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Synthesis and cytotoxic activities of transition-metal complexes of a binaphthyl-linked bipyridine ligand

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Abstract A binaphthyl-linked bipyridyl compound, 1,1′-bis(6-methyl-6′-oxymethylenyl-2,2′-bipyridine)binaphthyl, (**L**) has been synthesised and used as a ligand for the formation of Cu(II), Ni(II), and Co(II) complexes. The ligand and its transition-metal complexes were characterized by physico-chemical and spectroscopic methods. The complexes were also investigated for cytotoxic activity. The cytotoxicity of complexes, Cu**L**$(ClO_4)_2$, Ni**L**$(ClO_4)_2(H_2O)$, Co**L**$(ClO_4)_2$, were tested in vitro applying seven well-characterized human tumor cell lines, MCF7, EVSA-T, WIDR, IGROV, M19 MEL, A498, H226, and the microculture sulforhodamine B (SRB) test. All complexes show a very high cytotoxicity ($ID_{50} < 250$ ng/ml) in these cell lines.

Introduction

Binaphthylic compounds have received a great deal of attention as chiral ligands for transition metal catalysts [1, 2]. In recent times, they have also found use as chiral-building blocks in coordination and metallosupramolecular chemistry [3–5]. Considerable interest in these complexes arises from the field of bioinorganic chemistry [5, 6]. In addition, 2,2′-bipyridine is a well-known and important ligand. In earlier work, many chiral bipyridines have been prepared and used as chiral ligands in asymmetric reactions and the self-assembly field [7–10]. More recently, we have synthesized the macrocyclic and acyclic binaphthyl-bipyridyl-based ligands [11] and studied metal complexes of macrocyclic binaphthylic ligand containing two 1,1′-binaphthyl units linked through two bipyridine units, and their genotoxic activity [12]. To the best of our knowledge there are no other reports of the cytotoxic activity of binaphthylic ligands and their metal complexes. For this reason, we now report on the synthesis and characterization of the acyclic ligand 1,1′-bis(6-methyl-6′-oxymethylenyl-2,2′-bipyridine)binaphthyl, (L), which contains two bipyridine units connected through one 1,1′-binaphthyl unit, and its Cu(II), Ni(II), and Co(II) complexes (Fig. 1). Also presented are the cytotoxicities of the novel complexes against human tumor cell lines, MCF7, EVSA-T, WIDR, IGROV, M19 MEL, A498, H226, and the microculture sulforhodamine B (SRB) test.

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Experimental

The ligand (L) was synthesised as reported in the literature [11]. All reagents were used as purchased from commercial suppliers without further purification. The purity of L was checked by its melting point (92–94 °C), elemental analyses Calc. for $C_{44}H_{34}N_4O_2$: C, 81.2; H, 5.3; N, 8.6, Found: C, 80.8; H, 5.0; N, 8.6; molecular weight (Calcd. 650.74, MS *m/z*: 651 (M^+)), and ^{1}H NMR (300 MHz; $CDCl_3$): 2.63 (6H, s, CH_3), 5.27 (4H, s, CH_2), 6.74 (2H, d, *J* 7.3 Hz), 7.14 (2H, d, *J* 7.3 Hz), 7.34–7.39 (8H, m), 7.48 (2H, d, *J* 9.0 Hz), 7.66 (2H, t, *J* 7.6 Hz), 7.88 (2H, d, *J* 7.6 Hz), 7.96 (2H, d, *J* 9.0 Hz), 8.08 (2H, d, *J* 7.9 Hz), 8.16 (2H, d, *J* 7.9 Hz). δ_C (75.5 MHz; $CDCl_3$): 24.67 (CH_3), 72.05 (CH_2), 115.48, 115.61, 118.12, 118.53, 119.94, 120.92, 123.48, 124.01, 125.36, 125.70 (C), 126.68 (C), 128.17,

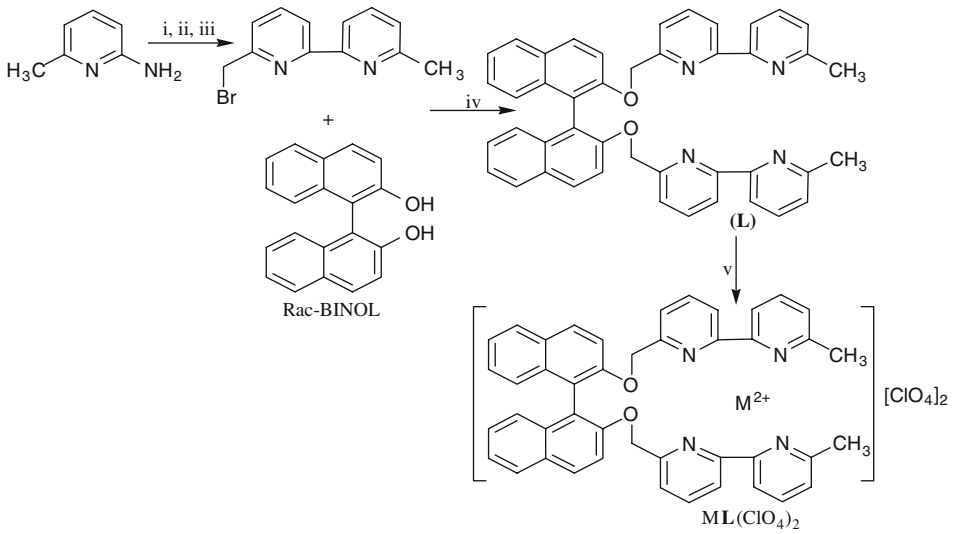

Fig. 1 Synthesis of the ligand **L,** 1,1′-bis(6-methyl-6′-oxymethylenyl-2,2′-bipyridine) binaphthyl and metal complexes $ML(ClO_4)_2$. (i) %47 HBr, Br_2, $NaNO_2$ then NaOH, (ii) $NiCl_2(PPh_3)_2$, Zn, Et_4NI, THF, under argon gas, (iii) NBS, CCl4, benzoyl peroxide, (vi) DMF, K_2CO_3, (v) MeOH, $M(ClO_4)_2$ {M=Cu^{2+}, Ni^{2+}, Co^{2+}}

128.31 (C), 129.694, 134.42 (C), 137.50, 154.09 (C), 155.58 (C), 157.42 (C), 157.99 (C).

Melting points were determined by using a Gallenkamp MPD350.BM2.5 digital melting point apparatus and are listed as uncorrected. The compounds were checked for purity by TLC on silica gel 60 F_{254} (Merck). Spectroscopic data were recorded on the following instruments: elemental analyses were performed on a CHNS-O Carlo Erba EA 1108 elemental analyser; IR spectra of the solid complexes were recorded in KBr on a Shimadzu 470 spectrophotometer; ^{1}H-NMR spectra (δ, ppm, Hz) were recorded on a Varian (300 MHz) in solvent $CDCl_3$; ^{13}C-NMR spectra (δ, ppm, Hz) were recorded on a Varian (75.5 MHz) in solvent $CDCl_3$; MS-FAB$^+$; Finnigan Mat 95 mass spectrometer. Conductivity measurements were carried out in ca. 10^{-3} mol/L anhydrous N,N-dimethylformamide solutions at 20 °C using a Metrohm 712 conductometer. The electronic absorption spectra of the complexes in DMF were measured in the range 200–1100 nm using a Shimadzu UV 160 A, Pharmacia (VIS) spectrophotometer. EPR and Ligand Field spectra were obtained at Leiden University (LIC) on a Bruker EPR at room temperature (powder) and on a Perkin-Elmer Lambda 900 spectrophotometer using the diffuse-reflectance technique, with MgO as a reference.

Preparation of the complexes

To **L** (0.065 g, 0.1 mmol) dissolved in MeOH (25 ml), the appropriate hydrated metal perchlorate salt (0.1 mmol) was added also dissolved in MeOH (25 ml). The reaction mixture was refluxed for 2 h and filtered hot. The filtrate was evaporated to half the volume and then allowed to cool by standing at room temperature. After slow evaporation of the solvent at 25 °C, colored complexes obtained were collected and washed MeOH. The reaction made with Cu(II) perchlorate salt was applied at room temperature.

Co**L**$(ClO_4)_2$: Color of complex: claret, yield: 0.067 g (72%); Selected IR data (KBr disc ν_{max}/cm^{-1}): 1596, 1571, 1504, 1468, 1433, 1366, 1324, 1251, 1203,1091, 816, 790, 752, 624; *m/z*: 808.1 [Co**L**$(ClO_4)]^+$, 354.6 [1/2Co**L**$]^{2+}$ Λ_M/Ω^{-1} mol^{-1} cm^2 (DMF): 158 (2:1); Electronic spectrum (DMF): 338, 296 nm; Calc. for $C_{44}H_{34}N_4O_{10}Cl_2Co$: Co, 6.5; C, 58.1; H, 3.8; N, 6.2, Found: Co, 6.6, C, 57.6; H, 3.7; N, 6.7.

Ni**L**$(H_2O)(ClO_4)_2$: Color of complex green, yield: 0.037 g (40%); Selected IR data (KBr disc ν_{max}/cm^{-1}): 3408, 1600, 1571, 1504, 1468, 1436, 1369, 1321, 1251, 1203,1091, 819, 790, 752, 624; *m/z*: 807.1 [Ni**L**$(ClO_4)]^+$, 707.2 [Ni**L**$]^{2+}$, 354.1 [1/2Ni**L**$]^{2+}$ Λ_M/Ω^{-1} mol^{-1} cm^2 (DMF): 167 (2:1); Electronic spectrum (DMF): 338, 294 nm; Calc. for $C_{44}H_{36}N_4O_{11}Cl_2Ni$: Ni, 6.3; C, 57.1; H, 3.8; N, 6.0, Found: Ni, 6.5, C, 57.0; H, 3.3; N, 5.8.

Cu**L**$(ClO_4)_2$: Color of complex: dark brown, yield: 0.046 g (50%); Selected IR data (KBr disc ν_{max}/cm^{-1}): 1596, 1571, 1504, 1465, 1436, 1376, 1324, 1251, 1212, 1145, 1088, 816, 793, 755, 624; *m/z*: 812.1 [Cu**L**$(ClO_4)]^+$, 713.2 [Cu**L**$]^{2+}$, 356.5 [1/2Cu**L**$]^{2+}$; Λ_M/Ω^{-1} mol^{-1} cm^2 (DMF): 164 (2:1); Electronic spectrum (DMF): 445, 292 nm; Calc. for $C_{44}H_{36}N_4O_{11}Cl_2Cu$: Cu, 7.0, C, 57.9; H, 3.8; N, 6.2, Found: Cu, 7.3; C, 58.0; H, 4.0; N, 5.8.

Activity

Cell lines

The human tumor cell lines, MCF7 breast cancer, EVSA-T breast cancer, WIDR colon cancer, IGROV ovarian cancer, M19 MEL melanoma, A498 renal cancer and H226 non-small cell lung cancer, were used. Cell lines WIDR, M19

MEL, A498, IGROV, and H226 belong to the currently used anticancer screening panel of the National Cancer Institute, USA [13]. The MCF7 cell line is estrogen receptor (ER)+/progesterone receptor (PgR)+ and the cell line EVSA-T is (ER)-/(PgR).

Prior to the experiments, a mycoplasma test was carried out on all cell lines and found to be negative. All cell lines were maintained in a continuous logarithmic culture in RPMI 1640 medium with Hepes and phenol red. The medium was supplemented with 10% FCS, penicillin 100 IU/ml, and streptomycin 100 μg/ml. The cells were mildly trypsinized for passage and for use in the experiments.

Biochemicals

RPMI and FCS were obtained from Life Technologies (Paisley, Scotland). SRB, DMSO, penicillin and streptomycin were obtained from Sigma (St. Louis MO, USA), TCA and acetic acid from Baker BV (Deventer, NL) and PBS from NPBI BV (Emer-Compascuum, NL).

SRB test

The test and reference compounds were dissolved to a concentration of 250000 ng/ml in full medium, by twentyfold dilution of a stock solution which contained 1 mg compound/200 μl. The compounds Cu**L**$(ClO_4)_2$, Ni**L**$(H_2O)(ClO_4)_2$ and Co**L**$(ClO_4)_2$ were dissolved in DMSO. Cytotoxicity was estimated by the microculture sulforhodamine B (SRB) test [14].

The experiment was started on day 0. On day 0, 150 μl of trypsinized tumor cells (1500–2,000 cells/well) were plated in 96-wells flat bottom microtiter plates (falcon 3072, BD). The plates were preincubated 48 h at 37 °C, 8.5% CO_2 to allow the cells to adhere. On day, a threefold dilution sequence of ten steps was made in full medium, starting with the 25,0000 ng/ml stock solution. Every dilution was used in quadruplicate by adding 50 μl to a column of four wells. This results in a highest concentration of 62,500 ng/ml present in column 12. Column two was used for the blank. To column 1 PBS was added to diminish interfering evaporation.

Results and discussion

In the present work, the acyclic ligand 1,1′-bis(6-methyl-6′-oxymethylenyl-2,2′-bipyridine)binaphthyl, (L), which contains two bipyridine units linked via a 1,1′-binaphthyl unit was prepared using our published procedure [11] as a cream solid in a good yield 85% (Fig. 1). Using this ligand (L), a number of novel mononuclear Cu(II), Ni(II), and Co(II) complexes were prepared (Fig. 1). The structures of the ligand and its metal complexes were investigated by physico-chemical and spectroscopic studies.

The proton and carbon-13 NMR spectra for the free ligand were consistent with the proposed formulation. The methyl protons were assigned to the most upfield signal in the spectra (δ 2.63). Methylene protons appeared as a singlet at δ 5.27. The FAB-mass spectrum of the ligand gave a parent ion at *m/z* 651, corresponding to the molecular weight of ligand **L** ($[M+H]^+$). The composition of the ligand was also confirmed by elemental analyses.

The infrared spectra of the complexes were compared with those of the free ligand. Upon comparison, it was found that the $\nu_{(C=N)}$ stretching vibration from bipyridine is found in the free ligand at 1619 cm^{-1}. This band is shifted to lower frequencies (19–23 cm^{-1}) on coordination, as a result of the interaction between the metal and the bipyridine nitrogen atoms in the all complexes. On the other hand, all complexes show single absorption bands at 1088–1091 and 624 cm^{-1} which were assigned to the ν_3 and ν_4 stretching modes of non-coordinated perchlorate ion [15]. For Ni**L**$(H_2O)(ClO_4)_2$ complex, a broad band around 3408 cm^{-1} is considered to originate from the O–H stretchings of the water molecule [16].

The molar conductance measurements in anhydrous DMF are found to be in the range 158–164 $\Omega^{-1}.cm^2.mol^{-1}$ corresponding to 1:2 electrolytic nature for all complexes [17]. Thus, the complexes may be formulated in solution as [Cu**L**]$(ClO_4)_2$, [Ni**L**]$(ClO_4)_2$, and [Co**L**]$(ClO_4)_2$.

The electronic spectra of the ligand and complexes exhibit an intense band around 300 nm region, which may be assignable to $\pi \rightarrow \pi^*$ transitions of the conjugated aromatic rings. The electronic spectra of the complexes are of little help in the present case, since the expected d → d transitions are masked by the tail of this strong charge-transfer band. The [Cu**L**]$(ClO_4)_2$ complex has broad band near 445 nm, which may be caused by the nitrogen → metal charge transfer (L → Cu charge transfer). The LF of the solid Cu compound shows, in addition to a strong LMCT at 470 nm, 2 band maxima (785 and 1550 nm), suggesting an octahedrally and a tetrahedrally based Cu(II) species, although a five-coordinate axially compressed structure cannot be excluded. The EPR of the powder shows an inverted spectrum with g// = 2.05 and g(perp) = 2.22, suggesting a compressed-axial geometry.

The API-ES mass spectrum of the complex, Ni**L**$(H_2O)(ClO_4)_2$, in the positive ion mode is structurally enlightening, since it displays a series of intermediate breakdown species. The loss of an anionic perchlorate ion from the neutral parent molecule generates the cationic $[NiL(ClO_4)]^+$, which is the first peak observed at *m/z* 807.1 in the mass spectra. The loss of a second perchlorate anion occurs to generate $[NiL]^{2+}$ at *m/z* 707.2. The final major

Table 1 ID_{50} values (ng/ml) of test compounds and the reference compounds in vitro using SRB as a cell viability test

	A498	EVSA-T	H226	IGROV	M19	MCF-7	WIDR
Cu**L**$(ClO_4)_2$	250	146	350	2017	180	51	330
Ni**L**$(H_2O)(ClO_4)_2$	45	19	57	624	29	4	63
Co**L**$(ClO_4)_2$	25	15	49	368	24	<3.2	31
DOX	90	8	199	60	16	10	11
CPT	2253	422	3269	169	558	699	967
5-FU	143	475	340	297	442	750	225
MTX	37	5	2287	7	23	18	<3.2
ETO	1314	317	3934	580	505	2594	150
TAX	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2

peak of interest arises at 354.2 in the spectra from the separation of the half *m/z* unit of $[Ni\mathbf{L}]^{2+}$. The electrospray ionization mass spectra of Co**L**$(ClO_4)_2$ shows two intense peaks at *m/z* 808.1 and 354.6. The first peak corresponds to the $[Co\mathbf{L}(ClO_4)]^{+}$ cation, where the parent molecule has lost one perchlorate anion, to generate a positive ion. The loss of a second perchlorate generates half *m/z* unit of $[Co\mathbf{L}]^{2+}$ as a second peak. The electrospray ionization mass spectrum of Cu**L**$(ClO_4)_2$ shows identical breakdown patterns. The loss of an anionic perchlorate ion from the neutral parent molecule generates the cationic $[Cu\mathbf{L}(ClO_4)]^{+}$ species which is the first peak observed in the mass spectra. The loss of a second perchlorate anion generates $[Cu\mathbf{L}]^{2+}$. The separation of the half *m/z* unit of $[Cu\mathbf{L}]^{2+}$ also being observed as a major peak.

Activity

Results of the SRB absorbance and the corresponding dose-response curves of each test compound are given in Appendix 1. The ID_{50} values (ng/ml) of the compounds and some established cytotoxic drugs are given in Table 1.

On day 7, the incubation was terminated by washing the plate twice with PBS. Subsequently, the cells were fixed with 10% trichloroacetic acid in PBS and placed at 4°C for 1 h. After five washings with tap water, the cells were stained for at least 15 minutes with 0.4% SRB dissolved in 1% acetic acid. After staining the cells were washed with 1% acetic acid to remove the unbound stain. The plates were air-dried and the bound stain was dissolved in 150 µl 10 mM Tris-base. The absorbance was read at 540 nm using an automated microplate reader (Labsystems Multiskan MS). Data were used for construction of concentration-response curves and determination of the ID_{50} value by use of Deltasoft 3 software.

The compounds Cu**L**$(ClO_4)_2$, Ni**L**$(H_2O)(ClO_4)_2$, and Co**L**$(ClO_4)_2$ showed mostly a very high cytotoxicity ($ID_{50} < 250$ ng/ml).

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