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## Binding of transition metal complexes to guanine and guanine–cytosine: hydrogen bonding and covalent effects

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**Abstract** Density functional calculations and Atoms in Molecules analysis are used to investigate the role of covalent and hydrogen bondings in determining the binding of transition metal complexes to guanine, and the subsequent effect on pairing with cytosine. Hydrogen bonding is ubiquitous, and typically contributes ca. 10% to overall binding, a value that varies with the coordination site on guanine, as well as metal and ligands. Early transition metals show a clear preference for the O6 position, while later ones prefer N7, the crossover point coming at the vanadium group. Metallation at N7 causes a redistribution of hydrogen bonding strength between guanine and cytosine, but does not greatly affect the overall pairing energy. In contrast, metallation at O6 strongly reduces the pairing energy, as may be expected given the role of O6 in pairing guanine with cytosine. This effect can be quantified using electron density properties, and seems to be due to both electrostatic repulsion from the positive metal centre and a redistribution of electron density within guanine itself. Qualitative agreement with experimental mass spectroscopic results is obtained.

**Keywords** DNA damage · Anticancer drug · Electronic structure · Computational chemistry

### Introduction

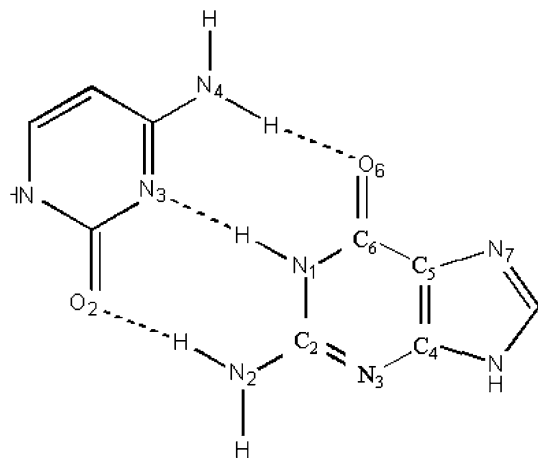
Drugs based on metal complexes are of increasing interest to medicinal chemists [1, 2]. The anti-tumour activity of platinum-based drugs is well known, but other metals such as gold, ruthenium, titanium and vanadium also show promise in this area [3, 4]. Among several modes of action, binding of metals to DNA is

one of the most important. Metal complexes interact with many different sites of nucleic acids, and can bind the canonical DNA structure or lead to non-canonical forms, such as triplexes, quadruplexes, junctions, etc. In general, the favoured sites are the negatively charged phosphate groups and electron-rich N and O atoms. For the latter, the following order has been established for transition metal ions at neutral pH: N7/O6 of guanine (G) > N3 of cytosine (C) > N7 of adenine (A) > N1(A) > N3(A,G) [5]. Perhaps the most studied transition metal drug is cisplatin, a widely used anti-tumour drug, which attacks the DNA as its main target, attacking both guanine and adenine [6], with a strong preference for the former [7]. Platination leads to large structural rearrangements in DNA, ultimately resulting in cell death through apoptosis, necrosis or both [8]. Among other transition metals V, Ti [9], Ru [10–12], gold [13, 14], Rh [15], Ir [16], Cu [17] and Co [18] are considered potentially active against tumours, though DNA may not be the critical target for all such metals. Thus, transition metals represent a source of possible new drugs for treating cancer or other diseases.

Here we focus on the binding of transition metals to guanine via both N7 and O6 sites (see Fig. 1), the most favourable for metallation of the free DNA bases, and also on the effect of complexation on the guanine–cytosine (GC) pair. Much theoretical work has been carried out on cisplatin's chemistry [19–22], including our recent DFT and Atoms in Molecules (AIM) study of the binding of cisplatin to purine bases [23]. Several studies of metals other than platinum have also been reported: Hobza and co-workers [24] showed that metallation ( $M = \text{Mg}^{2+}$ ,  $\text{Hg}^{2+}$ ) distorts the Watson–Crick pair in a similar manner to cisplatin, weakening the N–H···O6 H-bond and strengthening the O2···H–N2 H-bond. Importantly for our study, they also showed that Hartree–Fock calculations do not differ significantly from MP2 ones.

Poater et al. [25] studied binding of metal cations ( $M = \text{Cu}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Cu}^{2+}$ ) to guanine and its effect on GC, explaining distortions in terms of the donor–acceptor

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**Fig. 1** Numbering scheme for guanine–cytosine pair

character of the bases and detecting increased aromaticity of guanine and cytosine six-membered rings. Bowers and co-workers [26] reported use of ion mobility mass spectrometry to analyse gas-phase metal cation binding to DNA bases, suggesting that “soft” cations such as Pt bond to guanine N7, and hence stabilise Watson–Crick structures, whereas “hard” cations, such as  $\text{Li}^+$ ,  $\text{Cr}^{2+}$ ,  $\text{Mn}^{2+}$ , etc. prefer guanine O6, promoting globular structures of dinucleotide duplexed in which GC is disrupted.

Despite the large volume of work in literature, a systematic study of the effect of metallation on the GC pair has not been carried out. In order to fill this gap, in this work we propose a systematic analysis of all the transition metals, to investigate the properties of metal–guanine adducts and monitor the effect on the GC pair.

## Computational details

All ab initio and DFT calculations were performed using Gaussian03 [27]. As in Ref. [23], geometry optimisations were carried out without symmetry constraints at the HF level using the 6-31G(d,p) basis set [28] on C, H, O and N atoms and the SDD basis set and ECP [29] on metal. Following harmonic frequency calculation confirmation as minima or transition state, subsequent

single point energy and electron density calculations were performed using the B3LYP density functional [30, 31] with a DGDZVP basis set [32] on C, H, O, and N and SDD on metal. This combination of methods was shown to reliably reproduce binding energies of cisplatin to guanine and adenine, and also the energy of pairing guanine with cytosine. Binding energies were calculated via the difference in energy of complexes from those of isolated metal and guanine or guanine–cytosine fragments, where all fragments were fully optimised at the same level.

Extensive use was made of Bader’s [33] theory of Atoms in Molecules (AIM), which partitions molecules into constituent atoms on the basis of the electron density. In this work, we have concentrated solely on topological analysis of the density [34], i.e. finding those critical points in the electron density where the gradient of the density,  $\nabla\rho$ , is zero. Properties at such critical points (CPs), especially at (3, −1) or bond CPs (where the electron density has two positive curvatures, perpendicular to the bond, and one negative curvature, parallel to the bond) have found extensive use in characterising bonding interactions. The presence of such CPs, and the associated trajectories of  $\nabla\rho$  (collectively termed the molecular graph) allow rigorous identification of the interactions present within a molecule or complex [35]. Many studies have demonstrated approximately linear relations between H-bond stabilisation energy and both the increase in density at H⋯B bond CP and the decrease at A–H for a wide range of A–H⋯B systems. For instance, we recently showed a high-quality, family-independent method able to estimate H-bond energies, extensively employed in this work [23].

## Results and discussion

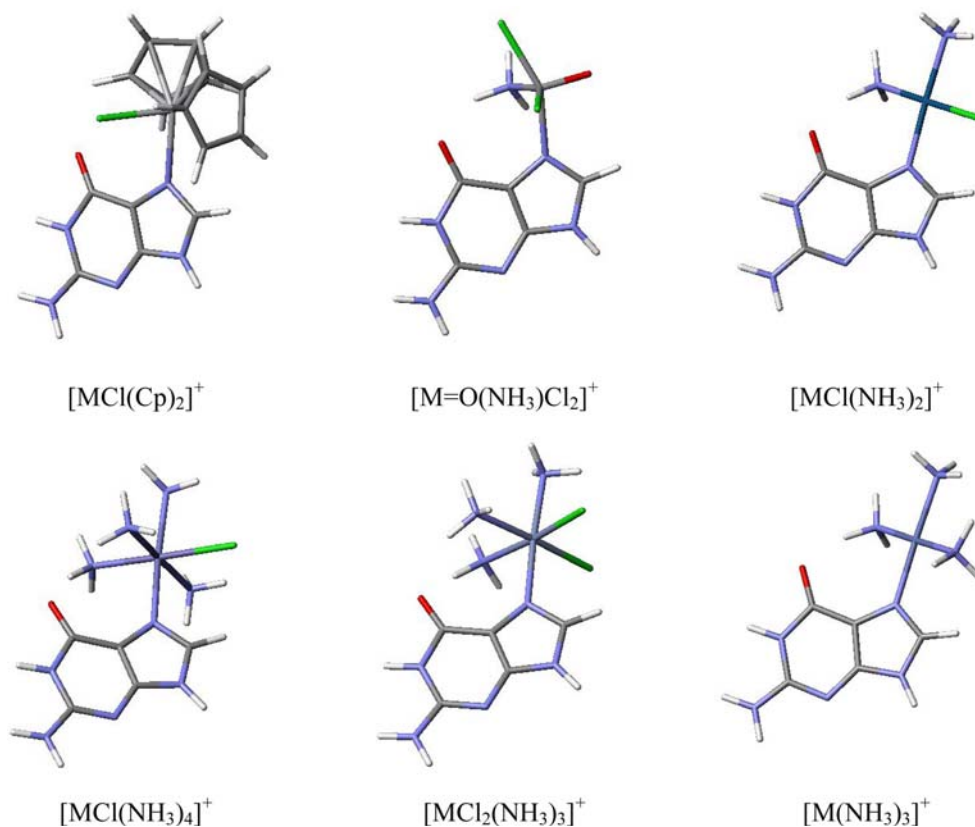
### Metal–guanine complexes

Table 1 and Fig. 2 report ligands, coordinations and electron configuration for each metal group studied. Ligands were chosen to obtain singly charged metals and, where possible, closed shell ions. In particular, all metals of the titanium group are  $[\text{M}^{\text{IV}}\text{Cl}(\text{Cp})_2]^+$ , since

**Table 1** Metal group elements features

Metal (oxidation state)	Electron configuration/spin state	Ligand(s)	Conformation
Ti(IV)	$d^0/\text{singlet}$	$[\text{Cl}(\text{Cp})_2]$	Tetrahedral
V(V)	$d^0/\text{singlet}$	$[(\text{Cl}_2\text{NH}_3)=\text{O}]$	Square pyramidal
Cr(II)	$d^3/\text{quartet}$	$[\text{Cl}_2(\text{NH}_3)_3]$	Octahedral
Mn(II)	$d^5/\text{sextet}$	$[\text{Cl}(\text{NH}_3)_4]$	Octahedral
Fe(II)	$d^6/\text{singlet}$	$[\text{Cl}(\text{NH}_3)_4]$	Octahedral
Co(I)	$d^8/\text{singlet}$	$[(\text{NH}_3)_3]$	Square planar
Ni(II)	$d^8/\text{singlet}$	$[\text{Cl}(\text{NH}_3)_2]$	Square planar
Cu(I)	$d^{10}/\text{singlet}$	$(\text{NH}_3)_3$	Square planar
		$(\text{NH}_3)$	Linear
Zn(II)	$d^{10}/\text{singlet}$	$[\text{Cl}(\text{NH}_3)_4]$	Octahedral

**Fig. 2** Ligands from Table 1, shown as complexes to N7 of guanine



such complexes are known to have anti-tumour activity [9]. The vanadium group elements considered are in the fifth oxidation state, as  $[M^V=O(NH_3)Cl_2]^+$  in approximate square pyramidal conformation. The Cr group metals are  $d^3$ , octahedral as  $[M^{II}Cl_2(NH_3)_3]^+$ . Fe, Zn and Mn group elements share the same ligands, as octahedral  $[MCl(NH_3)_4]^+$  complexes. The Co group metals are in the oxidation state I, with three  $NH_3$  ligands in square planar orientation. The Ni group metals are derived from cisplatin as square planar  $[M^{II}Cl(NH_3)_2]^+$ . For the Cu group, two different ligands were employed, namely square planar  $[M^I(NH_3)_3]^+$  for Cu and Ag and linear  $[M^I(NH_3)]$  for Au. In this way, we aim to eliminate the gross effect of changing the overall charge on the complex, and hence to study the more subtle effects of metal and ligand change.

Table 2 contains energetic, geometrical and electron density properties of all metal–guanine complexes studied in this work. As shown previously, Hartree–Fock theory provides reasonable geometries for metal–guanine adducts [23]. To further test this, Table 2 contains data from B3LYP/6-31G\*\* optimisations (Lan12DZ basis set and ECP on metal). The agreement between DFT and our combined HF optimised, DFT single point (HF/DFT) calculations is satisfactory for all the complexes. Only in the case of the Mn–guanine adduct does HF significantly underestimate the binding energy, probably due to the fact that the correlation becomes important in the case of the Mn(II) sextet state.

Despite this, both methods agree that the binding energy of this complex is very low. As a further test,  $S^2$  values for any non-singlet complexes were calculated, and in all cases are close to the ideal values (between 3.76 and 3.78 for the Cr group, and all exactly 8.75 for the Mn group), indicating little or no contamination from other spin states.

In general, metal–guanine complexes contain two types of interaction (see Fig. 3), namely the covalent M–X (X = N7 or O6) bond and intramolecular H-bonds. As shown in Ref. [23], the total binding energy may be decomposed into contributions from covalent energy ( $E_{cov}$ ) and intramolecular H-bond energy ( $E_{intra}$ ), allowing us to separately monitor these effects. Table 2 reports total, hydrogen bond and covalent energies calculated in this manner, along with bond lengths and electron densities of M–X of all the studied complexes.

Let us discuss briefly the nature of the intramolecular hydrogen bonding. As seen previously, bonds such N–H...O, N–H...Cl and N–H...N are ubiquitous in metal–guanine complexes. The strength of these interactions depends on both the nature of the metal and the ligand, ranging from 3 to 8 kcal/mol. For example, the  $[MCl(Cp)_2] \cdots$ guanine complexes (M = Ti, Zr, Hf) have weak C–H...N and C–H...O H-bonds, with energies no larger than ca. 4 kcal/mol. Similarly, N–H...O and N–H...N interactions for the Co group complexes average around 4 kcal/mol. In contrast, N–H...O and N–H...Cl H-bonds in the Fe group contribute 7 kcal/mol on average, while in the Ni group, the H-bond motifs noted

**Table 2** Energies, electron densities and bond length in the metal...guanine complexes

Group	Complex	Binding energy <sup>a</sup> (kcal/mol)	$E_{\text{cov}}$ (kcal/mol)	$E_{\text{intra}}$ (kcal/mol)	$r(\text{M-X})$ (Å)	$\rho(\text{M-X})$ (au)
Ti	Ti-N	36.40 (35.67)	33.20	3.17	2.342	0.048
	Ti-O	51.88	48.43	3.45	2.000	0.085
	Zr-N	48.52	46.08	2.44	2.424	0.049
	Zr-O	60.75	57.58	3.18	2.133	0.074
	Hf-N	50.35	47.91	2.44	2.116	0.076
V	Hf-O	63.26	58.95	4.31	2.401	0.051
	V-N	57.88 (56.44)	53.24	4.64	2.079	0.087
	V-O	58.98	55.29	3.69	1.921	0.105
	Nb-N	68.05	68.05	0.0	2.312	0.062, 0.029 <sup>b</sup>
	Nb-O	68.07	68.07	0.0	2.154	0.077, 0.016 <sup>b</sup>
	Ta-N	71.95	71.95	0.0	2.310	0.063, 0.035 <sup>b</sup>
	Ta-O	72.07	72.07	0.0	2.171	0.075, 0.024 <sup>b</sup>
Cr	Cr-N	61.74 (60.90)	53.79	7.95	2.148	0.063
	Cr-O	60.27	55.75	4.52	1.973	0.081
	Mo-N	65.16	58.26	6.90	2.262	0.064
	Mo-O	61.60	57.24	4.36	2.125	0.074
	W-N	68.14	61.30	6.84	2.259	0.068
	W-O	63.90	59.52	4.38	2.135	0.074
Mn	Mn-N	34.05 (44.01)	27.27	6.78	2.430	0.034
	Mn-O	36.76	31.86	4.90	2.203	0.046
	Tc-N	29.47	26.17	3.3	2.613	0.035
	Tc-O	29.61	25.56	4.03	2.408	0.043
	Re-N	26.07	19.03	7.03	2.866	0.025
	Re-O	21.30	17.09	4.21	2.627	0.032
Fe	Fe-N	46.18 (46.11)	38.78	7.40	2.222	0.043
	Fe-O	46.96	40.05	6.91	2.086	0.048
	Ru-N	53.20	46.34	6.86	2.249	0.058
	Ru-O	49.52	42.80	6.72	2.189	0.055
	Os-N	57.99	50.61	7.38	2.225	0.067
	Os-O	52.49	45.71	6.78	2.200	0.060
Co	Co-N	43.33 (43.20)	43.33	3.41	2.218	0.050
	Co-O	41.15	41.15	3.83	2.145	0.040
	Rh-N	50.75	50.75	3.92	2.218	0.063
	Rh-O	44.83	44.83	4.58	2.252	0.046
	Ir-N	59.06	59.06	4.27	2.153	0.082
	Ir-O	49.70	49.70	5.04	2.204	0.058
Ni	Ni-N	66.85 (67.60)	61.21	5.64	1.907	0.083
	Ni-O	62.32	56.44	5.88	2.017	0.077
	Pd-N	71.54	64.16	7.38	2.050	0.085
	Pd-O	62.28	56.73	5.55	2.081	0.094
	Pt-N	80.45 (80.69)	72.99	7.46	2.092	0.103
	Pt-O	68.69 (67.41)	63.00	5.69	2.059	0.093
Cu	Cu-O	25.83 (23.70)	21.10	4.73	2.522	0.023
	Ag-O	24.96	20.56	4.40	2.604	0.026
	Au-N	80.42	80.42		2.108	0.102
Zn	Au-O	70.13	70.13		2.125	0.086
	Zn-N	29.11 (30.67)	21.60	7.51	2.419	0.034
	Zn-O	30.61	24.34	6.27	2.178	0.046
	Cd-N	32.75	26.93	5.92	2.532	0.036
	Cd-O	33.27	28.58	4.69	2.362	0.043
	Hg-N	42.82	36.94	5.88	2.472	0.048
	Hg-O	34.04	31.11	2.93	2.376	0.050

<sup>a</sup>Calculated using combined HF/DFT approach, except for values in brackets which are the binding energies calculated at the at B3LYP/6-31G\*\* (Lanl2DZ). Positive values indicate stabilisation

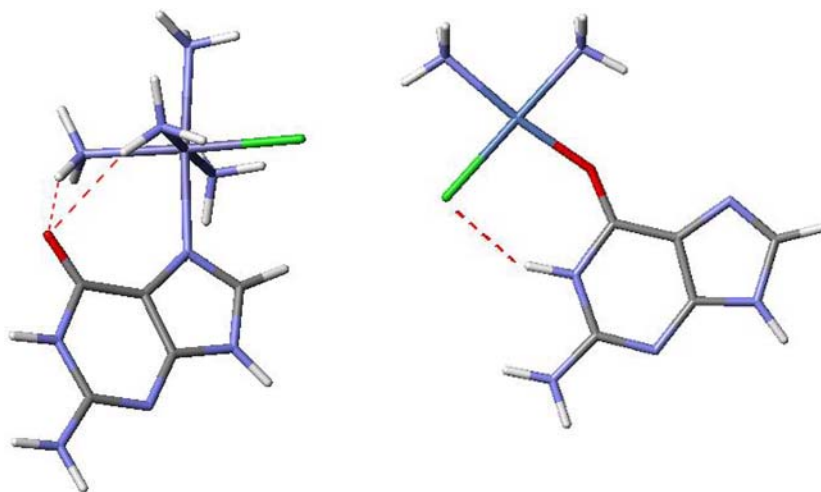
<sup>b</sup>Reported as the value for the principal interaction, then any secondary interaction (see text)

for cisplatin are conserved, with energies between 5.7 and 7.5 kcal/mol, the strongest being cisplatin's. Thus, generally H-bonding contributes ca. 10% to the total binding energy on average. However, in a few cases, no H-bonding was found, e.g. the binding energies of the Nb, Ta and Au complexes are purely from the metal-guanine interaction.

Table 2 also reports the covalent energies of the metal...guanine bonds, estimated as in Ref. [23] as the

total binding energy less the contribution from H-bonding. The results present a wide diversity of cases, mainly depending on the position of the metal in the periodic table. Thus, the covalent energies of the 54 studied complexes range between 20 and 80 kcal/mol, and the M-X length between 1.900 and 2.900 Å. Depending on the metal, the relative stability of M-O and M-N also varies. In such an intricate picture, it is still possible to spot interesting trends and tendencies

**Fig. 3** Intramolecular hydrogen bonds in Fe–N7 and Ni–O6 complexes



that can provide important information on the interaction of transition metals with DNA.

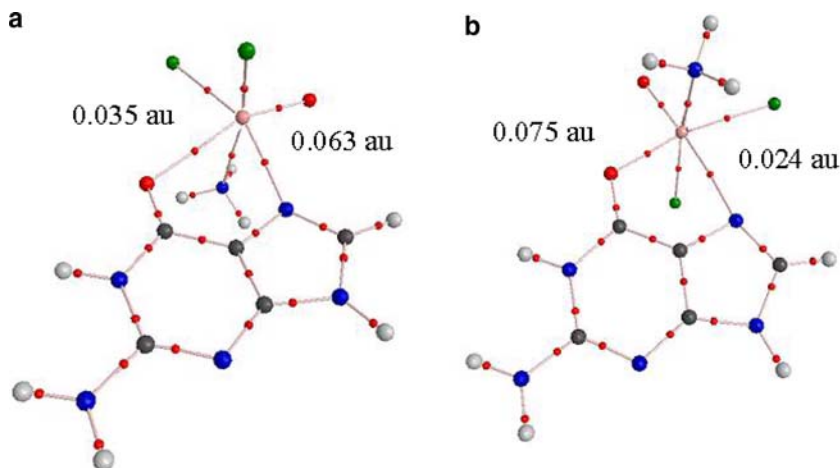
Stable complexes of the titanium group metals are found binding to both O6 and N7, having covalent energies between 40 and 80 kcal/mol. As is evident in Table 2, complexes at O6 are more strongly bound than at N7, with the average energy difference around 13 kcal/mol, in accord with the known preference for  $\text{Ti}^{\text{IV}}$  for hard oxygen ligands [36]. Moreover, the M–O bonds are shorter than the corresponding M–N. Interestingly, the total and covalent energies increase down the group, such that the Ti complexes are ca. 20 kcal/mol more weakly bound than the Hf complexes.

In the vanadium group, the total binding and the covalent energy increase compared to Ti, an effect that appears to be stronger for the N7 complexes, leading to almost equivalence of the energy of V–N and V–O, which differ by just 2 kcal/mol. The behaviour of Nb and Ta is intriguing, with M–N7 and M–O6 virtually isoenergetic (the difference is less than 0.1 kcal/mol on average). The metals are asymmetrically bound to N7 or O6, but also contain a secondary covalent interaction to

the other nucleophilic site on guanine, i.e. Nb–N and Ta–N complexes contain an interaction to O6. AIM confirms this point (see Fig. 4), and allows us to quantify each interaction: for instance, the electron density for the Ta–N complex at the Ta $\cdots$ O interaction is roughly half that of the Ta $\cdots$ N bond, while the Ta–O complex contains a weaker Ta $\cdots$ N interaction, with ca. 30% of the strength of the Ta $\cdots$ O bond. This is particularly interesting, as such chelating complexes of cisplatin, although weakly bound, are very active in destabilising DNA pairing bases [23, 37, 38].

Cr group metals show similar binding energies to both N7 and O6 of guanine, with covalent energies ranging between 54 and 60 kcal/mol. Again, these energies increase down the group: here the difference between, for instance, W–N and Cr–N is 7 kcal/mol. Also, the M $\cdots$ O6 bonds are clearly shorter than the corresponding M $\cdots$ N7. Metals of Mn group are the most weakly bound to guanine, with total binding energies less than 30 kcal/mol and bond length greater than 2.5 Å on average. Interestingly, unlike all the other groups studied, here the total binding energy and the

**Fig. 4** Molecular graphs of the Ta–N complex and Ta–O





covalent energy decrease down the group. Thus, Re–N and Re–O are the weakest complexes considered here, i.e. the covalent bonds are the longest and the covalent energy the smallest, 2.866 and 2.627 Å, and 19.03 and 17.09 kcal/mol, respectively.

Complexes of the Fe and Co group present similar features, e.g. the covalent energies of Fe–N and Co–N differ by less 3 kcal/mol on average. While Fe–O and Fe–N are essentially isoenergetic, Co shows a slight preference for N7. The heavier metals, though, show larger binding energies to N7, about 4 kcal/mol on average. Here, as seen before, the energy increases along the groups and thus larger metals are more strongly bound to guanine. The nickel group complex energies are clearly larger than any previous group and, perhaps not surprisingly, platinum complexes are the most strongly bound, about 10 kcal/mol more than nickel and palladium. All complexes follow platinum's known behaviour, and are bonded to N7 rather than O6.

Unexpectedly, stable M–N complexes of copper and silver could not be found, so only M–O complexes appear to be stable. Nevertheless, Cu–O and Ag–O covalent bonds are rather weak, 21.10 and 20.56 kcal/mol. In contrast, linear gold complexes are highly stable, 70.13 and 80.42 kcal/mol for Au–O and Au–N, respectively, such that these complexes are the only ones to rival platinum in overall stability. This may be relevant, as such gold complexes are known to have some anti-tumour activity. The metals of the zinc group form rather weak M–N(O) bonds, the strongest being Hg–N at just 36.94 kcal/mol. As before, the energy increases down the group, for instance Hg complexes are about 10 kcal/mol more stable than the corresponding Zn complexes.

An important property is the ability of the metal to form bonds to O6 of guanine, as it has been shown both experimentally and theoretically that metallation at this site may drastically disrupt the GC pairing, leading to the formation of globular DNA conformation [23, 26, 37, 38]. Figure 5 contains a schematic drawing of the relative preference for O6 and N7 for the complexes reported in Table 2. The height of the pyramids is proportional to  $\Delta_{N-O}$ , the energy difference between M–N and M–O complexes, with positive values indicating preference for N7.

Therefore, as discussed above, titanium group complexes clearly prefer O6, with  $\Delta_{N-O} = -13$  kcal/mol on average. Moving to vanadium, the energy difference is much less pronounced, the pyramids disappearing as the values approach zero. The situation is intriguing for both Mn and Cr groups, where the first row metals prefer O6 but the later rows showing the reversed trend and positive  $\Delta_{N-O}$ . Most other metals prefer the N7 position, albeit with a few interesting exceptions. For instance, Fe–O is slightly more stable than Fe–N by about 2 kcal/mol. Cu and Ag, unlike Au, do not bond to N7 at all, although the M–O6 complexes are rather weak. Finally, the Zn group elements prefer O6, except Hg, which prefers N7. From Fig. 5, another aspect becomes clear: in all cases,  $\Delta_{N-O}$  values increase down the

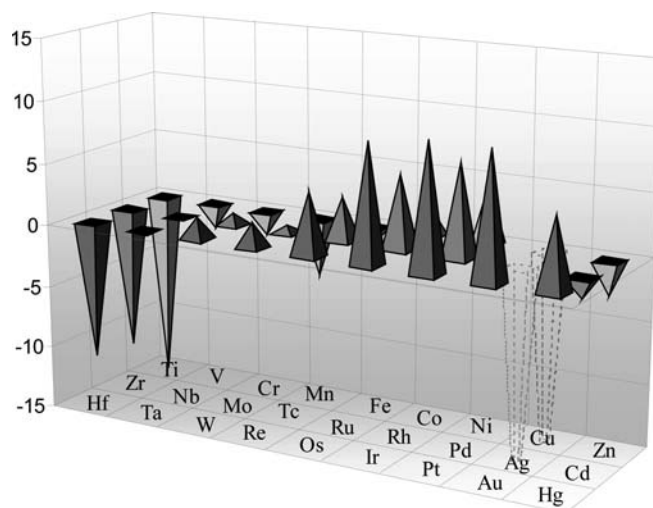


Fig. 5  $\Delta_{N-O}$  (kcal/mol) of transition metals: positive values indicate a preference for N7

group, and in some cases (e.g. Mn, Zn groups) change sign. This is easily explained in terms relative hardness: descending down the group, the number of valence electrons is constant, while the size increases. Thus, the metals become relatively softer, and tend to prefer coordination at N7 over O6.

#### Metal–guanine⋯cytosine adducts

Adding a cytosine molecule to the metal⋯guanine complexes in the standard Watson–Crick position hardly alters the pattern or strength of intramolecular H-bonding between metal and guanine, and so this will not be discussed in detail here. On the contrary, Table 3 shows that covalent energies are strongly affected by the presence of cytosine: we calculate an average increase in  $E_{cov}$  of 10 kcal/mol for all complexes considered, close to the value found solely for platinum complexes (11 kcal/mol) in Ref. [23]. As the increase in the energy is virtually constant, all trends discussed above are repeated. Strengthening of M–O and M–N bonds is also evident from AIM analysis, with both types of bond in general richer in electron density by 10–15%.

As reported previously, the AIM method predicts the strength of GC pairing, giving a value of 22.47 kcal/mol, cf. the experimental value of 21.0 kcal/mol [39]. Platination is known to perturb the relative strength of the H-bonds of the GC pair [23]. While the total energy is close to that of the free GC pair, those H-bonds in which guanine acts as a base are weaker, and those for which guanine is the proton donor are stronger, leading to significant deformation of the pair, as depicted in Fig. 6. The analysis of data in Table 3 and Figs. 6 and 7 reveal a similar pattern for all complexes considered here.

Figure 6 plots  $E_{GC}$  against  $E_{cov}$  for the M–X, bond, with a line representing the free GC pair. Metallation at N7, in accord with previous findings, causes a small

**Table 3**  $E_{\text{cov}}$  and hydrogen bond energies in the metal...GC complexes

Group	Complex	$E_{\text{cov}}$ (kcal/mol)	$\text{H}_4\cdots\text{O}_6$ (kcal/mol)	$\text{N}_1\text{H}_1\cdots\text{N}_3\text{-C}$ (kcal/mol)	$\text{N}_2\text{H}_2\cdots\text{O}_2$ (kcal/mol)	$E_{\text{GC}}$ (kcal/mol)
Ti	GC		8.37	7.54	6.56	22.47
	Ti-N	41.33	5.27	8.58	8.82	22.67
	Ti-O	61.10	2.67	2.12	10.91	15.70
	Zr-N	55.36	5.14	8.93	8.82	22.89
	Zr-O	73.45	0.00	5.29	10.80	16.09
V	Hf-N	57.07	5.18	8.95	8.96	23.09
	Hf-O	77.67	1.27	5.23	5.68	12.18
	V-N	63.81	4.45	10.02	11.48	25.95
	V-O	69.67	1.94	8.86	10.00	20.80
	Nb-chelate	81.46	3.08	10.65	9.28	23.01
Cr	Ta-chelate	85.09	3.58	10.95	9.32	23.85
	Cr-N	62.04	4.85	8.70	8.82	22.37
	Cr-O	63.52	1.64	4.57	11.3	17.34
	Mo-N	65.83	4.80	8.42	8.95	22.16
	Mo-O	65.78	1.95	5.00	10.61	17.57
Mn	W-N	70.96	4.79	8.57	7.07	20.44
	W-O	67.96	1.95	5.02	10.75	17.70
	Mn-N	32.50	5.39	8.31	8.56	22.26
	Mn-O	39.38	2.25	6.60	8.85	17.70
	Tc-N	29.19	5.37	8.19	8.42	21.99
Fe	Tc-O	32.82	2.83	6.84	8.61	18.28
	Re-N	24.5	5.24	8.17	8.43	21.84
	Re-O	28.6	4.56	7.3	8.61	20.50
	Fe-N	46.20	5.18	8.26	8.76	22.20
	Fe-O	49.89	1.90	5.71	9.25	16.86
Co	Ru-N	53.20	5.14	8.30	8.66	22.10
	Ru-O	51.44	2.51	6.50	8.92	17.93
	Os-N	57.69	5.18	8.02	8.75	21.96
	Os-O	53.41	2.87	6.71	8.88	18.46
	Co-N	47.57	5.85	8.38	8.35	22.58
Ni	Co-O	45.58	4.70	7.97	7.42	20.09
	Rh-N	54.70	5.64	8.36	8.19	22.19
	Rh-O	48.60	4.90	8.00	7.52	20.42
	Ir-N	62.30	5.61	8.34	8.52	22.47
	Ir-O	53.62	4.61	7.89	7.60	20.10
Cu	Ni-N	70.00	4.89	8.79	8.91	22.58
	Ni-O	69.66	3.41	7.62	9.84	20.87
	Pd-N	74.02	4.90	8.73	8.89	22.52
	Pd-O	65.83	3.28	6.92	9.14	19.34
	Pt-N	81.33	4.89	8.76	8.95	22.60
Zn	Pt-O	74.58	1.52	6.84	9.45	17.81
	Cu-O	20.78	4.41	7.95	7.42	20.78
	Ag-O	25.26	5.48	8.74	8.67	22.89
	Au-N	89.22	4.07	8.34	9.00	21.41
	Au-O	79.36	5.54	7.95	7.42	20.91
Hg	Zn-N	28.71	5.22	8.34	8.54	22.10
	Zn-O	34.97	2.76	6.59	8.82	18.17
	Cd-N	31.56	5.20	8.23	8.46	21.90
	Cd-O	38.66	3.12	6.90	8.61	18.63
	Hg-N	44.79	4.74	8.47	8.73	21.94
	Hg-O	43.76	3.66	8.77	9.49	21.92

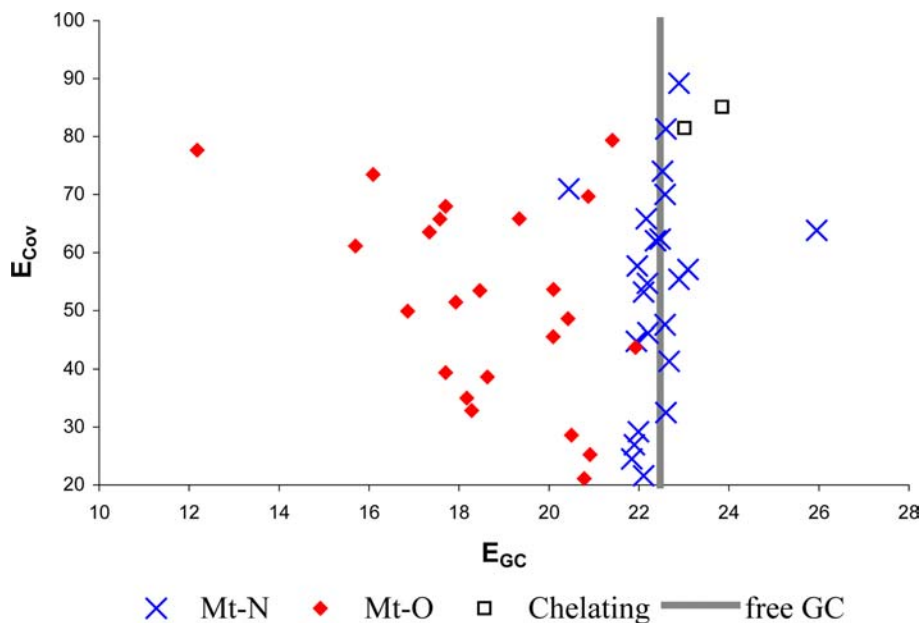
overall gain in the binding strength of GC, 1 kcal/mol on average, with almost all M-N complexes located on or close to the free GC line. Figure 7 decomposes this total pairing energy into contributions from each H-bond: Fig. 7a shows that the  $\text{N}_4\text{H}_4\cdots\text{O}_6$  bonds are weakened on average by around 40%. In contrast, the  $\text{N}_1\text{H}_1\cdots\text{N}_3$  and  $\text{N}_2\text{H}_2\cdots\text{O}_2$  bonds are strengthened by about 15 and 30%, respectively. Thus, although the total hydrogen bond energy hardly increases, the structure of the GC pair is strongly distorted. Among the M-N complexes, two show particularly interesting properties:  $E_{\text{GC}}$  for the V complex is 25.95 kcal/mol, considerably

larger than the free pair, while for the W complex  $E_{\text{GC}}$  is 20.44 kcal/mol, rather less than free GC. In contrast, most M-O complexes are found to be weaker than free GC, to the left of Fig. 6. Figure 7b shows that this is due to even greater weakening of  $\text{N}_4\text{H}_4\cdots\text{O}_6$ , 65% on average, and lesser enhancement of  $\text{N}_2\text{H}_2\cdots\text{O}_2$  when compared to metallation at N7. Complexation at O6 therefore uniformly leads to a loss of hydrogen bond energy and large distortions of Watson-Crick pairing.

Our data indicate that the effect on GC pairing depends more on the position of metallation rather than on the nature of the metal itself. Hobza and co-workers



**Fig. 6** Plot of  $E_{GC}$  vs  $E_{cov}$  (kcal/mol)



[24] suggested that in either case, distortion of the GC pair may be explained in terms of electrostatics. First, the metal produces a more negative partial charge on the O6 and more positive partial charges on the H<sub>1</sub>–N<sub>1</sub> and H<sub>2</sub>–N<sub>2</sub>. This explains the strengthened N<sub>1</sub>H<sub>1</sub>⋯N<sub>3</sub> and N<sub>2</sub>H<sub>2</sub>⋯O<sub>2</sub> H-bonds, but not the weakening of the N<sub>4</sub>–H<sub>4</sub>⋯O<sub>6</sub>. It has been suggested that the repulsion between the positive charge of metals and the partial positive charge of N–H on cytosine causes the loss of energy in N<sub>4</sub>H<sub>4</sub>⋯O<sub>6</sub>, though Poater et al. [25] proposed that the distortion of the Watson–Crick (WC) pair might have its origin in a change of donor/acceptor capability rather than electrostatic interactions.

To further investigate this, the molecular electrostatic potential (MEP) on the 0.001 au isodensity surface for some metal⋯guanine complexes were calculated (see Fig. 8). In general, the maximum MEP on this surface ( $V_{max}$ ) is located between the two N–Hs of guanine, and is related to the strengthening of N<sub>1</sub>H<sub>1</sub>⋯N<sub>3</sub> and N<sub>2</sub>H<sub>2</sub>⋯O<sub>2</sub> H-bonds. For example, in the Ti–O complex, the sum of the energies of those H-bonds is 13.03 kcal/mol and  $V_{max}$  = 0.1876 au, while in the Pt–N complex these values are 17.71 kcal/mol and 0.1991 au, rising to 18.06 kcal/mol and 0.2176 au for V–O. Thus, these data support the electrostatic argument noted above.

AIM analysis (Table 4) reveals some interesting properties of the metal–guanine complexes, which may explain the differences of O and N metallation. As noted previously [25], metallation affects all bonds of the six-membered ring of guanine, as well as C2–N2 and C6–O6. In particular, C6–O6 bonds are longer and have reduced electron density than in isolated guanine, while all other bonds except the C4–C5 are strengthened by the presence of the metal. The latter is virtually unchanged in the N complexes, and slightly weakened in O complexes. The position of the metal does not affect C2–N2, but strengthening of the ring bonds and weakening

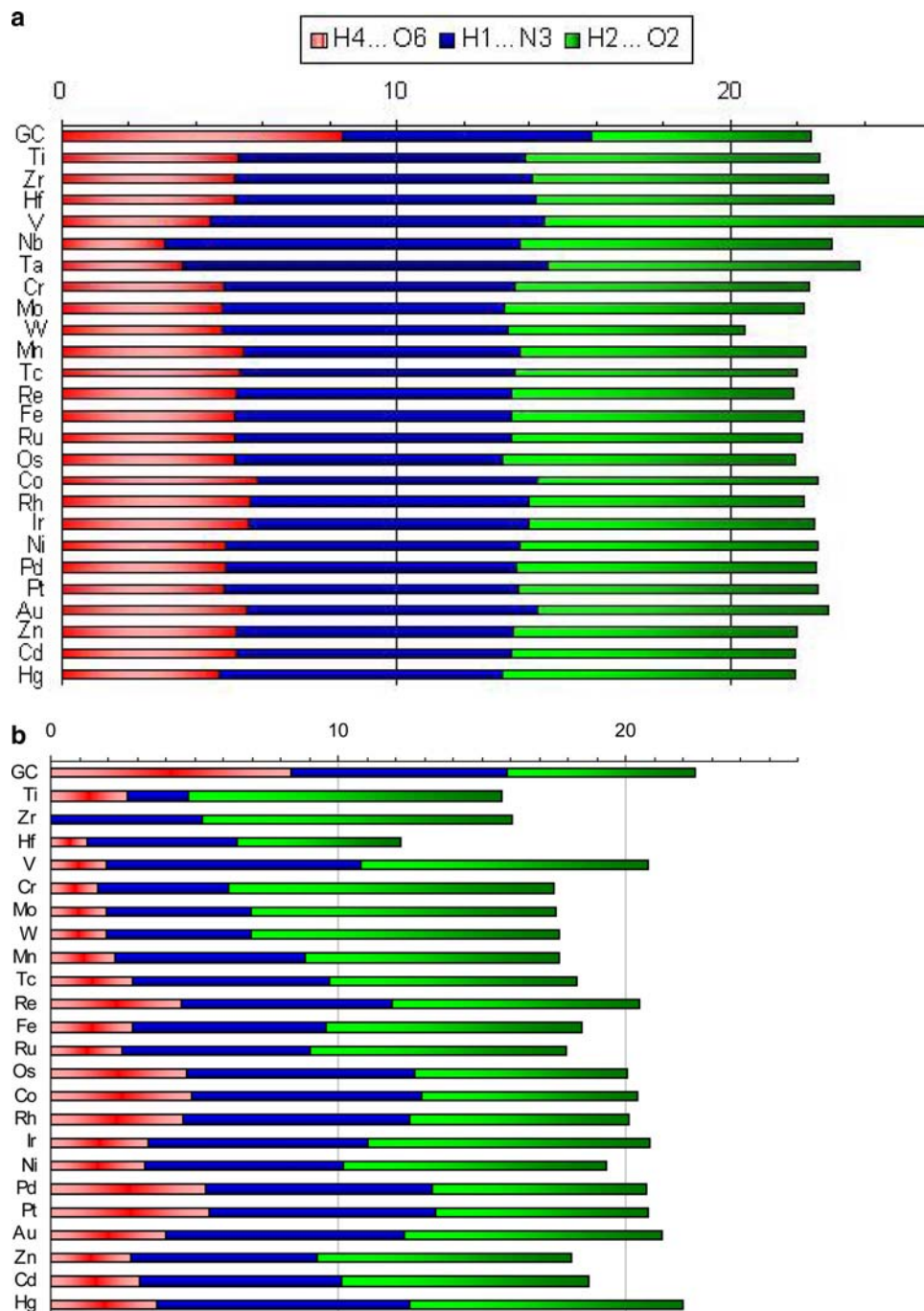
of C6–O6 are more pronounced for O rather than N metallation. O metallation decreases C6–O6  $\rho_c$  by between 15 and 20% and increases N1–C6 and C5–C6  $\rho_c$  by between 10 and 15%, changes which are uniformly less than 5% for N metallation.

This suggests an explanation of the different effects of O and N metallation on GC pairing. Complexation at N7 only slightly affects the electron density of guanine near the H-bonding position. Thus, distortion might be mainly due to the electrostatic effects reported above. In contrast, along with the electrostatic repulsion, O metallation induces large redistributions of density within the six-membered ring: the direct M–O interaction shifts density from the C6–O6 bond toward the metal, weakening this bond and reducing donor ability of O6. This further weakens N<sub>4</sub>H<sub>4</sub>⋯O<sub>6</sub>, and leads to overall loss of stability of the GC pair. Interestingly, the V and Ti group elements, those most strongly bound to O6, promote the largest shifts in density and H-bond energy among the complexes considered.

From the data reported here, the early metals are perhaps the most interesting examples. First, they show a clear preference for O over N complexation, most evident for the Ti group, where  $\Delta_{N-O}$  = –13 kcal/mol on average. Furthermore, the covalent energies are among the largest of the metals: Hf–O and V–O bonds are as strong as most platinum complexes reported in Ref. [23]. This is especially interesting, as M–O complexes appear to disrupt completely the Watson–Crick GC pair, with Ti, Zr and Hf reducing the GC pair binding by ca. 10 kcal/mol.

In addition, AIM analysis reveals an odd property of the Ta and Nb complexes. In both cases, as shown in Fig. 4, a “chelating” complex results: while in the isolated guanine complexes the bonding is asymmetric, addition of a cytosine leads to much more symmetrical complexes, with similar M–N and M–O electron densi-

**Fig. 7** **a** GC hydrogen bonds in the M–N<sub>GC</sub> complexes. **b** GC hydrogen bonds in the M–O<sub>GC</sub> complexes

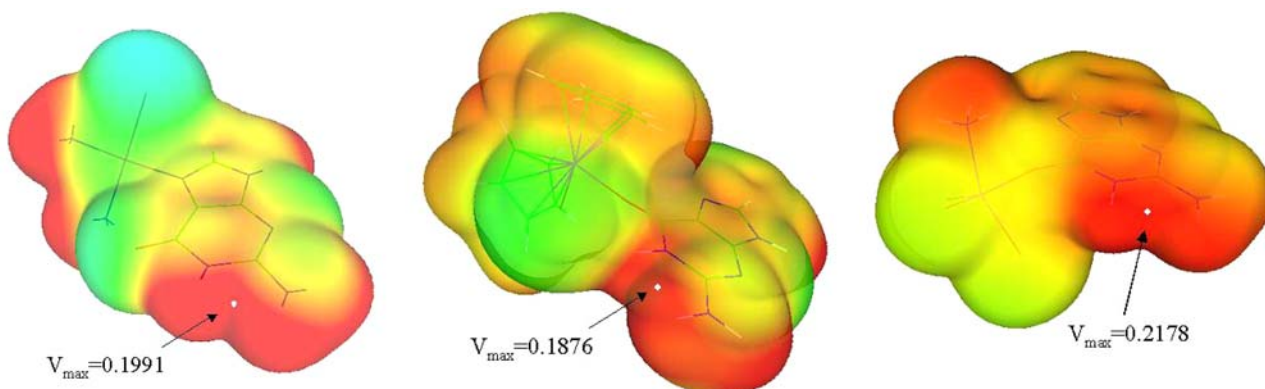


ties and bond lengths (the ratio  $\rho_{M-O}/\rho_{M-N}$  approaches unity). Therefore, as both bonds are relatively strong, this “chelating” mode strengthens the metal–guanine interaction. However, the effect of these complexes on GC pairing does not follow the pattern seen for chelating platinum complexes, with both Ta and Nb complexes having similar pairing energy to the free GC pair.

Although the mechanism is still unknown, several studies suggest Ru complexes to be potentially active against cancer. From Tables 2 and 3, Ru...guanine adducts are not particularly strong, nor indeed are any Fe group metals, with covalent energies of between 46 and

51 kcal/mol. Although data from Tables 2 and 3 suggest a preference for N7, metallation on O6 is also intriguing: the Fe group causes some of the strongest effects on pairing. From Fig. 6, the Fe group clusters away from the GC line and in the upper region, close to the Ti group. Here, along with electrostatic and electron density shift reasons, further repulsion may originate from interaction between the NH groups of the ligand and cytosine, which are extremely close.

It has been shown experimentally that gold complexes are potentially active in treating cancer [13, 40]. In vitro studies showed that Au–DNA adducts are weaker



**Fig. 8** 0.001 au isosurface electrostatic potential of Pt–N, Ti–O and V–O complexes

than corresponding Pt–DNA ones. In contrast, our data suggest that Au forms the most stable monofunctional adducts considered, with Au–N binding energy of 89.22 kcal/mol, around 8 kcal/mol larger for Pt. This apparent incongruence is resolved when we consider that, unlike the linear Au complexes, Pt-based drugs bind DNA in a bifunctional way, where the total binding energy is more than double that of monofunctional complexes. Interestingly, Au hardly changes the GC pairing energy, being 21.41 and 20.91 kcal/mol for the Au–N and Au–O, respectively.

Table 5 reports properties of Ti complexes in which the ligand set was varied, allowing us to investigate the

importance of ligands in metal⋯guanine (and GC) interactions. Along with the metallocene sandwich ( $\text{Cp}_2$ ) discussed above,  $[\text{Ti}(\text{NH}_3)_2\text{Cl}_3]^+$  and  $[\text{Ti}=\text{O}(\text{NH}_3)_2\text{Cl}]^+$  were considered, i.e. keeping the monocharged  $d^0$  nature of the complexes constant. Table 5 indicates that, independent of the ligands, Ti complexes prefer the O6 position of guanine. The  $\Delta_{\text{N-O}}$  is on average equal to  $-10.7$  kcal/mol, although this trend is reversed for  $[\text{Ti}=\text{O}(\text{NH}_3)_2\text{Cl}]^+$  complexes, where  $\Delta_{\text{N-O}}$  is slightly positive, a surprising finding that may be resolved by AIM analysis (see Fig. 9). In the N7 complex to guanine, an extra bond CP is present, representing a weak Ti–O interaction (distance = 2.926 Å, electron

**Table 4** Topological analysis of guanine ring (au)

	$\rho_{\text{N1-C6}}$	$\rho_{\text{C6-O6}}$	$\rho_{\text{C5-C6}}$	$\rho_{\text{N3-C4}}$	$\rho_{\text{C2-N2}}$
Guanine	0.2758	0.4174	0.2934	0.3255	0.3234
Ti–N	0.2924 (+6%)	0.4082 (–2)	0.2956 (+1)	0.3481 (+7)	0.3366 (+4)
Ti–O	0.3106 (+12)	0.3504 (–16)	0.3259 (+11)	0.3444 (+5)	0.3386 (+5)
V–N	0.2936 (+6)	0.4090 (–2)	0.3062 (+4)	0.3418 (+5)	0.3379 (+5)
V–O	0.3107 (+13)	0.3497 (–16)	0.3257 (+11)	0.3456 (+6)	0.3395 (+5)
Fe–N	0.2913 (+5)	0.4029 (–4)	0.2986 (+2)	0.3362 (+3)	0.3335 (+3)
Fe–O	0.3041 (+10)	0.3760 (–10)	0.3100 (+6)	0.3361 (+3)	0.3330 (+3)

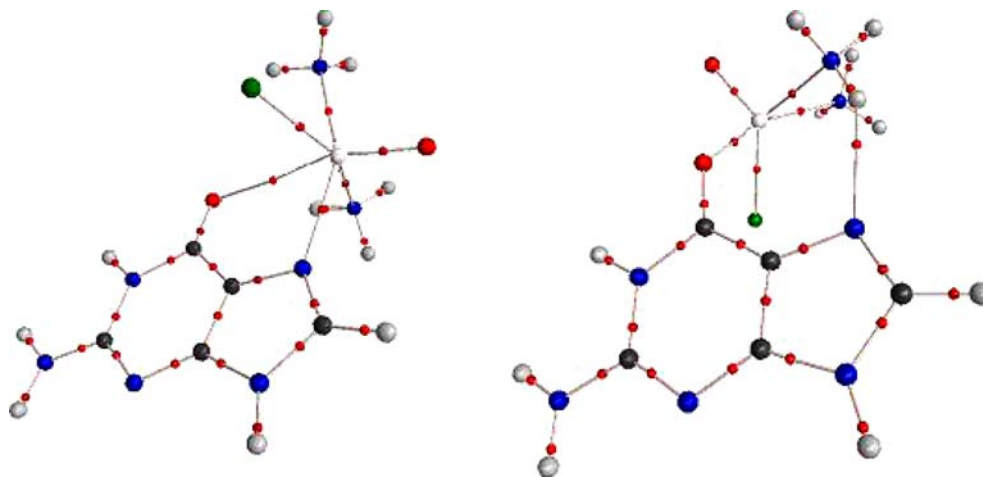
Values in parenthesis are variations from free guanine

**Table 5** Effect of ligand variation on Ti⋯G and Ti⋯GC complexes

	Ligand	Bonding	Binding energy	$E_{\text{intra}}$	$E_{\text{GC}}$	$E_{\text{cov}}$
Ti–G	$\text{Cp}_2\text{Cl}$	Ti–N7	36.40	3.17		33.20
	$\text{Cp}_2\text{Cl}$	Ti–O6	51.88	3.45		48.43
	$(\text{NH}_3)_2\text{Cl}_3$	Ti–N7	48.51	8.33		40.18
	$(\text{NH}_3)_2\text{Cl}_3$	Ti–O6	55.01	2.91		52.10
	$=\text{O}(\text{NH}_3)_2\text{Cl}$	Ti–N7	59.35	0.0		59.35
	$=\text{O}(\text{NH}_3)_2\text{Cl}$	Ti–O6	60.16	3.65		56.51
Ti–GC	$\text{Cp}_2\text{Cl}$	Ti–N7	67.34	3.33	22.67	41.33
	$\text{Cp}_2\text{Cl}$	Ti–O6	78.33	1.53	15.70	61.10
	$(\text{NH}_3)_2\text{Cl}_3$	Ti–N7	78.62	8.71	22.92	47.00
	$(\text{NH}_3)_2\text{Cl}_3$	Ti–O6	87.91	8.70	19.51	58.70
				1.00 <sup>a</sup>		
	$(\text{NH}_3)_2\text{ClO}$	Ti–N7	91.31	4.09	22.66	64.56
$(\text{NH}_3)_2\text{ClO}$	Ti–O6	95.49	1.90	21.23	72.36	

<sup>a</sup>This energy corresponds to cytosine–NH⋯Cl–metal interaction

**Fig. 9** Molecular graphs of  $[\text{Ti}=\text{O}(\text{NH}_3)_2\text{Cl}]\text{-N7}$  and  $[\text{Ti}=\text{O}(\text{NH}_3)_2\text{Cl}]\text{-O6}$  complexes



density = 0.011 au). In contrast, the O6 complex of guanine contains an  $\text{N-H}\cdots\text{N}$  H-bond, in common with all other complexes considered, including the  $\text{Ti-N}_{\text{GC}}$  complex in which the secondary  $\text{Ti-O}$  interaction is no longer present (distance = 3.481 Å). Thus, the  $[\text{Ti}=\text{O}(\text{NH}_3)_2\text{Cl}]^+$  complex presents some peculiar bonding modes, and analysis of the topology of the electron density allows explanation of its apparently anomalous behaviour.

H-bonding results in Table 5 include both intramolecular and GC results. The presence of the polar hydrogens of ammine ligands increases the strength of the former, especially in the N7 complexes. Similar to other N7 complexes, individual H-bonds are distorted but the overall pairing energy is conserved.  $\text{Ti-O6}$  complexation reduces the pairing energy, with  $\Delta_{\text{GC}}$  (the difference between free and metallated GC) varying between 1 and 7 kcal/mol. These data indicate that the ligand modulates the behaviour of the metal to some extent, but ultimately all Ti complexes reported in Table 5 behave qualitatively in a similar manner.

Bowers and co-workers [26] recently analysed gas-phase binding properties of metals to the dinucleotide duplex dCG-dCG using ion mobility mass spectrometry.

They showed that metals classified as hard acids led to globular structures with disruption of the GC pair, whereas soft metals stabilised the Watson-Crick structure. Table 6 compares our data with the results reported by Bowers. It should be noted that the experimental data are for bare  $\text{M}^{n+}$ , i.e. without any ligands, in contrast to our calculations, thus any comparison can only be qualitative. Nevertheless, we find broad agreement between the two approaches, with all the cases in which a globular structure is found showing substantial disruption of the GC pair ( $\Delta_{\text{GC}} < 0$ ), while those found to maintain pairing show either little or no disruption.

This agreement is not perfect, however; our results suggest complexation of  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  at O6, and hence substantial disruption. We therefore also performed optimisation of complexes of *naked*  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  complexes to the Watson-Crick GC pair. Both metals are doubly charged and adopt a chelating conformation, binding to both O6 and N7. Data obtained for these chelating structures agree much better with experiment: in both cases AIM analysis confirms that metallation leads to a stabilisation of the GC pairing, with  $\Delta_{\text{GC}} = +2.16$  and  $+3.20$  kcal/mol for  $\text{Zn}^{2+}$  and

**Table 6** Data from Bowers' work compared to theoretical predictions

	Experiment <sup>a</sup>		This work	
	Hard/soft	Structure	Coordination site	$\Delta_{\text{GC}}$ (kcal/mol) <sup>b</sup>
$\text{Cu}^+$	Soft	WC	O6	-1.70
$\text{Ag}^+$	Soft	WC	O6	+0.47
$\text{Cd}^{2+}$	Soft	WC	O6	-3.84
			N7/O6 <sup>c</sup>	+2.16 <sup>c</sup>
$\text{Pt}^{2+}$	Soft	WC	N7	+0.13
$\text{Zn}^{2+}$	Soft	WC	O6	-4.3
			N7/O6 <sup>c</sup>	+3.20 <sup>c</sup>
$\text{Fe}^{2+}$	Borderline	Globular	O6	-5.61
$\text{Ni}^{2+}$	Borderline	Globular	N7/O6	-1.8
$\text{Cr}^{2+}$	Hard	Globular	O6	-5.13
$\text{Mn}^{2+}$	Hard	Globular	O6	-4.77

<sup>a</sup>From Ref. [26]

<sup>b</sup>Variation in GC pairing energy in complex from free GC

<sup>c</sup>The calculations are referred to metal...guanine complexes with no ligand on the metal



$\text{Cd}^{2+}$ , respectively. Bowers and co-workers [26] also suggested that  $d^{10}$  metals promote Watson–Crick pairing, again in accord with our findings that the most stable  $d^{10}$  metal...guanine complexes show pairing energies ranging between 20.78 and 22.10 kcal/mol.

Finally, it is well known that the electron density at the bond CP is related to bond strength, at least for series of similar bonds [41], including our results for Pt–N and Pt–O bonds [23]. Here we are able to extend that outcome to a much more diverse set of complexes, a total of 108 data for 27 metals. We find that the close linear relation established for Pt–N and Pt–O bonds is rather less apparent for this data, with  $\rho_c$  for M–X bonds in metal...guanine and metal...GC complexes of all the transition metals correlating fairly well to  $E_{\text{cov}}$ , with  $r^2=0.80$ . Further studies are underway to establish whether such relations fall into families, e.g. hard/soft, for which more accurate relations may ensue.

## Conclusions

Analysis of more than 100 metal...guanine(cytosine) complexes leads to following conclusions:

1. As shown previously, calculations at B3LYP/DGDZVP(SDD)//HF/6-31G\*(SDD) are capable of reproducing high-level binding energies of transition metal purine complexes.
2. Hydrogen bonds such as N–H...O, N–H...N, N–H...Cl, C–H...O and C–H...N are important in all metal...guanine complexes studied, contributing ca. 10% of overall stabilisation.
3. The relative stability of M–O6 and M–N7 depends more on the nature of the metal than the choice of ligand. Early metals prefer O6, whereas more electron-rich metals prefer N7. Also, across rows relative stability decreases as M–N7 energies become larger than M–O6.
4. The effect of metallation on the GC pair is similar to that observed in platinum complexes, i.e.  $\text{N}_4\text{--H}_4\text{...O}_6$  is weakened, while  $\text{N}_1\text{--H}_1\text{...N}_3$  and  $\text{N}_2\text{--H}_2\text{...O}_2(\text{C})$  are enhanced, leading to large changes in the geometry of the  $\text{G} \equiv \text{C}$  pair. The overall pairing energy of GC is unchanged for the M–N complexes, but significantly reduced for M–O.
5. Titanium and vanadium group metals show particularly large covalent bond energies and strongly affect the GC pair, where the energy falls more than 10 kcal/mol.
6. The effect of metallation at N7 on GC pairing can be explained in terms of electrostatics, but metallation at O6 also induces large redistributions of electron density within guanine, leading to a loss of H-bond donor capability of O6.
7. The electron density at the metal...guanine bond correlates with the covalent energy, though without the accuracy found previously for Pt complexes alone.

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