Electronic supplementary information

Pegylated-polycaprolactone nano-sized drug delivery platforms loaded with biocompatible silver(I) complexes for anticancer therapeutics

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S1.1 Synthesis and characterization of compounds 1-3

To a suspension of $AgNO_3$ (0.3 mmol) in CH_2Cl_2, the amount of the corresponding phosphine, i.e. PPh_3 (0.6 mmol) for **1**, xantphos (0.3 mmol) for **2** and **3**, was added and the resulting mixture was stirred at room temperature for 2 h. To the resulting mixture an equimolar amount of the thioamide dmp2SH for **1** and **2** or the thioamidate $dmP2S^-$ (obtained by deprotonation of dmP2SH with a 0.1 M KOH solution in MeOH) for **3** was added. After filtration, crystallization of the resulting reaction mixtures by slow diffusion of hexane led to the formation of crystals of compounds **1-3** within ca. 1 week. The compounds were isolated as white/off-white microcrystalline solids.

$[Ag(dmp2SH)(PPh_3)_2]NO_3$ (1). Yield: 0.206 g (82%). FTIR (KBr), $\tilde{\nu}/cm^{-1}$: 3056(w), 2560(w), 1733(m), 1626(vs), 1566(vs), 1479(vs), 1435(vs), 1406(s), 1385(s), 1335(m), 1288(s), 1237(s), 1193(w), 1161(w), 1095(s), 1069(w), 1026(s), 998(m), 972(m), 919(m), 890(s), 824(s), 743(vs), 696(vs), 620(w), 598(w), 564(w), 539(m), 516(s), 501(s). ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm): 8.25 (s, 1H, NH), 7.65-7.58 (m, 6H, p-H, PPh_3), 7.53-7.40 (m, 12H, m-H, PPh_3), 7.35-7.25 ppm (m, 12H, o-H, PPh_3), 6.62 (s, 1H), 2.06 (s, 6H, CH_3).

$[Ag(dmp2SH)(xantphos)]NO_3$ (2). Yield: 0.186 g (70%). FTIR (KBr), $\tilde{\nu}/cm^{-1}$: 3046(w), 2969(w), 1626(vs), 1574(vs), 1480(s), 1444(vs), 1404(s), 1385(vs), 1305(s), 1240(s), 1185(m), 1081(m), 882(s), 837(m), 810(m), 745(vs), 700(vs), 511(s). ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm): 7.90 (s, 1H, NH), 7.76-7.74 (dd, 2H, xantphos) 7.50-7.38 (m,8H, o-H, phenyl), 7.37-7.27 (4H, p-H, phenyl), 7.23-7.19 (8H, m-H, phenyl), 6.94-6.91 ppm (t, 2H, phenyl), 6.76 (s, 1H), 6.62-6.59 (m, 2H, xantphos), 2.05 (s, 6H, CH_3), 1,57 (s, 6H, CH_3).

[Ag(dmp2S)(xantphos)] (3). Yield: 0.123 g (47%). FTIR (KBr), $\tilde{\nu}/cm^{-1}$: 3445(b), 3049(w), 1618(w), 1567(s), 1435(s), 1404(vs), 1336(w), 1242(vs), 1097(m), 1072(w), 1027(w), 999(w), 880(m), 790(m), 746(s), 696(s), 510(s). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.53-7.51 (d, 2H, xantphos), 7.34-7.31 (m, 8H, o-H, phenyl), 7.24-7.22 (d, 4H, p-H, phenyl), 7.16-7.13(t, 8H, m-H, phenyl),7.08-7.05 (t, 2H, xantphos), 6.57-6.55 (m, 2H, xantphos), 6.19 (s,1H) ,1.90 (s, 6H, CH_3), 1.55(s, 6H, CH_3).

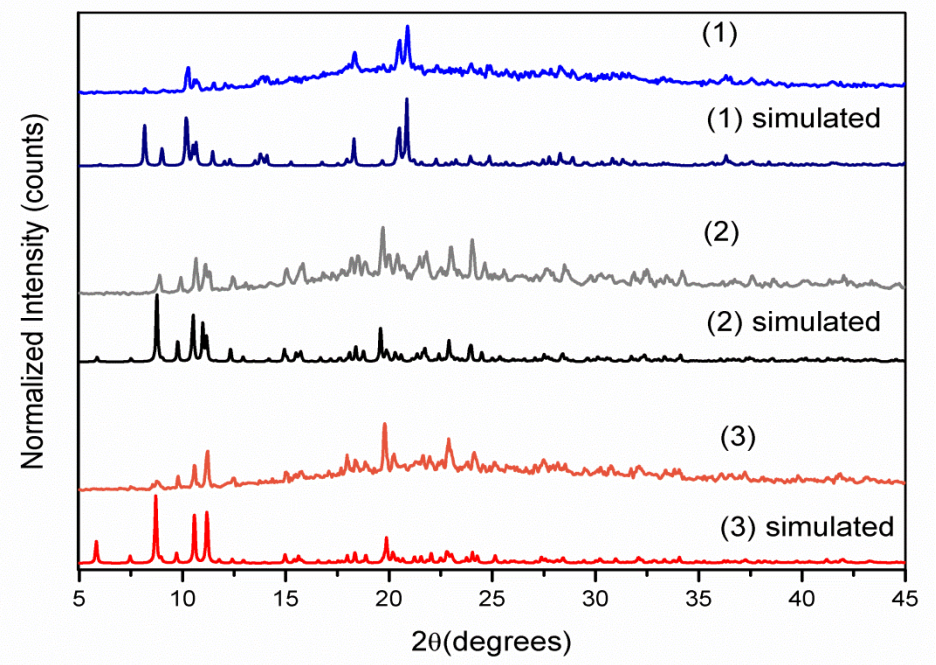


Figure S1. Powder X-ray diffraction patterns of compounds [Ag(dmp2SH)(PPh_3)$_2$](NO_3) (**1**) (blue), [Ag(dmp2SH)(xantphos)](NO_3) (**2**) (grey), and [Ag(dmp2S)(xantphos)] (**3**) (red).

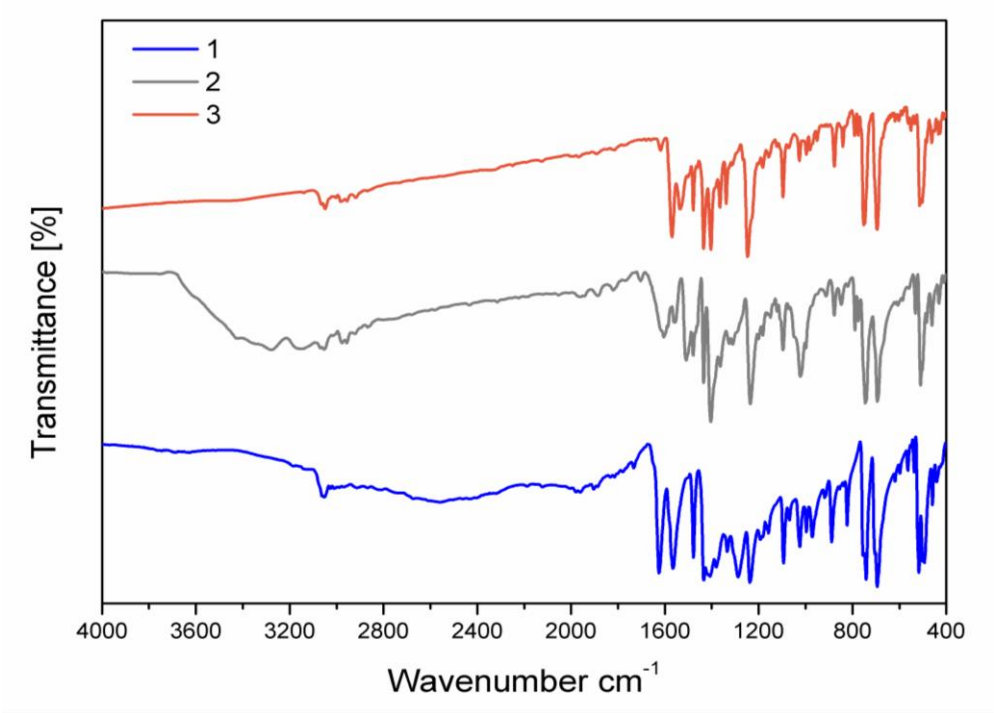
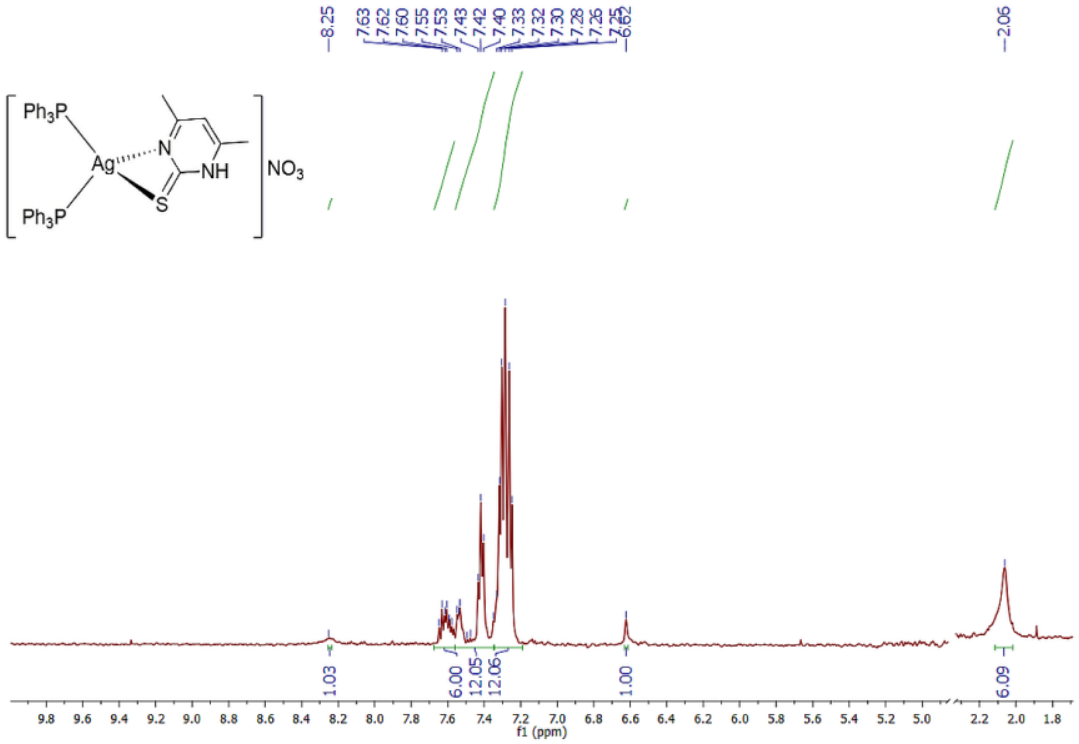
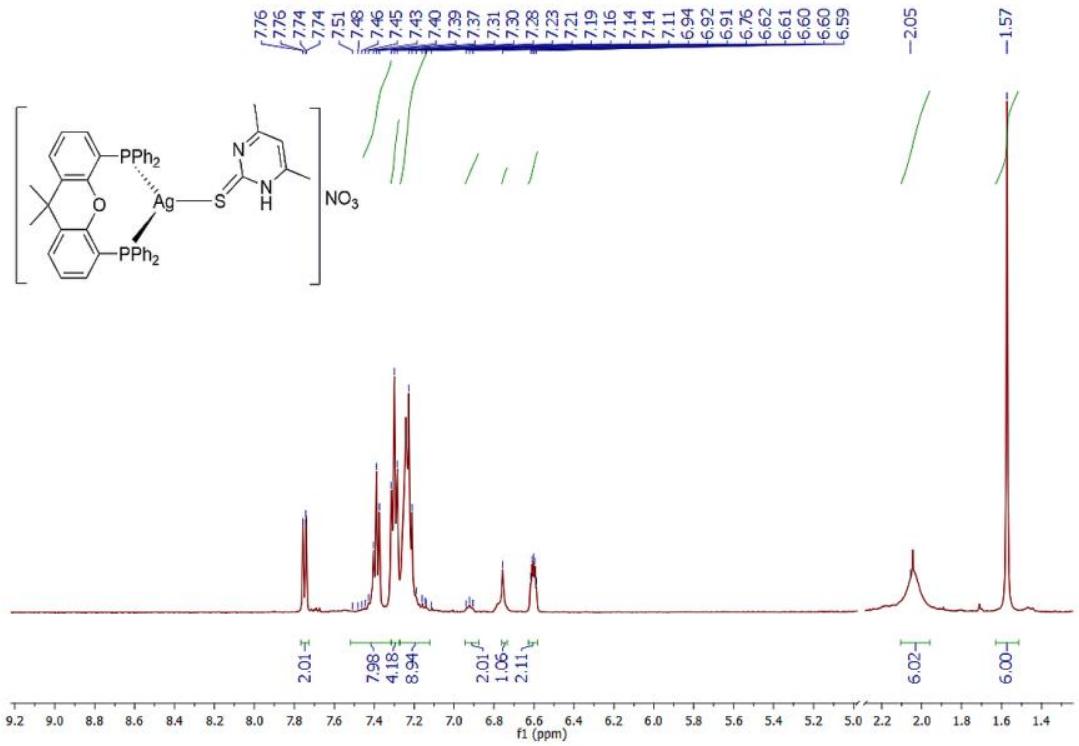


Figure S2. FTIR spectra of compounds [Ag(dmp2SH)(PPh_3)$_2$](NO_3) (**1**) (blue), [Ag(dmp2SH)(xantphos)](NO_3) (**2**) (grey), and [Ag(dmp2S)(xantphos)] (**3**) (red).


Ph3P
Ag
N
NH
S
Ph3P
NO3
8.25
7.63
7.62
7.60
7.55
7.53
7.43
7.42
7.40
7.33
7.32
7.30
7.28
7.25
6.62
2.06
1.03
6.00
12.05
12.06
1.00
6.09
f1 (ppm)

(a)


PPh2
Ag
S
N
N
H
O
PPh2
NO3
7.76
7.76
7.74
7.74
7.51
7.48
7.46
7.45
7.43
7.40
7.39
7.37
7.31
7.30
7.28
7.23
7.21
7.19
7.16
7.14
7.14
7.11
6.94
6.92
6.91
6.76
6.62
6.61
6.60
6.60
6.59
2.05
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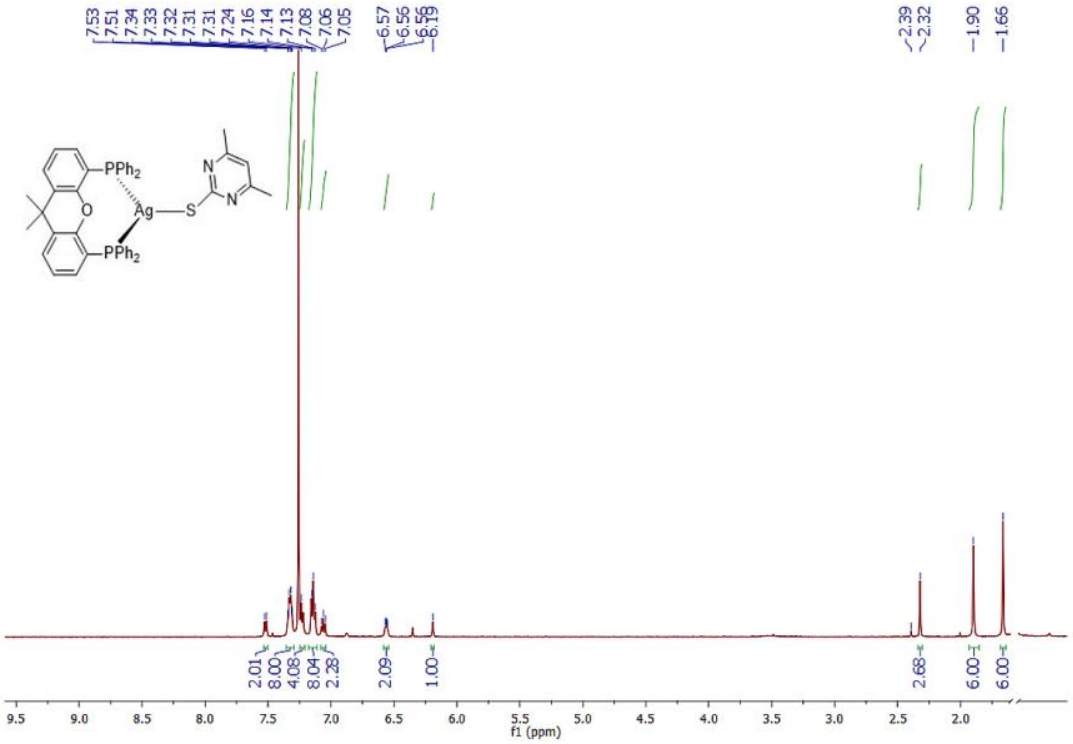
2.11
6.02
6.00
f1 (ppm)

(b)



(c)

Figure S3. ^{1}H NMR spectra of compounds: (a) [Ag(dmp2SH)(PPh_3)$_2$](NO_3) (**1**) in DMSO-d_6, (b) [Ag(dmp2SH)(xantphos)](NO_3) in DMSO-d_6 (**2**), (c) [Ag(dmp2S)(xantphos)] (**3**) in $CDCl_3$.

S1.2 Synthesis and characterization of mPEG-PCL copolymer

Accurately weighted amounts of mPEG and ε-CL in 1:1 molar ratio were placed into a three-necked round-bottom flask that was equipped with a mechanical stirrer, condenser, and N_2 inlet. The catalyst $Sn(Oct)_2$ (0.05 g/mL solution in toluene) was added at the concentration of 400 ppm based on the reactants. The reaction mixture was degassed and, after purging with N_2 several times, was inserted into a heated salt bath at 180 °C. The reaction was carried out under constant mechanical stirring (400 rpm) and N_2 atmosphere over a period of 3 h. To further increase the molecular weight of the produced copolymer and remove any unreacted monomers, high vacuum (~5.0 Pa) was then applied slowly to the system over a period of about 15 min. The reaction flask was then quenched to room temperature and the products were purified by dissolving in $CHCl_3$. The copolymer was precipitated in cold methanol, filtered twice and dried in a vacuum oven for 24 h, before storage in hermetically sealed vails.

FTIR (KBr), $\tilde{\nu}/cm^{-1}$: 3443(w), 2942(s), 2867(s), 1724(vs), 1470(m), 1419(w), 1396(w), 1297(s), 1247(vs), 1195(vs), 1148(w), 1110(s), 1042(m), 982(m), 839(w),729 (s). ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 4.22 (s, 2H), 4.07-4.05 (m, 2H), 3.65-3.50 (m, 2H), 2.36-2.18 (m, 2H), 1.66-1.57 (m, 2H), 1.42-1.39(m, 2H), 1.26-1.12 (m, 2H)

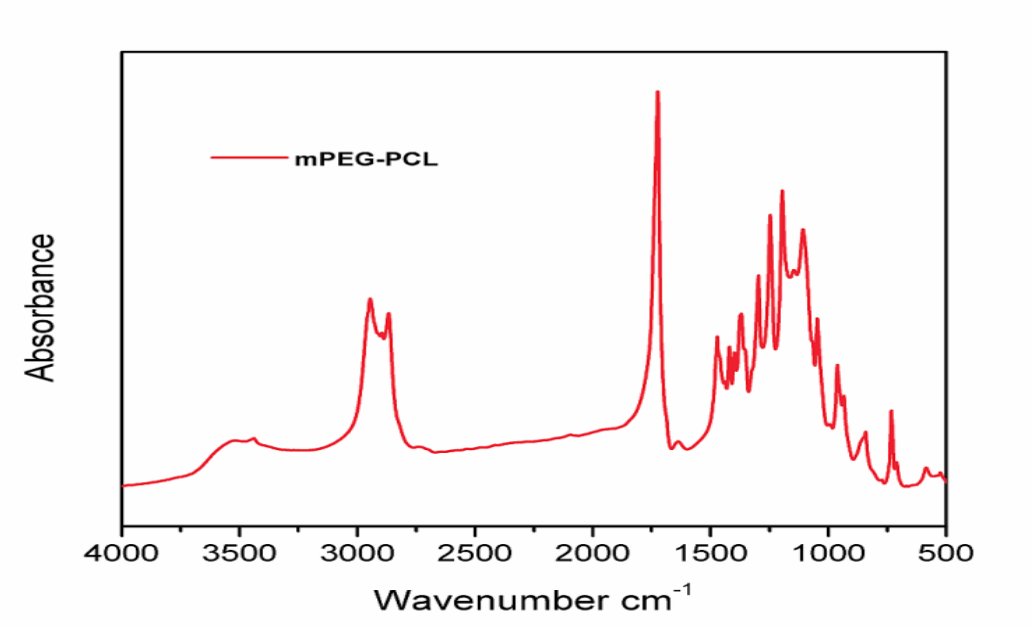


Figure S4. FTIR spectrum of **mPEG-PCL** copolymer.

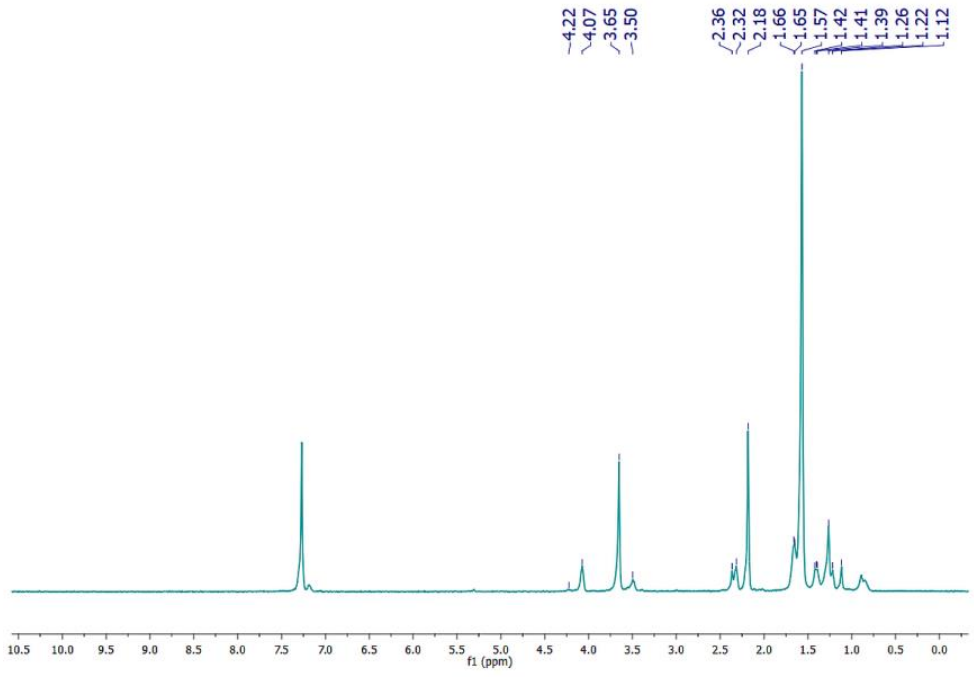


Figure S5. ^{1}H NMR spectrum of **mPEG-PCL** copolymer in $CDCl_3$.

S1.3 Synthesis and characterization of mPEG-PCL nanoparticles loaded with Ag(I) compounds

0.250 g of **mPEG-PCL** copolymer were dissolved in 5 mL of CH_2Cl_2 and stirred with a magnetic stirrer. 50 mg of each one of the compounds **1-3** were then added to the solution and sonicated for 1 min until complete dispersion. The aqueous phase (40 mL of 1% w/v PVA aqueous solution) was then added to the above oil phase and homogenized. The formed O/W emulsion was stirred (800 rpm) at room temperature under a fume hood until the evaporation of the organic solvent was completed. **[X]@mPEG-PCL** (X = 1, 2, 3) nanoparticles were then separated from the rest of the solution by centrifugation at 12,000 rpm for 15 min. Possible solvent or emulsifier residue was removed by three consecutive washes with ultrapure water. The resulting suspension was filtered (25 mm syringe filter, glass fiber, 1.00 μm), lyophilized (Scanvac, Coolsafe 110-4 Pro, Labogen, Scandinavia) and stored at ambient temperature until further study.

[1]@mPEG-PCL. Yield: 48.14%. FTIR (KBr), $\tilde{\nu}/cm^{-1}$: 3385(b), 2946(w), 2909(w), 2868(w), 1726(vs), 1656(w), 1572(s), 1534 (s), 1478(m), 1438(s), 1397(m), 1367(s), 1338(m), 1294(m), 1270(m), 1246(vs), 1190(s), 1097(m), 950(w), 840(m), 753(m), 696(m), 514(w). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 13.28 (s), 7.69-765 (m), 7.55-7.46 (m), 7.34-732(m),7.24-7.14 (m), 7.05 (s,1H) ,4.23-4.06 (m), 3.70-3.38(m), 2.32-2.29(m), 2.17(s), 1.67-1.21(m).

[2]@mPEG-PCL. Yield: 52.81%. FTIR (KBr), $\tilde{\nu}/cm^{-1}$: 3405(b), 2944(w), 2908(w), 2867(w), 1726(vs), 1584(s), 1471(w), 1435(w), 1421(m), 1403(m), 1368(m), 1295(m), 1271(m), 1243(s), 1198(s), 1108(m), 960(m), 842(w), 733(w). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.94 (s), 7.66-7.65 (d), 7.47-7.35(m), 7.19-7.05(m), 6.77 (s), 4.27 (s,), 4.08-4.05(m), 3.66(s), 2.53(s), 2.40-2.29(m), 2.17(s), 1.67-1.20(m)

[3]@mPEG-PCL. Yield: 64.32%). FTIR (KBr), $\tilde{\nu}/cm^{-1}$: 3303(b), 2946(w), 2908(w), 2868(w), 1726(vs), 1584(s), 1473(w), 1368(m), 1296(m), 1271(m), 1244(s), 1191(s), 1108(m), 1047(w), 960(m), 843(w), 733(w). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.52-7.47 (m), 7.08-7.05 (m), 6.99-6.97 (m) ,6.25 (s), 3.90-3.88(s), 3.47(s), 3.30-3.28(m), 3.20(s), 2.26(s), 2.14-1.98(m), 1.49-1.21(m).

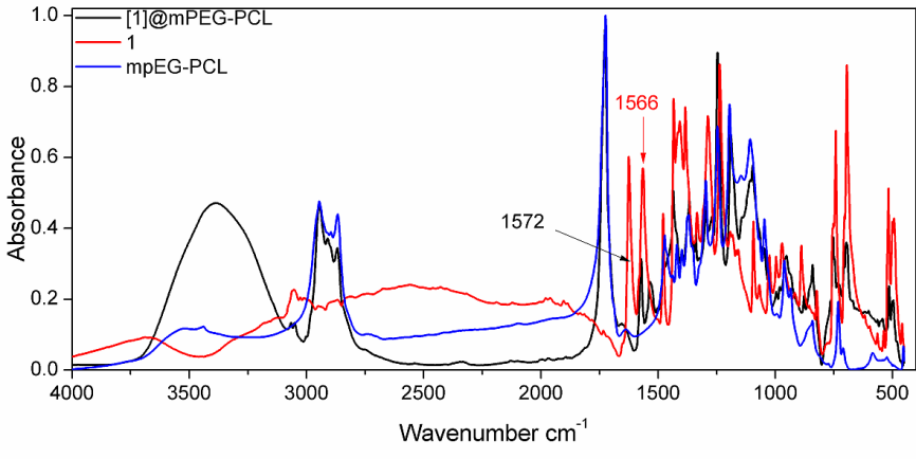
Yield, drug-loading and encapsulation efficiency. The determination of loading of compounds **1-3** ("drugs") into the **mPEG-PCL** copolymer nanoparticles (Drug Loading, DL) and their encapsulation efficiency (EE) was performed by dissolving 10 mg of the prepared **[X]@mPEG-PCL** (X = 1, 2, 3) nanoparticles in 10 mL of CH_2Cl_2. The resulting suspension was stirred for 24 h and filtered using PTFE hydrophobic filters of 0.45 nm pore size. Their content in the respective Ag(I) compounds was determined using a Shimadzu HPLC prominence system (Kyoto, Japan), consisting of a degasser (DGU-20A5, Kyoto, Japan), a liquid chromatograph (LC-20 AD, Kyoto, Japan), an autosampler (SIL-20AC, Kyoto, Japan), a UV/Vis detector (SPD-20A, Kyoto, Japan) and a column oven (CTO-20AC, Kyoto, Japan). A CNW Technologies Athena C18, 120 A, 5 μm, 250 mm × 4.6 mm analytical column was used, and the analysis was performed at 25°C. The mobile phase consisted of CH_3CN/H_2O (acidified with phosphoric acid at final pH = 3.0) 80/20 v/v, at a flow rate of 1.0 mL/min. UV detection was performed at 280 nm for **1** and **3**, and 320 nm for **2**, respectively. The injection volume was 20 μL. The calibration curve was created by diluting a stock CH_3CN solution of 500 ppm to concentrations of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, and 25.0 ppm.

The yield of the prepared nanoparticles, as well as drug loading (DL) and encapsulation efficiency (EE) were calculated based on the following equations:

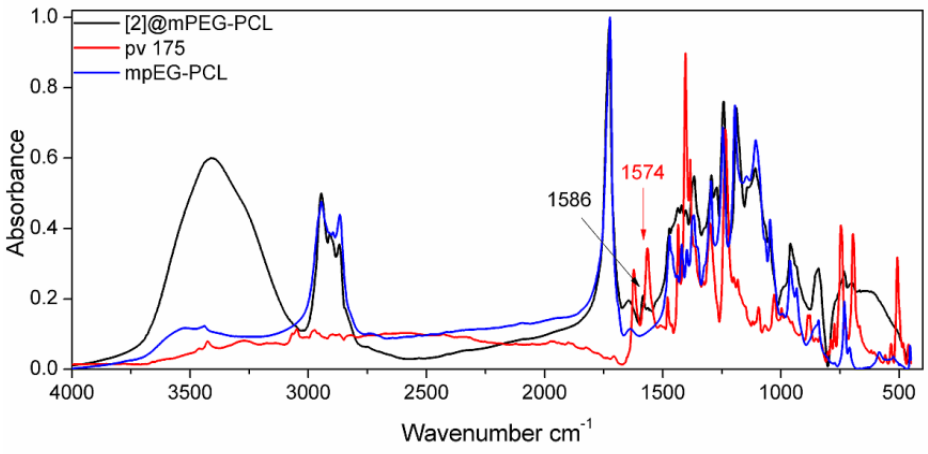
Yield (%) = [Weight of nanoparticles/Initial weight of polymer and drug]×100 (eq. 1)

DL (%) = [Weight of drug in nanoparticles/Weight of nanoparticles]×100 (eq. 2)

EE (%) = [Weight of drug in nanoparticles/Initial weight of drug]×100 (eq. 3)



(a)



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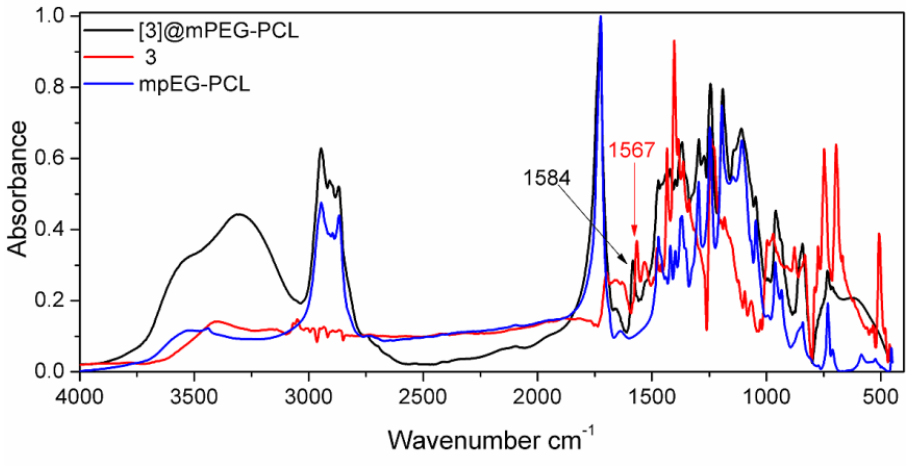

(c)

Figure S6. Overlay of FTIR spectra of **[X]@mPEG-PCL** (X = 1, 2, 3) nanoparticles and the corresponding compounds **1**-**3** in free form.

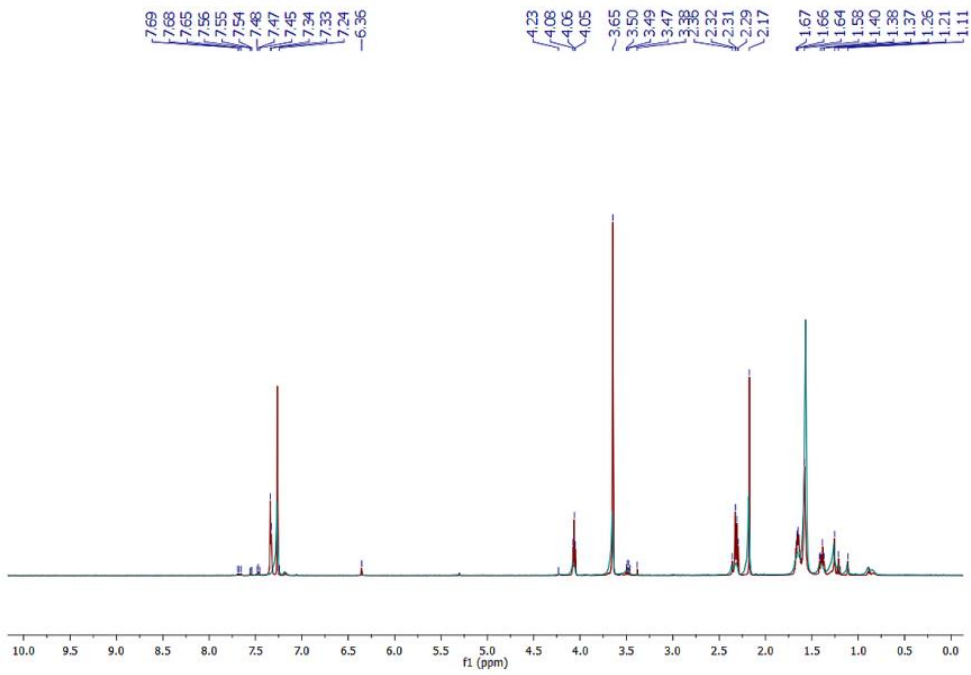

Figure S7. Overlay of ^{1}H NMR spectra of **[1]@mPEG-PCL** nanoparticles (red) and **mPEG-PCL** copolymer (blue) in $CDCl_3$.

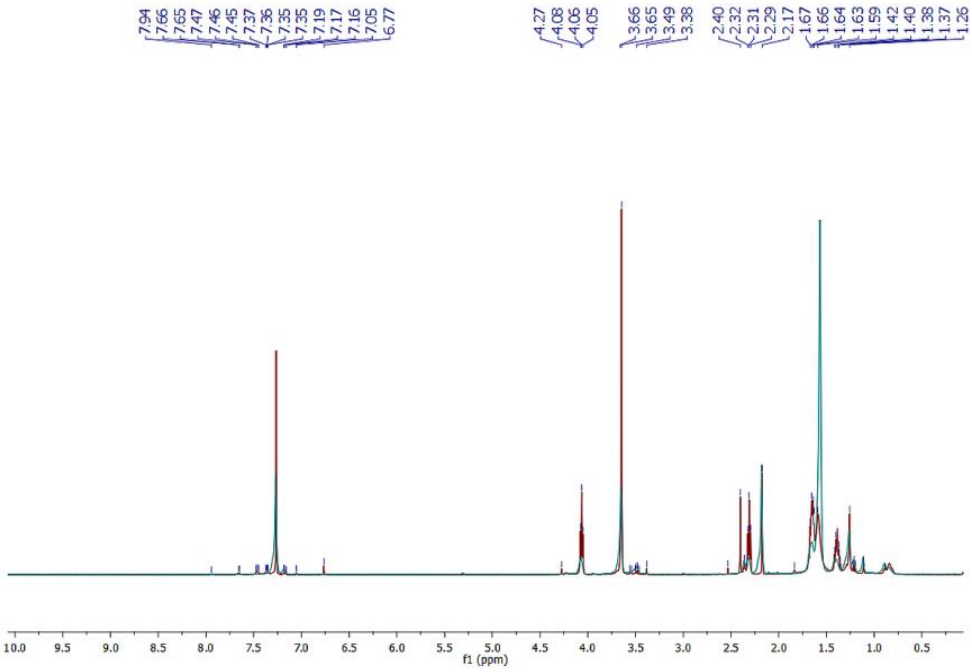

Figure S8. Overlay of ^{1}H NMR spectra of **[2]@mPEG-PCL** nanoparticles (red) and **mPEG-PCL** copolymer (blue) in $CDCl_3$.

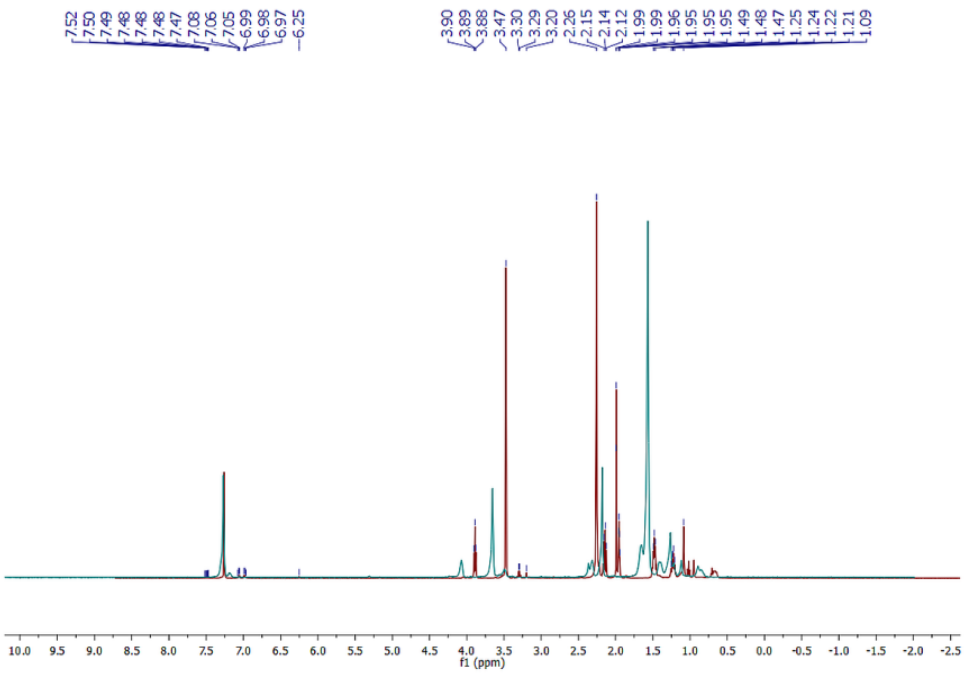

Figure S9. Overlay of ^{1}H NMR spectra of **[3]@mPEG-PCL** nanoparticles (red) and **mPEG-PCL** copolymer (blue) in $CDCl_3$.

S1.4. Release studies

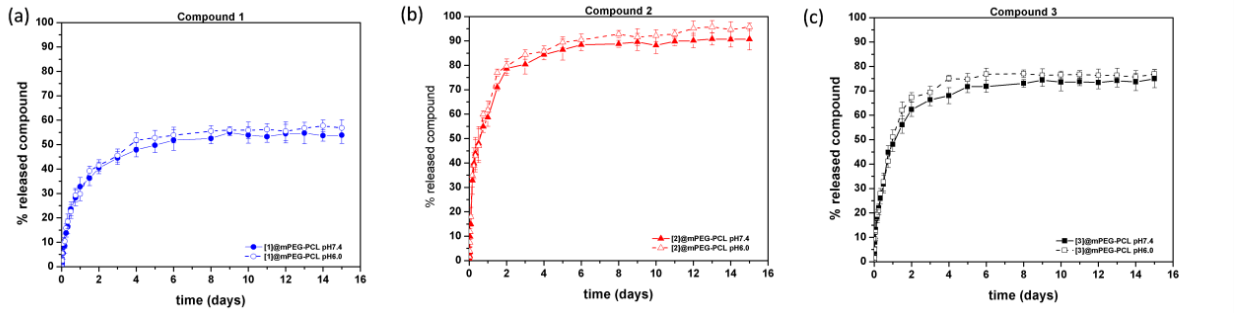

Figure S10. Comparative *in vitro* release profiles of **[X]@mPEG-PCL** (X = 1, 2, 3) nanoparticles at pH 7.4 (straight line) and pH 6.0 (dashed line) and 37 ºC.

S1.5. Cytotoxicity studies

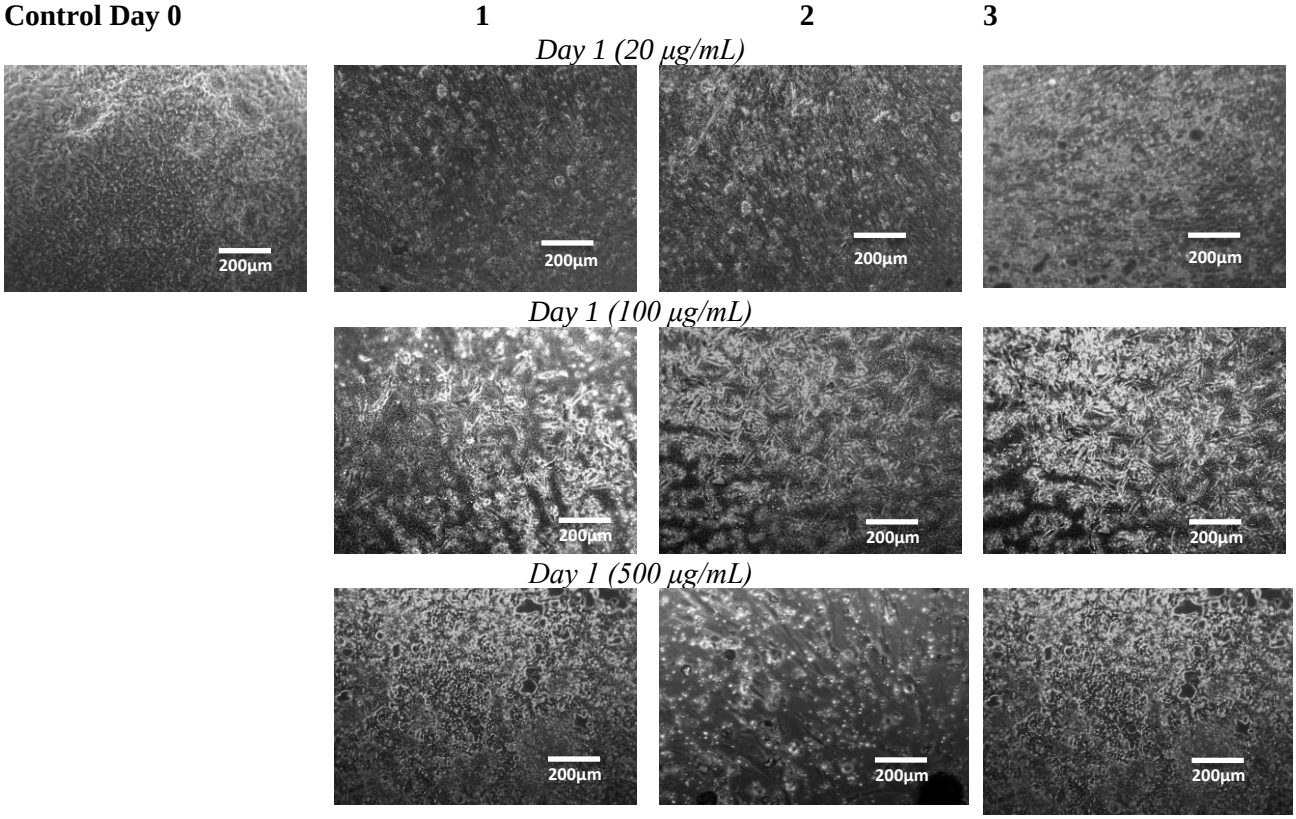

Figure S11. Optical microscopy images (×200µm) of morphological changes of HeLa cells on day 0 and day 1 after exposure of compounds **1**-**3** at concentrations of 20, 100 and 500 µg/mL.

S1.6 Transmission electron microscopy imaging

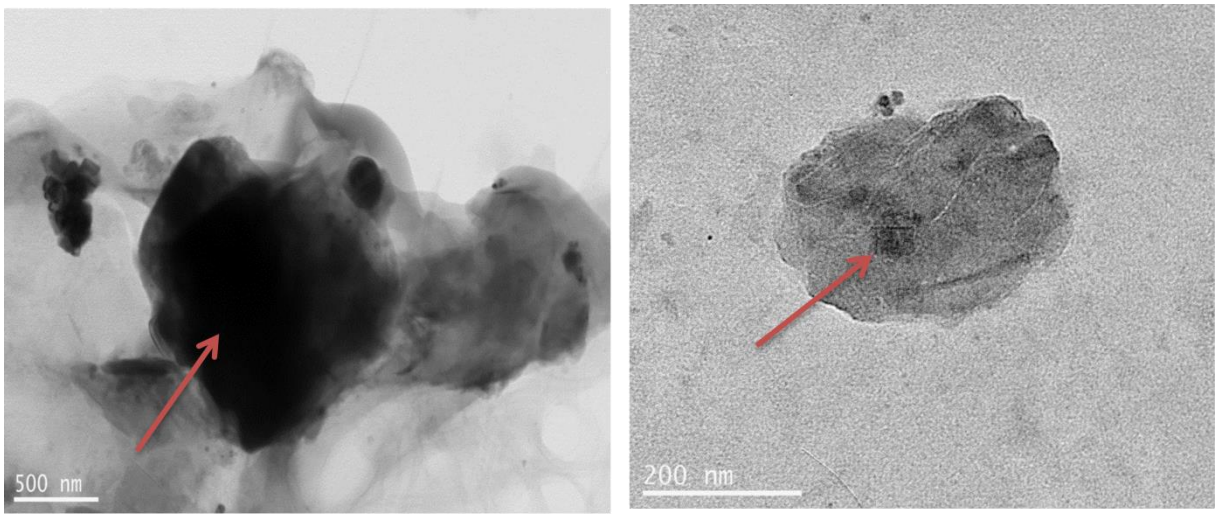

Figure S12. TEM images of **[X]@mPEG-PCL** (X = 1, 3) nanoparticles.