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PD(II)/PT(II)/AU(III)-BASED COMPLEXES AS RNA BINDERS

Five metal complexes bearing aminopyridyl-2,2'-bipyridine tetradentate ligands and possessing a quasi-planar geometry were challenged toward different types of nucleic acid molecules including RNA polynucleotides in the duplex or triplex form and an RNA Holliday four-way junction. Natural double helix DNA and a DNA G-quadruplex are also considered for comparison. The binding mechanism is studied in detail and showed high RNA affinity tuned by structural features.

Beyond the cornerstone anticancer metal complex cisplatin, some research groups focus now their attention on other coinage metal centres **[1, 2]**. Au(III) and Pd(II) have similar coordination but different chemical properties, which may result in different biological activities. The choice of the ligand plays a major role: for Au(III) to limit the tendency to be reduced to Au(I) or Au(0) in biological media, for Pd(II) to restrict the greater kinetic lability **[3, 4]**. In this work, we focus more on Pd(II) complexes, but within a set of metal complexes which also contain the Pt(II) and Au(III) counterparts for comparison purposes. As for the ligands, we selected tetradentate nitrogen ligands, as they were found to form stable Pd(II) complexes, with improved ability to reach their biological target **[5]**. Despite the efforts of the scientific community to unravel the activity of many metal complexes to be used as drugs, there is still room for mechanistic studies on solution equilibria, which may both enlighten the potential medicinal interest of a new complex and help to define robust structure/reactivity relationships. This is particularly true for DNA-binding studies, where the role of non-canonical structures (G4, i-motifs) stabilisers has been evidenced **[6, 7]**. This is even more true for RNA studies that are less abundant, compared with DNA, and which may concern both polynucleotides or oligomers forming pecu-

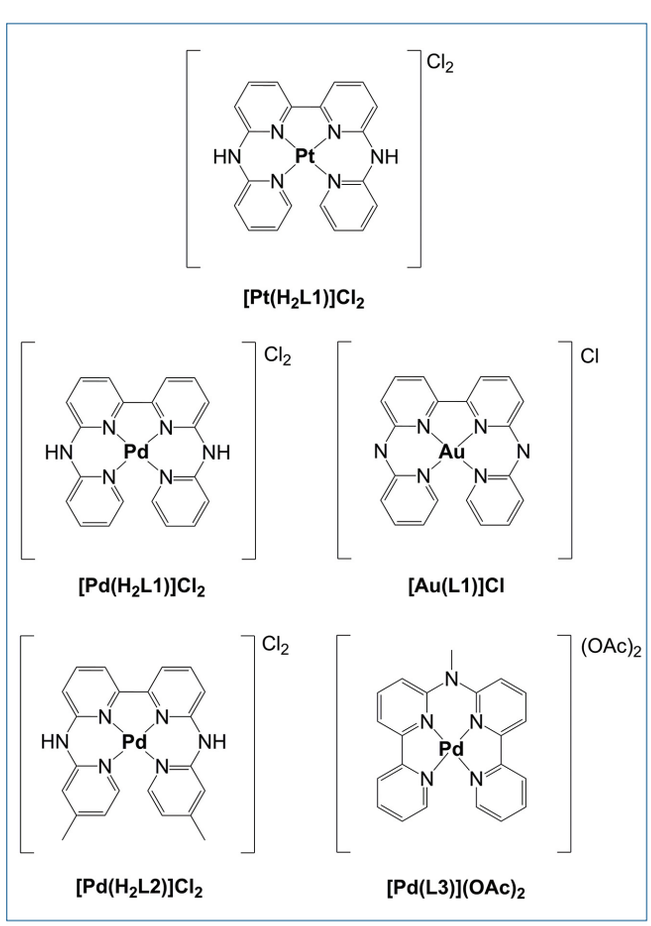

Fig. 1 - Molecular structures of the analysed complexes

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liar structures (as the Holliday four-way junction considered in this work). Mechanistic solution studies on the interaction between Pd(II) complexes and poly/oligo RNAs are quite rare whilst, to the best of our knowledge, RNA four-way junctions (4WJ) have never been analysed **[8, 9]**. RNA 4WJs belong to the different junctions which are common architectural features in RNA; they play a role in RNA folding which is a critical feature in the regulation of any RNA activity. RNA 4WJs show a higher diversity with respect to those based on DNA, with a continuous interconversion between parallel and antiparallel conformations, whereas DNA 4WJs typically adopt a fixed antiparallel structure **[10]**. The structures of the metal complexes considered in this work are shown in Fig. 1. $[Pt(H_2L1)]Cl_2$ complex (where H_2L1 is N-(6-(6-(pyridin-2-ylamino)-pyridin-2-yl)pyridin-2-yl)pyridin-2-amine) was discovered by some of the authors of the present work, to drive the crystallization of a DNA oligomer into a 4WJ-like motif **[11]**. The logic is to analyse how the change in the geometry/rigidity of the tetradentate ligand or a different metal centre may modulate the reactivity with different nucleotide substrates.

	K_{app} (10^4 M^{-1})				
	$[Pd(H_2L1)]^{2+}$	$[Pd(H_2L2)]^{2+}$	$[Pd(L3)]^{2+}$	$[Pt(H_2L1)]^{2+}$	$[Au(L1)]^{+}$
Auto-aggregation	*yes*	*yes*	*no*	*yes*	*no*
poly(rA)·poly(rU)	6.7±0.2	9.3±0.3	16±2	27±2	1.4±0.1
poly(rA)·2poly(rU)	2.5±0.5	12±1	50±5	17±3	5.4±0.5
CT-DNA	1.0±0.1	1.1±0.1	3.4±0.5	+∞	0.58±0.07
G-quadruplex	+∞	+∞	+∞	+∞	30±2

Tab. 1 - Binding constants for different metal/nucleic acid systems at 25.0 °C. NaCl 0.1 M, NaCac 2.5 mM, pH=7.0; for G-quadruplex the buffer is KCl 0.1 M, LiCac 2.5 mM, pH 7.0; +∞ = quantitative aggregation on the nucleotide/cooperative reaction

Polynucleotides binding

Spectrophotometric titrations were carried out with increasing amounts of the synthetic RNA duplex (poly(rA)poly(rU), made of one strand of adenine and one strand of uracil) or triplex (poly(rA)2poly(rU), a duplex with an additional strand of uracil) or natural DNA duplex (calf thymus DNA, or CT-DNA) added to the metal complex solution. The very significant changes observed in the absorbance profile indicate that some form of interaction does indeed take place for all systems. According to a simplified model, where a nucleotide reacting unit (base pair/triplet/G4) interacts with one complex molecule (dye, drug) the relevant binding constant, K_{app}, can generally be evaluated. K_{app} is an apparent equilibrium constant as it has to account also for possible metal complex self-aggregation. Tab. 1 reports the data collected; they immediately point out that the binding affinity is the intricate result of the interaction between the ligand, the metal centre, and the substrate. The affinity for the RNA polynucleotides was further investigated using melting tests. The stabilisation of poly(rA)2poly(rU), even if lower than the double-stranded counterparts, remained high. This fact, together with the high K_{app} values for poly(rA)2poly(rU), indicates that the third strand does not significantly prevent binding. As in the triplex, the wide groove is hindered by the third strand, this observation would suggest that the binding occurs principally in the minor groove. Interestingly, the affinity of all the complexes is lower for double-stranded DNA with respect to both double and triple-stranded RNAs (Tab. 1). The very similar changes in the metal complex spectral profile upon metal complex/nucleotide interaction suggest that the binding mode is mainly the same (total/partial intercalation) for DNA and RNAs with an efficacy tuned by geometrical changes. An exception is $[Pt(H_2L1)]^{2+}$, the polynucleotide-bound absorbance profile of which is greatly different between DNA and RNAs. This observation agrees with the existence of a quantitative/cooperative binding for the $[Pt(H_2L1)]^{2+}$/CT-DNA binding. Such features may be related to aggregation of the platinum complex on the polynucleotide template, which would occur on the DNA grooves and not in the RNAs ones.

DNA G-quadruplex binding

The affinity of this family of complexes for G4 structures (CTA-22 sequence, antiparallel DNA human telomere) was evaluated by means of absorbance titrations, melting tests and mass spectrometry experiments. With the exception of the $[Au(L1)]^{+}$/G4

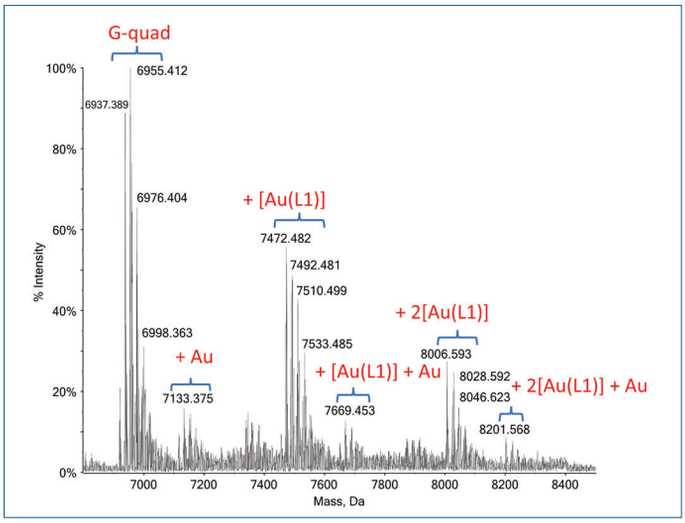
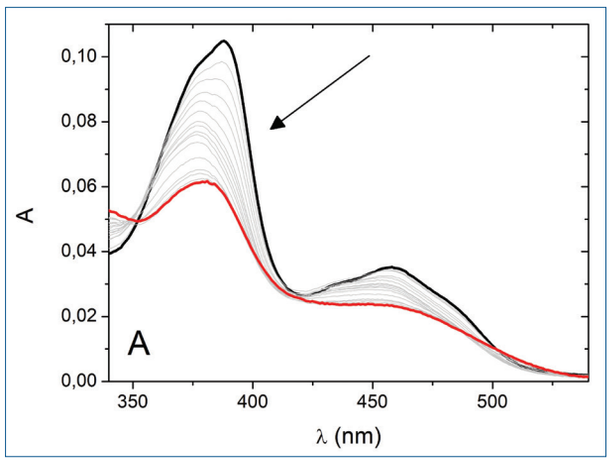


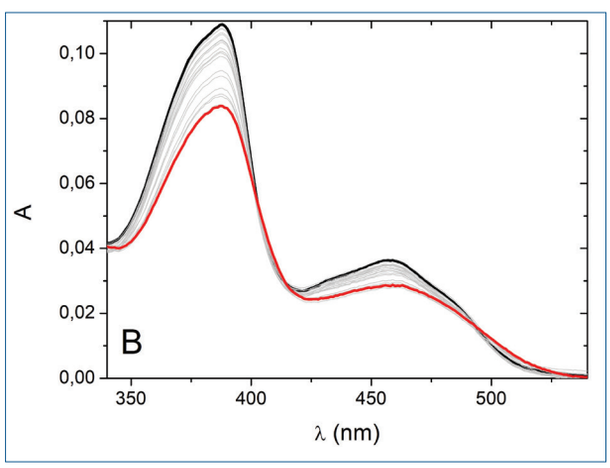
Fig. 2 - Mass spectrum of 10^{-6} M G4 incubated for 24 h with [Au(L1)]Cl in 100 mM ammonium acetate solution (pH 7.0) and in the presence of 60% EtOH. 3:1 metal complex/G4 molar ratio

system, the reaction turned out to be quantitative (Tab. 1). Melting tests showed some non-negligible stabilisation of the oligonucleotide upon adduct formation. This strong interaction was hence further inspected by means of ESI mass spectrometry. The spectrum of G4 alone shows a signals cluster at 6937.358, 6954.347, 6975.375 and 6998.323 Da that was in perfect agreement with the CTA-22 molecular mass plus up to four ammonium ions, which promote the G4 assembly and are accommodated inside the structure **[12, 13]**. Subsequently, the obtained G4 was incubated for 24h with each of the metal complexes and the mass spectra of the respective solutions were recorded. The experiments highlight that both adducts of the metal ion alone, or of 1, 2, or 3 metal complexes, can be observed interacting with the G4. Overall, both the nature of the metal centre and the ligand geometry influence the reactivity of this type of metal complexes. In fact, the reactivity of [Au(L1)]Cl was much more complicated (Fig. 2). Probably, the presence of the Au(III) centre could trigger some redox equilibria during the reaction with the biomolecules leading to the consequent release of Au(I) ions. This reactivity behaviour is characteristic and quite common for Au(I-II)-based compounds and it has been well described in the literature **[14]**.

RNA 4WJ interactions

Coming back to the main focus of our work, we switched from the RNA polynucleotides above to the analysis of the possible binding to peculiar RNA oli-





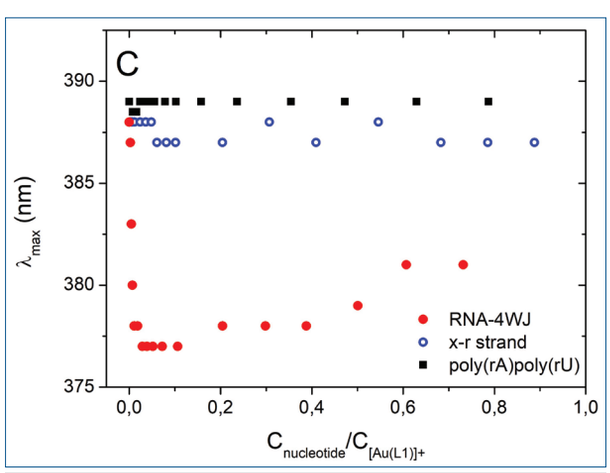


Fig. 3 - UV-vis titrations $[Au(L1)]^+$ (C_{Au}=6.36×10^{-6} M) with RNA oligonucleotides, CaCl2 182 μM, pH 7.0, T=25.0 °C. (A) $[Au(L1)]^+$/RNA-4WJ system, C_{RNA4WJ} from 0 M (——) to 4.65×10^{-6} M (.....); the arrow points to the $[Au(L1)]^+$ band which, interestingly, upon RNA-4WJ increase, undergoes first a blue and then a redshift.; (B) $[Au(L1)]^+$/xr strand system, $C_{xrstrand}$ from 0 M (——) to 4.54×10^{-6} M (.....); (C) position of the absorption maximum (nm) of $[Au(L1)]^+$ as a function of the nucleotide added for addition of RNA-4WJ (full red circles), x-r stand (open blue circles) or poly(rA)·poly(rU) (black full squares)

gos. RNA-4WJ deserve much interest as a possible specific anticancer drug target. They have an active role in RNA folding but, to the best of our knowledge, no information about metal complexes binding is available. Given the tendency of $[Pd(H_2L2)]^{2+}$ to lose its ligand in solution, this complex was discarded from our studies with the delicate RNA-4WJ substrate. The spectral profile changes indicate the presence of an interaction in all four cases. This is not surprising as the RNA-4WJ junction contains four double-stranded RNA appendices. Thus, some dye binding may occur here, as already evidenced by the experiments with poly(rA)poly(rU). On the other hand, a different behaviour with respect to what was already observed for the polynucleotide would suggest specific binding to the RNA-4WJ. Such an observation was made for the $[Au(L1)]^{+}$ complex, where the spectral evolution upon RNA-4WJ addition differed from what was observed for poly(rA) poly(rU) titrations. Also, binding to RNA-4WJ did not generate the same spectroscopic signature as when two only (x and r) coupled strands were added (Fig. 3A-C). There are two opposite shifts (first blue, then redshift) which suggest the formation of two types of adducts: Fig. 3C emphasises this behaviour by plotting the position of the maximum against the nucleotide content. The same panel shows that these shifts happen only for the RNA-4WJ and not for RNA polynucleotides nor x-r two-strands coupling. Melting tests were done on RNA-4WJ systems. The mixtures were heated, cooled down slowly until r.t. and then re-heated a second time. This procedure enables to enlighten possible peculiar adduct geometries which become accessible only when the junction is opened. The same experiments were repeated also using known reference dyes such as ethidium bromide (EB), thiazole orange (TO), coralyne and TMPyP4. Fig. 4 shows the results: it can be observed that there are two different behaviours. (A) EB, TO and Coralyne undergo reversible binding modes and produce a RNA-4WJ stabilisation which is reproducible over the two consecutive runs; (B) TMPyP4 first stabilises the system, then places itself in a position which strongly destabilises the RNA-4WJ in the second run. $[Pt(H_2L1)]^{2+}$ and $[Pd(L3)]^{2+}$ belong to type (A), whereas $[Pd(H_2L1)]^{2+}$ and $[Au(L1)]^{+}$ belong to type (B), probably correlated to peculiar interactions with RNA-4WJ cavity.

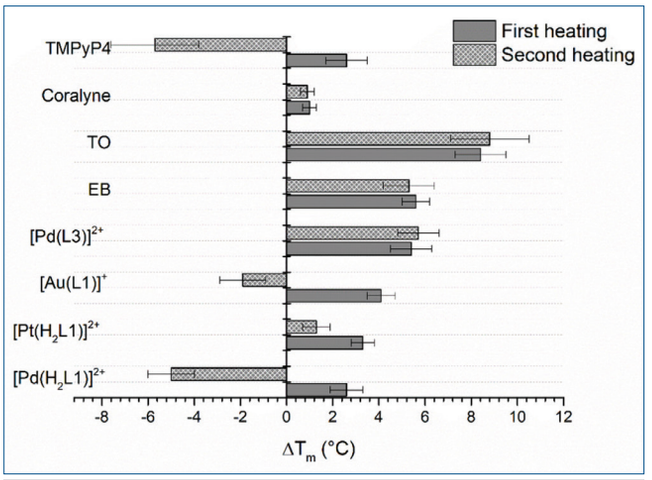


Fig. 4 - Melting temperature difference (ΔT_m, °C) between that of a drug/RNA-4WJ mixture and that of the RNA-4WJ alone; C_{drug}=20 μM, C_{RNA4WJ}=10 μM, $CaCl_2$ 182 μM, pH 7.0

Conclusions

According to this study, all the metal complexes interact with RNA (and DNA) fragments, but the exact features of this interaction are in each case the result of a complex structure-reactivity relationship (SAR) involving both the ligand, the metal centre, and the polynucleotide. The different metal centre and/or the ligand produces planar geometries with different distortion degrees. A higher planarity may produce two opposite effects: on the one hand, it favours intercalation between DNA base pairs, and on the other hand, it favours (also on the polynucleotide grooves) dye-dye aggregation processes which inhibit intercalation. The net effect will thus be a delicate compromise of these two opposite trends. Interestingly, the analysed metal complexes seem to react better with RNAs with respect to DNA, even in the triplex form. As for the binding with the RNA-4WJ, $[Pd(H_2L1)]^{2+}$ and $[Au(L1)]^{+}$ metal complexes seem to be species able to bind RNA at the junction. These species, similarly to $Pt(H_2L1)]^{2+}$/DNA-4WJ structure, may possess those specific molecular properties which trigger/stabilise a peculiar superstructure **[10]**. Further studies are ongoing to elucidate this point.

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Complessi di Pd(II), Pt(II) e Au(III) come leganti di RNA/DNA

In questo lavoro si analizzano le interazioni di cinque nuovi complessi metallici costitiuti da ligandi amminopiridil-2,2'-bipiridinici tetradentati con differenti tipologie di RNA, in particolare doppia e tripla elica e una RNA "Holliday four-way junction". La doppia elica di DNA naturale e DNA G-quadruplex sono studiate a fini comparativi. Il meccanismo di interazione è stato analizzato in dettaglio, osservando un'elevata affinità per l'RNA modulata dalla struttura dei complessi metallici.

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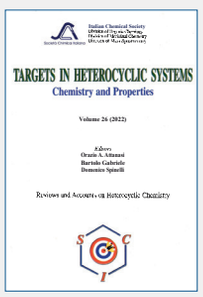

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