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Synthesis and anticancer activity of silver(I)-N-heterocyclic carbene complexes derived from the natural xanthine products caffeine, theophylline and theobromine

Electronic Supplementary Information (ESI)

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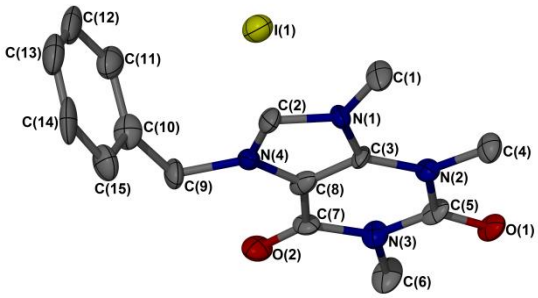
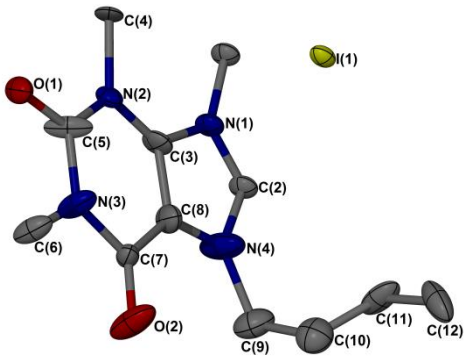
1. Molecular structures of imidazolium salts

2b



2c



2d

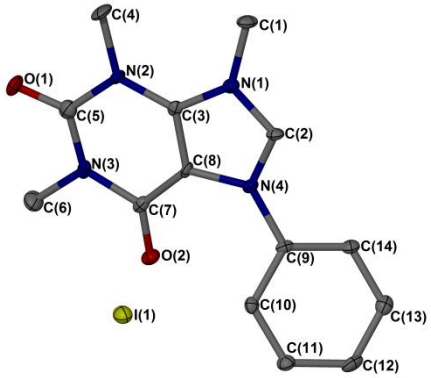


Fig. S1 Molecular structures of imidazolium iodide salts **2b**-**2d**. Hydrogen atoms have been omitted for clarity and ellipsoids are shown at 50 % probability.

2. NMR spectra for silver-NHC complexes

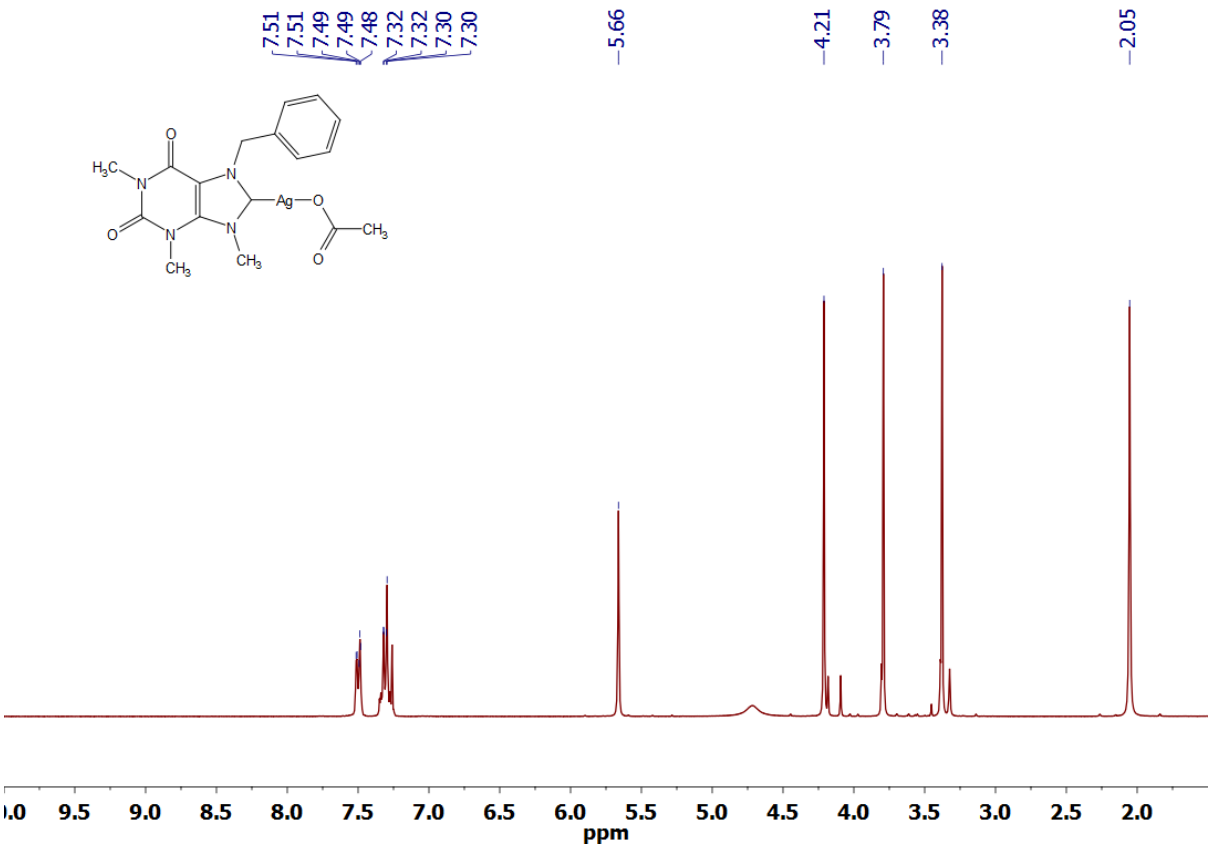


Fig. S2 ^{1}H NMR spectrum of complex **3b** (300MHz, $CDCl_3$).

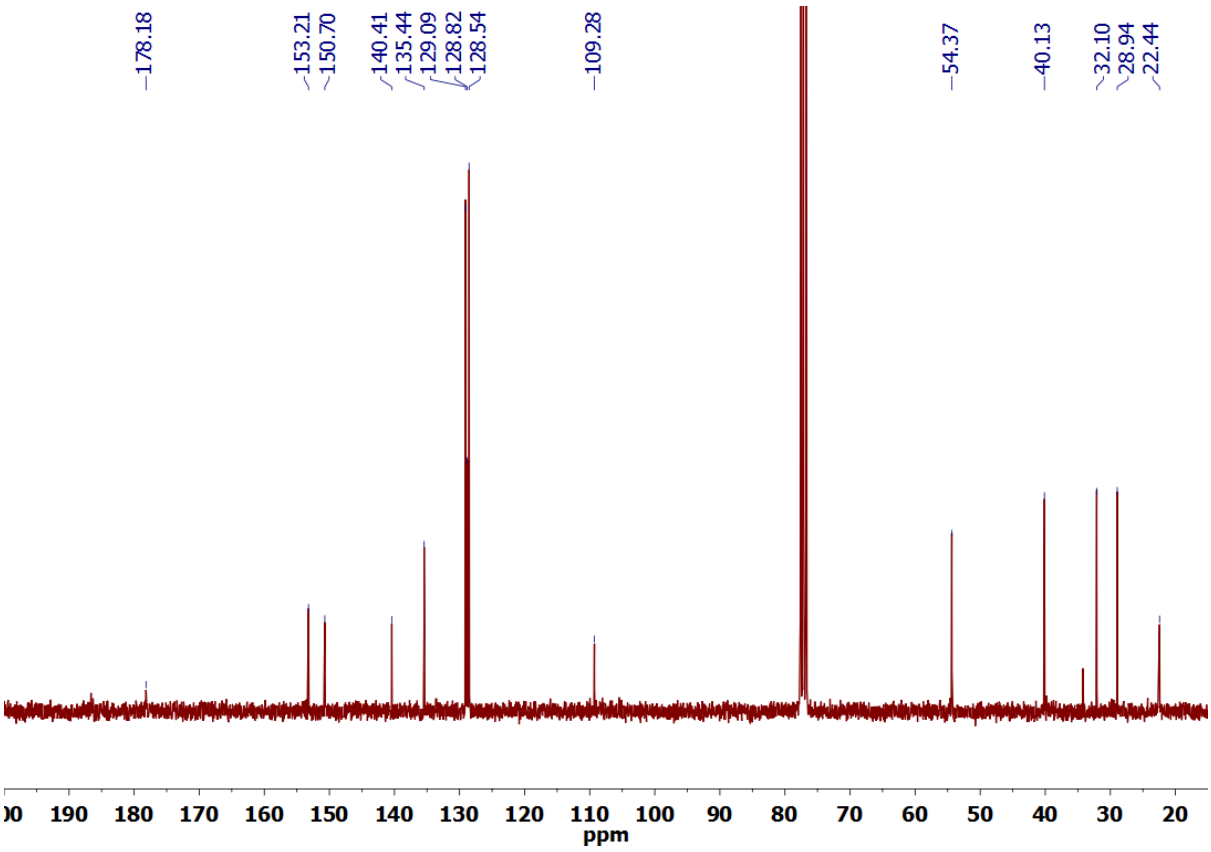


Fig. S3 $^{13}C\{^{1}H\}$ NMR spectrum of complex **3b** (75MHz, $CDCl_3$).

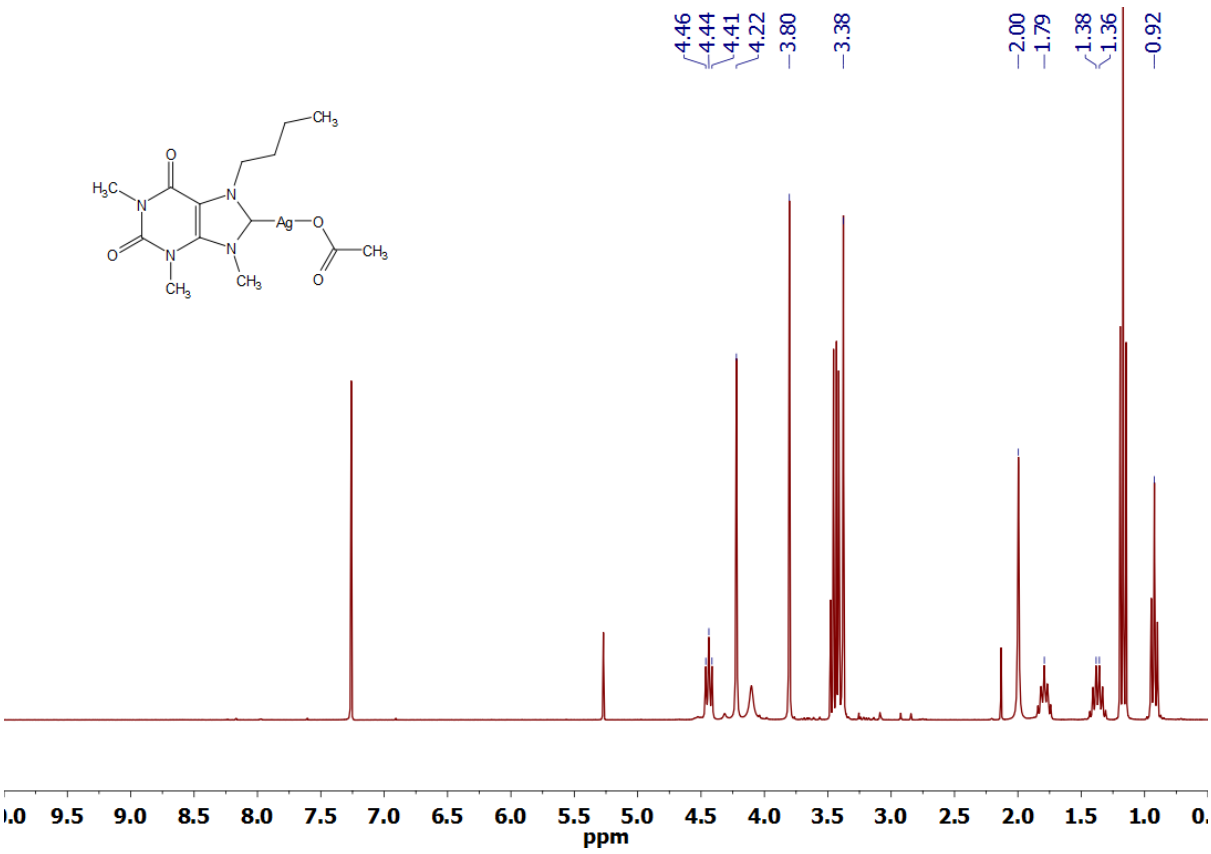


Fig. S4 ^{1}H NMR spectrum of complex **3c** (300MHz, $CDCl_3$).

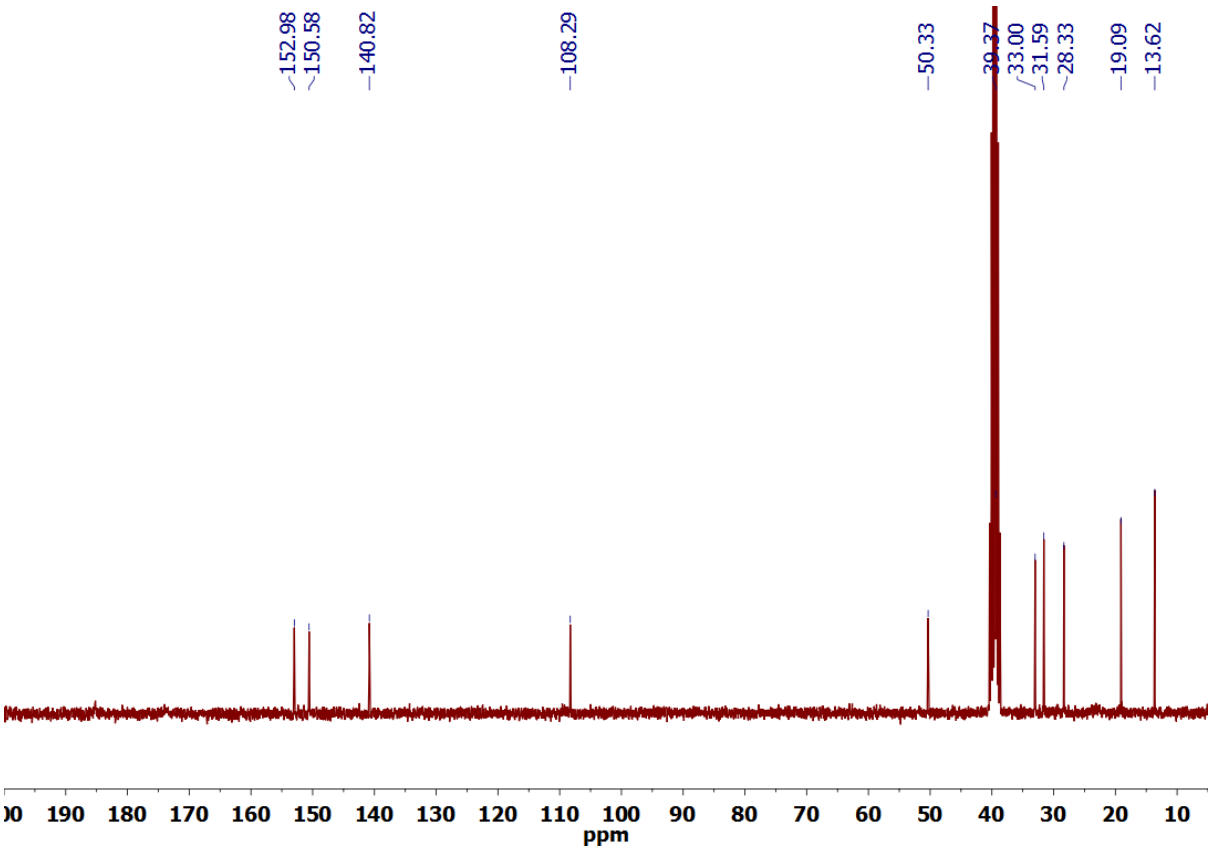


Fig. S5 $^{13}C\{^{1}H\}$ NMR spectrum of complex **3c** (75MHz, d_6-DMSO).

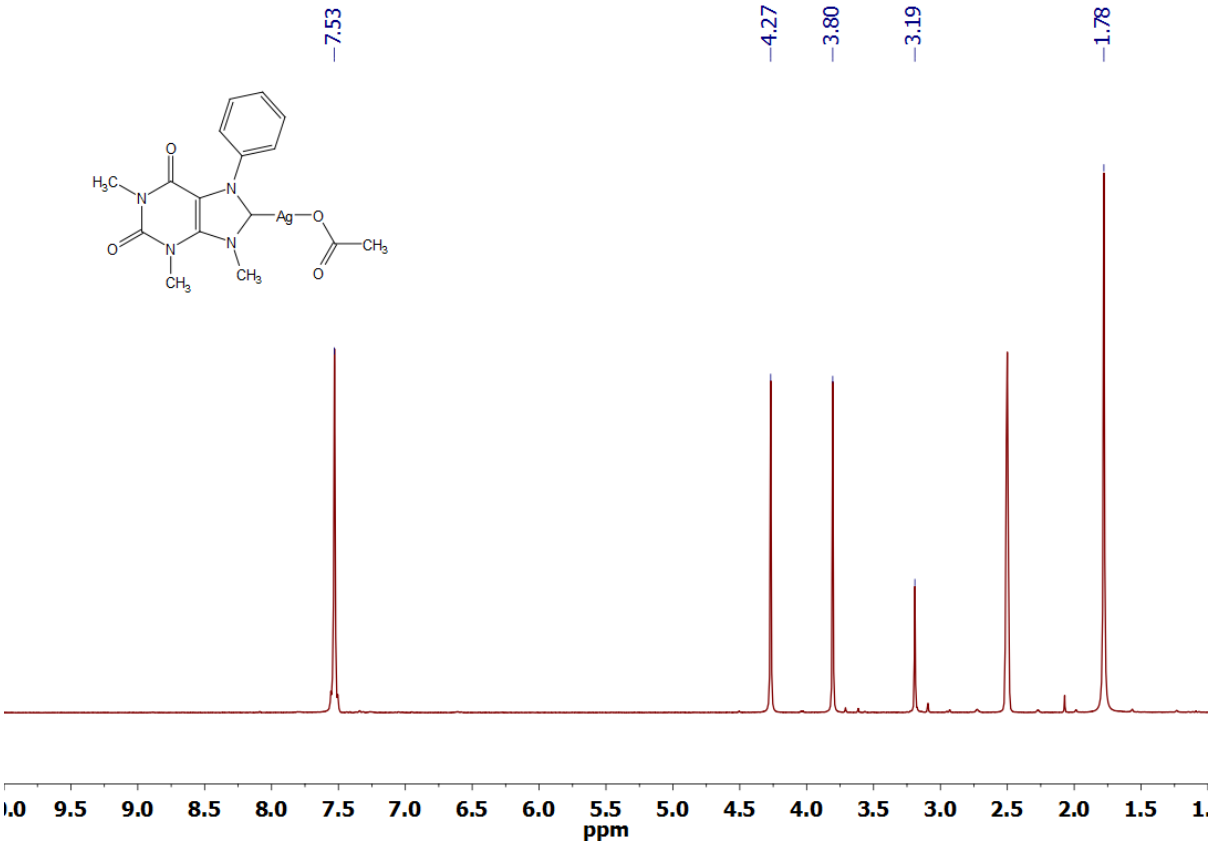


Fig. S6 ^{1}H NMR spectrum of complex **3d** (300MHz, d_6-DMSO with water suppression).

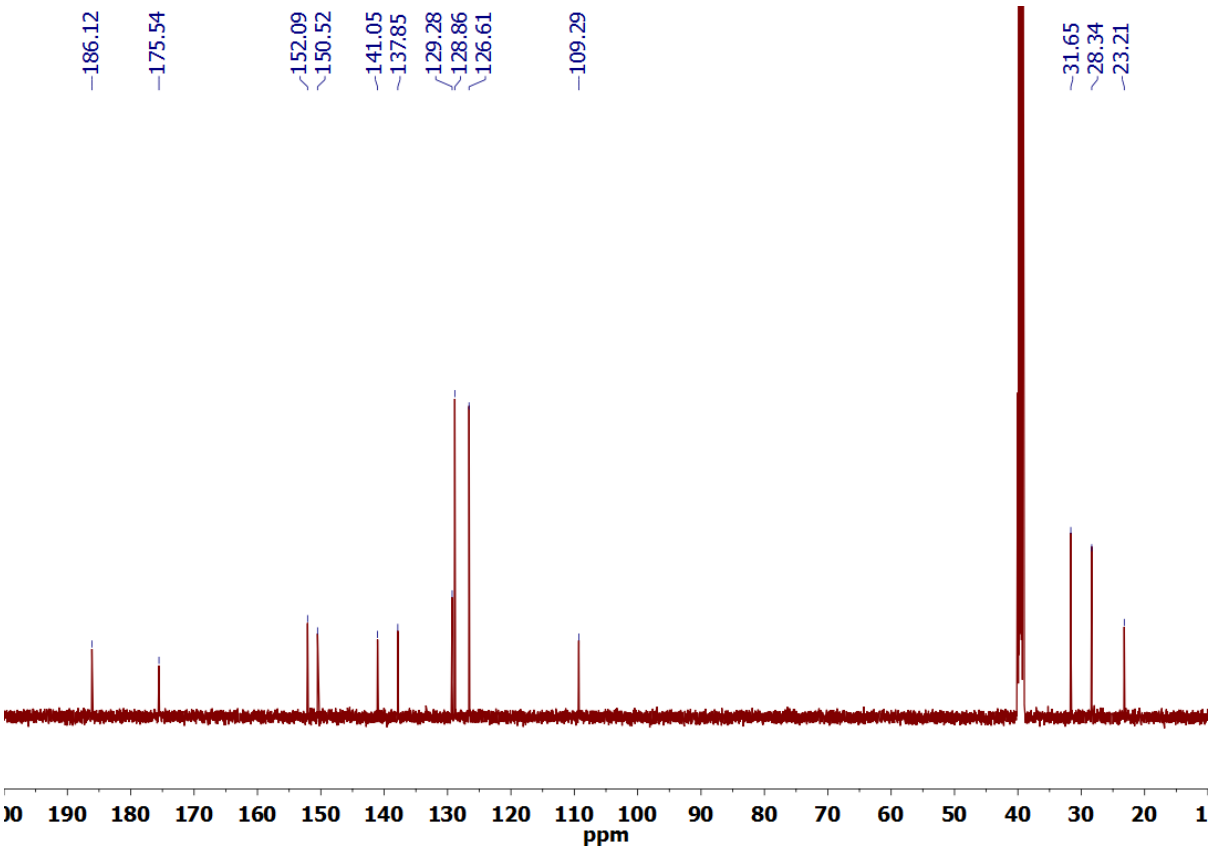


Fig. S7 ^{13}C{^{1}H} NMR spectrum of complex **3d** (75MHz, d_6-DMSO)**.**

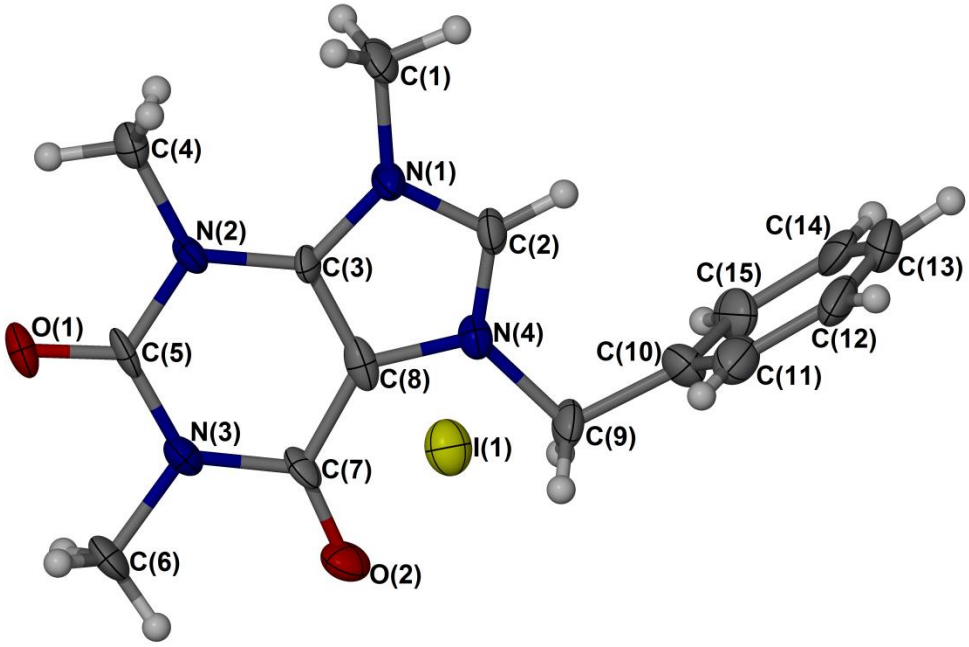
3. Crystallographic details

X-ray diffraction data were collected on an Agilent SuperNova diffractometer fitted with an Atlas CCD detector with Mo-Kα radiation (λ = 0.71073 Å) or Cu Kα radiation (λ = 1.5418 Å). Crystals were mounted under oil on glass or nylon fibres. Data sets were corrected for absorption using a multiscan method, and the structures were solved by direct methods using SHELXS-97 and refined by full-matrix least squares on F2 using ShelXL-97, interfaced through the program X-Seed. Molecular graphics for all structures were generated using POV-RAY in the X-Seed program.

Crystallographic details for 2b

Identification code	Ligand2b
Formula	$C_{15}H_{17}IN_4O_2$
Formula weight	412.23
Size	0.05 x 0.03 x 0.02 mm
Crystal morphology	Colourless fragment
Temperature	100.00(10) K
Wavelength	1.54184 Å
Crystal system	Orthorhombic
Space group	$Pna2_1$
Unit cell dimensions	a = 16.8371(11) Å α = 90°
	b = 10.7293(6) Å β = 90°
	c = 9.0448(6) Å γ = 90°
Volume	1633.95(18) $Å^3$
Z	4
Density (calculated)	1.676 Mg/m^3
Absorption coefficient	15.511 mm^{-1}
F(000)	816
Data collection range	$4.89 \leq \theta \leq 66.59°$
Index ranges	$-20 \leq h \leq 20$, $-12 \leq k \leq 10$, $-9 \leq l \leq 10$
Reflections collected	9250
Independent reflections	2736 [R(int) = 0.0828]
Observed reflections	1744 [$I > 2\sigma(I)$]

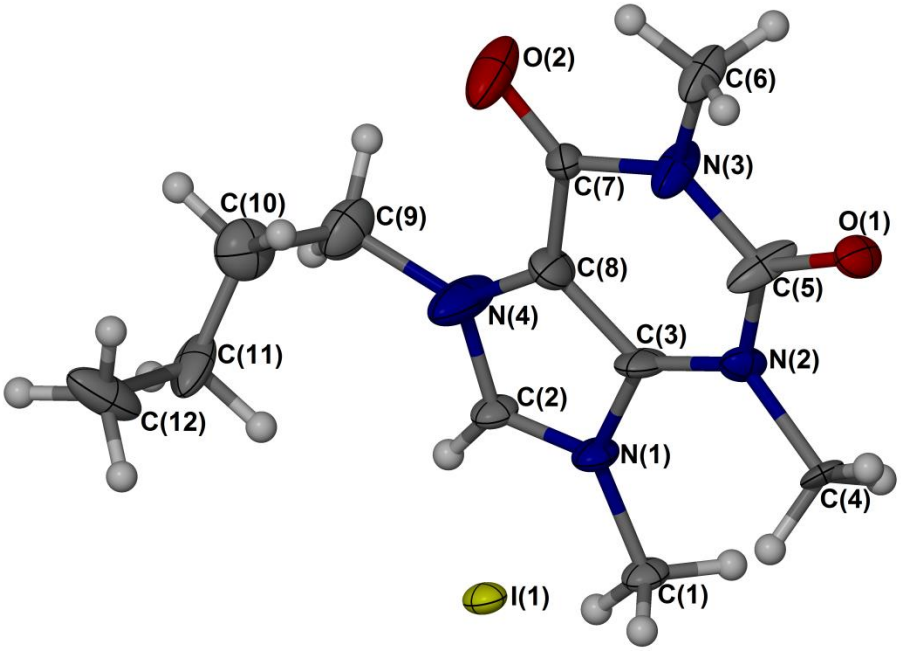
Absorption correction	multi-scan
Max. and min. transmission	0.7467 and 0.511
Refinement method	Full
Data / restraints / parameters	2736 / 1 / 202
Goodness of fit	1.017
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0491$, $wR_2 = 0.1024$
R indices (all data)	$R_1 = 0.0921$, $wR_2 = 0.1211$
Largest diff. peak and hole	0.838 and -0.829e.Å^{-3}
Absolute structure parameter	0.084(14)



Crystallographic details for 2c

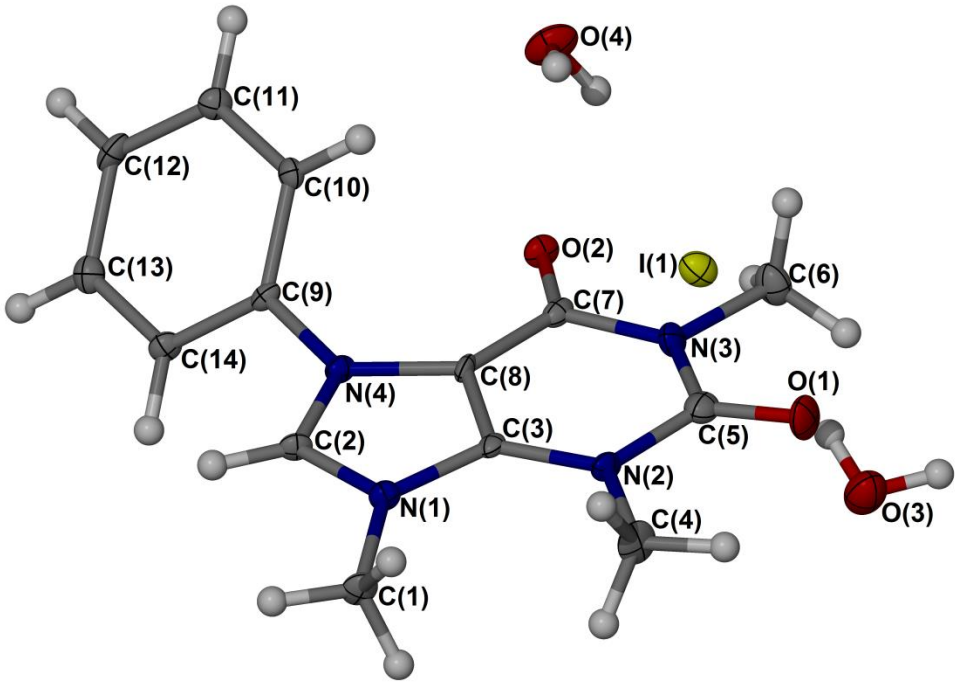
Identification code	Ligand2c
Formula	$C_{12}H_{19}IN_4O_2$
Formula weight	378.21
Size	0.21 x 0.17 x 0.06 mm
Crystal morphology	Colourless block
Temperature	99.98(14) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	*Cc*
Unit cell dimensions	$a = 9.4007(7)$ Å $\alpha = 90°$

	b = 22.7653(11) Å	β = 90.244(6)°
	c = 7.1881(5) Å	γ = 90°
Volume	1538.31(17) Å^3	
Z	4	
Density (calculated)	1.633 Mg/m^3	
Absorption coefficient	2.086 mm^{-1}	
F(000)	752	
Data collection range	3.35 ≤ θ ≤ 27.1°	
Index ranges	-8 ≤ h ≤12, -24 ≤ k ≤ 29, -9 ≤ l ≤ 8	
Reflections collected	4777	
Independent reflections	2369 [R(int) = 0.0372]	
Observed reflections	2249 [I >2σ(I)]	
Absorption correction	multi-scan	
Max. and min. transmission	0.885 and 0.6684	
Refinement method	Full	
Data / restraints / parameters	2369 / 289 / 159	
Goodness of fit	1.085	
Final R indices [I >2σ(I)]	R_1 = 0.0564, wR_2 = 0.1407	
R indices (all data)	R_1 = 0.0593, wR_2 = 0.1439	
Largest diff. peak and hole	2.111 and -1.313e.Å^{-3}	
Absolute structure parameter	0.28(8)	



Crystallographic details for 2d

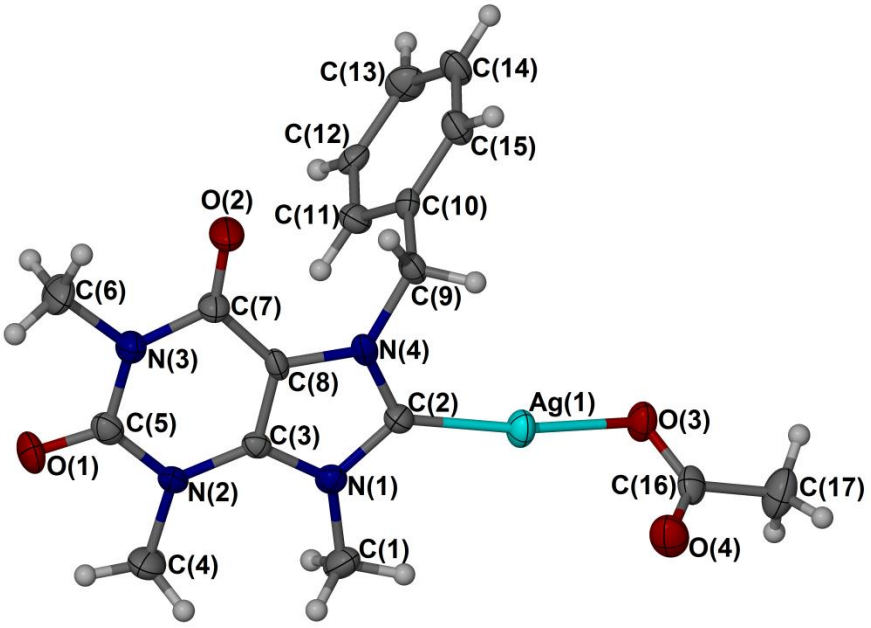
Identification code	Ligand2d
Formula	$C_{14}H_{19}IN_4O_4$
Formula weight	434.23
Size	0.17 x 0.06 x 0.04 mm
Crystal morphology	Colourless needle
Temperature	99.97(11) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/n$
Unit cell dimensions	a = 10.4060(11) Å α = 90°
	b = 7.2140(7) Å β = 89.954(9)°
	c = 21.938(2) Å γ = 90°
Volume	1646.9(3) $Å^3$
Z	4
Density (calculated)	1.751 Mg/m^3
Absorption coefficient	1.97 mm^{-1}
F(000)	864
Data collection range	$3.38 \leq \theta \leq 27.1°$
Index ranges	$-13 \leq h \leq 13$, $-9 \leq k \leq 9$, $-28 \leq l \leq 28$
Reflections collected	8263
Independent reflections	3604 [R(int) = 0.0537]
Observed reflections	2723 [$I > 2\sigma(I)$]
Absorption correction	multi-scan
Max. and min. transmission	0.9254 and 0.7306
Refinement method	Full
Data / restraints / parameters	3604 / 6 / 225
Goodness of fit	1.016
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0424, wR_2 = 0.0689
R indices (all data)	R_1 = 0.0668, wR_2 = 0.0775
Largest diff. peak and hole	0.847 and -0.865e.$Å^{-3}$



Crystallographic details for 3b

Identification code	Complex3b	
Formula	$C_{17}H_{19}AgN_4O_4$	
Formula weight	451.23	
Size	0.24 x 0.08 x 0.05 mm	
Crystal morphology	Colourless needle	
Temperature	200.01(10) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	$P\ \bar{1}$	
Unit cell dimensions	a = 7.9222(8) Å	α = 105.139(15)°
	b = 9.4296(18) Å	β = 96.986(10)°
	c = 12.3121(19) Å	γ = 92.438(11)°
Volume	878.6(2) $Å^3$	
Z	2	
Density (calculated)	1.706 Mg/m^3	
Absorption coefficient	1.178 mm^{-1}	
F(000)	456	
Data collection range	$3.3 \leq \theta \leq 28.38°$	

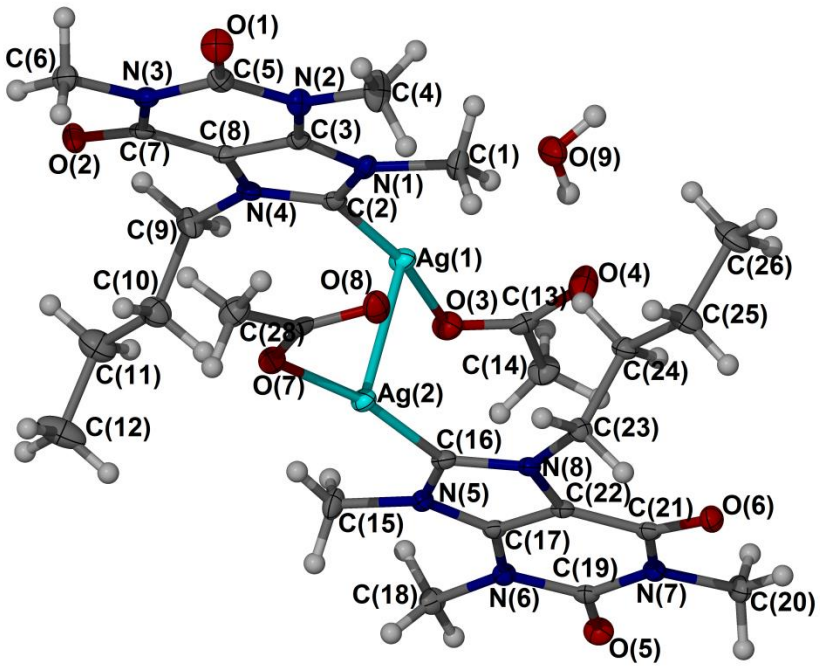
Index ranges	$-7 \le h \le 10$, $-12 \le k \le 12$, $-16 \le l \le 16$
Reflections collected	13274
Independent reflections	4315 [R(int) = 0.1124]
Observed reflections	3000 [$I > 2\sigma(I)$]
Absorption correction	multi-scan
Max. and min. transmission	0.9434 and 0.7652
Refinement method	Full
Data / restraints / parameters	4315 / 0 / 240
Goodness of fit	1.061
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0774, wR_2 = 0.1653
R indices (all data)	R_1 = 0.1181, wR_2 = 0.1792
Largest diff. peak and hole	1.865 and -0.922e.Å^{-3}



Crystallographic details for 3c

Identification code	Complex3c
Formula	$C_{28}H_{44}Ag_2N_8O_9$
Formula weight	852.45
Size	0.21 x 0.1 x 0.03 mm
Crystal morphology	Colourless needle
Temperature	100.0(2) K
Wavelength	0.71073 Å

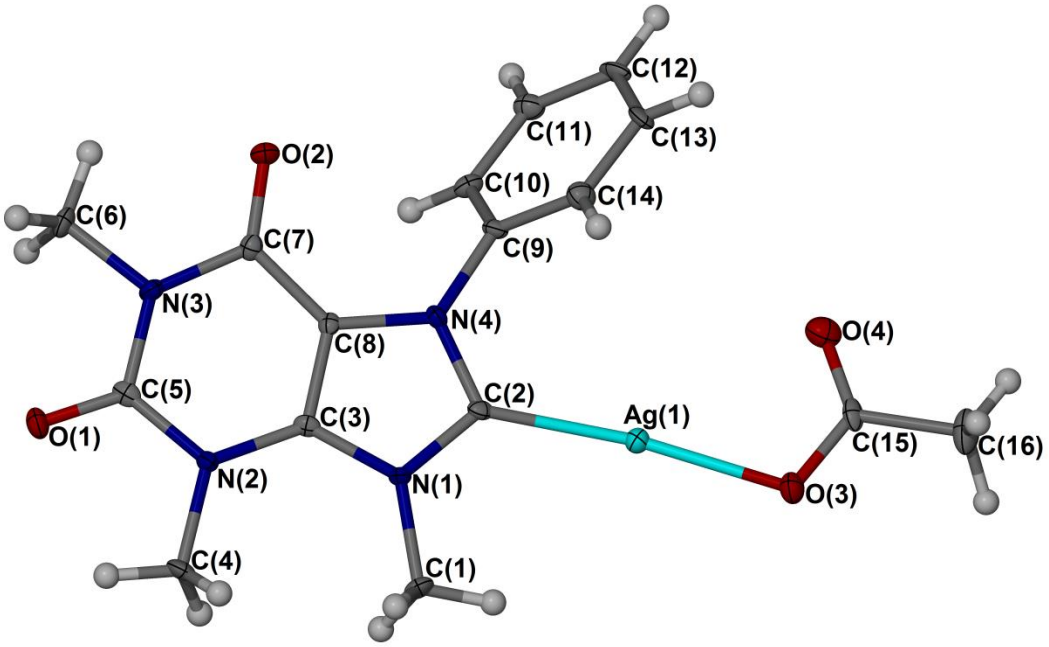
Crystal system	Triclinic	
Space group	$P\ \bar{1}$	
Unit cell dimensions	a = 9.0608(4) Å	α = 87.707(4)°
	b = 9.5485(5) Å	β = 84.198(4)°
	c = 19.4525(9) Å	γ = 82.717(4)°
Volume	1660.21(14) Å^3	
Z	2	
Density (calculated)	1.705 Mg/m^3	
Absorption coefficient	1.243 mm^{-1}	
F(000)	868	
Data collection range	$2.93 \leq \theta \leq 28.28°$	
Index ranges	$-12 \leq h \leq 12$, $-12 \leq k \leq 12$, $-25 \leq l \leq 25$	
Reflections collected	24918	
Independent reflections	8239 [R(int) = 0.0369]	
Observed reflections	6835 [$I > 2\sigma(I)$]	
Absorption correction	multi-scan	
Max. and min. transmission	0.9637 and 0.7803	
Refinement method	Full	
Data / restraints / parameters	8239 / 0 / 395	
Goodness of fit	1.022	
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0331, wR_2 = 0.0712	
R indices (all data)	R_1 = 0.0447, wR_2 = 0.0764	
Largest diff. peak and hole	1.221 and -0.572e.Å^{-3}	



Crystallographic details for 3d

Identification code	Complex3d	
Formula	$C_{16}H_{17}AgN_4O_4$	
Formula weight	437.21	
Size	0.2 x 0.12 x 0.06 mm	
Crystal morphology	Colourless plate	
Temperature	99.9(5) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	$P\ \bar{1}$	
Unit cell dimensions	a = 8.7170(6) Å	α = 82.181(6)°
	b = 9.9462(6) Å	β = 86.063(6)°
	c = 10.2712(9) Å	γ = 67.816(6)°
Volume	816.79(10) $Å^3$	
Z	2	
Density (calculated)	1.778 Mg/m^3	
Absorption coefficient	1.264 mm^{-1}	
F(000)	440	
Data collection range	$3.17 \leq \theta \leq 28.28°$	

Index ranges	$-11 \leq h \leq 11$, $-13 \leq k \leq 10$, $-9 \leq l \leq 13$
Reflections collected	6438
Independent reflections	4023 [R(int) = 0.0405]
Observed reflections	3452 [$I > 2\sigma(I)$]
Absorption correction	multi-scan
Max. and min. transmission	0.928 and 0.7861
Refinement method	Full
Data / restraints / parameters	4023 / 0 / 230
Goodness of fit	1.043
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.043, wR_2 = 0.0756
R indices (all data)	R_1 = 0.0531, wR_2 = 0.0836
Largest diff. peak and hole	0.743 and -0.78e.Å^{-3}



4. Chemosensitivity data

Cells were incubated in 96-well plates, at 2 x 103 cells per well in 200 µL of growth media (RPMI 1640 supplemented with 10 % foetal calf serum, sodium pyruvate (1mM) and L-glutamine (2mM)). Cells were incubated for 24 hours at 37 ºC in an atmosphere of 5 % CO_2 prior to drug exposure. Silver compounds and cisplatin were dissolved in dimethylsulfoxide at a concentration of 25 mM and diluted with medium to obtain drug solutions ranging from 25 µM to 0.049 µM. The final dimethylsulfoxide concentration

was 0.1% (v/v) which is non-toxic to cells. Drug solutions were applied to cells and incubated for 96 hours at 37 ºC in an atmosphere of 5 % CO_2. The solutions were removed from the wells and fresh medium added to each well along with 20 µL MTT (5mg / mL), and incubated for 4 hours at 37 ºC in an atmosphere of 5 % CO_2. The solutions were removed and 150 µL dimethylsulfoxide was added to each well to dissolve the purple formazan crystals. A plate reader was used to measure the absorbance at 540 nm. Lanes containing medium only, and cells in medium only (no drug), were used as blanks for the spectrophotometer and 100 % cell survival respectively. Cell survival was determined as the absorbance of treated cells divided by the absorbance of controls and expressed as a percentage. The concentration required to kill 50 % of cells (IC_{50}) was determined from plots of % survival against drug concentration. Each experiment was repeated 3 times and a mean value obtained.

Table S1 Response of eight cell lines to silver(I)-NHC complexes **3a**-**3e** and cisplatin. Values presented are IC_{50} µM ± SD (in parentheses) for three independent experiments.

Cell Line	cisplatin	3a	3b	3c	3d	3e
A357	1.2 (0.3)	34.5 (3.8)	21.4 (7.5)	27.4 (3.9)	11.5 (5.3)	12.4 (1.3)
HCT116	2.4 (0.3)	26.7 (9.9)	29.6 (3.5)	22.4 (4.8)	19.5 (2.3)	19.0 (5.1)
HT-29	0.6 (0.1)	41.8 (9.8)	29.9 (16.5)	28.5 (2.0)	21.4 (6.5)	20.7 (1.5)
LN229	0.7 (0.5)	29.2 (12.8)	18.4 (12.0)	46.5 (9.8)	11.2 (1.5)	7.4 (1.8)
Panc-1	2.6 (0.9)	31.7 (2.8)	29.7 (8.2)	16.9 (1.1)	7.6 (3.2)	23.5 (5.9)
SiHa	0.9 (0.3)	21.9 (3.8)	15.3 (2.6)	16.4 (0.5)	13.1 (3.7)	14.0 (2.2)
U87MG	0.9 (0.4)	33.7 (1.4)	22.8 (5.1)	14.0 (4.3)	17.6 (4.5)	22.1 (8.1)
U-251	1.0 (0.6)	54.8 (15.7)	26.7 (8.8)	51.4 (17.8)	14.2 (2.5)	29.6 (5.1)
Average	**1.29**	**34.34**	**24.23**	**27.94**	**14.51**	**18.59**

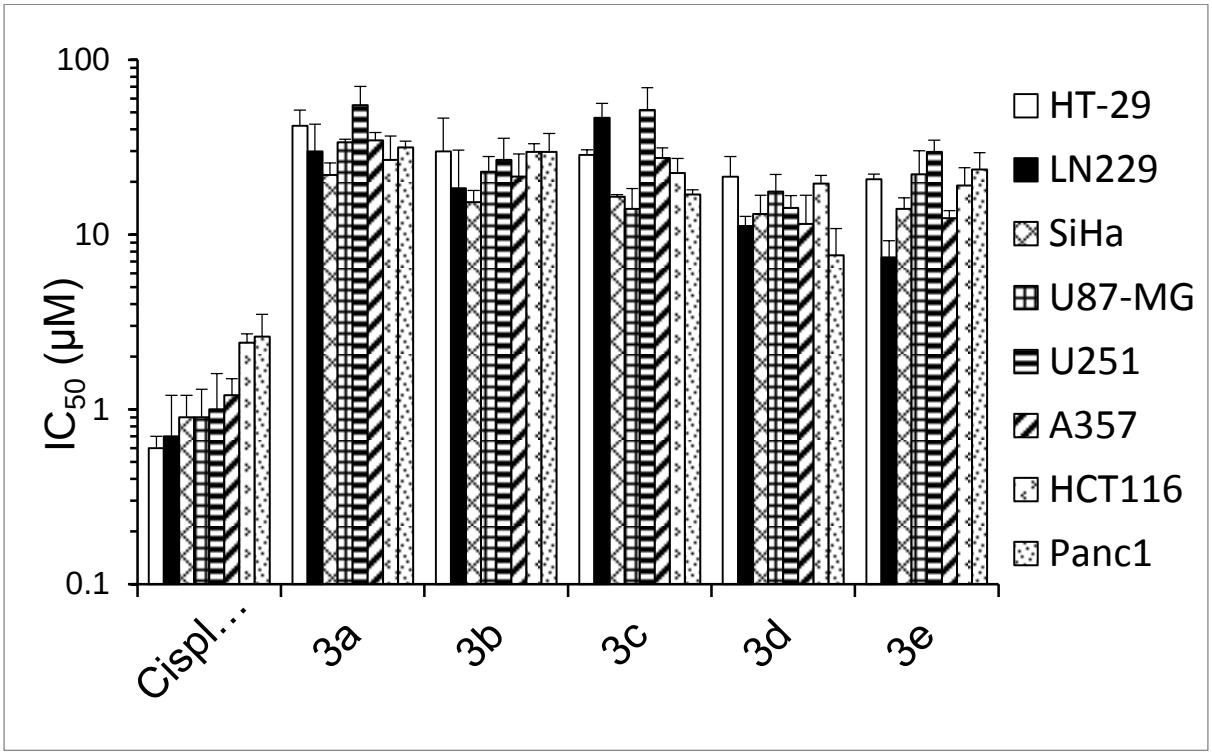


Figure S8 Response of eight cell lines to silver(I)-NHC complexes **3a**-**3e** and cisplatin. Values presented are IC_{50} (μM) ± SD for three independent experiments and are plotted on a logarithmic scale.

5. Log *P* data

Equal volumes of octanol and NaCl-saturated water were stirred at room temperature for 24 hours, and separated to give octanol-saturated water and water-saturated octanol. Five standard concentrations (5, 10, 20, 40 and 60 μM) of the complexes were prepared from the octanol-saturated water. Analysis using UV / *vis* spectroscopy was used to obtain a calibration curve of absorbance vs. concentration for each complex at its maximum absorbance. Accurate amounts of the complexes were dissolved in the octanol-saturated water (25 mL) to make up a concentration of 50 μM. 3 mL of octanol-saturated water containing the complex was placed in a centrifuge tube and 3 mL of water-saturated octanol was layered on top. Six samples prepared in this manner were shaken for 4 hours using a vibrax machine at 500 $gmin^{-1}$. The layers were separated and the octanol-saturated water layer was retained for analysis using UV / *vis* spectroscopy. The average concentration of the six runs was calculated using the calibration graph and maximum absorbance for each complex. Subtraction of the average concentration obtained from the concentration of an unshaken sample in octanol-saturated water gave the final $[C]_{org}$. The $[C]_{org}$ and $[C]_{aq}$ were used to determine the partition coefficient Log *P*.

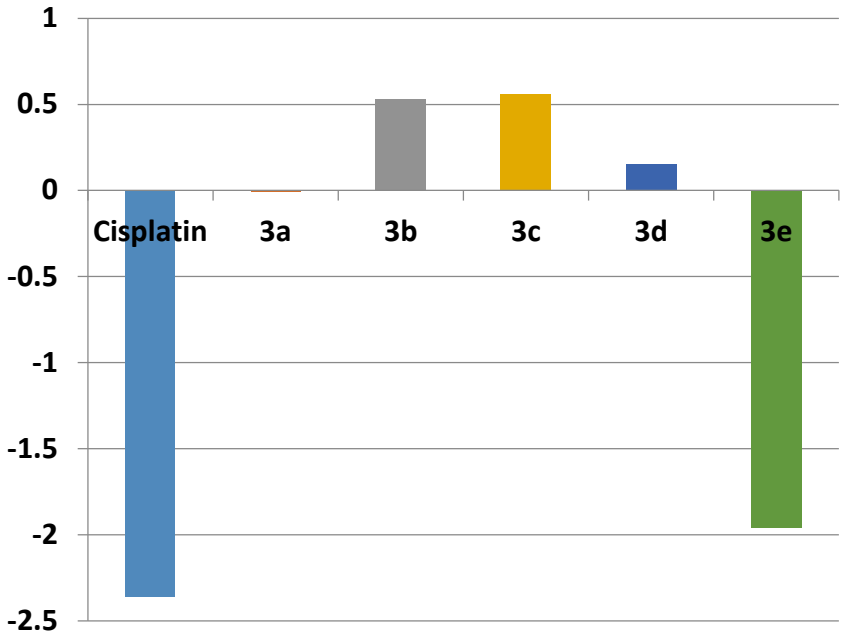


Fig. S9 Bar chart to show Log *P* values for complexes **3a**-**3e**.