On the G-Quadruplex Binding of a New Class of Nickel(II), Copper(II), and Zinc(II) Salphen-Like Complexes

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The involvement of non-canonical DNA structures, such as G-quadruplex (G4) DNA, in cancer development and progression has set the pace towards the renaissance of DNA-binding metal complexes. In this work, we report the DNA-binding of three Ni(II), Cu(II), Zn(II) complexes of a salphen-like N,N-donor ligand, bearing two imidazole groups condensed with a phenylenediamine moiety. Both duplex and G4 DNAs derived from human telomeres (hTel), and a sequence mimicking the promoter of the oncogene myc (c-myc) were studied. UV-Vis and circular dichroism spectroscopic binding studies pointed out that, while all the three complexes bind the selected oligonucleotides, the Cu(II) derivative is the strongest and G4-selective compound of the series. Lastly, FRET DNA melting assay results on the Cu(II) complex/hTel G4 system were interpreted by a loop-binding mechanism of interaction, corroborated by molecular dynamics (MD) simulations.

Metal-based compounds have generated wide interest in several fields of medicinal chemistry for their multiple application, spanning from bioimaging agents to potential drugs with antiparasitic, antibacterial, antirheumatic or anticancer effects, amongst all.[1] In this respect, the discovery of the potent anticancer effect of cisplatin has shed light on the use of metal complexes in therapies, and on DNA as a target for metallodrugs.[2] Several inorganic DNA-binding compounds have indeed shown interesting anticancer properties.[3,4] However, the development of DNA-targeting compounds has been hampered by the ubiquitous nature of the polynucleotide, mainly responsible for the seriousness of these compounds’ side effects. Several groups, seeking for a more selective approach, have then started to explore non-canonical DNA secondary structures as possible targets for anticancer drugs.[4] Of all of them, G-quadruplexes (G4 s) have rapidly caught the attention of the scientific community,[5] especially since their visualization in human cells in 2013.[6] These secondary structures, where tetrads of guanines are stacked to each other, seem to be involved in numerous crucial mechanisms for cancer development, such as telomeres integrity maintenance or oncogenes’ transcription control.[6] Consequently, several metal complexes were explored as G4-targeting molecules,[7] as their binding to these secondary structures could induce potential anticancer effects, e.g. by inhibiting telomere extensions or oncogene expression.[8] Of all the possible binding modes, the most explored is the π-stacking of an extended planar aromatic system onto a peripheral guanine tetrad, which grants a tight binding and the stabilization of the G4. In this context, salphen-like metal complexes have been widely investigated for their interesting and easily fine-tuned optical and binding properties, thus serving as an ideal scaffold for achieving this G4-binding.[7] Seeking for an improvement of the G4-DNA-binding capabilities and selectivity over B-DNA, different substituents have been introduced in the N,N bridged phenyl ring,[9] while the latter has also been replaced by an ethylene,[9] naphthalene,[10] or pyrimidine[11] moiety, for instance. Besides, different metal ions have been explored to fine-tune the structural, optical and DNA-binding properties of these compounds.[10,12]

Moreover, various positively charged groups have been introduced in the salicylaldehyde scaffold, to increase water solubility and strengthen the interaction with the G4-DNA by electrostatically binding to its negatively charged backbone.[10,13] With our surprise, however, no modification on the nature of the 2-hydroxy-aldehydic moiety has been reported so far. To fill this gap and increase our knowledge on the G4-binding of Schiff base metal complexes, we have synthesized three metal complexes of an N,N-donor ligand, named “phenim” (Figure 1), bearing two 5-membered aromatic rings in place of the 6-membered of the classic salphen ligands. This scaffold provided us with important information on the role played by the size of...
the aldehydic moiety in the G4-binding. Noteworthy, while Cu$^{2+}$ and Zn$^{2+}$ complexes of this ligand were already studied in 2018 for their catalytic properties towards benzyl alcohol oxidation,[14] to the best of our knowledge our report represents the first attempt so far to study the G4-binding properties of this novel class of Schiff base complexes.

Using a previously reported procedure,[15] we synthesized three phenim metal complexes having a Ni$^{2+}$ (1), Cu$^{2+}$ (2) or Zn$^{2+}$ (3) center (Figure 1a). Mass spectrometry confirmed the achievement of the three dicationic complexes (Figures S1–3); furthermore, a second abundant species, corresponding to the monocharged ion [ML–H]$^+$ upon loss of the imidazolium proton after ionization,[16] was also detected (Figures S1–3). $^1$H- and $^{13}$C-NMR in $d_2$-acetone of 3 further confirmed the Schiff base formation and its metal coordination, with the imino proton and carbon signals downfielded (respectively at 9.40 ppm and 149.19 ppm) as reported for similar compounds (Figure S4, Figure S5).[8b,11,15]

Any attempt in recording a $^1$H-NMR for 1 resulted in a non-resolved spectrum, whether using a coordinating or non-coordinating solvent. These data were interpreted in terms of coordination chemistry properties of our Ni$^{2+}$ complex in the triplet spin state in solution and therefore paramagnetic because of its distorted octahedral geometry. Such hypothesis is nicely corroborated by the solid-state structure of 1 determined by X-ray crystallography (Figure 1b), and by DFT calculations performed on both the singlet and triplet spin states, as detailed in the SI (Tables S1–8). Interestingly, although devoid of any positive side chains, are within the same range as those reported for similar Cu$^{2+}$ and Zn$^{2+}$ complexes.[8b,10–11]

Circular dichroism (CD) was used to assess whether the DNA-binding of 1–3 causes perturbation of the secondary structures of the three DNA sequences. The latter, indeed, in our experimental conditions typically show well distinct CD signals.[19] Upon addition of increasing amounts of our compounds, we found that, while 3 interacts with the CT-DNA inducing no conformational changes (Figure S7c), both 1 and 2 slightly modify the secondary structure of the oligonucleotide, as assessed by the marginal variations of its diagnostic CD bands (Figure S7a,b). Furthermore, an increase of the positive band and a decrease of the negative CD signal in hTelo solution was recorded in the presence of 1–3 (Figure S7d–e), suggesting a minor conformational change of its secondary structure, as already reported for other metal complexes.[9,12b,20] No significant changes were detected in c-myc solutions (Figure S7f–g).

Overall, in the presence of increasing amounts of each metal complex, only weak variations in the CD signals of the oligonucleotides could be recorded. Nevertheless, the appearance of induced CD (ICD) bands at ca. 300 nm for 1–3 (Figure S7) further confirms the binding occurrence of these complexes with the chiral DNA structure, which generates new CD chromophores.

As 2 has shown the most interesting G4-binding properties, we decided to further investigate on its binding to hTelo, chosen as our G4 model. Förster Resonance Energy Transfer (FRET) DNA melting assay pointed out that despite the tight binding towards hTelo, 2 could stabilize the G4 only by ca. 1.5 °C (Figure S8), much lower than other metal salphen complexes reported in the literature.[8a,9] Intrigued by this result, we have...
tried to provide an atomistic interpretation by performing a 300 ns molecular dynamics (MD) simulation on the 2/htelo complex. The root mean square deviation (RMSD) plot shows that the guanine bases (red line in Figure S9) rigidly remain in the same position along the simulation. In contrast, some variations occur when considering all the atoms of the oligonucleotide, mainly at ca. 70 and 200 ns (black line in Figure S9). Additionally, the structure obtained in the equilibrium phase suggests that the binding of 2 does not involve any interaction with the G4-tetrad. A representative snapshot is reported in Figure 3 and shows that 2 interacts via π–π stacking with flanking bases A3 and T20 of the loop that spans on top of 3'-end. Some organic and inorganic compounds with interesting G4-stabilization properties were already reported to interact with the G4-loops. However, differently from 2, these compounds could intercalate between the top loop and the 3'-end, by π–π stacking also with the G-tetrad. We therefore surmise that the lack of the interaction with the G-tetrad might account for the decreased stabilization capabilities of 2.

In conclusion, three complexes of nickel(II) (1), copper(II) (2) and zinc(II) (3), with square planar or octahedral geometry, have been synthesized and characterized to explore how the DNA binding properties of salphen-like metal compounds are influenced by the presence of two 5-membered rings in the aldehydic core. All compounds interact with duplex and G4-DNAs. However, this new ligand changed the electronic and structural properties of the nickel(II) complex 1, significantly reducing its expected G4-DNA binding affinity. On the contrary, the reduction in size, from the 6- to 5-membered ring, of the aldehydic core did not affect the DNA-binding affinity of 2 and 3 compared to the analogous salphen complexes. Finally, replacing the salphen with the phenim ligand reduces the G4-stabilizing capabilities of the Cu²⁺ phenim complex, 2. This is likely due to the loop-binding mechanism of interaction of 2 with hTelo, which does not involve any G-tetrads, according to the MD simulation results.

Overall, the use of a phenim ligand led to metal complexes (2 and 3) endowed of similar, yet unique, binding affinity towards the G4-DNAs if compared to the well-established salphen complexes. As such, this scaffold stands as a good candidate for further improving and exploring the G4-binding and stabilizing properties of salphen-like metal complexes. For instance, the introduction of positively charged arms in place of the imidazolium proton can foster the G4-binding of this class of compounds. Similarly, the extension of the aromatic system on the NN bridged-phenyl ring might allow the intercalation of
the metal complexes between the G-tetrad and top loops, thus improving their G4-stabilization capabilities.

Deposition Number 2058320 (for 1) contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.

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Conflict of Interest

The authors declare no conflict of interest.

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