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Mendeleev Commun., 2021, **31**, 207–209

Mendeleev Communications

First ^{97}Ru complex with pyridine-2,6-dicarboxamide conjugate for potential use as radiopharmaceutical

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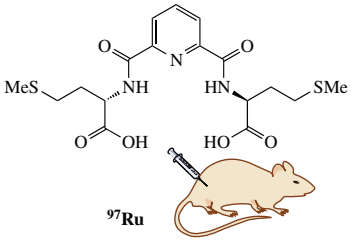
DOI: 10.1016/j.mencom.2021.03.020

A new peptidomimetic conjugate of natural methionine and pyridine-2,6-dicarboxylate was synthesized and evaluated as a potential ligand for ^{97}Ru radiopharmaceuticals. The distribution of this radiopharmaceutical in mice was investigated. A fundamental difference was found in the distribution and excretion of ruthenium complex and free ruthenium ion in the body, which suggests a difference in their transport pathways due to the stability of the complex in the body.



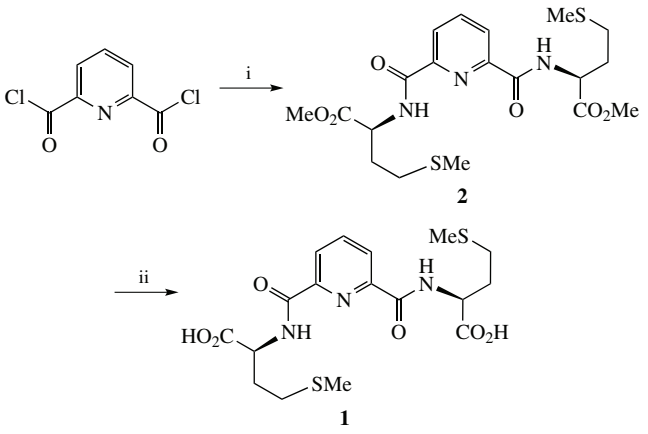
Keywords: ruthenium-97, mouse model, clearance, pyridine-2,6-dicarboxamide, conjugates, radioactivity.

Recently, much attention has been paid to compounds of Group VIII metals as chemotherapeutic agents. Among them, ruthenium is considered one of the most promising metals, mainly because it is less toxic to healthy tissues in comparison with platinum and its compounds (cisplatin, *etc.*).[1] In addition, while cisplatin and its analogs generally intercalate DNA, the mechanisms of action of ruthenium compounds are more diverse.[2] Moreover, ruthenium can bind to albumin and transferrin, which increases its bioavailability.[3] The best known ruthenium-based antitumor compounds are KP1019 [indazolium *trans*-tetrachloridobis(1*H*-indazole)ruthenate(III)], NAMI-A [imidazolium *trans*-tetrachlorido(1*H*-imidazole)(*S*-dimethylsulfoxide)ruthenate(III)] and RAPTA-C [dichloro(η^6-*p*-cymene)-(1,3,5-triaza-7-phosphaadamantane)ruthenium(II)], belonging to the indazole, imidazoline and η^6-arene complexes, respectively.[4]

Cancer cells are thought to have an increased ability to absorb ruthenium. Similar to copper, ruthenium is capable of reducing to ruthenium(II) under hypoxic conditions typical of tumors. The low valence of ruthenium increases its biological activity. It is possible to create versatile antitumor drugs by binding ruthenium with biologically active ligands that provide a known therapeutic effect.[4(a)] To design multifunctional radio- and chemotherapeutic drugs,[5] it is necessary to combine the antitumor activity of both a radionuclide and a binding ligand (or framework). For the production of radiopharmaceuticals using ruthenium radionuclides, the ^{97}Ru radionuclide with $t_{1/2}$ = 2.79 days is of greatest interest, for which various methods of production and separation have been described.[6] Transferrin is known to be one of the transporters in the body. The accumulation of ^{97}Ru transferrin complex in tumors is up to 3 times higher than that of other radionuclide complexes.[7] Recently, we have proposed[8] a fairly simple, commercially convenient and affordable method of separating ^{97}Ru. The parameters of this radionuclide first make it possible to diagnose organs in which the complex accumulates. The administration of ^{97}Ru without a carrier, that is, bound directly to the ligand without a measurable amount of ruthenium, can simultaneously enhance the effect on the tumor by direct destruction of cells. If a radionuclide with a carrier is administered, that is, introduced into a ready-made complex with a measurable amount of ruthenium ions, then an additional effect of inactive ruthenium ions is observed, which also have antitumor properties. Thus, it is possible to achieve a multitarget effect on cells within the framework of the previously proposed design.[5]

Here we report the first synthesis and biodistribution of a ^{97}Ru complex with a peptidomimetic ligand. Molecules derived from natural compounds attract much attention in drug development.[9] Moreover, amino acid-containing peptidomimetics show high potential in the development of self-assembled highly ordered supramolecular systems,[10] materials for recognizing chiral molecules,[11] mimicking the activity of nitrile hydratase (NHase, EC 4.2.1.84)[12] and anion receptors.[13] Artificial peptides with dicarboxylic acids exhibit nanomolar antimicrobial,[14] anti-inflammatory[15] and antineoplastic activity against MCF-7,[16] L-1210, Molt-4 and HL60 cells.[17] Additionally, copper and zinc complexes with peptidomimetics based on amino acid-conjugated pyridine-2,6-dicarboxamide showed promising activity in inhibiting the binding of HIV-EP1 metalloprotein (HIV enhancer binding protein) to the NF-κB recognition sequence of DNA by ejecting metal from the enzyme.[18] Pyridinedicarboxylate also acts as a scaffold for various supramolecules used for binding metals.[19]

To create potential ^{97}Ru radiopharmaceuticals, we propose peptidomimetic ligand **1** based on pyridine-2,6-dicarboxylic acid and natural L-methionine. The synthesis of simple peptidomimetic ligand **1** occurs in two stages, namely, acylation



Scheme 1 *Reagents and conditions*: i, (*S*)-MeS(CH$_2$)$_2$CH(NH$_2$)CO$_2$Me, Et$_3$N, THF; ii, NaOH, H$_2$O–EtOH.

of freshly prepared L-methionine methyl ester with pyridine-2,6-dicarbonyl dichloride to afford compound **2** and removal of methyl ester groups by mild alkaline hydrolysis of **2** (Scheme 1, for details, see Online Supplementary Materials). All compounds were characterized by multinuclear NMR (see Online Supplementary Materials). The signals were assigned using 2D COSY and NOESY spectra.

Contrary to the previously studied pyridine-2,6-dicarboxamide peptidomimetics,[10] ligand **1** does not form any ordered structures, which was demonstrated by 2D NOESY experiments (see Online Supplementary Materials). Thus, the interaction of ligand **1** with the ruthenium ion is not complicated by interligand supramolecular interactions.

The reaction of $^{97}Ru^{IV}$ chloride, which exists in the form of the $[^{97}Ru(H_2O)Cl_5]^-$ anion ($^{97}Ru^{IV}$) at a pH value close to neutral,[20] with chelator **1** was carried out in water at pH 7, and the reaction product was purified by radiochromatography.† To establish the comparative distribution of ^{97}Ru radionuclide, mice were injected with samples of $^{97}Ru^{IV}$ and its complex with amide **1**.‡ The results are shown in Figure 1.

The accumulation and clearance of radioactivity of the resulting complex are necessary indicators of its behavior in the body. Comparison of the behavior of ^{97}Ru associated with chelator **1** with that of unbound ruthenium in mouse organs shows a fundamental difference in their excretion (see Figure 1). The clearance of $^{97}Ru^{IV}$ was much faster than that of the complex, which accumulated for up to 90 h. $^{97}Ru^{IV}$ was excreted

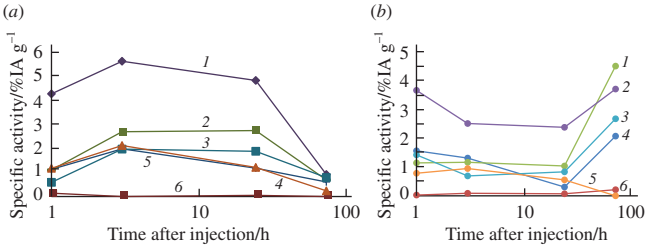


Figure 1 Distribution of specific activity (as percentage of injected activity) of (*a*) $^{97}Ru^{IV}$ and (*b*) the ^{97}Ru–**1** complex in (*1*) kidneys, (*2*) liver, (*3*) spleen, (*4*) lungs, (*5*) heart and (*6*) brain of mice as a function of time.

preferentially through the kidneys. Concerning accumulated radioactivity, the kidney/liver ratio was ~2, ~1.8 and 1 after 3, 24 and 72 h, respectively. For the complex, this ratio was 1.9, 2.4 and 0.8, respectively, which shows an increasing contribution of the liver. The slow penetration of small amounts of the complex into the brain probably indicates the existence of specific transporters. There was practically no difference between the accumulation of ruthenium chloride and its complex in the heart, where activity was not observed after 72 h in either case. $^{97}Ru^{IV}$ accumulated and was retained in the spleen and lungs for up to 24 h, after which it was excreted. A completely different case occurred with complex ^{97}Ru–**1**, which accumulated in these organs after 24 h, as it was in the liver, and its accumulation increased up to at least 90 h.

Thus, the data obtained allow us to conclude that there are other transport pathways for the complex as compared to those for $^{97}Ru^{IV}$. Obviously, this indirectly indicates the stability of the complex, which has its own transporters in the body, and as a result, the organ of preferential excretion has changed. We did not consider the accumulation of activity in bones, blood and muscles since bone/blood and bone/muscle tissue ratios for unbound ^{97}Ru are comparable to those for the accumulation of ^{99m}Tc.[7] Thus, a rather long traveling of the proposed complex through a number of body organs, along with the high capability of ruthenium to accumulate in tumors and metastases, gives confidence in the possibility of therapeutic and diagnostic use of the ^{97}Ru–**1** complex when designing a multifunctional radiopharmaceutical.

This work was supported by the Russian Science Foundation (grant no. 16-13-10451, for chemical synthesis) and the Russian Foundation for Basic Research (grant no. 19-08-00055, for animal distribution experiments). Authors are grateful to M. V. Lomonosov Moscow State University for the opportunity to use the NMR facilities of the Center for Magnetic Tomography and Spectroscopy.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.03.020.

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† Separation of ^{97}Ru was carried out according to the described[12] procedure, reaching a radiochemical yield of 94% and a radiochemical purity of 99%. The resulting solution was evaporated to dryness, the residue was dissolved in a minimum amount of bidistilled water, and the solution was diluted until pH ~ 7 to obtain the anionic form of ruthenium.[20] Thereafter, ligand **1** (25.5 mg, 0.06 mmol) was added, and the mixture was vigorously stirred at room temperature for 30 min. Then, thin layer chromatography was performed on Silufol plates using 95% ethanol as eluent. The chromatogram was cut into strips of 1 cm, and the activity was measured on a gamma spectrometer with a high-purity germanium GR 3818 detector at the ^{97}Ru line (215.7 keV). It was preliminarily established that the R_f values for ligand **1**, inactive $RuCl_4$ and its complex with **1** are 0.75, 0.35 and 0.175, respectively. The chromatogram was developed using iodine vapor. The R_f values of radioactive and non-radioactive compounds were the same.

‡ A solution (100 μl) of the ^{97}Ru–**1** complex and a control solution of $^{97}Ru^{IV}$ were injected intraperitoneally into laboratory white mice (males) weighing 28–33 g. At 1, 3, 24, 72 and 90 h after injection, the animals were sacrificed by cerebral dislocation. The injected activity of ^{97}Ru was 1.5 kBq per mouse in three iterations, and the activity of the ^{103}Ru impurity was less than 5 Bq. Other radionuclides were absent. Three mice were used for each point.

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Received: 29th October 2020; Com. 20/6350