Vitamin B_{12} (cobalamin, Cbl) has been an object of scientific interest for several decades. Due to its unique structure, which consists of a macrocyclic Co(III) complex having multiple side substituents characterized by the presence of multiple functional groups, it is an interesting molecule in terms of modification possibilities. Moreover, it plays a key role in metabolic processes, including the methionine cycle, in which it directly participates in the transfer of a methyl group. In addition, the proper functioning of the aforementioned cycle mediates DNA synthesis. For this reason, a high demand for Cbl is manifested by cells with a high frequency of divisions. One example is cancer cells. This knowledge has prompted scientists to use Cbl as a carrier of therapeutic (or diagnostic) compounds in targeted therapy. In the context of reducing the side effects of chemotherapy, cyanocobalamin (CblCN) was used as a carrier for Cisplatin, and pioneering research on this system was published by R. Alberto's group. This publication inspired the expansion of vitamin B_{12}'s carrier capabilities to other model Pt(II) complexes. Scientific reports on other transition metal complexes with anticancer properties prompted consideration of model CblCN-Pd(II) conjugates as well. An additional distinguishing aspect of Cbl is the ability to activate small biomolecules through ligand interaction at the axial position. Of particular note is nitric oxide (NO), which has a number of important functions in the body, such as cell signaling. Moreover, its action is strongly correlated with local concentration. High concentrations can induce pathological conditions and even lead to cell death. Therefore, another part of the study focused on the interaction of the NO donor with aquacobalamin to accumulate high concentrations of NO in cancer cells. The induction of strong nitroxidative stress could contribute to the death of this cell. S-nitrosohomocysteine (HcyNO) was chosen for the study. Experimental studies were extended by a theoretical workshop (DFT methods), by means of which the influence of the macrocyclic ring (corin - Kor, porphyrin - Porf) on the interaction with HcyNO was studied. The formation of cancer cells is influenced by many factors, such as dysfunctions in metabolic processes. Due to the involvement of both vitamin B_{12} and homocysteine in a single cycle, studies have been conducted on the possible interaction of these two molecules.

The main purpose of using vitamin B_{12} as a carrier is to increase cytostatic accumulation in cancer cells with reduced transport to healthy cells. An important point of this method is the persistence of the conjugates in body fluids. For this reason, three CblCN-Pt(II) conjugates were synthesized, which were distinguished by their coordination sphere on the Pt^{2+} ion. CblCN-$PtCl_2(H_2O)$ was synthesized by reacting CblCN with the $[PtCl_2(H_2O)_2]$ complex in aqueous solution at pH = 2. The Pt(II) compound was previously subjected to speciation studies, which made it possible to select the most reactive form. The next two conjugates,

CblCN-Pt(dien) and CblCN-Pt(terpy), required several stages of synthesis. First, two-step syntheses of [Pt(dien)Cl]Cl and [Pt(terpy)Cl]Cl were carried out, which were activated to $[Pt(dien)H_2O]^{2+}$ and $[Pt(terpy)H_2O]^{2+}$, respectively. The resulting Pt(II) complexes with organic ligands were reacted with CblCN (pH = 3 for $[Pt(dien)H_2O]^{2+}$ and pH = 4.2 for $[Pt(terpy)H_2O]^{2+}$). Due to the passivity of Pt(II) complexes and the long duration of the reaction, experiments were carried out at molar ratios of [Pt(II)]:[CblCN] = 1000:1. The presence of the resulting conjugates was confirmed by IR and MS-ESI spectroscopy techniques. In addition, for CblCN-Pt(dien) and CblCN-Pt(terpy), identification was carried out using ^{195}Pt-NMR. Purification of the conjugates from the reaction mixture was not possible, due to the presence of equilibrium systems. During isolation, the breakdown of the products took place. Kinetic studies due to the solubility limitations of Pt(II) complexes were narrowed down to the determination of the observable reaction rate constant. In the case of CblCN-$PtCl_2(H_2O)$, there is a very rapid substitution of a water molecule for Co^{3+}, followed by dissociation of a water molecule from the $[PtCl_2(H_2O)_2]$ complex ($k_{obs(2)} = (32.7 \pm 6.16) \cdot 10^{-6}$ s^{-1}). A similar reaction mechanism is observed for the formation of CblCN-Pt(terpy) ($k_{obs(1)} = 10^{-1}$ s^{-1}; $k_{obs(2)} = 2.18 \cdot 10^{-6}$ s^{-1}). Interesting results were obtained when studying the CblCN-Pt(dien) conjugate. Due to the formation of $[Pt(dien)]_n$ polymers, the kinetic analysis was performed in the γ band, since for the dz^2 orbital of the Co^{3+} ion the contribution of polymer diversity is small. Under the conditions of the experiment carried out, $k_{obs} = (2.00 \pm 0.048) \cdot 10^{-8}$ s^{-1}. The study was supplemented by molecular modeling using the DFT method. The results show that the most thermodynamically favored conjugate is CblCN-$PtCl_2(H_2O)$ (in particular, the trans-CblCN-$PtCl_2(H_2O)$ isomer), whose binding energy value in the aqueous phase is -18.15 kcal/mol. The information derived from experimental and theoretical studies can be taken both as a basis for optimizing carrier-drug binding stability and as a guide for developing synthetic procedures. The advantage of the CblCN-$PtCl_2(H_2O)$ conjugate, is its high durability, which in the physiological system can be further enhanced by the content of high concentrations of Cl^- anions in the plasma.

Dynamic research progress has shown that Pd(II) complexes also exhibit anticancer properties. The lack of literature reports on the possibility of CblCN-Pd(II) combinations gave rise to studies on new conjugates of vitamin B_{12} with selected Pd(II) complexes. Four two-center complexes were synthesized: CblCN-$PdCl(H_2O)_2$, CblCN-$PdCl_3$, CblCN-Pd(dien), CblCN-Pd(terpy). The selection of ligands in the coordination sphere of Pd(II) was determined by determining the effect of these ligands on the stability of the new conjugates. Analogous to Pt(II) compounds, Pd(II) complexes were subjected to speciation experiments, which yielded

the first two complexes: $[PdCl(H_2O)_3]^+$ (aqueous solution at pH = 2) and $[PdCl_3(CH_3OH)]^-$ (methanol). The [Pd(terpy)Cl]Cl and [Pd(dien)Cl]Cl complexes were obtained by two-step synthesis and then activated (aqueous solution at pH = 4.1) to $[Pd(terpy)H_2O]^{2+}$ and $[Pd(dien)H_2O]^{2+}$. Reactions between CblCN and Pt(II) complexes were carried out at molar ratios of 1:10. Identification of the products was carried out by IR spectroscopy, MS-ESI. In addition, the presence of CblCN-$PdCl(H_2O)^2$ conjugate was confirmed using ^{15}N-NMR. Purification of the synthesis products from the reaction mixture was unsuccessful due to the disintegration of the conjugates during the procedures performed. Kinetic studies were performed for [Pd(II)]:[CblCN] systems from 10:1 to 100:1 at 25°C. The kinetic curves studied for all conjugates are monoexponentially in nature. In the case of CblCN-$PdCl(H_2O)_2$, CblCN-PdCl3 and CblCN-Pd(dien), the dependence in the kobs function of [Pd(II)] is linear, and the determined equilibrium constants of the reaction take values of 56 ± 3 M^{-1}, 208 ± 7 M^{-1}, 107 ± 12 M^{-1}, respectively. Interesting results were obtained for CblCN-Pd(terpy). In contrast to the Pd(II) conjugates described above, the value of kobs decreases with increasing concentration of $[Pd(terpy)H_2O]^{2+}$ reaching the minimum value of the function (K = 53 M^{-1} for the conjugate formation reaction), which was related to the model of the reaction of N-methylimidazole with Cbl-H_2O. Moreover, computer studies were performed using the DFT method. Optimization of the geometry of the conjugates and determination of their binding energies showed that CblCN-$PdCl_3$, CblCN-Pd(dien) and CblCN-Pd(terpy) should be thermodynamically favored ($E_{bin} < 0$). In contrast, the formation of CblCN-$PdCl(H_2O)_2$ is thermodynamically unlikely ($E_{bin} > 0$), but the values are small and do not exclude the possibility of forming a conjugate. The CblCN-$PdCl_3$ conjugate appears to be the most promising candidate from the point of view of potential therapeutic applications. This is supported primarily by its relatively high stability, which seems sufficient to maintain the bridging bond during transport into the cell. Also important is the high concentration of Cl^- ions in the plasma, which will favorably influence the persistence of the conjugate with a large amount of these ligands in the Pd(II) coordination sphere.

Thiols and nitrosothiols play important functions in the living organism, and homeostasis of these compounds is crucial to the proper functioning of the body. Literature data prove that vitamin B_{12} is capable of interacting with naturally occurring compounds from this group, such as cysteine, glutathione and nitrosoglutathione. The lack of information on the reactions between homocysteine and nitrosohomocysteine and vitamin B_{12} has contributed to the initiation of research on these interactions. The reaction between aquacobalamin ($CblH_2O$), and homocysteine (Hcy) was carried out under near-physiological conditions (pH = 7.4), with

an excess of Hcy. Identification of the final synthesis product was carried out using MS-ESI spectroscopy. The results indicated the formation of Cbl-Hcy. Kinetic studies were carried out under pseudo-primary conditions at the following ratios of Cbl-H_2O concentrations (for a fixed concentration of 5.10^{-5} M) to homocysteine: 1:10; 1:20; 1:40; 1:60; 1:80; 1:100. Experiments were also carried out under temperature dependence (25 - 45^{o}C). The obtained curve of the dependence of absorbance changes on time is characterized by a simple course and can be described by a single-exponential function. The temperature dependence allowed the determination of the reaction activation parameters: $\Delta H^{\ddagger}$ = 78.10 ± 1.05 kJ/mol, $\Delta S^{\ddagger}$ = 42.98 ± 1.21 J/mol·K, $\Delta G^{\ddagger}$310K = 91.42 ± 1.66 kJ/mol and E^{a} = 80.66 ± 1.05 kJ/mol. The conducted tests showed that there was no effect of oxygen on the course of the reaction. Next, the effect of Hcy on Cbl-NO was checked. Spectroscopic results (UV-VIS) confirmed the possibility of interaction between these molecules and suggest multi-step processes, which may result in the formation of the following compounds: Cbl-Hcy, Cbl-NO^{-} and Cbl-NO_2. These data allow us to argue how important nitroxidative stress and high cellular Hcy concentrations are on Cbl in terms of the proper functioning of the methionine cycle. In the next stage, spectroscopic studies focused on the possibility of interaction of HcyNO with aquaporin. During their course, the effect of oxygen on the interaction of selected compounds was taken into account. The results showed that the carried out reaction between HcyNO and CblH_2O is complex and multi-step regardless of the O_2 concentration in the reaction system. However, the preference for the influence of the donor atom (S or N) is already related to the O_2 concentration. Its presence in the system contributes to the predominantly Cbl-N-Hcy formation as a transition product (stage I). In contrast, under anaerobic conditions, Cbl-S-Hcy and Cbl-N-Hcy have comparable contributions to stage I of the process. Under equilibrium conditions with air, the simplest course of the reaction between HcyNO and Cbl is observed compared to conditions where the O_2 content was at the extremes. The complex course of the reaction suggests changes in the reaction environment during the course of the reaction. Under anaerobic conditions, subsequent steps may be the result of O_2 diffusion into the system. A process under anaerobic conditions appears to be an interesting result in the context of anticancer therapy. The strong contribution of the reaction, in which the product is Cbl-Hcy, indicates the release of NO, high concentrations of which may manifest anticancer properties.

Comprehensive interest in macrocyclic systems like corrin (Kor) and porphyrin (Porf) in the context of biomedical applications prompted this study of the effects of these systems on interactions with Hcy and HcyNO. For this purpose, computer methods (DFT) were used. For Hcy interactions, connections to cobalt ions (Co^{+}, Co^{2+}, Co^{3+}) via S were considered. The

results showed no interaction with Co^{+}, and the most energetically favorable conjugate is Porf(Co^{III})(Im)-Hcy. In addition, the higher durability is supported by the shortest S-Co bond. In the perspective of biomedical applications, this result seems promising for the treatment of Homocystinuria, in which excess Hcy could be bound by Porf(Co^{III})(Im)-(H_2O). Regarding the interaction of HcyNO with Kor and Porf, the most thermodynamically favored conjugate is Porf(Co^{II})-N-HcyNO (E_{bin} = -8.84 kcal/mol, aqueous phase). Moreover, the bond length between N, and Co^{2+} is relatively small (1.99 Å). Considering the results obtained for Porf(Co^{II})-N-HcyNO, this system in connection with the favorable value of E_{bin} can be used to accumulate HcyNO in cancer cells. The accumulation of NO donors in these cells can contribute to an increase in NO concentrations and, consequently, to nitroxidative stress, which will contribute to tumor death.

The studies presented in this paper shed new light on the applicability of vitamin B_{12}. Both in the context of targeted transport of model compounds with possible anticancer properties, and to regulate NO homeostasis and regulate metabolic processes related to methionine synthesis, vitamin B_{12} appears to be a promising candidate.

Kraków (2023)