SYNTHESIS, STRUCTURE AND BIOLOGICAL ACTIVITY OF THREE PLATINUM(II) COMPLEXES BEARING ALKYLEUGENOXYACETATE (ALKYL: ETHYL OR PROPYL) AND HETEROCYCLIC AMINE

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Abstract

Three organometallic complexes of platinum(II), $[PtCl_2(Eteug)(C_9H_7N)]$ (P1), $[PtCl(Eteug)(C_9H_6NO)]$ (P2) and $[PtCl(Preug)(C_9H_6NO)]$ (P3), have been synthesized for the first time. The structures of P1, P2 and P3 were determined by platinum analysis, EDX, IR, ^1H NMR, ^{13}C NMR and NOESY NMR spectra. In these complexes, the allyl group of ethyl eugenoxyacetate (Eteug) and propyl eugenoxyacetate (Preug) coordinate with platinum(II) in an η^2 manner, quinoline (C_9H_7N) coordinates with Pt(II) via N atom, deprotonated 8-hydroxyquinoline (C_9H_7NO) coordinates with Pt(II) via N and O atoms. In all the complexes, the N atom occupies the *trans*-position in comparison to the allyl group. All P1, P2 and P3 exhibit a potent cytotoxicity on Hep-G2 human cancer cell line with small IC_{50} values of 6.97, 0.31 and 1.27 μg/ml, respectively.

Keywords: Alkyleugenoxyacetate, platinum(II) complexes, heterocyclic amine, cytotoxicity.

1. INTRODUCTION

Since FDA approval of Cisplatin for the treatment of meta static ovarian and testicular cancers was granted in 1978, it has urged researchers in developing new platinum complexes for medical applications. Although over thousand complexes have been prepared and tested thus far, only two platinum drugs are approved for clinical use world wide being Caboplatin and Oxaliplatin. Nevertheless, they all have toxic side effects and are not universally effective in all cancer types [1-4]. Therefore, there is still an urgent demand for development new platinum-drugs with improved properties, for example, broader spectrum of activity and reduced toxicity.

Recently, we have synthesized many platinum(II) complexes containing natural aryl olefin such as safrole, methyl eugenol and methyl eugenoxyacetate, some of which were tested and found to be potentially inhibitory on Hep-G2 and RD human cancer cell lines [5, 6]. As a continuation of our research on aryl olefin-platinum complexes, we herein describe the synthesis, structure and cytotoxic properties against Hep-G2 cell line of three platinum(II) complexes bearing alkyleugenoxyacetate (alkyl:ethyl or propyl) and quinoline or 8-hydroxyquinoline.

2. EXPERIMENTAL

2.1. Synthesis

Trans-$[PtCl_2(Eteug)(C_9H_7N)]$ (denoted P1) wasprepared as follow:1.1 mmol quinoline in an aqueous ethanol solution (5 ml, 1:1 v/v) was added gradually to a mixture of 590.5 mg (1 mmol) $K[PtCl_3(Eteug)]$(prepared according to the synthetic protocol of Da et al [5]), 8 ml water and 8 ml ethanol. The mixture was stirred at room temperature for two hours. After cooling in an ice bath at about 5 ^oC for 30 minutes, precipitate was filtered off, washed with a solution of 50% by volume aqueous ethanol (2 x 2 ml) and cool ethanol (2 x 2 ml), then dried in a vacuum at 45 ^oC for 2 h. Yielded 85 %.

$[PtCl(Eteug)(OC_9H_6N)]$ (denoted P2)was prepared as follow: 1.1 mmol 8-hydroxyquinolinein an aqueous ethanol solution (5 ml, 1:1 v/v) was added gradually to a mixture of 590.5 mg (1 mmol) $K[PtCl_3(Eteug)]$, 8 ml water and 8 ml ethanol. The mixture was stirred at room temperature for two hours, then glue-like solution appeared and was separated from the reaction mixture. Pouring 10ml ethanol into the glue-like solution and shaking for 10 minutes, the fresh precipitated yellow powder was filtered off, washed with ethanol (3 x 2ml), then dried in a vacuum at 45 ^oC for 2 h. Yielded 85%.

[PtCl(Preug)(OC_9H_6N)] (denoted P3) was prepared starting from 604.5 mg (1 mmol) K[$PtCl_3$(Preug)] (prepared according to the synthetic protocol of Chi *et al.* [7]) and 1.1 mmol 8-hydroxyquinolinein an aqueous propanol solution (1:1 v/v) according to the procedure for preparation of P1. Yielded 90 %.

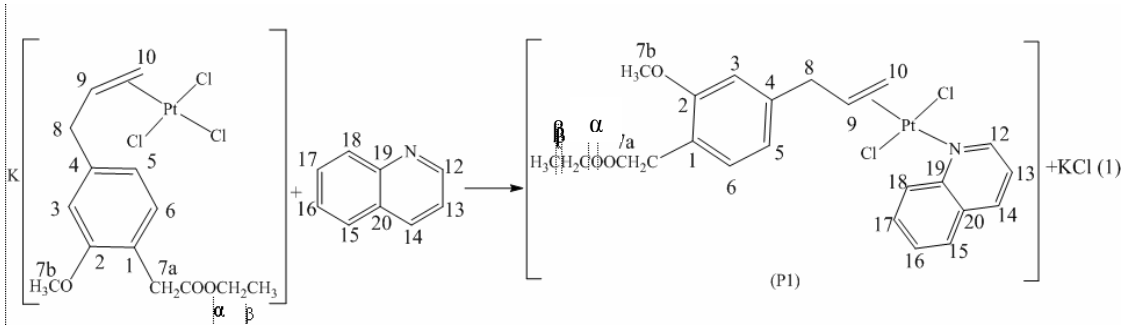
2.2. Apparatus and methods

Pt was analyzed according to the weight method [5] at Faculty of Chemistry - Hanoi National University of Education. The EDX spectra were recorded on a JED-2300 instrument at Institute of Material Science, Vietnam Academy of Science and Technology. The IR spectra were recorded on IMPACT-410 NICOLET spectrometer in KBr discs in the range 400-4000 cm^{-1}; the ^1H NMR, ^{13}C NMR, NOESY NMR spectra were recorded on a Bruker AVANCE 500 MHz, all at 298-300 K, with TMS as the internal standard at Institute of Chemistry, Vietnam Academy of Science and Technology.

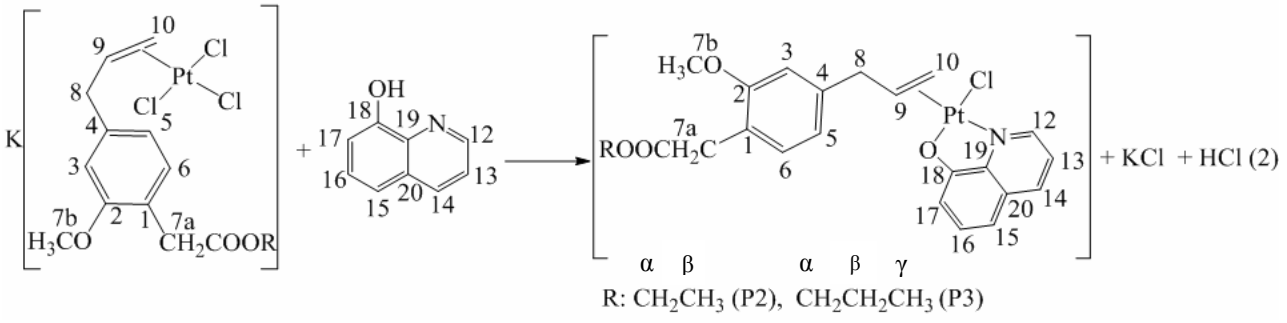
The anticancer activities were tested at Institute of Chemistry of Natural Compounds - Vietnam Academy of Science and Technology, using method which is applied by the National cancer Institute of America (NCI). IC50 values were calculated based on OD values taken on an Elisa instrument at 515-540 nm.

3. RESULTS AND DISCUSSION

Complex *trans*-[$PtCl_2$(Eteug)(C_9H_7N)] (P1) was prepared by replacement a Cl ligand from K[$PtCl_3$(Eteug)] by a quinoline ligand in high yield, 85 %. According to the *trans*-effect, the Cl ligand in the opposite position to Eteug is replaced by quinoline. The neutral complex P1 precipitates out and can be easily isolated. The reaction equation is described as shown in scheme (1):



However, the interaction between 8-hydroxyquinoline and K[$PtCl_3$(Alkeug)] gave no *trans*-[$PtCl_2$(Alkeug)(HOC_9H_6N)] but [PtCl(Alkeug)(OC_9H_6N)](Alkeug: Eteug (P2) or Preug (P3)) with the yield of 85 % and 90 %, respectively in which 8-hydroxyquinoline is deprotonated and coordinates with Pt(II) via N and O atoms as displayed in scheme (2). The analogous coordination of 8-hydroxyquinoline with Pt(II) was also observed when it interacted with K[$PtCl_3$(morpholine)] producing [PtCl(morpholine)(OC_9H_6N)] [8]. The numeration of the examined compounds in schemes (1) and (2) are used only for the analysis of the NMR spectra.



The composition of each complex as determined by platinum analysis by weight method and EDX spectra showed good agreement between the theoretical and actual values. The analytical results and IR spectra (ν) are summarized in table 1.

The IR spectra of P1, P2 and P3 show bands for the presence of Alkeug and amines. For instance, all P1, P2 and P3 display a intense band at around 1750

cm^{-1} corresponding to the $\nu_{C=O}$ band of Alkeug. In addition, the absence of a band at around 1640 cm^{-1} from the C=C double bond of allyl group in non-coordinated Alkeug [5, 7] and the appearance of $\nu_{(Pt-C=C)}$ band at around 440 cm^{-1} indicate the allyl group coordinates in an η^2 manner. Besides, in IR spectra of P2, P3 there is no evidence of ν_{OH}, indicating that 8-hydroxyquinoline has been deprotonated on coordination to Pt(II). The Pt-N, Pt-O stretching vibrations in P1, P2, and P3 are observed in the region 510÷540 cm^{-1} show that quinoline and 8-oxyquinoline have coordinated with Pt(II).

Table 1: Mass percentage of Pt; Atomic ratio of Pt:Cl and main bands in IR spectra of examined complexes

Compounds	% Pt (Found/ Calc.)	Atomic ratio of Pt:Cl (Found/Calc.)	Main bands in IR spectra of examined complexes, cm^{-1}						
			ν_{CH} aromatic	ν_{CH} aliphatic	$\nu_{C=O}$	$\nu_{C=C}$, $\nu_{C=N}$	ν_{C-C}, ν_{C-O}	ν_{Pt-O}, ν_{Pt-N}	$\nu_{(Pt-C=C)}$
trans-[$PtCl_2$(Eteug)(C_9H_7N)] (P1)	29.64 / 30.23	1.05 : 1.92 / 1 : 2	3067	2975 2925 2854	1756	1594 1515	1212 1029	510	444
[PtCl(Eteug)(OC_9H_6N)] (P2)	45.76 / 46.64	1.10 : 0.97 / 1 : 1	3065	2980 2937	1743	1576 1505	1218 1031	540	452
[PtCl(Preug)(OC_9H_6N)] (P3)	45.23 / 46.13	1.10 : 0.91 / 1 : 1	3084	2962 2891	1761	1572 1504	1210 1033	520	414

The assignment of the ^{1}H NMR signals is based on their chemical shift, spin-spin splitting patterns and NOESY spectra. The results are listed in tables 2 and 3. For example, in Fig. 1 the signals of all the protons in P3 have been unambiguously assigned.

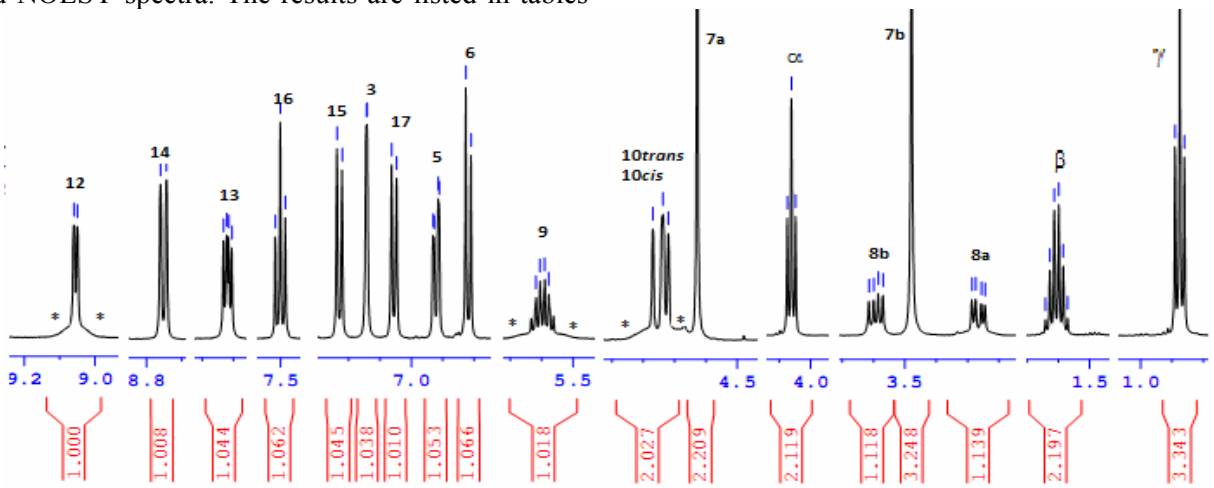


Figure 1: The expanded ^{1}H NMR signals of P3

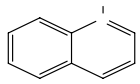
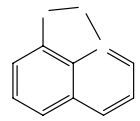
Table 2: ^{1}H NMR signals of Ankeug in P1, P2 and P3, δ (ppm), *J* (Hz)

Complex (Solvent)	H3	H5	H6	H7a	H7b	H8a	H8b	H9	H10*cis*	H10*trans*	Hα	Hβ	Hγ
P1 $(CD_3)_2CO$	7.28 d ^4J 1.5	7.11 dd ^3J 8.0 ^4J 1.5	7.02 d ^3J 8.0	4.78 s	3.78 s	3.31 dd ^2J 15.0 ^3J 5.0	3.60 dd ^2J 15.0 ^3J 4.5	5.88 m $^2J_{PtH}$ 72	4.72 dd ^3J 8.0; ^2J 1.5 $^2J_{PtH}$ 70	4.80 d ^3J 14.0 $^2J_{PtH}$ 70	4.23 q ^{3}J 7.0	1.27 t ^{3}J 7.0	-
P2 $(CD_3)_2CO$	7.14 d ^4J 1.5	6.92 dd ^3J 8.0 ^4J 1.5	6.82 d ^3J 8.0	4.60 s	3.48 s	3.27 dd ^2J 15.0 ^3J 6.0	3.59 dd ^2J 15.0 ^3J 6.0	5.59 m $^2J_{PtH}$ 71	4.73 d ^3J 8.0 $^2J_{PtH}$ 70	4.76 d ^3J 14.5 $^2J_{PtH}$ 70	4.15 q ^3J 7.0	1.20t ^3J 7.0	-
P3 $(CD_3)_2CO$	7.14 d ^4J 1.5	6.92 dd ^3J 8.0 ^4J 1.5	6.82 d ^{3}J 8.0	4.63 s	3.48 s	3.27 dd ^2J 15.0 ^3J 6.0	3.59 dd ^2J 15.0 ^3J 6.0	5.59 m $^2J_{PtH}$ 72	4.73 d ^3J 8.0 $^2J_{PtH}$ 70	4.76 d ^3J 15.0 $^2J_{PtH}$ 70	4.06t ^3J 7.0	1.60m	0.88 t ^3J7.5

Upon coordination to Pt(II), the resonances of the ethylenic protons (H9, H10*cis* and H10*trans*, table 2) are upfield in comparison to those of non-coordinated Alkeug with $\Delta\delta \approx 0.3$ ppm [5, 7]. The ^{195}Pt satellites from H9, H10*cis*, and H10*trans* are clear (indicated with * in figure 1) with the distance between them, $^{2}J_{PtH}$, 71-72 Hz (table 2), showing that the allyl of Alkeug is an η^2 coordinated olefin. Additionally, for non-coordinatinated Alkeug, two protons H8 give rise to a doublet at 3.29 ppm with ^{3}J = 7.0 Hz [5, 7], but in the spectra of P1÷P3 we observed one doublet of doublets for H8a centered at 3.31 ppm for P1 and 3.27 ppm for P2, P3 and another doublet of doublets for H8b centered at 3.60 ppm for P1 and 3.59 ppm for P2, P3. This is expected since upon coordination to Pt(II), rotations around C4-C8 and C8-C10 bonds do not occur on the NMR timescale at the recorded temperature. Thus H8a and H8b are in different space positions.

Moreover, chemical shifts of almost all protons of Alkeug in P2, P3 are smaller than those of P1. This may be explained that these protons fall into a shielding zone of O atom which is only present in P2 and P3 but not in P1.

Table 3: ^{1}H NMR signals of non-coordinated amines and coordinated amines in P1, P2 and P3, δ (ppm), *J* (Hz)

Amine		H12	H13	H14	H15	H16	H17	H18
	Free	8.80	7.30	8.00	7.70	7.40	7.60	8.05
	P1	9.02, br	7.82 dd ^{3}J 8.0 ^{3}J 5.0	8.68 d ^{3}J 8.0	8.14 d ^{3}J 8.0	7.77 t ^{3}J 8.0	7.88 t ^{3}J 8.0	8.81 br
	Free	8,76	7,38	8,10	7,29	7,44	7,18	-
	P2	9.05 dd ^{3}J 5.0 ^{4}J 1.0 $^{3}J_{PtH}$ 33	7.81 dd ^{3}J 8.5 ^{3}J 5.0	8.74 dd ^{3}J 8.5 ^{4}J 1.0	7.22 d ^{3}J 8.0	7.50 t ^{3}J 8.0	7.05d ^{3}J 8.0	-
	P3	9.05 d ^{3}J 5.0 $^{3}J_{PtH}$ 33	7.82 dd ^{3}J 8.5 ^{3}J 5.0	8.75 d ^{3}J 8.5	7.22 d ^{3}J 8.0	7.50 t ^{3}J 8.0	7.05d ^{3}J 8.0	-

The proton resonances of quinoline and 8-oxyquinoline are listed in table 3. As expected, chemical shifts of the protons in coordinated amines (table 3) are larger than in free amines. In ^{1}H NMR spectrum of P1 the ^{195}Pt satellites from H12 are broadened beyond detection. In signals from H12 of P2 and P3, the ^{195}Pt satellites are clear (indicated with * in figure 1). The distance between two satellites, $^{3}J_{PtH,}$ is 33 Hz (table 3). This magnitude of $^{3}J_{PtH}$ is in reasonable agreement with the *trans*-configuration of complexes [$PtCl_2$(amine)(pyridine)] [9]. In other words, the N atom of 8-oxyquinoline occupies the *trans*-position in comparison with the allyl group in coordination plan of P2 and P3.

Table 4: ^{13}C NMR signals of Eteug and amines in P1 and P2, δ (ppm)

Comp.	C1	C2	C3	C4	C5	C6	C7a	C7b	C8	C9	C10	Cα	Cβ	Cγ	C=O
P1	146.5	150.9	115.0	133.6	122.1	116.1	67.2	56.3	40.1	102.7	70.4	61.4	14.5	-	169.8
P2	142.5	146.6	114.4	133.9	121.5	115.9	67.0	55.7	39.6	98.9	68.9	61.2	14.4	-	169.6
P3	142.5	146.6	114.4	133.9	121.5	115.8	66.9	55.7	39.6	99.0	68.8	66.8	22.6	10.5	169.7

	C12	C13	C14	C15	C16	C17	C18	C19	C20
P1	153.6	123.1	141.3	129.1	129.4	116.1	128.9	153.6	131.2
P2	150.6	122.1	147.3	114.9	131.5	115.4	169.2	146.0	132.0
P3	150.6	122.1	147.2	114.9	131.5	115.4	169.2	146.0	132.0

The ^{13}C NMR (table 4) further confirmed the structure of the platinum complexes. The assignment of the ^{13}C NMR signals is based on their chemical shift, experienced rules and ^{13}C NMR spectra of analogous complexes [6]. The results are listed in table 4. The ^{13}C NMR spectra of P1, P2 and P3 show resonances for the presence of Alkeug and amines in the complexes. For example, the characteristic signal for the carbonyl group, C=O, of Ankeug appears at around 169.7 ppm (table 4). In the ^{13}C NMR spectra of the complexes, all the signals except C9, C10 generally shift down field with respect to their position in the free ligand due to coordination of the ligand. Particularly, the resonances of the ethylenic

carbons (C9, C10) are upfield (table 4). This indicates that Alkeug coordinates with Pt(II) at ethylenic double bond of allyl group resulting rehybridization from sp^2 to some sp^3 character of the carbon atoms [10] and H9, H10 may fall into a shielding zone of Pt atom. To determine the configuration of the complexes, NOESY spectra were studied. There is no cross peak between Eteug protons and quinoline protons in the spectrum of P1and between Alkeug protons and 8-oxyquinoline protons, especially H12 in the spectra of P2 and P3. This shows that quinoline in P1 and pyridine ring of 8-oxiquinoline in P2, P3 are at the *trans*-position in comparison to the ethylenic double bond of Alkeug as shown in schemes (1) and (2).

Basing on the analytical results and the analysis of the IR, ^{1}H NMR, ^{13}CNMR, NOESY spectra of P1, P2 and P3 above, we have determined the structures of them as described in schemes (1) and (2).

Complexes P1, P2 and P3 were tested for *in vitro* cytotoxicity on human cancer cellHep-G2. The results show that all P1, P2 and P3exhibit notable cytotoxic activity against this cell with the IC_{50} values of 6.97, 0.31 and 1.27 µg/ml, respectively.

4. CONCLUSION

In this paper, we present the preparation, structure and evaluation for cytotoxic activity against Hep-G2 human cancer cell line of three complexes *trans*-$[PtCl_2(Eteug)(C_9H_7N)]$ (P1), $[PtCl(Eteug)(C_9H_6NO)]$ (P2) and $[PtCl(Preug)(C_9H_6NO)]$ (P3). These complexes were characterized by EDX, IR, ^{1}H NMR, ^{13}C NMR and NOESY spectroscopy. The characterization results reveal that Alkeugcoordinates with Pt(II) at ethylenic double bond of allyl group, quinoline (C_9H_7N) coordinates with Pt(II) via N atom, deprotonated 8-hydroxyquinoline (C_9H_7NO) coordinates with Pt(II) via N and O atoms. Particularly, the ^{1}H NMR and NOESY spectra show that the N atom in each complex, P1, P2, P3 occupies the *trans*-position in comparison to the allyl group. All P1, P2 and P3 exhibit notable cytotoxicity on Hep-G2 human cancer cell line with IC_{50} values of 6.97, 0.31 and 1.27 µg/ml, respectively.

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