Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Modification of the Duocarmycin Pharmacophore enables CYP1A1 Targeting for Biological Activity

Klaus Pors,[a] Paul M. Loadman,[a] Steven D. Shnyder,[a] Mark Sutherland,[a] Helen M. Sheldrake,[a] Meritxell Guino,[b] Konstantinos Kiakos,[c] John A. Hartley,[c] Mark Searcey,[b] and Laurence H. Patterson*,[a]

[a] Institute of Cancer Therapeutics, University of Bradford, West Yorkshire BD7 1DP, U.K.

[b]Cancer Research UK Drug-DNA Interactions Research Group, UCL Cancer Institute, London, WC1E 6BT, U.K.

[c]School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, Norfolk NR4 7TJ, U.K.

* L.H.Patterson@Bradford.ac.uk

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Materials and Methods

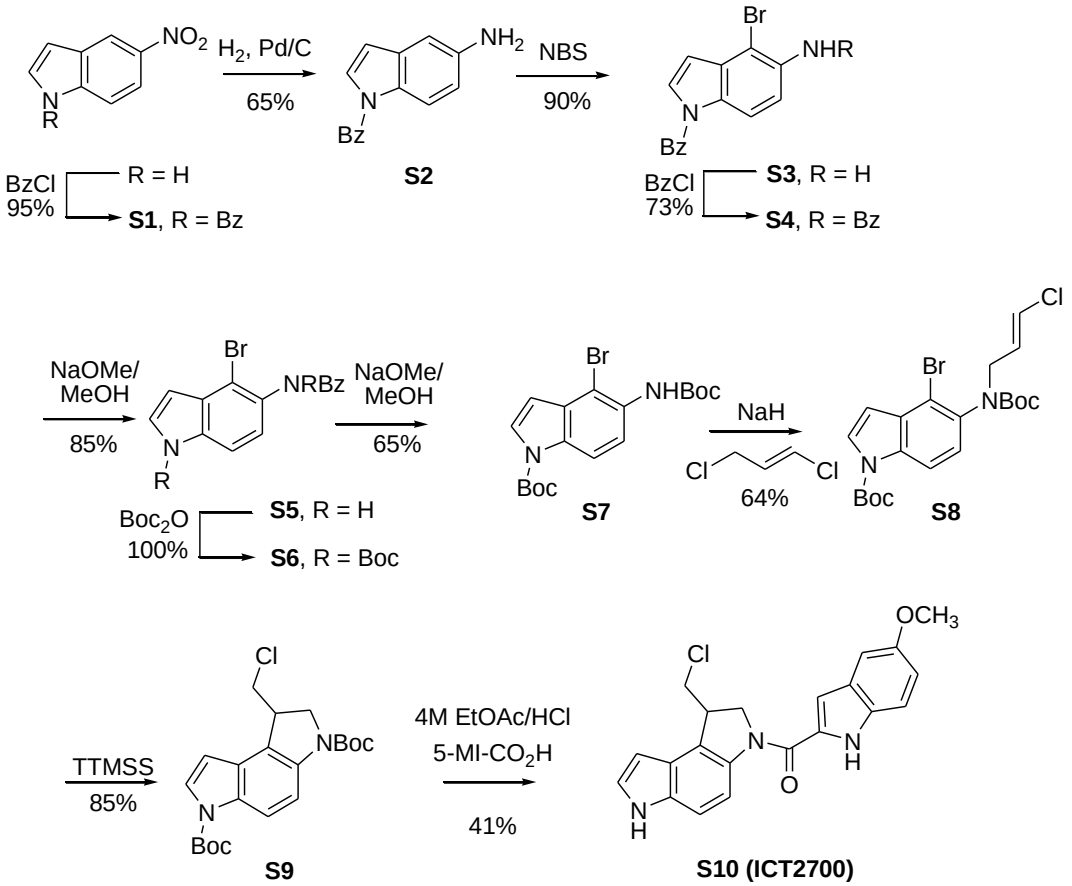
Experimental Details - Chemistry

Chemicals and Reagents

All chemicals were obtained from Aldrich (Poole, Dorset), Lancaster (Morecambe, Lancashire) and VWR (Poole, Dorset). Anhydrous solvents were from Aldrich and were used without further purification. All other solvents were supplied by VWR. Silica for column chromatography: particle size 35-70 µm and 20-35 µm, was supplied by VWR. Aluminium backed thin layer chromatography plates were supplied by VWR.

Sample Analysis

Melting points were determined with a Stuart scientific SMP3 melting point apparatus. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Advance AM 400 (400 MHz) spectrometer. NMR spectra were processed using a Bruker XWIN NMR 3.5 program. Carlo Erba CHN1108 Elemental Analyser was employed for the elemental analysis. Fast Atom Bombardment (FAB+) and Mass spectra sample identification using FAB techniques were obtained on a ZAB SE instrument.



Scheme S1. Synthetic route to compound **S10** (de-OH CPI-MI, ICT2700)

1-benzoyl-5-nitro-1*H*-indole [S1].

A solution of 5-nitroindole (10 g, 62 mmol) in anhyd. CH_2Cl_2 (150 mL) was treated with DMAP (7.57 g, 62 mmol), Et_3N (8.6 mL, 62 mmol) and benzoyl chloride (11.5 mL, 99.2 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction was quenched by addition of sat. $NaHCO_3$ (50 mL) and the aqueous phase was subsequently extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were then combined, dried over $MgSO_4$, concentrated *in vacuo* and filtered through SiO_2 affording a pale yellow solid **S1** (15.6 g, 95%); R_f 0.49 (30% EtOAc in hexane); mp = 157.8-158.7 ºC; IR (neat) υ_{max} 1695,

1513, 1440, 1315, 1273, 1199, 1189, 1064, 896, 881, 795, 709 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ 8.54 (d, 1H, *J* = 2.3 Hz, Ar*H*), 8.49 (d, 1H, *J* = 9.2 Hz, Ar*H*), 8.28 (dd, 1H, *J* = 9.2, 2.3 Hz, Ar*H*), 7.79-7.76 (m, 2H, Ar*H*), 7.69-7.65 (m, 1H, Ar*H*), 7.59-7.55 (m, 2H, Ar*H*), 7.50 (d, 1H, *J* = 3.7 Hz, Ar*H*), 6.77 (dd, 1H, *J* = 3.7, 0.5 Hz, Ar*H*N); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ 168.5 (*C*=O), 144.4 (Ar*C*), 139.1 (Ar*C*NO_2), 133.4 (Ar*C*), 132.8 (Ar*C*H), 130.63 (Ar*C*), 130.4 (Ar*C*H), 129.4 (2C, Ar*C*H), 128.9 (2C, Ar*C*H), 120.1 (Ar*C*H), 117.2 (Ar*C*H), 116.5 (Ar*C*H), 108.8 (Ar*C*H); MS (ES+) *m/z* calculated for $C_{15}H_{10}N_2O_3$ [M] 266.25. Found [M+1] 266.2.

5-amino-1-benzoyl-1*H*-indole [S2].

1-benzoyl-5-nitro-1*H*-indole **S1** (3 g, 11.3 mmol) was dissolved in anhyd. THF (90 mL) and degassed for 15 minutes with N_2. Pd/C (0.6 g, 10% w/w) was added and the reaction was stirred at room temperature for 6 h under a positive pressure of H_2. Filtration through Celite and concentration *in vacuo* gave a crude solid that needed further purification. Column chromatography (SiO_2, 30% EtOAc in hexane) yielded a yellow solid **S2** (1.75g, 65%); R_f 0.18 (30% EtOAc in hexane); mp = 119.9-120.5 °C; IR (neat) υ_{max} 3432, 3344, 1657, 1455, 1345, 1280, 1186, 1066, 869, 692 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ 8.22 (d, 1H, *J* = 8.7 Hz, Ar*H*), 7.72-7.70 (m, 2H, Ar*H*), 7.58-7.48 (m, 3H, Ar*H*), 7.19 (d, 1H, *J* = 3.7 Hz, Ar*H*), 6.86 (d, 1H, *J* = 2.3 Hz, Ar*H*), 6.77 (dd, 1H, *J* = 8.7, 2.3 Hz, Ar*H*), 6.45 (d, 1H, *J* = 3.7 Hz, Ar*H*N), 3.69 (br s, 2H, N*H*$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ 168.2 (*C*=O), 143.2 (Ar*C*NH_2), 134.9 (Ar*C*), 132.1 (Ar*C*), 131.6 (Ar*C*H), 129.9 (C, Ar*C*), 129.0 (2C, Ar*C*H), 128.5 (2C, Ar*C*H), 128.1 (Ar*C*H), 117.2 (Ar*C*H), 114.0 (Ar*C*H), 108.3 (Ar*C*H), 105.7 (Ar*C*H); MS (ES+) *m/z* calculated for $C_{15}H_{12}N_2O$ [M] 236.27. Found [M+1] 237; Analysis calculated for $C_{15}H_{12}N_2O$: C, 76.25; H, 5.12; N, 11.86. Found: C, 76.30; H, 5.15; N, 11.63.

5-amino-1-benzoyl-4-bromo-1*H*-indole [S3].

5-amino-1-benzoyl-1*H*-indole **S2** (1.9 g, 8.04 mmol) was dissolved in anhyd. THF (80 mL) and cooled to 0 °C. The reaction mixture was treated with a solution of NBS (1.43 g, 8.04 mmol) in anhyd. THF (20 mL) and a few drops of concentrated H_2SO_4 and then allowed to warm to room temperature and then stirred for 2 h under N_2. The reaction was quenched with sat. $NaHCO_3$ (75 mL) and the aqueous phase was subsequently extracted with EtOAc (3 × 75 mL). The organic extracts were then combined, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography (SiO_2, 30% EtOAc in hexane) afforded a yellow solid **S3** (2.2g, 90%); R_f 0.44 (30% EtOAc in hexane); mp = 145.5-146.7 ºC; IR (neat) υ_{max} 3417, 3339, 1670, 1618, 1579, 1527, 1474, 1434, 1378, 1348, 1296, 1272, 1199, 1181, 1066, 894, 815, 771, 717, 696 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, ppm) δ 8.18 (d, 1H, *J* = 8.7 Hz, Ar*H*), 7.73-7.70 (m, 2H, Ar*H*), 7.60-7.51 (m, 3H, Ar*H*), 7.25 (d, 1H, *J* = 3.9 Hz, Ar*H*), 6.84 (d, 1H, *J* = 8.7 Hz, Ar*H*), 6.57 (d, 1H, *J* = 3.9 Hz, Ar*H*N), 4.09 (br s, 2H, NH_2); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ 168.4 (*C*=O), 140.8 (Ar*C*NH_2), 134.4 (Ar*C*N), 132.3 (Ar*C*), 131.9 (Ar*C*H), 129.5 (Ar*C*), 129.1 (2C, Ar*C*H), 128.6 (2C, Ar*C*H), 128.3 (Ar*C*H), 116.3 (Ar*C*H), 114.0 (Ar*C*H), 108.2 (Ar*C*H), 99.9 (Ar*C*Br); HRMS *m/z* calculated for $C_{15}H_{11}BrN_2O$ [M+1] 315.0128, 317.0108. Found [M+1] 315.0114, 317.0048.

1-benzoyl-5-benzoylamino-4-bromo-1*H*-indole [S4].

5-amino-1-benzoyl-4-bromo-1*H*-indole **S3** (2.15 g, 6.8 mmol) in anhyd. CH_2Cl_2 (60 mL) was treated with DMAP (0.83 g, 6.8 mmol), Et_3N (0.95 mL, 6.8 mmol) and benzoyl chloride (1.26 mL, 10.9 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction was quenched by addition of sat. $NaHCO_3$ (50 mL) and the aqueous phase was subsequently extracted with $CHCl_3$ (3 × 50 mL). The organic extracts were then combined,

dried over $MgSO_4$ and concentrated *in vacuo* to afford a solid. Recrystallisation from $CHCl_3$ gave an off-white solid **S14** (2.09, 73%); R_f 0.45 (30% EtOAc in hexane); mp = 230.7-231.7 ºC; IR (neat) υ_{max} 3258, 3165, 1674, 1654, 1637, 1536, 1519, 1423, 1368, 1325, 1193, 1175, 1066, 894, 716 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, ppm) δ 8.52 (d, 1H, *J* = 9.0 Hz, Ar*H*),8.47 (br s, 1H, N*H*), 8.37 (d, 1H, *J* = 9.0 Hz, Ar*H*), 8.00-7.98 (m, 2H, Ar*H*), 7.77-7.74 (m, 2H, Ar*H*), 7.64-7.53 (m, 6H, Ar*H*), 7.40 (d, 1H, *J* = 3.7Hz, Ar*H*), 6.68 (dd, 1H, *J* = 3.7, 0.5 Hz, Ar*H*); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ 168.4 (*C*=O), 165.3 (*C*=O), 134.8 (Ar*C*), 134.0 (Ar*C*), 132.9 (Ar*C*), 132.3 (Ar*C*), 132.1 (Ar*C*H), 131.6 (Ar*C*), 129.3 (2C, Ar*C*H), 128.9 (2C, Ar*C*H), 128.8 (Ar*C*H), 128.7 (2C, Ar*C*H), 127.2 (3C, Ar*C*H), 119.3 (Ar*C*H), 116.0 (Ar*C*H), 108.3 (Ar*C*H), 99.6 (Ar*C*Br); HRMS *m/z* calculated for $C_{22}H_{15}BrN_2O_2$ [M+1] 419.0390. Found [M+1] 419.396.

5-Benzoylamino-4-bromo-1*H*-indole [S5].

A solution of 1-benzoyl-5-benzoylamino-4-bromo-1*H*-indole **S4** (2 g, 4.78 mmol) in MeOH (250 mL) was treated with NaOMe 2 M in MeOH (5 mL) and stirred for 10 min. The reaction was subsequently quenched with H_2O (250 mL) and CH_2Cl_2 (100 mL). The two phases were then separated and the aqueous phase extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$). The solution was filtered through SiO_2 and concentrated *in vacuo* to give the title compound as an off-white solid **S5** (1.26, 85%); R_f 0.45 (50% EtOAc in hexane); mp = 188.9-190.1 °C; IR (neat) υ_{max} 3380, 3293, 2918, 2850, 1654, 1524, 1490, 1440, 1340, 1317, 1265, 1182, 857, 767, 702 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, ppm) δ 8.37 (br s, 2H, N*H*), 8.25 (d, 1H, *J* = 8.9 Hz, Ar*H*), 7.99 (d, 1H, *J* = 6.9 Hz, Ar*H*), 7.99 (d, 1H, *J* = 8.1 Hz, Ar*H*), 7.58-7.51 (m, 3H, Ar*H*), 7.39 (d, 1H, *J* = 8.9 Hz, Ar*H*), 7.28 (t, 1H, *J* = 2.9 Hz, Ar*H*), 6.60-6.59 (m, 1H, Ar*H*); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ 165.5 (*C*=O), 135.1 (Ar*C*), 133.1 (Ar*C*), 132.8 (Ar*C*), 131.9 (Ar*C*H), 128.9 (2C, Ar*C*H),

128.7 (Ar*C*), 127.1 (2C, Ar*C*H), 125.6 (Ar*C*H), 117.6 (Ar*C*H), 110.6 (Ar*C*H), 103.3 (Ar*C*H), 100.2 (Ar*C*Br); HRMS *m/z* calculated for $C_{15}H_{11}BrN_2O$ [M+Na] 336.9952. Found [M+Na] 336.9966.

5-(Benzoyl-*tert*-butoxycarbonylamino)-4-bromo-indole-1-carboxylic acid *tert*-butyl ester [S6].

A solution of 5-benzoylamino-4-bromo-1*H*-indole **S5** (1.26 g, 4 mmol) in anhyd. THF (40 mL) was treated with Boc_2O (2.6 g, 12 mmol) and DMAP (1.47 g, 8 mmol) and the solution stirred at room temperature under N_2 for 1 h. The reaction mixture was quenched by addition of 5% HCl (30 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (3 × 30 mL) and combined organic extracts concentrated *in vacuo* of the . Purification by column chromatography (50% EtOAc in hexane) gave 2 g (quantitative) of the title compound **S6** as an off-white solid; R_f 0.51 (30% EtOAc in hexane); mp = 167.6-168.1 °C; IR (neat) υ_{max} 3359, 3147, 2978, 2922, 2851, 1731, 1677, 1464, 1366, 1337, 1233, 1139, 1099, 836, 722 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, ppm) δ □8.16 (d, 1H, *J* = 8.5 Hz, Ar*H*), 7.82 (d, 1H, *J* = 7.5 Hz, Ar*H*), 7.81 (d, 1H, *J* = 8.5 Hz, Ar*H*), 7.67 (d, 1H, *J* = 4.2 Hz, Ar*H*), 7.52-7.43 (m, 3H, Ar*H*), 7.25 (d, 1H, *J* = 8.5 Hz, Ar*H*), 6.70 (d, 1H, *J* = 4.2 Hz, Ar*H*), 1.67 (s, 9H, (C*H*$_3$)$_3$), 1.25 (s, 9H, (C*H*$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ □172.0 (*C*=O), 152.6 (*C*=O), 149.3 (*C*=O), 146.8 (Ar*C*), 136.9 (Ar*C*H), 134.7 (Ar*C*), 132.9 (Ar*C*), 132.3 (Ar*C*), 131.4 (Ar*C*H), 128.2 (2C, Ar*C*H), 128.1 (Ar*C*H), 125.7 (Ar*C*H), 115.6 (Ar*C*), 114.9 (Ar*C*H), 107.8 (Ar*C*H), 85.2 (*C*(CH$_3$)$_3$), 84.5 (*C*(CH$_3$)$_3$), 83.6 (Ar*C*H), 28.1 (3C, C(*C*H$_3$)$_3$), 27.5 (3C, C(*C*H$_3$)$_3$); HRMS *m/z* calculated for $C_{25}H_{27}BrN_2O_5$ [M+1] 515.1176. Found [M+1] 515.1161.

4-Bromo-5-*tert*-butoxycarbonylamino-indole-1-carboxylic acid *tert*-butyl ester [S7].

A solution of 5-(benzoyl-*tert*-butoxycarbonylamino)-4-bromo-indole-1-carboxylic acid *tert*-butyl ester **S6** (2 g, 4.00 mmol) in MeOH (400 mL) was treated with NaOMe 2 M in MeOH (10 mL) and stirred for 1 h. The reaction was subsequently quenched with H_2O (300 mL) and CH_2Cl_2 (150 mL). The two phases were then separated and the aqueous phase extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were dried ($MgSO_4$). After two column chromatography purifications (20% EtOAc in hexane) the desired compound **S7** was afforded as a white solid (1.06 g, 65% yield); R_f 0.61 (20% EtOAc in hexane); mp = 122.9-123.6 °C; IR (neat) υ_{max} 2978, 2929, 1736, 1518, 1368, 1323, 1278, 1227, 1157, 1142, 1099, 837 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, ppm) δ 8.04 (s, 2H, 2 × Ar*H*), 7.60 (d, 1H, *J* = 3.8 Hz, Ar*H*), 7.93 (s br, 1H, N*H*), 6.58 (d, 1H, *J* = 3.8 Hz, Ar*H*), 1.67 (s, 9H, (CH_3)$_3$), 1.55 (s, 9H, (CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ 152.9 (*C*=O), 149.4 (*C*=O), 131.5 (2 × Ar*C*), 131.2 (Ar*C*), 129.6 (Ar*C*), 126.9 (Ar*C*H), 117.5 (Ar*C*H), 114.6 (Ar*C*H), 107.2 (Ar*C*H), 84.2 (*C*(CH$_3$)$_3$), 80.9 (*C*(CH$_3$)$_3$), 28.4 (3C, C(*C*H$_3$)$_3$), 28.2 (3C, C(*C*H$_3$)$_3$); HRMS *m/z* calculated for $C_{18}H_{23}BrN_2O_4$ [M+1] 411.0914. Found [M+1] 411.0912.

4-Bromo-5-[*tert*-butoxycarbonyl-(3-chloro-allyl)amino]-indole-1-carboxylic acid-*tert*-butyl ester [S8].

A solution of 4-bromo-5-*tert*-butoxycarbonylamino-indole-1-carboxylic acid *tert*-butyl ester **S7** (1 g, 2.4 mmol) in anhyd. DMF (75 mL) was cooled to 0 °C and treated with NaH (60%, 290 mg, 7.2 mmol) portionwise over 15 min. The resulting suspension was stirred at 0 °C for 15 min. The reaction mixture was subsequently treated with 1,3-dichloropropene (0.67 mL, 7.2 mmol), warmed to room temperature and stirred for 1 h under N_2. The reaction was quenched with an aq. solution of NaCl and the two phases separated. The aqueous phase was further extracted with EtOAc (3 × 100 mL) and the combined organic extracts dried ($MgSO_4$), filtered and concentrated *in vacuo*. Column chromatography (10% EtOAc in

hexane) gave 0.75 g (64%) of the title compound **S8** as a clear colourless oil; R_f 0.33 (10% EtOAc in hexane); IR (neat) υ_{max} 2975, 1700, 1461, 1370, 1270, 1151, 1099, 733 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, ppm) δ 8.06 (m, 1H, Ar*H*), 7.65 (s, 1H, Ar*H*), 7.12-7.06 (m, 1H, Ar*H*), 6.65 (d, 1H, *J* = 3.7 Hz, Ar*H*), 6.07-6.01 (m, 2H, =C*H*), 4.57 (d, 0.25H, *J* = 5.5 Hz, N-CH_2), 4.53 (d, 0.25H, *J* = 4.4 Hz, N-CH_2), 4.44 (dd, 0.5H, *J* = 15.13, 6.4 Hz, N-CH_2), 4.36 (d, 0.25H, *J* = 5.5 Hz, N-CH_2), 4.32 (d, 0.25H, *J* = 4.4 Hz, N-CH_2), 3.92 (q, 0.5H, *J* = 7.3 Hz, N-CH_2), 1.67 (br s, 9H, (CH_3)$_3$), 1.33 (br s, 9H, (CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ 154.5 (*C*=O), 149.3 (*C*=O), 132.0 (Ar*C*N), 128.9 (Ar*C*H), 127.8 (Ar*C*), 127.2, 127.1 (=*C*H), 126.0, 125.5 (=*C*H), 121.4 (Ar*C*), 120.3 (Ar*C*H), 115.6 (Ar*C*Br), 114.4 (Ar*C*H), 107.8 (Ar*C*H), 84.5, 84.4 (*C*(CH$_3$)$_3$), 80.4 (*C*(CH$_3$)$_3$), 49.3, 46.3 (*C*H$_2$N), 28.4 (3C, C(*C*H$_3$)$_3$), 28.2. 28.1 (3C, C(*C*H$_3$)$_3$); HRMS *m/z* required for $C_{21}H_{26}BrClN_2O_4$ [M+1] 485.0840, 487.0845. Found [M+1] 485.0837, 487.0817.

8-Chloromethyl-7,8-dihydro-3,6-diaza-as-indacene-2,3,6-tricarboxylic acid 3,6-di-*tert*-butyl ester [S9].

A solution of 4-bromo-5-[*tert*-butoxycarbonyl-(3-chloro-allyl)amino]-indole-1-carboxylic acid-*tert*-butyl ester **S8** (0.75 g, 1.54 mmol) in anhyd. toluene (50 mL) was degassed for 1 h and subsequently treated with AIBN (58 mg, 0.35 mmol) and TTMSS (0.5 mL, 1.65 mmol). The reaction mixture was heated at 90 °C for 5 h then cooled to room temperature and concentrated *in vacuo*. Column chromatography (10% EtOAc in hexane) gave 530 mg (85%) of the title compound **S9** as a white solid; R_f 0.27 (10% EtOAc in hexane); mp = 88.4-90.0 °C; IR (neat) υ_{max} 2975, 1730, 1693, 1483, 1433, 1394, 1367, 1337, 1292, 1140 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, ppm) δ □8.06 (d, 2H, *J* = 8.1 Hz, Ar*H*), 7.61 (d, 1H, *J* = 3.5 Hz, Ar*H*), 6.46 (d, 1H, *J* = 3.5 Hz, Ar*H*), 4.13 (d, 2H, *J* = 9.3 Hz, CH_2), 3.89 (d, 2H, *J* = 7.0 Hz, C*H*, CH_2), 3.54 (t, 1H, *J* = 10.5 Hz, CH_2), 1.66 (br s, 9H, (CH_3)$_3$), 1.59 (br s, 9H, (CH_3)$_3$); ^{13}C

NMR ($CDCl_3$, 100 MHz, ppm) δ 152.2 (*C*=O), 149.4 (*C*=O), 134.9 (Ar*C*), 132.5 (Ar*C*), 129.6 (Ar*C*), 128.4 (Ar*C*), 127.5 (Ar*C*H), 115.3 (Ar*C*H), 111.9 (Ar*C*H), 104.0 (Ar*C*H), 82.8 ($C(CH_3)_3$), 79.2 ($C(CH_3)_3$), 52.5 (CH_2Cl), 46.7 (CH_2N), 42.2 (*C*H), 28.5 (3C, $C(CH_3)_3$), 28.2 (3C, $C(CH_3)_3$); MS (ES) *m/z* required for $C_{21}H_{27}ClN_2O_4$ [M] 406.90. Found [M^+] 406.9. Anal. Calcd for $C_{21}H_{27}ClN_2O_4$: C, 61.99; H, 6.69; N, 6.88. Found: C, 62.03; H, 6.54; N, 6.82.

(1-chloromethyl-1,6-dihydro-2H-3,6-diaza-as-indacen-3-yl)-(6-methoxy-1H-indol-2-yl)-methanone [S10, de-OH-CPI-MI, ICT2700]

A solution of **S9** (118 mg, 0.62 mmol) in 3 mL of 4 M HCl-EtOAc was stirred at 25 ^oC for 1 h. The solvent was removed *in vacuo*. The residue was dissolved in anhydrous DMF (10 ml), 5-methoxyindole-2-carboxylic acid (44 mg, 0.23 mmol) and EDCI (118 mg, 0.62 mmol) added, and the mixture was stirred at 25 ^oC for 21 h. The reaction mixture was poured into a solution of brine (30 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Column chromatography (EtOAc-PE 2:8→1:1) provided the title compound **S10 (ICT2700)** (50 mg, 41%) as an off-white solid; R_f 0.09 (EtOAc:PE, 3:7); mp = 192-194 °C (dec); ^1H NMR (600 MHz, $CDCl_3$) δ 9.47 (1H, brs), 8.34 (1H, brs), 8.28-8.33 (1H, brm), 7.37 (1H, d, *J* 8.6) 7.35 (1H, d, *J* 8.8), 7.29 (1H, brs), 7.13 (1H, brs), 7.01 (1H, brs), 6.99 (1H, d, *J* 8.9), 6.51 (1H, brs), 4.73 (1H, t, *J* 9.7), 4.66 (1H, dd, *J* 2.9, 10.3), 4.11-4.15 (1H, m), 4.09 (1H, dd, *J* 2.4, 11.3), 3.87 (3H, s, OCH_3), 3.61 (1H, t, *J* 10.8); ^{13}C NMR (67.5 MHz, CD_3COCD_3) δ 161.4 (C), 156.4 (C), 139.0 (C), 136.3 (C), 133.8 (C), 133.4 (C), 130.3(C), 128.3 (C), 126.0 (CH), 123.1 (C), 117.3 (CH), 114.7 (CH), 114.4 (CH), 112.6 (CH), 106.8 (CH), 104.0 (CH), 100.8 (CH), 56.7 (CH_3), 56.2 (CH_2), 48.7 (CH_2), 45.4 (CH); *m/z* (ES-) 380 (33), 378 (100), 327 (16), 325 (13), 212 (27), 115 (50); (ES+) 382 (35), 381 (65), 380 (100), 379 (78). Anal. Calcd for $C_{23}H_{19}ClN_2O_2$: C, 66.52; H, 4.68; N, 10.84.

Experimental Details - Biology

Chemosensitivity

Cell lines. A2780, A2780/adr and A2780/cis human ovarian adenocarcinoma cell lines (ECACC, Salisbury, UK) were cultured in RPMI 1640 cell culture medium supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine and 10% fetal bovine serum (all from Sigma).

CHO lines were a gift from the late Dr T Friedberg, University of Dundee.

Growth Inhibition Assays. Cell growth inhibition was assessed using the MTT assay (*1*).

1×10^4 cells/ml were inoculated into each well of a 96-well plate and incubated overnight at 37 °C in a humidified atmosphere containing 5% CO_2. Compounds were dissolved in DMSO and then diluted in complete cell culture medium to give a broad range of concentrations, such that the maximum final DMSO concentration was not greater than 0.1%. Medium was removed from each well and replaced with compound or control solutions, and the plates then incubated for a further 96 h. After 96 h culture medium was removed and 200 µL of 0.5 $mgml^{-1}$ MTT solution (Sigma) in complete medium added to each well. Following a further 4 h incubation, the solution was removed from each well and 150 µL of DMSO added to solubilise the formazan crystals resulting from MTT conversion. Absorbance values for the resulting solutions were read at 550 nm on a microplate reader and cell survival calculated from the ratio of absorbance of treated cells to control cells. Results were expressed in terms of IC_{50} values (concentration of compound required to kill 50% of cells) and all experiments were performed in triplicate.

Cell Uptake Study

Measurement of cellular uptake

A2780 cells were routinely maintained at 37 ^{o}C in a humidified, CO_2-enriched (5%) environment and cultured in RPMI 1640 supplemented with 10% foetal calf serum, sodium pyruvate (2 mM) and L-glutamine (2 mM). A2780 cells were harvested in exponential growth and resuspended in complete supplemented RPMI at a concentration of 1×10^7 cells/ml and incubated at 37 ^{o}C.

ICT2700 and ICT2740 were initially dissolved in DMSO and stock solutions were stored at -80 ^{o}C prior to biological evaluation. ICT2700 and ICT2740 were added to the cell suspension giving a final concentration of 10 or 100 µM ensuring that the final DMSO concentration was < 0.1%.

Following 15 min incubation with compound at 37 ^{o}C a sample of cell suspension (50 µl) was taken. Samples were centrifuged (10,000g x 1 min) and the cell free supernatant (20 µL) deproteinised by the addition of acetonitrile (40 µL). The remaining pellet containing cells was washed with Hanks Balanced Salt Solution and resuspended in 50 µL acetonitrile, centrifuged (10,000g x 1 min) and 10 µL injected for analysis. Quantitation by LC/MS enabled the weight of drug per sample to be calculated. This was adjusted for original sample volume and expressed in µM concentrations. The cell pellet volume was estimated to be 15 µL by assuming a mean cell diameter of 17.5 µm and calculating the total volume from the equation $4/3x\pi r^3$.

Chromatographic analysis of ICT2700 and ICT2740

LC / MS analysis was carried out using a Waters ZMD (Micromass, Manchester, UK) quadrupole mass spectrometer and Waters Alliance 2695 (Milford, MA, USA) quaternary

pump chromatography system. The Waters Alliance 2695 is attached in series to the Waters ZMD mass spectrometer as described below.

The mass spectrometer was operated in positive ion electrospray mode with a voltage of +3.00 kV applied to the capillary. A solvent flow of 1.0 ml/min (split 1:10) with a nitrogen gas flow of 400 l/min and a source temperature of 180 ^{o}C was employed to produce stable spray conditions. The cone voltage was set at 25 V to give clear mass spectra from these samples. The mass spectra were continuously scanned from m/z 250 to m/z 700 per second throughout the entire HPLC separation. Masslynx software (V4.1, Micromass Ltd., Manchester, UK) was used to analyse the mass spectral data and produce total ion chromatograms (TICs) for the separation. ICT2700 (m/z 380.1) and the dechlorinated metabolite CPI-MI (m/z 360.1) were monitored using UV/VIS (329nm) and a SIR (Single Ion Recording) channel.

Separation was achieved using gradient of 50% B to 70% B over 15 min rising to 90% between 15 and 25 min and remaining at 90% B until 30 min before returning to 50% B for re-equilibration (mobile Phase A contains 10% methanol 0.1% formic acid; mobile Phase B contains 90% methanol 0.1% formic acid). A flow rate of 1.0 ml/min was used throughout with a Hichrom RPB column (25 cm × 4.6 mm id) for the separation. A Waters 996 Photodiode Array Detector (λ = 330 nm) with Masslynx software (V4.1) was used for spectral analysis of the peaks of interest.

MRM analysis of CHO1A1 in CHO cells

Multiple Reaction Monitoring (MRM) analysis of CYP1A1 in the CYP1A1-expressing CHO cells was carried out by first identifying a peptide that is unique to CYP1A1 (*2*)· This was found by tryptic digestion of recombinant human CYP1A1 and identifying a predominant

peptide fragment specific to CYP1A1. The MRM peptide identity was confirmed by acquiring a full MSMS spectrum of IQEELDTVIGR, the relevant peptide.

Sample Preparation

Peptides:

The peptide sequence IQEELDTVIGR was identified as a unique signature peptide for CYP1A1 (*2*) and prepared as a 1 mM stock solution in DMSO and diluted with phosphate buffered saline (pH 7.4) when required as an authentic standard for analysis of CYP1A1 in CHO transfected cells.

CHO Cell preparation:

CHO wild type and CYP1A1 expressing cells (1×10^6 cells) were pelleted by centrifugation and subjected to 2 freeze thaw cycles at –80 ^{o} C vortexing on each defrost. The pellet was then sonicated on ice, frozen and defrosted again. After vortexing, the cell walls were sheared by syringing through a narrow bore needle several times.

The S9 fraction was obtained by centrifugation at 9000g for 20 min. The supernatant was next transferred to an ultracentrifuge tube and centrifuged at 100,000g for 1 h to obtain a microsomal pellet. The pellet was resuspended in 1 mL phosphate buffered saline (pH 7.4) before repeating the ultracentrifugation step. The pellet was resuspended in 60 µL urea (8 M in 100 mM tris) and a volume of 30 µL was used for analysis.

Protein digestion for HPLC/MS/MS analysis:

Protein digestion was performed using standard methodology. Briefly 5 x v/v of urea (8 M in 100 mM Tris) and 1 part EDTA (5 mM) per 15 parts per sample was added to all samples to be analysed by LC/MS/MS. Dithiothreitol (DTT) at 200 mM (100 mM tris) was added at 1 in

6 of original sample volume. Samples were incubated at room temperature for 1 h. Iodoacetamide at 200 mM (100 mM Tris) was added at 1 in 1.5 of the original sample volume and samples incubated at room temperature (21 ^{o}C) for 1 h. Additional DTT at 200 mM (100 mM Tris) was added at 1 in 1.5 of original sample volume. Samples were next incubated at room temperature for 1 h.

To facilitate the trypsinisation process, the urea in the sample was diluted by addition of water so that the final urea concentration was 0.6 M. Trypsin at 1 mg/ml was added at 1 in 2 original volume to the samples. Samples were incubated at 37 ^{o}C overnight.

Solid phase extraction:

C18 solid phase cartridges (ISOLUTE C18, 100 mg/1 mL, Biotage AB, Sweden) were primed with one cartridge volume of 100% acetonitrile followed by one cartridge volume of 0.1% TFA. The sample was then applied to the column before washing through with one column volume of 0.1% TFA. The cartridge was then dried under vacuum before elution in 1 mL of 100% acetonitrile. Using a centrifugal evaporator, the samples were evaporated to dryness and then reconstituted in 30 µl mobile phase A. The sample (10 µL) was injected onto the HPLC/MS/MS system for analysis.

Sample Analysis: Sample injection was via a Waters (Milford, MA) Acquity UPLC system, with samples maintained at 8 °C in the dark, attached in series to a Waters Acquity PDA UV absorbance detector and a Waters Quattro Premier XE MS/MS. MassLynx Software was used for analysis of peaks of interest.

Chromatographic conditions

Chromatographic analysis of samples was performed on a Waters C18 10 cm Acquity

column (10 cm × 2.1 mm) (Milford, MA, USA) with a flow rate of 0.2 ml/min. Mobile phase A consisted of 5% acetonitrile and 95% dH_2O containing 0.015% trifluoroacetic acid (TFA). Mobile phase B consisted of 57% acetonitrile and 43% dH_2O containing 0.015% TFA. Initial conditions were 40% B rising to 80% B by 9 min, returning to 40% B over a further 0.5 min.

The following conditions were used to establish reproducible signals on the mass spectrometer. The analysis was in ESI+ mode and the peptide gave optimal transitions of m/z 636.5 to 242.2, 773.5, 571.5 and 1031.4 with the cone set at 35 kV and collision energy set at 22kv.

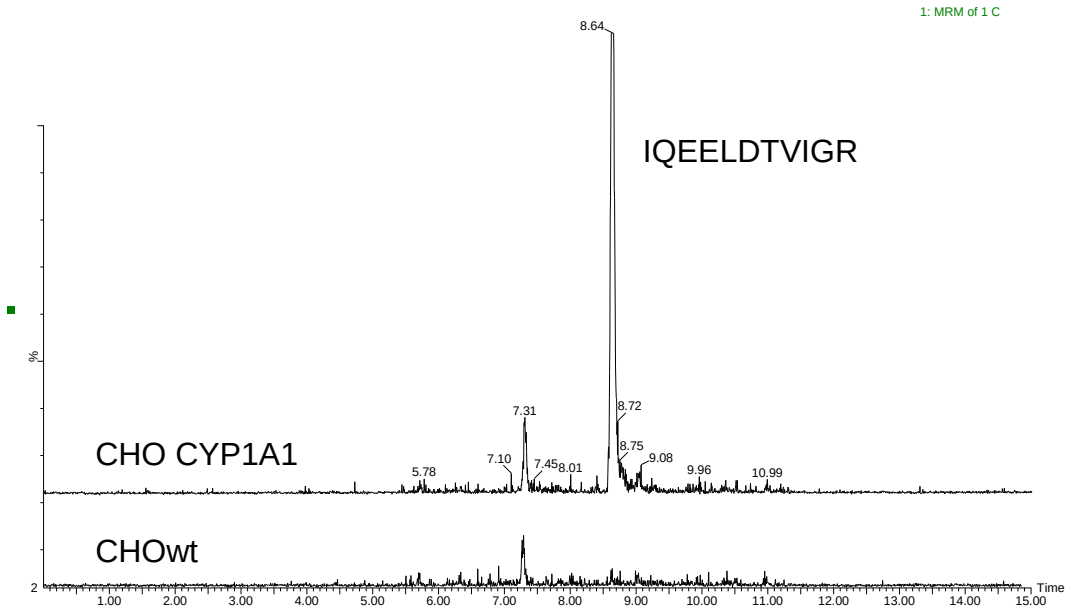


Figure S1 Multiple Reaction Monitoring (MRM) analysis shows a CYP1A1 unique peptide in the CHO CYP1A1 cells but not CHO wild-type. The analysis was in ESI+ mode with the cone set at 35kV and collision energy set at 22 kv. Under these conditions double charged IQEELDTVIGR peptide was confirmed by MSMS analysis and by co-elution with the authentic peptide standard.

Ethoxyresorufin O-de-ethylation (EROD) *Activity Assay*

The principle of the 7-EROD activity assay for CYP1A1 oxidative activity is the O-deethylation of non fluorescent ethoxyresorufin to produce resorufin, a fluorescent derivative as described by Burke *et al*. (*3*). Briefly the assay was carried out on microsomal fractions from CHO CYP1A1 and CHOwt cells grown to confluence in MEM medium (without ribonucleotides and deoxy-ribonucleotides) supplemented with 10% FCS and 30 nM methotrexate. Cells were harvested and resuspended in 0.1 M KP_i (pH 7.4)/0.1 mM EDTA/20% glycerol. After lysis by sonication (3 × 5 sec, cycle 6, power 40%) using a Status US70 homogeniser (Scientific Laboratory Supplies), the sample was centrifuged at 9000g at 4 °C for 15 min. The supernatant was further centrifuged at 100 000g at 4 °C for 60 min. The resulting microsomal pellet was resuspended in buffer (as described above). EROD (100 µM) and NADPH (2 mM) were incubated with sufficient microsomal protein to enable detection of the fluorescent product with time. Fluorescence was measured at an excitation wavelength of 560 nm and emission wavelength of 590 nm.

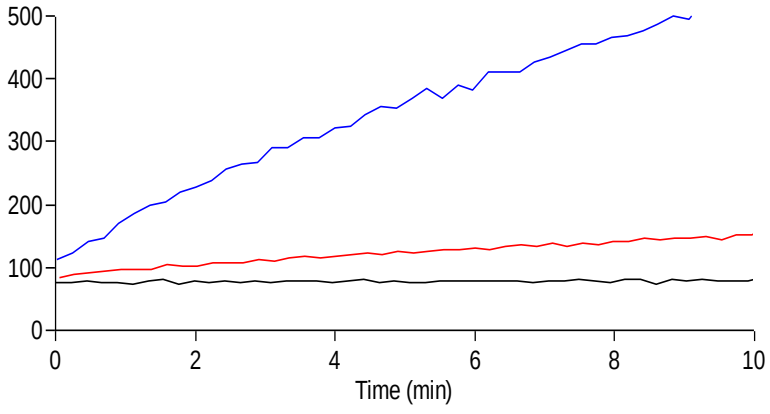


Figure S2. 7-EROD activity assay. Black line: 80 µg microsomes from CHOwt cells. Red and blue line: 4.6 and 23 µg microsomes from CHO CYP1A1 cells.

CYP1A1 Bactosome Metabolism of ICT2700 using LC-MS

Bactosome incubations

The presence of ICT2700, the hydroxylated metabolite ICT2740 and CPI-MI, the spirocyclized product was measured following incubation of ICT2700 for 0-45 min with CYP1A1 bactosomes (6.3 pmoles P450/55 µg protein), NADPH (2 mM), PBS (pH 7.4). At various time points between 0-45 min, a 20 µL sample was taken and the incubation was stopped by the addition of ice cold acetonitrile (40 µL) and centrifuged at 5000 rpm for 5 min. 10 uL of the supernatant was injected for analysis by LC/MS

Analytical method

As described under "Chromatographic analysis of ICT2700 and ICT2740".

Thermal Cleavage Assay

A thermally-induced cleavage assay was used to specifically detect purine-N3 alkylations as previously described (***4***). Briefly, a 208-bp sequence of pUC18 plasmid DNA was PCR amplified using primers 5′-CTCACTCAAAGGCGGTAATAC-3′ and 5′-TGGTATCTTTATAGTCCTGTCG-3′, the latter being 5′-end radiolabeled with [γ-^{32}P] ATP (5000 Ci/mmol, Amersham) using T4 polynucleotide kinase (New England Biolabs). 4 pmol of each primer was used for the exponential amplification from 10 ng of the pUC18 plasmid template DNA. The resulting PCR product was detected by agarose gel electrophoresis, isolated and purified using the Geneclean III kit (MP Biomedicals) following the manufacturer's standard protocol. Drug–DNA incubations were carried out in TEOA buffer [25 mmol/L triethanolamine, 1 mmol/L EDTA (pH, 7.2)] at 37 °C for 2 h, in a total volume of 50 µL, using 10 µL of the purified probe per reaction, enough to yield an activity of at least

200 counts/s. Following precipitation and lyophilisation, dried DNA pellets were heated at 90 °C for 30 min in a total volume of 100 µL sodium citrate buffer [1.5 mmol/L sodium citrate, 15 mmol/L NaCl (pH, 7.2)]. Following a further precipitation samples were re-suspended in 5 µL formamide loading buffer and subjected to electrophoresis on 6% denaturing polyacrylamide gels.

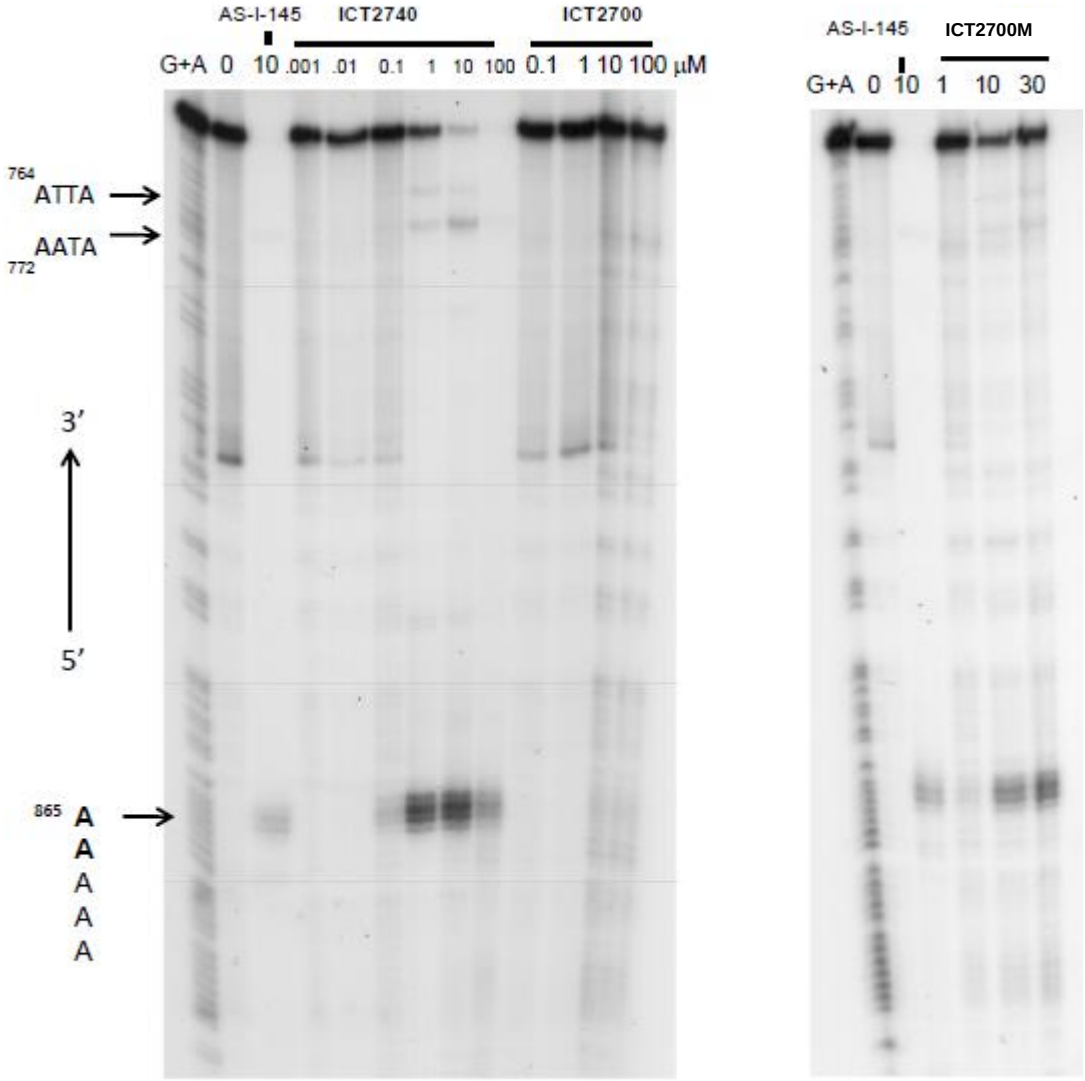


Figure S3. Thermal cleavage gel showing purine N-3 lesions on the bottom strand of a 5'-^{32}P labelled 208 bp fragment of pUC18, defined from 749 to 956. 0 is the untreated control. Arrows indicate the position and sequence context of the alkylated adenines (in bold). Drug- DNA incubations were for 2 h at 37 °C.

[1] T. Mosmann, *Immunol. Methods* ***65***, 55 (1983).
[2] C. W. Sutton, M. Sutherland, S. D. Shnyder, L. H. Patterson, *Proteomics* **10**, 327 (2010).
[3] M. D. Burke, R. A. Prough, R. T. Mayer, *Drug Metab. Dispos.* **5**, 1 (1977).
[4] K. Kiakos *et al.*, *Mol. Cancer Ther*. **6**, 2708 (2007).