Reviews

Antitumor activities of ferrocene compounds

L. V. Snegur,[a]* V. N. Babin,[a] A. A. Simenel,[a] Yu. S. Nekrasov,[a] L. A. Ostrovskaya,[b] and N. S. Sergeeva[c]

[a]A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences,
28 ul. Vavilova, 119991 Moscow, Russian Federation.
Fax: +7 (499) 135 5083. E-mail: snegur@ineos.ac.ru
[b]N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences,
4 ul. Kosygina, 119991 Moscow, Russian Federation.
E-mail: larros@list.ru
[c]P. A. Herzen Research Institute of Oncology,
2 3rd Botkinskii pr., 125284 Moscow, Russian Federation.
Fax: +7 (495) 945 7415. E-mail: prognoz.06@mail.ru

Data on the antitumor activities of ferrocene compounds and the results concerning the mechanisms of their action published between 1995 and 2010 are discussed. The biomedical aspects of the study of ferrocene derivatives are briefly analyzed.

Key words: ferrocene, antitumor activity.

The concept of cytotoxic action of antitumor drugs is commonly accepted in the modern medicine and pharmacology. The effects of the most chemotherapeutic agents are caused, first of all, by their toxicity against tumor cells, resulting in cell death and tumor tissue necrosis and removal from the organism with participation of the immunity system. This is the principle mechanism of action of antitumor drugs of key classes: alkylating agents (cyclophosphamide, embichin, nitrosoureas), antimetabolites (methotrexate, mercaptopurine, 5-fluorouracil), antibiotics (adriamycin), activators of oxidative metabolism (procarbazine), intercalators (rubomycin).[1] The key target for the cytostatics is the DNA of actively proliferating tumor cells (Table 1).

However, many chemotherapeutic agents prove to be toxic even relative to normal proliferating cells of the body. The lack of specificity typical of action of most antitumor agents is caused by the insufficient difference between the therapeutically effective (ED_{50} or ED_{90}) and lethal doses (LD_{50}) of the agents; as a consequence, the therapeutic indices (TI = ED_{50}/LD_{50}) are rather low. The lack of specificity of most of known chemotherapeutic agents results in the development of side effects manifested as serious toxic consequences (suppression of haemopoiesis, pronounced disorder of liver and kidney function, *etc.*), which considerably worsen the quality of patient´s life, often being the direct cause of the fatal outcome. Therefore, development of novel antitumor agents with low toxicity, strong activity

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 12, pp. 2113—2124, December, 2010.

Table 1. Molecular mechanisms of the biological action of antitumor agents

Antitumor agents	Biochemical consequences
Cytotoxic agents	
Alkylating agents	Alkylation of DNA nucleic bases and subsequent chain cross-linking
Antimetabolites	Incorporation in DNA and some types of RNA
Antibiotics, alkaloids and other substances of plant origin	Intercalation between DNA base pairs, DNA cleavage, depression of DNA synthesis
Synthetic antitumor agents of various groups	DNA cross-linking
Hormone agents and hormone antagonists	Specific binding to estrogen receptors of tumor cells, retardation of cell division
Enzymes	Disruption of aspargine metabolism (for L-asparagnase agent)
Cytokines	Activation of macrophages, killer cells, T-lymphocytes

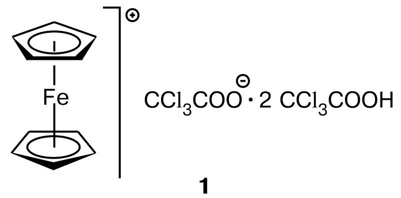
and with a principle novel mechanism of action other than the cytotoxic or cytostatic effects is a topical challenge.

A highly important initial stage in the chemical carcinogenesis is abnormal alkylation of DNA purine bases.[2,3] On treatment with alkylating agents, this process gives 3-alkyladenine and 7-alkylguanine.[4] On treatment with intercalating carcinogens of the class of polycyclic aromatic hydrocarbons and azoaromatic derivatives, alkylation may be mediated by the result of cell metabolic response to the carcinogenic attack.[5] Since upon N-alkylation to position 7, purines get a positive charge mainly located on the imidazole fragment, the nucleosidic bond with ribose becomes destabilized. This results in elimination of purine bases, *i.e.*, DNA depurinization.[6] Depurinization can also occur without preliminary alkylation, because the basicity of the pyrimidine nitrogen in the imidazole fragment (pK_a 7.4)[7] makes its protonation quite possible. However, the rate of this reaction is normally low. Meanwhile, it was shown that depurinization is faster during carcinogenesis.[6]

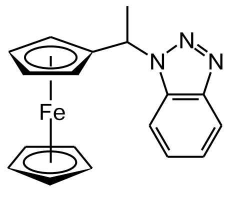
The elimination of purine bases (first of all, guanine) gives rise to a C'_1 carbocation center, and this stimulates further transformations, most important of which are skeletal rearrangements, resulting in the cleavage of the sugar-phosphate backbone. This means that single-strand breaks appear in the DNA structure, which is a rather trivial event for the genome. This type of damage is successfully eliminated by the DNA excision repair system,[8] which heals these damages. However, if the number of single-strand breaks becomes relatively large, the rate of functioning of this repair system is no longer adequate to ensure the genome integrity. In this case, recombination repair system[9,10] based on migration of whole DNA fragments followed by their incorporation into the damaged sites comes at the forefront. However, no matter how fast the two repair systems work, under certain conditions in the presence of closely spaced breaks, ionic contacts between DNA and histons may be lost, *i.e.*, rather extended regions of free DNA with single-strand breaks may appear. One consequence of this is release of DNA fragments to the cytoplasm,[11,12] which is considered by some researchers as a typical sign of malignant transformation of cells.[13] All this may lead to uncontrolled DNA recombination processes and finally may be genetically fixed.[4]

The available theoretical studies[14] and results of biochemical investigations of ferrocenium salts[15] and ferrocenyl alkyl azoles[16—18] provide grounds for the development of new-generation agents with low toxicity. The principle of action of these agents is not always based on cytotoxic or cytostatic effects but implies the possibility of other pathways of inhibition of tumor growth related by both the direct action on the tumor cells[16—18] and the mediated action on the immune system.[19]

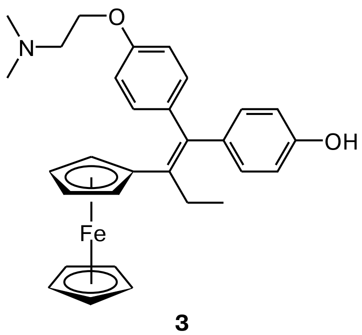
Therefore, the line of research concerned with the antitumor properties of metallocenes and, especially, ferrocene derivatives, which has been actively developed in the last 20—25 years, is of obvious interest. The biologically active metallocenes: ferrocenium trichloroacetate (**1**),[15] 1-[1-ferrocenyl(ethyl)]benzotriazole (**2**),[16—18] and ferrocifen (**3**)[20] with antiproliferative activity, are shown below.



1



2



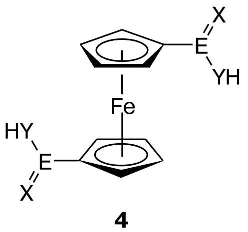
3

This review discusses the data concerning the antitumor activities of ferrocene compounds and the possible mechanisms of their action published from 1995 to 2010 and gives brief analysis of the biomedical aspects of the studies of ferrocene compounds. The review also considers some earlier results that are directly related, in our opinion, to the problem in question, including the data that have not been mentioned in earlier reviews; some aspects of the generally accepted views on the initial stages of chemical carcinogenesis, which are important, in our opinion, for the understanding of the possible mechanisms of action of ferrocene compounds.

In the mid-1970s, when there was no experimental evidence, the concept of antitumor activity of ferrocene compounds was elaborated theoretically, and in 1979 one of the first theoretical publications[14] appeared where the authors substantiated the potential antitumor activity of neutral functionalized ferrocene compounds and ferrocenium salts. This work proposed a model mechanism of malignant transformation of cells induced by chemical carcinogens. The authors considered the first stages of influence of polycyclic hydrocarbons and aromatic amines on the genetic material, chromatin (DNA complexed with basic proteins, histones). According to the proposed model, the impact of carcinogens on chromatin induces the development of uncontrolled pathological recombination processes in the genome, which is the basis for the malignant transformation of the cell.

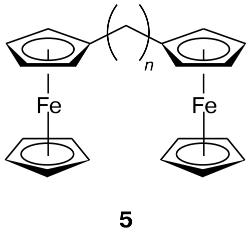
In view of these considerations, the authors[14] formulated the key principles for the design of antitumor molecules. These compounds should prevent the uncontrolled recombination, and, in the authors´ opinion, one of the mechanisms of their action is to create a hindrance to direct contacts between the phosphate groups of DNA molecules of different chromartin strands at break points. Such molecules should, first, be able to spatially separate the "illegal contacts" that arise upon exposure to carcinogenic factors at the DNA cleavage sites; to this end, the compounds must have a rigid cage, not to be "crumpled" by chromatin; second, they must have functional groups able to effectively contact (*i.e.*, form non-hydrolyzable bonds) with the DNA phosphate groups at the cleavage sites.

In conformity of this model, as a possible line of the search for new potentially active drugs, it was proposed to study the antitumor activity of ferrocene compounds **4** containing various functional groups, binuclear ferrocene derivatives **5** and ferrocenium salts.[14] This subsequently underlay our synthetic and biological research.[16–18]



4

E = P, As; X = O, S, Se, F, Cl;
Y = O, S



5

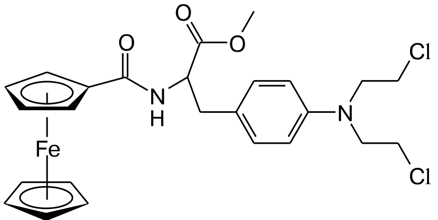
$n = 1, 2$

During the next 30 years, the situation changed crucially. By 2010 several reviews have been published and book chapters have appeared considering various aspects of the antitumor activities of organometallic compounds,[21–26] and the possible mechanisms of antitumor effects of metallocenes.[25] In these reviews the attention was focused on ferrocene compounds including those based on polymers.[26]

Five years after the publication of an early theoretical work,[14] the first experimental proof for this approach was obtained, indicating antitumor activity of ferrocene compounds.[15] These results appeared in press nearly at the same time as our synthetic exploration[27] related to the search for biologically active compounds and, undoubtedly, this stimulated our biological studies.[28,16a,17a]

Currently, studies of the antitumor activities of organometallic compounds, in particular, metallocenes, are vigorously carried out all over the world including Russia, Japan, Republic of South Africa, USA, European countries, Pakistan, Brazil. A broad range of metallocene derivatives and π-cyclopentadienyl-π-arene transition metal (Ti, Zr, Hf, V, Mo, Fe, Co, Ru) complexes have been investigated; functional derivatives of ferrocene are studied extensively. The search for active compounds among various metal complexes was also stimulated by the appearance of the first effective metal-containing cytostatic agent, *cis*-diamminodichloroplatinum,[29] *cis*-$(NH_3)_2PtCl_2$, in clinical practice.

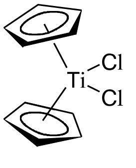
The logic of the first studies on the synthesis of metallocene derivatives with the potential antitumor activities was quite natural. In compounds known to show antitumor activity, either the aromatic phenyl fragment was replaced by aromatic ferrocenyl group $(C_5H_5)Fe(C_5H_4)$—, or a ferrocenyl group was introduced as an additional substituent. This direction has not been exhausted even now. Jordanian chemists prepared ferrocenyl analogs of cisplatin, which showed cytotoxic activities *in vitro* against the P388 leukosis cells at the same level of cisplatin and 5-fluorouracil.[30] However, the first attempts were unsuccessful.[31–34] Thus modification of embichin and sarcolysin, which are alkylating type antitumor drugs, by introducing the ferrocenyl substituent resulted in a sharp decrease of the antitumor effect (this was estimated as retardation of the tumor growth on S37 sarcoma, S180 sarcoma, Ca755 carcinoma, Lewis lung carcinoma, and L1210 leukemia) and in some cases, this even stimulated the tumor development.[33] This may be related to the increase in the drug lipophilicity caused by introduction of the ferrocenyl fragment into hydrophilic compounds, and this could change the pharmacokinetics. Meanwhile, this modification considerably decreased the acute toxicity of the drugs. For ferrocenyl-modified sarcolysin **6**, which contains the *bis*(β-chloroethyl)amine alkylating group,[33] the LD_{50} value was more than 1500 mg kg^{-1} (*versus* LD_{50} of 25 mg kg^{-1} for the parent sarcolysin); however, the researchers did not notice or appreciate this fact due to the lack of antitumor effect.



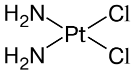
6

Screening of the antitumor activities of (ferrocenyl)-polyamines $Fc(CH_2)_nNH(CH_2)_nNH_2$ ($n \le 4$) (compounds were administered to animals as hydrobromides) revealed no activity (P388 lymphoid leukemia) in any of the compounds; these amines proved to be toxic, LD_{50} being 12.5 mg kg^{-1} or even higher.[34] Thus, in the earlier attempts to prepare potential antitumor drugs based on metallocenes, the introduction of a ferrocenyl fragment into compounds known to have substantial, moreover, clinically significant antitumor effect, caused a decrease in this effect. These results evoked skepticism as regards the possibility of using metallocene structures in oncology.

A new phase of investigations was related to the antitumor activity of titanocene dichloride **7** (see Refs 35—37). The researchers were guided by the structural analogy between this compound and cisplatin **8** (*cis*-diamminodichloroplatinum).



7



8

On the Ehrlich ascites carcinoma (EAC) mouse model, the drug used in the 30—60 mg kg^{-1} dose increased the average life span of the animals by 400% compared to the control mice without considerable toxic effects, despite the presence of a titanium atom in the molecule.

Thus, the antitumor activity of a metallocene was demonstrated for the first time. Subsequently metallocene dihalides, Cp_2MX_2, and related compounds with the central Group IVB-VIB metal atom have been studied.[36—40] Certain regularities in the efficiency of these compounds were identified. The series of antitumor activity (based on the central atom) was composed:

$$V > Ti > Nb > Ta \approx Mo \approx W >> Hf, Zr.$$

Thus, titanium and vanadium derivatives are most active, while the compounds of heavier hafnium and zirconium are absolutely inactive.

In view of these results, the authors of the review[23] drew the conclusion about the diagonal relationship of the activities depending on the position of the central atom in the Periodic Table of elements.

Subsequently, it is the compounds of the titanocene dichloride and vanadocene dichloride series that have been studied most comprehensively.[41] Toxicological characteristics of $(C_5H_5)_2TiCl_2$ and $(C_5H_5)_2VCl_2$ were determined. A comparison with cisplatin showed the absence of renal toxicity or adverse effect against the hemapoietic organs, although slight hepatotoxicity and embryotoxicity were detected.[42—44] This was followed by reports on preclinical trials of titanocene dichloride,[45] the results of phase I clinical trials;[45] later, it was reported that clinical investigations of the drug were terminated.[20b,46]

Studies of titanocene dichloride derivatives modified by benzyl substituents at the Cp rings are currently in progress.[47] They were found to affect the immune system and are suspected to accelerate apoptosis.[48]

The first experimental results supporting the antitumor activity of ferrocene compounds were obtained in a study of ferrocenium salts with "hard" anions, tetrachloroferrate(II), picrate, and trichloroacetate (the parent ferrocene and inorganic iron(III) salts were inactive).[15a] In 1980 it was mentioned incidentally, without any specifics, that N^6-(ferrocenylmethyl)adenine suppresses cell division when present in low doses.[49] The above ferrocenium salts increased the life span of animals by 70—100% relative to the control animals; the studies were carried out on the mouse EAC model.[5] With a relatively high dependence of the efficiency on the dose, the ferrocenium salts proved to have moderate toxicity (LD_{50} of 240 to 400 mg kg^{-1}). (Note for comparison that metal-containing agents used in clinical practice have the following lethal doses: cisplatin has LD_{50} of 13 mg kg^{-1} [50] and ferroceronum, which is the sodium salt of *ortho*-carboxybenzoylferrocene (used in the therapy of iron deficiency pathologies) has LD_{50} of 60 mg kg^{-1} (see Ref. 51)). This results was rather non-trivial, because there was not any structural analogy with known antitumor agents from the chemical standpoint.

The cited publications[15,22,25—35] largely stimulated the search for new ferrocene compounds with antiproliferative activity. During the period from 1984 to 1995, the research related to ferrocenium salts was continued;[15,52—54] poly(alkyl)ferrocenium salts were investigated;[55] the first ferrocenyl-nucleosides were reported, which, however, were inactive in *in vitro* experiments with respect to the L1210 leukemia cell culture;[56] we started the studies of ferrocenyl alkyl azoles.[28,57,58,16a,25] As regards the use of ferrocenium salts in clinical practice, they have not been commercialized.*

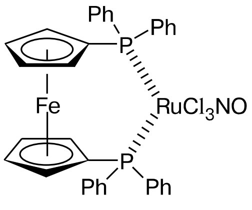
---

* Private communication of Professor E. W. Neuse, a co-author of the first publication on the antitumor effects of ferrocenium salts.[15a]

The efficiency of ferrocenium salts was demonstrated against solid (dense) tumors[22] such as B16 melanoma, Lewis lung carcinoma, large intestine Ca38 colon carcinoma. However, these salts had relatively low efficiency against L1210 and P388 leukemia. Pronounced antiproliferative activity of the compounds was found against xenotransplants of human tumor in test with human tumor transfer under the mouse renal capsule.[58,18b] We found that not only 100% inhibition of the tumor growth but also the implantants regression took place. In the case of epidermoid lung carcinoma (two different histological forms of cancer were studied), the tumor regression reached 10 and 15% upon the use of *sym*-1,1´-diethylferrocenium triiodide. The observed effect is comparable with the effect of cisplatin, used in clinical practice, which induced tumor regression by 23% in the same experiment (on dermoid lung cancer) (see Ref. 18b). Ferrocenium triiodide, $Fc^+I_3^-$ (daily dose 1.5 mg kg^{-1}) had a 100% growth-inhibiting action on the esophageal cancer in a subcapsular test *in vivo*; then a 30% regression of the tumor was observed.[18b] The effects of 1,1´-dimethylferrocenium, $Me_2Fc^+BF_4^-$, and decamethylferrocenium, $Me_{10}Fc^+BF_4^-$, tetrafluoroborates on the tumor tissue cultures were investigated.[55,60,61] It was found that the introduction of two methyl groups decreases the activity as compared with unsubstituted ferrocenium FcH^+ with respect to the Yoshida ascites tumor. However, the decamethyl homolog displayed fairly high activity (in a concentration of 1.0 μmol L^{-1}) with respect to L1210 leukemia. The activity of ferrocenium salts was also studied by a clonogenic test *in vitro*,[61] which takes account of antitumor agent-induced dynamics of colony-forming units (dividing stem cells). It was shown that a 100 μg mL^{-1} concentration of the salt provides high inhibiting activity (for ferrocenium tetrachloroferrate $FcH^+FeCl_4^-$, the inhibiting concentration providing a 50% effect, IC_{50}, is 40 μg mL^{-1}). We studied the activity of ferrocenium and 1,1´-diethylferrocenium triiodides in *in vitro* and *in vivo* experiments.[53,54,16a] It was found, for example, that 1,1´-diethylferrocenium triiodide in a dose of 12.5 mg kg^{-1} inhibits the growth of adenocarcinoma 755 by 80% and increases the life span of animals by 90%. Ferrocenium triiodide reduces considerably (almost 10-fold compared with the control) the virus titer in the Rauscher virus-induced leukemia mouse model.[16a]

The antitumor activity is inherent in not only ferrocenium salts but also in other stable organoiron cations having sandwich structures. In particular, this activity was exhibited by salts of dibenzeneiron(II) homologs, $[bis(\eta^6\text{-mesitylene})Fe]^{2+}$, and arenecyclopentadienyliron(II) $[(\eta^5\text{-}C_5H_5Fe\text{-}\eta^6\text{-}C_7H_8]^+$ (see Ref. 55). Against this background, neutral ferrocene and its homologs were a sharp contrast, as they did not show any antitumor effect. Ruthenocene and its derivatives did not show any noticeable activity at that time either.[62—65] Note that subsequently, ruthenium—arene organometallic complexes demonstrated antimetastatic and tumor growth-inhibiting properties.[66] The current studies are concerned with the transport of multinuclear ruthenium(II)—arene complexes[67] and with antiproliferative properties of organoruthenium, -rhodium, and -iridium half-sandwich complexes with *N*,*N*-chelating ligands,[68(a)] which, however, demonstrated a lower cytotoxicity in *in vitro* experiments than cisplatin.

A study of the nitrosyl ruthenium complex with symmetrically substituted bis-(diphenylphosphine)ferrocene (**9**) against a breast cancer cell culture (MDA-MB-231 line) showed an IC_{50} value of 10 μmol L^{-1} (see Ref. 68b). This exceeds the cytotoxicity of both the initial nitrosyl-ruthenium trichloride, $RuCl_3NO \cdot 2H_2O$, for which IC_{50} > 200 μmol L^{-1} (*i.e.*, the absence of activity was detected) and the proper (diphenylphosphine)ferrocene with IC_{50} of 97 μmol L^{-1} or cisplatin with IC_{50} of 63 μmol L^{-1} on exposure for 48 h (MTT test). The researchers cited[68b] consider the initial (diphenylphosphine)ferrocene to be a cytotoxic agent whose activity considerably increases upon the coordination to inactive nitrosoruthenium trichloride.

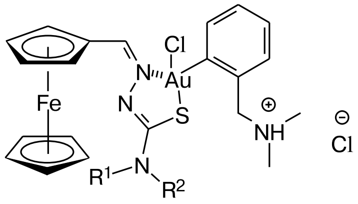


9

This brings about a significant question of what is responsible for the difference in activity: the charge, the solubility, or other factors, for example, the difference between the redox potentials. In a clonogenic test *in vitro* of moderately water-soluble and potentially anionogenic ferrocene derivatives including ferrocenylacetic acid, $FcCH_2COOH$, and ferrocenylmethylthiomalic acid, $FcCH_2SCH(COOH)CH_2COOH$, both compounds were found to inhibit the colony formation of human lung cancer cells and PC-9 lung carcinoma cells to even a greater extent than ferrocenium salts.[61] However, unlike ferrocenium salts, these processes develop within a certain, quite extended period rather than just after incubation with the agent and have prolonged action, which suggests a metabolic activation stage, namely, oxidation to ferrocenium salts (in the case of ferrocenylacetic acid) or the formation of the $FcCH_2^+$ cation (in the case of ferrocenylmethylthiomalic acid).

Of certain interest are works on the synthesis of platinum(II) and some other metal complexes with mono- and bidentate ferrocene-containing ligands.[63—65,69] These works can be considered as development of the idea of varying the properties of cytostatic agents by introducing ferrocene-containing substituents. The results attest to a lower activity of the complexes compared to cisplatin, not to speak of the second-generation derivatives such as carboplatin. However, since these compounds showed rather high activity, on the one hand, and lower toxicity compared with cisplatin, on the other hand, one can expect that this line of search for antitumor has a large potential. Among the synthesized potentially antitumor compounds, note *cis*-dichloro-bis(1-ferrocenylethylamine)-platinum(II), copper and gold complexes with 1,1′-(diphenylphosphino)ferrocene,[70,71] other ligands,[72] platinum ferrocenylmalonate complexes,[73] which are active against adriamycin-resistant P388 mouse leukemia.

Recently, the cytotoxicity of three ferrocene complexes with gold-containing ligands (**10**) was determined in experiments *in vitro* on HeLa-229 sarcoma cells;[72] it was almost two orders of magnitude lower than the cytotoxicity of cisplatin under the studied conditions, IC_{50} was 34 μmol L^{-1} for the complexes with the ethyl substituent (R^1 = H; R^2 = Et) and IC_{50} was 0.45 μmol L^{-1} for cisplatin. Also, it was found that the maximum cell growth inhibition E_{max} reaches 80 to 90% for Au-ferrocene complexes, which is comparable with cisplatin. In particular, for the compound isolated as the acetone complex ($R^1 = R^2$ = H), E_{max} is 89%, and for cisplatin, E_{max} is 62%.



10

It is noteworthy that the complexes based on gold(III) and platinum(II) are isoelectronic (d^8) and iososteric (planar chiral). Therefore, these complexes attract attention of researchers by analogy with cisplatin.

Since approximately 1995, publications started to appear that shed light on the mechanisms responsible for the antitumor effects of compounds. The studies involved compounds that had previously shown antitumor effects. It was found by gel electrophoresis that ferrocenium salts, namely, tetrabromoferrates of ferrocenium, $Fc^+FeBr_4^-$, monoacetyl-, $CH_3C(O)Fc^+FeBr_4^-$, and 1,1′-(diacetyl)-ferrocenium, $[CH_3C(O)(C_5H_4)]_2Fe^+FeBr_4^-$, and propionylferrocenium, $CH_3CH_2C(O)Fc^+FeBr_4^-$, in which the ferrocene fragments carry a positive charge, react with DNA to induce separation of DNA double strands.[74] This was interpreted in a traditional way[75] by assuming that ferrocenium salts act indirectly, hydroxyl radicals OH• formed under the action of other radicals (ferrocenium salts) being the active species. The degree of DNA cleavage (DNA-cleaving activity) increased in the series[74]

$$Fc^+FeBr_4^- < CH_3C(O)Fc^+FeBr_4^- <$$

$$< [CH_3C(O)(C_5H_4)]_2Fe^+FeBr_4^- < CH_3CH_2C(O)Fc^+FeBr_4^-;$$

this is well correlated with the inhibition dose ID_{50}, which is equal to 19, 16, 8, and 6 μg mL^{-1}, respectively. The ID_{50} value is the dose that provides a 50% retardation of the cell growth. The lower the ID_{50} value (*in vitro* experiment was carried out on mouse B16 melanoma cells), the more toxic the compound. The cytotoxocity was observed for 1,1′-(diacetyl)ferrocenium and propionylferrocenium salts with ID_{50} of 8 and 6 μg mL^{-1}, respectively, which is comparable with the toxicity of the pharmaceutical drug cisplatin, *i.e.*, it is sufficiently high.

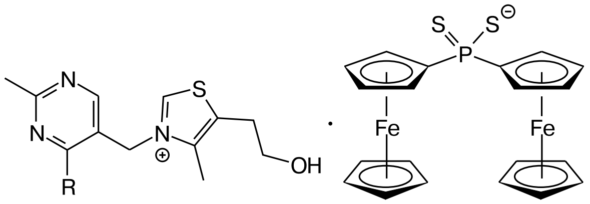
One more point of interest is that one can see how the introduction of the carbonyl group into the ferrocene side chain, namely, in the α-position to the cyclopentadienyl ring, correlates with (increases) the cytotoxocity. This pronounced increase in the toxicity was observed in our *in vivo* studies of 1-[1-ferrocenyl(ethyl)]benzotriazole **2**, which contained, apart from the major compound, an impurity of acetylferrocene $FcC(O)CH_3$. The same effect might be involved to some extent in the case of ferrocerone, sodium *ortho*-carboxybenzoylferrocene tetrahydrate, $FcC(O)-C_6H_4(CO_2Na)$-*o*$\cdot 4H_2O$, an antianemic agent, which is 7.5 times more toxic than ferrocene (the ferrocene LD_{50} is 450 mg kg^{-1}, the ferrocerone LD_{50} is 60 mg kg^{-1})[51].

Sodium (diferrocenyl)dithiophosphinic acid $Na^+(Fc_2PS_2)^-$, in which the ferrocene fragments are parts of the anion, *i.e.*, have an overall negative charge, showed no antitumor activity *in vivo* against Ehrlich ascites carcinoma model upon subcutaneous administration.[76] In *in vitro* studies of the intensity of DNA synthetic processes by the ^3H-thymidine test (the ^3H-thymidine or antiproliferative test is used to estimate the intensity of incorporation into DNA of tritium-labeled thymidine nucleoside, ^3H-thymine-ribose) on sarcoma 37, hepatoma mh-22a, and P388 leukosis tumor cells, it was found that ferrocene derivatives of thiamine (**11a**) and (hydroxy)thiamine (**11b**)* activate the synthesis of DNA, which is especially noticeable on short-term treatment (3 h).[76]

On subcutaneous administration of thiamine- and (hydroxy)thiamine(diferrocenylduithiophosphinates) (**11a,b**), both compounds were found to have low toxicity with LD_{50} exceeding 1000 mg kg^{-1} (exact LD_{50} values could not be determined due to sparing solubility of the agents in water).[76]

---

* 4-Methyl-3-[2-methyl-4-aminopyrimidyl-5)methyl]-5-(2-hydroxyethyl)thiazolium (thiamine is Vitamin B_1, R = NH_2); 4-methyl-3-[2-methyl-4-hydroxypyrimidyl-5)methyl]-5-(2-hydroxyethyl)thiazolium ((hydroxy)thiamine is Vitamin B_1 antagonist, R = OH, has antitumor activity).



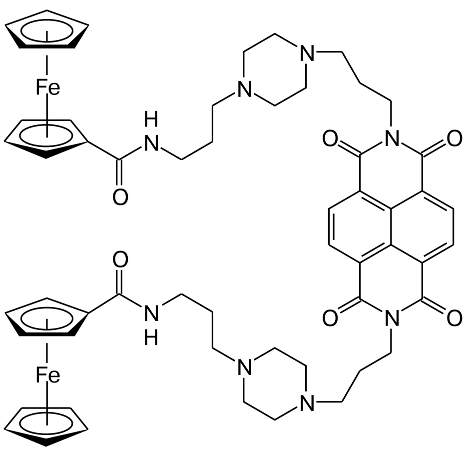
11a,b

R = NH$_2$ (**11a**), OH (**11b**)

A study of the effect of 1-[1-ferrocenyl(ethyl)]benzotriazole **2** on incorporation of ^{3}H-thymidine into DNA of human ovarian cancer cells, *i.e.*, on the activity of DNA synthetic processes[16b,77] (concentrations: $2 \cdot 10^{-6}$, $2 \cdot 10^{-5}$, 10^{-4}, $2 \cdot 10^{-4}$, $4 \cdot 10^{-4}$ mol L^{-1}; measurements were performed 3, 24, and 72 h after treatment of cells with the agent) showed that introduction of the test compounds induces dose-dependent inhibition of tritium label incorporation into DNA of tumor cells, *i.e.*, suppresses the DNA synthesis. This allows considering DNA as a key target of the ferrocenyl(alkyl)azole agents, which show acute cytotoxocity in high doses and antitumor effect in low doses, this effect increasing following increase in the dose.

Thus, ferrocene derivatives influence the DNA synthesis: cationic ferrocenium salts and neutral ferrocenyl alkyl azoles inhibit, while compounds containing ferrocene groups as anions activate the DNA synthesis.

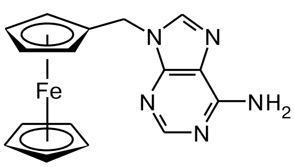
It was found[78,79] that a bulky molecule containing a planar naphthalene fragment connected to two terminal ferrocene fragments by hydrocarbon chains, diferrocenyl(naphthalene)diimide **12**, intercalates between two base pairs of double-stranded DNA (the authors used an adenine-thymine oligonucleotide dA$_{20}$dT$_{20}$ model) to give a complex that is more stable than classical intercalators. The rate constants for dissociation of this compound from double-stranded DNA (k_d 0.06 s^{-1}) and denatured, *i.e.*, single-stranded, DNA (k_d 4.20 s^{-1}) were determined; they differ by a factor of 70 (denatured DNA expels the intercalator faster than intact DNA). The authors concluded that single- and double-stranded DNA can be kinetically distinguishable by diferrocenyl(naphthalene)diimide **12**.



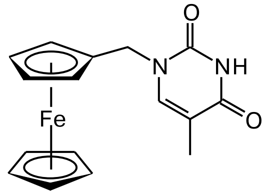
12

The intercalation properties of the proper polycyclic naphthalene compounds are well studied. In the case of ferrocene-containing naphthalene, it is the naphthalene plane that probably intercalates into DNA, but additional intercalation interactions of ferrocene fragments cannot be ruled out either, as the ligand—ligand distances in ferrocene are equal to the distances between the nearest base pairs in DNA (~0.34 nm).

Some representatives of ferrocene-modified nucleic bases. For example, *N*^{6}-(ferrocenylmethyl)adenine, when present in a very low concentration (10^{-6} mol L^{-1}), is known to suppress cell division.[49] The *in vivo* antitumor effects of 9-*N*-ferrocenyl(methyl)adenine (**13**), FcCH$_2$Ad,[77] 1-*N*-ferrocenyl(methyl)thymine (**14**), FcCH$_2$Tm,[18d] and *S*-ferrocenyl(methyl)thiopyrimidine (**15**)[18e] against animal solid tumor systems such as Ca755 carcinoma, B16 melanoma, Lewis lung carcinoma (LLC), and L1210 and P388 leukemias were studied. This showed a quite pronounced antitumor activity of the studied compounds, in particular, *S*-ferrocenyl(methyl)thiopyrimidine,[18e] which caused up to 95% inhibition of tumor growth (TGI) as compared with the control. This efficiency is rather good, being comparable with the action of well-known cytostatics, cisplatin and cyclophosphamide. Moreover, synergistic therapeutic action was observed for the combined use of, for example, ferrocenyl(methyl)thymine and cyclophosphane in experiments on Ca755 and LLC models.[18d] These results are of prime importance for the future studies of ferrocene compounds as promising drugs for tumor

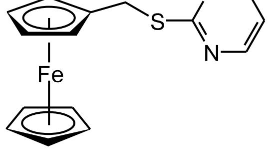


13



14

TGI 70% (2.5 mg kg^{-1} dose)
LD50 of 40 mg kg^{-1}



15

TGI 95% (20.0 mg kg^{-1} dose)

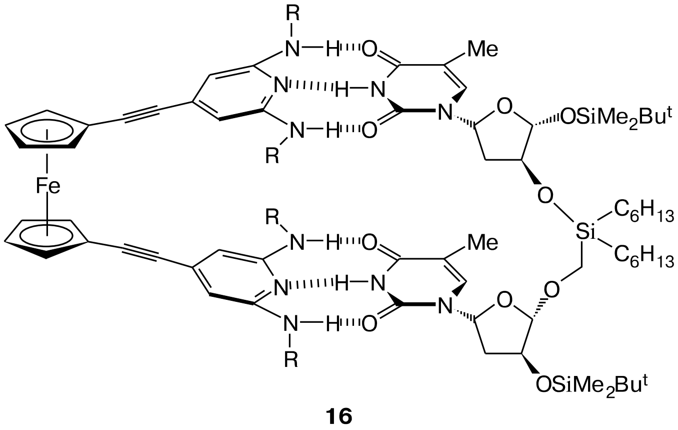
polychemotherapy. It should be emphasized that the greatest antitumor effects were attained on administration of low doses of compounds, 2.5 mg kg^{-1} for the thymine derivative, 20.0 mg kg^{-1} for Fc-thiopyrimidine derivative, which may be attributable to the absence of immune response at low doses.

Significant results were obtained in a study of the antitumor activities of 1-[1-ferrocenyl(ethyl)]benzotriazole, Fc(Et)BTr, bis(ferrocenylethyl)benzotriazolium salt, $[Fc(Et)]_2BTr^+BF_4^-$, and two ferrocenium salts, ferrocenium triiodide, $Fc^+I_3^-$, and symmetrically substituted diethylferrocenium triiodide, 1,1´-$Et_2Fc^+I_3^-$, using a subcapsular test on the material of human tumor surgery.**[18b,77]** In this experiment, 100% inhibition of the esophageal cancer growth was observed upon the use of benzotriazolium and unsubstituted ferrocenium salts and 1-[1-ferrocenyl-(ethyl)]benzotriazole; the xenotransplant regression reached 36, 30, and 16%, respectively. A highly encouraging situation were observed with respect to lung cancer of different histological types (also material from surgery). This is especially true for 1-[1-ferrocenyl(ethyl)]benzotriazole the effect of which increased considerably with increase in the dose in the dose range studied (0.5 to 4.5 mg kg^{-1}) to reach 45% of tumor regression at a total dose of 18.0 mg kg^{-1}, which is comparable with the action of cyclophosphamide, an alkylating type agent. Moreover, 1-[1-ferrocenyl(ethyl)]benzotriazole have practically never stimulated other neoplastic growth.

In 1991 a paper appeared devoted to the synthesis of, according to the authors, the first metallocene nucleosides.**[56]** It was shown in relation to L1210 leukemia that the cytotoxicity (ID_{50}) of 5-(2-ferrocenylethenyl)uridine is 2000 times lower than that of the antitumor antibiotics adriamycin, the ID_{50} inhibition dose of the ferrocene derivative was 30 μmol and that of adriamycin was 0.015 μmol. The ID_{50} inhibition dose of ferrocenylmethanol tetrachloroferrate, $(Fc^+CH_2OH)FeCl_4^-$, was 31 μmol, which barely differs from ID_{50} of ferrocenylnucleoside; the ferrocene-modified nucleoside and the simple ferrocenylmethanol salt had equal activities.

Evidence for the formation of strong complexes of ferrocene compounds with nucleotides was obtained.**[80]** While developing synthetic receptors for dinucleotides, Japanese scientists synthesized a heteroannular compound **16** based on ferrocene in which the pyridine substituents are triple-bonded to the cyclopentadienyl rings. In the molecular design of receptors for dinucleotides, the authors proceeded from the approximate equality of the distances between the cyclopentadienyl rings in ferrocene and the closest nucleic base pairs in DNA (~0.34 nm). This correspondence was first noted by Babin *et al.***[14]** The association constant K_a for the complex between the ferrocene receptor and the lipophilic analog of (thymidyl)thymidine (compound in which the phosphoric acid residue is replaced by a silicon fragment with two *n*-hexyl substituents) was determined to be $1.2 \cdot 10^5$ L mol^{-1}. The free energy change upon complexation was $\Delta G_{298} = -29.0$ kJ mol^{-1}, which was regarded**[80]** as being high for artificial receptors of thymine—thymine dinucleotide derivatives. Thus, the possibility of complexation through hydrogen bonding between the pyridine nitrogen and amino-group hydrogen of the ferrocene compound, on the one hand, and hydrogen and oxygen atoms of the nucleic bases, on the other hand, was demonstrated.

The data on the antitumor activities of ferrocene compounds are not exhausted by examples of low-molecular-mass monomeric compounds. In 1993 we studied the antitumor activities of semioxidized ferrocenylenemethylene oligomer $—(—FcCH_2—Fc^+CH_2—)—_n (PF_6)_{n/2}$, $n = 15$ (see Ref. 16a). It was found in *in vivo* experiments on the methylcholanthrene MX-11-induced tumor model that the agent reduces the mortality rate of animals. Two groups of control animals were used: the animals that were administered physiological saline and the positive control group that received ferrocenium triiodide, $Fc^+I_3^-$. Two stages can be distinguished in the kinetic dependences of the animal mortality *vs.* time: first, the mortality rate of animals is retarded (upon the administration of the oligomer, the mortality rate was 5 times lower than in the control group and 3.3 times lower than in the group receiving



16

the ferrocenium salt) and then is accelerated as compared with the control group, *i.e.*, the situation is reversed in the second stage. Thus, it was shown that the action of the ferrocenylenemethylene oligomer is ambiguous. This can be naturally explained by assuming that in the first stage, the oligomer in its initial form inhibits the tumor process. Meanwhile, metabolic transformation of the oligomer takes place as chain rupture, oxidation of nonoxidized ferrocene nuclei, and replacement of cyclopentadienyl ligands. Moreover, the released iron ions are transported to the cytoplasm and can activate the redox processes in cells. This accounts for acceleration of the tumor process. It is noteworthy that the instant where the kinetics changes (inflection point) arrives later for the oligomer than for the ferrocenium triiodide, *i.e.*, extended action of the oligomer is realized.

Ferrocene-containing oligomers with non-oxidized metal (ferrocene is covalently bonded to the polymer chain) were studied in order to elucidate the influence of the metal oxidation state on the antitumor activity of the compounds.[81—83] Water-soluble conjugates were synthesized by coupling 4-ferrocenylbutanoic acid $\mathrm{Fc(CH_2)_3COOH}$ with various polyaspartamides (asparagine $\mathrm{H_2N{-}C(O){-}CH_2{-}CH(NH_2)COOH}$ derivatives) having additionally primary amino groups for coupling. The choice 4-ferrocenylbutanoic acid as the active agent is due to low redox potential (E^0 0.172 V in aqueous ethanol[84,85]) relative the parent ferrocene (E^0 0.199 V) or other ferrocenylcarboxylic acids,[84,85] which facilitates oxidation in the biological media. Peptide-containing compounds, polyaspartamides, were used to enhance the biocompatibility and promote entering the tumor cells, since asparagine is needed for the vital activity of tumor cells, which cannot synthesize asparagine by their own. Five ferrocene-containing oligomers were tested *in vitro* on HeLa cells (epithelial cells of cancer origin).[81—83] Compounds with tertiary amino groups sensitive to protonation at physiological pH were found to have the highest cytotoxic activity, that is, they retard the cell growth to a higher extent. The lowest activity was found for poly(ethylene oxide)-containing conjugates with ferrocenylbutanoic acid fragments. Thus, like the monomers, oligomeric ferrocene compounds have antitumor action.

Works on the synthesis of ferrocene-containing peptides based on S-ferrocenylmethyl-*L*-cysteine, S-ferrocenyl(ethyl)-*D*-penicillamine, and *N*-ferrocenyl(methyl)-histidine and *N*-ferrocenyl(methyl)-*L*-tryptophane have been started.[86]

After 25 years since the first results on ferrocenium were obtained and thoroughly studied,[15,22,87] the researchers turn again to these compounds in an attempt to elucidate the mechanism of their antitumor activity. Italian authors reported an integrated approach to the activity problem.[88] They study simple neutral ferrocene compounds, in particular, ferrocenylcarboxylic (FcCOOH) and ferrocenylacetic acids ($\mathrm{FcCH_2COOH}$) and the corresponding salts (in the radical cation form) including ferrocenylcarboxylic ($\mathrm{FcCOOH]^{+\cdot}PF_6^-}$) and ferrocenylacetic acid ($\mathrm{FcCH_2COOH]^{+\cdot}PF_6^-}$) hexafluorophosphates, ferrocenium salt ($\mathrm{Fc^{+\cdot}PF_6^-}$), ferrocene as a water-soluble complex with modified β-cyclodextrin (β-CD). β-Cyclodextrin has been modified, as we suppose, to reduce its hydrophilic properties in order to obtain a more stable complex with hydrophobic ferrocene. To this end, the hydroxy groups in β-CD were converted to methoxy groups. This approach to increasing the water solubility of Fc compounds has long been known and appears quite useful as applied to the antitumor activity problem. It was found that the oxidized form of ferrocene is more active as the neutral form. Relying on these results, the authors[88a] assumed that the cytotoxic activity of the compounds does not involve their direct reaction with DNA but is associated with the ability of ferrocenium salts to generate reactive oxygen species, which react with the cell DNA and thus induce its cleavage. There is certain doubt concerning some results (for example, ESR measurements to not seem to be quite correct as no results of control experiments are presented); therefore, these conclusions are only one of the possible explanations.

To sum up, note the following facts that are known to date: 1) metallocene dihalides and ferrocenium salts as well as ferrocenyl(alkyl)azoles and Fc-polyaspartamides exhibit antitumor activities; 2) in terms of toxicity, they can be classified into three groups: $\mathrm{Cp_2MX_2}$, $\mathrm{LD_{50}} <$ < 100—250 mg kg^{-1}; $\mathrm{Fc^+X^-}$, $\mathrm{LD_{50}} \approx$ 200—450 mg kg^{-1}; FcAlkAz, the maximum tolerated dose of 630—1500 mg kg^{-1}; 3) ferrocene is accumulated in the thymus gland rich in DNA; 4) titanocene dichloride and vanadocene dichloride, as well as 1-[1-ferrocenyl(ethyl)]benzotriazole inhibit DNA synthesis but have little influence on RNA or protein synthesis; 5) mono- и diacetylferrocenium salts break the DNA double helix; 6) diferrocenyl(naphthalene)diimide intercalates between the nucleic bases of the DNA double helix. Thus, analysis of the data obtained by now indicates that

1) sandwich type bulky structures, metallocenes, show antitumor activities;

2) manifesting of this activity depends on the presence or the possibility to acquire a positive charge by the molecule;

3) for the activity to be manifested, some degree of water solubility is needed, although water-insoluble metallocenes also exert antineoplastic action.

Thus, the possibility of developing a new ferrocene-based class of drugs for treating tumor diseases is considered. Synthetic approaches have been thoroughly worked out, experimental studies of the antitumor activities of a number of agents have been performed, and some aspects of the mechanism of compound action have been studied. Presumably, the subsequent targeted synthesis of ferrocene-containing compounds, performing the set of preclinical testing of the agents would help to identify the agents that show high antitumor efficiency and an original mechanism of action and are promising for clinical trials.

This work was financially supported by the Russian Academy of Sciences (Program of the Presidium of the RAS "Fundamental Sciences for Medicine"), the Division of Chemistry and Material Sciences of the Russian Academy of Sciences (Fundamental Research Program "Medical and Biomolecular Chemistry"), and by the Russian Foundation for Basic Research (Project No. 09-03-00535).

References

1. (a) M. D. Mashkovskii, *Lekarstvennye sredstva* [*Pharmaceuticals*] Meditsina, Moscow, 1993, **2**; (b) VIDAL´ Handbook *Lekarstvennye preparaty v Rossii* [*Pharmaceuticals in Russia*], 6th edition, AstraFarmServis, Moscow, 2000; (s) D. A. Kharkevich, *Farmakologiya*, Geotar Meditsina, Moscow, 2000, p. 582 (in Russian).
2. P. N. Magee, E. Faber, *Biochem. J.*, 1962, **83**, 114.
3. K. Y. Lee, W. Lijinsky, *J. Nat. Cancer Inst.*, 1966, **37**, 401.
4. P. F. Swan, P. N. Magee, *Biochem. J.*, 1971, **125**, 841.
5. E. C. Miller, J. A. Miller, *Ann. N.Y. Acad. Sci.*, 1969, **163**, 731.
6. M. M. Vilenchik, *Zakonomernosti moleculeyarno-geneticheskogo deistviya kantserogennykh uglevodorodov* [*Characteristic Features of the Molecular Genetic Action of Carcinogenic Hydrocarbons*], Nauka, Moscow, 1977 (in Russian).
7. H. Sigel, *J. Am. Chem. Soc.*, 1975, **97**, 3209.
8. D. K. Granner, *Organization and Replication of DNA*, in R. K. Murray, D. K. Granner, P. A. Mayes, V. W. Rodwell, *Harper´s Biochemistry*, Appleton & Lange, Norwalk—Connecticut—San Mateo—California, 21nd ed., 1988.
9. E. Farber, *Cancer Res.*, 1970, **33**, 2537.
10. H. W. Thielman, *Eur. J. Biochem.*, 1976, **61**, 501.
11. M. A. Girgis, *Compt. Rend. Acad. Sci.*, 1967, **D-265**, 245.
12. Ya. G. Erenpreis, *Arkhiv patalogich. anatomii i gistologii* [*Pathology and Histology Archive*], 1972, **34**, 31 (in Russian).
13. Yu. S. Larin, *Dokl. Akad. Nauk SSSR*, 1969, **184**, 701 [*Dokl. Chem.* (*Engl. Transl.*), 1969].
14. V. N. Babin, A. V. Dubinin, P. M. Raevskii, A. F. Sviridov, A. L. Sherman, *Model´ khimicheskogo carcinogeneza (molekulyarnye aspekty)* [*Chemical Carcinogenesis Model (Molecular Aspects)*], in *Modeli. Algoritmy. Prinyatie reshenii*, Nauka, Moscow, 1979, p. 153 (in Russian).
15. (a) P. Köpf-Maier, H. Köpf, E. W. Neuse, *Angew. Chem.*, 1984, **96**, 446; *Angew. Chem., Int. Ed.*, 1984, **23**, 456; (b) P. Köpf-Maier, H. Köpf, E. W. Neuse, *J. Cancer. Res. Clin. Oncol.*, 1984, **108**, 336; (c) P. Köpf-Maier, H. Köpf, E. W. Neuse, Ger. Offen, DE, 1985, No. 3403443.
16. (a) L. V. Popova (Snegur), V. N. Babin, Yu. A. Belousov, Yu. S. Nekrasov, A. E. Snegireva, N. P. Borodina, G. M. Shaposhnikova, O. B. Bychenko, P. M. Raevskii, N. B. Morozova, A. I. Ilyina, K. G. Shitkov, *Appl. Organomet. Chem.*, 1993, **7**, 85; (b) L. V. Snegur, Yu. S. Nekrasov, V. V. Gumenyuk, N. B. Morozova, Zh. V. Zhilina, I. K. Sviridova, I. A. Rodina, N. S. Sergeeva, K. G. Shchitkov, V. N. Babin, *Ross. Khim. Zhurn.*, 1998, **42**, 178 [*Mendeleev Chem. J.* (*Engl. Transl.*), 1998, **42**, 151]; (c) Yu. S. Nekrasov, Zh. V. Zhilina, V. V. Gumenyuk, L. V. Snegur, N. S. Sergeeva, V. N. Babin, *Ross. Khim. Zhurn.*, 1997, **41**, 117 [*Mendeleev Chem. J.* (*Engl. Transl.*), 1999, **41**, 172].
17. (a) K. G. Shchitkov, L. V. Snegur, N. B. Morozova, A. I. Il´ina, V. N. Babin, V. I. Borisov, I. G. Rusakov, A. E. Snegireva, N. P. Borodina, G. M. Shaposhnikova, RF Pat. No. 2098096, 1993; (b) L. V. Snegur, V. N. Babin, N. V. Polyakova, V. I. Boev, A. F. Kolomiets, Yu. S. Nekrasov, K. G. Shchitkov, A. A. Pankratov, N. B. Morozova, A. I. Il´ina, RF Pat. No. 2089507, 1996.
18. (a) L. V. Snegur, A. A. Simenel, Yu. S. Nekrasov, E. A. Morozova, Z. A. Starikova, S. M. Peregudova, Yu. V. Kuzmenko, V. N. Babin, L. A. Ostrovskaya, N. V. Bluchterova, M. M. Fomina, *J. Organomet. Chem.*, 2004, **689**, 2473; (b) L. V. Snegur, Yu. S. Nekrasov, N. S. Sergeeva, Zh. V. Zhilina, V. V. Gumenyuk, Z. A. Starikova, A. A. Simenel, N. B. Morozova, I. K. Sviridova, V. N. Babin, *Appl. Organomet. Chem.*, 2008, **22**, 139; (c) A. A. Simenel, S. V. Samarina, L. V. Snegur, Z. A. Starikova, L. A. Ostrovskaya, N. V. Bluchterova, M. M. Fomina, *Appl. Organomet. Chem.*, 2008, **22**, 276; (d) A. A. Simenel, E. A. Morozova, L. V. Snegur, S. I. Zykova, V. V. Kachala, L. A. Ostrovskaya, N. V. Bluchterova, M. M. Fomina, *Appl. Organomet. Chem.*, 2009, **23**, 219; (e) A. A. Simenel, G. A. Dokuchaeva, L. V. Snegur, A. N. Rodionov, M. M. Ilyin, S. I. Zykova, L. A. Ostrovskaya, N. V. Bluchterova, M. M. Fomina, V. A. Rikova, *Appl. Organomet. Chem.*, 2011, **25**, 70.
19. R. Kovjazin, T. Eldar, M. Patya, A. Vanichkin, H. M. Lander, A. Novogrodsky, *FASEB J.*, 2003, **17**, 467.
20. (a) G. Jaouen, S. Top, A. Vessières, G. Leclercq, M. J. McGlinchey, *Curr. Med. Chem.*, 2004, **11**, 2505; (b) A. Vessiires, S. Top, W. Beck, E. Hillard, G. Jaouen, *Dalton Trans.*, 2006, 529; (c) M. Gormen, D. Plaïuk, P. Pigeon, E. A. Hillard, M. -A. Plamont, S. Top, A. Vessiires, G. Jaouen, *Tetrahedron Lett.*, 2010, **51**, 118.
21. K. E. Dombrovski, W. Baldwin, J. E. Sheats, *J. Organomet. Chem.*, 1986, **302**, 281.
22. P. Köpf-Maier, H. Köpf, *Chem. Rev.*, 1987, **87**, 1137.
23. J. Haiduc, C. Silvestry, *Coord. Chem. Rev.*, 1990, **99**, 253.
24. N. Motohashi, R. Meyer, S. R. Gollapudi, K. R. Bhattiprolu, *J. Organomet. Chem.*, 1990, **398**, 205.
25. V. N. Babin, P. M. Raevskii, K. G. Shchitkov, L. V. Snegur, Yu. S. Nekrasov, *Ross. Khim. Zhurn.* (*Zhurn. Ross. khim. o-va im. D. I. Mendeleeva*), 1995, **39**, 19 [*Mendeleev Chem. J.* (*Engl. Transl.*), 1995, **39**, 17].
26. (a) E. W. Neuse, *J. Inorg. Organomet. Polym. Materials*, 2005, **15**, 3; (b) X. Wu, M. L. Go, in *Metallotherapeutic Drugs and Metall-Based Diagnostic Agents: The Use of Metals in Medicine*, Eds M. Gielen and E. R. T. Tiekink, 2005, John Wiley and Sons, Ltd; (c) *Bioorganometallics: Biomolecules*, *Labeling*, *Medicine*, Ed. G. Jaouen, WILEY-VCH, Weinheim, 2006.
27. (a) N. S. Kochetkova, V. I. Boev, V. N. Babin, R. B. Materikova, L. V. Popova (Snegur), V. M. Bondareneko, A. s. SSSR 320 212, 1985, No. 1; (b) N. S. Kochetkova, V. I. Boev, L. V. Popova (Snegur), V. N. Babin, *Izv. Akad. Nauk SSSR. Ser. Khim.*, 1985, 1397 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.* (*Engl. Transl.*), 1985, **34**, 1278].
28. L. V. Popova (Snegur), V. N. Babin, R. B. Materikova, V. I. Boev, A. E. Snegireva, N. P. Borodina, G. M. Shaposhni-

kova, O. B. Bychenko, *V IUPAC Symposium on Organo-Metallic Chemistry directed towards Organic Synthesis*, OMCOS-V—Italy—Florence, 1989, pS2—28.

29. B. Rosenberg, L. Van Camp, J. E. Trosko, V. H. Mansour, *Nature*, 1969, **222**, 385.
30. T. A. K. Al-Allaf, L. J. Rashan, *Appl. Organometal. Chem.*, 1999, **13**, 63.
31. F. D. Popp, S. Roth, J. Kirbay, *J. Med. Chem.*, 1963, **6**, 83.
32. F. D. Popp, *J. Pharm. Sci.*, 1973, **62**, 679.
33. G. N. Yashchenko, A. A. Shashmurina, G. M. Anoshina, L. A. Gorelova, N. G. Evstigneeva, L. V. Alekseeva, L. B. Radina, *Khim.-Farm. Zhurn.*, 1978, **12**, 68 [*Pharm.-Chem. J.*, 1978, **12**, 68].
34. V. J. Fiorina, R. J. Dubois, S. Brynes, *J. Med. Chem.*, 1978, **21**, 393.
35. P. Köpf-Maier, *Angew. Chem. Int. Ed.*, 1979, **91**, 509.
36. P. Köpf-Maier, H. Köpf, *Z. Naturforsch.*, 1979, **34c**, 805.
37. P. Köpf-Maier, M. Leitner, R. Voigtlander, H. Köpf, *Z. Naturforsch.*, 1979, **34c**, 1174.
38. P. Köpf-Maier, M. Leitner, H. Köpf, *J. Inorg. Nucl. Chem.*, 1980, **42**, 1789.
39. P. Köpf-Maier, B. Hesse, H. Köpf, *J. Cancer. Res. Clin. Oncol.*, 1980, **96**, 43.
40. P. Köpf-Maier, A. Moorman, H. Köpf, *Eur. J. Cancer. Oncol.*, 1985, **21**, 853.
41. P. Köpf-Maier, *Proc. Sem. Tumor Xenografts in Anticancer Drug Devel.*, Milano, 1986 (cited from Ref. 22).
42. P. Köpf-Maier, P. Erkenswick, *Toxicology*, 1984, **33**, 171.
43. P. Köpf-Maier, *Toxicology*, 1985, **37**, 111.
44. P. Köpf-Maier, P. Funke-Kaiser, *Toxicology*, 1986, **38**, 81.
45. P. Köpf-Maier, *Eur. J. Clin. Pharm.*, 1994, **47**, 1.
46. M. Tacke, Book of Abstracts, *International Conference on Organometallic and Coordination Chemistry*, September 2—8, 2008, N. Novgorod, Russia, S18.
47. K. Strohfeldt, M. Tacke, *Chem. Soc. Rev.*, 2008, **37**, 1174.
48. (a) O. Oberschmidt, A.-R. Hamauske, C. Pampillun, K. Strohfeldt, N. J. Sweeney, M. Tacke, *Anti-Cancer Drugs*, 2007, **18**, 317; (b) M. Tacke, *Anti-Cancer Drugs*, 2007, **18**, 311; (c) I. Fichtmer, C. Pampillun, N. J. Sweeney, K. Strohfeldt, M. Tacke, *Anti-Cancer Drugs*, 2006, **17**, 333; (d) G. Kelter, N. Sweeney, K. Strohfeldt, H.-H. Fiebig, M. Tacke, *Anti-Cancer Drugs*, 2005, **16**, 1091.
49. S.-C. Chen, *J. Organomet. Chem.*, 1980, **202**, 183.
50. M. N. Hughes, *The Inorganic Chemistry of Biological Processes*, John Wiley and Sons, Chichester—New York—Brisbane—Toronto, 2nd ed.
51. *Ferrotseron*, USSR Ministry of Medical Industry, Moscow, 24 pp. (in English).
52. P. Köpf-Maier, H. Köpf, E. W. Neuse, US Pat. No. 4851430, 1989.
53. N. B. Morozova, L. V. Popova (Snegur), A. I. Il´ina, V. N. Babin, K. G. Shchitkov, V. I. Borisov, I. G. Rusakov, N. I. Dunyakhina, A. E. Snegireva, N. S. Sergeeva, O. I. Skotnikova, N. P. Borodina, G. M. Shaposhnikova, in *Khimioterapiya v lechenii onkologicheskikh bol´nykh* [*Chemotherapy in the Therapy of Cancer Patients*], RF Ministry of Health, Moscow, 1993, p. 91 (in Russian).
54. N. B. Morozova, L. V. Popova (Snegur), A. I. Il´ina, V. N. Babin, K. G. Shchitkov, Yu. S. Nekrasov, V. I. Borisov, I. G. Rusakov, T. K. Kulabukhova, A. E. Snegireva, V. I. Boev, N. S. Sergeeva, O. I. Skotnikova, N. P. Borodina, G. M. Shaposhnikova, RF Pat. No. 2025125, 1991 (in Russian).
55. A. Houlton, R. M. G. Roberts, J. Silver, *J. Organomet. Chem.*, 1991, **418**, 107.
56. P. Meunier, I. Ouattara, B. Gautheron, J. Tirouflet, D. Camboli, J. Besancon, F. Boulay, *Eur. J. Med. Chem.*, 1991, **26**, 351.
57. K. G. Shchitkov, L. V. Popova, N. B. Morozova, V. N. Babin, A. I. Il´ina, I. G. Rusakov, A. E. Snegireva, N. S. Sergeeva, O. I. Skotnikova, N. P. Borodina, V. I. Boev, O. B. Bychenko, G. M. Shaposhnikova, *Khimioterapiya v lechenii onkologicheskikh bol´nykh* [*Chemotherapy in the Therapy of Cancer Patients*], RF Ministry of Health, Moscow, 1993, p. 95 (in Russian).
58. L. V. Snegur, Ph.D. Thesis, A. N. Nesmeyanov Institute of Organoelement Compounds, Moscow, 1993 (in Russian).
59. G. Caldwell, M. G. Meirim, E. W. Neuse, C. E. J. van Rensburg, *Appl. Organomet Chem.*, 1998, **12**, 793.
60. M. Wenzel, J. Wu, E. Liss, E. W. Neuse, *Z. Naturforsch.*, 1988, **43c**, 963.
61. E. W. Neuse, F. Kanzawa, *Appl. Organomet. Chem.*, 1990, **4**, 19.
62. E. W. Neuse, *Polym. Adv. Technol.*, 1998, **9**, 786.
63. M. Wenzel, E. Nipper, W. Klose, *J. Nucl. Med.*, 1977, **18**, 367.
64. A. Taylor, M. Wenzel, *Naturwiss*, 1977, **18**, 273.
65. M. Wenzel, M. Scheider, E. Liss, *Z. Naturforsch.*, 1979, **34**, 670.
66. W. H. Ang, P. J. Dyson, *Eur. J. Inorg. Chem.*, 2006, 4003.
67. A. Nazarov, Ch. Hartinger, B. Keppler, P. Dyson, *Appl. Organomet. Chem.*, 2008, **22**, 300.
68. (a) M. Gras, B. Therrien, G. Süss-Fink, A. Casini, F. Edafe, P. J. Dyson, *J. Organomet. Chem.*, 2010, **695**, 1119; (b) G. Von Poelhsitz, A. L. Bogado, M. P. de Araujo, H. S. Selistre-de-Araújo, J. Ellena, E. E. Castellano, A. A. Batista, *Polyhedron*, 2007, **26**, 4707.
69. E. W. Neuse, M. C. Meirim, N. F. Bloom, *Organometallics*, 1988, **7**, 2562.
70. G. Pilloni, R. Graziani, B. Longato, *Inorg. Chim. Acta*, 1991, **190**, 165.
71. D. T. Hill, G. R. Girard, F. L. McCabe, *Inorg. Chem.*, 1989, **28**, 3529.
72. J. S. Casas, M. V. Castaño, M. C. Cifuentes, J. C. García-Monteagudo, A. Sánchez, J. Sordo, U. Abram, *J. Inorg. Biochem.*, 2004, **98**, 1009.
73. A. Rosenfeld, J. Blum, D. Gibson, A. Ramu, *Inorg. Chim. Acta*, 1992, **201**, 219.
74. H. Tamura, M. Miwa, *Chem. Lett.*, 1997, 1177.
75. N. M. Emanuel´, *Kinetika eksperimental´nykh opukholevykh protsessov* [*Kinetics of Experimental Tumor Processes*], Nauka, Moscow, 1977, 416 pp (in Russian).
76. D. A. Oparin, V. D. Makhaev, V. D. Vil´chevskaya, T. I. Zimatkina, Zh. V. Motylevich, S. M. Zimatkin, S. V. Zabrodskaya, A. I. Krylova, Yu. Yu. Gorelikova, *Khim.-Farm. Zhurn.*, 1996, **30**, 11 [*Pharm.-Chem. J.*, 1996, **30**, 11].
77. L. V. Snegur, Doctoral Thesis (Chemistry), A. N. Nesmeyanov Institute of Organoelement Compounds, Moscow, 2002.
78. S. Takenaka, Y. Uto, M. Takagi, H. Kondo, *Chem. Lett.*, 1998, 989.
79. (a) S. Takenaka, Y. Uto, H. Saita, M. Yokoyama, H. Kondo, W. D. Wilson, *J. Chem. Soc., Chem. Commun.*, 1998, 1111;

(b) S. Sato, S. Takenaka, *J. Organomet. Chem.*, 2008, **693**, 1177.

80. M. Inouye, M. Takase, *Angew. Chem., Int. Ed.*, 2001, **40**, 1746.
81. T. Smit, J. R. Snyman, E. W. Neuse, L. Bohm, C. E. van Rensburg, *Anti-Cancer Drugs*, 2005, **16**, 501.
82. M. G. Meirim, E. W. Neuse, G. Caldwell, *J. Inorg. Organometal. Polym.*, 1997, **7**, 71.
83. T. Smit, E. W. Neuse, P. Becker, R. Anderson, C. E. J. van Rensburg, *Drug Davelop. Res.*, 2006, **66**, 204.
84. N. F. Blom, E. W. Neuse, H. G. Tomas, *Transition. Met. Chem.*, 1987, **12**, 301.
85. J. C. Swarts, E. W. Neuse, G. J. Lamprecht, *J. Inorg. Organometal. Polym.*, 1994, **4**, 143.
86. S. Cowell, Zh. Zhilina, M. Kavarana, I. Alves, V. J. Hruby, 2nd International Symposium, 17th American Peptide Symposium, San Diego, California, 2001, Program and Abstracts, p. 371.
87. S. G. Ward, R. C. Taylor, P. Köpf-Maier, H. Köpf, J. Balzarini, E. De Clercq, *Appl. Organomet. Chem.*, 1989, **3**, 491.
88. (a) D. Osella, M. Ferrali, P. Zanello, F. Laschi, M. Fontani, C. Nervi, G. Cavigiolio, *Inorg. Chim. Acta*, 2000, **306**, 42; (b) G. Tabbi, C. Cassino, G. Cavigiolio, D. Colangelo, A. Ghiglia, I. Viano, D. Osella, *J. Med. Chem.*, 2002, **45**, 5786.

Received October 4 , 2010;
in revised form November 12, 2010