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Tuned structure and DNA binding properties of metal complexes base on a new 4-acylpyrazolone derivative

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It is a common knowledge that the spatial structure of substrates is the major influencing factor for DNA binding. To tune the binding affinity of DNA, a new 4-acylpyrazolone derivative ligand, (2-hydroxy-N'-((5-hydroxy-3-methyl-1-(4nitrophenyl)-4,5-dihydro-1H-pyrazol-4-yl)(phenyl)methylene)benzohydrazide) (H₂L), as well as its three complexes has been prepared and well characteristized. Reaction of H₂L with CuCl₂ resulted a mononuclear compound with tetracoordinated quadrilateral plane, [Cu(HL)Cl] (1). When H₂L was coordinated to Cu(OAc)₂, a dinuclear Cu(II) compound with chemical formula of [Cu₂L₂(CH₃OH)₂]-CH₃OH (2) was obtained and the coordination geometry of Cu(II) is a square pyramid. Upon assembled H₂L with Mn(OAc)₂, a quite different dinuclear compound with chemical compisition of [Mn₂L₂(O CH₃)₂(H₂O)₂]-CH₃OH (3) were afforded where Mn(III) displayed distorted octahedron configurations. DNA binding studies were performed on H₂L and its three complexes by means of the electron absorption titration and EB-DNA competition experiments, and the results indicate they all bind DNA in an intercalation mode and their binding affinity follows the order of $1 > 2 > 3 > H_2L$. In addition, the Time Dependent Density Functional Theory (TD-DFT) calculation were performed for H₂L and its three complexes to better clarifies the electronic transitions in the UV-Vis spectra.

Introduction

Since Avery and others completed the famous pneumococcal transformation experiment and concluded that DNA is genetic material, researches in the field of DNA have long captivated chemists¹ and those reported showed that the biological activity of transition metal complexes has become an important field and has been proved to play important roles in medicine, pharmacy and diagnostics.^{2,3} In particular, transition metal complexes can serve as an important DNA targeting compound and play unique roles in biochemical research.⁴⁻⁶ Therefore, it is particularly necessary to study the interaction between transition metal complexes and specific DNA sequences. In general, transition metal complexes act as DNA binding agents and their biological activity exhibit diversity. The pattern of compounds binding to DNA follows three non-covalent modes: embedding, groove bonding and electrostatic interaction.⁷ For example, Manoj Munde et al. optimized the interaction in the DNA minor groove complex by inducing a suitable conformation of the heterocycle,⁸ Chirgadze found electrostatic feature of Zn(II) complex in the binding of factor Zif268 from Mus musculus with operator DNA⁹ and many researchers have reported Pt(II), Yb(III) and Ag(I) complexes can affect DNA by intercalating between CT-DNA double helix.¹⁰⁻¹³ Among these three methods, the embedding mode of $\pi \rightarrow \pi$ interaction between the delocalized π system of aromatic ring and the π system of base is the strongest one with wide application. In such transition metal complexes that have been documented, copper and manganese acts as an essential trace mineral for human body. More importantly, manganese is an important component of several important physiological enzymes in the body and copper has the function of cutting nucleic acids, thus the study of its complexes is also a hot spot in the field of biological activity. 14,15

Pyrazoles are a very important class of drug synthesis intermediates, which play important roles in the fields of antitumor and anti-virus due to their high efficiency, low toxicity and structural diversity.^{16,17} Since Knott discovered that antipyrine containing pyrazole ring show analgesic and anti-inflammatory effects in 1883, a variety of pyrazole compounds with superior biological activity have been reported. In the past few decades, the synthesis of pyrazole derivatives has become a hot research topic for developing drugs with higher biological activity.^{18,19} Among them, pyrazolone derivatives with various functional groups are simple in synthesis, stable in nature, and convenient in structural modification. Numerous studies demonstrated that the pyrazolone based compounds contains a plurality of coordination atoms such as N and O, which has strong coordination ability and lavish chelating modes. In addition, their metal compounds have a certain electron transfer function, magnetic exchange, photoluminescence and strong antibacterial, antiviral and other biological activities.^{20,21} In recent years, the research on these compounds has gradually increased, and metal organic compounds involving 4-acylpyrazolone have been developed, which extends a new way of conjecture for researches on pyrazolone.22-24

Based on the above considerations, two Cu(II) transition compounds (1 and 2) and one Mn(III) compound (3) based on a new 4-acylpyrazolone derivative as shown in Scheme 1, 2-hydroxy-

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⁵⁷ Electronic Supplementary Information (ESI) available: Crystallographic data and details of refinements for 1, 2 and 3 (Table S1). The selected bond lengths and angles for 1, 2 and 3 (Table S2). Crystallographic files in CIF format have been deposited with the Cambridge Crystallographic Data Center with deposition numbers CCDC 1968400-1968402.See DOI: 10.1039/x0xx00000x

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N'-((5-hydroxy-3-methyl-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-4-yl)(phenyl)methylene)benzohydrazide (H_2L), were prepared by using different metal ions and anions for tuning the structure as well as DNA binding properties. Crystal structure analysis shows that **1** is a mononuclear compound with planar quadrilateral configuration and the coplanarity is strong. However, **2** is a dinuclear five-coordinated Cu(II) compound with two copper coordination units bridged by salicylhydrazide hydroxyl groups of the ligand by replacing chloride anion in **1** with acetate **3** is a guite different dinuclear compound with twoOMn(III) secondination sphere bridged by methoxyl which was resulted by further replacing Cu(II) with Mn(III). DNA binding studies performed on the 4-acylpyrazolone ligand and its three complexes by electron absorption titration and EB-DNA competition experiments indicate they all bind DNA in the intercalation mode and their binding affinity follows the order of $1 > 2 > 3 > H_{2}L$.



Scheme 1. The synthetic route of the ligand H_2L

Experimental Details

Synthesis of the ligand. Salicylhydrazide (3 mmol, 0.46 g) were added in 10 min to a 20 mL ethanol solution of 4-benzoyl-3-methyl-1-(4-nitrophenyl)-1H-pyrazol-5(4H)-one (3 mmol, 0.97 g), and then glacial acetic acid (1 mL) was added dropwise, the mixture was stirred and heated at reflux for 4 h. After cooling the mixture to room temperature, 80 mL H_2O was added to obtain brown precipitate which was separated by filtration and further recrystallized with ethanol to give a pale yellow solid H_2L .

H₂L: 0.71 g, Yield: 53 %; 215~219 °C;. Anal. data, Calc. For C₂₄H₁₉N₅O₅: C, 63.02 %; H, 4.19 %; N, 15.31 %. Found: C, 62.87 %, H, 4.18%, N, 15.39. δ: 1.60 (s, 3H), 6.99 (dd, 2H), 7.27 (d, 1H), 7.49 (m, 3H), 7.61 (m, 5H), 7.82 (d, 1H), 8.27 (d, 2H), 9.44 (bs, 1H), 11.79 (bs, 1H), 15.64 (s, 1H). IR(KBr, cm⁻¹): 3274 (br, w), 3065 (w), 1639 (m), 1595 (m), 1523 (s).

Synthesis of the complexes 1, 2 and 3. H_2L (45.7 mg, 0.1 mmol) was added to a 20 mL CH₃OH to obtain a suspension, then CuCl₂·2H₂O (17.4 mg, 0.1 mmol) was dissolved in methanol and added to this suspension to obtain a clear solution and set aside for slow evaporation. After 2~3 weeks, dark brown single crystals (1) suitable for crystal analysis were collected by filtration, washed with methanol, and dried in air. Yield: 33 mg, 52.86% based on CuCl₂·2H₂O. The preparation of 2 and 3 is similar to that of complex 1 except for the metal salts used. The metal salts used in the preparation of 2 and 3 were Cu(OAc)₂·H₂O and Mn(OAc)₂·4H₂O, respectively, and the yields of 2 and 3 were 66.54% and 57.25% respectively.

Anal. Calcd for Cu₂C₄₈H₃₄Cl₂N₁₀O₁₀ (**1**): C, 52.38%; H, 3.70%; N, 12.99%. Found: C, 52.27%; H, 3.90%; N, 12.88%. IR (KBr, cm⁻¹): 3065 (w), 1604(s), 1562 (m), 1523 (s), 595 (w), 525 (m).

Anal. Calcd for $Cu_2C_{52}H_{48}N_{10}O_{14}$ (**2**): C, 55.35%; H, 4.67%; N, 12.46%. Found: C, 55.04%; H, 4.54%; N, 12.46%. IR (KBr, cm⁻¹): 3065 (w), 1604(s), 1571 (m), 1523 (s), 604 (w), 525 (m).

Anal. Calcd for $MnC_{26}H_{26}N_5O_8$ (**3**): C, 56.36%; H, 5.93%; N, 12.84%. Found: C, 56.43%; H, 5.95%; N, 12.79%. IR (KBr, cm⁻¹): 3065 (w), 1604(s), 1566 (m), 1523 (s), 605 (w), 537 (m).

Instrument, testing methods and details of X-ray single crystal diffraction analysis are available in the Supporting Information. The refinement data, selected bond distances and angles are listed in Table S1 and Table S2 respectively. The hydrogenbonding distances (Å) and angles (°) of **1**, **2** and **3** are listed in Table S3.

Results and discussion

Synthesis and Characterization.

H₂L was prepared as orange solid in four steps from the commercially available hydrazine hydrate (80%), methyl salicylate, ethyl acetoacetate, benzoyl chloride and 4nitrophenylhydrazine hydrochloride as depicted in Scheme 1. The ¹H NMR spectrum confirmed the identity of H_2L by the presence of well separated broad peaks at 9.44, 11.79 and 15.64 ppm corresponding to one acylhydrazone and two phenoxy protons as well as one singlet peak at 1.60 ppm which is characteristic of methyl group in pyrazole ring. The single crystals of 1, 2 and 3 which were obtained by slow evaporation of the resulted methanol solution of H₂L and metal salts under room temperature were rather insoluble in most solvents except in DMSO, DMF and methanol. The IR spectra of H₂L and the three metal compounds were shown in Figure 1. In the infrared spectra of H₂L, the phenolic hydroxyl group of the ligand is designated as the band at 3065 cm⁻¹. A broad band at 3274 cm⁻¹ is assigned to u_{OH} of the enolic group which suggests the enolic form of the ligand in the solid state. The acylhydrazone $u_{C=0}$, $u_{C=N}$ and the pyrazole ring $u_{C=N}$ are at 1639, 1595 and 1523 cm⁻¹, respectively. Compared with the infrared spectrum of the H₂L, it can be clearly observed in 1 that the disappearance of the characteristic peak of the enol group at 3274 cm⁻¹, indicating that the oxygen participates in the coordination. Also, the

acylhydrazone $u_{C=0}$ and $u_{C=N}$ shift 35 cm⁻¹ and 33 cm⁻¹ approximately to lower wave number respectively, indicating the coordination of this oxygen and nitrogen.²⁵ The pyrazole ring $u_{C=N}$ remains unchanged, indicating that the ring nitrogen does not take part in coordination. Moreover, two new vibrational absorption peaks appear at 595 and 525 cm⁻¹ in the infrared spectrum of **1**, which can be attributed to u_{M-O} and u_{M-N} .²⁶ Similar changes occurred in compound **2** and **3**. However, in the IR spectra of **2**, the vibrational absorption peak attributed to phenolic hydroxyl groups of salicylhydrazone at 3065 cm⁻¹ in H₂L, **1** and **3** was undetectable, suggesting that phenolic hydroxyl groups are involved in coordination in **2**. These results can be verified in the crystal structure analysis described below.



X-ray structure description

Crystal structure of 1. X-ray structural analysis reveals that **1** is mononuclear and crystallizes in the triclinic system of the P-1 space group. The crystal structure of **1** with atomic numbering scheme is shown in Figure 2a. Its asymmetric unit contains one crystallographically independent Cu(II) ion, one ligand anion HL⁻ and one Cl⁻ ion. Each Cu(II) atom is chelated by nitrogen and

oxygen atoms on acylhydrazone (N2 and O2) and oxygen atoms on pyrazolone (O3) of ligand HL⁻ with^Othe^Oremain Mg^Oreuten coordination site fullfilled by Cl⁻ ion (Cl1). The coordination polyhedron of Cu1 is calculated as a planar quadrilateral by continuous shape measures method with a deviation of 0.34 % as shown in Figure 2b. In the formed quadrilateral plane, the Cu-O2 and Cu-O3 bond distance are 1.944(2) Å and 1.904(2) Å; the Cu-N2 and Cu-Cl1 bond distances are 1.988(3) Å and 2.216(10) Å. The angle of N2–Cu1–Cl1 is 173.05(8)° and the angle of O3–Cu1– O2 is 170.87(11)°, which are not much different from the theoretical value of 180°. Meanwhile, the geometric index τ_4 is 0.114 ($\tau_4 = [360-(\alpha + \beta)]/141$, where α and β are the two largest angles in the coordination environment).^{27,28}

In addition, there are significant hydrogen bonding interactions between the molecules. As shown in Figure 2c, two adjacent mononuclear Cu(II) units were connected by the C5-H5…O2 hydrogen bond to form a central symmetric dimer, in which the C-H on the benzene ring acts as the hydrogen bond donor, and the carbonyl oxygen of salicylhydrazone acts as the hydrogen bond acceptor. The distance of C5---O2 is 3.341 (5) Å and the angle of C5–H5…O2 is 175.19(2)°. Then, with the aid of the C11–H11…O4 hydrogen bond, adjacent dimers can be joined together to form an infinite one-dimensional chain where the C-H on the benzene ring of the salicylhydrazide as hydrogen bonding donor and the oxygen atoms on nitro groups as hydrogen bond acceptor (Figure 2d). The distance of C11...O4 is 3.333(6) Å and the angle of C11–H11…O4 is 125.03(3)°. Finally, the 1D supramolecular chain further forms an infinite 2D supramolecular framework along the ac plane through the interaction of C2–H2···N3 hydrogen bonds, as shown Figure 2e. The distance of C2…N3 is 3.457(5) Å and the angle of C2–H2…N3 is 167.55 (3)°.



Figure 2. a) View of the coordination environment of Cu1 center in **1** with thermal ellipsoids at 30% probability (All H atoms have been omitted for clarity); b) The coordination polyhedron of Cu1 in **1**; c) The supramolecular dimer of **1** constructed by C–H···O hydrogen bonds (Symmetry code: 1-x, 2-y, -z); d) The 1D supramolecular chain of **1** constructed by C–H···O hydrogen bonds (Symmetry code: A = 1-x, 2-y, -z; B = -1+x, -1+y, 1+z; C = -x, 1-y, 1-z; D = -2+x, -2+y, 2+z; E = -1-x, -y, 2-z); e) The 2D supramolecular architecture constructed by C–H···N hydrogen bonds along the *ac* plane (Symmetry code: A = 1-x, 2-y, -z; B = -1+x, -1+y, 1+z; C = -x, 1-y, 1-z; D = -2+x, -2+y, 2+z; E = -1+x, -1+y, 1+z; C = -x, 1-y, 1-z; D = 1-x, 2-y, 1-z; E = x, y, 1+z; F = -x, 1-y, 2+z).

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Crystal structure of 2. To study the effect of different anions on Cu(II) coordination toward H_2L , Cu(OAc)₂·H₂O was used to replace CuCl₂·2H₂O to perform the same experiment. Quite interestingly, a dinuclear compound with chemical composition of [Cu₂L₂(CH₃OH)₂]·CH₃OH (2) in which two Cu(II) coordination subunits was bridged by phenolic hydroxyl groups of salicylhydrazone was obtained. As shown in Table S1, 2 also crystallizes in the triclinic system of the P-1 space group. The asymmetric unit contains one crystallographically independent Cu(II) ion, one deprotoned ligand anion L²⁻, one coordinated methanol and one crystalline methanol (Figure 3a). Among the five coordinated atoms of Cu(II), O3, O4, and N5 atoms are provided by one ligand anion L²⁻, O5 atom are from another ligand anion L²⁻ and O6 is provided by a coordinated methanol molecule. The Cu-O3/O4 bond distances are 1.902 (2)/1.922 (2) Å, the Cu-O6/N5 bond distances are 1.950 (2)/1.926(2) Å, and the Cu-O5 bond distances is 2.686(2) Å. According to the geometric index of τ_5 varies from 0 to 1, corresponding structure varies from regular square pyramidal geometry to trigonal bipyramidal. By calculation, the geometric index τ_5 of **2** is 0.002 $(\tau_5 = |\alpha - \beta|/60$, where α is the angle of O6–Cu1–N5 and β is the angle of O3-Cu1-O4 in the coordination environment).^{29,30}

Therefore, the coordination polyhedron of Cu(II) in 2 is a slightly distorted square pyramid (Figure 3b) which is ascertained of a deviation of 6.02 % for the ideal square pyramid calculated by continuous shape measures method. Notably, as shown in Figure 3c, two crystallographically independent Cu(II) ions are bridged by two phenolic oxygen atoms (O5) to form a dinuclear unit with a Cu-Cu distance of 6.052(3) Å. Due to three hydrogen bonds of O7-H7...O1, C6-H6...O1 and O6-H6a...O7, the dinuclear molecule is connected to an infinite onedimensional chain (Figure 3d), wherein the ligand nitro oxygen (O1) acted as a hydrogen bond acceptor, C-H on the benzene ring and O-H on the coordinated methanol molecule serve as donors, and oxygen on the crystalline methanol acts as both a hydrogen bond acceptor and donor. The distance of O7…O1, C6…O1 and O6…O7 are 2.877(5), 3.460(3) and 2.651(4) Å. The angle of O7–H7…O1, C6–H6…O1 and O6–H6a…O7 are 159.01(1)°, 158.61 (4)° and 162.82(2)°. Since the two coordination reactions are performed under exactly the same conditions, the change of structure between 1 and 2 can be attributed to the electronic properties and spatial effects of anions used.



Figure 3. a) View of the coordination environment of Cu1 center in **2** with thermal ellipsoids at 30% probability (All H atoms have been omitted for clarity. Symmetry code for O5 : 1-x, -y, -z); b) The coordination polyhedron of Cu1 in **2** Symmetry code for O5 : 1-x, -y, -z); c) The dinuclear structural unit of **2** (Symmetry code: 1-x, -y, -z); d) The 1D supramolecular chain of **2** constructed by C–H···O and O–H···O hydrogen bonds (Symmetry code: A = 1-x, -y, -z; B = 1-x, -1-y, 1-z; C = x, -1+y, 1+z; D = 1-x, -2-y, 2-z; E = x, -2+y, 2+z).

Crystal structure of 3. To further study the effect of different metal ions on the crystal structure, the same experiment was carried out by replacing Cu(OAc)₂·H₂O with Mn(OAc)₂·4H₂O. As depicted also in Table S1, the introduction of manganese ions with hard Lewis acidity output a binuclear complex 3 with a chemical formula of [Mn₂L₂(OCH₃)₂(H₂O)₂] · CH₃OH which is quite different from 2 where two Mn(III) coordination subunits are connected by two methoxyl originated from methanol solvents. 3 crystallizes in the monoclinic system of P2₁/c space group and its asymmetric unit contains one Mn(III) ion, one ligand anion L^{2-} , two methoxyl and one water molecule (Figure 4a). As a result of coordination preferation of manganese ions, Mn(III) in 3 is six coordinated and its coordination sphere is fulfilled by five oxygen atoms and one N atom provided by one ligand anion L²⁻ (O2, O3, and N2), two methoxyl (O7 and O7A) and one water molecule (O6). In the coordination polyhedron of 3 (Figure 4b), the Mn-O2/O3/O7A /N2 bond distances are 1.912(3)/1.915(3)/

1.872(3)/1.990(4) Å, the Mn–O6/O7 bond distances are 2.324(4)/2.172(3) Å. The angles of O2–Mn1–O3/O7A–Mn1–N2 are 168.56(2)°/169.90(2)° and the axial angle of O6–Mn1–O7 are 177.26(2)°, which are slightly off the ideal value of 180°. The angles of O6–Mn1–N2 and O7–Mn1–N2 are 90.66(2)° and 91.74(1)°. The dihedral angle between the planes of N2-Mn1-O2 and O3-Mn1-O7A is 12.01°. Therefore, the coordination polyhedron of **3** can be described as a slightly distorted octahedron³¹ which could be verified by a deviation of 2.36 % from the ideal octahedron calculated by continuous shape measure method. Furthermore, as shown in Figure 4c, two crystallographically independent Mn(III) ions are bridged by oxygen atoms (O7, O7A) of methoxyl to form a dinuclear structural unit with a Mn…Mn distance of 3.108(2) Å. With the help of intramolecular hydrogen bonds, the adjacent dinuclear units were further connected to give a one-dimensional supramolecular chain with crystalline methanol molecules acted

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both as acceptor and donor, coordinated water and nitrogen atom from pyrazole functioned as donor and acceptor for the two hydrogen bonds (O6–H6B···O8, O8–H8···N3) (Figure 4d). The distance of O6…O8 and O8…N3 are 2.818(6) and 2.815(6) ແດ້ The angle for O6–H6B…O8 and O8–H8^{.D}N3 ີສາ& 360.82(3)⁹⁹480 166.58(3)° respectively.

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Figure 4. a) View of the coordination environment of Mn1 center in **3** with thermal ellipsoids at 30% probability (Crystalline methanol All H atoms have been omitted for clarity, Symmetry code for O7A: 1-x, 1-y, 1-z); b) The coordination polyhedron of Mn1 in **3** (Symmetry code for O7A: 1-x, 1-y, 1-z); c) The dinuclear structural unit of **3** (Symmetry code: 1-x, 1-y, 1-z); d) The 1D supramolecular chain of the **3** constructed by O–H…O and O–H…N hydrogen bonds (Symmetry code: A = 1-x, 1-y, 1-z; B = -1+x, y, z; C = -x, 1-y, 1-z; D = 2-x, 1-y, 1-z; E = 1+x, y, z).

From the above discussion, We can see that the electronic properties of both metal ions and anions do affect the conformation and coordination behavior of ligand H_2L although the bond length data of pyrazolone group of H_2L in 1, 2 and 3 show that all the carbonyl group of pyrazolone group in the ligand present as enol form (Table S2).³² As depicted in Figure 5, the angles between the coordination plane of the central metal ion and the three benzene rings in the ligand H_2L are 10.29°, 62.73°, and 12.55° in compound 1. And that in compound 2 and 3 are 23.18°, 66.31°, 9.37° and 8.94°, 86.37°, 21.00°

respectively. In addition, in the formation of the compound 1, H_2L took off one proton of hydroxyl groups of pyrazolone and acted as a tridentate monovalent anionic ligand to chelate one Cu(II) ion. By contrast, in compound 2, H_2L not only loses the hydroxyl hydrogen of pyrazolone, but also removes the hydrogen on acylhydrazone and acted as the divalent tetradentate anion ligand for chelating and bridging two Cu(II) ions. However, H_2L is a divalent tridentate anion ligand for chelating one Mn(III) ion although its hydroxyl hydrogen on pyrazolone and acylhydrazone also disappeared.



Figure 5. The diagram of the angles between the coordination plane of the central metal ion and the three benzene rings in the ligand H₂L between in 1 (a), 2 (b) and 3 (c).

Theoretical calculations

To further understand the electronic structures of H_2L , 1, 2 and 3, DFT calculations are performed. In the singlet state, we optimized the molecular geometry by the Gaussian 09 with the level of B3LYP. 6-31G basis sets for Non-metal atoms and LANL2DZ basis sets for metal atom were used to perform the calculation. The optimized structures of H_2L , 1, 2 and 3 are shown in Figure S1 and their cartesian coordinates are depicted

in Supporting Information. According to the experimental and calculated bond distances of the three metal complexes listed in Table 1, it is apparent that the optimal bond distance calculated is in good agreement with that of the experiment. The deviation between the calculated and experimental bond distances in **1** ranged from 0.0089 Å to 0.0804 Å, 0.0115 Å to 0.2137 Å in **2**, and 0.0315 Å to 0.3508 Å in **3**, which could be attributed to the calculated geometry in isolated state, while the experimental

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geometry is an atom filled with lattice and with the presence of intermolecular forces.³³

The surface plots of some selected molecular orbitals of the H₂L and its three metal complexes are shown in Figure 6. The calculated molecular orbital energy of the LUMO+2, LUMO+1, LUMO, HOMO, HOMO-1, and HOMO-2 for H₂L are -1.177, -1.892, -2.685, -6.198 -6.696, and -6.885 eV, respectively. The calculated molecular orbital energy of the LUMO and HOMO for 1 are -4.826 and -6.412 eV. The calculated molecular orbital energy of the LUMO and HOMO for 2 are -4.541 and -5.098 eV. While that for 3 are -3.330 and -5.649 eV. For 1, the graph of the highest occupied molecular orbital (HOMOs) indicates that the molecular orbital electron cloud is mainly present in the orbits of the salicylhydrazone fragment. The graph of the lowest unoccupied molecular orbital (LUMOs) indicates that the LUMOs are delocalized and electron clouds are mainly distributed in the orbits of Cu(II). In the case of 2, the HOMOs and LUMOs are delocalized and reside on the orbitals of the entire ligand. For 3, the HOMOs reside mainly on the orbitals of the Mn(III) with the LUMOs spread over the orbitals of the entire molecule. The total energy of the H₂L, 1, 2 and 3 are -1575.285, -3674.651, - 3772.915 and -3740.169 au, respectively. The HOMO-LUMO orbital energy of ligands and complexes are alphegative, and the total energy of the three metal complexes are all much lower than the ligand, which proves H₂L and its three metal complexes are all stable and the stability should be in the order of 2 > 3 > $1 > H_{2}L^{34}$

Table 1. Experimental and calculated bond distances (Å) in the 1, 2 and 3.					
Complex	Bond lengths	Experimental	Calculated		
1	Cu1-03	1.904(2)	1.84928		
	Cu1-02	1.944(2)	1.86360		
	Cu1–N2	1.988(3)	1.93773		
	Cu1–Cl1	2.216(1)	2.22498		
2	Cu1-03	1.902(2)	2.02364		
	Cu1-04	1.922(2)	2.00514		
	Cu1-06	1.950(2)	2.16810		
	Cu1–N5	1.926(2)	2.08653		
	Cu1-05	2.686(2)	2.69758		
3	Mn1–02	1.912(3)	1.97066		
	Mn1–03	1.915(3)	1.87849		
	Mn1–N2	1.990(4)	1.95850		
	Mn1–07A	1.872(3)	2.09879		
	Mn1–06	2.324(3)	2.11491		
	Mn1–07	2.172(3)	1.82115		



UV-visible absorption spectra

UV-Vis absorption spectroscopy is usually used as a tool for analyzing and inferring the composition content and structure of a substance. Therefore, we measured the UV-Vis spectra of H₂L, **1**, **2** and **3** in 5.0 \times 10⁻⁵ mol·L⁻¹ methanol solution. As shown in Figure 7, the UV absorption peaks of 1, 2 and 3 are significantly different from that of H_2L . The absorption spectra of H_2L shows an absorption peak at 351 nm, which is attributed to the $\pi \rightarrow \pi^*$ transition in the ligand, and its molar absorption coefficient value (ϵ) is 2.49 × 10⁴ L mol⁻¹ cm⁻¹, indicating that this ligand has a strong ability to absorb light. The UV absorption peaks of complexes 1, 2 and 3 were at 368 nm, 358 nm and 372 nm, respectively. Compared with the UV-Vis spectra of H₂L, those of

three complexes all were red-shifted, which may be due to the perturbation caused by metal ion coordination.





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To better clarify the UV-visible absorption spectra of H₂L, 1, 2, and 3, we performed TD-DFT calculations and simulated UV-Vis spectra based on the optimal geometry of the ligand and its complexes in methanol.³⁵ The calculated vertical electronic excitation energy (eV) and oscillator strength (f) are summarized in Table 2. The experimental UV absorption peak of H₂L and the three metal complexes can be explained by TD-DFT calculation. The experimental absorption peak of H₂L is located at 351 nm, and the corresponding simulated absorption peak at 355 nm is attributed to the transitions of (69%) HOMO–3 \rightarrow LUMO, (16%) HOMO-2 \rightarrow LUMO and (13%) HOMO-1 \rightarrow LUMO, which is intraligand charge transfer (ILCT) characters. As for the three metal complexes, the simulated peak of 1 at 376 nm and that of experiment at 368 nm is attributed to (69%) HOMO-1 \rightarrow LUMO+1 and (10%) HOMO-1 \rightarrow LUMO+2 transitions, the simulated peak of 2 at 365 nm and that of experiment at 358 nm is attributed to (14%) HOMO-4 \rightarrow LUMO+1 and (56%) HOMO- $1 \rightarrow$ LUMO+3 transitions and the simulated peak of **3** at 348 nm and that of experiment at 372 nm is attributed to (18%) HOMO-4 \rightarrow LUMO+2, (17%) HOMO–3 \rightarrow LUMO+3 and (48%) HOMO \rightarrow LUMO+5 transitions. Obviously, the UV spectra simulated by H₂L

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and its three metal complexes are in good agreement with the experimental spectra. DOI: 10.1039/C9NJ05948B





Excitation(eV) (eV)	λ _{ov} (nm)	Osc strength (f)	Key transitions	Character	λexpt
HaL	Xex ()		hey dansidono	endroter	nenpt
3.4005	364.61	0.1848	(67%) HOMO–2 → LUMO	H ₂ L(π)→H ₂ L(π*)	-
			(69%) HOMO–3 → LUMO	H ₂ L(π)→H ₂ L(π*)	
3.4914	355.12	0.2473	(16%) HOMO–2 → LUMO	H ₂ L(π)→H ₂ L(π*)	351
		(13%) HOMO–1 \rightarrow LUMO	H₂L(π)→H₂L(π*)		
3.8787	319.66	0.3499	(66%) HOMO \rightarrow LUMO+1	$H_2L(\pi) \rightarrow H_2L(\pi^*)$	-
1					
2.8687 432.20	0.1544	(60%) HOMO \rightarrow LUMO+1	H ₂ L(π)→H ₂ L(π*)	-	
		(19%) HOMO \rightarrow LUMO+2	$H_2L(\pi) \rightarrow H_2L(\pi^*)$	-	
3.2680	379.38	0.3447	(48%) HOMO \rightarrow LUMO+2	H₂L(π)→H₂L(π*)	-
3.2941 376.39	0.2764	(69%) HOMO–1 \rightarrow LUMO+1	$M(\pi) \rightarrow H_2L(\pi^*)$	368	
		(10%) HOMO–1 \rightarrow LUMO+2	$M(\pi) \rightarrow H_2L(\pi^*)$		
2					
3.3914 365.58	0.6441	(14%) HOMO–4→ LUMO+1	$H_2L(\pi) \rightarrow H_2L(\pi^*)$	358	
		(56%) HOMO–1→ LUMO+3	H ₂ L(π)→H ₂ L(π*)		
3.5862 345.73	0.2866	(25%) HOMO–3→ LUMO+2	$H_2L(\pi) \rightarrow H_2L(\pi^*)$	-	
		(45%) HOMO–2→ LUMO+4	H₂L(π)→H₂L(π*)	-	
		(18%) HOMO–5→ LUMO+2	$H_2L(\pi) \rightarrow H_2L(\pi^*)$	_	
3					
3.5597 348.30		(18%) HOMO–4→ LUMO+2	H₂L(π)→H₂L(π*)		
	348.30	3.30 0.9139	(17%) HOMO–3→ LUMO+3	$M(\pi) \rightarrow H_2L(\pi^*)$	372
			(48%) HOMO→ LUMO+5	$M(\pi) \rightarrow H_2L(\pi^*)$	
3.6345 341.13	341 13	3 0.0926	(54%) HOMO−1→ LUMO+4	$M(\pi) \rightarrow H_2L(\pi^*)$	-
	341.13		(39%) HOMO→ LUMO+5	M(π)→ H₂L (π*)	_

DNA-binding Experiments

Electronic absorption titration. UV-Vis spectroscopy is one of the most effective means for understanding the mode and affinity of molecular and DNA binding. After adding equal concentrations of CT-DNA in 2.5 mL H₂L, 1, 2, and 3 (3×10^{-5} M) Tris-HCl/NaCl buffer solution, the intensity of the absorption band at 340~380 nm ($\pi \rightarrow \pi^*$) decreased and a slight red shift occurred (Figure 9a, c, e and g). With the amount of DNA from 0 uL to 150 uL, the absorption band of H₂L, 1, 2, and 3 shows a hypochromism of about 22.29%, 56.76%, 40.59% and 38.48%, respectively. At the same time, the absorption bands of H₂L, 1, 2, and 3 all show red shifts of about 3~4 nm at the maximum absorption point. It has been known from previous reports that the hypochromic change with a red shift in the UV spectrum of the complex is an evidence of intercalative mode of binding, and the extent of hypochromicity of the absorption band can be taken as a measurement of intercalative binding strength.^{36,37} Therefore, the changes of UV-Vis absorption of H₂L, 1, 2 and 3 indicate that the complex binds to CT-DNA through an intercalation mode. The binding constant of H₂L, 1, 2 and 3 with CT-DNA (K_b) was determined by monitoring the changes of the absorbance (Figure 9b, d, f and h). The intrinsic binding constant K_b of H₂L, 1, 2, and 3 are 5.68 × 10³ M⁻¹ (R = 0.991 for 15 points), 4.17 × 10⁴ M⁻¹ (R = 0.998 for 15 points), 2.43 × 10⁴ M⁻¹ (R = 0.997

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for 15 points) and 9.24 × 10³ M⁻¹ (R = 0.987 for 15 points), respectively. Obviously, the K_b values of ligand and its three metal complexes are higher than those of classical intercalators, and their binding affinity follows the order of $1 > 2 > 3 > H_2L$.^{38,39} Generally speaking, there are two reasons for the above results: a) The charge transfer of the ligand to the metal ion by

coordination of H_2L with the metal atom reduces the charge

density on the pyrazole ring, the benzene ring_{New} tracted which facilitates the insertion of the pyrazole of the benzene ring into the DNA base pair;⁴⁰ b) The steric hindrance of the crystal structure also affects the binding affinity to DNA.⁴¹ The planarity of the three metal complexes is superior to that of the ligand and the planarity of the three metal complexes follows the order of 1 > 2 > 3, hence their embedding ability is 1 > 2 > 3.



Figure 9. Electronic spectra of H₂L (a), 1 (c), 2 (e), and 3 (g) in Tris-HCl/NaCl buffer (pH = 7.2) upon addition of CT-DNA (0~150 uL); Plots of [DNA] / ($\epsilon_a - \epsilon_f$) vs. [DNA] for H₂L (b), 1 (d), 2 (f), and 3 (h) (Arrow shows the emission intensity changes upon increasing DNA concentration). [H₂L], [1], [2] and [3] = 3 × 10⁻⁵ M, [DNA] = 2.5 × 10⁻³ M.

EB-DNA fluorescence competition experiment. To further investigate the binding properties of H₂L, 1, 2 and 3 with DNA, EB-DNA fluorescence experiments were performed in Tris-HCl/NaCl buffer (pH = 7.2). 10 uL of EB (2.2×10^{-3} M) and 10 uL of CT-DNA (2.5 ×10⁻³ M) solution were added to 2.5 mL of Tris-HCl / NaCl buffer, followed by dropping the sample solution to observe the changes in the fluorescence spectrum (Figure 10a, c, e and g). The selected fluorescence spectrum with emission range between 550 and 650 nm were excited at a wavelength of 520 nm.⁴² Upon addition of H₂L or its complexes 1, 2 or 3, the emission intensity of the EB-DNA system is significantly reduced, indicating that both the ligand and its three complexes could replace EB of the EB-DNA system. Significantly, Ethidium bromide (EB) is an aromatic fluorescent compound that can be embedded in nucleobase molecules to detect DNA, so H_2L and the three complexes are most likely to bind DNA in an intercalated manner.43 In addition, the fluorescence quenching data were further analyzed by the Stern-Volmer relation. As shown in Figure 10b, d, f and h, the K_{sv} values for H₂L, 1, 2 and 3 are $3.45 \times 10^3 \text{ M}^{-1}$ (R = 0.993 for 8 points), $1.51 \times 10^4 \text{ M}^{-1}$ (R = 0.997 for 13 points), $1.16 \times 10^4 \text{ M}^{-1}$ (*R* = 0.991 for 13 points) and $8.71 \times 10^3 \text{ M}^{-1}$ (R = 0.998 for 13 points), respectively. Fluorescence quenching efficiency shows the binding affinity of H₂L and its three complexes to DNA which follow the order of $1 > 2 > 3 > H_2L$.⁴⁴⁻⁴⁶ Such a trend is consistent with the previous absorption spectral results. In combination with the previous analysis of the complex structure, we deduced that the degree of their ability to bind to DNA is dependent on the coplanarity of the compound. As mentioned in the structural analysis, the degree of coplanarity of the three complexes is in the order of 1> 2> 3 and the coplanarity of free ligand H₂L is inferior to its complexes.



Figure 10. Emission spectra of EB bound to CT-DNA excited at 520 nm in the presence of H_2L (a), 1 (c), 2 (e), and 3 (g); Fluorescence quenching curves of EB bound to CT-DNA induced by H_2L (b), 1 (d), 2 (f), and 3 (h). [H_2L], [1], [2] and [3] = 3 × 10⁻⁵ M, [DNA] = 2.5 × 10⁻⁵ M.

Conclusions

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To sum up, three transition compounds (1, 2 and 3) based on a new 4-acylpyrazolone derivative, 2-hydroxy-N'-((5 hydroxy-3methyl-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-4-yl)(phenyl)methylene)benzohydrazide (H₂L), were prepared by using different metal ions and anions for tuning the structure as well as DNA binding properties. Crystal structure analysis shows that 1 is a mononuclear compound with planar quadrilateral configuration and its coplanarity is strong. Howerer, 2 is a dinuclear five-coordinated Cu(II) compound with two copper coordination units plane bridged by salicylhydrazide hydroxyl groups of the ligand by replacing chloride anion in 1 with acetate. 3 is a quite different dinuclear compound with two Mn(III) coordination sphere bridged by methoxyl which was resulted by further replacing Cu(II) with Mn(III). DNA binding studies performed on the pyrazolone ligand and its three complexes by electron absorption titration and EB-DNA competition experiments indicate they all bind DNA in an intercalation mode and their binding affinity follows $1 > 2 > 3 > H_2L$. The results of this experiment indicate that steric hindrance of compound played pivotal roles in DNA binding. Furthermore, the structural optimization of H₂L, 1, 2 and 3 by DFT was performed, and TD-DFT calculations were performed on this basis to gain a better understanding of the electronic transitions in the UV-Vis spectra.

Conflicts of interest

There are no conflicts to declare.

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