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Thermal behavior and biological activity of $[Co_2(Cl)_2\,tpmc](BF_4)_2$ complex

ABSTRACT

A large number of interesting Co (II) complexes with macrocyclicligands have been synthesized and the recognition of its complexes as important bioactive compounds in vitro and in vivo has aroused growing interest in these agents as potential drugs for therapeutic use in various diseases. Numerous available information on their bioinorganic properties and mode of action in several biological systems, combined with the new possibilities imposed by the development of medical chemistry, opens space for the development of a new generation of highly active drugs with minimized side effects which could add significantly to the current clinical research and practice. In this paper we attempt to present some properties of the earlier isolated the first Co(II)tpmc complex for which crystal structure confirmed chair conformation of macrocycle. Complex with formula $[Co_2(Cl)_2tpmc](BF_4)_2$ (tpmc = N,N′,N″,N‴- tetrakis(2-pyridylmethyl)-1,4,8,11 - tetraazacyclotetradecane), was studied on thermal behaviour and biological activity. TG-DTA analysis indicates that complex decomposition in a single step in the range of 365 -435 °C. Investigated cytotoxic activity against two human cancer cell lines: HeLa (human cervix adenocarcinoma) and K562 (human myelogenous leukaemia). Complex was also preliminary assayed in vitro toward bacteria, fungi and mould together with the starting material for the synthesis (ligands, simple salts and solvents) as test substances. Investigated complex showed a moderate activity against strains of bacteria and were inactive against the tested fungi and mould. Minimal inhibitory concentration suppressing the visible growth of bacteria was determined. Biological investigations show the complex has significant cytotoxic potential.

Key words: *Co(II) complex, tpmc, microbiological activity, cytotoxic activity, thermal behavior.*

1. INTRODUCTION

Macrocyclic ligands with metal ions usually create construction of stable complexes of different structures, catalytic, redox, etc. characteristics. This complexes have important implications for a range of chemical and biochemical applications [1]. One of the attractive macrocyclic ligands is the tpmc, a fully *N*-substituted cyclam with pendant 2-pyridylmethyl groups.

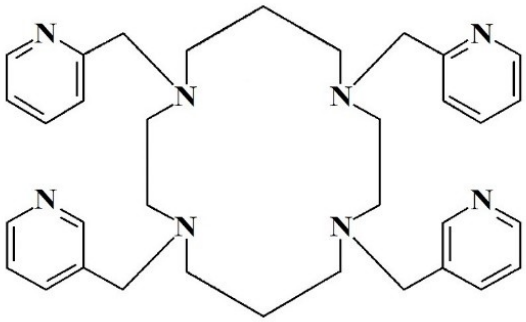

Figure 1. Structure of macrocyclic ligand N,N',N'',N'''-tetrakis(2-pyridilmethyl)-1,4,8,11-tetraazacyclotetradecane (tpmc).

Slika 1. Struktura makrocikličnog liganda N,N',N'',N'''-tetrakis (2-piridilmetil) -1,4,8,11-tetraazaciklotetradekana (tpmc).

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The fundamental role of cobalt and the recognition of its complexes as important bioactive compounds in vitro and in vivo aroused an everincreasing interest in these agents as potential drugs for therapeutic intervention in various diseases. The vast array of information available for their bioinorganic properties and mode of action in several biological systems, combined with the new opportunities offered by the flourishing technologies of medicinal chemistry, is creating an exciting scenario for the development of a novel generation of highly active drugs with minimized side effects which could add significantly to the current clinical research and practice.

The mixed-ligand Co(II) complexes with tpmc and another additional ligand were intensively studied [2-7] but up to now the X-ray analysis has been performed for only three of them $[Co_2(ox)tpmc](ClO_4)_2 \cdot 3H_2O$, $[Co_2(Cl)_2tpmc](BF_4)_2$ and $[Co_2(\mu\text{-}pht)_2tpmc](ClO_4)_2 \cdot H_2O$ [4,6,8].

A great number of termodinamically stable Co(II) complexes with tpmc and other ligands (mono- or dicarboxylates, pseudohalides (NCO, NCS, NCSe) *etc*.) in macrocyclic enviroment are isolated [5-7,9]. Also, the biological activity of these complexes was examined. In most cases, the complexes showed cytotoxic activity as well as the activity of these compounds against some microorganisms.

Continuing previous research, the aim of the this study was to investigate the previously synthesized $[Co_2(Cl)_2tpmc](BF_4)_2$ complex, its potential cytotoxic effect on human cancer cell lines and microbiological activity. The investigate complex was earlier tested on some cultures of microorganisms *M. lysodeikticus*; S.A, *S. aureus*; *E. coli*; *B. subtilis* and showed selective antibacterial activity towards Gram (+) bacteria *Staphylococcus P*. [10]. Now, we wanted to expand this research. Also, to study thermal stability of the complex to examine the possible relationships between the thermal data and the biological activity.

2. EXPERIMENTAL

Materials and Methods

Complex $[Co_2(Cl)_2tpmc](BF_4)_2$ and ligand tpmc were prepared by described procedure [10]. All other chemicals were of p.a. grade and were used as supplied.

The simultaneous TG-DTA experiments were carried out using a SDT 2960 thermal analyzer in air (flow rate = 90 mL min^{-1}) from 20 to 580°C (heating rate = 15°C min^{-1}) using platinum cups.

Cytotoxicity assay

Stock solutions of the test compounds were made up in dimethyl sulfoxide (DMSO) at 10 mM, filtered through Millipore filters (0.22μm), and diluted for use in the nutrient medium to the relevant working concentrations. For all of the cells used, the nutrient medium was RPMI 1640 without phenol red, supplemented to final concentration with L-glutamine (3mM), streptomycin (100 mg/mL), and penicillin (100 IU/mL), fetal bovine serum (10%; FBS; 56°C heat-inactivated due to inactivation of cholinesterases and system complement [11] and HEPES (25mM), adjusted to pH 7.2 (bicarbonate solution). For cell survival determinations, the 3 (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was dissolved in phosphate buffered saline, pH 7.2 (to 5 mg/mL), and filtered through Millipore filters (0.22 μm) before use.

The HeLa (human cervix adenocarcinoma) cells were cultured as monolayers in the nutrient medium (see above), while the K562 (human myelogenous leukemia) cells were maintained as suspension cultures in the same nutrient medium. All of these cells were grown at 37 °C in 5% CO_2 and a humidified air atmosphere.

The HeLa cells were seeded (2,000 cells per well) into 96-well microtiter plates, and 20 h later, after cell adherence, five different concentrations of the test compounds were added to the wells. The final test compound concentrations were from 12.5 μM to 200μM. Only nutrient medium was added to the cells in the control wells. For the K562 cells the test compounds were added to cell suspensions (3,000 cells per well) 2h after cell seeding, to the same final concentrations applied to the HeLa cells. All of the experiments were carried out in triplicate. Nutrient medium with the corresponding concentrations of the test compounds, but void of cells, was used as the blank.

Cell survival was determined by the MTT test according to the method of Mosmann [12] and modified by Ohno and Abe [13] at 72h after the test compound additions. Briefly, 20μL MTT solution (5 mg/mL in phosphate buffered saline) was added to each well. The samples were incubated for a further 4 h at 37°C in 5% CO_2 with a humidified atmosphere. Then, 100μL 10% sodium dodecylsulfate (SDS) was added to each of the wells, and the absorbance of the cell medium from each well was measured at 570 nm the next day. To calculate the cell survivals (%), the absorbance at 570 nm of each of the samples with cells grown in the presence of the test compounds were divided by the absorbance of the control sample (the absorbance of cells grown in nutrient medium only), after the subtraction of the blank sample

absorbance. IC50 was determined from the graph as the concentration of compound which decrease survival of treated cells by 50%. IC50 values for each compound were obtained by numerical analysis of data obtained.

Antimicrobial test

The biological activity of the investigated complex was examined by screening five different cultures of microorganisms. All these microorganisms are known as human pathogens, causing different types of diseases and infections. For the preliminary antimicrobial test, the agar well diffusion method was applied. Antimicrobial activity of complex was performed using Staphylococcus saprophyticus ATCC 15305, Staphylococcus xylosus ATCC 29991, Bacillus cereus ATCC 10987 and fungal strains Candida albicans ATCC 24433 and Aspergillus niger ATCC 12066. Bacteria were cultivated on Mueller-Hinton agar and fungi on Sabouraud dextrose agar. Inoculation was performed by mixing 0,1mL of the microorganism suspension in physiological solution (0,8mg/mL NaCl) with 20mL of the molten cold medium [8]. In the inoculated agar plates the holes (Ø0,8 cm) were formed and 100µL of the tested solutions (1mg/mL in DMSO) were separately introduced in the holes.The complex didn't show antifungal activity. Incubation temperature was: 37°C for bacteria and 28°C for fungi.

Minimal inhibitory concentration (MIC) values were determined using agar dilution method [13] and the antibacterial activities of the complex was quantified [14,15] in accordance with the CLSI recommendations (Clinical and Laboratory Standards Institute 2006) [16]. Apart from the complex, tpmc and starting salts were tested. The initial concentration of the complexes was 8 mg/mL in DMSO. This solution was doubly diluted to give concentrations in the range 8–0.125mg/mL. 0.5mL of the solution of the tested substances was mixed with 9.5 mL of melted and cooled nutrition agar. The bacteria were seeded on the surface of the agar plate. After incubation for 24 h, the MIC values were determined as the lowest concentration of the complex preventing visible growth of the bacteria. All investigations were carried out in triplicate.

3. RESULTS AND DISCUSSION

In the previous paper, we described the crystal structure of the complex $[Co_2(Cl)_2tpmc](BF_4)_2$ [10]. Each metal is *exo* coordinated by two rings (NCH_2CH_2N) and two pendant-N atoms in the complex cation $[Co_2(Cl)_2tpmc]^{2+}$ (Figure 2). X-ray analysis has shown that the cobalt (II) magnetic centers in the binuclear complex are sufficiently separated from each other (5.710 Å) and both bridging systems (Co-N-C-C-C-N-Co) are ineffective for the magnetic exchange interaction. The nearest coordination sphere of the cobalt center is composed of 4 nitrogens and fifth coordination site is occupied by chloro ligand. The crystals of the investigated complex $[Co_2(Cl)_2tpmc](BF_4)_2$ are isomorphic with the those of $[Cu_2(Br)_2tpmc](ClO_4)_2$ [17]. In both of them tpmc ligand adopts the *chair* conformation and the fifth coordination site is ocupied by monodentate non-bridging ligand.

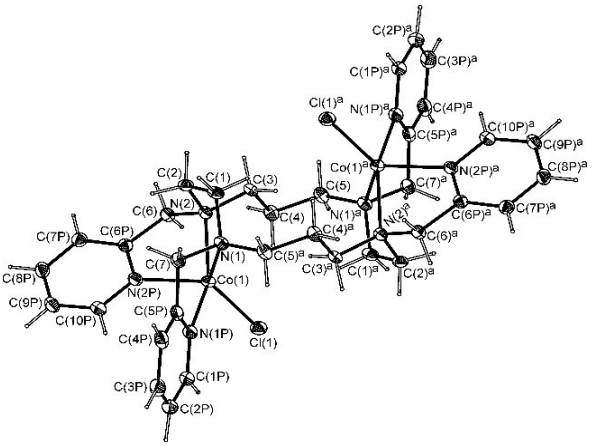

Figure 2. The ORTEP drawing of the $[Co_2(Cl)_2tpmc]^{2+}$.

Slika 2. ORTEP dijagram za $[Co_2(Cl)_2tpmc]^{2+}$.

The practical applicability of the newly synthesized compound depends on their thermal stability. Therefore, thermal studies of coordination compounds with potential biological activity are routinely included in their characterization [18-20].

Thermal studies

The thermal decomposition of the complex $[Co_2(Cl)_2tpmc](BF_4)_2$ is shown in the Figure 3.

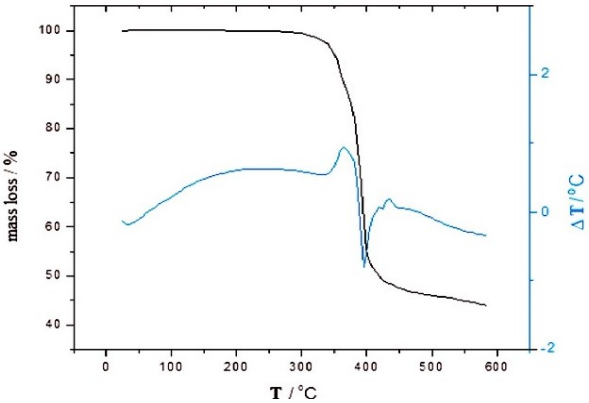

Figure 3. Simultaneous TG-DTA of $[Co_2(Cl)_2tpmc](BF_4)_2$.

Slika 3. Simultana TG-DTA analiza $[Co_2(Cl)_2tpmc](BF_4)_2$.

This complex is stable up to 310°C. The complex decomposes exothermally at 365°C. The curve of this complex reveals that decomposes at one step showing endothermic peak at 395°C and two exothermic peaks at 365 and 435 °C in DTA.

Endothermic and exothermic decomposition of complex cation and ligand tpmc and merge with each other in the range of 365–435°C. The final products are most probably belongs to corresponding Co(II) complex with cyclam and with -NH_2 groups, $[Co_2(NH_2)_4cyc]$ (cyc=1,4,8,11-tetraazacyclotetradecane), mass loss (found 42,5%, calculate 41%) or mixture of cobalt carbonates and oxides.

The composition of final products is those which best fit with the observed mass losses in the TG study. Thermogravimetric results are in good agreement with the corresponding DTA data.

Cytotoxicity

The cytotoxic activity was determined according to the dose values of the complex, required to reduce the cell survival by 50 % (IC50). The IC50 values obtained in this study are given in Table 1. The cytotoxicity curves from the MTT assay show the survival of HeLa, and K 562 cells grown for 72h in the presence of increasing concentrations of complex.

Table 1. Concentrations (μM) of the test compound that induced 50% decrease (IC_{50}) in malignant cell

Tabela 1. Koncentracije (μM) testiranih jedinjenja koje izazivaju smanjenje malignih ćelija za 50% (IC_{50})

Compound	IC_{50} ± SD /μM	
	HeLa	K562
$[Co_2Cl_2tpmc](BF_4)_2$	17.88±2.36	24.51±5.33
tpmc	>200	>200
$NaBF_4$	>200	>200
$CoCl_2{\cdot}6H_2O$	>200	>200
Cisplatin	10.9±3.5	7.9±2.5

The *in vitro* cytotoxicity of the complex, $CoCl_2{\cdot}6H_2O$, $NaBF_4$ and ligand tpmc was evaluated using cis-platin as referent cytostatic drug. The IC50 values of the complex were 17.88 against HeLa and 24.51μM against K562, while cis-DDP lied in the range 10.9 -7.9μM. The tpmc, $CoCl_2{\cdot}6H_2O$ and $NaBF_4$ showed significantly lower activity (IC50 >200 μM) for all tested cell lines. Based on this, it can be concluded that the cytotoxic effect originates from the complex itself.

According to the data in Table 1, the cytotoxic effects of the complex are lower than those of cis-platin. Nevertheless, the inhibition of cell proliferation by these binuclear Co(II) complex is notable.

All data are results from three independent experiments, each carried out in triplicate.

Antibacterial activity

Two experiments were conducted. Test organisms were Staphylococcus saprophyticus ATCC 15305, Staphylococcus xylosus ATCC 29991, Bacillus cereus and fungal strains Candida albicans ATCC 24433 and mould Aspergillus niger ATCC 12066. As a control group the free salt of Co(II) which was used as starting substances in the synthesis, as well as ligand (tpmc) and solvent were tested. Preliminary test performed with bacterial and fungal strains indicated that the complex had no antifungal activity. The second test was performed for quantification of antibacterial activity by agar dilution method (MIC determination). Selective antibacterial activity against Gram (+) bacteria of the Staphylococcus was detected. Noticeably, the tested complex showed similar antibacterial activity toward both investigated Staphylococcus strains. Under the same conditions and the same concentrations, the control group were inactive at concentrations up to 400 lg/mL, indicating that the activity of the complex, where it is found, originated from himself. Different antibacterial activities of complexes and tpmc could be explained by chelation effects of the macrocyclic ligand, which reduce the polarity of the metal ions and may result in increasing uptake of compounds through the bacterial cell membrane. The MIC values of the tested compound are given in Table 2.

Table 2. Minimum inhibitory concentration (MIC), in ìg/mL of the complex in DMSO

Tabela 2. Minimalna inhibitorna koncentracija (MIC) kompleksa u DMSO, u ìg/mL

Complex	S.S.*	S.X.*	B.C.*	C.A.*	A.N.*
$[Co_2Cl_2(tpmc)](BF_4)_2$	100	150	200	>200	>200

* S.S. Staphylococcus saprophyticus, S.X. Staphylococcus xylosus; B.C. Bacillus cereus; C.A, Candida albicans; A.N, Aspergillus niger.

4. CONCLUSIONS

TG-DTA analysis showed that the $[Co_2(Cl)_2tpmc](BF_4)_2$ complex was stable up to 310°C, decomposed in one step in the range 365-435°C showing an endothermic peak at 395°C and two exothermic peaks at 365 and 435°C in DTA. The tested complex was found to have significantly higher cytotoxic activity (lower IC50 values) than their components tested as a control. IC50 complex values were 17.88 relative to HeLa and 24.51μM relative to K562, while cis-DDP lay in the range of 10.9–7.9μM. In addition, selective antibacterial activity against Gram (+) bacteria of the Staphylococcus saprophyticus and Staphylococcus xylosus was detected.

Acknowledgments

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IZVOD

TERMIČKO PONAŠANJE I BIOLOŠKA AKTIVNOST $[Co_2(Cl)_2$ TPMC$](BF_4)_2$ KOMPLEKSA

Poznavanje biološke uloge kobalta i prepoznavanje njegovih kompleksa kao važnih bioaktivnih jedinjenja razlozi su sve veće zainteresovanosti za potencijalnu terapijsku primenu ovih kompleksa kod različitih bolesti. Brojne dostupne informacije o njihovim osobinama i načinu delovanja u nekoliko bioloških sistema, u kombinaciji sa novim mogućnostima koje nameće razvoj medicinske hemije, otvaraju prostor za razvoj nove generacije visoko aktivnih lekova sa minimalnim nuspojavama. U ovom radu smo ispitali neke osobine ranije izolovanog Co (II) tpmc kompleksa, prvog kod koga je rendgenska strukturna analiza potvrdila da se makrciklični ligand nalazi u konformaciji stolice. Ispitivane su termogravimetrijske I biološke osobine kompleksa $[Co_2(Cl)_2$tpmc$](BF_4)_2$ (tpmc = N, N´, N´´, N´´´-tetrakis (2-piridilmetil) -1,4,8,11-tetraazaciklotetradekan). TG-DTA analiza ukazuje da je razlaganje kompleksa u jednom koraku u rasponu temperature od 365 -435 ° C. Ispitivana je citotoksična aktivnost na dve ljudske ćelijske linije karcinoma: HeLa (adenokarcinom grlića maternice) i K562 (mijelogena leukemija). Antimikrobno djelovanje kompleksa kvantifikovano je određivanjem minimalne inhibitorne koncentracije (MIC) korišćenjem bakterija Staphylococcus saprophyticus, Staphylococcus xylosus, Bacillus cereus, soja kvasca Candida albicans i plesni Aspergillus niger. Biološka ispitivanja su pokazala da kompleks ima značajan citotoksični potencijal I umerenu aktivnost prema bakterijama soja Staphylococcus.

Ključne reči: *Co(II) kompleks, tpmc, mikrobiološka aktivnost, citotoksičnost, termička stabilnost*

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