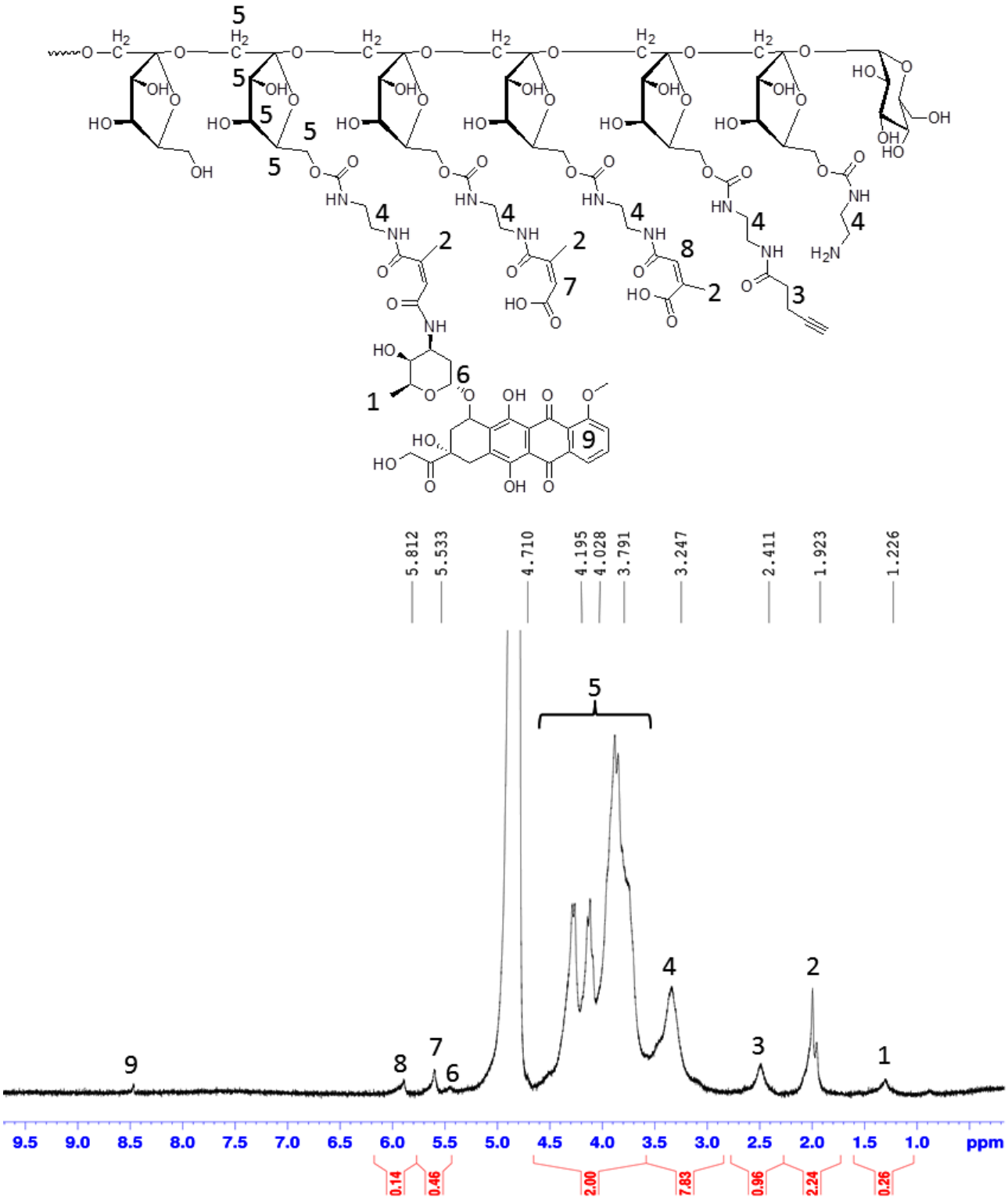

Figure S1. ^{1}H NMR of the INU-EDA-P,C-Doxo conjugate

EXPERIMENTAL PART

Materials

Human colon cancer HCT-116 cells were cultivated at 37°C and 5% CO_2 in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine. Human normal bronchial epithelial 16HBE cells and human liver cancer cell lines SK-Hep-1 were cultured in DMEM medium containing 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine and 0.25 µg/ml amphotericin B (only for 16HBE) at 37°C and 5% CO_2.

Methods

Doxorubicin release in plasma

The in vitro doxorubicin release from INU-EDA-P,C-Doxo was estimated incubating samples in human plasma at 37±0.1 °C. In particular, 2 mg of INU-EDA-P,C-Doxo was dispersed in 1 ml of plasma and kept at 37 °C under stirring. At suitable time intervals, ranging from 1 to 24 h, 2 mL of ethanol were added in order to precipitate plasma proteins. After immediate mixing and centrifugation for 5 min at 10 000 rpm at 4 °C, supernatants were filtered through a 0,2µm RC syringe filter and analysed by HPLC using C6-phelyl column and ACN/0.4 M ammonium phosphate buffer at pH 4±0.1 32:68 as mobile phase (flow 0.8 mL min^{-1}).. The amount of equivalent doxorubicin hydrochloride was calculated using a standard curve obtained with doxorubicin hydrochloride standard solutions (ranging from 0.01 to 0.1 mg/ml). Every experiments were carried out in triplicate.

Cytotoxicity of INU-EDA-P,C-Doxo

16HBE, HCT-116 and SK-Hep-1 cells were seeded in 96-well plates at a density of 6500 cells/well and cultivated for 24h at 37°C and 5% CO_2. Then, cell culture media was replaced by 200 µL of fresh media with the desired concentration (final doxorubicin concentration: 50; 25; 5; 0,5 µM) of

INU- EDA-P,C-Doxo. Untreated cells were used as negative control (100% viability). Cell viability was evaluated using Cell Counting Kit-8 (CCK-8) (Sigma-Aldrich) after 24h and 48h of treatment. The water-soluble tetrazolium salt (WST-8) was added to each well of the plates (1:10 dilution in complete medium); that were incubated for 2 h at 37 °C. The absorbance was recorded at 460 nm using a microplate reader DU-730 Life Science spectrophotometer (Beckman Coulter).

RESULTS AND DISSCUSSION

Doxorubicin release and the stability of INU- EDA-P,C-Doxo in plasma was assessed to simulate the intravenous administration of the conjugate in vivo. Figure S2 shows the cumulative amount of doxorubicin hydrochloride present in plasma at each time considered within 48 h from the incubation. It can be noticed that about 8% of the payload is released in 10 h, reaching a plateau after about 12 h. Perhaps, this behaviour can be due to the release of the drug available on the surface of the assembled conjugate, which is limited, strongly hydrated and thus hydrolytically cleavable. This result clearly points out that the system is quite stable in plasma and so can perform well as drug delivery system after intravenous administration, improving drug efficacy, selectivity and its bioavailability.

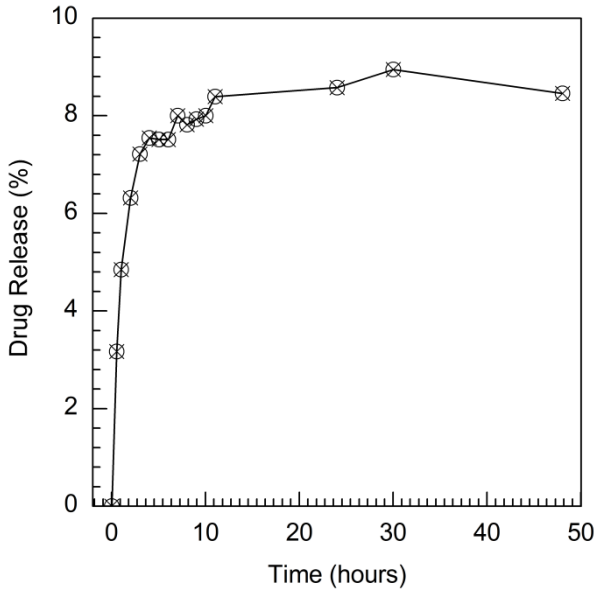

Figure S2. Doxorubicin release in human plasma.

In order to evaluate a possible discrimination effect of the nanoparticles on normal and tumor cells, cell viability assay was performed on 16HBE (normal) and HCT-116 and Sk-Hep-1 (cancer) cell lines treated with different amount of INU-EDA-P,C-Doxo.

After 24h of incubation (Figure 1), it is possible to evaluate a different viability between the normal and the tumor cells: the doxorubicin at 25 and 50μM, linked to the nanoparticles, induce higher mortality effects on both cancer cell lines respect to the normal cells.

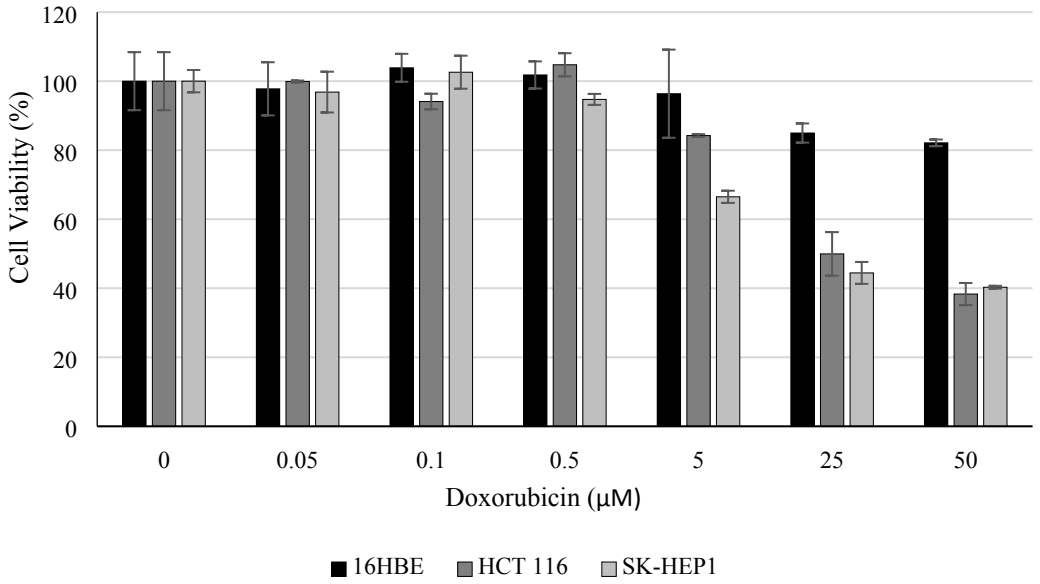

Figure S3. MTS assay on 16HBE (Black), HCT 116 (Grey) and SK-HEP-1 (light grey) for INU-EDA-P,C-Doxo at 24h.

After 48h of treatment (Figure 2), the difference is more evident: HCT-116 and Sk-Hep-1 show a viability 3 times and 12 times lower than 16HBE at 25 μM and this variance is higher at 50 μM of doxorubicin.

Therefore, it is evident that the nanoparticles preferentially kill tumor cells instead of the normal one, irrespective of the cancer cell lines used. The different effect, probably, depends of the

dissimilar pH registered in the cytoplasm and in the different compartments (lysosomes, endosomes) of the tumor cells respect to the normal one.

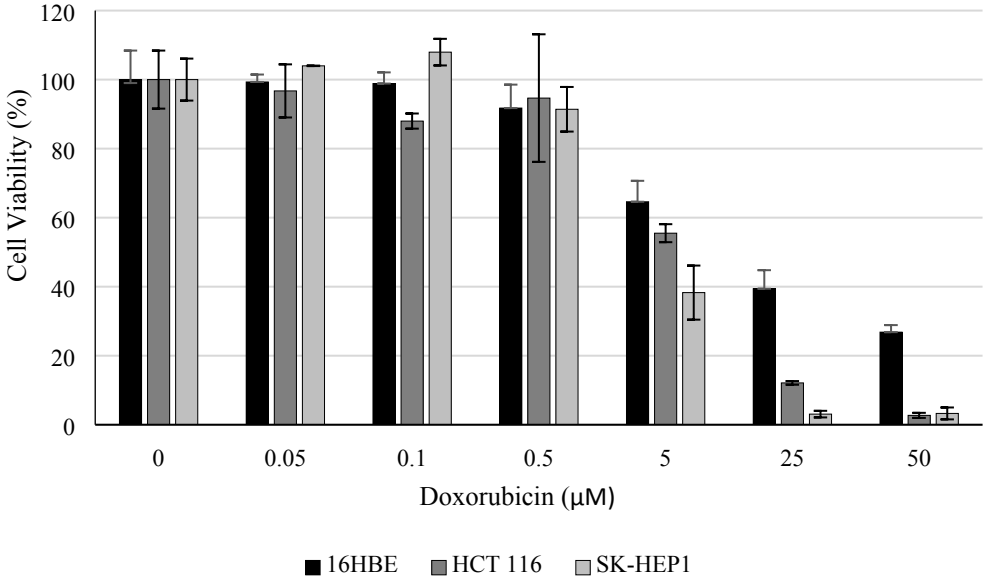

Figure S4. MTS assay on 16HBE (Black), HCT 116 (Grey) and SK-HEP-1 (light grey) for a INU-EDA-P,C-Doxo at 48h.