

Supporting Information for

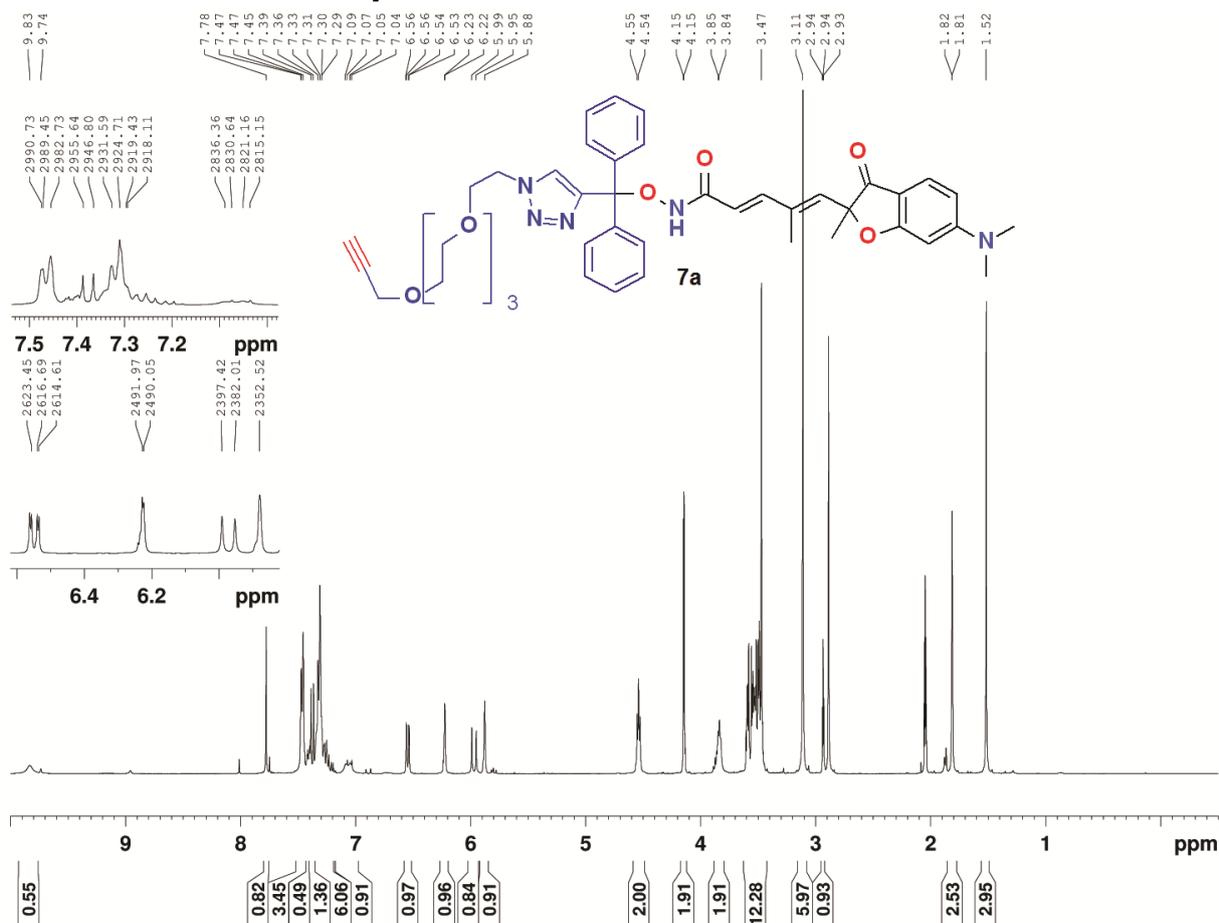
Histone deacetylase inhibitors delivery using nanoparticles with intrinsic passive tumor targeting properties for tumor therapy.

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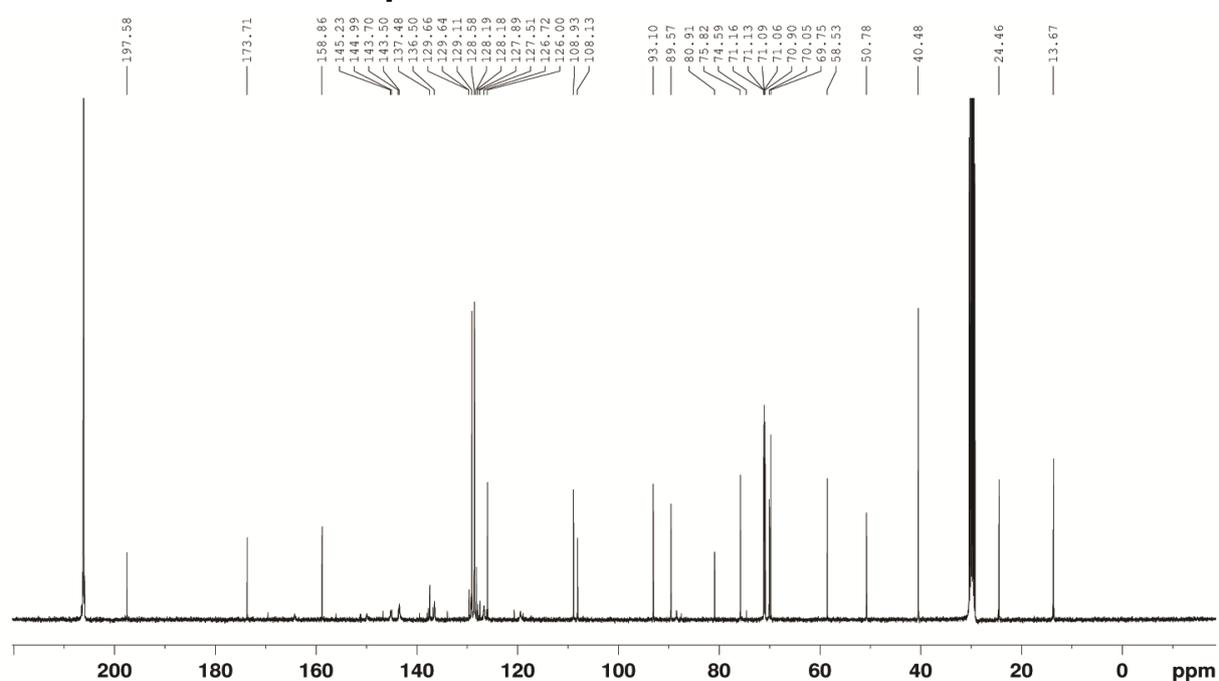
Table content

1.	¹ H NMR of compound 4a.....	2
2.	¹³ C NMR of compound 4a.....	2
3.	¹ H NMR of compound 4b.....	3
4.	¹³ C NMR of compound 4b.....	3
5.	HPLC of compound 4b.....	4
6.	HPLC of compound 2.....	4
7.	Table S1. Release kinetic of compounds 2 from its prodrug 4b at pH 3, 4.3, 5 and 7.3 expressed in percent.	5
8.	Figure S1. Example of HPLC monitoring of 4b at pH= 4.3.....	6
9.	Figure S2. Transmission electron microscopy images of the synthesized nanoparticles 8.....	7
10.	Figure S3. Groups description and schedule of the in vivo experimental protocol.....	8
11.	Figure S4. Quantification of NPs11 accumulation in different organs over time.	8
12.	Figure S5. Effect of compound 2 and NPs 8 on histone H3 acetylation in tumors.	9
13.	Figure S6. Effect of compound 2 and NPs 8 on histone H3 acetylation in liver and in kidneys.	10

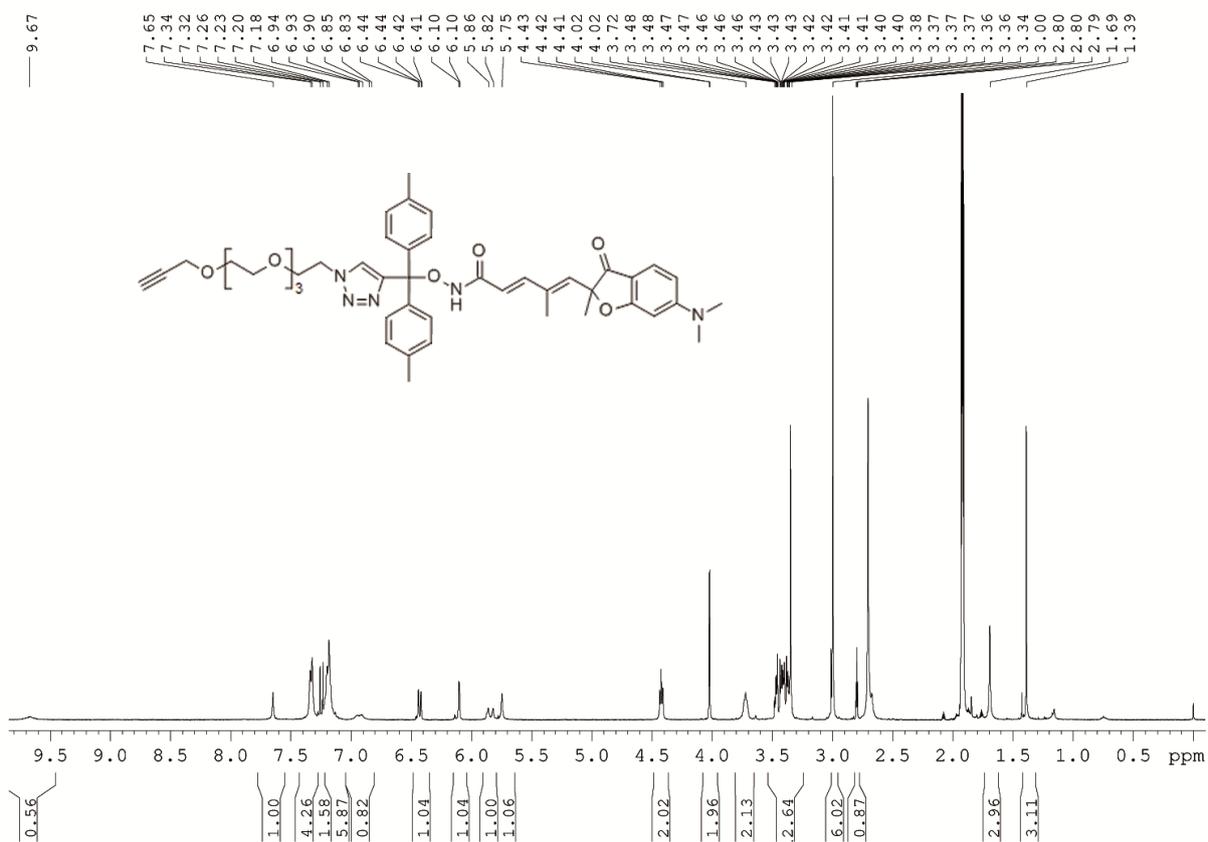
1. ¹H NMR of compound 4a.



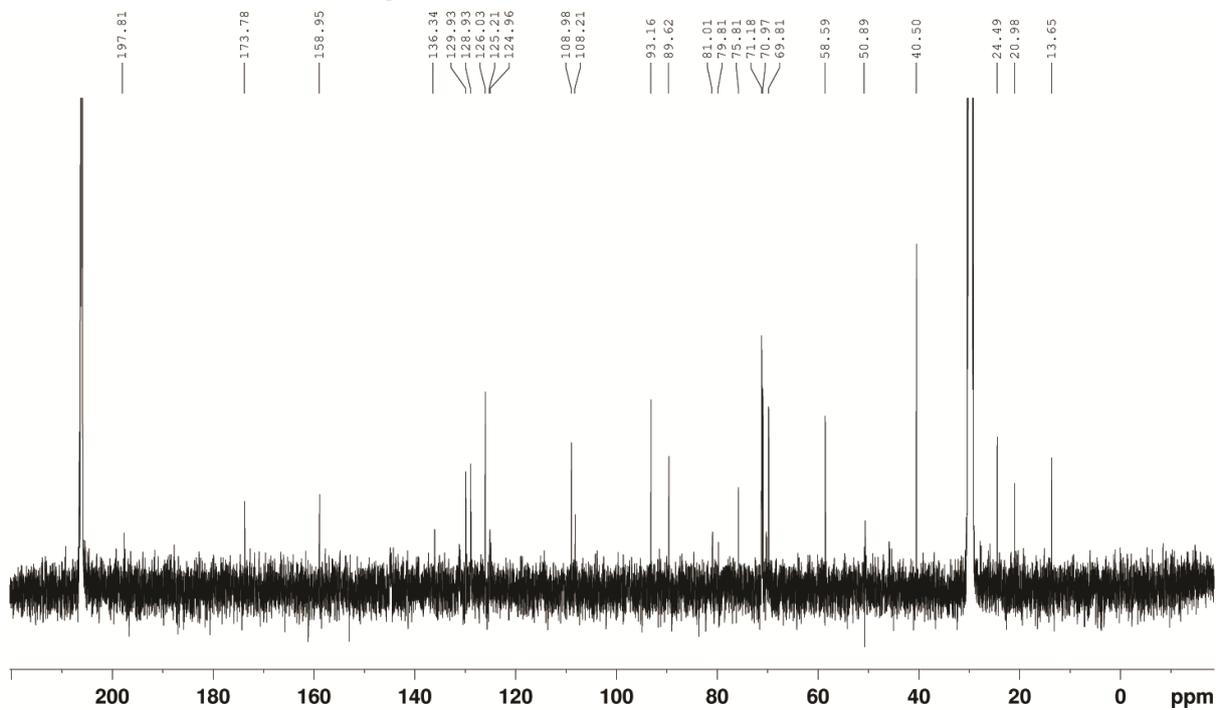
2. ¹³C NMR of compound 4a.



3. ^1H NMR of compound 4b.



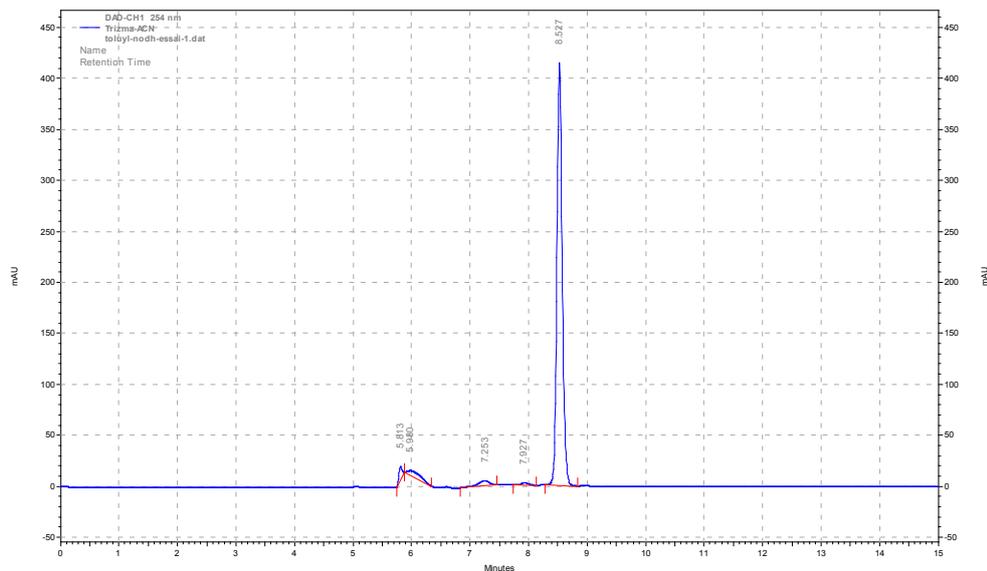
4. ^{13}C NMR of compound 4b.



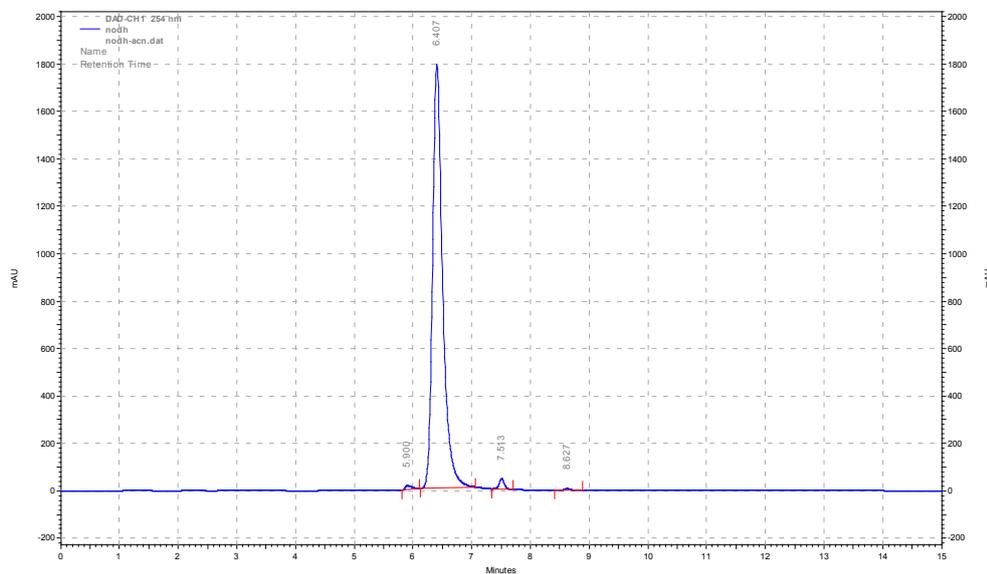
5. HPLC of compound 4b.

Composition of eluting system for the determination of the half-life.

Time (min)	% ACN	%H ₂ O+0.1% TFA	Flow (mL/min)
0.0	85.0	15.0	0.250
5.0	85.0	15.0	0.500
10.0	85.0	15.0	1.000
15.0	85.0	15.0	1.000



6. HPLC of compound 2.



7. Table S1. Release kinetic of compounds 2 from its prodrug 4b at pH 3, 4.3, 5 and 7.3 expressed in percent.

The quantity of released compound 2 versus its prodrug 4b is expressed in relative percentage

pH 3

time (min.)	0	24	43	76	94	164	193	246	300	376	1260
2	0.00	6.75	6.53	21.35	29.92	42.02	47.76	63.82	73.03	74.77	87.52
4b	100.00	93.25	93.47	78.65	70.08	57.98	52.24	36.18	26.97	25.23	12.48

pH 4.3

time (min)	0	17	34	51	71	97	113	188	286	415	1376
2	0,00	2,88	4,49	6,05	7,61	9,28	11,60	18,46	27,06	37,17	63,59
4b	100,00	97,12	95,51	93,95	92,39	90,72	88,40	81,54	72,94	62,83	36,41

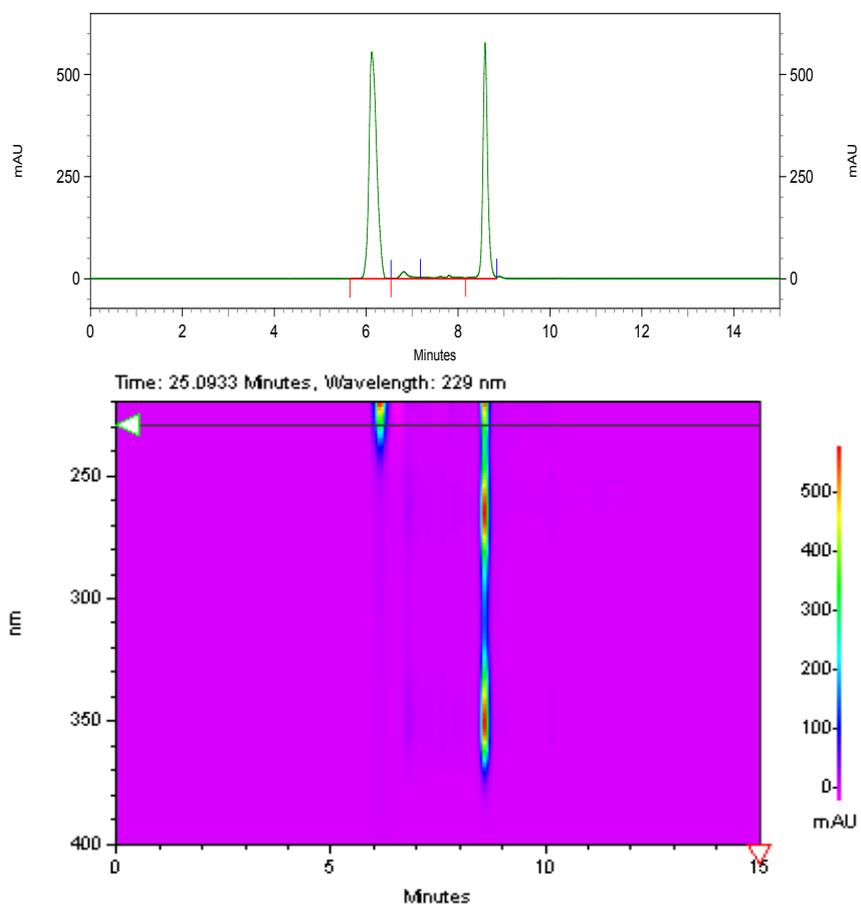
pH 5.0

time (min)	0	30	122	1080	1370
2	0,00	7,10	10,00	38,56	52,42
4b	100,00	92,90	90,00	61,44	47,58

pH 7.3

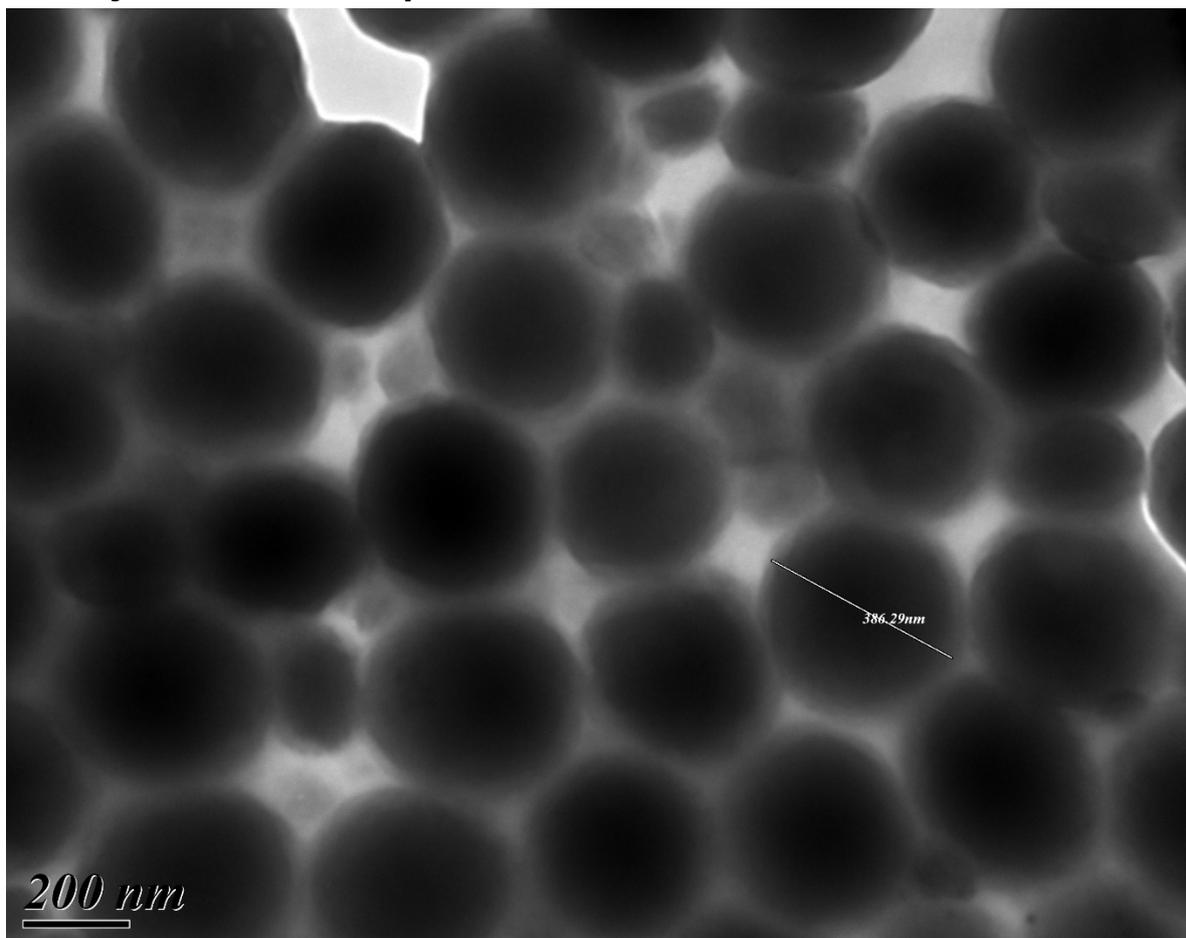
time(min)	0	271	1380	2760	4380	11520
2	0,00	1,22	14,36	26,60	48,31	77,57
4b	100,00	98,78	85,64	73,40	51,69	22,43

8. Figure S1. Example of HPLC monitoring of 4b at pH= 4.3

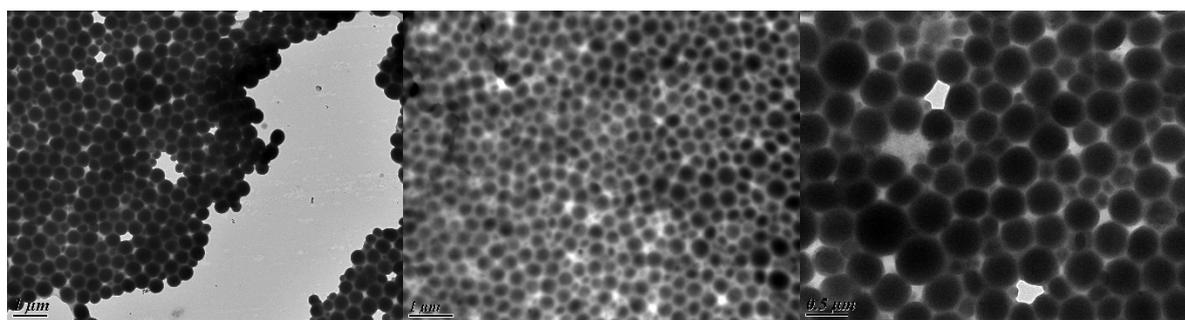


Measure made at t = 17 min

9. Figure S2. Transmission electron microscopy images of the synthesized nanoparticles 8.



80000X-0006



15000X-0004

20000X-0000

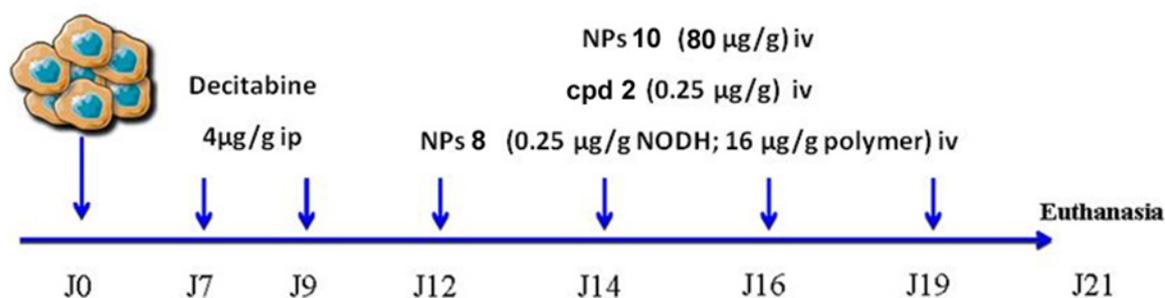
40000X-0005

TEM pictures were performed with a Hitachi H7650 microscope operating at an accelerating voltage of 120 kV. For the particle size and morphology observation, samples diluted about 100 times were deposited on a 200 mesh carbon film-coated copper grids surface (3 x 5 μ l).

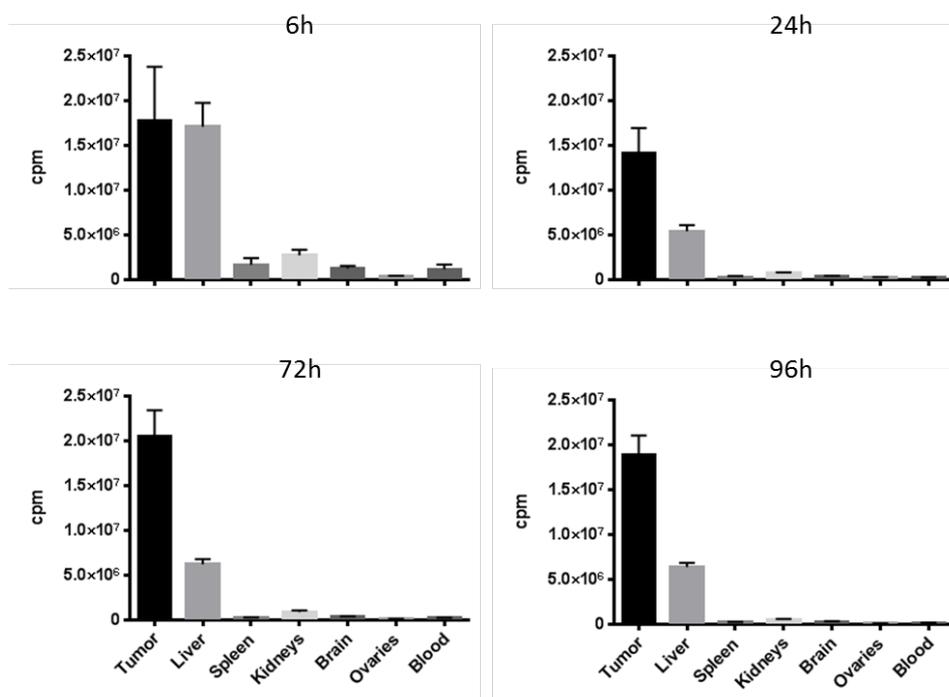
10. Figure S3. Groups description and schedule of the *in vivo* experimental protocol.

- 4 groups :
- Untreated
 - Decitabine 4 μ g/g ip + NPs 10 (80 μ g/g) iv
 - Decitabine 4 μ g/g ip + cpd 2 (0.25 μ g/g) iv
 - Decitabine 4 μ g/g ip + NPs 8 (0.25 μ g/g NODH; 16 μ g/g polymer) iv

AK7 tumor cells

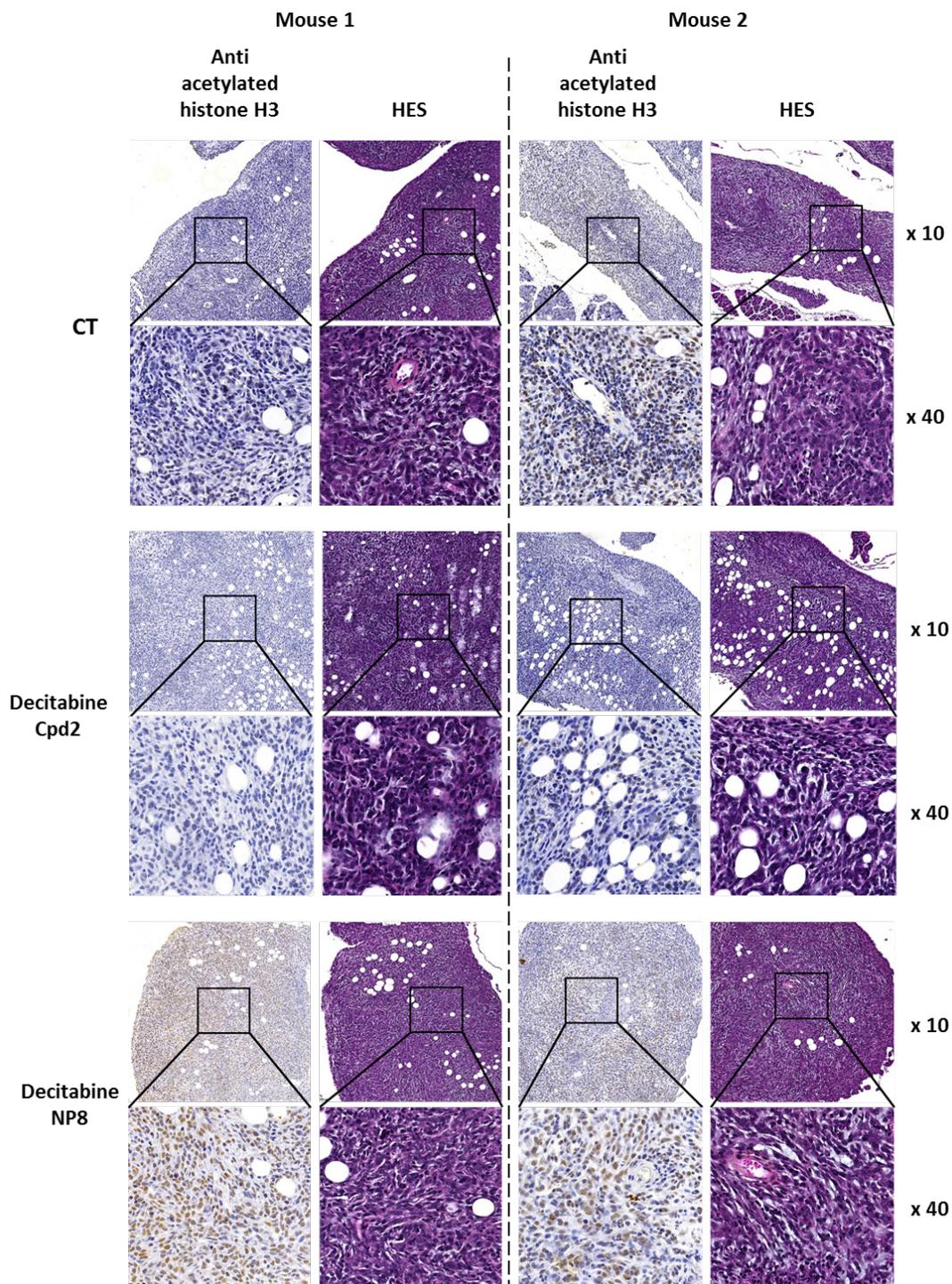


11. Figure S4. Quantification of NPs11 accumulation in different organs over time.



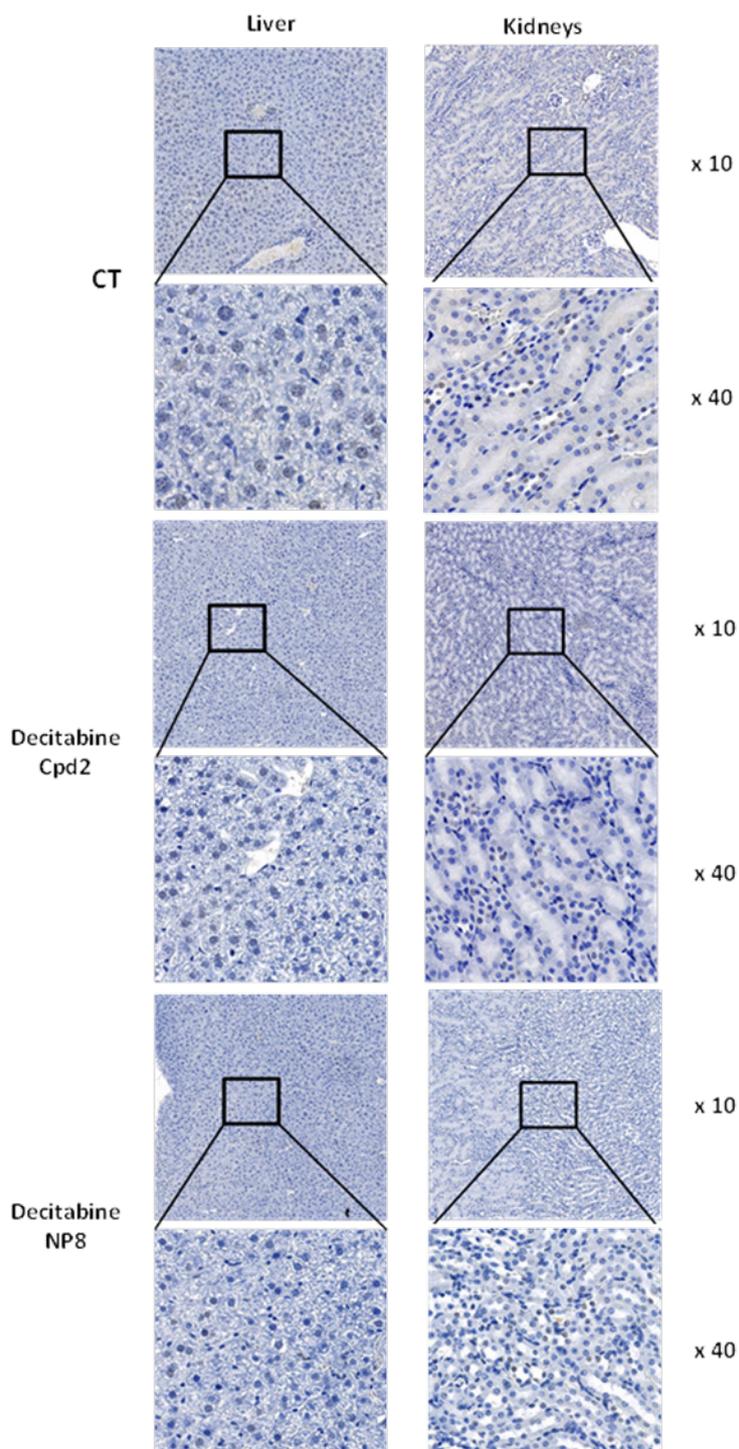
Quantification of the biodistribution of NPs 11 in different organs. C57Bl/6 mice bearing orthotopic AK7 tumors were injected with 60 μ g of NPs 11 per g of mice in the tail vein. The graphics represent the quantification of NPs 11 accumulation in dissected tumor, liver, spleen, kidneys, brain, ovaries and in blood over time. Values are means \pm S.E.M. of results obtained on 5 mice.

12. Figure S5. Effect of compound 2 and NPs 8 on histone H3 acetylation in tumors.



Immunohistochemistry using anti-acetylated histone H3 antibody and HES staining of tumors. Tissues were fixed in 4% CH₂O in PBS, embedded in paraffin, cut into 5- μ m sections. Immunohistochemistry and histology were performed on tissues slices (paraffin-embedded) by Cellular and Tissue Imaging Core Facility of Nantes University (MicroPICell) using anti-acetylated histone H3 antibody (Active Motif, 1/100) or hematoxylin, eosin and safran staining (HES), respectively. Pictures were obtained using a NanoZoomer 2.0HT (Hamamatsu).

13. Figure S6. Effect of compound 2 and NPs 8 on histone H3 acetylation in liver and in kidneys.



Immunohistochemistry of liver and kidneys using anti-acetylated histone H3 antibody. Tissues were fixed in 4% CH₂O in PBS, embedded in paraffin, cut into 5- μ m sections. Immunohistochemistry and histology were performed on tissues slices (paraffin-embedded) by Cellular and Tissue Imaging Core Facility of Nantes University (MicroPICell) using anti-acetylated histone H3 antibody (Active Motif, 1/100). Pictures were obtained using a NanoZoomer 2.0HT (Hamamatsu).