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QSAR STUDY OF CO-ORDINATION COMPLEXES OF RUTHENIUM ON NUCLEIC ACIDS AS AN ANTI-CANCEROUS DRUGS

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Abstract
In this paper, we have studied about the Anti-Cancerous Property of Ruthenium complexes with nucleic acids with the help of different types of linkages like hydrogen bonding, electronic effects as well as hydrophobic interactions. Some other parameters also used for producing different types of complexes like varying chemical hardness of receptor molecule, electro negativities etc.

Keywords: Nucleic Acids, Ruthenium Complexes ; Quantitative Structure-Activity Relationships.

Introduction

For years, many attentions have been focused on the interaction of octahedral Ru (II) complexes with DNA owing to their potential utility as DNA probes, molecular light switches and chemotherapy drugs and photodynamic therapy for tumors [1-13]. For one thing, DNA has long been considered the main target for anticancer drugs. In general, Ru (II) complexes can bind to DNA in three non- covalent modes: intercalation binding, groove binding and electrostatic binding. It's know that complexes with an enlarged aromatic ligand(intercalating ligand) can bind to DNA with high affinity(104~106), while those complex such as $Ru(bpy)_3^{2+}$ can bind to DNA mostly in electrostatic [14].

For the last decade, a number of ruthenium complex with 2-phenylimidazo [4,5-*f*][1,10]-phenanthroline (PIP) and its derivates as intercalating ligand have been synthesized and their DNA binding properties have been investigated thoroughly. Ji et al. indicate that the binding affinity of these complexes depended not only on the conformations of DNA, but also on the structures of intercalative ligands [15-18]. The factors included the enlarged aromatic ring, intramolecular hydrogen bond and the planarity properties of intercalative ligand will enhance the binding affinity of these ruthenium to DNA, and the electronic effects (the donor/acceptor electron properties of substituent group on intercalative litgand) is also one factor influencing the bind of complexes to DNA [19,20].

A recently studies try to explain the DNA-binding affinity by computational calculations with density functional theory (DFT). It's shown that the energy of these complex' frontier molecular orbit, that is the highest occupied molecular orbit (HOMO) and the lowest unoccupied molecular orbit (LUMO) is varied and when they intercalating in the DNA base pairs, the energy of the transition conformation will different. According to the frontier molecular theory, an electron will transfer more easily from a high HOMO to a lower LUMO, and resulting those complex have the lowest LUMO (in generally, the HOMO of DNA is higher than that of ruthenium complexes) will bind to DNA the strongest [21-23]. But this is still confused since the binding energy of complexes to DNA is not known and the optimal conformation of the supermolecular complex-DNA is also not been illuminated.

In 1930's, Hammett indicated that the activity of an organic reaction is in relating to the substituent effects [24-26]. Based on these, Fujita and Hansch developed a linear Hansch equation to elucidate the relationship between the bioactivity/physical activity and the structure of organic molecules [27], and which is so called the linear free energy relationships and thus have been utilized extensively in agrochemistry, pharmaceutical chemistry, toxicology [28] for its excellent predictable ability. More recently, Prasanna S et al. successful discerned the structural and physicochemical requirements for selective COX-2 over COX-1 inhibition among the fused pyrazole ring systems by Hansch method [29]. However, there are still no reports focused on quantity structure-activity relationship on DNA-binding properties of ruthenium(II) complexes [30,31].

In this paper, a series of Ru (II) complexes with electron-donor or electron- acceptor substituents in the intercalative ligands, $[Ru(phen)_2(o\text{-}MOP)]^{2+}$1, $[Ru(phen)_2(o\text{-}MP)]^{2+}$2, $[Ru(phen)_2(o\text{-}CP)]^{2+}$3 and $[Ru(phen)_2(o\text{-}NP)]^{2+}$4 (**Scheme 1**) were synthesized and characterized. The DNA-binding properties of these complexes have been investigated by the spectroscopic and viscosity experiments. The quantity structural-activity relationship of these ruthenium complexes, as well as some other analogues has also been investigated.

Experiment Section

Chemicals

CT-DNA was purchased from the Sino-American Biotechnology Company. All reagents and solvents were purchased commercially (AR, Acros Inc., and Sigma Inc., etc.) and used without further purification unless otherwise noted. Doubly

distilled water was used to prepare buffers. The concentration of calf thymus DNA was determined spectrophotomertrically using the molar absorptivity 6600 $M^{-1} \cdot cm^{-1}$ (260 nm) (The ratio of UV absorbance at 260 and 280 nm is in the range of 1.8-1.9:1).

Synthesize and characterization

$[Ru(phen)_2Cl_2] \cdot 2H_2O$ were prepared following the literature procedure [24]. Ru(II) complexes 1, 2, 3 and 4 were synthesized by refluxing $Ru(phen)_2Cl_2$ and o-MOP (o-MP, o-CP or o-NP) in ethylene glycol under an argon atmosphere with high yield. Each complex was obtained as a PF_6- salt and purified with column chromatography.

Ru (II) complexes 1, 2 and 3 emit fluorescence in Tris-buffer in the range of 500 – 700 nm at room temperature, with the maximum at 589, 588 and 589, respectively, and only a very weak fluorescence was observed for complexes 4 at the same conditions (the maximum is at 588 nm).

2-(2-methoxylphenyl) imidazo [4,5–f][1,10] phenanthroline(o- MOP) (1a): The ligand 2-(2-methoxylphenyl) imidazo [4,5-*f*][1,10] phenanthroline (o-MOP) was prepared by the method similar to that in reference [32], and with some modification.

A solution of phenanthraquinone (0.26 g, 1.2 mmol), o-anisaldehyde (0.24 g, 1.8 mmol) and ammonium acetate (1.9 g, 25 mmol) in 10 cm^3 glacial acetic acid was refluxed for 2 hour. The cooled deep red solution was diluted with 25 cm^3 water, and neutralized with ammonmium hydroxide. Then the mixture was filtered and the precipitates were washed with water and acetone, then dried and purified by chromatography over 60-80 mesh SiO_2 using methanol as an eluent, yields: 0.35 g, 84%. Calculated for $C_{20}H_{14}N_4O \cdot H_2O$ (%): C: 69.7; H: 4.69; N: 16.3; Found(%): C: 69.3; H: 4.66; N: 16.2. ES-MS (in DMSO, *m/z*): 326.7 (calc. 326.4).

2-(2-methylphenyl) imidazo [4,5–f][1,10] phenanthroline(o- MP) (2a)
2-(2-chlorophenyl) imidazo [4,5–f][1,10] phenanthroline(o-CP) (3a)
2-(2-nitrophenyl) imidazo [4,5–f][1,10] phenanthroline(o-NP) (4a)

Physical measurements

Microanalyses were carried out on an Elementar Vario EL elemental analyser. Electrospray mass spectra (ESI-MS) were recorded on a LCQ system (Finnigan MAT, USA). The spray voltage, tube lens offset, capillary voltage and capillary temperature were set at 4.50 kV, 30.00 V, 23.00 V and 200 °C, respectively, and the quoted m/z values are for the major peaks in the isotope distribution. Emission spectra were measured on a Shimadzu RF-5000 spectrofluorophotometer and UV-Visble absorption was recorded on a Shimadzu UVPC-3000 spectrophotometer. Viscosity experiments were performed on an Ulbbelodhe viscometer, immersed in a thermostatted water-bath maintained at 30.0 ± 0.1°C. Data were presented as (η/η0)1 / 3 vs. the concentration of [Ru]/[DNA]. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t > 100 s) corrected for the flow time of buffer alone (t0), i.e., η=t-t0.DNA-binding properties of Ru (II) complexes

Electronic absorption spectra: In general, the complex binding to DNA in an intercalation mode exhibits a red and hypochromism shift in the absorption spectra, and the extents of spectral change are closely correlative to the DNA-binding affinities of these complexes. The spectral shifts in an intercalation mode are usually greater than those in groove binding mode. In the presence of double helix calf thymus DNA (CT- DNA), the electronic absorption spectra for all of these complexes exhibit obviously hypochromism, and the hypochromism values for 1, 2, 3 and 4 at MLCT absorption band (452~455 nm) are 12, 9, 9 and 21%, respectively.

Results and Discussion

In order to clarify the DNA-binding affinities of these complexes, the intrinsic binding constants were calculated according to equation (1) [32], through a plot of

[DNA]/ Σa-Σf vs. [DNA]

[DNA]/ Σa-Σf=[DNA]/Σb-Σf+1/Kb(Σa-Σf) (1)

where [DNA] is the concentration of DNA in base pairs, Σa, Σf and Σb are respectively the apparent extinction coefficient (Aobsd/[M]), the extinction coefficient for free metal (M) complex and the extinction coefficient for the metal(M) complex in the fully bound form. In plots of [DNA]/ Σa-Σf versus [DNA], Kb is given by the ratio of slope to intercept. The calculated values for 1, 2, 3 and 4 at MLCT absorption band are 1.1, 0.35, 0.53 and 1.7 × 105 M-1, respectively. These values are

smaller than those for [Ru(bpy)2dppz]2+(> 106 M-1) [33] and [Ru(ip)2dppz]2+(2.1 × 107 M-1) [34]. Such DNA-binding constants suggest that the interaction of these complexes with DNA should be in an intercalation mode.

Emission spectra: The interaction of Ru (II) complexes with double helix CT-DNA was monitored via luminescence. All ruthenium complexes 1-4 emit luminescence in the range 500-700 with the maxium near 600 nm at room temperature. Upon the addition of CT-DNA, the emission spectra of all of these complexes enhanced obviously . The emission of complex 4 exhibits pronounced enhancement, and its emission intensity increases steadily to8.5 times relative to that of the original and reaches saturation at ca. [DNA] / [Ru]=8:1. However, the emission intensities increase by 2.2, 1.7 and 1.5 for complexes 1, 2 and 3, respectively. The enhancement of emission intensities of these complexes can be attributed to the hydrophobic environment inside the DNA helix, which reduces the accessibility of water molecules and makes the mobility of the complexes be restricted at the binding site.

Viscosity behaviors: The viscosity experiments, being sensitive to the change of length of double helix DNA, were considered as one of the most unambiguous methods to determine the binding mode of complex to DNA in absence of crystal data [35]. In general, the relative viscosity of DNA in presence of complex in an intercalation mode will be increased, because the intercalative ligand will separate the base pairs of DNA, and thus lengthen the DNA helix. On the contrary, a partial and/or non-classical intercalation of complex will reduce the relative viscosity of DNA, since the binding ligand may bend (or kink) the DNA helix and reduce its effective length [36]. The experiments on relative viscosity of rod-like CT-DNA in the presence of complexes 1, 2, 3 and 4, as well as $[Ru(bpy)_3]^{2+}$, were carried out.

The viscosity of DNA remains almost unchanged upon addition of $[Ru(bpy)_3]^{2+}$, which is consistent with an electrostatic association. However, in the presence of Ru (II) complexes 1, 2, 3 and 4 respectively, the relative viscosity of rod-like DNA was increased, because the stacking interaction of these complexes with the base pairs of DNA lengthens the DNA helix, indicating these complexes can bind to DNA in intercalation mode.

Quantitative structure-activity relationships on ruthenium(II) complexes

Quantitative Structure-Activity Relationships was carried out on MatLab 6.5 for these newly synthesized ruthenium(II) complexes, as well as some congers from references.

Firstly, we try to draw a plot with the logKb versus electronic parameter (σ) on Origin 6.0, and the corresponding equation is as follows:

LogKb=0.6726 (± 0.1751)σ+4.5001 (± 0.09799) (2)

n=7; r=0.8643; SD=0.1683; p=0.0121

Outlinear:

X=-OCH3, Y=-H; X=-CH3, Y=-OH; X=-H, Y=-Phen; X=-H, Y=-OH; X=-H, Y=-OCH3

Considering there is hydrogen bond exists in some of these ruthenium complexes, we import indicative variable (IH-bonding) to indicate hydrogen bond exists in the intercalative ligand. The value of IH-bonding is 1 if there is hydrogen bond, egardless it's intramolecular or intermolecular hydrogen bond, and the value of IH-bonding is 0 if there is not, thus we get model 2:

LogKb=0.2770σ+0.2818I+4.6366 (3)

n=12; R=0.7441; F=5.5824; p=0.0265

It's obviously the hydrogen bond contribute to the DNA binding of these ruthenium complexes, since the coefficient IH-bonding is positive. Encouraged, we considered that the hydrophobic parameter may also contribute to the DNA- binding properties of these complexes, thus we obtained the model 3:

LogKb=0.2429π+0.0429π2+0.2907σ+0.6389I+4.3491 (4)

n=12; R=0.9338; F=11.9134; p=0.0030

In this model 3, it's obviously the coefficient of electronic parameter(σ) is positive, indicating the electron acceptor group on intercalative ligand of ruthenium complexes will enhance the binding affinity of ruthenium complexes to DNA, while an electron donor group will decrease the binding affinity. The positive coefficient for the hydrophobic parameter(π) indicate a hydrophobic group in the intercalative ligand will increase the DNA-binding affinity of ruthenium complexes, while hydrophilic group will decrease the DNA-g affinity.

Conclusion

A series of ruthenium(II) have been synthesized, and the bindingbehavior of these ruthenium(II) complexes with calf-thymus DNA have been investigated, and the results show that these complexes can bind to DNA in intercalating mode. The further studies on the quantity structure-activity relationship of these ruthenium complexes, as well as some from reference was investigated, and a QSAR equation was obtained: logKb=0.2429π+0.0429π2+ 0.2907σ+0.6389I+4.3491 (n=12; R=0.9338; F = 11.9134; p = 0.0030). It's shown that the DNA-binding affinity of ruthenium complexes in studied depended on the electronic effect, hydrophobic effect and hydrogen bond, and an electron withdraw group in the intercalative ligand will increase the DNA-binding affinity of ruthenium complexes, while an electron Donor group will decrease the DNA-binding affinity. In addition, hydrogen bond is important to obtain a high DNA-binding ruthenium complex.

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