Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Electronic supplementary information

A polymer–(multifunctional single-drug) conjugate for combination therapy

*Dongfang Zhou, Shasha He, Yuwei Cong, Zhigang Xie, Xuesi Chen, Xiabin Jing and Yubin Huang**

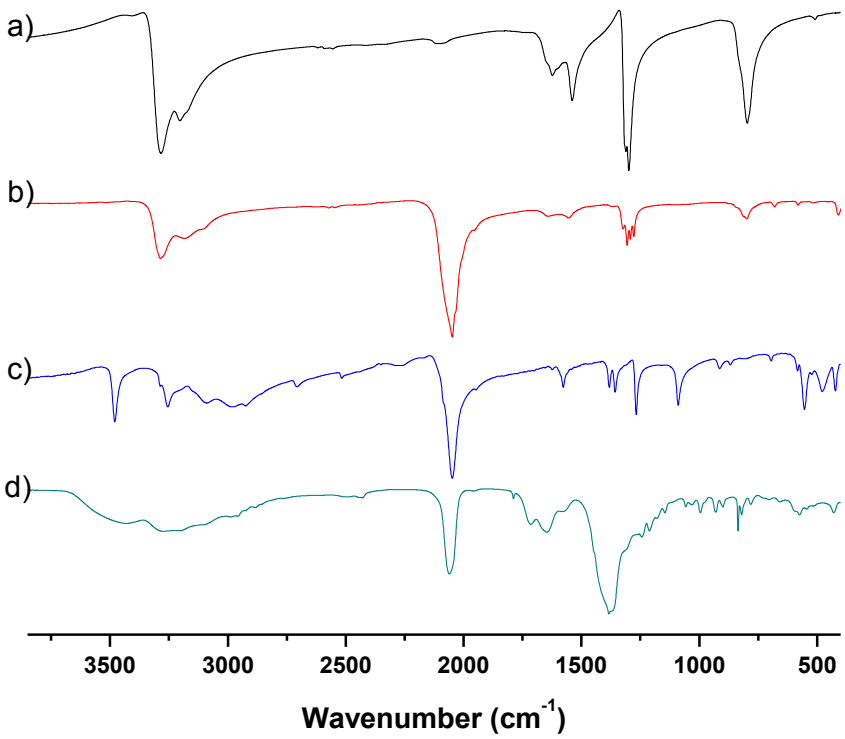
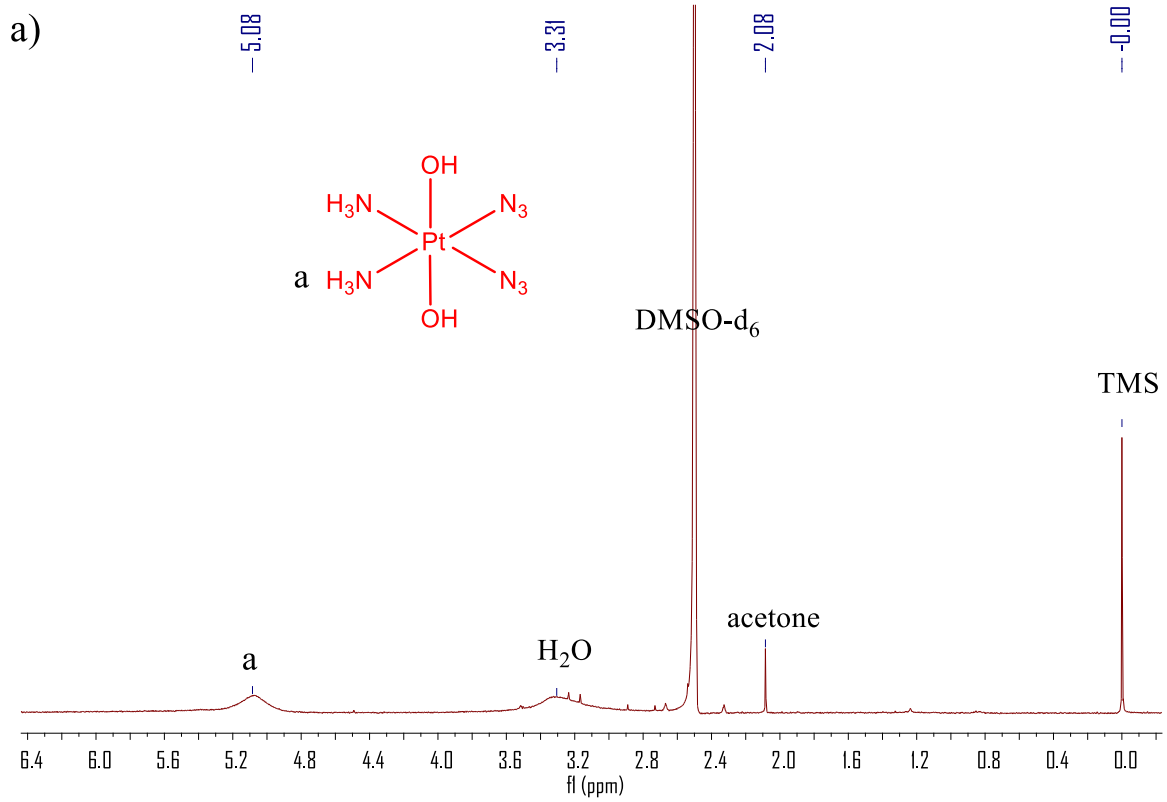
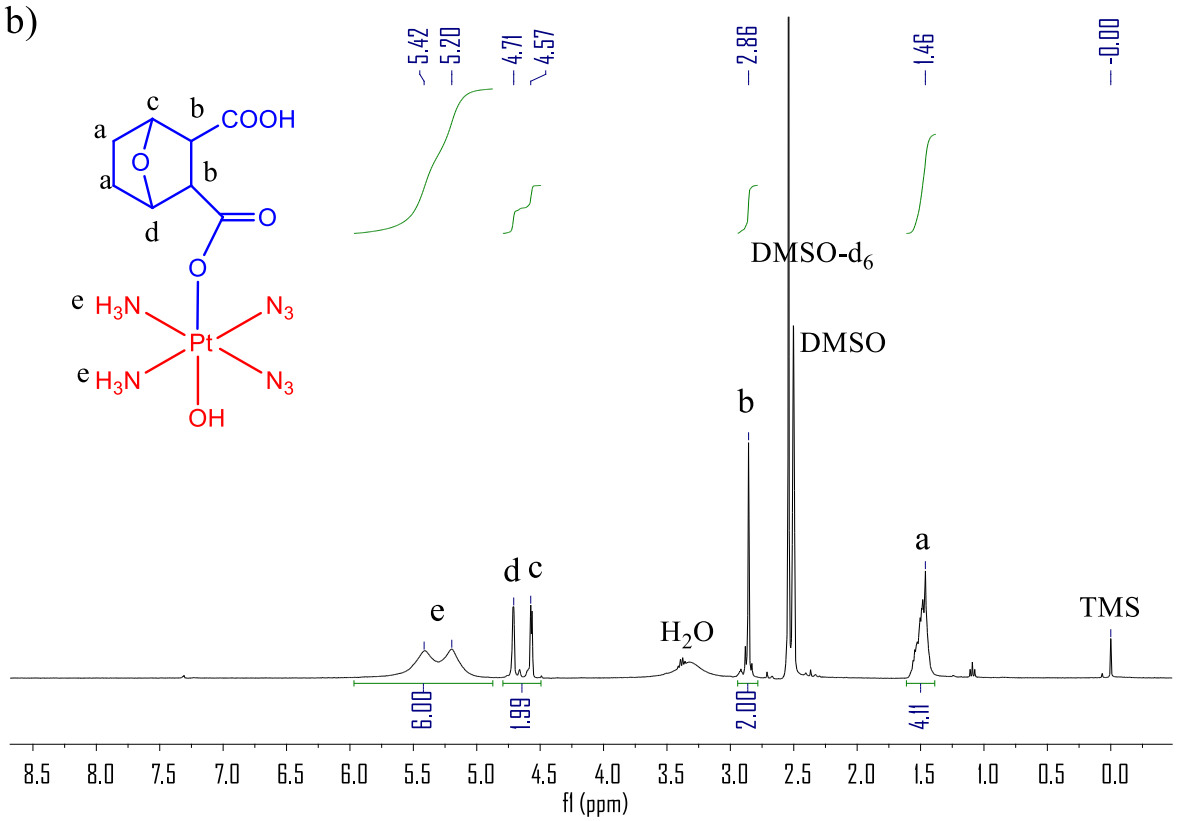


Fig. S1 FTIR spectra of a) cisplatin, b) c,c-[$Pt(NH_3)_2(N_3)_2$], c) $CIS(N_3)$ and d) Z-DMC-$CIS(N_3)$.

The park at 2050 cm^{-1} (azide stretching) proves the structure of c,c-[$Pt(NH_3)_2(N_3)_2$]. $CIS(N_3)$, which is obtained by oxidizing c,c-[$Pt(NH_3)_2(N_3)_2$] with H_2O_2, displays a sharp and intense peak at 3480 cm^{-1} (OH stretching) and a new Pt-OH stretch at 550 cm^{-1}, respectively, compared with cisplatin. After reacting with demethylcantharidin, the 3480 cm^{-1} band is weakened, and there appear two peaks (1702 cm^{-1} and 1645 cm^{-1}) characteristic of the coordinated carboxyl group (1645 cm^{-1}) and free carboxyl group (1702 cm^{-1}) in Z-DMC-$CIS(N_3)$, respectively, confirming the structure of Z-DMC-$CIS(N_3)$. The observed absorption at 1645 cm^{-1} of the coordinated carboxylate is in good agreement with previously published data for analogous complexes (1633-1669 cm^{-1}). Comparison of the three FTIR spectra of cisplatin, $CIS(N_3)$ and Z-DMC-$CIS(N_3)$ indicate that $CIS(N_3)$ reacted with demethylcantharidin to afford Z-DMC-$CIS(N_3)$.


a)
5.08
3.31
2.08
0.00
OH
H3N
N3
Pt
a
H3N
N3
OH
DMSO-d6
TMS
acetone
H2O
a
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
f1 (ppm)


b)
5.42
5.20
4.71
4.57
2.86
1.46
0.00
c
b
COOH
a
a
O
b
d
O
O
e
H3N
N3
Pt
e
H3N
N3
OH
DMSO-d6
DMSO
b
a
d c
e
H2O
TMS
6.00
1.99
2.00
4.11
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

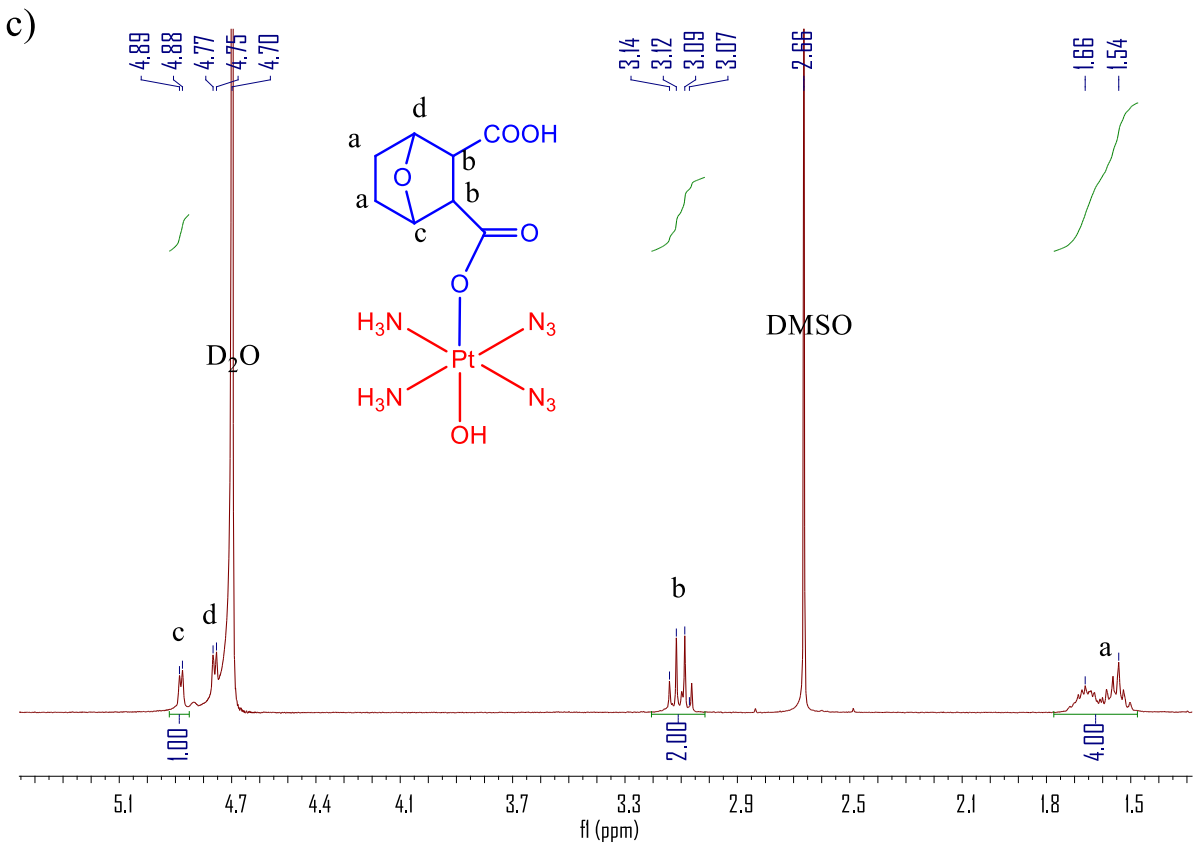


Fig. S2 ^{1}H NMR (400MHz) spectra of a) CIS(N_3) (DMSO-d_6), b) Z-DMC-CIS(N_3) (DMSO-d_6) and c) Z-DMC-CIS(N_3) (D_2O).

CIS(N_3) has the characteristic peak of NH_3 at 5.08 ppm. After it is reacted with demethylcantharidin to form Z-DMC-CIS(N_3), the chemical shift in DMSO-d_6 of NH_3 moves to 5.31 ppm (d, 6H; NH_3), and new chemical shifts of demethylcantharidin residue appear at 1.46 ppm (m, 4H; CH_2CH_2), 2.86 ppm (s, 2H; CHCH), 4.57 ppm (s, 1H; OCH) and 4.71 (s, 1H; OCH). Also, the chemical shifts in D_2O at 1.60 ppm (m, 4H; CH_2CH_2), 3.11 ppm (m, 2H; CHCH) and 4.74 ppm (d, 1H; OCH), 4.88 ppm (d, 1H; OCH) confirm the structure of Z-DMC-CIS(N_3).

Table S1 Elemental analysis of Z-DMC-CIS(N_3)

$C_8H_{16}N_8O_6Pt$	Anal. Calc.	Found[a)]
C	18.64	18.95
H	3.13	3.04
N	21.74	22.01

[a)] Detected by elemental analyzer.

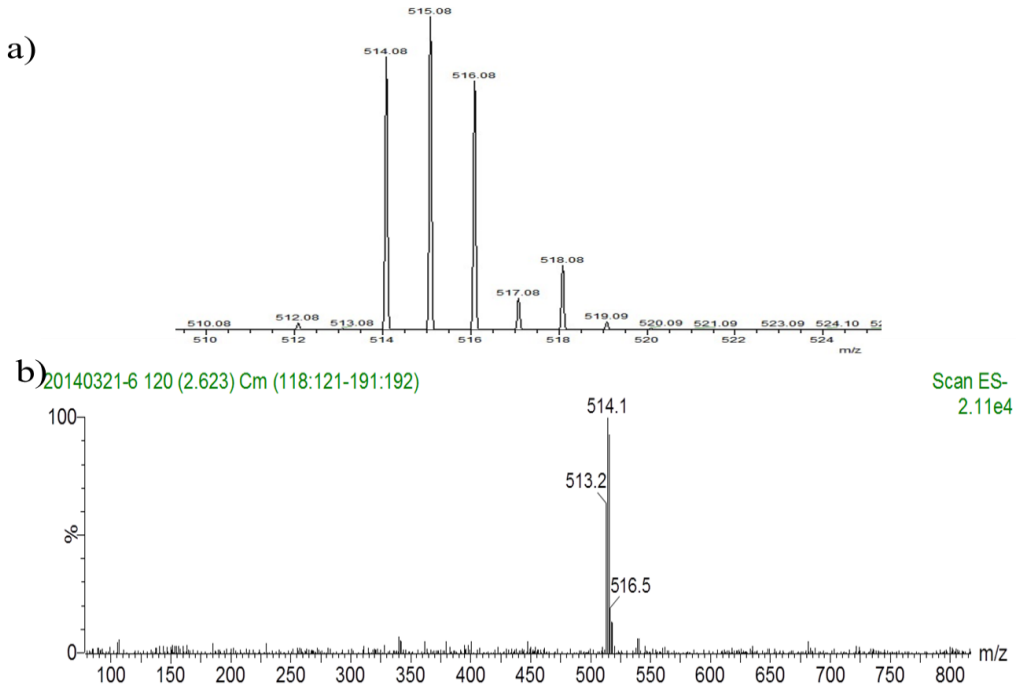


Fig. S3 a) Theoretical and b) measuring ESI-MS spectra of Z-DMC-CIS(N_3).

The theoretical isotope distribution of Z-DMC-CIS(N_3) is shown in Fig. S3a. The major peak at m/z 515.08 could be attributed to its molecular ion. It corresponded to the m/z 514.1 determined by ESI-MS (Fig. S3b), deprotonated Z-DMC-CIS(N_3) (negative mode). This agreement proved the successful synthesis of Z-DMC-CIS(N_3).

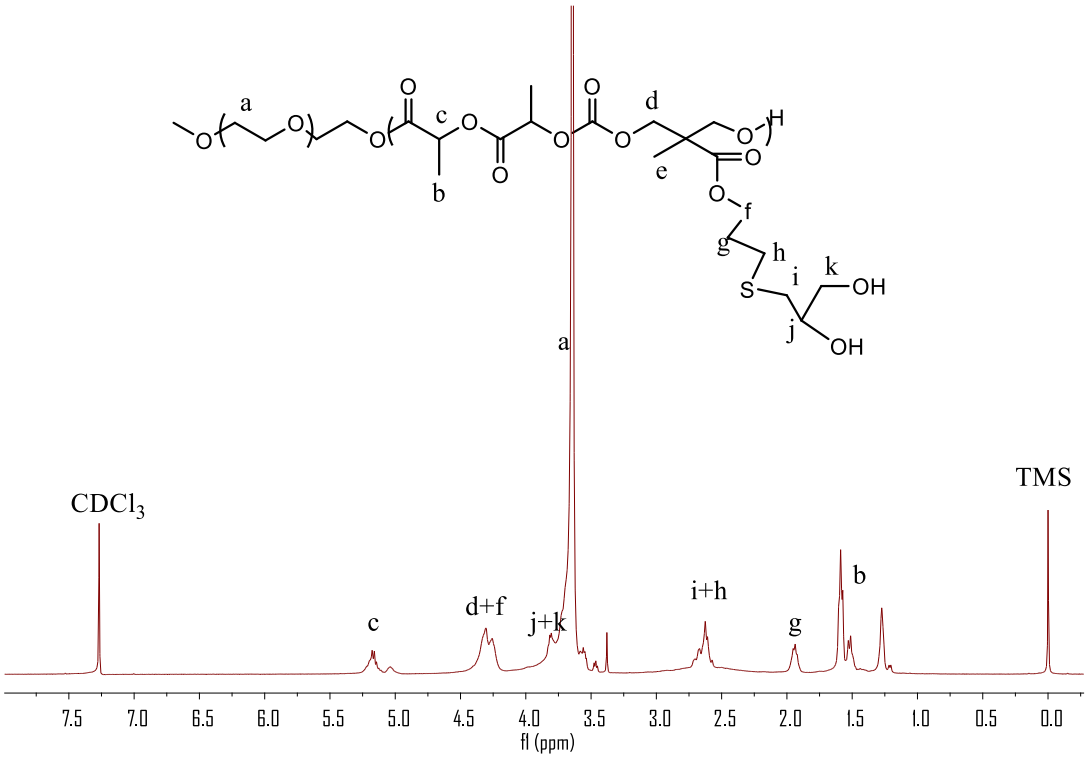


Fig. S4 ^{1}H NMR (400 MHz) spectrum of mPEG-b-P(LA-co-MPD) ($CDCl_3$).

Biodegradable copolymer mPEG-*b*-P(LA-*co*-MPD) with pendant hydroxy groups is chosen as the carrier polymer for Z-DMC-CIS(N_3). Its preparation consists of the following steps as shown briefly: (1) LA and MAC were ring-opening polymerized with PEG_{5k} as a macroinitiator in the presence of $ZnEt_2$ as catalyst to obtain mPEG-*b*-P(LA-*co*-MAC); (2) mPEG-*b*-P(LA-*co*-MAC) was reacted with monothioglycerol under UVA (365nm) irradiation in the presence of DMPA as catalyst in CH_2Cl_2 to give hydroxy groups pendent block copolymer mPEG-*b*-P(LA-*co*-MPD). The ^{1}H NMR spectrum of mPEG-*b*-P(LA-*co*-MPD) is shown in Fig. S4.

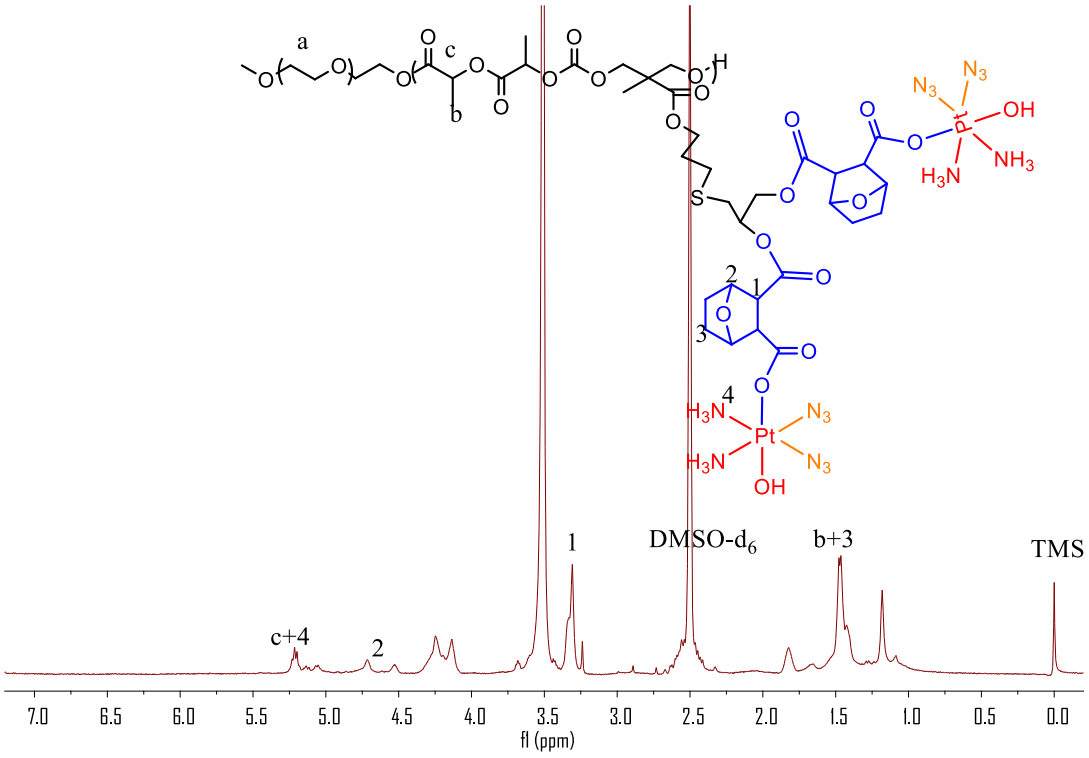


Fig. S5 ^{1}H NMR (400 MHz) spectrum of P-Z-DMC-CIS(N_3) (DMSO-d_6).

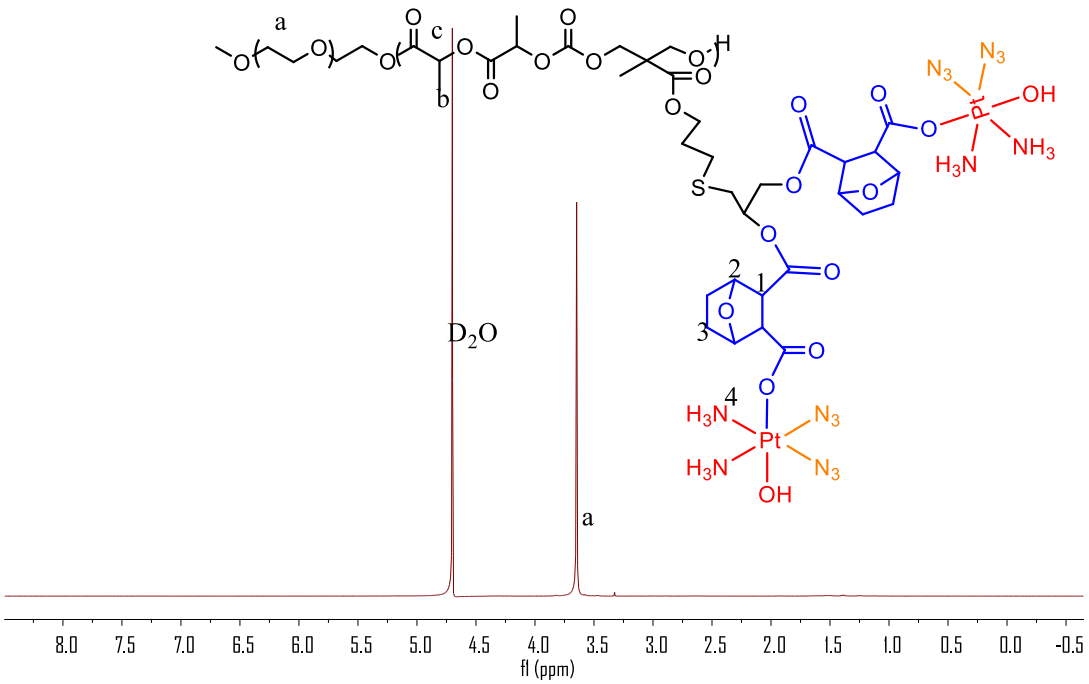


Fig. S6 ^{1}H NMR (400MHz) spectrum of P-Z-DMC-CIS(N_3) micelles (D_2O).

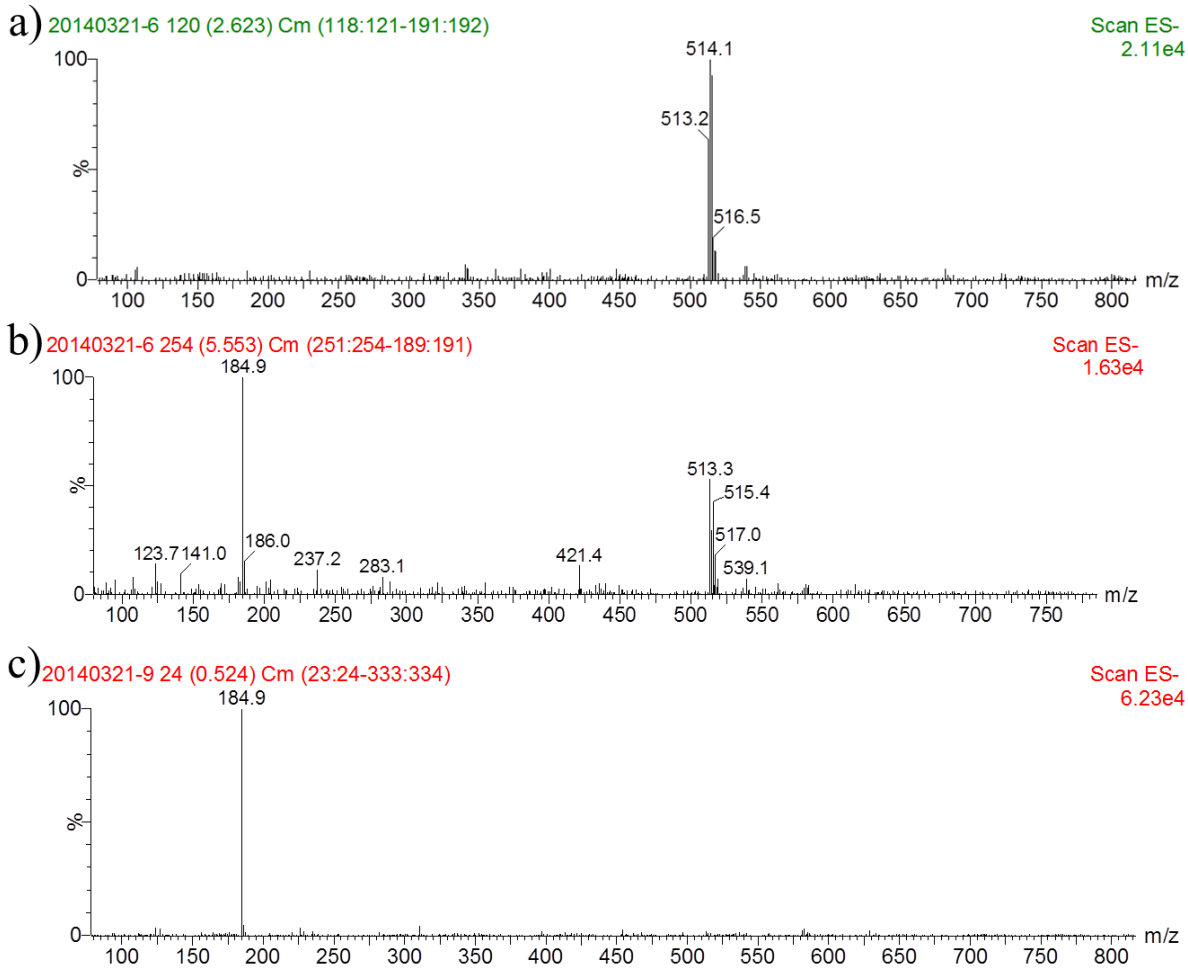


Fig. S7 ESI-MS spectra of Z-DMC-CIS(N_3) after UVA irradiation at a) 0 min, b) 30 min and c) 1 h.

Z-DMC-CIS(N_3) showed a molecular ion peak at m/z 514.1 (de-protonated Z-DMC-CIS(N_3)). The relative abundance of Z-DMC-CIS(N_3) decreased dramatically upon 30 min UVA irradiation. A molecular ion, corresponding to DMC appeared at m/z 184.9, denoting fast release of the axially coordinated DMC of Z-DMC-CIS(N_3) and reduction from Pt(IV) to Pt(II). After 1 hour UVA irradiation, the molecular ion peak of Z-DMC-CIS(N_3) is no longer present and only DMC can be detected.

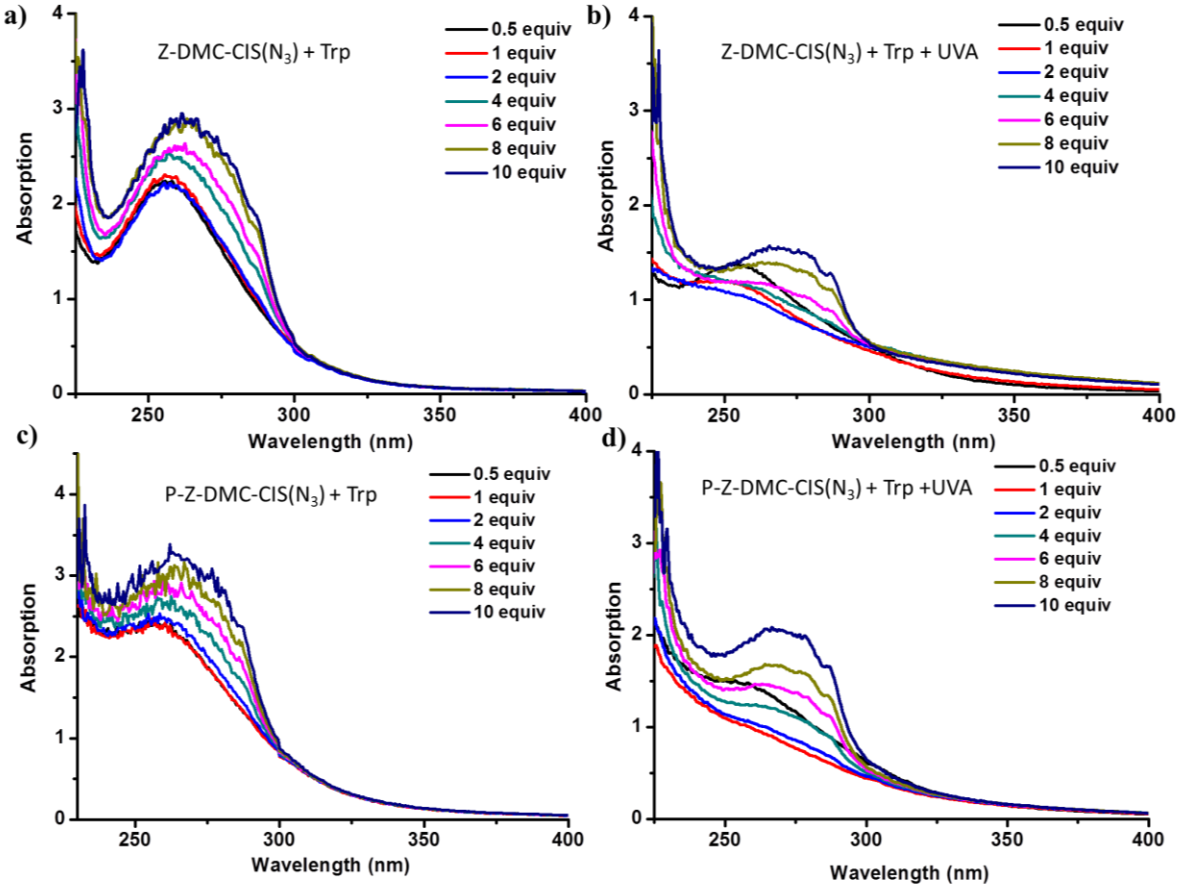


Fig. S8 UV-vis absorbance spectra of Z-DMC-CIS(N_3) (a, b) and P-Z-DMC-CIS(N_3) (c, d) (20 μM Pt) incubated with various mol equivalents of Trp in the absence (a, c) and presence (b, d) of UVA irradiation.

Spectra after 30 min UVA irradiation showed a decrease in intensity of the LMCT transition. The spectra suggest that photodecomposition of Z-DMC-CIS(N_3) and P-Z-DMC-CIS(N_3) still occurs in the presence of L-Trp. Note: all solutions of L-Trp alone were photostable under the same conditions.

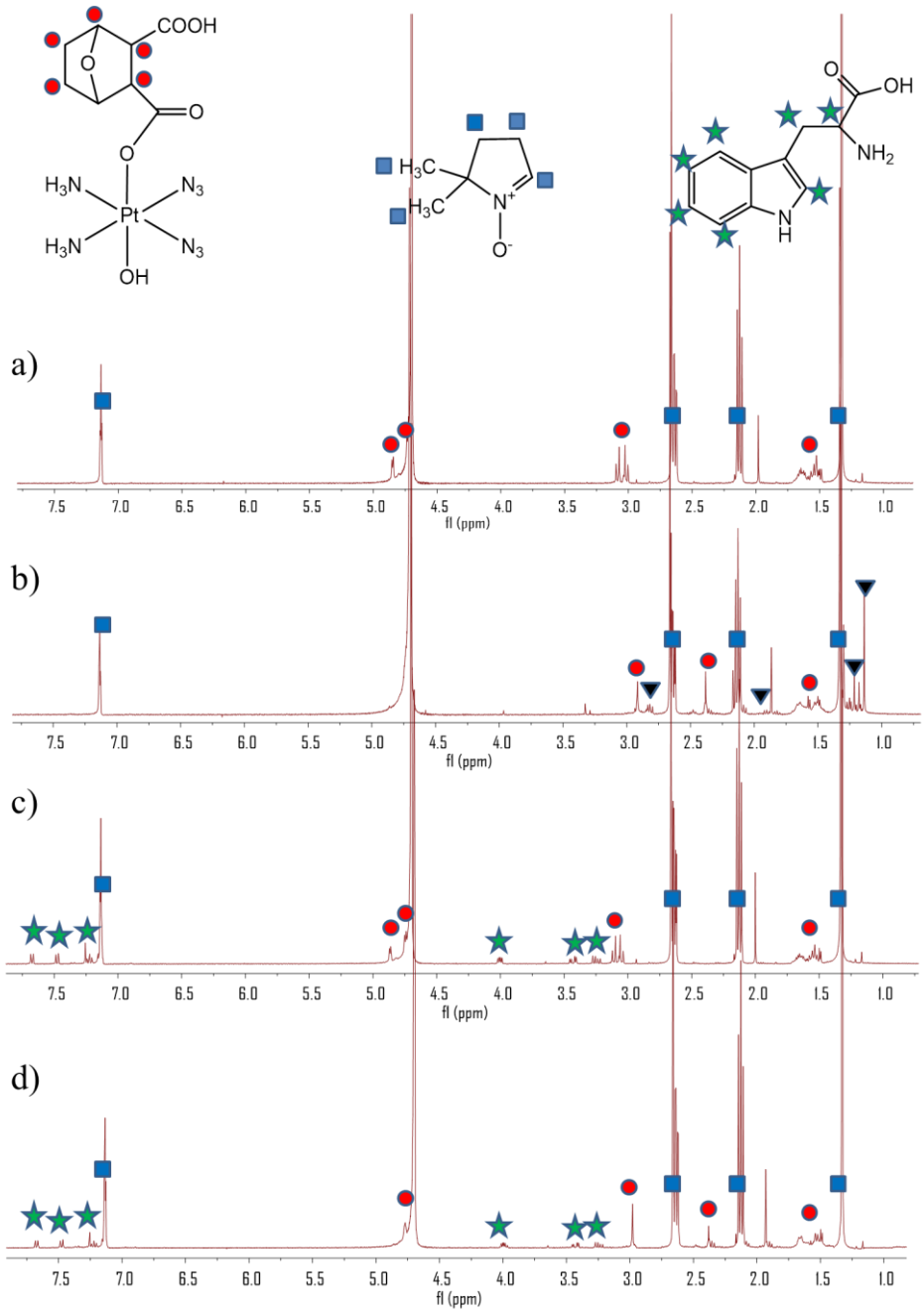


Fig. S9 ^{1}H NMR spectra of a D_2O solution of Z-DMC-CIS(N_3) (4 mM) and DMPO (8 mM) in the absence and presence of 1 mM L-Trp. a) Dark solution, b) Irradiated solution, c) Dark solution in the presence of 1 mM L-Trp, d) Irradiated solution in the presence of 1 mM L-Trp. Assignments: colored circles (●), DMC peaks; colored squares (■), DMPO peaks; colored stars (★), L-Trp; filled triangles (▼), DMPO photoproducts.

Spectrum d shows the decrease in intensity of L-Trp peaks indicating its involvement in the reaction with the formed azidyl radicals. This is consistent with the unchanged DMPO peaks and lack of formation of DMPO photoproducts in the presence of L-Trp.

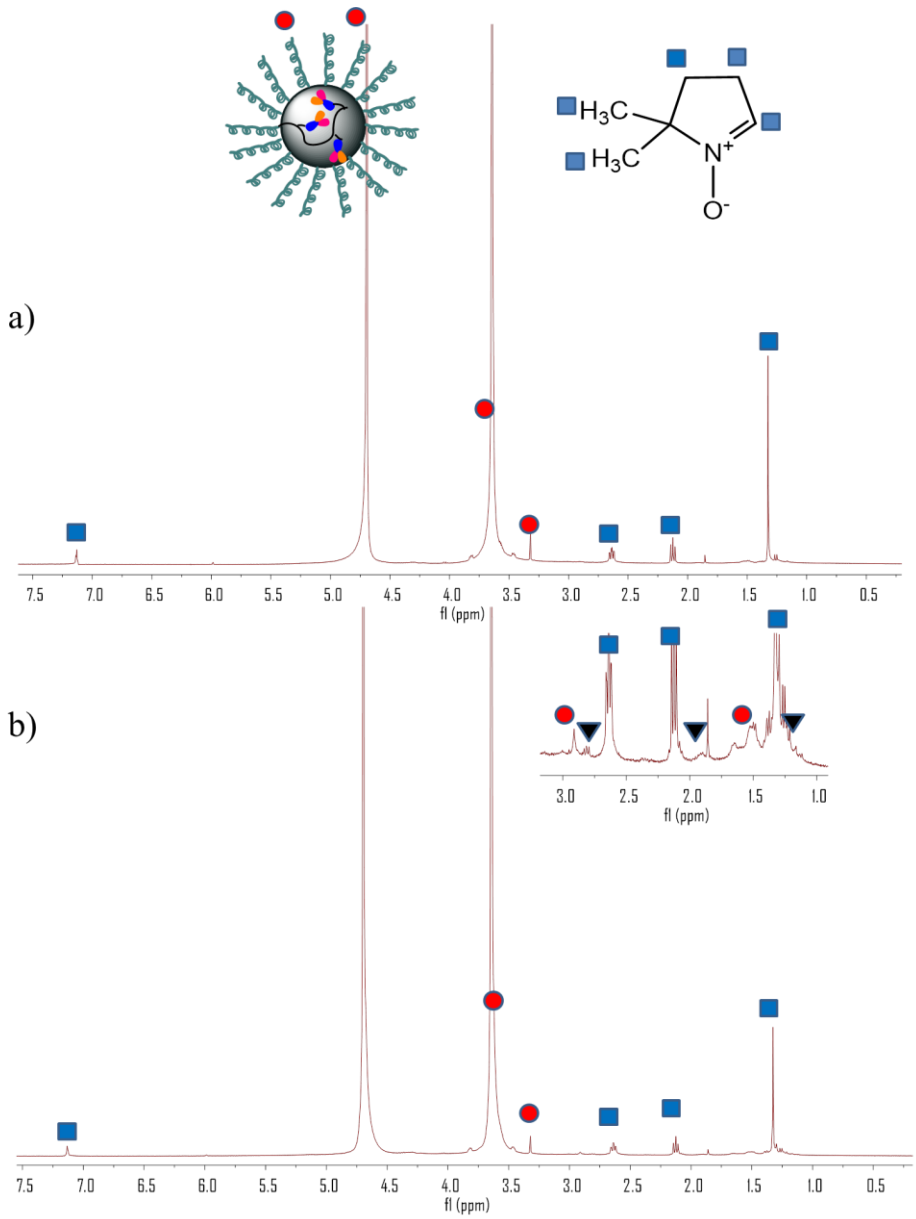


Fig. S10 ^{1}H NMR spectra of a D_2O solution of P-Z-DMC-CIS(N_3) (4 mM Pt) and DMPO (8 mM) a) in the absence and b) presence of UVA irradiation. Assignments: colored circles (●), DMC peaks; colored squares (■), DMPO peaks; filled triangles (▼), DMPO photoproducts.

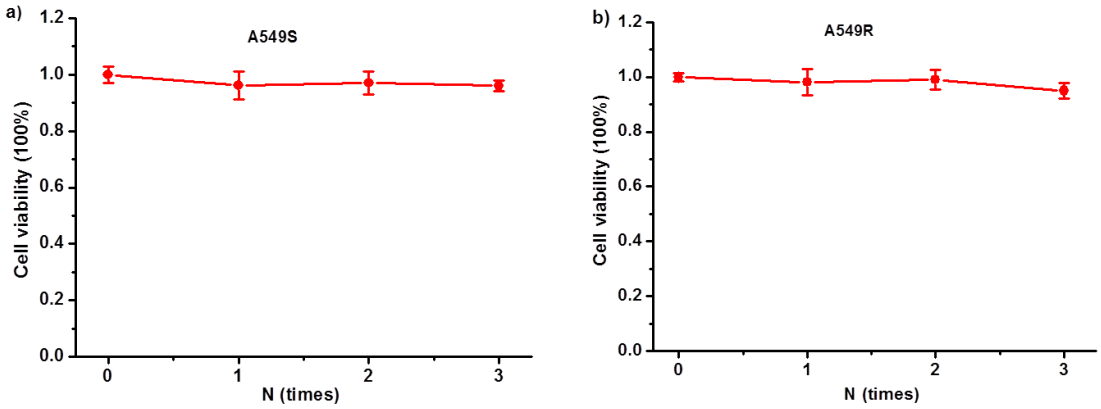


Fig. S11 Cell viability of A549S (a) and A549R (b) cells without any drug in the presence of intermittent UVA irradiation once a day (365 nm, 5 J/cm^2 each time).

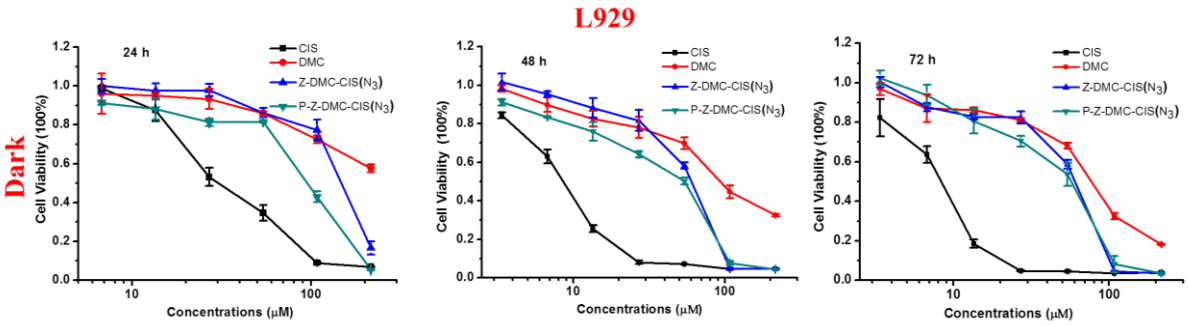


Fig. S12 *In vitro* cytotoxicity of cisplatin, DMC, Z-DMC-CIS(N_3) and P-Z-DMC-CIS(N_3) against L929 cells for 24 h, 48 h and 72 h in the dark.

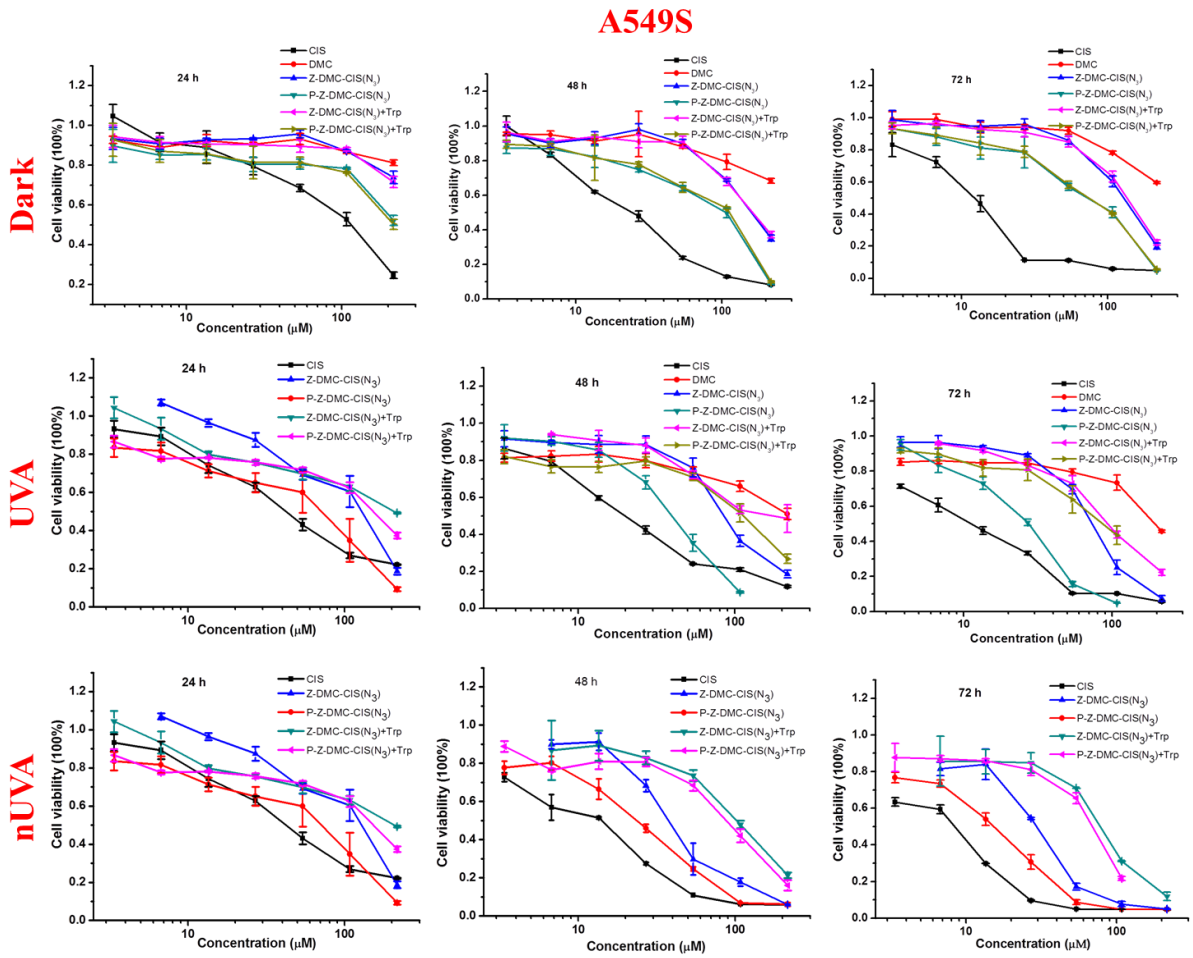


Fig. S13 *In vitro* cytotoxicity of cisplatin, DMC, Z-DMC-CIS(N_3) (with or without 500 μM Trp) and P-Z-DMC-CIS(N_3) (with or without 500 μM Trp) against A549S cells for 24 h, 48 h and 72 h in the absence and presence of intermittent UVA irradiation (365 nm, 5 J/cm^2 each time).

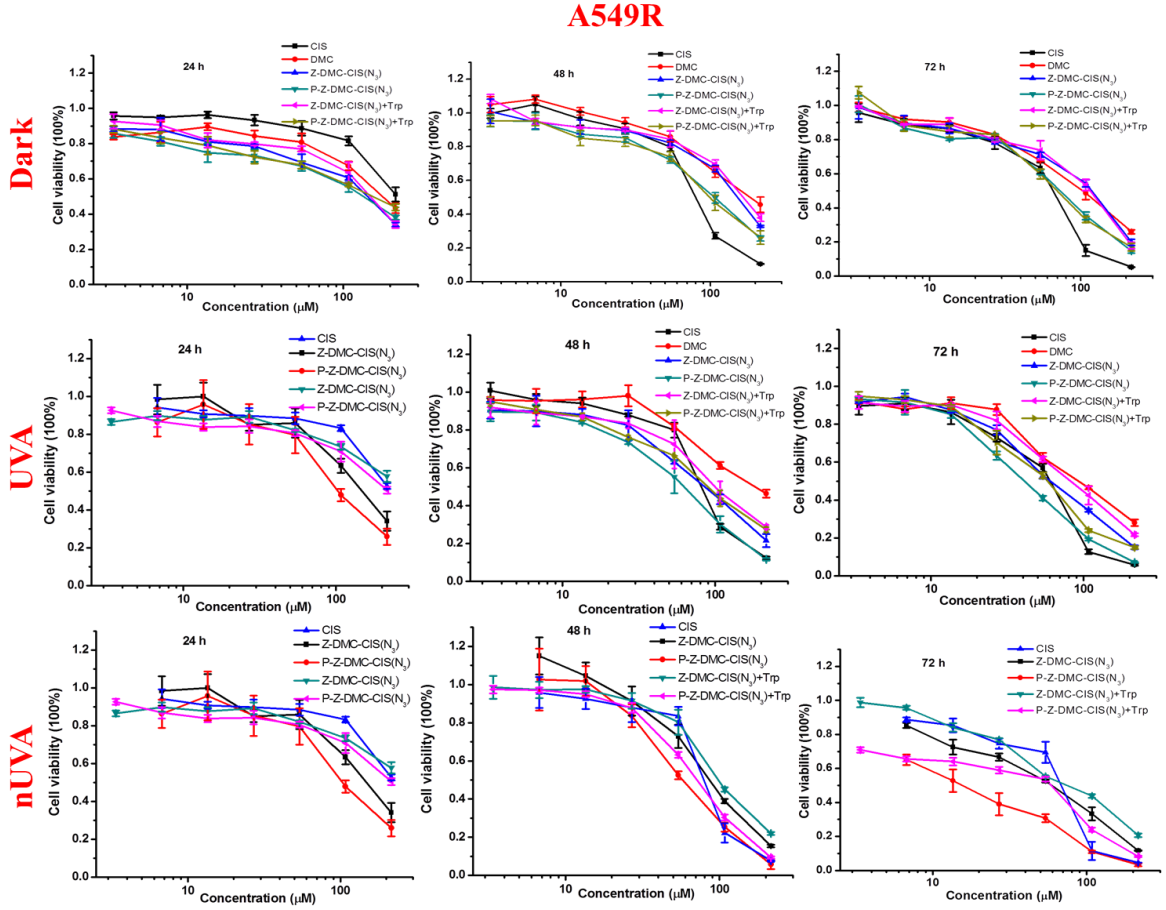


Fig. S14 *In vitro* cytotoxicity of cisplatin, DMC, Z-DMC-CIS(N_3) (with or without 500 μM Trp) and P-Z-DMC-CIS(N_3) (with or without 500 μM Trp) against A549R cells for 24 h, 48 h and 72 h in the absence and presence of intermittent UVA irradiation (365 nm, 5 J/cm^2 each time).

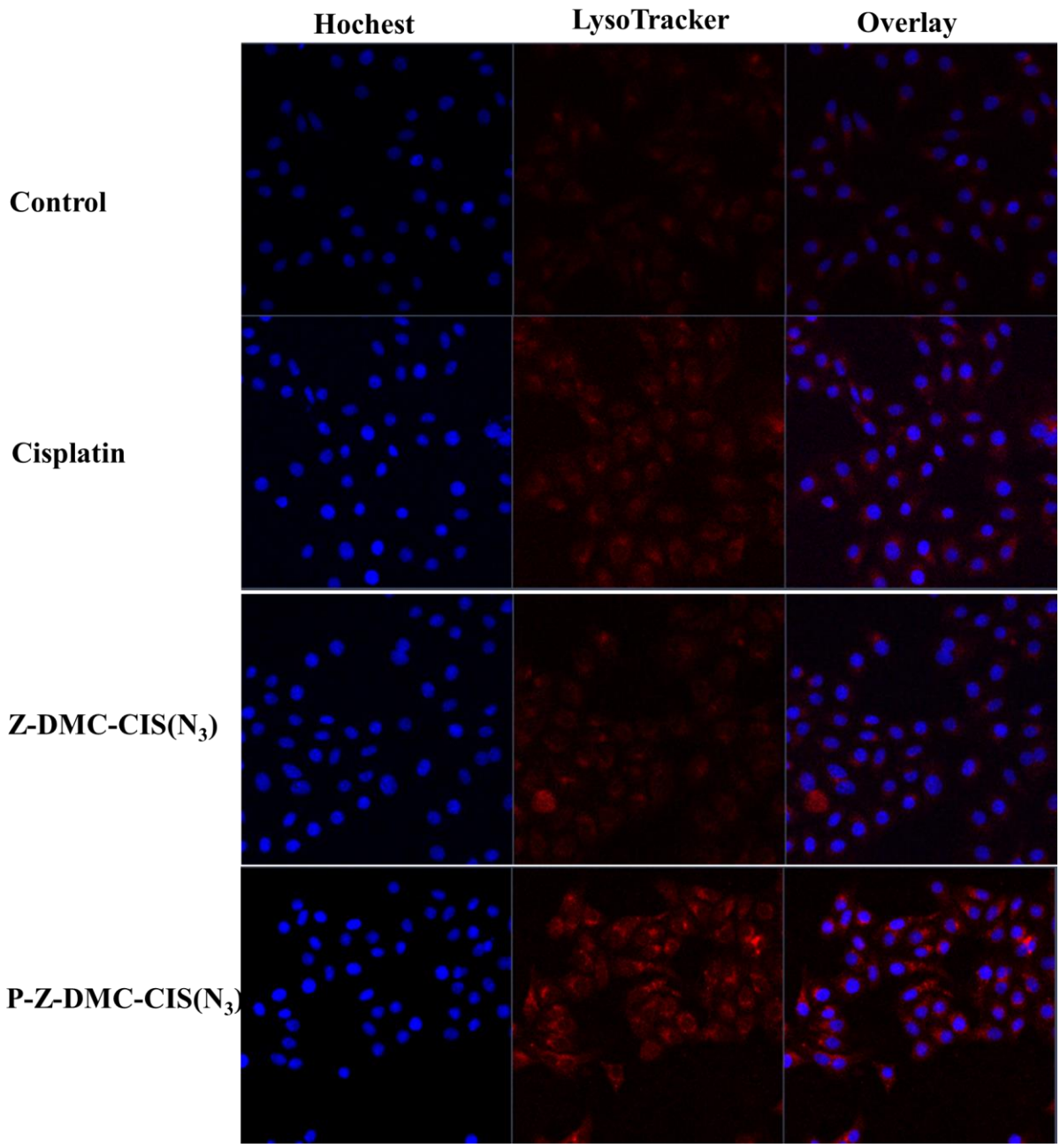


Fig. S15 CLSM of A549S cells incubated with cisplatin, Z-DMC-CIS(N_3) and P-Z-DMC-CIS(N_3).

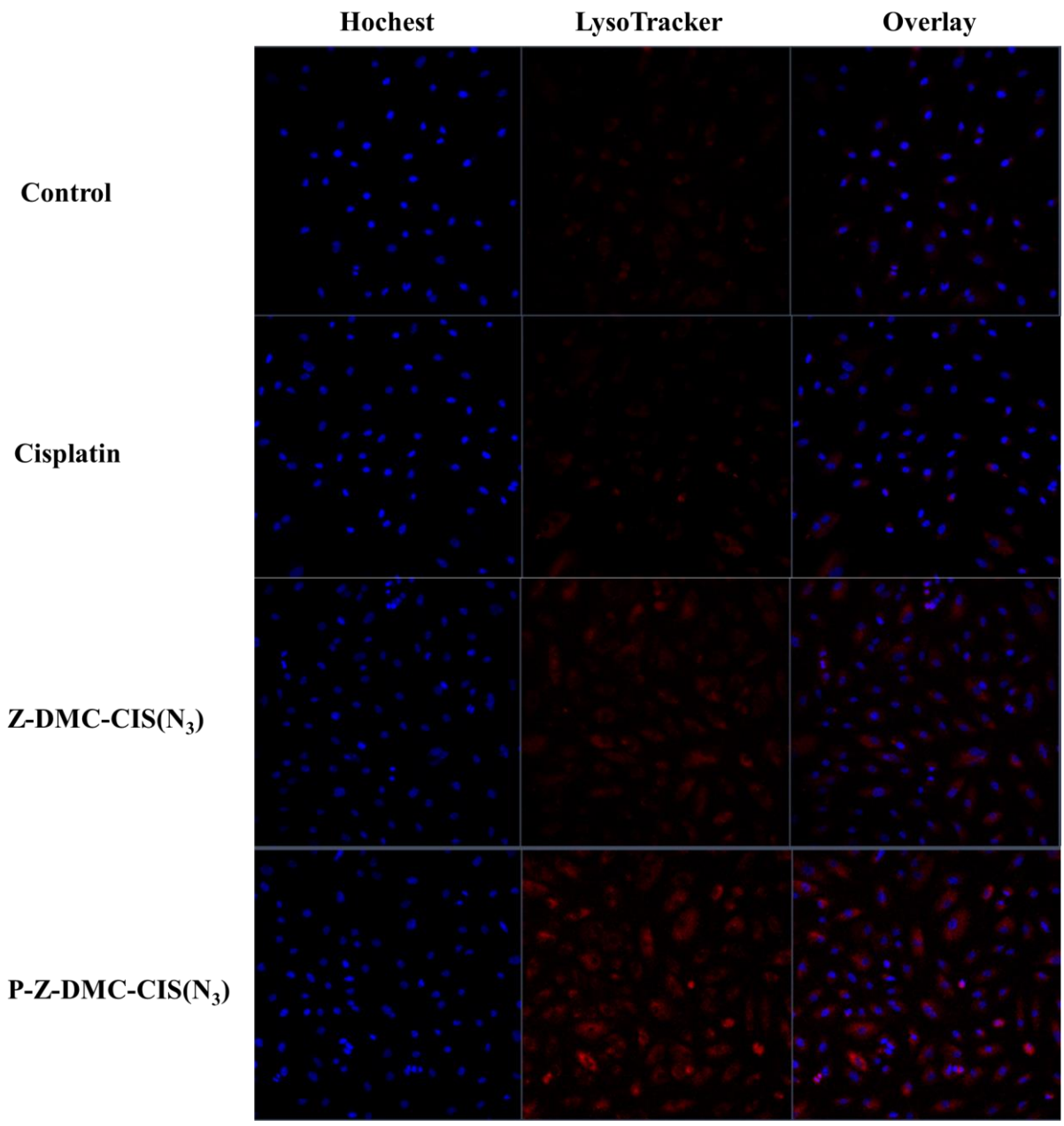


Fig. S16 CLSM of A549R cells incubated with cisplatin, Z-DMC-CIS(N_3) and P-Z-DMC-CIS(N_3).