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Targeting cytotoxicity and tubulin polymerization by metal–carbene complexes on a purine tautomer platform†

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This communication describes the synthesis, structural investigation and tubulin binding of purine rare imino-tautomer based Ag(I) and Hg(II)–carbene complexes. These complexes exhibit cytotoxicity through tubulin interaction by binding to a site close to the GTP binding site.

The biological action of organometallic compounds has elicited considerable interest in the past and it continues to attract close scrutiny due to the diverse mechanisms of action, especially in anticancer chemotherapy.[1] In addition to the conventional nucleic acid target, organometallic anticancer compounds are being designed to seek alternate macromolecular targets and pathways. Compounds built around cyclopentadienyl cores, metal–arene interactions, polynuclear clusters and N-heterocyclic carbenes (NHCs), are promising candidates against a variety of cancer cell lines. Their promise is centered around a predictable three dimensional structure, fine-tuning of their properties *via* the choice of metal ions and transport to desired sites using conventional drug delivery strategies.[2]

In particular, metal–NHCs offer a versatile platform for discovering novel metallodrugs as their synthesis is relatively simple and it can support different metal ions such as Ag, Au, Pt, Pd, Cu, Ni, and Ru, thus allowing faster optimization of the structural requirements and biological activity, in a divergent fashion.[3] The anticancer action of metal–NHCs is of contemporary interest as these complexes exert favourable biological action through a number of mechanisms such as the activation of apoptosis, depolarization of the mitochondria inner membrane potential, nuclear translocation of apoptosis-inducing factors and caspase-12 and inhibition of cysteine-dependent protein tyrosine phosphatases and thioredoxin reductase, to name a few.[2]

Imidazolium moieties carrying synthetic ligands are the preferred scaffolds to stabilize carbenoid species, thus the majority of reports focus on the modification of this heterocycle for NHC synthesis.[3a,4] Notably, despite the presence of an imidazole-like ring in purine heterocycles, reports of carbene generation on natural purine nucleobases are rare, and most reports concern xanthine and caffeine.[5] Recent advances in organometallic anticancer drugs and the possibility of using natural purines, such as adenine and guanine, gave us an impetus to design adenine-based carbenes as antitumor agents.[6]

We have investigated metalated adenine analogues for the generation of higher order supramolecular frameworks and surface patterning and the synthesis of polymeric adenine templates, and their use in catalysis and DNA cleavage.[7] The present study describes a novel methoxyadenine rare tautomer platform for stabilizing silver- and mercury-based carbenoid species, for possible cytotoxicity and interaction with microtubules. The structural tunability of the ligand and metal ion stabilizing carbene was considered as a beneficial feature to exploit this framework in these studies.

It was envisaged that the two benzyl rings on the imidazole ring in ligand **1** would render the C8 carbon susceptible for carbene generation, which in turn could react with Ag or Hg to create the desired organometallic species. Consequently, C8–H abstraction was achieved using Ag_2O and $Hg(OAc)_2$, where the metal oxide or acetate acted as a base as well as a metal center, stabilizing the formation of the N-heterocyclic carbene. The proposed structures of the C8 centered metallodimers, **1A** and **1B**, are shown in Scheme 1. The syntheses of ligand **1** and complexes **1A** and **1B** are described in the ESI.† The formation of these complexes was confirmed by spectroscopic methods. ^{1}H NMR spectra of **1A** and **1B** clearly revealed complete disappearance of the C8–H signals present at δ 9.26 in ligand **1**

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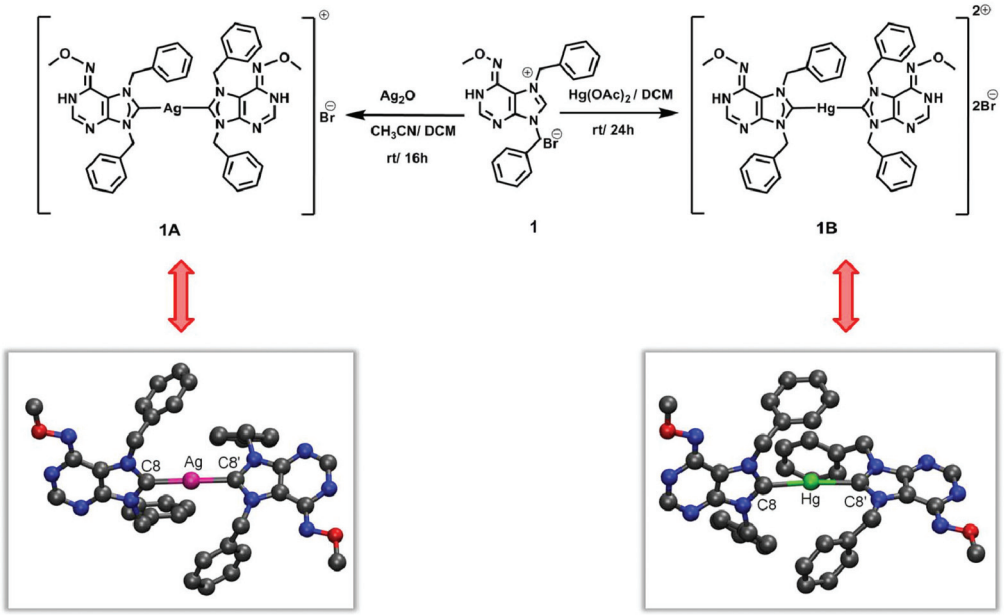

Scheme 1 Synthesis of silver carbene (**1A**) and mercury carbene (**1B**) complexes from ligand **1**. Insets: DFT-optimized geometries of **1A** and **1B**.

(Fig. S2†). This was further confirmed by ^{13}C NMR where a peak corresponding to C8 at δ 136.37 shifted to δ 178.52 for **1A** and 175.80 for **1B**, characteristic of the corresponding metal carbene signal (Fig. S3†). HRMS data also confirmed the formation of carbene complexes **1A** and **1B** (Fig. S4†).

Complexes **1A** and **1B** did not crystallize despite trying multiple crystallization conditions. Thus, we resorted to Density Functional Theory (DFT) calculations to gain insight into the structural features of these carbene complexes. DFT calculations for both complexes were performed by employing B3LYP exchange-correlation functional,[8] with a 6-31G(d,p) basis set for all non-metallic atoms and a LANL2DZ basis set for both Ag and Hg ions[9] (see the ESI† for geometry optimizations). Normal mode analysis of the vibrational frequencies was performed and the absence of any negative frequency confirmed the structure to be at minima (at least local). These calculations considered basis set superposition errors (BSSE) in order to calculate accurate energies in the counterpoise approximation.[10] Geometry optimization of **1A** and **1B** afforded nearly linear carbene complexes, with a C8–Ag–C8′ bond angle of 178.63° and a C8–Hg–C8′ bond angle of 170.62° (Scheme 1). A slight elongation in the C8–N bond was observed, while the N7–C8–N9 bond angle decreased by 3.13° in **1A** and by 1.9° in **1B** (Table S2†). All of the DFT calculations were performed using Gaussian 09 (see ESI†).

Silver and other metal complexes have been studied for multiple biological effects such as antiseptic activity, inhibition of inflammation and antibacterial and antitumor action.[11] We became interested in assessing the possible anticancer action of **1A** and **1B**, as recent studies reported the effect of silver complexes on caspase-independent cancer cell apoptosis through a mitochondrial apoptosis inducing factor pathway.[12] Mitochondrial targeting and the lack of genotoxicity further augmented the scope of their biological action.

We assessed the anticancer properties of ligand **1** and its metal complexes (**1A** and **1B**) by MTT assay, which uses cytosolic redox enzymes for the reduction of tetrazolium dye as an indicator of cell viability, in A549 lung cancer and MCF7 breast cancer cell lines, for 24 h. Curcumin, a known anticancer compound, was used as a control.[13] **1**, **1A** and **1B** (40 μM) exhibited similar cytotoxicity (loss of viable cells) against the A549 adenocarcinoma cell line, which was comparable to the curcumin control (40 μM) (Fig. 1a). However, for the MCF7 cell line, **1**, **1A** and **1B** (40 μM) show ~20%, ~60% and ~90% cytotoxicity, respectively, when compared to ~70% cytotoxicity exhibited by curcumin (40 μM) *i.e.*, the MCF7 cell line is 80%, 40%, 10% and 30% viable after treatment with **1**, **1A**, **1B** and curcumin, respectively, (Fig. 1b). These results suggest that while **1** is least cytotoxic to the MCF7 cell line, the cytotoxicity of **1A** is comparable to that of curcumin with **1B**, exhibiting excellent cytotoxicity. At this time, it can be proposed that this difference in cytotoxicity could be attributed to specificity for a particular cell line. After determining the cytotoxicity of these compounds, changes in the morphology of the cancer cell lines were studied after 24 h conjugate uptake and the ensuing changes in cellular morphology were compared, as evident from the differential interference contrast images (Fig. 2, S5 and S6†). Cell deformation alludes to the potential anticancer activity of **1A** and **1B**.

We decided to probe the possible mechanism for morphological changes by these conjugates. It is well known that microtubules, an important constituent of the cytoskeleton, are key targets for anticancer drug action. Moreover, microtubules are also one of the vital cytoskeleton filaments, which

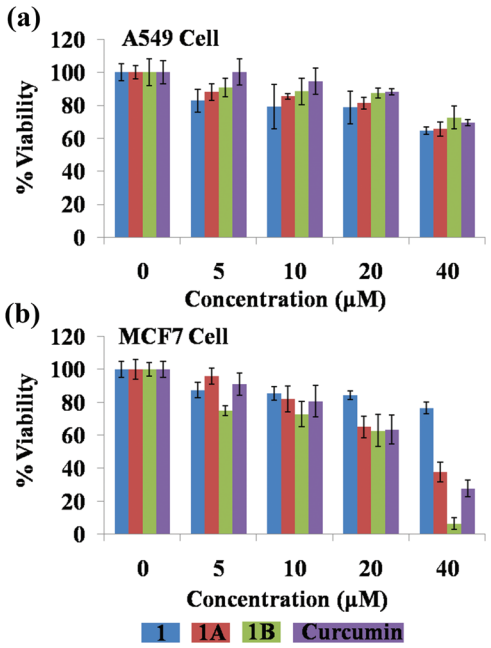

Fig. 1 The percentage viability of the cancer cell lines after treatment with **1**, its metal complexes (**1A** and **1B**) and curcumin (as a control) was assessed by MTT assay in: (a) A549 and (b) MCF7 cell lines for 24 h.

maintain cell structure. Therefore, the ability of these conjugates to perturb microtubule networks inside cancer cells was assessed. As imagined, the microtubule networks in A549 and MCF7 cell lines were dramatically affected after treatment with the conjugates for 24 h, which resulted in drastic morphological changes, suggesting this as a possible mechanism in eliciting an anticancer response (Fig. 3, S7†). Based on these results, we surmise that the anticancer activity of our compounds is perhaps a consequence of disruption of the microtubule networks leading to deformation of cellular morphology.

The effect of these conjugates on microtubule dynamics was further investigated by following *in vitro* microtubule assembly/polymerization, *via* a turbidity assay, in the presence of **1**, **1A**, **1B** and curcumin, at 37 °C for 40 min. Curcumin is known to induce significant depolymerization of microtubules.[14] A significant increase in turbidity, when measured at 350 nm, is observed during *in vitro* microtubule polymerization.[15] Interference with this process was inferred from the turbidity data (Fig. 4a), where **1A** offered the greatest inhibition and relative inhibition was determined to be **1A** > curcumin > **1** > **1B**. Thus, the anticancer activity of **1A** and **1B** could be ascribed to their ability to target microtubule polymerization.

Anti-cancer activities of our conjugates prompted us to qualitatively interrogate their interaction with tubulin focusing on the probable binding site, nature of binding and the amino acids supporting this interaction. As the adenine imino tautomer exhibits structural similarity to guanine, we decided to investigate the possibility of our tautomer occupying the GTP binding site in β-tubulin through docking studies.[16] It is known that GTP binds to both α and β-tubulin monomers, where β-tubulin bound GTP is hydrolyzed during microtubule assembly.[17] Docking studies performed to assess the binding of **1** in proximity to the GTP binding site suggested that **1** probably binds close to the Asn117 residue of β-tubulin (Fig. 4b and c), similar to GTP/GDP binding. Incidentally, this site is proximal to the GTP/GDP binding pocket of β-tubulin (Fig. 4d).[17] Such an interaction could possibly be ascribed to the structural similarity between GTP and rare purine tautomer, **1**. Thus, it could be proposed that **1** exerts its action through hydrophobic and hydrogen bonding interactions with β-tubulin.

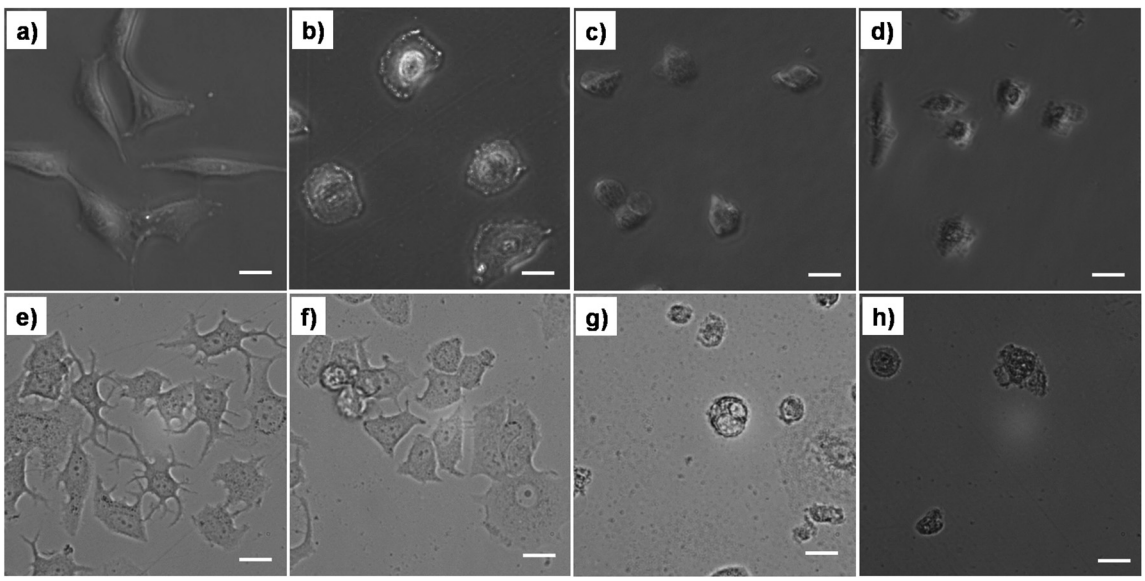

Fig. 2 Changes in cell morphology after treatment with **1** and its complexes for 24 h. Upper panel: (a) untreated A549 cells; (b) with **1**; (c) with **1A**; (d) with **1B**. Lower panel: (e) untreated MCF7 cells; (f) with **1**; (g) with **1A**; (h) with **1B** [scale bar = 20 μm].

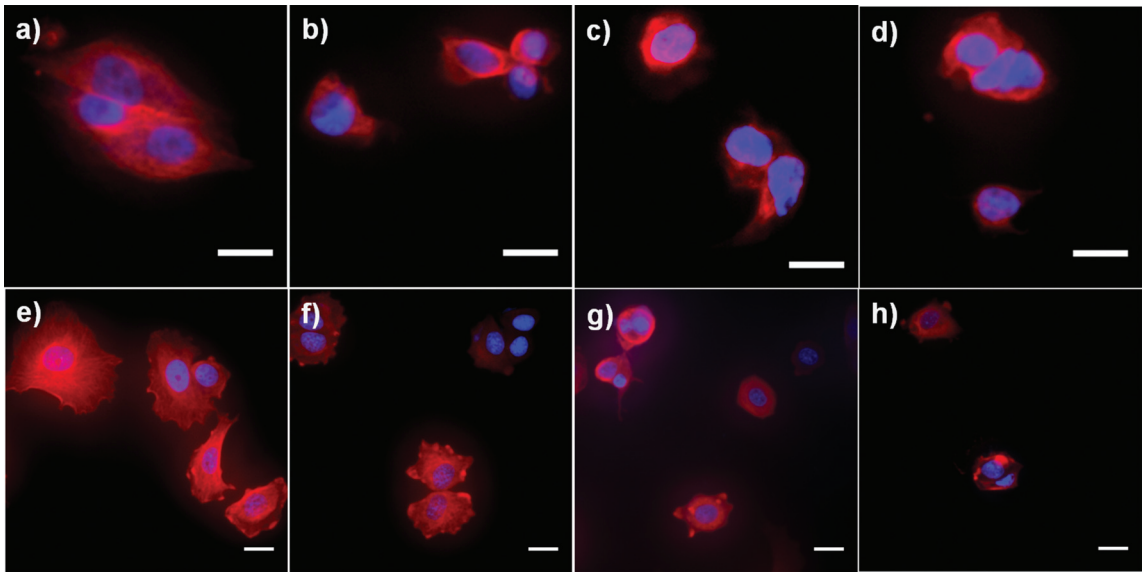

Fig. 3 Changes in the cellular microtubule network on treatment with **1** and its complexes for 24 h. Upper panel: (a) untreated A549 cells; (b) with **1**; (c) with **1A**; (d) with **1B**. Lower panel: (e) untreated MCF7 cells; (f) with **1**; (g) with **1A**; (h) with **1B**. [Scale bar = 20 μm; tubulin labelled by antibody (red) and nucleus stained by DAPI (blue)].

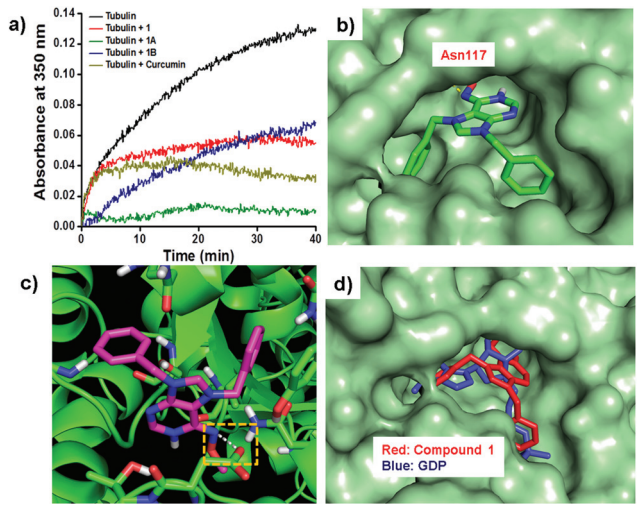

Fig. 4 (a) Turbidity assay indicating the inhibition of tubulin polymerization by curcumin, **1**, **1A** and **1B**. (b) Docked image depicting the interaction of **1** with Asn117 in β-tubulin. (c) Binding of **1** with the Asn117 residue of β-tubulin through H-bonding. (d) Docking studies illustrate a similar binding pocket for **1** and guanine nucleotide, in the β-tubulin subunit.

In conclusion, we have presented carbene generation on a rare purine tautomer, its characterization by analytical methods, its structure *via* DFT calculations, and its potential anticancer activity against lung carcinoma and breast cancer cell lines, A549 and MCF7, respectively. **1**, **1A** and **1B** altered the cellular morphology by inhibiting tubulin polymerization *via* noncovalent interactions in the tubulin binding site. Given their structural similarity to guanine, **1** and its metal complexes are implicated in blocking the GTP binding site. We surmise that this approach may present a new avenue for the discovery of microtubule polymerization inhibitors. Further investigations in this direction are currently in process.

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