Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

Coordination Polymer Particles as Potential Drug Delivery Systems

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SUMMARY

S1. Experimental procedures for preparation of Zn(bix) spheres, encapsulation of fluorescent drugs in the Zn(bix) spheres, *in vitro* release studies and *in vitro* cytotoxicity assays

MATERIALS

All reagents were purchased from ALDRICH and used without further purification unless otherwise indicated. All HPLC quality solvents used were purchased from ROMIL, and used without further purification.
The 1,4-bis(imidazol-1-ylmethyl)benzene (bix) was synthesized according to literature procedures P. K. Dhal, F. H. Arnold, *Macromolecules* 1992, *25*, 7051.

PREPARATION OF Zn(bix) SPHERES

Zn(bix) spheres were fabricated by addition of an aqueous solution (5 mL) of $Zn(NO_3)_2 \cdot 6H_2O$ (150 mg, 0.5 mmol) to an ethanolic solution (25 mL) of bix ligand (121 mg, 0.5 mmol) under stirring at room temperature. Immediately, the precipitation of a white solid was observed. After 1 minute, ethanol (50 mL) was added into the reaction mixture to stabilize the spheres. The resulting spheres were then purified by centrifugation and washing three times with ethanol. Zn(bix) particles were collected as a white solid or redispersed in ethanol to obtain a white colloid. Zn(bix) spheres with different average sizes were prepared using the same synthetic methodology and by simply modifying the concentration of both $Zn(NO_3)_2 \cdot 6H_2O$ and bix ligand. Anal. (%) Calcd. for $C_{14}H_{14}O_6ZnN_6$: C, 39.31; H, 3.30; N, 19.65; Found: C, 40.25; H, 3.57; N, 19.52. IR (ATR, cm^{-1}): 2980 (s), 1618 (s), 1524 (s), 1426 (m), 1332 (s), 1092 (s), 954 (m), 654 (m).

ENCAPSULATION IN Zn(bix) SPHERES

Drug/Zn(bix) spheres were obtained by addition of an aqueous solution (5 mL) of $Zn(NO_3)_2 \cdot 6H_2O$ (150 mg, 0.5 mmol) to an ethanolic solution (25 mL) of Bix (121 mg, 0.5 mmol) containing the drug (DOX, DAU, CPT and SN38) (3.3×10^{-3} M) under vigorous stirring at room temperature. For all drugs, the resulting encapsulated metal-organic systems were purified by centrifugation and washed three times with ethanol. The encapsulated systems were finally redispersed in ethanol or phosphate buffer to obtain the corresponding colloidal solutions.

IN VITRO RELEASE STUDIES

A dialysis bag (cut-off molecular weight: 3500) containing the Drug/Zn(bix) spheres (c ~ 6 mg/mL) dispersed in phosphate buffered saline solution (PBS; pH = 7.4) was placed in 100 mL of PBS (pH = 7.4; dialysate) at 37 ºC under light stirring. Throughout the experimental procedure, the solution inside the dialysis bag was also agitated by magnetic stirring. To determine the increase of Drug concentration diffused through the dialysis bag, 2 mL of external PBS solution were taken from the dialysate at prefixed times, and each aliquot was analyzed by fluorescence spectroscopy. To minimize the effects of Drug decomposition in solution, every four hours the external solution of PBS was changed.

CHARACTERIZATION

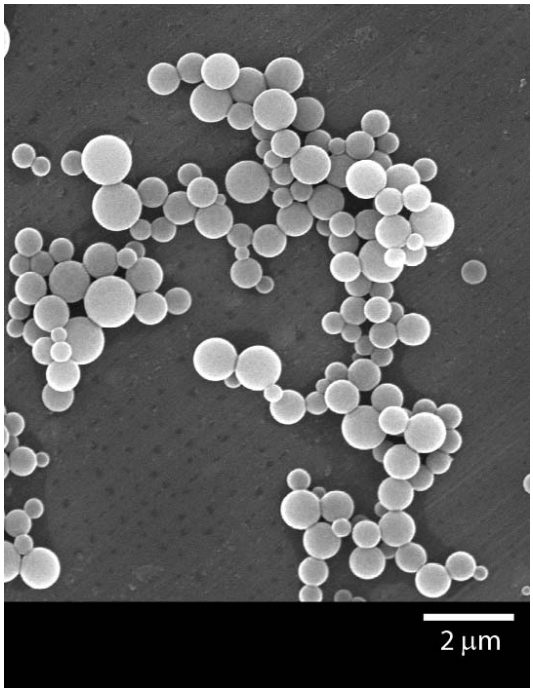
Scanning electron microscopy (SEM) images were collected on a scanning electron microscope (HITACHI S-570) at acceleration voltages of 10-15 kV. Aluminium was used as support. Transmission electron microscopy (TEM) images were obtained with a JEOL JEM 2010F. The measurements were performed at room temperature and a voltage of 200 kV. Optical and fluorescence images were obtained using a Zeiss Axio Observer Z-1 inverted optical/fluorescence microscope with motorized XY stage, Hg lamp excitation source, AxioCam HRc digital camera and standard filters. Fluorescence emission spectra of Drugs/Zn(bix) were measured at $\lambda_{exc.}$= 470 nm in PBS by means of a custom-made spectrofluorimeter, where a Brilliant (Quantel) pulsed laser is used as excitation source, and the emitted photons are detected in an Andor ICCD camera coupled to a spectrograph.

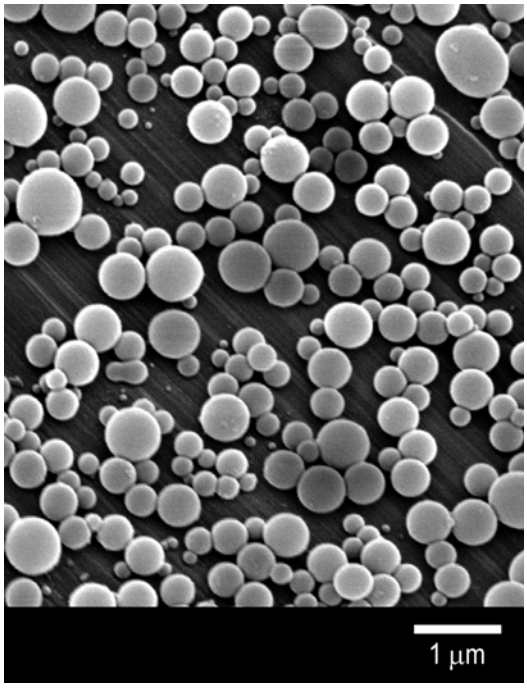
IN VITRO CYTOTOXICITY ASSAYS

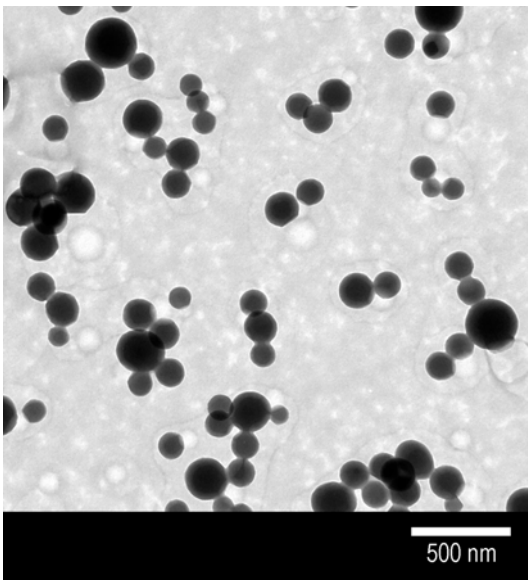
In order to determine the cytotoxic effects of the metal-organic systems, we first seed the acute promyelocytic leukemia cell line (HL-60, ATCC No. CCL-240) into 96-well plates in a volume of 100 µl with 1 x 10^4 cells per well. Afterwards, the effect of the Zn(bix) and DOX/Zn(bix) spheres in cell viability was evaluated by redispersion of the capsules in culture medium to obtain the final concentrations tested (100 µM, 10 µM, 5 µM, 1 µM, 0,5 µM, 0,1 µM, 0,05 µM and 0,01 µM) in a volume of 100 µl/well, and subsequent addition of these solutions to the seed cells for incubation times of 24 h and 48 h at 37ºC. After the incubation times, 20 µl of MTT solution (EZ4U kit, cell proliferation and cytotoxicity assay, from Biomedica) were added to each well, and after 3 hours of reaction, the cell viability was determined by measuring the absorbance at 450 nm using the *Victor*^{3} multilabel plate reader from PerkinElmer. The cytotoxicity experiments were performed at least three times with four repeated incubations per well plate. From the cytotoxicity assays, viability measurements of cells at variable dose of spheres were used to calculate the IC_{50} in order to obtain the concentration of spheres that gives a response half way between the minimum and the maximum values of viability. For this goal, the

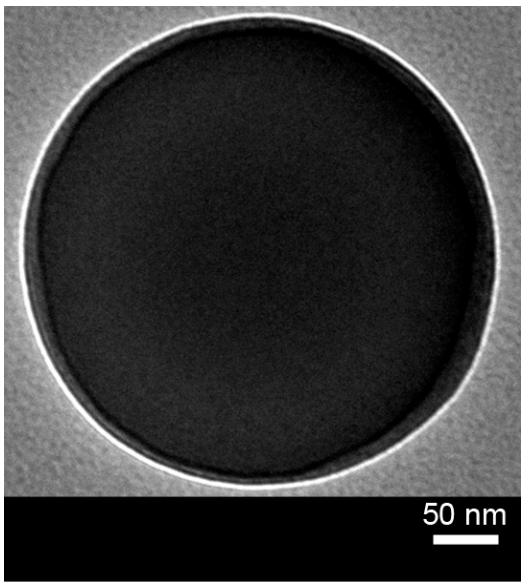
data was analyzed by the software GraphPad Prism 5 to use a nonlinear regression with a pharmacological model of dose response stimulation with variable slope. This model assumes that the dose response curve has a standard slope, equal to a Hill slope (or slope factor) of 1.0.

S2. SEM (Scanning Electron Microscope) and TEM (Transmission Electron Microscope) images of DOX/ZnBix spheres.



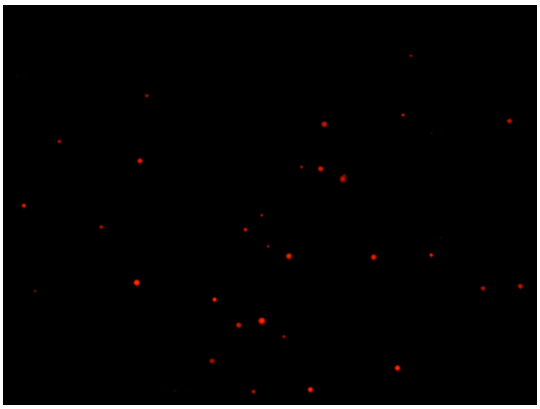




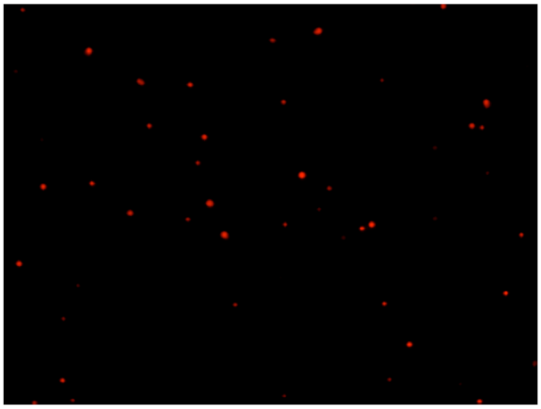


S3. Fluorescence optical microscope images of DOX/Zn(bix), DAU/Zn(bix), CPT/Zn(bix) and SN38/Zn(bix) spheres collected at: DOX/Zn(bix) and DAU/Zn(bix): λ_{exc}= 540 - 552 nm and λ_{em} > 590 nm; CPT/Zn(bix): λ_{exc}= 359 - 371 nm and λ_{em} > 397 nm; SN38/Zn(bix): λ_{exc}= 450 - 490 nm and λ_{em} > 515 nm.

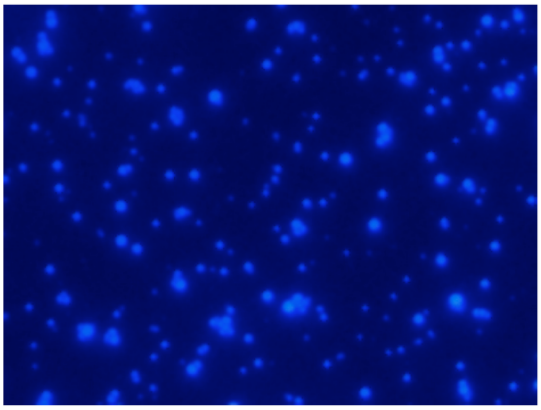
DOX/Zn(bix)



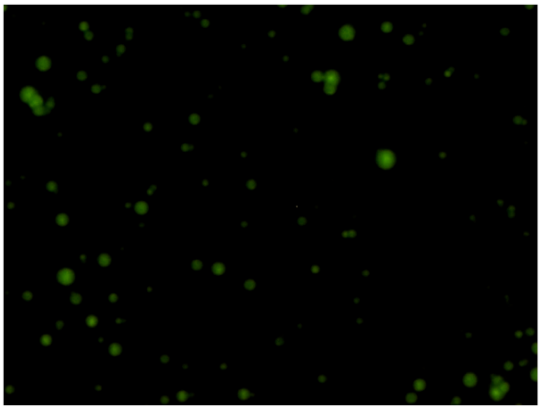
DAU/Zn(bix)



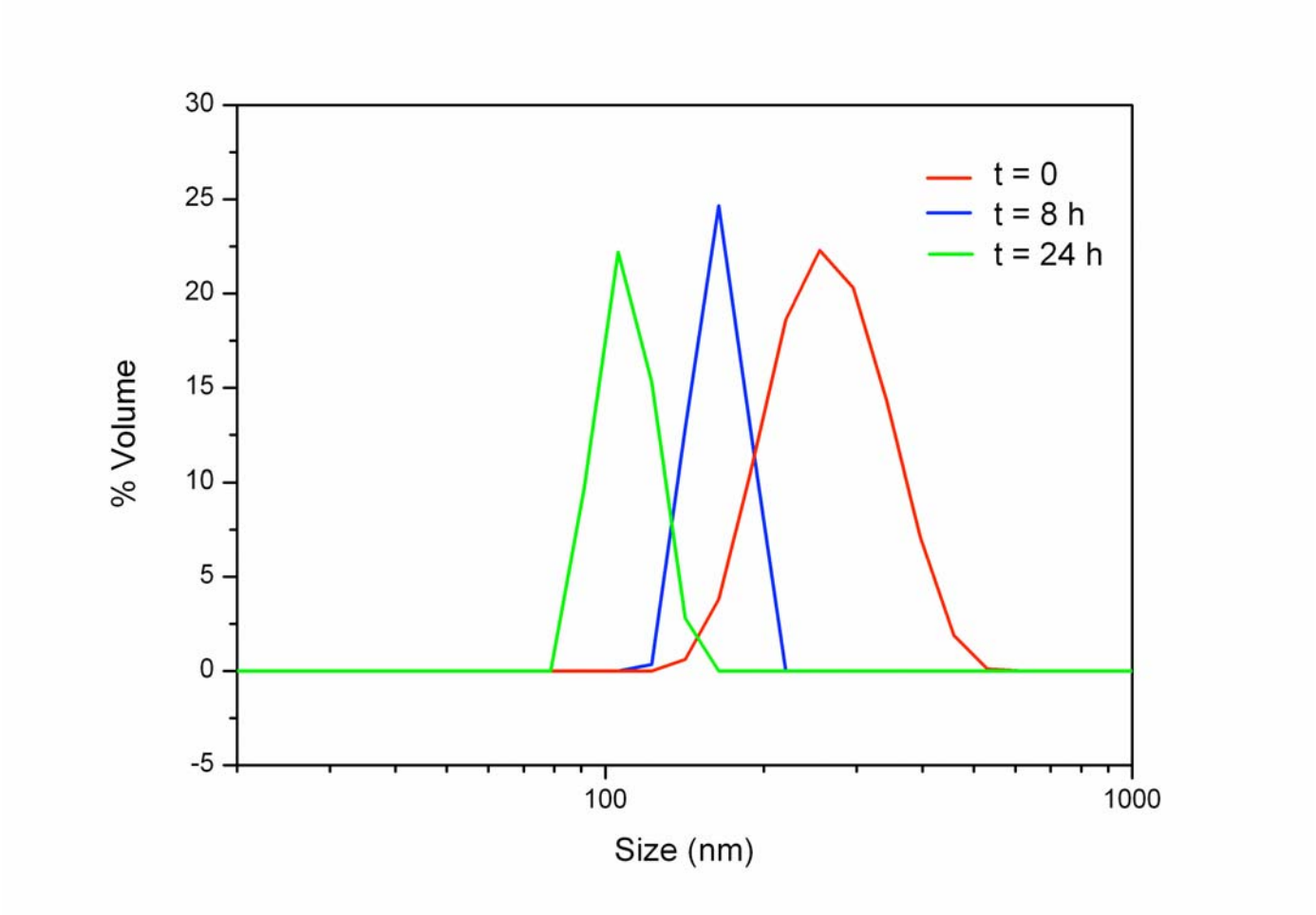
CPT/Zn(bix)



SN38/Zn(bix)



S4. Dynamic light scattering (DLS) measurements of a dispersion of DOX/Zn(bix) spheres in PBS at 37 ºC at different times (0, 8 and 24 hours).



S5. In vitro cytotoxicity assay curves after 48 hours for HL60 cells obtained by plotting the cell viability percentage against Zn(bix) and DOX/Zn(bix) concentration.