

SYNTHESIS, CHARACTERIZATION AND CYTOTOXIC ACTIVITY OF Pt(II)COMPLEX OF CAMPHOR 4-METHYL THIOSEMICARBAZONE

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Received: 12 March 2018; Accepted for publication: 14 May 2018

ABSTRACT

The new complex of Pt(II) with camphor 4-methyl thiosemicarbazone was synthesized and characterized by means of MS, ¹H-NMR and IR spectroscopies. Results show that, the molecular formula of new Pt(II) complex is [Pt(C₁₂H₂₀N₃S)]₂. The Pt(II) complex is of four coordinate. The result of *in vitro* anti-cancer activity of the complex has shown that the complex of Pt(II) with camphor 4-methyl thiosemicarbazone exhibit inhibitor on Hep-G2 and RD cancer cells with IC₅₀ values of 7.74 and 7.61 μg.mL⁻¹. These results indicated a potential of new Pt(II)complex in biomedical application.

Keywords: camphor 4-methyl thiosemicarbazone, complex of Pt(II).

1. INTRODUCTION

The anticancer drugs base on Platinum complexes are the mainstay of chemotherapy regimens in clinic. However, the efficacy of platinum drugs is badly affected by systemic toxicities and drug resistance, and the pharmacokinetics of most platinum drugs is largely unknown [1, 2, 3]. In recent years, platinum complexes with bioactive molecules, natural compounds, targeting groups or nonmaterial's has been interested by chemical and biomedical researchers [4, 5, 6]. The motivation comes from some of the following demands: improve the selectivity or minimize the systemic toxicity of the drugs, enhance the cellular accumulation of the drugs, overcome the tumor resistance to the drugs, visualize the drug molecules *in vitro* or *in vivo*, achieve a synergistic anticancer effect between different therapeutic modalities, or to add extra functionality to the drugs [5, 6]. The development of drug delivery systems in the last several decades has provided a variety of methods including the synthesis new Pt(II), Pt(IV) complexes, the incorporation of drugs into liposome's, lipid emulsions, and polymeric micelles to reduce side effects, to increase their solubility, and to prolong circulation time as well [6]. Camphor has bioactivity, it has been used in traditional medicine from time immemorial. The coordination of camphor and platinum could create new compounds with high bioactivity. In

this paper, we present the new results of Pt(II) complex with camphor 4-methyl thiosemicarbazone.

2. CHEMICALS AND METHODS

2.1. Chemicals

Camphor, 4-methyl thiosemicarbazide, acetic acid and ethanol were purchased from Merck, $K_2[PtCl_4]$ was purchased from Sigma - Aldrich.

2.2. Methods

2.2.1. Synthesis of camphor 4-methyl thiosemicarbazone (H4methiocam)

The H4methiocam was prepared from 4-methyl thiosemicarbazide and camphor (1:1 molar ratio). The mixture of reactants (2.1 g, 20 mmol 4-methyl thiosemicarbazide and 3.04 g, 20 mmol camphor) was dissolved in warm ethanol – water solvent (120 mL ethanol and 80 mL water) and anhydrous acetic acid was added until pH reached 4. This mixture was stirred and reflux at 70 °C for 6 h. After cooling to room temperature, crystalline product was isolated and washed with water and dried over P_2O_5 . H4methiocam was obtained as a white powder. Yield (3.52g, 74 %).

2.2.2. Synthesis of Pt(II) complex (Pt-4methiocam).

To synthesize Pt-4methiocam, a solution of $K_2[PtCl_4]$ (0.415 g, 1 mmol) in water (50 mL) was added to a solution of camphor 4-methyl thiosemicarbazone (0.478 g, 2 mmol) in ethanol (100 mL) at 30 °C under stirring for 1 h. The reaction mixture was kept at room temperature for 24 h. Afterwards, the precipitate was filtered and washed several times with water and dried over P_2O_5 . The Pt-4methiocam was obtained as a yellow powder. Yield (0.623 g, 93 %).

2.2.3. Structure determination

Mass spectroscopy with electrospray ionization technique (ESI-MS) was used in order to confirm the formula of new compounds (Agilent 1100 LC/MSD Trap). IR spectra were recorded with a FTIR Shimadzu spectrophotometer using KBr discs. 1H -NMR spectra were obtained with a Bruker 500 MHz spectrometer and the chemical shifts are given in units of δ relative to TMS as an internal standard using DMSO- d_6 as the solvent.

2.2.4. Cytotoxicity assay

The cytotoxicity assay was performed based on the method of Skehan et al. [7] and Likhiwitayawuid et al. [8] using sulforhodamine B(SRB). Ellipticine was used as the positive reference.

3. RESULTS AND DISCUSSION

3.1. The result of spectra

Mass spectra of H4methiocam and Pt-4methiocam are shown in Fig.1, ESI/MS data in Table 1.

As seen in the MS spectra (Fig. 1(a,b)), the appearance of a cluster of peaks with m/z (MH^+) = 240, 241, 242 of H4methiocam (Fig. 1(a)) and a cluster of peaks with m/z = 795, 796, 797 of Pt-4methiocam (Fig. 1(b)) were consistent with the molecular formula of ligand $C_{12}H_{21}N_3S$ and the complex $Pt(C_{12}H_{21}N_3S)_2$ calculated from different isotopes.

Table 1. MS data and compound's molecular formula.

Sample	m/z , $[M+H]^+$	M	Molecular formula
H4methiocam	240	239	$C_{12}H_{21}N_3S$
Pt-4methiocam	672	671	$Pt(C_{12}H_{20}N_3S)_2$

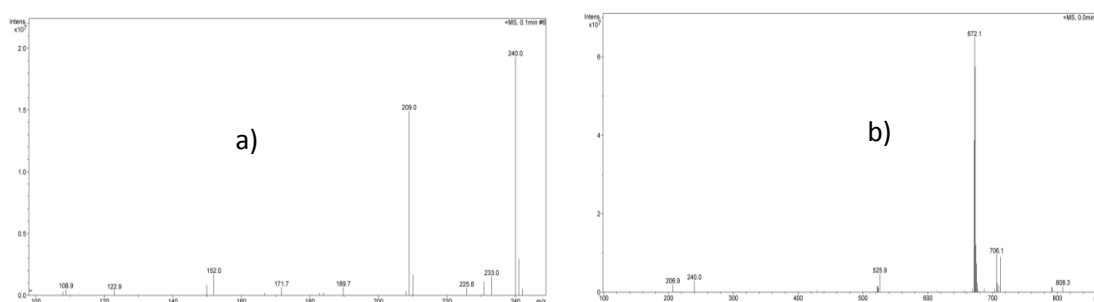


Figure 1. Mass spectra of H4methiocam (a) and Pt-4methiocam (b).

The 1H -NMR spectrum of H4methiocam (Fig. 2(a)) exhibited a singlet at 9.68 ppm attributed to NH-hydrazine proton. The presence of NH signal indicated the presence of H4methiocam in the thione form. The proton signal of the NH-amide appeared at 7.96 ppm. Signals at 1.27 ppm to 1.77 ppm were assigned to 9H of 3 CH_3 groups (of camphor) and signals in range 1.80 to 2.93 ppm were assigned to protons of CH and CH_2 . The signal at 3.33 ppm was assigned to 3H of CH_3 (of CH_3-N). The H signal of NH-hydrazine (NHC=S group) from Pt-4thiocam complex's spectrum (Figure 2(b)) was changed to confirm the deprotonation of the ligand due to coordination with Pt(II) via S and N. The signals of other protons appeared in similar range in ligand's spectrum.

The IR spectrum of H4methiocam (Fig.3a) showed absorption bands at 3448 and 3182 cm^{-1} due to stretching frequencies for NH-amide and NH-hydrazine. The band due to the $-SH$ group was not observed in 2500-2600 cm^{-1} and the presence of band at 852 cm^{-1} due to $\nu(C=S)$ suggested the existence of thiosemicarbazone in the thione form. The absorptions band for $-CN$ appeared at 1593 cm^{-1} . The IR spectrum of Pt-4methiocam (Fig. 3b) showed absorption band at 3313 cm^{-1} due to stretching frequencies for NH-amide, while the absorption for NH at region 3000–3200 cm^{-1} was absent. The $\nu(C=S)$ band at 852 cm^{-1} in the spectrum of the ligand shifted to 812 cm^{-1} in the spectrum of the complex, indicated that the existence of ligand is in the thiol form and deprotonation on complexation and that Pt(II) coordinated with the thiolate sulfur. The $\nu(C=N)$ band of the thiosemicarbazone at 1533 cm^{-1} shifted to 1537 cm^{-1} in the spectrum of the complex, indicated the coordination of the azomethine nitrogen. This result was confirmed by the presence of new bands at 611 and 405 cm^{-1} due to $\nu_{(Pt-N)}$ and $\nu_{(Pt-S)}$. These spectra suggested

that after deprotonation the ligand coordinated with the Pt(II) via S and N. Selected IR bands for the ligand (H4methiocam) and complex (Pt-4methiocam) are given in Table 2.

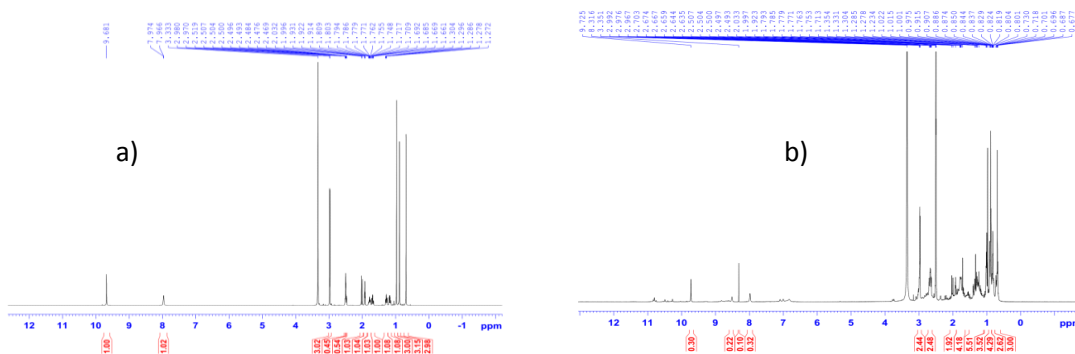


Figure 2. ¹H-NMR spectra of H4methiocam (a) and Pt-4methiocam (b).

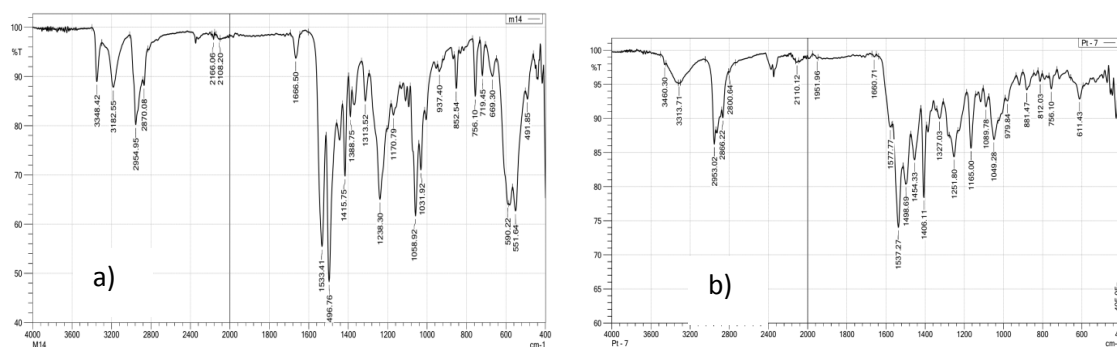


Figure 3. IR spectra of H4methiocam (a) and Pt-4methiocam (b).

Table 2. Selected IR bands of the H4methiocam and Pt-4methiocam

Compounds \ ν, cm^{-1}	NH	CN	NN	CS	Pt-X (X= N, S)
H4methiocam	3448, 3182	1533	1058	852	-
Pt-4methiocam	3313	1537	1049	812	611, 405

Based on the above analysis, reasonable structures of H4methiocam ligand and Pt-4methiocam complex are depicted in Fig. 4.

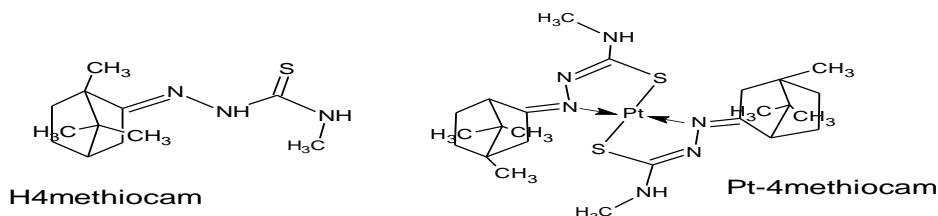


Figure 4. Structures of the H4methiocam and Pt-4methiocam

3.2. Cytotoxicity

The complex (Pt-4methiocam) was tested for the cytotoxic activity in order to evaluate inhibition on Hep-G2 and RD cell lines. The results show that the Pt-4methiocam inhibited to both Hep-G2 and RD cell lines with IC_{50} values of 7.74 and 7.61 g/mL^{-1} (Table 3).

Table 3. The cytotoxic activity of Pt-4thiocam on Hep-G2 and RD cells.

Sample	Concentration (g/mL)	Hep-G2		RD	
		Cell Survival (%)	IC_{50} (g/mL)	Cell Survival (%)	IC_{50} (g/mL)
Elipticine (refrence)	5	0	-	0	-
DMSO	-	100	-	100	-
Pt-4methiocam	10	0	7.74	0	7.61

4. CONCLUSION

In conclusion, the complex of camphor 4-methyl thiosemicarbazone with Pt(II) was successfully synthesized from $K_2[PtCl_4]$ and camphor 4-methyl thiosemicarbazone in ethanol-water solvent. The analysis data from MS, IR, and 1H -NMR spectra showed that the molecular formula of complex of Pt(II) with camphor 4-metyl thiosemicarbazone is $[Pt(C_{12}H_{20}N_3S)_2]$. The Pt(II) complex is four coordinate. The new complex displayed a high activity, it inhibits to both Hep-G2 and RD cancer cell lines with IC_{50} values of 7.74 and 7.61 $\mu g/mL^{-1}$. These results suggest a possibility of developing Pt-4methiocam as one of the potential chemotherapeutic agents.

Acknowledgments. This work was financially supported by the Ministry of Education and Training of Vietnam (MOET) under Code. B2017 - TDV - 01(PTHT).

REFERENCES

1. Indrani Pal, Falguni Basuli and Samaresh Bhattacharya - Thiosemicarbazone complexes of the platinum metals. A story of variable coordination modes, Indian Acad. Sci. (Chem. Sci.) **114** (4) (2002) 255–268.
2. Lorena Giovagnini, Luca Ronconi, Donatella Aldinucci, Debora Lorenzon, Sergio Sitran, and Dolores Fregona - Synthesis, Characterization, and Comparative in Vitro Cytotoxicity Studies of Platinum(II), Palladium(II), and Gold(III) Methylsarcosinedithiocarbamate Complexes, J. Med. Chem. **48** (5) (2005)1588–1595.
3. Justin J. Wilson and Stephen J. L. - Synthetic Methods for the Preparation of Platinum Anticancer Complexes, Chem. Rev. **114** (8) (2014) 4470–4495.
4. Utku S., Topal M., Dogen A., and Serin M. S. - Synthesis, characterization, antibacterial and antifungal evaluation of some new platinum(II) complexes of 2-phenylbenzimidazole ligands, Turkish Journal of Chemistry **34** (3) (2010) 427–436.
5. Poonia N., Kumar M. S., Arora D., and Mahadevan N. - Development of cisplatin loaded poly (d, l-lactide-co-glycolide)-poly(ethylene glycol) immunonanoparticles for epidermal growth

- factor receptor (EGFR) positive pancreatic cancer cells, *International Journal of Recent Advances in Pharmaceutical Research* **3** (2011) 14–24.
6. Timothy C. J., Kogularamanan Suntharalingam, and Stephen J. L. - The Next Generation of Platinum Drugs: Targeted Pt(II) Agents, Nanoparticle Delivery, and Pt(IV) Prodrugs (Review), *Chem. Rev.* **116** (5) (2016) 3436–3486.
 7. Skehan P., Storeng R., Scudiero D., Monks A, McMahon J., Vistica D., Warren JT., Bokesch H., Kenney S., Boyd MR - New colorimetric cytotoxicity assay for anticancer agents. *Eur. J. Cancer* **27** (1991) 1162-1168.
 8. Kittisak Likhitwitayawuid, Cindy K. Angerhofer, Geoffrey A. Cordell, John M. Pezzuto, and Nijisiri Ruangrunsi - Cytotoxic and antimalarial bisbenzylisoquinoline alkaloids from *Sephania evecata*, *Journal of Natural Products* **56** (1) (1993) 30-38.