SYNTHESIS OF NOVEL Pt COMPLEXES WITH α-GLYOXIMES, SCHIFF BASES, AND THEIR PHYSICAL-CHEMICAL AND BIOLOGICAL STUDY

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Abstract

Platinum-complexes permanently play an important role in the medical treatment of tumor cells. Since Pt has a soft-type Lewis acidic character, it forms the most stable complexes with S, P, N and I donor atoms, resulting planar (cisplatine) or octahedral (satraplatine) arrangement of ligands.

In our research, new platinum complexes were synthesized with α-glyoximes, such as $[Pt(diethyl\text{-}glyoxH)_2(amine)_2]$, where glyoxH = mono deprotonated glyoxime, amine = imidazole, 2-amino-pyrimidine or 3-hydroxy-aniline, and with Schiff bases, such as $[Pt(3\text{-}heptanone)_2(en)]$, $[Pt(3\text{-}heptanone)_2(1,2\text{-}pn)]$, $[Pt(3\text{-}heptanone)_2(1,3\text{-}pn)]$ (en = ethylenediamine, pn = propylenediamine). The Schiff bases were obtained with the condensation reaction between 3-heptanone and the corresponding diamines.

The molecular structure of our products has been investigated by IR, UV–VIS and NMR spectroscopy, MS, thermoanalytical measurements (TG-DTG-DTA), and powder XRD. The biological activity study of compounds revealed their possible application in medical point of view since some of them proved to act as antibacterial agents and potential anticancer drugs. On the other hand, some members of the family of complexes can play catalytic role in organic chemistry transformations.

Introduction

After cisplatin was discovered by Barnett Rosenberg due to its anti-tumor effect, and was introduced to the market as drug in 1978, beside the second generation Pt-drugs, as carboplatin, oxaliplatin [1, 2], it became the most important agent in cancer treatment.

During the study of relationships between the activity and the structure of Pt-complexes by Cleare and Hoeschele, they noted that the prerequisite of the metal complex to be an efficient drug is the ability of the platinum center to coordinate a bidentate amine ligand in cis position, or alternatively, two amine components containing minimum one NH group. Furthermore, the complexes must also contain two medium bonded leaving groups (e.g. chloride, sulfate, citrate, oxalate) [3].

The anti-tumor effect of cisplatin can be explained by the platinum coordination to the DNA, which makes a distortion in the helicoidal structure, resulting to the inhibition of

replication and transcription of DNA. Finally, this process leads to the cell circuit stop and apoptosis [4].

It is also worth mentioning that Schiff bases are used in many fields, as in paint industry, however, due to their biological activity, they deserve more interest as antibacterial, antiviral, and anti-inflammatory agents. Some derivatives have anti-tumor effect, too.

Experimental

Used materials: $PtCl_4$, MeOH, EtOH, HCOOH, 3,4-hexanedione, hydroxylamine-hydrochloride, KOH, 3-heptanone, ethylenedianine, 1,2-propylene-diamine, 1,3-propylene-diamine, imidazole, 2-amino-pyrimidine, 3-hydroxy-aniline.

Methods: - *Preparation of diethyl-glyoxime*

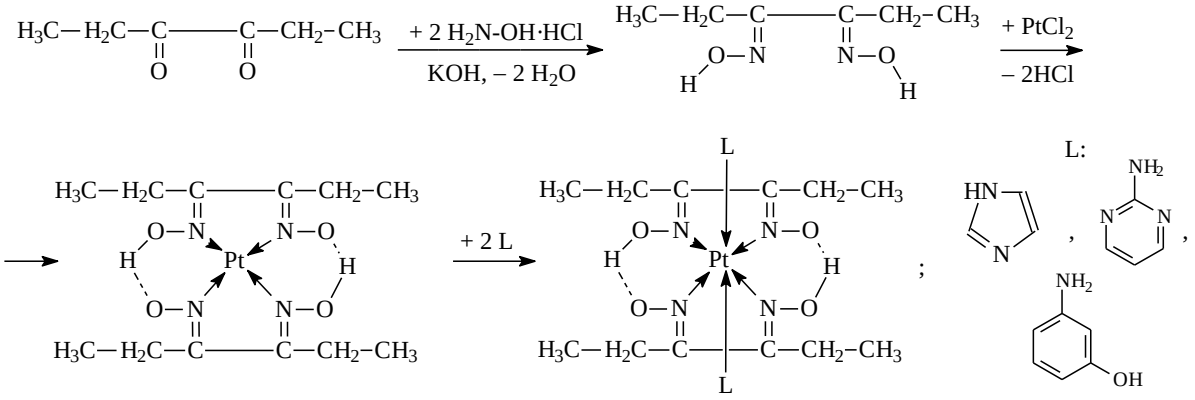
In the first step 3,4-hexanedione was reacted with hydroxylamine-hydrochloride dissolved in water. To the hydroxylamine-hydrochloride solution equimolar amount of KOH was added in order to liberate the free amine from its salt. The reaction mixture was heated for 2–3 hours, and then the precipitated product was filtered off. After recrystallization from EtOH or MeOH, it was dried on air.

- *Synthesis of [Pt(diethyl-glyoxH)$_2$(amine)$_2$] complexes*

The platinum salt was reduced with formic acid before its use in the complex synthesis:

$$PtCl_4 + HCOOH \rightarrow PtCl_2 + CO_2 + 2\ HCl$$

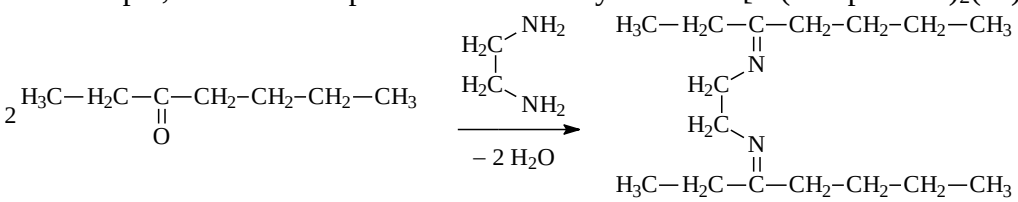
Diethyl-glyoxime was dissolved in EtOH or MeOH, than added to the aqueous solution of the reduced platinum salt ($PtCl_2$). The mixture was heated for 2–3 hours. After cooling, the formed [Pt(diethyl-glyoxH)$_2$] was filtered, washed with EtOH–water mixture (1:1), and dried on air. For the synthesis of complexes, the reaction mixture of the diethyl-glyoxime and $PtCl_2$ was heated for 1 hour, than the corresponding amine (imidazole, 2-amino-pyrimidine, 3-hydroxy-aniline) was added, and heated further for 2 hours. After cooling the product was filtered, washed with EtOH–water mixture (1:1), and then dried on air. The reactions:

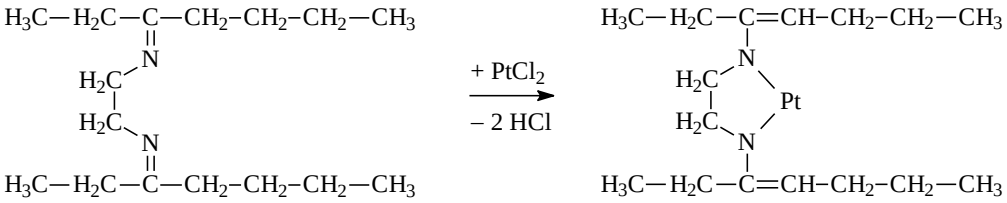


- *Synthesis of [Pt(3-heptanone)$_2$(A)] (A = en, 1,2-pn, 1,3-pn) complexes*

The Schiff bases were prepared by the condensation reaction between 3-heptanone and the corresponding diamine (ethylenediamine, 1,2- and 1,3-propylenediamine) in EtOH solution. The mixture was heated at 70–80 °C for 2–3 hours. The solution obtained was directly used for the complex syntheses by adding the reduced platinum salt ($PtCl_2$) dissolved in water. The product was filtered, washed with EtOH–water mixture (1:1), and then dried on air.

For example, the reactions performed for the synthesis of [Pt(3-heptanone)$_2$(en)] are:





Results and discussion

Microscopic characterization and the yield of prepared complexes are presented in Table 1.

Table 1. Microscopic characterization, calculated molecular weight and the yield of prepared complexes.

Nr.	Compound	Calc. mol. weight	Yield (%)	Microscopic characterization
1.	[Pt(diethyl-glyoxH)$_2$]	481.41	59	Brown, triangle-based prisms
2.	[Pt(diethyl-glyoxH)$_2$ (imidazole)$_2$]	617.56	56	Brown, square-based long crystals
3.	[Pt(diethyl-glyoxH)$_2$ (2-amino-pyrimidine)$_2$]	671.61	54	Brown, long needles, triangle-based prisms
4.	[Pt(diethyl-glyoxH)$_2$ (3-hydroxy-aniline)$_2$]	699.66	42	Black, shining, triangle-based prisms
5.	[Pt(3-heptanone)$_2$(en)]	445.50	5	Black, irregular microcrystals
6.	[Pt(3-heptanone)$_2$(1,2-pn)]	459.53	18	Black, triangle-based crystals
7.	[Pt(3-heptanone)$_2$(1,3-pn)]	459.53	13	Black, irregular microcrystals

Infrared spectroscopic study

The mid-IR spectra were recorded with a Bruker Alpha FTIR spectrometer (Platinum single reflection diamond ATR), at room temperature, in the wavenumber range of 4000–400 cm^{-1}, and the far-IR range of 650–150 cm^{-1}, respectively, on a Perkin–Elmer System 2000 FTIR spectrometer, operating with a resolution of 4 cm^{-1}. The samples were measured in solid state (in powder form), respectively, in polyethylene pellets. The most important IR values for the selected complexes are presented in Table 2.

Table 2. IR data of the selected complexes.

Comp. / cm^{-1}	[Pt(diEt-glyoxH)$_2$]	[Pt(diEt-glyoxH)$_2$ (imidazole)$_2$]	[Pt(diEt-glyoxH)$_2$ (2-am.-pyrimid.)$_2$]	[Pt(diEt-glyoxH)$_2$ (3-HO-aniline)$_2$]
υ_{C-H}	2975, 2938, 2876 m	2975, 2938, 2876 m	2975, 2937, 2875 m	2974, 2937, 2876 w
$\upsilon_{C=N}$	1535 s	1532 vs	1535 s	1534 s
δ_{CH2}	1459 s	1447 s	1459 s	1448 s
δ_{CH3}	1360 m	1360 s	1359 m	1358 w
υ_{N-O}	1241 vs	1242 vs	1241 vs	1240 vs
υ_{N-OH}	1107 s	1110 s	1106 s	1106 s
τ_{O-H}	916 vs	918 vs	916 vs	915 vs
γ_{C-H}	712 s	715 s	712 s	710 s
υ_{Pt-N}	518 s	515, 512, 509 s	518, 515 s	516, 513, 501 s
δ_{N-Pt-N}	358 vs	356 vs	328 vs	324 vs

(Abbreviations: vs = very strong, s = strong, m = medium, w = weak)

Mass spectrometry

Mass spectra of the samples were recorded by an Agilent/Technologies 6320 Mass Spectrometer using electrospray ionization (ESI). The samples were dissolved in MeOH. In the spectra we could detect the molecular ions and some decomposition fragments.

Thermoanalytical measurements (TG-DTG-DTA)

Thermal measurements were performed with a 951 TG and 910 DSC calorimeter (DuPont Instruments), in Ar or N_2 at a heating rate of 10 Kmin^{-1} (sample mass 4–10 mg).

In the case of [Pt(diethyl-glyoxH)$_2$(amine)$_2$] complexes the first decomposition steps are belonging to the leaving amine groups up to 300 °C. Subsequently the decomposition of

glyoxime groups begins, which is accompanied by a big exothermic peak. This behavior can be explained with the presence of oxygen in the molecule as shown, for example, in Figure 1. The general decomposition mechanism is the following:

$$[Pt(diethyl\text{-}glyoxH)_2(amine)_2] \rightarrow [Pt(diethyl\text{-}glyoxH)_2(amine)] \rightarrow [Pt(diethyl\text{-}glyoxH)_2] \rightarrow$$
$$\rightarrow [Pt(diethyl\text{-}glyoxH)] \rightarrow PtO$$

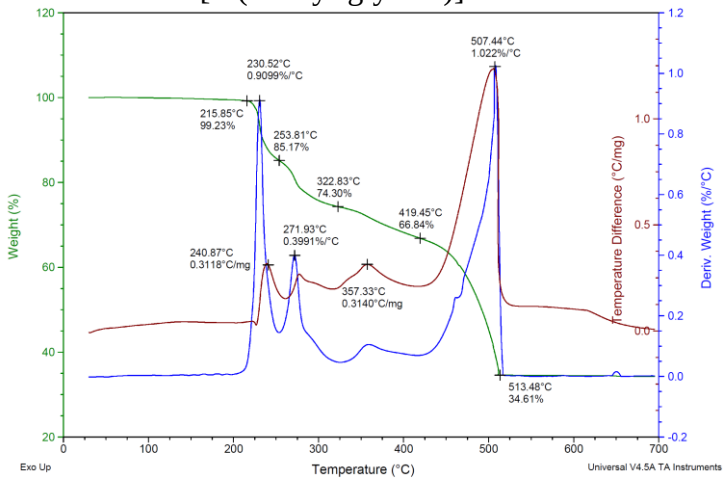


Figure 1. Thermal decomposition of $[Pt(diethyl\text{-}glyoxH)_2(imidazole)_2]$

NMR spectroscopic study

The NMR spectra (^1H and ^{13}C NMR) were recorded in DMSO-d_6 in 5mm tubes at RT on a Bruker DRX 500 spectrometer at 500 MHz, using TMS as internal reference. In the ^1H NMR spectra the aliphatic protons appear at 1.1–2.8 ppm in all complexes, the aromatic protons appear between 7.7–8.3 ppm. In the ^{13}C NMR spectra the aliphatic carbons appear at 10–20 ppm, and the double bonded carbons appear at 158 ppm.

Powder X-ray diffraction measurements

Powder XRD measurements were carried out on PANalytical X'pert Pro MPD X-ray diffractometer. The crystal structure of the complexes was studied with this method. As being new compounds their diffractograms are not deposited in the Cambridge database.

UV–VIS spectroscopy

The electronic spectra were recorded with Jasco V-670 Spectrophotometer in 10% EtOH/water solutions containing substrate in 10^{-4} mol/l concentration. For a few complexes the characteristic wavelengths are the followings: $[Pt(diethyl\text{-}glyoxH)_2]$ – 271 nm, 320 nm, 470 nm; $[Pt(diethyl\text{-}glyoxH)_2(2\text{-}amino\text{-}pyrimidine)_2]$ – 198 nm, 286 nm. Using Britton-Robinson buffer solutions the electronic spectra were also recorded as a function of pH, and then the acidity constants were calculated with the formula below:

$$K_a = 10^{-pKa},\ pK_a = pH + \lg\frac{\boldsymbol{A} - \boldsymbol{A}_{max}}{\boldsymbol{A}_{min} - \boldsymbol{A}},$$ where A is the absorbance.

The obtained values are listed in Table 3.

Table 3. The acidity constants for selected complexes.

Compound	λ	pH	A	A_{max}	A_{min}	pK_a	K_a
[Pt(diethyl-glyoxH)$_2$]	448	10.48	0.020123	0.0282773	0.0143296	10.63068	$2.34 \cdot 10^{-11}$
	323	10.48	0.108823	0.112591	0.0803457	9.604606	$2.49 \cdot 10^{-10}$
	273	9.71	0.31477	0.339324	0.285667	9.638467	$2.3 \cdot 10^{-10}$
[Pt(diEt-glyoxH)$_2$ (2-am.-pyrimid.)$_2$]	268	10.48	0.458324	0.475482	0.290584	9.48983	$3.24 \cdot 10^{-10}$
	195	11.31	2.51446	2.63318	2.31618	11.08724	$8.18 \cdot 10^{-12}$

Biological study

The antimicrobial effects of complexes were studied for Gram-negative and Gram-positive (especially B. Subtilis) germs. The observation was made with the disk method. Filtering paper disks were impregnated with a concentrate probe solution, and sterilized (with UV-radiation or in autoclave), then putted on the germ substrate. After a 24 hour-incubation we investigated whether the studied compound blocked the growth of the germ substrate. When there was no growth of germ substrate around the disks, the case was called as inhibition zone. The Gram staining [5] is an empirical solution for dividing the germs into two parts (Gram-negative and Gram-positive) in accordance with the physical and chemical properties of the cell-wall. The results are included in Table 4. The complexes were dissolved in DMSO in 2 mmol/l concentration.

The results are included in table 4. The complexes were solved in DMSO, and the concentration was 2 mmol/l.

Table 4. The inhibition zone dimension as a function of the quantity of complexes.

Compound	5 µl	10 µl	20 µl	30 µl
[Pt(diethyl-glyoxH)$_2$(imidazole)$_2$]	-	9 mm	12,7 mm	16,33 mm
[Pt(diethyl-glyoxH)$_2$(3-hydroxy-aniline)$_2$]	-	7,83 mm	13,66 mm	14,66 mm

Conclusion

In this work new platinum complexes were synthesized and their physico-chemical properties were studied. Decomposition mechanism was determined with the thermoanalytical method. The antibacterial effect of compounds was investigated, and in the future we propose to study their anti-tumor effect, too.

Acknowledgement

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