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Synthesis, characterization, DNA interactions and antiproliferative activity on glioblastoma of iminopyridine platinum(II) chelate complexes

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ABSTRACT

A series of iminopyridine platinum chelate compounds has been prepared and characterized by NMR spectroscopy and single-crystal X-ray diffraction. The complexes were evaluated in C6 tumoral cells as an in vitro model for glioblastoma multiforme. The DNA-binding properties of these complexes were studied by UV–Vis absorption and fluorescence spectroscopy and Density Functional Theory calculations were performed in an effort to rationalize the observed properties at the molecular level. The most promising drug candidate displayed a similar potency in inducing cell death to the clinically used reference compound and showed significant inhibition of glioblastoma cell proliferation. Moreover, this compound had a safer profile than cisplatin on non-tumoral cells.

1. Introduction

Serendipity led to cisplatin becoming a breakthrough in cancer treatment [1,2]. Following the discovery of this compound in the mid-1960s, significant effort aimed at the development of alternative cisplatin derivatives was triggered and this led to clinically approved platinum-based anticancer drugs such as carboplatin and oxaliplatin, along with others like picoplatin, which is in clinical trials [3,4].

The accumulation of platinum ions in the body has deleterious effects. The two major problems associated with the use of cisplatin derivatives are the severe toxic side effects [5–7] and the intrinsic or acquired resistance manifested in various types of cancers [8]. Thus, in the last few decades – and due to the need to obtain more efficient drugs with minimal side effects – the effectiveness of numerous platinum coordination compounds has been evaluated by 'trial and error'. As a result of these efforts it could be inferred that the success of platinum coordination compounds as drugs depends on the 'match' between the effect of platinum against a particular tumor cell and the electronic and kinetic effects of the ligands [9]. However, the well-established cause of the antitumoral activity of cisplatin must also be taken into account for the design of potential antitumoral compounds. In this respect, DNA is considered to be the primary intracellular target [10–12]. Hydrolysis of the chlorido ligands coordinated in cisplatin leads to the formation of the active cisplatin derivative, which coordinates to DNA and generates a kink in the helix [13]. This model, however, is being challenged by numerous studies demonstrating that cisplatin toxicity could be originated rather from multiple sources, including modifications of proteins, small peptides, lipids and RNA, than solely from modifications of chromosomal DNA [14–17]. Despite the growing interest in other targets, significant progress in the field of platinum-based anticancer agents is expected through research based in part on a mechanistic understanding of the DNA-binding and pharmacological effects of cisplatin derivatives.

Over the past 30 years, platinum-based drugs have dominated the treatment of cancers by chemical agents [18]. In fact, among the currently used chemotherapeutics, platinum complexes are held in high regard, with approximately 60% of treatment schemes utilising cisplatin and its analogues [19]. Oxaliplatin and picoplatin are successful examples of such compounds [20]. In oxaliplatin, which is active in patients with colorectal cancer, both of the NH_3 units in cisplatin have been replaced by (1*R*,2*R*)-cyclohexane-1,2-diamine (*R*,*R*-dach) while

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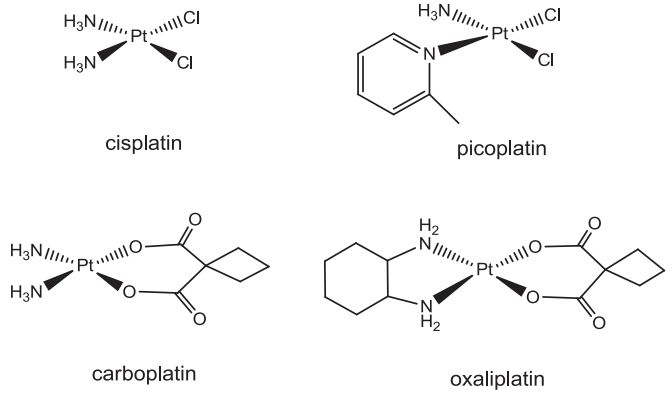


Fig. 1. Structure of cisplatin and its analogues.

picoplatin, which is in clinical development for the treatment of patients with solid tumors, contains a 2-methylpyridine in place of one NH_3 ligand. Despite these structural modifications, cisplatin still plays a pivotal role in the systemic treatment of a variety of solid tumors (Fig. 1).

Glioblastoma multiforme (GBM) is the most common form of brain tumor in adults. However, current treatment of this type of tumor with surgery, chemotherapy and radiotherapy is unsatisfactory [21]. Temozolomide (TMZ) is an active agent for recurrent GBM due to its spontaneous conversion to monomethyl triazenoimidazole carboxamide [22]. However, the survival benefit of this drug is quite modest and GBM is resistant to most other standard cancer chemotherapeutics such as cisplatin, although the cause of this resistance is not fully understood [23]. Irinotecan, etoposide and cisplatin have been used in the treatment of GBM and they have shown efficacy as adjuvant chemotherapy. Since the approval of TMZ, this compound has mostly been used as a treatment after progression or recurrence [24]. Consequently, there is clearly an unmet clinical need for new treatments that are able to arrest the rapid development of the disease through the action of new drugs that have antiproliferative activity on glioblastoma cells.

Several key characteristics must be taken into account in the search for platinum-based drugs derived from cisplatin, namely saline solubility and stability, facile transport through cellular membranes, relatively stable DNA-binding ability, and selectivity and specificity towards cancer cells. Furthermore, the ease of preparation and cost associated with the synthesis of new drugs are other issues to be considered. With these premises in mind, the aim of the work described here was to investigate the biophysical properties and in vitro cytostatic activities of new chelating cisplatin derivatives as potential therapeutic agents for GBM. The glioma cell line C6 was selected as an in vitro model for glioblastoma since it shares several histopathological tumor markers and genetics with human GBM [25]. Cisplatin-based therapy is considered as the second line against GBM since drug resistance and undesirable side effects, including neurotoxicity, limit its efficacy [26]. The synthesis and characterization of new cisplatin derivatives as potential candidates and a spectroscopic study of the interaction between the drugs and DNA are discussed in detail. In addition, a series of molecular descriptors related to the absorption and distribution properties of the synthesized compounds have been calculated at the Density Functional Theory (DFT) level.

2. Results and discussion

2.1. Synthesis and characterization

A bulky carrier ligand coordinated to the platinum(II) core could reduce the level of undesired substitution reactions in the square-planar complexes [27]. Iminopyridine ligands were chosen as scaffolds to generate chelate cisplatin analogues due to the ease with which their characteristics can be tuned. These ligands were conveniently prepared by a condensation reaction between the appropriate amine and ketone, as similar to iminopyridine ligands previously described [28].

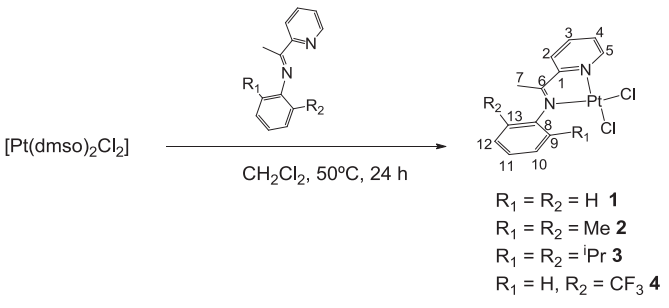
The synthesis of the platinum chelate compounds **1**–**4** is straightforward (Scheme 1). For these compounds the metal precursor *cis*-$[Pt(DMSO)_2Cl_2]$ was reacted with 1 M equivalent of the appropriate iminopyridine ligand in dichloromethane and the products were obtained as orange solids in high yields. All complexes are air stable in the solid state and are soluble in organic solvents such as acetone, methanol or dichloromethane.

The products **1**–**4** were characterized by spectroscopy, elemental analysis and, in the cases of **1**–**3**, by X-ray crystallography. The NMR characteristics of the complexes are consistent with those reported for related Pt(II) complexes containing these types of ligands [29–35]. All of the signals in the ^1H and ^{13}C NMR spectra were observed with the expected chemical shifts and assignment was performed on the basis of 2D–^1H ^{13}C HSQC spectra ligands (see Fig. S1 as a representative characterization of the series in the Supporting information). The ^1H NMR spectra of compounds **1**–**4** in DMSO-d_6 contained resonances at relatively high frequency and these correspond to protons of the pyridyl ring. In compound **3** the isopropyl groups became magnetically inequivalent and this change can be attributed to hindered rotation of the aryl rings. The stabilities of the new compounds were tested in DMSO-d_6 by ^1H NMR spectroscopy and the compounds were unchanged after three days in solution at room temperature.

The molecular structures of the novel complexes were confirmed by single-crystal X-ray structure analysis. ORTEP views of compounds **1**–**3** are shown in Fig. 2 along with the numbering systems used in the crystallographic study. Bond lengths and angles and crystallographic details are collected in Tables S1 and S2 in the Supporting information, respectively. Complexes **1**–**3** crystallize in the monoclinic P21/c space group. All of the complexes have a monomeric structure in the solid state with an almost identical square planar geometry. The ligands chelated the metal via the pyridinic and iminic nitrogen atoms, exhibiting a bidentate coordination mode and forming a five-membered chelate ring. As in related systems, the plane of the N-aryl group lies approximately perpendicular to the metal coordination plane [36–37]. The N–Pt–N chelating angles are comparable in the three compounds and such values are often observed in group 10 complexes with iminopyridines [29–37].

2.2. Cytotoxicity studies

The great success of cisplatin in fighting tumor cell, and its limitations, have motivated efforts to develop new antitumor agents with improved therapeutic properties. In these regard, the use of iminopyridine ligands as scaffold to generate cisplatin derivatives is scarce. Brunner et al. [34] and Conrad et al. [31] reported the synthesis of several iminopyridine Pt(II) complexes and tested their antitumor activity respect to the hormone independent human mammary carcinoma cell line MDA-MB 231, and cisplatin-sensitive and cisplatin-resistant human ovarian cells OV2008 and C13, respectively. Both research



Scheme 1. Synthesis of platinum chelate compounds **1**–**4**.