Introduction

Cancer is one of the most feared diseases known to mankind. Therefore, the development of new and more efficient drugs has continuously attracted a great deal of attention. There are a number of known anticancer drugs targeting different metabolic pathways, such as alkylating agents (busulfan, melphalan, chlorambucil), anti-metabolites (asparaginase, 5-fluorouracil, methotrexate) or DNA linking agents (carboplatin, cisplatin or oxoplatin). Cisplatin, $\text{cis}[-\text{Pt(NH}_3\text{)}_2\text{Cl}_2]$, is the most commonly used and researched drug for a variety of cancers. Despite its high toxicity (due to being a reductive environment), cisplatin can be activated into Pt(II) antitumour agents (apoptosis) due to the resultant inability of DNA to replicate. It must be noted that cisplatin does not act very specifically, and affects all cells as it cannot distinguish between cancerous and healthy cells. Even though its clinical effectiveness is relatively high, it comes with many side-effects including nephrotoxicity and neurotoxicity, together with possible development of drug resistance over time. Many trials have targeted the synthesis of so-called “warheads” that can target the unique metabolic pathways of tumour cells (such as the glucose-based respiration that causes them to offer a reductive environment), thereby increasing specificity. Non-toxic Pt(IV) species can be activated into Pt(II) antitumour agents in vivo by reducing agents such as glutathione.16,7,14b,22 Thus, Pt(IV) based compounds can be successfully used as cisplatin prodrugs. An example of such a Pt(IV) complex is satraplatin, which can be orally administered and becomes active after reduction by ascorbate and glutathione (GSH) in the malignant cells.8

Another approach to circumvent the shortcomings of cisplatin is through targeted drug delivery systems.16,9 A variety of cancer types in clinical trials and was finally approved as an anti-tumour drug by the FDA (Food and Drug Administration) in 1978.4 Cisplatin is capable of forming intra- and inter-strand cross-links with nucleic acids of DNA. This leads to cell death (apoptosis) due to the resultant inability of DNA to replicate.5

This work demonstrates synthetic strategies for the incorporation of an anticancer drug, cisplatin, and a Pt(IV) cisplatin prodrug into two zirconium-based metal–organic-frameworks (MOFs): UiO66 and UiO66-NH$_2$. Cisplatin was chosen due to its reported high potency in killing ca. 95% of different cancers. Two approaches for its incorporation were investigated: conjugation and encapsulation. In the conjugation route, a Pt(IV) cisplatin prodrug was incorporated into UiO66-NH$_2$ utilising its amine group in an amide-coupling reaction. In the second case, cisplatin was encapsulated into the large cavities of both MOFs. The presence of platinum was confirmed by energy-dispersive X-ray spectroscopy and microwave plasma-atomic emission spectroscopy. The cytotoxicity of the formulations was assessed on the A549 lung cancer cell line. The results show that the system in which cisplatin is conjugated to UiO66-NH$_2$ is more efficient in inducing cell death than the materials where cisplatin is encapsulated into the pores of the MOFs. This is consistent with the higher drug loading achieved with the conjugation technique. One disadvantage of cisplatin therapy is that it may lead to thrombosis and, as a consequence, to heart attack and cardiac arrest. To ameliorate this potential side effect, we investigated the incorporation of NO (which has been widely researched for its antithrombotic properties) into the drug-loaded MOFs. All the cisplatin or pro-drug loaded MOFs are able to entrap and then release NO. Furthermore, the amount of NO released from these formulations is much greater than from the pure MOFs. As a result, the drug delivery systems developed in this work have potentially potent double functionality.
of systems have been designed to release the drug only inside a tumour cell, and to leave healthy cells untouched. Carbon nanotubes,\textsuperscript{10} liposomes,\textsuperscript{16,17} polymers\textsuperscript{18,19} and nano-sized metal phosphates\textsuperscript{20} or oxides\textsuperscript{21} are all under investigation as suitable drug carriers. In addition to these systems, metal–organic frameworks (MOFs) have recently come to the fore as drug delivery systems, and may potentially be of use in cancer therapy.\textsuperscript{22}

MOFs are a comparatively new class of materials: they were first synthesised by Robson in 1989.\textsuperscript{13} They offer great potential in many applications, for example CO\textsubscript{2} capture and hydrogen storage,\textsuperscript{16} gas separation and purification,\textsuperscript{17} heterogeneous catalysis,\textsuperscript{18} luminescence,\textsuperscript{19} MRI imaging\textsuperscript{20} and biomedicine.\textsuperscript{21} MOFs are porous materials with tunable surface areas and a wide range of pore sizes.\textsuperscript{22} Methods exploiting their adsorption capacities for drug storage and delivery are hence of increasing interest.\textsuperscript{21} In this work, two biocompatible MOFs based on Zr and 1,4-benzenedicarboxylate building blocks, UiO66 (Fig. 1)\textsuperscript{14} and UiO66-NH\textsubscript{2}, were employed as cisplatin delivery devices.

In addition to the problems of non-specificity identified above, anticancer therapy using cisplatin may lead to thrombosis:\textsuperscript{24} the formation of blood clotting that may cause hypoxia and in extreme cases tissue death, heart attacks and strokes. Entrapment of nitric oxide (NO) – known for its anti-thrombosis, anti-inflammatory and anti-bacterial effects\textsuperscript{24,25} – in the cisplatin-loaded MOFs, could mitigate this risk. Previous studies have shown that NO can be stored and released on demand by the MOFs HKUST-1, CPO-27-Mg and CPO-27-Ni.\textsuperscript{26} Nitric oxide itself has also been reported to cause cancer cell death.\textsuperscript{27} Thus, preparing MOFs loaded with both cisplatin and NO should permit the production of dual-functionality systems without compromising the anticancer efficacy of the former.

In this work we examined whether we could successfully encapsulate cisplatin in the UiO66 [Zr\textsubscript{6}O\textsubscript{4}O\textsubscript{4}(OH)\textsubscript{4}BDC\textsubscript{6}] (BDC = 1,4-benzenedicarboxylate see Fig. 1) and UiO66-NH\textsubscript{2} [Zr\textsubscript{6}O\textsubscript{4}O\textsubscript{4}(OH)\textsubscript{4} (BDC-NH\textsubscript{2})\textsubscript{6}] (BDC-NH\textsubscript{2} = 2-amino-1,4-benzenedicarboxylate) MOFs, utilising their pores. Both UiO66 and UiO66-NH\textsubscript{2} have very high porosities, offering octahedral (11 Å radius) and tetra- hedral (8 Å radius) cages\textsuperscript{28,29} that could accommodate cisplatin, which is ca. 5 Å in size.\textsuperscript{26} UiO66 and UiO66-NH\textsubscript{2} have the same basic structure, but the latter has a free amine group on the organic linker. This means that while both systems can take cisplatin up into their pores, UiO66-NH\textsubscript{2} can also potentially form covalent bonds with a guest through this amine group. For the latter, we used a platinum prodrug with a carboxylic group, cis,cis,trans-[Pt\textsuperscript{IV}(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}(O\textsubscript{2}CCH\textsubscript{2}CH\textsubscript{2}CO\textsubscript{2}H)(OH)] (Fig. 2), and UiO66-NH\textsubscript{2}. The idea was to conjugate the Pt(n) prodrug to UiO66-NH\textsubscript{2} by covalent bonds similar to peptide bonds.

The major aim of our study was to determine which of these approaches – encapsulation or conjugation – is more efficient for drug delivery. To ameliorate some of the common side effects of cisplatin therapy, bifunctional systems loaded with nitric oxide were also prepared. We believe this study sheds more light on using MOFs as drug delivery systems and specifically their potential supportive roles in cancer treatments.

**Experimental section**

All materials for MOF synthesis and cisplatin, cis-[Pt(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}], were obtained from Sigma Aldrich and used without further purification. UiO66, UiO66-NH\textsubscript{2} and cis,cis,trans-[Pt\textsuperscript{IV}(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}(O\textsubscript{2}CCH\textsubscript{2}CH\textsubscript{2}CO\textsubscript{2}H)(OH)] were prepared according to literature procedures.\textsuperscript{31}

**MOF synthesis**

**UiO66.** A mixture of zirconium(IV) chloride (82 mg, 0.35 mmol) and 1,4-benzenedicarboxylic acid (58 mg, 0.35 mmol) in dimethyl formamide (DMF; 5 mL) was acidified using HCl (37 wt\%, 805 μL, 9.7 mmol) and acetic acid (concentrated, 605 μL, 10.57 mmol). The solution was sealed inside a 23 mL Teflon-lined steel autoclave and heated at 220 °C under autogenous pressure for 24 hours. The UiO66 product was collected by vacuum filtration, washed with DMF and dried in vacuum. Yield: 117 mg, 84%.

**UiO66-NH\textsubscript{2}.** A mixture of zirconium(IV) chloride (82 mg, 0.35 mmol) and 2-amino-1,4-benzenedicarboxylic acid (63 mg, 0.35 mmol) in DMF (5 mL) was acidified using HCl (37 wt\%, 805 μL, 9.7 mmol) and acetic acid (concentrated, 605 μL, 10.57 mmol). The solution was sealed inside a 23 mL Teflon-lined steel autoclave and heated at 120 °C under autogenous pressure for 24 hours. The UiO66-NH\textsubscript{2} product was collected by

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**Fig. 1** The crystal structure of UiO66, based on CCSD deposition file 733458. Zr (green), O (red) and C (black) are represented by coloured spheres.

**Fig. 2** The structure of the cisplatin prodrug used in this work.
vacuum filtration, washed with DMF and dried in vacuum. Yield: 130 mg, 90%.

Encapsulation method

For this method both MOFs (UiO66 and UiO66-NH₂) were used. The procedure was as follows: MOF powders (ca. 350 mg) were dehydrated under dynamic vacuum overnight and then immersed in a solution of cisplatin, cis-[Pt(NH₃)₂Cl₂] (35 mL, at 80% of saturation solution, 2 mg mL⁻¹, (6.66 mM) in deionised water). This corresponded to a theoretical loading of 29.8 mg of cisplatin per 100 mg of dehydrated MOF. The encapsulation continued for 48 hours under stirring at room temperature. The samples were centrifuged and allowed to dry in air.

Prodrug synthesis

A prodrug of cisplatin, cis,cis,trans-[Pt⁷⁺(NH₃)₂(Cl)₂(O₂CCH₂CH₂CO₂H)(OH)] (see Fig. 2), was synthesised in the following procedure. A suspension of cisplatin (0.4 g, 1.33 mmol) in H₂O (12 mL) at 60 °C was oxidized with H₂O₂ (20 mL) added dropwise. The reaction was left for 4 h, and the resultant bright yellow solution left to cool overnight. Yellow crystals (yield: 234 mg, 53%) were recovered by filtration and washed with ice cold water. A more detailed procedure can be found in the literature.⁶⁻¹³ The product (202 mg, 0.6 mol) was then reacted with succinic anhydride (60 mg, 0.6 mol) at 70 °C in a DMF (5 mL) suspension for 24 h and then cooled to room temperature. DMF was removed under vacuum and the residual suspension (1 mL) was dissolved in acetone, and a pale yellow solid precipitated with diethyl ether. Yield: 180 mg, 70%.

Incorporation of the prodrug into UiO66-NH₂ (conjugation method)

The prodrug (Fig. 2) was conjugated to UiO66-NH₂ using the EDC/NHS method in an aqueous solution. A detailed procedure can be found in the literature. In brief, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC: HCl 0.038 g, 0.20 mmol) and N-hydroxysuccinimide (NHS 0.023 g, 0.20 mmol) were dissolved in de-ionized water (15 mL) under stirring. Next, the prodrug (0.70 g, 0.16 mmol) was added into the aqueous solution. After the solution became clear, MOF UiO66-NH₂ (0.140 g) was added and the reaction mixture stirred at room temperature for 24 h. Finally, the solid product was recovered by vacuum filtration, washed with water and left to dry in air. Elemental analysis of the prodrug: calculated: [C] = 11.06%, [H] = 2.76%, [O] = 6.45%, found: [C] = 10.75, [H] = 2.67%, [O] = 6.45%.

Nitric oxide loading

In order to activate (remove solvent from) the MOF powders (0.015 g per glass vial), they were first placed under vacuum (2.3 x 10⁻³ bar) during which time ca. 30% of the mass was lost. They were then heated to 120 °C while still under dynamic vacuum and held at this temperature overnight, leaving a fully activated material. The samples were subsequently cooled to room temperature and exposed to ca. 2 atm of dry NO (99.5%, Air Liquide) for 45 min. The vials were next evacuated and exposed to dry argon, before being flame sealed. This cycle of evacuation and argon flushing was repeated three times in order to remove any residual physisorbed NO from the surfaces of the MOF and glassware.

Drug release experiments

The drug-loaded UiO66-NH₂ and UiO66 powders were formulated into pellets using a hand press in order ensure reproducibility in the drug release experiments. The pellets contained 25 wt% of the drug-loaded MOF, with the remaining 75% being Tetlon. In each experiment, two pellets of 20 mg each, were added to 10 mL of a pH 7.4 TRIS buffer (prepared from 100 mL 0.1 M TRIS, 84 mL 0.1 M HCl, and 12 mL deionised H₂O) at 37 °C. Aliquots of 0.5 mL were removed after the following times: 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 24 h. Cisplatin release was quantified in terms of the amounts of Pt in solution, using an Agilent MP4100 microwave plasma-atomic emission spectrometer (MP-AES). Experiments were performed in duplicate. All calculations for the extent of release are related to the amount of active powder in a pellet.

Cell culture

The A549 lung cancer cell line (ATCC) was stimulated for 24 h with the MOF formulations. The growth media used for cell culture was Gibco RPMI 1640 supplemented with penicillin (100 μg mL⁻¹), streptomycin (100 μg mL⁻¹), L-glutamine (292 μg mL⁻¹) (all Life Technologies) and 10% v/v heat-inactivated fetal bovine serum (FBS; Gibco). This is henceforth referred to as “complete RMPI”. Cells were incubated at 37 °C (5% CO₂) and passaged in this medium until required for stimulation.

For the latter, 2% FBS in complete RPMI was used for cell seeding. Cells were harvested with the TrypLE Express Enzyme (1×; Life Technologies) and seeded at a concentration of 40 000 cells per mL in a 96-well flat bottomed plate, with 100 μL of cell suspension added to each well. Suspensions of the MOF formulations were prepared with a concentration of 1 mg/100 μL and aliquots of 10, 30 and 50 μL were used to stimulate the cells. Complete RPMI was added to even up the volume in wells to 150 μL over the plate. This corresponded to 100 μg, 300 μg and 500 μg of MOF per well respectively. A cisplatin solution was prepared as a positive control, with a concentration of 1 mg mL⁻¹ (3.33 mM). The aliquots used for cell stimulations were the same as those for MOF powders: 10 μL (222 μM), 30 μL (666 μM) and 50 μL (1110 μM).

The Alamar Blue cell viability assay was used to evaluate cell viability after 24 h exposure to the MOFs. Resazurin solution (5 mM in RPMI) was added at 10% of the well volume (15 μL to 150 μL well volume), and incubated for 4 h. The fluorescence of each well was quantified using a SpectraMax Multi-Mode Microplate reader (Molecular Devices) with excitation/emission wavelengths set at 555/585 nm. After 4 hours, a linear relationship between fluorescence intensity and cell number was observed. The standard curve was constructed as follows: fluorescence of untreated cells corresponded to 100% viability and 0% cells (RPMI media alone) to 0% viability, with additional calibration points at 75%, 50% and 25%.
Material characterization and instrumentation

Powder X-ray diffraction (PXRD) patterns were collected on a PANalytical Empyrean diffractometer using Cu Kα radiation. Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) was performed on a JEOL JSM-5600 instrument at a 20 keV excitation energy. Thermogravimetric analysis (TGA) was conducted on a Discovery instrument (TA Instruments) using approximately 2–3 mg of the sample, which was heated at 10 °C min⁻¹ to 800 °C under a flow of N₂ gas (25 mL min⁻¹). IR spectra were recorded over the region of 600–4000 cm⁻¹ on a Shimadzu ATR spectrophotometer.

NO release measurements were performed using a Sievers NOA 280i chemiluminescence analyzer. Calibration of the instrument was performed by passing air through a zero filter (Sievers < 1 ppb NO) and 91 ppm NO gas (AP, balance nitrogen). The flow rate was set to 200 mL min⁻¹ with a cell pressure of 8.5 Torr and an oxygen pressure of 6.1 psi. In order to trigger and measure the NO release, dry nitrogen gas was humidified by passing it over a solution of LiCl (sat.) to give 11% R.H.

Results and discussion

Encapsulation of cisplatin in the pores of UiO66-NH₂ and UiO66

Successful preparation of the MOFs was confirmed by X-ray diffraction, with the patterns of the obtained materials being identical to those reported in the literature. The particle size of the MOFs was assessed by SEM to be around 500 mm. SEM images of UiO66-NH₂ (left) and UiO66 (right) are shown in Fig. 3.

Table 1  Cisplatin loading calculated based on EDX analysis

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<tr>
<th>MOF</th>
<th>Pt : Zr ratio (mol)</th>
<th>Corresponding to cisplatin loading wt%</th>
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<tr>
<td>UiO66-NH₂-prodrug</td>
<td>1.00 : 1.76</td>
<td>30.7</td>
</tr>
<tr>
<td>UiO66-NH₂ encapsulated</td>
<td>1.00 : 15.91</td>
<td>4.9</td>
</tr>
<tr>
<td>UiO66 encapsulated</td>
<td>1.00 : 17.38</td>
<td>4.7</td>
</tr>
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Table 1 shows the Pt : Zr ratios of the different MOFs and the corresponding cisplatin loading. The Pt : Zr ratio of UiO66-NH₂-prodrug is 1.00 : 1.76, which corresponds to 30.7 wt% loading (expressed w.r.t cisplatin).

Conjugation of the prodrug to the amine group of UiO66-NH₂

The amide-coupling reaction allows for the direct incorporation of a non-toxic Pt(IV) prodrug to the MOF using its amine group. The Pt(IV) prodrug can be easily reduced in the oxygen-poor environment typical of tumour cells to give cytotoxic Pt[II] species. The UiO66-NH₂ integrity was retained after the prodrug loading process: this is clear from the powder X-ray diffraction data in Fig. 4, where the pattern is observed to be unchanged post-incorporation. Attempts were made to reduce the Pt(IV) prodrug with ascorbic acid and quantify the amount of cisplatin released by 195Pt NMR, but the signal to noise ratio was low and the results therefore inconclusive. However, EDX analysis clearly demonstrates pro-drug conjugation (see Table 1). These data show that the ratio of Pt : Zr in the prodrug-conjugated UiO66-NH₂ is 1 : 1.76, which corresponds to 30.7 wt% loading (expressed w.r.t cisplatin) and indicates that approximately every second amine group has successfully been functionalised with the pro-drug.

Infrared spectroscopy (Fig. 6) shows bands at around 1580 and 1730 cm⁻¹ corresponding to amide groups. The band at 1750 cm⁻¹ in UiO66-NH₂, which can serve as a proof of a successful conjugation, has disappeared in UiO66-NH₂-prodrug. This indicates that the amide-coupling reaction has occurred.

TGA was performed in order to assess the thermal stability of the formulations. The data suggest that the MOF with a conjugated cisplatin prodrug is not as thermally stable as the unmodified UiO66-NH₂, and starts decomposing at 300 °C, (50 °C lower than unmodified UiO66-NH₂; Fig. 7).
Drug release. Cisplatin release data are given in Fig. 8. UiO66 releases 22.73 μg of cisplatin per mg of MOF in the first 24 hours, approximately four times more than the amount released from UiO66-NH2 (5.88 μg cisplatin per mg MOF). However, the EDX analysis shows that the cisplatin loading is similar in both MOFs (4.9 wt% in UiO66-NH2 and 4.7 wt% in UiO66). The results possibly indicate a relatively strong interaction between cisplatin and the amine group in UiO66-NH2 MOF, preventing release of the former. As a result, after 24 h only 12.5% of loaded cisplatin in the active MOF powder is being released in the case of UiO66-NH2 while in UiO66 the release amount is 48%.

Cell viability studies

The lung cancer cell line A549 was stimulated with different MOF formulations and cell viability was examined after 24 h exposure using the Alamar Blue assay. This cell line was selected for in vitro studies because cisplatin is commonly used to treat lung cancer. The data are presented as mean ± SEM (standard error of the mean) from two independent experiments, with each set of conditions run in triplicate in each experiment. Statistical analysis was performed by Repeated Measures ANOVA and Sidak’s multiple comparisons test using GraphPad Prism v6.05 software. Differences between means were considered statistically significant when \( P < 0.05 \) (*), \( P < 0.01 \) (**), \( P < 0.001 \) (***) or \( P < 0.0001 \) (****). “\( P \)” is the probability of obtaining the observed effect purely due to chance. \( P < 0.05 \) is the conventional threshold for a statistically significant result, and indicates that there is only a 5% of chance that the conclusion drawn is in fact false. Subsequent levels of significance commonly used in statistics are \( P < 0.01 \), \( P < 0.001 \), \( P < 0.0001 \), which denote 1%, 0.1% and 0.01% chance, respectively. The lower the \( P \) value obtained, the higher the level of significance of the observed effect and, consequently, the greater our confidence that it is true.

Fig. 9(a) shows the viability of cancer cells in response to UiO66 and UiO66-NH2 encapsulated with cisplatin at three different concentrations. We observed that cisplatin loaded UiO66 significantly decreased cell viability compared to UiO66 alone. Conversely, the analogous UiO66-NH2 systems did not induce significant changes in cell viability. This may be due to the fact that cisplatin bind to amine groups in the MOF, and is thus not available for release and interaction with cells. These findings agree with the results from cisplatin release (discussion vide supra).

In Fig. 9(b), we compared the cytotoxic efficacy of UiO66-NH2 with encapsulated cisplatin and UiO66-NH2 conjugated with the cisplatin prodrug. It appears that the latter performed better in inducing cell death, particularly at higher concentrations where statistically significant outcomes were observed. This is expected to be a result of the higher drug loading in the conjugated system, as well as the binding between cisplatin and the amide groups of UiO66-NH2. The conjugated UiO66-NH2 system shows approximately the same cytotoxicity as the encapsulated UiO66
at higher concentrations, but is less effective at low concentrations. This can be ascribed to the fact that the UiO66-encapsulated cisplatin is freely able to exit the MOF, while for the prodrug to be active hydrolysis of the amide linkage is required. This makes the conjugated system require more time to become active, but offers promise for sustained release and selectivity for cancerous cells only.

In all cases we observe a distinct dose-dependent effect of the drug-loaded MOFs on cell viability. It is clear that these systems are biologically functional, and thus have potential as drug delivery systems.

**Nitric oxide adsorption**

While UiO66 and UiO66-NH₂ have high pore volumes, they do not have any open metal sites with which to effectively bind nitric oxide, and in the two systems only the amine group in UiO66-NH₂ can perform this function. The amine group has in this work been utilized for conjugation with the prodrug to form a peptide bond. Nevertheless, cisplatin and the prodrug themselves offer open sites on their amine groups and Pt centres.

NO-loading and NO release from the untreated MOFs and all three drug-loaded materials were performed in triplicate. The release profiles are depicted in Fig. 10 and the absolute quantities of NO involved are summarized in Table 2. The incorporation of cisplatin into the pores of the UiO66 material significantly increased the amount of NO loaded and released from the system, since the cisplatin complex offers two amine groups and a metal site to which NO can bind.

The unmodified UiO66-NH₂ shows more NO release than UiO66 due to the presence of NH₂ groups, which can form the diazeniumdiolate group (NONOate) with NO. Again, the encapsulation of cisplatin into the pores of the UiO66 material significantly increased the amount of NO loaded and released from the system, since the cisplatin complex offers two amine groups and a metal site to which NO can bind.

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In contrast, when UiO66-NH₂ is conjugated with the cisplatin prodrug there is almost no change in NO release performance. This is thought to be because: (i) although the introduction of the prodrug provides additional amine groups, it also occupies the NH₂ groups of the MOF, and (ii) the bulky nature of the prodrug complex (see Fig. 1) may present steric hindrance for the coordination of NO molecules to its amine groups, reducing the ability of incoming NO to bind to them.
In addition, the cisplatin loaded MOFs were successfully loaded with NO, with the aim of preventing the thrombotic effects that can occur with cisplatin therapy. Nitric oxide release is unaffected by the conjugation of the prodrug to UiO66-NH2. However, MOFs loaded with cisplatin present much higher NO release capacities than the pure materials, due to the open sites available for NO binding on cisplatin.

To conclude, our results demonstrate a successful approach for the synthesis of a bifunctional material containing Pt-based anticancer agents and nitric oxide as both an antitumour and antithrombotic agent.

**Acknowledgements**

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**References**


**Conclusions**

Using a solvothermal method, in this work we first synthesized the MOFs UiO66 and UiO66-NH2. We next loaded them with cisplatin using two approaches – encapsulation of cisplatin to both MOFs and conjugation of a cisplatin prodrug to UiO66-NH2. The prodrug investigated, cis,cis,trans-[Pt²⁺(NH₂)₂(Cl)(O₂CCH₂CH₂CO₂H)(OH)] is expected to allow the selective targeting of tumour cells because it is only reduced to an active Pt(n) species under the highly reducing conditions typical of such cells. The results obtained show that for UiO66-NH2 conjugation allows higher loading than encapsulation (30.7 wt% against 4.9 wt%), and that this translates into greater cytotoxicity in an *in vitro* assay. Considering the encapsulated systems, the amount of release of cisplatin from UiO66 is significantly higher than from UiO66-NH2, even though EDX results suggest that the drug loading is similar in both systems. This may be due to an interaction of cisplatin with amine groups of the UiO66-NH2 MOF.

![Fig. 10 Total NO release from UiO66-NH2 (blue), UiO66-NH2 conjugated with the prodrug (green), and with cisplatin encapsulated (red), and from UiO66 loaded with cisplatin (purple). Inset: NO release from pure UiO66.](image-url)

<table>
<thead>
<tr>
<th>MOF</th>
<th>Total NO (µmoles per g of MOF)</th>
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<tr>
<td>UiO66</td>
<td>11.3 ± 1.87</td>
</tr>
<tr>
<td>UiO66-NH2</td>
<td>9.27 ± 3.95</td>
</tr>
<tr>
<td>UiO66-prodrug (conjugated)</td>
<td>7.69 ± 0.43</td>
</tr>
<tr>
<td>UiO66-cisplatin (encapsulated)</td>
<td>16.5 ± 4.25</td>
</tr>
<tr>
<td>UiO66-NH2-cisplatin (encapsulated)</td>
<td>22.7 ± 5.33</td>
</tr>
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This table shows the amounts of nitric oxide released from the MOFs, as mean ± SEM.


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