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ONE-POT SOLVOTHERMAL SYNTHESIS AND X-RAY STRUCTURE OF A METHYLATED TERPYRIDINE DERIVATIVE AS DNA BINDER AND ANTICANCER AGENT

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Abstract – A new terpyridine derivative [mterpy]I {(mterpy = 2-[4-(4-methylphenyl)-2,2'-bipyridin-6-yl]-1-methylpyridinum) iodide} was synthesized and characterized by single crystal X-ray crystallography, and π-π stacking interaction was detected in the structure. The new methylated polypyridine ligand [mterpy]I was easily obtained under solvothermal condition. Meanwhile, the product exhibited evident *in vitro* antiproliferation activity towards cancer cells in methyl-thiazolyl-tetrazolium (MTT) assays.

INTRODUCTION

In recent decades, terpyridine substituted derivatives, as multidentate polypyridine ligands with efficient and stable chelating ability towards many metal ions, have been intensively explored because of their versatility as building blocks for many interesting functional complexes and materials.[1,2] Terpyridine and its derivatives with plentiful potential application, such as DNA binding agents,[3] topoisomerase I inhibitors[4] and antitumor agents,[5] are of important ligands for constructing metal complexes. Specifically, Pt(II) terpyridine complexes as planar intercalators,[6] have been researched for interest in recent years due to their promising biological activity.[7-9] There were a variety of methods for terpyridine modification, among which methylation was not widely used. Generally, the redox behavior of a compound probably changed significantly after methylation, leading to some new interesting characteristics. It has been reported that some ruthenium(II) complexes base on the methylated polypyridine showed particular

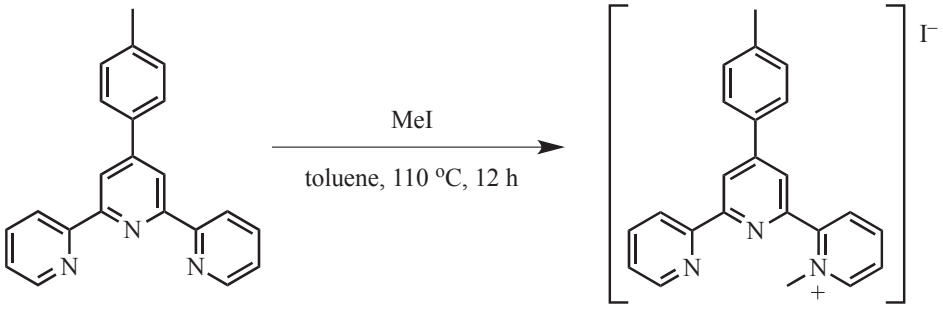
spectroscopic and electrochemical properties.[10-13] However, the properties of methylated polypyridine ligands themselves, especially some biological activities, were often neglected.

In order to develop new ligands and new modifications of known ligands for the preparation of noval molecular complexes, we have successfully synthesized a ligand by a simple method. In particular, we synthesized a monomethylated polypyridyl ligand, [mterpy]I {(mterpy = 2-[4'-(4-methylphenyl)-2,2'-bipyridin-6-yl]-1-methylpyridinum) iodide}, via a one-pot solvothermal approach. The structural details of [mterpy]I was revealed by single crystal X-ray diffraction analysis. And the double-stranded DNA binding ability and *in vitro* cytotoxicity against cancer cell lines were investigated.

RESULTS AND DISCUSSION

CHEMICAL

The methylation of polypyridyl derivatives has been reported previously,[14,15] using iodomethane as the reactant under reflux conditions. In this work, another more advantageous approach was introduced (Scheme 1). Herein, the reactants terpy (4'-(4-methylphenyl)-2,2':6',2''-terpyridine)) and 3.5 equiv. of iodomethane in toluene (5 mL) were sealed in a 10 mL Teflon-lined stainless-steel container, and the reaction was further carried out in the oven set at 110 °C. To the best of our knowledge, this was the first time that in situ solvothermal method was used for the methylation of polypyridyl compound without stirring. Moreover, pale yellow crystals of [mterpy]I suitable for X-ray diffraction studies were directly obtained by cooling of the reaction system in the container. As a result, the methylation reaction was accomplished by simple conditions and convenient separation process.



terpy [mterpy]I

Scheme 1. One-pot solvothermal synthesis of the new compound [mterpy]I

STRUCTURE

The new compound [mterpy]I was characterized by single crystal X-ray crystallography. Crystallographic data and structural refinement parameters are listed in Table 1. Selected bond distances and angles are listed in Table S1. The obtained bond lengths and bond angles are similar to some reported related

terpyridine derivatives.[14,15] An ORTEP perspective view of the ligand structure is shown in Figure 1 (left). The ligand crystallized in the $P2_1/c$ monoclinic space group, and its molecular conformation can be depicted by the dihedral angles between the pyridine rings. As shown, within one mterpy unit, the dihedral angles between the middle pyridine ring1 (N1, C1-C5) and another two connected pyridine rings, ring2 (N2, C6-C10) and ring3 (N3, C11-C15) are 10.67° and 47.84° respectively. This indicates that the $[mterpy]^+$ moiety is of negligible planar character as a whole. Nevertheless, the most interesting feature of the crystal structure is the mode of π-π electron interactions between pyridine rings of neighbouring molecules (Figure 1, right). Two molecules stacked in a head to head fashion, with a distance of 3.862 Å between the bipyridyl units from two monomers, suggesting that π-π stacking interactions are involved in stabilising the structure.

Table 1. Crystal and structure refinement data for [mterpy]I

	[mterpy]I
Chemical formula	$C_{23}H_{20}N_3I$
Formular weight (g mol^{-1})	465.32
Crystal system	Monoclinic
Space group	*P* 1 21/*c* 1
a (Å)	14.0056(8)
b (Å)	13.1159(7)
c (Å)	11.7562(6)
α (°)	90
β (°)	105.479(6)
γ (°)	90
V ($Å^3$)	2081.2(2)
Z	4
D_c (g cm^{-3})	1.485
θ range (°)	3.018-26.998
μ (mm^{-1})	1.550
F (000)	928.0
Crystal size (mm)	0.28×0.31×0.35
Temperature (K)	296
Reflections collected	9311
Independent reflections	3181
Goodness-of-fit (GOF)	1.014
Largest difference in peak and hole (e $Å^{-3}$)	0.570, -0.695
$R_1{}^a$ $wR_2{}^b$ ($I > 2\sigma(I)$)	0.0621, 0.0866
$R_2{}^a$ $wR_2{}^b$ (all data)	0.0375, 0.0742

$^aR_1 = \Sigma\|F_o| - |F_c\|/\Sigma|F_o|$. $^bwR_2 = |\Sigma w(|F_o|^2 - |F_c|^2)/\Sigma|w(F_o)^2|^{1/2}$,
Where $w = 1/[s^2(Fo^2) + (0.0189P)^2]$ where $P = (Fo^2+2Fc^2)/3$

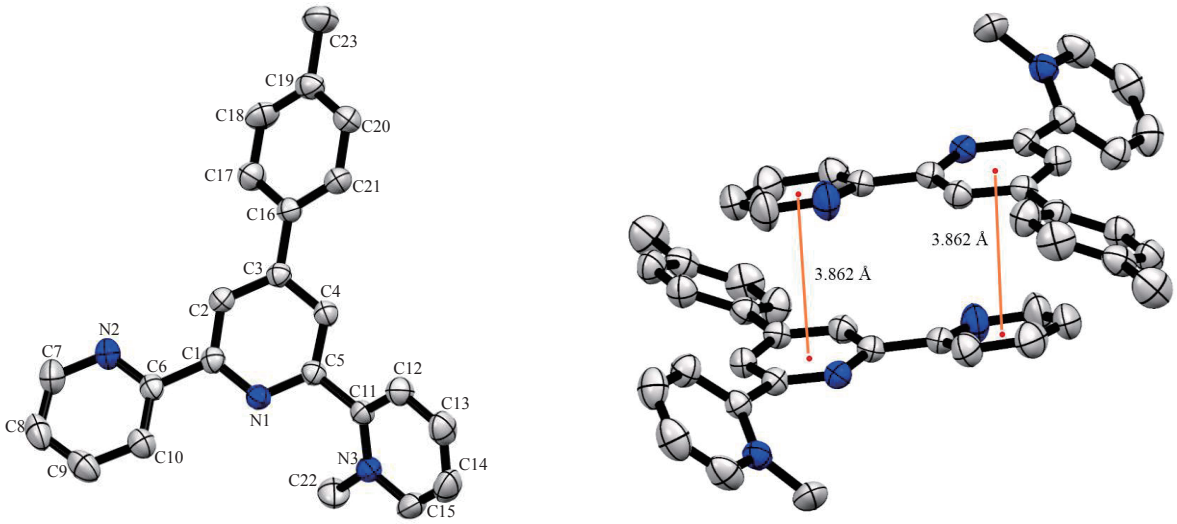


Figure 1. (left) The X-ray structure of the cation moiety of [mterpy]I. (right) Two related moleculars connected by π-π stacking in the structure

DNA BINDING

Competitive DNA-binding studies with ethidium bromide (EB) were used to reveal the binding abilities between [mterpy]I and CT DNA. Neither the compound nor DNA exhibited perceptible fluorescence. However, EB is a good DNA intercalator that emits intense fluorescence upon binding to DNA. EB binding to DNA in the absence of the compound yielded maximum fluorescence emission. Binding of the [mterpy]I should displace EB from DNA, causing the fluorescence emission to decrease. As shown in Figure 2, the addition of [mterpy]I at increasing concentrations induced a dramatic decrease of the fluorescence intensity of the emission band from the CT DNA-EB system, where the compound induced the reductions of up to 50%. The Stern-Volmer plot of DNA-EB illustrate that the quenching of EB bound to DNA by [mterpy]I, resulting the K_{sv} value of 1.39×10^4 M^{-1}. The results from above DNA

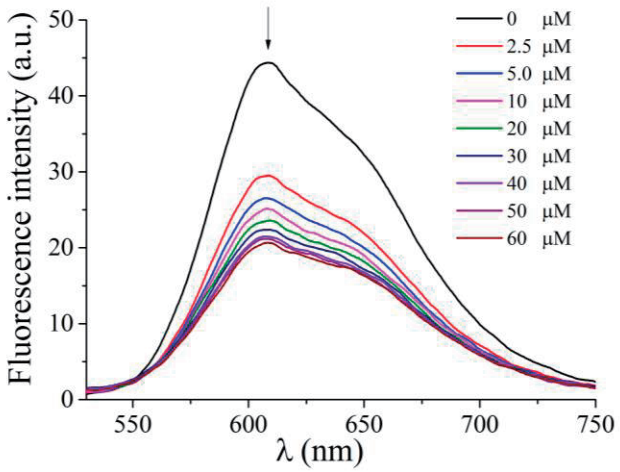


Figure 2. Fluorescence quenching of EB (20 μM) bound to CT-DNA (26 μM) by [mterpy]I in PBS buffer

binding assays indicate strong binding affinities are occurred between [mterpy]I and CT DNA, and intercalation together with electrostatic interaction is probably the predominant binding mode between them.[16]

ANTITUMOR ACTIVITY ASSAYS

The antiproliferative activity of [mterpy]I against HepG2 cancer cell lines were evaluated by MTT assays. Cisplatin was used as a positive control in these studies. The half maximal inhibitory concentrations (IC_{50}) were determined and found to be 74.3 μM and 31.8 μM for [merty]I and cisplatin respectively (Table 2). Based on these values, the in *vitro* antiproliferative activities of the [merty]I was not as potent as cisplatin. Even so, our results suggest that suitable modification could be promising strategies for developing polypyridyl derivatives as interesting DNA binders and potential anticancer agents.

Table 2. IC_{50} values of the [mterpy]I and cisplatin towards HepG2 cell lines[a]

compound	IC_{50} [μM]
[mterpy]I	74.3 ± 6.7
cisplatin	31.8 ± 3.2

[a] IC_{50} values are drug concentrations necessary for 50% inhibition of cell viability. Data are presented as means ± standard deviations of at least three independent experiments and the drug treatment period was 48 h.

EXPERIMENTAL

All chemical solvents were purchased from commercial sources and used without future purification. Ethidium bromide (EB), pBR322 DNA and calf thymus DNA (CT-DNA) were purchased from Sangon Biotech (Shanghai, China). Nuclear magnetic spectra were recorded on a Bruker AVANCE 400 spectrometer under ambient condition. High resolution mass spectrometric analysis was carried out on a Waters Xevo G2-XS Q-TOF instrument. Bio-Rad Sub-Cell GT electrophoresis system was used for the electrophoresis experiment and JUNYI scanner (JY04s-3c) was used for gel imaging. Stock solutions (10 mM) of [mterpy]I was prepared in DMSO, which were further diluted using buffer or cell culture medium until working concentrations were achieved.

Synthesis of 2-[4'-(4-methylphenyl)-2,2''-bipyridin-6-yl]-1-methylpyridinum iodide, [mterpy]I

A mixture of terpy[8,17] (323 mg, 1 mmol) and MeI (220 μL, 3.5 mmol) in toluene (5 mL) was sealed in a 10 mL Teflon-lined stainless-steel container and heated without stirring at 110 °C for 12 h. The reaction was cooled to ambient temperature, yielding light yellow crystals that suitable for X-ray diffraction analysis. Meanwhile, solvent was removed by centrifugation and the solid products was further washed

by toluene (5 mL) and Et_2O (5 mL), and dried *in vacuo*. Yield: 265 mg (57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (d, J = 6.5 Hz, 1H), 8.87 (d, J = 1.6 Hz, 1H), 8.82–8.74 (m, 2H), 8.51 (d, J = 8.0 Hz, 1H), 8.43 (d, J = 8.0 Hz, 1H), 8.35–8.23 (m, 2H), 8.00 (t, J = 7.4 Hz, 1H), 7.91 (d, J = 8.0 Hz, 2H), 7.55 (t, J = 6.2 Hz, 1H), 7.42 (d, J = 7.9 Hz, 2H), 4.42 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.54, 153.43, 151.64, 149.75, 149.47, 148.98, 147.10, 145.43, 139.50, 137.09, 132.79, 129.75, 129.51, 127.08, 126.49, 124.48, 123.06, 120.64, 118.19, 47.01, 20.28. HR–MS (Acetonitrile) *m/z*: calcd 338.1657 for $C_{23}H_{20}N_3$, found 338.1636 for $[mterpy]^+$.

X-Ray crystallography

Diffraction data were collected by using an Agilent Gemini EOS diffractometer equipped by a CCD detector with Mo Kα radiation (λ = 0.71073 Å). Absorption corrections were applied through a multi-scan program SADABS. All calculations were performed using the SHELX214 program package.[18] Non-hydrogen atoms were refined by the full-matrix least squares technique in the anisotropic approximation and hydrogen were refined calculated geometrically riding on their carrier atoms. Crystallographic data for the structures in this paper have been deposited with the Cambridge Crystallographic Database Center, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the deposition numbers CCDC 1854401 for [mterpy]I (Fax: +44-1223-336-033; E-mail: deposit@ccdc.cam.ac.uk).

Fluorescence DNA titration

The competitive binding of the tested compound to CT DNA was determined with ethidium bromide (EB) displacement assay. The CT DNA-EB (20 μM EB, 26 μM CT DNA) conjugation was prepared in PBS buffer. By adding various concentrations of compound solution stepwise, the constants (K_{sv}, M^{-1}) have been calculated and the quenching efficiency for each compound was evaluated according to the classical Stern-Volmer equation,[19] $I_0/I = 1 + K_{sv}[Q]$, where I_0 and I are the fluorescence intensities of CT DNA solution in the absence and presence of quencher, [Q] is the concentration of quencher ([mterpy]I) and K_{sv} is the Stern-Volmer constant.

Cytotoxicity test

A standard MTT assay was employed to evaluate the potential cytotoxicities of compounds against HepG2 cell lines. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS 100 μg mL^{-1} streptomycin, and 100 U mL^{-1} penicillin. The cells were cultured in a humidified incubator, which provided an atmosphere of 5% CO_2 and 95% air at a constant temperature of 37 °C. In a typical experiment, the cells were seeded in a 96-well

flat-bottomed microplate at 10,000 cell per well in growth medium solution. The medium was then changed to growth medium with or without test compounds with serial concentrations. The microplate was then incubated at 37 °C with 5% CO_2 in a humidified incubator for 48 h. After 48 h, MTT (20 μL, 5 mg/mL) was added to each well. The microplate was reincubated at 37 °C in 5% CO_2 for another 4 h. Then the medium was carried out and 150 μL DMSO was added into each well, the microplates were shaken for 10 min. The absorbance at the wavelength of 570 nm was measured by a microplate reader (reference wavelength: 630 nm). The IC_{50} value of each compound (the concentration that is required to reduce the absorbance by 50% relatively to the controls) was analysed and calculated by SPSS.

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