Turk J. Pharm. Sci. 5 (1) 45-52, 2008

Short Communication

SYNTHESIS OF PLATINUM(II) COMPLEXES OF 1-ARYL-3-(BENZIMIDAZOLE-2-YL)-PYRAZOLE-4-KETOXIMES AND STUDIES ON THEIR CYTOTOXIC EFFECTS

Kadriye BENKLİ[1]*, İsmail KAYAGİL[1], Zerrin İNCESU-SELLER[2], Gühergül ULUÇAM[3], Şeref DEMİRAYAK[1]

[1]Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 26470, Eskişehir,TURKEY
[2]Anadolu University, Faculty of Pharmacy, Department of Biochemistry, 26470, Eskişehir,TURKEY
[3]Trakya University, Faculty of Art and Science, Department of Chemistry, 22030, Edirne, TURKEY

Abstract

In this research, synthesis of some complexes in the presence of Pt(II) were studied. The structure of the compounds were elucidated by IR, ^{1}H-NMR, Mass and Elemental Analyses.

The cytotoxicity of Pt-complex was evaluated. The cellular responses through MTT method were measured in normal rat embryo fibroblast line F2408 and H-ras active-rat embryo fibroblast line 5RP7.

Keywords : *Metal complexes, ketoximes, MTT*

1-Aril-3-(benzimidazol-2-il)-pirazol-4-ketoksimlerin Pt(II) komplekslerinin sentezleri ve bu bileşiklerin sitotoksik etkileri üzerine çalışmalar

Bu çalışmada bazı 1-Aril-3-(benzimidazol-2-il)-pirazol-4-ketoksim'lerin Pt(II) kompleksleri sentezlenmiştir. Bileşiklerin yapıları IR, ^{1}H-NMR, Kütle ve elementel analiz ile aydınlatılmıştır.

Pt(II) komplekslerinin sitotoksik etkileri araştırılmıştır. Hücresel cevaplar, normal sıçan embriyo fibroblastı F2408 ve aktif sıçan embriyo fibroblastında, MTT metodu kullanılarak ölçülmüştür.

Anahtar Kelimeler : *Metal kompleksleri, ketoksimler, MTT*

***Correspondence :** Tel.: +90 222 3350581/3780 ; Fax: +90 222 3350750
E-mail: kbenkli@anadolu.edu.tr

INTRODUCTION

The most drugs used nowadays show important side effects, such as resistance of cancer cells to the cytostatic agents. Therefore the new strategies are developed in synthesis of new cyctotoxic and therapeutic agents and in some cases the complexes by metal-ligand bonding derivatives were synthesized. Especially cisplatin is highly effective anticancer drug. The efficiency of cisplatin is based on its ability to bind to genomic DNA. The two chlorine atoms of the cisplatin molecules are replaced by N atoms of adjacent purine bases[1] and most of the well-known platinum anticancer complexes have at least one N-H group, which is responsible for important hydrogen-bond donor properties[2]. Ligands possessing the hydroxyimino groups together with other powerful donor groups can be very efficient chelating agents which are be able to facilitate the stabilisation of high oxidation states of 3d-metals.

Otherways, oxime compounds and other some compounds bearing the C=NOH group cause such biological effects as oxidative DNA cleavage[3], an increase in the targeting of specific nuclear bases of DNA[4], and a change in the antitumor action because of their lipophilicity[5]. Furthermore, the possibility of a synergic effect between a metal ion, as Pt(II), and oxime ligands may lead to an improvement in antitumor activity.

To get an insight into these mechanisms and knowledges, we now report on the synthesis and characterization of some cisplatin analogues with 1-aryl-3-(benzimidazole-2-yl)-pyrazole-4-ketoximes[6-10] for obtained new therapeutic agents. Toxic effects of these compounds were studied by MTT assay. In the anticancer effect studies, the possible inhibitory effects of these compounds were measured on 5RP7 cancer cells[11,12].

EXPERIMENTAL

Melting points were determined by using a Gallenkamp apparatus and given uncorrected. Spectroscopic data were recorded on the following instruments; IR: Shimadzu 435 IR spectrophotometer; ^{1}H NMR: DPX 400 NMR spectrometer; Elemental analyses were performed on a Leco CHNS analyser and all the analyses for C, H, N were within ± 0.4 % of the theoretical values.

Synthesis of the ligands (1,2)

1-Aryl-3-(benzimidazole-2-yl)-pyrazole-4-carboxaldehydes (**1**) were prepared by using hydrazones in condition of Wilsmeier-Hack reaction[6]. The oxime derivatives (**2**) were obtained by reacting the appropriate compounds with hydroxylamine hydrochloride in the presence of sodium acetate in ethanol by using literature method[7].

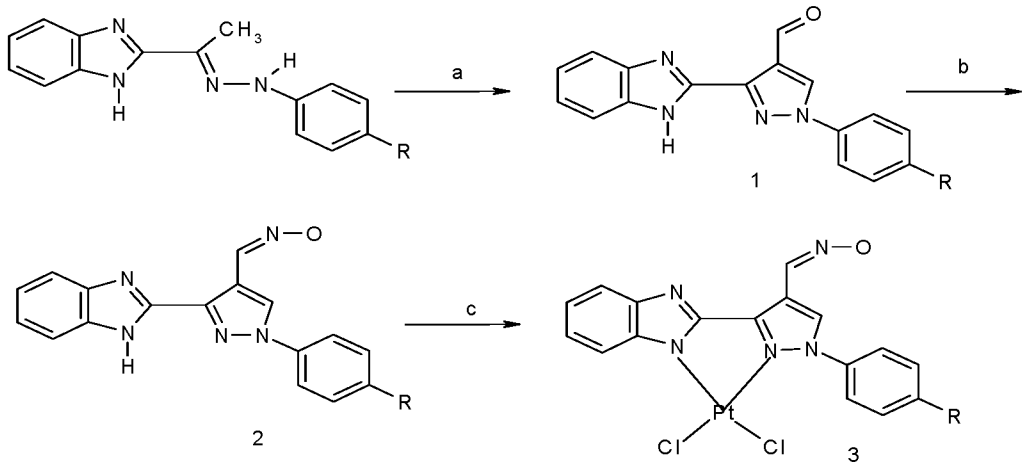
Preparation of the Pt(II) complexes (3)

1-Aryl-3-(benzimidazole-2-yl)-pyrazole-4-ketoxime derivatives (0.5 mmol) in EtOH-H_2O and K_2PtCl_4 (0.5 mmol) in H_2O (5 ml) were stirred at 25°C for 8-12h. The solution was filtered and then kept in the refrigerator for 24 h. The precipitated complexes were filtered. After washing several times with distilled water and diethyl ether, the final products were dried in vacuo (yield ~50%). Column chromatography was used for purifying of some compounds. Reactions were shown in the Scheme 1 and the physicochemical properties of the compounds were shown in the Table 1.

2a IR (KBr)νmax(cm^{-1}): 3425-3386 (O-H), 3117-2786 (N-H), 1635 (C=N), 995 (N-O). ^{1}H-NMR σ(ppm): 4.97 (1H, s, -CH-), 6.83-7.32 (10H, m, Ar-H), 9.50 (1H, s, NH), 11.25, (1H, s, OH).

3a IR (KBr)νmax(cm^{-1}): 3558 (O-H), 1648 (C=N), 1093 (N-O), 327 (Pt-Cl), 298 (Pt-N). ^{1}H-NMR σ(ppm): 5.34 (1H, s, -CH-), 6.94-8.02 (10H, m, Ar-H), 11.97, (1H, s, OH).

2b IR (KBr)νmax(cm^{-1}): 3442-3390 (O-H), 3127-3012 (N-H), 1654 (C=N), 999 (N-O). ^{1}H-NMR σ(ppm): 5.18 (1H, s, -CH-), 6.98-7.65 (10H, m, Ar-H), 9.87 (1H, s, NH), 11.64, (1H, s, OH).
3b IR (KBr)νmax(cm^{-1}): 3560 (O-H), 1648 (C=N), 1093 (N-O), 339 (Pt-Cl), 286 (Pt-N). ^{1}H-NMR σ(ppm): 5.64 (1H, s, -CH-), 6.97-8.12 (10H, m, Ar-H), 12.34, (1H, s, OH).



a : DMF / $POCl_3$ b : H_2N-OH/NaOAc-EtOH c : K_2PtCl_4 / EtOH-H_2O

Scheme 1. Synthesis of compounds

Table 1. Physicochemical properties of compounds.

Comp	R	m.p.(°C)	Yield (%)	Mol. Form.
2a	H	244	85	$C_{17}H_{12}N_5O$
3a	H	>350	48	$Pt(C_{17}H_{11}N_5O)Cl_2$
2b	Cl	275	72	$C_{17}H_{11}ClN_5O$
3b	Cl	>350	56	$Pt(C_{17}H_{10}ClN_5O)Cl_2$

Cell Culture

Normal rat embryo fibroblast-like cells (F2408) and H-ras oncogene active fibroblast cells (5RP7) were maintained in Dulbecco Modified Eagle Medium (DMEM) (Sigma) supplemented with 10% (v/v) foetal calf serum (FCS) (Gibco), penicillin/streptomycin at 100 units/ml and glutamine as adherent monolayers. Cells were incubated at 37°C under 5% CO_2/95% air in a humidified atmosphere.

Cytotoxic valuation

F2408 and 5RP7 cells were treated with compounds to study the cytotoxic effects. Individual wells of 96-well microtiter plate were inoculated with 200 µl of 10% FCS medium containing $1x10^3$ cells/ml. After 24 or 48 h of exposure to the drug at various concentrations (0,1-0,02-0,01-0,002-0,001-0,0002 mg/ml), the cells were used for the cytotoxicity evaluation.

The cytotoxicity response of these cells was determined with the MTT (3-(4,5-dimethylthiazole-2yl)-2,5diphenyl-2H-tetrazolium bromide) method. Briefly, 200µl of freshly prepared 5µg/ml MTT solution, was added to each well after drug treatment. The cells were incubated 2h and washed with phosphate buffered saline (PBS). After removal of the buffer, dimethyl sulfoxide (DMSO) was added and suspension was obtained. The optical density (OD)

was determined by using a Dynatech. MR5000 (Dynatech Lab,USA) plate reader at the wavalength of 370 nm. Each dose of compounds was repeated 4 times per experiment. The IC_{50} value were defined as the concentration of test compound resulting in a %50 reduction of absorbance compared to untreated cells in the MTT assay. Cytotoxicity of compounds against F2408 and 5RP7 cell lines were shown in the Table 2.

Table 2. Cytotoxicity of compounds against F2408 and 5RP7 cell lines.

Comp	Cytotoxicity IC_{50}, mg/ml F2408	5RP7
Cl-Ligand	0.02	0.0004
Cl-Pt	0.03	NE
H-Ligand	0.03	0.003
H-Pt	0.04	0.01

RESULTS AND DISCUSSION

Synthesis

The structure of the compounds were elucidated by spectral data and elemental analyses. The IR spectrum of ligands showed three bands between 3442-3380 cm^{-1} due to ν(O-H), 3127-2786 cm^{-1} due to ν(N-H), 1654-1635 cm^{-1} due to ν(C=N), which are different from the spectrum of platinum complexes. No bands exist in the 2700-3400 cm^{-1} region, which are due to N-H vibrations in the IR spectrum of the complexes. New bands in the low frequency regions at 339-327 cm^{-1} and 319-286 cm^{1} assignable to ν(Pt-N), ν(Pt-Cl) were observed.

In the ^{1}H-NMR spectra, the peaks of aromatic protons were observed about 6.54-8.12 ppm as multiplets. The peaks of O-H protons of the ligands were obtained between 11.25-11.64 ppm, while they were obtained between 11.95-12.34 in the spectrum of complexes. The peaks of N-H protons of the ligands were measured between 9.36-9.87 ppm, which disappeared in the spectrum of complexes.

Cytotoxicity

The cytotoxicity of Pt-complex was evaluated. The cellular responses through MTT method were measured in normal rat embryo fibroblast line F2408 and H-ras active-rat embryo fibroblast line 5RP7 at the concentrations of 0,0001-0,1 mg/ml for 24h and 48h. The results are shown in Figure 1 and 2.

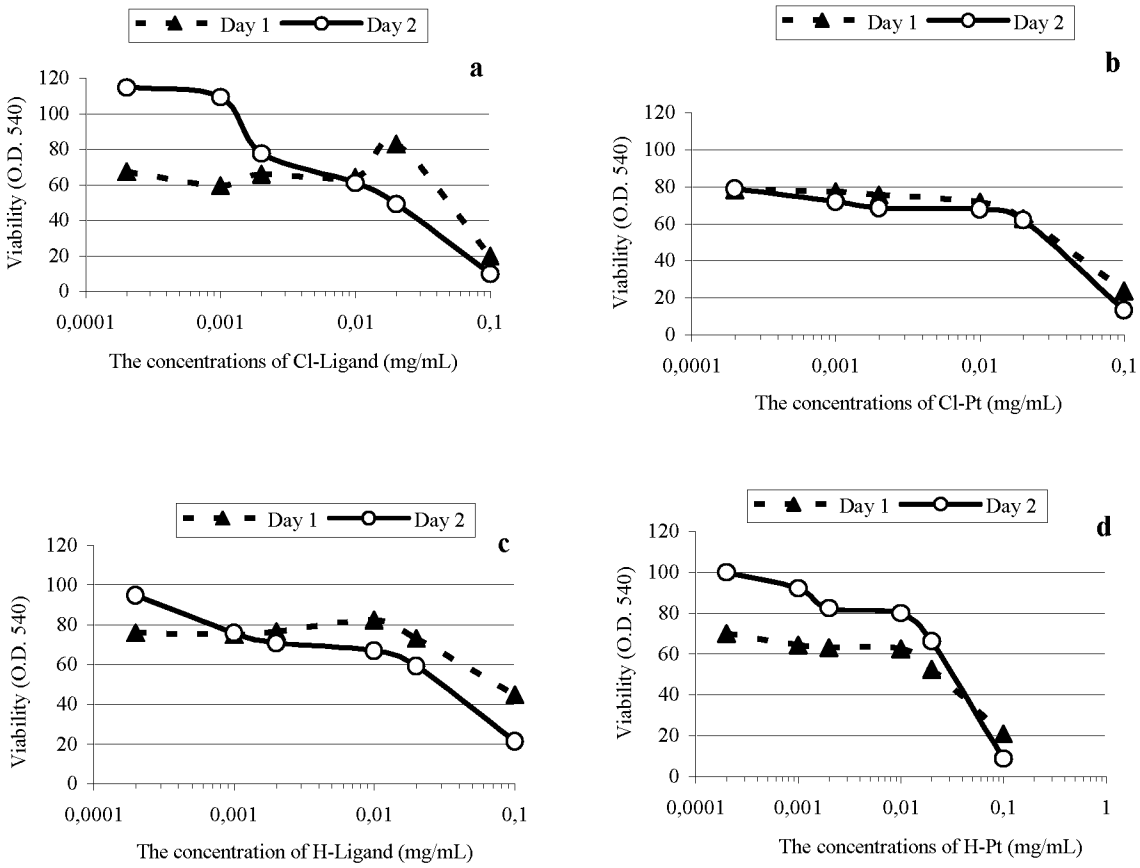


Figure 1: Toxicity of compounds (Cl-Ligand, Cl-Pt; H-Ligand, H-Pt) for F2408 cells. (a, b) cells were treated with either Cl-Ligand or Cl-Pt ; (c,d) with either H-Ligand or H-Pt, respectively. At each time point, MTT assay was performed for quantization of viable cells. Cells were seeded at 1x103 /ml and incubated with 0.0002, 0.001, 0.002, 0.01, 0.02, 0.1 mg/ml concentrations of compounds. Results are the mean of quadruplicate wells.

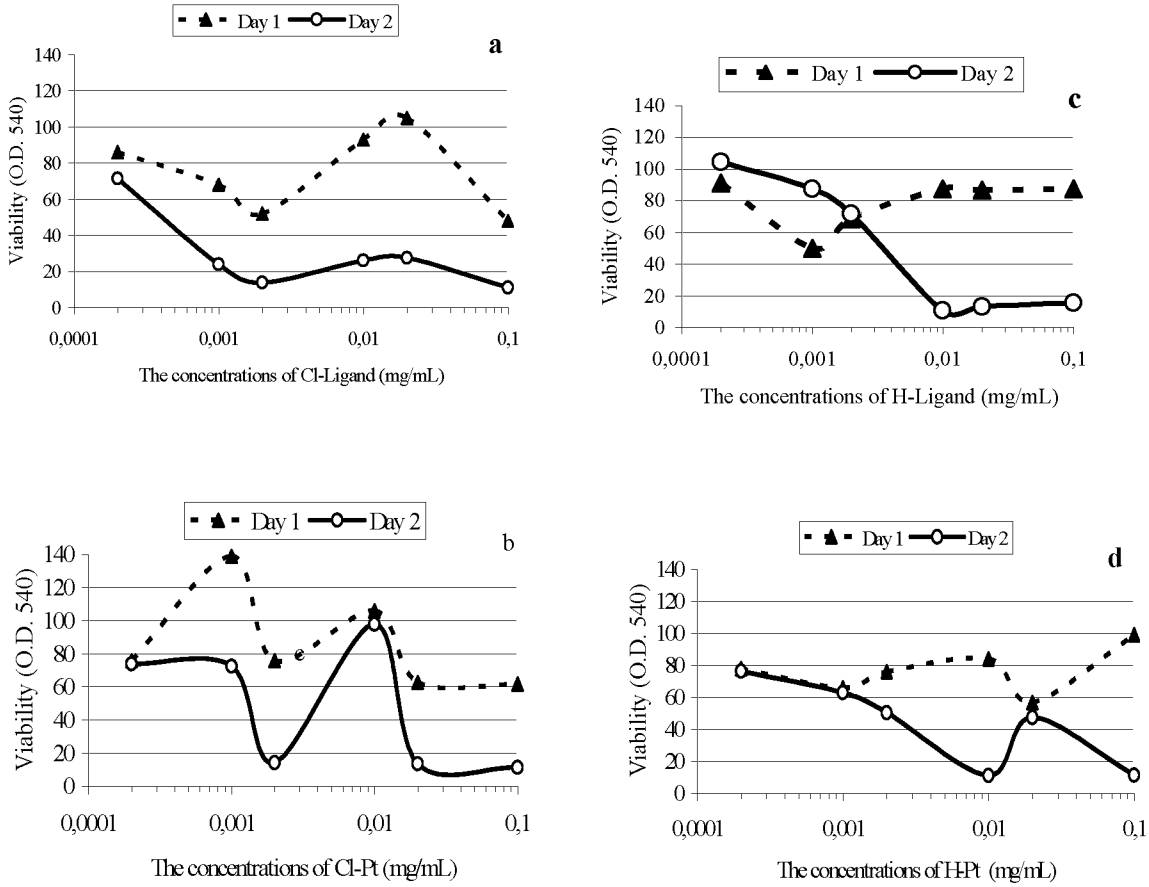


Figure 2: Toxicity of compounds for 5RP7 cancer cells. At each time point, MTT assay was performed for quantization of viable cells. Results are the mean of quadruplicate wells.

Except of the higher dose (1.0 mg/ml), the introduction of Pt complex at either Cl or H produced derivatives with less cytotoxic activity in F2408 cell line. In contrast, all compounds are more toxic for cancer rat embryo fibroblast after 48h incubation. Greater activity was observed in Cl-Ligand, H-Ligand and H-Pt compounds at the concentration range of 0.001-0.1 mg/ml. H-Pt compound showed 40% cytotoxicity at 0,001 μg/ml, in comparison to control compound (H-Ligand) and control cell line (F2408) at 24 h. Unlike Cl-Pt, Cl-ligand showed a dose-dependent cytotoxic effects on 5RP7 cancer cell lines.

ACKNOWLEDGEMENT

Authors are gratefull to Anadolu University, Commission of Scientific Research Projects, for financially support.

REFERENCES

1. **Mock, C., Puscasu, I., Rauterkus, M. J., Tallen, G., Wolff, J. E. A., Krebs, B.,** "Novel Pt(II) anticancer agents and their Pd(II) analogues: syntheses, crystal structures, reactions with nucleobases and cytotoxicities" *Inorg. Chim. Acta*, 319, 109-116, **2001**.

2. **Reedijk, J.**, "New clues for platinum antitumor chemistry: Kinetically controlled metal binding to DNA" *PNAS*, 100 /7) 3611-3616, **2003.**

3. **Saglam, N., Colak, A., Serbest, K., Dülger, S., Güner, S., Karaböcek, S., Beldüz, A. O.**, "Oxidative cleavage of DNA by homo- and heteronuclear Cu(II)-Mn(II) complexes of an oxime-type ligand" *Biomet.*, 15 (4) 357-365, **2002**.

4. **Hambley, T. W., Ling, E. C., O'Mara, S., McKeage, M. J., Russell, P. J.**, "Increased targeting of adenine-rich sequences by (2-amino-2-methyl-3-butanone oxime)dichloroplatinum(II) and investigations into its low cytotoxicity" *Biol. Inorg. Chem.*, 5, 675-681, **2000**.

5. **Failes, T. W., Hall, M. D., Hambley, T. W.**, "The first examples of platinum amine hydrogenate complexes: structures and biological activity" *J. Chem. Soc. Dalton Trans.*, 1596-1600, **2003**.

6. **Kira, M. A., Nofal, Z. M., Gadalla, K. Z.,** "The vilsmeier-haack reaction-VI reaction of phosphoryl chloride-dimethylformamide with schiff bases and azines", *Tetrahedron Lett.*, 4215-4222, **1970**.

7. **Massolini, G., Carmellino, M. L., Baruffini, A.,** "Fungicidal activity of arylfurylketoximes", *Farmaco*, 51 (4), 287-292, **1996**.

8. **Altman, J., Wilchek, M., Warshawsky, A.,** "Platinum(II) complexes with 2,4-diaminobutyric acid, ornithine, lysine and 4,5-diaminovaleric acid", *Inorg. Chim. Acta*, 107, 165-168, **1985**.

9. **Spassovska, N.H., Pelova, R. G., Wołowiec, S., Jeżowska-Trzebiatowska, B.,** "Platinum(II) complexes of pyrimidine-derived ligands", *Inorg. Chim. Acta*, 106, 171-176, **1985**.

10. **Algul, O., Ozcelik, B., Abbasoglu, U., Gumus, F.,** "Synthesis, characterization and genotoxicity of platinum(II) complexes with substituted benzimidazole ligands", *Turk J. Chem.*, 29, 607-615, **2005**.

11. **Merante, F., Raha, S., Reed, J.K., Proteau, G.,** "The simultaneous isolation of RNA and DNA from tissues and cultured cells" *Methods Mol. Biol.*,58, 3-9, **1996**.

12. Scioscia K. A., Snyderman C. H., Rueger R., Reddy J., D'Amico F., Comsa S., Collins B., "Role of arachidonic acid metabolites in tumor growth inhibition by nonsteroidal antiinflammatory drugs", *Am J Otolaryngol*, 18, 1-8, **1997**.

Received: 14.11.2006
Accepted: 4.06.2007