloroform/methanol 90/10 (v/v) mixture, to provide **7** (103 mg, 81%). MS (ESI, m/z) 321 $[M+H]^+$. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.80 (t, *J* = 1.38 Hz, 1H), 7.63 (s, 4H), 7.60 (dd, *J* = 1.51, 2.51 Hz, 1H), 7.24 (dd, *J* = 1.51, 2.51 Hz, 1H), 6.06 (br. s, 1H), 3.95 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ (ppm) = 166.5, 155.9, 131.9, 131.7, 126.6, 125.4, 125.3, 125.3, 124.2, 122.8, 117.1, 116.1, 90.3, 88.7, 52.6.

**Methyl 3-((4-(trifluoromethyl)phenyl)ethynyl)-5-(((trifluoromethyl)sulfonyl)oxy)-benzoate (8)**. Trifluoromethanesulfonic anhydride (54 μL, 0.32 mmol) was added to a solution of **7** (93 mg, 0.29 mmol) and triethylamine (61 μL, 0.43 mmol) in anhydrous dichloromethane (2 mL) at –20 °C under inert atmosphere. The reaction mixture was removed from cooling bath and left to stir at 23 °C for 2.5 h. The solution was diluted with dichloromethane (3 mL), washed with water (1 mL), saturated aqueous sodium bicarbonate solution (1 mL), dried with sodium sulfate and evaporated to dryness under reduced pressure. The residue following evaporation was subjected to column chromatography (silica gel), eluting with ethyl acetate/hexane 20/80 (v/v) mixture. The combined fractions containing **8** were evaporated to dryness to afford product (128 mg, 98%). MS (ESI, m/z) 453 $[M+H]^+$. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.25 (t, *J* = 1.38 Hz, 1H), 7.92 (dd, *J* = 1.38, 2.38 Hz, 1H), 7.66 (s, 4H), 7.63 (s, 1H), 3.99 (s, 3H).

***tert*-Butyl 4-(3'-(methoxycarbonyl)-5'-((4-(trifluoromethyl)phenyl)ethynyl)-[1,1'-biphenyl]-4-yl)piperidine-1-carboxylate (10)**. A mixture of triflate **8** (25 mg, 56 μmol), *tert*-butyl 4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperidine-1-carboxylate (**9**, 22 mg, 56 μmol), tetrakis(triphenylphosphine)palladium(0) (127 mg, 0.11 mmol), potassium carbonate (15 mg, 0.11 mmol) and DMF (1 mL) was degassed and heated to 90 °C under the atmosphere of inert gas for 8 h. After cooling to 23 °C, the solvent was removed under reduced pressure. The residue was resuspended in ethyl acetate (3 mL), washed with water (2 × 1 mL) and dried with sodium sulfate. Ethyl acetate was removed under reduced pressure, and the residue was subjected to column chromatography (silica gel), eluting with chloroform/methanol 95/5 (v/v) mixture to obtain the title compound **10** (21 mg, 67%). MS (ESI, m/z) 564 $[M+H]^+$. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.86 (s, 1H), 7.56 (m, 4H), 7.46 - 7.49 (m, 1H), 7.29 (m, 5H),

5.36 (m, 2H), 3.94 (s, 3H), 2.84 (br. s., 2H), 2.71 (t, *J* = 12.05 Hz, 1H), 1.86 (m, 2H), 1.68 (m, 2H), 1.50 (s, 9H).

**Methyl 3-amino-5-bromobenzoate (13).** 3-Bromo-5-aminobenzoic acid **12** (1.0 g, 4.62 mmol) was stirred in methanol (15 mL) with ice cooling, and the yellow solution was treated with thionyl chloride (4.00 mL, 55.0 mmol) dropwise over 20 min. The resulting mixture was allowed to warm up to r.t. and left stirring overnight. The reaction mixture was quenched with aqueous saturated $NaHCO_3$ solution at 0°C. The solvent was then removed under vacuum, and the residue was suspended in ethyl acetate (200 mL). The organic phase was washed with brine (100 mL), dried ($Na_2SO_4$) and concentrated *in vacuo* to afford the title compound as a yellow solid (1.08 g, 98%). MS (ESI, m/z) 231 $[M+H]^+$; ESI-HRMS calcd. m/z for $C_8H_8BrNO_2$ 229.9817, found 229.9818 $[M+H]^+$. HPLC purity 98.8 % ($R_t$ = 12.3 min), m.p. 84-89˚C. $^1$H NMR (400 MHz, MeOD): δ (ppm) = 7.10 (t, *J* = 1.6 Hz, 1H), 6.83 (t, *J* = 1.6 Hz, 1H), 6.57 (t, *J* = 1.6 Hz, 1H), 3.46 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ (ppm) = 116.0, 147.7, 132.6, 122.9, 122.3, 121.6, 114.6, 52.3.

**Methyl 3-bromo-5-((*tert*-butoxycarbonyl)amino)benzoate (14).** To a solution of **13** (3.73 g, 16.2 mmol) in $CH_2Cl_2$ (40 mL), $Boc_2O$ (4.2 g, 19.4 mmol) and 4-(dimethylamino)pyridine (1.9 g, 16.2 mmol) were sequentially added with ice cooling bath. The resulting mixture was allowed to stir at 0 °C for 2 h. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel chromatography using as eluent hexane/ethyl acetate (75:25) to afford the title compound as a white solid (4.3 g, 80 %). MS (ESI, m/z) 331 $[M+H]^+$; ESI-HRMS calcd. m/z for $C_{13}H_{16}BrNO_4$ 329.0263, found 329.0260 $[M+H]^+$. HPLC purity 99.6 % ($R_t$ = 20.14 min), m.p. 140-143˚C. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.98 (s, 1H), 7.82 (s, 1H), 7.80 (s, 1H), 6.60 (s, 1H), 3.91 (s, 3H), 1.52 (s, 9H). $^{13}$C NMR (100 MHz, MeOD): δ (ppm) = 165.6, 153.3, 141.3, 132.1, 125.4, 124.7, 121.9, 117.5, 51.5, 27.2.

**Methyl 3-((*tert*-butoxycarbonyl)amino)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (15).** A solution of **14** (1 g, 3 mmol), bis(pinacolato)diboron (914 mg, 3.6 mmol), KOAc (885 mg, 9 mmol) in dry 1.4-dioxane (25 mL) was degassed with $N_2$ for 30 min. Then, $PdCl_2$(dppf) (220 mg g, 0.3 mmol) was added. The reaction mixture was heated at 95 °C for 2 h. After cooling, the resulting mixture was suspended in ethyl acetate and filtered through Celite. The solvent was removed under reduced pressure leaving a black residue, which was purified by silica gel chromatography using as eluent hexane:ethyl acetate75:25). The title compound was obtained as a white solid (992 mg, 88 %), m.p. 181-183˚C. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.16 (t, *J* = 2.0 Hz, 1H), 8.14 (t, *J* = 2.0 Hz, 1H), 7.90 (t, *J* = 2.0 Hz, 1H), 6.53 (s, 1H), 3.90 (s, 3H), 1.52 (s, 9H), 1.34 (s, 12H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ (ppm) = 166.9, 152.6, 138.2, 130.0, 128.9, 122.2, 84.1, 52.1, 28.3, 24.9. MS (ESI, m/z) 378 $[M+H]^+$; ESI-HRMS calcd. m/z for $C_{19}H_{28}BNO_6$ 376.2046, found 376.2049 $[M+H]^+$.

**4-(4-Bromophenyl)-1,2,3,6-tetrahydropyridine (17).** 4-(4-Bromophenyl)piperidin-4-ol **16** (1.0 g, 3.90 mmol) was carefully added to $CF_3COOH$ (2.99 mL, 39 mmol), and the resulting mixture was heated at 90 °C for 3 h. After cooling, the solvent was removed under vacuum to give the title product as a white solid (0.90 g, 97 %), m.p. 214-218˚C. MS (ESI, m/z) 239 $[M+H]^+$; ESI-HRMS calcd. m/z for $C_{11}H_{12}BrN$ 238.0231, found 238.0230 $[M+H]^+$. $^1$H NMR (400 MHz, MeOD): δ (ppm) = 7.54 (d, *J* = 8.6 Hz, 2H), 7.41 (d, *J* = 8.6 Hz, 2H), 6.34 – 6.00 (m, 1H), 3.85 (dd, *J* = 2.7 Hz, 2H), 3.48 (t, *J* = 6.1 Hz, 2H), 2.93 – 2.60 (m, 2H). $^{13}$C NMR (100 MHz, MeOD): δ (ppm) = 138.1, 134.6, 131.4, 126.6, 121.7, 116.4, 42.0, 40.7, 23.3.

**4-(4-Bromophenyl)piperidine (18).** To a solution of 4-(4-bromophenyl)-1,2,3,6-tetrahydropyridine **17** (0.90 g, 3.78 mmol) in dry MeOH (20 mL) and $Et_3N$ (2 mL) was added

Rh/C catalyst (0.060 g, J.Bishop & Co. Platinum). The resulting reaction mixture was stirred at r.t. in a hydrogen atmosphere (100 psi) for 24 h. The mixture was filtered through a cake of Celite, and the filtrate was concentrated to give the title compound as a white solid (0.91 g, 98 %), m.p. 109-113˚C. MS (ESI, m/z) 241 $[M+H]^+$. $^1$H NMR (400 MHz, MeOD): δ (ppm) = 7.31 (2H, *J* = 8.0, d), 7.13 (2H, *J* = 8.0, d), 3.09-3.06 (2H, m), 2.64-2.70 (2H, m), 2.55-2.56 (1H, m), 1.61-1.70 (2H, m), 1.55-1.59 (2H, m). $^{13}$C NMR (100 MHz, MeOD): δ (ppm) = 145.3, 131.2, 128.5, 128.2, 126.4, 119.4, 45.6, 41.4, 32.7.

**Methyl 5-((*tert*-butoxycarbonyl)amino)-4'-(piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylate (19).** A suspension of **15** (0.514 g, 01.3 mmol), $K_2CO_3$ (0.565 g, 4.0 mmol) in dry DME (10 mL) was stirred for 15 min. **18** (0.555 g, 1.9 mmol) was added, and the yellow suspension was degassed with $N_2$ for 40 min. Then, $Pd(Ph_3P)_4$ (0.078 g, 0.068 mmol) was added to the resulting mixture while flushing $N_2$ for an additional 5 min. The reaction was heated at 85 °C for 7 h; after cooling the mixture was filtered through Celite, and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography using as eluent $CH_2Cl_2$: MeOH: $Et_3N$ (9:1:0.1) to afford to the title compound as a white solid (0.55 g, 70 %), m.p. 161-165˚C. MS (ESI, m/z) 411 $[M+H]^+$; ESI-HRMS calcd. m/z for $C_{24}H_{30}N_2O_4$ 411.2284, found 411.2285 $[M+H]^+$. $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.84 (m, 2H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.04-7.06 (m, 1H), 6.67 (br. s, 1H), 3.85 (s, 3H), 3.28-3.37 (m, 2H), 2.78-2.83 (m, 2H), 2.65-2.68 (m, 1H), 1.85-1.90 (m, 4H), 1.47 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ (ppm) = 166.9, 152.8, 146.0, 142.0, 139.3, 137.9, 131.5, 128.6, 128.4, 127.3, 126.8, 126.2, 122.6, 118.0, 61.1, 52.2, 46.7, 42.7, 42.3, 33.8, 29.7, 28.4.

**Methyl 5-((*tert*-butoxycarbonyl)amino)-4'-(1-(2,2,2-trifluoroacetyl)piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylate (20).** To a suspension of **19** (0.538 g, 1.3 mmol) in dry $Et_2O$ (2.5 mL) at 0 °C and $N_2$ atmosphere, $Et_3N$ (0.43 mL, 3.1 mmol) and trifluoroacetic anhydride (0.34 mL, 2.4 mmol) were added, and the resulting mixture was stirred for 1 h. The organic solvent was removed under reduced pressure, and the resulting orange oil was used in the next step without any further purification. MS (ESI, m/z) 507 $[M+H]^+$; ESI-HRMS calcd. m/z for $C_{26}H_{29}F_3N_2O_5Na$ 529.1926, found 529.1935 $[M+Na]^+$.

**Methyl 5-amino-4'-(1-(2,2,2-trifluoroacetyl)piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylate (21).** To a solution of **20** (0.538 g, 1.06 mmol) in $CH_2Cl_2$ (12 mL), $F_3CCO_2H$ (2.44 mL, 31.8 mmol) was added, and the resulting mixture was stirred overnight. A saturated solution of $NaHCO_3$ was gradually added and the aqueous layer was extracted with $CH_2Cl_2$ (3 x 30 mL). The collected organic fractions were dried over $Na_2SO_4$, filtered and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography using as eluent hexane/ethyl acetate (60:40) to give yellow oil (0.37 g, 70%). MS (ESI, m/z) 407 $[M+H]^+$; ESI-HRMS calcd. m/z for $C_{21}H_{22}F_3N_2O_3$ 407.1583, found 407.1576 $[M+H]^+$.

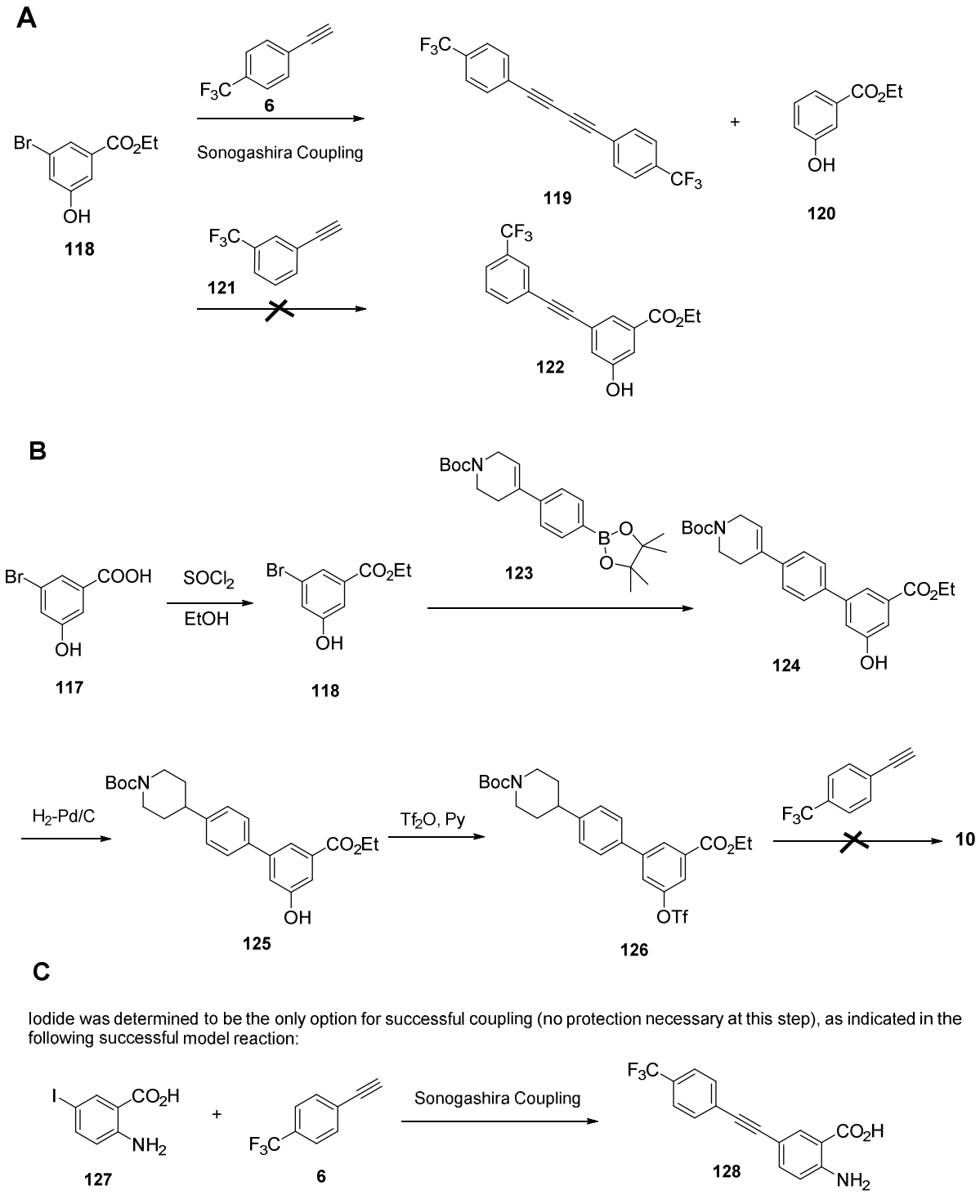
$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.65 (t, *J* = 1.5 Hz, 1H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.33 (t, *J* = 1.5 Hz, 1H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.06 (t, *J* = 1.5 Hz, 1H), 4.72 (m, 1H), 4.16 (d, *J* = 13.0 Hz, 1H), 3.91 (s, 3H), 3.86 (s, 1H), 3.27 (td, *J* = 2.4, 13.0 Hz, 1H), 3.01 – 2.72 (m, 2H), 2.02 (d, *J* = 13.0 Hz, 2H), 1.75 (qd, *J* = 4.0, 13.0 Hz, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ (ppm) = 167.1, 146.7, 143.7, 142.1, 139.1, 138.9, 131.6, 127.5 (t, $J_{C-F}$ = 41 Hz), 122.7, 121.2, 120.3, 118.7, 117.8, 114.8, 52.1, 46.4, 44.2, 42.1, 33.6, 32.6.

**Methyl 5-azido-4'-(1-(2,2,2-trifluoroacetyl)piperidin-4-yl)-[1,1'-biphenyl]-3-carboxylate (22).** To a solution of **21** (0.121 g, 0.29 mmol) in a 3:7 mixture of $H_2O$ and acetonitrile (10 mL), *p*-toluenesulfonic acid (0.509 g, 2.6 mmol) was added, and the mixture was stirred for 5 min. $NaNO_2$ (0.184 g, 2.6 mmol) was then added, and the yellow solution was stirred at room

temperature. The course of the reaction was followed by TLC (hexane : ethyl acetate = 60:40), and the reaction was allowed to continue until the starting material disappeared. $NaN_3$ (0.030 g, 0.47 mmol) was added at room temperature, and the reaction mixture was stirred overnight. $Et_2O$ was added, and the phases were separated. The organic phase was dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give an orange oil that was purified by silica gel chromatography using as eluent hexane : ethyl acetate (70:30) to afford the title compound as a yellow oil (0.11 g, 83%). $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.03 (t, *J* = 1.6 Hz, 1H), 7.68 (t, J= 1.6 Hz, 1H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.37 (t, *J* = 1.6 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 2H), 4.80 – 4.63 (m, 1H), 4.17 (dd, *J* = 3.1, 14.2 Hz, 1H), 3.96 (s, 3H), 3.27 (td, *J* = 2.4, 13.3, 13.7 Hz, 1H), 2.90 (td, *J* = 4.2, 12.5 Hz, 2H), 2.02 (d, *J* = 14.2 Hz, 2H), 1.76 (qd, *J* = 4.2, 12.5 Hz, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ (ppm) = 166.6, 155.9 , 144.9 , 143.2 , 141.5 , 138.1 , 132.7 , 127.8 , 125.1 , 122.1 , 118.7 , 52.9 , 46.8 , 44.6 , 42.5 , 34.0 , 33.0.

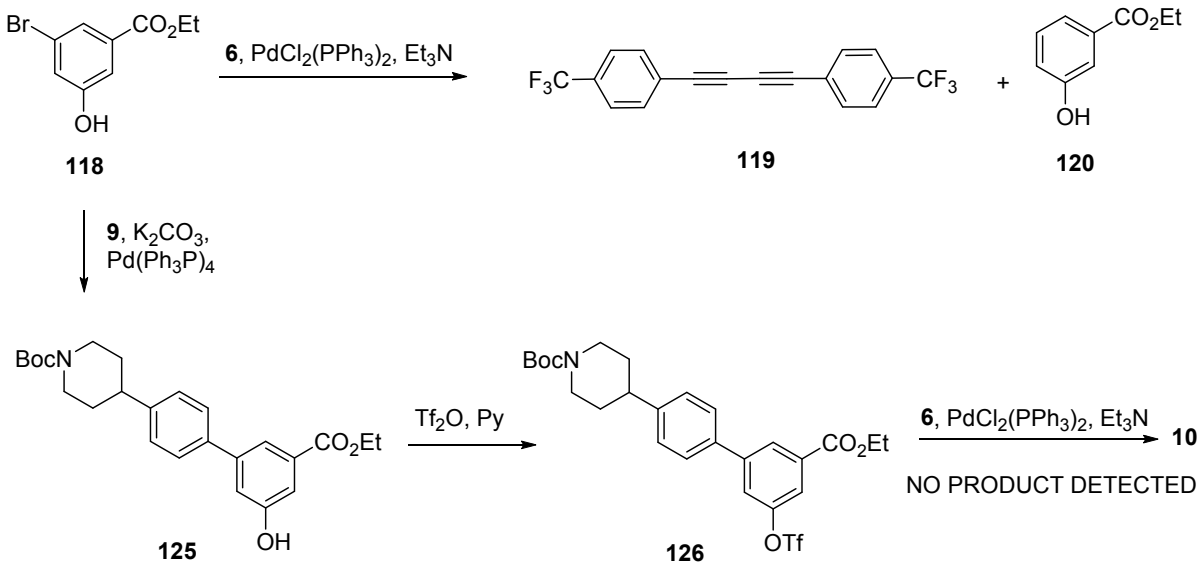
## Scheme S1

Aryl bromide (A) and triflate (B) failed to produce Sonogashira products with p-$CF_3$-Ph-acetylene



**Scheme S2**

**Attempted (unproductive) chemical synthesis of compound 10**




**Reaction between aryl bromide 118 and alkyne 6 under Sonogashira coupling conditions**. Thionyl chloride (1.42 mL, 20 mmol) was added dropwise to a solution of 3-bromo-5-hydroxybenzoic acid (**117**, 2.15 g, 10 mmol) in ethanol (30 mL) at 0 °C. The solution was heated to reflux under nitrogen atmosphere until all the starting material was consumed. The solvent was removed by under reduced pressure. The crude product was the purified by silica gel with an ethyl acetate : hexane system (3:7) to produce **118**. (2.04 g, 90%). $^{1}$H NMR (400 MHz $CDCl_3$): δ (ppm) = 7.73 (s, 1H), 7.56–7.54 (m, 1H), 7.27–7.24 (m, 1H), 6.32 (s, 1H), 4.42–4.36 (m, 2H), 1.43–1.39 (m, 3H). $^{13}$C NMR (100 MHz $CDCl_3$): δ (ppm) = 165.8, 156.7, 132.8, 124.7, 123.34–122.78; 115.5, 115.5, 61.8, 14.2.

The ester **118** (0.53 g, 2.2 mmol), alkyne **6** (0.39 g, 2.4 mmol), $PdCl_2(PPh_3)_2$ (76 mg, 0.11 mmol), copper(I) iodide (21 mg, 0.11 mmol) and triethylamine (0.9 mL, 6.5 mmol) were suspended in anhydrous DMF (2 mL). The mixture was heated to 60 °C for 2 h under $N_2$ atmosphere. After cooling to room temperature, the reaction mixture was diluted with water (10 mL) and neutralized with aqueous HCl (0.1 M), extracted with ethyl acetate (3 × 5 mL). The combined extracts were washed with water (2 mL), brine (2 mL), dried with sodium sulfate and evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with hexane : ethyl acetate (3:1) to afford two products: 1,4-bis(4-(trifluoromethyl)phenyl)buta-1,3-diyne (**119**, 0.380 g, 95% from **6**) and ethyl 3-hydroxybenzoate (**120**, 0.41 g, 98% from **118**).

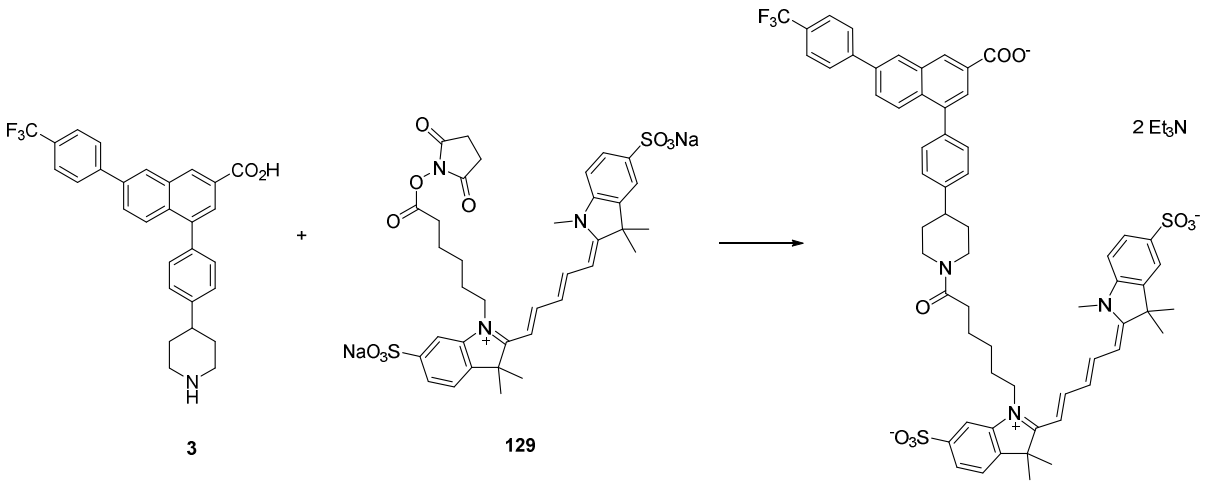
**1,4-Bis(4-(trifluoromethyl)phenyl)buta-1,3-diyne (119).** $^{1}$H NMR (400 MHz $CDCl_3$): δ (ppm) = 7.57–7.84 (m, 8H); $^{13}$C NMR (100 MHz $CDCl_3$): δ (ppm) = 132.8, 125.5, 125.5, 125.4, 125.4, 80.9, 77.3, 76.7, 75.6.

**Ethyl 3-hydroxybenzoate (120).** $^{1}$H NMR (400 MHz $CDCl_3$): δ (ppm) = 7.72 (t, $J$ = 1.51 Hz, 1H), 7.59–7.66 (m, 1H), 7.52–7.58 (m, 1H), 7.24 (t, $J$ = 2.13 Hz, 1H), 6.80 (s, 1H), 4.34–4.43 (m, 3H), 1.35–1.42 (m, 4H).

***tert*-Butyl 4-(3'-(ethoxycarbonyl)-5'-hydroxy-[1,1'-biphenyl]-4-yl)piperidine-1-carboxylate (125)**. A mixture of ester **118** (0.5g, 2 mmol), boronate **9** (0.77 g, 2 mmol), potassium carbonate (0.65, 4.63 mmol), tetrakis(triphenylphosphine)palladium(0) (0.10 g, 0.09 mmol), toluene (11 mL), ethanol (0.29 mL), and water (0.29 mL) was degassed heated to reflux for 13 h under $N_2$ atmosphere. The solvents were then removed under reduced pressure. The residue was resuspended in water (10 mL). Aqueous solution was extracted with ethyl acetate (3 × 5 mL). The combined extracts were dried over $Na_2SO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with ethyl acetate : hexane (1:3) to afford the title compound **125**. (0.26 g, 62%) $^1$H NMR (400 MHz $CDCl_3$): δ (ppm) = 7.80–7.85 (m, 1H), 7.57–7.55 (m, 2H), 7.50–7.49 (m, 1H), 7.30–7.26 (m, 3H), 6.00 (s, 1H), 4.43–4.37 (m, 2H), 4.30–4.26 (m, 2H), 2.87–2.81 (m, 2H), 2.75–2.7 (m, 1H), 1.89–1.85 (m, 2H), 1.72–1.60 (m, 2H), 1.51 (m, 9H), 1.42–1.4 (m, 3 H).

***tert*-Butyl 4-(3'-(ethoxycarbonyl)-5'-(((trifluoromethyl)sulfonyl)oxy)-[1,1'-biphenyl]-4-yl)piperidine-1-carboxylate (126)**. Phenol **125** (0.13 g, 0.31 mmol) was dissolved in pyridine (0.52 mL). Triflic anhydride (102 μL, 0.60 mmol) was then added to the solution at 0 °C and the solution was stirred for additional 2 h at room temperature. After the reaction was completed, water (3 mL) was added to quench the reaction and the organic products were extracted with ethyl acetate (3 × 1 mL). The combined extracts were washed with brine (1 mL), dried with sodium sulfate and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with hexane : ethyl acetate (4:1) to afford **126** (0.08 g, 46%). $^1$H NMR (400 MHz $CDCl_3$ ): δ (ppm) = 8.29–8.28 (m, 1H), 7.88–7.87 (m, 1H), 7.65–7.63 (m, 1H), 7.56–7.54 (m, 2H), 7.35–7.32 (m, 2H), 4.46–4.40 (m, 2H), 4.30–4.27 (m, 2H), 2.87–2.80 (m, 2H), 2.76–2.68 (m, 1H), 1.88–1.84 (m, 2H), 1.72–1.64 (m. 2H), 1.62–1.61 (m, 9H), 1.50–1.40 (m, 3H).

**Scheme S3. Chemical synthesis of fluorescent compound 130**




Furthermore, we explored a different fluorescent antagonist analogue for possible use in screening. Fluorescent antagonist derivative **130** containing a cyanine-5 (Cy5) fluorophore was prepared for comparison to **4**. The structure and synthesis of **130** are described in the Supporting information (Scheme S3).

Fluorescent derivative **130** was tested in a functional assay of antagonism of the agonist-induced inhibition of cAMP production in the presence of 30 μM forskolin in Chinese hamster ovary (CHO-K1) cells stably expressing the hP2Y$_{14}$R (P2Y$_{14}$R-CHO cells, using an EC$_{80}$ concentration of agonist **2** of 316 nM).[1] Under these conditions, the IC$_{50}$ values for **130** was 299±23 nM (n=3). Therefore, compound **4** was retained as the preferred fluorescent tracer due to its more favorable affinity (K$_i$ 0.08 nM)[14] in comparison to **130**.

**4-(4-(1-(6-(3,3-Dimethyl-6-sulfonato-2-((1E,3E,5Z)-5-(1,3,3-trimethyl-5-sulfonatoindolin-2-ylidene)penta-1,3-dien-1-yl)-3*H*-indol-1-ium-1-yl)hexanoyl)piperidin-4-yl)phenyl)-7-(4-(trifluoromethyl)phenyl)-2-naphthoate (130)**.

A solution of **129** (5 mg, 6.6 μmol) in DMF (0.1 mL) was added to a solution of **3** (4.03 mg, 7.9 μmol) and ammonium bicarbonate (0.1 M, 0.9 mL) in DMF (0.9 mL) and stirred for 12 h at 0 °C. The reaction mixture was diluted with water (10 mL) and freeze-dried to remove the solvents. The residue was subjected to HPLC (Column: Luna® 5 μm C18(2) 100 Å, LC Column 250 x 4.6 mm, Phenomenex, Inc., Torrance, CA), eluting with aqueous triethylammonium acetate buffer (10 mM)-$CH_3CN$ 40/60. Fractions containing the title product **130** were combined and freeze-dried to obtain a deep-purple solid.

1. Barrett, M. O.; Sesma, J. I.; Ball, C. B.; Jayasekara, P. S.; Jacobson, K. A.; Lazarowski, E. R.; Harden, T. K. A selective high-affinity antagonist of the P2Y$_{14}$ receptor inhibits UDP-glucose–stimulated chemotaxis of human neutrophils. *Mol. Pharmacol.* **2013**, *84*, 41–49.

**Figure S1.** Comparison between the binding site of agonist- (panel A, pink surface) and antagonist-bound (panel B, green surface) hP2Y$_{14}$R homology models after 10 ns of MD membrane simulations.

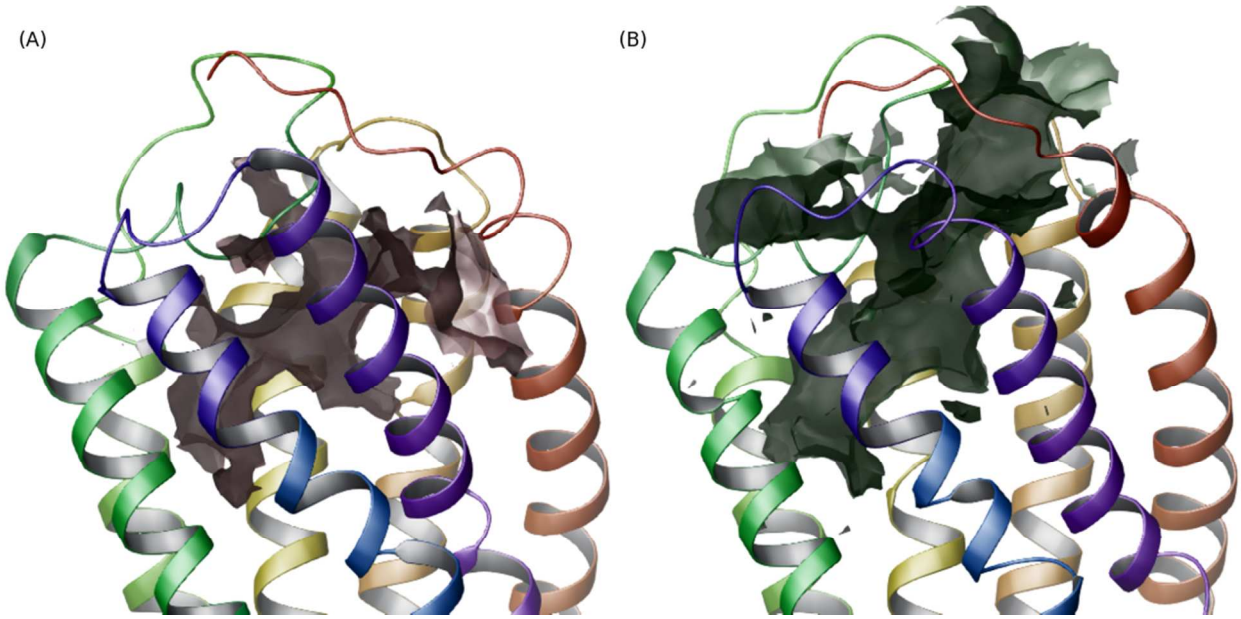


**Figure S2.** Major conformational changes occurred after 10 ns of antagonist-bound MD simulation in the hP2Y$_{14}$R homology model (green). Transmembrane domains experiencing largest movement with respect to the agonist-bound structure (pink) are displayed as cartoon. Directions of movement are indicated by arrows.

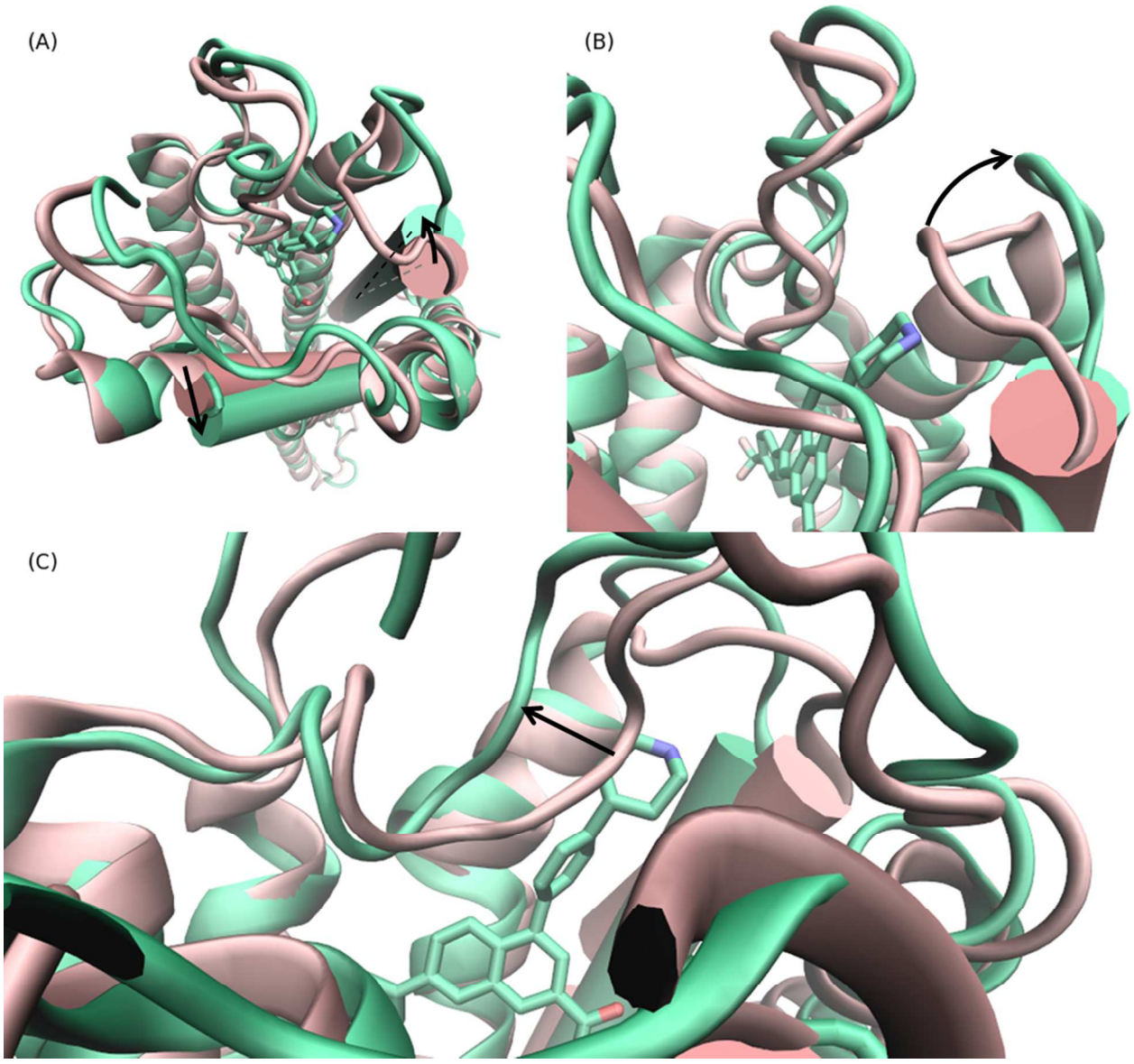
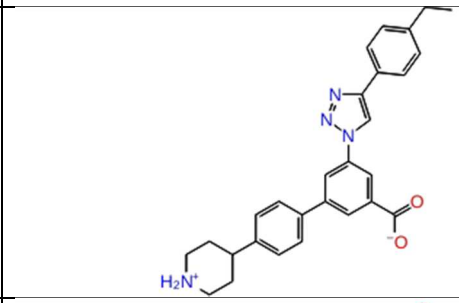
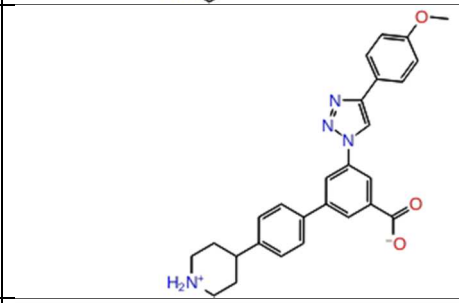
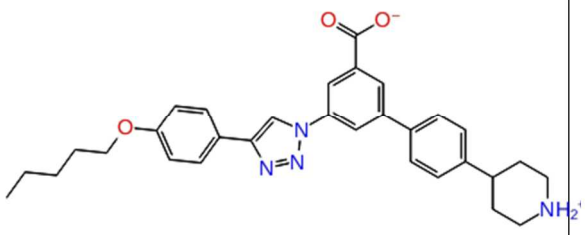
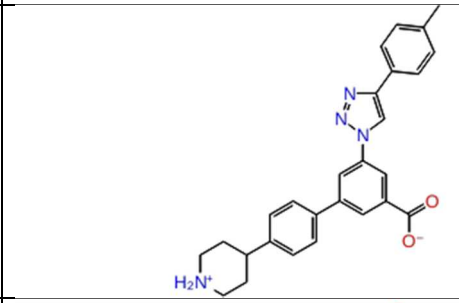
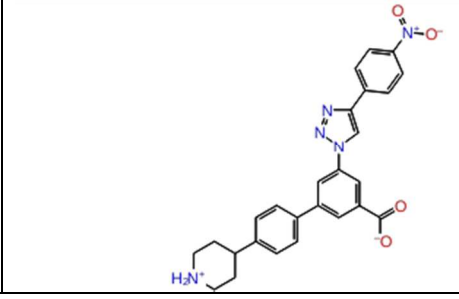
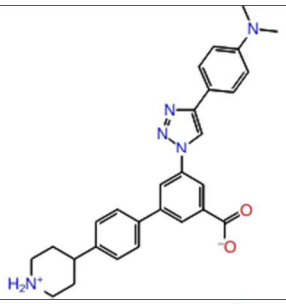
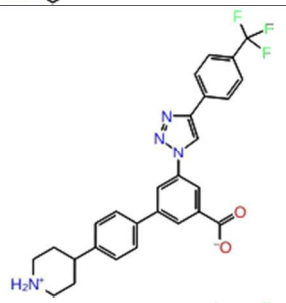
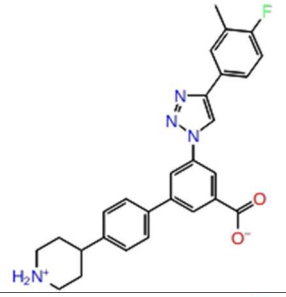
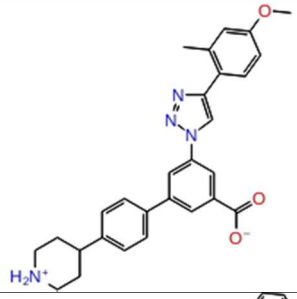
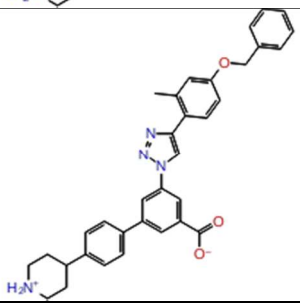


**Table S1**. Library of 57 triazole derivatives used for the docking on hP2Y$_{14}$R.

| Cpd ID | Structure | Molecular formula | Molecular weight |
|---|---|---|---|
| 01 |  | C28H28N4O2 | 452.561 |
| 02 |  | C27H26N4O3 | 454.533 |
| 03 |  | C31H34N4O3 | 510.642 |
| 04 |  | C27H26N4O2 | 438.534 |
| 05 |  | C26H23N5O4 | 469.504 |

| | | | |
|---|---|---|---|
| 06 |  | C28H29N5O2 | 467.576 |
| 07 |  | C27H23F3N4O2 | 492.505 |
| 08 |  | C27H25F1N4O2 | 456.524 |
| 09 |  | C28H28N4O3 | 468.560 |
| 10 |  | C34H32N4O3 | 544.659 |

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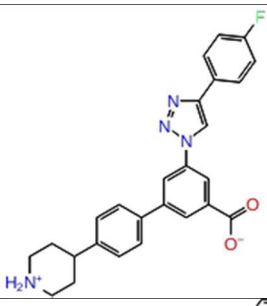
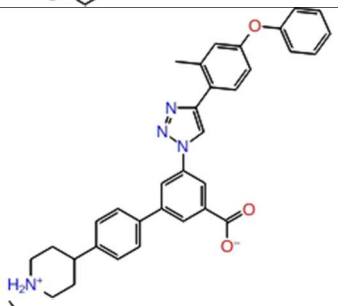
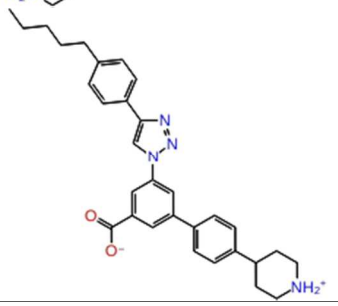
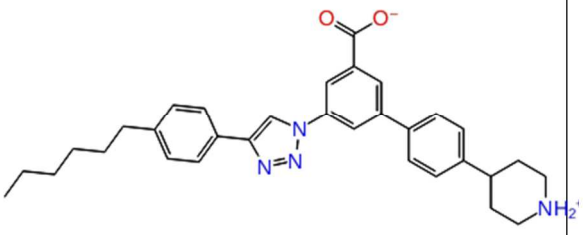
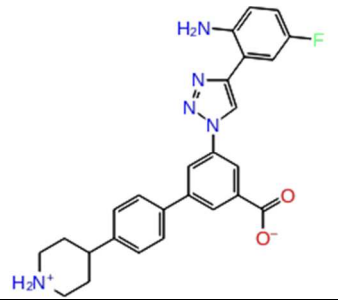
|---|---|---|---|
| 11 |  | C26H23F1N4O2 | 442.497 |
| 12 |  | C33H30N4O3 | 530.632 |
| 13 |  | C31H34N4O2 | 494.642 |
| 14 |  | C32H36N4O2 | 508.669 |
| 15 |  | C26H24F1N5O2 | 457.512 |

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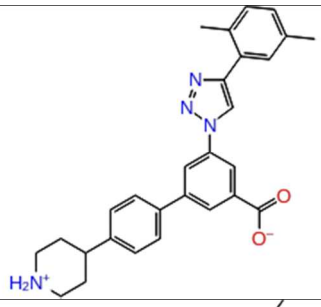
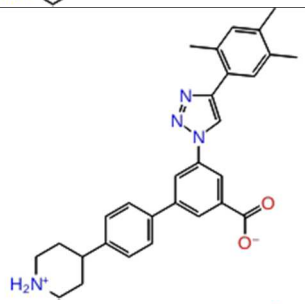
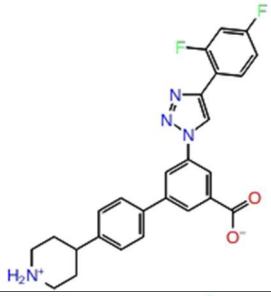
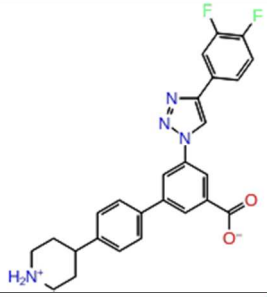
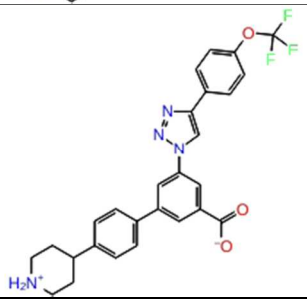
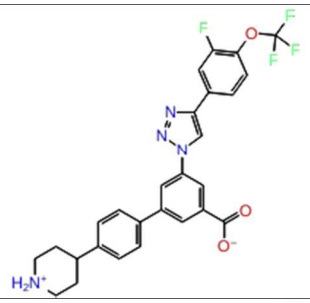
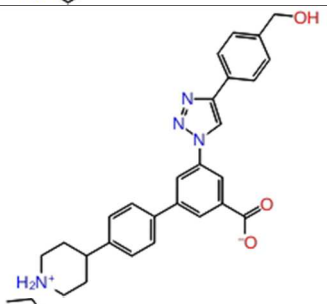
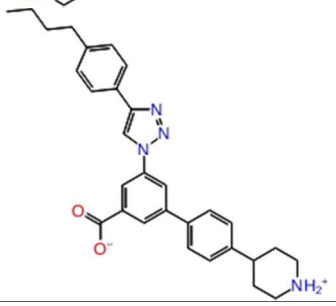
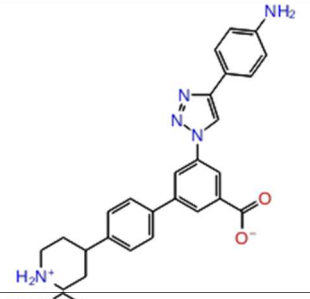
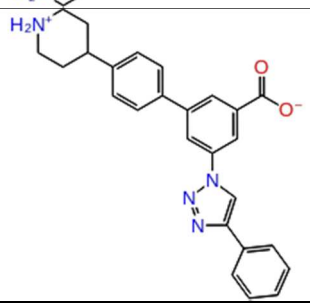
|---|---|---|---|
| 16 |  | C28H28N4O2 | 452.561 |
| 17 |  | C29H30N4O2 | 466.588 |
| 18 |  | C26H22F2N4O2 | 460.488 |
| 19 |  | C26H22F2N4O2 | 460.488 |
| 20 |  | C27H23F3N4O3 | 508.505 |

| | | | |
|---|---|---|---|
| 21 |  | C27H22F4N4O3 | 526.495 |
| 22 |  | C27H26N4O3 | 454.533 |
| 23 |  | C30H32N4O2 | 480.615 |
| 24 |  | C26H25N5O2 | 439.521 |
| 25 |  | C26H24N4O2 | 424.507 |

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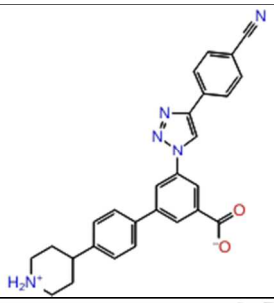
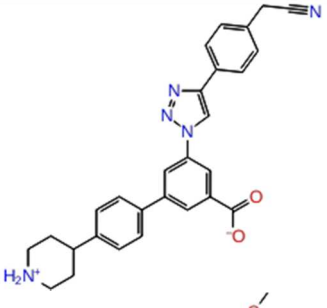
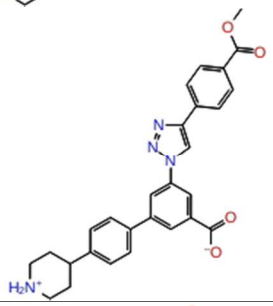
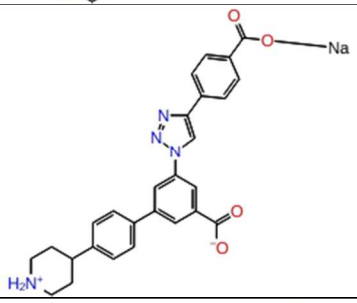
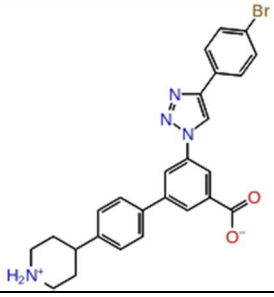
| 26 |  | C27H23N5O2 | 449.517 |
| 27 |  | C28H25N5O2 | 463.544 |
| 28 |  | C28H26N4O4 | 482.544 |
| 29 |  | C27H23N4Na1O4 | 490.499 |
| 30 |  | C26H23Br1N4O2 | 503.408 |

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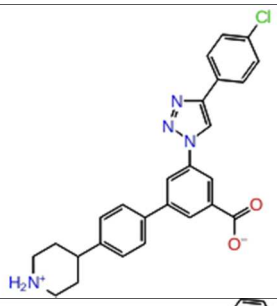
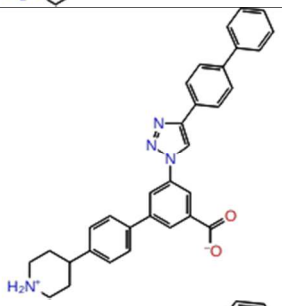
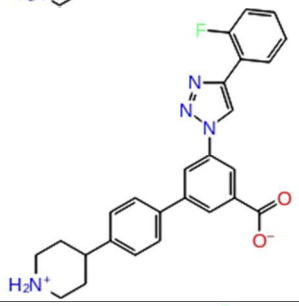
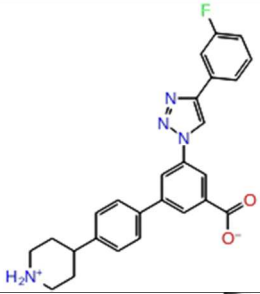
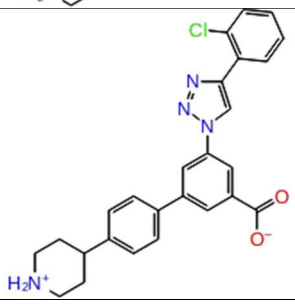
| 31 |  | C26H23Cl1N4O2 | 458.952 |
| 32 |  | C32H28N4O2 | 500.606 |
| 33 |  | C26H23F1N4O2 | 442.497 |
| 34 |  | C26H23F1N4O2 | 442.497 |
| 35 |  | C26H23Cl1N4O2 | 458.952 |

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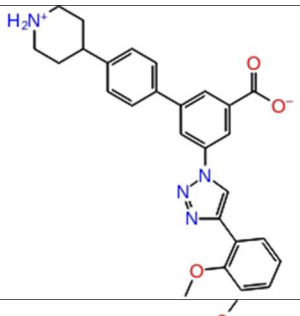
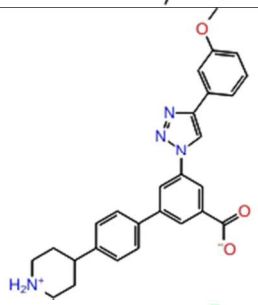
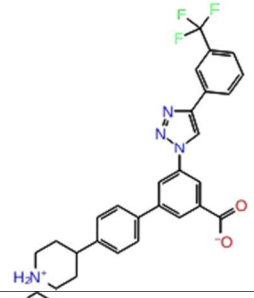
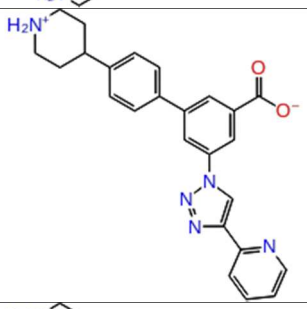
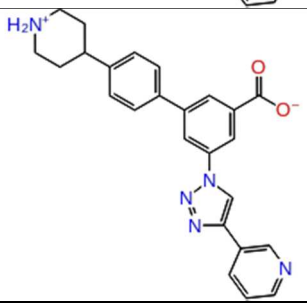
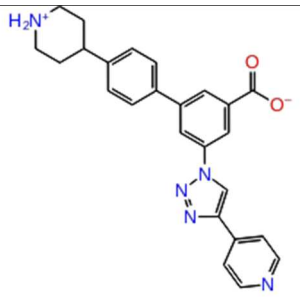
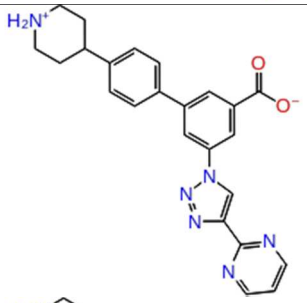
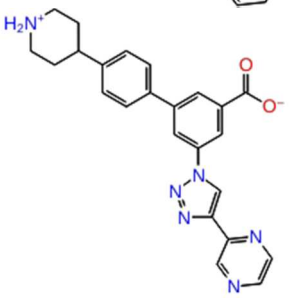
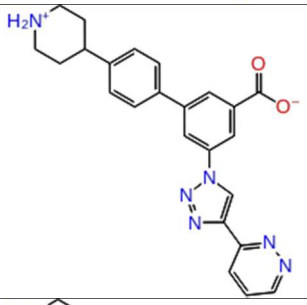
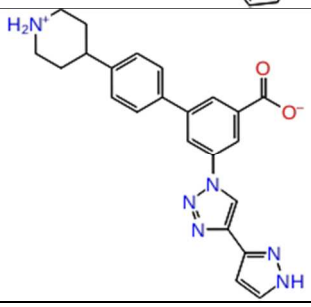
|---|---|---|---|
| 36 |  | C27H26N4O3 | 454.533 |
| 37 |  | C27H26N4O3 | 454.533 |
| 38 |  | C27H23F3N4O2 | 492.505 |
| 39 |  | C25H23N5O2 | 425.494 |
| 40 |  | C25H23N5O2 | 425.494 |

| | | | |
|---|---|---|---|
| 41 |  | C25H23N5O2 | 425.494 |
| 42 |  | C24H22N6O2 | 426.482 |
| 43 |  | C24H22N6O2 | 426.482 |
| 44 |  | C24H22N6O2 | 426.482 |
| 45 |  | C23H22N6O2 | 414.471 |

| | | | |
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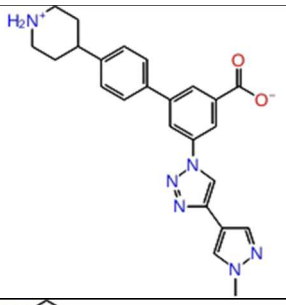
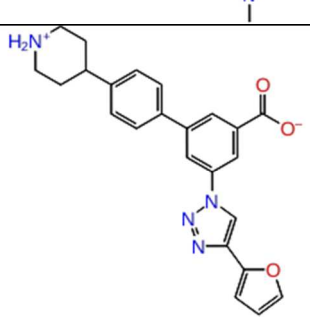
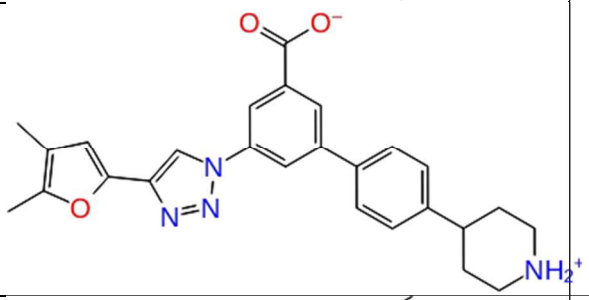
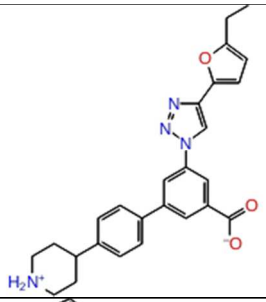
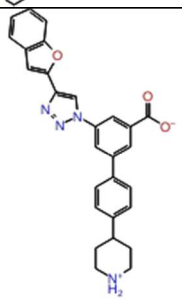
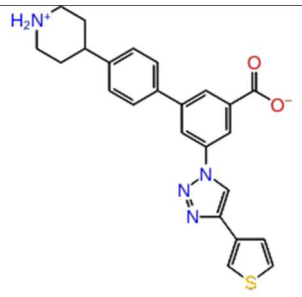
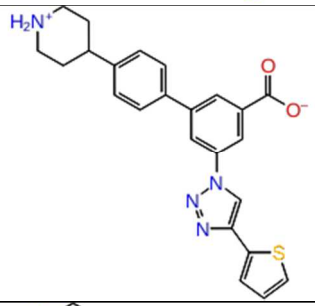
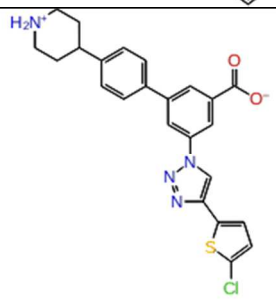
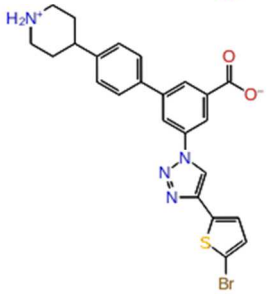
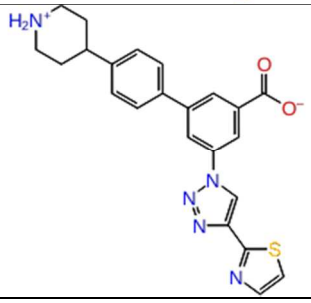
| 46 |  | C24H23N6O2 | 428.498 |
| 47 |  | C24H22N4O3 | 414.468 |
| 48 |  | C26H26N4O3 | 442.522 |
| 49 |  | C26H26N4O3 | 442.522 |
| 50 |  | C28H24N4O3 | 464.528 |

| | | | |
|---|---|---|---|
| 51 |  | C24H22N4O2S1 | 430.533 |
| 52 |  | C24H22N4O2S1 | 430.533 |
| 53 |  | C24H21Cl1N4O2S1 | 464.978 |
| 54 |  | C24H21Br1N4O2S1 | 509.434 |
| 55 |  | C23H21N5O2S1 | 431.520 |

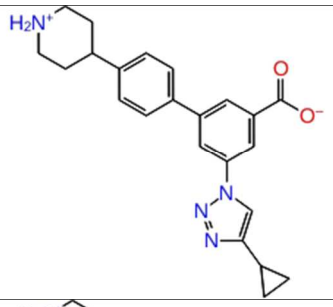
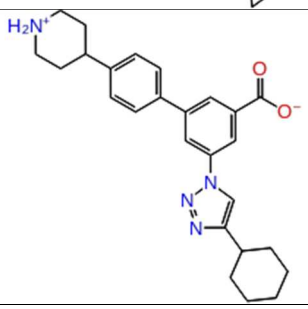
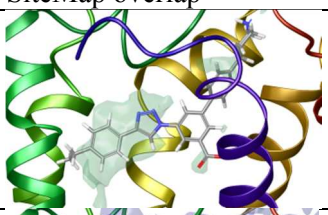
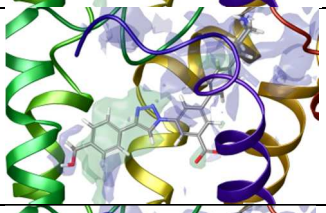
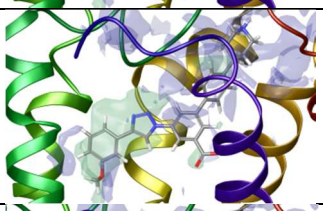
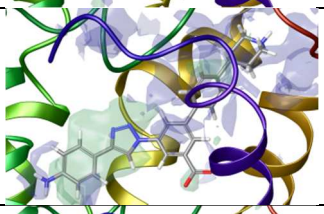
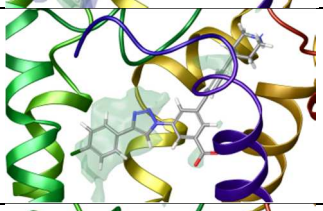
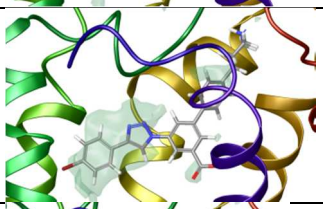
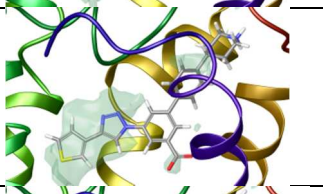
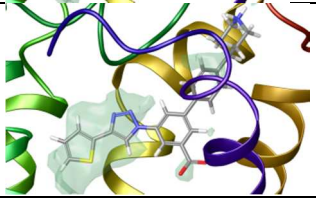
| | | | |
|---|---|---|---|
| 56 |  | C23H24N4O2 | 388.473 |
| 57 |  | C26H30N4O2 | 430.555 |

**Table S2**. Overlap with hP2Y$_{14}$R interaction sites and selected triazole derivatives.

| Compound | ID | SiteMap overlap | Selection criteria | Docking Score (kcal/mol) |
|---|---|---|---|---|
| **66** | 01 |  | Ethyl group docks in hydrophobic region | -9.509 |
| **67** | 22 |  | Hydroxyl group docks in H-bond donor region | -9.778 |
| **68** | 37 |  | Methoxy group docks in hydrophobic pocket | -9.067 |
| **69** | 24 |  | -$NH_2$ group docks in H-bond donor region | -9.051 |
| **70** | 31 |  | Chloro docks in hydrophobic region | -8.779 |
| **71** | 30 |  | Bromo docks in hydrophobic region | -8.723 |
| **72** | 51 |  | Thienyl establishes π–π interaction with Phe191 | -8.519 |
| **73** | 52 |  | Thienyl establishes π–π interaction with Tyr102 | -8.271 |

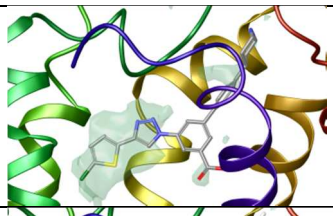
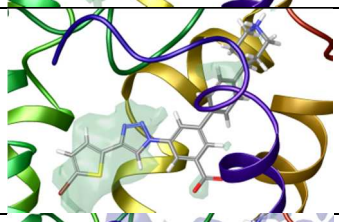
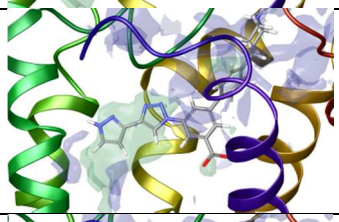
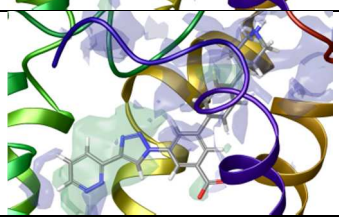
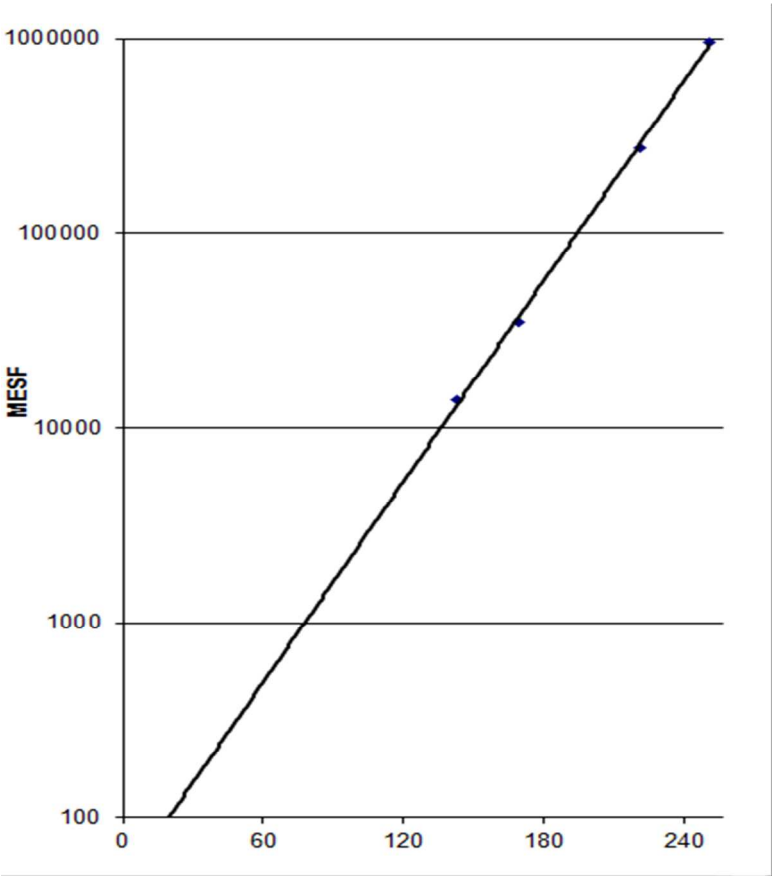
| | | | | |
|---|---|---|---|---|
| **74** | 53 |  | Thienyl establishes π–π interactions, Chloro fits in hydrophobic region | -8.575 |
| **75** | 54 |  | Thienyl establishes π–π interactions, Bromo fits in hydrophobic region | -8.193 |
| not synthesized (see Table 1) | 45 |  | -NH docks in H-bond donor region | -7.610 |
| not synthesized (see Table 1) | 44 |  | π–cation interaction with Arg253 | -8.112 |

**Figure S3. Fluorescence standards with assigned MESF (Quantum Alex Fluor 488, Bangs Laboratories, Fishers, IL)**

One drop from each of the 4 fluorescent intensity populations was added to 0.5 ml PBS and lightly vortexed. After adjusting gains, optics, lasers and threshold and gating the single population, with flow rate 200-500 events per second, PMT voltage was adjusted to the defined baseline. The fluorescent intensities were recorded increasing PMT voltage to obtain well-separated, sharply-defined peaks, all of them in the analysis window. Analyzing the results with linear regression using QuickCal v 2.3 software (Bang Laboratories, Inc.), we got a curve where X axis represents assigned MESF, Y axis means MFI. To calculate the MESF from the MFI of the samples in this study, the samples were measured under exactly the same conditions and extrapolated the assigned MFI from the calibration curve.



**Effect of Compound 65 at $P2Y_1$ and $P2Y_6$ receptors**

The effects of compound **65** at $P2Y_1R$ and $P2Y_6R$ were determined using intracellular calcium assay using fluorescent FLIPR calcium 5 assay kit in FLIPR Tetra instrument (Molecular Devices, Sunnyvale, CA).

**Method**

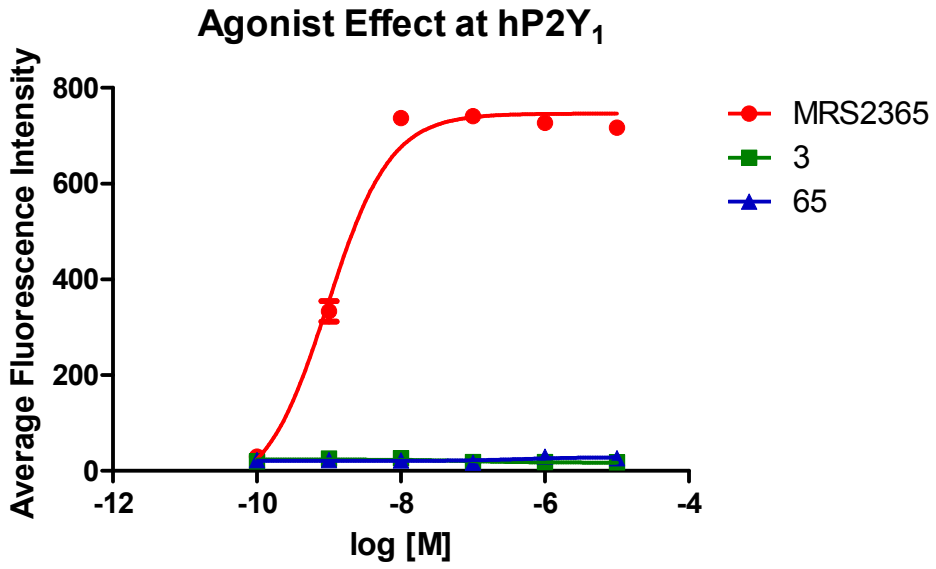
1321N1 human astrocytoma cells overexpressing either $hP2Y_1R$ or $hP2Y_6R$ were plated in black 96-well plates and grown overnight at 37° C. On the day of the assay, the media was removed and replaced with FLIPR Calcium 5 dye and incubated for 1 h. For agonist assays, cells were directly treated with increasing concentration of agonists from 1 nM to 10 µM and the change in intracellular calcium is measured by the change in fluorescent intensities. For antagonists assay, the compounds (10 µM) were added to the cells and incubated for 30 minutes followed by the addition of standard agonist and measuring the change in intracellular calcium.

**Results**

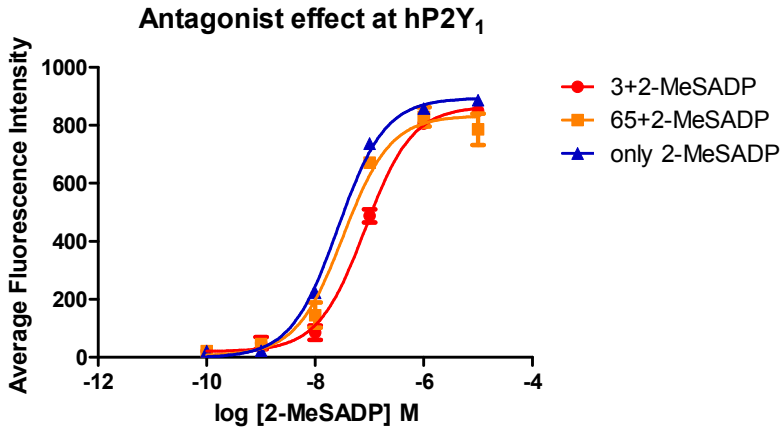
Both $P2Y_1$ and $P2Y_6$ receptors are associated with Gq protein signaling which leads to changes in intracellular calcium levels. Hence, calcium assay was used to study the effect of the Compound **65** and compound **3**,a known $P2Y_{14}R$ antagonist, at these receptors. As you can see, Compound 65 and compound 3 didn't have any significant effect on either $hP2Y_1$ receptors (Fig S2 &S3) or at $hP2Y_6$ receptors (Fig S4 & S5). This shows that compound 65 does not have any effect on other P2Y receptors.

Figure S4.

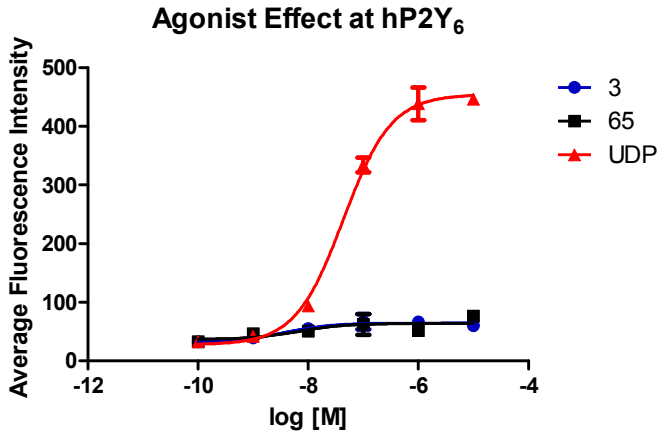
A



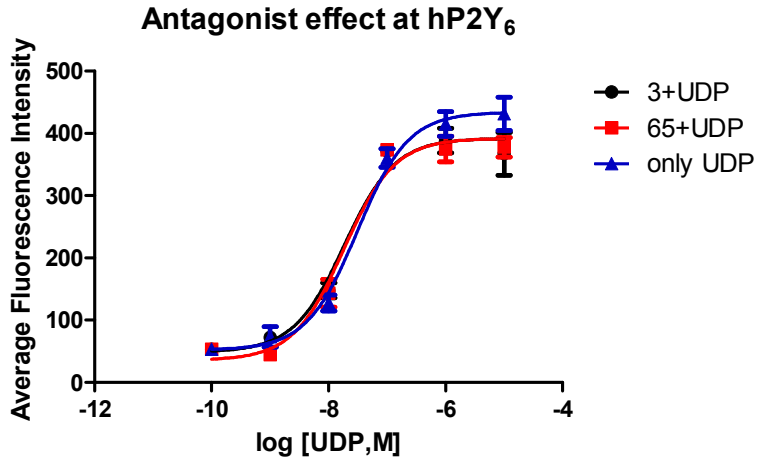
B


Antagonist effect at hP2Y1
Average Fluorescence Intensity
1000
800
600
400
200
0
-12
-10
-8
-6
-4
log [2-MeSADP] M
3+2-MeSADP
65+2-MeSADP
only 2-MeSADP

C


Agonist Effect at hP2Y6
Average Fluorescence Intensity
500
400
300
200
100
0
-12
-10
-8
-6
-4
log [M]
3
65
UDP

D


Antagonist effect at hP2Y6
Average Fluorescence Intensity
500
400
300
200
100
0
-12
-10
-8
-6
-4
log [UDP,M]
3+UDP
65+UDP
only UDP