Electronic Supplementary Material (ESI) for MedChemComm.

Synthesis and growth-inhibitory activities of imidazo[5,1-*d*]-1,2,3,5-tetrazine-8-carboxamides related to the anti-tumour drug temozolomide, with appended silicon, benzyl and heteromethyl groups at the 3-position

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ELECTRONIC SUPPLEMENTARY INFORMATION

CONTENTS

4-Oxo-3-phenethyl-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide (13b)

Phenethyl isocyanate (441 mg, 3.0 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (274 mg, 2.0 mmol) in dry dimethylsulfoxide (2.5 mL) at room temperature under nitrogen. The resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of ice and the solid product (pale pink) was removed by filtration, washed with water and ethyl acetate, and recrystallised from chloroform to give the title compound (259 mg, 46%); (Found: $M+H^+$, 285.1161. $C_{13}H_{12}N_6O_3+H^+$ requires 285.1100); v_{max}/cm^{-1} 3132,1743, 1668; δ_H (400 MHz; d_6-DMSO) 8.83 (1 H, s, C<u>H</u>), 7.81 (1 H, br s, NH<u>H</u>), 7.68 (1 H, br s, N<u>H</u>H), 7.21-7.33 30 (5 H, m, Ar<u>H</u>), 4.53 (2 H, t, J 7.4, C<u>H</u>$_2$), 3.14 (2 H, t, J 7.4, C<u>H</u>$_2$); δ_c (100 MHz; d_6-DMSO) 162.0, 139.4, 138.2, 134.7, 131.2, 129.3, 129.2, 129.0. 127.1, 50.4, 31.1; Found C 55.0, H 4.3, N 29.4. Calc, for $C_{13}H_{12}N_6O_3$ C 54.9, H 4.3, N 29.6%

3-(2-Methoxylbenzyl)-4-oxo-3,4-dihyroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide (13c)

ortho-Methoxybenzyl isocyanate (390 mg, 2.4 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (274 mg, 2.0 mmol) in dry dimethylsulfoxide (2.5 mL) at room temperature under nitrogen. The resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of ice and the solid product (off-white) removed by filtration, washed with water and ethyl acetate then recrystallised from chloroform/hexane (343 mg, 57%); (Found: $M+H^+$, 301.1100. $C_{13}H_{12}N_6O_3+H^+$ requires 301.1049); v_{max}/cm^{-1} 3460, 3094, 1728, 1683, 1589, 1454; δ_H (400 MHz; d_6-DMSO) 8.84 (1 H, s, C<u>H</u>), 7.81 (1 H, br s, NH<u>H</u>), 7.69 (1 H, br s, N<u>H</u>H), 7.30-7.33 (1 H, m, Ar<u>H</u>), 7.27 (1 H, d, *J* 7.5, 1.4, Ar<u>H</u>), 7.06 (1 H, d, *J* 7.8, Ar<u>H</u>), 6.91 (1 H, td, *J* 7.5, 0.8, Ar<u>H</u>), 5.45 (2 H, s, C<u>H</u>$_2$Ar); δ_C (100 MHz; d_6-DMSO) 162.0, 157.1, 139.6, 134.9, 131.4, 129.7, 129.4, 129.1,

123.7, 120.7, 111.3, 56.0, 47.9; Found C 51.6, H 3.9, N 28.0. Calc, for $C_{13}H_{12}N_6O_3$ C 52.0, H 4.0, N 28.0%.

3-(3-Methoxybenzyl)-4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide (13d)

meta-Methoxybenzyl isocyanate (390 mg, 2.4 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (274 mg, 2.0 mmol) in dry dimethylsulfoxide (2.5 mL) at room temperature under nitrogen. The resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of ice and the solid product (off-white) removed by filtration, washed with water and ethyl acetate, and recrystallised from chloroform (335 mg, 56%); (Found: $M+H^+$, 301.1091. $C_{13}H_{12}N_6O_3+H^+$ requires 301.1049); ν_{max}/cm^{-1} 3092, 1730, 1678, 1601; δ_H (400 MHz; d_6-DMSO), 8.84 (1 H, s, C<u>H</u>) 7.82 (1 H, br s, N<u>H</u>H), 7.70 (1 H, br s, NH<u>H</u>), 7.28-7.32 (1 H, s, Ar<u>H</u>), 7.00-7.02 (1 H, m, Ar<u>H</u>), 6.89- 6.92 (1 H, m, Ar<u>H</u>), 5.49 (2 H, s, C<u>H</u>$_2$Ar), 3.76 (3 H, s, Me); δ_c (100 MHz; d_6-DMSO) 162.0, 159.9, 139.7, 137.6, 134.9, 131.4, 130.1, 129.5, 120.4, 114.0, 113.7, 55.6, 52.2; Found C 51.3, H 3.9, N 27.8. Calc, for $C_{13}H_{12}N_6O_3$ C 52.0, H 4.0, N 28.0%.

3-(2,4-Dimethoxybenzyl)-4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide (13f)

To 2,4-dimethoxyphenylacetic acid (3.92 g, 20 mmol) in dry toluene (100 mL) was added dry triethylamine (2.93 mL, 4.2 mmol) and diphenyl phosphorylazide (4.31 mL, 4 mmol). The mixture was stirred at room temperature for 0.5 h, and then heated at reflux for a further 3 h. After cooling, the mixture was concentrated under reduced pressure and purified by distillation using an oil pump to give 2,4-dimethoxybenzyl isocyanate (2.31 g, 60%); ν_{max}/cm^{-1} 2243.

2,4-dimethoxybenzyl isocyanate (717 mg, 4.4 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (548 mg, 4 mmol) in dry dimethylsulfoxide (5 mL) at room temperature under nitrogen. The resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of ice and the solid product (purple) removed by filtration, washed with water and ethyl acetate and recrystallised from chloroform/hexane to give the *title compound* (613 mg, 46%); v_{max}/cm^{-1} 3473, 3121, 1734, 1697, 1605, 1589; δ_H (400 MHz; d_6-DMSO) 8.81 (1 H, s, CH), 7.80 (1 H, br s, NHH), 7.68 (1 H, br s, NHH), 7.22 (1 H, d, *J* 8.4, ArH), 6.60 (1 H, d, *J* 2.4, ArH), 6.48 (1 H, dd, *J* 8.4, 2.4, ArH), 5.37 (2 H, s, CH_2Ar), 3.80 (3 H, s, OMe), 3.76 (3 H, s, OMe); δ_C (100 MHz; d_6-DMSO) 162.0, 161.1, 158.5, 139.4, 134.8, 131.2, 130.7, 129.3, 115.9, 105.1, 98.9, 56.1, 55.8, 47.5.

3-(3,4-Dimethoxybenzyl)-4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide (13g)

To 3,4-dimethoxyphenylacetic acid (785 mg, 4.0 mmol) in dry toluene (22.5 mL) was added dry triethylamine (425 mg, 4.2 mmol) and diphenyl phosphorylazide (0.86 mL, 4.0 mmol). The reaction mixture was stirred at room temperature for 0.5 h, and then heated at reflux for a further 3 h. After cooling, the mixture was concentrated under reduced pressure and the crude product (3,4-dimethoxybenzyl isocyanate) was used in the next step.

Crude 3,4-dimethoxybenzyl isocyanate (4 mmol) was added to a suspension of 5-diazoimidazole-4-carboxamide (274 mg, 2.0 mmol) in dry dimethylsulfoxide (2.5 mL) at room temperature under nitrogen. The resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of ice and the solid product was removed by filtration and purified by column chromatography to give the as a *title compound* colourless solid (68 mg, 10%); v_{max}/cm^{-1} 1734, 1686, 1616; δ_H (400 MHz; d_6-DMSO) 8.83 (1 H, s, CH), 7.82 (1 H, br s, NHH), 7.70 (1 H, br s, NHH), 7.03 (1 H, d, *J* 1.9, ArH), 6.91-6.98

(2 H, m, ArH), 5.42 (2 H, s, CH_2Ar), 3.74 (3 H, s, OMe), 3.73 (3 H, s, OMe); δ_C (100 MHz; d_6-DMSO) 162.0, 149.2, 149.1, 139.6, 134.9, 131.3, 129.4, 128.3, 121.1, 112.4, 112.1, 56.0, 56.0, 52.2.

4-Oxo-3-(3,4,5-trimethoxybenzyl)-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide (13h)

3,4,5-Trimethoxybenzyl isocyanate (536 mg, 2.4 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (274 mg, 2.0 mmol) in dry dimethylsulfoxide (2.5 mL) at room temperature under nitrogen. The resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of ice and the solid product removed by filtration, washed with water and ethyl acetate, and then recrystallised from chloroform/hexane to give an off-white powder 323 mg, 45%); (Found: $M+H^+$, 361.1300. $C_{15}H_{16}N_6O_5+H^+$ requires 361.1260); ν_{max}/cm^{-1} 1740, 1690, 1589; δ_H (400 MHz; d_6-DMSO) 8.84 (1 H, s, CH), 7.81 (1 H, br s, NHH), 7.68 (1 H, br s, NHH), 6.75 (2 H, s, ArH), 5.42 (2 H, s, CH_2Ar), 3.76 (6 H, s, 3,5-OMe), 3.64 (3 H, s, 4-OMe); δ_C (100 MHz; d_6-DMSO) 162.0, 153.4, 139.7, 137.6, 134.9, 131.7, 131.3, 129.4, 105.9, 60.4, 56.4, 52.5; Found C 49.5, H 4.4, N 23.2. Calc, for $C_{15}H_{16}N_6O_5$ C 50.0, H 4.5, N 23.3%.

4-Oxo-3-(thiophen-2-ylmethyl)-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide (13j)

To 2-thiopheneacetic acid (2.84 g, 20 mmol) in dry toluene (22.5 mL) was added dry triethylamine (2.93 mL, 4.2 mmol) and diphenyl phosphorylazide (4.31 mL, 4.0 mmol). The reaction mixture was stirred at room temperature for 0.5 h, and then heated at reflux for a further 3 h. After cooling, the mixture was concentrated under reduced pressure and purified

by distillation using an oil pump to give thiophen-2-ylmethyl isocyanate (350 mg, 12%) as a colourless oil.

Thiophen-2-ylmethyl isocyanate (350 mg, 2.5 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (314 mg, 2.3 mmol) in dry dimethylsulfoxide (2.5 mL) at room temperature under nitrogen. The resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of ice and the solid product (off-white) was removed by filtration, washed with water and ethyl acetate and air dried to give the title compound (405 mg, 64%); ν_{max}/cm^{-1} 3094, 1732, 1690, 1607; δ_H (400 MHz; d_6-DMSO) 8.85 (1 H, s, C<u>H</u>) 7.83 (1 H br s, N<u>H</u>H), 7.71 (1 H, br s, NH<u>H</u>), 7.55 (1 H, dd, *J* 5.1, 1.0, Ar<u>H</u>), 7.25 (1 H, dd, *J* 3.5, 1.0, Ar<u>H</u>), 7.03 (1 H, dd, *J* 5.1, 3.5, Ar<u>H</u>), 5.68 (2 H, s, C<u>H</u>$_2$Ar).

4-Oxo-3-(thiophen-3-ylmethyl)-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carboxamide (13k)

To 3-thiopheneacetic acid (2.84 g, 20 mmol) in dry toluene (22.5 mL) was added dry triethylamine (2.93 mL, 4.2 mmol) and diphenyl phosphorylazide (4.31 mL, 4 mmol). The mixture was stirred at room temperature for 0.5 h, and then heated at reflux for a further 3 h. After cooling, the mixture was concentrated under reduced pressure and purified by distillation using an oil pump to give thiophen-3-ylmethyl isocyanate (1.14 g, 40%) as a colourless oil.

Thiophen-3-ylmethyl isocyanate (306 mg, 2.2 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (274 mg, 2.0 mmol) in dry dimethylsulfoxide (2.5 mL) at room temperature under nitrogen. The resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of ice and the solid product (off-white) removed by filtration, washed with water and ethyl acetate and air dried (235 mg, 42%); ν_{max}/cm^{-1} 3094, 1728, 1688, 1604; δ_H (400 MHz; d_6-DMSO) 8.85 (1 H, s, C<u>H</u>), 7.83 (1 H, br s,

NHH), 7.70 (1 H, br s, NHH), 7.55-7.58 (2 H, m, ArH), 7.17 (1 H, dd, *J* 4.9, 1.3, ArH), 5.50 (2 H, s, CH_2Ar); δ_C (100 MHz; d_6-DMSO) 162.0, 139.5, 136.7, 134.9, 131.4, 129.5, 128.0, 127.3, 124.4, 48.0.

3-(3-Methyl-isoxazol-5-ylmethyl)-4-oxo-3,4-dihydro-imidazo[5,1-d][1,2,3,5]tetrazine-8-carboylic acid amide (13l)

5-lsocyanatomethyl-3-methyl-isoxazole (735 mg, 5.32 mmol) was diluted in dimethylsufoxide (1.5 mL) and added to a solution of 5-diazoimidazole-4-carboxamide (250 mg, 1.83 mmol) in dimethylsulfoxide (2.0 mL). The reaction mixture was stirred at room temperature for 16 h before the addition of sufficient ice water to cause precipitation of the crude product which was collected by vacuum filtration and washed with diethyl ether to give the *title compound* as a yellow solid (503 mg, 98 %); δ_H (400 MHz; d_6-DMSO) 8.86 (1 H, s), 7.84 (1 H, s), 7.71 (1 H, s), 6.51 (1 H, s), 5.64 (2 H, s), 2.21 (3 H, s).

3-(3-Methyl-[1,2,4]oxadiazol-5-ylmethyl)-4-oxo-3,4-dihydro-imidazo[5,1-d][1,2,3,5]-tetrazine-8-carboxylic acid amide (13m)

Hydrazine hydrate (75 μL, 64% in water) was added to a solution of 3-methyl [1,2,4]oxadiazol-5-yl)-acetic acid methyl ester (160 mg, 1.02 mmol) in EtOH (1.0 mL). The resultant mixture was heated to reflux for 1 h whereupon it was cooled to room temperature and concentrated in vacuo. Diethyl ether was added to the mixture which was then cooled to 0 °C. The resulting yellow precipitate was collected by filtration and washed with cold diethyl ether to give (3-methyl-[1,2,4]oxadiazol-5-yl)-acetic acid hydrazide (quantitative yield) in suitably pure form to be used without any further purification.

An aqueous solution of sodium nitrite (84 mg, 1.2 mmol) was added to a solution of (3-methyl-[1,2,4]oxadiazol-5-yl)-acetic acid hydrazide (1.02 mmol) at 0 ˚C in a 1:1 mixture of

dichloromethane:hydrochloric acid (1 M HCl; 1.5 mL). The reaction mixture was stirred at this temperature for 5 min before being poured onto crushed ice. The crude azide was extracted with dichloromethane and then dried over $MgSO_4$, filtered and concentrated in vacuo. Toluene (1.5 mL) was added to the crude residue and the resultant mixture heated to 80 °C for 1 h whereupon it was cooled and concentrated in vacuo to give a crude orange residue corresponding to 5-isocyanatomethyl-3-methyl-[1,2,4]oxadiazole (quantitative yield); ν_{max}/cm^{-1} 2261, 1593, 1495, 1435, 1325; δ_H (400 MHz; $CDCl_3$) 4.56 (2 H, s), 2.36 (3 H, s).
The isocyanate was diluted in dimethylsulfoxide (1.5 mL) and added to a solution of 5-diazoimidazole-4-carboxamide (355 mg, 2.58 mmol) in dimethylsulfoxide (3.5 mL). The reaction mixture was stirred at room temperature for 10 min before the addition of sufficient ice water to cause precipitation of the crude product which was collected by vacuum filtration and washed with ethanol and then diethyl ether to give the *title compound* as a yellow solid (399 mg, 56 % yield); δ_H (400 MHz; d_6-DMSO) 8.92 (1 H, s), 7.88 (1 H, s), 7.74 (1 H, s), 5.89 (2 H, s), 2.34 (3 H, s); MS (ES+): 277.08 ($M+H^+$, 100).

4-Oxo-3-(1H-[1,2,3]triazol-4-ylmethyl)-3,4-dihydro-imidazo[5,1-d][1,2,3,5]tetrazine-8-carboxylic acid amide (13n)

To an aqueous suspension of 3-propargyl-8-carbamoylimidazotetrazin-4-one (100 mg, 0.46 mmol), $CuSO_4.5H_20$ (5.7 mg, 0.023 mmol) in water:*tert*-butanol (1:1; 1 mL) was added sodium ascorbate (14 mg, 0.069 mmol) and trimethylsilyl azide (105 μL, 0.80 mmol). The reaction mixture was stirred at room temperature for 72 h, whereupon it was poured onto crushed ice. The resultant precipitate was filtered, washed with ethyl acetate and then diethyl ether to give the *title compound* (25 mg, 42%) as a purple solid; δ_H (400 MHz; d_6-DMSO) 15.1 (1 H, br s), 8.86 (1 H, s), 7.92 (1 H, br s), 7.81 (1 H, s), 7.69 (1 H, s), 5.61 (2 H, s); MS (ES+): 545.2 ($(2M+Na)^+$, 5), 262.1 25 ($M+H^+$, 80), 126.1 (85), 85.1 (100).

(R)-3-(1-Phenylethyl)-8-carbamoylimidazo[5,1-d][1,2,3,5]tetrazine-4-one (13o)

(R)-(+)-1 -PhenyIethyI isocyanate (0.6 mL, 4.2 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (0.5 g, 3.65 mmol) in dry dimethylsulfoxide (5 mL) at room temperature under nitrogen. The reaction mixture was stirred at the dark at room temperature under nitrogen. After 16 h, the reaction mixture was poured onto ice and extracted with dichloromethane (3 x 25 mL). The combined organics were washed with water (25 mL) and brine (25 mL), dried over sodium sulfate, and evaporated to dryness. The resulting solid was triturated with ethyl acetate then purified by flash column chromatography (SiO_2, 3:7 acetonitrile in dichloromethane) to furnish the desired product as a colourless solid (577 mg, 56%); LCMS (ES+) M/z 285 ($M+H^+$) at 2.67 minutes; δ_H (400 MHz; d_6-DMSO) 8.79 (1 H, s). 7.78 (1 H, br s), 7.66 (1 H, br s), 7.46 (2 H, m), 7.29-7.39 (3 H, m), 6.11 (1 H, q), 1.89 (3 H, d).

(S)-3-(1-Phenylethyl)-8-carbamoylimidazo[5,1-d][1,2,3,5]tetrazine-4-one (13p)

(S)-(-)-1-PhenyIethyI isocyanate (0.6 mL, 4.2 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (0.5 g, 3.65 mmol) in dry dimethylsulfoxide (5 mL) at room temperature under nitrogen. The reaction mixture was stirred at the dark at room temperature under nitrogen. After 16 h, the reaction mixture was poured onto ice and the resulting precipitate was purified by flash column chromatography (SiO_2, 3:7 acetonitrile in dichloromethane) to furnish the desired product as a colourless solid (678 mg, 65%); LCMS (ES+) m/z 285 $(M+H)^+$ at 2.73 minutes; δ_H (400 MHz; d_6-DMSO) 8.79 (1 H, s), 7.78 (1 H, br s), 7.66 (1 H, br s), 7.46 (2 H, m), 7.29-7.39 (3 H, overlapping m), 6.11 (1 H, q), 1.89, (3 H, d).

(S)-3-(1-Phenylpropyl)-8-carbamoylimidazo[5,1-d][1,2,3,5]tetrazin-4-one (13q)

(S)-(-)-1-PhenylpropyI isocyanate (0.6 mL, 4.2 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (0.5 g, 3.65 mmol) in dry dimethylsulfoxide (5 mL). The reaction mixture was stirred at the dark at room temperature under nitrogen. After 16 h, the reaction mixture was poured onto ice and extracted with dichloromethane (3 x 25 mL). The combined organic extracts were washed with water (25 mL) and brine (25 mL), dried over $NaSO_4$, and evaporated to dryness. The residue was purified by reverse phase chromatography (Cl 8 silica, gradient 0-100% acetonitrile in water), and then dried by co-evaporation with toluene to furnish the desired product as a pink solid (359 mg, 33 %); LCMS (ES+) m/z 299 $(M+H)^+$ at 2.62 minutes; δ_H (400 MHz; d_6-DMSO) 8.78 (1 H, s), 7.78 (1 H, br s), 7.66 (1 H, br s), 7.48 (2 H, m), 7.30-7.42 (3 H, overlapping m), 5.82 (1 H, q), 2.41 (1 H, m), 2.32 (1 H, m), 0.94 (3 H, t).

(R)-3-(1-(4-Methoxylphenyl)ethyl)-8-carbamoylimidazo[5,1-d][1,2,3,5]tetrazin-4-one (13r)

(R)-(+)-1-(4-Methoxyphenyl)ethylisocyanate (0.445 mL, 3.83 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (0.5 g, 3.65 mmol) in dry dimethylsulfoxide (5 mL). The reaction mixture was stirred at the dark at room temperature under nitrogen. After 16 h, the reaction mixture was poured onto ice and the resulting precipitate was washed with ether and purified by flash column chromatography (SiO_2, gradient 20-100% acetonitrile in dichloromethane) to furnish the desired product as a colourless solid (189 mg, 16%); LCMS (ES+) m/z 315 $(M+H)^+$ at 2.37 minutes; δ_H (400 MHz; d_6-DMSO) 8.78 (1 H, s), 7.76 (1 H, br s), 7.64 (1 H, br s), 7.39 (2 H, m), 6.91 (2 H, m), 6.05 (1 H, q), 3.72 (3 H, s), 1.85 (3 H, d).

(+/-)-3-(1-(4-Bromophenyl)ethyl)-8-carbamoylimidazo[5,1-d][1,2,3,5]tetrazin-4-one (13s)

4-Bromo-α-methylbenzyl isocyanate (0.32 mL, 2.21 mmol) was added dropwise to a suspension of 5-diazoimidazole-4-carboxamide (264 mg, 1.92 mmol) in dry dimethylsulfoxide (5.0 mL). The reaction mixture was stirred in the dark at room temperature under nitrogen. After 18 h, the reaction mixture was poured onto ice and the resulting precipitate was purified by flash column chromatography (SiO_2, gradient 0-100% acetonitrile in dichloromethane) to furnish the *title compound* as a colourless solid (525 mg, 75%); LCMS (ES+) m/z 363/365 $(M+H)^+$ at 2.77 minutes; δ_H (400 MHz; d_6-DMSO) 8.78 (1 H, s), 7.78 (1 H, br s), 7.64 (1 H, br s), 7.55 (2 H, m), 7.40 (2 H, m), 6.06 (1 H, q), 1.84 (3 H, d).

5-(3-(Prop-2-yn-1-yl)triaz-2-en-1-yl)-1*H*-imidazole-4-carboxamide, (14a)

To a suspension of 5-diazoimidazole-4-carboxamide (0.5 g, 3.65 mmol) in ethyl acetate (10 mL) under argon kept in dark with aluminium foil, propargylamine (0.7 mL, 0.91 mmol) was added dropwise. The reaction was stirred for 18 h at room temperature. The resulted precipitate was filtered off and washed with ethyl acetate. The pink solid was dried under high vacuum, then grounded with pestle and mortar, triturated with ethyl acetate to give the *title compound* as a pink solid (624 mg, 88%); (Found: $M+H^+$, 193.0832. $C_7H_7N_6O+H^+$ requires 193.0835), (Found: $M+Na^+$, 215.0652. $C_7H_7N_6O+Na^+$ requires 215.0653); δ_H (400 MHz; d_6-DMSO) 13.03 – 12.35 (1 H, br s), 10.94 (1 H, br s), 7.57 (1 H, s), 7.50 (1 H, br s), 7.35 (1 H, br s), 4.25 (2 H, s), 3.18 (1 H, s).

5-(3-Benzyltriaz-2-en-1-yl)-1*H*-imidazole-4-carboxamide (14b)

To a suspension of 5-diazoimidazole-4-carboxamide (0.5 g, 3.65 mmol) in ethyl acetate (10 mL) under argon in the dark, benzylamine (1.2 ml, 11.0 mmol) was added dropwise. The reaction mixture was stirred for 18 h at room temperature. The resulted precipitate was

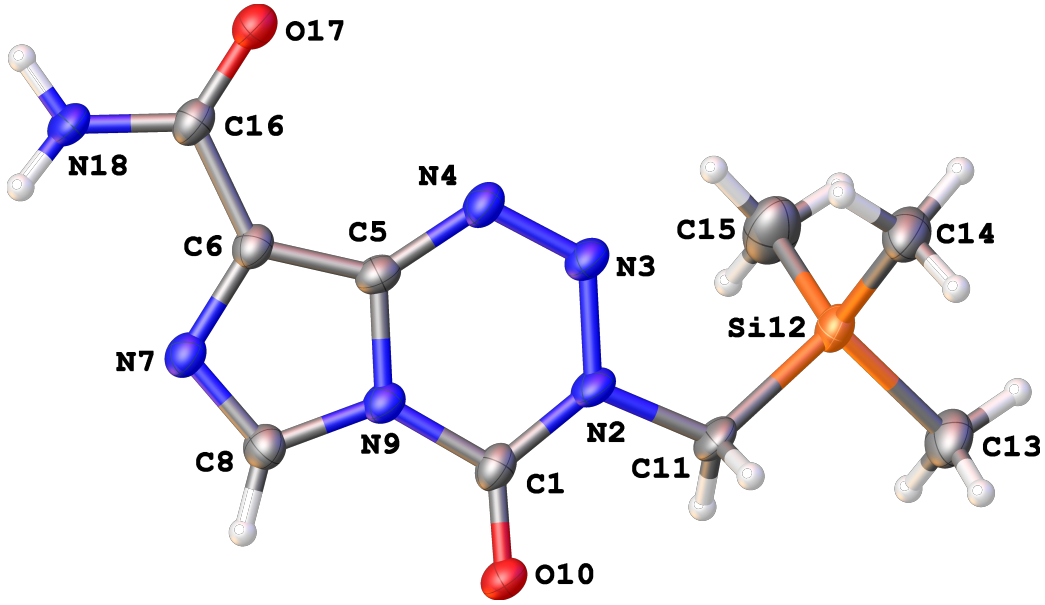
filtered off and washed with ethyl acetate and air dried to give the *title compound* as a pink solid (846 mg, 95 %); (Found: $M+H^+$, 245.1150. $C_{11}H_{12}N_6O+H^+$ requires 245.1145), (Found: $M+Na^+$, 267.0971. $C_{11}H_{12}N_6O+Na^+$ requires 267.0965); δ_H (400 MHz; d_6-DMSO) 11.39 (1 H, br s), 11.10 (1 H, br s), 7.52 (1 H, s), 7.32 (6 H, m), 7.00 (1 H, s), 4.66 (2 H, s).

Crystallography

A suitable crystal was selected and in fomblin film on a micromount on a SuperNova, Dual, Cu at zero, Atlas diffractometer. The crystal was kept at 120(2) K during data collection. Using Olex2,[1] the structure was solved with the olex2.solve[2] structure solution program using Charge Flipping and refined with the ShelXL[3] refinement package using Least Squares minimisation.

1. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. and Puschmann, H. *J. Appl. Cryst.* 2009, **42**, 339-341.
2. Bourhis, L.J., Dolomanov, O.V., Gildea, R.J., Howard, J.A.K., Puschmann, H. *Acta Cryst.* 2015, **A71**, 59-75.
3. Sheldrick, G.M. *Acta Cryst.* 2008, **A64**, 112-122.

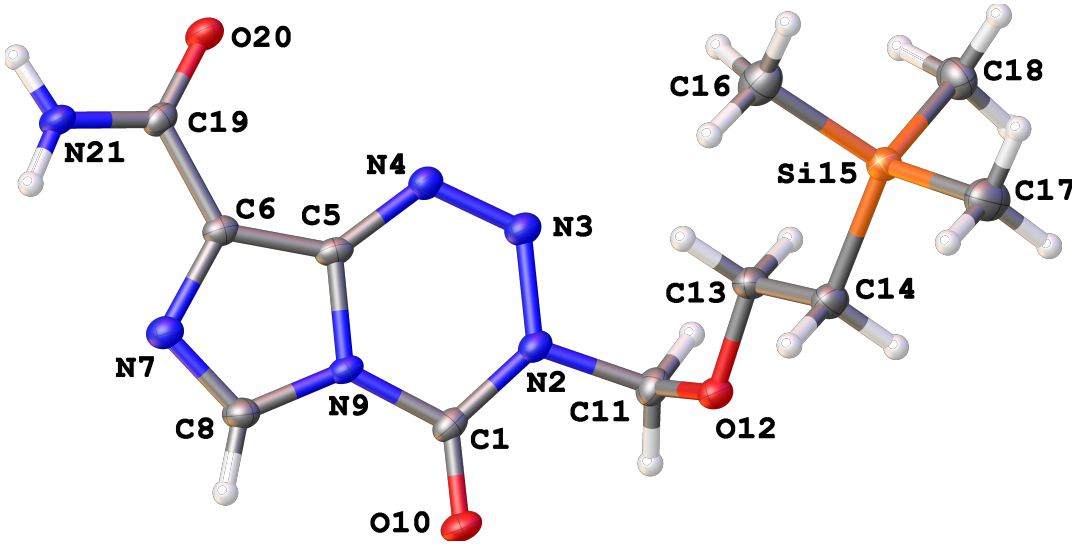
Figure S1. 3-Trimethylsilylmethylimidazotetrazine (9): CCDC 1582898



Crystal Data

Empirical formula	$C_9H_{14}N_6O_3Si$
Formula weight	266.35
Temperature/K	120(2)
Crystal system	triclinic
Space group	P-1
a/Å	6.0637(7)
b/Å	6.3295(8)
c/Å	17.0569(17)
α/°	82.559(9)
β/°	81.097(9)
γ/°	72.642(10)
Volume/Å^3	614.88(12)
Z	2
ϱ_{calc}g/cm^3	1.439
μ/mm^{-1}	1.768
F(000)	280.0
Crystal size/mm^3	0.3771 × 0.2509 × 0.0598
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	10.54 to 149.226
Index ranges	-7 ≤ h ≤ 7, -7 ≤ k ≤ 7, -7 ≤ l ≤ 21
Reflections collected	2327
Independent reflections	2327 [R_{int} = ?, R_{sigma} = 0.0336]
Data/restraints/parameters	2327/2/173
Goodness-of-fit on F^2	1.074
Final R indexes [I>=2σ (I)]	R_1 = 0.0825, wR_2 = 0.2166
Final R indexes [all data]	R_1 = 0.0905, wR_2 = 0.2225
Largest diff. peak/hole / e Å^{-3}	0.93/-0.48

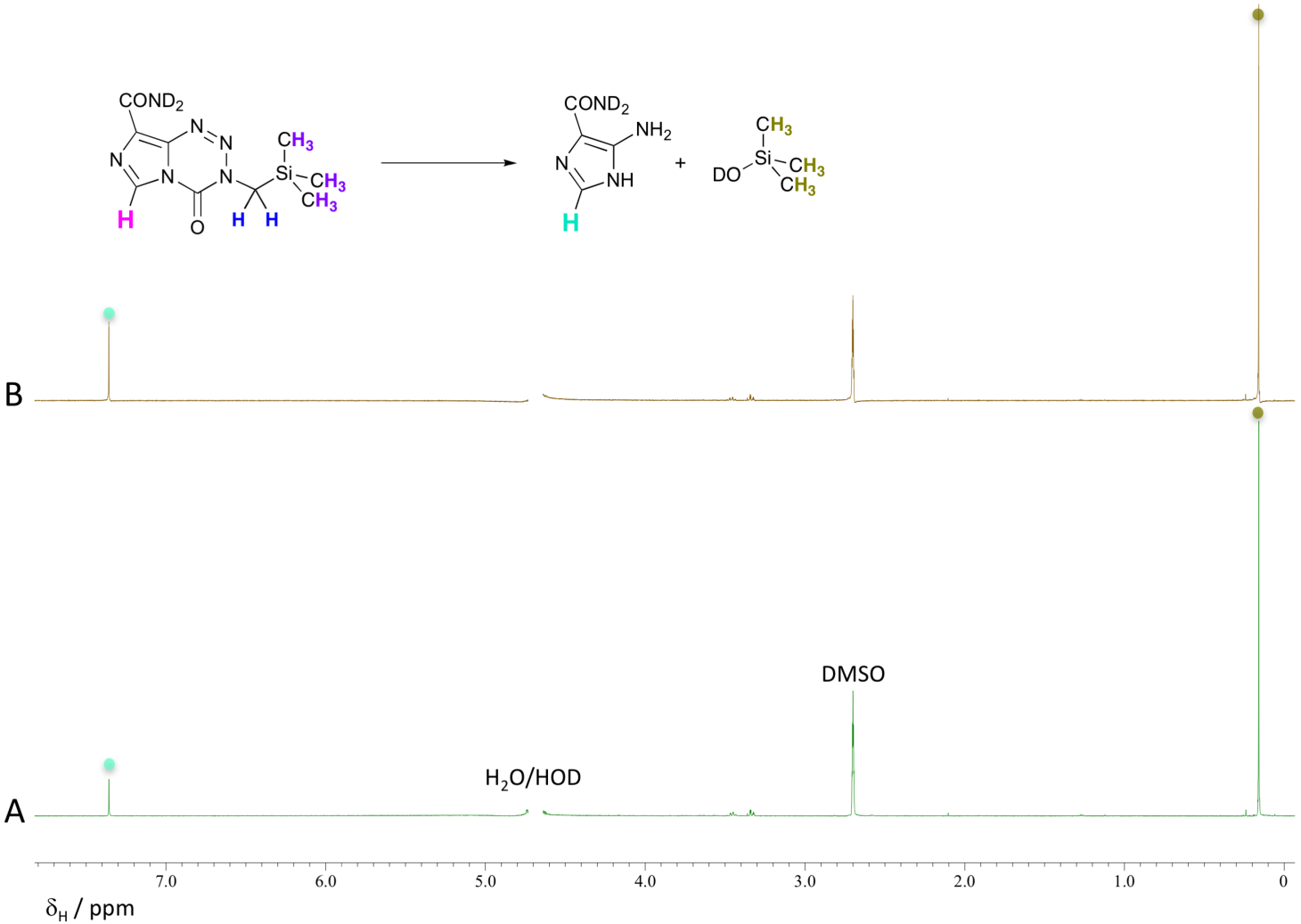
Figure S2. 3-(2-Trimethylsilylethoxy)methylimidazotetrazine (10): CCDC 1582899



Crystal Data

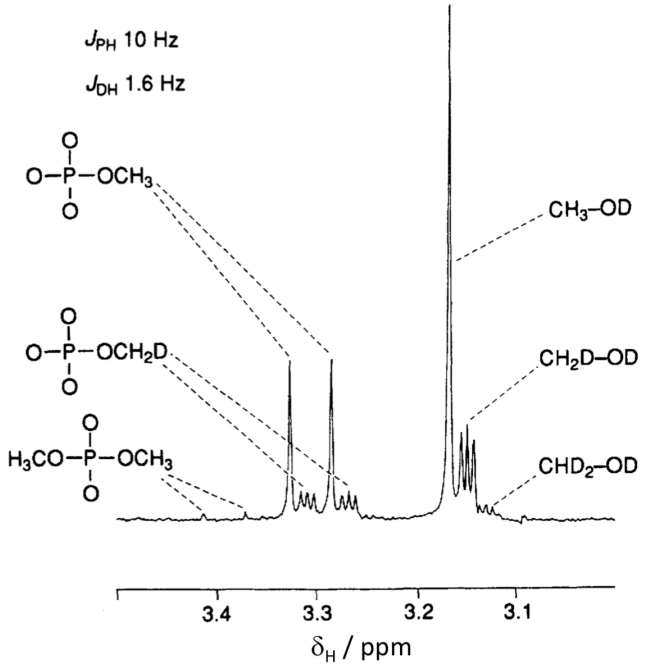
Empirical formula	$C_{11}H_{18}N_6O_3Si$
Formula weight	310.40
Temperature/K	120(2)
Crystal system	triclinic
Space group	P-1
a/Å	7.5168(10)
b/Å	9.0109(15)
c/Å	11.4120(15)
α/°	88.485(12)
β/°	78.814(11)
γ/°	86.275(12)
Volume/Å^3	756.61(19)
Z	2
ϱ_{calc}g/cm^3	1.362
μ/mm^{-1}	1.565
F(000)	328.0
Crystal size/mm^3	0.3666 × 0.2579 × 0.0469
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	9.838 to 148.232
Index ranges	-8 ≤ h ≤ 8, -10 ≤ k ≤ 11, -14 ≤ l ≤ 13
Reflections collected	5157
Independent reflections	2945 [R_{int} = 0.0278, R_{sigma} = 0.0395]
Data/restraints/parameters	2945/0/193
Goodness-of-fit on F^2	1.041
Final R indexes [I>=2σ (I)]	R_1 = 0.0410, wR_2 = 0.1096
Final R indexes [all data]	R_1 = 0.0452, wR_2 = 0.1139
Largest diff. peak/hole / e Å^{-3}	0.47/-0.28

Figure S3: Product peak assignments for reaction of TMS-methylimidazotetrazine 9 in deuterated phosphate buffer



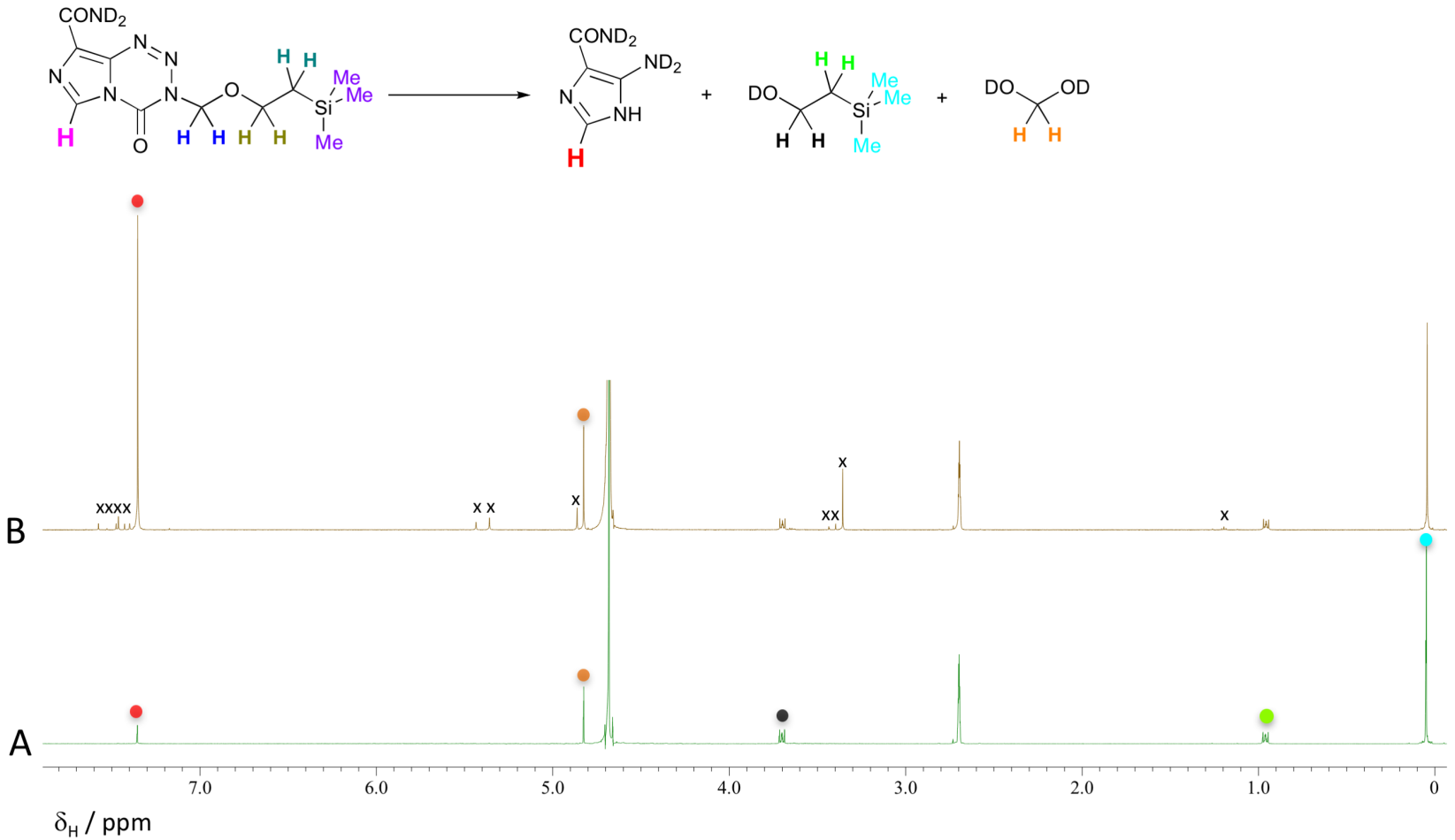
A, High resolution ^{1}H NMR spectrum recorded at the end of a kinetic run. B, Spectrum of the same sample after addition of authentic AIC (**4**).

Figure S4: Reaction products of TMZ in deuterated phosphate buffer



^{2}H-Methyl products produced in the reaction of TMZ in neutral deuterated phosphate buffer. Note the preponderance of C***H***$_3$-methyl products in contrast to imidazotetrazine **9**. Image adapted from R.T. Wheelhouse and M.F.G. Stevens, *J Chem Soc, Chem Commun*, 1993,1177.

Figure S5: Product peak assignments for reaction of SEM-imidazotetrazine 10 in deuterated phosphate buffer

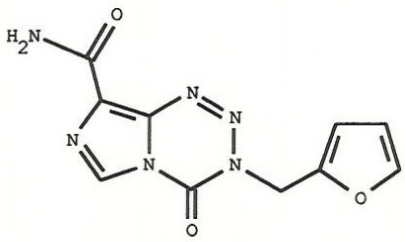


A, High resolution ^{1}H NMR spectrum recorded at the end of a kinetic run. B, Spectrum of the same sample after addition of authentic AIC (**4**) and formaldehyde hydrate. x = Artefacts from the reaction of AIC with excess formaldehyde hydrate

Figure S6: NCI60 panel data for compound 13i

File: 1 Entry: 21

NSC Number D688216-T



(MOLF) Molecular Formula: C10H8N6O3
(MW) Molecular Weight: 260
(CAMT) Total Amount in Inventory: 29 MG

Inventory Record - NSC/Sample Number D688216-T/D1
(COMI) Supplier's Compound Identification: DL36

National Cancer Institute Developmental Therapeutics Program

Mean Graphs

NSC: D- 688216 -T / 1	Units: Molar	SSPL:	Exp. ID:9607MD50-22
Report Date: July 18, 1996		Test Date: July 8, 1996	

Panel/Cell Line	Log_{10} GI50	Log_{10} TGI	Log_{10} LC50
Leukemia			
CCRF-CEM	-4.74	-4.26	> -4.00
HL-60(TB)	-6.42	-4.46	> -4.00
K-562	> -4.00	> -4.00	> -4.00
MOLT-4	-4.00	> -4.00	> -4.00
RPMI-8226	> -4.00	> -4.00	> -4.00
Non-Small Cell Lung Cancer			
A549/ATCC	> -4.00	> -4.00	> -4.00
EKVX	> -4.00	> -4.00	> -4.00
HOP-62	> -4.00	> -4.00	> -4.00
HOP-92	> -4.00	> -4.00	> -4.00
NCI-H226	> -4.00	> -4.00	> -4.00
NCI-H23	> -4.00	> -4.00	> -4.00
NCI-H322M	> -4.00	> -4.00	> -4.00
NCI-H460	> -4.00	> -4.00	> -4.00
NCI-H522	-4.66	-4.28	> -4.00
Colon Cancer			
COLO 205	> -4.00	> -4.00	> -4.00
HCC-2998	> -4.00	> -4.00	> -4.00
HCT-116	> -4.00	> -4.00	> -4.00
HCT-15	> -4.00	> -4.00	> -4.00
HT29	> -4.00	> -4.00	> -4.00
KM12	> -4.00	> -4.00	> -4.00
SW-620	> -4.00	> -4.00	> -4.00
CNS Cancer			
SF-268	> -4.00	> -4.00	> -4.00
SF-295	> -4.00	> -4.00	> -4.00
SF-539	> -4.00	> -4.00	> -4.00
SNB-19	> -4.00	> -4.00	> -4.00
SNB-75	-4.07	> -4.00	> -4.00
U251	> -4.00	> -4.00	> -4.00
Melanoma			
LOX IMVI	-4.05	> -4.00	> -4.00
M14	> -4.00	> -4.00	> -4.00
SK-MEL-2	> -4.00	> -4.00	> -4.00
SK-MEL-28	> -4.00	> -4.00	> -4.00
SK-MEL-5	> -4.00	> -4.00	> -4.00
UACC-257	> -4.00	> -4.00	> -4.00
UACC-62	> -4.00	> -4.00	> -4.00
Ovarian Cancer			
IGROV1	> -4.00	> -4.00	> -4.00
OVCAR-3	> -4.00	> -4.00	> -4.00
OVCAR-4	> -4.00	> -4.00	> -4.00
OVCAR-5	> -4.00	> -4.00	> -4.00
OVCAR-8	> -4.00	> -4.00	> -4.00
SK-OV-3	> -4.00	> -4.00	> -4.00
Renal Cancer			
786-0	> -4.00	> -4.00	> -4.00
A498	> -4.00	> -4.00	> -4.00
ACHN	> -4.00	> -4.00	> -4.00
CAKI-1	> -4.00	> -4.00	> -4.00
RXF 393	> -4.00	> -4.00	> -4.00
SN12C	> -4.00	> -4.00	> -4.00
TK-10	> -4.00	> -4.00	> -4.00
UO-31	> -4.00	> -4.00	> -4.00
Prostate Cancer			
PC-3	> -4.00	> -4.00	> -4.00
DU-145	> -4.00	> -4.00	> -4.00
Breast Cancer			
MCF7	> -4.00	> -4.00	> -4.00
MCF7/ADR-RES	> -4.00	> -4.00	> -4.00
MDA-MB-231/ATCC	> -4.00	> -4.00	> -4.00
HS 578T	> -4.00	> -4.00	> -4.00
MDA-MB-435	> -4.00	> -4.00	> -4.00
MDA-N	> -4.00	> -4.00	> -4.00
BT-549	> -4.00	> -4.00	> -4.00
T-47D	> -4.00	> -4.00	> -4.00
MG_MID	-4.07	-4.02	-4.00
Delta	2.35	0.44	0.00
Range	2.42	0.46	0.00

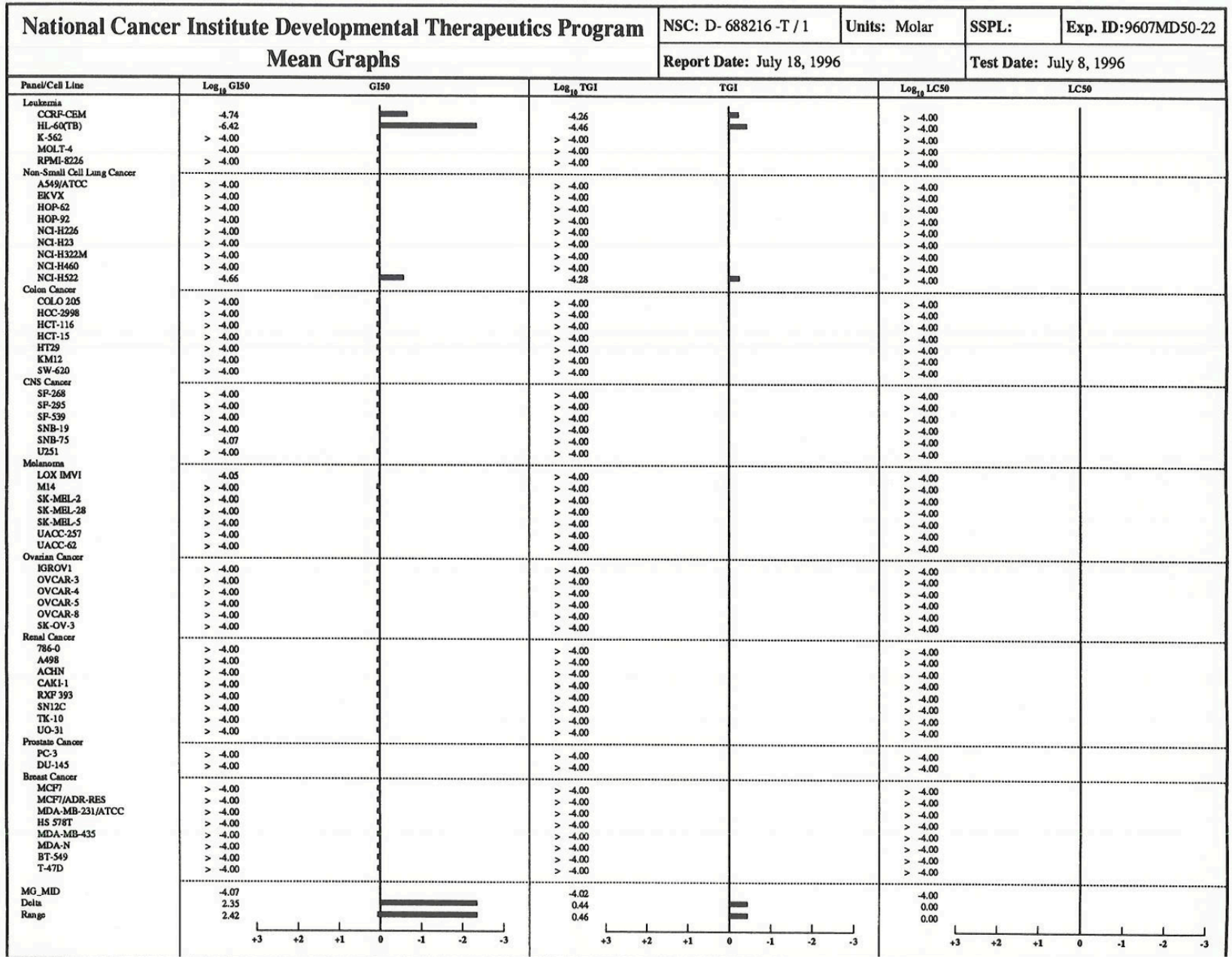


Figure S7: Reaction of benzylimidazotetrazine 13b in deuterated phosphate buffer

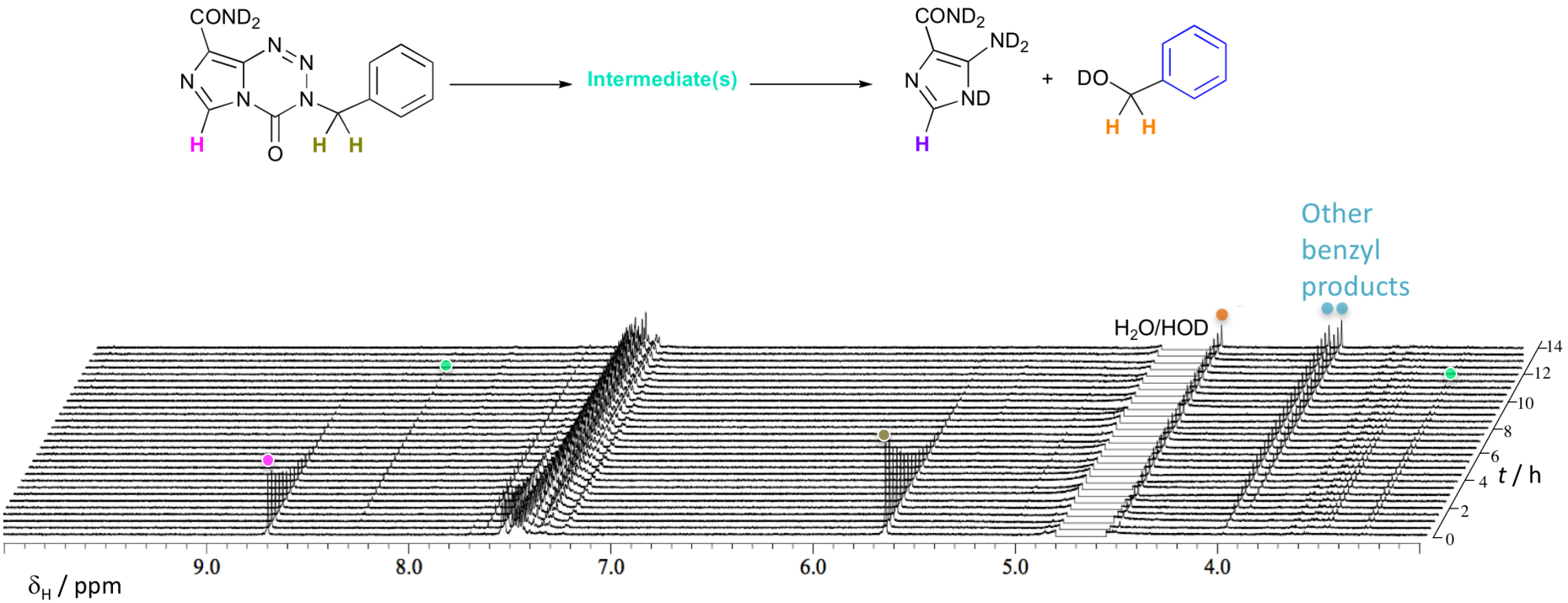


Figure S8: Reaction of benzylimidazotetrazine 13b in deuterated phosphate buffer

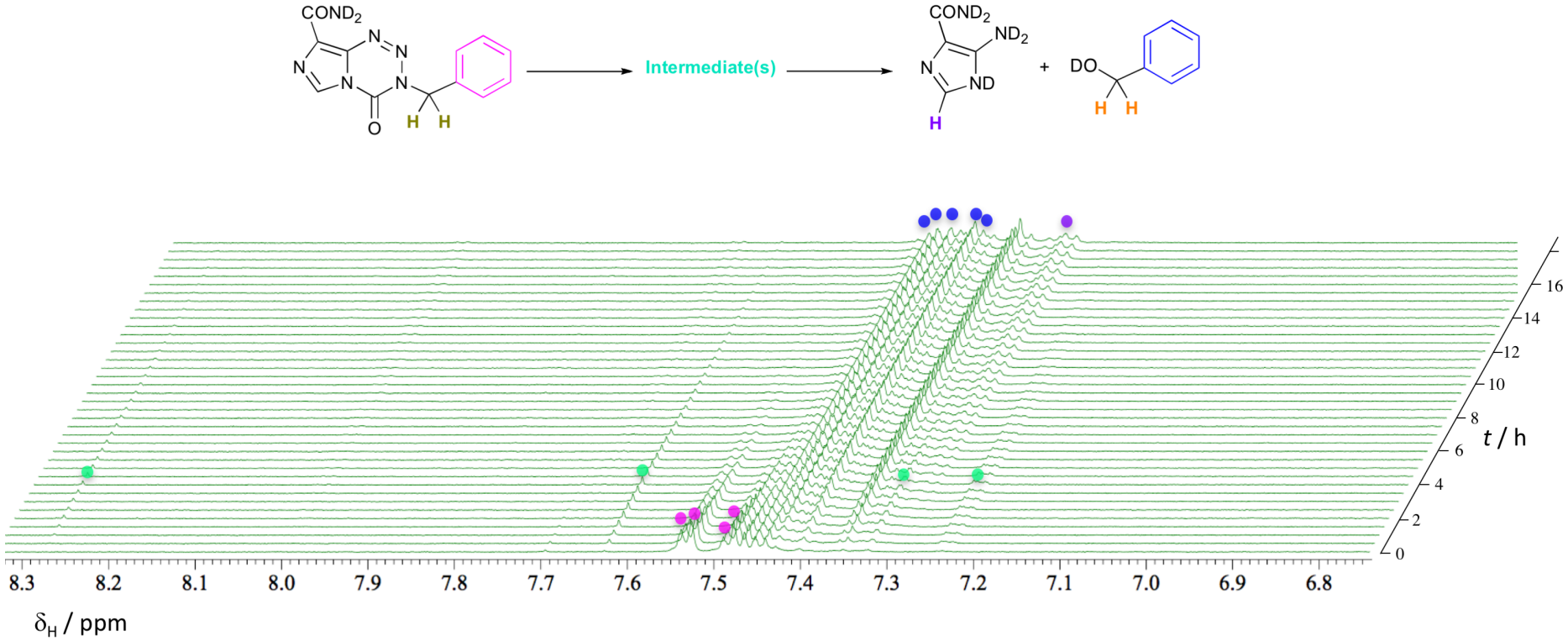
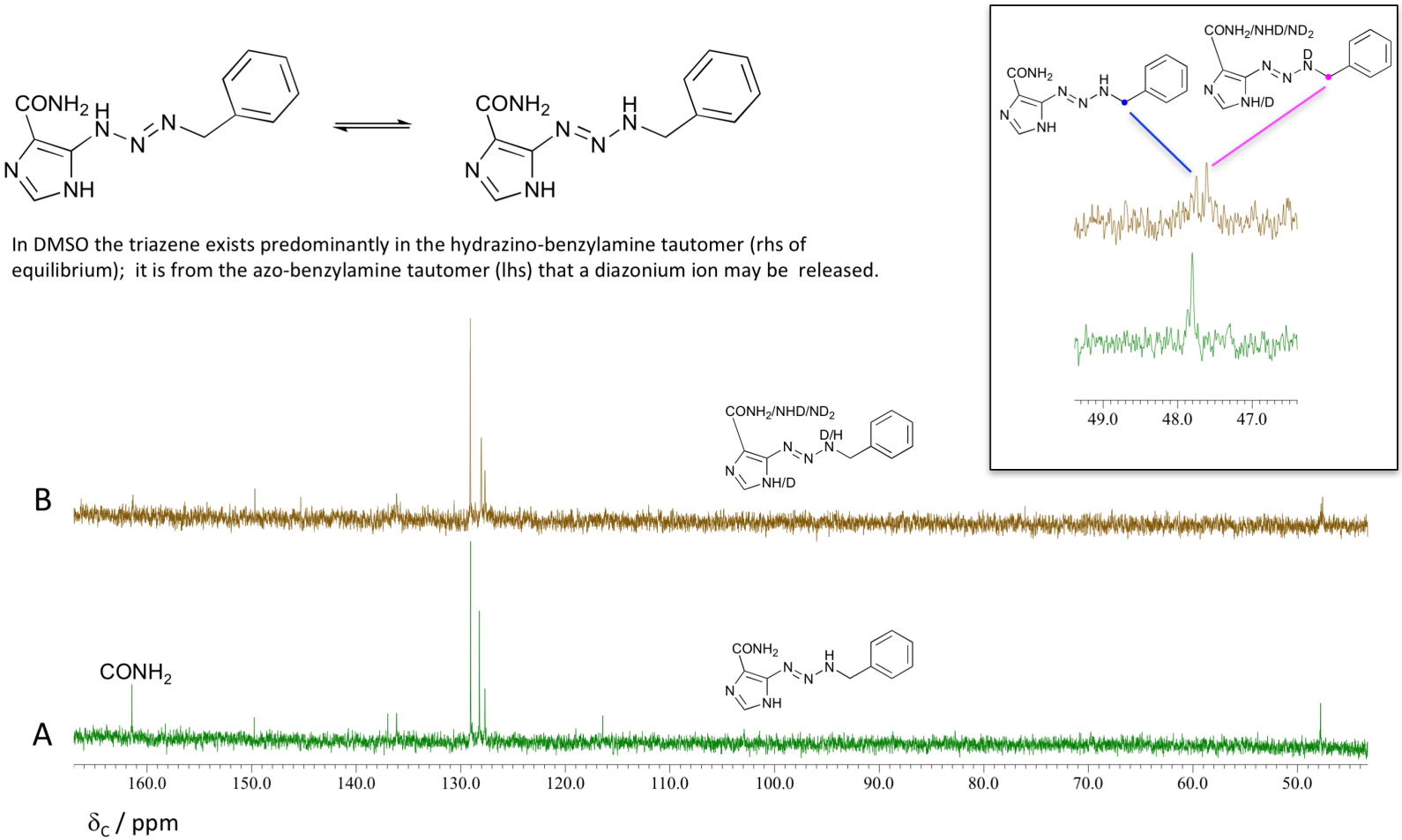


Figure S9: Investigation of tautomerism in benzyltriazene 14b



A, ^{13}C CPD NMR spectrum of triazene **14c** in d_6-DMSO (0.75 ml). B, Spectrum of a similar sample after addition of H_2O:D_2O (50 μl, 1:1). Inset, Expansion showing splitting of the methlyene resonance at 47.8 ppm by the isotopic shift due to neighbouring deuterium exchange at *N*1. Note that after 8 h acquisition, some degradation of the triazene was apparent.