Supporting Information

Antitumour imidazotetrazines. Synthesis and chemistry of 4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide (nor-temozolomide): an intermediate for the preparation of the antitumour drug temozolomide and analogues, avoiding the use of isocyanates.

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General procedure:
IR spectra were recorded on a Shimadzu FT-IR8400S. NMR spectra were recorded on a Bruker Avance 400 instrument at 400.13 MHz (1H), 100.62 MHz (13C), 40.54 MHz (15N) in DMSO-d6 or CDC13 with tetramethylsilane as reference; coupling constants are reported in Hz. The LC/MS system consisted of an Agilent Technologies 1200 series LC connected to a 6110 Single Quadrupole MS with ESI source. High resolution mass spectra were recorded on a Micromass LCT spectrometer using electrospray. Elemental analyses were carried out at MEDIAC Ltd., Chobham, Surrey, UK. Merck silica gel 60 was used for flash chromatography. Unless otherwise stated, all commercially available reagents were used without further purification.

**Tert-butyl (8-carbamoyl-4-oxoimidazo[5,1-d][1,2,3,5]tetrazin-3(4H)-yl)methylcarbamate 11**

Ethyl chloroformate (573 µL, 5.99 mmol, 1.05 eq.) followed by triethylamine (556 µL, 5.99 mmol, 1.05 eq.) were added dropwise to a stirred solution of N-(tert-butoxycarbonyl) glycine in THF (20 mL) at 0 °C. The mixture was stirred for 45 minutes before the addition of an aqueous solution (5 mL) of sodium azide (557 mg, 8.57 mmol, 1.5 eq.) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and was then diluted with water. The crude acyl azide was extracted four times with toluene and the combined organic extracts were successively washed with a saturated solution of sodium bicarbonate (twice), 1M HCl and water. The solution of acyl azide in toluene was dried over MgSO4 at 0 °C and was then heated slowly (CAUTION) with stirring until nitrogen gas evolution was observed, which occurred at 58 °C (oil bath temperature). The temperature of the oil bath was maintained at 63 °C for 1.5 h and was then increased slowly to 70 °C. The solution was stirred at this temperature for 30 minutes and was concentrated under reduced pressure to give 1.1 g of tert-butyl isocyanatomethylcarbamate 10 as a 1:1.42 mixture with toluene (ratio determined by 1H NMR, 3.63 mmol of isocyanate, 64% yield). The isocyanate was used without further purification in the next step. IR (νmax, cm⁻¹): 3308.0-3371.7 (w (br)), 2980.1 (w), 2245.2 (s), 1699.3 (s (br)), 1494.9 (s), 1367.6 (s), 1246.1 (s), 1153.5 (s), 947.1 (s), 846.8 (s), 729.1 (s). δH (CDCl3): 5.20 (s (br), 1H, NH), 4.53 (s (br), 2H, CH2), 1.36 (s, 9H, tBu).

1.02g of the crude isocyanate 10 (3.37 mmol, 1.32 eq.) was added dropwise in the dark under nitrogen to a stirred suspension of 5-diazoimidazole-4-carboxamide (350 mg, 2.55 mmol) in dry DMSO (4 mL) and the mixture was stirred overnight. The resulting solution was poured into ice and the precipitate was filtered, washed successively with water, ethyl acetate and diethyl ether to give 0.47g of the title compound as a pale pink solid (60%yield). δH (DMSO-d6): 8.85 (s, 1H, CH), 8.05 (s (br), 1H, NH), 7.83 (s, 1H, CONH2), 7.70 (s, 1H, CONH2), 5.49 (d, 2H, J=6.3Hz, CH2), 1.38 (s, 9H, tBu). δC (DMSO-d6): 161.4 (CONH2), 155.0 (C=O), 138.6 (C3), 134.1 (C1), 131.0 (C9), 129.1 (C8), 78.9 (C(=Bu)), 54.8 (CH2), 28.0 (tBu). IR (νmax, cm⁻¹): 3354.3 (w), 3163.4 (w), 3099.7 (w), 2984.0 (w), 1739.9 (s), 1709.0 (s), 1687.8 (s), 1614.5 (s) 1520.0 (s), 1460.2 (m), 1365.7 (m), 1269.2 (m), 1244.1 (s), 1221.0 (m), 1167.0 (s (br)), 1126.5 (m), 1049.3 (m), 1018.4 (m), 906.6 (m), 734.9 (s), 713.7 (s). LCMS: 94% pure, m/z (ES⁻): 332.0 (M+Na). HRMS (ES⁻): Found: 310.1319. C3H7N2O2 (M⁻) requires 310.1258.

**4-Oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide 1b**

A suspension of tert-butyl (8-carbamoyl-4-oxoimidazo[5,1-d][1,2,3,5]tetrazin-3(4H)-yl)methylcarbamate 11 (2.93 g, 9.47 mmol) in 3N HCl (130 mL) was stirred at room temperature overnight and was then kept at 4 °C for 4 h. The precipitate was filtered and washed successively with water, ethyl acetate and diethyl ether to give 1.68 g of the title compound as a pale pink solid (98% yield). δH (DMSO-d6): 14.95 (s, 1H, NH), 8.77 (s, 1H, CH), 7.75 (s, 1H, CONH2), 7.65 (s, 1H, CONH2), δC (DMSO-d6): 161.6 (CONH2), 139.0 (C3), 134.5 (C1), 130.3 (C9), 128.5 (C8). δH (DMSO-d6): 15.5 (s, 1H), -35.4 (s, 1H), -107.4 (d, J=11.3Hz, IN), -180.6 (s, 1H), -199.5 (d, J=3.7Hz, IN), -275.1 (s, 1H). IR (νmax, cm⁻¹): 3543.4 (w), 3446.91 (w), 3132.5 (m), 2704.3-3257.9 (m (br)), 1749.5 (s), 1672.3 (s), 1633.8 (s), 1601.0 (s), 1479.5 (m), 1442.8 (m), 1410.0 (m), 1242.2 (m), 1222.9 (s), 1151.5 (m), 1066.9 (m), 939.4 (m), 833.3 (m), 727.2 (s), 700.2 (s), 682.8 (s), 634.6 (s). HRMS (ES⁻): Found: 181.0440. C3H7N2O2 (M⁻) requires 181.0468. Found: C: 30.95; H: 2.89; N: 43.31. 8.08H2O requires C: 30.87; H: 2.90; N: 43.20%

**Representative procedure for the alkylation of 8: Synthesis of temozolomide 1a**

A 60% dispersion of sodium hydride in mineral oil (16.3 mg, 0.408 mmol, 1.05 eq.) was added in one portion to a slurry of nor-temozolomide 1b (70 mg, 0.389 mmol) in dry DMP (4.5 mL) at 0 °C. The resulting suspension was stirred 3 minutes before the addition of methyl iodide (48 µL, 0.778 mmol, 2 eq.). The mixture became rapidly a green solution and was stirred at 0 °C for 30 minutes and then at room temperature for 2 h. The mixture was concentrated to dryness under high vacuum and the crude product was absorbed on silica. The crude product was purified by flash chromatography using DCM:MeOH 95:5 as eluant and two fractions were collected. The first fraction (46 mg, 61 % yield) corresponded to compound 1a. δH (DMSO-d6): 8.82 (s, 1H, CH), 7.80 (s, 1H, CONH2), 7.67 (s, 1H, CONH2), 3.86 (s, 3H, CH3). The 1H NMR data were consistent with the data of an original sample of temozolomide, as shown by an NMR spectrum of a mixture of temozolomide and the product obtained above. The second fraction (18 mg) was identified as a 1:0.70 mixture of compound 1a and one of its regioisomers.

**Deuterated temozolomide 1c:**

δH (DMSO-d6): 8.82 (s, 1H, CH), 7.78 (s, 1H, CONH2), 7.66 (s, 1H, CONH2), δC (DMSO-d6): 162.0 (CONH2), 139.7 (C3), 135.1 (C9), 131.0 (C8), 129.8 (C7). Note: the septet for CD3 was not observed. IR (νmax, cm⁻¹): 3437.3 (m), 3122.9 (m), 2984.0 (w), 1739.9 (s), 1709.0 (s), 1687.8 (s), 1614.5 (s)
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1734.1 (s), 1676.2 (s), 1602.9 (m (br)), 1589.4 (m (br)), 1484.6 (m), 1398.4 (m), 1359.9 (m (br)), 1267.3 (s), 1126.5 (s (br)), 985.7 (m), 846.8 (m), 815.9 (m), 800.5 (m), 733.0 (s), 709.8 (s). HRMS (ESI\(^+\)): Found: 198.0803. C\(_{9}\)H\(_{14}\)N\(_{2}\)O\(_{2}\) (MH\(^+\)) requires 198.0813. The analytical data were consistent with the reported data of an original sample synthesized from 5-diazoimidazole-4-carboxamide and deuteriated methyl isocyanate,\(^{1,9}\)

10. Representative procedure for the alkylation of nor-temozolomide 8 under Mitsunobu conditions: Synthesis of 3-benzyl-4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide 1e.

11. Diisopropyl azodicarboxylate (210 µL, 1.112 mmol, 4 eq.) was added dropwise at 0°C to a slurry of nor-temozolomide 1b (50 mg, 0.278 mmol), polymer supported triphenylphosphine (3 mmol/g, 371 mg, 1.112 mmol, 4 eq.) and benzyl alcohol (106 mL, 1.112 mmol, 4 eq.) in dry DMF.

12. 1 mL under nitrogen, and the resulting mixture turned dark green after 2 h. The mixture was stirred for 2 days at room temperature and was then diluted with acetonitrile. The suspension was filtered over Celite® and the filtrate was concentrated to dryness under high vacuum. The product was dissolved in MeCN:MeOH, absorbed on silica and purified by flash chromatography (gradient elution DCM:MeOH 98:2 to 95:5) to give the title compound as a white solid (19mg, 25%). δ\(_1\)H (DMSO-\(d_6\)): 8.82 (s, 1H, CH), 7.80 (s (br), 1H, CONH\(_2\)), 7.67 (s (br), 1H, CONH\(_2\)), 7.39-7.45 (m, 2H, ArCH), 7.30-7.39 (m, 3H, ArCH), 5.50 (s, 2H, CH\(_2\)). HRMS (ESI\(^+\)): Found: 271.0974. C\(_{12}\)H\(_{15}\)N\(_{2}\)O\(_{2}\) (MH\(^+\)) requires 271.0938. The analytical data were consistent with the data of an original sample synthesized from 5-diazoimidazole-4-carboxamide and benzyl isocyanate.\(^{1,9}\)

3-(Hydroxymethyl)-4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide 9

1. From nor-temozolomide 1b

A suspension of nor-temozolomide 1b (500 mg, 2.78 mmol) in 37% aqueous formaldehyde (10 mL) was stirred at room temperature overnight. The suspension was filtered and the product was washed with water, ethyl acetate and diethyl ether to give 459 mg of the title product as a pink solid (79%).

δ\(_1\)H (DMSO-\(d_6\)): 8.86 (s, 1H, CH), 7.82 (s, 1H, CONH\(_2\)), 7.70 (s, 1H, CONH\(_2\)), 7.22 (t, 1H, OH, J=7.7Hz), 5.62 (d, 2H, CH\(_2\), J=7.7Hz). δ\(_{13}\)C (DMSO-\(d_6\)): 161.5 (C=O), 138.8 (C\(_6\)), 134.2 (C\(_4\)), 131.0 (C\(_9\)), 129.3 (C\(_{10}\)), 71.9 (CH\(_3\)). IR (v\(_{\text{max}}\) cm\(^{-1}\)): 3444.9 (m), 3344.68 (w), 3325.4 (w), 3128.6 (w), 2980.12 (w)

δ\(_1\)H (DMSO-\(d_6\)): 8.82 (s, 1H, CH), 7.81 (s, 1H, CONH\(_2\)), 7.68 (s, 1H, CONH\(_2\)), 3.87 (s, 3H, CH\(_3\)). LCMS: 91% pure; m/z: 217.4 (M+Na\(^+\)).

4-Oxo-3-(prop-2-ynyl)-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide 1d.

δ\(_1\)H (DMSO-\(d_6\)): 8.86 (s, 1H, CH), 7.82 (s, 1H, CONH\(_2\)), 7.70 (s, 1H, CONH\(_2\)), 5.14 (d, 2H, J=2.5Hz, CH\(_2\)), 3.52 (t, 1H, J=2.5Hz, CH). δ\(_{13}\)C (DMSO-\(d_6\)): 161.4 (CONH\(_2\)), 138.5 (C\(_8\)), 134.2 (C\(_{10}\)), 131.3 (C\(_9\)), 129.2 (C\(_6\)), 77.3 (C=CH), 76.5 (C=CH), 38.4 (CH\(_3\)). IR (v\(_{\text{max}}\) cm\(^{-1}\)): 3425.7 (w (br)), 3286.8 (w), 3163.4 (w (br)), 3092.0 (w), 1736.0 (s), 1672.3 (s), 1608.7 (s), 1484.8 (m (br)), 1365.7 (m), 1346.4 (m), 1309.7 (w), 1276.9 (w), 1255.7 (m), 1070.5 (s), 1035.8 (m), 955.3 (m), 891.1 (m), 848.7 (m), 738.8 (s), 719.5 (s). LCMS: crude product 91% pure; m/z: 459.2 (2M+Na\(^{2+}\)), 241.4 (M+Na\(^{+}\) (100), 219.2 (M\(^{+}\)).

HRMS (ESI\(^+\)) Found: 219.0631. C\(_8\)H\(_7\)N\(_2\)O\(_2\) (MH\(^+\)) requires 219.0652. Found: C, 43.70; H, 2.75; N, 38.61
C₈H₆N₆O₂ requires C, 44.04; H, 2.77; N, 38.52

**Ethyl 2-(8-carbamoyl-4-oxoimidazo[5,1-d][1,2,3,5]tetrazin-3(4H)-yl)acetate If.**

δ_H (DMSO_d₆): 8.93 (s, 1H, CH), 7.89 (s, 1H, CONH₂), 7.75 (s, 1H, CONH₂), 5.23 (s, 2H, CH₂), 4.20-4.23 (q, 2H, J=7.1Hz, CH₂), 1.22-1.26 (t, 3H, J=7.1Hz, CH₃). LCMS: 97%

pure; m/z: 289.4 (M+Na⁺), 267.5 (MH⁺). The NMR data were consistent with the reported data of an original sample synthesized from 5-diazoimidazole-4-carboxamide 3 and ethyl isocyanoacetate.
Comparison of analytical data for compounds 1a, 1b and compound reported in ref 12.

$^{13}$C chemical shifts of compounds 1a, 1b and compound reported ref.12

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<th>C-8</th>
<th>C-8a</th>
<th>CH$_3$</th>
<th>CONH$_2$</th>
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<td>134.5</td>
<td>-</td>
<td>161.6</td>
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$^{15}$N chemical shifts of compounds 1a, 1b and compound reported in ref.12

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<th>N-3</th>
<th>N-5</th>
<th>N-7</th>
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Isolated product spiked with authentic sample of Temozolomide.
starting material due to decomposition of the sample in DMSO

formaldehyde due to decomposition of the sample in DMSO