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RESEARCH ARTICLE

In vitro Cytotoxic Activities of Platinum(II) Complexes Containing 2-Acetoxymethyl and 2-(2′-Hydroxyethyl)benzimidazole Ligands

Gökçen EREN*, Fatma GÜMÜŞ*°, Şükran YILMAZ**

In vitro Cytotoxic Activities of Platinum(II) Complexes Containing 2-Acetoxymethyl and 2-(2′-Hydroxyethyl) benzimidazole Ligands

Summary

Two platinum(II) complexes with the structures $[PtL_2Cl_2]$ (L=2-acetoxymethyl (1) and 2-(2′-hydroxyethyl) (2) benzimidazole as carrier ligands) were evaluated for their in vitro cytotoxic activities against the human MCF-7 breast (Estrogen receptor (+), ER(+)), MDA-MB-231 breast (Estrogen receptor (-), ER(-)), Caco-2 colon, and SK-Hep-1 liver cancer cell lines. The biological studies have shown that Compound 1 which has more lipophylic group on the second position of its benzimidazole ligand is more active than compound 2 and the complexes tested have found to be more active against MDA-MB-231 (ER(-)) cell line than MCF-7 (ER(+)) cell lines.

Key Words: *Benzimidazole, cytotoxic activity, MCF-7, MDA-MB-231, platinum complexes.*

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2-Asetoksimetil ve 2-(2′-Hidroksietil)benzimidazol Ligandlarını Taşıyan Platin(II) Komplekslerinin in vitro Sitotoksik Aktivitesi

Özet

Yapıları $[PtL_2Cl_2]$ (taşıyıcı ligand olarak L=2-asetoksimetil (1) ve 2-(2′-hidroksietil) (2) benzimidazol) olan iki platin(II) kompleksinin in vitro sitotoksik etkileri, insan MCF-7 meme (Östrojen reseptör (+), ER(+)), MDA-MB-231 meme (Östrojen reseptör (-), ER(-)), Caco-2 kolon ve SK-Hep-1 karaciğer kanser hücre hatları kullanılarak araştırıldı. Biyolojik çalışmalar, taşıdığı benzimidazol ligandının ikinci konumunda daha lipofilik grup taşıyan Bileşik 1'in, Bileşik 2'ye göre daha fazla sitotoksik etkili ve her iki bileşiğin de MDA-MB-231 (ER(-)) üzerine olan etkisinin, MCF-7 (ER(+)) hücre hattı üzerine olan etkisinden fazla olduğunu gösterdi.

Anahtar Kelimeler: *Benzimidazol, sitotoksik aktivite, MCF-7, MDA-MB-231, platin kompleksleri.*

* Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Ankara, Turkey
** Cell and Virus Bank Department, Foot and Mouth Disease Institute, Ankara, Turkey
° Corresponding Author E-mail: fgumus@gazi.edu.tr

INTRODUCTION

Cisplatin is one of the most widely used antineoplastic agents in the treatment of solid tumour and haematological malignancies, including cancers of the testes, ovary, bladder, head and neck, oesophagus, stomach and lung, as well as lymphoma and osteosarcoma (1). However, the efficiency of cisplatin and other platinum-based drugs, such as carboplatin and oxaliplatin are often accompanied by toxic side effects and tumor resistance, which in turn leads to secondary malignancies (2).

Breast cancer is a major health problem among women in the world. The successful treatment of this disease is limited by the fact that especially MCF-7 and MDA-MB-231 breast cancer cells can become resistant to cisplatin treatment (3,4). Cisplatin has also limited efficacy as monotherapy in breast cancer patients (5). Therefore, there is a need to design new chemotherapeutic agents.

One of the other most common cancers is hepatocellular carcinoma (HCC), and the third most common cause of cancer death with a high degree of malignancy and poor prognosis (6,7). Its incidence is increasing worlwide due to the dissemination of hepatitis B and C virus infection (8,9). A majority of patients are surgically unresectable at the time of diagnosis, and even for those surgery is possible, the risk of recurrence is extremely high. Consequently, chemotherapy is an important strategy for most HCC patients. But the effectiveness of cisplatin for HCC is unsatisfactory because of multidrug resistance (10).

Furthermore, colon adenocarcinoma is intrinsically refractory to cisplatin, whereas in initially sensitive tumors acquired resistance is often developed in the course of therapy, by virtue of different cellular mechanisms (11).

In the research of new platinum complexes avoiding toxicity and resistance, special attention has been paid to the replacement of one or both NH_3 carrier ligands of cisplatin by other N-donor ligand(s). The use of sterically demanding diamines as carrier ligands as an alternative compound to cisplatin can slow or block repair enzymes (12). It is well-known that benzimidazole nucleus is a constituent of many bioactive heterocyclic compounds and benzimidazole derivatives are structural isosters of naturally occurring nucleotides, which allow them to interact easily with the biopolymers of the living system which is responsible for their numerous biological activities and functions (13).

In previous studies, taking into consideration the fact that variations in the chemical structure of the ammine groups of the cisplatin can have significant effects on the cytotoxic activity and toxicity of platinum complexes, and the properties of benzimidazole nucleus we synthesized some platinum complexes with the 2-substituted benzimidazole ligands. It was determined that some of these platinum complexes have in vitro cytotoxic activities on RD (14), HeLa (15-19), MCF-7 (15-20), MDA-MB-231 (19) and Hep-2 (18) cell lines.

In this paper, two Pt(II) complexes with the carrier ligands 2-acetoxymethyl (**1**) or 2-(2'-hydroxyethyl) (**2**) benzimidazole which were synthesized previously by us and found to be cytotoxic against HeLa and MCF-7 human cancer cell lines (17), were evaluated for their preliminary in vitro cytotoxic activities on the human SK-Hep-1 (Liver adenocarcinoma cell line) and the human Caco-2 (Colonic carcinoma cell line) cancer cell lines. In order to compare the in vitro cytotoxic activities of the compounds **1** and **2** against the estrogen receptor positive (ER(+)) and estrogen receptor negative (ER(-)) breast cancer cells, the cytotoxic activities of the compounds on the human MCF-7 (ER(+)) and MDA-MB-231 (ER(-)) breast cacer cell lines were also performed.

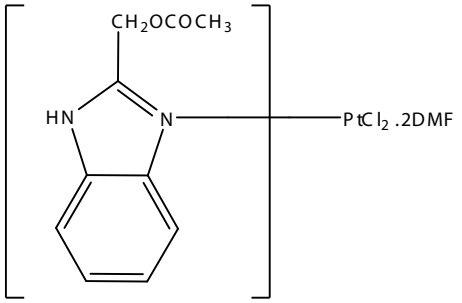
MATERIAL AND METHODS

Chemistry

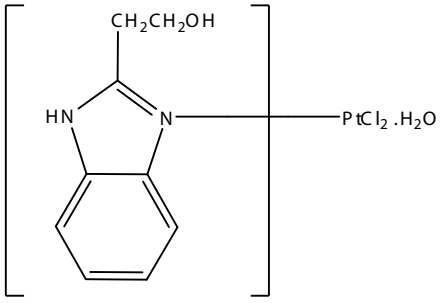
Synthesis and detailed structural analyses of platinum(II) complexes **1** and **2** were reported in our previous paper (17). Chemical structures of the complexes are given in Figure 1.

Cell Lines and Growth Conditions

Cisplatin (CAS 15663-27-1) used as reference compound was obtained from Sigma. The human MCF-7 breast, MDA-MB-231 breast, Caco-2 colon, and SK-Hep-1 liver cancer cell lines used in this study were obtained from the Cell Culture Collection (HUKUK numbers: 00092502, 02031201, 98052301, 91091816, respectively) of Institute for Foot and Mouth Disease (IFMD) (Turkey). The cells were grown as monolayer cultures in T75 flasks, subcultured three times at 37 ^{o}C in an atmosphere



Compound 1



Compound 2

Figure 1. Chemical structures of the compounds **1** and **2**.

of 5% CO_2 in air and 100% relative humidity and maintained at third passage. Three of cell suspensions were prepared at concentration of $8.7x10^3$ cell/mL and dispensed onto 96 well cell culture plates (100 µL per well). The multiwell plates were incubated at 37 °C, 5% CO_2 in air for 24 h. After 24 h, the culture medium was removed from the wells and equal volumes (100 µL) of the serial dilutions (80, 40, 20, 10, 5, 1 and 0.5 µM) of the test compounds were added into each well. After 72 h incubation periods, cytotoxicity was evaluated colorimetrically by MTT assay (21). Three experiments were carried out for each compounds.

RESULTS AND DISCUSSION

As an extension of our previous studies on benzimidazole Pt(II) complexes, in the present study two platinum(II) complexes with 2-acetoxymethyl (**1**) or 2-(2'-hydroxyethyl) (**2**) benzimidazole carrier ligands which were synthesized before by us (17) were evaluated for their in vitro cytotoxic activities against the human MCF-7 breast (ER(+)), MDA-MB-231 breast (ER(-)), Caco-2 colon, and SK-Hep-1 liver cancer cell lines. Cisplatin was used as reference compound. IC_{50} values of the compounds **1**, **2** and cisplatin were calculated from the dose-survival curves for the growth inhibition of all the cell lines used for the measuring time point, 72 h by using GraphPad Prism 6 software.

The compounds **1** and **2** were found to be inactive in human colon cancer cell line Caco-2 (Data not shown).

Our biological studies have shown that compound **1** which has IC_{50} value of 19.78 µM was found to be more active than compound **2** (IC_{50}=50.02 µM) against Sk-Hep-1 cell lines while cisplatin has IC_{50} value of 9.71 µM (Table 1). It has been also observed that the treatment with compounds **1** and **2** decreased the number of viable cells in ER(-) breast cancer cells (IC_{50} values of compound **1** and **2** are 13.60 and 40.51 µM, respectively) more than ER(+) breast cancer cells used (IC_{50} values of compound **1** and **2** are 48.15 and >80 µM, respectively) which is similar to that observed in case of cisplatin.

It is clear that with higher LogP value, compound **1** is more active that **2** in all cell lines tested (LogP values of 2-acetoxymethylbenzimidazole and

Table 1. IC_{50} (µM) of compounds 1, 2 and cisplatin for cytotoxic activity on Sk-Hep-1, MDA-MB-231 and MCF-7 cell lines.

Cell Lines	IC_{50} (µM)		
	Compound 1	Compound 2	Cisplatin
Sk-Hep-1	19.78±1.18	50.02±1.22	9.71±1.42
MDA-MB-231	13.60±1.33	40.51±1.36	9.19±1.44
MCF-7	48.15±1.15	>80	39.47±1.21

2-(2'-hydroxyethyl)benzimidazole are 1.39 and 0.89, respectively). It can be suggested that the substitution of the second position of benzimidazole which is selected as carrier ligand in this study, with more hydrophobic acetoxymethyl group leads to the enhancement in cytotoxicity. LogP values of the compounds **1**, **2,** and their carrier-ligands were calculated by using Molecular Operating Environment (MOE) 2011.10 software (22).

It can be concluded that, in this study two main results have been obtained: i) Compound **1** which has more lipophylic group on the second position of its benzimidazole ligand is more active than compound **2** (LogP values of complexes **1** and **2** are 4.74 and 3.74, respectively). ii) The complexes tested have found to be more active against MDA-MB-231 (ER(-)) cell line than MCF-7 (ER(+)) cell lines. The future work lies in the design of compounds with enchanced lipophylicity of benzimidazole ligands, may lead to have new Pt(II) complexes having better cytotoxic activities.

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