Supporting Information

Identification of Aminopyridazine-derived Anti-neuroinflammatory Agents Effective in an Alzheimer's Mouse Model

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KEY WORDS: Alzheimer's disease; neuroinflammation; microglia; Morris water maze; anti-neuroinflammatory agent; animal model;

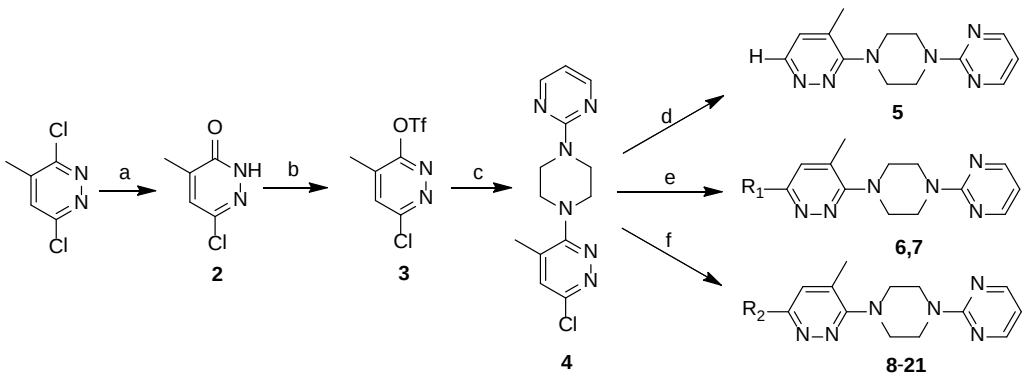
CONTENT

1. Synthesis and characterization of compounds 1b and 4-21

1.1. General

Reagents were obtained from commercial sources and were used as received. Dry solvents were obtained according to the standard procedures, and reactions were conducted under dry N_2 unless otherwise noted. The product solutions were evaporated in vacuo using a rotatory evaporator. The reactions were monitored by thin layer chromatography (TLC) using Qing Dao Hai Yang GF254 silica gel plates visualized with ultraviolet (UV) light (254 nm), iodine steam or phosphomolybdic acid (PMA), and column chromatography was performed using silica gel (200-300 mesh). ^{1}H Nuclear magnetic resonance (NMR) data were acquired with a Bruker NMR AVANCE 400 (400 MHz) and ^{13}C NMR spectra were recorded using a Bruker NMR AVANCE 500 (500 MHz); all samples were dissolved in $CDCl_3$ if not stated otherwise. Chemical shifts are given in δ relative to tetramethylsilane (TMS) in parts per million (ppm), and coupling constants (*J*) are in hertz (Hz). Low-resolution mass spectra (MS) and compound purity data were acquired on a Waters ZQ LC/MS single quadrupole system equipped with an electrospray ionization (ESI) source, a UV detector (220 nm and 254 nm), and an evaporative light scattering detector (ELSD). High performance liquid chromatography (HPLC) analysis revealed a purity of >95% for all compounds.

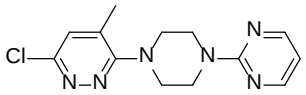
1.2. Synthetic route of target compounds



Scheme 1. Synthesis of target compounds **5-21**

Reagents and conditions: (a) acetic acid, reflux (56.5%); (b) trifluoromethanesulfonic anhydride, triethylamine, DCM, -10 ºC to 0 ºC; (c) 1-(2-pyrimidyl)piperazine, DMF, 0 ºC (76.5% over two steps); (d) H_2, Pd/C, MeOH, rt, 24 h (64%); (e) R_1=Me (**6**), MeMgCl, NMP, ferric acetylacetonate, Et_2O, THF, 0.5 h, rt (76.4%); R_1=*i*-Pr (**7**), *i*-PrMgCl, NMP, ferric acetylacetonate, Et_2O, THF, 0.5 h, rt (84.2%); (f) $R_2B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, DME (31.5-79.9%).

6-chloro-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (4)

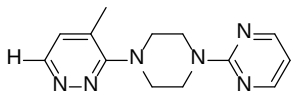


100 g (0.69 mol) of 6-chloro-4-methylpyridazin-3(2*H*)-one, prepared according to the literature[1, 2], and 211 mL (1.52 mol, 2.2 equiv.) of triethylamine was dissolved in 300 mL of anhydrous dichloromethane in an inert atmosphere. The mixture was kept at -10 ºC, and 405 g (1.44 mol, 2.08 equiv.) of trifluoromethanesulfonic anhydride in 200 mL of dichloromethane was added over 2 h. The mixture was allowed to warm to ambient temperature and stirred for an additional 2 h. The reaction was quenched with 500 mL of water, and the organic phase was separated. The organic phase was washed with 1 N HCl (100 mL×2) and brine (100 mL), dried over $MgSO_4$ and filtered. The solvent was removed in vacuo to afford crude 6-chloro-4-methylpyridazin-3-yl trifluoromethanesulfonate (**3**) as brown oil. The crude product (**3**) was dissolved in DMF (200 mL) and cooled in an ice bath. Triethylamine (115 mL, 0.83 mmol, 1.2 equiv.) was added, followed by 2-(piperazin-1-yl)pyrimidine (136 g, 0.83 mmol, 1.2 equiv.) in DMF (150 mL). The mixture was allowed to warm to room temperature over 1 h and was stirred at this temperature for 18 h. It was diluted with ethyl acetate (500 mL), washed with water (500 mL×2) and brine (200 mL), dried ($MgSO_4$), and concentrated under vacuum. The residue was purified by flash chromatography to afford 6-chloro-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (**4**) as a white powder (153 g, 76.5% yield over two steps).

^{1}H NMR ($CDCl_3$): δ 8.33 (d, J = 4.8 Hz, 2H), 7.21 (d, J = 0.4 Hz, 1H), 6.53 (t, J = 4.8Hz, 1H), 3.98 (dd, J_1 = 6.8 Hz, J_2 =2.8 Hz, 4H), 3.35 (t, J = 4.8 Hz, 4H), 2.35 (d, J

= 0.8Hz, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 163.0, 161.7, 157.7, 151.3, 134.1, 130.1, 110.3, 49.5, 43.6, 18.1. High-resolution mass spectra (HRMS) calcd for $C_{13}H_{15}ClN_6$: [M + H] +, 291.1120; found, 291.1118.

4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (5)

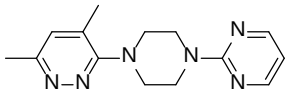


A mixture of 6-chloro-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (**4**, 581 mg, 2.00 mmol) and 10% Pd/C (145 mg, 25 wt %) was hydrogenated with H_2 at room temperature for 8 h and then filtered through Celite. The filtrate was evaporated, and the residue was purified by flash column chromatography to give 328 mg (64% yield) of compound **5** as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.72 (d, J = 4.8 Hz, 1H), 8.33 (d, J = 4.4 Hz, 2H), 7.17 (d, J = 4.8 Hz, 1H), 6.52 (t, J = 4.4 Hz), 3.98 (t, J = 5.2 Hz, 4H), 3.37 (t, J = 5.2 Hz, 4H), 2.35 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 163.8, 161.8, 157.7, 147.1, 131.0, 129.1, 110.2, 49.5, 43.7, 18.1. High-resolution mass spectra (HRMS) calcd for $C_{13}H_{16}N_6$: [M + H] +, 257.1509; found, 257.1510.

1.3. General procedure the synthesis of compounds 6 and 7

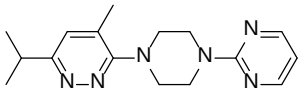
To a solution of 6-chloro-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (**4**, 581 mg, 2.00 mmol) and iron (III) acetylacetonate (50 mg) in tetrahydrofuran (50 mL) was added alkylmagnesium chloride (4.0 mmol) via a syringe at room temperature. The resulting mixture was stirred for 2 h, diluted with ethyl acetate and carefully quenched with a few drops of 10% HCl.[3] The mixture was washed with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification on silica gel afforded compounds **6-7**.

6-dimethyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (6)



76.4% yield, ^{1}H NMR ($CDCl_3$): δ 8.32 (d, J = 4.8 Hz, 2H), 7.03 (s, 1H), 6.53 (t, J = 4.8Hz, 1H), 3.99 (t, J = 5.2 Hz, 4H), 3.32 (t, J = 5.2 Hz, 4H), 2.56 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 162.2, 161.8, 157.7, 155.4, 131.1, 129.7, 110.1, 49.7, 43.8, 21.3, 17.8. High-resolution mass spectra (HRMS) calcd for $C_{14}H_{18}N_6$: [M + H] +, 271.1666; found, 271.1663.

6-isopropyl-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (7)

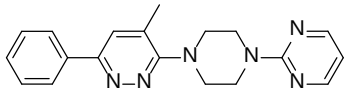


84.2% yield, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (d, J = 4.8 Hz, 2H), 7.05 (s, 1H), 6.50 (t, J = 4.8 Hz, 1H), 3.97 (t, J = 5.2 Hz, 4H), 3.33 (t, J = 5.2 Hz, 4H), 3.15 (m, 1H), 2.33 (s, 3H), 1.32 (d, J = 7.2 Hz, 6H).^{13}C NMR (500 MHz, $CDCl_3$): δ 163.6, 162.4, 161.8, 157.7, 131.3, 127.5, 110.1, 49.6, 43.7, 34.0, 22.3, 18.0. High-resolution mass spectra (HRMS) calcd for $C_{16}H_{22}N_6$: [M + H] +, 299.1906; found, 299.1980.

1.4. General procedure for the synthesis of compounds 1b, 8-21

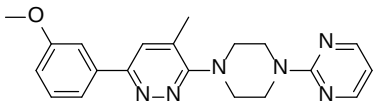
Briefly, 600 mg (2.07 mmol) of 3-chloro-4-methyl-6-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (**4**), 277 mg (2.27 mmol) of arylboronic acid, 856 mg (6.20 mmol) of potassium carbonate and 120 mg (0.103 mmol) of tetrakis(triphenylphosphine)palladium were placed in a reaction tube with 6 mL of DME, and the reaction was capped and heated at 110ºC for 48 h.[4] The solution was cooled to ambient temperature and filtered through Celite. The filtrate was concentrated under reduced pressure to give an oily residue. After purification of the crude product by column silica gel chromatography, compounds **1** and **8-21** were obtained.

4-methyl-6-phenyl-3-(4-(pyrimidin-2-yl) piperazin-1-yl)pyridazine (1b)



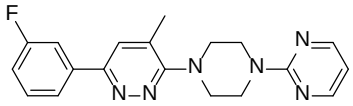
50% yield, ^{1}H NMR ($CDCl_3$): δ 8.35(d, J = 4.8 Hz, 2H), 8.03 (d, J = 9.2 Hz, 2H), 7.60 (s, 1H), 7.48 (m, 3H), 6.54 (t, J = 4.8 Hz, 1H), 4.02 (t, J = 5.2 Hz, 4H), 3.44 (t, J= 5.2 Hz, 4H), 2.43 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 162.6, 161.8, 157.7, 154.9, 136.4, 131.1, 129.2, 128.8, 126.9, 126.5, 110.2, 49.5, 43.7, 18.5.
High-resolution mass spectra (HRMS) calcd for $C_{19}H_{20}N_6$: $[M + H]^+$, 333.1822; found, 333.1825

6-(3-methoxyphenyl)-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (8)



74.2% yield, ^{1}H NMR ($CDCl_3$): δ 8.35 (d, J = 4.8 Hz, 2H), 7.74 (s, 1H), 7.59 (s, 1H), 7.51 (d, J = 7.6 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 6.98 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H), 6.53 (t, J = 4.8 Hz, 1H), 4.02 (t, J = 5.2 Hz, 4H), 3.89 (s, 3H), 3.44 (t, J = 5.2 Hz, 4H), 2.42 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 162.7, 161.8, 160.1, 157.7, 154.5, 137.8, 132.1, 131.1, 129.7, 127.0, 118.7, 115.8, 111.1, 110.2, 55.4, 49.5, 43.7, 18.5.
High-resolution mass spectra (HRMS) calcd for $C_{20}H_{22}N_6O$: $[M + H]^+$, 363.1928; found, 363.1929.

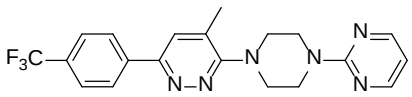
6-(3-fluorophenyl)-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (9)



72.3% yield, ^{1}H NMR ($CDCl_3$): δ 8.36 (d, J = 4.8 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.67-7.71 (m, 1H), 7.62, (s, 1H), 7.46-7.51 (m, 1H), 7.13-7.18 (m, 1H), 6.55 (t, J = 4.8 Hz, 1H). 4.05 (t, J = 4.8 Hz, 4H), 3.47 (t, J = 5.2 Hz, 4H), 2.47 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 164.3, 162.8, 162.3, 161.7, 157.8, 153.9, 138.0, 138.0, 132.7, 130.7, 130.6, 128.1, 122.28, 122.26, 116.58, 116.41, 113.8, 113.6, 110.3, 49.5, 43.7, 18.7. High-resolution mass spectra (HRMS) calcd for $C_{20}H_{22}N_6O$: $[M + H]^+$,

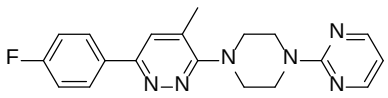
351.1728; found, 351.1729.

4-methyl-6-(4-(trifluoromethyl)phenyl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl) pyridazine (10)



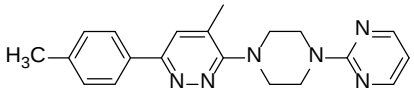
31.5% yield, ^{1}H NMR ($CDCl_3$): δ 8.35 (d, J = 4.8 Hz, 2H), 8.16 (d, J = 8.0 Hz, 2H), 7.74 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 0.8 Hz, 1H), 6.54 (t, J = 4.8 Hz, 1H), 4.03(t, J = 5.2 Hz, 4H), 3.48 (t, J = 5.2 Hz, 4H), 2.45 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 162.9, 161.8, 157.8, 153.3, 139.8, 131.2, 131.1, 127.0, 126.7, 125.80, 125.77, 110.3, 49.4, 43.7, 18.6. High-resolution mass spectra (HRMS) calcd for $C_{20}H_{19}F_3N_6$: [M + H] $^{+}$, 401.1696; found, 401.1693.

4-methyl-6-(4-fluorophenyl)-3-(4-(pyrimidin-2-yl) piperazin-1-yl)pyridazine (11)



79.9% yield, ^{1}H NMR ($CDCl_3$): δ 8.34 (d, J = 4.8 Hz, 2H), 8.02 (m, 2H), 7.55 (s, 1H), 7.16 (t, J = 8.8 Hz, 2H), 6.53 (t, J = 4.8 Hz, 1H), 4.02 (t, J = 5.2 Hz, 4H), 3.44 (t, J = 5.2 Hz, 4H), 2.43 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 164.6, 162.7, 162.5, 161.8, 157.7, 153.9, 132.5, 131.3, 128.3, 128.2, 126.6, 115.9, 115.7, 110.2, 49.5, 43.7, 18.4. High-resolution mass spectra (HRMS) calcd for $C_{19}H_{19}FN_6$: [M + H] $^{+}$, 351.1728; found, 351.1728.

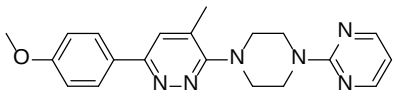
4-methyl-6-(p-tolyl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (12)



69.2% yield, ^{1}H NMR ($CDCl_3$): δ 8.33(d, J = 4.8 Hz, 2H), 7.93 (d, J = 8.0 Hz, 2H), 7.55 (s, 1H), 7.27 (d, J = 8.4 Hz, 1H), 6.51 (t, J = 4.8 Hz, 1H), 4.00 (t, J=5.2 Hz, 4H), 3.41 (t, J = 5.2 Hz, 4H), 2.39 (s, 6H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 162.4, 161.7,

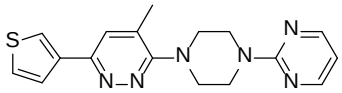
157.7, 154.8, 139.2, 133.6, 131.0, 129.5, 126.5, 126.3, 110.1, 49.5, 43.7, 21.2, 18.3. High-resolution mass spectra (HRMS) calcd for $C_{20}H_{22}N_6$: $[M + H]^+$, 347.1979; found, 347.1979.

4-methyl-6-(4-methoxyphenyl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (13)



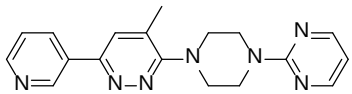
51.5% yield, ^{1}H NMR ($CDCl_3$): δ 8.35 (d, J = 4.8 Hz, 2H), 8.00 (d, J = 8.4 Hz, 2H), 7.54 (s, 1H), 7.01 (d, J = 8.4 Hz, 2H), 6.53 (t, J = 4.8 Hz, 1H), 4.02 (t, J = 4.8 Hz, 4H), 3.87 (s, 3H), 3.42 (t, J = 5.2 Hz, 4H), 2.42 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 162.3, 161.8, 160.7, 157.7, 154.6, 131.2, 129.0, 127.7, 126.3, 114.2, 110.1, 55.3, 49.6, 43.8, 18.4. High-resolution mass spectra (HRMS) calcd for $C_{20}H_{22}N_6O$: $[M + H]^+$, 363.1928; found, 363.1925.

4-methyl-6-(thiophen-3-yl)-3-(4-(pyrimidin-2-yl) piperazin-1-yl)pyridazine (14)



57.2% yield, ^{1}H NMR ($CDCl_3$): δ 8.34 (d, J = 4.8 Hz, 2H), 7.88 (d, J = 1.2 Hz, 1H), 7.77 (d, J = 4.8 Hz, 1H), 7.49 (s, 1H), 7.41 (m, 1H), 6.52 (m, 1H), 4.01 (t, J = 5.2 Hz, 4H), 3.41 (t, J = 4.8 Hz, 4H), 2.40 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 162.3, 161.8, 157.7, 151.5 138.9, 131.2, 126.7, 126.5, 125.9, 123.0, 110.1, 49.5, 43.7, 18.3. High-resolution mass spectra (HRMS) calcd for $C_{17}H_{18}N_6S$: $[M + H]^+$, 339.1386; found, 339.1385.

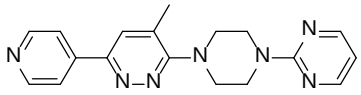
4-methyl-6-(pyridin-3-yl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (15)



35.7% yield, ^{1}H NMR ($CDCl_3$): δ 9.17 (d, J = 1.2 Hz, 1H), 8.67 (d, J = 3.6 Hz, 1H), 8.43 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 4.8 Hz, 2H), 7.62 (s, 1H), 7.43 (dd, J_1 = 12.0 Hz,

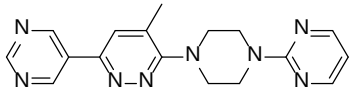
J_2 = 4.8 Hz, 1H), 6.54 (t, J = 4.8 Hz, 1H), 4.03 (t, J = 4.8 Hz, 4H), 3.46 (t, J = 5.2 Hz, 4H), 2.45 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 162.9, 161.8, 157.7, 152.3, 150.2, 147.6, 133.9, 132.2, 131.3, 126.7, 123.8, 110.3, 49.4, 43.7, 18.6. High-resolution mass spectra (HRMS) calcd for $C_{18}H_{19}N_7$: $[M + H]^+$, 334.1775; found, 334.1775.

4-methyl-6-(pyridin-4-yl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (16)



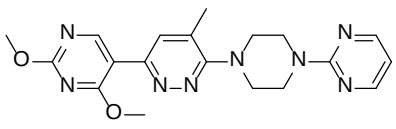
37.4% yield, ^{1}H NMR ($CDCl_3$): δ 8.73 (d, J = 4.0 Hz, 2H), 8.35 (d, J = 4.8 Hz, 2H), 7.93 (d, J = 6.0 Hz, 2H), 7.63 (s, 1H), 6.54 (t, J = 4.4 Hz, 1H), 4.02 (t, J = 5.2 Hz, 4H), 3.49 (t, J = 5.2 Hz, 4H), 2.45 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 163.3, 161.8, 157.7, 152.1, 150.5, 143.7, 130.9, 126.9, 120.4, 110.3, 49.3, 43.6, 29.7, 18.7. High-resolution mass spectra (HRMS) calcd for $C_{18}H_eN_7$: $[M + H]^+$, 334.1775; found, 334.1773.

4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)-6-(pyrimidin-5-yl)pyridazine (17)



79.6% yield, ^{1}H NMR ($CDCl_3$): δ 9.35 (s, 2H), 9.26, (s, 1H), 8.98 (s, 1H) 8.33 (d, J = 4.8 Hz, 2H), 7.60 (s, 1H), 6.53 (t, J = 4.8 Hz, 1H), 4.02 (t, J = 4.8Hz, 4H), 3.47 (t, J = 4.8 Hz, 4H), 2.45 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 163.1, 161.7, 158.7, 157.7, 154.4, 149.7, 131.3, 130.0, 126.4, 110.3, 49.3, 43.6, 18.8. High-resolution mass spectra (HRMS) calcd for $C_{17}H_{18}N_8$: $[M + H]^+$, 335.1727; found, 335.1729.

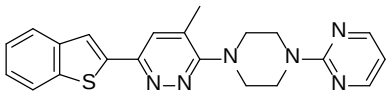
4-methyl-6-(2,4-dimethoxypyrimidin-5-yl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)py ridazine (18)



66.7% yield, ^{1}H NMR ($CDCl_3$): δ 9.01 (s, 1H), 8.34 (d, J = 4.8 Hz, 2H), 7.70 (s, 1H), 6.53 (t, J = 4.8 Hz, 1H), 4.08 (s, 3H), 4.06 (s, 3H), 4.01(d, J = 5.2 Hz, 4H), 3.43 (d, J

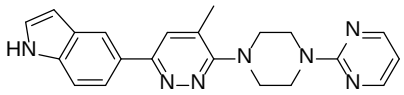
= 5.2 Hz, 4H), 2.41 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 168.1, 165.3, 162.3, 161.8, 159.2, 157.7, 150.3, 130.2, 132.0, 112.0, 110.2, 55.0, 54.2, 49.4, 43.7, 18.5. High-resolution mass spectra (HRMS) calcd for $C_{19}H_{22}N_eO_2$: $[M + H]^+$, 395.1939; found, 395.1937.

4-methyl-6-(benzothiophen-2-yl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (19)



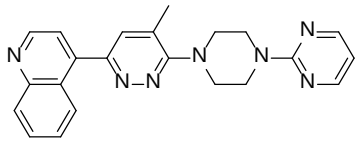
69.7% yield, ^{1}H NMR ($CDCl_3$): δ 8.35 (d, J = 4.4 Hz, 2H), 7.86 (dd, J_1 = 6.0 Hz, J_2 = 2.8 Hz, 1H), 7.77 (dd, J_1 = 5.2 Hz, J_2 = 2.8 Hz, 1H), 7.60 (s, 1H), 7.35 (dd, J_1= 6.0 Hz, J_2=3.2 Hz, 1H), 6.78 (s, 1H), 4.00 (t, J = 4.8 Hz, 4H), 3.45 (t, J = 4.8 Hz, 4H), 2.43 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 161.6, 158.6, 157.7, 148.0, 142.6, 140.41, 139.96, 136.4, 124.8, 124.1, 123.8, 122.6, 122.1, 113.5, 110.3, 44.6, 43.2, 21.7. High-resolution mass spectra (HRMS) calcd for $C_{21}H_{20}N_6S$: $[M + H]^+$, 389.1543; found, 389.1546.

5-(5-methyl-6-(4-(pyrimidin-2-yl)piperazin-1-yl) pyridazin-3-yl)-1H-indole (20)



78.4% yield, ^{1}H NMR ($CDCl_3$): δ 8.39 (br s, 1H), 8.35 (d, J = 4.8 Hz, 2H), 8.27 (s, 1H),7.99 (d, J = 5.2 Hz, 1H), 7.67 (t, J = 9.6 Hz 2H), 7.50 (m, 3H)6.63 (s, 1H), 6.53 (t, 4.8 Hz, 1H)4.02 (t, J= 4.8Hz, 4H), 3.44 (t, J = 5.2 Hz, 4H), 2.44 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 162.2, 161.8, 157.7, 156.2, 136.6, 132.0, 131.3, 128.5, 127.0, 125.1, 121.0, 119.2, 111.5, 110.1, 113.4, 50.0, 43.8, 18.4. High-resolution mass spectra (HRMS) calcd for $C_{21}H_{21}N_7$: $[M + H]^+$, 372.1931; found, 372.1933.

4-(5-methyl-6-(4-(pyrimidin-2-yl)piperazin-1-yl) pyridazin-3-yl)quinolone (21)



35% yield, ^{1}H NMR ($CDCl_3$): δ 9.33 (s, 1H), 8.53(s, 1H),8.36 (d, J = 4.8 Hz, 2H), 8.09 (t, J_1 = 6.0 Hz, J_2 = 4.8Hz, 1H), 7.7-7.5 (m, 3H), 6.92 (s, 1H), 6.56 (t, J = 4.8 Hz, 1H), 4.04 (t, J = 5.6 Hz, 4H), 3.87 (t, J_1 = 5.6 Hz, J_2 = 4.8 Hz, 4H), 2.08 (s, 3H) ^{13}C NMR (500 MHz, $CDCl_3$): δ 161.7, 159.7, 157.8, 153.0, 151.2, 143.6, 138.5, 134.7, 130.8, 128.7, 128.4, 128.0, 127.4, 124.5, 112.8, 110.3, 44.7, 43.4, 19.6.
High-resolution mass spectra (HRMS) calcd for $C_{22}H_{21}N_7$: $[M + H]^+$, 384.1931; found, 384.1934.

2. In vitro assay protocols

2.1. Cell culture condition

Microglia cell-based assays for the concentration-dependent activity of the compounds were performed as previously described.[5-9] The murine microglial cell line BV-2 (Cell Resource Center of Chinese Academy Of Medical Science, Beijing, China) was cultured at 37°C, 7% CO_2 in αMEM containing 10% fetal calf serum (Hyclone, UT, U.S.), 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco, CA, U.S.).

2.2. Inhibition activity of IL-1β synthesis

Two days prior to treatment, the cells were trypsinized and plated in 96-well plates at a density of 2×10^4 cells/well. After 48 h, the cells were rinsed with PBS, and 80 μl of serum-free MEM was added. For each drug, one 96-well plate was used and ten different experimental conditions were tested (six wells per each condition): (1) control cells in plain αMEM; (2) cells treated with media containing LPS (100 ng/ml); (3–9) cells treated with LPS (100 ng/ml)+one of seven different concentrations of

drug; and (10) cells treated with the highest concentration of drug in the absence of LPS. The drugs and LPS solutions were added to the BV-2 cells sequentially. The drug solutions (10 μl) were added to the cells first, and then 10 μl of a 1 μg/ml LPS solution was added to all wells except for (1) and (10), where 10 μl media was added. The cells were incubated for 16 h at 37°C. The levels of IL-1β in cell lysates were measured by ELISA (R&D systems, MN, U.S.) according to the manufacturers' instructions.

3. In vivo studies of compound 14[10]

3.1. Materials and methods

3.1.1. Materials

Compound **14** was dissolved in 0.5% carboxymethylcellulose (CMC)-saline solution to the desired 2.5 mg/kg concentration. Donepezil (Eisai Pharmaceutical, Suzhou, China) was used as the positive control drug in this study. $A\beta_{1-42}$ (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile saline (0.9% NaCl) at a concentration of 1 mM, then sealed and stored at -20 ºC. $A\beta_{1-42}$ was incubated at 37 °C for 5 days before the injection to aggregate.

3.1.2. Animals

Healthy male Kunming mice (8 weeks old; body weight, 18 - 22 g at the start of experiments; Experimental Animal Center of Shenyang Pharmaceutical University, Shenyang, China) were used throughout the study. Animals were housed in a room maintained at a controlled temperature (23±1 ºC) and relative humidity of 45±15% with a 12 h light/12 h dark cycle and were provided ad libitum access to standard laboratory food and water. Behavioral experiments were performed in a sound-attenuated and air-regulated experimental room to which mice were habituated

for ≥1 h. All animal studies were performed in strict accordance with the National Institutes of Health Guide for the use and care of laboratory animals and the guidelines established by the Chinese Society of Laboratory Animal Sciences.

3.2. Treatment

Mice were randomly divided into four groups (n=10/group). The Aβ model group received interacerebroventricular (i.c.v.) injection of 3 μl of aggregated $A\beta_{1-42}$ (equivalent to 410 pmol/mouse). The mice were anesthetized with 3.5% chloral hydrate (i.p.) and fixed on a stereotactic instrument (Narishige, Tokyo, Japan). $A\beta_{1-42}$ was implanted into the right ventricle (A, -0.5 mm; L, 1 mm from the bregma; V, 3 mm from the skull). As a control, the sham-operated group of mice were injected i.c.v. with the vehicle (saline) only. The vehicle itself failed to induce any behavioral and neurochemical changes. The Aβ+compound **14** (2.5 mg/kg body weight) group consisted of Aβ model mice with per oral (p.o.) administration of compound **14** at 2.5 mg/kg. The Aβ + donepezil (0.65 mg/kg) group was given p.o. donepezil at 0.65 mg/kg. The Aβ model group and sham-operated group also received p.o. administration of the same volume of 0.5% CMC-saline solution. All compounds were administered at a volume of 0.1 ml/10 g body weight once a day from the day of i.c.v. Aβ until the end of behavioral testing.

3.3. Morris water maze tests

The experiment was carried out 9-13 days after the $A\beta_{1-42}$ injection. The apparatus was a circle water tank (100 cm diameter and 44 cm height), divided into four equal quadrants. During testing, the tank was filled with water (23±1 °C). A transparent platform was set inside the tank and its top was submerged 1 cm below the water surface in the center of one among the four quadrants of the maze in the reference memory test. The movements of the animals in the tank were monitored with a video tracking system (purchased from Chinese Academy of Medical Sciences).

3.3.1. Reference memory tests

The reference memory test was conducted three times a day for 4 consecutive days. In each trial, the mouse was placed in the water at one of the three starting positions that were equally spaced around the rim of the tank. The sequence of the positions was selected randomly. If the mouse found the platform within 60 s, it was allowed to remain there for 10 s and then returned to its home cage. If the mouse could not find the platform within 60 s, the trial was terminated and a maximum score of 60 s was assigned. The swimming distance, escape latency and swimming speed to the platform were recorded by a computer.

3.3.2. Probe tests

After the 12th reference memory test training trial on day 13 after the $A\beta_{1-42}$ injection, the platform was removed from the pool and each mouse underwent a 60 s spatial probe trial. The swimming percentage of path length and the time spent in the target quadrant where the platform was located during training were recorded.

3.4. Statistical analysis

All analyses were performed using IBM SPSS Statistics 17.0 software. All data are expressed as the means ±S.E.M.. Significant differences among the experimental groups were tested using one-way analysis of variance ANOVA followed by the Bonferroni test. Escape latency and swimming distance in the Morris water maze were determined by two-way ANOVA with repeated measures (trial blocks). Values of $P < 0.05$ were considered statistically significant.

4. Brain uptake assay

Compound 14 was administered to mice (20g-30g) by oral gavage using 2.5mg/kg compound in 0.5% carvoxymethylcellulose vehicle. At 0, 15, 30, 60, 120, 180, 240, 300, 360 min after administration, blood was collected in heparinized tubes from anesthetized animals and plasma obtained by centrifugation. After perfusion, brains

were immediately harvested, weighed and homogenized in 0.1% formic acid and deproteinized with ice-cold acetonitrile. The obtained mixture was then centrifuged to remove precipitated protein, and the brain homogenate supernatants were further diluted with 0.1% formic acid. The plasma samples were acidified with 0.1% formic acid. The solid phase extraction followed by HPLC analysis was used to quantify the amount of compound in the plasma and brain supernatants with compound 14 used as an internal standard.

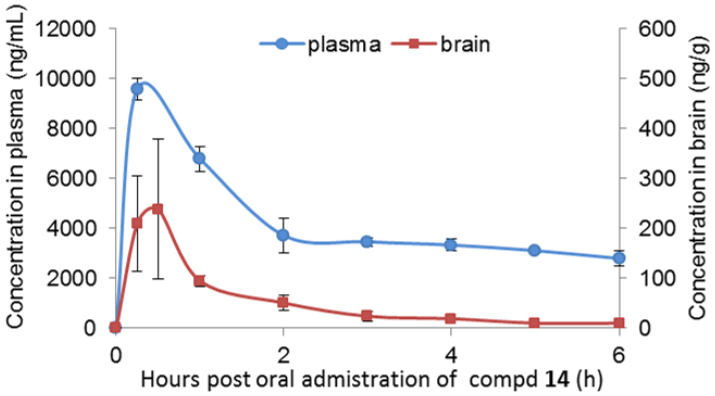


Figure S1. Brain and plasma level of compound 14 after single dose oral administration of 2.5mg/kg. (●, concentration of compound in plasma, ng/mL; ■, concentration of compound 14 in brain, ng/g)

5. References

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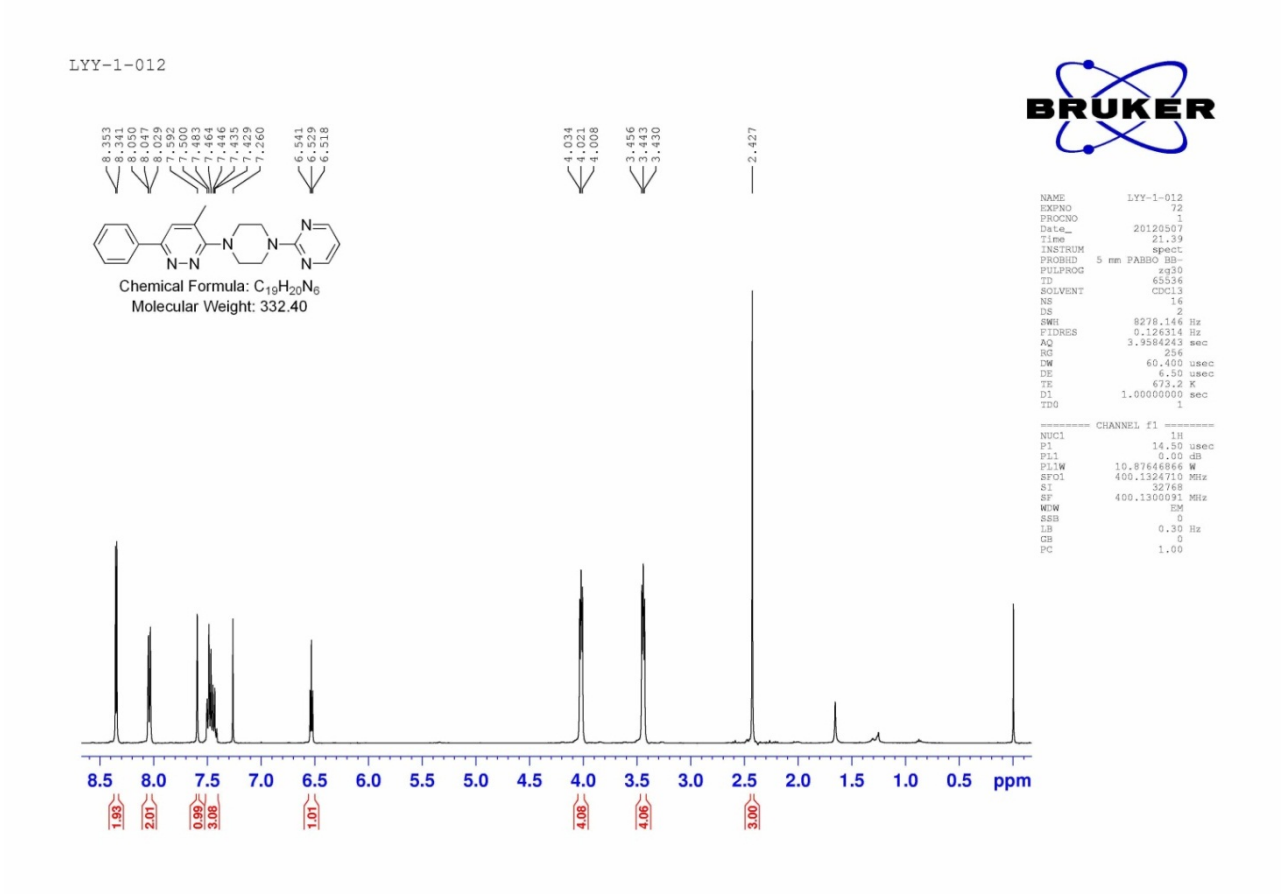
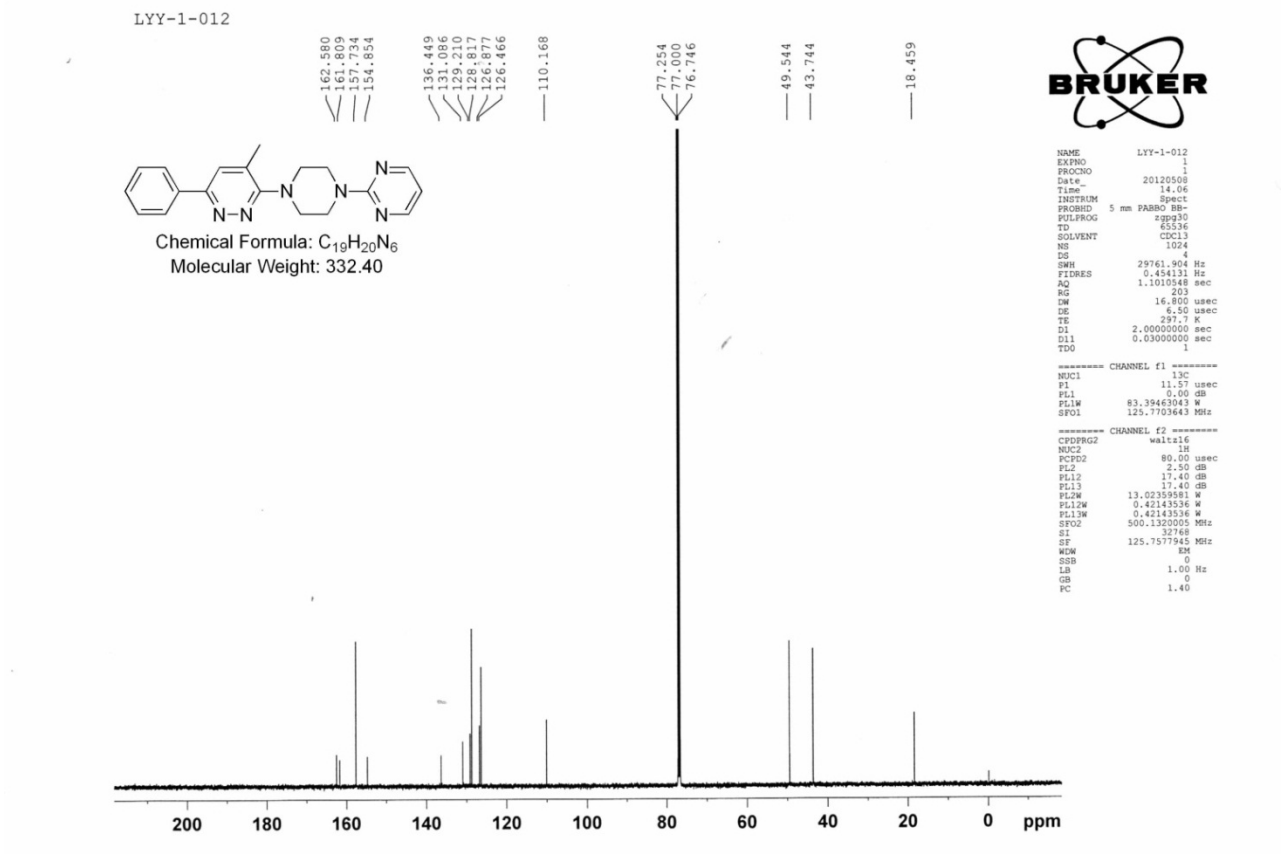
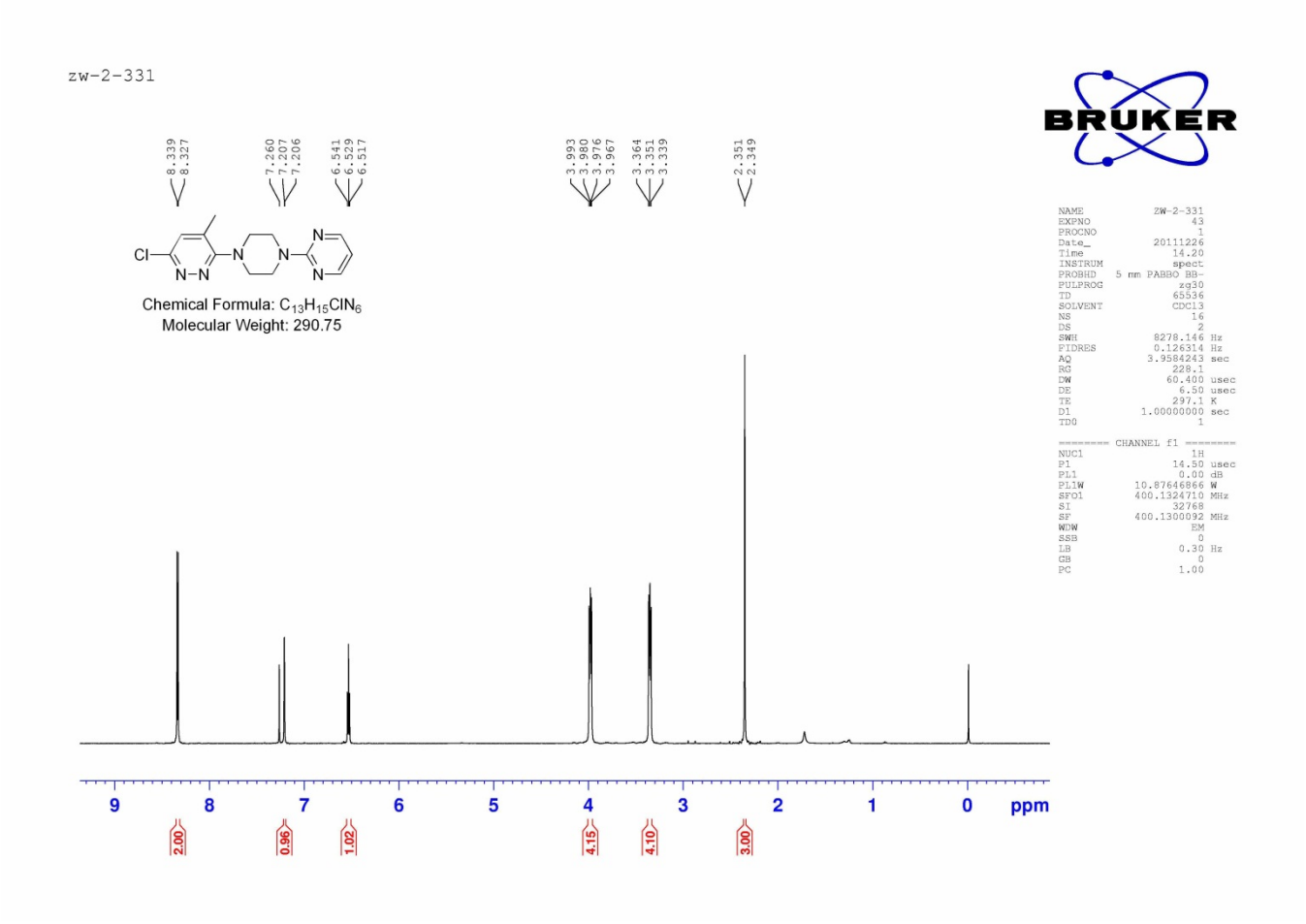
6. NMR spectra of compounds 1b, 4-21

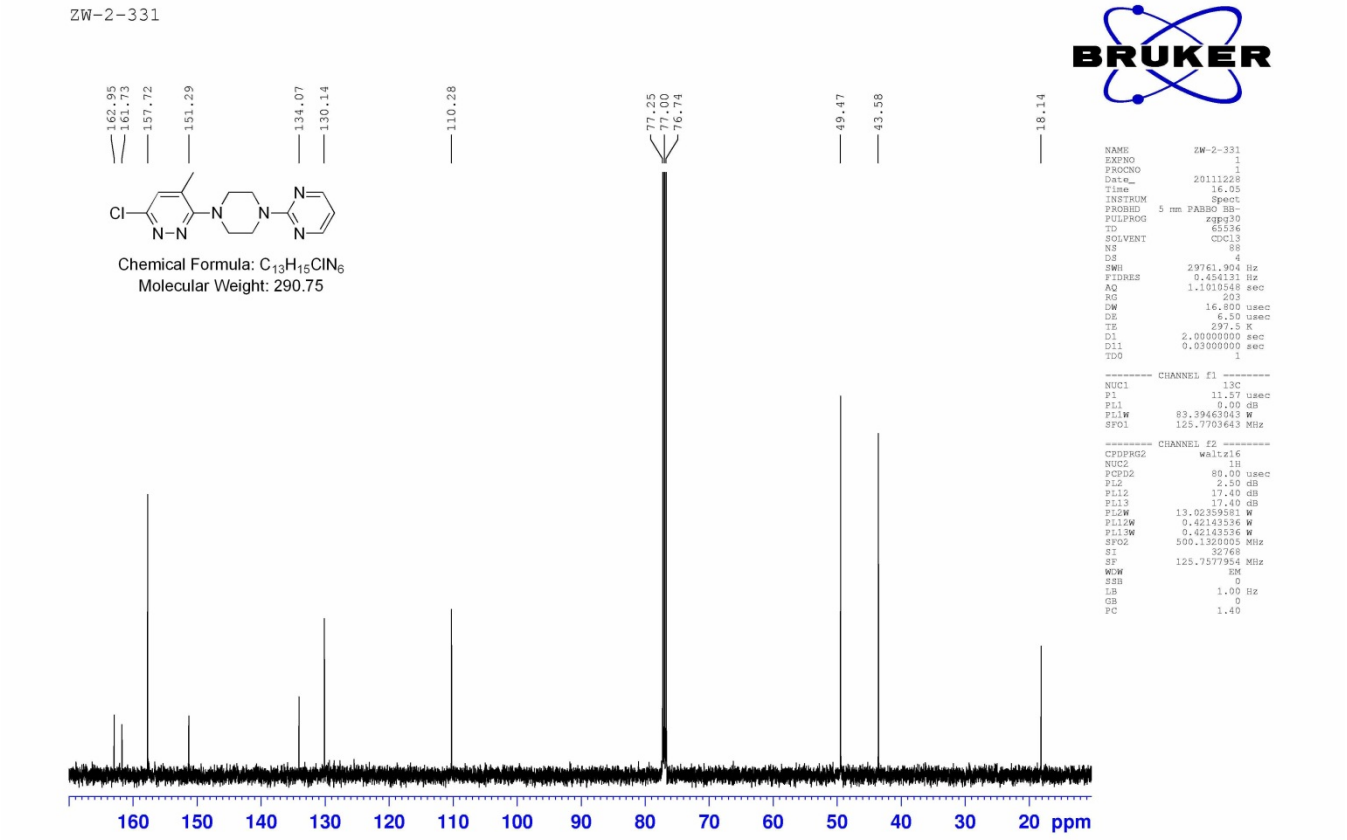
4-methyl-6phenyl-3-(4-(pyrimidin-2-yl) piperazin-1-yl)pyridazine (1b)



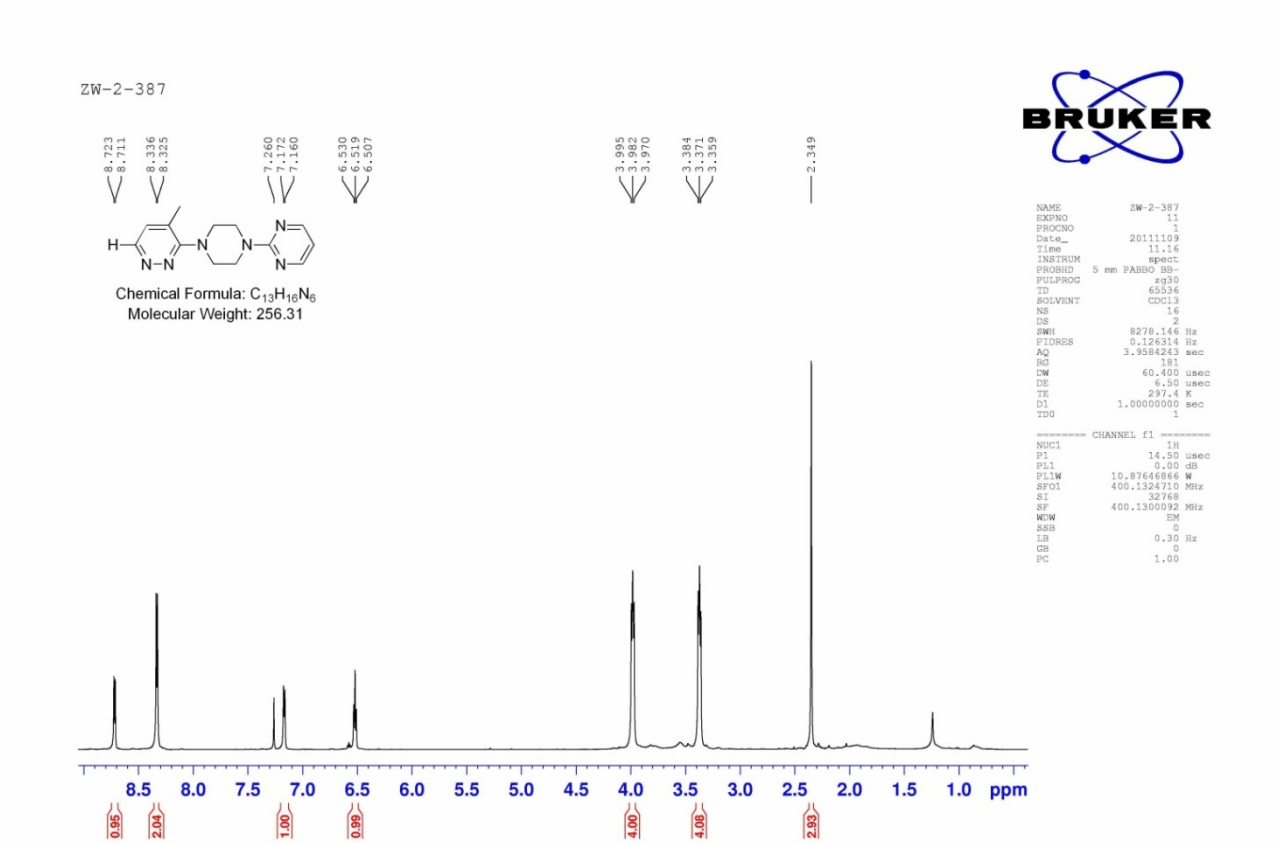


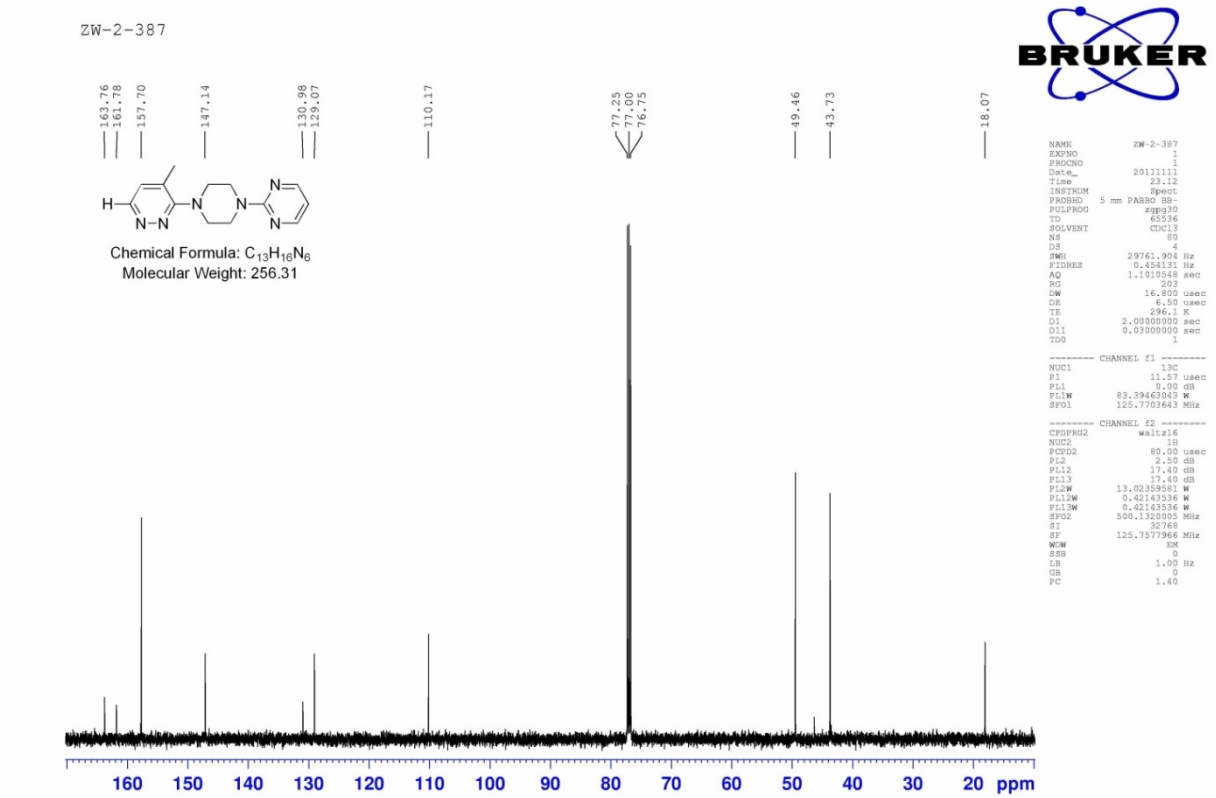
6-chloro-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (4)



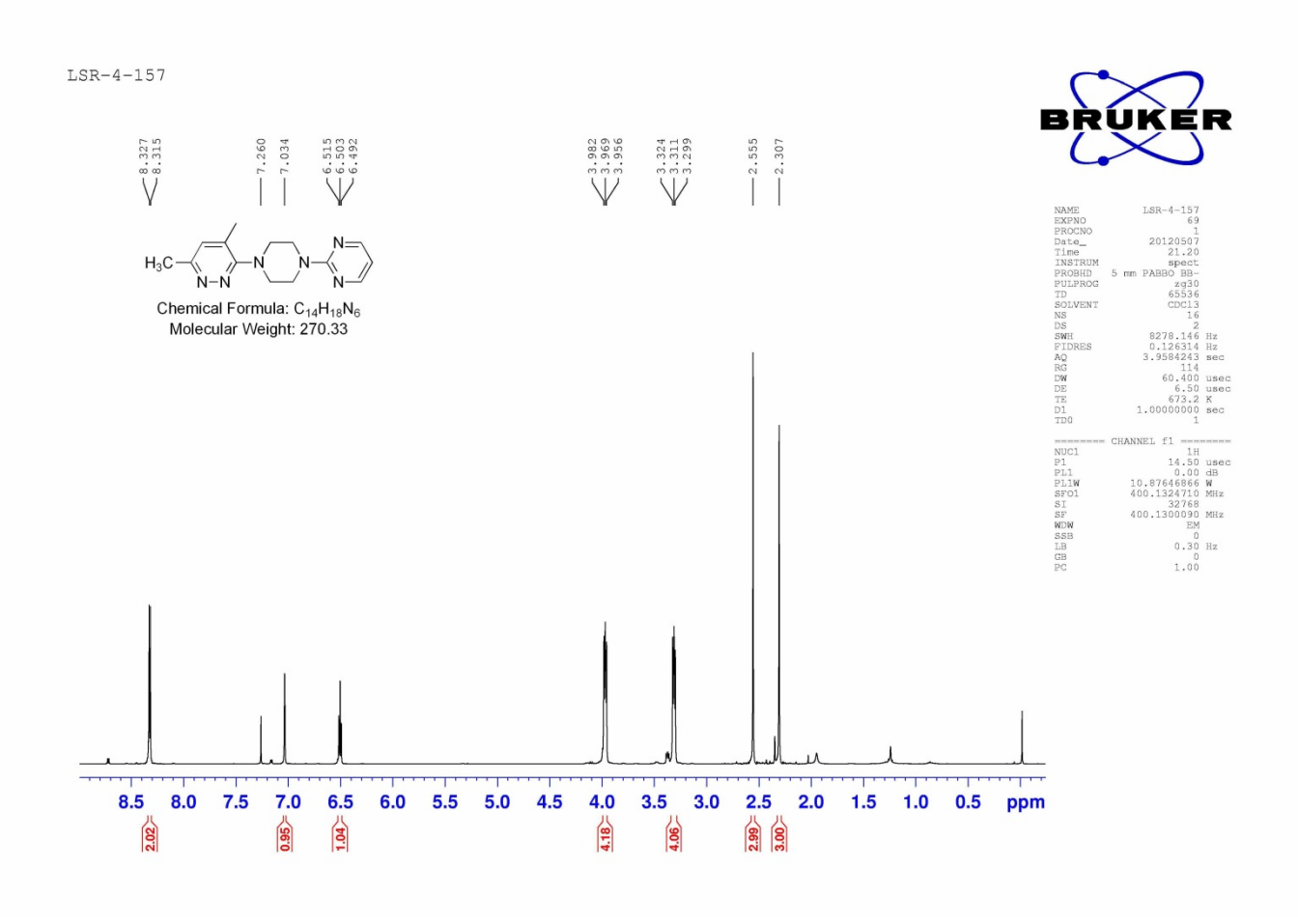


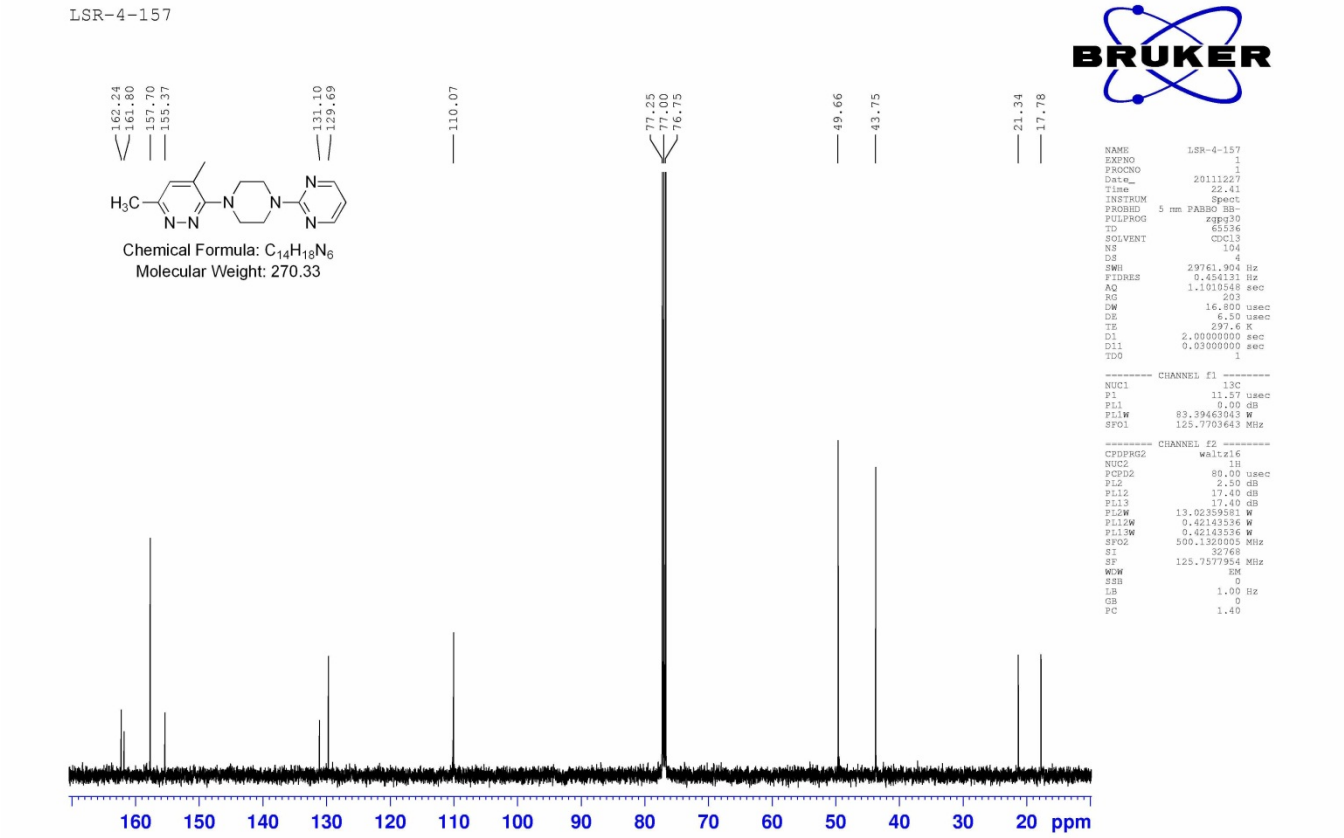
4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (5)



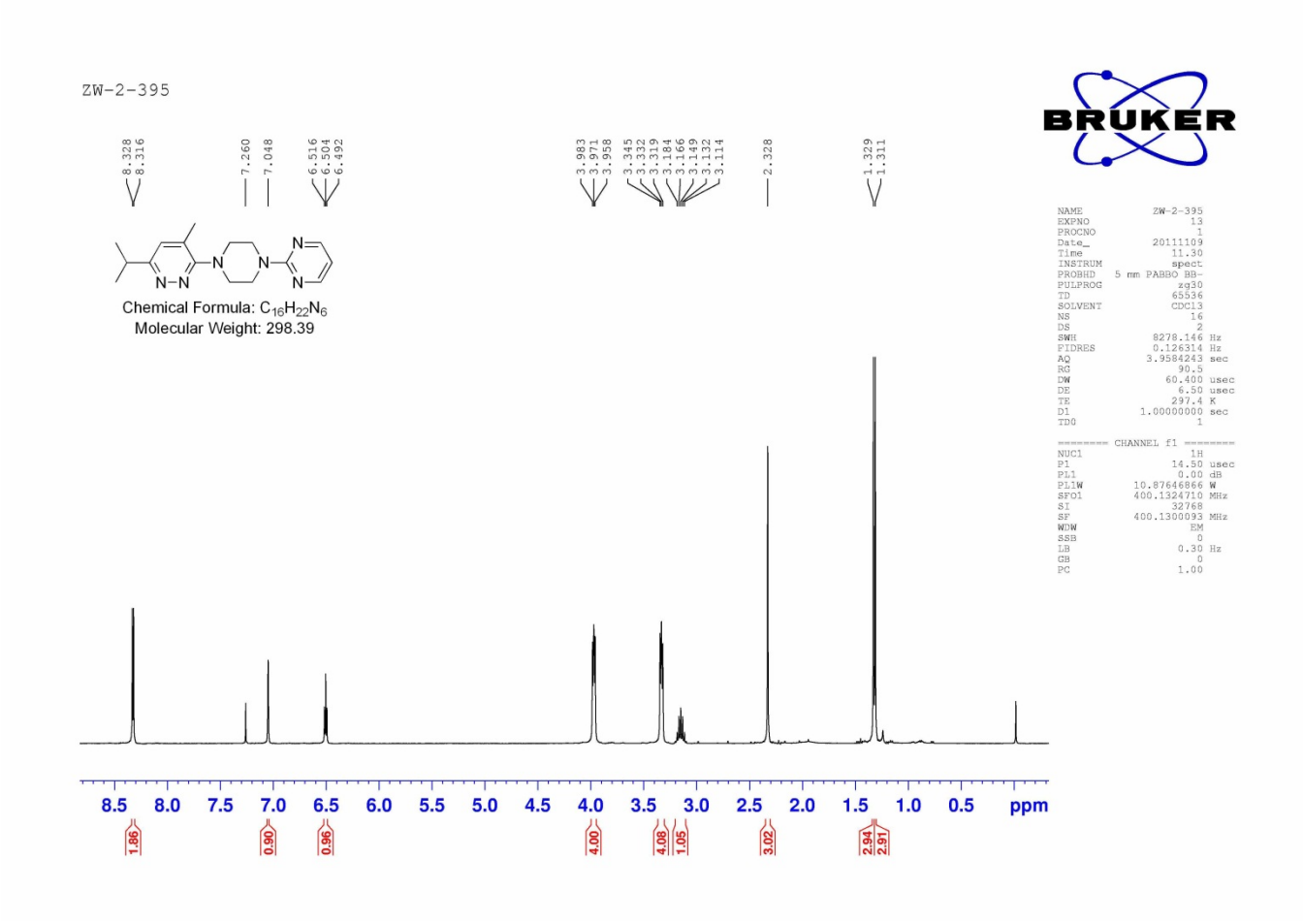


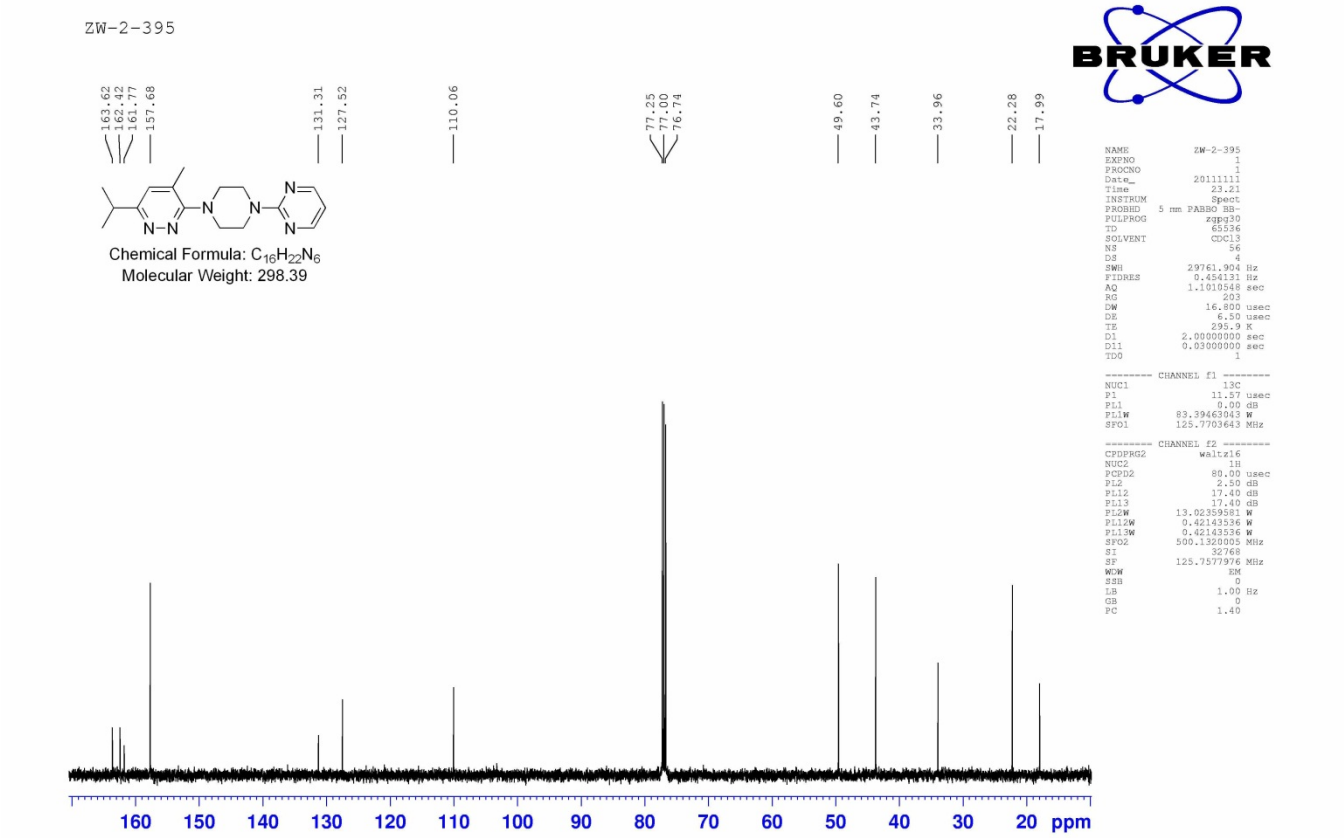
6-dimethyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (6)



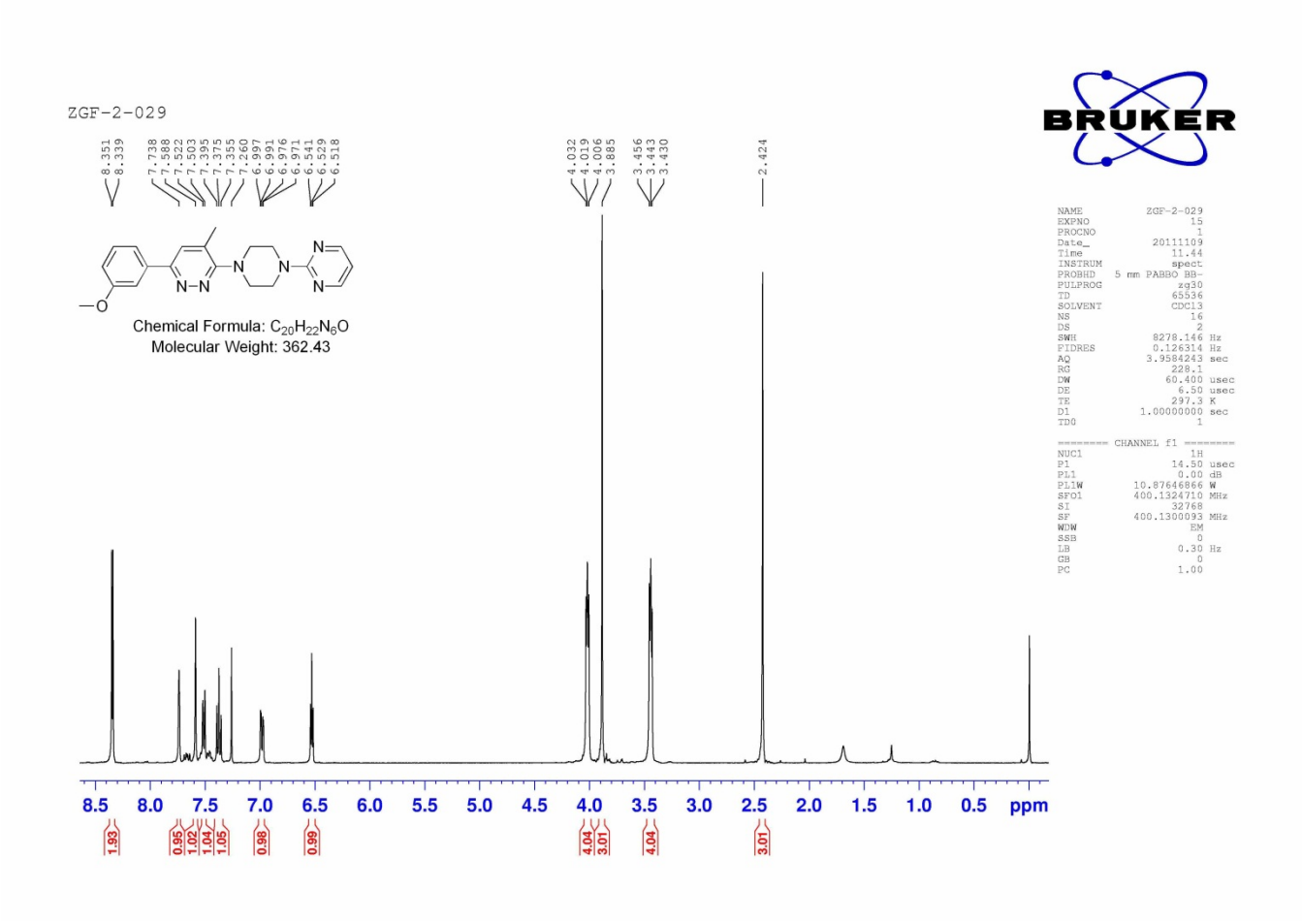


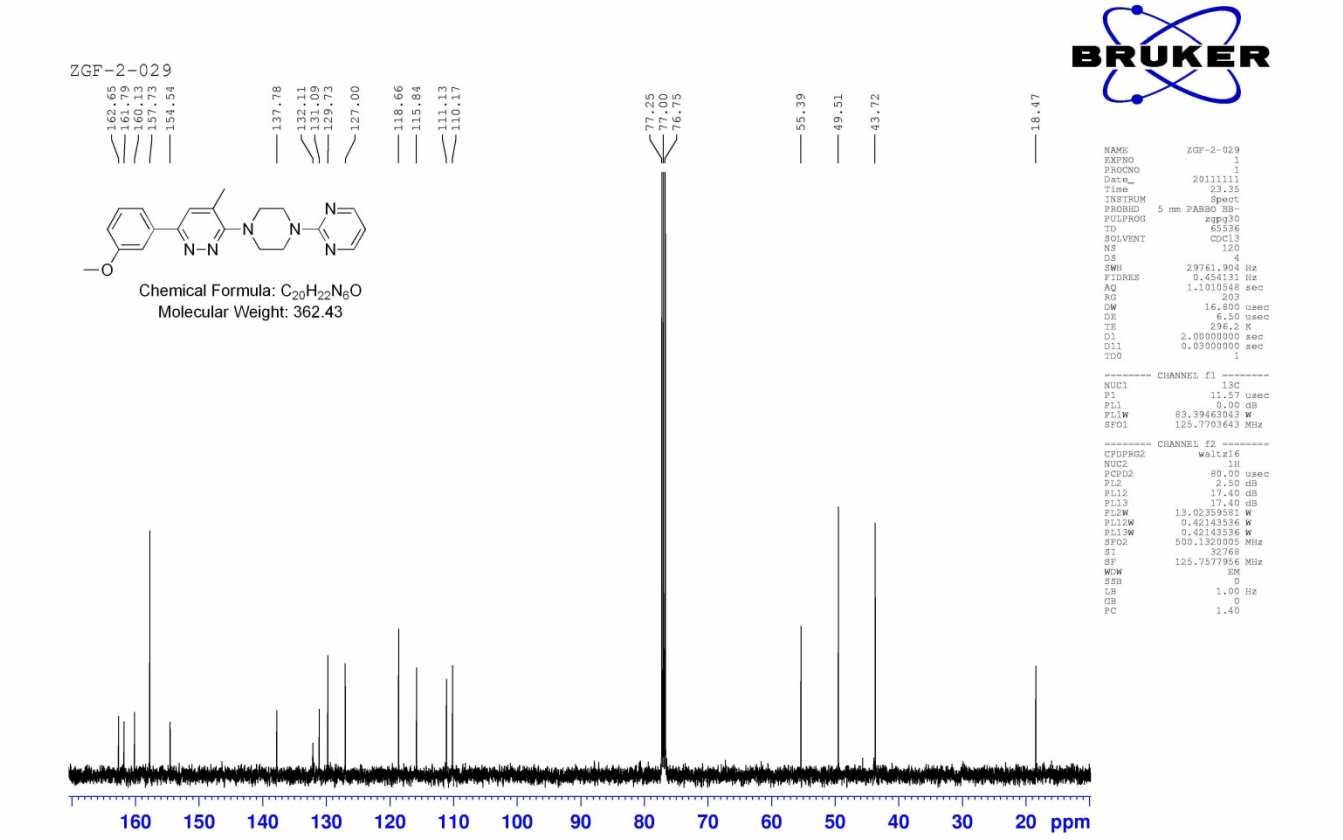
6-isopropyl-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (7)



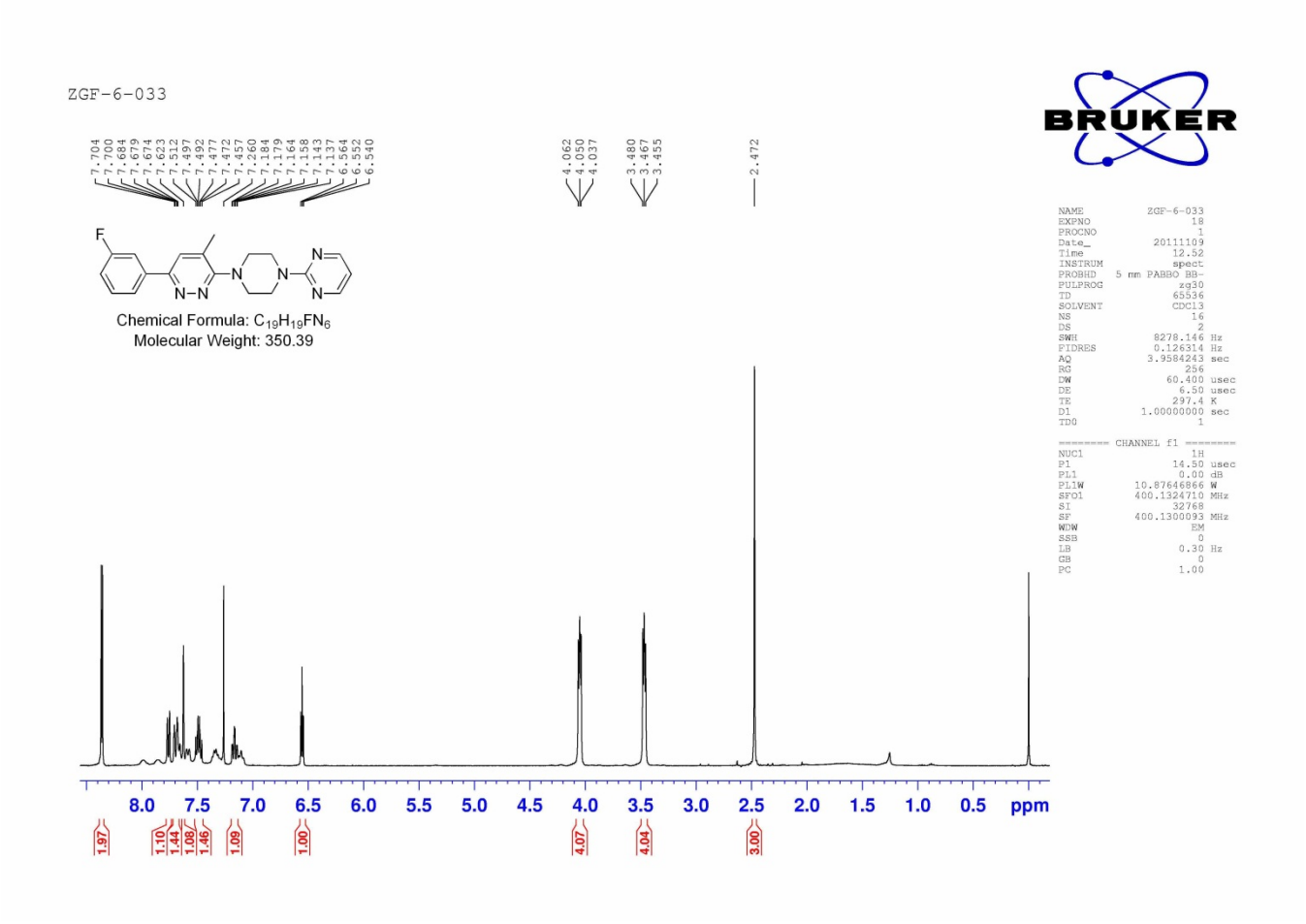


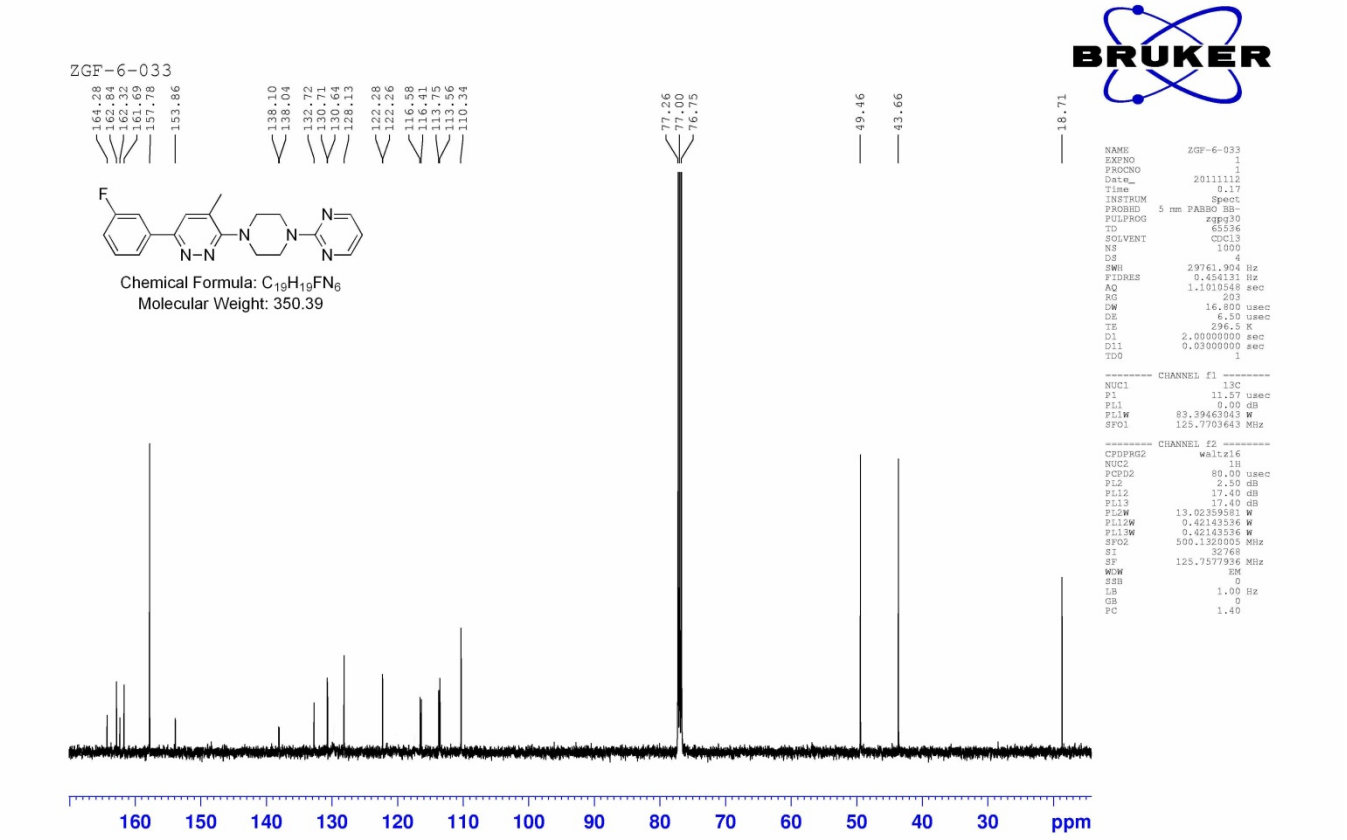
6-(3-methoxyphenyl)-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (8)



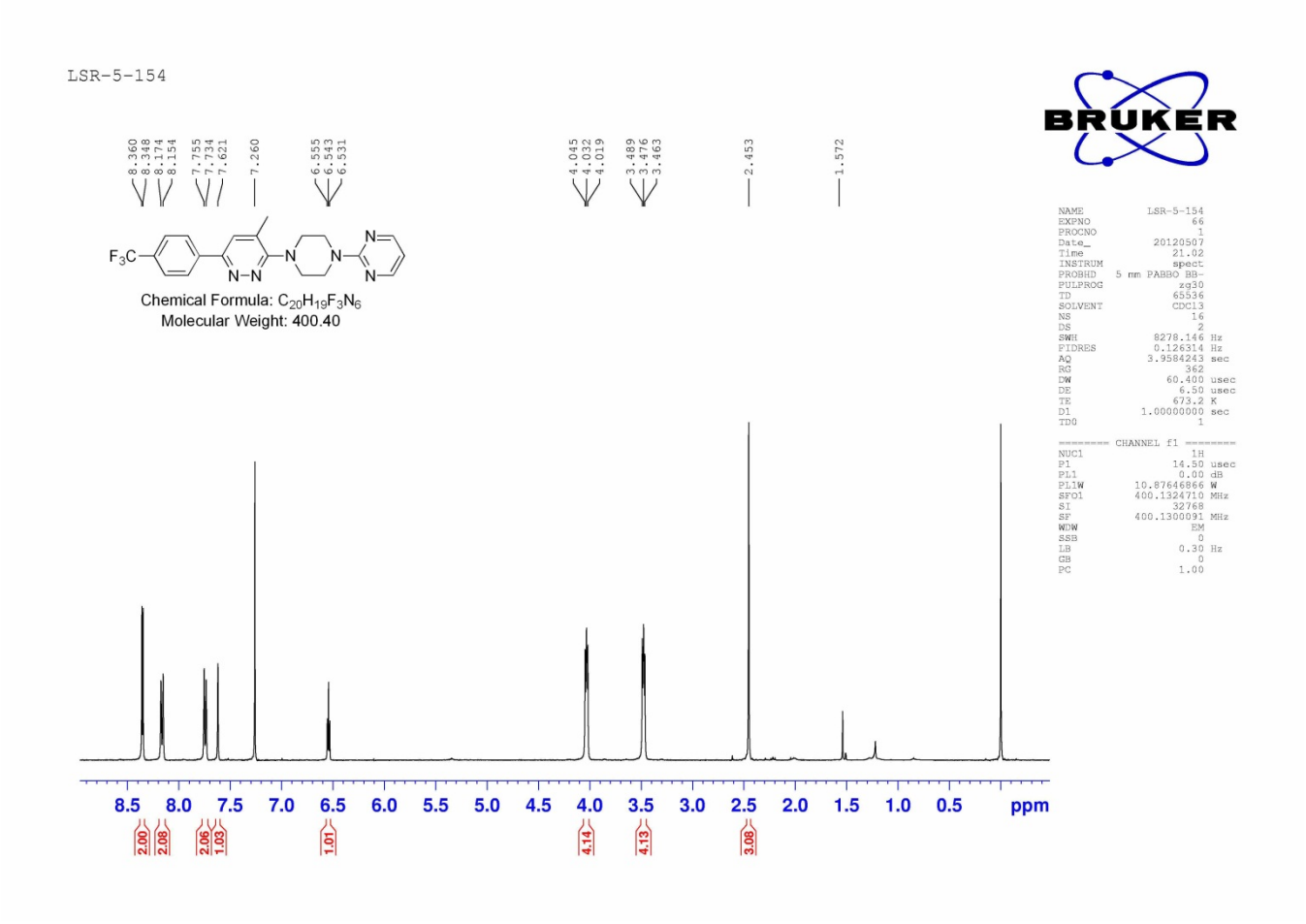


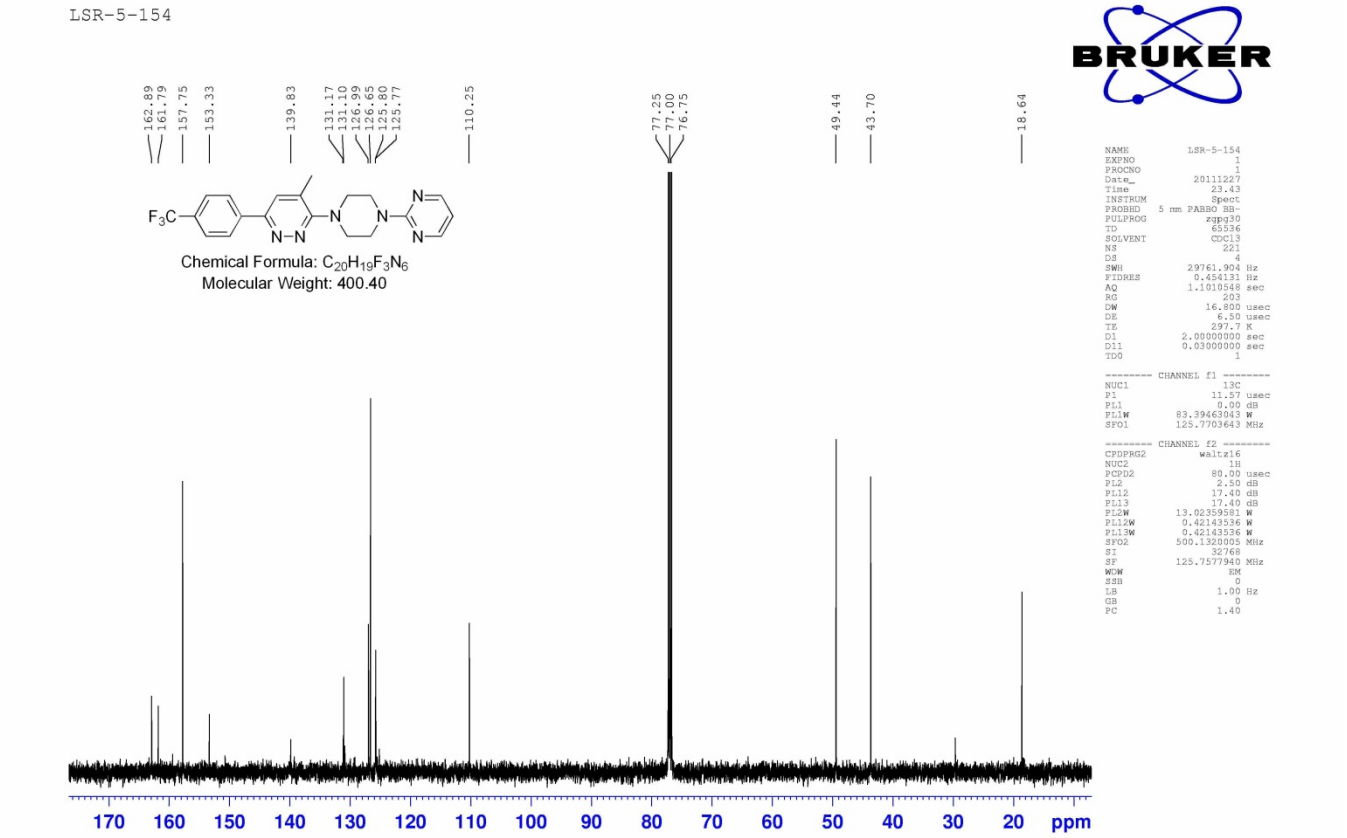
6-(3-fluorophenyl)-4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (9)



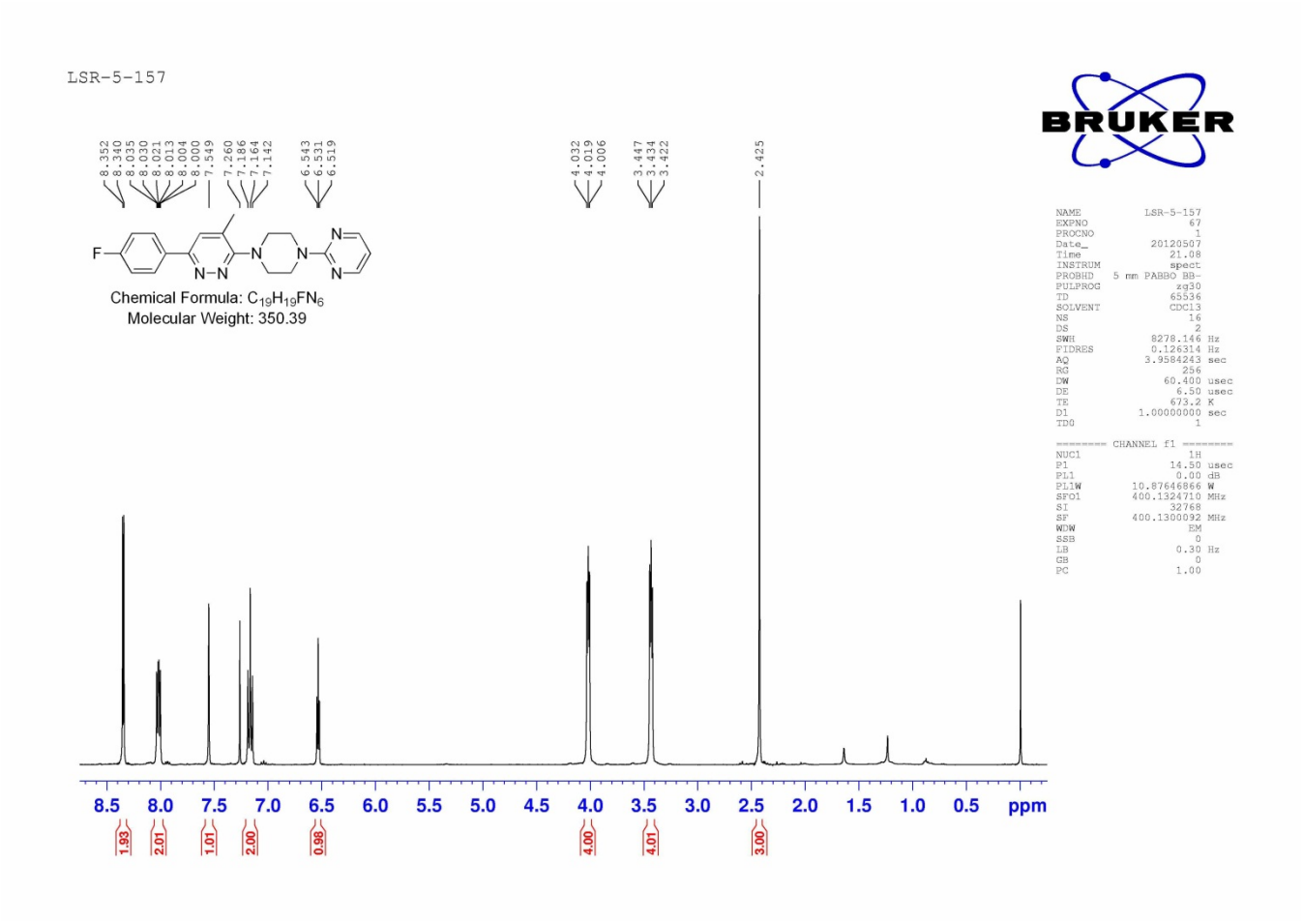


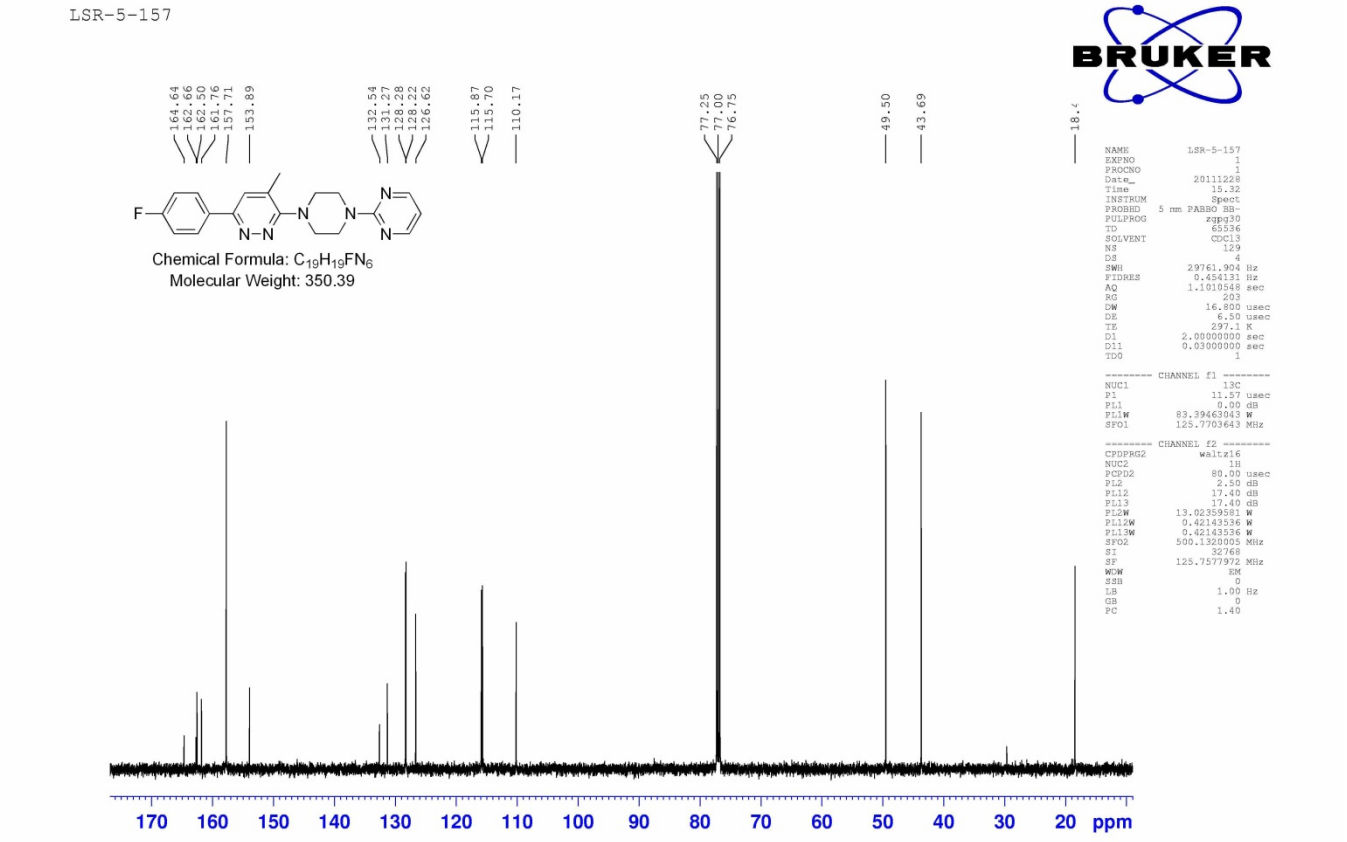
4-methyl-6-(4-(trifluoromethyl)phenyl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (10)



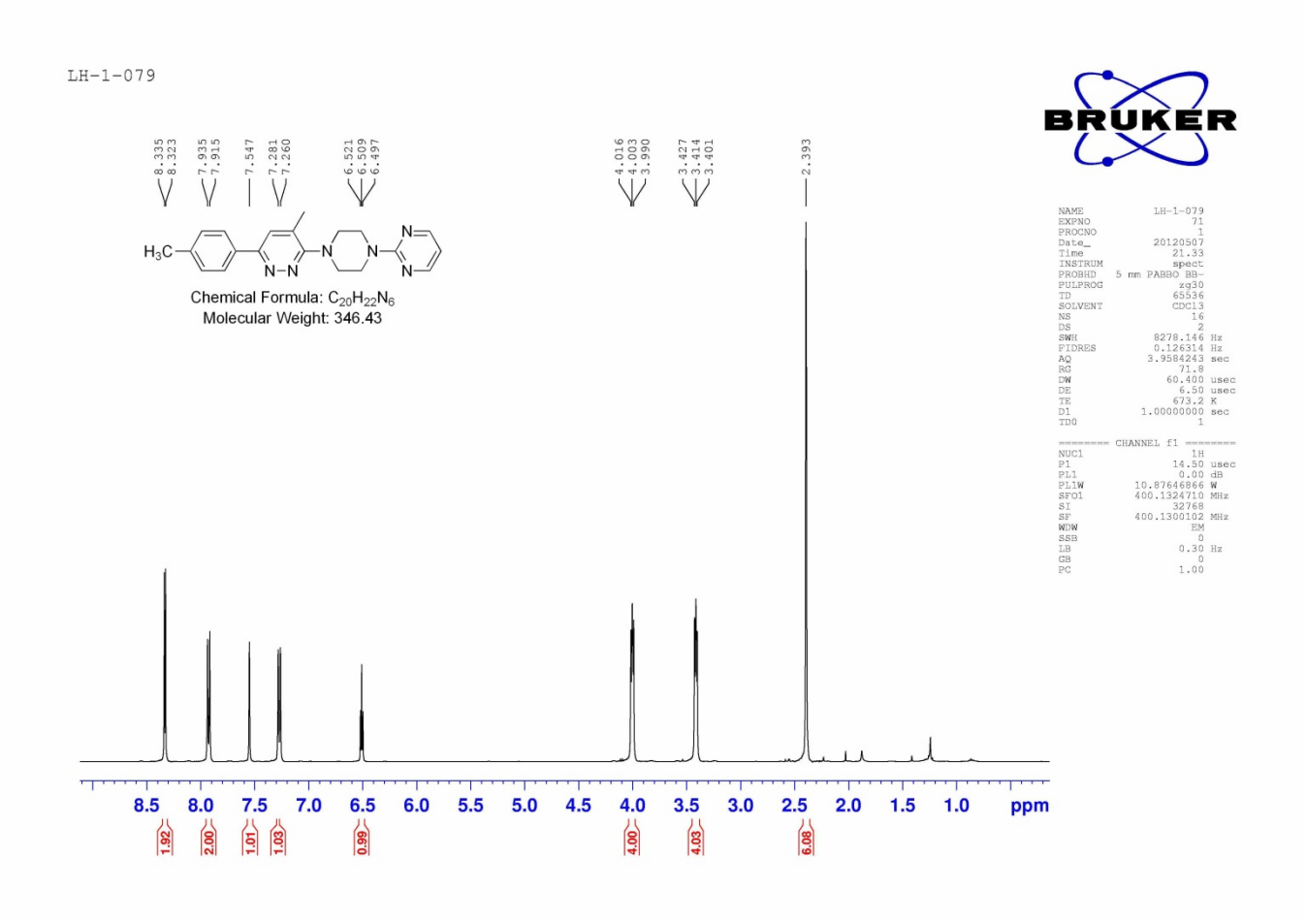


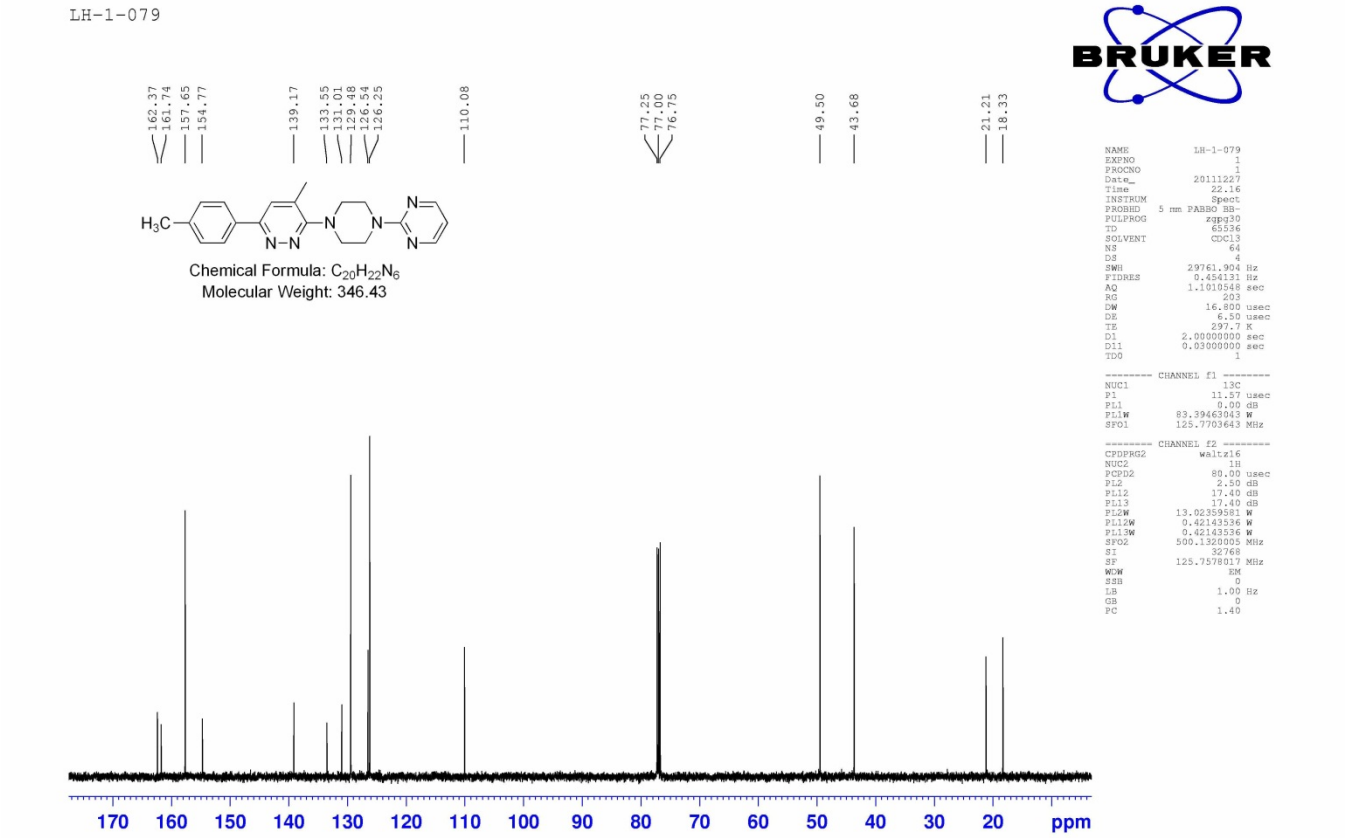
4-methyl-6-(4-fluorophenyl)-3-(4-(pyrimidin-2-yl) piperazin-1-yl)pyridazine (11)



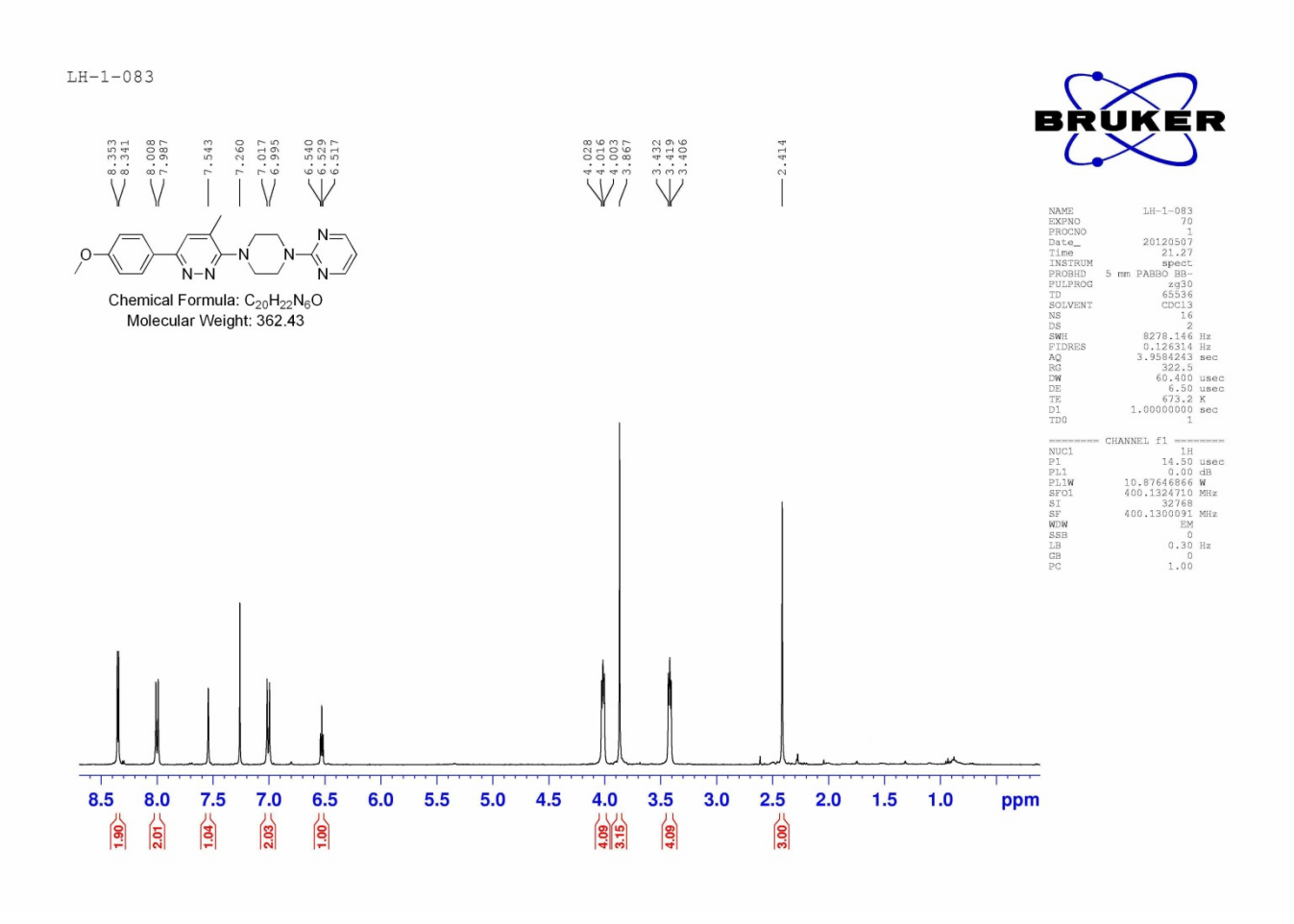


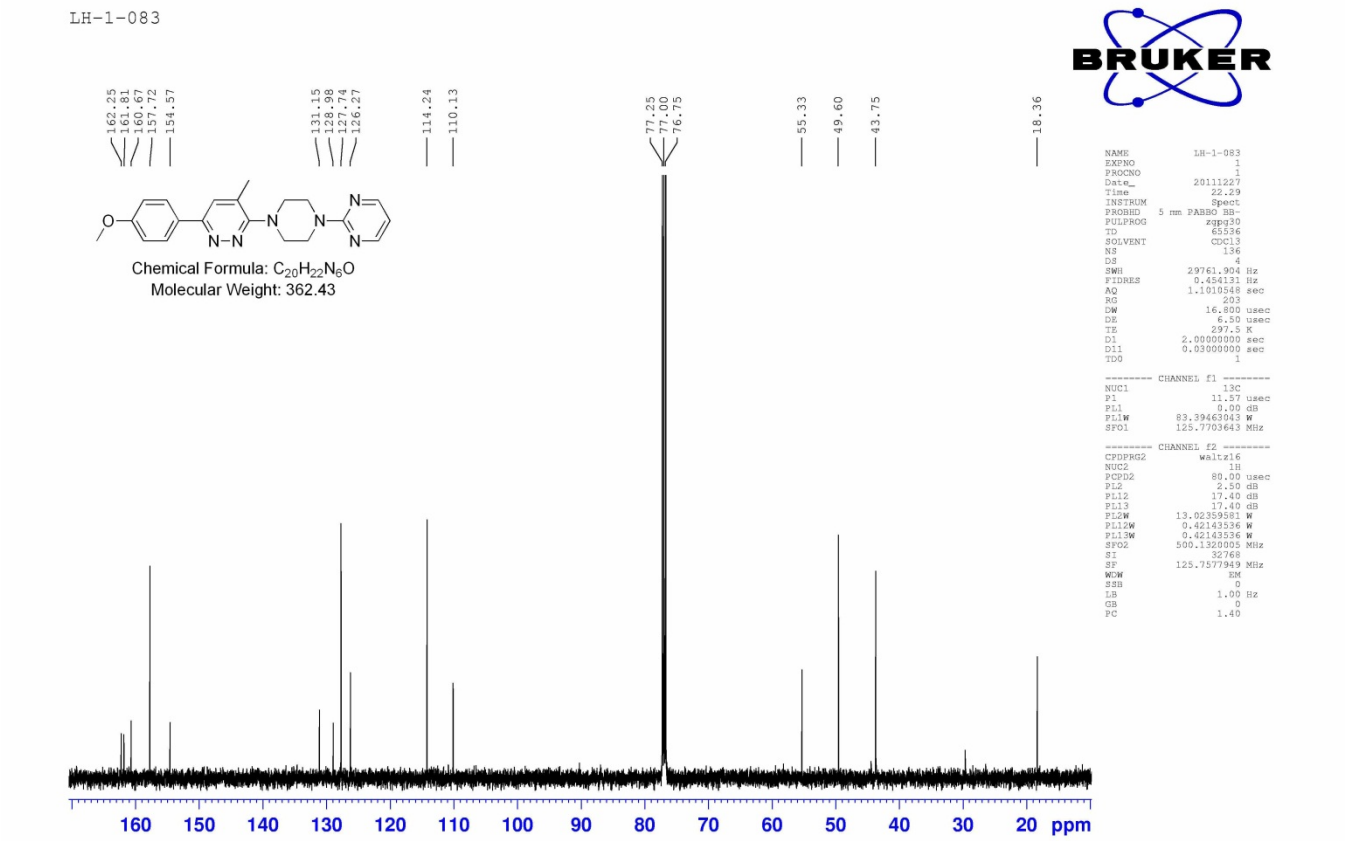
4-methyl-6-(p-tolyl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (12)



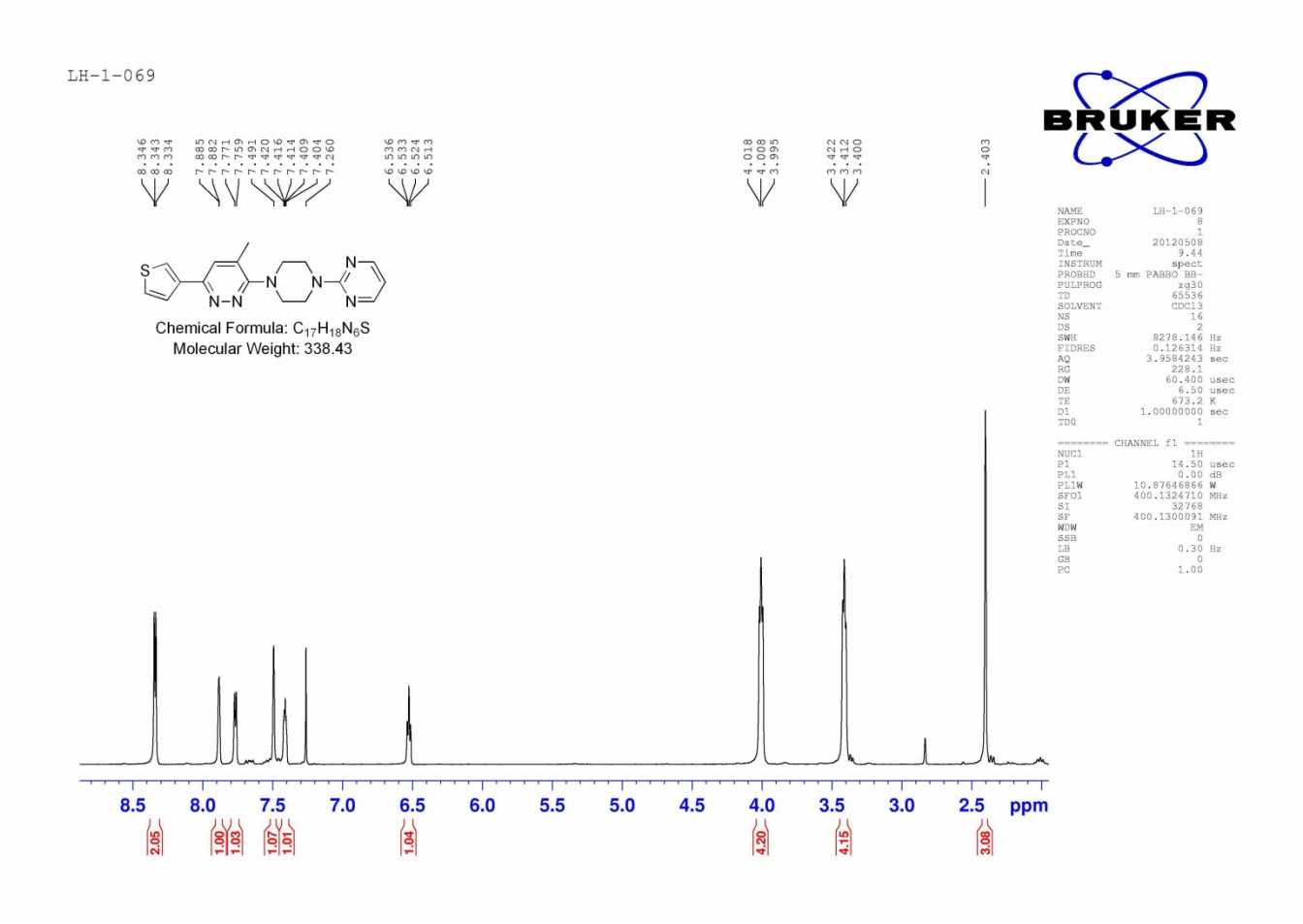


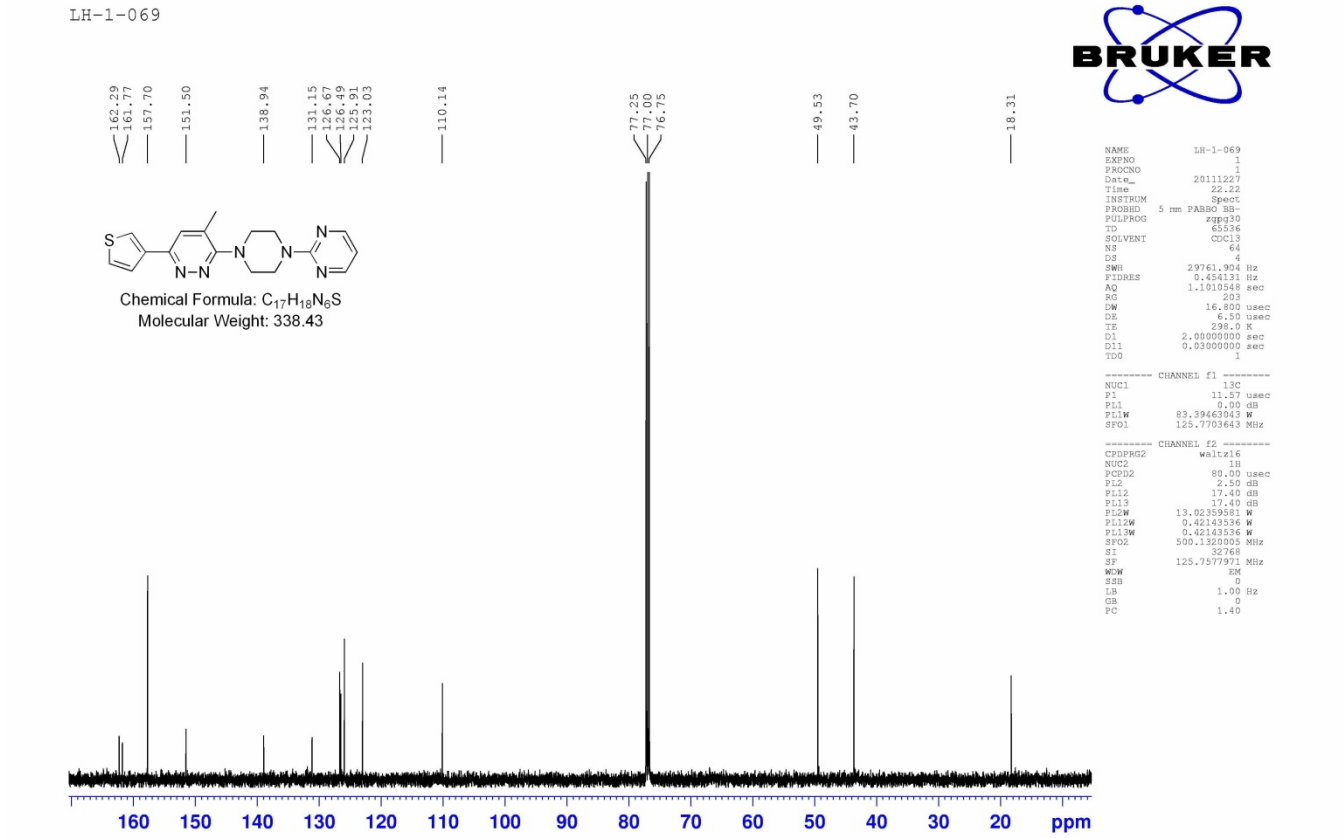
4-methyl-6-(4-methoxyphenyl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (13)



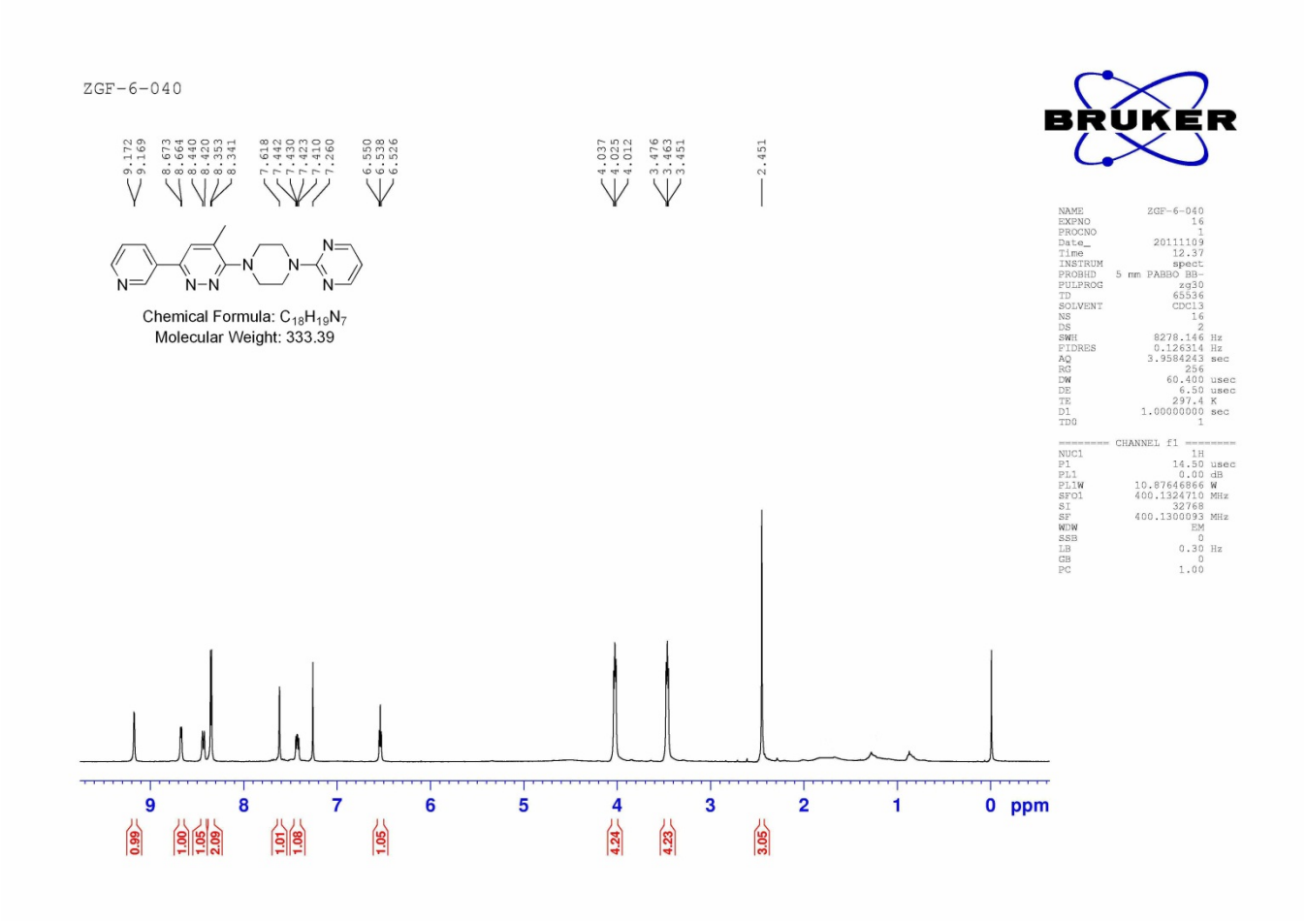


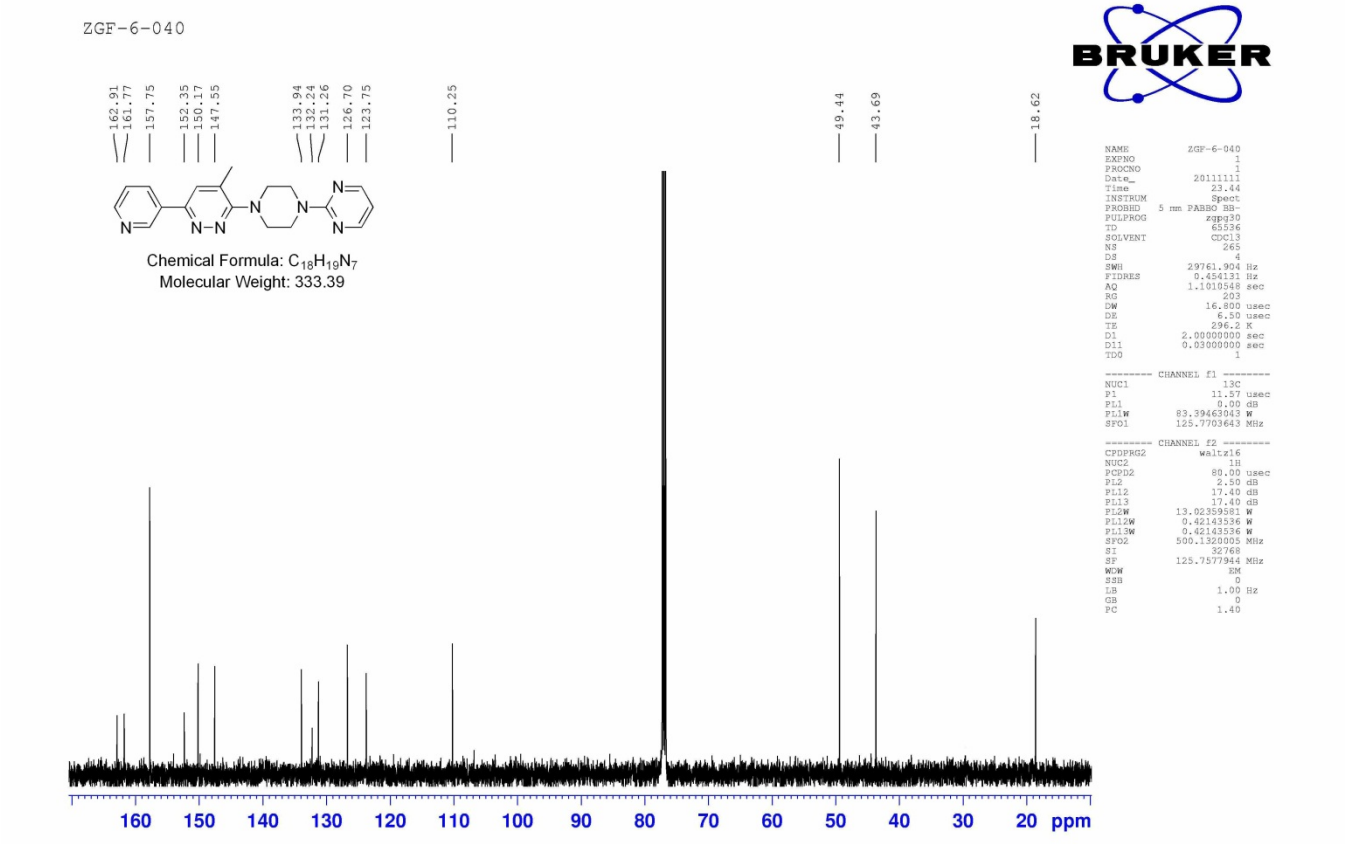
4-methyl-6-(thiophen-3-yl)-3-(4-(pyrimidin-2-yl) piperazin-1-yl)pyridazine (14)



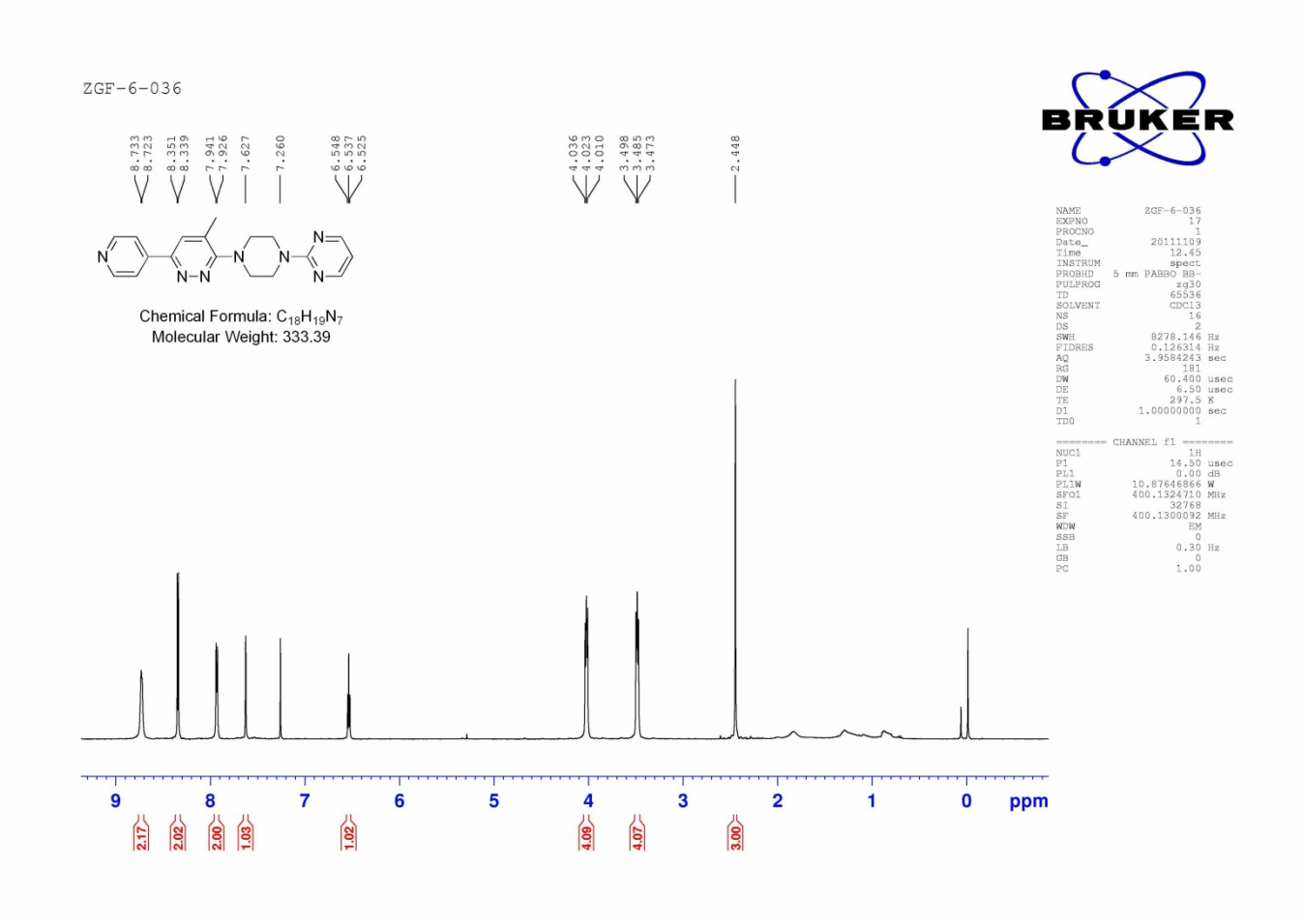


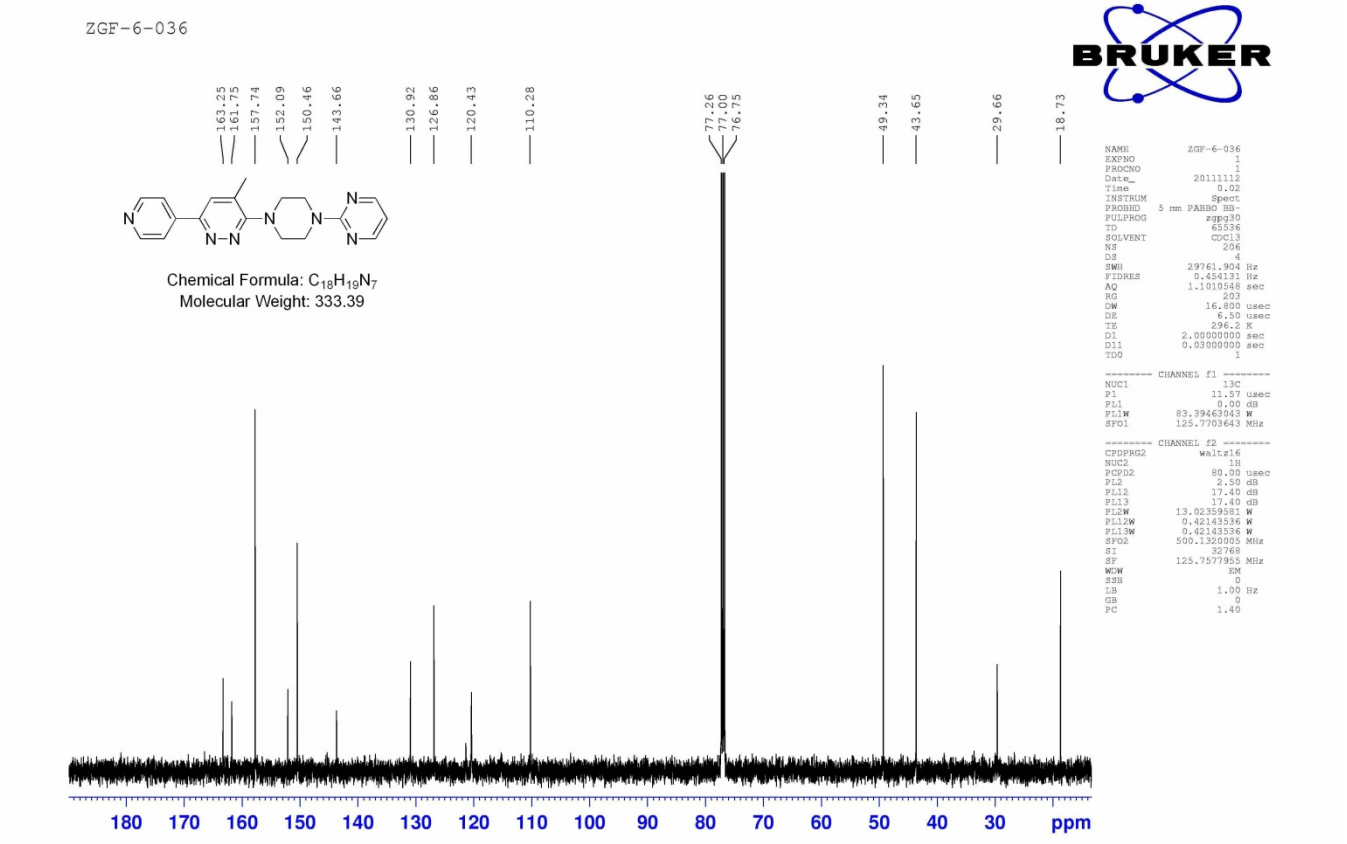
4-methyl-6-(pyridin-3-yl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (15)



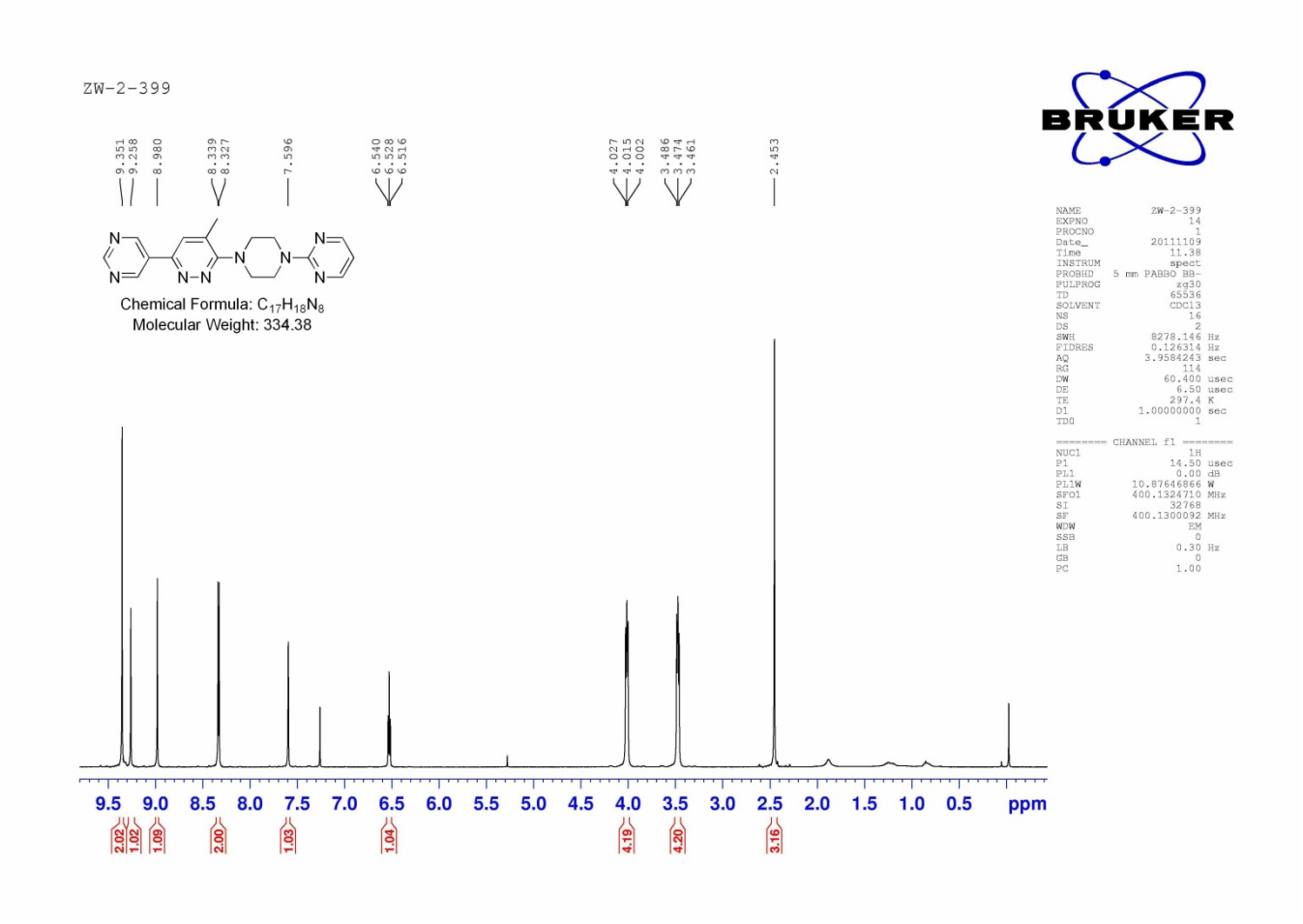


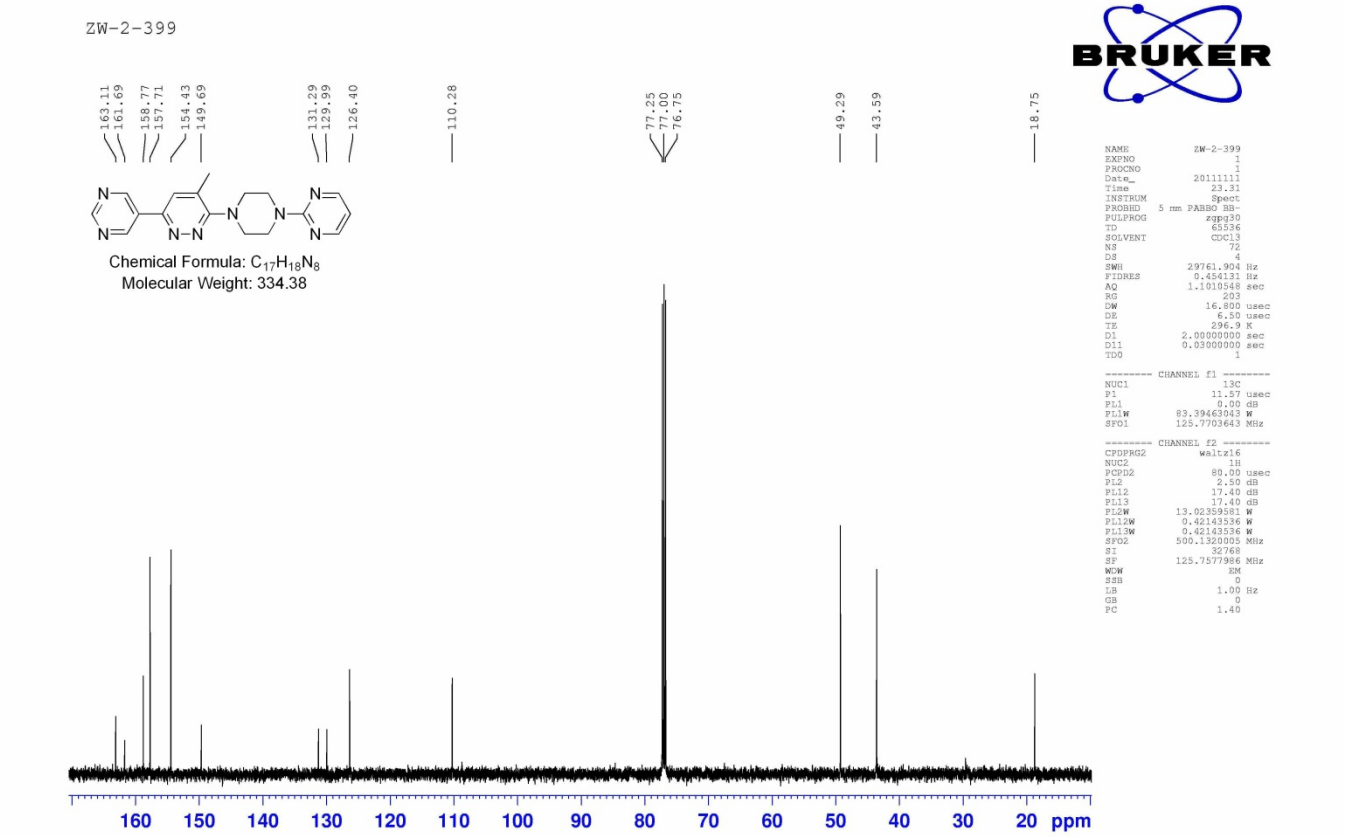
4-methyl-6-(pyridin-4-yl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (16)



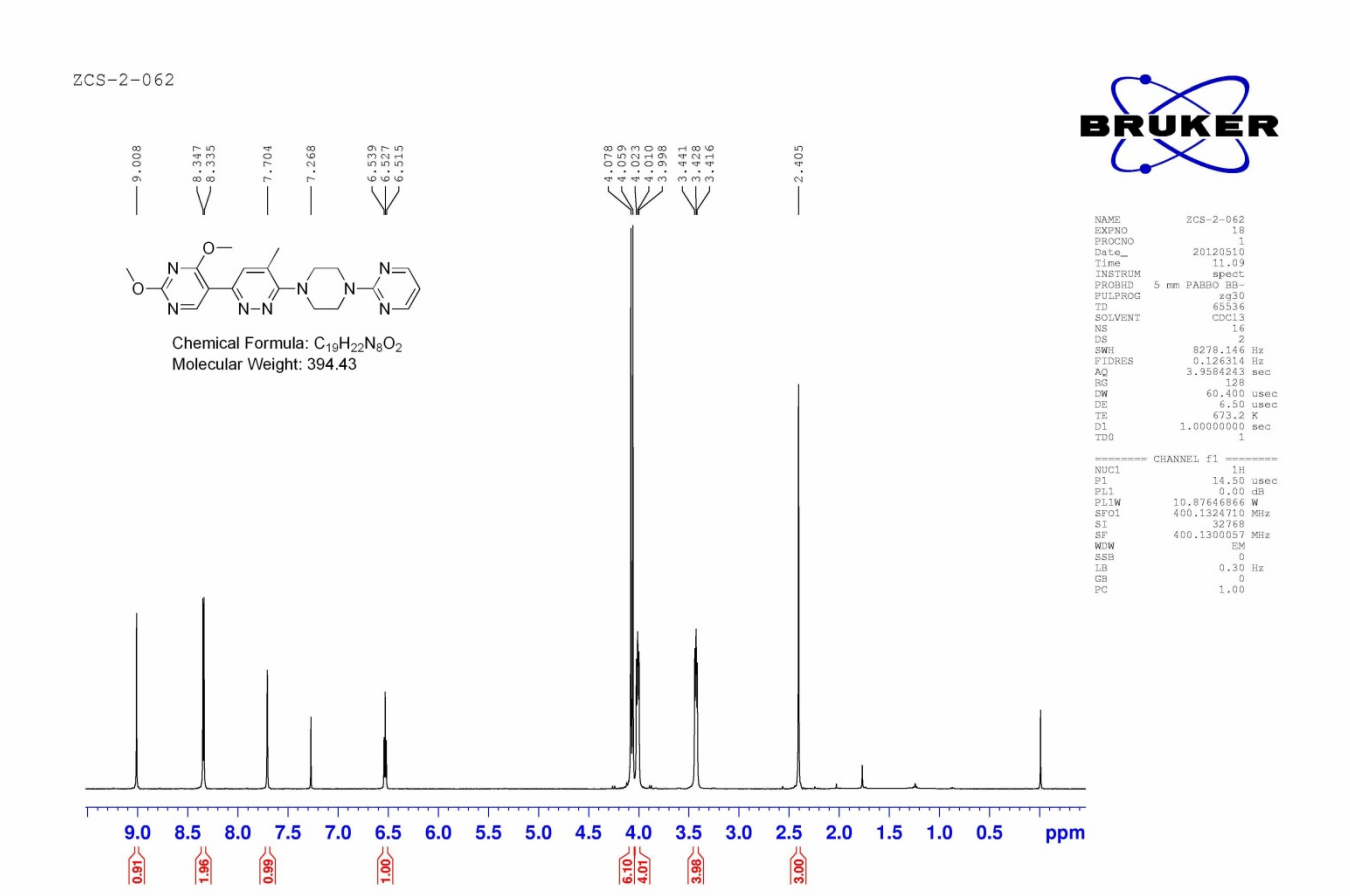


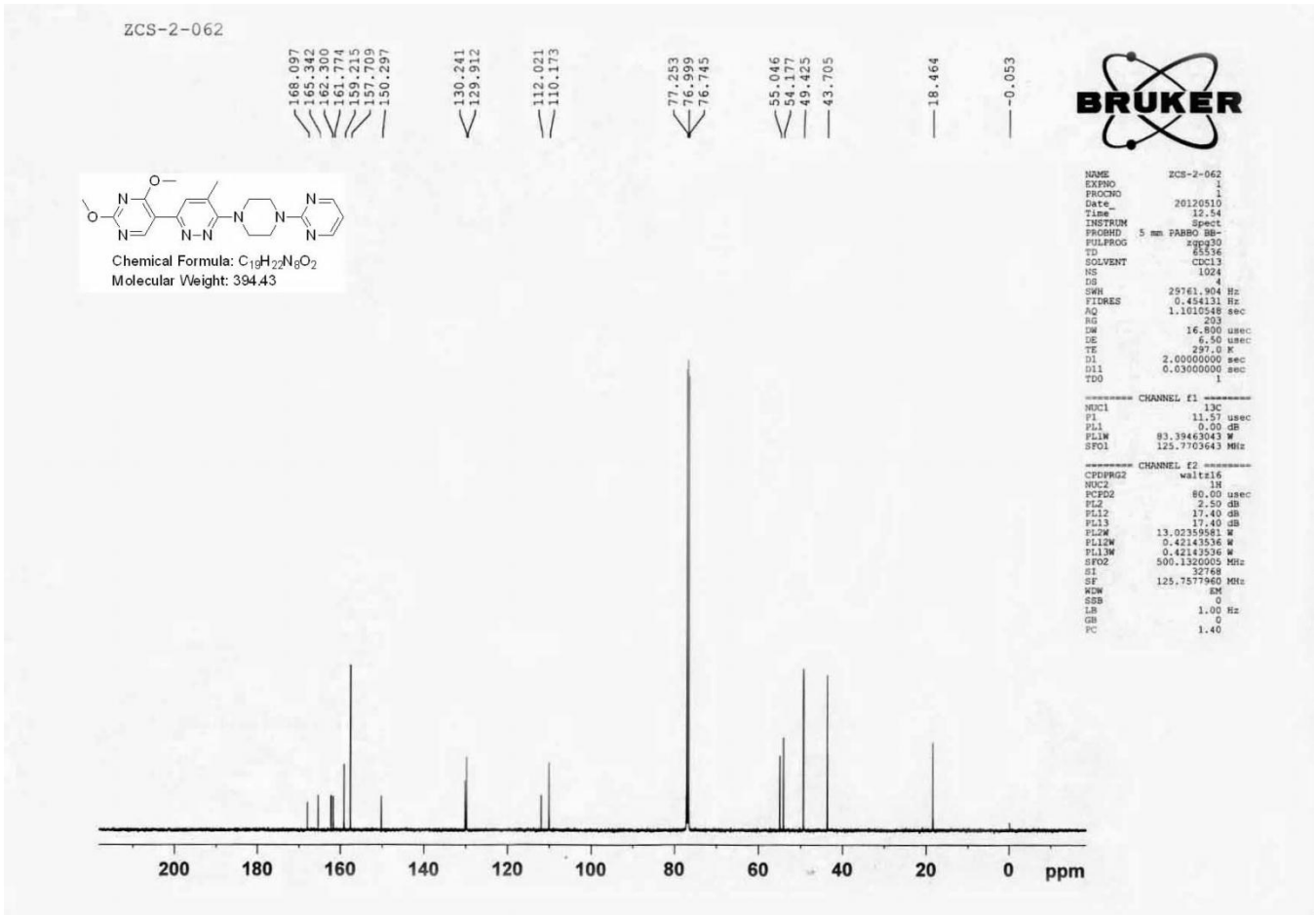
4-methyl-3-(4-(pyrimidin-2-yl)piperazin-1-yl)-6-(pyrimidin-5-yl)pyridazine (17)



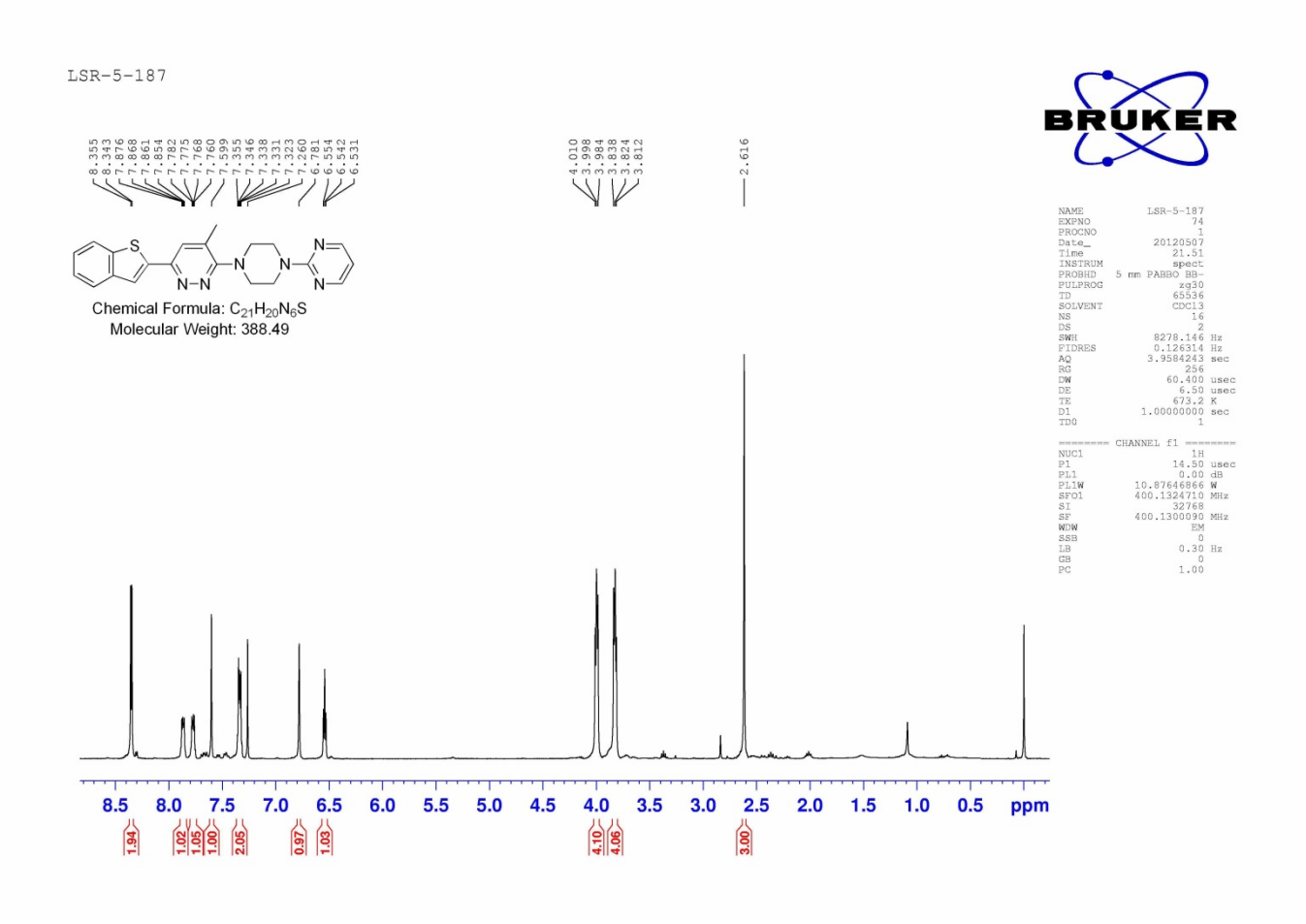


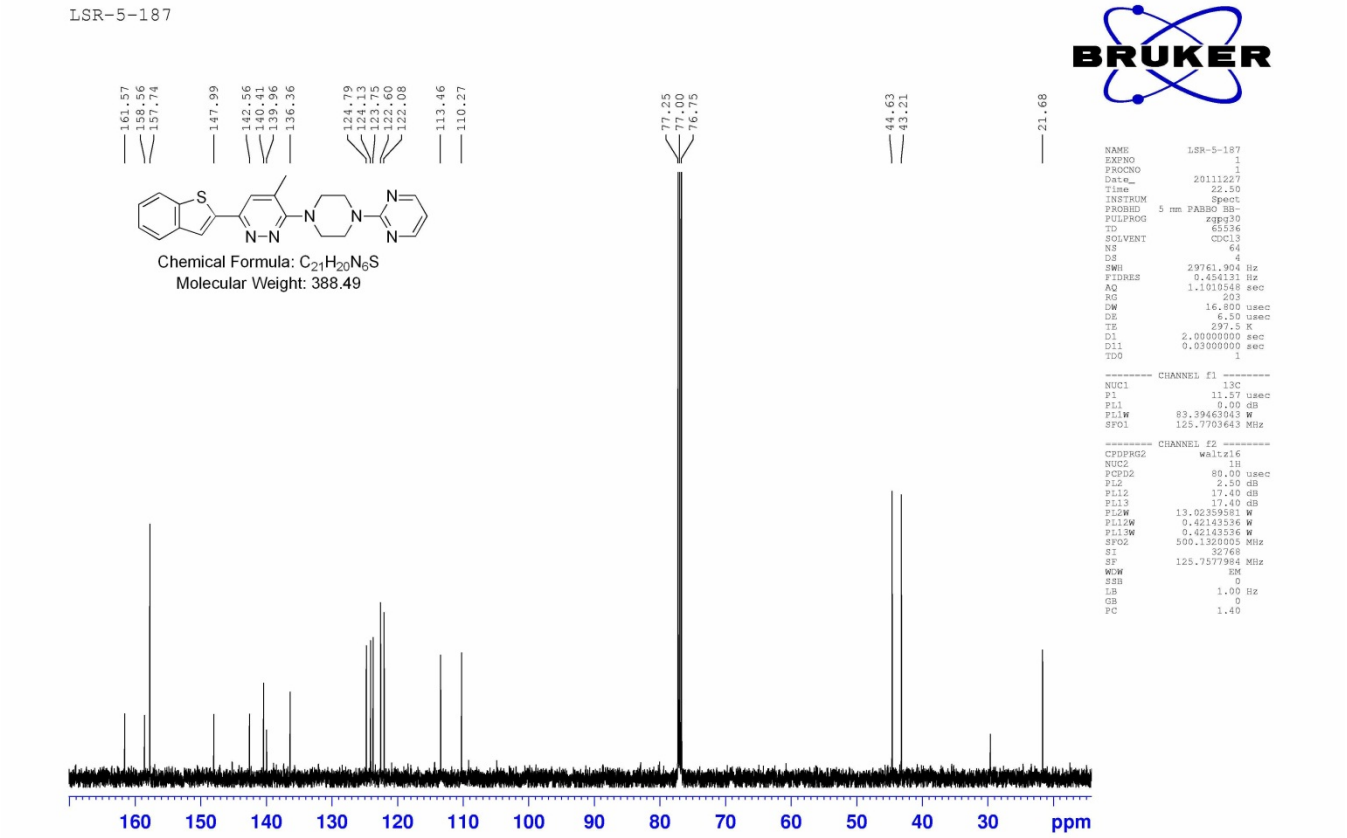
4-methyl-6-(2,4-dimethoxypyrimidin-5-yl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (18)



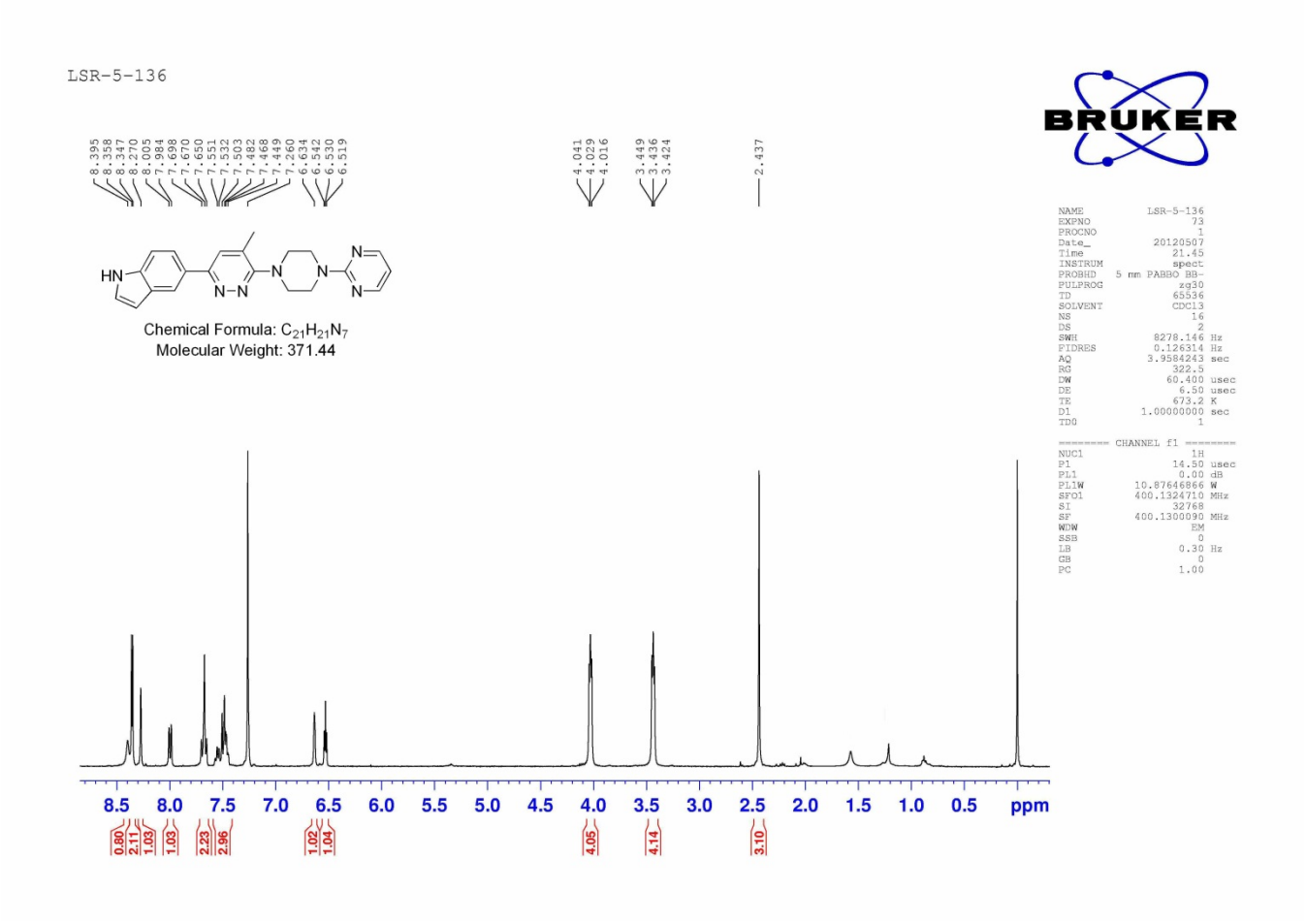


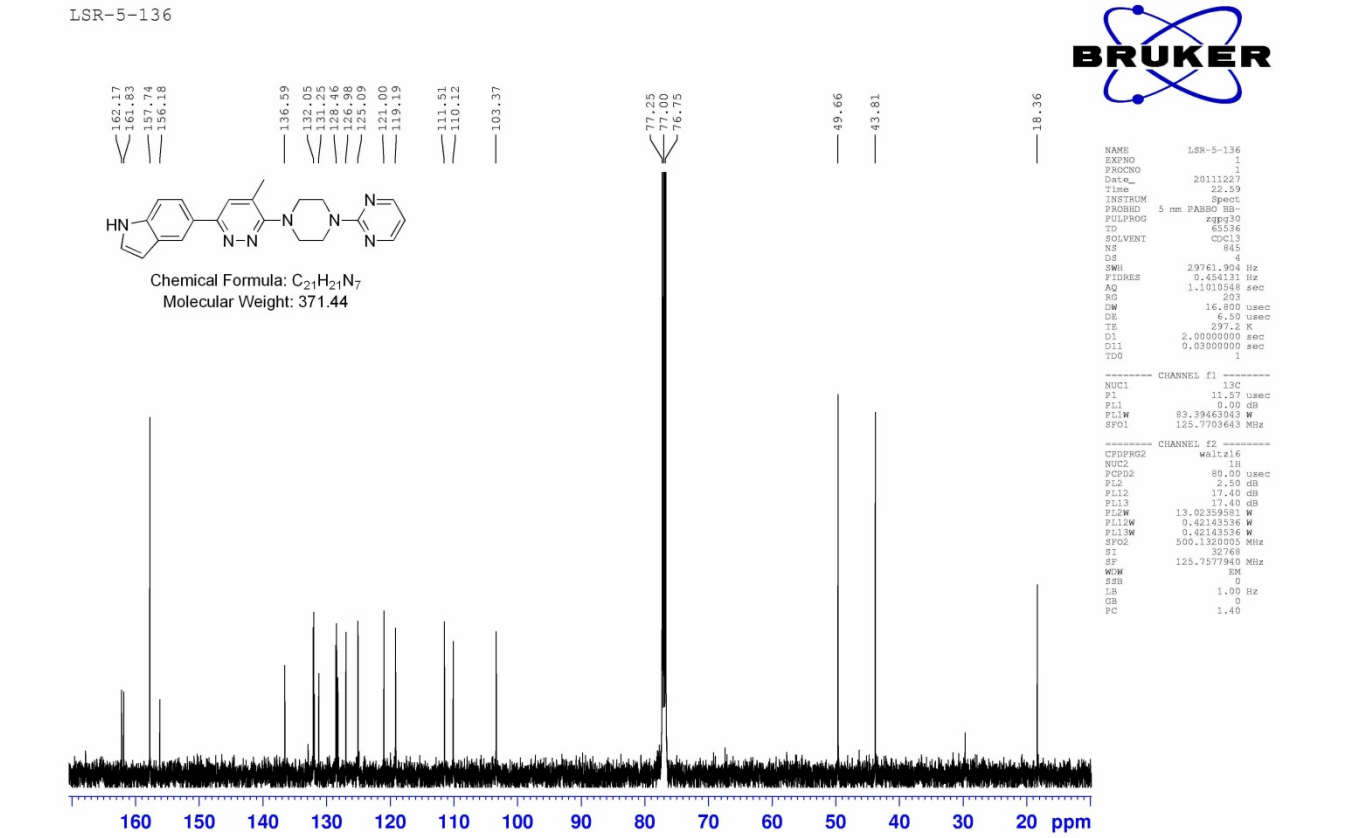
4-methyl-6-(benzothiophen-2-yl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)pyridazine (19)





5-(5-methyl-6-(4-(pyrimidin-2-yl)piperazin-1-yl) pyridazin-3-yl)-1H-indole (20)





4-(5-methyl-6-(4-(pyrimidin-2-yl)piperazin-1-yl) pyridazin-3-yl)quinolone (21)