Supporting information

Novel caffeine derivatives with antiproliferative activity

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1. Chemistry experimental materials and methods

All commercial reagents used were of the highest available purity from Sigma-Aldrich (Czech Republic). Solvents for synthesis were obtained from Penta chemicals Co. Acetonitrile, phosphate buffer saline pellets (PBS) and formic acid for LC-MS analyses were purchased from Sigma-Aldrich in LC-MS grade purity (Czech Republic). Ultrapure water of ASTM I type for LC-MS analyses were prepared with Barnstead SmartPure 3 UV/UF apparatus (Germany). The reactions were monitored by TLC (Merck silica gel 60 F254 analytical plates; detection UV 254). Purity of all products was tested by determination of their uncorrected melting points (Melting Point Apparatus – Stuart SMP30). The ^{1}H NMR and ^{13}C NMR spectra of new compounds were recorded in $CDCl_3$ or DMSO-*d6* solution at ambient temperature on a Varian S500 spectrometer (499.87 MHz for 1H, and 125.71 MHz for 13C)

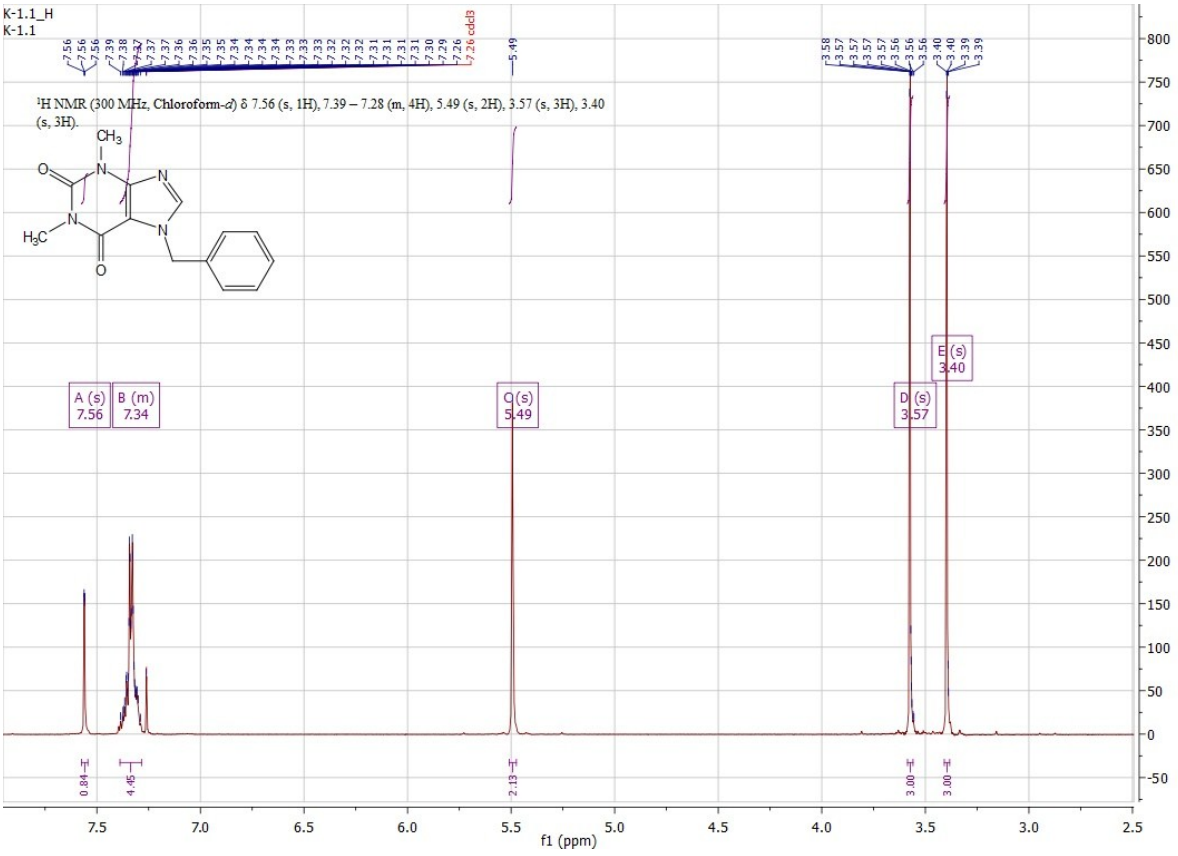
and Varian Mercury VX BB 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C). For ^{1}H δ are given in parts per million (ppm) relative to $CDCl_3$ (δ = 7.26) or DMSO–*d6* (δ = 2.50) and for ^{13}C relative to $CDCl_3$ (δ = 77.16) or DMSO-*d6* (δ = 39.52). The coupling constants (*J*) are expressed in Hertz.

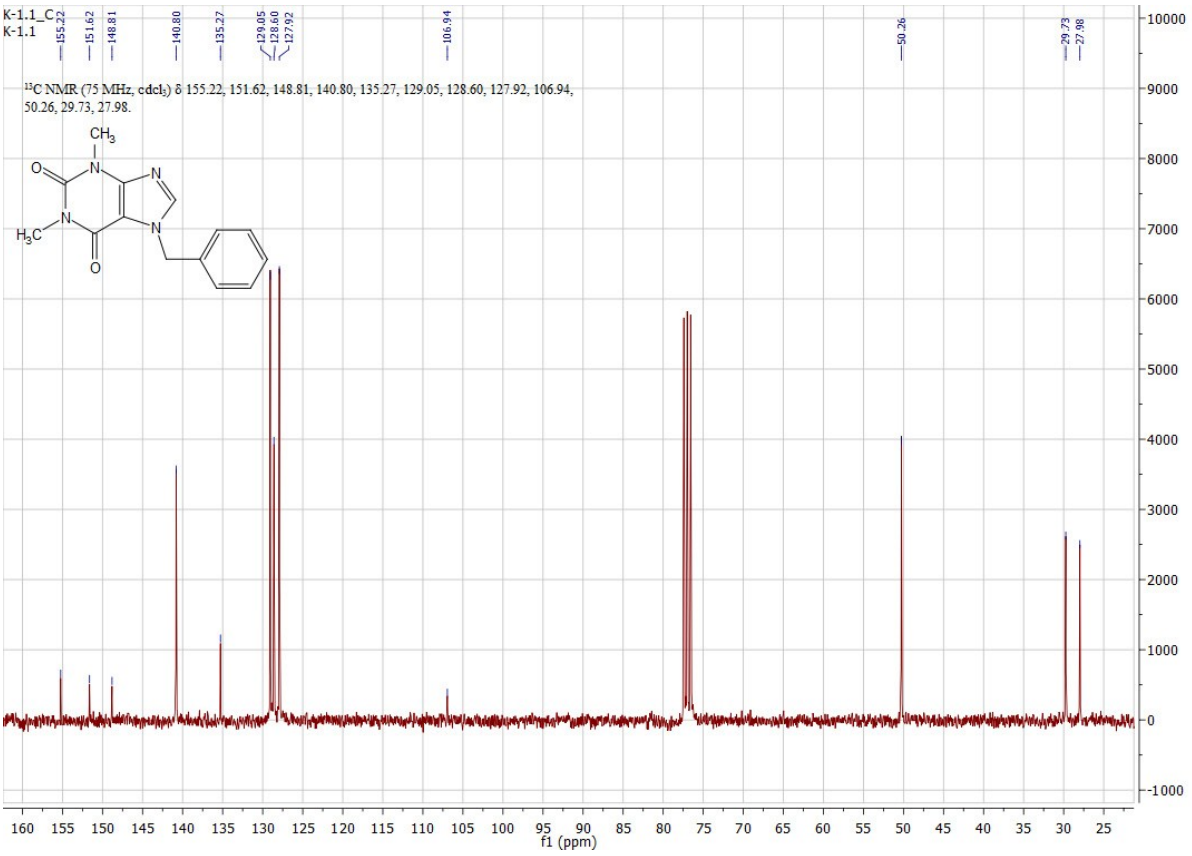
High resolution mass spectra (HRMS) and uncalibrated purity of the compounds were measured bya Dionex UltiMate 3000 analytical LC-MS system coupled with a Q Exactive Plus hybrid quadrupole-orbitrap spectrometer (both produced by ThermoFisher Scientific, Bremen, Germany). The LC-MS system consisted of a binary pump HPG-3400RS connected to a vacuum degasser, a heated column compartment TCC-3000, an autosampler WTS-3000 equipped with a 100µL loop and a VWD-3000 ultraviolet detector. A Waters Atlantis dC18 (2.1 x 100mm/3µm) column was used as the stationary phase. Acetonitrile and water used in the analyses were acidified with 0.1% (v) of formic acid. Ions for mass spectrometry (MS) were generated by heated electro-spray ionization (HESI) working in positive mode, with the following settings: sheath gas flow rate 40, aux gas flow rate 10, sweep gas flow rate 2, spray voltage 3.2 kV, capillary temperature 350°C, aux gas temperature 300°C, S-lens RF level 50. The full-scan MS analyses monitored ions within m/z range 100 – 1500, operating on a resolution level of 70 000. Compounds **1-19**, **21-25** and **27** were dissolved in methanol and injected into the LC-MS system in volume of 1µL. 1. Elution was carried out by a linear gradient method mixing acetonitrile (with 0.1% (v/v) of formic acid) and water (with 0.1% (v/v) of formic acid): 0 – 1 min: 10% acetonitrile, 1 – 4 min: 10% – 100% acetonitrile, 4 – 5 min: 100% acetonitrile, 5 – 7.5 min: 10% acetonitrile. The flow-rate in the gradient elution was set to 0.4 mL/min. The chromatograms and mass spectra were processed on Chromeleon 6.80 and Xcalibur 3.0.63 software, respectively. For prediction of compounds' partitioning, a chromatographic column with Immobilized Artificial Membrane (IAM) - Rexchrom IAM.PC.DD.2 (150 x 4.6 x 12um 300Å) was used. Elution was carried out isocratically by phosphate-buffer-saline (PBS, 10mM, ph = 7.4) and acetonitrile (4:1). The flow-rate was 1.1mL/min and detection performed by UV-detector operating at 210nm.

*1.1. General synthesis of novel caffeine derivatives (**1**-**17**).*

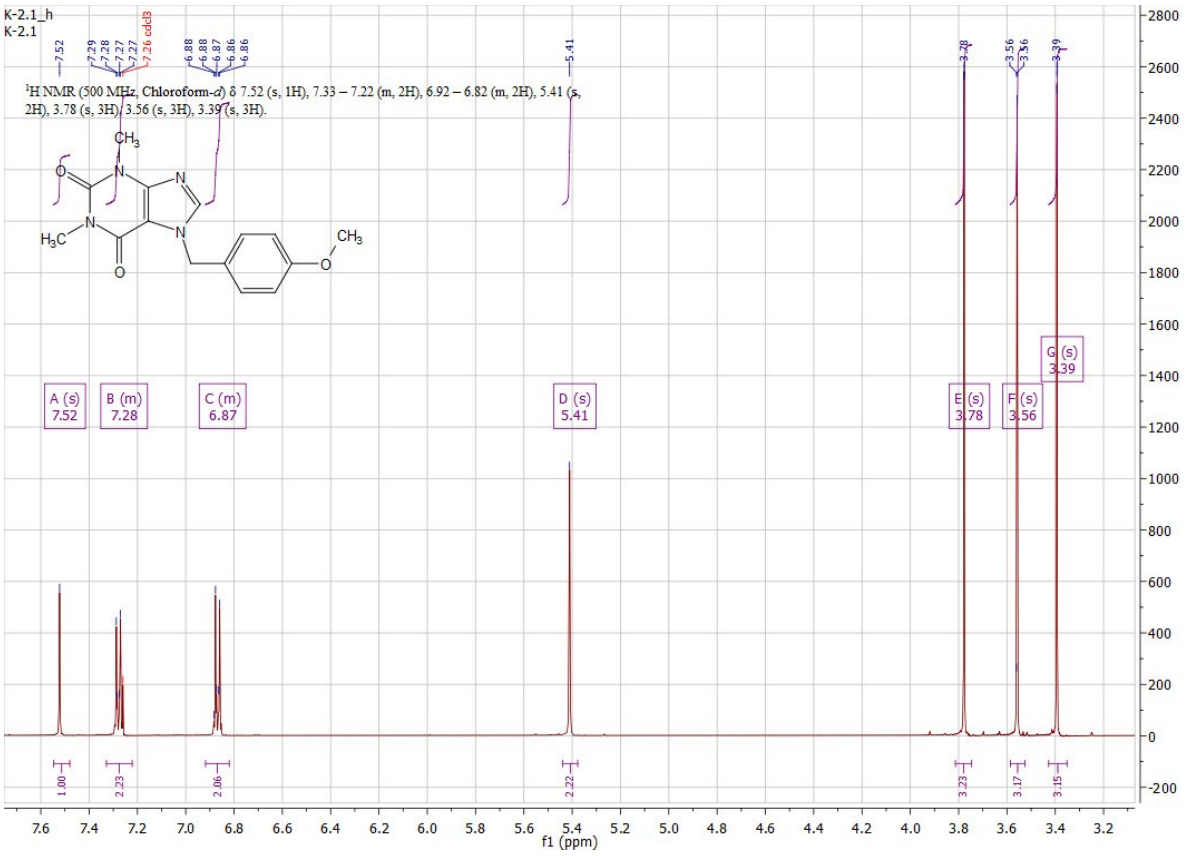
To a suspension of theophylline (1.0 g, 5.55 mmol) and potassium carbonate (0.86 g) in DMF (15 mL), the appropriate benzyl bromide, cyclohexylmethyl bromide or (1-bromoethyl)benzene (6.11 mmol) was added dropwise. The reaction mixture was heated at 100 °C for 4 hours. After cooling down, 30 mL of water was added. The resulting solid was filtered and washed with water (2 × 30 mL) and diethylether (2 × 30 mL) and dried under vacuum.

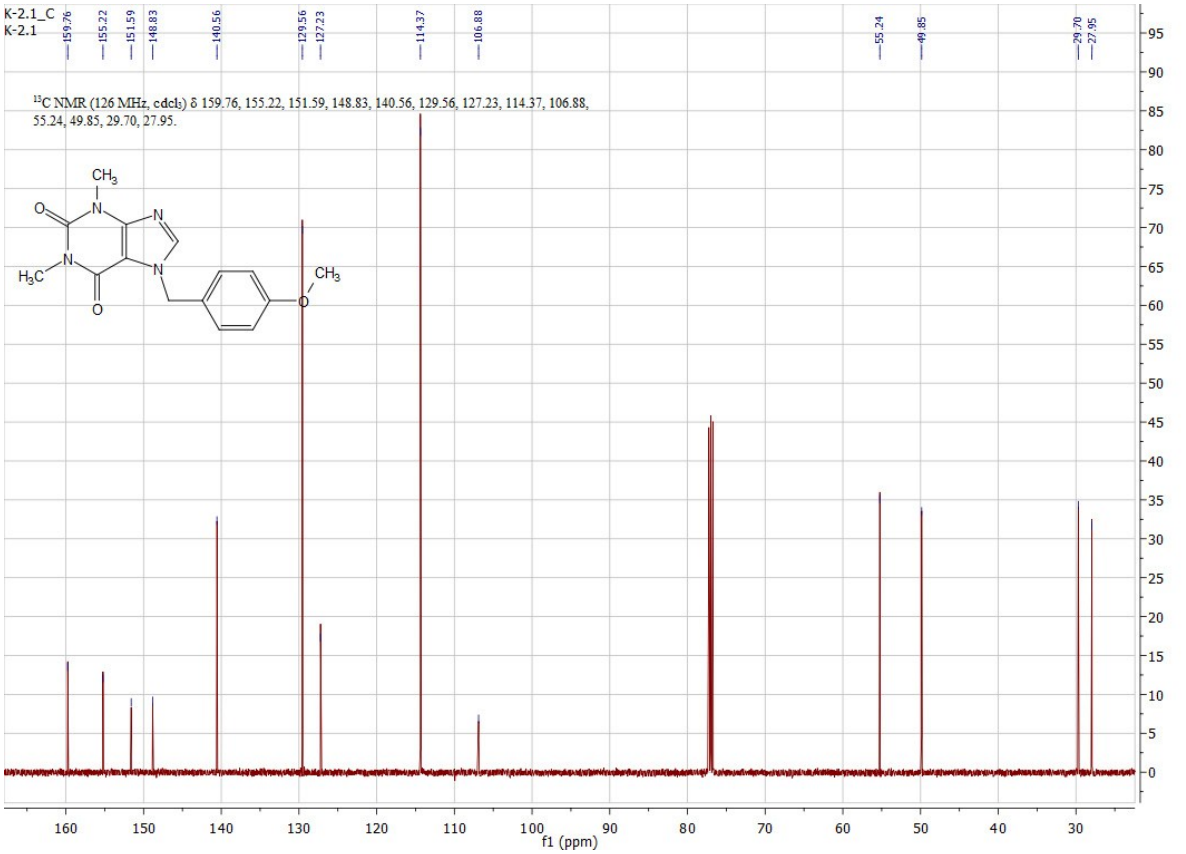
*1.1.1. 7-benzyl-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**1**).* White solid (1.38 g, 92 %); m.p. 158-159 °C; ^{1}H NMR (300 MHz, Chloroform-d) δ 7.56 (s, 1H), 7.39 – 7.28 (m, 4H), 5.49 (s, 2H), 3.57 (s, 3H), 3.40 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 155.22, 151.62, 148.81, 140.80, 135.27, 129.05, 126.60, 127.92, 106.94, 50.26, 29.73, 27.98; HRMS: *m/z* $[M+H]^+$ 271.1184 (calculated for: $[C_{14}H_{15}N_4O_2]^+$ 271.1189).



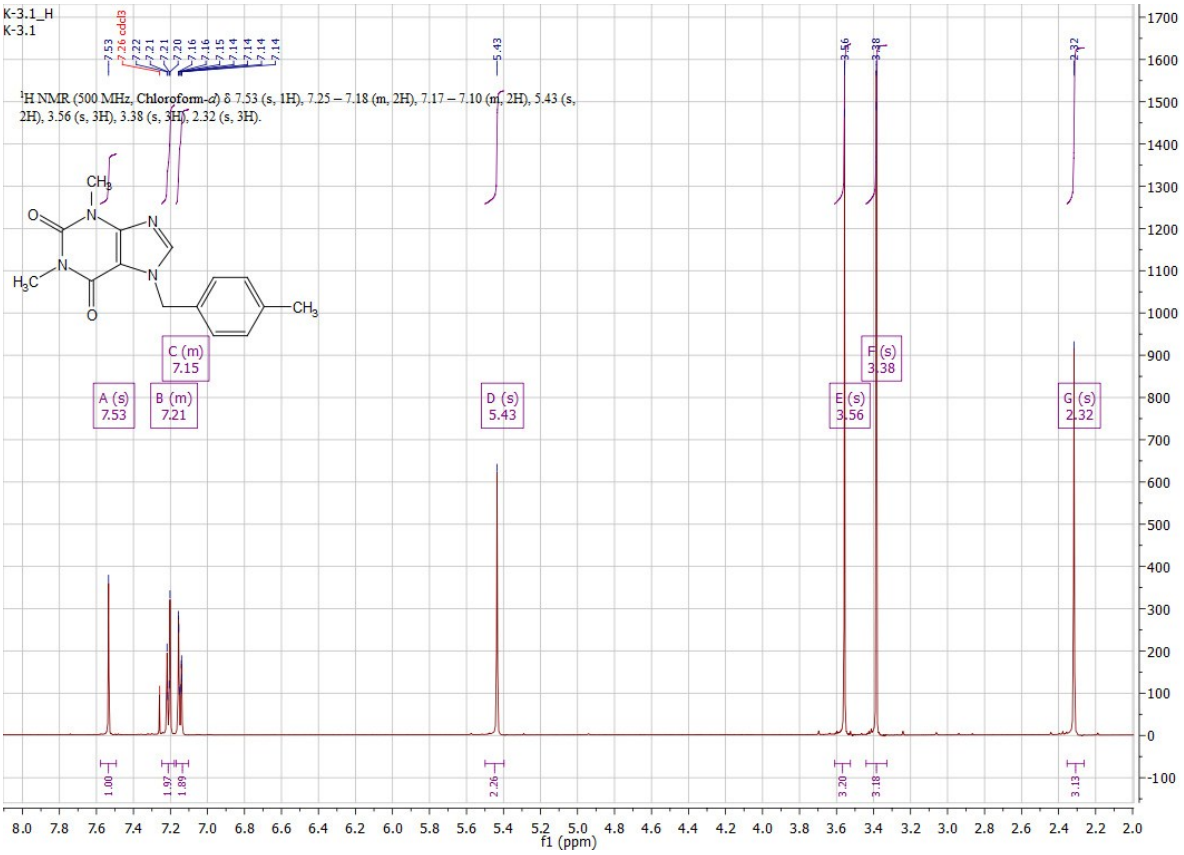


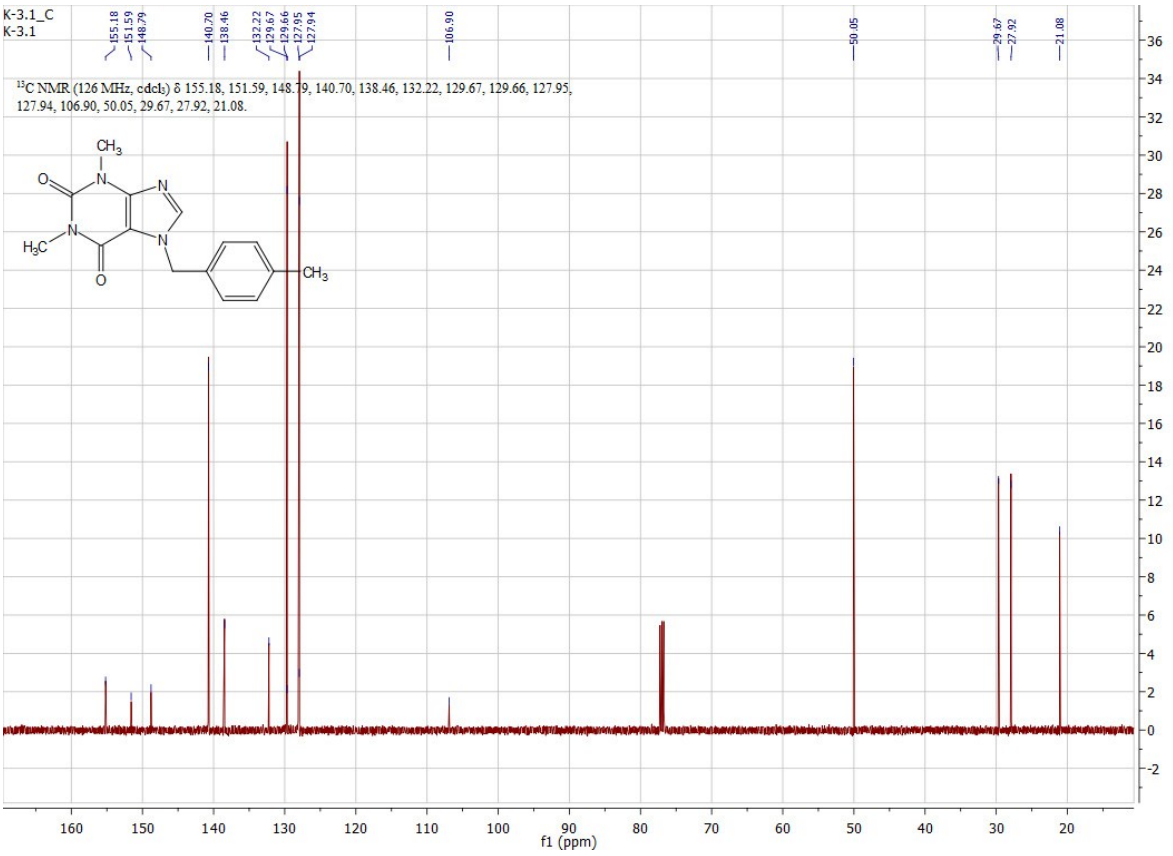
*1.1.2. 7-[(4-methoxyphenyl)methyl]-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**2**).* White solid (1.03 g, 62 %); m.p. 155-156 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.52 (s, 1H), 7.33-7.22 (m, 2H), 6.92-6.82 (m, 2H), 5.41 (s, 2H), 3.78 (s, 3H), 3.56 (s, 3H), 3.39 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 159.76, 155.22, 151.59, 148.83, 140.56, 129.56, 127.23, 114.37, 106.88, 55.24, 49.85, 29.70, 27.95; HRMS: *m/z* $[M+H]^+$ 301.1290 (calculated for: $[C_{15}H_{17}N_4O_3]^+$ 301.1293).


K-2.1_h
K-2.1
7.52
7.29
7.28
7.27
7.27
7.26 cdcl3
6.88
6.88
6.87
6.86
6.86
5.41
3.78
3.56
3.56
3.39
^{1}H NMR (500 MHz, Chloroform-d) δ 7.52 (s, 1H), 7.33 – 7.22 (m, 2H), 6.92 – 6.82 (m, 2H), 5.41 (s, 2H), 3.78 (s, 3H), 3.56 (s, 3H), 3.39 (s, 3H).
A (s) 7.52
B (m) 7.28
C (m) 6.87
D (s) 5.41
E (s) 3.78
F (s) 3.56
G (s) 3.39
1.00
2.23
2.06
2.22
3.23
3.17
3.15
f1 (ppm)

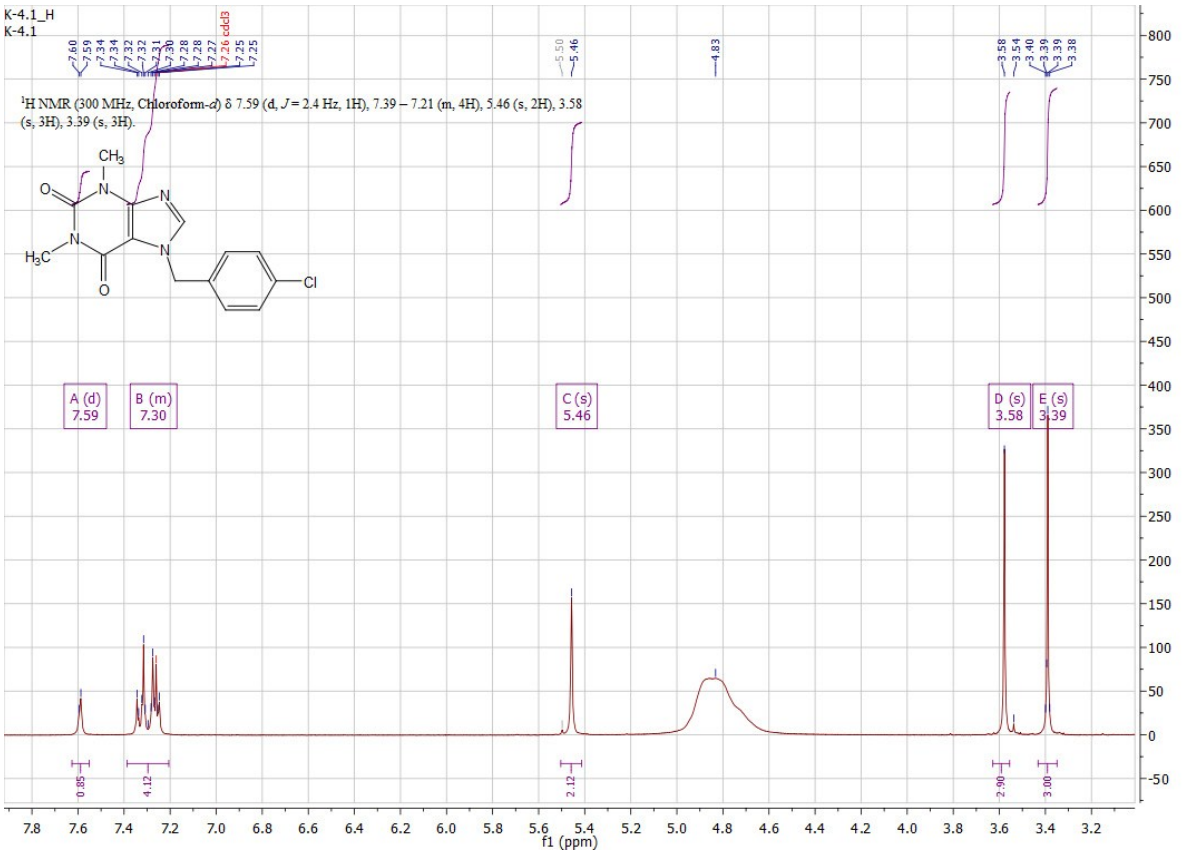

K-2.1_C
K-2.1
159.76
155.22
151.59
148.83
140.56
129.56
127.23
114.37
106.88
55.24
49.85
29.70
27.95
^{13}C NMR (126 MHz, cdcl$_3$) δ 159.76, 155.22, 151.59, 148.83, 140.56, 129.56, 127.23, 114.37, 106.88, 55.24, 49.85, 29.70, 27.95.
f1 (ppm)

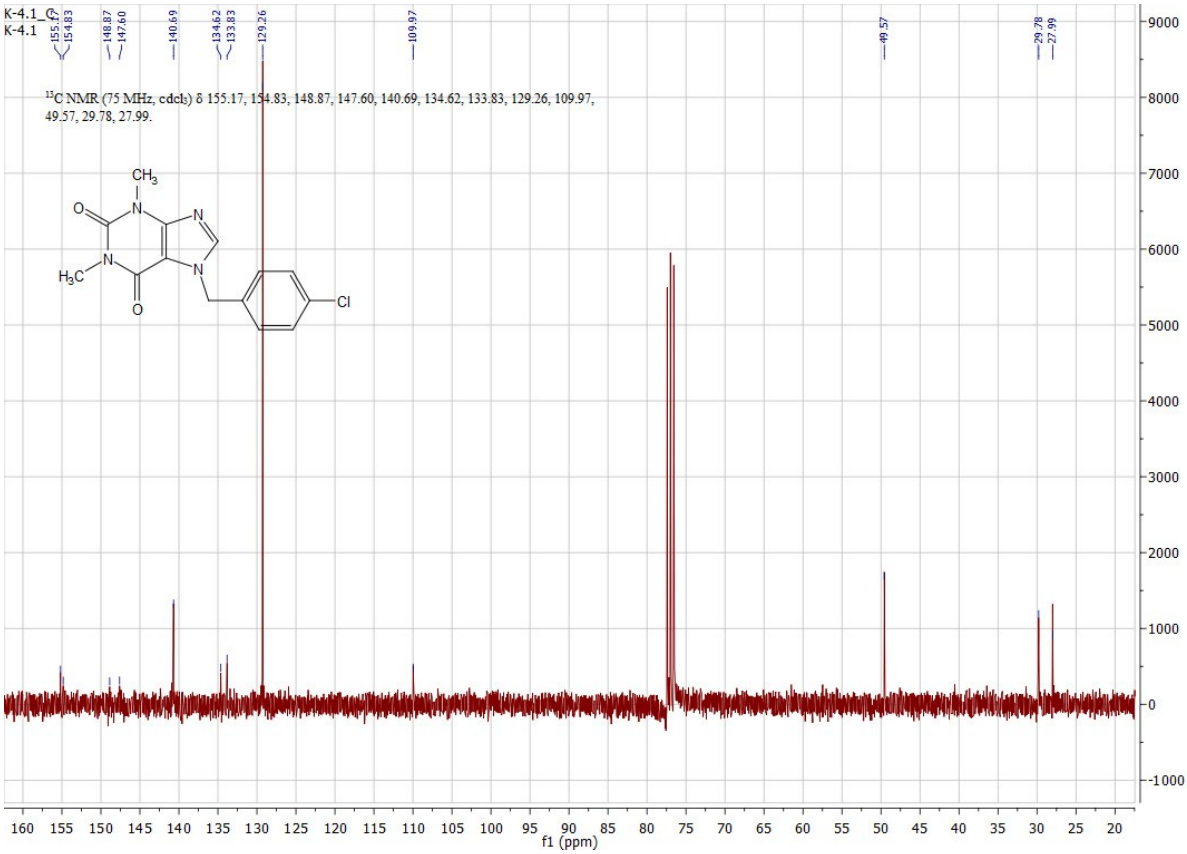
*1.1.3. 1,3-dimethyl-7-[(4-methylphenyl)methyl]-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**3**).* White solid (1.47 g, 92 %); m.p. 214-218 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.53 (s, 1H), 7.25 - 7.18 (m, 2H), 7.17 - 7.10 (m, 2H), 5.43 (s, 2H), 3.56 (s, 3H), 3.38 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 155.18, 151.59, 148.79, 140.70, 138.46, 132.22, 129.67, 127.95, 106.90, 50.05, 29.67, 27.92, 21.08; HRMS: *m/z* $[M]^+$ 285.1340 (calculated for: $[C_{15}H_{17}N_4O_2]^+$ 285.1346).



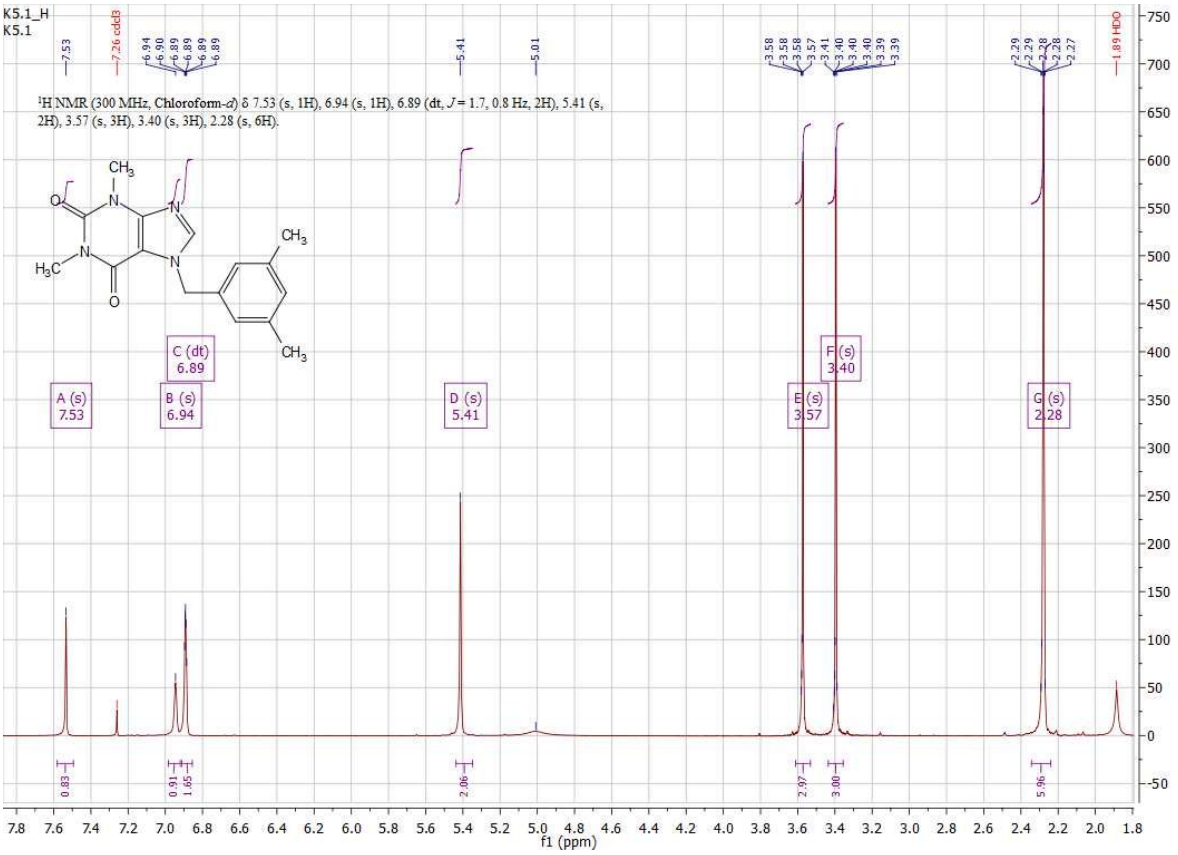


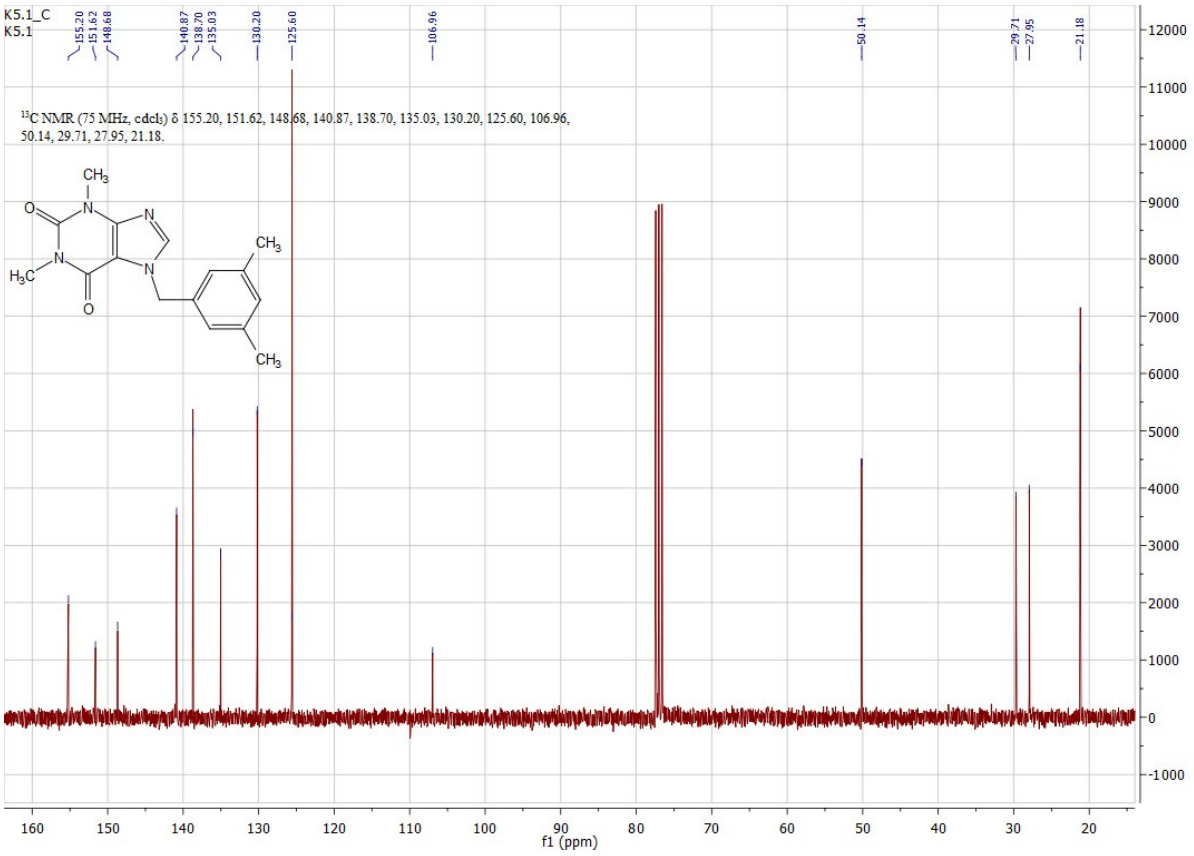
*1.1.4. 7-[(4-chlorophenyl)methyl]-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**4**).* White solid (1.09 g, 64 %); m.p. 227-231 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.59 (s, 1H), 7.39 – 7.21 (m, 4H), 5.46 (s, 2H), 3.58 (s, 3H), 3.39 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 155.17, 154.83, 148.87, 140.69, 134.62, 133.83, 129.26, 109.97, 49.58, 29.78, 27.99; HRMS: *m/z* $[M+H]^+$ 305.0796 (calculated for: $[C_{14}H_{14}ClN_4O_2]^+$ 305.0800).


K-4.1_H
K-4.1
^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.59 (d, *J* = 2.4 Hz, 1H), 7.39 – 7.21 (m, 4H), 5.46 (s, 2H), 3.58 (s, 3H), 3.39 (s, 3H).
A (d) 7.59
B (m) 7.30
C (s) 5.46
D (s) 3.58
E (s) 3.39
f1 (ppm)

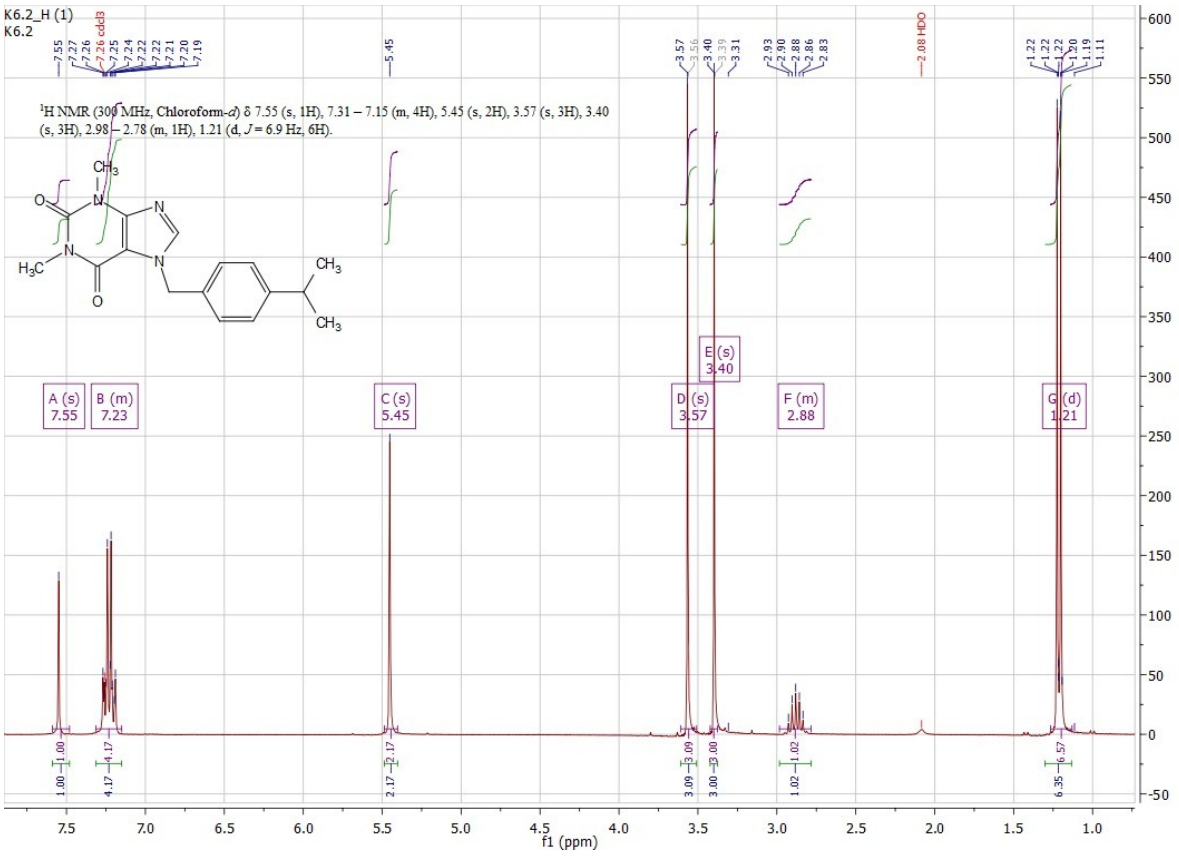

K-4.1_C
K-4.1
^{13}C NMR (75 MHz, cdcl$_3$) δ 155.17, 154.83, 148.87, 147.60, 140.69, 134.62, 133.83, 129.26, 109.97, 49.57, 29.78, 27.99.
f1 (ppm)

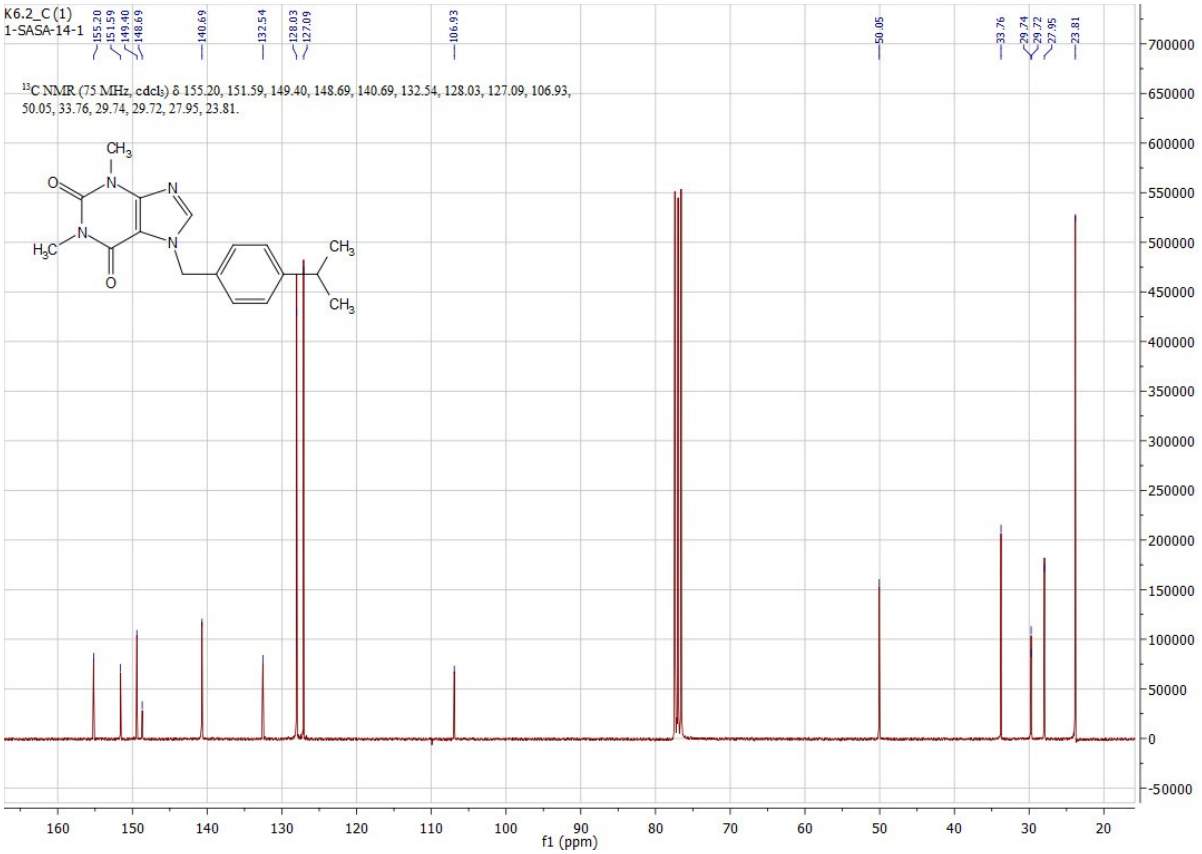
*1.1.5. 7-[(3,5-dimethylphenyl)methyl]-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**5**).* White solid (1.15 g, 69 %); m.p. 136-138 °C; ^{1}H NMR (300 MHz, Chloroform-d) δ 7.53 (s, 1H), 6.94 (s, 1H), 6.89 (dt, J = 1.7, 0.8 Hz, 2H), 5.41 (s, 2H), 3.57 (s, 3H), 3.40 (s, 3H), 2.28 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 155.20, 151.62, 148.68, 140.87, 138.70, 135.03, 130.20, 125.60, 106.96, 50.14, 29.71, 27.95, 21.18.; HRMS: *m/z* $[M+H]^+$ 299.1499 (calculated for: $[C16H19N4O2]^+$ 299.1503).



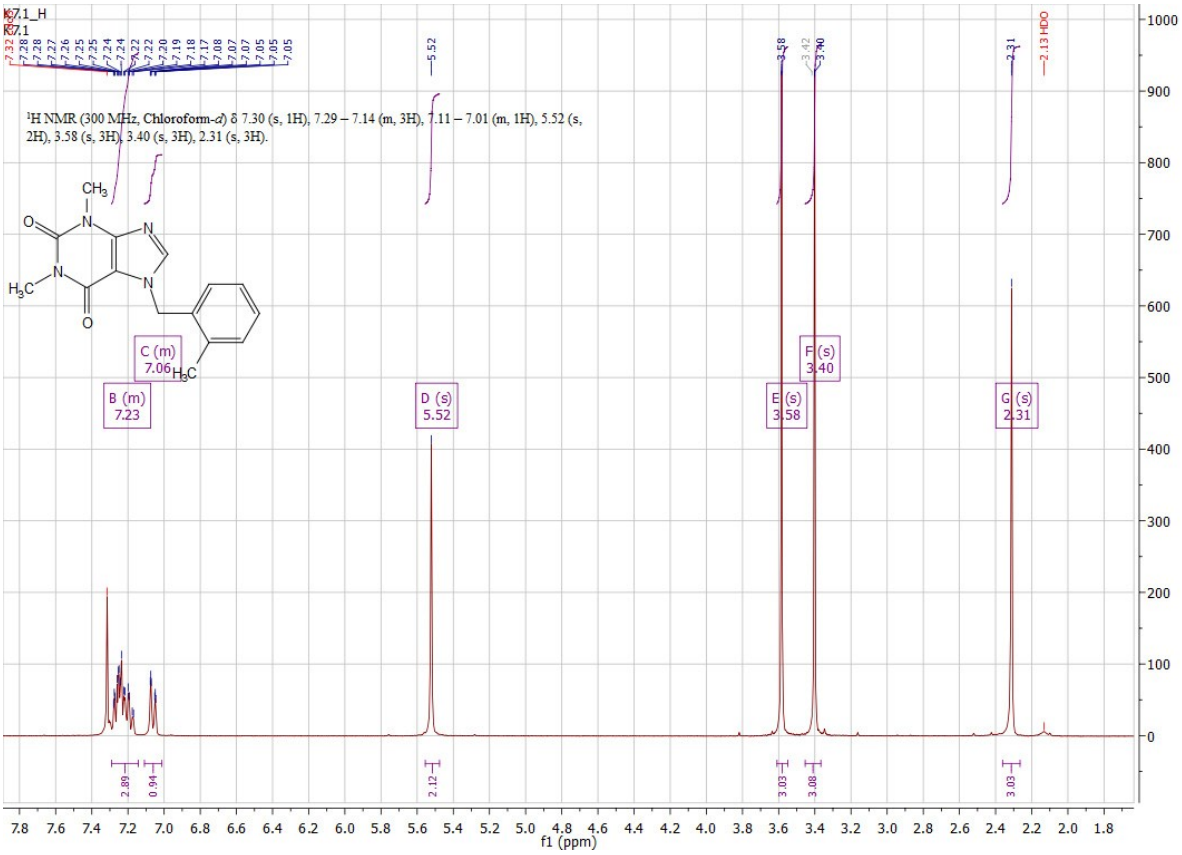


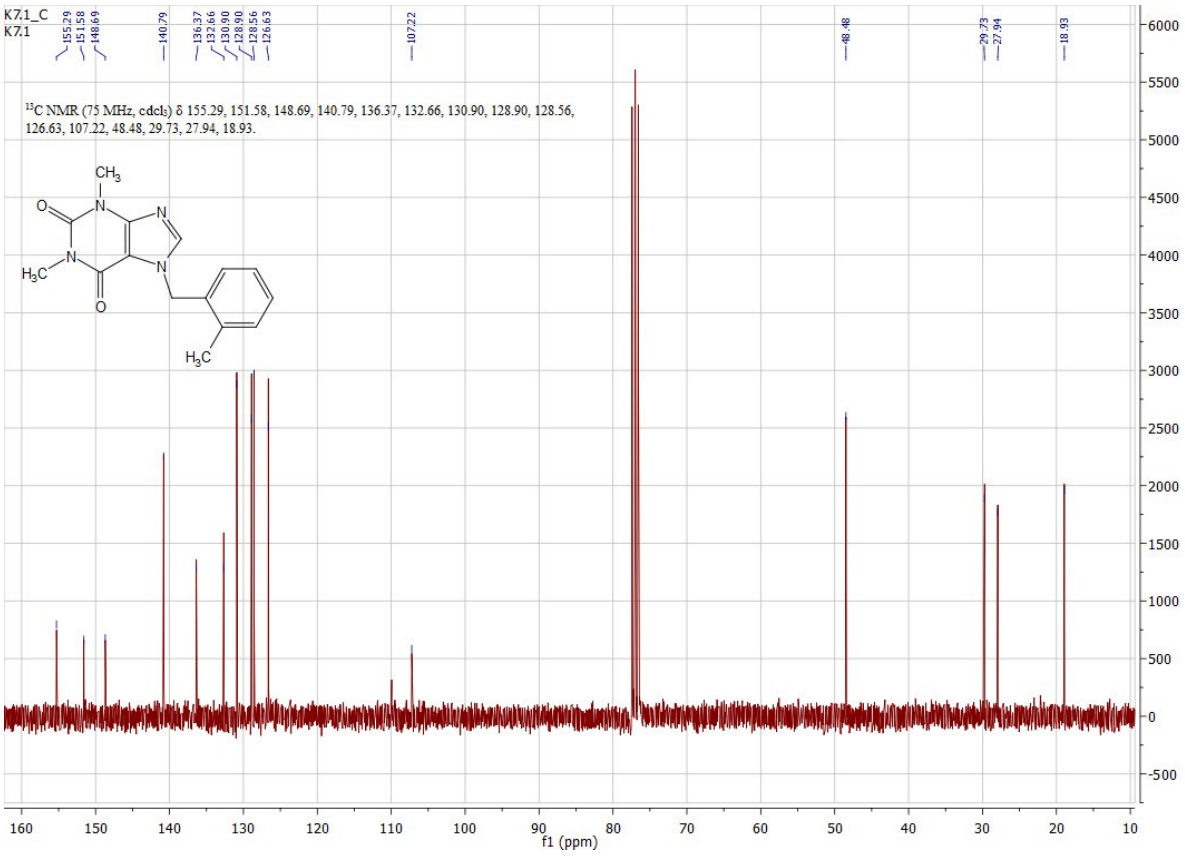
1.1.6. 1,3-dimethyl-7-{[4-(propan-2-yl)phenyl]methyl}-2,3,6,7-tetrahydro-1H-purine-2,6-dione ***(6)***. White solid (1.17 g, 67 %); m.p. 129-131 °C; ^{1}H NMR (300 MHz, Chloroform-d) δ 7.55 (s, 1H), 7.31 – 7.15 (m, 4H), 5.45 (s, 2H), 3.57 (s, 3H), 3.40 (s, 3H), 2.98 – 2.78 (m, 1H), 1.21 (d, J = 6.9 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 155.20, 151.59, 149.40, 148.69, 140.69, 132.54, 128.03, 127.09, 106.93, 50.05, 33.76, 29.74, 27.95, 23.81; HRMS: *m/z* $[M+H]^+$ 313.1660 (calculated for: $[C_{17}H_{21}N_4O_2]^+$ 313.1659).


K6.2_H (1)
K6.2
^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.55 (s, 1H), 7.31 – 7.15 (m, 4H), 5.45 (s, 2H), 3.57 (s, 3H), 3.40 (s, 3H), 2.98 – 2.78 (m, 1H), 1.21 (d, *J* = 6.9 Hz, 6H).
A (s) 7.55
B (m) 7.23
C (s) 5.45
D (s) 3.57
E (s) 3.40
F (m) 2.88
G (d) 1.21
f1 (ppm)

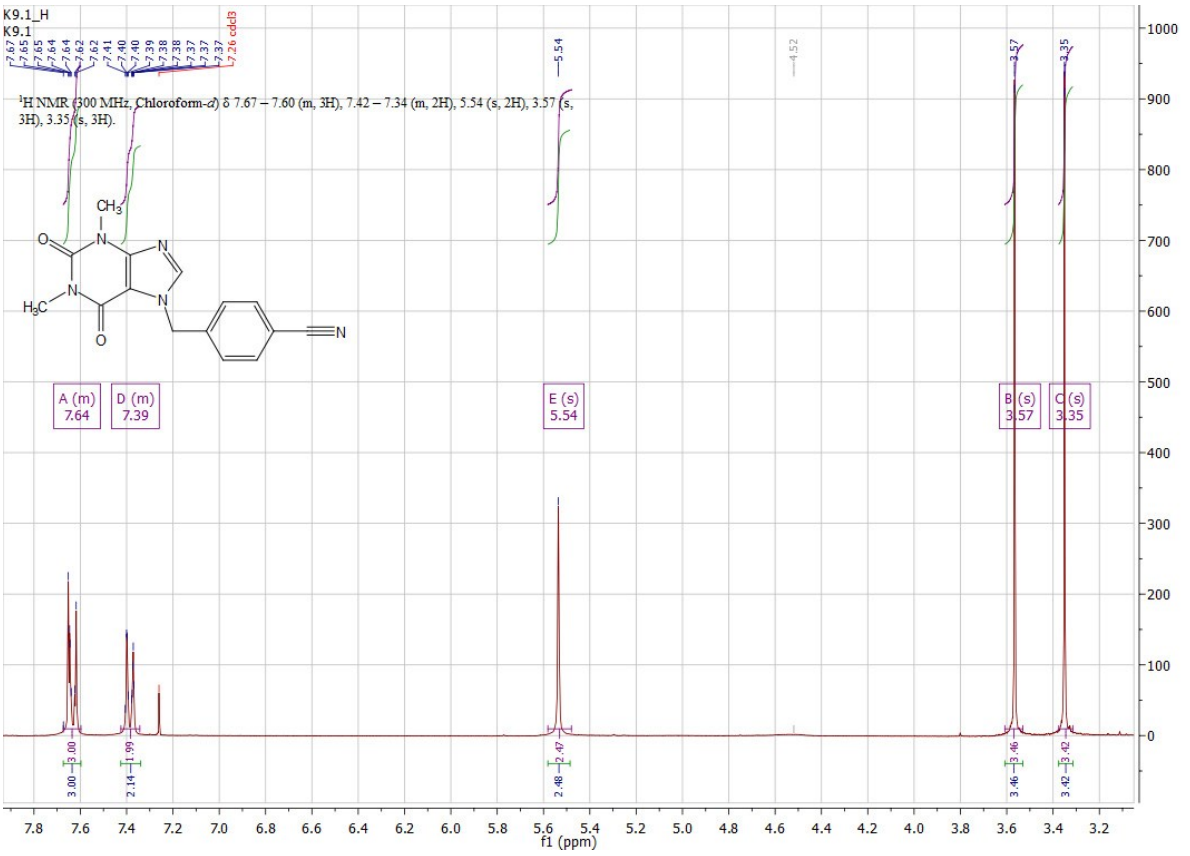

K6.2_C (1)
1-SASA-14-1
^{13}C NMR (75 MHz, cdcl$_3$) δ 155.20, 151.59, 149.40, 148.69, 140.69, 132.54, 128.03, 127.09, 106.93, 50.05, 33.76, 29.74, 29.72, 27.95, 23.81.
f1 (ppm)

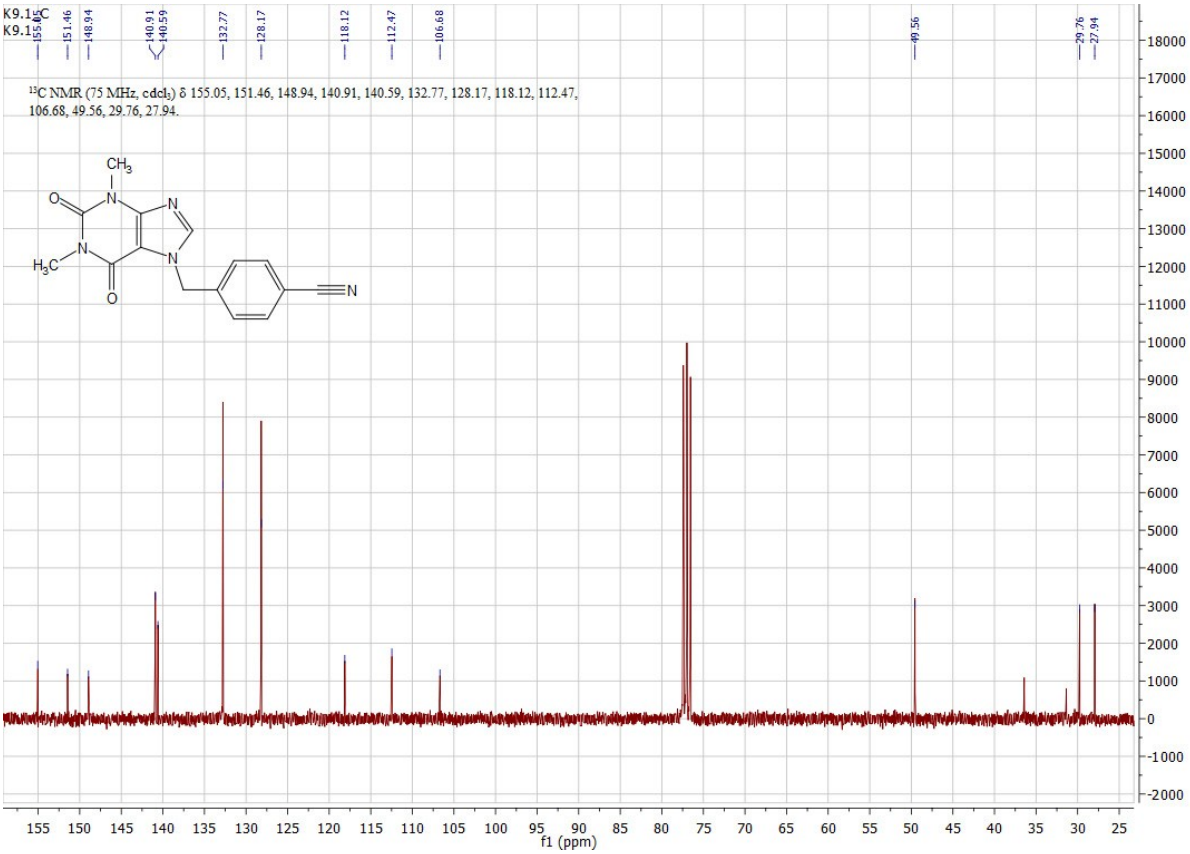
*1.1.7. 1,3-dimethyl-7-[(2-methylphenyl)methyl]-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**7**).* White solid (1.36 g, 86 %); m.p. 156-158 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.30 (s, 1H), 7.29 – 7.14 (m, 3H), 7.11 (m, 1H), 5.52 (s, 2H), 3.58 (s, 3H), 3.40 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 155.29, 151.58, 148.69, 140.79, 136.37, 132.66, 130.90, 128.90, 128.56, 126.63, 107.22, 48.48, 29.73, 27.94, 18.93; HRMS: *m/z* $[M+H]^+$ 285.1346 (calculated for: $[C_{15}H_{17}N_4O_2]^+$ 285.1346).



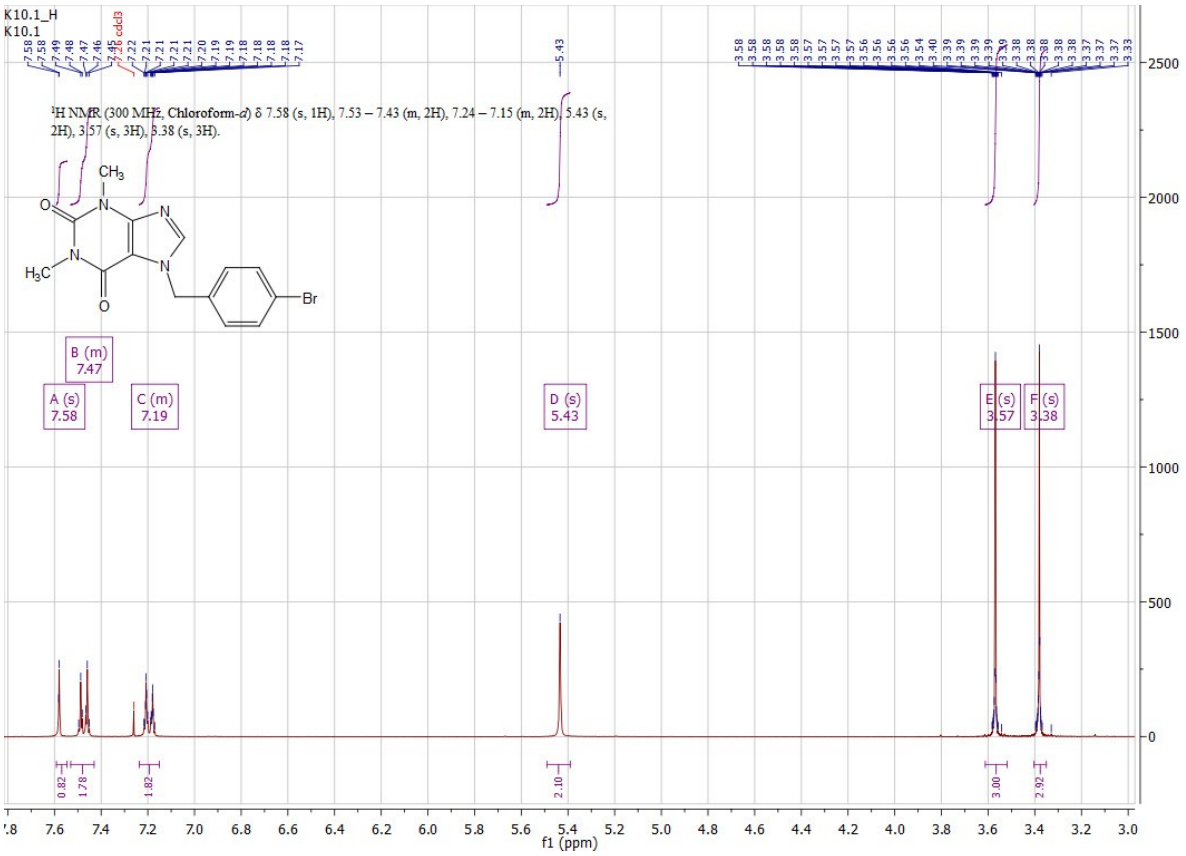


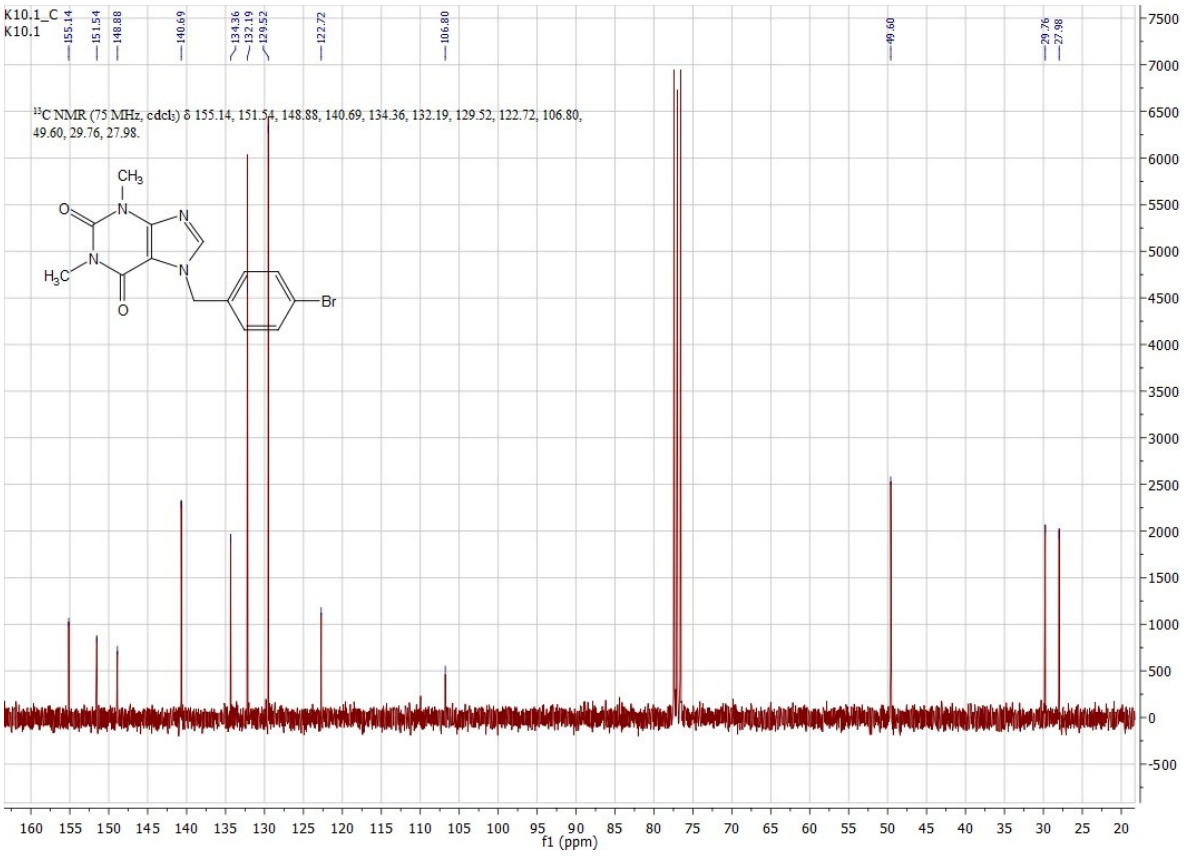
*1.1.8. 4-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)methyl]benzonitrile (**8**).* White solid (1.39 g, 84 %); m.p. 250-252 °C; ^{1}H NMR (300 MHz, Chloroform-d) δ 7.67 – 7.60 (m, 3H), 7.42 – 7.34 (m, 2H), 5.54 (s, 2H), 3.57 (s, 3H), 3.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 155.05, 151.46, 148.94, 140.91, 140.59, 132.77, 128.17, 118.12, 112.47, 106.68, 49.56, 29.76, 27.94; HRMS: *m/z* $[M+H]^+$ 296.1151 (calculated for: $[C_{15}H_{14}N_5O_2]^+$ 296.1142).


K9.1_H
K9.1
^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.67 – 7.60 (m, 3H), 7.42 – 7.34 (m, 2H), 5.54 (s, 2H), 3.57 (s, 3H), 3.35 (s, 3H).
A (m) 7.64
D (m) 7.39
E (s) 5.54
B (s) 3.57
C (s) 3.35
f1 (ppm)

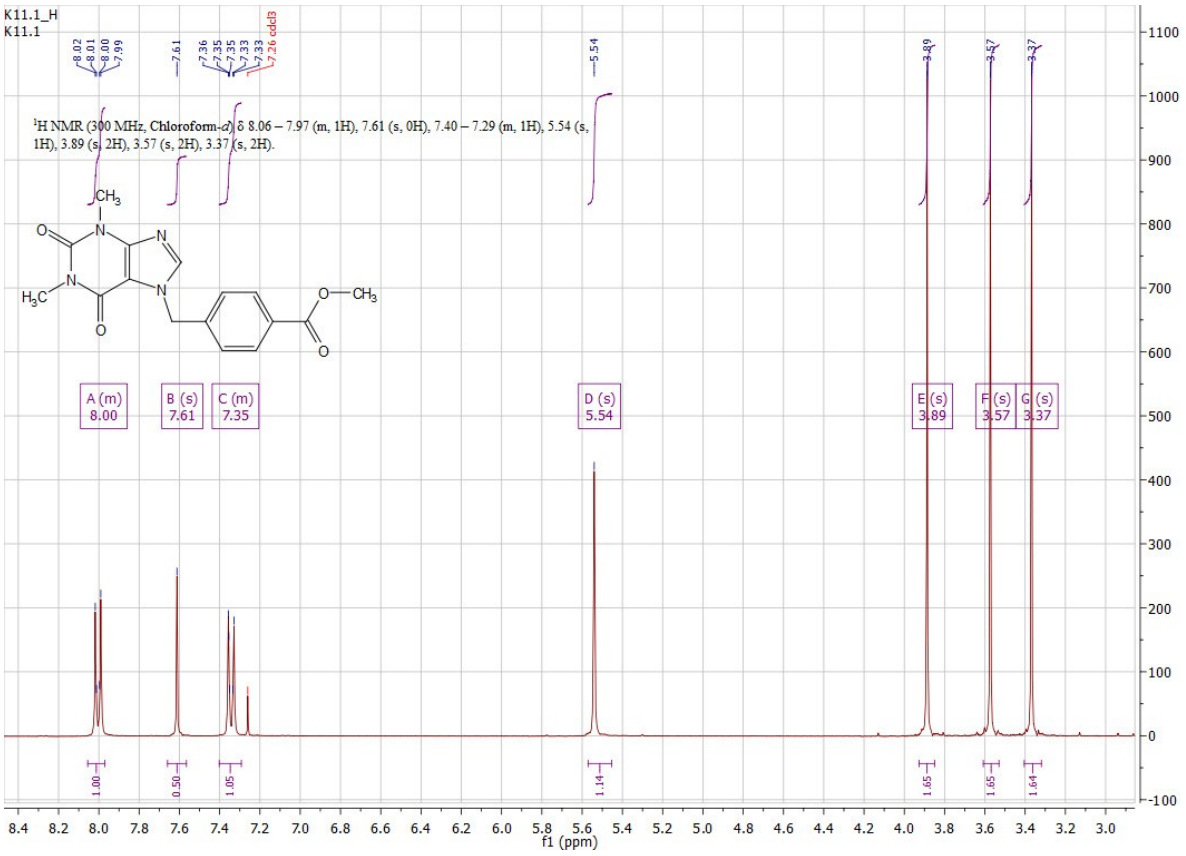

K9.1_C
K9.1
^{13}C NMR (75 MHz, cdcl$_3$) δ 155.05, 151.46, 148.94, 140.91, 140.59, 132.77, 128.17, 118.12, 112.47, 106.68, 49.56, 29.76, 27.94.
f1 (ppm)

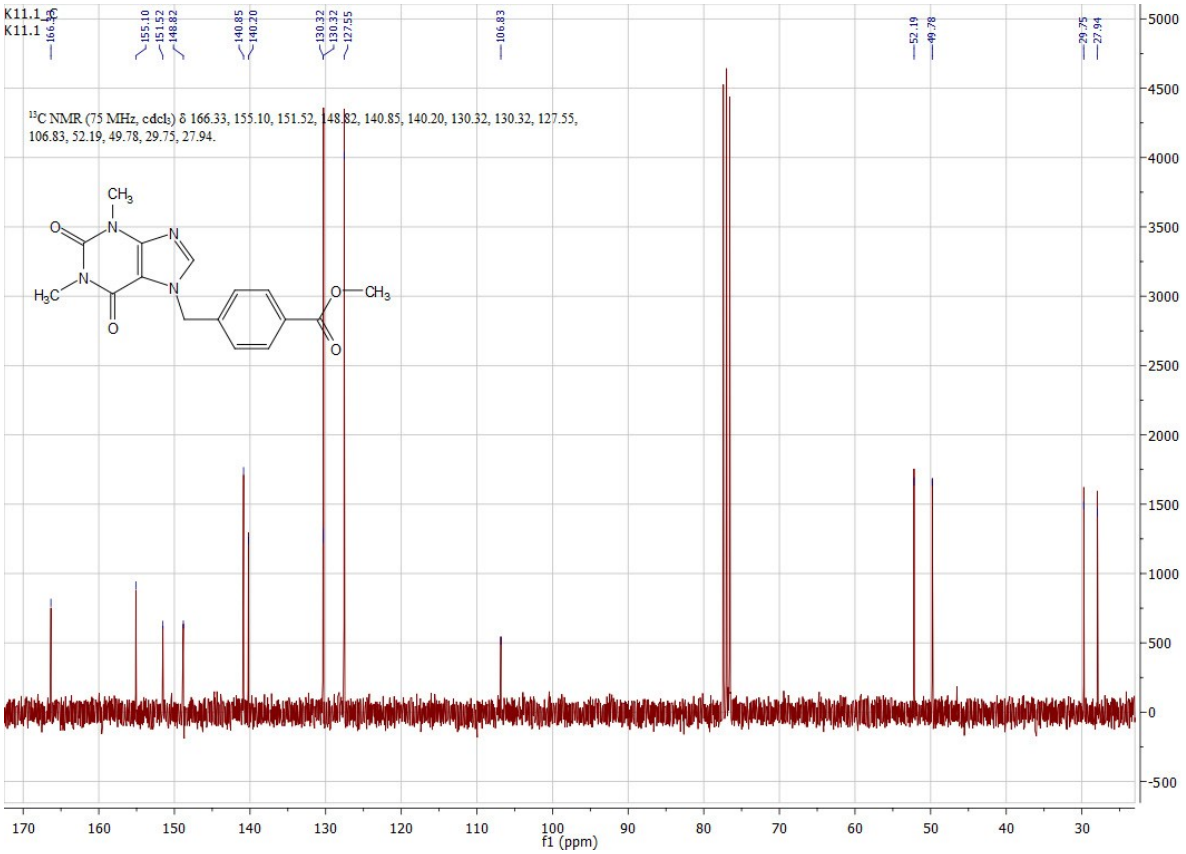
*1.1.9. 7-[(4-bromophenyl)methyl]-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**9**).* White solid (1.68 g, 86 %); m.p. 200-204 °C; ^{1}H NMR (300 MHz, Chloroform-d) δ 7.58 (s, 1H), 7.53 – 7.43 (m, 2H), 7.24 – 7.15 (m, 2H), 5.43 (s, 2H), 3.57 (s, 3H), 3.38 (s, 3H).; ^{13}C NMR (75 MHz, cdcl3) δ 155.14, 151.54, 148.88, 140.69, 134.36, 132.19, 129.52, 122.72, 106.80, 49.60, 29.76, 27.98; HRMS: *m/z* $[M+H]^+$ 349.0304 (calculated for: $[C_{14}H_{14}BrN_4O_2]^+$ 349.0295).



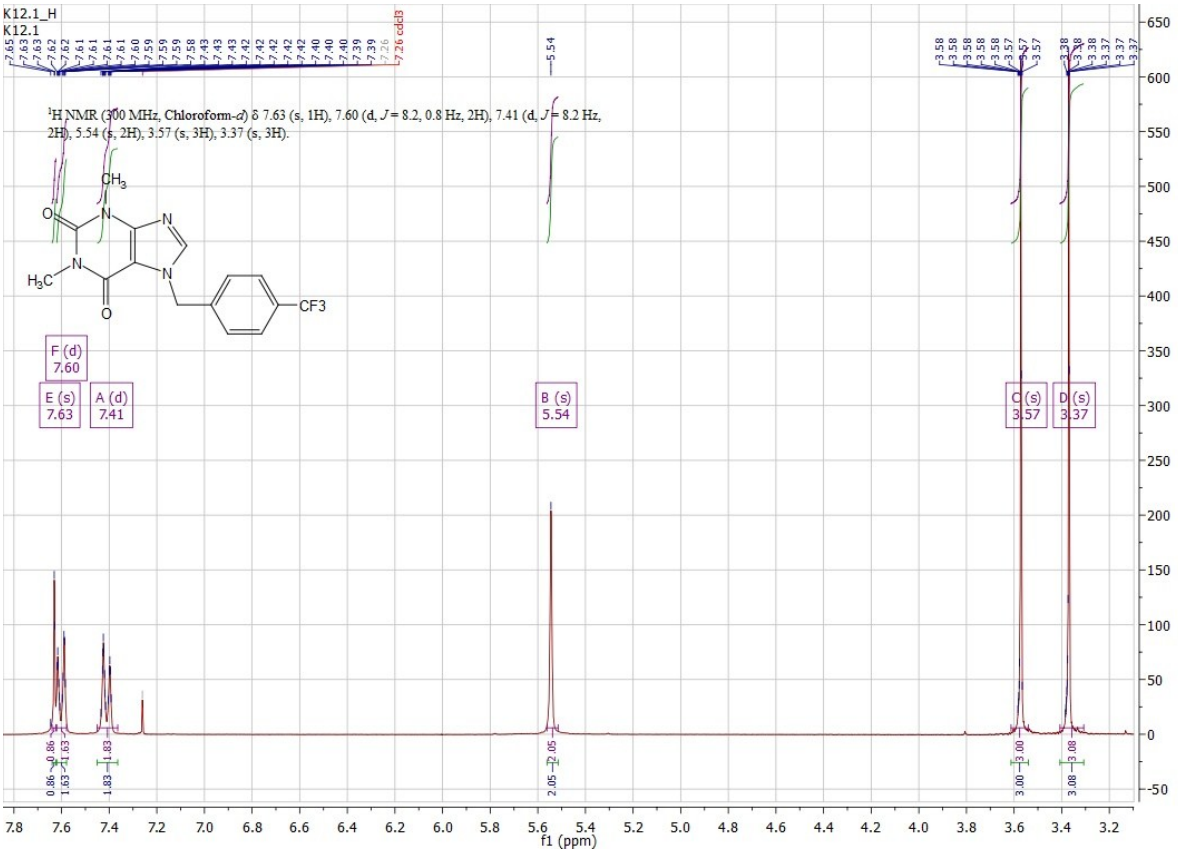


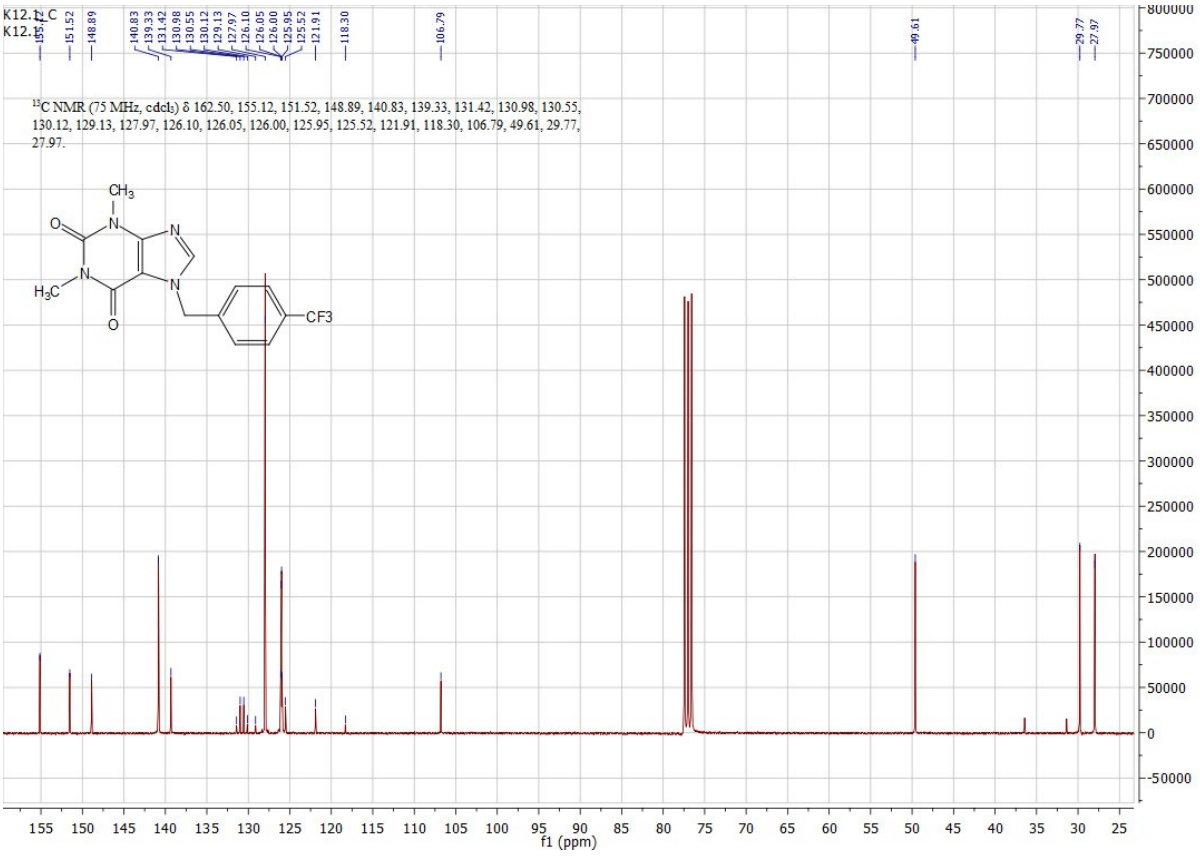
*1.1.10. methyl 4-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)methyl]benzoate (**10**).* White solid (1.48 g, 81 %); m.p. 190-193 °C; ^{1}H NMR (300 MHz, Chloroform-d) δ 8.06 – 7.97 (m, 1H), 7.61 (s, 0H), 7.40 – 7.29 (m, 1H), 5.54 (s, 1H), 3.89 (s, 2H), 3.57 (s, 2H), 3.37 (s, 2H); ^{13}C NMR (75 MHz, cdcl3) δ 166.33, 155.10, 151.52, 148.82, 140.85, 140.20, 130.32, 130.32, 127.55, 106.83, 52.19, 49.78, 29.75, 27.94; HRMS: *m/z* $[M+H]^+$ 329.1238 (calculated for: $[C_{16}H_{17}N_4O_4]^+$ 329.1244).


K11.1_H
K11.1
8.02
8.01
8.00
7.99
7.61
7.36
7.35
7.35
7.33
7.33
7.26 cdcl3
5.54
3.89
3.57
3.37
^{1}H NMR (300 MHz, Chloroform-d) δ 8.06 – 7.97 (m, 1H), 7.61 (s, 0H), 7.40 – 7.29 (m, 1H), 5.54 (s, 1H), 3.89 (s, 2H), 3.57 (s, 2H), 3.37 (s, 2H).
A (m) 8.00
B (s) 7.61
C (m) 7.35
D (s) 5.54
E (s) 3.89
F (s) 3.57
G (s) 3.37
1.00
0.50
1.05
1.14
1.65
1.65
1.64
f1 (ppm)

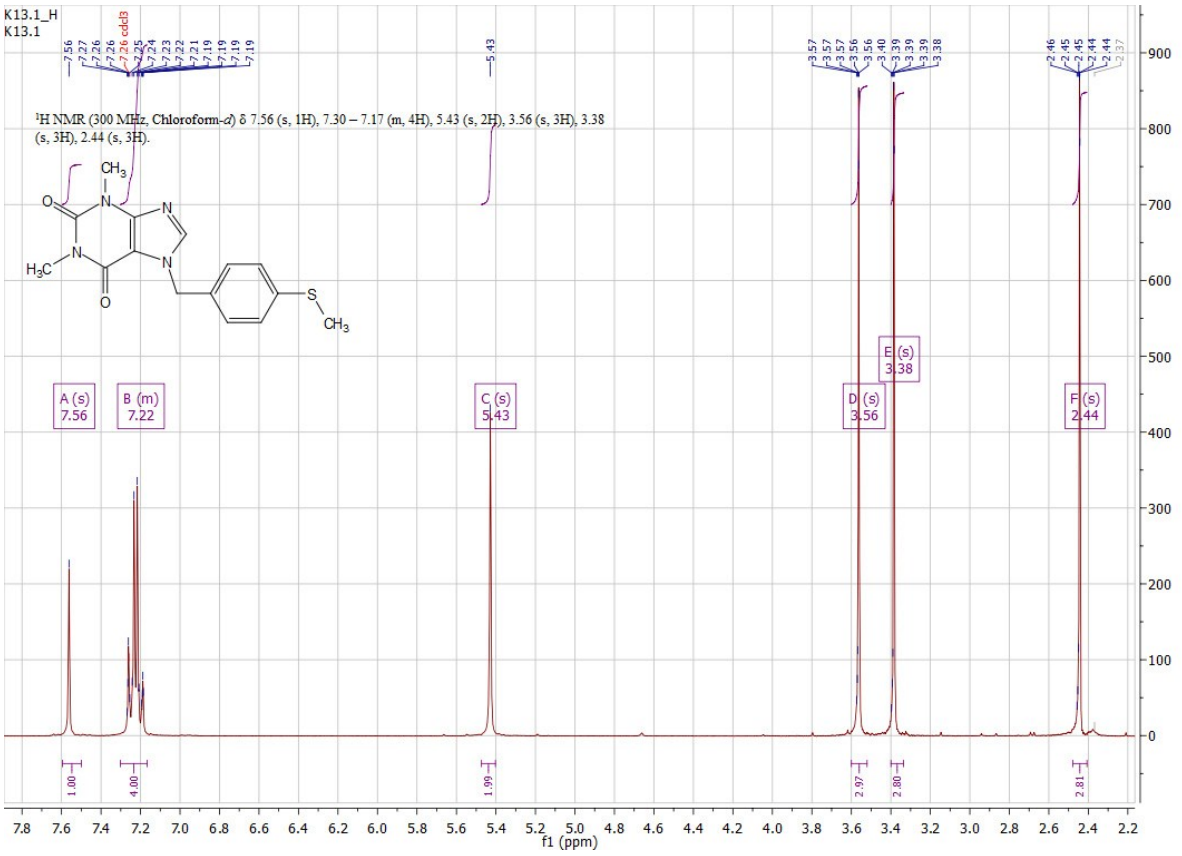

K11.1_C
K11.1
166.33
155.10
151.52
148.82
140.85
140.20
130.32
130.32
127.55
106.83
52.19
49.78
29.75
27.94
^{13}C NMR (75 MHz, cdcl$_3$) δ 166.33, 155.10, 151.52, 148.82, 140.85, 140.20, 130.32, 130.32, 127.55, 106.83, 52.19, 49.78, 29.75, 27.94.
f1 (ppm)

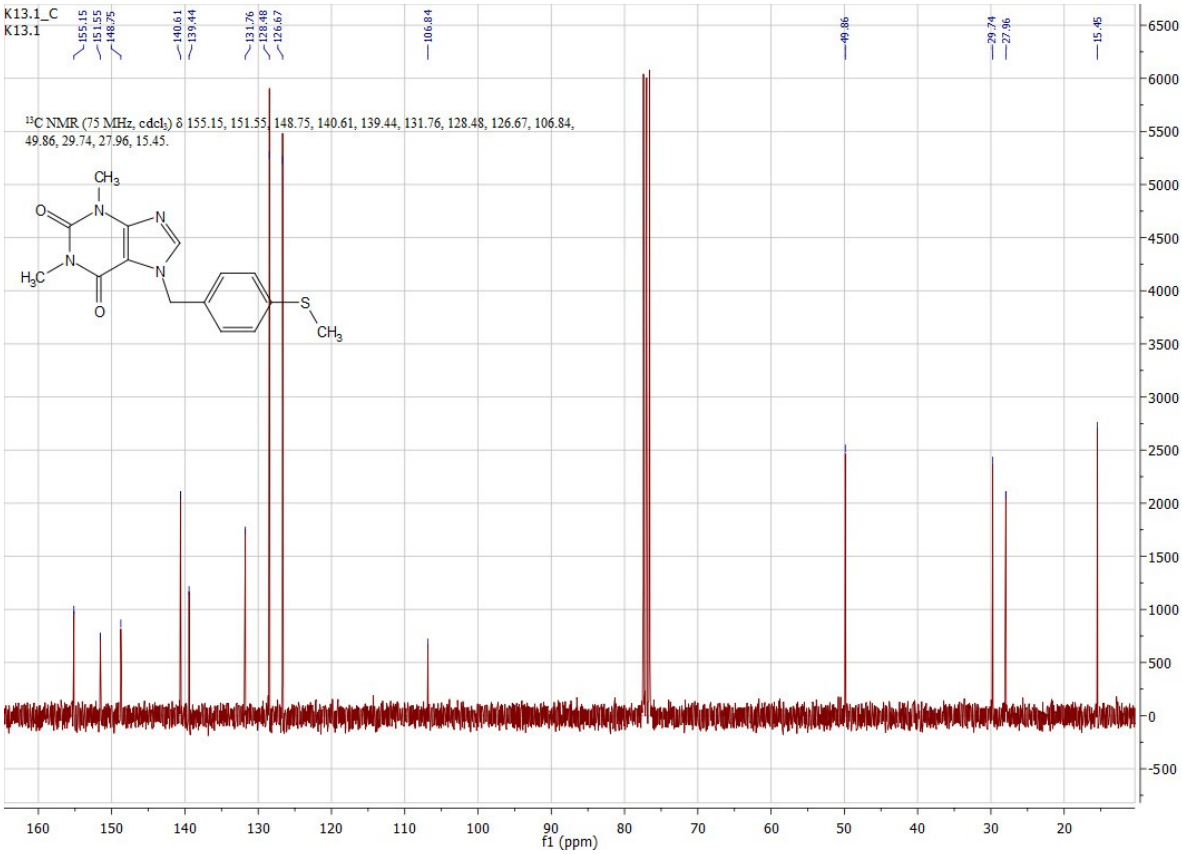
*1.1.11. 1,3-dimethyl-7-{[4-(trifluoromethyl)phenyl]methyl}-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**11**).* White solid (1.30 g, 69 %); m.p. 199-201 °C; ^{1}H NMR (300 MHz, Chloroform-d) δ 7.63 (s, 1H), 7.60 (d, J = 8.2, 0.8 Hz, 2H), 7.41 (d, J = 8.2 Hz, 2H), 5.54 (s, 2H), 3.57 (s, 3H), 3.37 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 155.29, 151.69, 149.05, 141.00, 139.49, 130.77 (q, *J* = 32.7 Hz), 127.97, 126.02 (q, *J* = 3.7 Hz), 123.72 (q, *J* = 272.3 Hz), 155.29, 151.69, 149.05, 141.00, 139.49; HRMS: *m/z* $[M+H]^+$ 339.1060 (calculated for: $[C_{15}H_{14}F_3N_4O_2]^+$ 339.1063).



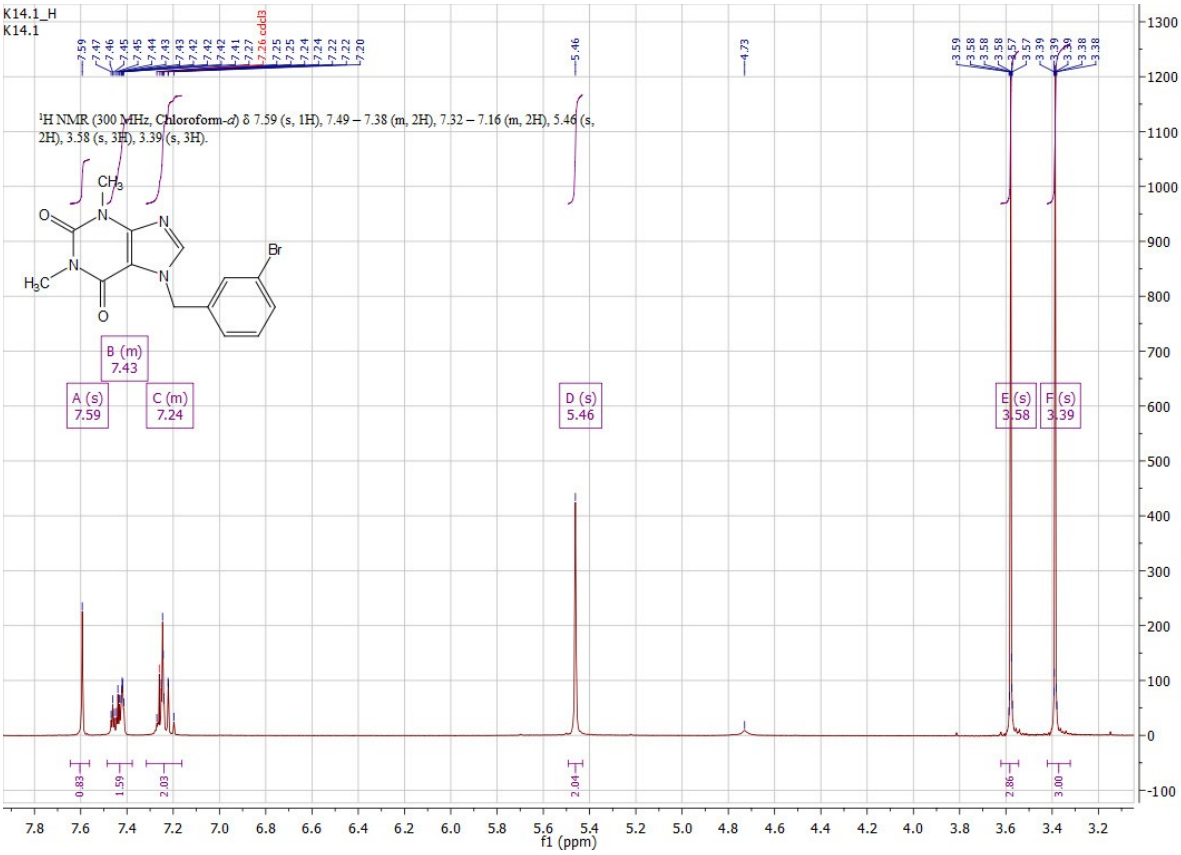


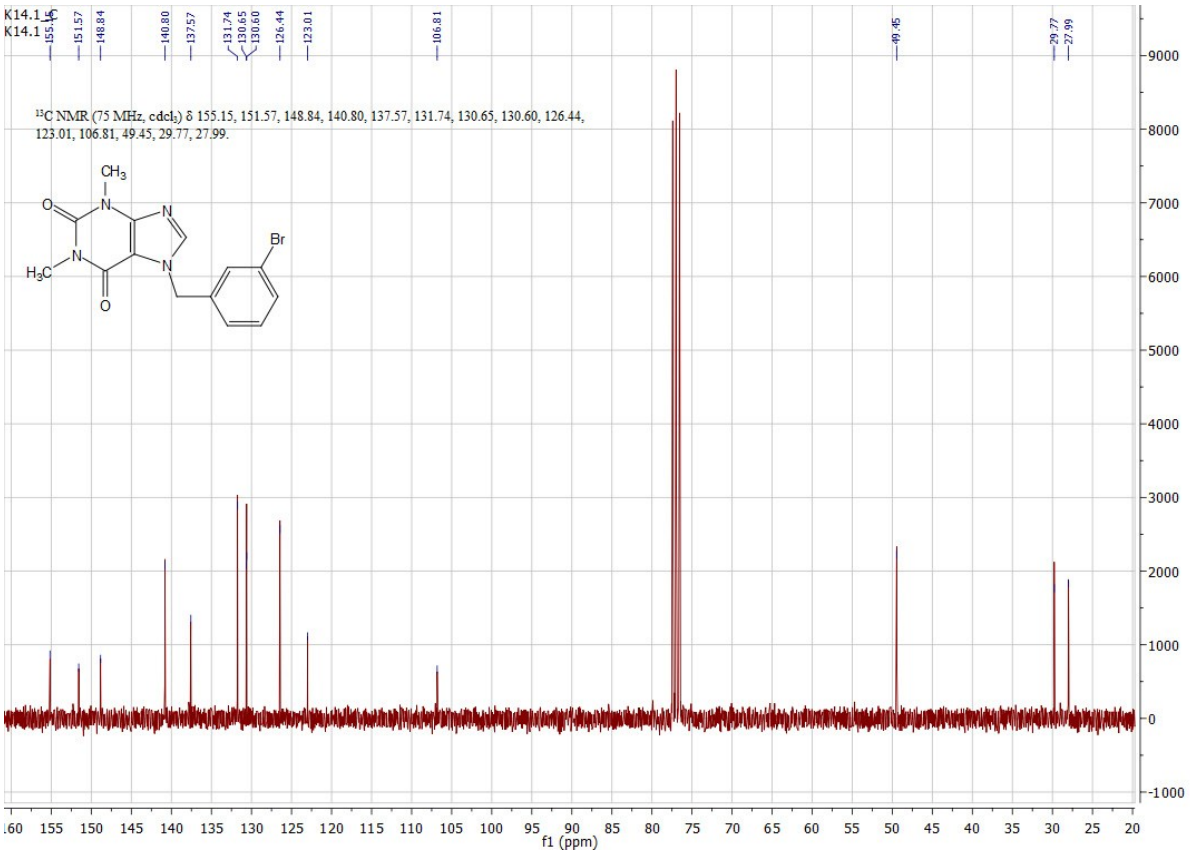
1.1.12. 1,3-dimethyl-7-{[4-(methylthio)phenyl]methyl}-2,3,6,7-tetrahydro-1H-purine-2,6-dione ***(12)***. White solid (1.27 g, 66 %); m.p. 123-125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.56 (s, 1H), 7.30-7.17 (m, 4H), 5.43 (s, 2H), 3.56 (s, 3H), 3.38 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.15, 151.55, 148.75, 140.61, 139.44, 131.76, 128.48, 126.67, 106.84, 49.86, 29.74, 27.96, 15.45; HRMS: *m/z* $[M+H]^+$ 317.1064 (calculated for: $[C_{15}H_{17}N_4O_2S]^+$ 317.1067).


K13.1_H
K13.1
¹H NMR (300 MHz, Chloroform-d) δ 7.56 (s, 1H), 7.30 – 7.17 (m, 4H), 5.43 (s, 2H), 3.56 (s, 3H), 3.38 (s, 3H), 2.44 (s, 3H).
A (s) 7.56
B (m) 7.22
C (s) 5.43
D (s) 3.56
E (s) 3.38
F (s) 2.44
f1 (ppm)

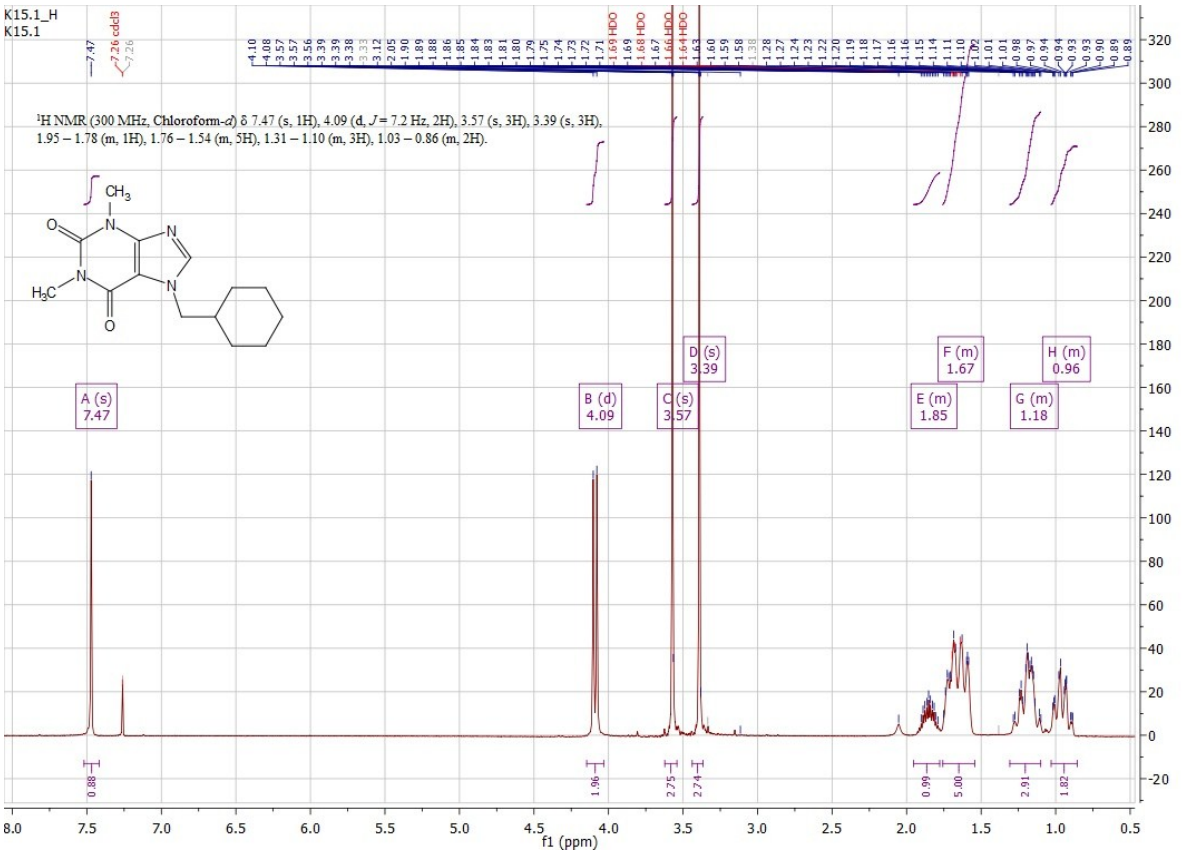

K13.1_C
K13.1
¹³C NMR (75 MHz, cdcl₃) δ 155.15, 151.55, 148.75, 140.61, 139.44, 131.76, 128.48, 126.67, 106.84, 49.86, 29.74, 27.96, 15.45.
f1 (ppm)

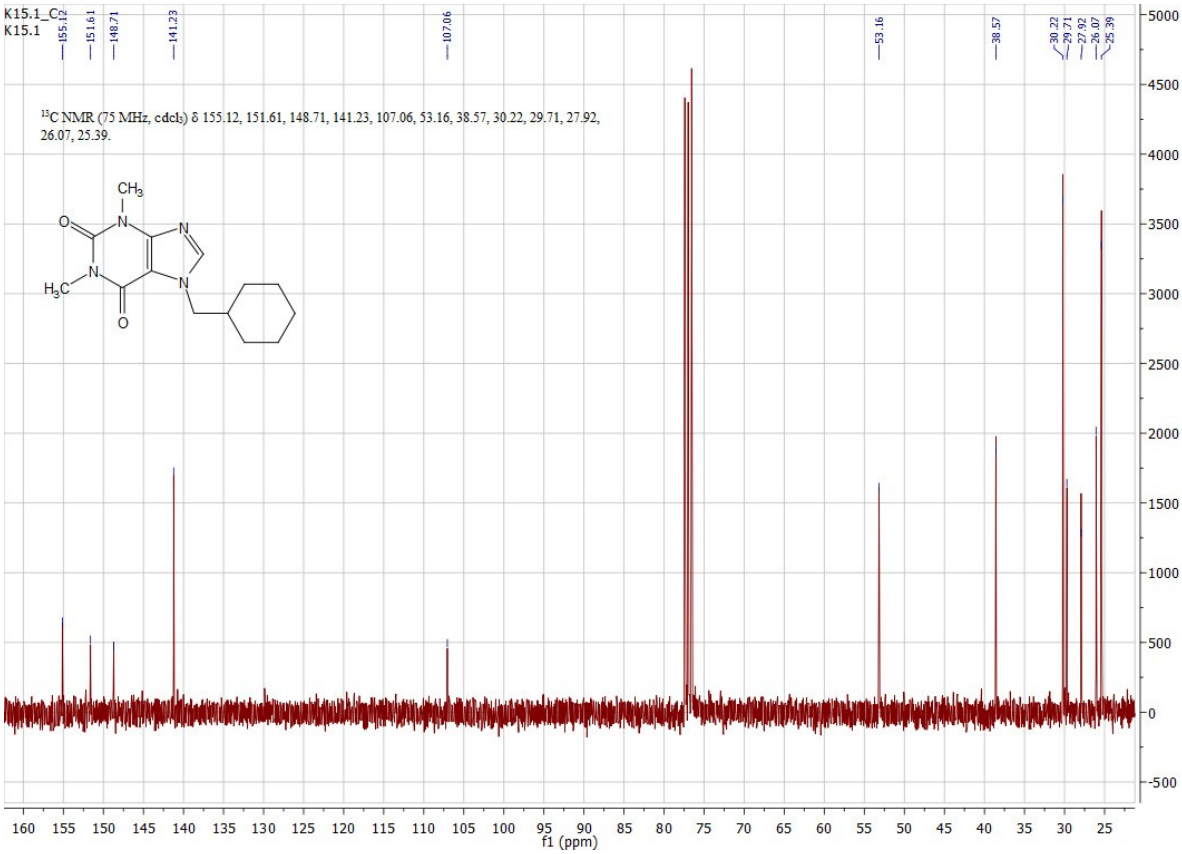
*1.1.13. 7-[(3-bromophenyl)methyl]-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**13**).* White solid (1.24 g, 64 %); m.p. 150-151 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.59 (s, 1H), 7.49-7.38 (m, 2H), 7.31-7.16 (m, 2H), 5.46 (s, 2H), 3.58 (s, 3H), 3.39 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 155.15, 151.57, 148.84, 140.80, 137.57, 131.74, 130.65, 130.60, 126.44, 123.01, 106.81, 49.45, 29.77, 27.99; HRMS: *m/z* $[M+H]^+$ 349.0295 (calculated for: $[C_{14}H_{14}BrN_4O_2]^+$ 349.0295).



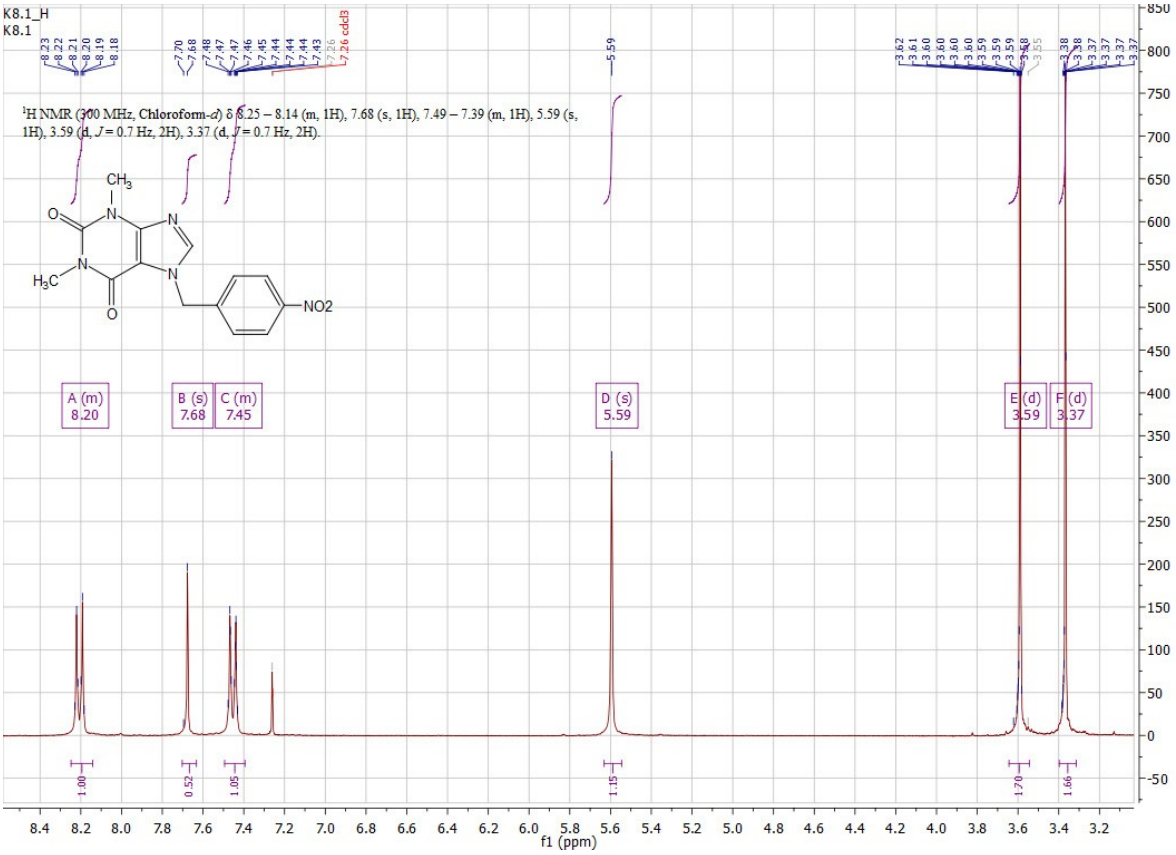


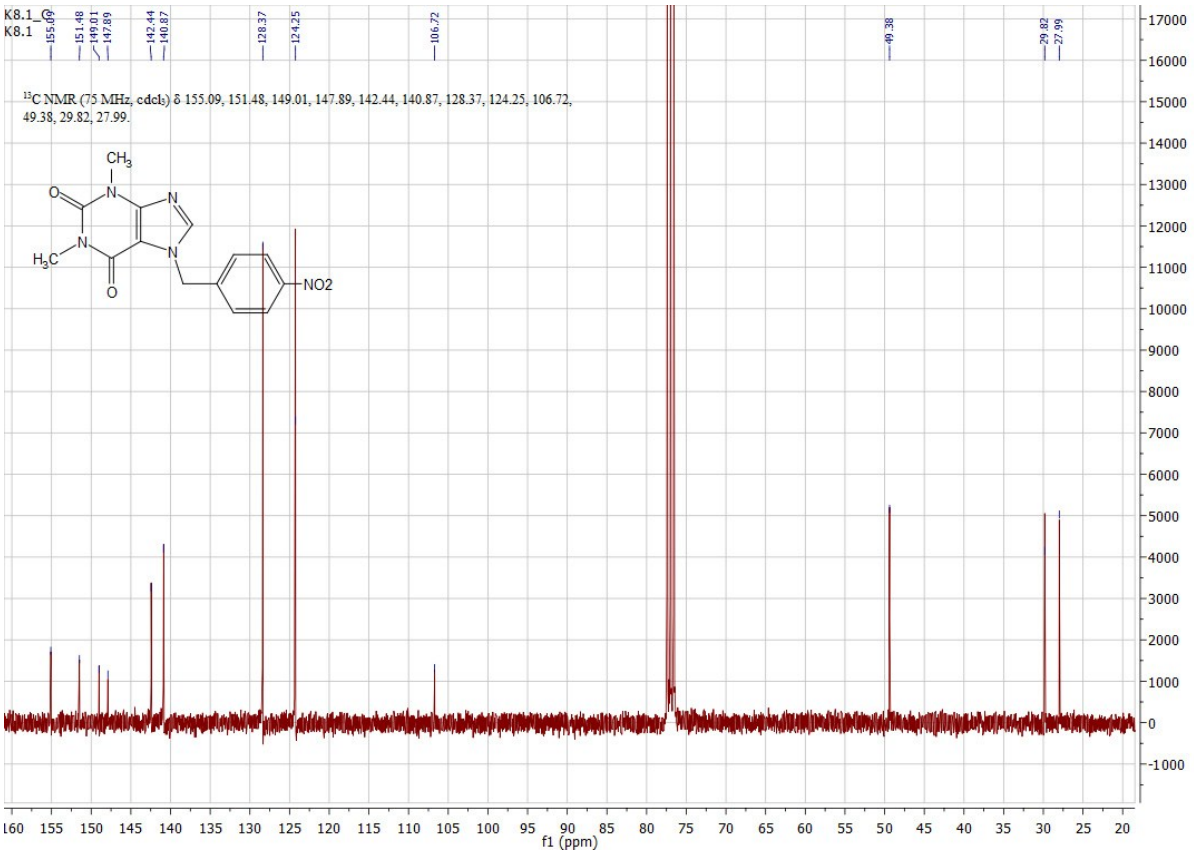
*1.1.14. 7-(cyclohexylmethyl)-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**14**).* White solid (0.24 g, 16 %); m.p. 104-105 °C; ^{1}H NMR (300 MHz, Chloroform-d) δ 7.47 (s, 1H), 4.09 (d, J = 7.2 Hz, 2H), 3.57 (s, 3H), 3.39 (s, 3H), 1.95 – 1.78 (m, 1H), 1.76 – 1.54 (m, 5H), 1.31 – 1.10 (m, 3H), 1.03 – 0.86 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 155.12, 151.61, 148.71, 141.23, 107.06, 53.16, 38.57, 30.22, 29.71, 27.92, 26.07, 25.39; HRMS: *m/z* $[M+H]^+$ 277.1658 (calculated for: $[C_{14}H_{21}N_4O_2]^+$ 277.1659).


K15.1_H
K15.1
^{1}H NMR (300 MHz, Chloroform-d) δ 7.47 (s, 1H), 4.09 (d, J = 7.2 Hz, 2H), 3.57 (s, 3H), 3.39 (s, 3H), 1.95 – 1.78 (m, 1H), 1.76 – 1.54 (m, 5H), 1.31 – 1.10 (m, 3H), 1.03 – 0.86 (m, 2H).
A (s) 7.47
B (d) 4.09
C (s) 3.57
D (s) 3.39
E (m) 1.85
F (m) 1.67
G (m) 1.18
H (m) 0.96
f1 (ppm)

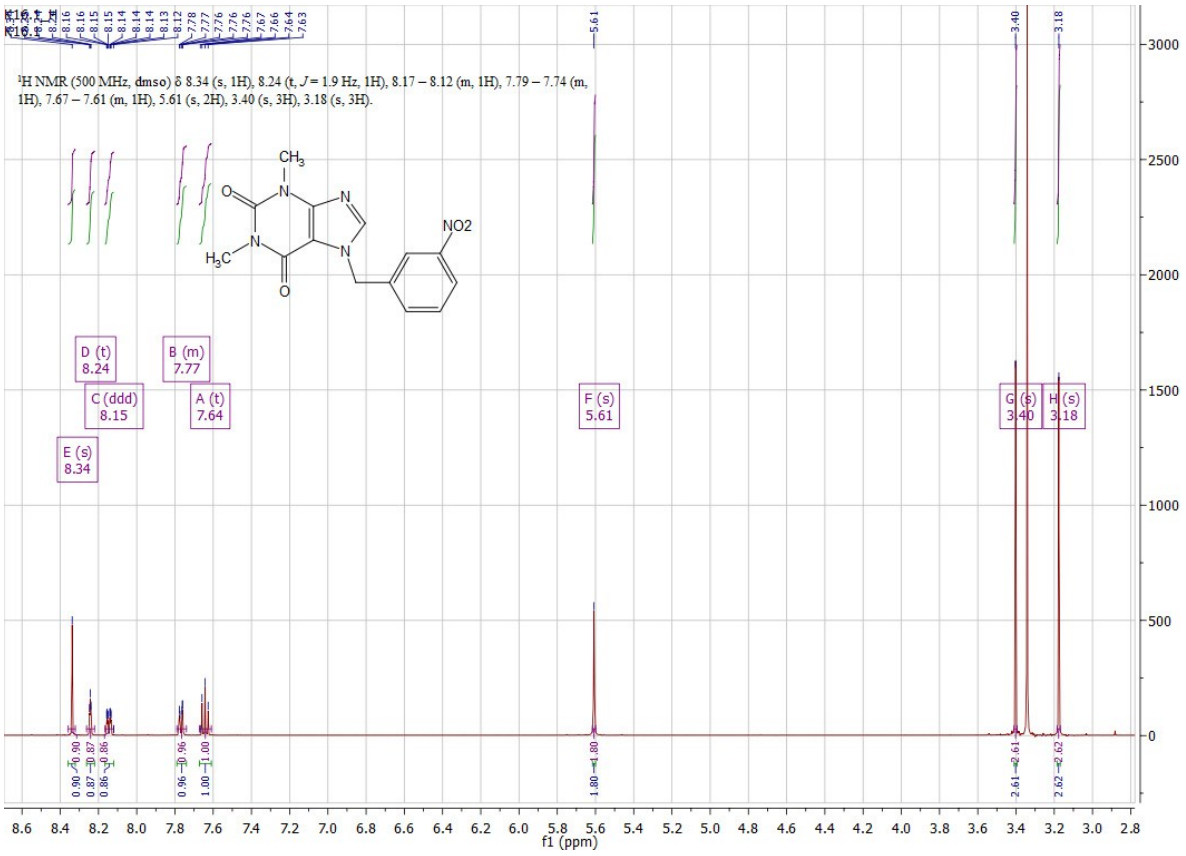

K15.1_C
K15.1
^{13}C NMR (75 MHz, cdcl$_3$) δ 155.12, 151.61, 148.71, 141.23, 107.06, 53.16, 38.57, 30.22, 29.71, 27.92, 26.07, 25.39.
f1 (ppm)

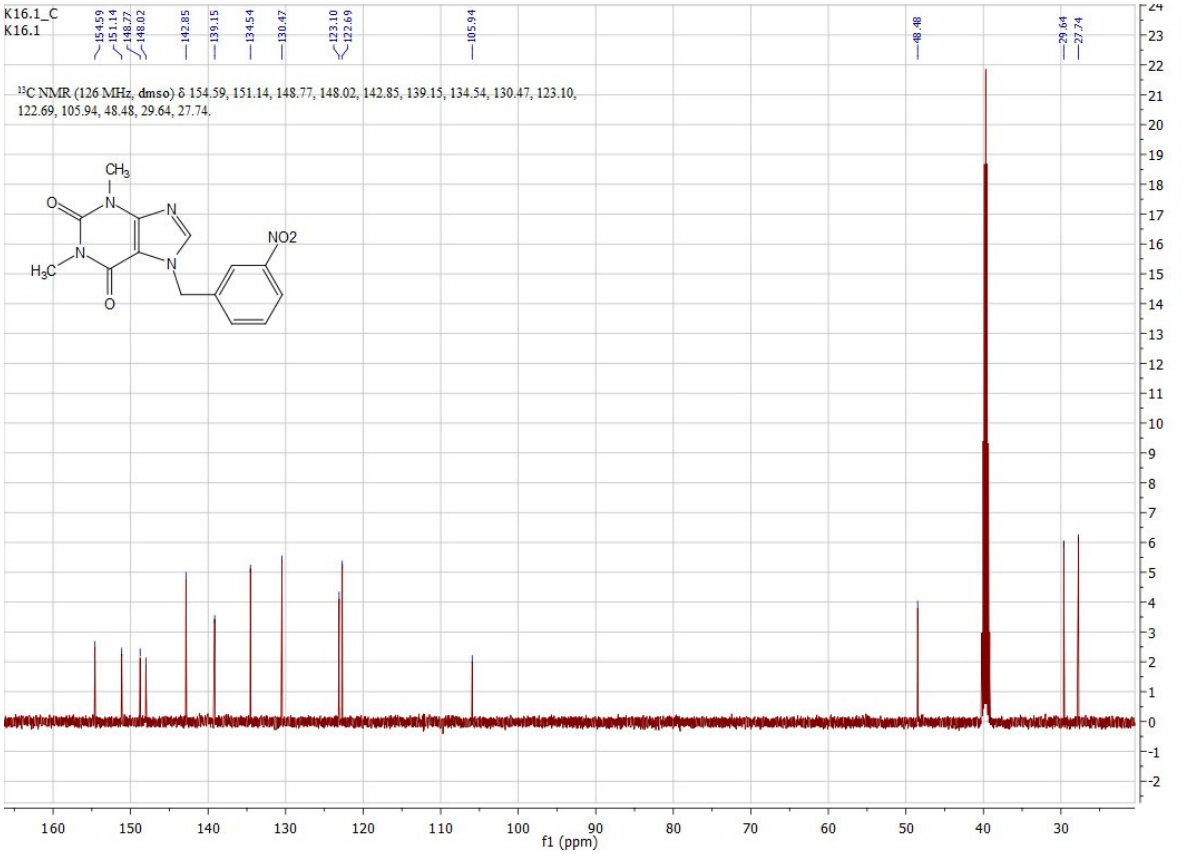
*1.1.15. 1,3-dimethyl-7-[(4-nitrophenyl)methyl]-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**15**).* Yellow solid (1.50 g, 85 %); m.p. 232-235 °C; ^{1}H NMR (300 MHz, Chloroform-d) δ 8.25 – 8.14 (m, 1H), 7.68 (s, 1H), 7.49 – 7.39 (m, 1H), 5.59 (s, 1H), 3.59 (d, J = 0.7 Hz, 2H), 3.37 (d, J = 0.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 155.09, 151.48, 149.01, 147.89, 142.44, 140.87, 128.37, 124.25, 106.72, 49.55, 29.82, 27.99; HRMS: *m/z* $[M+H]^+$ 316.1038 (calculated for: $[C_{14}H_{14}N_5O_4]^+$ 316.1040).



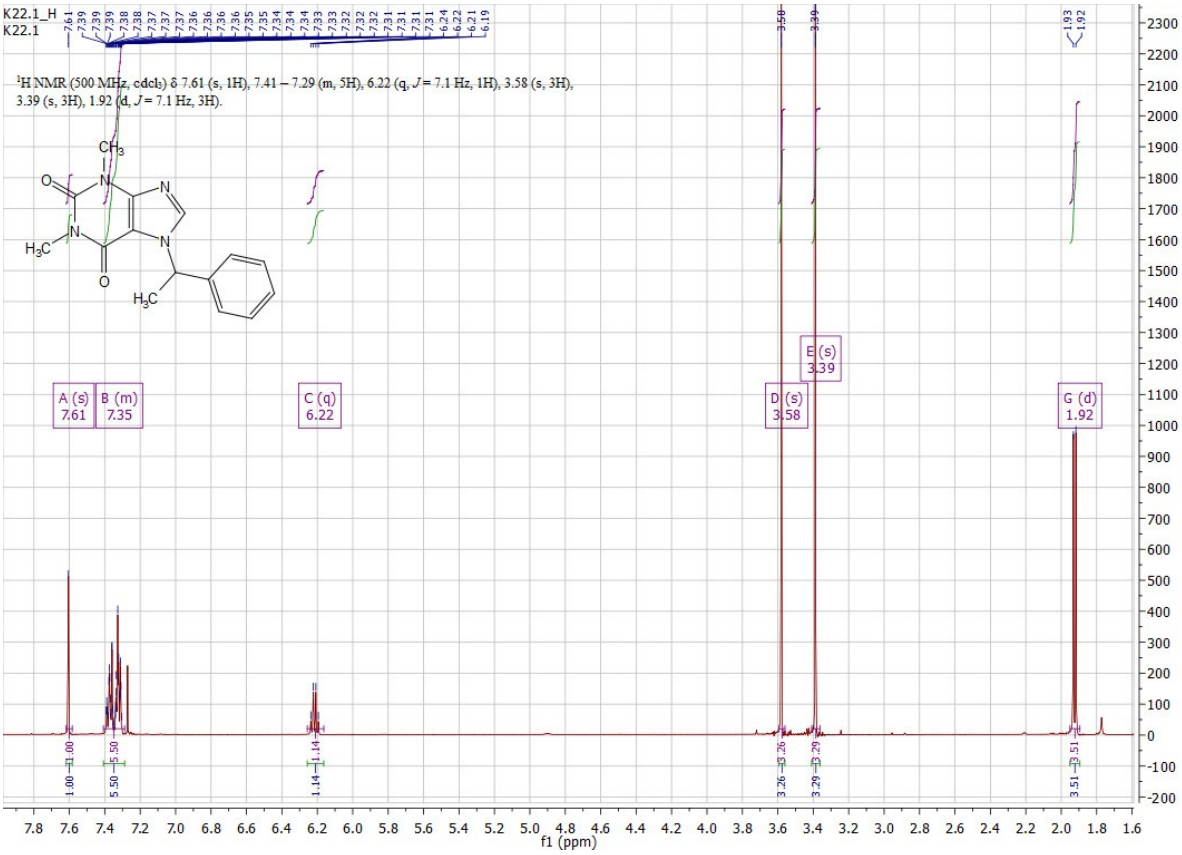


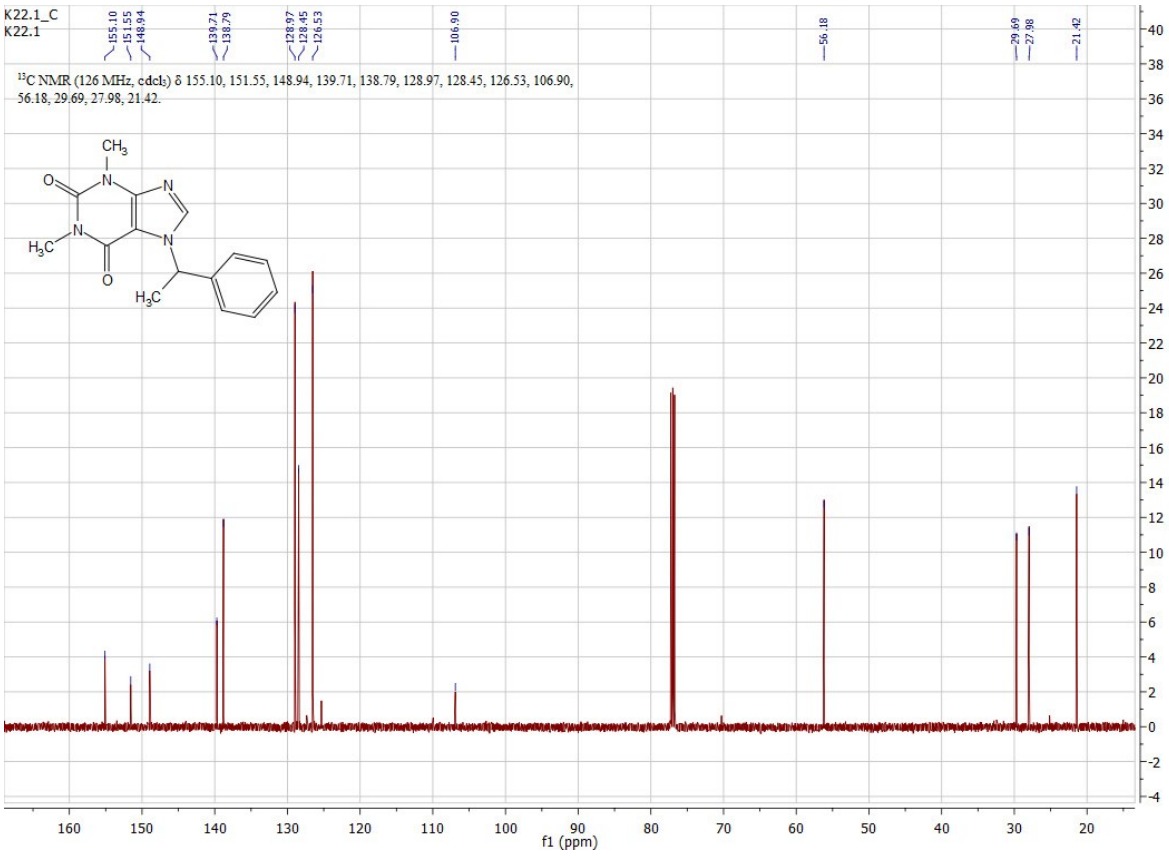
*1.1.16. 1,3-dimethyl-7-[(3-nitrophenyl)methyl]-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**16**).* White solid (1.30 g, 74 %); m.p. 186-188 °C; ^{1}H NMR (500 MHz, DMSO) δ (ppm) 8.34 (s, 1H), 8.24 (t, *J* = 1.9 Hz, 1H), 8.17 – 8.12 (m, 1H), 7.79 – 7.74 (m, 1H), 7.67 – 7.61 (m, 1H), 5.61 (s, 2H), 3.40 (s, 3H), 3.18 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ (ppm) 154.59, 151.14, 148.77, 148.02, 142.85, 139.15, 134.54, 130.47, 123.10, 122.69, 105.94, 48.48, 29.64, 27.74; HRMS: *m/z* 316.1037 $[M+H]^+$ (calculated for: $[C_{14}H_{14}N_5O_4]^+$ 316.1040).


¹H NMR (500 MHz, dmso) δ 8.34 (s, 1H), 8.24 (t, J = 1.9 Hz, 1H), 8.17 – 8.12 (m, 1H), 7.79 – 7.74 (m, 1H), 7.67 – 7.61 (m, 1H), 5.61 (s, 2H), 3.40 (s, 3H), 3.18 (s, 3H).
D (t) 8.24
B (m) 7.77
C (ddd) 8.15
A (t) 7.64
E (s) 8.34
F (s) 5.61
G (s) 3.40
H (s) 3.18
f1 (ppm)


K16.1_C
K16.1
¹³C NMR (126 MHz, dmso) δ 154.59, 151.14, 148.77, 148.02, 142.85, 139.15, 134.54, 130.47, 123.10, 122.69, 105.94, 48.48, 29.64, 27.74.
f1 (ppm)

*1.1.17. 1,3-dimethyl-7-(1-phenylethyl)-2,3,6,9-tetrahydro-1H-purine-2,6-dione (**17**).* White solid (0.96 g; 60 %); m.p. 96-100 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.61 (s, 1H), 7.41 – 7.29 (m, 5H), 6.22 (q, *J* = 7.1 Hz, 1H), 3.58 (s, 3H), 3.39 (s, 3H), 1.92 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, CDCl3) δ (ppm) 155.10, 151.55, 148.94, 139.71, 138.79, 128.97, 128.45, 126.53, 106.90, 56.18, 29.69, 27.98, 21.42; HRMS: *m/z* 285.1346 $[M+H]^+$ (calculated for: $[C_{15}H_{17}N_4O_2]^+$ 285.1346).

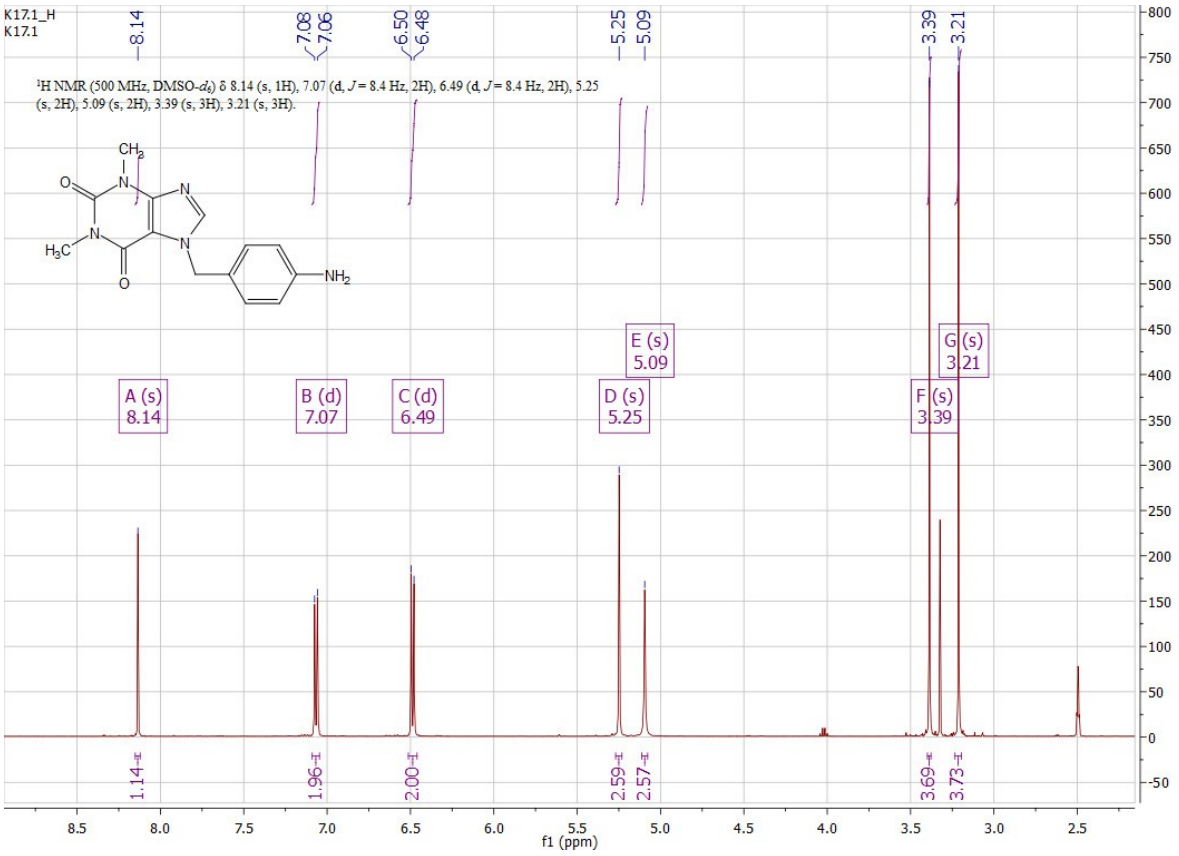


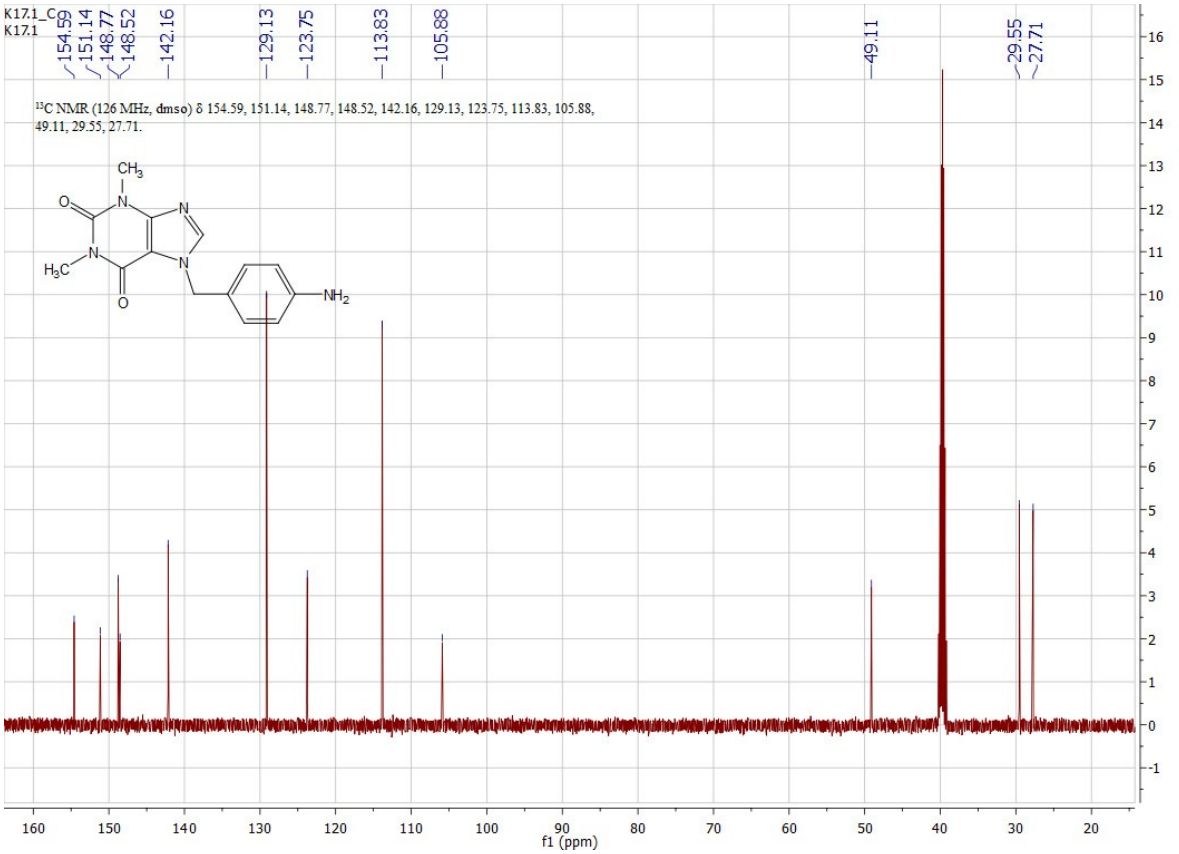


*1.2. Synthesis of amine derivatives (**18, 19**).*

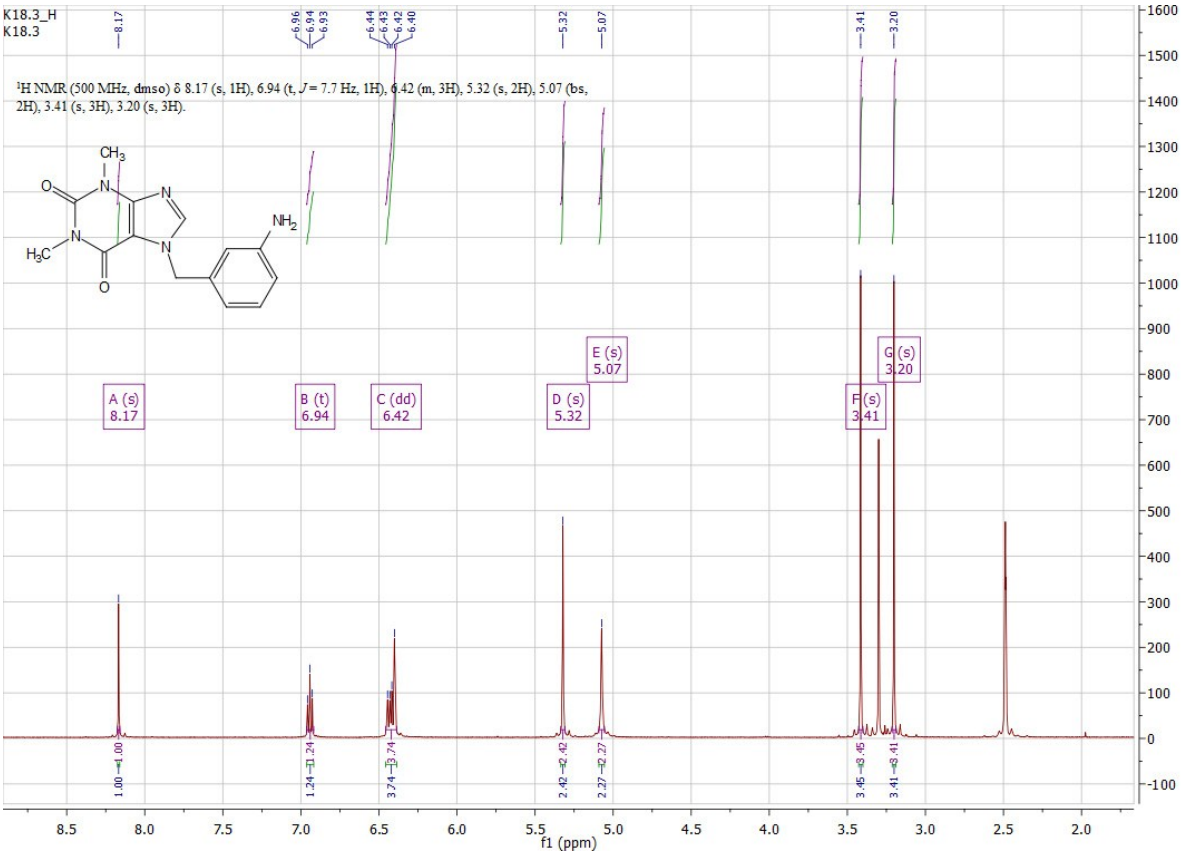
Zinc powder (1.03 g, 15.7 mmol) was added to a solution of **15** or **16** (500 mg, 1.57 mmol) in AcOH (20 mL) and stirred at room temperature overnight. The reaction mixture was extracted with DCM and water. The combined organic layers were evaporated under reduced pressure, and the residue was purified by column chromatography using EtOAc/MeOH/Et_3N (60:1:0.2) as eluent.

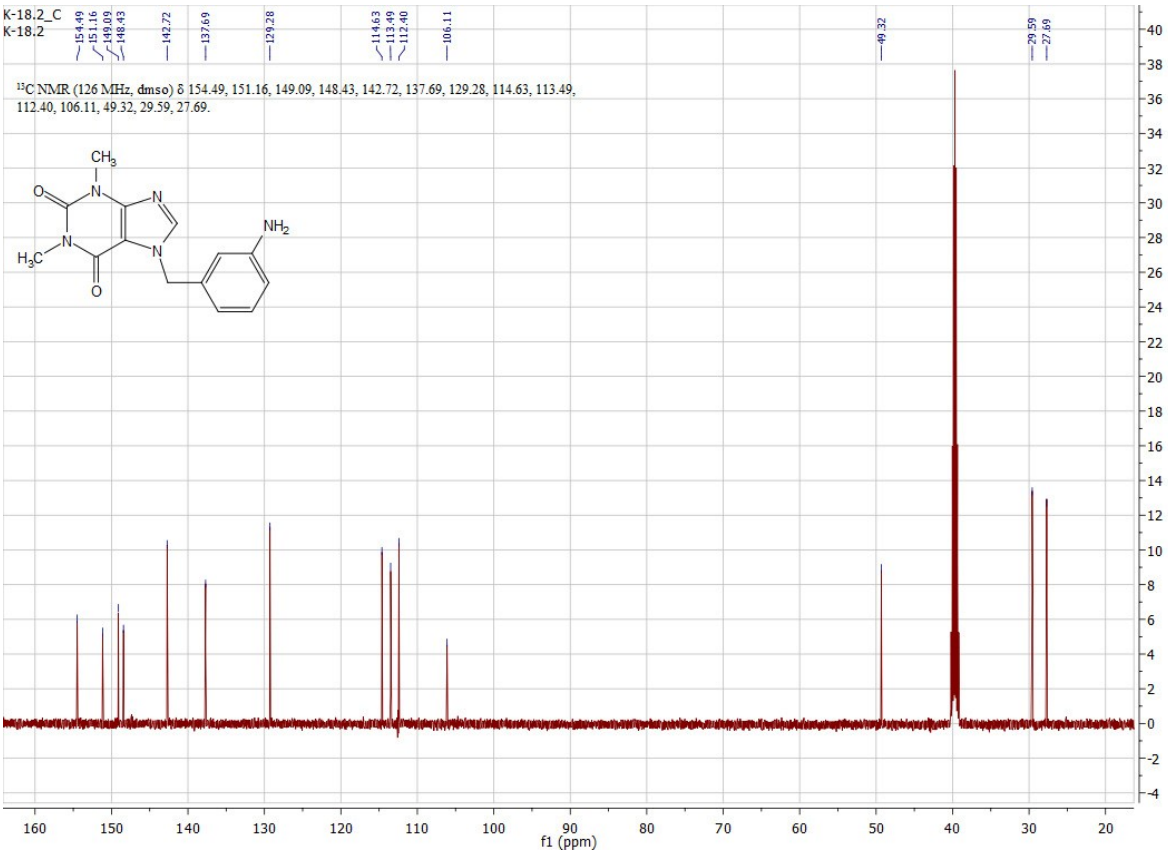
*1.2.1. 7-[(4-aminophenyl)methyl]-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**18**).* White solid (0.41 g, 91 %); 180-185 °C; ^{1}H NMR (500 MHz, DMSO) δ 8.14 (s, 1H), 7.07 (d, *J* = 8.4 Hz, 2H), 6.49 (d, *J* = 8.4 Hz, 2H), 5.25 (s, 2H), 5.09 (s, 2H), 3.39 (s, 3H), 3.21 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ (ppm) 154.59, 151.14, 148.77, 148.52, 142.16, 129.13, 123.75, 113.83, 105.88, 49.11, 29.55, 27.71; HRMS: *m/z* 286.1300 $[M+H]^+$ (calculated for: $[C_{14}H_{16}N_5O_2]^+$ 286.1299).


K17.1_H
K17.1
8.14
7.08
7.06
6.50
6.48
5.25
5.09
3.39
3.21
¹H NMR (500 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.07 (d, J = 8.4 Hz, 2H), 6.49 (d, J = 8.4 Hz, 2H), 5.25 (s, 2H), 5.09 (s, 2H), 3.39 (s, 3H), 3.21 (s, 3H).
A (s) 8.14
B (d) 7.07
C (d) 6.49
D (s) 5.25
E (s) 5.09
F (s) 3.39
G (s) 3.21
1.14
1.96
2.00
2.59
2.57
3.69
3.73
f1 (ppm)


K17.1_C
K17.1
154.59
151.14
148.77
148.52
142.16
129.13
123.75
113.83
105.88
49.11
29.55
27.71
¹³C NMR (126 MHz, dmso) δ 154.59, 151.14, 148.77, 148.52, 142.16, 129.13, 123.75, 113.83, 105.88, 49.11, 29.55, 27.71.
f1 (ppm)

*1.2.2. 7-[(3-aminophenyl)methyl]-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione (**19**).* White solid (0.40 g, 90 %); m.p. 188-192 °C; ^{1}H NMR (500 MHz, DMSO) δ (ppm) 8.17 (s, 1H), 6.94 (t, *J* = 7.7 Hz, 1H), 6.42 (m, 3H), 5.32 (s, 2H), 5.07 (bs, 2H), 3.41 (s, 3H), 3.20 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ (ppm) 154.49, 151.16, 149.09, 148.43, 142.72, 137.69, 129.28, 114.63, 113.49, 112.40, 106.11, 49.32, 29.59, 27.69; HRMS: *m/z* 286.1296 $[M+H]^+$ (calculated for: $[C_{14}H_{16}N_5O_2]^+$ 286.1299).





*1.3. Synthesis of 2-chloro-N-{4-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)methyl]phenyl}acetamide intermediate (**20**).*

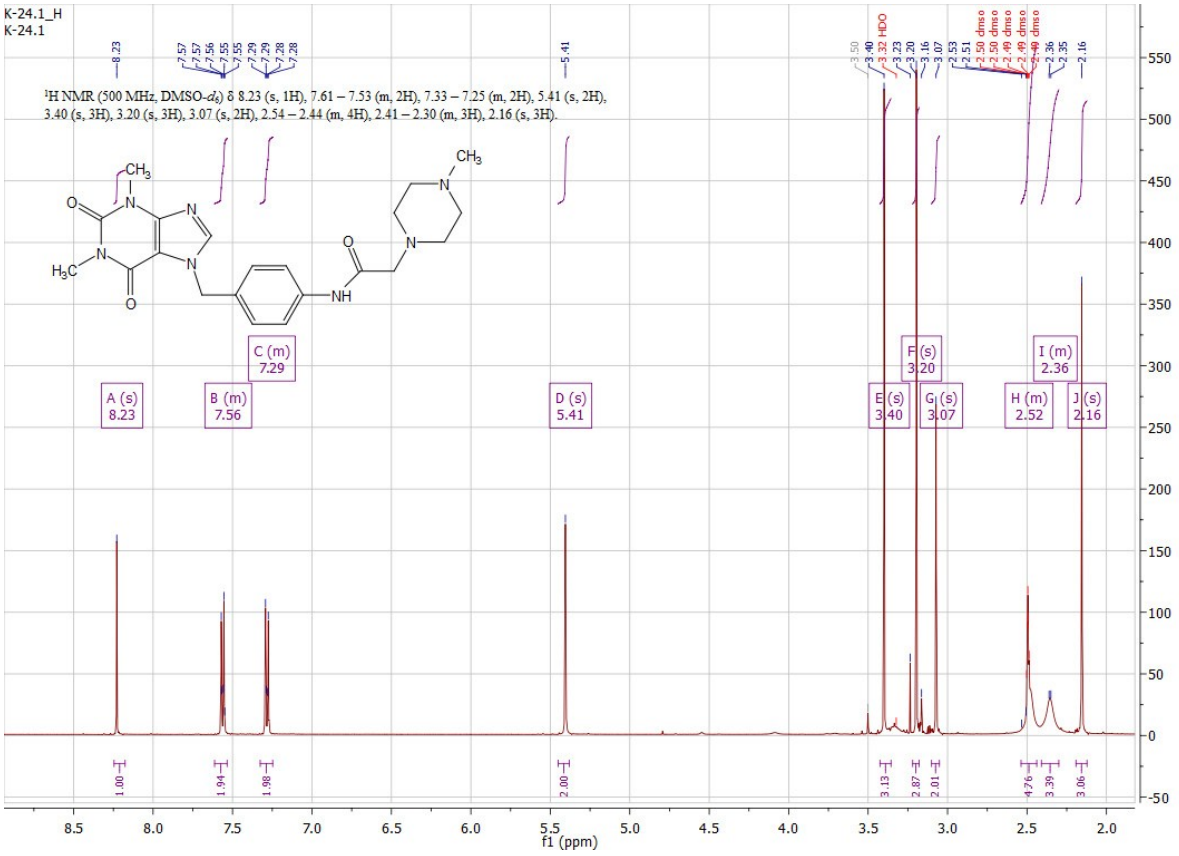
To a solution of **18** (2 g, 7.01 mmol) and triethylamine (2.15 mL, 15.4 mmol) in THF (10 mL), chloroacetyl chloride (0.61 mL, 7.7 mmol) was added dropwise at 0 °C and the resulting mixture was stirred at r.t. for 12 h. The solvent was then removed under reduced pressure, and the solid residue was triturated with a saturated aqueous solution of $NaHCO_3$ (30 mL) and filtered. The solid was washed successively with water and dried to yield 2.63 g of crude **20**. The intermediate of grey color was used for subsequent reactions without further purification.

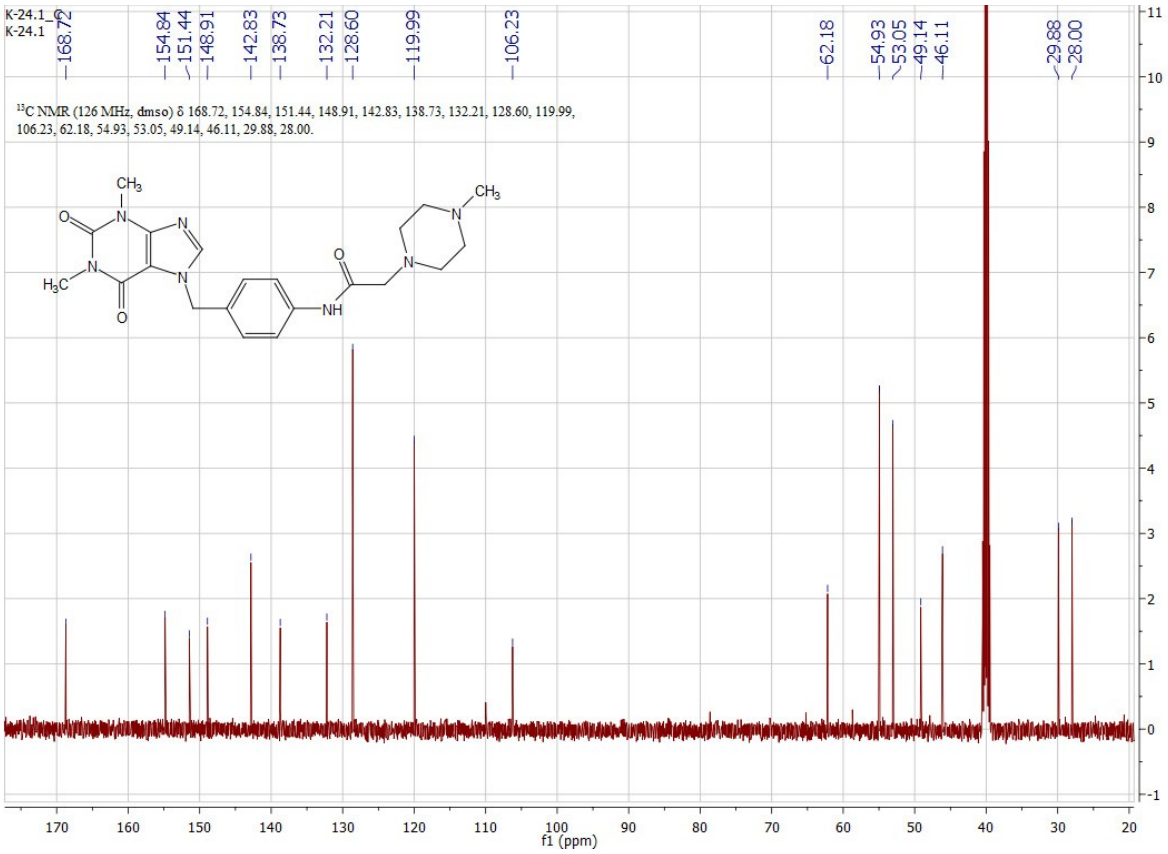
*1.4. Synthesis of novel caffeine derivatives with polar substituents at position 4- of the benzyl moiety (**21**-**25**).*

To a stirred solution of **20** (0.5 g, 1.38 mmol) in THF (10 mL) appropriate secondary amine (4.15 mmol) was added dropwise at r.t. The reaction mixture was then heated up-to 50 °C and stirred

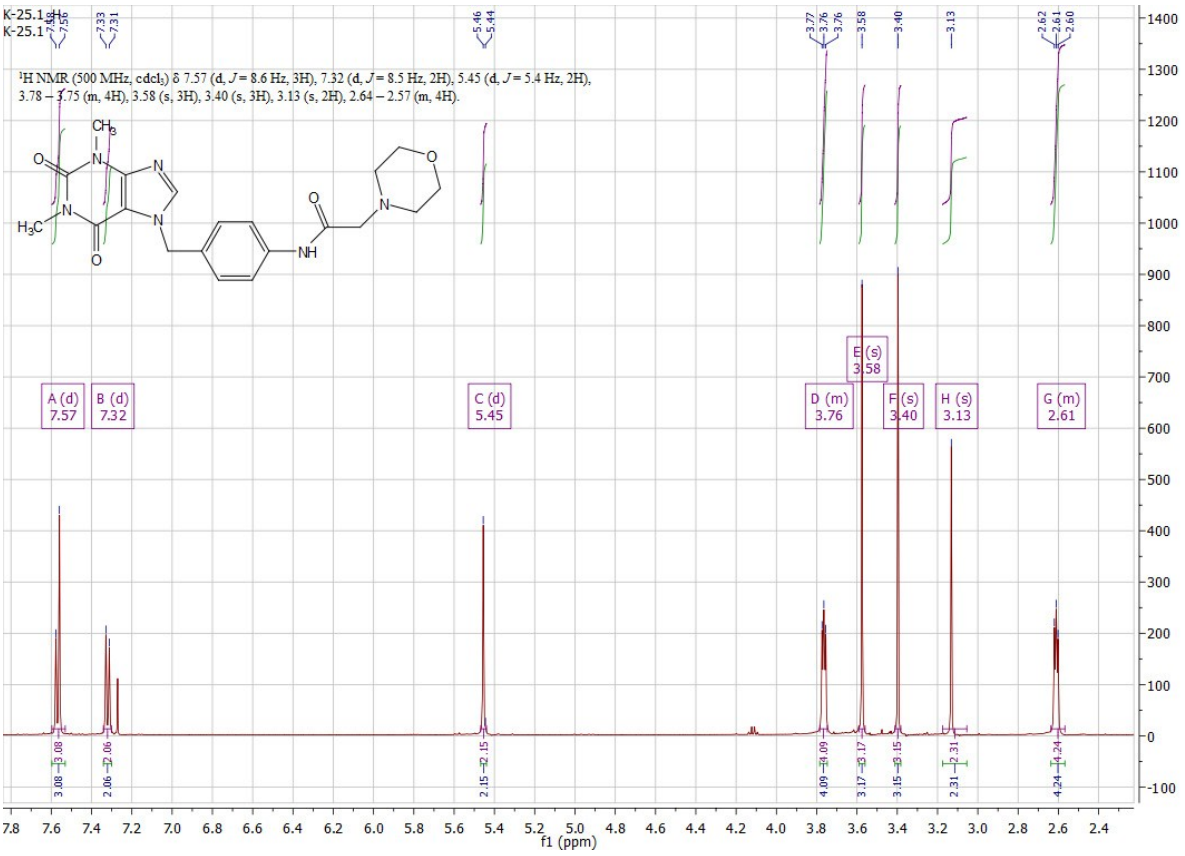
overnight following solvent removal. The crude product was purified by column chromatography using EtOAc/MeOH/Et_3N 60:1:0.2 as eluent. Oily residues were then recrystallized from diethyl ether to give beige solids.

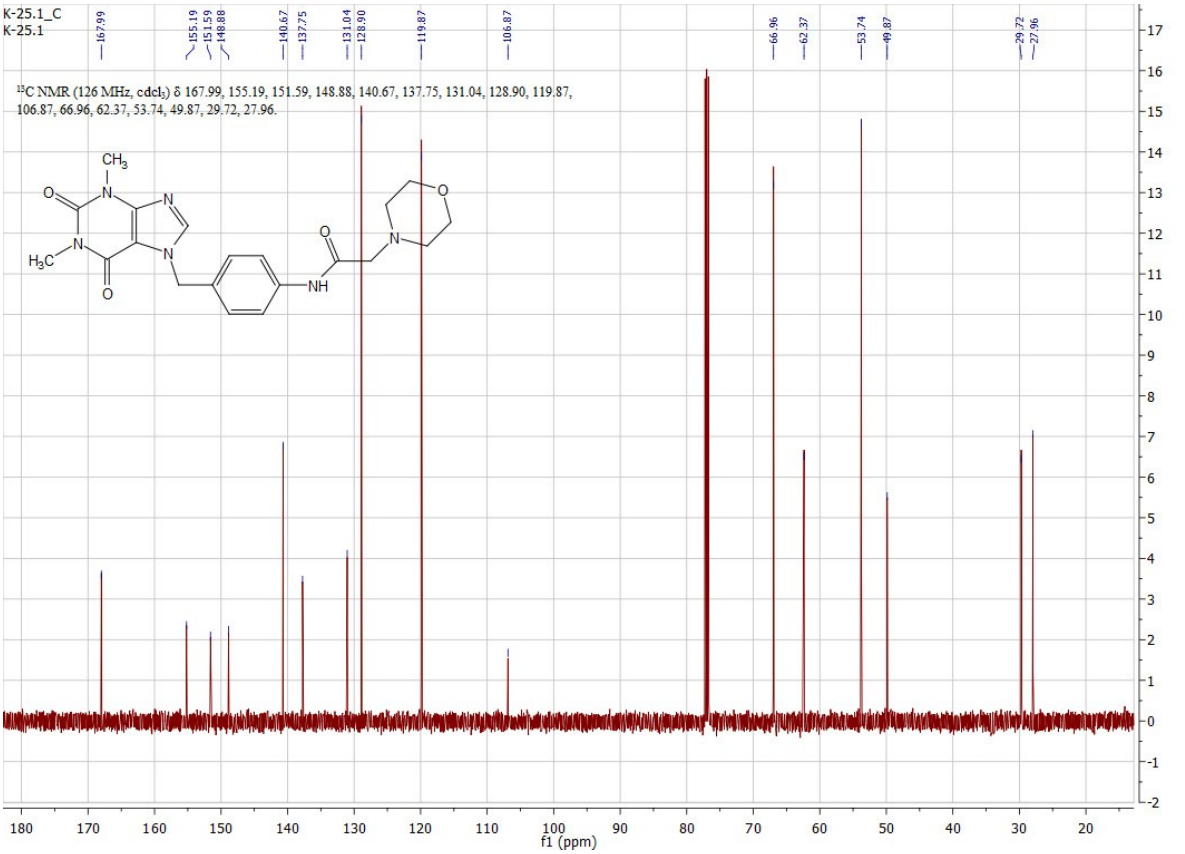
*1.4.1. N-{4-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)methyl]phenyl}-2-(4-methylpiperazin-1-yl)acetamide (**21**).* Beige solid (0.25 g, 43 %); m.p. 180-181 °C; ^{1}H NMR (500 MHz, DMSO) δ (ppm) 9.68 (s, 1H), 8.23 (s, 1H), 7.58 – 7.55 (m, 2H), 7.30 – 7.27 (m, 2H), 5.41 (s, 2H), 3.40 (s, 3H), 3.20 (s, 3H), 3.07 (s, 2H), 2.49 (s, 4H), 2.35 (s, 4H), 2.16 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ (ppm) 168.72, 154.84, 151.44, 148.91, 142.83, 138.73, 132.21, 128.60, 119.99, 106.23, 62.18, 54.93, 53.05, 49.14, 46.11, 29.88, 28.00; HRMS: *m/z* 426.2246 $[M+H]^+$ (calculated for: $[C_{21}H_{28}N_7O_3]^+$ 426.2248).



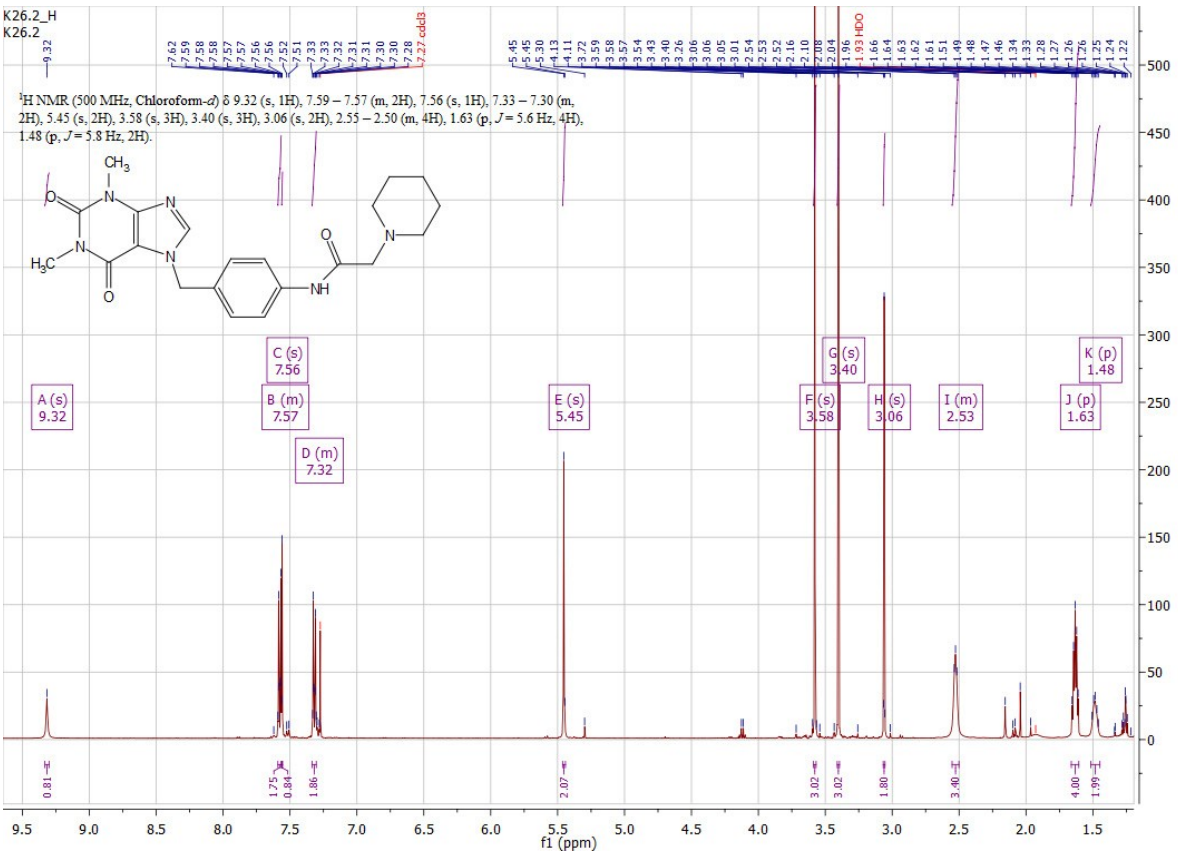


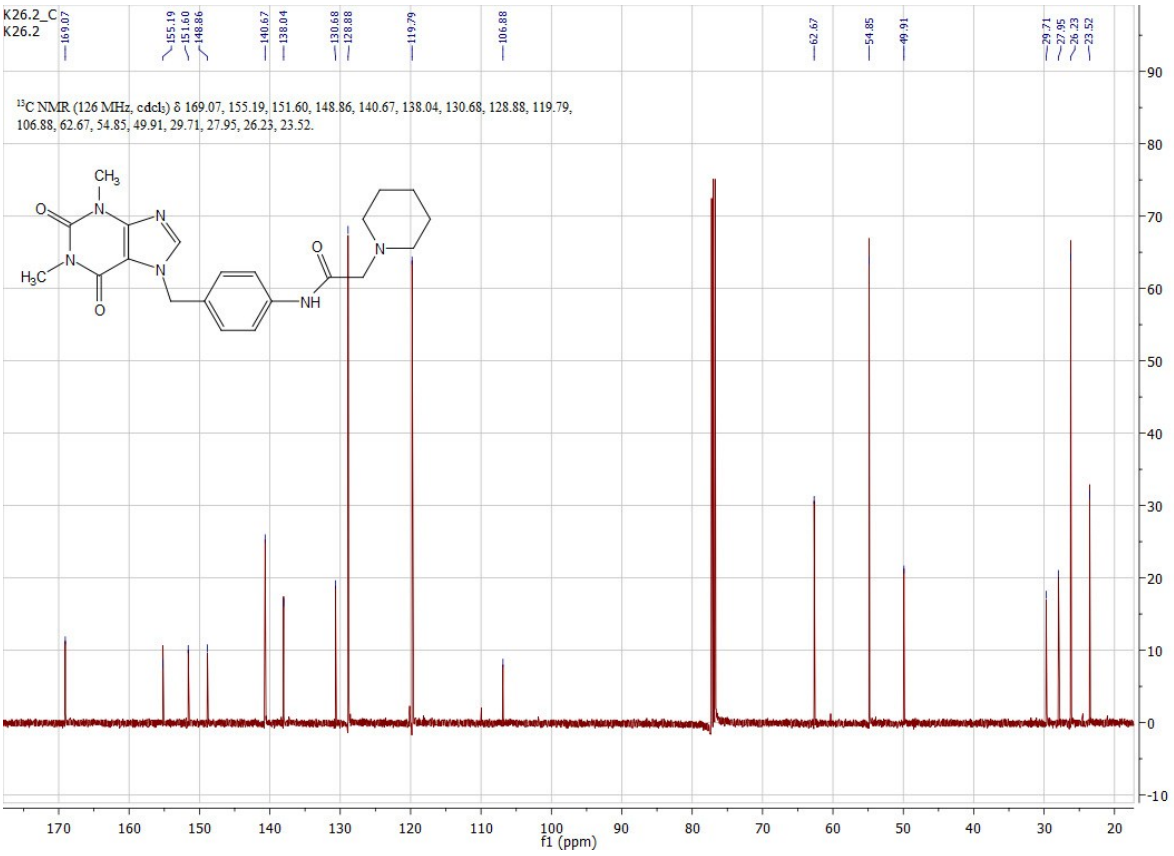
*1.4.2. N-{4-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)methyl]phenyl}-2-(morpholin-4-yl)acetamide (**22**).* Beige solid (0.18 g, 32 %); m.p. 228-231 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.57 (d, *J* = 8.6 Hz, 3H), 7.32 (d, *J* = 8.5 Hz, 2H), 5.45 (d, *J* = 5.4 Hz, 2H), 3.78 – 3.75 (m, 4H), 3.58 (s, 3H), 3.40 (s, 3H), 3.13 (s, 2H), 2.64 – 2.57 (m, 4H); ^{13}C NMR (126 MHz, CDCl3) δ (ppm) 167.99, 155.19, 151.59, 148.88, 140.67, 137.75, 131.04, 128.90, 119.87, 106.87, 66.96, 62.37, 53.74, 49.87, 29.72, 27.96; HRMS: *m/z* 413.1928 $[M+H]^+$ (calculated for: $[C_{20}H_{25}N_6O_4]^+$ 413.1932).


K-25.1_H
K-25.1
^{1}H NMR (500 MHz, cdcl$_3$) δ 7.57 (d, J = 8.6 Hz, 3H), 7.32 (d, J = 8.5 Hz, 2H), 5.45 (d, J = 5.4 Hz, 2H), 3.78 – 3.75 (m, 4H), 3.58 (s, 3H), 3.40 (s, 3H), 3.13 (s, 2H), 2.64 – 2.57 (m, 4H).
A (d) 7.57
B (d) 7.32
C (d) 5.45
D (m) 3.76
E (s) 3.58
F (s) 3.40
H (s) 3.13
G (m) 2.61
f1 (ppm)

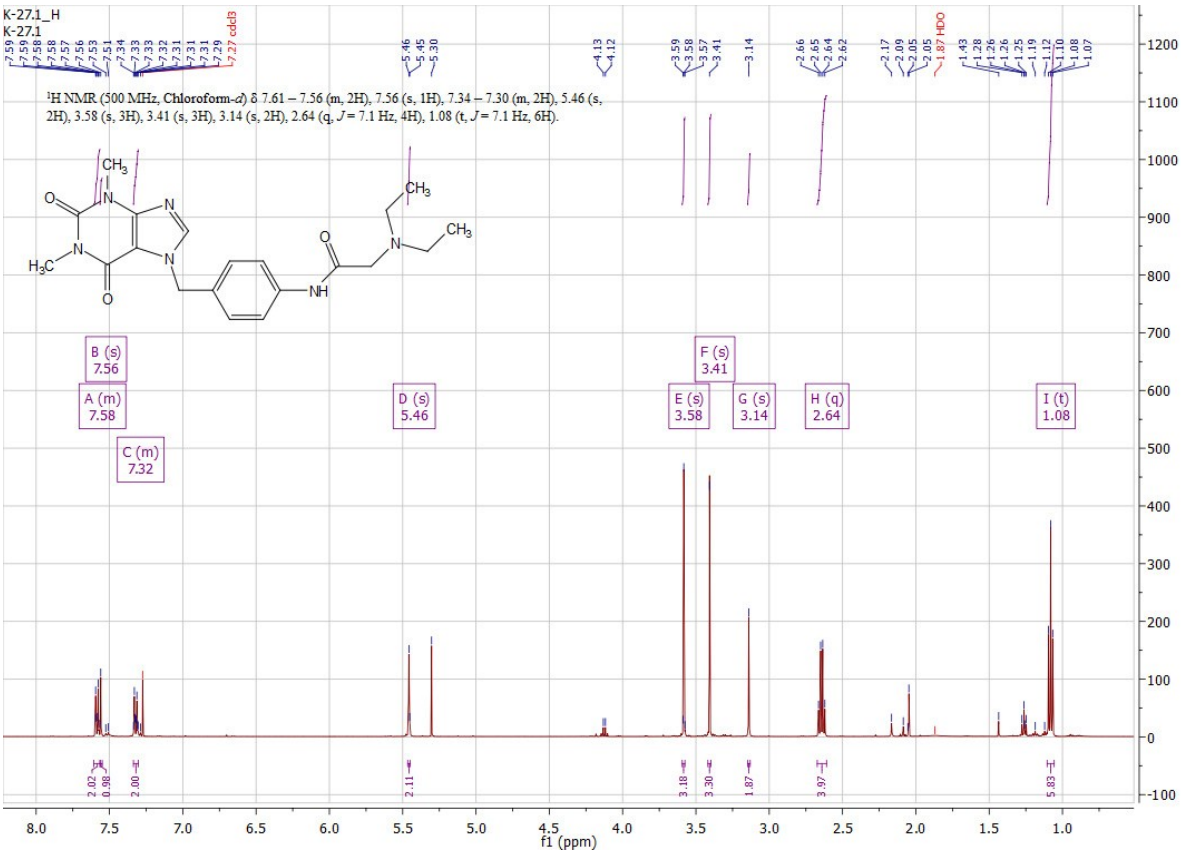

K-25.1_C
K-25.1
^{13}C NMR (126 MHz, cdcl$_3$) δ 167.99, 155.19, 151.59, 148.88, 140.67, 137.75, 131.04, 128.90, 119.87, 106.87, 66.96, 62.37, 53.74, 49.87, 29.72, 27.96.
f1 (ppm)

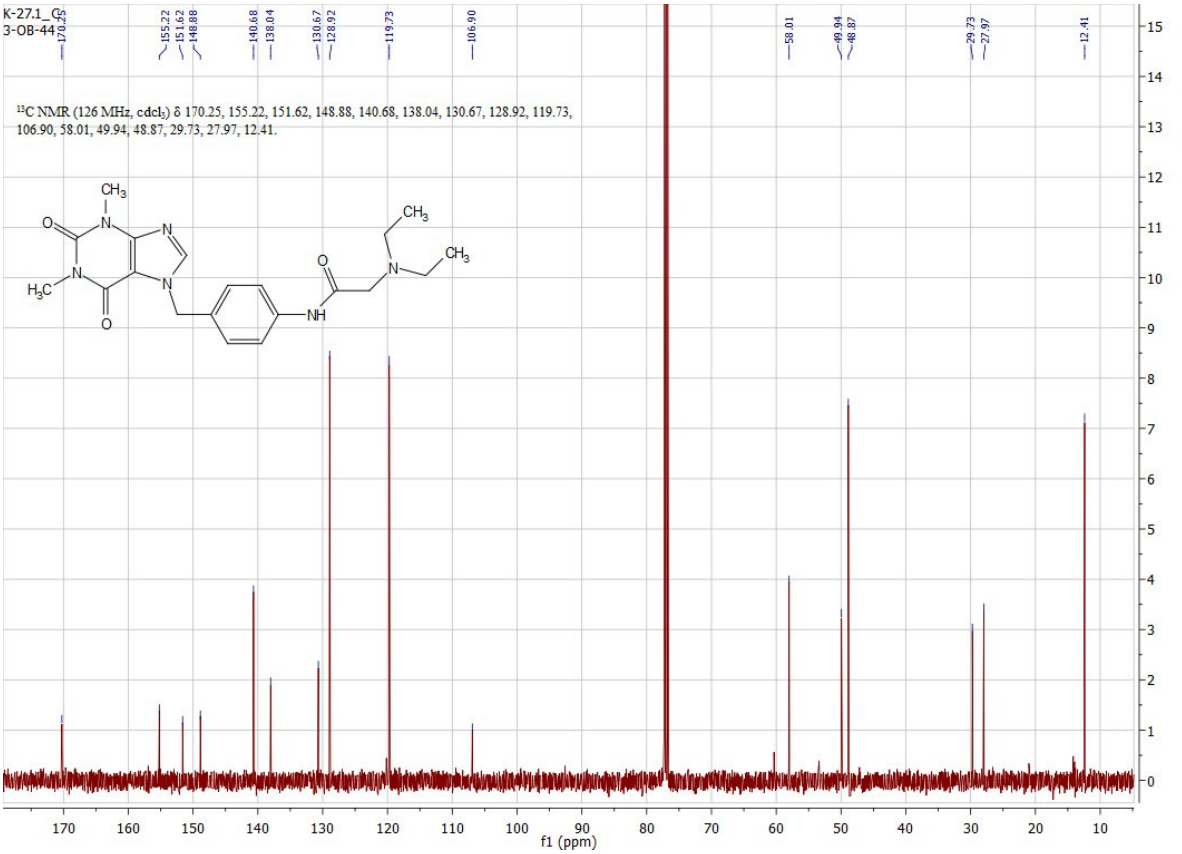
*1.4.3. N-{4-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)methyl]phenyl}-2-(piperidin-1-yl)acetamide (**23**).* Beige solid (0.15 g, 26 %); m.p. 183-186 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.30 (s, 1H), 7.58 – 7.55 (m, 2H), 7.54 (s, 1H), 7.32 – 7.29 (m, 2H), 5.44 (s, 2H), 3.56 (s, 3H), 3.39 (s, 3H), 3.05 (s, 2H), 2.55 – 2.48 (m, 4H), 1.62 (p, *J* = 5.6 Hz, 4H), 1.47 (p, *J* = 5.8 Hz, 2H); ^{13}C NMR (126 MHz, CDCl3) δ (ppm) 169.07, 155.19, 151.60, 148.86, 140.67, 138.04, 130.68, 128.88, 119.79, 106.88, 62.67, 54.85, 49.91, 29.71, 27.95, 26.23, 23.52; HRMS: *m/z* 411.2144 $[M+H]^+$ (calculated for: $[C_{21}H_{27}N_6O_3]^+$ 411.2139).



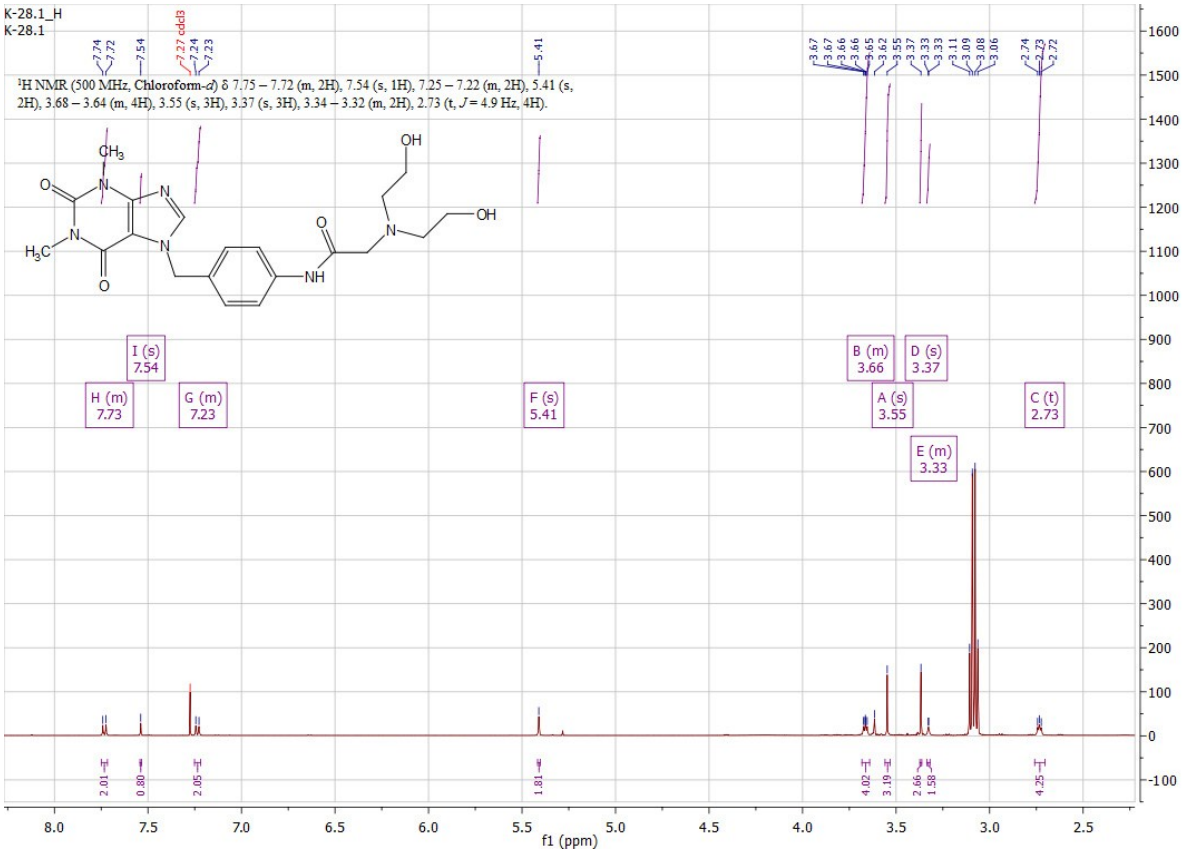


*1.4.4. 2-(diethylamino)-N-{4-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)methyl]phenyl}acetamide (**24**).* Beige solid (0.16 g, 29 %); m.p. 167-172 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.46 (s, 1H), 7.60 – 7.57 (m, 2H), 7.56 (s, 1H), 7.34 – 7.30 (m, 2H), 5.46 (s, 2H), 3.58 (s, 3H), 3.41 (s, 3H), 3.14 (s, 2H), 2.64 (q, J = 7.1 Hz, 4H), 1.08 (t, J = 7.1 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 170.25, 155.22, 151.62, 148.88, 140.68, 138.04, 130.67, 128.92, 119.73, 106.90, 58.01, 49.94, 48.87, 29.73, 27.97, 12.41; HRMS: *m/z* 399.2139 $[M+H]^+$ (calculated for: $[C_{20}H_{27}N_6O_3]^+$ 399.2139).


K-27.1_H
K-27.1
^{1}H NMR (500 MHz, Chloroform-d) δ 7.61 – 7.56 (m, 2H), 7.56 (s, 1H), 7.34 – 7.30 (m, 2H), 5.46 (s, 2H), 3.58 (s, 3H), 3.41 (s, 3H), 3.14 (s, 2H), 2.64 (q, J = 7.1 Hz, 4H), 1.08 (t, J = 7.1 Hz, 6H).
B (s) 7.56
A (m) 7.58
C (m) 7.32
D (s) 5.46
F (s) 3.41
E (s) 3.58
G (s) 3.14
H (q) 2.64
I (t) 1.08
f1 (ppm)


K-27.1_C
3-OB-44
^{13}C NMR (126 MHz, cdcl$_3$) δ 170.25, 155.22, 151.62, 148.88, 140.68, 138.04, 130.67, 128.92, 119.73, 106.90, 58.01, 49.94, 48.87, 29.73, 27.97, 12.41.
f1 (ppm)

*1.4.5. 2-[bis(2-hydroxyethyl)amino]-N-{4-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)methyl]phenyl}acetamide (**25**).* Beige solid (0.22 g, 37 %); m.p. 110-112 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.75 – 7.72 (m, 2H), 7.54 (s, 1H), 7.25 – 7.22 (m, 2H), 5.41 (s, 2H), 3.68 – 3.64 (m, 4H), 3.55 (s, 3H), 3.37 (s, 3H), 3.34 – 3.32 (m, 2H), 2.73 (t, *J* = 4.9 Hz, 4H); Low solubility of the compound disabled to measure ^{13}C NMR spectrum; HRMS: *m/z* 431.2037 $[M+H]^+$ (calculated for: $[C_{20}H_{27}N_6O_5]^+$ 431.2037).

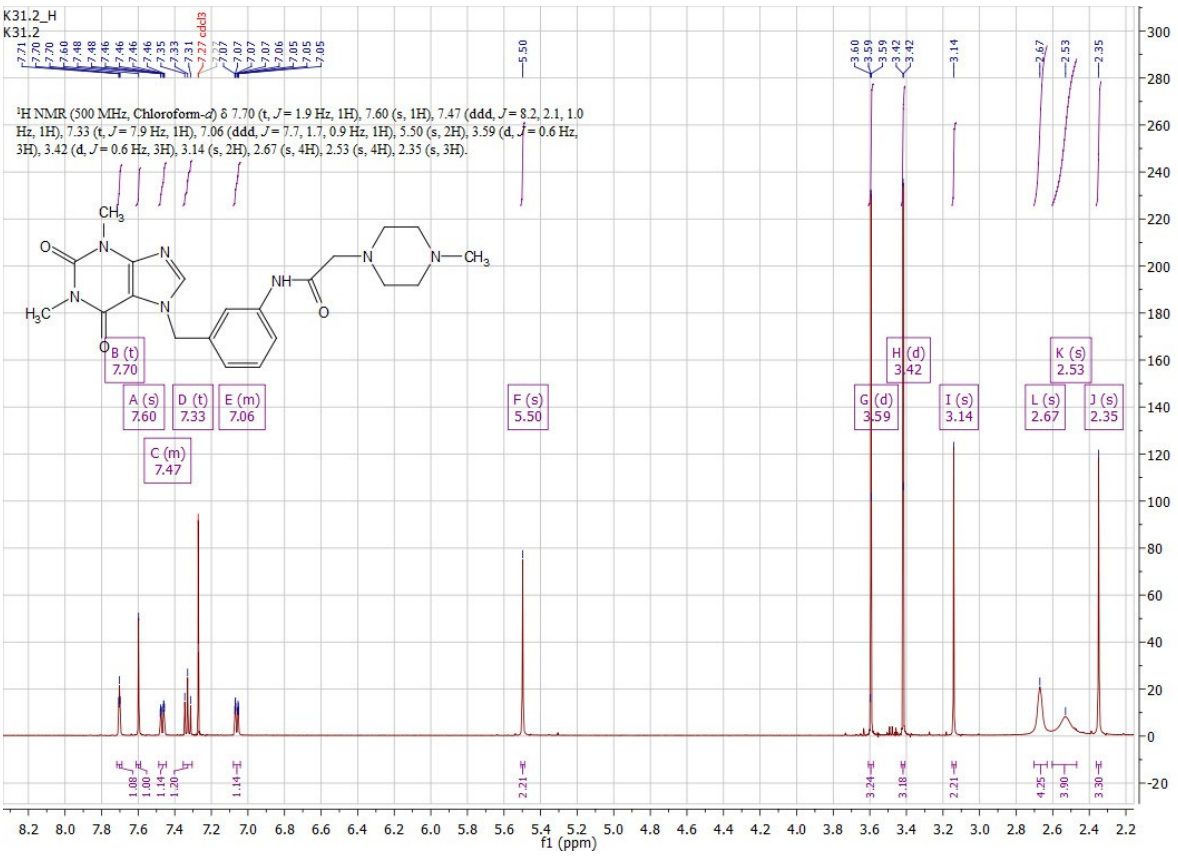


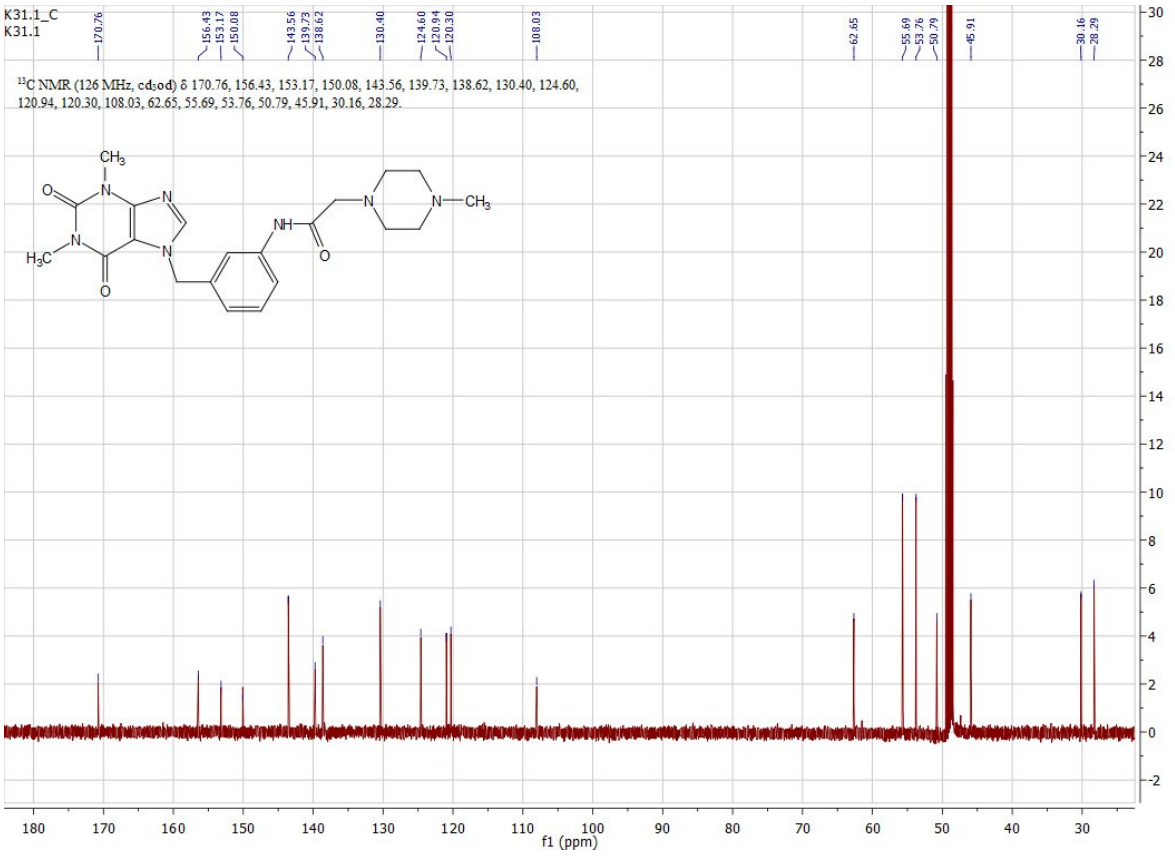
*1.5. Synthesis of 2-chloro-N-{3-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)methyl]phenyl}acetamide intermediate (**26**).*

To a solution of **19** (2 g, 7.01 mmol) and triethylamine (2.15 mL, 15.4 mmol) in THF (10 mL), chloroacetyl chloride (0.61 mL, 7.7 mmol) was added dropwise at 0 °C and the resulting mixture was stirred at r.t. for 12 h. The solvent was then removed under reduced pressure, and the solid residue was triturated with a saturated aqueous solution of $NaHCO_3$ (30 mL) and filtered. The solid was

washed successively with water and dried to yield 2.32 g of crude **26**. The intermediate of brown color was used for subsequent reactions without further purification.

*1.6. Synthesis N-{3-[(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-yl)methyl]phenyl}-2-(4-methylpiperazin-1-yl)acetamide (**27**)* To a stirred solution of **26** (0.63 g, 1.75 mmol) in THF (10 mL) appropriate secondary amine (5.25 mmol) was added dropwise at r.t. The reaction mixture was then heated to 50 °C and stirred overnight following solvent removal. The crude product was purified by column chromatography using EtOAc/MeOH/Et_3N 60:1:0.2 as eluent. Oily residues were then recrystallized from diethyl ether to give white solid (0.19, 25 %); m.p. 162-165 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.60 (d, J = 2.0 Hz, 1H), 7.51 (ddd, J = 8.2, 2.2, 1.1 Hz, 1H), 7.30 (t, J = 7.9 Hz, 1H), 7.11 (dt, J = 7.8, 1.2 Hz, 1H), 5.52 (s, 2H), 3.52 (s, 3H), 3.17 (s, 3H), 2.69 – 2.52 (m, 8H), 2.32 (s, 3H); ^{13}C NMR (126 MHz, cd3od) δ 170.76, 156.43, 153.17, 150.08, 143.56, 139.73, 138.62, 130.40, 124.60, 120.94, 120.30, 108.03, 62.65, 55.69, 53.76, 50.79, 45.91, 30.16, 28.29; HRMS: *m/z* 426.2245 $[M+H]^+$ (calculated for: $[C_{21}H_{27}N7O3]^+$ 425.2175).





2. Testing of biological effect

2.1. Cell cultivation

Selected human tumor cell lines A2780 (ovarian carcinoma), A549 (lung carcinoma), AGS (gastric adenocarcinoma), COLO-201 (colorectal adenocarcinoma), HeLa (cervix adenocarcinoma), HT-29 (colorectal adenocarcinoma), Jurkat (acute T cell leukemia), MCF-7 (breast adenocarcinoma), MOLT-4 (acute lymphoblastic leukemia), PANC-1 (pancreas epithelioid carcinoma) and SW-480 (colorectal adenocarcinoma) were purchased from ATCC (Manassas, USA) or Sigma-Aldrich (St. Louis, USA) and cultivated according to provider´s culture method guidelines. Starting experiments, each cell line was seeded at previously established optimal density (1.10^3 to 30.10^3 cells per well) in a 96-well plate and cells were allowed to settle overnight.

2.2. Cell proliferation assay and growth percent calculation

Caffeine derivatives (**1**-**27**) were dissolved in DMSO (Sigma-Aldrich, St. Louis, MO, USA) and stock solutions were further diluted with appropriate cultivation media; the concentration of DMSO in final medium was 0.1-0.3 %. At the end of the cultivation period, WST-1 proliferation assay (Roche Applied Science, Switzerland) was performed according to the manufacturer´s protocol and the absorbance measured using TecanInfinite200 (Tecan, Switzerland).

2.2.1. Primary screening

Cells were treated for 48 hours with derivatives or caffeine in the final concentration of 20 µM. ATR inhibitor VE-821 (Tinib Tools, Czech Republic) at a concentration of 10 µM was used as positive control and DMSO in concentration of 0.2 % was used as negative control. For primary screening each value is the mean of the absorbance measured in 4 wells and represents the percentage of control, non-treated cells (100%). The growth percent (GP) value was calculated for each derivative tested. GP represents the mean of proliferation decrease in percent of all the 11 cell lines treated with the same derivative. To evaluate the sensitivity of cell lines, the growth percent value was also calculated for each cell line as a mean of proliferation decrease in percent after caffeine derivative treatment (**1**-**27**) in the appropriate cell line (Fig. S1, Tab. S2).

2.2.2. Determination of the half maximal inhibitory concentration (IC_{50})

The cells were seeded at a concentration 5 x 10^3 cells/well in 200 µl culture medium containing various concentration (0,1; 1; 5; 10; 20; 50; 100; 200 µM) of compound 6 and VE-821 and incubated in 5 % CO_2 at 37 °C for 48 h. Cytotoxicity was evaluated as 50% inhibition concentration (IC_{50}) value using the statistic software GraphPad Prism (GraphPad Software, Inc., USA). Experiments were performed at least three times at each drug concentration per experiment (Tab. S1).

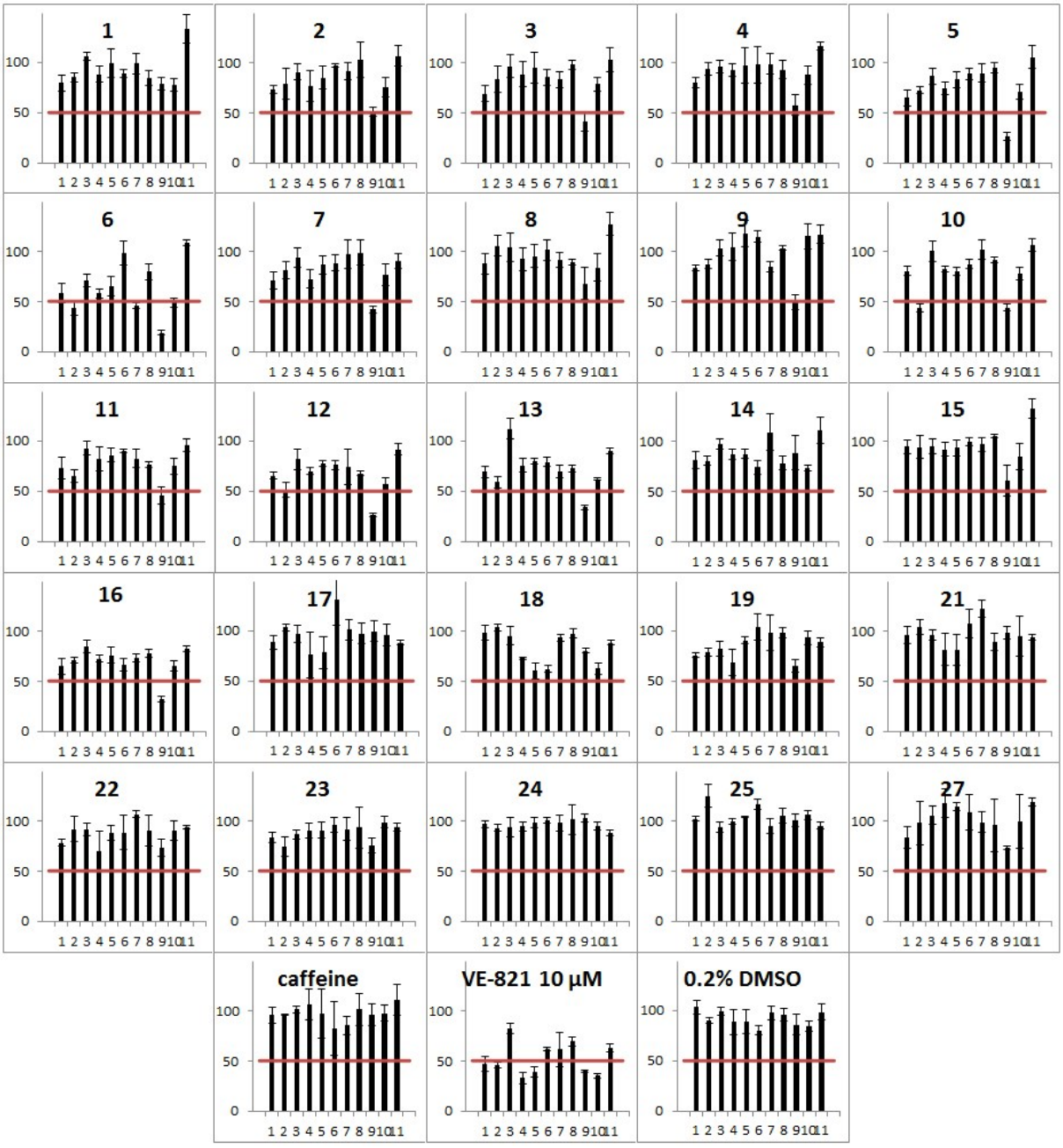


Figure S1. Decrease in cell proliferation after caffeine derivative treatment. Each graph represents proliferation of cells cultivated with indicated caffeine derivative (**1**-**27**) in percent related to untreated control cells (100%). Numbers 1-11 define appropriate cell line (1.Jurkat, 2.MOLT-4, 3.A549, 4.AGS, 5.COLO-201, 6.HT-29, 7.PANC-1, 8.SW-480, 9.A2780, 10.HeLa, 11.MCF-7). Red line borders the 50% value. Error bars indicate ± SD.

Table S1. IC_{50} values of **6** and VE-821 in A2780 and MOLT-4 cell lines.

	A2780 **6**	A2780 VE-821	MOLT-4 **6**	MOLT-4 VE-821
IC_{50} (µmol/l)	**21**	**14**	**31**	**26**
95% Confidence Intervals	16 to 29	9 to 22	24 to 41	16 to 40
Goodnes of fit (R^2)	0,9342	0,876	0,9294	0,8674

2.3. Verification of inhibitory effect on ATM/ATR

Exponentially growing HL-60 cells were suspended in a culture medium at a maximal concentration of 1×10^5/mL. The standard ATR inhibitor VE-821 (10 µM) and caffeine derivative **6** (20, 100 and 200 µM) were added 30 min before irradiation. Aliquots of cell suspension were plated into 25 cm^2 flasks (TPP, Trasadingen, Switzerland or NUNC, Roskilde, Denmark) and were irradiated at room temperature by a dose of 6 Gy using a ^{60}Co γ-ray source (Chisotron Chirana, Prague, Czech Republic). After irradiation, flasks were incubated in 5% CO2 atmosphere under 37 ºC. Non irradiated control cells were handled in the same way, except that irradiation was omitted.

Whole-cell lysates (Cell Lysis Buffer, Cell Signaling Technology, Danvers, MA, USA) were prepared 4 hours after the irradiation, and quantification of the protein content was performed using BCA assay (Sigma-Aldrich, St. Louis, MO, USA). The lysates were loaded into each lane of a polyacrylamide gel. After electrophoretic separation, the proteins were transferred to a PVDF membrane (Bio-Rad, Hercules, CA, USA). Non-specific binding of the membranes was blocked for 1 hour in a Tris-buffered saline containing 0.05% Tween 20 and 5% non-fat dry milk. The membranes were washed twice with TBST, each time for 5 minutes, and once with TBS, again for 5 minutes. Incubation with a primary antibody (β-actin – Sigma-Aldrich, St. Louis, MO, USA; Chk1_serine 345, Chk2_ threonine 68 – Cell Signaling, Danvers, MA, USA) was performed at 4 °C overnight. The following day the membranes were washed five-times with TBST, each time for 5 minutes, and once with TBS, for 10 minutes, and then incubated with an appropriate secondary antibody (DakoCytomation, Glostrup, Denmark) for one hour at room temperature. Band detection was performed using a chemiluminiscence detection

kit (Roche, Basel, Switzerland). To ensure equal protein loading, each membrane was reprobed and β-actin was detected. Band density was quantified using a GeneTools image analysis system (Syngene, Cambridge, UK) and normalised to β-actin.

Table S2. The one-dose primary screening results expressed as % of inhibition, against the proliferation of control cells (100%). Mean and SD of three independent experiments. Concentration of each derivate 0.02 mmol/l, the volume of DMSO in cultivation medium 0.1-0.3%.

	cell line/ derivative	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**21**	**22**	**23**	**24**	**25**	**27**	**caffeine**	**DMSO 0.2%**	**VE-821 10 µM**	**mean GP per line**
1	**Jurkat**	79	73	69	80	65	58	71	87	84	80	73	65	69	81	94	64	88	98	75	96	78	83	97	102	83	95	103	47	**80**
		8	*4*	*8*	*5*	*8*	*9*	*8*	*10*	*3*	*5*	*11*	*4*	*6*	*8*	*7*	*7*	*7*	*7*	*2*	*8*	*4*	*5*	*3*	*2*	*11*	*8*	*7*	*8*	
2	**MOLT-4**	85	78	83	93	72	43	81	105	87	43	65	51	59	80	94	71	103	103	79	104	91	75	93	124	97	96	90	45	**83**
		5	*16*	*14*	*7*	*3*	*7*	*8*	*10*	*5*	*4*	*6*	*8*	*6*	*5*	*12*	*3*	*3*	*3*	*4*	*7*	*13*	*10*	*3*	*12*	*22*	*1*	*3*	*3*	
3	**A549**	106	91	96	96	86	71	93	103	103	100	93	82	112	96	95	84	97	95	82	96	91	86	94	93	105	101	99	82	**94**
		4	*8*	*11*	*7*	*8*	*6*	*10*	*14*	*8*	*10*	*7*	*10*	*10*	*5*	*7*	*6*	*8*	*9*	*7*	*6*	*7*	*4*	*10*	*5*	*9*	*3*	*4*	*5*	
4	**COLO-201**	88	76	88	92	74	58	72	92	104	82	82	70	76	87	91	72	76	73	68	81	70	90	94	99	117	106	88	33	**84**
		8	*15*	*13*	*6*	*7*	*5*	*9*	*11*	*13*	*3*	*12*	*3*	*7*	*5*	*7*	*3*	*23*	*1*	*13*	*16*	*20*	*7*	*5*	*3*	*15*	*15*	*12*	*5*	
5	**HT-29**	99	84	94	96	83	65	86	95	117	80	86	78	79	87	93	75	78	60	90	81	88	91	98	103	114	97	89	39	**88**
		14	*11*	*15*	*18*	*8*	*10*	*9*	*11*	*13*	*4*	*7*	*3*	*3*	*5*	*8*	*8*	*16*	*8*	*3*	*15*	*7*	*8*	*5*	*1*	*4*	*24*	*12*	*5*	
6	**SW-480**	88	97	85	97	89	98	88	101	114	87	90	76	79	74	99	66	130	62	104	107	88	95	100	116	108	83	80	62	**93**
		4	*2*	*8*	*18*	*6*	*12*	*8*	*10*	*6*	*5*	*2*	*4*	*5*	*7*	*4*	*6*	*25*	*3*	*13*	*14*	*17*	*7*	*3*	*5*	*17*	*27*	*5*	*2*	
7	**AGS**	99	91	83	99	89	45	96	91	84	102	82	74	69	109	96	73	101	93	98	122	106	92	98	94	98	85	97	61	**91**
		11	*9*	*8*	*10*	*9*	*3*	*14*	*8*	*5*	*10*	*9*	*17*	*6*	*18*	*7*	*4*	*10*	*3*	*18*	*8*	*4*	*12*	*8*	*8*	*10*	*9*	*7*	*17*	
8	**PANC-1**	84	103	98	92	95	79	98	89	103	91	76	68	73	78	105	77	97	97	98	89	90	93	101	105	95	101	96	69	**91**
		7	*18*	*5*	*9*	*5*	*8*	*12*	*3*	*2*	*3*	*2*	*2*	*3*	*7*	*2*	*4*	*11*	*5*	*5*	*8*	*15*	*20*	*15*	*8*	*26*	*16*	*7*	*5*	
9	**A2780**	79	51	41	57	26	19	42	68	49	43	46	26	34	88	60	32	100	80	65	98	73	75	103	100	73	96	86	39	**62**
		7	*5*	*9*	*10*	*4*	*3*	*3*	*16*	*7*	*3*	*9*	*2*	*2*	*17*	*15*	*3*	*11*	*3*	*6*	*6*	*9*	*8*	*4*	*6*	*2*	*10*	*10*	*1*	
10	**HeLa**	78	75	78	88	71	49	76	83	115	77	75	57	62	73	85	65	96	62	93	95	89	98	95	106	99	97	85	35	**82**
		6	*9*	*7*	*9*	*8*	*5*	*11*	*14*	*13*	*6*	*8*	*6*	*1*	*3*	*13*	*5*	*11*	*5*	*7*	*20*	*10*	*6*	*4*	*4*	*26*	*8*	*5*	*3*	
11	**MCF-7**	133	106	103	116	105	108	90	127	117	106	96	91	90	111	132	82	88	88	88	93	94	94	88	95	118	110	98	62	**103**
		14	*10*	*12*	*4*	*11*	*2*	*8*	*12*	*9*	*7*	*6*	*5*	*3*	*13*	*10*	*3*	*2*	*2*	*4*	*3*	*2*	*4*	*3*	*4*	*4*	*15*	*8*	*4*	
mean GP per derivative		**93**	**84**	**83**	**92**	**78**	**63**	**81**	**95**	**98**	**81**	**78**	**67**	**73**	**88**	**95**	**69**	**96**	**83**	**86**	**96**	**87**	**88**	**96**	**103**	**101**	**97**	**92**	**52**	