Supporting Information for

Development of small-molecule fluorescent probes targeting neutrophils *via N*-formyl peptide receptors

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1. Synthetic procedures, characterisation details and spectral data

General details. Unless otherwise stated, all reagents were purchased from chemical suppliers and used without further purification, room temperature corresponds to ambient temperature and yields refer to spectroscopically and chromatographically pure compounds. Reactions were monitored by thin layer chromatography (TLC) performed on commercially available glass plates pre-coated with Merck silica gel 60 F254 or Merck silica gel 60 RP-18 F254s. Visualisation was performed by the quenching of UV fluorescence (λ_{max}= 254/365 nm) and by staining with iodine or potassium permanganate. All flash chromatography was performed using slurry packed Merck 9325 Keiselgel 60 or Aldrich C18-reverse phase silica gel. Melting points were obtained using a Gallenkamp Melting Point apparatus and are uncorrected. NMR spectra were recorded using an internal deuterium lock at ambient probe temperatures on a Bruker Avance (400 MHz) instrument. Chemical shifts (δ) are quoted in ppm, to the nearest 0.01 ppm (for ^{1}H NMR spectra), or 0.1 ppm (for ^{13}C NMR spectra), and are referenced to the residual non-deuterated solvent peak. Coupling constants (J) are reported in Hertz (Hz) to the nearest 0.1 Hz. Data are reported as follows: chemical shift, multiplicity (br= broad; s= singlet; d= doublet; t= triplet; m= multiplet, or as a combination of these, e.g. dd, dt, etc.), integration, assignment and coupling constant(s). Assignments were determined either on the basis of unambiguous chemical shift or coupling pattern, and analogy to fully interpreted spectra for related compounds. Liquid chromatography–mass spectrometry (LC-MS) experiments were performed on a Waters Aquity UPLC I-CLASS coupled with Waters LCT Premier (operating in ES^+ or ES^- mode). High resolution masses (HRMS) for accurate mass determination were performed on the same equipment, and samples were referenced against leucine enkaphalin or sulfadimethoxine. For analytical HPLC, a Waters BEH Acquity C18 (50mm x 2.1mm) column was used and the mobile phase was composed of solvent A (99.9% Water, 0.1% Formic Acid) and solvent B (99.9% Acetonitrile, 0.1% Formic Acid) used in a linear gradient (time= 0 min, 95%A and 5%B; time= 3.2 min, 5%A and 95%B; time= 3.5 min, 95%A and 5%B; total run time 4 min; for compound **17**, time= 0 min, 95%A and 5%B; time= 5 min, 95%A and 5%B; time= 30 min, 5%A and 95%B; time= 35 min, 95%A and 5%B; total run time 35 min). The sample solutions were prepared at a concentration of 0.1 mg/1 mL. The injection volume was 10µL, the flow rate was 0.5mL/min, the column temperature was 40 °C and UV detection was carried out at 5 fixed wavelengths within the range 210-550 nm. The values of retention time (t_R) are given in

minutes. Electron spray ionisation (ESI) conditions were as follow: 2kV (ES^+) and 2.5kV (ES^-) capillary voltage; 30 V (ES^+) and 150 V (ES^+) sample cone voltage; 2.1kV MCP Voltage; 350 °C desolvation temperature; 120 °C source temperature; 10 L/h cone gas flow (N_2); 400 L/h desolvation gas flow (N_2). Mass values are reported within the error limits of ±5 ppm mass units. UV–vis spectra of the probes were recorded using a Perkin-Elmer Lambda 25 UV-vis spectrophotometer, and fluorescence emission spectra were obtained on a Varian Cary Eclipse fluorescence spectrofluorometer, using 0.1-0.5 mg/mL sample solutions and 1.0 cm path-length quartz cuvettes (1.0-3.0 mL) at 25°C. Spectra were not corrected for light intensity or detector sensitivity. Data were recorded on-line and analysed by Excel software. 6-Methyl-4,5-dihydropyridazin-3(2*H*)-one **1**, 4-(3-methoxybenzyl)-6-methylpyridazin-3(2*H*)-one **5** and amino-BODIPY **15** were prepared by adopting previously reported procedures [37,44].

4-(Anthracen-9-yl-methyl)-6-methylpyridazin-3(2*H*)-one, 2.

Compound **1** [37] (200.0 mg, 1.79 mmol, 1 eq.) and 9-anthracenecarboxaldehyde (405.0 mg, 1.96 mmol, 1.2 eq.) were sequentially added to a solution (7.0 mL) of KOH in absolute EtOH (5%, w/v). The reaction was refluxed under stirring for 2.5 h. After cooling, the mixture was concentrated *in vacuo*, diluted with ice-cold water (10.0 mL), and acidified with 6N HCl until pH= 3. The suspension was extracted with CH_2Cl_2 (3 x 25.0 mL), and the organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The resulting residue was purified by column flash chromatography using cyclohexane/ethyl acetate 3:1 as eluent, to obtain **2** as brownish solid. Yield = 71.1% (382.5 mg, 1.27 mmol); mp= 158-160 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ 10.50 (exch br s, 1H, NH), 8.50 (s, 1H, Ar), 8.07-8.09 (m, 2H, Ar), 8.01-8.03 (m, 2H, Ar), 7.50-7.52 (m, 4H, Ar), 6.13 (s, 1H, Ar), 4.89 (s, 2H, CH_2), 1.96 (s, 3H, CH_3). LC-MS (ESI): m/z calcd. for $C_{20}H_{16}N_2O$ 300.4 (MW), found 301.1 $[M+H]^+$; t_R= 2.2. HRMS (ESI): m/z calcd. for $C_{20}H_{17}N_2O$ 301.1341 $[M+H]^+$, found 301.1352.

2-[5-(Anthracen-9-yl-methyl)-3-methyl-6-oxopyridazin-1(6*H*)-yl]-*N*-(4-bromophenyl)acetamide, 4.

K_2CO_3 (352.0 mg, 2.55 mmol, 2 eq.) was added to a stirred solution of intermediate **2** (382.5 mg, 1.27 mmol, 1 eq.) in anhydrous acetonitrile (10.0 mL). After 10 min at 50 °C h, *N*-(4-bromophenyl)-2-chloroacetamide **3** [43] (472.1 mg, 1.90 mmol, 1.5 eq.) was added,

and the reaction was carried out at reflux for 3.5 h. The mixture was then cooled at room temperature to obtain **4** as a yellowish precipitate, which was filtered under vacuum, washed with acetonitrile (50.0 mL) and water (150.0 mL), and purified by crystallisation from EtOH. Yield = 69.1% (450.0 mg, 0.88 mmol); mp = 280-281 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ 9.05 (exch br s, 1H, NH), 8.51 (s, 1H, Ar), 8.06-8.10 (m, 2H, Ar), 7.97-8.01 (m, 2H, Ar), 7.44-7.53 (m, 8H, Ar), 6.17 (s, 1H, Ar), 5.03 (s, 2H, *CH*$_2$Ar), 4.91 (s, 2H, CH_2CO), 1.99 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ 165.4 (C), 161.2 (C), 146.3 (C), 142.6 (C), 137.0 (C), 132.1 (2CH), 131.7 (2C), 131.1 (CH), 130.6 (2C), 129.5 (2CH), 128.4 (C), 127.6 (CH), 126.8 (2CH), 125.4 (2CH), 124.1 (2CH), 121.6 (2CH), 117.1 (C), 58.4 (CH_2), 27.7 (CH_2), 21.0 (CH_3). LC-MS (ESI): m/z calcd. for $C_{28}H_{22}BrN_3O_2$ 512.4 (MW), found 512.1/514.1 with a correct isotopic ratio 1:1 of ions species $[M+H]^+$; t_R= 2.8. HRMS (ESI): m/z calcd. for $C_{28}H_{23}BrN_3O_2$ 512.0974 $[M+H]^+$, found 512.0966. UV-vis (PBS buffer, 10 μM): λ/nm 357, 377, 398. Fluorescence (PBS buffer, PBS buffer): λ_{max}(ex) 377 nm, λ_{max}(em) 497 nm.

2-[(5-(Dimethylamino)naphthalen-1-yl)sulfonyl]-4-(3-methoxybenzyl)-6-methylpyridazin-3(2*H*)-one, 7.

Dansyl chloride **6** (281.0 mg, 1.04 mmol, 1 eq.) and Et_3N (0.44 mL, 3.13 mmol, 3 eq.) were added to a solution of intermediate **5** [37] (200.0 mg, 0.87 mmol) in anhydrous acetonitrile (3.0 mL). The reaction was stirred at room temperature for 16 h. After evaporation of the solvent, the residue was extracted with brine and CH_2Cl_2 (3 x 25.0 mL). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. Lastly, the crude product was purified by column flash chromatography using cyclohexane/ethyl acetate 2:1 as eluent to obtain **7** as an orange solid. Yield = 11.5% (46.5 mg, 0.10 mmol); mp= 51-52 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ 8.64 (d, 1H, Ar, *J*= 8.8 Hz), 8.36-8.41 (m, 2H, Ar), 7.56-7.61 (m, 2H, Ar), 7.27 (t, 1H, Ar, *J*= 8.0 Hz, overlapped with $CDCl_3$ signal), 7.20 (d, 1H, Ar, *J*= 7.2 Hz), 6.98 (s, 1H, Ar), 6.85 (d, 1H, Ar, *J*= 8.8 Hz), 6.77-6.80 (m, 2H, Ar), 4.06 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 2.89 (s, 6H, 2 x NCH_3), 2.51 (s, 3H, CCH_3). ^{13}C-NMR ($CDCl_3$) δ 160.0 (C), 159.9 (C), 159.3 (C), 137.4 (2C), 134.5 (2C), 131.9 (CH), 131.3 (CH), 130.3 (CH), 130.0 (CH), 129.9 (2C), 128.7 (CH), 121.7 (2CH), 115.1 (2CH), 112.7 (2CH), 55.2 (CH_3), 45.5 (CH_3), 35.0 (CH_2), 21.5 (CH_3). LC-MS (ESI): m/z calcd. for $C_{25}H_{25}N_3O_4S$ 463.5 (MW), found 464.2 $[M+H]^+$; t_R= 2.7. HRMS (ESI): m/z calcd. for $C_{25}H_{26}N_3O_4S$ 464.1644 $[M+H]^+$, found 464.1630. UV-vis (PBS buffer, 10 μM): λ/nm 299. Fluorescence (PBS buffer, 10 μM): λ_{max}(ex) 299 nm, λ_{max}(em) 511 nm.

3-(6-Oxo-1,4,5,6-tetrahydropyridazin-3-yl)propanoic acid, 9.[48]

To an ice-cold solution of 4-oxoheptanedioic acid **8** (550.0 mg, 3.14 mmol, 1 eq.) in EtOH (8.0 mL), hydrazine hydrate (60% in water, 0.19 mL, 3.77 mmol, 1.2 eq.) was added drop-wise and the solution was stirred at 60 °C for 1.5 h. The solvent was evaporated, and the residue was extracted with brine (5.0 mL) and CH_2Cl_2 (3 x 15.0 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under vacuum. Lastly, the crude product was purified by crystallisation from EtOH to yield **9** as an amorphous white solid. Yield = 67.7% (362.0 mg, 2.13 mmol); mp = 186-187 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ 12.08 (exch br s, 1H, OH), 10.47 (exch, 1H, NH), 2.45-2.48 (m, 4H, $NCO(CH_2)_2$), 2.42 (t, 2H, CH_2CH_2COOH, *J*= 8.4 Hz), 2.23-2.28 (m, 2H, CH_2COOH).

3-[5-(3-Methoxybenzyl)-6-oxo-1,6-dihydropyridazin-3-yl]propanoic acid, 10.

To a stirred solution of **9** (360.0 mg, 2.11 mmol, 1 eq.) in 10.0 mL of KOH/absolute EtOH (5% w/v), 3-methoxybenzaldehyde (0.33 mL, 2.74 mmol, 1.3 eq.) was added, and the reaction was refluxed for 5 h. After cooling, the mixture was evaporated under vacuum, diluted with ice-cold water (5.0 mL), acidified with 6N HCl (pH= 3), and extracted with CH_2Cl_2 (3 x 25.0 mL). Removal of the solvent resulted in the crude product, which was purified by column flash chromatography using CH_2Cl_2/MeOH (gradient 95:5→90:10) as eluent to obtain **10** as yellowish solid. Yield = 36.5% (222.0 mg, 0.77 mmol); mp = 145-146 °C (EtOH). ^{1}H NMR (CD_3OD) δ 7.26-7.21 (m, 1H, Ar), 7.08 (s, 1H, Ar), 6.86-6.80 (m, 3H, Ar), 3.83 (s, 2H, ArCH_2), 3.79 (s, 3H, CH_3), 2.85 (t, 2H, N=CCH_2CH_2, J= 7.1 Hz), 2.66 (t, 2H, N=CCH_2CH_2, J= 7.1 Hz). ^{13}C NMR (DMSO-d_6) δ 173.50 (C), 160.65 (C), 159.31 (C), 146.29 (C), 141.89 (C), 139.57 (C), 130.64 (CH), 129.43 (CH), 121.18 (CH), 114.80 (CH), 111.73 (CH), 54.93 (CH_3), 34.69 (CH_2), 31.14 (CH_2), 28.75 (CH_2). LC-MS (ESI): m/z calcd. for $C_{15}H_{16}N_2O_4$ 288.3 (MW), found 289.1 $[M+H]^+$; t_R= 1.4. HRMS (ESI): m/z calcd. for $C_{15}H_{17}N_2O_4$ 289.1188 $[M+H]^+$, found 289.1188.

Ethyl 3-{5-[(3-methoxyphenyl)methyl]-6-oxo-1,6-dihydropyri dazin-3-yl}propanoate，11.

600 mg (2.08 mmol) of compound **10** was dissolved in 30 mL of anhydrous ethanol, 4 drops of concentrated sulfuric acid was added. The reaction was refluxed for 4h. Then the solution was concentrated under vacuum and diluted by dichloromethane. The organic phase was washed twice with water and dried over Na_2SO_4. The organic solvent was removed under vacuum and the solid residue was purified by flash column chromatography (eluent: hexane/ethyl acetate, 3:2) to obtain **11** as a pale-yellow solid. Yield = 76% (503 mg, 1.59 mmol); mp = 74-76 °C. ^{1}H NMR ($CDCl_3$) δ 7.29-7.25 (m,1H, Ar), δ 6.88-6.69 (m, 4H, Ar); δ 4.11 (q, 2H, O*CH*$_2$CH$_3$, J= 7.0), 3.87 (s, 2H, Ar-*CH*$_2$), 3.81 (s, 3H, OCH_3), 2.83 (t, 2H, N=CCH$_2$*CH*$_2$, J= 7.1), 2.64 (t, 2H, N=C*CH*$_2$CH$_2$, J= 7.1), 1.23 (t, 3H, OCH$_2$*CH*$_3$, J= 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ 172.6 (C), 161.7 (C), 160.1 (C), 147.4 (C), 143.7 (C), 138.5 (C), 130.9 (CH), 130.0 (CH), 121.9 (CH), 115.4 (CH), 112.5 (CH), 60.8 (CH_2), 55.4 (CH_3), 35.5 (CH_2), 31.7 (CH_2), 29.4 (CH_2), 14.3 (CH_3). HRMS(ESI): m/z calcd. for $C_{17}H_{20}N_2O_4$ 317.1501 $[M+H]^+$, found 317.1505.

Ethyl 3-{1-[2-(4-bromophenylamino)-2-oxoethyl]-5-(3-methoxybenzyl)-6-oxo-1,6-dihydropyridazin-3-yl}propanoate, 12.

K_2CO_3 (511 mg, 3.70 mmol, 3 eq.) was added to a stirred solution of compound **11** (390 mg, 1.23 mmol, 1.0 eq.) and N-(4-bromophenyl)-2-chloroacetamide **3** [43] (368 mg, 1.48 mmol, 1.2 eq.) in 10 mL of acetonitrile. The reaction was refluxed for 6 h. Then the solution was diluted with dichloromethane and extracted twice with water. The organic phase was dried, and the solvent was removed under vacuum. The solid residue was purified by flash column chromatography (hexane/dichloromethane/ethyl acetate, 8:9:3) to obtain **12** as pale-yellow solid. Yield=69% (447 mg, 0.85 mmol); mp = 53-54 °C. ^{1}H NMR ($CDCl_3$) δ 8.92 (s,1H, CONH), 7.39–7.35 (m, 4H, Ar), 7.28–7.24 (m, 1H, Ar), 6.84–6.77 (m, 4H, Ar), 4.91 (s, 2H, NCH$_2$CO), δ 4.09 (q, 2H, OCH$_2$CH$_3$, J= 7.1), 3.89 (s, 2H, Ar), 3.79 (s, 3H, OCH_3), 2.87 (t, 2H, N=CCH$_2$CH$_2$, J= 7.1 Hz), 2.68 (t, 2H, N=CCH$_2$CH$_2$, J= 7.1 Hz), 1.25 (t, 3H, OCH$_2$CH$_3$, J= 7.1 Hz). ^{13}C NMR ($CDCl_3$) δ 172.6 (C), 165.3 (C), 161.2 (C), 160.1 (C), 147.6 (C), 143.5 (C), 138.3 (C), 136.9 (C), 131.9 (CH), 130.8 (2CH), 130.0 (CH), 121.8 (2CH), 121.6 (CH), 117.0 (C), 115.4 (CH), 112.4 (CH), 60.9 (CH_2), 58.4 (CH_3), 55.4 (CH_3), 36.2 (CH_2), 31.6 (CH_2), 29.5 (CH_2), 14.3 (CH_3). HRMS(ESI): m/z calcd. for $C_{25}H_{26}BrN_3O_5$ 528.1134, 530.1114 $[M+H]^+$, found 528.1135, 530.1117.

3-{1-[2-(4-Bromophenylamino)-2-oxoethyl]-5-(3-methoxybenzyl)-6-oxo-1,6-dihydropyridazin-3-yl}propanoic acid, 13.

360 mg (0.68 mmol) of compound **12** was dissolved in 10 mL of tetrahydrofuran and 10 mL of 2 M NaOH solution was added. The reaction was kept at room temperature overnight. After completion of the reaction, the solution was neutralized by 1 N hydrochloric acid. The aqueous solution was then extracted with dichloromethane three times. The organic phase was collected and dried over Na_2SO_4. Subsequently, the solvent was removed under vacuum to obtain **13** as pale-yellow solid. Yield= 100% (340 mg, 0.68 mmol); mp = 133-135 °C (EtOH). ^{1}H NMR (CD_3OD) δ 7.55–7.47 (m, 4H, Ar), 7.26 (t, 1H, Ar, J=7.9 Hz), 7.11 (s, 1H, CH), 6.88–6.83 (m, 3H, Ar), 4.97 (s, 2H, NCH_2CO), 3.89 (s, 2H, Ar-CH_2), 3.81 (s, 3H, OCH_3), 2.90 (t, 2H, N=C*CH_2*CH_2, J=7.1), 2.69 (t, 2H, N=CCH_2*CH_2*, J=7.1 Hz). ^{13}C NMR (CD_3OD) δ 176.3 (C), 167.4 (C), 162.4 (C), 161.5 (C), 149.0 (C), 144.0 (C), 140.2 (C), 138.8 (C), 132.9 (2CH), 132.3 (CH), 130.7 (CH), 122.8 (2CH), 122.6 (CH), 117.7 (C), 115.9 (CH), 113.4 (CH), 56.6 (CH_2), 55.6 (CH_3), 36.6 (CH_2), 32.4 (CH_2), 30.4 (CH_2). LC-MS (ESI): m/z calcd. for $C_{23}H_{22}BrN_3O_5$ 500.3 (MW), found 500.1/502.1 $[M+H]^+$, 517.1/519.1 $[M+NH_4]^+$, 522.1/524.1 $[M+Na]^+$, 497.9/499.9 $[M-H]^-$ all with a correct isotopic ratio 1:1 of ions species; t_R= 1.4. HRMS(ESI): m/z calcd. for $C_{23}H_{22}BrN_3O_5$ 500.0821, 502.0801 $[M+H]^+$, found 500.0811, 502.0786.

5-({2-[3-(1-(2-[(4-bromophenyl)amino]-2-oxoethyl)-5-(3-methoxybenzyl)-6-oxo-1,6-dihydropyridazin-3-yl)propanamido]ethyl}amino)naphthalene-1-sulfonic acid, 16.

HOBt (7.6 mg, 0.06 mmol), Et_3N (0.011 mL, 0.08 mmol, 1.3 eq.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 0.014 mL, 0.08 mmol, 1.3 eq.) were sequentially added to a stirred solution of **13** (40.0 mg, 0.08 mmol, 1.3 eq.) in 1.0 mL of anhydrous DMF. A solution of 5-[(2-aminoethyl)amino]naphthalene-1-sulfonic acid (EDANS) **14** (14.9 mg, 0.06 mmol, 1eq.) in anhydrous DMF (4 mL) was added, and the mixture was stirred under N_2 for 48 h at room temperature. After evaporation of the solvent, the residue was and extracted with brine and CH_2Cl_2 (3 x 15 mL). The organic layers were combined, dried over Na_2SO_4, and evaporated under vacuum to obtain a crude residue, which was purified by column flash chromatography using CH_2Cl_2/MeOH 90:10 as eluent to obtain **16** as a brownish solid. Yield = 92.4% (41.5 mg, 0.05 mmol); mp = 60-70 °C (dec). ^{1}H-NMR

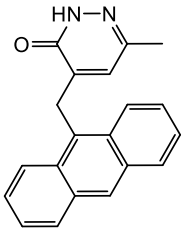
(CD_3OD) δ 8.14-8.20 (m, 2H, Ar), 8.02 (d, 1H, Ar, *J*= 8.4 Hz), 7.50 (d, 2H, Ar, *J*= 8.8 Hz), 7.42 (d, 2H, Ar, *J*= 8.8 Hz), 7.29-7.38 (m, 2H, Ar), 7.23 (t, 1H, Ar, *J*= 8.0 Hz), 6.99 (s, 1H, Ar), 6.79-6.83 (m, 3H, Ar), 6.56 (s, 1H, Ar, *J*= 7.6 Hz), 4.59 (s, 2H, NCH_2CO), 3.76 (s, 3H, OCH_3); 3.77 (s, 2H, CH_2Ar), 3.52 (t, 2H, CH_2NH, *J*= 5.6 Hz), 3.28 (t, 2H, CH_2NH, *J*= 5.6 Hz), 2.89 (t, 2H, CH_2CH_2CONH, *J*= 6.8 Hz), 2.81 (exch br s, 1H, SO_3H), 2.58 (t, 2H, CH_2CH_2CONH, *J*= 6.8 Hz) . ^{13}C-NMR (CD_3OD, signals marked with * correspond to additional peaks due to the presence of a minor rotamer) δ 175.7(C), 167.4 (C), 162.1 (C), 161.4 (C), 161.3 (C*), 148.8 (C), 145.4 (C), 144.0 (C), 141.8 (C), 140.2 (C), 138.8 (C), 132.8 (2CH), 132.0 (CH), 131.4 (C), 130.7 (CH), 128.6 (CH), 128.54 (CH*), 126.7 (CH), 125.4 (C), 125.3 (CH), 123.6 (CH), 122.8 (2CH), 122.6 (CH), 120.4 (C), 118.6 (CH*), 117.7 (CH*), 116.4 (CH*), 116.3 (CH), 116.0 (CH), 113.5 (CH*), 113.3 (CH), 105.1 (CH*), 104.9 (CH), 56.4 (CH_2), 55.9 (CH3*), 55.6 (CH_3), 45.5 (CH_2), 45.0 (CH_2*), 40.3 (CH_2*), 39.7 (CH_2), 36.5 (CH_2), 34.4 (CH_2), 31.0 (CH_2). LC-MS (ESI): m/z calcd. for $C_{35}H_{34}BrN_5O_7S$ 748.6 (MW), found 748.2/750.2 with a correct isotopic ratio 1:1 of ions species $[M+H]^+$ and 746.1/748.1 with a correct isotopic ratio 1:1 of ions species $[M-H]^-$; t_R= 2.1. HRMS (ESI): m/z calcd. for $C_{35}H_{35}BrN_5O_7S$ 748.1441 $[M+H]^+$, found 748.1481, 750.1464. UV-Vis (PBS buffer, 10 μM): λ/nm 250, 334. Fluorescence (PBS buffer, 10 μM): λ_{max}(ex) 334 nm, λ_{max}(em) 505 nm.

3-{1-[2-(4-bromophenylamino)-2-oxoethyl]-5-(3-methoxybenzyl)-6-oxo-1,6-dihydropyridazin-3-yl}-*N*-(5,5-difluoro-1,3,7,9,10-pentamethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-2-yl)propanamide, 17.

Triethylamine (24.2 μL, 0.174 mmol, 3.5 eq.) was added to a solution of **13** (29 mg, 0.058 mmol, 1 eq.) in 10 mL of anhydrous tetrahydrofuran under N_2 atmosphere. The solution was kept at -5 °C for 30 min, followed by addition of 6.9 mg (0.064 mmol, 1.1 eq.) of ethyl chloroformate at 0℃. One hour later, the ice bath was removed, and 22 mg (0.0794 mmol, 1.4 eq.) of **15** were added. After 16 h at room temperature, the organic solvent was removed under vacuum, and the solid residue was purified by flash column chromatography (dichloromethane/methanol, 100:1) performed on aluminum oxide (neutral, 150 mesh, Merk) to obtain **17** as orange solid. Yield = 85%; mp = 136-137 °C. ^{1}H NMR (DMSO-d_6) δ 7.37 (s, 1H, CONH); 7.53~7.48 (m, 4H, Ar), 7.24~7.21 (m, 2H, Ar), 6.85-6.23 (m, 3H, Ar), 6.23 (s, 1H, H-pyrrole), 4.84 (s, 2H, NCH_2CO), 3.78 (s, 2H, Ar-CH_2), 3.71 (s, 3H, OCH_3), 2.88 (t, 2H, N=CCH_2CH_2, J= 7.2 Hz), 2.66 (t, 2H, N=CCH_2CH_2, J= 7.2

Hz), 2.58 (s, 3H, Ar-*CH*$_3$), 2.42 (s, 3H, Ar-*CH*$_3$), 2.41 (s, 3H, Ar-*CH*$_3$), 2.20 (s, 3H, Ar-*CH*$_3$), 2.14 (s, 3H, Ar-*CH*$_3$). ^{13}C-NMR (101 MHz, DMSO) δ 170.70 (C), 165.28 (C), 159.62 (C), 159.34 (C), 152.98 (C), 149.20 (C), 146.43 (C), 142.63 (C), 141.69 (C), 141.56 (C), 139.35 (C), 138.11 (C), 134.92 (C), 131.65 (2×CH), 131.46 (C), 130.62 (CH), 129.45 (2×CH), 129.19 (C), 128.06 (C), 121.24 (CH), 121.16 (CH), 120.95 (CH), 115.00 (C), 114.83 (CH), 111.85 (CH), 55.17 (CH_2), 54.91 (CH_3), 35.15 (CH_2), 32.94 (CH_2), 29.59 (CH_2), 16.86 (CH_3), 16.27 (CH_3), 14.07 (CH_3), 13.54 (CH_3), 11.87 (CH_3). t_R = 16.43 min. HRMS(ESI): m/z calcd. for $C_{37}H_{38}BBrF_2N_6O_4$ 781.2097, 783.2076 $[M+Na]^+$, found 781.2216, 783.2206. UV-vis (methanol, 1 µM): λ/nm 500. Fluorescence (methanol, 1 µM): λ_{max}(ex) 500 nm, λ_{max}(em) 536 nm.

4-(Anthracen-9-yl-methyl)-6-methylpyridazin-3(2H)-one, **2**



$C_{20}H_{16}N_2O$
Exact Mass: 300,1263
Molecular Weight: 300,3610

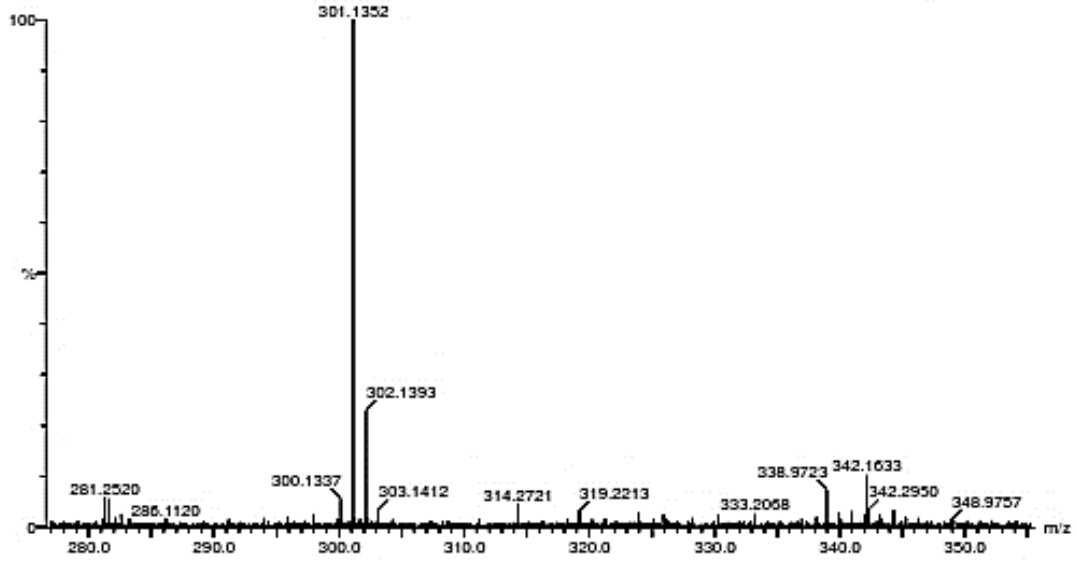
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

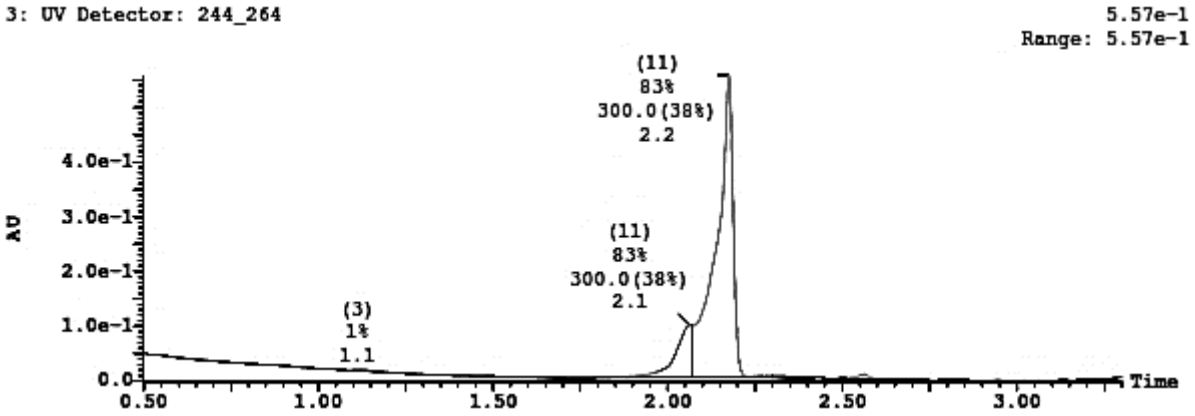
Monoisotopic Mass, Even Electron Ions
236 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 20-20 H: 0-200 N: 0-10 O: 0-10 Na: 0-1

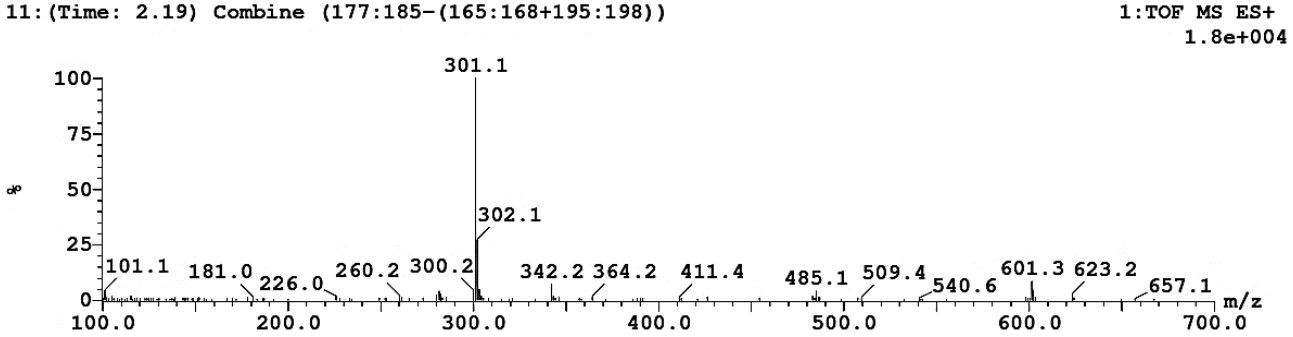
A.CILIBRIZZI AC130C
ms17507 175 (2.115) Cm (175)

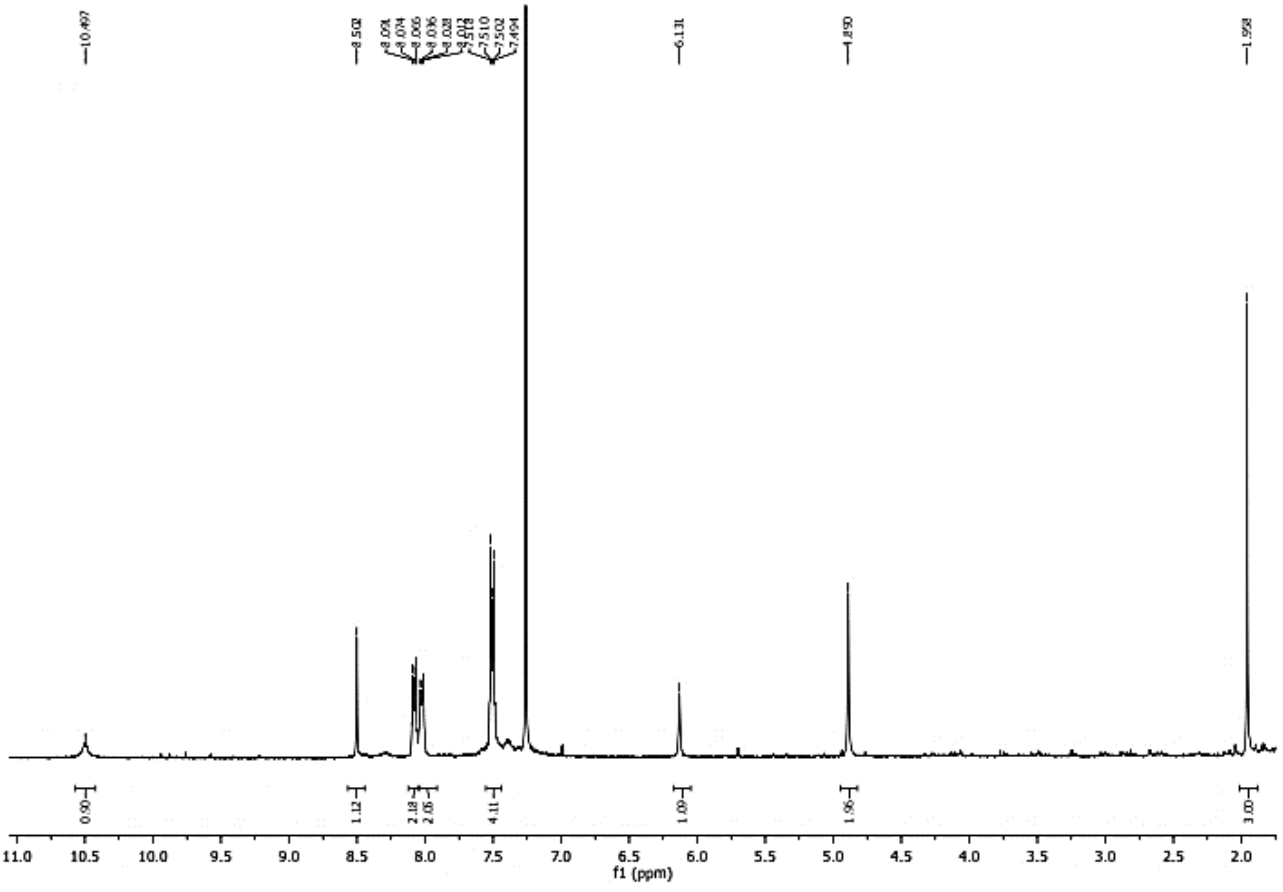
1: TOF MS ES+
7.95e+002



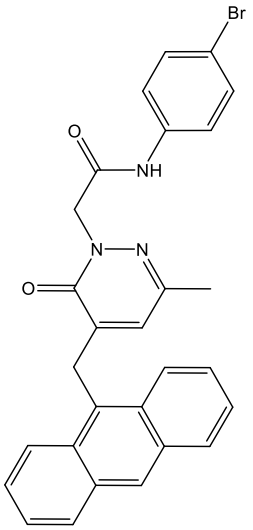
Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
301.1352	301.1341	1.1	3.7	13.5	103.2	0.0	C20 H17 N2 O


3: UV Detector: 244_264
5.57e-1
Range: 5.57e-1
(11)
83%
300.0(38%)
2.2
(11)
83%
300.0(38%)
2.1
(3)
1%
1.1
AU
4.0e-1
3.0e-1
2.0e-1
1.0e-1
0.0
0.50
1.00
1.50
2.00
2.50
3.00
Time


11:(Time: 2.19) Combine (177:185-(165:168+195:198))
1:TOF MS ES+
1.8e+004
301.1
302.1
101.1
181.0
226.0
260.2
300.2
342.2
364.2
411.4
485.1
509.4
540.6
601.3
623.2
657.1
%
m/z
100.0
200.0
300.0
400.0
500.0
600.0
700.0


10.497
8.502
8.074
8.074
8.066
8.036
8.028
7.518
7.510
7.502
7.494
6.131
1.890
1.958
0.90
1.12
2.18
2.05
4.11
1.09
1.96
3.07
f1 (ppm)

2-[5-(Anthracen-9-yl-methyl)-3-methyl-6-oxopyridazin-1(6H)-yl]-N-(4-bromophenyl) acetamide, **4**



$C_{28}H_{22}BrN_3O_2$
Exact Mass: 511,0895
Molecular Weight: 512,4070

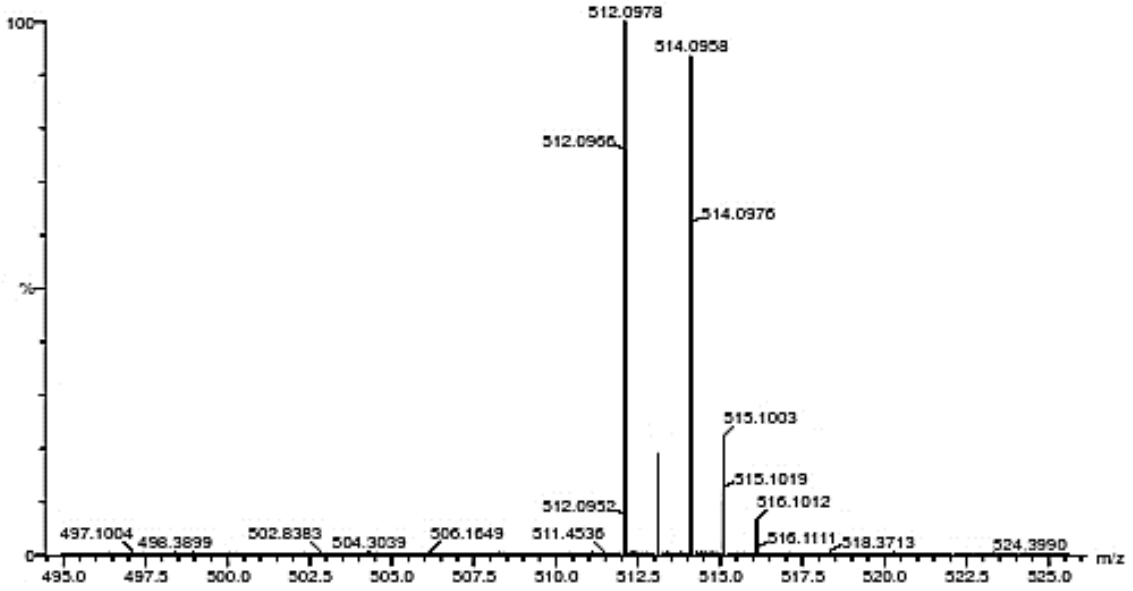
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

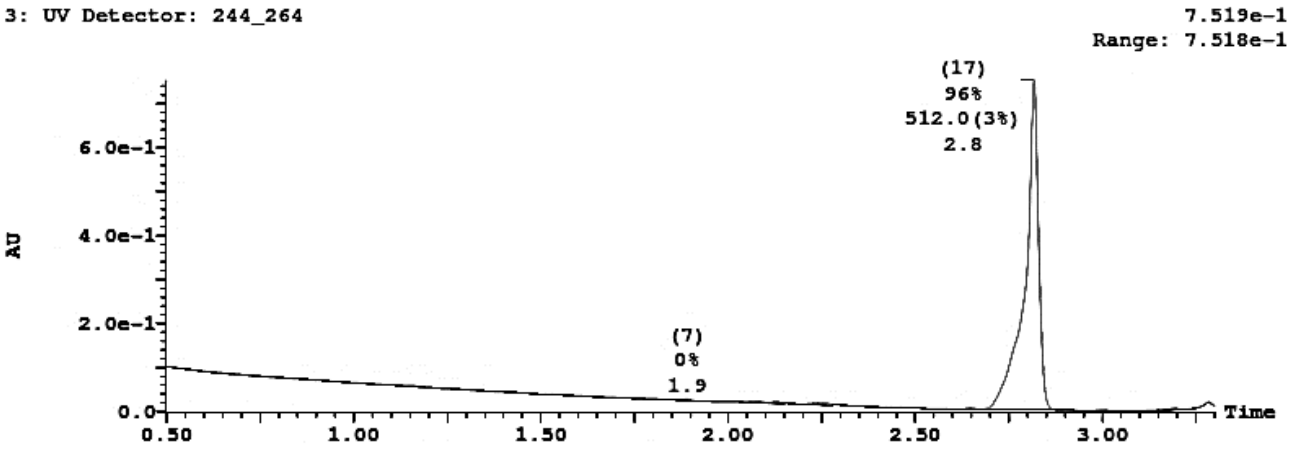
Monoisotopic Mass, Even Electron Ions
237 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 28-28 H: 0-200 N: 0-10 O: 0-10 Na: 0-1 Br: 1-1

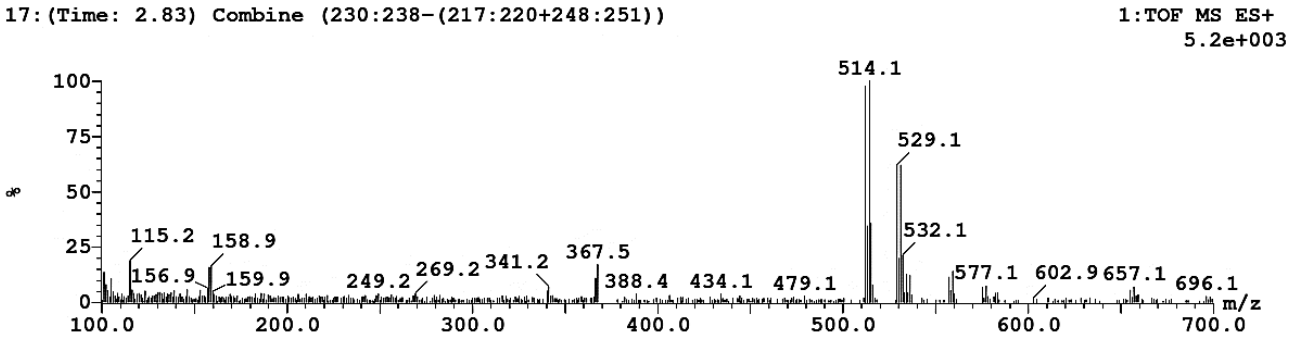
A.CILIBRIZZI AC135 PPT
ms17536a 235 (2.843) Cm (230:238)

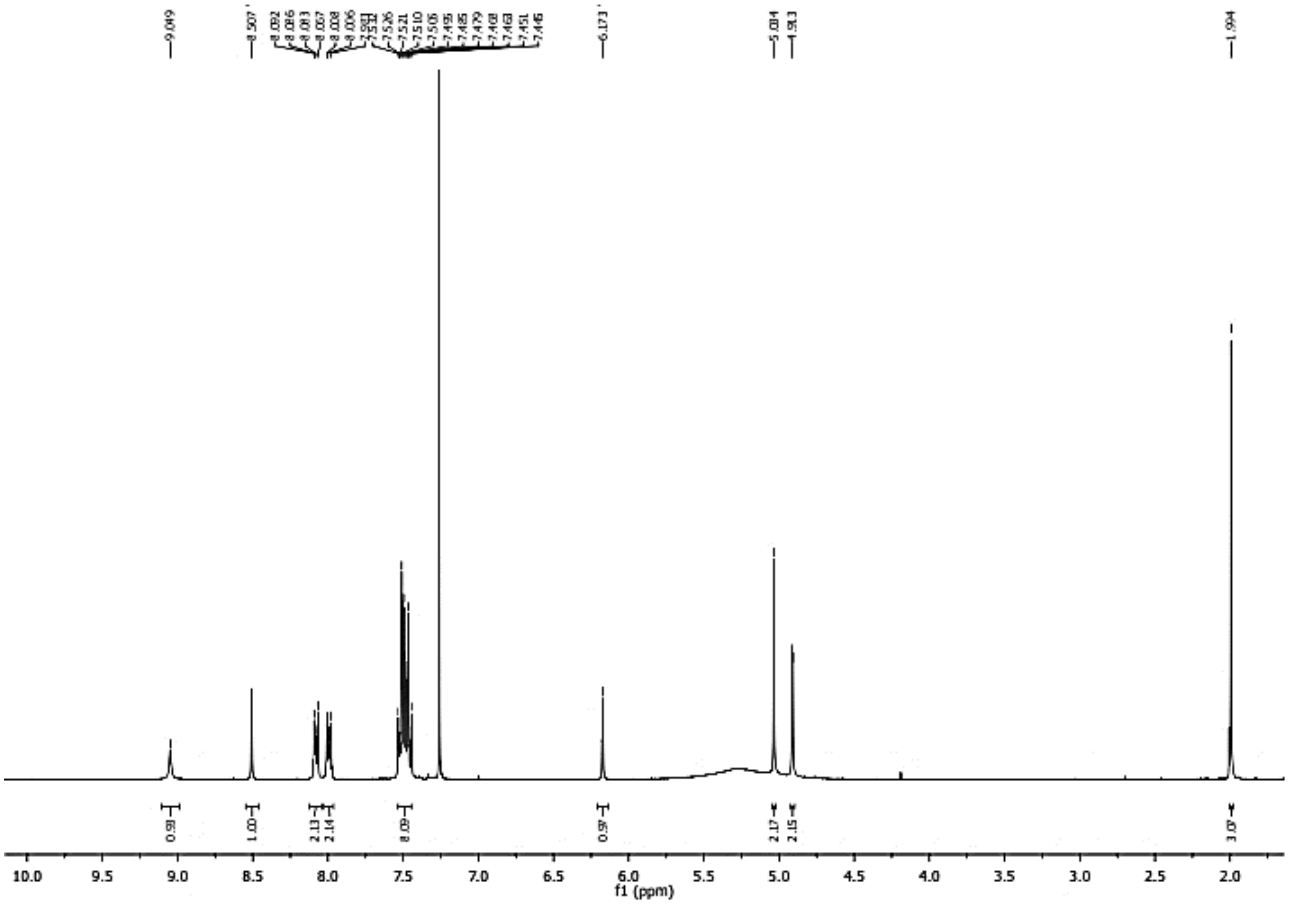
1: TOF MS ES+
2.88e+003

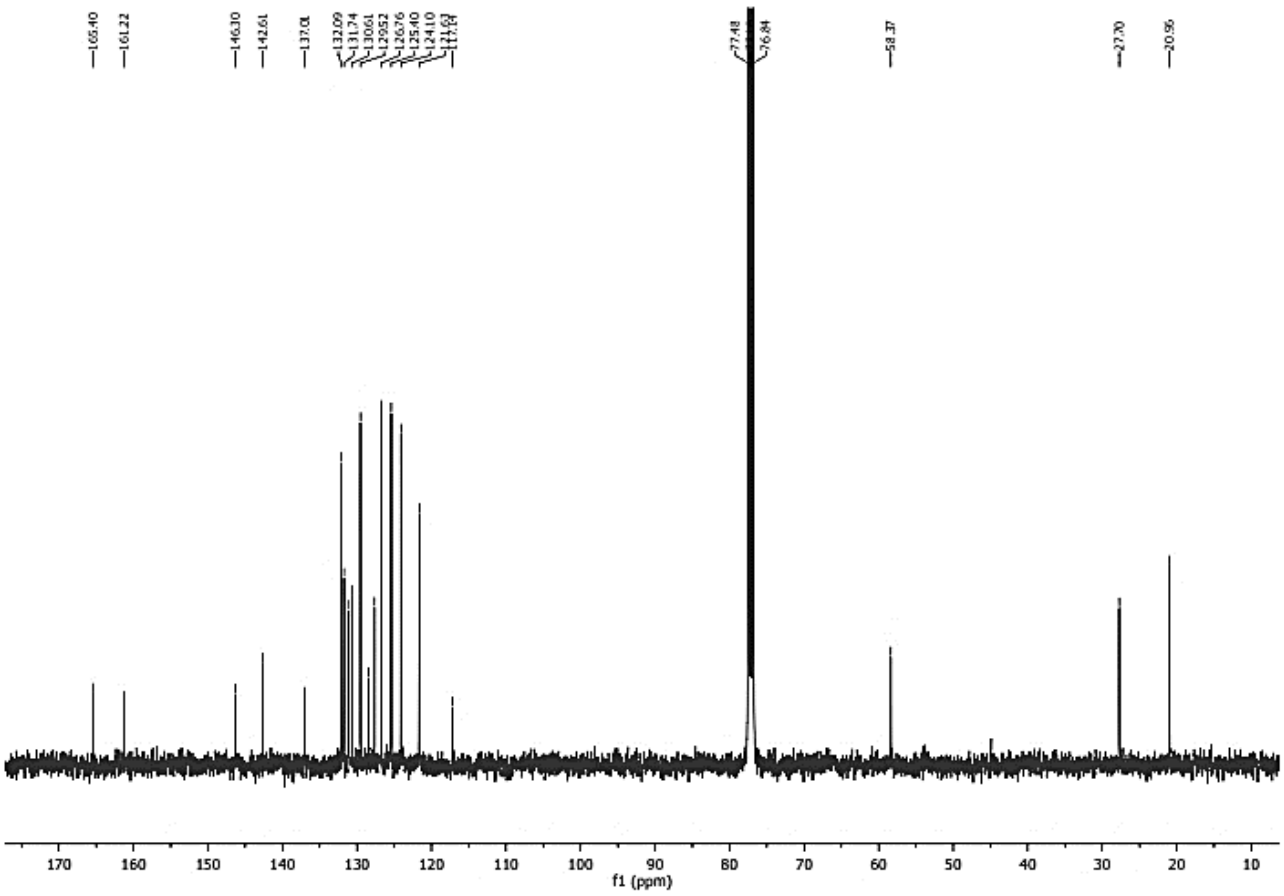


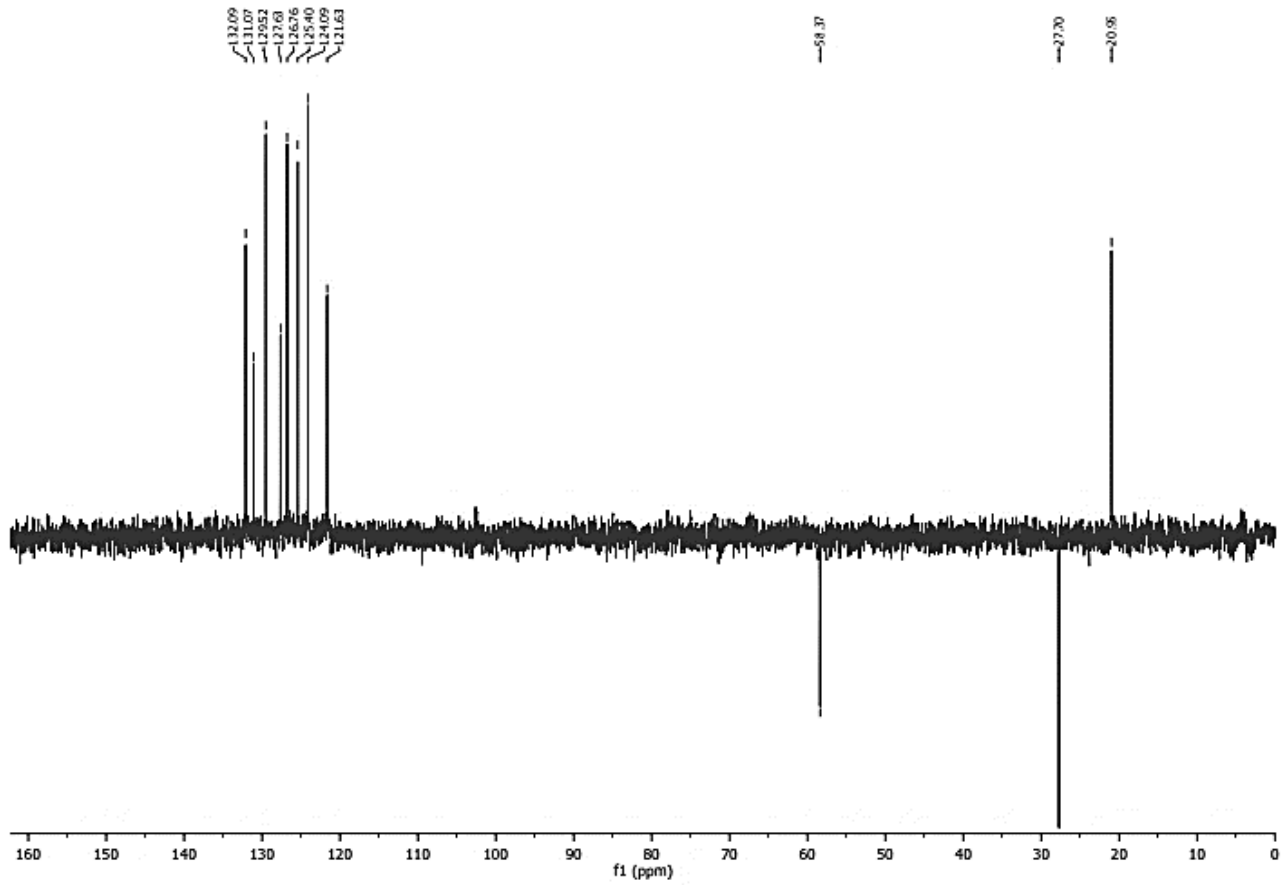
Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
512.0966	512.0974	-0.8	-1.6	18.5	446.1	0.0	C28 H23 N3 O2 Br


3: UV Detector: 244_264
7.519e-1
Range: 7.518e-1
(17)
96%
512.0(3%)
2.8
(7)
0%
1.9
AU
Time

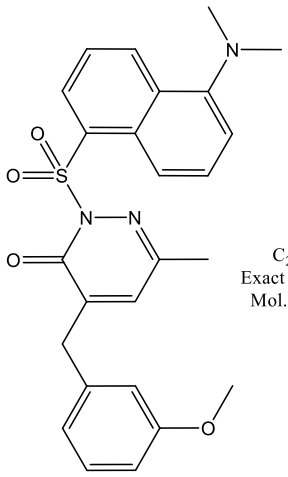

17:(Time: 2.83) Combine (230:238-(217:220+248:251))
1:TOF MS ES+
5.2e+003
514.1
529.1
532.1
115.2
158.9
156.9
159.9
249.2
269.2
341.2
367.5
388.4
434.1
479.1
577.1
602.9
657.1
696.1
%
m/z


f1 (ppm)


165.40
161.22
146.30
142.61
137.01
132.09
131.74
130.61
129.52
126.76
125.40
124.10
121.63
77.48
76.84
58.37
27.70
20.95
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


132.09
131.07
129.52
127.61
126.76
125.40
124.09
121.63
58.37
27.70
20.95
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

2-[(5-(Dimethylamino)naphthalen-1-yl)sulfonyl]-4-(3-methoxybenzyl)-6-methylpyridazin-3(2H)-one, ***7***



$C_{25}H_{25}N_3O_4S$
Exact Mass: 463,1566
Mol. Wt.: 463,5487

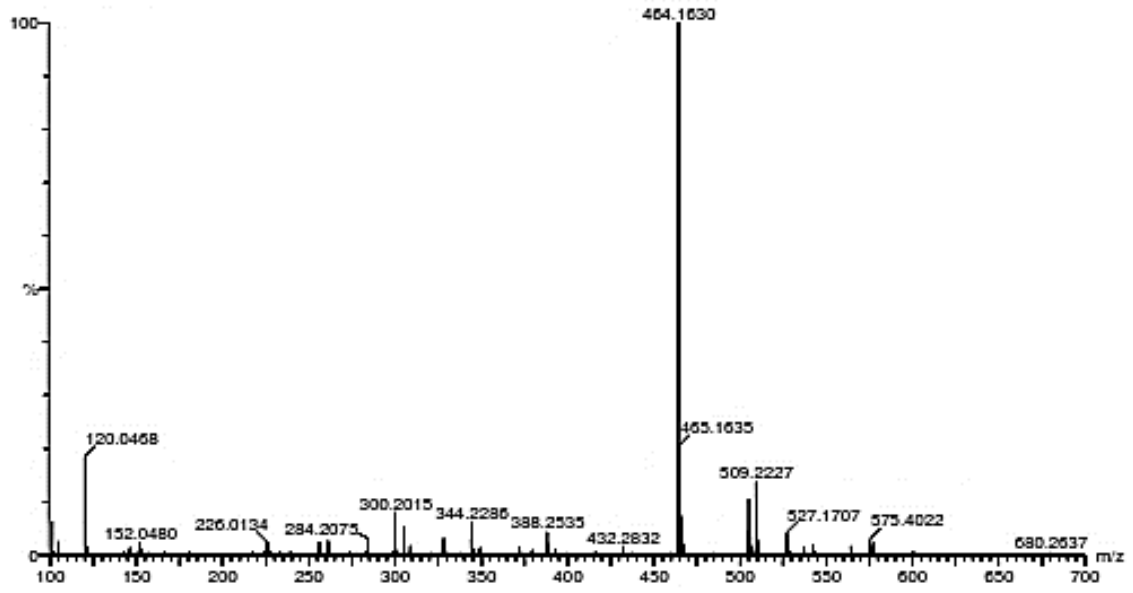
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

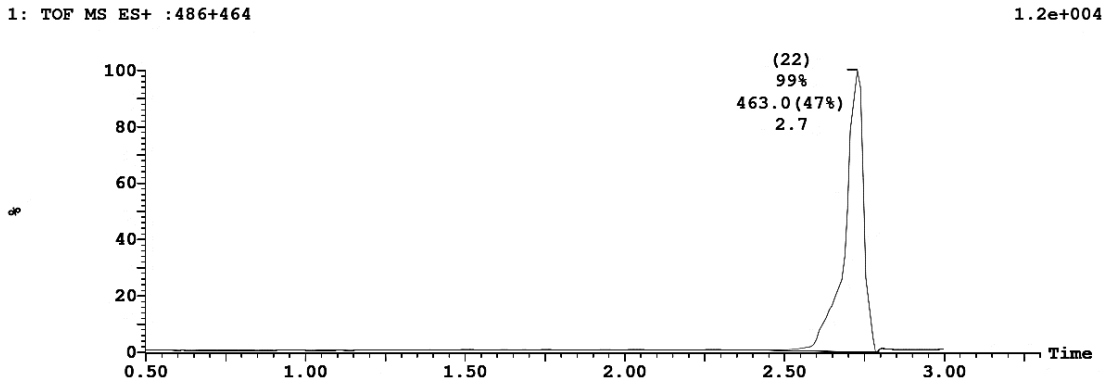
Monoisotopic Mass, Even Electron Ions
472 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-25 H: 0-200 N: 0-10 O: 0-10 Na: 0-1 S: 0-1

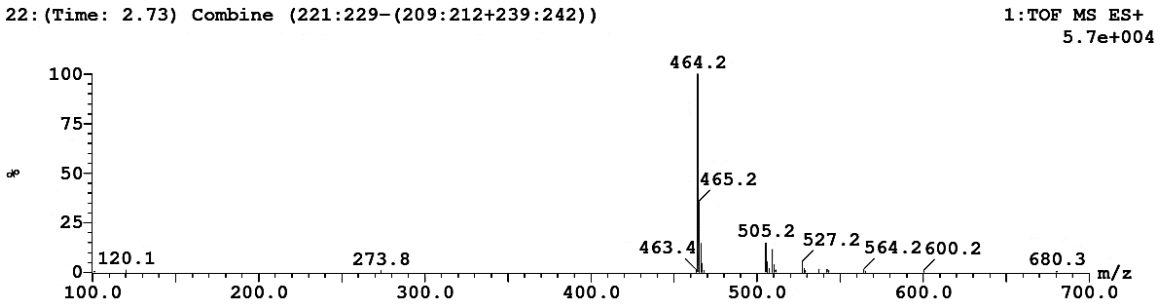
A.CILIBRIZZI AC119 2ND
ms17099 227 (2.746) Cm (227:229)

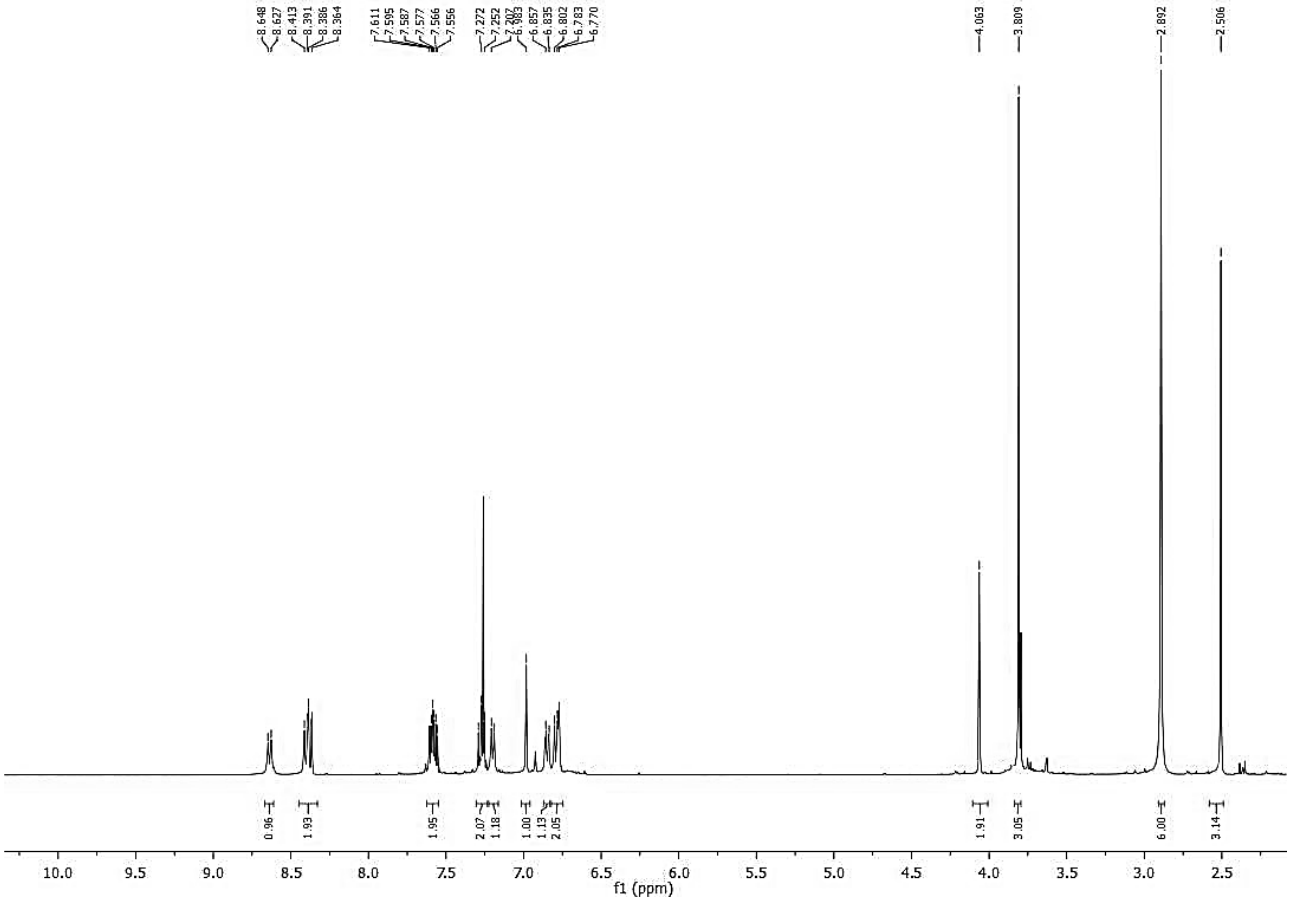
1: TOF MS ES+
1.00e+004

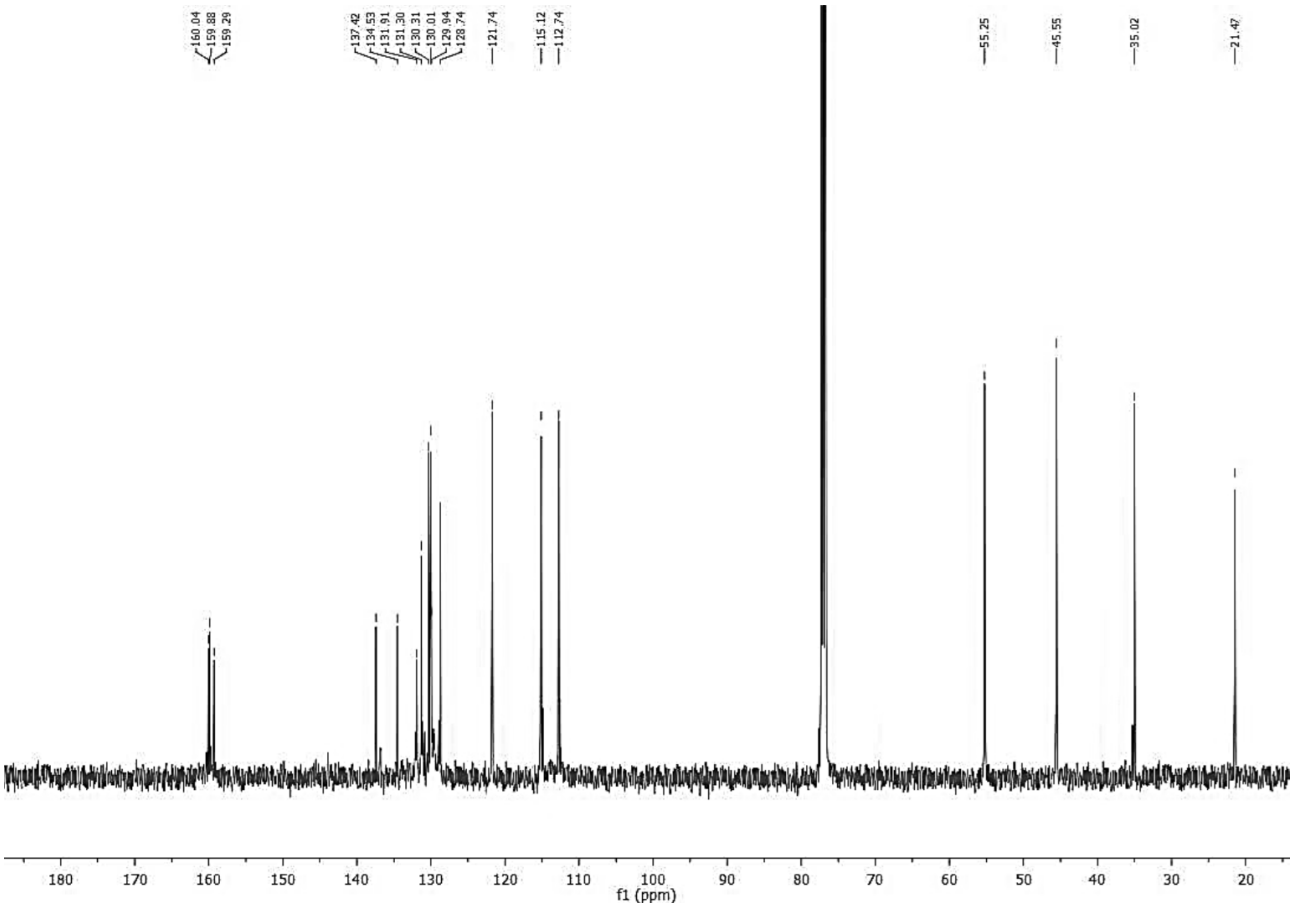


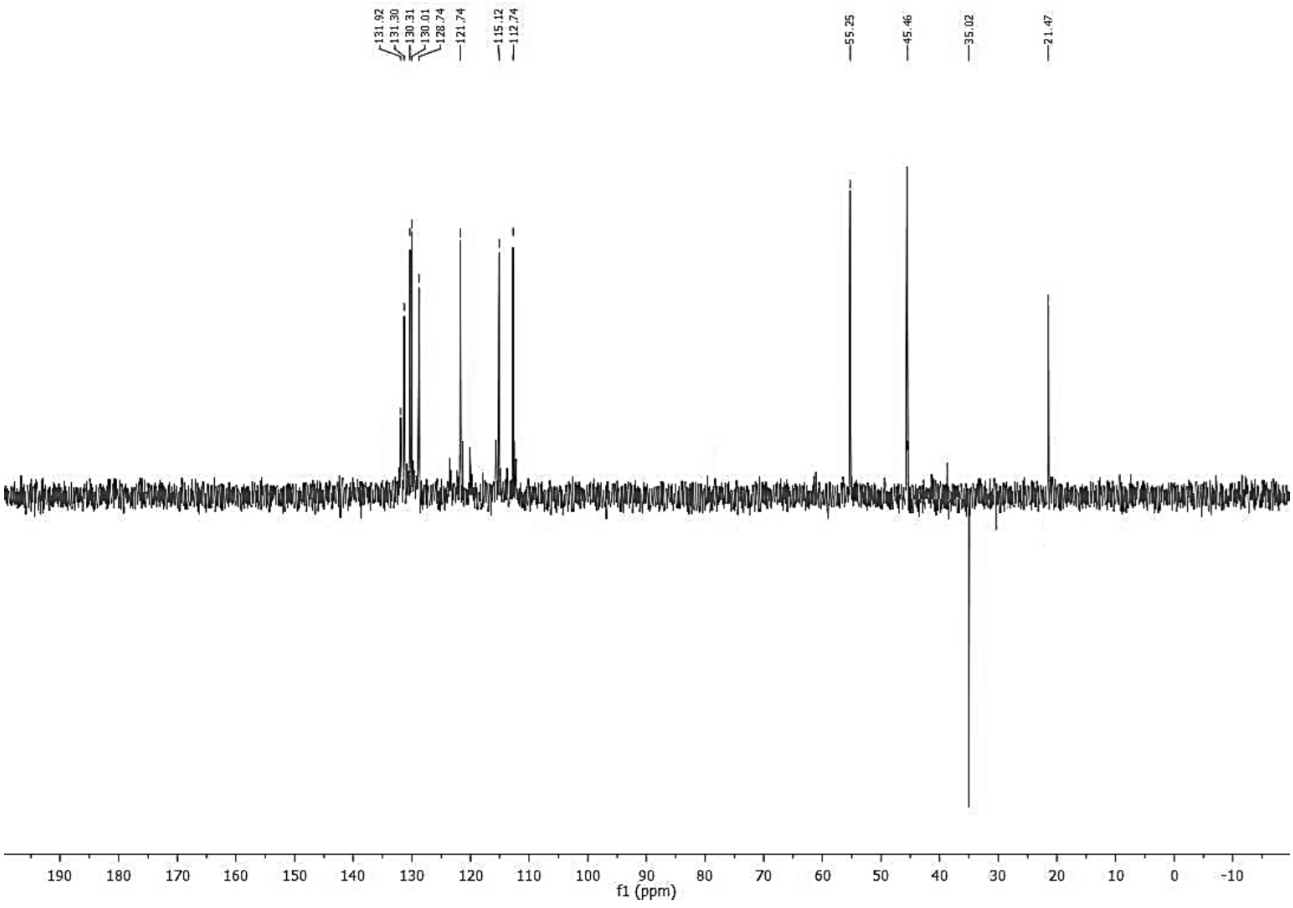
Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
464.1630	464.1644	-1.4	-3.0	14.5	365.5	0.0	C25 H26 N3 O4 S


1: TOF MS ES+ :486+464
1.2e+004
(22)
99%
463.0(47%)
2.7
%
Time

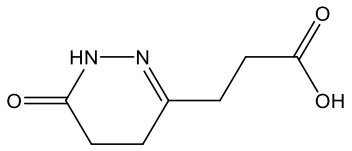

22:(Time: 2.73) Combine (221:229-(209:212+239:242))
1:TOF MS ES+
5.7e+004
%
464.2
465.2
505.2
527.2
564.2
600.2
680.3
463.4
120.1
273.8
m/z


f1 (ppm)

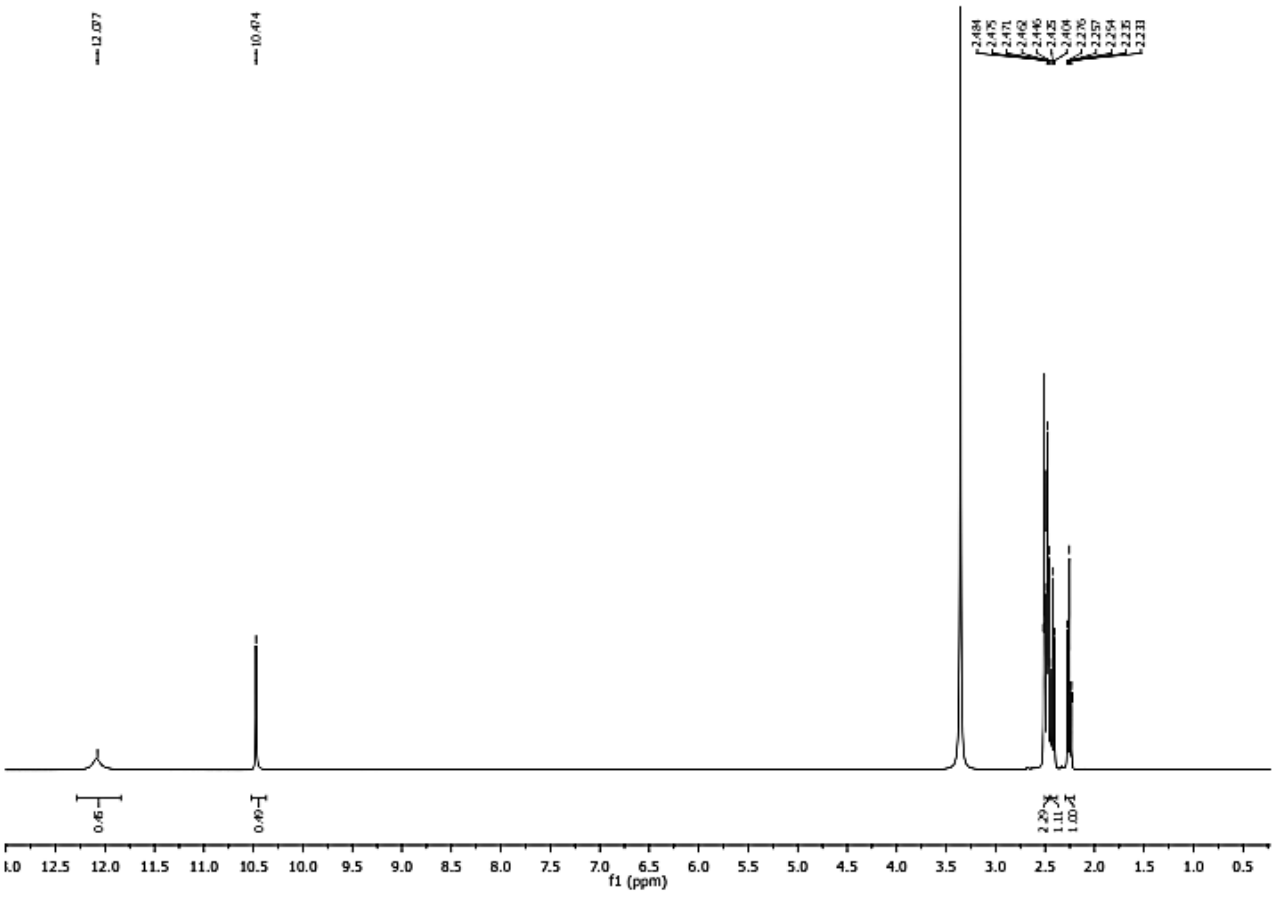

160.04
159.88
159.29
137.42
134.53
131.91
131.30
130.31
130.01
129.94
128.74
121.74
115.12
112.74
55.25
45.55
35.02
21.47
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)


131.92
131.30
130.31
130.01
128.74
121.74
115.12
112.74
55.25
45.46
35.02
21.47
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

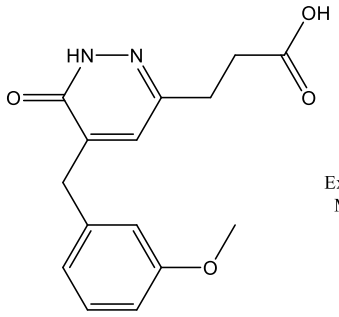
3-(6-Oxo-1,4,5,6-tetrahydropyridazin-3-yl)propanoic acid, **9**



$C_7H_{10}N_2O_3$
Exact Mass: 170,0691
Molecular Weight: 170,1680



3-[5-(3-methoxybenzyl)-6-oxo-1,6-dihydropyridazin-3-yl]propanoic acid, **10**



$C_{15}H_{16}N_2O_4$
Exact Mass: 288,11
Mol. Wt.: 288,30

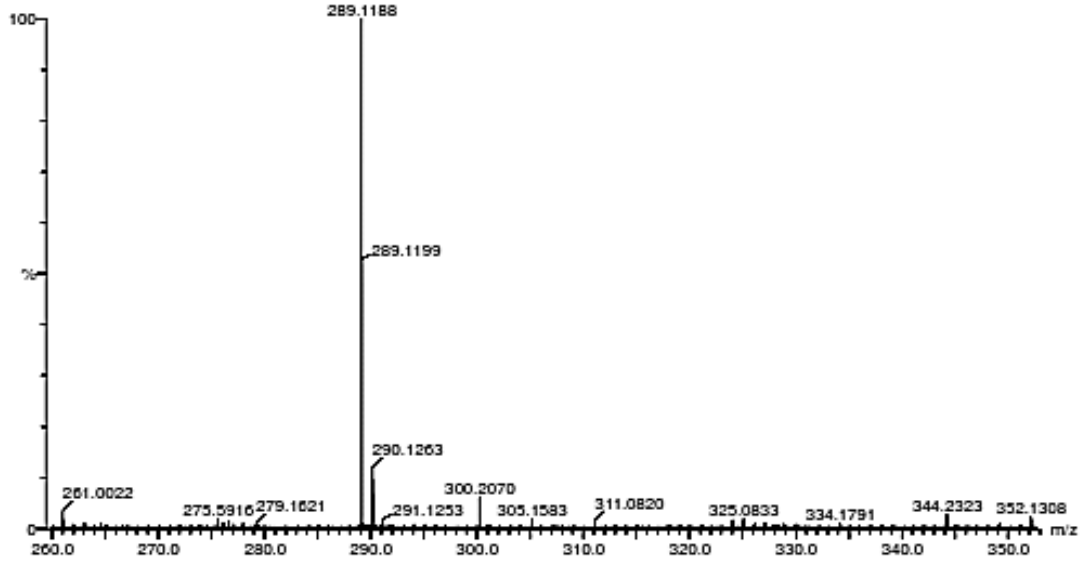
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

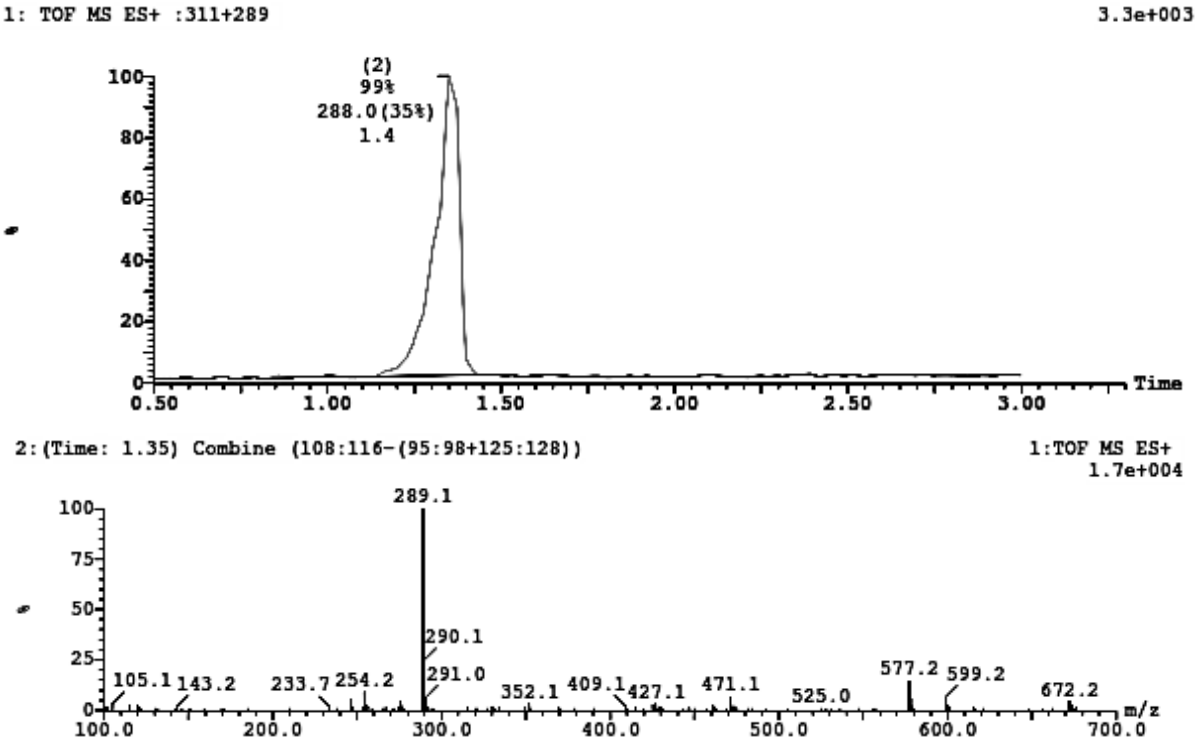
Monoisotopic Mass, Even Electron Ions
229 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 15-15 H: 0-200 N: 0-10 O: 0-10 Na: 0-1

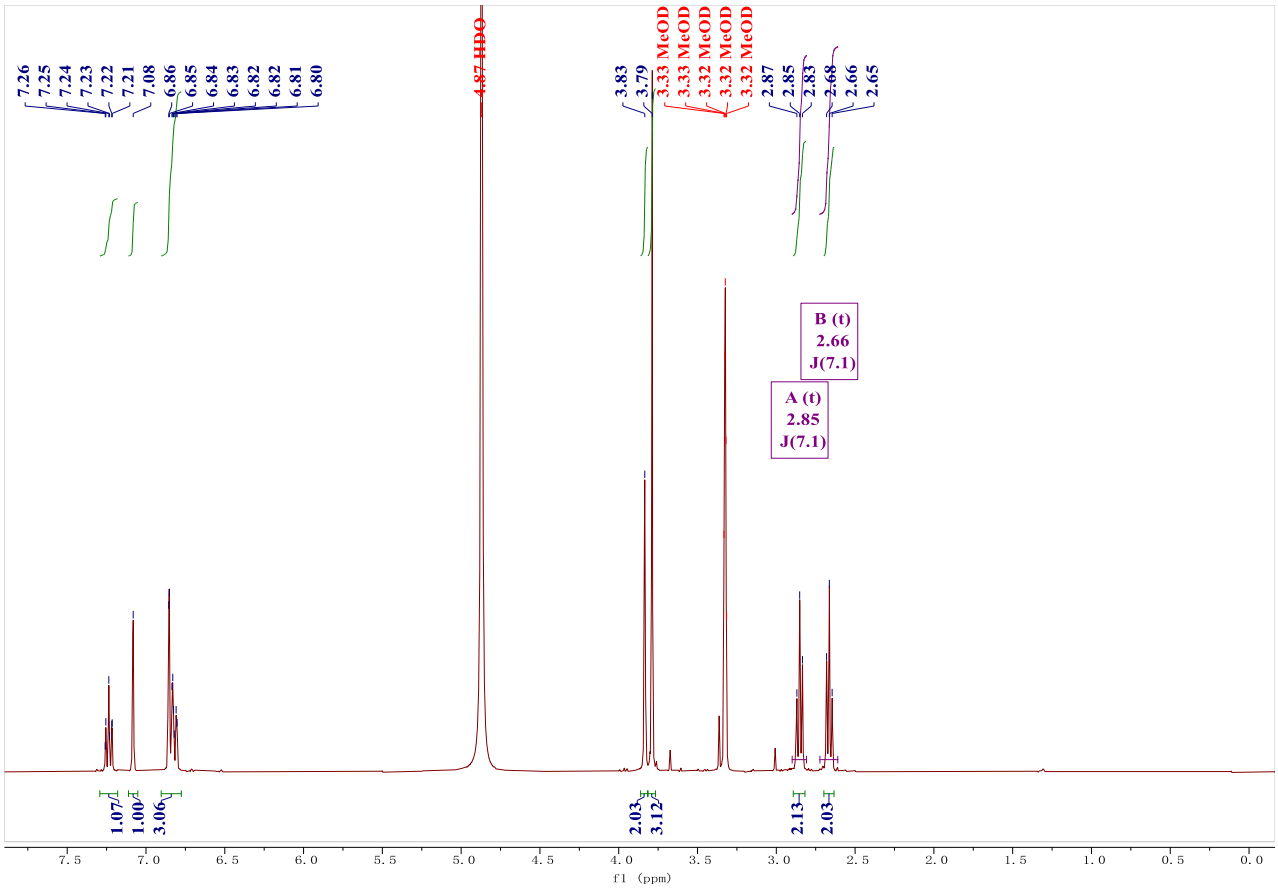
A/CILIBRIZZI AC133C
ms17684 107 (1.294) Cm (105:107)

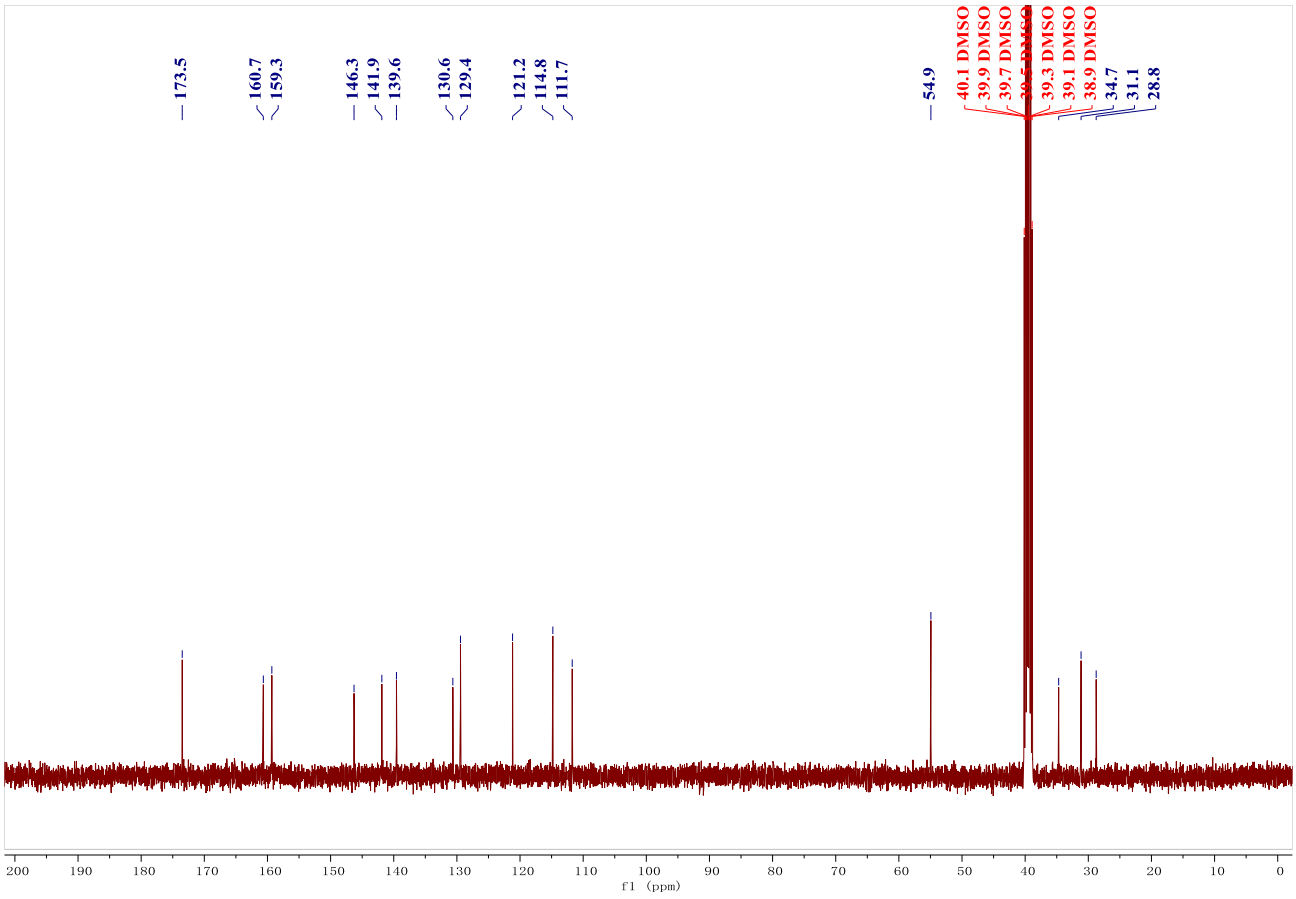
1: TOF MS ES+
1.72e+003



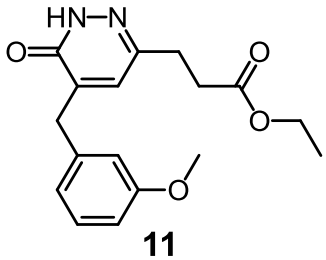
Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
289.1188	289.1188	0.0	0.0	8.5	314.6	0.0	C15 H17 N2 O4


1: TOF MS ES+ :311+289
3.3e+003
(2)
99%
288.0(35%)
1.4
Time
2:(Time: 1.35) Combine (108:116-(95:98+125:128))
1:TOF MS ES+
1.7e+004
289.1
290.1
291.0
105.1
143.2
233.7
254.2
352.1
409.1
427.1
471.1
525.0
577.2
599.2
672.2
m/z


7.26
7.25
7.24
7.23
7.22
7.21
7.08
6.86
6.85
6.84
6.83
6.82
6.82
6.81
6.80
4.87 HDO
3.83
3.79
3.33 MeOD
3.33 MeOD
3.32 MeOD
3.32 MeOD
3.32 MeOD
2.87
2.85
2.83
2.68
2.66
2.65
B (t)
2.66
J(7.1)
A (t)
2.85
J(7.1)
1.07
1.00
3.06
2.03
3.12
2.13
2.03
f1 (ppm)



Ethyl 3-{5-[(3-methoxyphenyl)methyl]-6-oxo-1,6-dihydropyri dazin-3-yl}propanoate, **11**

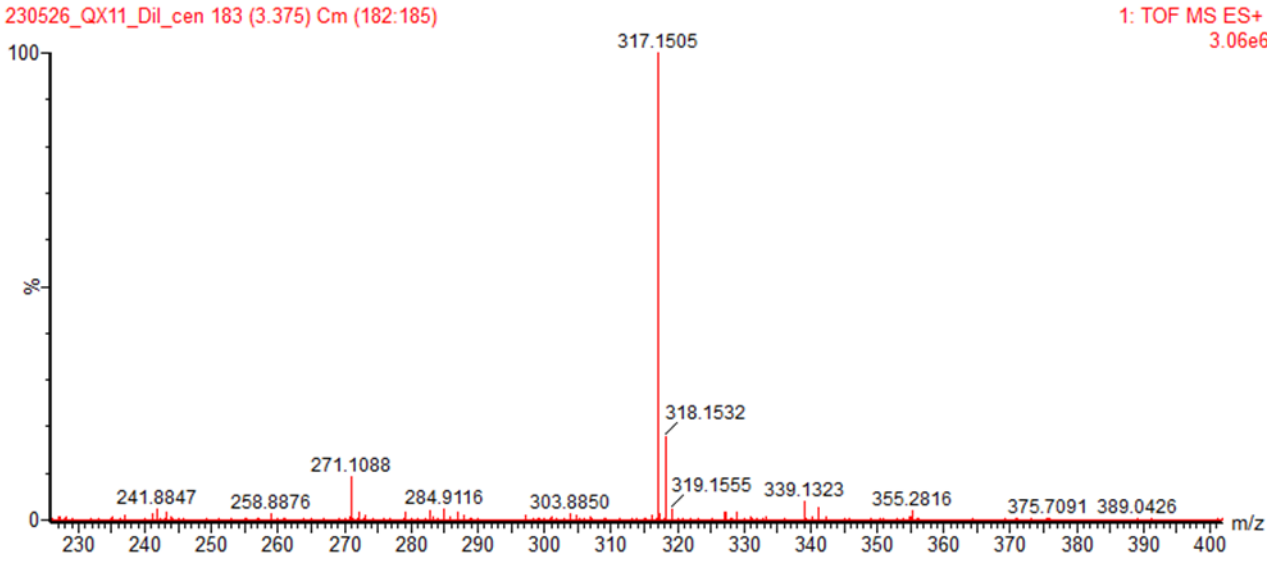


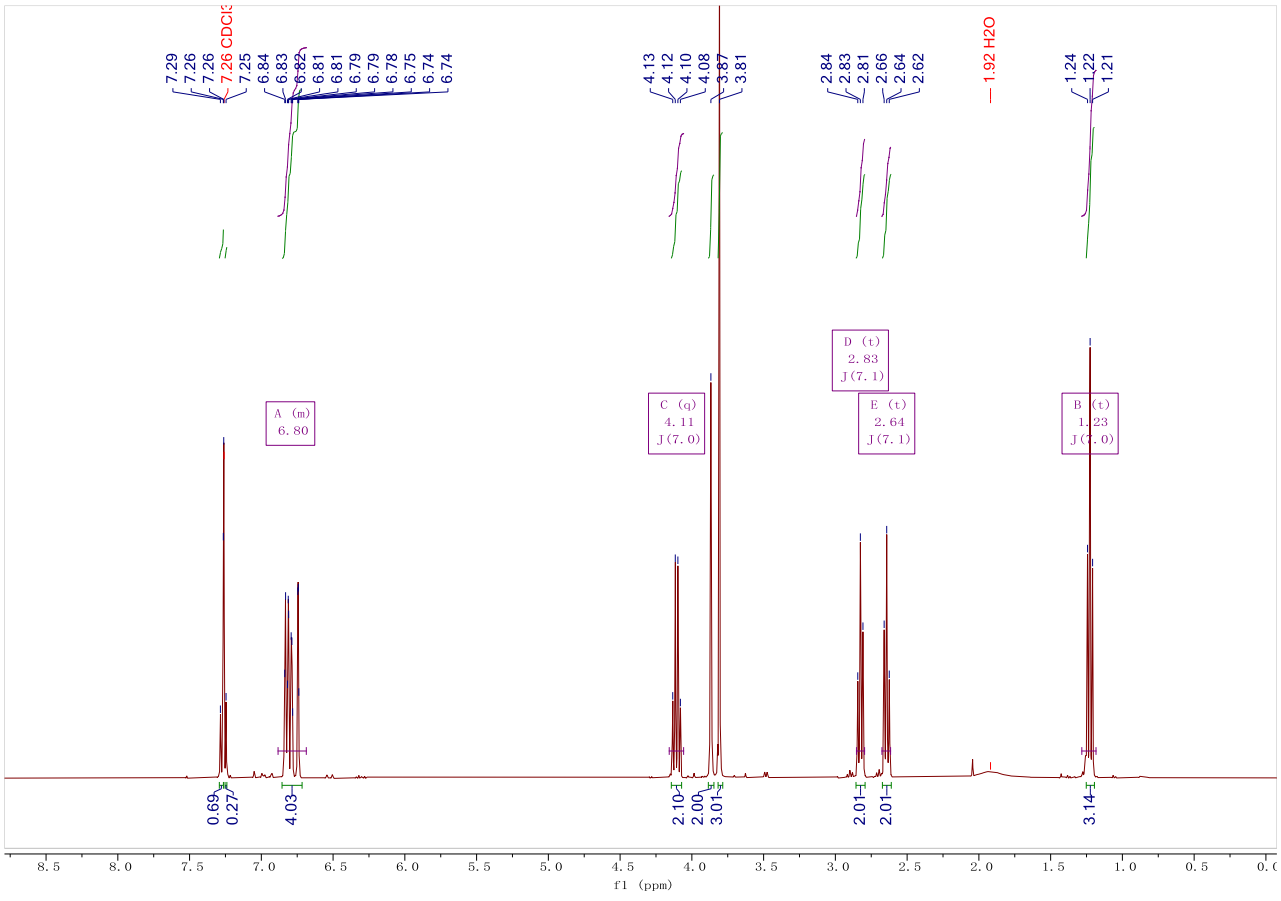
11

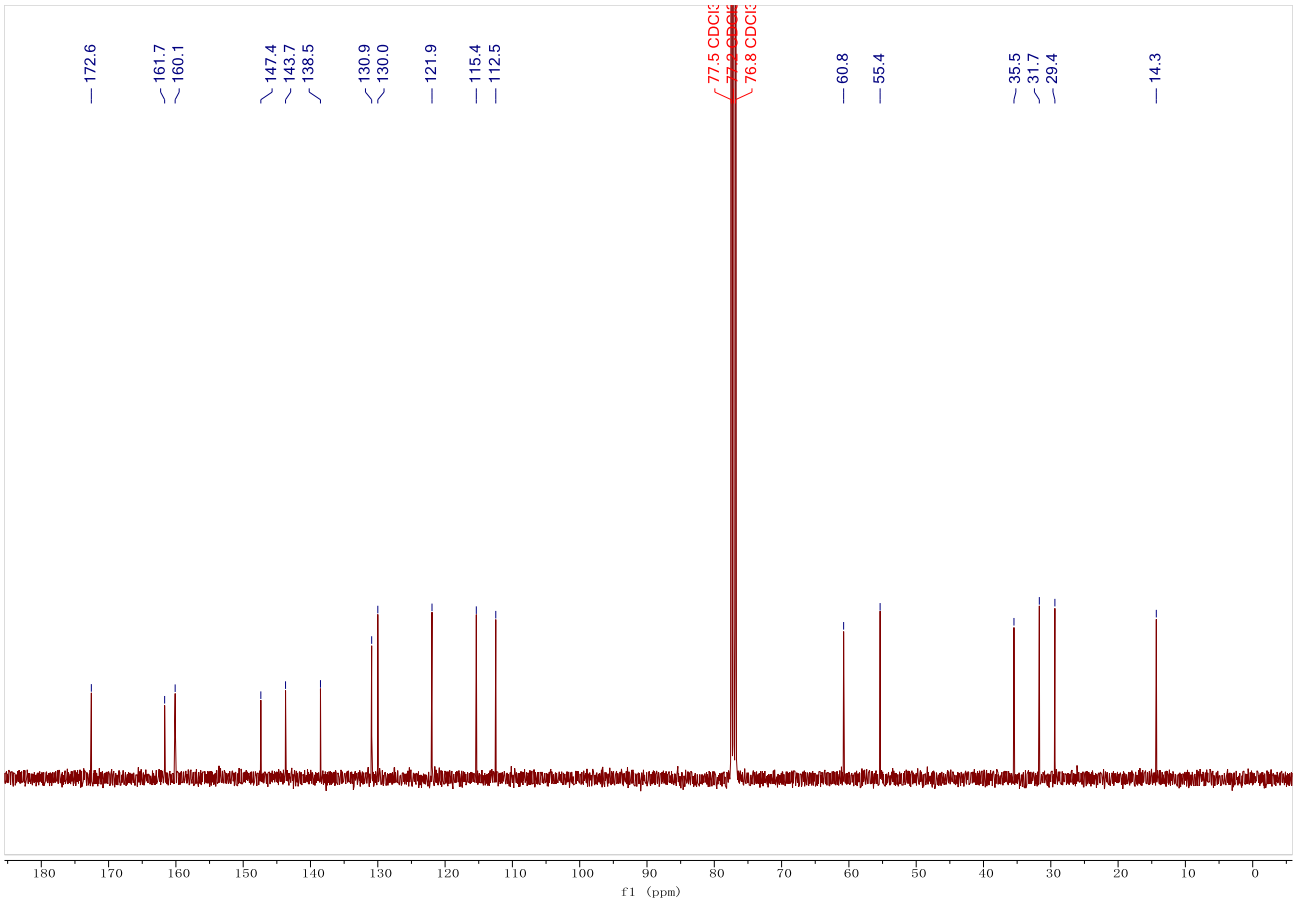
Chemical Formula: $C_{17}H_{20}N_2O_4$

Exact Mass: 316.1423

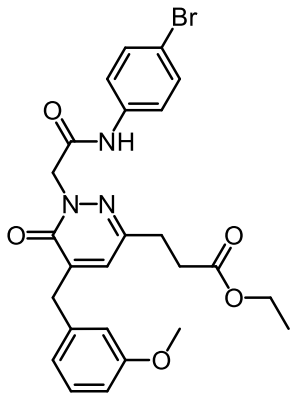
Molecular Weight: 316.3570


230526_QX11_Dil_cen 183 (3.375) Cm (182:185)
1: TOF MS ES+
3.06e6
317.1505
318.1532
271.1088
319.1555
339.1323
355.2816
241.8847
258.8876
284.9116
303.8850
375.7091
389.0426
m/z


7.29
7.26
7.26
7.26 CDCl3
7.25
6.84
6.83
6.82
6.81
6.81
6.79
6.79
6.78
6.75
6.74
6.74
4.13
4.12
4.10
4.08
3.87
3.81
2.84
2.83
2.81
2.66
2.64
2.62
1.92 H2O
1.24
1.22
1.21
A (m) 6.80
C (q) 4.11 J(7.0)
D (t) 2.83 J(7.1)
E (t) 2.64 J(7.1)
B (t) 1.23 J(7.0)
0.69
0.27
4.03
2.10
2.00
3.01
2.01
2.01
3.14
f1 (ppm)

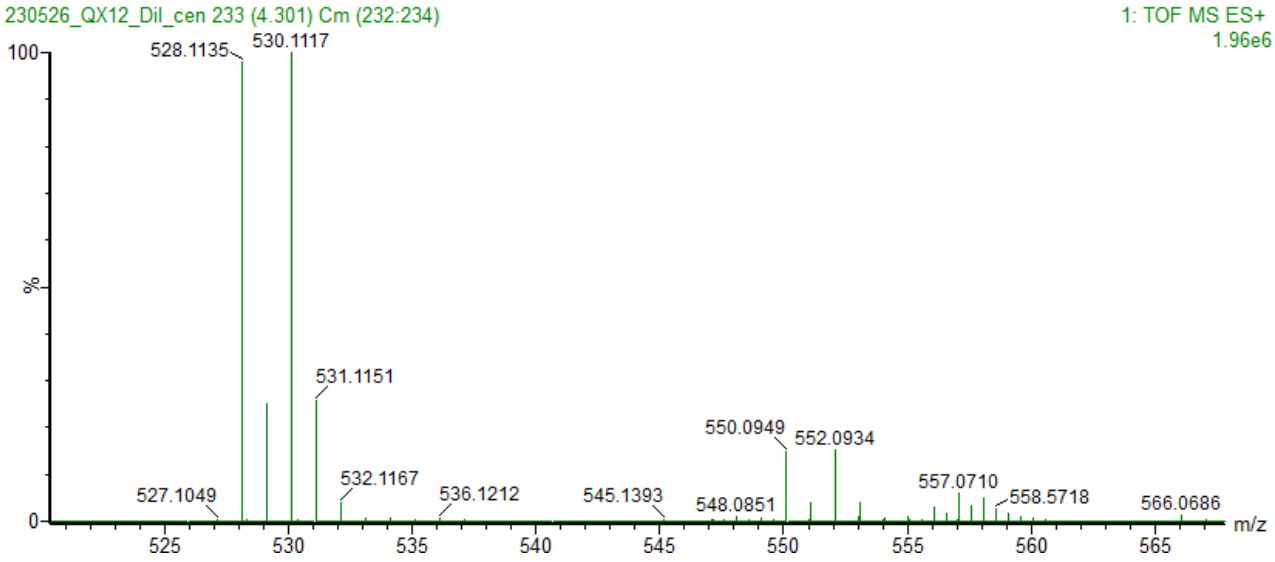


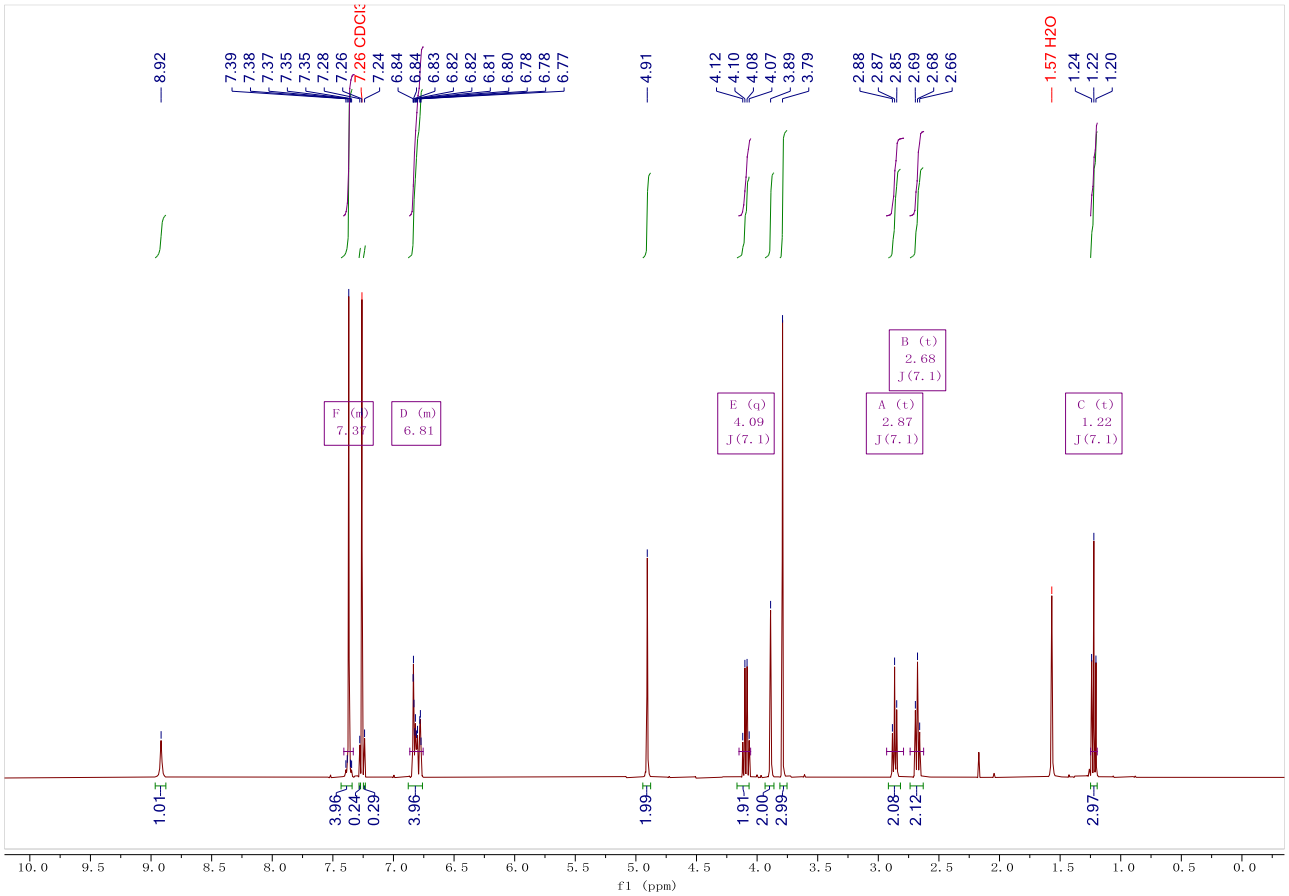
Ethyl 3-{1-[2-(4-bromophenylamino)-2-oxoethyl]-5-(4-methoxybenzyl)-6-oxo-1,6-dihydropyridazin-3-yl}propanoate, **12**

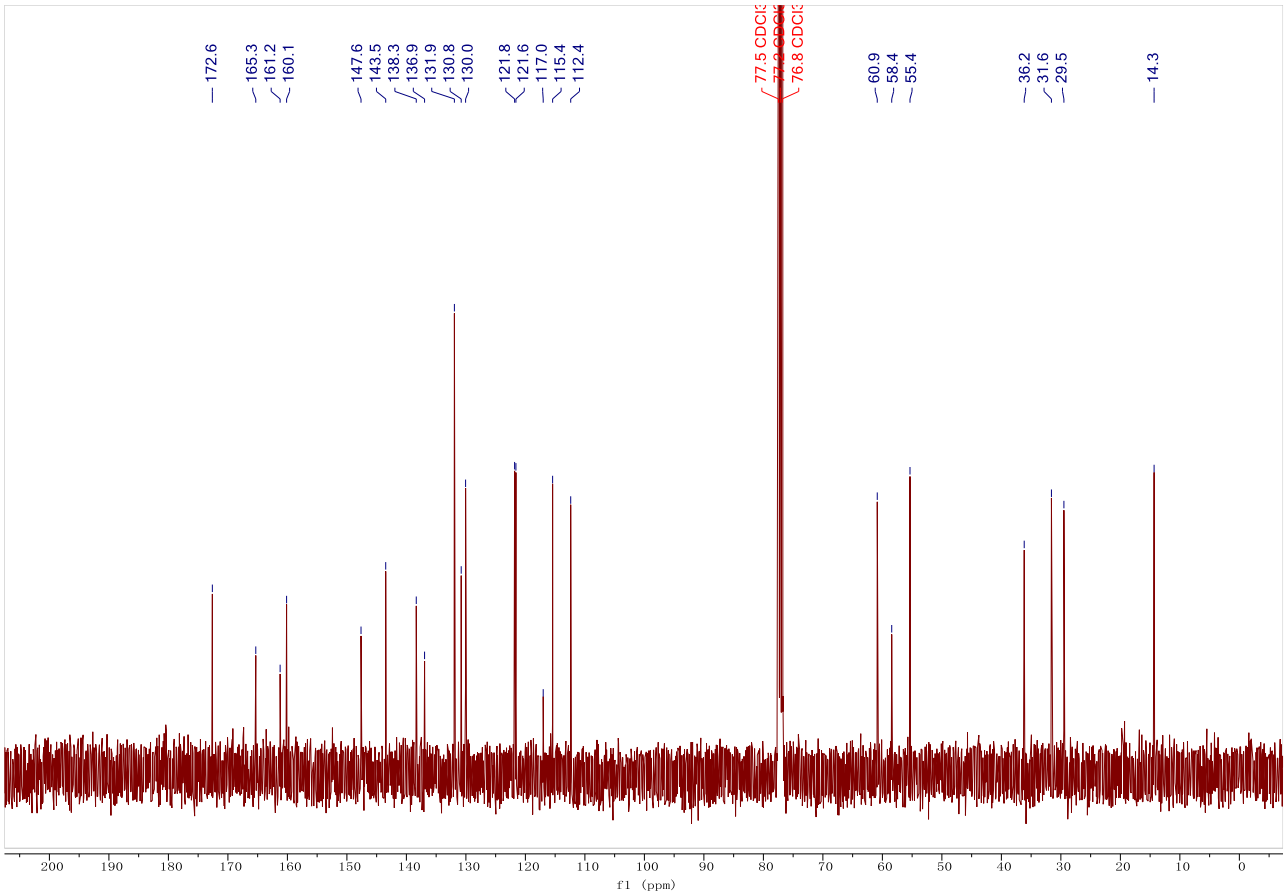


12

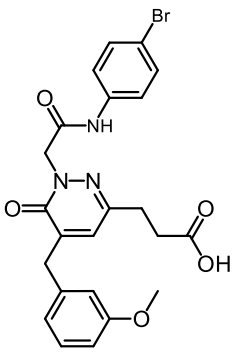
Chemical Formula: $C_{25}H_{26}BrN_3O_5$
Exact Mass: 527.1056
Molecular Weight: 528.4030


230526_QX12_Dil_cen 233 (4.301) Cm (232:234)
1: TOF MS ES+
1.96e6
100
%
0
528.1135
530.1117
531.1151
550.0949
552.0934
532.1167
557.0710
527.1049
536.1212
545.1393
548.0851
558.5718
566.0686
m/z
525
530
535
540
545
550
555
560
565


8.92
7.39
7.38
7.37
7.35
7.28
7.26
7.26 CDCl3
7.24
6.84
6.84
6.83
6.82
6.82
6.81
6.80
6.78
6.78
6.77
4.91
4.12
4.10
4.08
4.07
3.89
3.79
2.88
2.87
2.85
2.69
2.68
2.66
1.57 H2O
1.24
1.22
1.20
F (m) 7.37
D (m) 6.81
E (q) 4.09 J(7.1)
B (t) 2.68 J(7.1)
A (t) 2.87 J(7.1)
C (t) 1.22 J(7.1)
1.01
3.96
0.24
0.29
3.96
1.99
1.91
2.00
2.99
2.08
2.12
2.97
f1 (ppm)

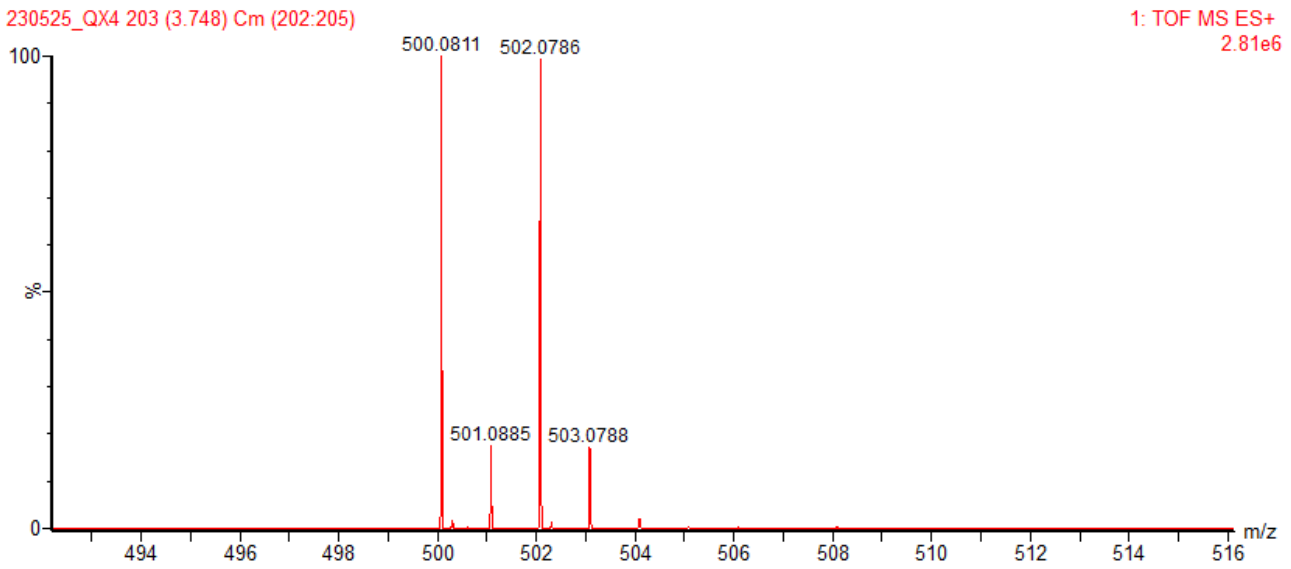


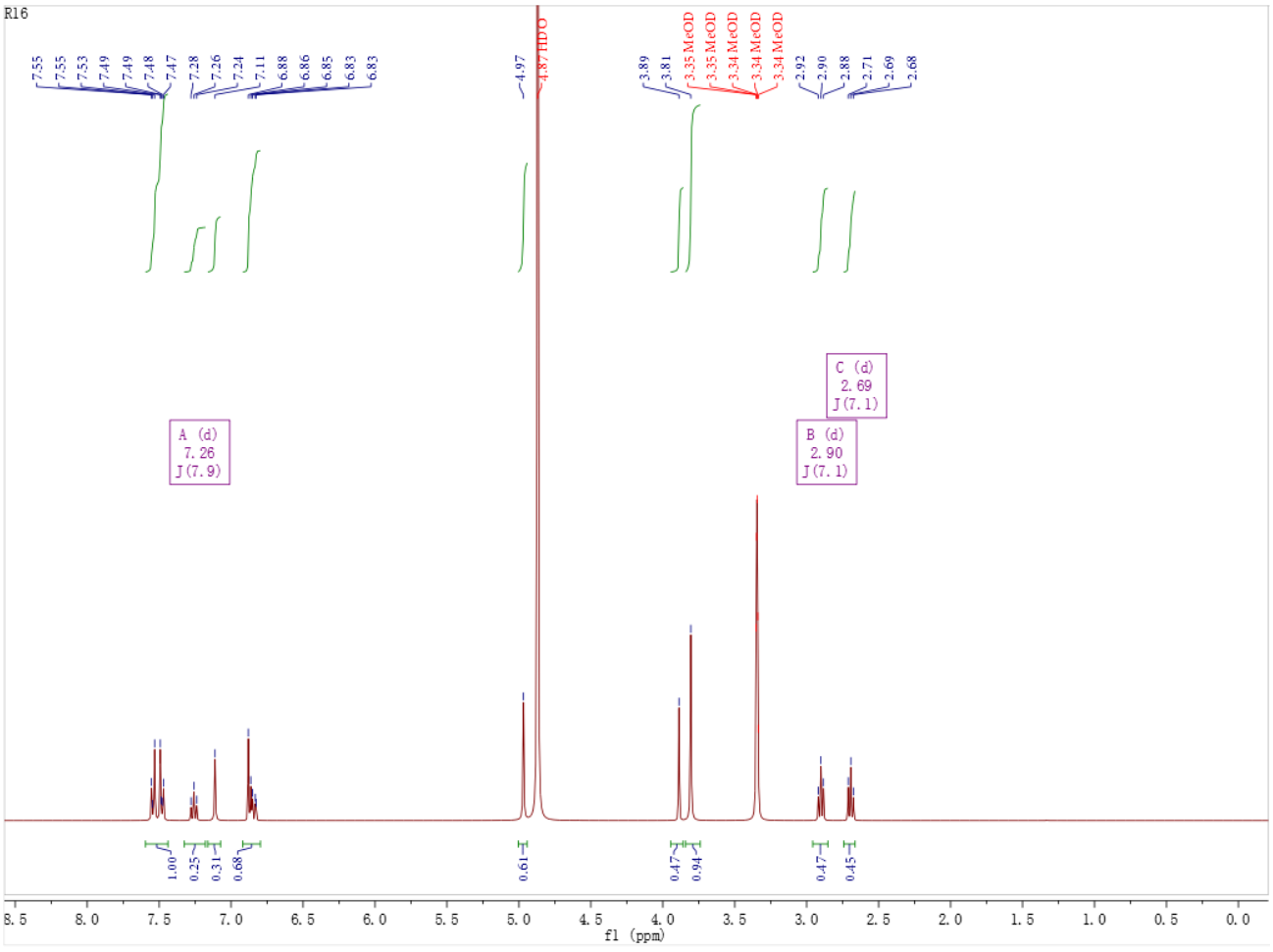
3-{1-[2-(4-bromophenylamino)-2-oxoethyl]-5-(3-methoxybenzyl)-6-oxo-1,6-dihydro pyridazin-3-yl}propanoic acid, **13**

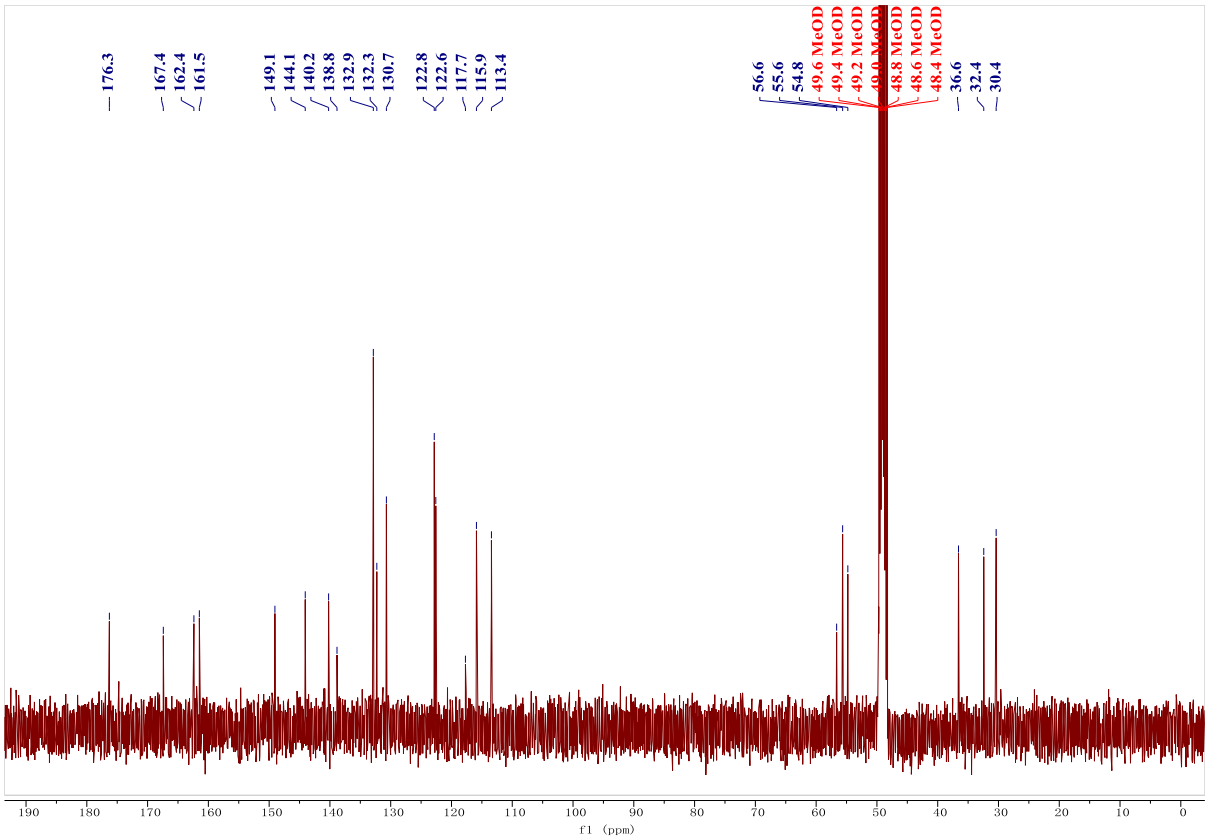


13

Chemical Formula: $C_{23}H_{22}BrN_3O_5$
Exact Mass: 499.0743
Molecular Weight: 500.3490


230525_QX4 203 (3.748) Cm (202:205)
1: TOF MS ES+
2.81e6
100
%
0
500.0811
502.0786
501.0885
503.0788
494
496
498
500
502
504
506
508
510
512
514
516
m/z


R16
7.55
7.55
7.53
7.49
7.49
7.48
7.47
7.28
7.26
7.24
7.11
6.88
6.86
6.85
6.83
6.83
4.97
4.87 HDO
3.89
3.81
3.35 MeOD
3.35 MeOD
3.34 MeOD
3.34 MeOD
3.34 MeOD
2.92
2.90
2.88
2.71
2.69
2.68
A (d)
7.26
J(7.9)
B (d)
2.90
J(7.1)
C (d)
2.69
J(7.1)
1.00
0.25
0.31
0.68
0.61
0.47
0.94
0.47
0.45
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



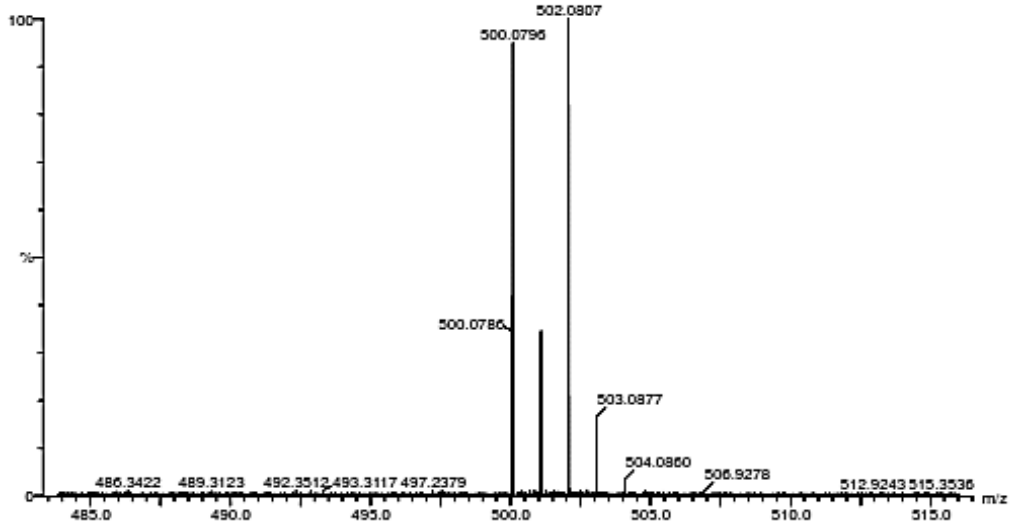
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

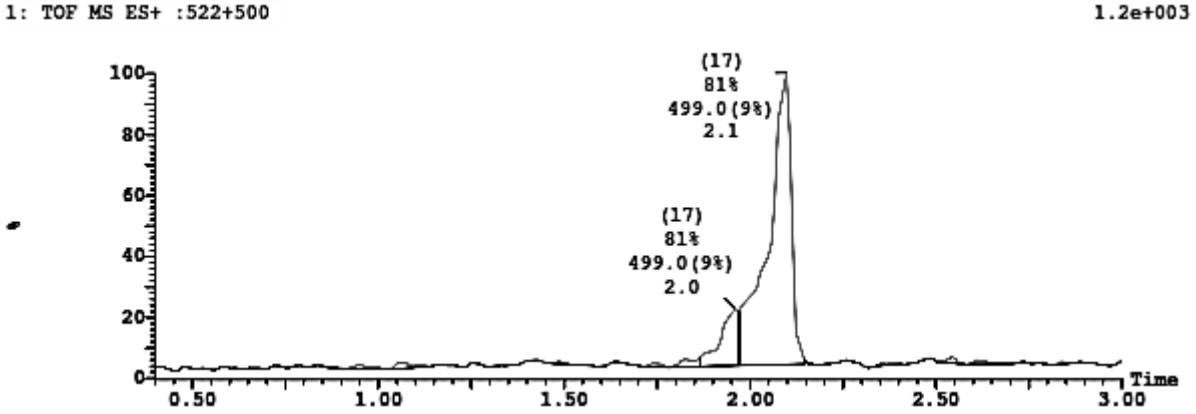
Monoisotopic Mass, Even Electron Ions
235 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 23-23 H: 0-200 N: 0-10 O: 0-10 Na: 0-1 Br: 1-1

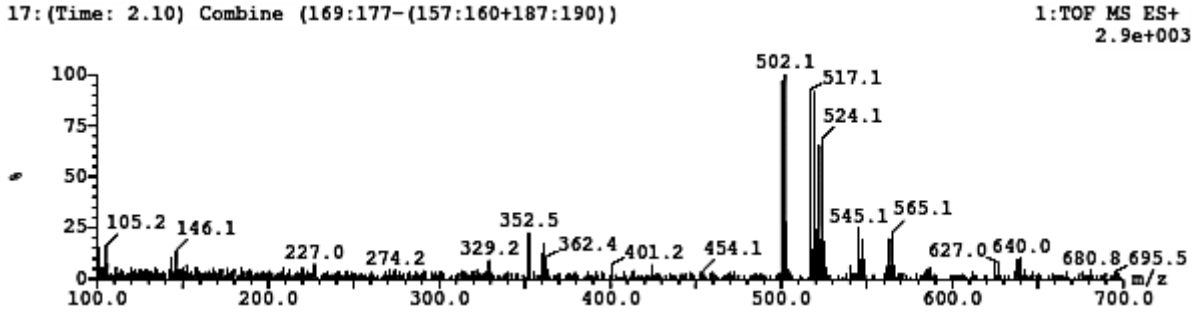
A.CILIBRIZZI AC137D
MS18224A 173 (2.098) Cm (170:179)

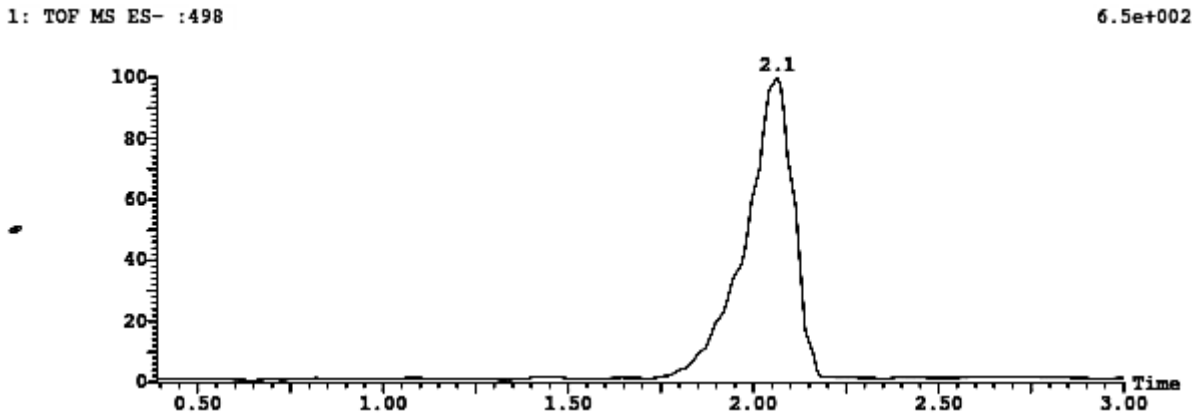
1: TOF MS ES+
1.41e+003

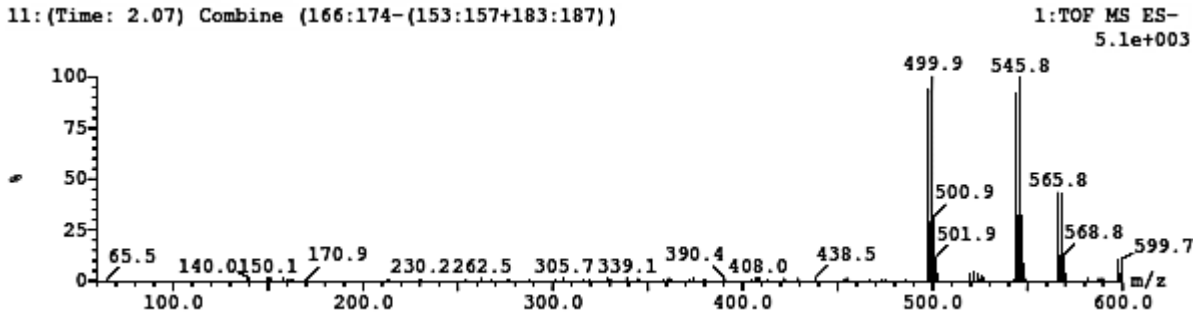


Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
500.0796	500.0821	-2.5	-5.0	13.5	384.5	0.0	C23 H23 N3 O5 Br

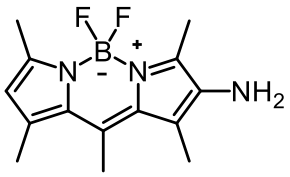

1: TOF MS ES+ :522+500
1.2e+003
(17)
81%
499.0(9%)
2.1
(17)
81%
499.0(9%)
2.0
Time


17:(Time: 2.10) Combine (169:177-(157:160+187:190))
1:TOF MS ES+
2.9e+003
502.1
517.1
524.1
105.2
146.1
352.5
545.1
565.1
227.0
274.2
329.2
362.4
401.2
454.1
627.0
640.0
680.8
695.5
m/z


1: TOF MS ES- :498
6.5e+002
2.1
Time

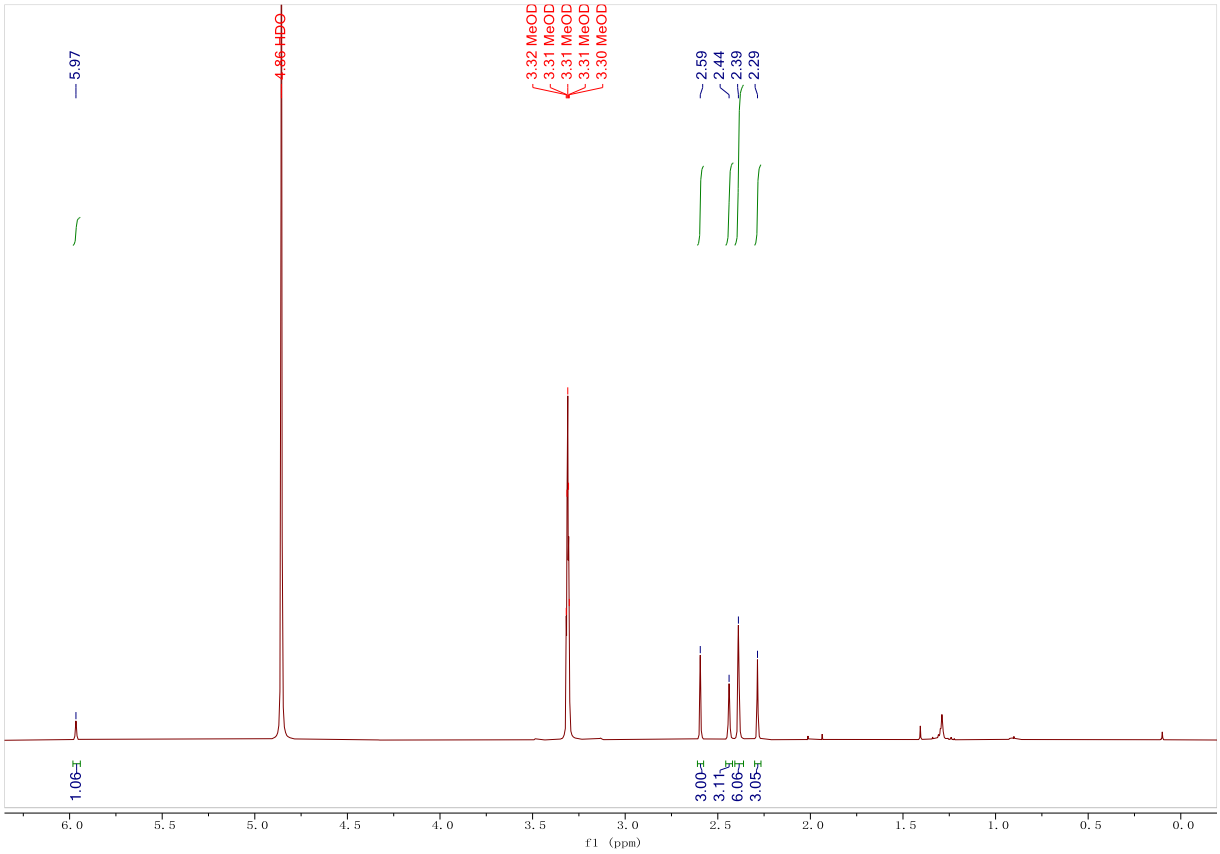

11:(Time: 2.07) Combine (166:174-(153:157+183:187))
1:TOF MS ES-
5.1e+003
499.9
545.8
500.9
565.8
501.9
568.8
599.7
65.5
140.0
150.1
170.9
230.2
262.5
305.7
339.1
390.4
408.0
438.5
m/z

5,5-Difluoro-1,3,7,9,10-pentamethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-2-amine, **15**

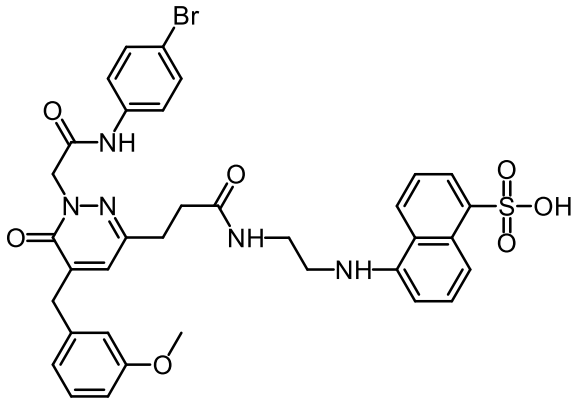


Chemical Formula: $C_{14}H_{18}BF_2N_3$
Exact Mass: 277.1562
Molecular Weight: 277.1258

15



3-{1-[2-(4-Bromophenylamino)-2-oxoethyl]-5-(3-methoxybenzyl)-6-oxo-1,6-dihydro pyridazin-3-yl}propanamidoethyl-2-(5-aminonaphthalene)-1-sulfonic acid, **16**



Chemical Formula: $C_{35}H_{34}BrN_5O_7S$

Exact Mass: 747.1362

Molecular Weight: 748.6490

16

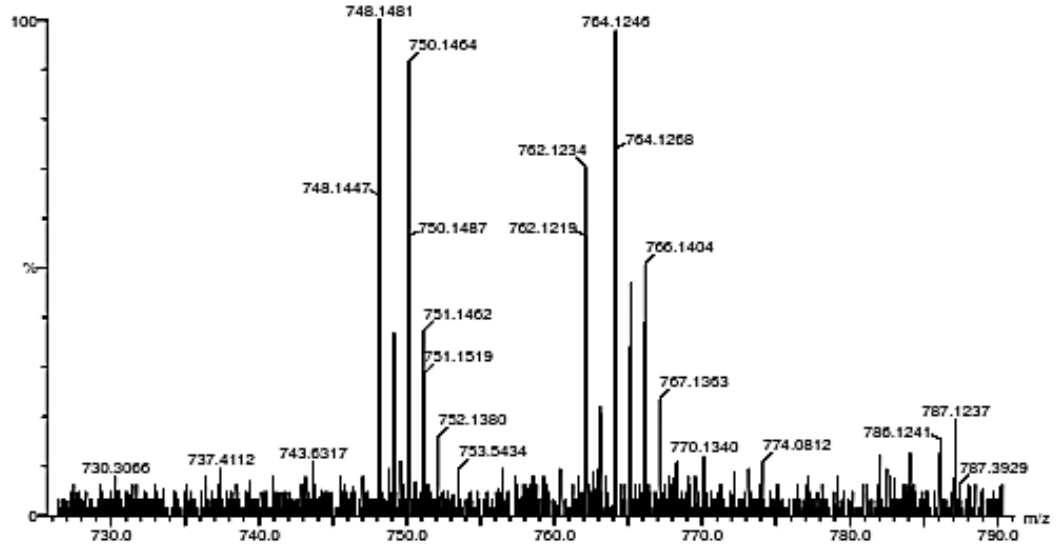
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 8.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

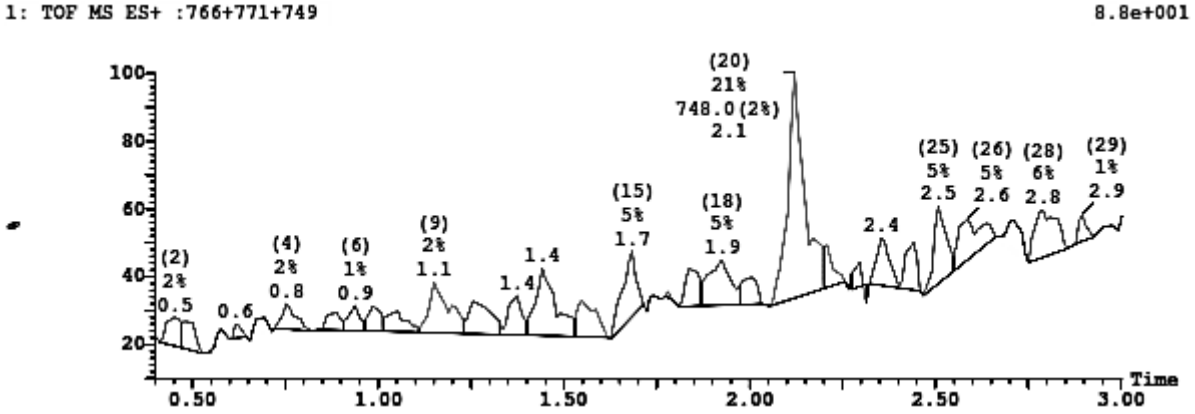
Monoisotopic Mass, Even Electron Ions
345 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 35-35 H: 0-200 N: 0-10 O: 0-15 Na: 0-1 S: 1-1 Br: 1-1

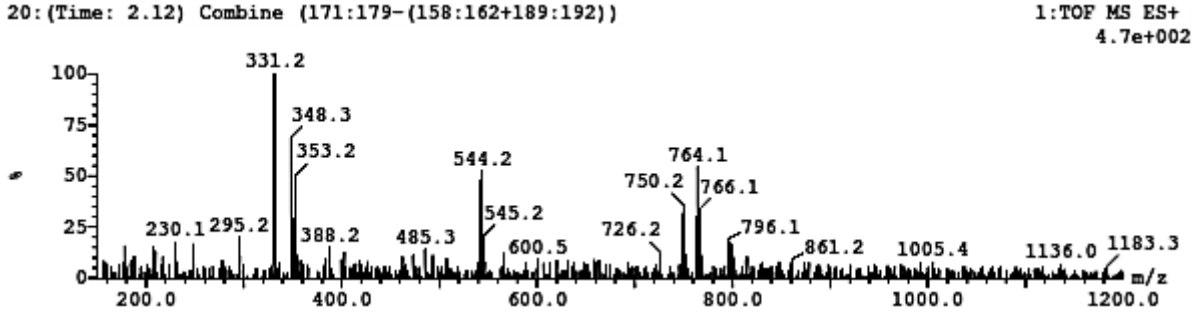
A.CILIBRIZZI AC160
ms21329 175 (2.121) Cm (174:175)

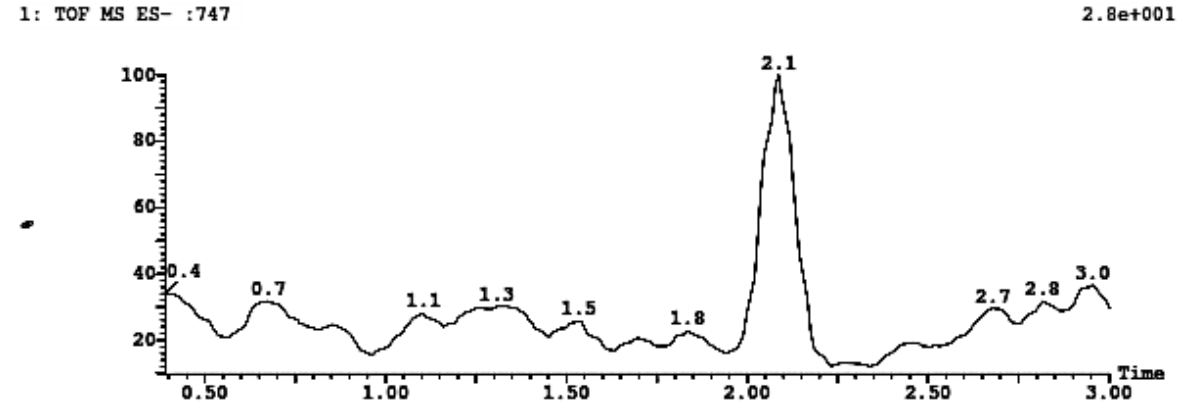
1: TOF MS ES+
6.47e+001

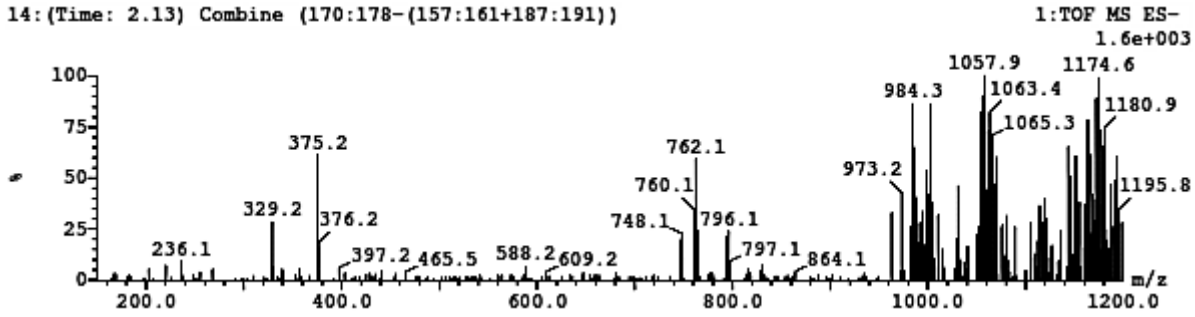


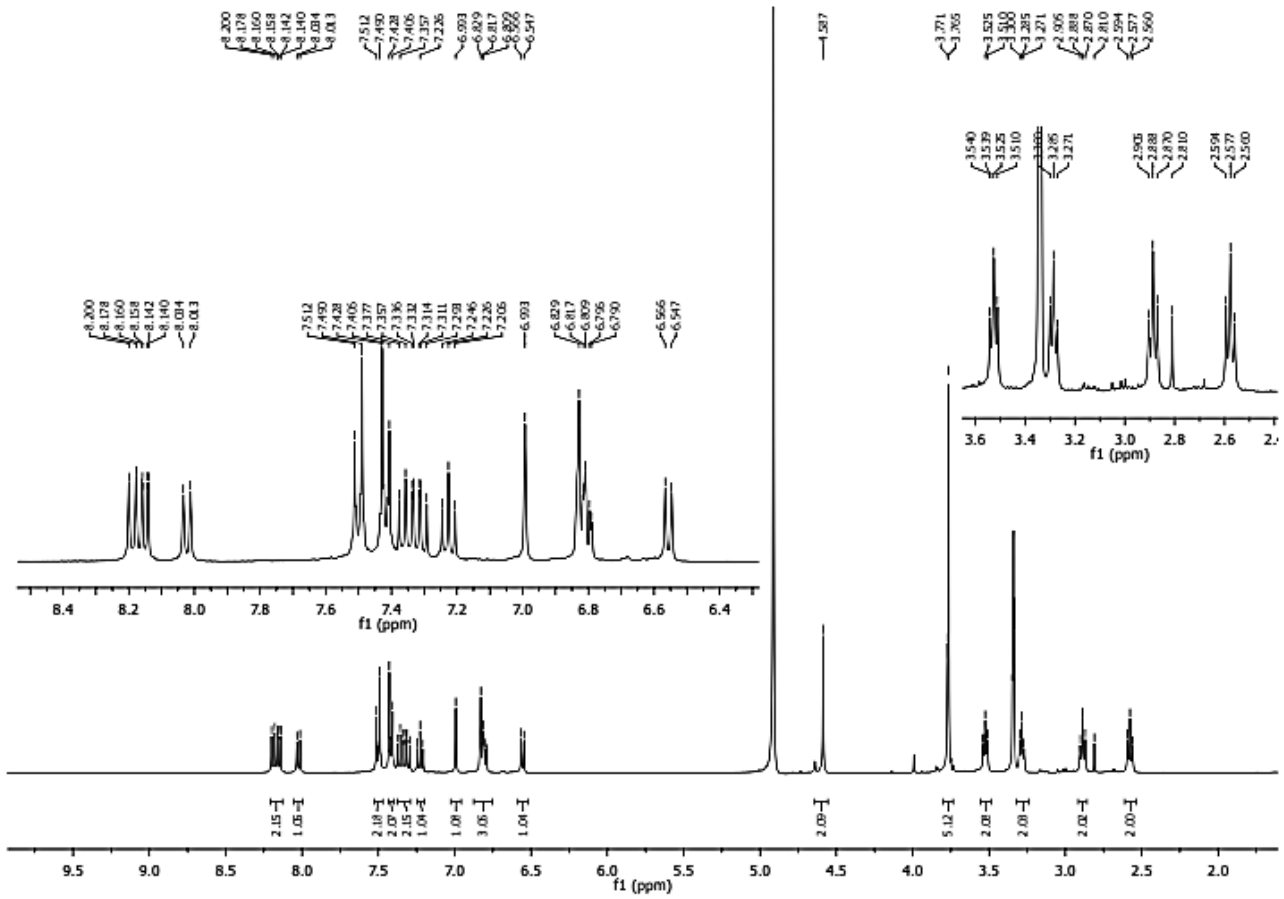
Minimum:				-1.5			
Maximum:		5.0	8.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
748.1447	748.1441	0.6	0.8	20.5	88.5	0.0	C35 H35 N5 O7 S Br

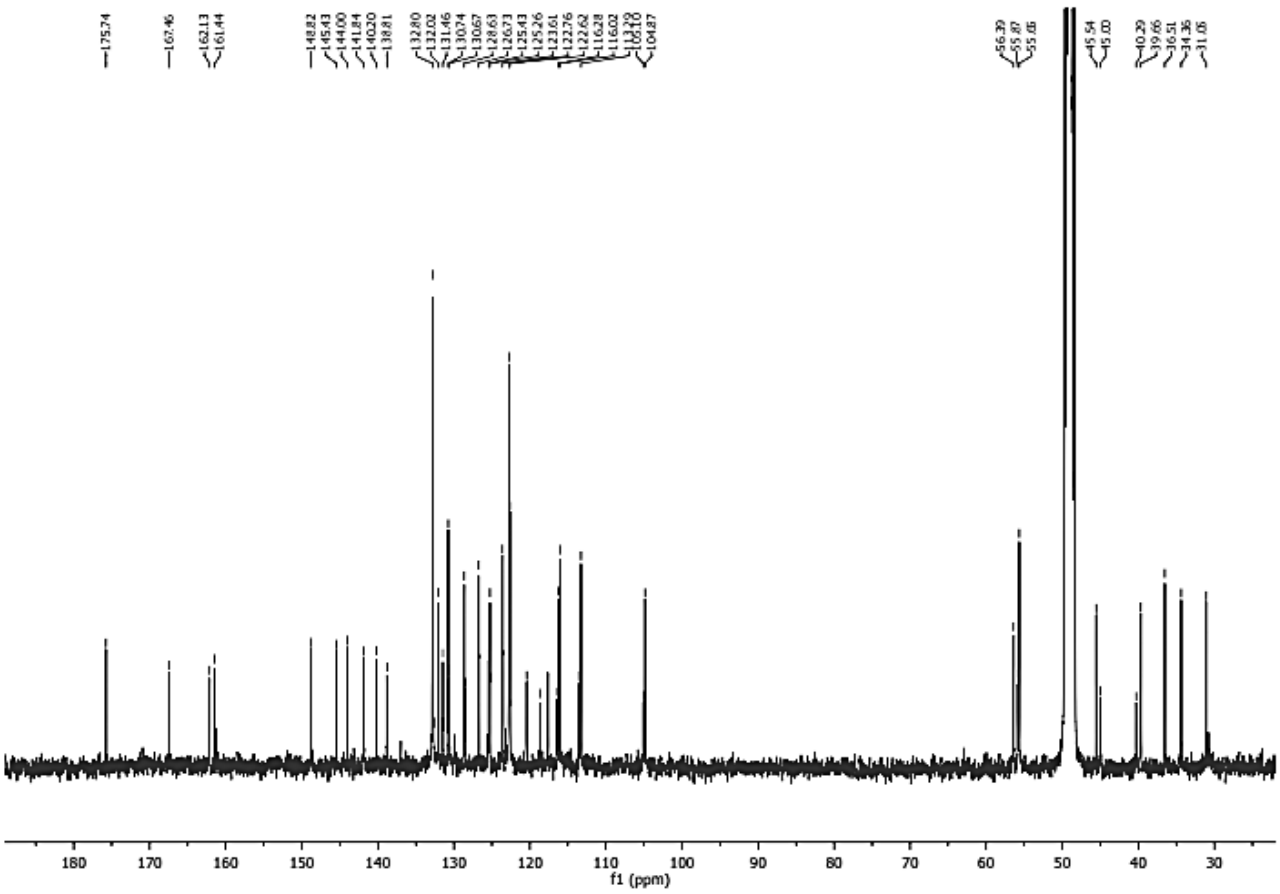

1: TOF MS ES+ :766+771+749
8.8e+001
(20)
21%
748.0(2%)
2.1
(25)
5%
2.5
(26)
5%
2.6
(28)
6%
2.8
(29)
1%
2.9
(15)
5%
1.7
(18)
5%
1.9
(2)
2%
0.5
(4)
2%
0.8
(6)
1%
0.9
(9)
2%
1.1
0.6
1.4
1.4
2.4
Time

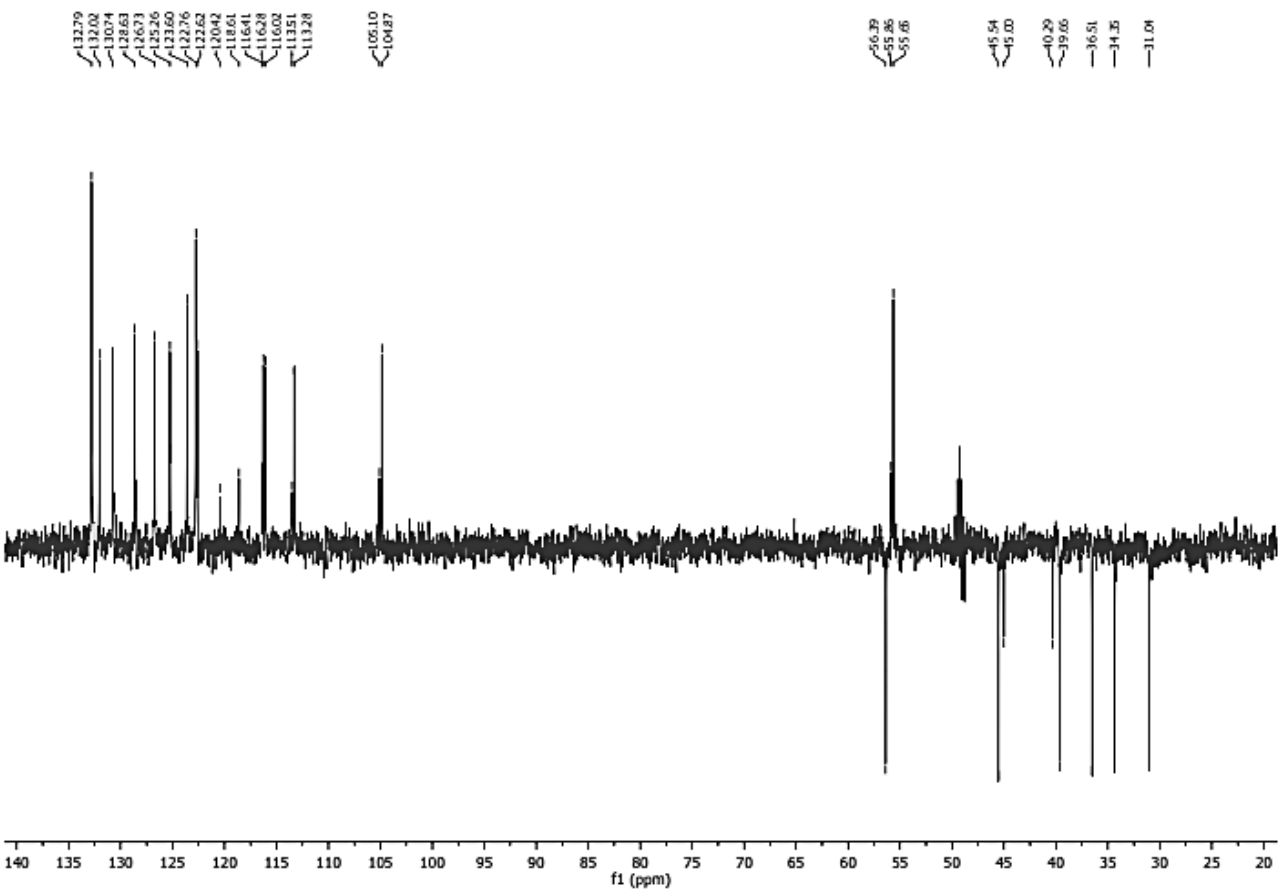

20:(Time: 2.12) Combine (171:179-(158:162+189:192))
1:TOF MS ES+
4.7e+002
331.2
348.3
353.2
544.2
764.1
750.2
766.1
545.2
230.1
295.2
388.2
485.3
600.5
726.2
796.1
861.2
1005.4
1136.0
1183.3
m/z


1: TOF MS ES- :747
2.8e+001
2.1
0.4
0.7
1.1
1.3
1.5
1.8
2.7
2.8
3.0
Time

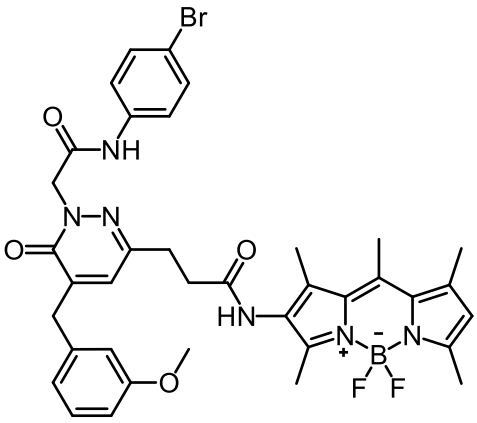

14:(Time: 2.13) Combine (170:178-(157:161+187:191))
1:TOF MS ES-
1.6e+003
1057.9
1174.6
984.3
1063.4
1180.9
1065.3
375.2
762.1
973.2
1195.8
760.1
329.2
376.2
748.1
796.1
236.1
397.2
465.5
588.2
609.2
797.1
864.1
m/z


f1 (ppm)


f1 (ppm)

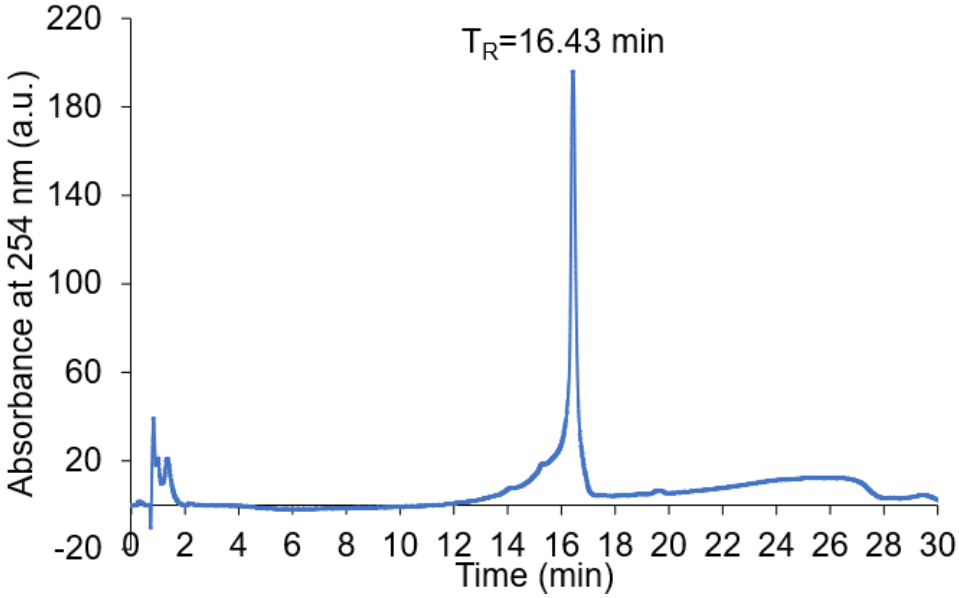

132.79
132.02
130.74
128.63
126.73
125.26
123.60
122.76
122.62
120.42
118.61
116.41
116.28
116.02
113.51
113.28
105.10
104.87
56.39
55.86
55.66
45.54
45.00
40.29
39.66
36.51
34.35
31.04
140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)

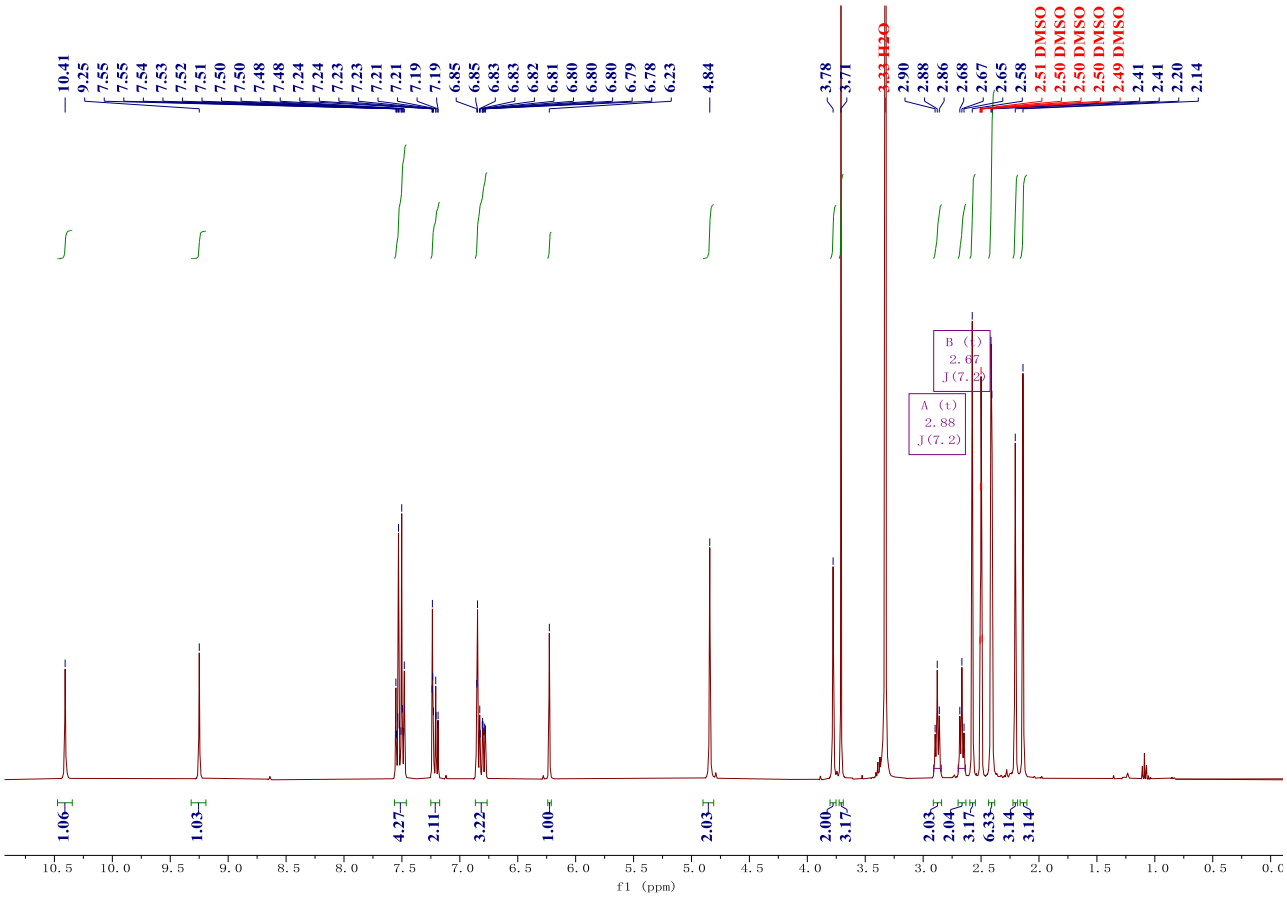
3-{1-[2-(4-bromophenylamino)-2-oxoethyl]-5-(3-methoxybenzyl)-6-oxo-1,6-dihydropyridazin-3-yl}-N-(5,5-difluoro-1,3,7,9,10-pentamethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-2-yl)propanamide, **17**

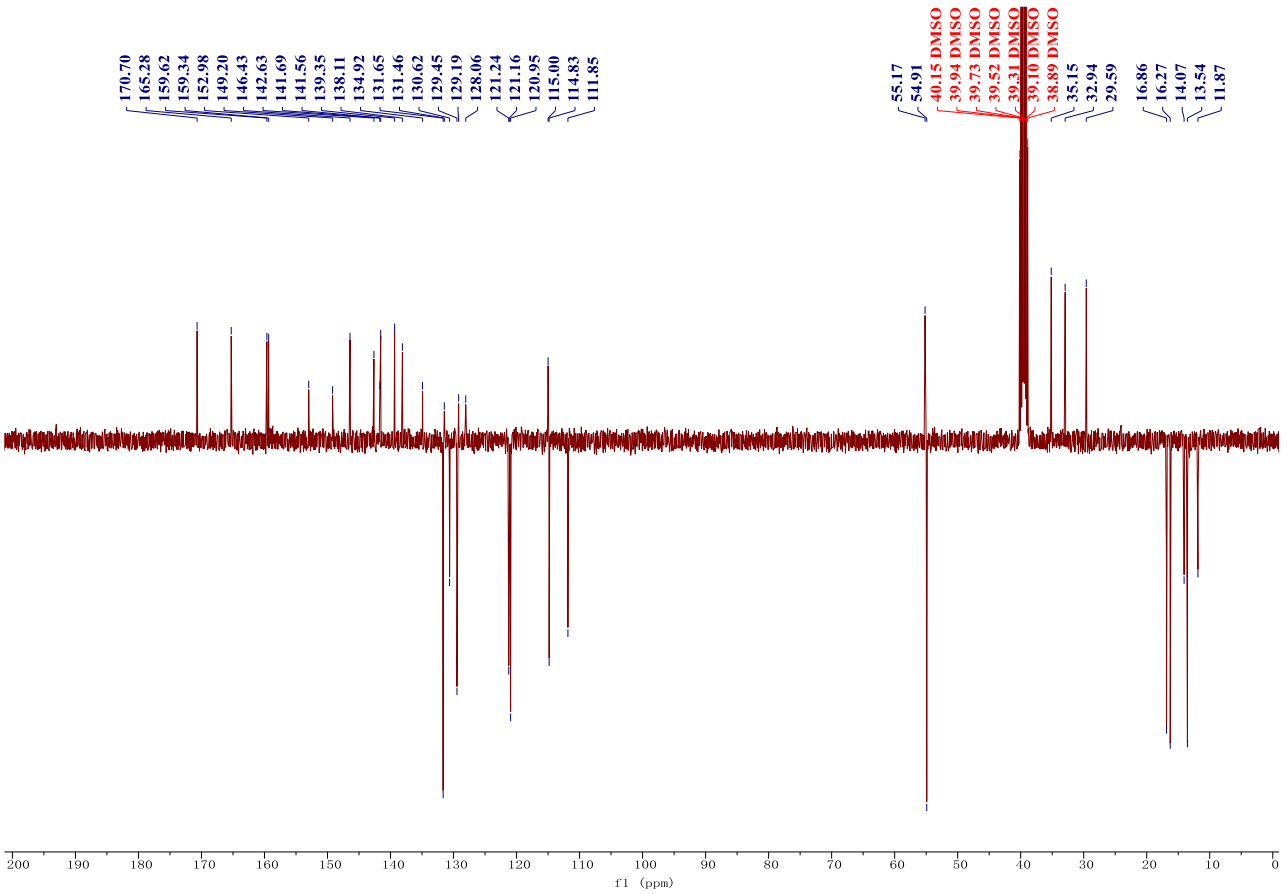


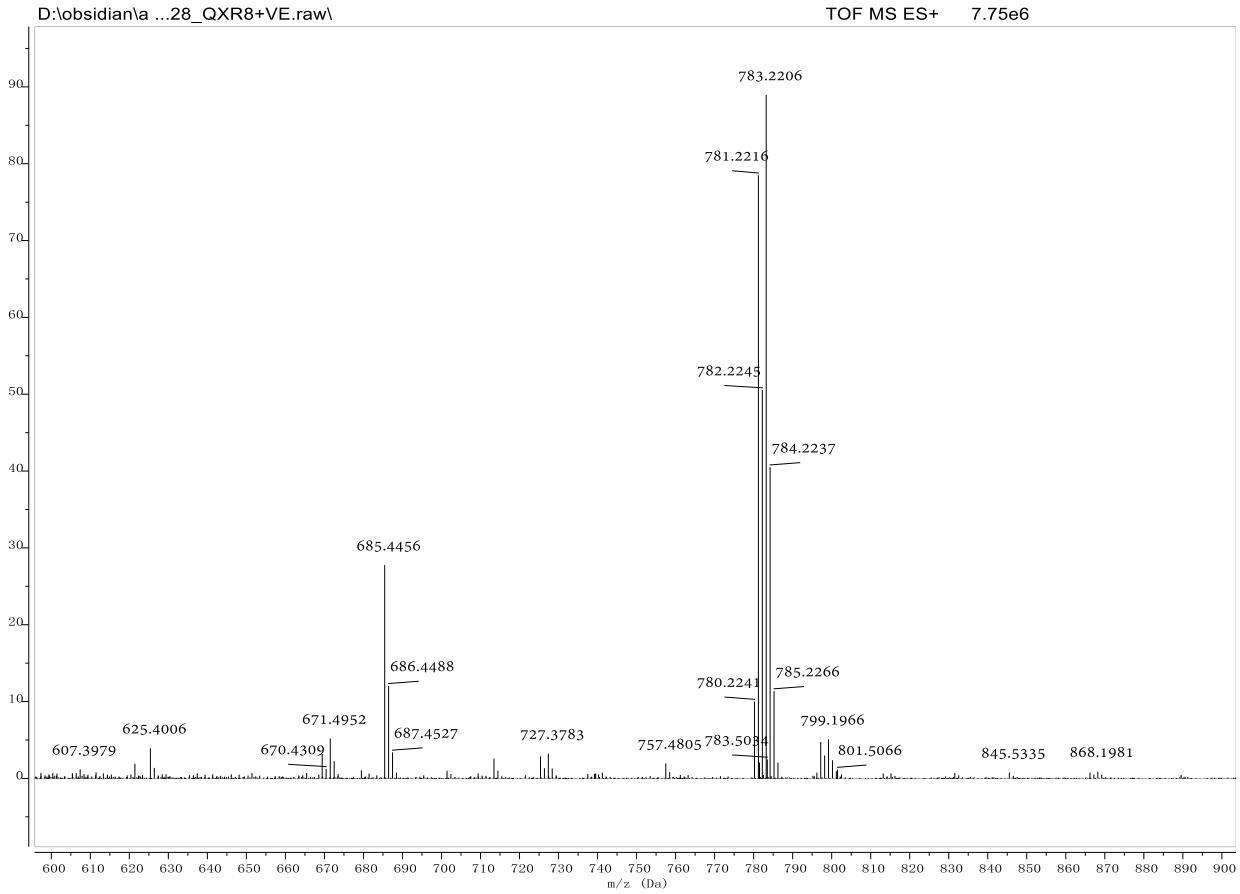
17

Chemical Formula: $C_{37}H_{38}BBrF_2N_6O_4$
Exact Mass: 758.2199
Molecular Weight: 759.4598




10.41
9.25
7.55
7.55
7.54
7.53
7.52
7.51
7.50
7.50
7.48
7.24
7.24
7.23
7.23
7.21
7.21
7.19
7.19
6.85
6.83
6.83
6.82
6.81
6.80
6.80
6.80
6.79
6.78
6.23
4.84
3.78
3.71
3.33 H2O
2.90
2.88
2.86
2.68
2.67
2.65
2.58
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.41
2.41
2.20
2.14
B (t)
2.67
J(7.2)
A (t)
2.88
J(7.2)
1.06
1.03
4.27
2.11
3.22
1.00
2.03
2.00
3.17
2.03
2.04
3.17
6.33
3.14
3.14
f1 (ppm)


170.70
165.28
159.62
159.34
152.98
149.20
146.43
142.63
141.69
141.56
139.35
138.11
134.92
131.65
131.46
130.62
129.45
129.19
128.06
121.24
121.16
120.95
115.00
114.83
111.85
55.17
54.91
40.15 DMSO
39.94 DMSO
39.73 DMSO
39.52 DMSO
39.31 DMSO
39.10 DMSO
38.89 DMSO
35.15
32.94
29.59
16.86
16.27
14.07
13.54
11.87
f1 (ppm)


D:\obsidian\a ...28_QXR8+VE.raw\
TOF MS ES+ 7.75e6
783.2206
781.2216
782.2245
784.2237
685.4456
686.4488
780.2241
785.2266
625.4006
671.4952
687.4527
727.3783
799.1966
607.3979
670.4309
757.4805
783.5034
801.5066
845.5335
868.1981
m/z (Da)

2. Optical profiles of probes

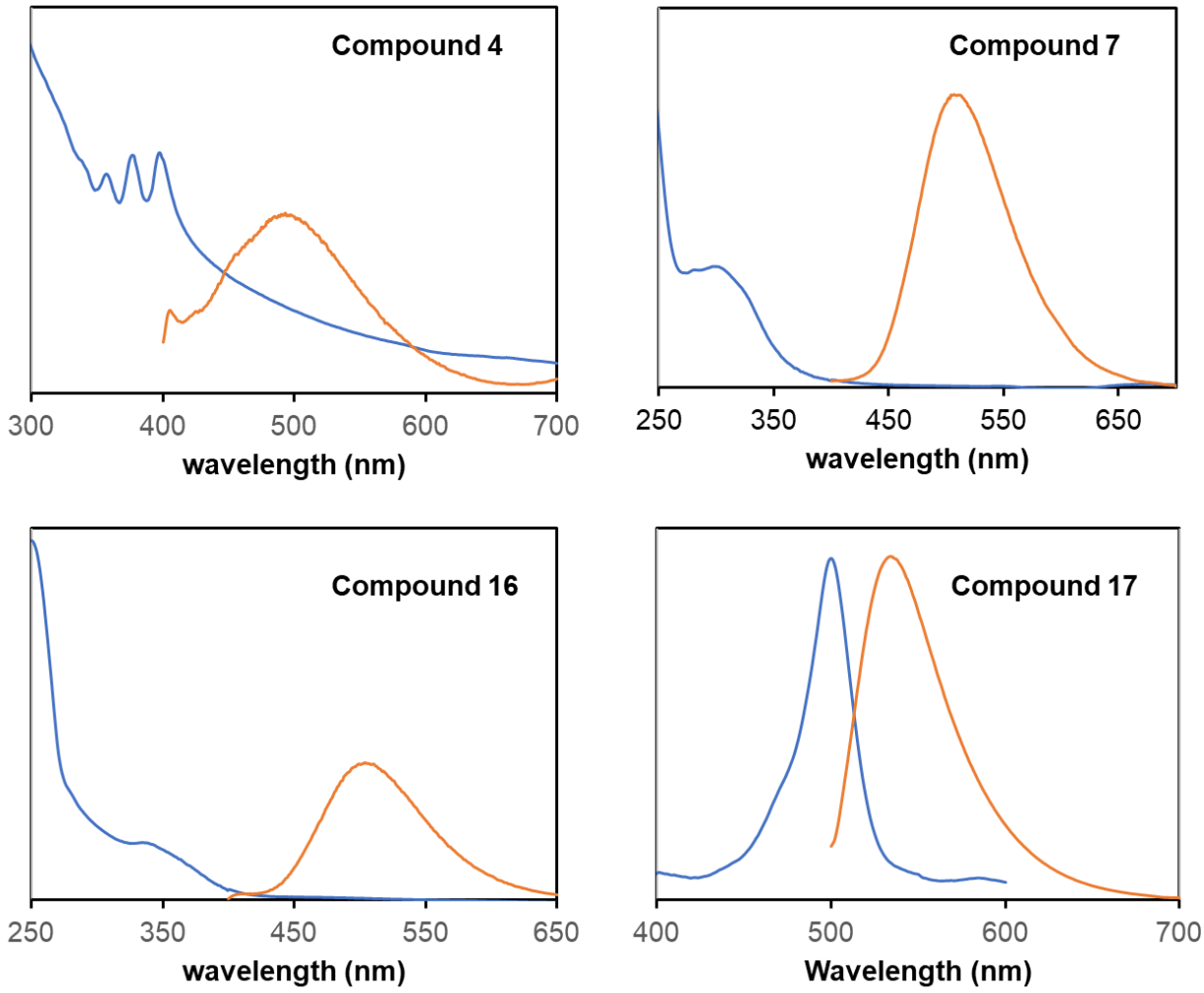


Fig. S2.1. The absorbance (blue) and emission (orange) profile for compound **4**, **7**, **16** and **17**.

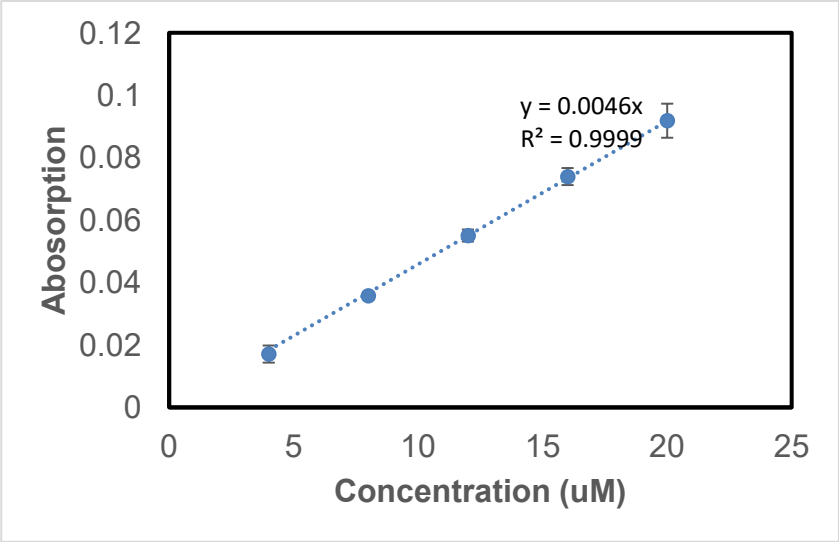


Fig. S2.2. The maximal absorption of compound **16** at different concentrations. The molar extinction coefficient was calculated to be 4600 M^{-1}•cm^{-1} by linear regression.

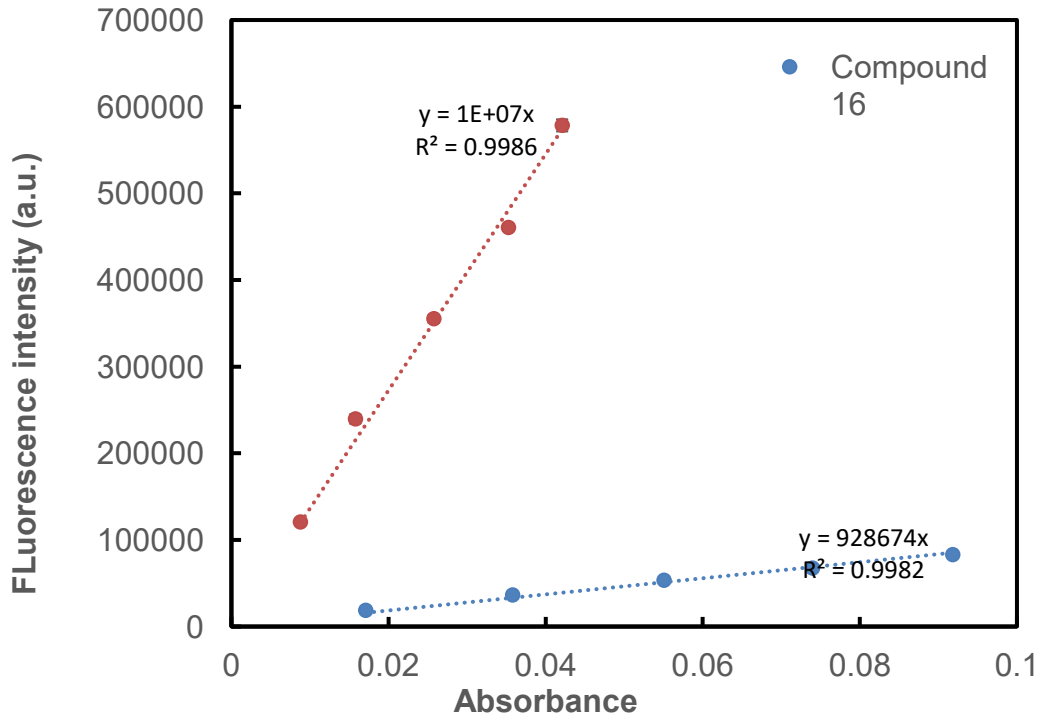


Fig. S2.3. The linear plot of fluorescence versus absorbance for fluorescein (the standard for relevant quantum yield calculation) and compound **16**. The quantum yield (QY) of compound **16** was given by the equation: $QY_{Compound\ 16}=QY_{Quinine\ sulphate}•Slope_{Compound\ 16}/Slope_{Quinine\ sulphate}$.

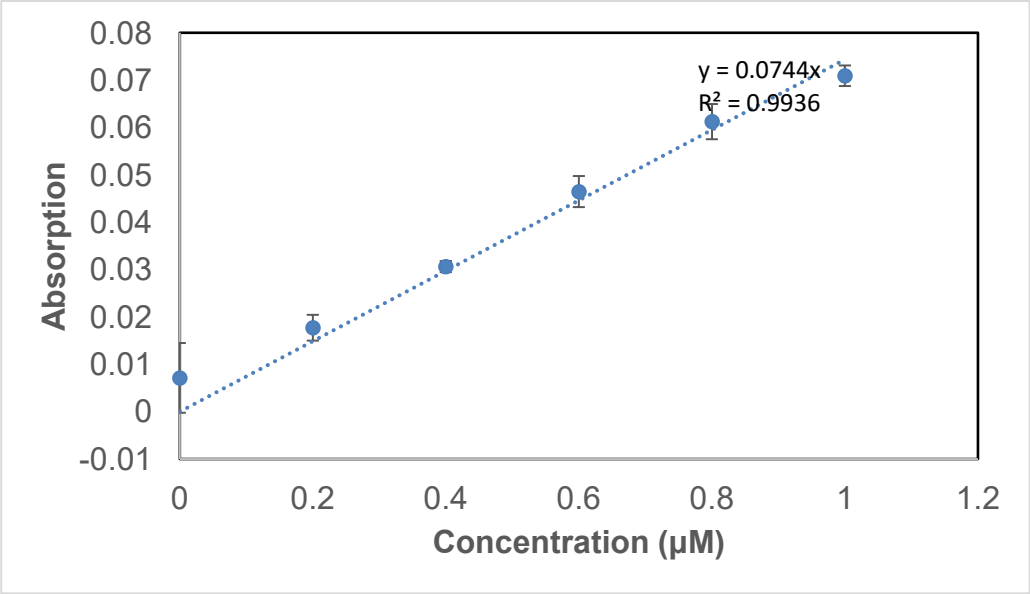


Fig S2.4. The maximal absorption of compound 17 at different concentrations. The molar extinction coefficient was calculated to be 74400 $M^{-1} \cdot cm^{-1}$ by linear regression.

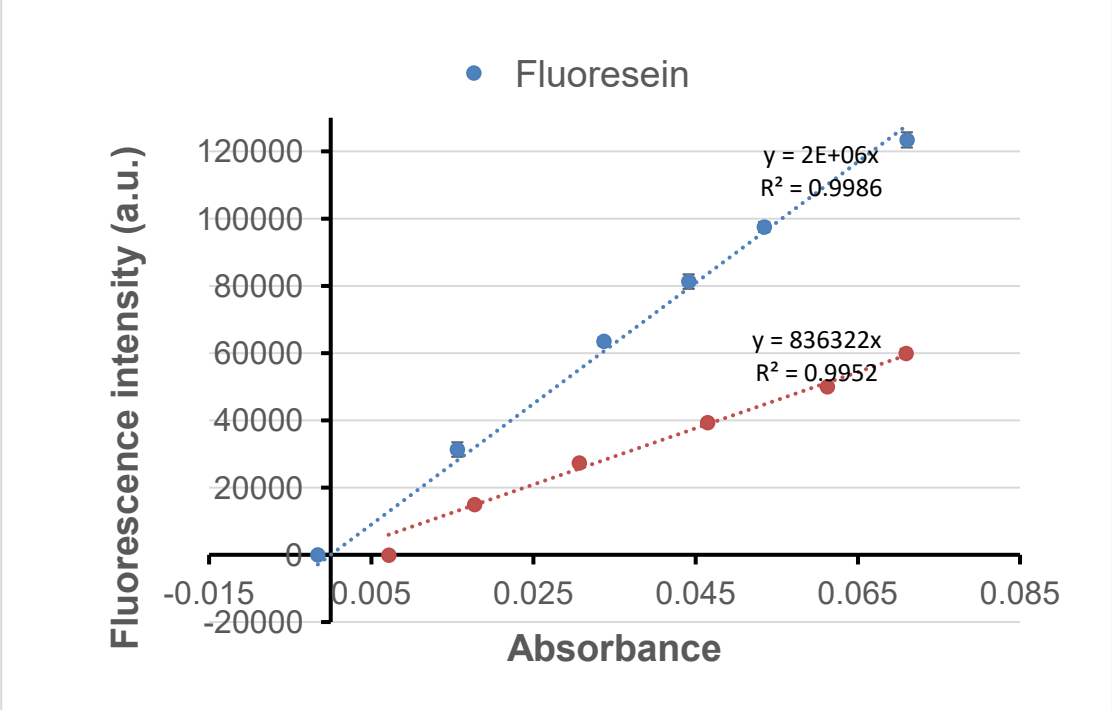


Fig S2.5. The linear plot of fluorescence versus absorbance for fluorescein (the standard for relevant quantum yield calculation) and compound **17**. The quantum yield (QY) of compound **17** was given by the equation: $QY_{Compound\ 17}=QY_{Fluoresein} \cdot Slope_{Compound\ 17} / Slope_{Fluoresein}$.

3. Representative dose–response curves from Ca^{2+} experiments (for 16 and 17)

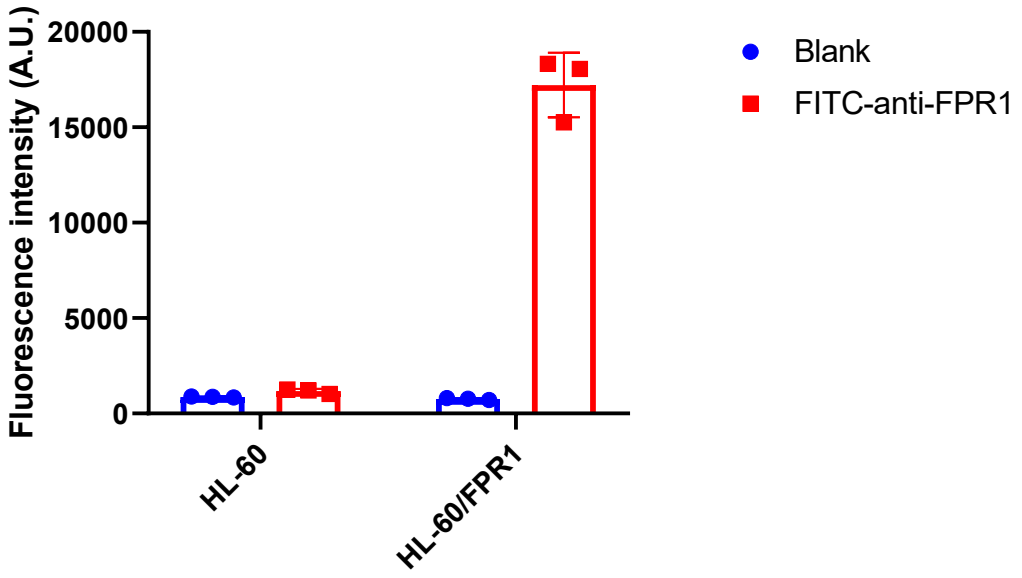


Fig. S3.1. Flow Cytometry analysis for the expression levels of FPR1 in FPR1-transfected HL-60 cells *vs*. wild-type HL-60 cells. FITC-anti-FPR1 is the FPR1 antibody used in the test, from BioLegend, San Diego, CA, USA, cat# 391603.

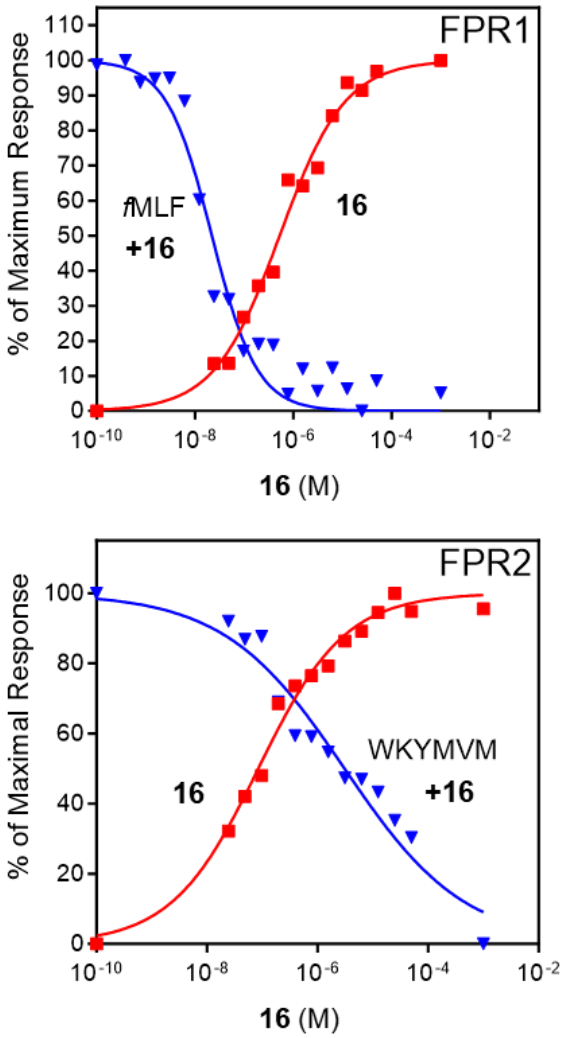


Figure S3.2. Evaluation of agonist and antagonist effects of compound **16** in FPR1-HL60 and FPR2-HL60 Cells. FPR1-HL60 (upper panel) and FPR2-HL60 (lower panel) cells were treated with the indicated concentrations of compound **16** or 1% DMSO (negative control not shown), and $[Ca^{2+}]_i$ was measured to evaluate agonist activity (red lines and symbols). To evaluate antagonist activity, the cells were incubated with compound **16** or 1% DMSO (negative control not shown) for 10 min, followed by activation with 5 nM *f*MLF (upper panel, blue line and symbols) or WKYMVM (lower panel, blue line and symbols) and subsequent monitoring of $[Ca^{2+}]_i$, as described. The data shown are presented as the mean ± SD from one experiment that is representative of three independent experiments with similar results.

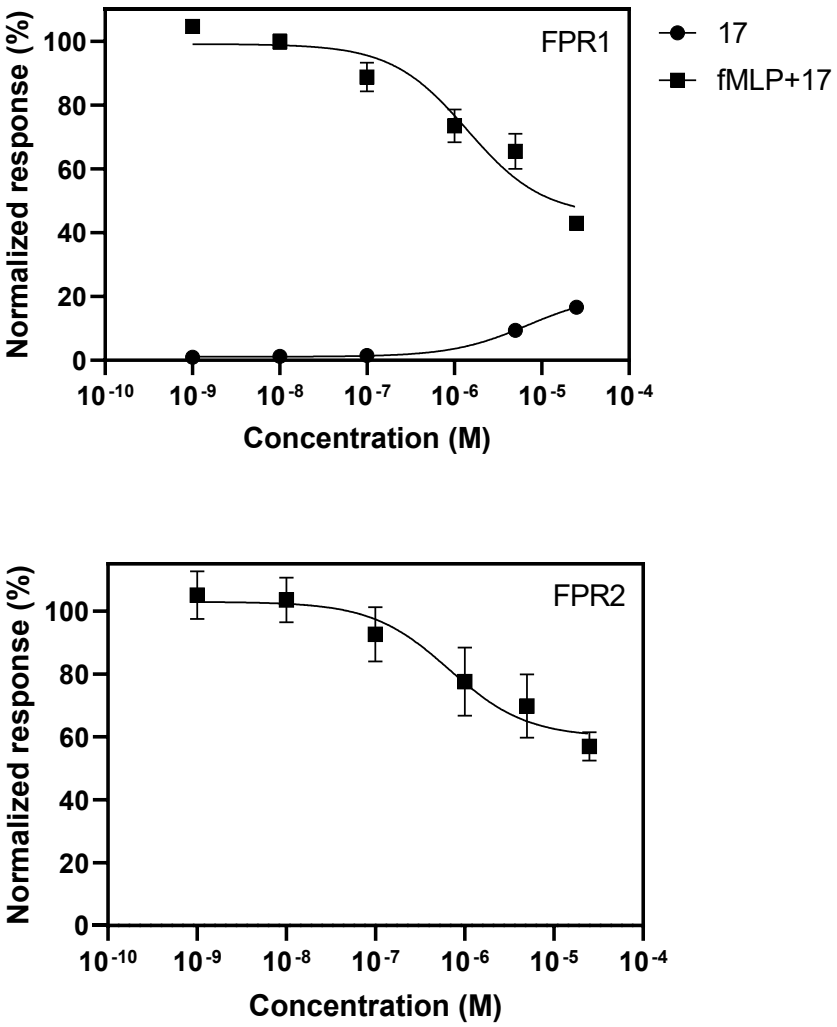


Figure S3.3. Evaluation of agonist and antagonist effects of compound **17** in FPR1-HL60 and FPR2-HL60 Cells. FPR1-HL60 (upper panel) and FPR2-HL60 (lower panel) cells were treated with the indicated concentrations of compound **17** or 1% DMSO (negative control not shown), and $[Ca^{2+}]_i$ was measured to evaluate agonist activity (round symbols). To evaluate antagonist activity, the cells were incubated with compound **17** or 1% DMSO (negative control not shown) for 30 min, followed by activation with 5 nM *f*MLF (upper panel, square symbols) or WKYMVM (lower panel, square symbols) and subsequent monitoring of $[Ca^{2+}]_i$, as described. The data shown are presented as the mean ± SD from one experiment that is representative of three independent experiments with similar results.

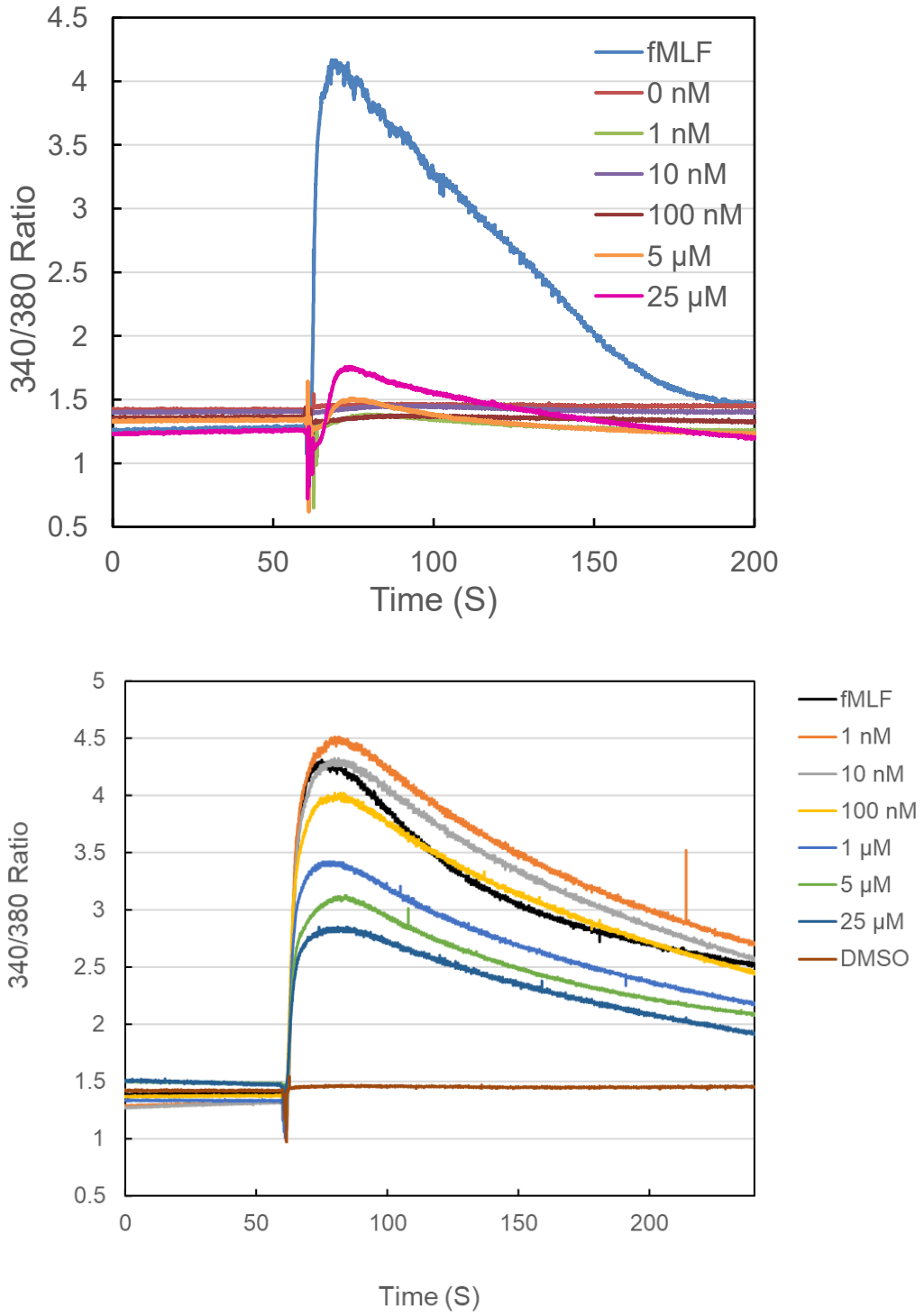


Figure S3.4. Ca^{2+} response curves of compound **17** showing the agonist effect (upper panel) and antagonist effect (lower panel) on FPR1-HL60. For agonist activity, the cells were incubated for 1 min before the treatment of indicated concentrations of compound **17** or 5 nM *f*MLF. For antagonist activity, cells were pre-treated for 30 min with indicated concentrations of compound **17** before the $[Ca^{2+}]_i$ measuring**.** the cells were incubated for 1 min before the treatment of indicated concentrations of 5 nM *f*MLF. The $[Ca^{2+}]_i$ was indicated by the 340/380 ratio of Fura-2 and was measured in real-time.

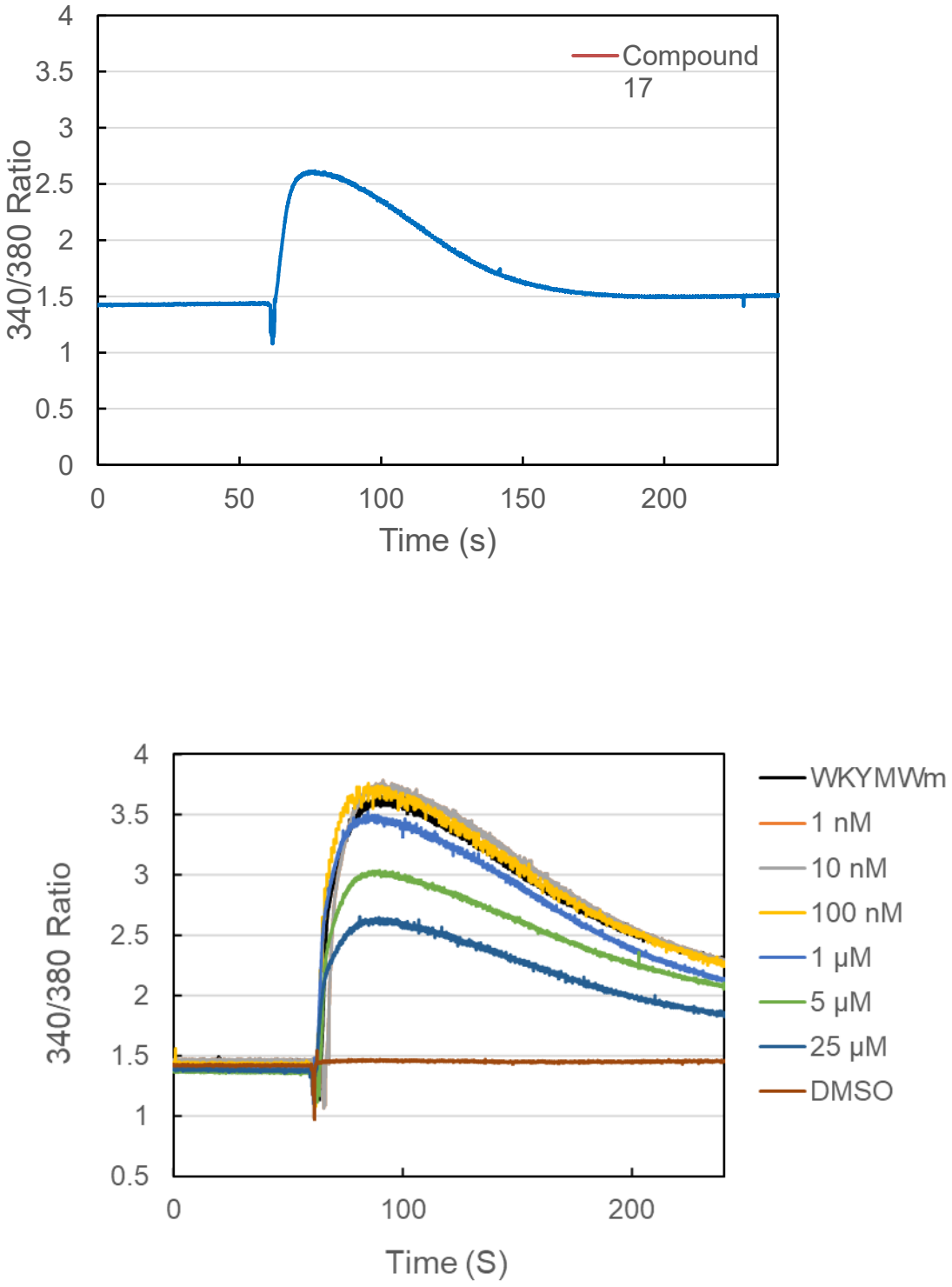


Fig. S3.5. Ca^{2+} response curves of compound **17** showing the agonist effect (upper panel) and antagonist effect (lower panel) on FPR2-HL60. For agonist activity, the cells were incubated for 1 min before the treatment of 25 μM compound **17** or 5 nM WKYMWm. For antagonist activity, cells were pre-treated with indicated concentrations of compound **17** before the $[Ca^{2+}]_i$ measuring**.** the cells were incubated for 1 min before the treatment of indicated concentrations of 5 nM WKYMWm.The $[Ca^{2+}]_i$ was indicated by the 340/380 ratio of Fura-2 and was measured in real-time.

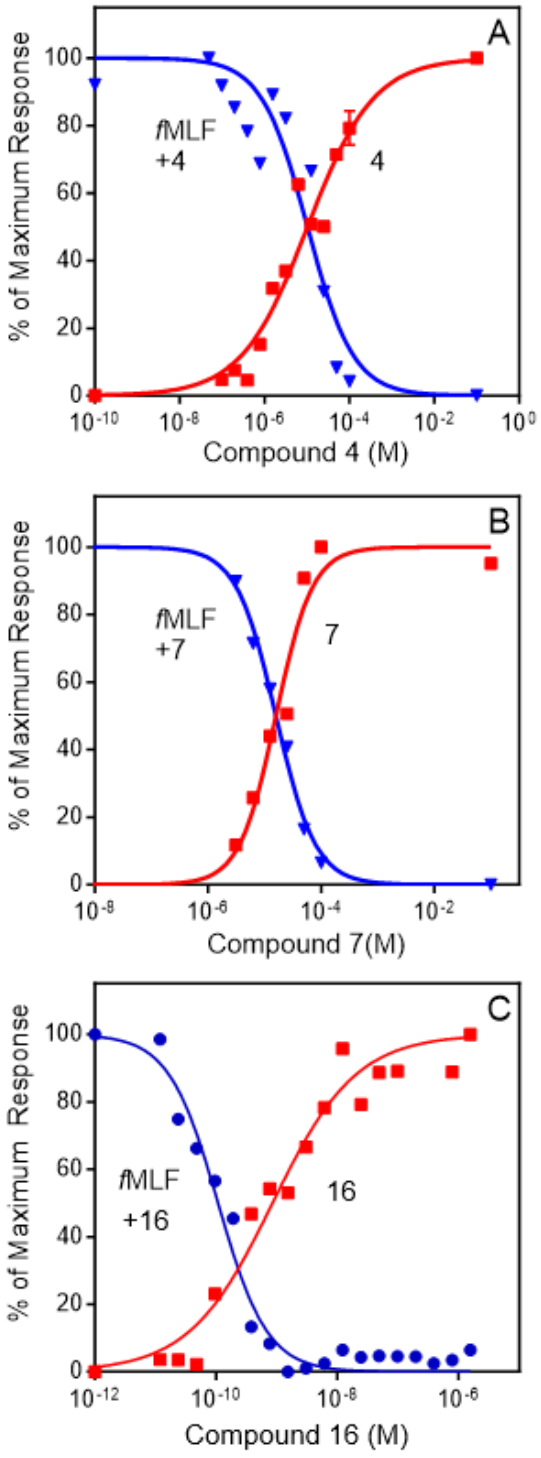


Figure S3.6. Evaluation of agonist and antagonist effects of probes on human neutrophils. Human neutrophils were treated with the indicated concentrations of compounds **4** (Panel A), **7** (Panel B), or **16** (Panel C), or 1% DMSO (negative control not shown), and $[Ca^{2+}]_i$ was measured to evaluate agonist activity (red lines and symbols). To evaluate antagonist activity, neutrophils were incubated with compounds **4** (Panel A), **7** (Panel B), or **16** (Panel C), or 1% DMSO (negative control not shown) for 10 min, followed by activation with 5 nM *f*MLF and subsequent monitoring of $[Ca^{2+}]_i$, as described. The data shown are presented as the mean ± SD from one experiment that is representative of three independent experiments with similar results