European Journal of Medicinal Chemistry 114 (2016) 141–152

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Toward overcoming cisplatin resistance via sterically hindered platinum(II) complexes

Haiyan Yu [a], Shaohua Gou [a, b, *], Zhimei Wang [a, b], Feihong Chen [a, b], Lei Fang [a, b, **]

[a] Pharmaceutical Research Center and School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China
[b] Jiangsu Province Hi-Tech Key Laboratory for Bio-medical Research, Southeast University, Nanjing 211189, China

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form
22 February 2016
Accepted 24 February 2016
Available online 27 February 2016

Keywords:
Platinum(II) complexes
Overcoming cisplatin resistance
Steric hindrance

ABSTRACT

A number of platinum(II) complexes with steric hindrance derived from (1*R*,2*R*)-N^1-benzylcyclohexane-1,2-diamine derivatives were designed and prepared. Biological assay indicated that most complexes showed antitumor activity against the tested cancer cell lines, especially those with chloride anions as leaving groups had compatible or superior activity to cisplatin and oxaliplatin. Complex **2a**, as the most potent agent, is also sensitive to cisplatin resistant SGC7901/CDDP cancer cell line, which has been subsequently studied by cellular uptake, flow cytometry, gel electrophoresis and western blot assays. The steric hindrance resulting from a pending 2-fluorobenzyl moiety of the ligand might be the key factor for its ability to overcome cisplatin resistant cancer cells.

1. Introduction

Since the discovery of cisplatin by Rosenberg in 1965, the application and research of platinum-based anticancer drugs have achieved rapid development [1–3]. Cisplatin as a broad-spectrum anticancer agent has shown great advantages in the treatment of some solid tumors, especially in the treatment of cervix, ovary, testicle, neck and head [4,5]. However, some side effects, including acquired or intrinsic drug resistance, serious toxicity and low solubility in water, have greatly limited its clinical application [6]. Thus, the non-classical antitumor platinum(II) complexes such as sterically hindered platinum(II) complexes [7], trans-platinum complexes [8], bi- and multi-nuclear platinum(II) complexes [9,10] have been investigated. So far, several mechanisms have been involved in cisplatin resistance, including reduced accumulation of cellular Pt and increased repair of DNA damage [11–13]. The interaction of cisplatin with biological molecules has been found to be one of the key reasons for the drug resistance [14,15] due to its high affinity to the sulfur-containing binding sites. It is well known that sulfur-containing biomolecules are correlated with the resistance of platinum-based drugs [16–18]. In order to overcome the resistance of cisplatin and reduce its side effects, different carrier ligands have been designed and applied to platinum complexes. Oxaliplatin, a third-generation platinum drug with 1*R*,2*R*-diaminocyclohexane (DACH) as carrier ligand, has shown great activity against many tumors including those resistant to cisplatin [19–21]. The high stability of the DACH-platinum fragment in oxaliplatin compared with the ammine in cisplatin leads to a different reaction behavior [22–24], thus, researchers have made great efforts on designing and synthesis of oxaliplatin derivatives [21–27]. Recent studies demonstrated that the introduction of steric hindrance to amine carrier ligand has a strong influence on the activity of the resulting platinum complexes [28]. Therefore, by changing the nature of the amine ligand, it is possible to obtain platinum(II) complexes with potent antitumor activity, which may be active toward cisplatin resistant cell lines as well.

In our recent research, a number of different alkyl groups as sterically hindered moieties have been introduced to DACH skeleton, which were used as carrier ligands to prepare several kinds of platinum(II) complexes [29–32]. The related studies indicated that the platinum(II) complexes containing such ligands showed potent antitumor activity and possessed somewhat different mechanism from cisplatin and oxaliplatin in reaction with DNA, and exhibited distinct cell cycle blocking and cell apoptosis. However, the *N*-monosubstituted alkyl group is flexible, which seems not feasible to offer fixed spatial resistance and may also result in the formation of stereo isomers [29]. Hence, a rigid benzyl group has been introduced to the DACH framework by connecting to one of the nitrogen

* Corresponding author. Pharmaceutical Research Center and School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China.
** Corresponding author. Pharmaceutical Research Center and School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China.
E-mail address: sgou@seu.edu.cn (S. Gou).

http://dx.doi.org/10.1016/j.ejmech.2016.02.060
0223-5234/

atoms (Scheme 1) [33–35], and several new ligands as shown in Fig. 1 have been obtained, which have been used to prepare a series of platinum(II) complexes bearing the steric hindrance(Scheme 2). Considering the significant feature of fluorine atom in drugs, we selected it as a substituent on the phenyl ring. Herein reported are these platinum(II) complexes (Fig. 2) of the ligands and their biological evaluation against a few human cancer cell lines including the typical compounds against cisplatin resistant cancer cell lines. Moreover, chemical and biological properties of the typical compounds have been studied to explicit their preliminary mechanism of action as well as the function of spatial resistance caused by the ligand.

2. Results and discussion

2.1. Synthesis of ligands and their complexes

The preparation of the ligand (**L**) was carried out starting from 1*R*, 2*R*-diaminocyclohexane via several synthetic steps (Scheme 1), in which *N*-mono Boc protected DACH (**I**) was used as the starting material. The unprotected amino group first underwent Schiff base condensation with benzaldehyde, followed by *in situ* reduction with sodium boronhydride, and then the Boc group was removed through hydrolysis in the presence of hydrochloric acid to give **L** in its hydrochloride salts. **LF^1**–**LF^3** were prepared by the same way except *o*-fluorobenzaldehyde, *m*-fluorobenzaldehyde and *p*-fluorobenzaldehyde were used as starting materials, respectively.

All ligands (**L**, **LF^1**, **LF^2** and **LF^3**) were characterized by elemental analysis, IR, ESI-MS, ^{1}H and ^{13}C NMR spectra, which were in agreement with the chemical structure proposed. The specific optical rotations of these ligands were as left-handed as that of DACH.

Complexes **1a**–**4a** were directly prepared by the reaction of the corresponding ligand and K_2PtCl_4. The rest complexes were obtained by using complexes **1a**–**4a** as starting materials to react with the corresponding silver dicarboxylate, respectively [29]. As expected, a chiral center at N^1 position of DACH can be formed after coordination of the ligand with a metal atom in the synthesized platinum(II) complexes. The diastereoisomeric mixture could be produced with one pendant benzyl moiety (at N^1 position) in the pseudo axial position above or below the resulting PtN_2O_2 square plane. Like the pending alkyl group we reported formerly [29], *S* configuration at N^1 atom of the complex with a pendant benzyl group is more thermodynamically stable than the corresponding *R* configuration resulting from a steric effect of the ligand by molecular calculation, which has also been confirmed by the crystallographic data of analogous metal complexes [29,36,37].

In the infrared spectra of the complexes, the strong absorption at 3100 cm^{-1} was attributed to the N–H stretching vibration that was significantly red shifted in comparison to the corresponding ligand. The C=O stretching vibration of complexes appeared between 1568 and 1616 cm^{-1}, blue shifting compared with the single carboxylate group, which is characteristic of coordinated carboxylate ligands. The absorption near 1250 cm^{-1} was attributed to the Ar–F bonding which is an obvious sign to distinguish complexes **1a**, **1b**, **1c**, **1d** and **1e** from others.

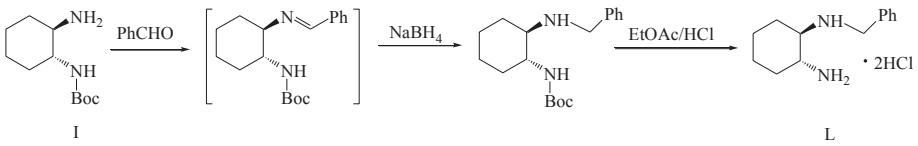
NMR spectroscopy has been applied to determine the framework of the synthesized metal complexes. In the ^{1}H NMR spectra, the signals of hydrogen atoms in DACH appeared in a range of 1.24–2.50 ppm, and the chemical shifts of NH*CH* and NH_2*CH* on DACH of the complexes moved to highfield area compared with the corresponding ligand. ^{13}C NMR spectra were in agreement with the proposed chemical structure of the complexes, particular those of complexes with a fluorine atom showed splitting carbon peaks in the area of aromatic carbons as expected. Besides, a representative ^{195}Pt NMR spectrum (complex **2a**) was recorded.

All the ESI-MS spectra of the complexes gave main peaks corresponding to $[M+H]^+$, $[M-H]^-$ or $[M-Cl]^-$ ions, which were composed of a few isotopic peaks owing to the presence of platinum isotopes, suggesting the bonding between Pt(II) ions and the ligands.

2.2. In vitro cytotoxicity assay

The cytotoxicity of all metal complexes against three human cancer cell lines was evaluated by MTT method with cisplatin and oxaliplatin as positive control. As shown in Table 1, complexes **1a**–**4a** with chloride anions as leaving groups showed strong anticancer activity comparable or even superior to cisplatin and oxaliplatin, whereas other complexes with dicarboxylates as leaving groups, except individual cases, exhibited moderate or low cytotoxicity against HepG2, SGC7901, and A2780 cell lines. Among all compounds, complex **2a** exhibited the strongest activity against all the tested three cancer cell lines, remarkably superior to cisplatin and oxaliplatin. It was 3.71 ± 0.48-fold, 1.56 ± 0.25-fold and 3.66 ± 0.56-fold as potent as cisplatin and 34.34 ± 3.49-fold, 9.63 ± 1.54-fold, and 8.64 ± 1.34-fold as potent as oxaliplatin towards HepG2, SGC7901, and A2780, respectively. In contrast to complexes **2a**–**4a** with a ligand containing a fluoro substituent on the phenyl ring, complex **1a** was less active, which was about one fifth as effective as cisplatin on SGC7901 and A2780, and one tenth as active as cisplatin on HepG2. Although weaker than those of complex **2a** and cisplatin, the cytotoxicity of complexes **3a** and **4a** was stronger than that of oxaliplatin against almost all the tested cancer cell lines, demonstrating the importance and unique function of the fluorine atom of the ligand in our complexes. However, complexes **1b**-**4e** which substituted the leaving group from chloride anions to dicarboxylate species would result in the conspicuous decrease of the antitumor activity of the complexes compared with their parent compounds. This can be due to the slow dissociation ability of chelating dicarboxylate from the metal atom in comparison to chloride anions.

In terms of their strong inhibiting ability on the above cancer cell lines, complexes **1a**–**4a** were further investigated against cisplatin resistant cancer cell line (SGC7901/CDDP) and human normal liver cell line (LO2). As showed in Table 2, complex **2a** has the potential to overcome cisplatin resistance, as its resistance index (RF) can reach 1.39, which is better than cisplatin and oxaliplatin. While complexes **1a**, **3a** and **4a** were less effective against SGC7901/CDDP cell than complex **2a**, even junior to that of oxaliplatin. These results indicated that the position of the fluoro



Scheme 1. Synthesis of ligand **L**.