Discovery of orally bioavailable purine-based inhibitors of the low molecular weight protein tyrosine phosphatase (LMPTP)

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Supporting Information

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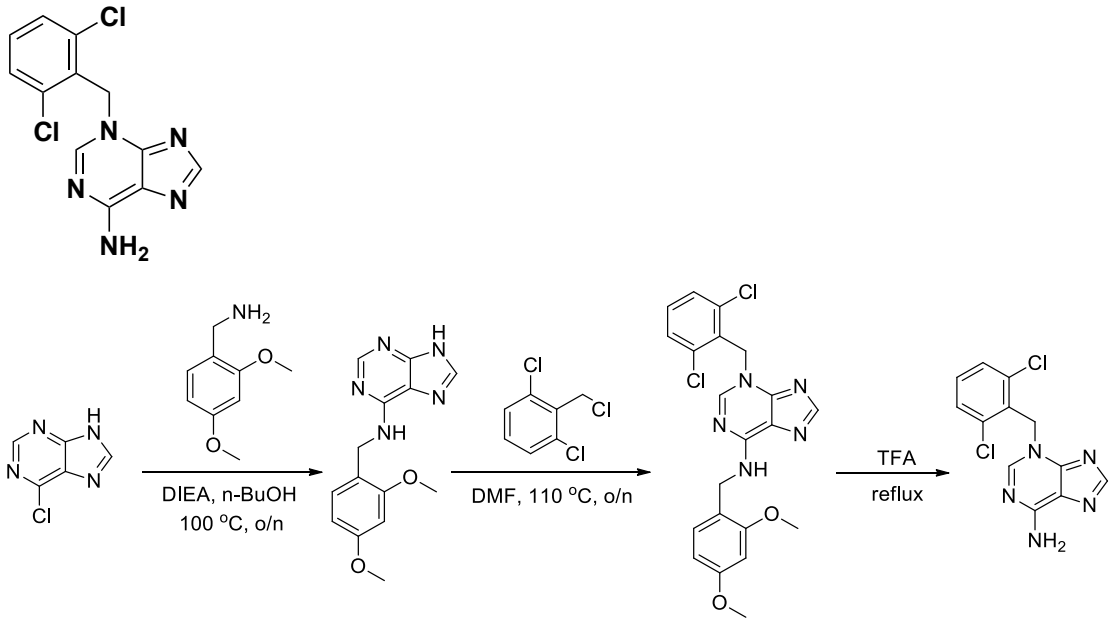
Chemistry

All reactions involving air and moisture-sensitive reagents and solvents were performed under a nitrogen atmosphere using standard chemical techniques. Anhydrous solvents were purchased and freshly used from Sigma-Aldrich or EMD Biosciences. All organic reagents were used as purchased. All starting materials and intermediates were purchased from Sigma Aldrich (St. Louis, MO) unless otherwise indicated. Analytical thin-layer chromatography was performed on Partisil K6F silica gel 60 Å, 250 μm. Microwave-assisted reactions were performed using a CEM Discovery system. ^{1}H and ^{13}C chemical shifts are reported in δ values in ppm in the corresponding solvent. All solvents used for chromatography on the synthetic materials were Fisher Scientific HPLC grade, and the water was Millipore Milli-Q PP filtered. LCMS analysis of synthetic materials was completed on a Waters Autopurification system, which consists of a 2767 sample manager, a 2545 binary gradient module, a system fluidics organizer, a 2489 UV/vis detector, and a 3100 mass detector, all controlled with MassLynx software. A Sunfire Analytical C18 5 μm column (4.6 × 50 mm) and stepwise gradient {10% [(MeCN + 0.1% TFA) in (water + 0.1% TFA)] to 98% [(MeCN + 0.1% TFA) in (water + 0.1% TFA)] for 6.5 min.} was used for analytical LCMS of final compounds. The final compounds were purified by silica gel flash chromatography with ethyl acetate/hexanes as the eluant. All NMR spectra for the synthetic materials were recorded on a Bruker Avance II

300, 400 or DRX-500 MHz instrument. The MestReNova 7 program was used to process and interpret NMR spectra. High Resolution Mass Spectrometry (HRMS) spectra were carried out on an Agilent 6224A Accurate-Mass Time-of-Flight (TOF) LC/MS system with electron spray ionization (ESI). All synthesized final compounds were determined to be ≥ 95% unless otherwise noted (by UV at 254 nm) pure by HPLC. Compound identity was verified by ^{1}H NMR and HRMS, and additionally by ^{13}C NMR for selected analogs.

3: 3-(2,6-Dichloro-benzyl)-3H-purin-6-ylamine



Step 1

To a mixture of 6-chloro-3H-purine (10.0 g, 64.7 mmol) in n-BuOH (50 mL) was added DIEA (16.7 g, 129.4 mmol) and 2,4-dimethoxy-benzylamine (13.0 g, 77.6 mmol) at room temperature. The reaction mixture was then heated to 100 °C and stirred overnight. The reaction mixture was cooled to room temperature and concentrated. The residue was washed with ethyl acetate (30 mL x3) and dried *in vacuum* to give (2,4-dimethoxy-benzyl)-(3H-purin-6-yl)-amine (17.7 g, yield: 92.9 %) as a white solid. ESI: calculated for $C_{14}H_{15}N_5O_5$ =285.12. Observed m/z $[M+H]^+$= 286.3.

Step 2

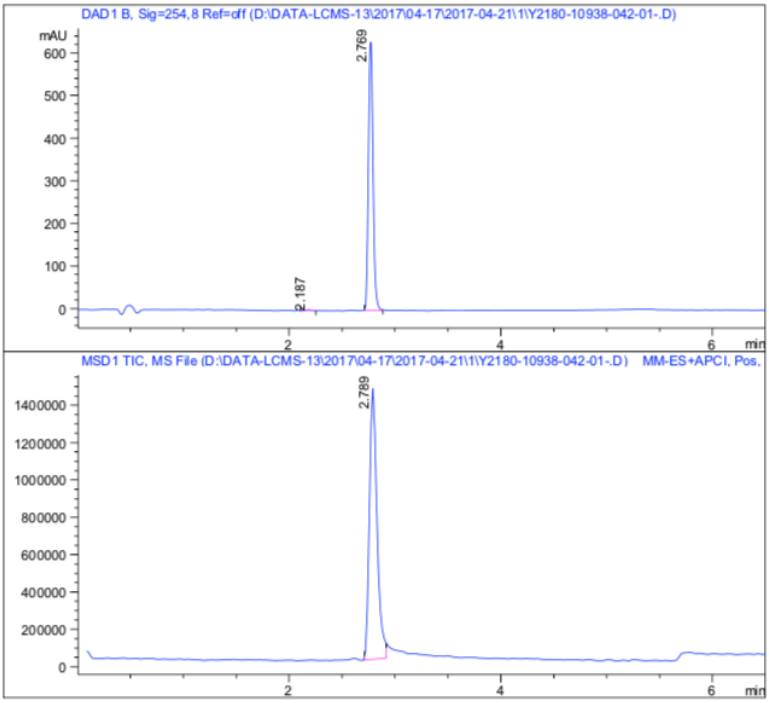
To a mixture of (2,4-dimethoxy-benzyl)-(3H-purin-6-yl)-amine (18.0 g, 63.0 mmol) in DMF (50 mL) was added 1,3-dichloro-2-chloromethyl-benzene (12.2 g, 63.0 mmol) at room temperature. The reaction mixture was then heated to 110 °C and stirred overnight. The reaction mixture was cooled to room temperature and filtered. The filter cake was washed with MeOH (10 mL x3) and dried under vacuum to give [3-(2,6-dichloro-benzyl)-3H-purin-6-yl]-(2,4-dimethoxy-benzyl)-amine (23.4 g, yield: 84.0 %) as a white solid. ESI: calculated for $C_{21}H_{19}Cl_2N_5O_2$ =443.09. Observed m/z $[M+H]^+$= 444.4.

Step 3

A mixture of [3-(2,6-dichloro-benzyl)-3H-purin-6-yl]-(2,4-dimethoxy-benzyl)-amine (540 mg, 1.22 mmol) in TFA (10 mL) was stirred at reflux (80 °C) for 8 hr. Solvent was removed and the residue was diluted with aqueous $NaHCO_3$ (30 mL) and DCM (30 mL), stirred for 10 min at r. t. and filtered to give 3-(2,6-dichloro-benzyl)- 3H-purin-6-ylamine (180 mg, yield: 67.9 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6): δ 8.11 (s, 1H), 7.89 -7.93 (b, 2H, NH2), 7.69 (s, 1H),

7.56 (s, 1H), 7.55 (s, 1H), 7.43 – 7.47 (dd, 1H), 5.75 (s, 2H); ^{13}C NMR (400 MHz, DMSO-d6): δ 155.8, 155.7, 149.1, 148.4, 143.2, 143.2, 133.6, 128.5, 126.7, 120.5, 37.5; HRMS: calculated for $C_{12}H_9N_4Cl_2$ $[M+H]^+$= 295.1474 Observed m/z $[M+H]^+$ = 295.1479.

HPLC purity: 99%.



Integration Results

Signal 1: DAD1 B, Sig=254,8 Ref=off

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2	2.769	BB	636.729	99.675	0.047	1915.577	99.570

Signal 2: MSD1 TIC, MS File

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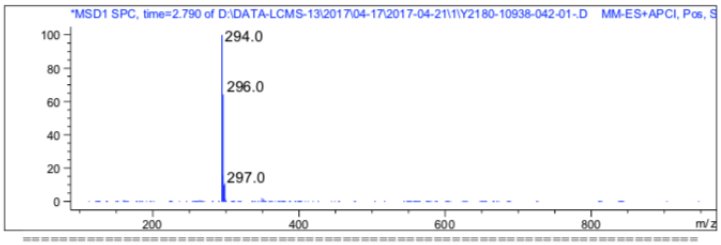
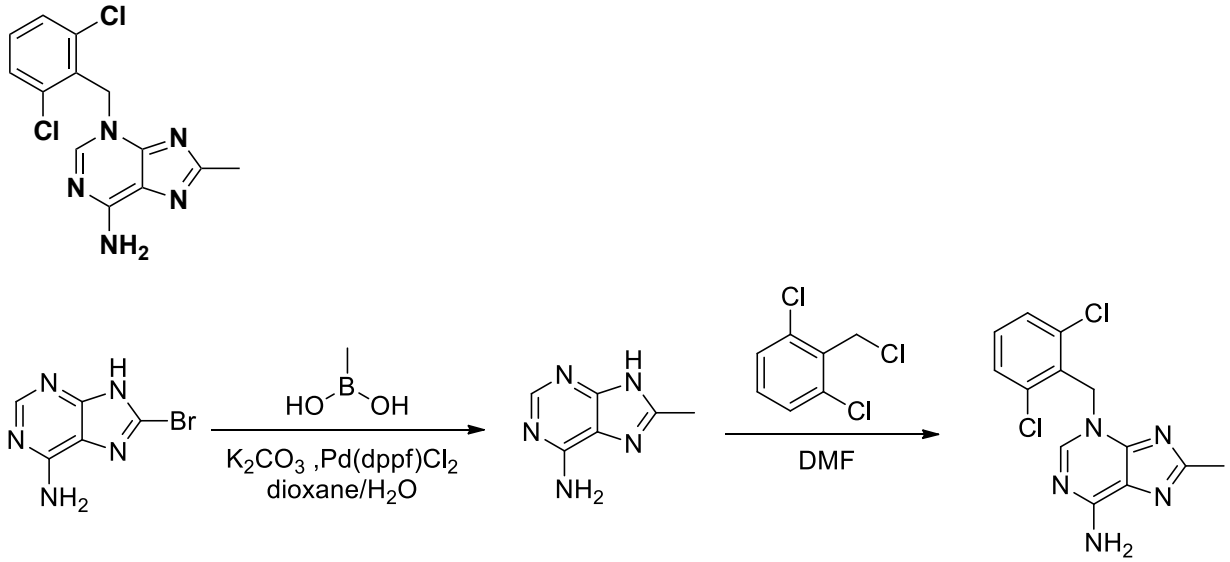


4a: 3-(2,6-Dichloro-benzyl)-8-methyl-3H-purin-6-ylamine



Step 1

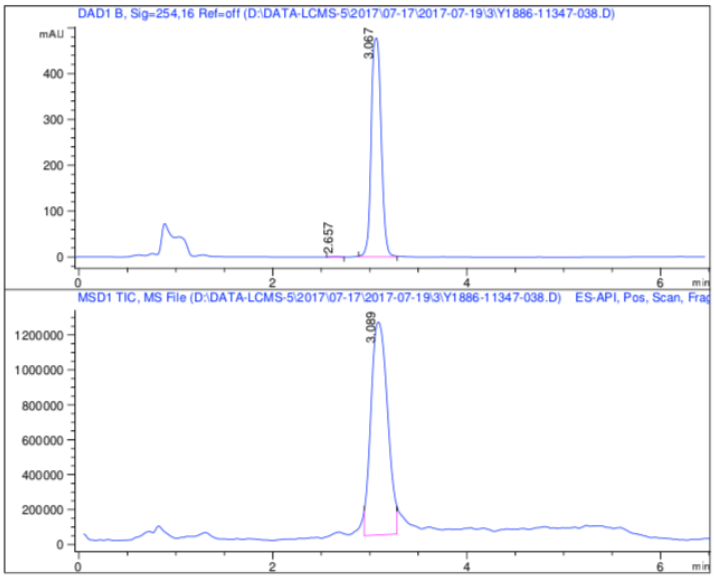
A flask charged with 8-bromo-9H-purin-6-ylamine (500 mg, 2.3 mmol), methylboronic (200 mg, 3.5mmol), $Pd(dppf)Cl_2$ (84 mg, 0.115 mmol) and K_2CO_3 (938 mg, 0.9 mmol) in dioxane/H_2O (50 mL/10 mL) was degassed and filled with N_2. The mixture was stirred at 95 ^oC overnight. Solvent was removed and the residue was purified with prep-TLC (DCM/MeOH = 10/1) to give 8-methyl-9H-purin-6-ylamine (150 mg, yield: 43 %) as yellow solid. ESI: calculated for $C_6H_7N_5$ =149.07. Observed m/z $[M+H]^+$= 150.0.

Step 2

A mixture of 8-methyl-9H-purin-6-ylamine (150 mg, 1.0 mmol) and 1,3-dichloro-2-chloromethyl-benzene (214 mg, 1.1 mmol) in DMF (3 mL) was stirred at 85 ^oC overnight. The mixture was cooled and filtered. The filtrate was purified by prep-HPLC (NH_4HCO_3 system) to give 3-(2,6-dichloro-benzyl)-8-methyl-3H-purin-6-ylamine (2 mg, yield: 5 %) as white solid.

^1H NMR (400 MHz, DMSO-d6): δ 7.82 (s, 1H), 7.69 -7.74 (b, 2H, NH_2), 7.60 (s, 1H), 7.59 (s, 1H), 7.51 – 7.58 (dd, 1H), 5.70 (s, 2H), 2.38 (s, 3H); HRMS: calculated for $C_{13}H_{11}N_5Cl_2$ $[M+H]^+$= 309.1739; Observed m/z $[M+H]^+$ = 309.1745.

HPLC purity: 99%.

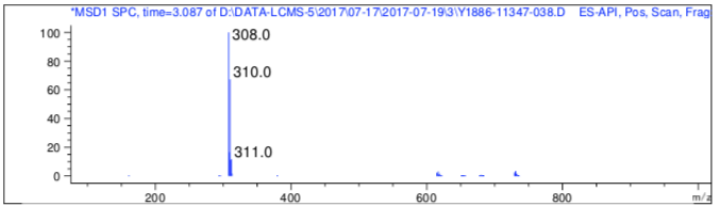


Integration Results

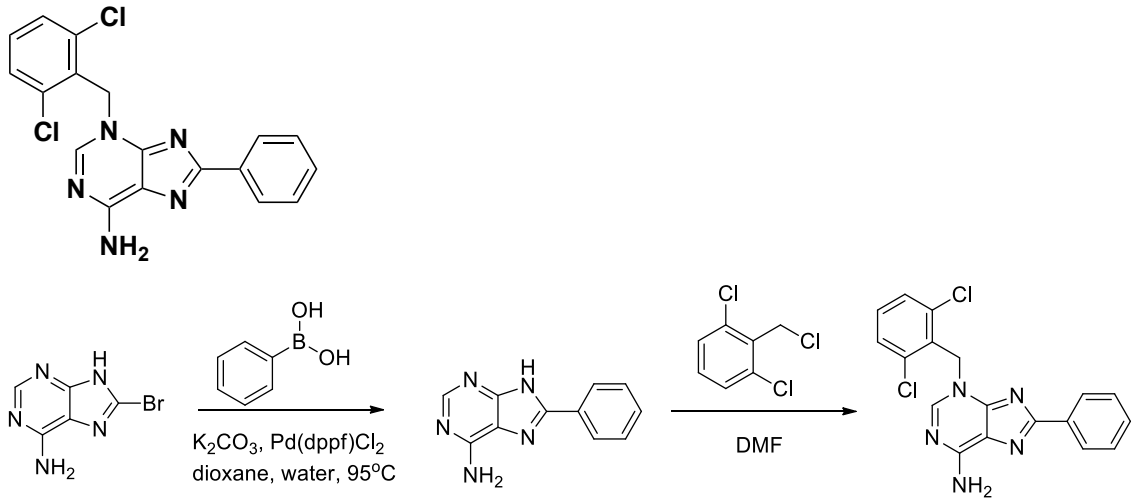
Signal 1: DAD1 B, Sig=254,16 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	2.657	MM	0.824	0.172	0.123	6.091	0.179
2	3.067	BB	477.658	99.828	0.113	3401.608	99.821

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.089	MF	1.224e+006	100.000	0.198	1.452e+007	100.000



4b: 3-(2,6-dichlorobenzyl)-8-phenyl-3H-purin-6-amine



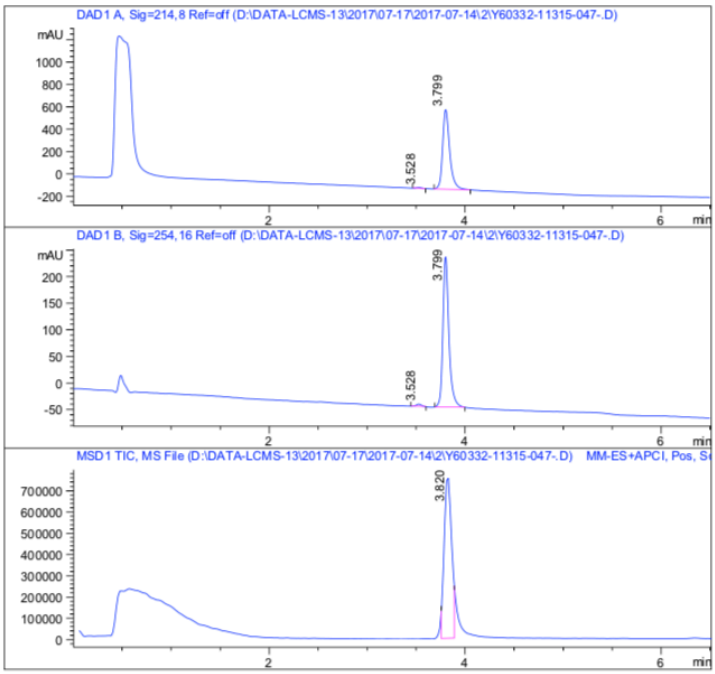
Step 1

A flask charged with 8-bromo-9H-purin-6-amine (500 mg, 2.3 mmol), phenylboronic acid (427 mg, 3.5 mmol), $Pd(dppf)Cl_2$ (84 mg, 0.115 mmol), and K_2CO_3 (952 mg, 6.9 mmol) in dioxane (20 mL） and water (4 mL) was degassed and filled with N_2. The reaction was stirred at 95 ^oC under nitrogen atmosphere overnight. Solvent was removed and the residue was purified by prep-TLC (DCM/MeOH = 10/1） to give 8-phenyl-9H-purin-6-amine (170 mg, yield: 35 %) as a brown solid. ESI: calculated for $C_{11}H_9N_5$ =211.08. Observed m/z $[M+H]^+$= 212.1.

Step 2

A mixture of 8-phenyl-9H-purin-6-amine (20 mg, 0.095 mmol) and 1,3-dichloro-2-(chloromethyl)benzene (18.5 mg, 0.095 mmol) in DMF (1 mL) was stirred at 85 ^oC overnight. Solvent was removed and the residue was purified by prep-HPLC (NH_4HCO_3 system) to give 3-(2,6-dichlorobenzyl)-8-phenyl-3H-purin-6-amine (2.8 mg, yield: 8 %) as a white solid.

^1H NMR (400 MHz, DMSO-d6): δ 7.87 (s, 1H), 8.17 – 8.28 (m, 2H), 7.93 -7.99 (b, 2H, NH_2), 7.58 (s, 1H), 7.56 (s, 1H), 7.40 – 7.46 (ddm, 3H), 7.26 – 7.33 (t, 1H, J = 7.3 Hz), 5.78 (s, 2H); HRMS: calculated for $C_{18}H_{13}N_5Cl_2$ $[M+H]^+$= 371.2433; Observed m/z $[M+H]^+$ = 371.2445. HPLC purity: 99%.



Integration Results

Signal 1: DAD1 A, Sig=214,8 Ref=off

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1	3.528	MM	7.458	1.031	0.060	26.896	0.743
2	3.799	MM	716.203	98.969	0.084	3593.468	99.257

Signal 2: DAD1 B, Sig=254,16 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.528	MM	4.328	1.498	0.064	16.521	1.351
2	3.799	MM	284.685	98.502	0.071	1206.243	98.649

Signal 3: MSD1 TIC, MS File

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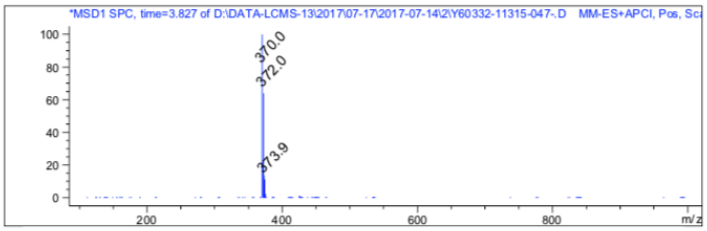
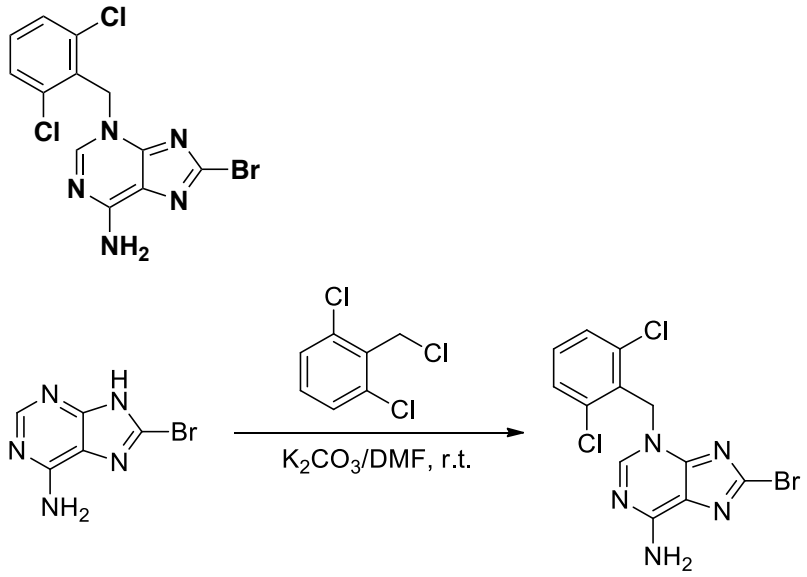
1	3.820	MF	774578.125	100.000	0.086	4.018e+006	100.000



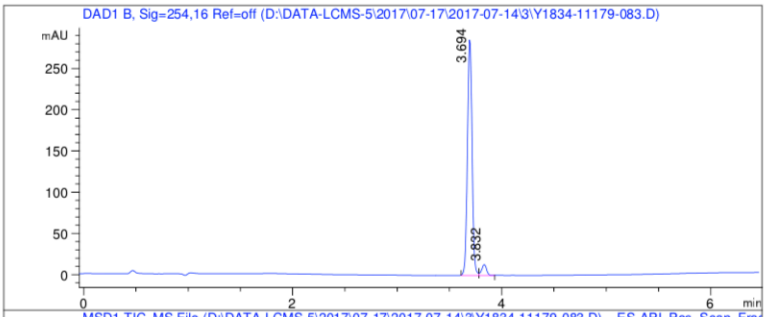
4c: **8-Bromo-3-(2,6-dichloro-benzyl)-3H-purin-6-ylamine**

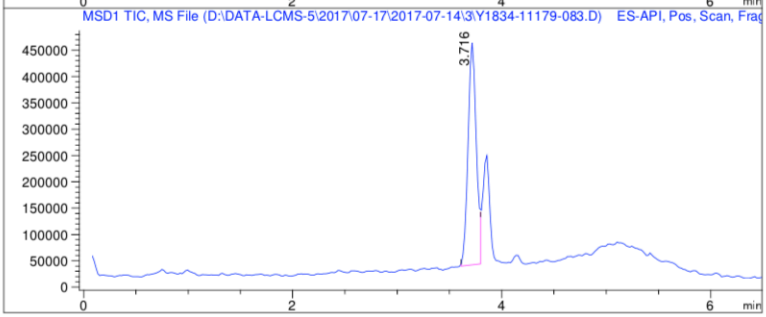


A mixture of 8-bromo-9H-purin-6-ylamine (50 mg, 0.233 mmol), 1,3-dichloro-2-chloromethyl-benzene (46 mg, 0.233 mmol) and K_2CO_3 (65 mg, 0.467 mmol) in DMF (2 mL) was stirred for 30 min at rt. The mixture was filtered and the filtrate was purified by prep-HPLC (NH_4OAc system) to give 8-bromo-3-(2,6-dichloro-benzyl)-3H-purin-6-ylamine (10.2 mg, yield: 12%) as a white solid.

^{1}H NMR (400 HMz, DMSO-d6): δ 8.23 – 8.28 (b, 1H, NH_2), 8.12 – 8.15 (b, 1H, NH_2), 7.58 (s, 1H), 7.60 (s, 1H), 7.56 (s, 1H), 7.40 – 7.46 (m, 1H), 5.70 (s, 2H); HRMS: calculated for $C_{12}H_8N_5BrCl_2$ $[M+H]^+$= 374.0434; Observed m/z $[M+H]^+$ = 374.0455.

HPLC purity: 95%.



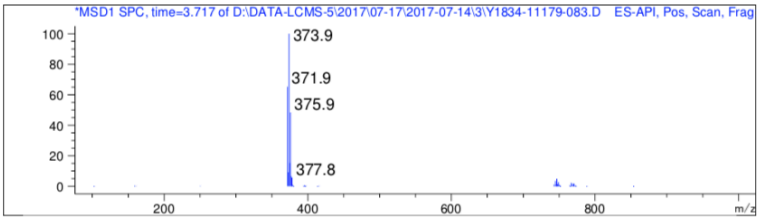


Integration Results

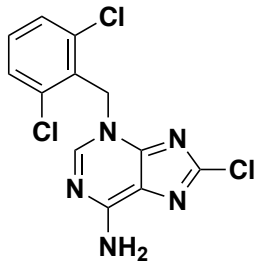
Signal 1: DAD1 B, Sig=254,16 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.694	BV	286.796	95.617	0.048	884.667	95.429
2	3.832	VB	13.145	4.383	0.052	42.372	4.571

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.716	BV	422220.781	100.000	0.084	2.293e+006	100.000



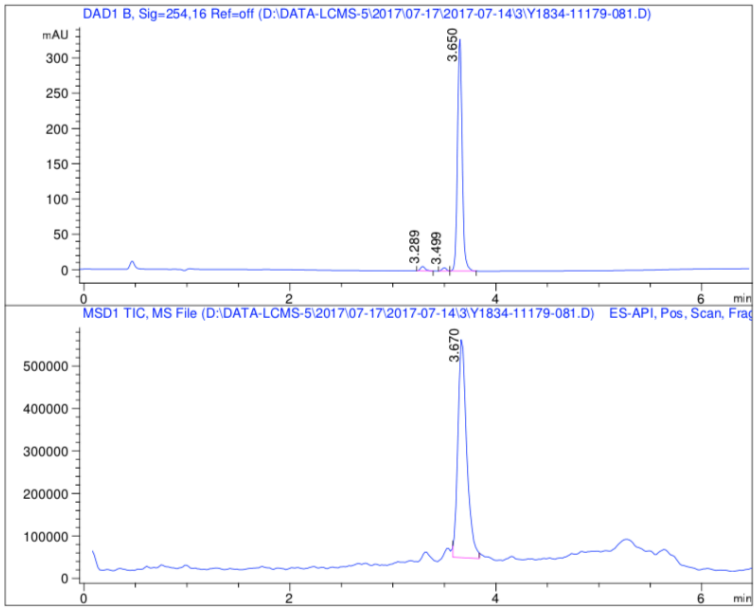
4d: 8-Chloro-3-(2,6-dichloro-benzyl)-3H-purin-6-ylamine



A mixture of 8-chloro-9H-purin-6-ylamine (50 mg, 0.233 mmol) and 1,3-dichloro-2-chloromethyl-benzene (46 mg, 0.233 mmol) in DMSO (1.5 mL) was heated at 90 ^{o}C for 16 hr. The mixture was filtered and the filtrate was purified by prep-HPLC (NH_4HCO_3 system) to give 8-chloro-3-(2,6-dichloro-benzyl)-3H-purin-6-ylamine (3.1 mg, yield: 4.1%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.25 – 8.17 (b, 2H, NH_2), 8.08 (s, 1H), 7.58 (s, 1H), 7.56 (s, 1H), 7.45 – 7.51 (bm, 1H), 5.71 (s, 2H); HRMS: calculated for $C_{12}H_8N_5Cl_3$ $[M+H]^+$= 329.5924; Observed m/z $[M+H]^+$ = 329.5930.

HPLC purity: 97%.



Integration Results

Signal 1: DAD1 B, Sig=254,16 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.289	BB	6.270	1.846	0.051	20.817	1.928
2	3.499	BV	4.592	1.352	0.046	13.491	1.249
3	3.650	VB	328.739	96.801	0.051	1045.620	96.823

#	R.T.	Type	Height	Height%	Width	Area	Area %
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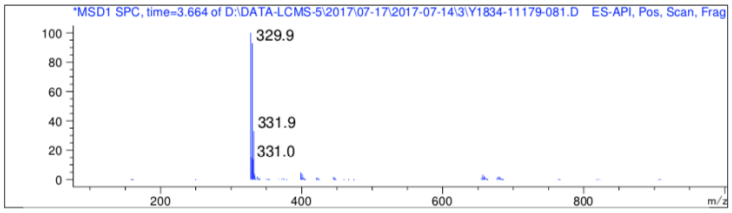
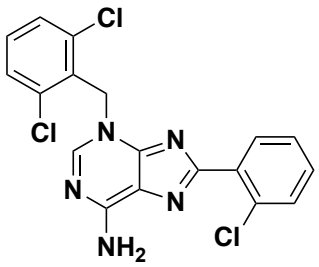


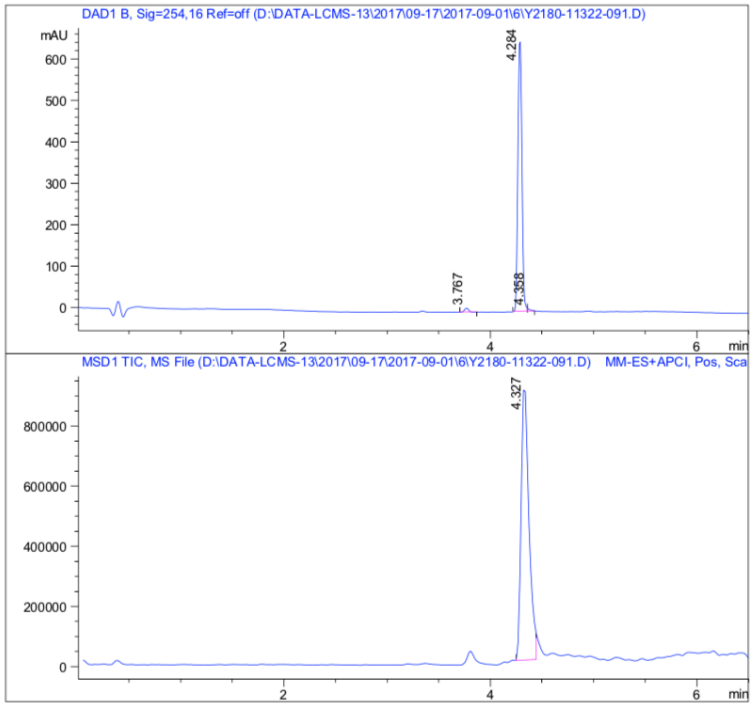
5a: 8-(2-Chloro-phenyl)-3-(2,6-dichloro-benzyl)-3H-purin-6-ylamine



The title compound was prepared in a similar fashion as **5c**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.24 – 8.28 (b, 1H, NH_2), 8.16 (s, 1H), 8.08 – 8.10 (b, 1H, NH2), 8.09 (d, 1H, J = 5.3 Hz), 7.57 (s, 1H), 7.55 (s, 1H), 7.43 – 7.46 (m, 2H), 7.38 – 7.41 (m, 2H), 5.79 (s, 2H); HRMS: calculated for $C_{18}H_{12}N_5Cl_3$ $[M+H]^+$= 405.6884; Observed m/z $[M+H]^+$ = 405.6890.

HPLC purity: 98%.



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Integration Results

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Signal 1: DAD1 B, Sig=254,16 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.767	BB	9.495	1.402	0.047	26.507	1.454
2	4.284	MF	660.346	97.537	0.045	1784.331	97.876
3	4.358	FM	7.180	1.061	0.028	12.219	0.670

Signal 2: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
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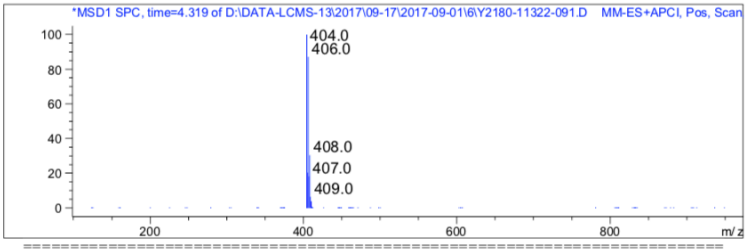

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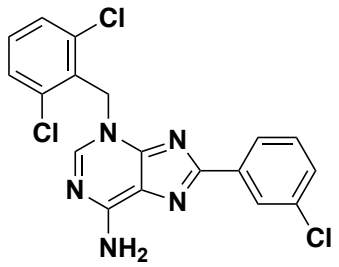
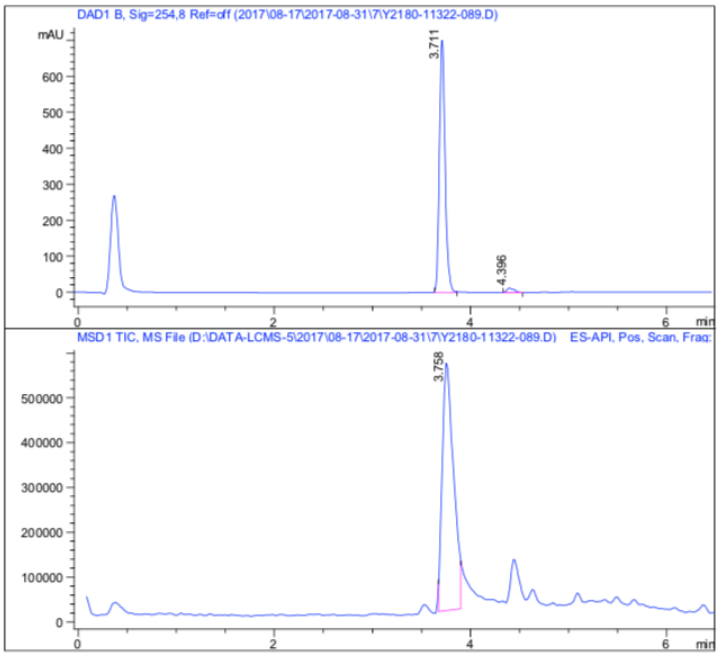
5b: 8-(3-Chloro-phenyl)-3-(2,6-dichloro-benzyl)-3H-purin-6-ylamine



The title compound was prepared in a similar fashion as **5c**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.24 – 8.29 (b, 1H, NH_2), 8.16 (s, 1H), 8.08 – 8.10 (b, 1H, NH_2), 8.07 (d, 1H, J = 5.3 Hz), 7.57 (s, 1H), 7.54 (s, 1H), 7.4 – 7.48 (m, 2H), 7.38 – 7.41 (m, 2H), 5.79 (s, 2H); HRMS: calculated for $C_{18}H_{12}N_5Cl_3$ $[M+H]^+$= 405.6884; Observed m/z $[M+H]^+$ = 405.6893.

HPLC purity: 98%.



Integration Results

Signal 1: DAD1 B, Sig=254,8 Ref=off

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1	3.711	BB	700.672	98.389	0.061	2826.335	98.046
2	4.396	BB	11.473	1.611	0.069	56.320	1.954

Signal 2: MSD1 TIC, MS File

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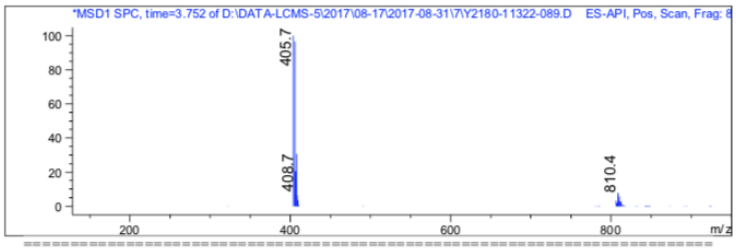
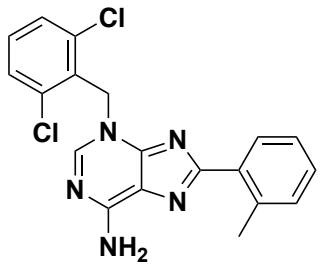


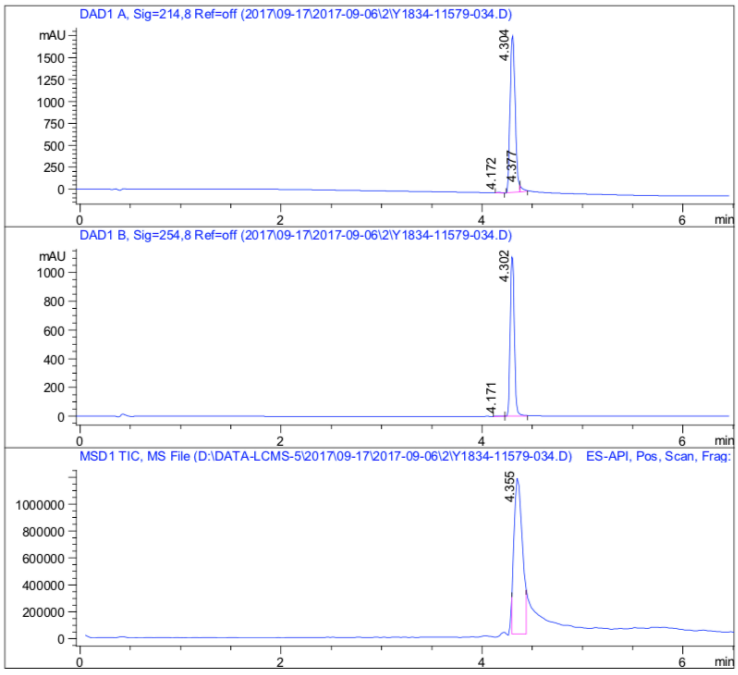
5d: 3-(2,6-Dichloro-benzyl)-8-o-tolyl-3H-purin-6-ylamine



The title compound was prepared in a similar fashion as **5c**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.30 (s, 1H), 8.06 – 8.10 (b, 2H, NH_2), 7.95 (s, 1H), 7.53 (s, 1H), 7.51 (s, 1H), 7.43 – 7.48 (m, 1H), 7.19 (m, 3H), 5.80 (s, 2H), 2.55 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d6): δ 169.7, 162.8, 148.4, 143.2, 133.6, 131.9, 131.5, 130.9, 129.1, 128.5, 126.7, 111.2, 37.5, 18.9; HRMS: calculated for $C_{19}H_{15}N_5Cl_2$ $[M+H]^+$= 385.2699; Observed m/z $[M+H]^+$ = 385.2691.

HPLC purity: 97%.



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Integration Results

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Signal 1: DAD1 A, Sig=214,8 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.172	MM	8.271	0.440	0.054	26.855	0.420
2	4.304	MF	1796.036	95.584	0.058	6243.476	97.588

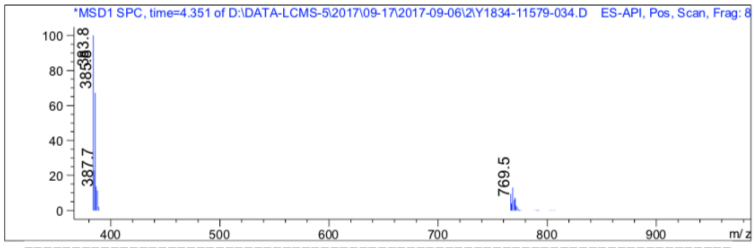
#	R.T.	Type	Height	Height%	Width	Area	Area %
3	4.377	FM	74.715	3.976	0.028	127.471	1.992

Signal 2: DAD1 B, Sig=254,8 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.171	BV	1.727	0.155	0.048	5.005	0.159
2	4.302	VB	1116.236	99.845	0.045	3149.061	99.841

Signal 3: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.355	MM	1.174e+006	100.000	0.094	6.596e+006	100.000



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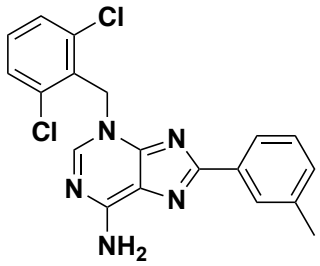
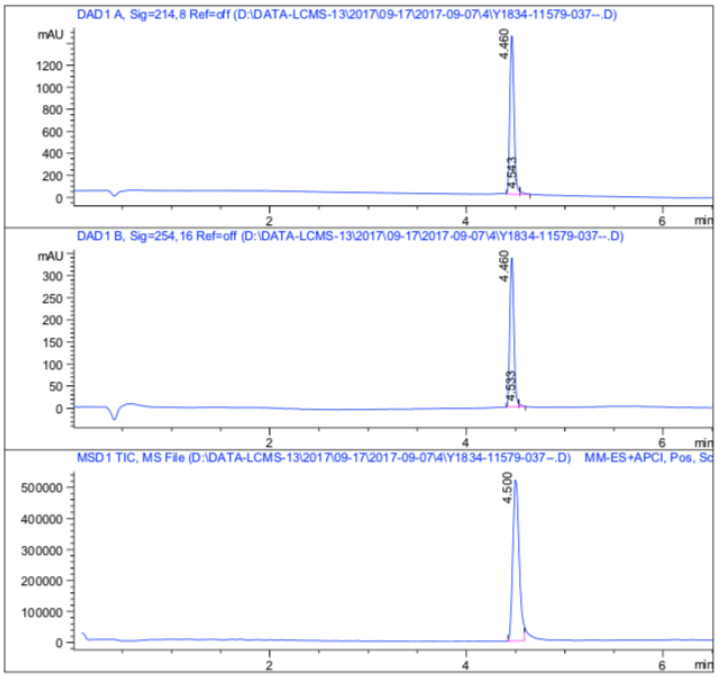
5e: 3-(2,6-Dichloro-benzyl)-8-m-tolyl-3H-purin-6-ylamine



The title compound was prepared in a similar fashion as **5c**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.15 (s, 1H), 8.04 (s, 1H), 7.94 – 7.98 (bm, 3H, NH_2 plus 1H), 7.58 (s, 1H), 7.56 (s, 1H), 7.45 (t, 1H), 7.29 (t, 1H), 7.13 (t, 1H), 5.79 (s, 2H), 2.36 (s, 3H); HRMS: calculated for $C_{19}H_{15}N_5Cl_2$ $[M+H]^+$= 385.2699; Observed m/z $[M+H]^+$ = 385.2689.

HPLC purity: 98%.



Integration Results

Signal 1: DAD1 A, Sig=214,8 Ref=off

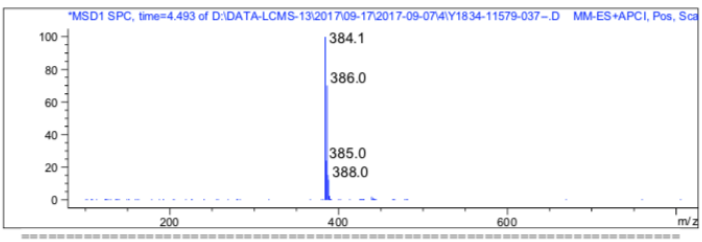
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.460	MF	1451.556	98.162	0.049	4260.187	98.445
2	4.543	FM	27.179	1.838	0.041	67.275	1.555

Signal 2: DAD1 B, Sig=254,16 Ref=off

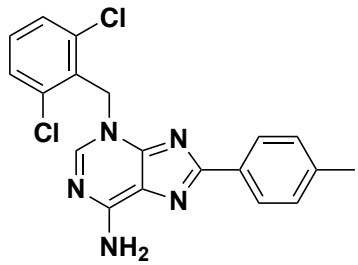
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.460	MF	339.430	98.153	0.047	966.791	98.885
2	4.533	FM	6.387	1.847	0.028	10.902	1.115

Signal 3: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.500	MM	542935.625	100.000	0.072	2.343e+006	100.000

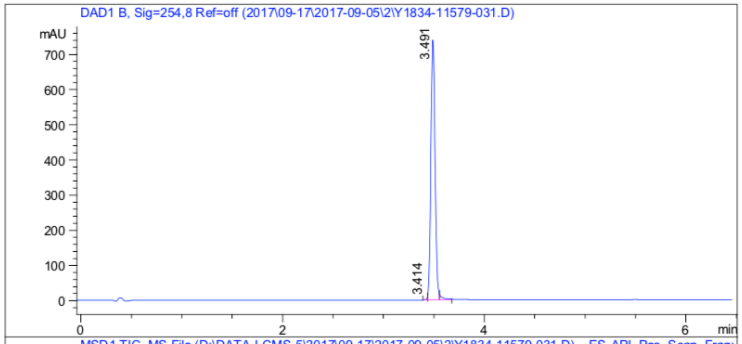


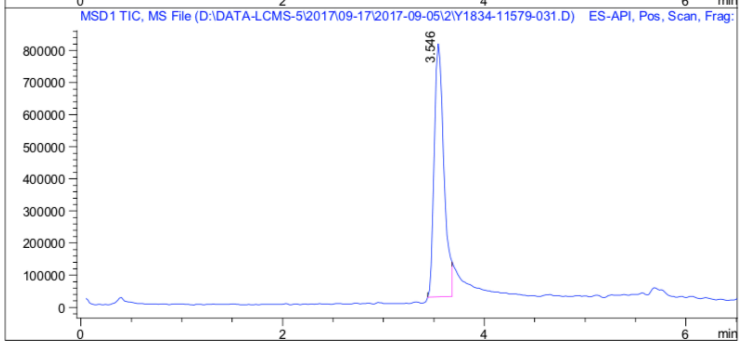
5f: 3-(2,6-Dichloro-benzyl)-8-p-tolyl-3H-purin-6-ylamine



The title compound was prepared in a similar fashion as **5c**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.16 (s, 1H), 8.06 (d, 2H, J = 8 Hz), 7.89 (b, 2H, NH_2), 7.58 (s, 1H), 7.56 (s, 1H), 7.45 (t, 1H), 7.29 (t, 1H), 7.21 (d, 2H, J = 8 Hz), 5.78 (s, 2H), 2.33 (s, 3H); HRMS: calculated for $C_{19}H_{15}N_5Cl_2$ $[M+H]^+$= 385.2699; Observed m/z $[M+H]^+$ = 385.2696.
HPLC purity: 98%.





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Integration Results

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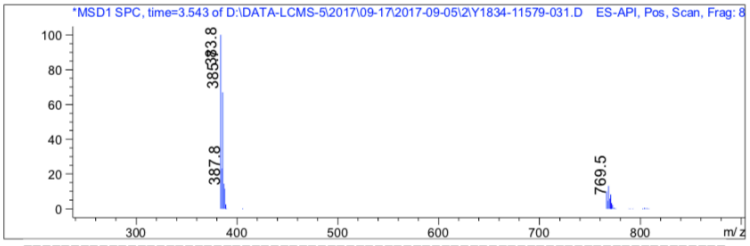
Signal 1: DAD1 B, Sig=254,8 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.414	MF	5.988	0.783	0.025	8.849	0.405
2	3.491	MF	743.296	97.239	0.048	2147.375	98.232

#	R.T.	Type	Height	Height%	Width	Area	Area %
3	3.557	FM	15.114	1.977	0.033	29.794	1.363

Signal 2: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.546	MF	795663.813	100.000	0.110	5.243e+006	100.000



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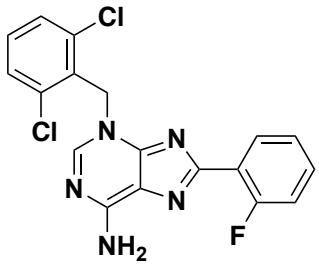
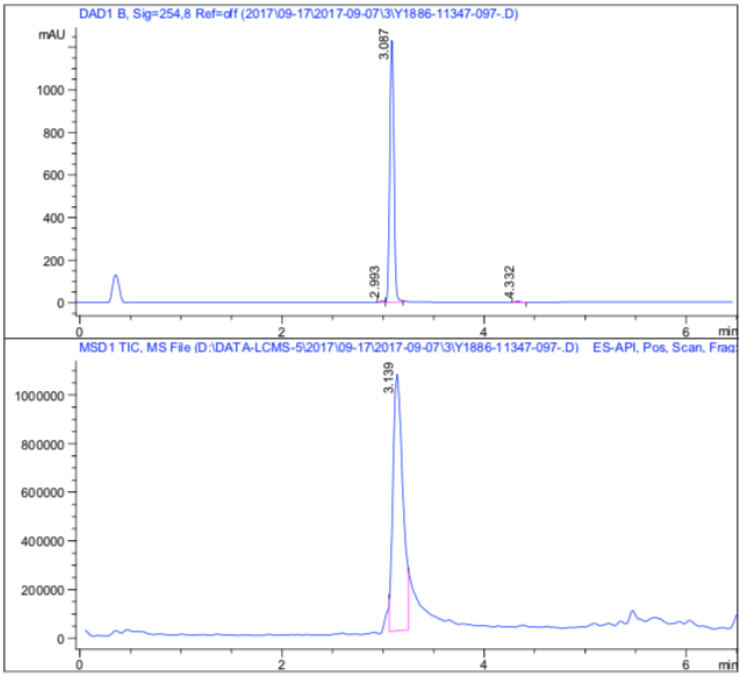
5g: 3-(2,6-Dichloro-benzyl)-8-(2-fluoro-phenyl)-3H-purin-6-ylamine



The title compound was prepared in a similar fashion as **5c**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.18 (s, 1H), 8.05 – 8.15 (m, 2H), 8.03 -8.05 (b, 2H, NH_2), 7.57 (s, 1H), 7.55 (s, 1H), 7.43 – 7.47 (m, 1H), 7.35 – 7.37 (m, 1H), 7.20 – 7.25 (m, 1H), 5.79 (s, 2H); HRMS: calculated for $C_{18}H_{12}N_5FCl_2$ $[M+H]^+$= 389.2338; Observed m/z $[M+H]^+$ = 389.2349.

HPLC purity: 99%.



Integration Results

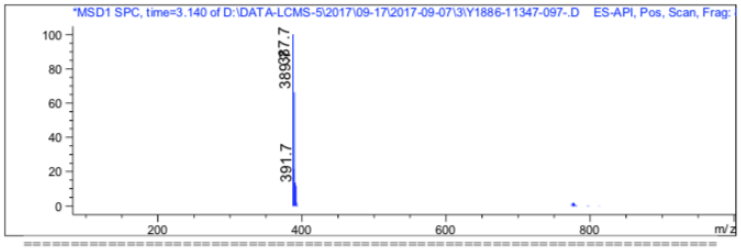
Signal 1: DAD1 B, Sig=254,8 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	2.993	BV	8.067	0.644	0.042	20.437	0.576
2	3.087	VB	1239.169	98.955	0.045	3515.108	99.049

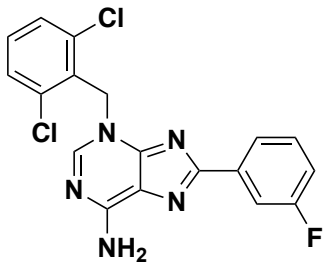
#	R.T.	Type	Height	Height%	Width	Area	Area %
3	4.332	BB	5.013	0.400	0.043	13.298	0.375

Signal 2: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.139	MM	1.062e+006	100.000	0.112	7.132e+006	100.000



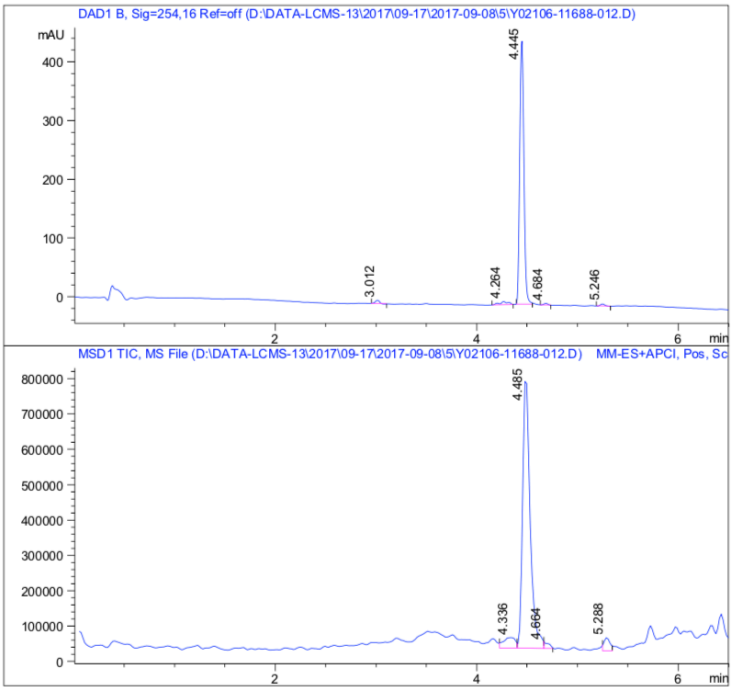
5h: 3-(2,6-dichlorobenzyl)-8-(3-fluorophenyl)-3H-purin-6-amine



The title compound was prepared in a similar fashion as **5c**.

^{1}H NMR (400 MHz, DMSO-d6): δ 9.32 – 9.38 (b, 1H, NH_2), 8.75(s, 1H), 8.63 – 8.69 (b, 1H, NH_2), 7.96 (d, 1H, J = 7.6 Hz), 7.87 (d, 1H, J = 9.6 Hz), 7.61 - 7.69 (m, 1H), 7.59 (s, 1H), 7.57 (s, 1H), 7.38 – 7.48 (m, 2H), 5.87 (s, 2H); HRMS: calculated for $C_{18}H_{12}N_5FCl_2$ $[M+H]^+$= 389.2338; Observed m/z $[M+H]^+$ = 389.2330.

HPLC purity: 95%.

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                    Integration Results
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Signal  1: DAD1 B, Sig=254,16 Ref=off
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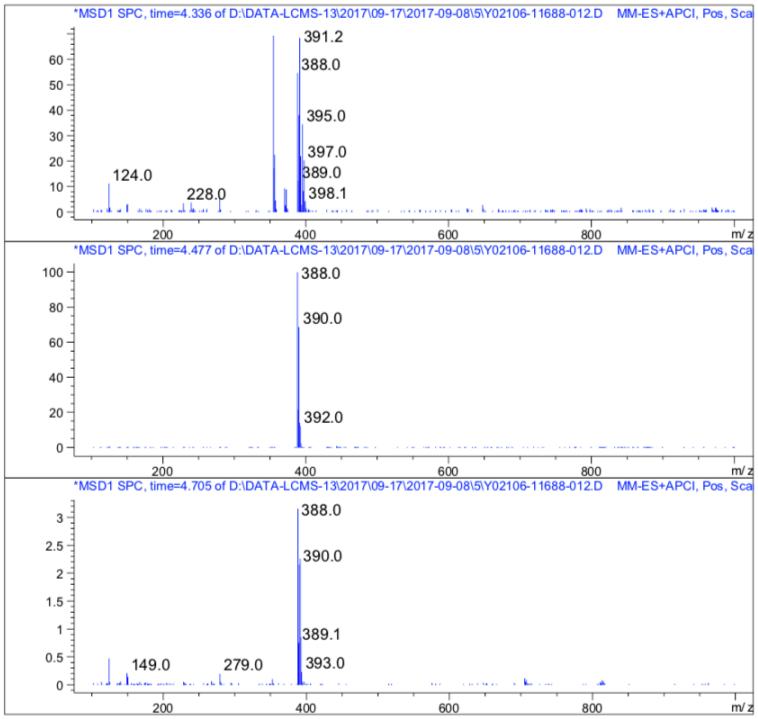
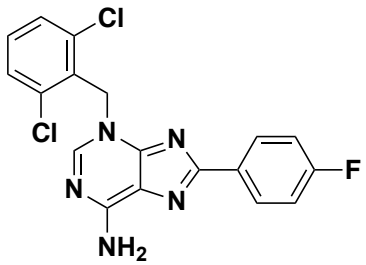
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.012	BB	5.881	1.255	0.048	17.336	1.263
2	4.264	MM	5.672	1.210	0.101	34.271	2.497
3	4.445	BB	450.731	96.165	0.046	1302.196	94.892
4	4.684	MM	2.961	0.632	0.049	8.726	0.636
5	5.246	BB	3.462	0.739	0.045	9.762	0.711

Signal 2: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.336	MF	29993.133	3.417	0.136	245344.359	5.540
2	4.485	MF	792320.625	90.256	0.084	3.984e+006	89.955
3	4.664	FM	17715.281	2.018	0.051	54654.223	1.234
4	5.288	MM	37833.289	4.310	0.064	144822.438	3.270



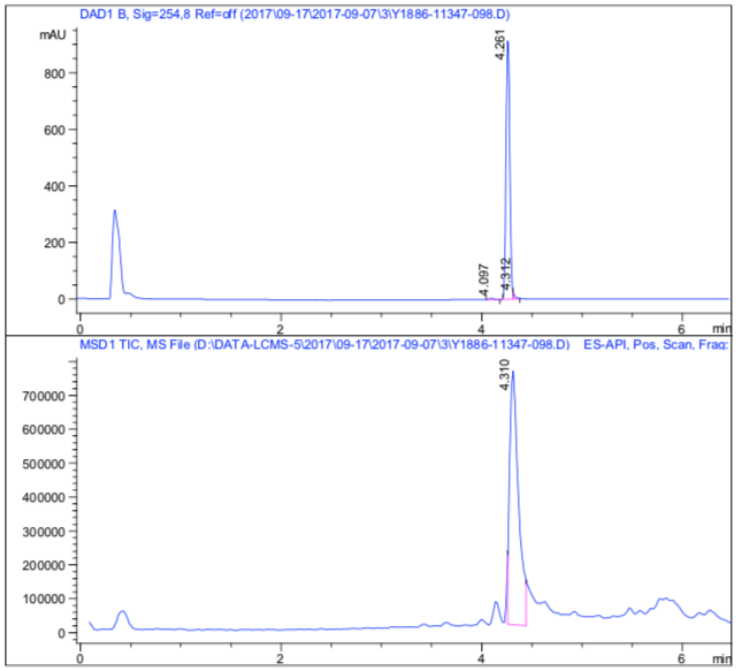
5i: 3-(2,6-Dichloro-benzyl)-8-(4-fluoro-phenyl)-3H-purin-6-ylamine



The title compound was prepared in a similar fashion as **5c**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.20 (s, 1H), 8.16 – 8.20 (m, 2H), 7.81 -7.92 (b, 2H, NH_2), 7.57 (s, 1H), 7.55 (s, 1H), 7.42 – 7.46 (m, 1H), 7.20 – 7.25 (m, 2H), 5.78 (s, 2H); HRMS: calculated for $C_{18}H_{12}N_5FCl_2$ $[M+H]^+$= 389.2338; Observed m/z $[M+H]^+$ = 389.2351.

HPLC purity: 98%.



Integration Results

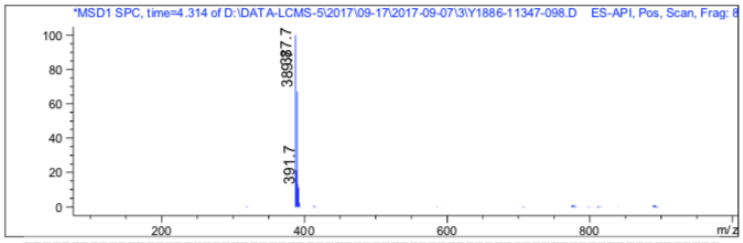
Signal 1: DAD1 B, Sig=254,8 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.097	BB	4.919	0.519	0.044	13.430	0.542
2	4.261	MF	920.309	97.015	0.044	2434.981	98.254

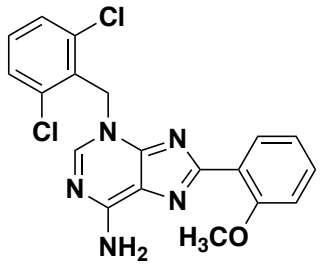
#	R.T.	Type	Height	Height%	Width	Area	Area %
3	4.312	FM	23.401	2.467	0.021	29.844	1.204

Signal 2: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.310	MM	757365.000	100.000	0.099	4.503e+006	100.000



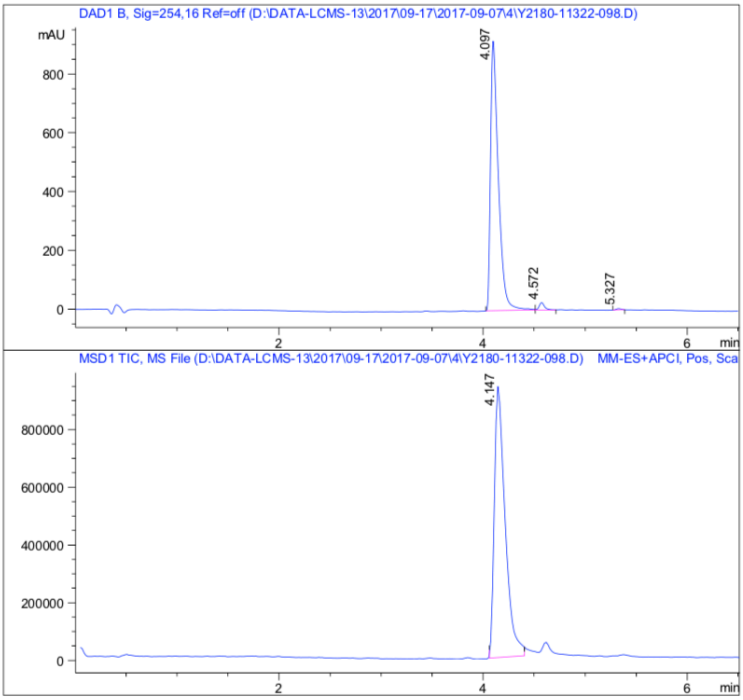
5j: 3-(2,6-Dichloro-benzyl)-8-(2-methoxy-phenyl)-3H-purin-6-ylamine



The title compound was prepared in a similar fashion as **5c**.

^{1}H NMR (400 MHz, DMSO-d6): δ 10.52 – 10.60 (b, 1H, NH_2), 8.28 – 8.33 (b, 1H, NH_2), 8.78 (s, 1H), 8.19 (d, 1H, J = 8 Hz), 7.59 (s, 1H), 7.57 (bs, 2H), 7.43 – 7.50 (m, 2H), 7.26 (d, 1H, 8.4 Hz), 7.25 (t, 1H, J = 7.4 Hz), 5.89 (s, 3H);

HRMS: calculated for $C_{19}H_{15}N_5OCl_2$ $[M+H]^+$= 401.2693; Observed m/z $[M+H]^+$ = 401.2685.
HPLC purity: 98%.



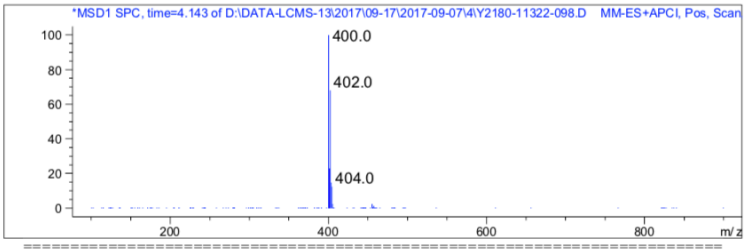
Integration Results

Signal 1: DAD1 B, Sig=254,16 Ref=off

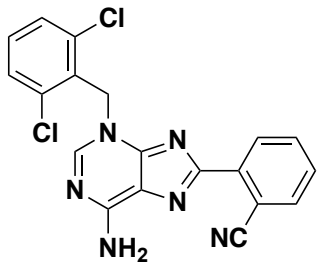
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.097	MF	920.817	96.757	0.089	4916.000	97.634
2	4.572	FM	26.018	2.734	0.064	100.679	2.000
3	5.327	MM	4.849	0.509	0.063	18.474	0.367

Signal 2: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	4.147	MM	951425.937	100.000	0.121	6.936e+006	100.000



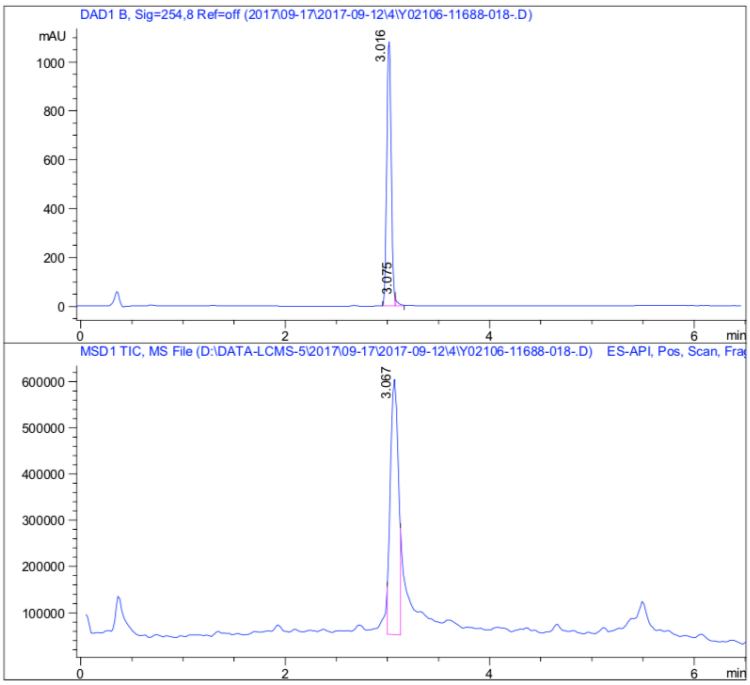
5k: 2-(6-amino-3-(2,6-dichlorobenzyl)-3H-purin-8-yl)benzonitrile



The title compound was prepared in a similar fashion as **5c**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.22 (d, 1H, J = 7.6 Hz), 8.08 – 8.16 (sb, 3H), 7.84 (d, 1H. J = 7.6), 7.58 (s, 1H), 7.56 (s, 1H), 7.42 – 7.46 (m, 1H), 7.20 – 7.25 (m, 2H), 5.78 (s, 2H);

HRMS: calculated for $C_{19}H_{12}N_6Cl_2$ $[M+H]^+$= 396.2528; Observed m/z $[M+H]^+$ = 396.2540.
HPLC purity: 99%.



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Integration Results

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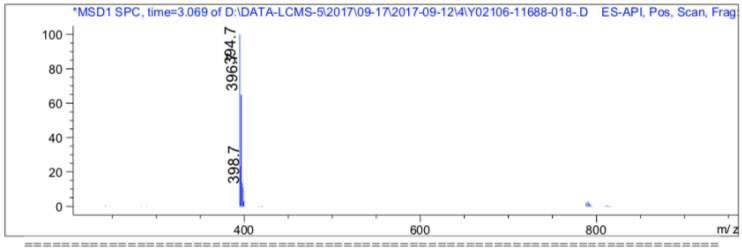
Signal 1: DAD1 B, Sig=254,8 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.016	MF	1088.078	96.926	0.050	3272.168	98.520
2	3.075	FM	34.508	3.074	0.024	49.145	1.480

Signal 2: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.067	MM	558247.750	100.000	0.087	2.913e+006	100.000



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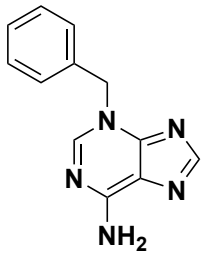
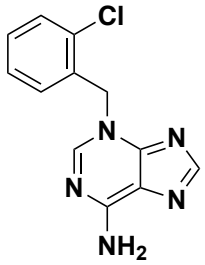
6a: 3-Benzyl adenine

The title compound is commercially available from Sigma Aldrich.



^{1}H NMR (400 MHz, DMSO-d6): δ 8.70 (s, 1H), 8.64 (s, 1H) 7.96 – 8.05 (b, 2H, NH_2), 7.26 – 7.40 (m, 5H), 5.64 (s, 2H);
HRMS: calculated for $C_{12}H_{11}N_5$ $[M+H]^+$= 226.2572; Observed m/z $[M+H]^+$ = 226.2581.

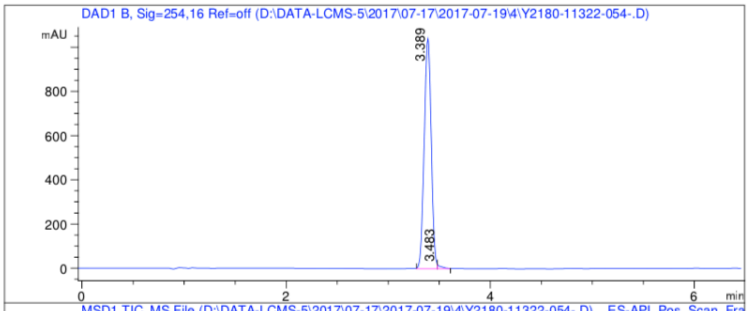
6b: 3-(2-Chloro-benzyl)-3H-purin-6-ylamine

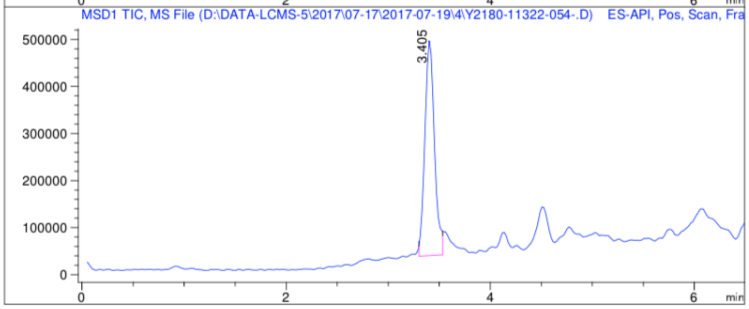


The title compound was prepared in a similar fashion as **3**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.44 (s, 1H), 8.71 (s, 1H), 7.96 – 8.05 (b, 2H, NH_2), 7.52 (d, 1H, J = 8 Hz), 7.35 (t, 1H, J = 8 Hz), 7.30 (t, 1H, J = 7.4 Hz), 7.28 (t, 1H, J = 7.4 Hz), 7.06 (d. 1H, J = 7.4 Hz), 5.64 (s, 2H);

HRMS: calculated for $C_{12}H_{10}N_5Cl$ $[M+H]^+$= 260.7023; Observed m/z $[M+H]^+$ = 260.7031.
HPLC purity: 99%.



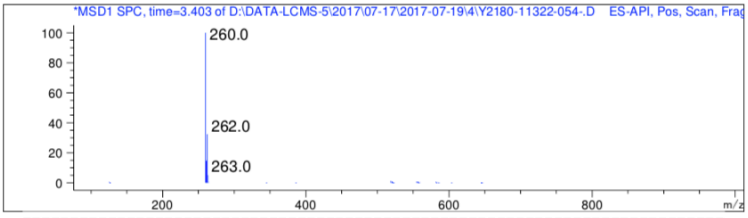


Integration Results

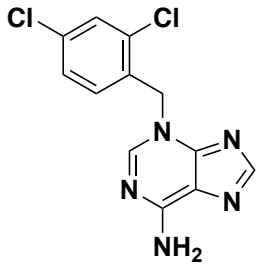
Signal 1: DAD1 B, Sig=254,16 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.389	MF	1041.840	98.418	0.080	4984.024	99.091
2	3.483	FM	16.745	1.582	0.046	45.732	0.909

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.405	MF	461730.094	100.000	0.107	2.972e+006	100.000



6c: 3-(2,4-Dichloro-benzyl)-3H-purin-6-ylamine

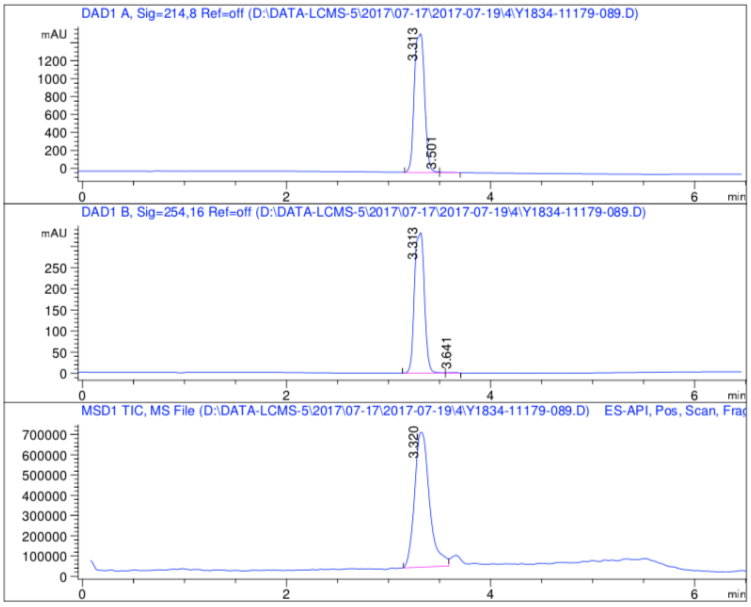


The title compound was prepared in a similar fashion as **3**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.45 (s, 1H), 7.96 – 8.03 (b, 2H, NH_2), 7.70 (s, 2H), 7.78 (dd, 1H, J = 2 Hz), 7.13 (d, 1H, J = 8.4 Hz), 5.59 (s, 2H);

HRMS: calculated for $C_{12}H_9N_5Cl_2$ $[M+H]^+$= 295.1474; Observed m/z $[M+H]^+$ = 295.1481.

HPLC purity: 99%.



Integration Results

Signal 1: DAD1 A, Sig=214,8 Ref=off

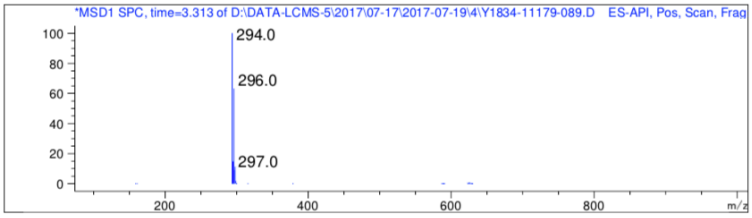
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.313	MF	1545.378	99.698	0.117	10839.181	99.761
2	3.501	FM	4.675	0.302	0.074	25.994	0.239

Signal 2: DAD1 B, Sig=254,16 Ref=off

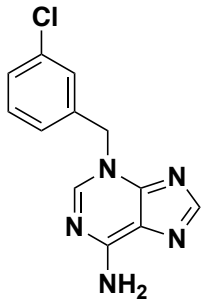
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.313	BV	331.798	99.580	0.093	2245.921	99.625
2	3.641	MF	1.399	0.420	0.101	8.450	0.375

Signal 3: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.320	BV	672144.750	100.000	0.150	6.797e+006	100.000



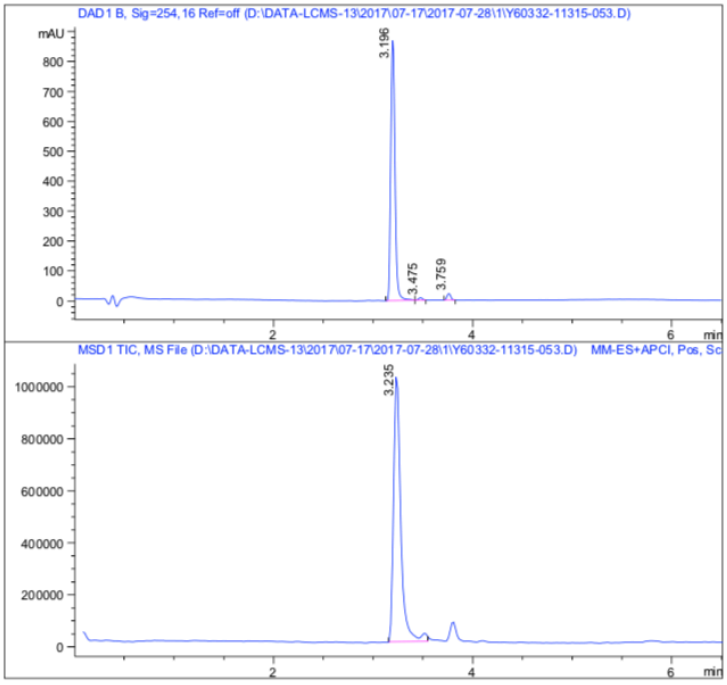
6d 3-(3-chlorobenzyl)-3H-purin-6-amine



The title compound was prepared in a similar fashion as **3**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.55 (s, 1H), 7.93 – 7.91 (b, 2H, NH_2), 7.70 (s, 1H), 7.51 (s, 1H), 7.40 (m, 3H), 5.50 (s, 2H);

HRMS: calculated for $C_{12}H_{10}N_5Cl$ $[M+H]^+$= 260.7023; Observed m/z $[M+H]^+$ = 260.7030.
HPLC purity: 97%.



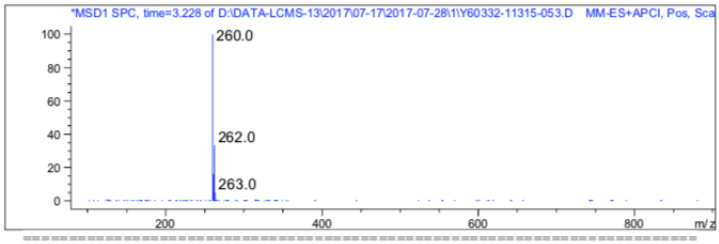
Integration Results

Signal 1: DAD1 B, Sig=254,16 Ref=off

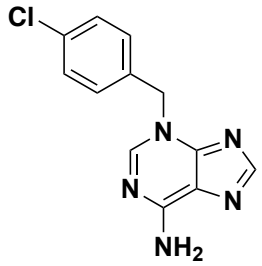
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.196	MF	874.924	96.600	0.049	2576.586	96.734
2	3.475	FM	9.113	1.006	0.056	30.441	1.143
3	3.759	MM	21.686	2.394	0.043	56.543	2.123

Signal 2: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.235	MM	1.054e+006	100.000	0.082	5.172e+006	100.000



6e: 3-(4-Chloro-benzyl)-3H-purin-6-ylamine

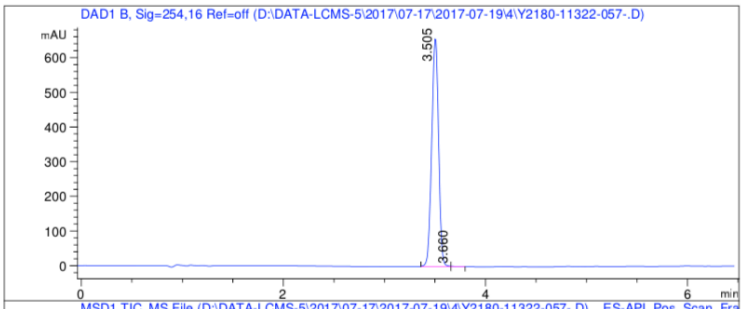


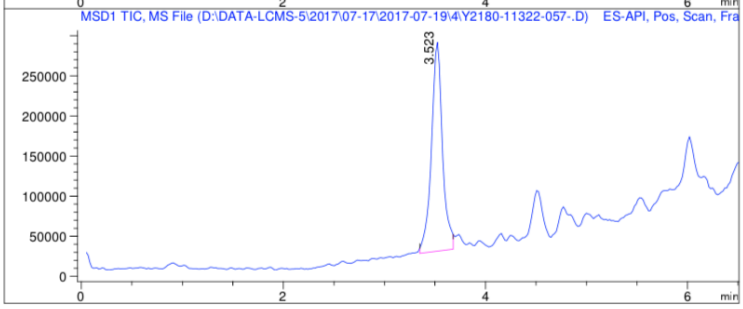
The title compound was prepared in a similar fashion as **3**.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.55 (s, 1H), 7.96 (brs, 2H), 7.89 (s, 1H), 7.49 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 5.49 (s, 2H).

^{1}H NMR (400 MHz, DMSO-d6): δ 8.57 (s, 1H), 7.91 – 7.96 (b, 2H, NH_2), 7.89 (s, 1H), 7.49 (d, 2H, J = 7.4 Hz), 7.40 (d, 2H, J = 7.4 Hz), 5.78 (s, 2H);

HRMS: calculated for $C_{12}H_{10}N_5Cl$ $[M+H]^+$= 260.7023; Observed m/z $[M+H]^+$ = 260.7029.
HPLC purity: 99%.





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Integration Results

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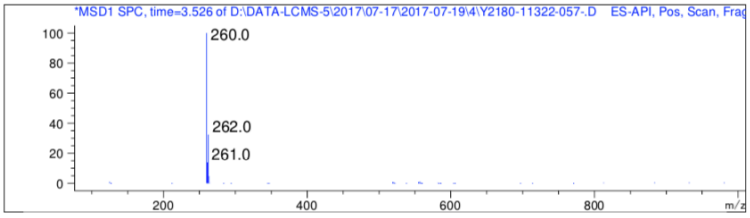
Signal 1: DAD1 B, Sig=254,16 Ref=off

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.505	MF	660.155	99.787	0.079	3116.650	99.829
2	3.660	FM	1.407	0.213	0.063	5.340	0.171

Signal 2: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.523	MF	263087.875	100.000	0.125	1.977e+006	100.000



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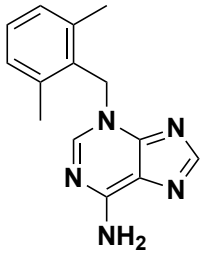
6f: 3-(2,6-dimethylbenzyl)-3H-purin-6-amine

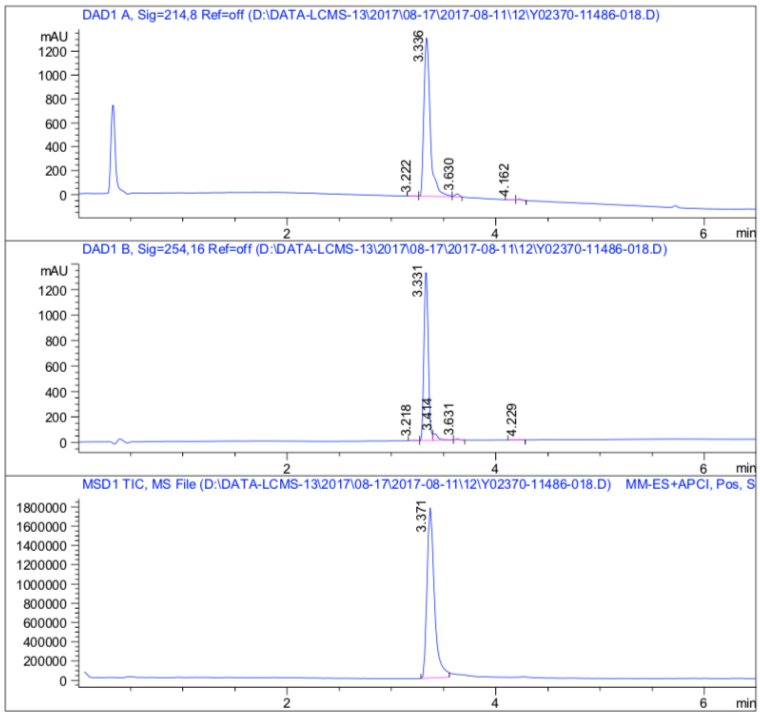


The title compound was prepared in a similar fashion as **3**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.00 – 7.11 (b, 2H, NH_2), 7.94 (s, 1H), 7.84 (s, 1H), 7.21 (m, 1H), 7.12 (s, 1H). 7.11 (s, 1H), 5.56 (s, 2H), 2,30 (s, 6H);

HRMS: calculated for $C_{14}H_{15}N_5$ $[M+H]^+$= 254.3104; Observed m/z $[M+H]^+$ = 254.3109.

HPLC purity: 97%.

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                          Integration Results

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 Signal  1: DAD1 A, Sig=214,8 Ref=off
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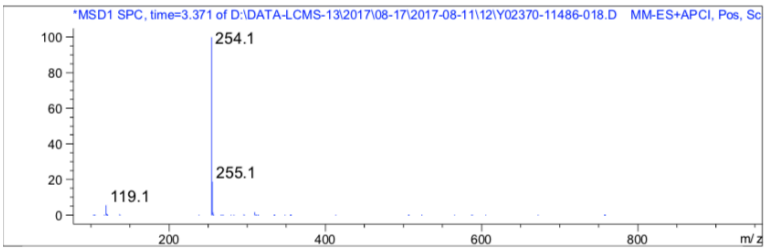
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.222	MF	4.921	0.357	0.065	19.216	0.333
2	3.336	FM	1334.131	96.667	0.070	5620.905	97.417
3	3.630	FM	25.759	1.866	0.053	81.418	1.411
4	4.162	MF	4.731	0.343	0.060	17.028	0.295
5	4.228	FM	10.592	0.767	0.049	31.398	0.544

Signal 2: DAD1 B, Sig=254,16 Ref=off

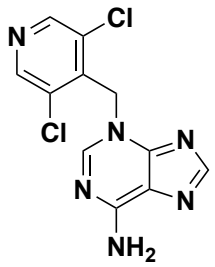
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.218	MF	3.059	0.217	0.059	10.807	0.267
2	3.331	FM	1338.956	95.049	0.047	3802.251	93.984
3	3.414	FM	52.558	3.731	0.059	184.661	4.564
4	3.631	FM	9.788	0.695	0.051	29.928	0.740
5	4.229	MM	4.344	0.308	0.069	17.986	0.445

Signal 3: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.371	MF	1.795e+006	100.000	0.081	8.720e+006	100.000



6g: 3-((3,5-dichloropyridin-4-yl)methyl)-3H-purin-6-amine

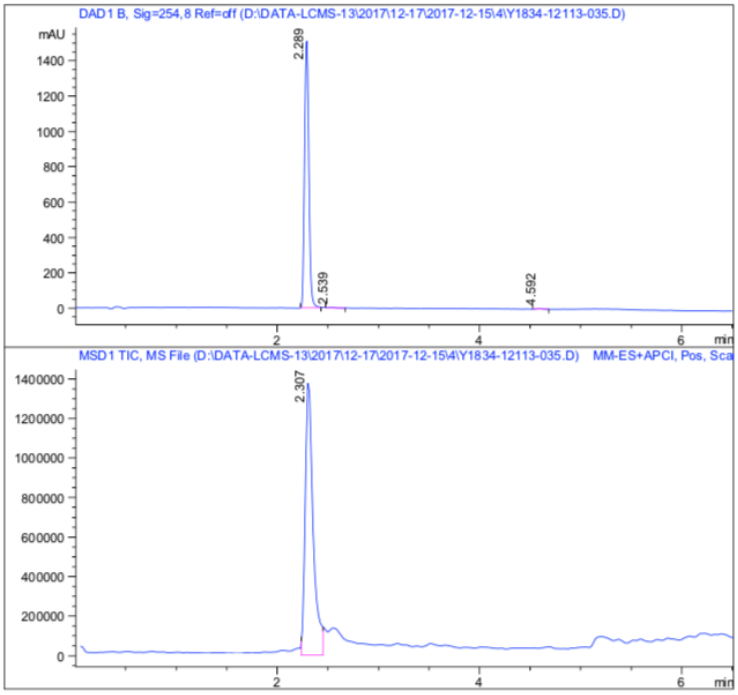


The title compound was prepared in a similar fashion as **3**.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.00 (b, 2H, NH_2), 7.94 (s, 1H), 7.84 (s, 1H), 7.21 (m, 1H), 7.12 (s, 1H). 7.11 (s, 1H), 5.56 (s, 2H), 2,30 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d6): δ 155.8, 149.1, 148.4, 147.0, 146.1, 133.3, 120.5, 37.5;

HRMS: calculated for $C_{11}H_8N_6Cl_2$ $[M+H]^+$= 296.1354; Observed m/z $[M+H]^+$ = 296.1361.

HPLC purity: 99%.



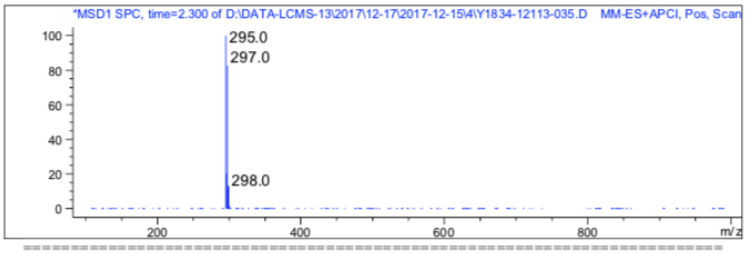
Integration Results

Signal 1: DAD1 B, Sig=254,8 Ref=off

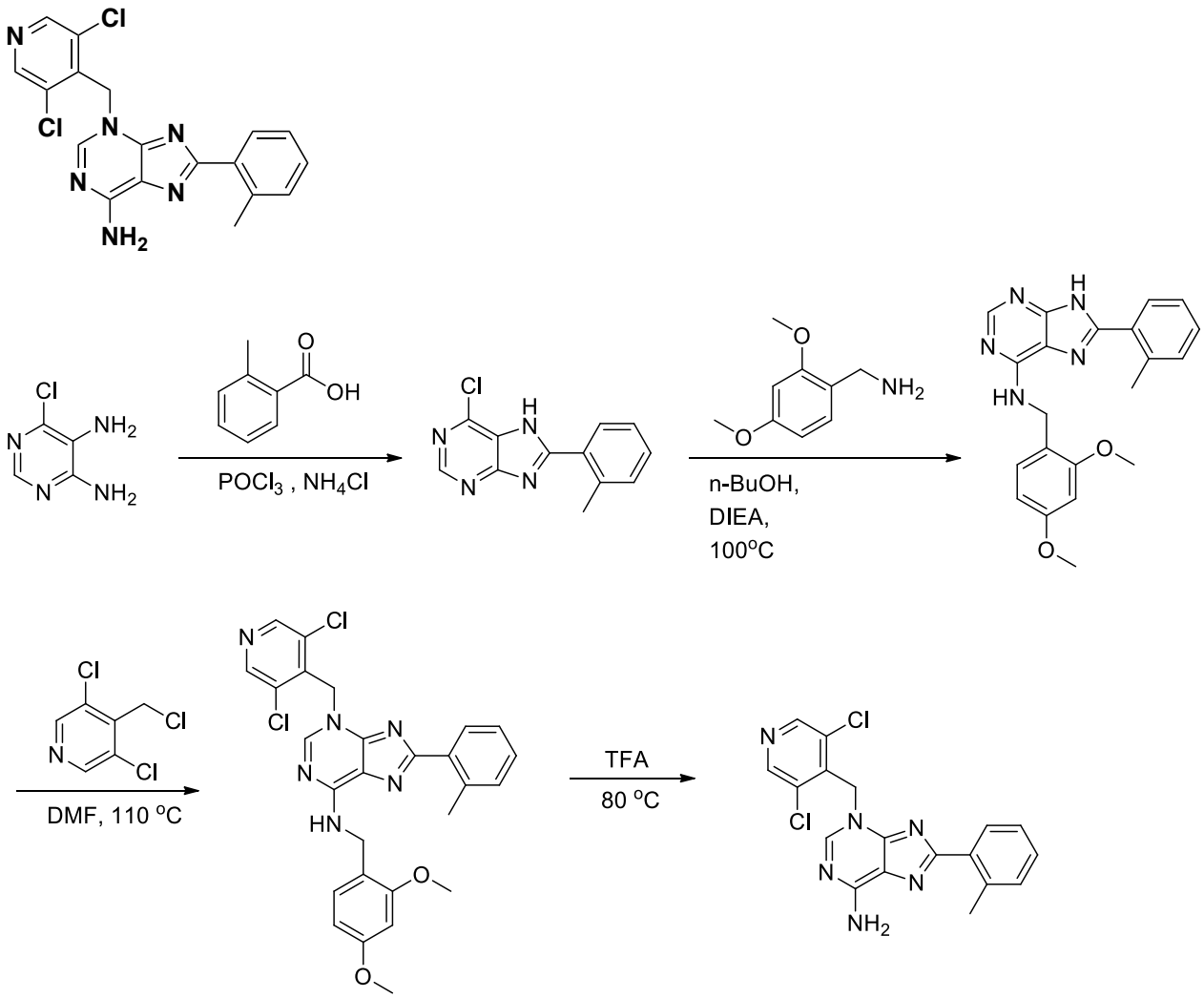
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	2.289	BB	1527.228	99.387	0.047	4524.237	99.080
2	2.539	BB	5.463	0.356	0.072	27.246	0.597
3	4.592	BB	3.957	0.258	0.058	14.759	0.323

Signal 2: MSD1 TIC, MS File

#	R.T.	Type	Height	Height%	Width	Area	Area %
1	2.307	MF	1.427e+006	100.000	0.088	7.567e+006	100.000



6h: 3-((3,5-dichloropyridin-4-yl)methyl)-8-(o-tolyl)-3H-purin-6-amine



Step 1

To a solution of 6-chloropyrimidine-4,5-diamine (500 mg, 3.47 mmol) in $POCl_3$ (10 mL) was added 2-methylbenzoic acid (473 g, 3.47 mmol) and NH_4Cl (551 mg, 10.41 mmol) at room temperature. The reaction mixture was then heated to 110 °C and stirred overnight. After cooling down to room temperature, the reaction was evaporated to remove $POCl_3$. The residue was dissolved in water (20 mL) and neutralized with $NH_3.H_2O$ to pH 7-8. The suspension was filtered and the filtered cake was dried to give 6-chloro-8-(o-tolyl)-7H-purine (749 mg, yield: 88.4%) as a yellow solid.

ESI: calculated for $C_{12}H_9ClN_4$ =244.05. Observed m/z $[M+H]^+$= 245.2.

Step 2

To a solution of 6-chloro-8-(o-tolyl)-7H-purine (749 mg, 3.07 mmol) in n-BuOH (10 mL) was added dropwise DIEA (792 mg, 6.14 mmol) and 2,4-dimethoxy-benzyl amine (512 mg, 3.07 mmol) at room temperature. The reaction mixture was then heated to 100 °C and stirred overnight. After cooling down to room temperature, the reaction mixture was

concentrated. The residue was washed with ethyl acetate (20 mL x 3) and dried to give N-(2,4-dimethoxybenzyl)-8-(o-tolyl)-9H-purin-6-amine (522 mg, yield: 45.4%) as a yellow solid.

ESI: calculated for $C_{21}H_{21}N_5O_2$ =375.17. Observed m/z $[M+H]^+$= 376.4.

Step 3

To a solution of N-(2,4-dimethoxybenzyl)-8-(o-tolyl)-9H-purin-6-amine (370 mg, 0.99 mmol) in DMF (10 mL) was added 3,5-dichloro-4-(chloromethyl)pyridine (385 mg, 1.97 mmol) at room temperature. The reaction mixture was then heated to 110 °C and stirred overnight. After cooling down to room temperature, the reaction was filtered and the filter cake was washed with MeOH (10 mL x 3) and dried to give 3-((3,5-dichloropyridin-4-yl)methyl)-N-(2,4-dimethoxybenzyl)-8-(o-tolyl)-3H-purin-6-amine (635 mg, yield: 100%) as a yellow solid.

ESI: calculated for $C_{27}H_{24}Cl_2N_6O_2$ =534.13. Observed m/z $[M+H]^+$= 535.3.

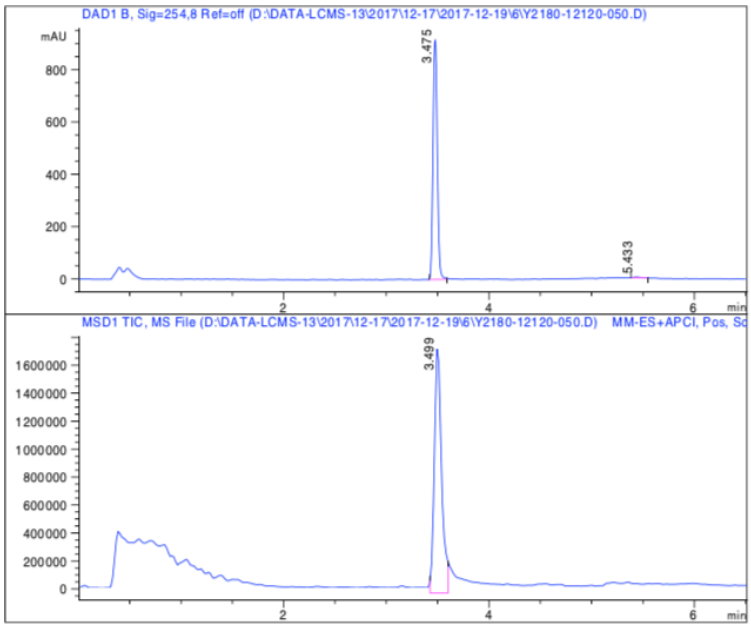
Step 4

A solution of 3-((3,5-dichloropyridin-4-yl)methyl)-N-(2,4-dimethoxybenzyl)-8- (o-tolyl)-3H-purin-6-amine (500 mg, 0.94 mmol) in TFA (10 mL) was stirred at 80 °C for 4 hr. After cooling down to room temperature, the reaction mixture was concentrated. The residue was washed with aqueous ($NaHCO_3$ 10 mL x 3) and DCM (10 mL x 3) and dried to give 3-((3,5-dichloropyridin-4-yl)methyl)-8-(o-tolyl)-3H-purin-6-amine (162 mg, yield: 46.4%) as a gray solid.

^{1}H NMR (400 MHz, DMSO-d6): δ 8.65 (s, 2H), 8.52 (s, 1H), 8.01 – 8.03 (b, 2H, NH_2), 7.18 (m, 3H), 5.81 (s, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d6): 169.7, 155.8, 148.4, 147.0, 146.1, 138.9, 133.3, 131.5, 130.9, 130.5, 125.8, 111.2, 37.5, 18.9;

HRMS: calculated for $C_{18}H_{14}N_6Cl_2$ $[M+H]^+$= 386.2581; Observed m/z $[M+H]^+$ = 386.2588.

HPLC purity: 99%.



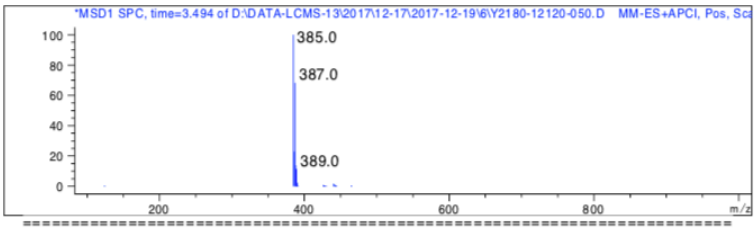
Integration Results

Signal 1: DAD1 B, Sig=254,8 Ref=off

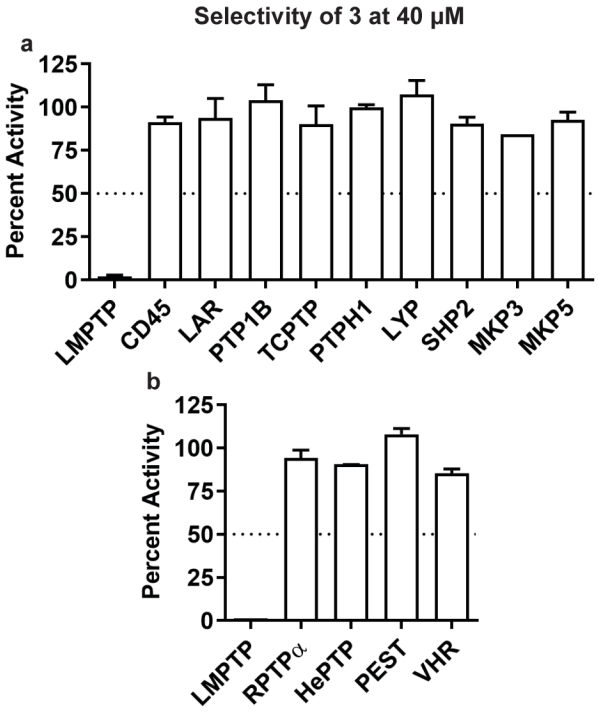
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.475	BB	923.616	99.670	0.046	2660.602	99.502
2	5.433	MM	3.058	0.330	0.073	13.311	0.498

Signal 2: MSD1 TIC, MS File

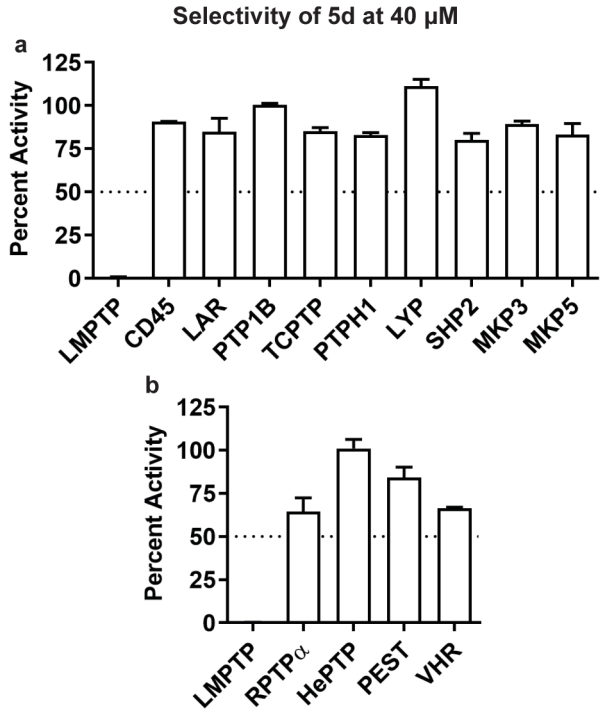
#	R.T.	Type	Height	Height%	Width	Area	Area %
1	3.499	MF	1.803e+006	100.000	0.079	8.548e+006	100.000



Selectivity of 3 & 5d

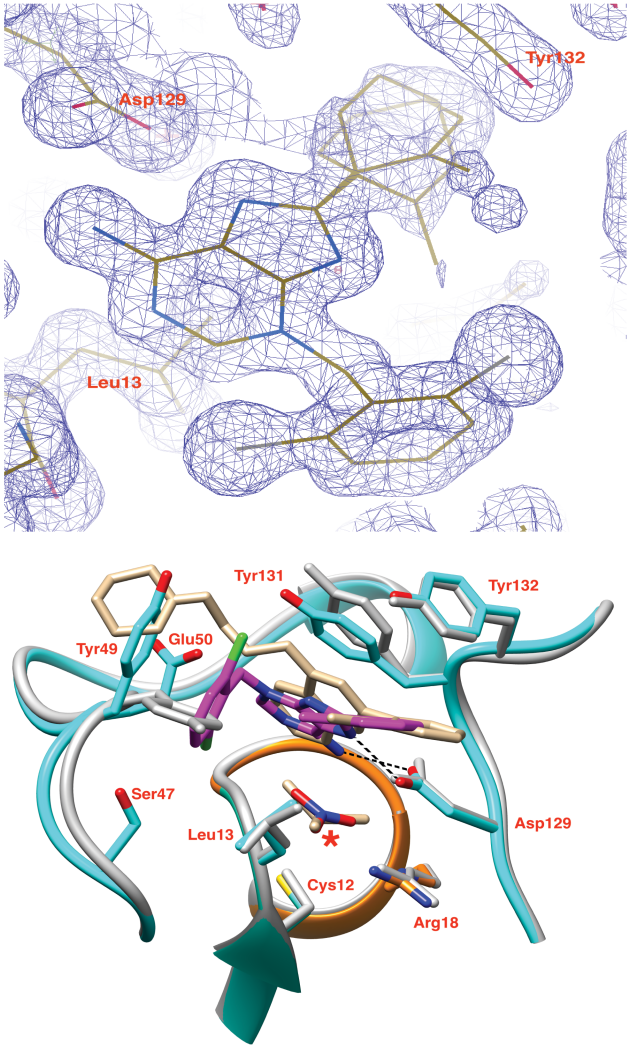


Supplemental Figure 1. Selectivity of analog **3**. (**a-b**) PTPs were incubated with (**a**) 0.4 mM 3-O-methylfluorescein phosphate (OMFP) or (**b**) 5 mM para-nitrophenylphosphate (pNPP) in the presence of dimethyl sulfoxide (DMSO) or 40 μM **3**. Mean±SEM % activity of PTPs incubated with inhibitors compared to DMSO is shown. Dotted line indicates 50% activity. Data from 2 independent experiments performed in triplicate is shown.



Supplemental Figure 2. Selectivity of analog **5d**. (**a-b**) PTPs were incubated with (**a**) 0.4 mM 3-O-methylfluorescein phosphate (OMFP) or (**b**) 5 mM para-nitrophenylphosphate (pNPP) in the presence of dimethyl sulfoxide (DMSO) or 40 µM **5d**. Mean±SEM % activity of PTPs incubated with inhibitors compared to DMSO is shown. Dotted line indicates 50% activity. Data from 2 independent experiments performed in triplicate is shown.

Crystal Structure of 5d



Supplemental Figure 3. Co-crystal structure of LMPTP inhibitors. *Upper,* wireframe representation of **5d** bound to LMPTP in its 2Fo-Fc electron density map contoured at 1.0 σ. The aminopurine (middle), dichlorophenyl (bottom) and o-tolyl (top, in both modeled conformations) are visible. Nearby residues are labeled. The picture was generated with coot. *Lower*, details of the binding of **5d** in the active site and comparison with compd **18**. LMPTP active site (cyan with P-loop in orange) is shown in ribbon representation with key side chains and bound **5d** (magenta) shown. The nitrate ion is marked by *. The structure of compd **18** and the VO_3 group (tan) in the active site (grey) from 5JNW are superimposed for comparison. Dashed lines indicate hydrogen bonds from **5d** to the two oxygen atoms of D129. For the structures of **5d** and **18** only one alternative conformation is shown for clarity. PDB: 7KH8.

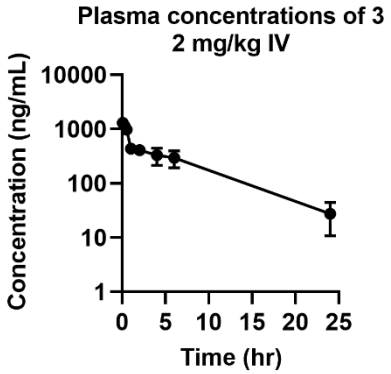
Supplemental Table 1. Crystal structure data collection and refinement statistics

Data Collection	**Overall (highest resolution shell)**
Resolution (Å)	50-1.3 (1.35-1.30)
Wavelength (Å)	1.1808
Space Group	$P2_12_12_1$
Unit Cell	
a, b, c (Å)	55.0, 59.0, 95.1
α, β, γ (°)	90, 90, 90
Unique Reflections	72357 (4521)
Completeness (%)	94.1 (59.5)
Redundancy	9.5 (4.7)
I/σ(I)	11.3 (3.8)
R_{meas}/R_{pim}	0.078/0.024 (0.302/0.134)
Wilson B Factor (Å^2)	14.6
Refinement	**Overall (highest resolution shell)**
Resolution (Å)	50-1.3 (1.34-1.30)
Reflections Used/Free	68550/3739 (3431/210)
R_{work} /R_{free}	0.125/0.161 (0.270/0.291)
Non-Hydrogen Protein Atoms	2661
Solvent Atoms	466
Average B Factors (Å^2)	21.6
Protein Main/Side Chain A	13.7/19.9
Protein Main/Side Chain B	16.6/23.3
Solvent	32.1
r.m.s.d. Bond Lengths (Å)	0.011
r.m.s.d. Bond Angles (°)	1.65
Ramachandran Plot	
Favored (%)	98.0
Allowed (%)	2.0
Disallowed (%)	0

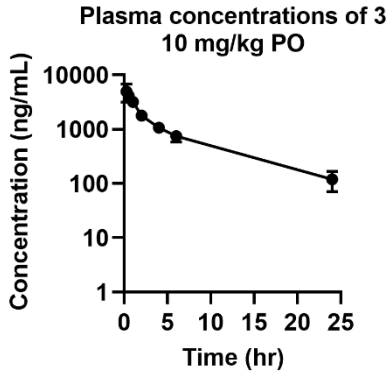
***In Vivo* Pharmacokinetics**

Compounds were dosed as a 1 mg/mL solution in 75% PEG400/25% water for IV dosing or as a 0.5% methylcellulose suspension for PO dosing unless indicated otherwise. 7 time points were measured: 0.25, 0.5, 1, 2, 4, 6 and 24 hours post dose unless otherwise indicated. Three animals were used per time point (C57BL/6 mice). Compound concentration was measured by LC/MS/MS. WinNonLin was used to calculate PK parameters.

Compound 3: Mouse PK

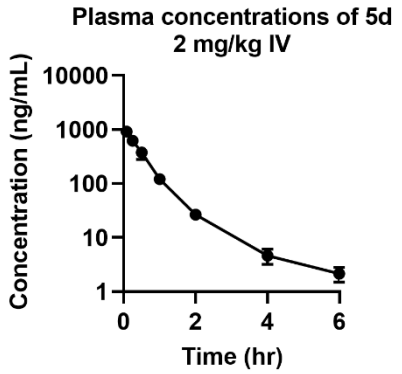


IV Time (hr)	Animal Study No.			Mean IV	SD
	101M	102M	103M		
0.08	1420.3	1089.0	1381.5	1296.9	181.1
0.25	1274.9	975.1	1177.1	1142.4	152.9
0.50	925.2	927.1	1080.6	977.6	89.2
1.00	468.1	419.2	420.4	435.9	27.9
2.00	449.7	467.1	310.8	409.2	85.7
4.00	302.4	454.8	228.7	328.6	115.3
6.00	256.6	408.9	216.7	294.1	101.4
24.00	20.1	15.8	46.7	27.5	16.7
PK Parameters	**101M**	**102M**	**103M**	**Mean IV**	**SD**
HL λz ($T_{1/2}$, hr)	5.02	4.01	8.48	5.83	2.35
C_{max} (ng/ml)	1420.3	1089.0	1381.5	1296.9	181.1
AUC_{last} (ng*hr/ml)	5146.8	6890.7	4711.7	5583.1	1153.2
$AUC_{INF\ pred}$ (ng*hr/ml)	5293.0	6983.2	5286.3	5854.2	977.8
MRT_{last} (hr)	4.66	4.87	5.61	5.05	0.50
Vz_{pred} (L/kg)	2.74	1.66	4.63	3.01	1.51
Cl_{pred} (L/hr/kg)	0.38	0.29	0.38	0.35	0.05
λz Calculation Time Range (hr)	2-24	4-24	4-24	NA	NA

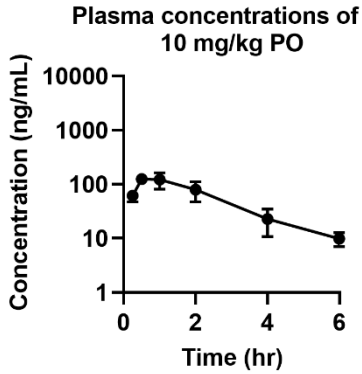


PO Time (hr)	Animal Study No.			Mean PO	SD
	301M	302M	303M		
0.25	7035.6	4387.5	3528.6	4983.9	1828.0
0.50	5125.8	3703.8	3631.4	4153.7	842.7
1.00	3717.8	3128.1	2725.7	3190.5	499.0
2.00	1622.6	1732.7	1978.8	1778.0	182.4
4.00	901.1	1139.2	1150.4	1063.6	140.8
6.00	564.3	811.7	867.4	747.8	161.3
24.00	75.3	171.9	111.3	119.5	48.8
PK Parameters	**301M**	**302M**	**303M**	**Mean PO**	**SD**
HL λz ($T_{1/2}$, hr)	5.81	7.59	5.99	6.47	0.98
T_{max} (hr)	0.25	0.25	0.50	0.33	0.14
C_{max} (ng/ml)	7035.6	4387.5	3631.4	5018.2	1787.6
AUC_{last} (ng*hr/ml)	17026.2	19373.4	19232.9	18544.2	1316.5
$AUC_{INF\ pred}$ (ng*hr/ml)	17650.0	21241.5	20192.2	19694.6	1846.8
MRT_{last} (hr)	3.90	5.34	4.90	4.71	0.74
$Vz_{F\ pred}$ (L/kg)	4.75	5.16	4.28	4.73	0.44
$Cl_{F\ pred}$ (L/hr/kg)	0.57	0.47	0.50	0.51	0.05
λz Calculation Time Range (hr)	4-24	4-24	4-24	NA	NA
Bioavailability (%)	60.99	69.40	68.90	66.43	4.72

Compound 5d: Mouse PK

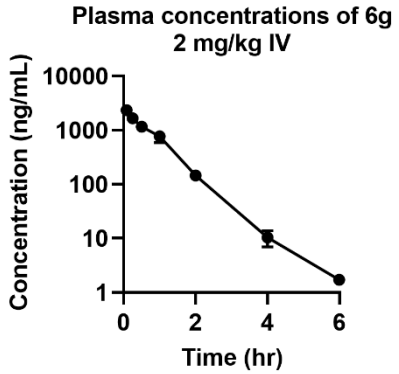


IV Time (hr)	Animal Study No.			Mean IV	SD	CV (%)
	M01	M02	M03			
0.0830	1090	764	892	**915**	**164**	**17.9**
0.250	776	545	545	**622**	**133**	**21.4**
0.500	480	297	348	**375**	**94.4**	**25.2**
1.00	125	107	131	**121**	**12.5**	**10.3**
2.00	28.7	28.6	22.8	**26.7**	**3.38**	**12.7**
4.00	4.79	6.00	3.14	**4.64**	**1.44**	**30.9**
6.00	2.33	2.70	1.42	**2.15**	**0.659**	**30.6**
24.0	BQL	BQL	BQL	**ND**	**ND**	**ND**
PK Parameters	**M01**	**M02**	**M03**	**Mean IV**	**SD**	**CV (%)**
Rsq adj	0.905	0.933	0.926	--	--	--
No. points used for $T_{1/2}$	7.00	3.00	7.00	**ND**	--	--
C_0 (ng/mL)	1291	904	1139	**1111**	**195**	**17.5**
$T_{1/2}$ (hr)	0.652	1.17	0.623	**0.817**	**0.310**	**38.0**
Vd_{ss} (L/kg)	2.09	3.54	2.61	**2.75**	**0.736**	**26.8**
Cl (mL/min/kg)	57.3	77.4	71.9	**68.9**	**10.4**	**15.1**
T_{last} (hr)	6.00	6.00	6.00	**6.00**	--	--
$AUC_{0\text{-}last}$ (ng*hr/mL)	638	469	508	**538**	**88.3**	**16.4**
$AUC_{0\text{-}inf}$ (ng*hr/mL)	640	474	510	**541**	**87.5**	**16.2**
$MRT_{0\text{-}last}$ (hr)	0.585	0.695	0.590	**0.623**	**0.0619**	**9.93**
$MRT_{0\text{-}inf}$ (hr)	0.607	0.762	0.605	**0.658**	**0.0900**	**13.7**
AUC_{Extra} (%)	0.342	0.966	0.251	**0.520**	**0.389**	**74.9**
$AUMC_{Extra}$ (%)	3.91	9.75	2.86	**5.51**	**3.71**	**67.5**

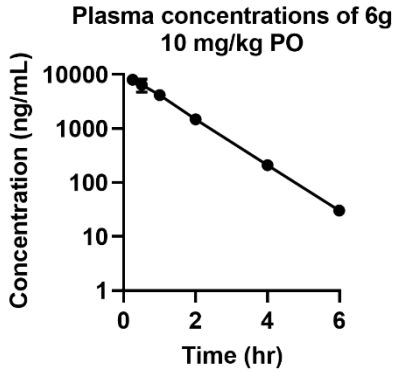


PO Time (hr)	Animal Study No.			Mean PO	SD	CV (%)
	M04	M05	M06			
0.250	71.0	68.3	45.0	**61.4**	**14.3**	**23.3**
0.500	149	115	109	**124**	**21.6**	**17.3**
1.00	162	80.3	125	**122**	**40.9**	**33.4**
2.00	78.6	47.5	112	**79.4**	**32.3**	**40.6**
4.00	15.1	16.8	36.6	**22.8**	**12.0**	**52.3**
6.00	6.92	10.1	12.6	**9.87**	**2.85**	**28.8**
24.0	BQL	BQL	BQL	**ND**	**ND**	**ND**
PK Parameters	**M04**	**M05**	**M06**	**Mean PO**	**SD**	**CV (%)**
Rsq adj	0.918	0.962	1.000	--	--	--
No. points used for $T_{1/2}$	3.00	4.00	3.00	**ND**	--	--
C_{max} (ng/mL)	162	115	125	**134**	**24.8**	**18.5**
T_{max} (hr)	1.00	0.500	1.00	**0.833**	**0.289**	**34.6**
$T_{1/2}$ (hr)	1.14	1.64	1.27	**1.35**	**0.261**	**19.3**
T_{last} (hr)	6.00	6.00	6.00	**6.00**	--	--
$AUC_{0\text{-}last}$ (ng*hr/mL)	327	228	382	**312**	**78.1**	**25.0**
$AUC_{0\text{-}inf}$ (ng*hr/mL)	339	252	405	**332**	**76.8**	**23.1**
$MRT_{0\text{-}last}$ (hr)	1.69	1.91	2.17	**1.92**	**0.243**	**12.6**
$MRT_{0\text{-}inf}$ (hr)	1.89	2.52	2.49	**2.30**	**0.360**	**15.6**
AUC_{Extra} (%)	3.36	9.52	5.70	**6.20**	**3.11**	**50.2**
$AUMC_{Extra}$ (%)	13.6	31.6	17.9	**21.0**	**9.37**	**44.6**
Bioavailability (%)	--	--	--	**18.6**	--	--

Compound 6g: Mouse PK

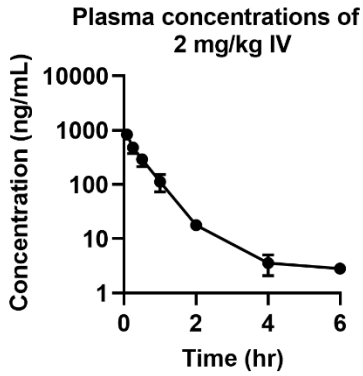


IV Time (hr)	Animal Study No.			Mean IV	SD	CV (%)
	M01	M02	M03			
0.0830	2190	2650	2240	**2360**	**252**	**10.7**
0.250	1560	1910	1500	**1657**	**221**	**13.4**
0.500	1090	1360	1040	**1163**	**172**	**14.8**
1.00	669	973	662	**768**	**178**	**23.1**
2.00	145	155	135	**145**	**10.0**	**6.90**
4.00	13.9	10.1	7.06	**10.4**	**3.43**	**33.1**
6.00	1.88	1.89	1.36	**1.71**	**0.303**	**17.7**
24.0	BQL	BQL	BQL	**ND**	**ND**	**ND**
PK Parameters	**M01**	**M02**	**M03**	**Mean IV**	**SD**	**CV (%)**
Rsq adj	0.996	0.986	0.987	--	--	--
No. points used for $T_{1/2}$	3.00	7.00	7.00	**ND**	--	--
C_0 (ng/mL)	2592	3118	2734	**2815**	**272**	**9.67**
$T_{1/2}$ (hr)	0.638	0.547	0.540	**0.575**	**0.0547**	**9.52**
Vd_{ss} (L/kg)	0.883	0.676	0.856	**0.805**	**0.112**	**13.9**
Cl (mL/min/kg)	19.2	15.4	19.9	**18.2**	**2.42**	**13.3**
T_{last} (hr)	6.00	6.00	6.00	**6.00**	--	--
$AUC_{0\text{-}last}$ (ng*hr/mL)	1734	2160	1671	**1855**	**266**	**14.3**
$AUC_{0\text{-}inf}$ (ng*hr/mL)	1735	2162	1672	**1857**	**266**	**14.3**
$MRT_{0\text{-}last}$ (hr)	0.760	0.727	0.712	**0.733**	**0.0245**	**3.34**
$MRT_{0\text{-}inf}$ (hr)	0.766	0.731	0.716	**0.737**	**0.0257**	**3.49**
AUC_{Extra} (%)	0.0997	0.0690	0.0633	**0.0774**	**0.0196**	**25.3**
$AUMC_{Extra}$ (%)	0.901	0.641	0.600	**0.714**	**0.163**	**22.9**

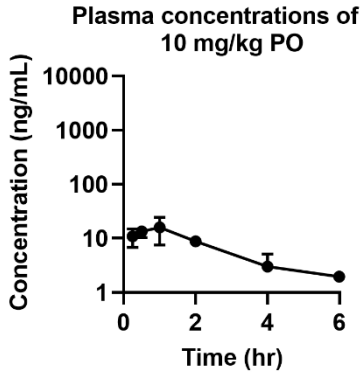


PO Time (hr)	Animal Study No.			Mean PO	SD	CV (%)
	M04	M05	M06			
0.250	9670	6680	7700	**8017**	**1520**	**19.0**
0.500	6330	4790	8310	**6477**	**1765**	**27.2**
1.00	4170	3360	4970	**4167**	**805**	**19.3**
2.00	1430	1550	1480	**1487**	**60.3**	**4.05**
4.00	217	224	190	**210**	**18.0**	**8.54**
6.00	31.4	35.7	24.0	**30.4**	**5.92**	**19.5**
24.0	BQL	BQL	BQL	**ND**	**ND**	**ND**
PK Parameters	**M04**	**M05**	**M06**	**Mean PO**	**SD**	**CV (%)**
Rsq adj	1.000	1.000	1.000	--	--	--
No. points used for $T_{1/2}$	3.00	3.00	3.00	**3.00**	--	--
C_{max} (ng/mL)	9670	6680	8310	**8220**	**1497**	**18.2**
T_{max} (hr)	0.250	0.250	0.500	**0.333**	**0.144**	**43.3**
$T_{1/2}$ (hr)	0.726	0.735	0.673	**0.711**	**0.0338**	**4.75**
T_{last} (hr)	6.00	6.00	6.00	**6.00**	--	--
$AUC_{0\text{-}last}$ (ng*hr/mL)	9806	8188	10511	**9501**	**1191**	**12.5**
$AUC_{0\text{-}inf}$ (ng*hr/mL)	9839	8225	10534	**9533**	**1184**	**12.4**
$MRT_{0\text{-}last}$ (hr)	1.11	1.25	1.10	**1.15**	**0.0844**	**7.32**
$MRT_{0\text{-}inf}$ (hr)	1.13	1.28	1.11	**1.17**	**0.0907**	**7.73**
AUC_{Extra} (%)	0.334	0.460	0.221	**0.339**	**0.120**	**35.4**
$AUMC_{Extra}$ (%)	2.08	2.55	1.39	**2.00**	**0.582**	**29.0**
Bioavailability (%)	--	--	--	**103**	--	--

Compound 6h: Mouse PK

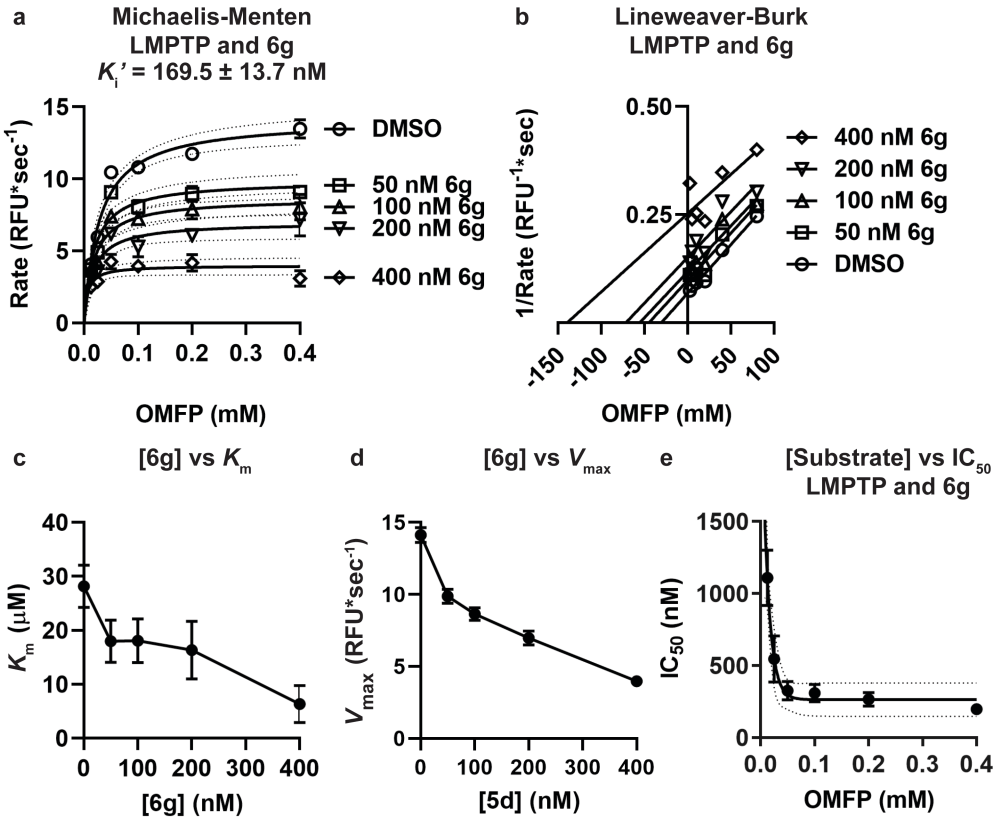


IV Time (hr)	Animal Study No.			Mean IV	SD	CV (%)
	M01	M02	M03			
0.0830	981	665	865	**837**	**160**	**19.1**
0.250	595	378	470	**481**	**109**	**22.6**
0.500	377	232	265	**291**	**76.0**	**26.1**
1.00	158	82.1	99.4	**113**	**39.8**	**35.2**
2.00	16.6	15.7	21.2	**17.8**	**2.95**	**16.5**
4.00	2.10	3.57	5.04	**3.57**	**1.47**	**41.2**
6.00	BQL	2.89	2.77	**2.83**	**ND**	**ND**
24.0	BQL	BQL	BQL	**ND**	**ND**	**ND**
PK Parameters	**M01**	**M02**	**M03**	**Mean IV**	**SD**	**CV (%)**
Rsq adj	0.966	0.851	0.893	**--**	**--**	**--**
No. points used for $T_{1/2}$	6.00	7.00	3.00	**ND**	**--**	**--**
C_0 (ng/mL)	1258	881	1171	**1103**	**198**	**17.9**
$T_{1/2}$ (hr)	0.438	0.743	1.36	**0.848**	**0.471**	**55.5**
Vd_{ss} (L/kg)	2.61	5.17	4.33	**4.04**	**1.30**	**32.3**
Cl (mL/min/kg)	83.2	125	100	**103**	**21.2**	**20.6**
T_{last} (hr)	4.00	6.00	6.00	**ND**	**--**	**--**
$AUC_{0\text{-}last}$ (ng*hr/mL)	543	358	447	**450**	**92.6**	**20.6**
$AUC_{0\text{-}inf}$ (ng*hr/mL)	545	362	452	**453**	**91.6**	**20.2**
$MRT_{0\text{-}last}$ (hr)	0.513	0.632	0.631	**0.592**	**0.0686**	**11.6**
$MRT_{0\text{-}inf}$ (hr)	0.523	0.687	0.720	**0.643**	**0.105**	**16.4**
AUC_{Extra} (%)	0.244	0.857	1.20	**0.768**	**0.486**	**63.3**
$AUMC_{Extra}$ (%)	2.16	8.82	13.3	**8.10**	**5.62**	**69.3**



PO Time (hr)	Animal Study No.			Mean PO	SD	CV (%)
	M04	M05	M06			
0.250	15.6	8.58	8.40	**10.9**	**4.11**	**37.8**
0.500	16.0	14.0	10.1	**13.4**	**3.00**	**22.4**
1.00	25.6	9.76	12.5	**16.0**	**8.47**	**53.1**
2.00	10.4	7.25	8.63	**8.76**	**1.58**	**18.0**
4.00	5.43	1.80	1.77	**3.00**	**2.10**	**70.1**
6.00	2.72	BQL	1.20	**1.96**	**ND**	**ND**
24.0	BQL	BQL	BQL	**ND**	**ND**	**ND**
PK Parameters	**M04**	**M05**	**M06**	**Mean PO**	**SD**	**CV (%)**
Rsq adj	0.999	0.944	0.782	--	--	--
No. points used for $T_{1/2}$	3.00	3.00	3.00	**3.00**	--	--
C_{max} (ng/mL)	25.6	14.0	12.5	**17.4**	**7.17**	**41.3**
T_{max} (hr)	1.00	0.500	1.00	**0.833**	**0.289**	**34.6**
$T_{1/2}$ (hr)	2.07	1.19	1.41	**1.55**	**0.457**	**29.4**
T_{last} (hr)	6.00	4.00	6.00	**ND**	--	--
$AUC_{0\text{-}last}$ (ng*hr/mL)	56.3	26.0	31.1	**37.8**	**16.2**	**42.9**
$AUC_{0\text{-}inf}$ (ng*hr/mL)	64.4	29.1	33.5	**42.3**	**19.2**	**45.4**
$MRT_{0\text{-}last}$ (hr)	2.08	1.53	1.90	**1.84**	**0.279**	**15.2**
$MRT_{0\text{-}inf}$ (hr)	2.95	1.98	2.35	**2.42**	**0.490**	**20.2**
AUC_{Extra} (%)	12.6	10.6	7.27	**10.2**	**2.69**	**26.5**
$AUMC_{Extra}$ (%)	38.4	30.7	24.8	**31.3**	**6.80**	**21.7**
Bioavailability (%)	--	--	--	**2.90**	--	--

MOA of 6g



Supplemental Figure 4. Analog **6g** inhibits LMPTP with an uncompetitive MOA. (**a**–**b**) Activity of 20 nM human LMPTP-A on increasing concentrations of OMFP in the presence of increasing concentrations of **6g**. (**a**) Mean±SEM reaction rate vs. OMFP concentration is shown. Lines show fitting to the Michaelis-Menten equation with 95% confidence interval. Mean±SEM K_i' is shown. (**b**) Lineweaver-Burk plot of data from (**a**). Lines show fitting to a linear regression. (**c-d**) Mean±SEM K_m (**c**) and V_{max} (**d**) values for each concentration of inhibitor from the Michaelis-Menten curves in (**a**) are shown. (**e**) IC_{50} values were calculated for **6g** on 20 nM human LMPTP-A-catalyzed hydrolysis of increasing concentrations of OMFP. Mean±SEM IC_{50} from 3 independent experiments performed in triplicate is shown. Lines show fitting to the one-phase decay equation with 95% confidence interval shown. (**a-e**) Data from 3 independent experiments performed in triplicate is shown.