Synthesis of Cis-Diammine (1,1-cyclobutane dicarboxylate) Platinum(II)

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ABSTRACT: *Platinum-based anticancer drugs are chemotherapeutic agents to treat cancer. Carboplatin (cis diammine cyclobutane dicarboxylate platinum (II)) is a second generation drug that has less toxic than cisplatin, allowing for high dosages. In the first stage, 1,3-Cyclobutane dicarboxylic acid, a key intermediate for preparation of carboplatin, have been synthesized in good yields using different methodology to achieve high purity then carboplatin was synthesized from reaction of prepared ligand with cisplatin. Purity of prepared carboplatin was confirmed by High-Performance Liquid Chromatography (HPLC). Platinum(II) complex carboplatin, have been characterized with* ^{1}H *and* ^{13}C *nuclear magnetic resonance spectra and infrared spectroscopy.*

KEYWORDS: *1,3- cyclobutane dicarboxylic acid; Platinum complexes; Carboplatin; Anticancer*.

INTRODUCTION

In the next two decades, the world is expected to see around 20 million cases of cancer. Therefore, all efforts are needed to face such a problem. Platinum-based cancers are chemotherapeutic agents to treat cancer. Strategies for improving platinum-based anticancer drugs usually involve changes in the neutral spectator ligands (which are usually nitrogenous), in the nature of the anions and oxidation state of the metal (Pt(II) , Pt(IV)). Different platinum based anticancer drugs are shown in Fig. 1.

The platinum(II) complex known as cisplatin [*cis*- $(NH_3)_2PtCl_2$] is one of the most effective drugs to treat ovarian, head and neck esophageal and nonsmall lung cancers [1]. Cisplatin (cis-diammine dichloroplatinum (II)) was synthesized in 1845. Its cytotoxic properties were unrecognized until 1965 when *Rosenberg* and his colleagues observed inhibition of bacterial growth by an electric current [2,3]. Cisplatin was investigated in several clinical trials in the early 1970s and became available for clinical use in 1978. However, cisplatin causes severe side effects of which renal toxicity and peripheral neuropathy are dose limiting.[4] Furthermore the limited water solubility narrow range of treatable cancers have fuelled researchers to develop less toxic platinum analogues. As a result, carboplatin has replaced cisplatin in many chemotherapeutic regimens. Carboplatin (cis diammine cyclobutane dicarboxylate platinum (II)) is a second generation drug that has less toxic than cisplatin, allowing for high dosages. Unfortunately, it is only active in treating the same type of tumors as cisplatin. Oxaliplatin, developed by *Kidani et al.*, [5] is a third-generation platinum antitumor drug following cisplatin and carboplatin, and has been used worldwide in combination

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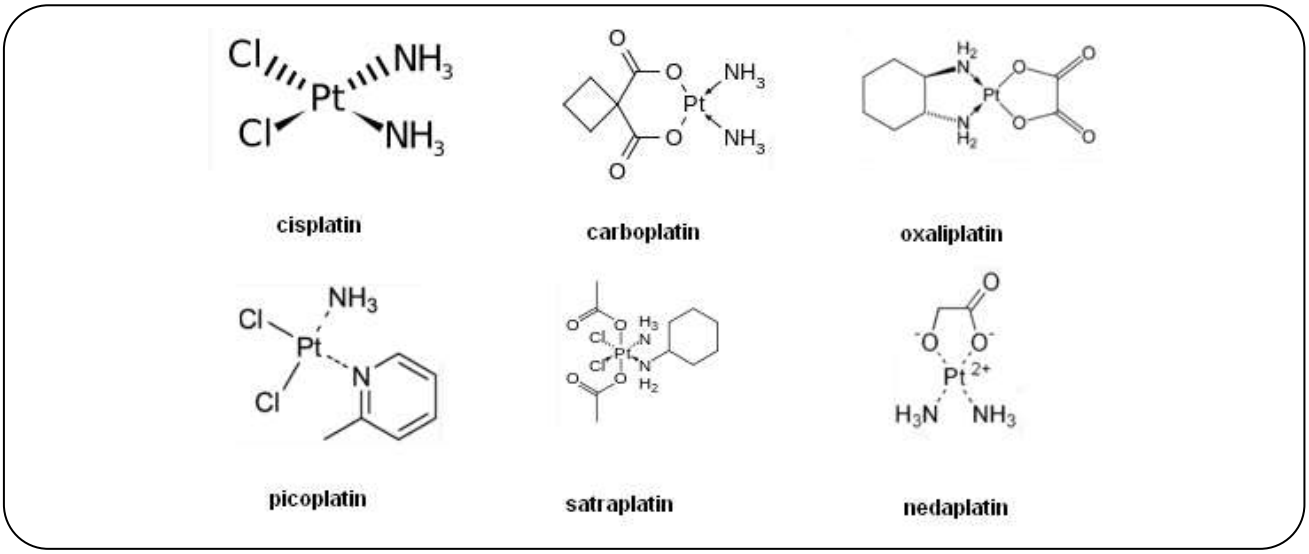


Fig. 1: platinum-based anticancer drugs.

chemotherapeutic treatments of metastatic colorectal cancer [6,8]. By comparison to cisplatin, carboplatin can be administered at higher doses because of its lower toxicity profile. Although less toxic, carboplatin has a similar spectrum of activity and exhibits cross-resistance to cisplatin, which is a result of the same non-leaving group amine ligands [9,10].

Oxaliplatin is also currently being explored for its potential as a treatment option after failure of cisplatin or carboplatin therapy, owing to its activity in cisplatin-resistant tumor models [11]. Oxaliplatin differs from carboplatin importantly in that two amine groups of the latter are replaced by (1R,2R)-diamino cyclohexane (DACH), which is largely credited for the unique anticancer properties of oxaliplatin.

In the present article, we report the synthesis and characterization of carboplatin drugs. This research is mainly concerned on synthesis of cyclobutane 1,1dicarboxylic acid ligand as a precursor for carboplatin drug.

EXPERIMENTAL SECTION

Preparation of $K_2[PtCl_6]$

Potassium chloride (0.15 g) dissolved in 2 mL water was slowly added under stirring to a solution of hexachloroplatinic acid $H_2PtCl_6.6H_2O$ (0.5 g) in 5 mL of water. To this methanol (7 ml) was added and the mixture was allowed to cool for 15 min in an ice bath. The yellow salt was filtered and washed with methanol and ether. The solid was dried in air.

Preparation of $K_2[PtCl_4]$

To a solution of potassium hexachloroplatinate (100 mg) in 1 mL of water $N_2H_2.2HCl$ (0.01 g) was added in small quantities. The mixture was heated up to 65˚C for about 2 h. The temperature was then raised to 90˚C to ensure completion of the reaction. A little excess of the $K_2[PtCl_6]$ was taken initially to prevent the complete reduction to metallic platinum. The mixture was then filtered to remove unreacted $K_2[PtCl_6]$ and washed with ice cold water.

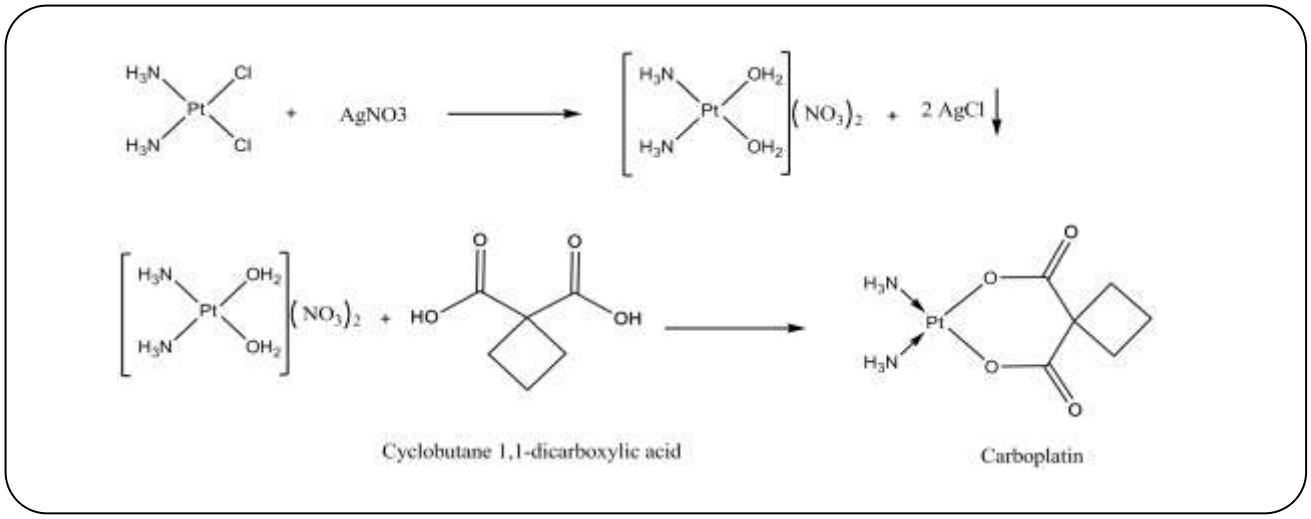
Preparation of cisplatin

0.02 g of NH_4Cl was dissolved in above filterate. Aqueous ammonia (3 M) was added to this until the pH reaches a value of 7. The solution was refrigerated for about 48 h. yellow solid separated out and recrystallised from 0.1 N HCl. Yellow crystals were washed with cold water and dried in air.

Synthesis of carboplatin

a. Synthesis of cyclobutane 1,1 dicarboxylic acid

A 250-mL three round-bottomed flask with a condenser, and dropping funnel was assembled. (0.02 mol) dimethyl malonate and (0.021 mol) 1,3dibromopropane was added and kept at 60-65˚C. 4.7 g potassium tertiary butoxide in 80 ml methanol was added dropwise in 60˚C. After the addition the mixture was refluxed for 2 h. Water was added to the reaction mixture for sodium bromide to dissolve. Methanol



Scheme 1: pathway to synthesis of carboplatin.

was evaporated in rotary and the ester was purified with steam distillation. Only heavy esters will remain in reaction flask. 400 mL of product was collected**.** The solution was transferred to a separatory funnel and the organic ester layer was separated. The remaining aqueous layer was washed with diethylether. Then diethylether was evaporated. In the next step ester is hydrolyzed to acid. Ester was dissolved in ethanol 2.5 gr potassium hydroxide was added and reaction mixture was refluxed for 2 h. Then ethanol was evaporated and the residue was dissolved in hot water Then HCl was added dropwise until pH reaches 3-4. To remove carbon dioxide, solution was boiled for a few minutes and its pH turns basic with ammonia. Amount of barium chloride was added to remove excess malonate as barium malonate perticipate. Then 10 ml HCl in added and the mixture is transferred to separatory funnel and washed three times with diethylether. The ether phase was dried under calcium chloride after evaporation white residue was remained which was recrystallized in ethylacetate. White cyclobutane dicarboxylic acid crystals were obtained. mp:156-158 ˚C.

b.Synthesis of carboplatin

0.01 mol cisplatin and 0.02 mol silver nitrate was dissolved in 100 mL water and was stirred for 3 h in dark. Then 0.1 g charcoal was added and was filtered off at 55-60 ℃ together with AgCl. The filtrate was concentrated under vacuum and was cooled to 0 ˚C for 2h to obtain white crystals. cis diamio diaqua platin nitrate crystals were filtered and dried in air. In last step 0.01 mol cis diamino diaqua platin nitrate and 0.015 mol cyclobutane 1,1 dicarboxylic acid were mixed in 50 °C for 10 h. The reaction mixture was filtered and the filtrate was kept at 0 ˚C to obtain carboplatin crystals. yield 65%, mp: 228-230 ˚C (Scheme 1).

RESULT AND DISCUSSION

Carboplatin is a colourless, crystalline powder, sparingly soluble in water, very slightly soluble in acetone and in alcohol. It melts at about 200 °C, with decomposition. Carboplatin, on the other hand, contains a relatively stable chelating CBDCA (CBDCA = 1,1-cyclobutane–dicarboxylato) ligand as its leaving group which its preparation and characterization is main concern of this research.

Characterization of 1,1-cyclobutane–dicarboxylato) ligand and carboplatin

a.CHN analysis

CHN analysis of carboxylic ligand and carboplatin is tested with Eager 300.

b.NMR spectroscopy

Three different hydrogens are observed in H-NMR and four different carbons are seen in C-NMR of the complex which confirms the structure.

c.IR spectroscopy

In the IR spectrum of carboxylic ligand has a strong band at 2400-3400 cm-1 assigned to O-H vibration of carboxylic acid. The C-O vibration is also observed

Table 1: CHN analysis of carboxylic ligand and carboplatin.

	Ligand Theorotical	Experimental	Carboplatin Theoretical	Experimental
N	-	-	7.55	7.53
C	50	49.98	19.41	19.40
H	5.59	5.53	3.26	3.22

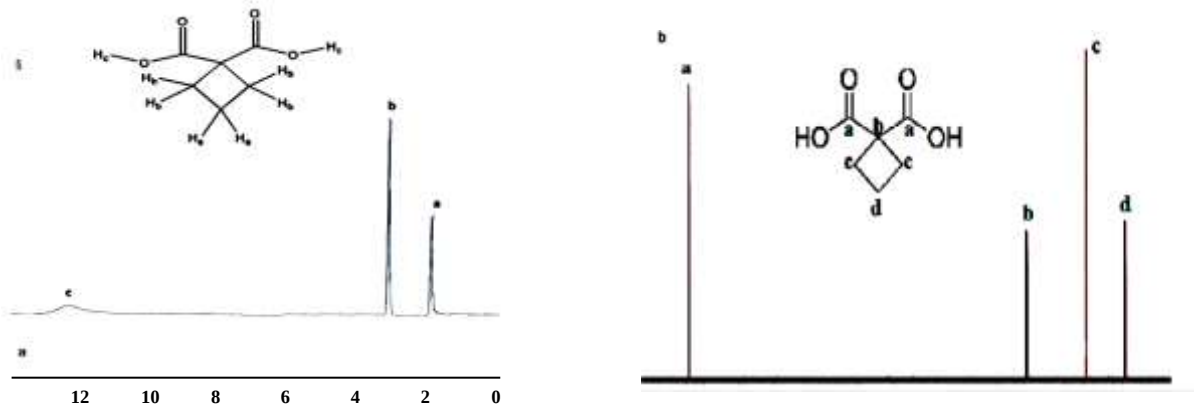


Fig. 2 :(a) H-NMR and (b) C-NMR of carboplatin.

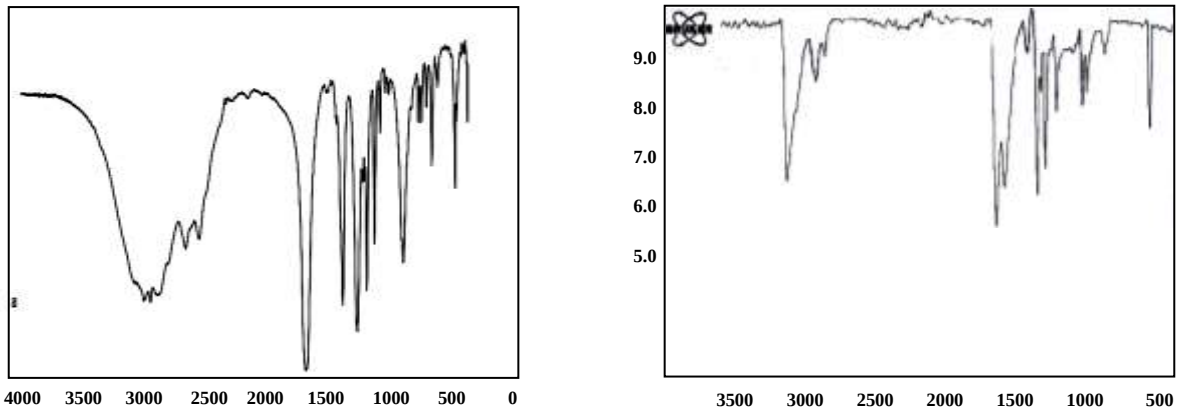


Fig. 3 : IR spectroscopy of (a) carboxylic ligand (b) carboplatin

with the presence of bands at 1000 -1500cm-1. The bands at 2900 and 3000 cm-1 are due to C-H aliphatic groups. For carboplatin complex the bands at 3258 and 456 cm^{-1} are due to Pt N-H and Pt-N stretching vibrations, respectively. These results are in concordance with previously published studies of platinum(II) and platinum(IV) complexes.

d.HPLC analysis

The analysis of cisplatin and carboplatin were carried out by High-Performance Liquid Chromatography (HPLC). Chromatographic conditions were established to obtain, an adequate separation of eluted compounds. The separation factor value depends on such factors as composition of the mobile phase, composition of the stationary phase and temperature. The system was equipped with a K-2800 UV/Vis Photo Diode Array (PDA). Two mobile phases were employed. The first contained a mixture of methanol-water (3% v/v) and pH 2.5 adjusted with methanesulphonic acid (cisplatin) and the second contained a mixture of of acetonitrile

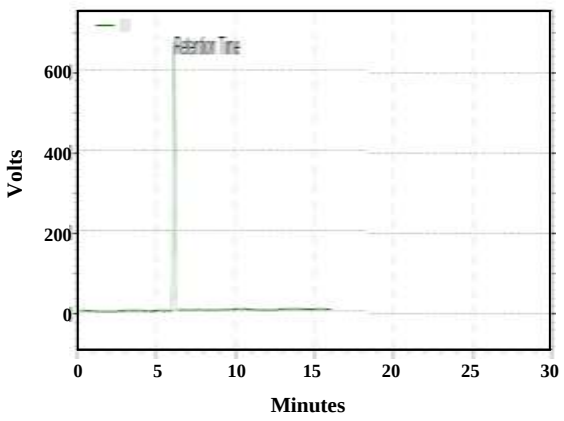


Fig. 4: Hplc spectrum of carboplatin (retention time= 6.33).

and water (13% v/v) (carboplatin). Spectrophotometric detections were measurements at 305, 200, 230 nm respectively. Runs were carried out at a flow rate of 2 mL min-1. Hplc spectrums of carboplatin is shown in Fig. 4.

CONCLUSIONS

Carboplatin drug was synthesized in good yield in two steps. At first preparation of cyclobutane 1,1dicarboxylic acid ligand is mainly concerned. This compound is made of dimethyl malonate and 1,3dibromopropane in presence of potassium tertiary butoxide in methanol. After high purification of this ligand, it was added to cis diamio diaqua platin nitrate in 50 ˚C to synthesis carboplatin. The analysis of this compound is carried out with hplc in presence of acetonitrile and water as mobile phase and Uv/Vis Photo Diode Array (PDA) detector.

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