Research Article

Applied Organometallic Chemistry

Received: 24 April 2007 Revised: 2 November 2007 Accepted: 6 November 2007 Published online in Wiley Interscience: 23 January 2008

(www.interscience.com) DOI 10.1002/aoc.1362

Ferrocenylalkyl azoles: bioactivity, synthesis, structure

Lubov' V. Snegur[a]*, Yury S. Nekrasov[a], Nataliya S. Sergeeva[b], Zhanna V. Zhilina[a], Vera V Gumenyuk[a], Zoya A. Starikova[a], Alexander A. Simenel[a], Nataliya B. Morozova[b], Irina K. Sviridova[b] and Valery N. Babin[a]

The toxicity of ferrocenylethyl benzotriazole (1) and other ferrocene compounds including ferrocenylmethyl benzimidazoles (4,5,6,11), ferricenium salts (3,9,10) and ferrocenylmethyl adenine (7), was studied. All ferrocene complexes under investigation showed low or medium toxicities. On the basis of an earlier model of chemical carcinogenesis, the antitumor activity of ferrocenylalkyl azoles 1, 8 and ferricenium salts 9, 10 was studied *in vivo* in the so-called sub-capsular test on human tumors. This effectiveness was compared with that of cisplatin. A series of ferrocenylalkyl azoles were synthesized by interacting azoles either with α-hydroxyalkyl ferrocenes $FcC(OH)R_1R_2$ in organic solvent in the presence of aqueous HBF_4 in quantitative yields or with trimethyl(aminomethyl)ferrocene iodide in an aqueous-basic medium in good yields. The X-ray determinations of molecular and crystal structures of α-(1-benzotriazolyl)ethylferrocene (1) and α-(1-naphthatriazolyl)ethylferrocene (12) were performed. **Copyright © 2008 John Wiley & Sons, Ltd.**

Keywords: ferrocene derivatives; ferrocenylalkyl azoles; α-ferrocenylalkylation; benzimidazole; benzotriazole; X-ray crystal structure; toxicity; *in vivo* antitumor experiments; a model of chemical carcinogenesis

Introduction

Chemical aspects of biological activity play an important role in the research for new active compounds. The results of investigations, theoretical[1,2] and experimental,[3] testifying to antitumor (antineoplastic) effects of ferrocene derivatives were published in the 1980s. Kopf-Maier and co-workers[3] were the first to discover the antiproliferative efficiency of ferrocene compounds by the example of ferricenium salts. The investigations that followed included a variety of biological tests of ferricenium salts.[4] Several years previously, Babin *et al*. formulated principles for the molecular design of antitumor compounds and theoretically predicted that ferricenium salts, ferrocene derivatives with different substituents and diferrocenes could demonstrate antitumor activity.[1] During the following three decades the number of metallocene compounds with antitumor effects has been enlarged and now includes alkylferrocenes,[5] ferrocenylalkyl azoles,[6–8] macromolecular ferrocene bioconjugates[9] and ferrocene-modified cisplatin.[10] Several detailed reviews devoted to the bio-organometallic chemistry of ferrocene compounds have been published.[6,11–14] Recently, some preliminary mechanistic investigations also appeared.[15,16]

In this paper a development of our biological work[2,6–8,17] is presented. Azoles including imidazole, pyrazole and adenine, which are the central ingredients in many drugs, have been chosen as the objects for chemical modification by ferrocene. Ferrocene compounds, additionally to antitumor and antianemic[18,19] properties, also demonstrate membrane permeability,[20] and low toxicity.[2–4,8,21,22] Ferrocene-modified heterocycles and also ferricenium salts were synthesized for biological tests. Those were (Fig. 1) ferrocene derivatives of benzotriazole $FcCH(CH_3)$BTr (**1**), naphthatriazole $FcCH(CH_3)$NaphthaTr (**12**), benzimidazoles (**4, 11**) and polyfluoroimidazoles (**5** and **6**), adenine Fc-CH_2-Ad (**7**), as well as ferricenium salts-ferricenium triiodide (**9**), symmetrically substituted 1,1′-diethyl ferricenium triiodide (**10**) and 1,1′,3,3′-(tetra-*tert*.butyl)ferricenium triiodide (**3**), 1*N*-benzotriazolyl ethylferricenium tetrachloroferrate (**2**) obtained by one-electron oxidation of **1** and finally *bis*-(ferrocenylethyl)benzotriazolium tetrafluoroborate (**8**).

Acute toxicities were defined by Prozorovsky's express method[23] using the increasing doses of the substances. For those preparations where the determination of LD_{50} turned out to be impossible due to the small solubility of the complexes in water, maximum tolerated doses (MTD) were found. All studied compounds **1**–**11** belong to medium toxicity (LD_{50} 178–300 mg kg^{-1} for ferricenium salts **2, 3, 9** and benzotriazolium salt **8**) or low toxicity (MTD 400–1500 mg kg^{-1} for uncharged compounds **1, 4–7, 11**) series (Table 1). The antitumor activities of benzotriazole derivatives **1, 8** and ferricenium salts **9, 10** were studied *in vivo* using subrenal capsular assay (SCA; Table 2). The best results were shown by compound **1**. With this compound, the inhibition of tumor growth was shown to be up to 100% and then the regression achieved was 45%. These results are comparable with those of cisplatin. The X-ray structural data for 1*N*-(ferrocenylethyl)benzotridazole (**1**)

* *Correspondence to: Lubov' V. Snegur, A. N. Nesmeyanov Institute of OrganoElement Compounds, Russian Academy of Sciences, 28 Vavilov St., 119991 Moscow, Russian Federation. E-mail: snegur@ineos.ac.ru*

a *A. N. Nesmeyanov Institute of OrganoElement Compounds, Russian Academy of Sciences, 28 Vavilov St., 119991 Moscow, Russian Federation*

b *P. A. Herzen Research Institute of Oncology, 3 Botkinskiy lane, 125284 Moscow, Russian Federation*

Copyright © 2008 John Wiley & Sons, Ltd.

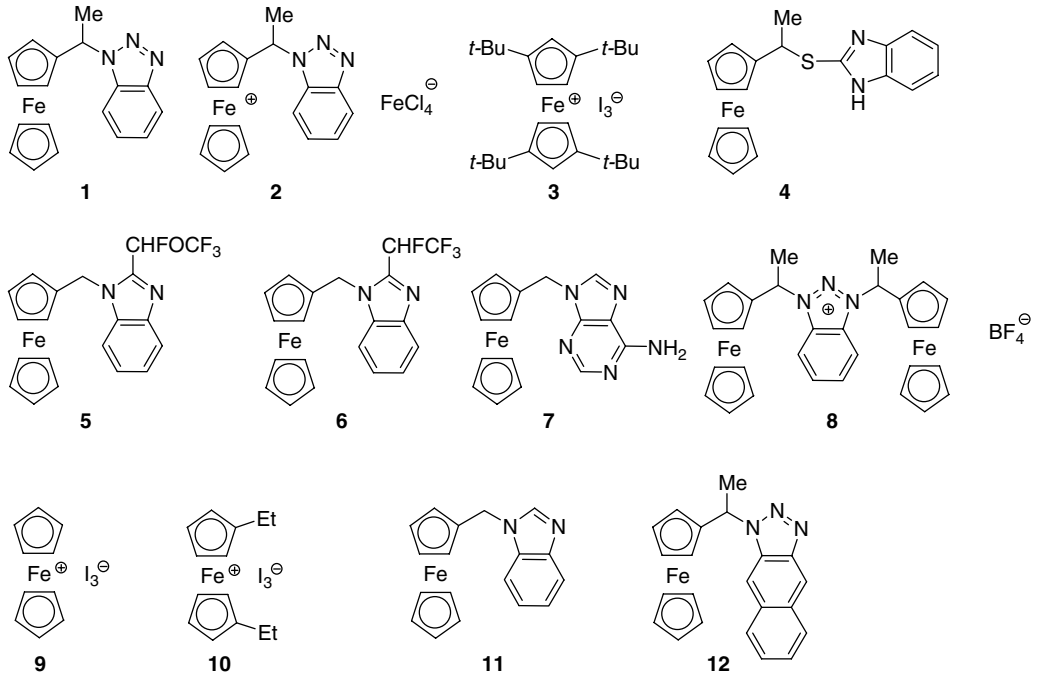


Figure 1. Azolyl alkylferrocenes, ferricenium salts and *bis*-(ferrocenylalkyl) benzotriazolium salt.

Table 1. Acute toxicities (LD_{50}) and maximum tolerated dose of ferrocene compounds

No.	Compound	LD_{50} (mg kg^{-1})	MTD (mg kg^{-1})	Synthesis [Lit.]
1	Fc-CH(CH_3)BTr	–	630	[8,25], Exp. part
2	[Fc-CH(CH_3)BTr]$^+FeCl_4^-$	178		Exp. part
3	1,1′,3,3′-^{t}Bu$_4$Fc$^+I_3^-$	216		Exp. part
4	Fc-CH_2-(2-S-BimH)	–	800	Exp. part,[34]
5	Fc-CH(CH_3)-(CHF-O-CF_3)-Bim	–	1000	[29]
6	Fc-CH_2-2-(CHF-CF_3)-Bim	–	1500	[29]
7	Fc-CH_2-Ad	–	1500	Exp. part
8	{[Fc-CH(CH_3)]$_2$BTr}$^+BF_4^-$	300		[2,35]
9	Fc$^+I_3^-$	178[33]		[2,36]
10	1,1′-Et$_2$Fc$^+I_3^-$	–	800	[2]
11	FcCH$_2$Bim		400[8]	[8]
12	Fc-CH(CH_3)NaphthaTr	Will be defined	–	Exp. part
13	Ferrocene, (η^5-C_5H_5)$_2$Fe	420		
14	Fc$^+CCl_3CO_2^-$	240[3]		[3]
15	Fc-C(O)-C_6H_4COO]$^-Na^+$	60[32,]		[37]
	Cisplatin, *cis*-Pt(NH_3)$_2Cl_2$	12–15		
	Cis-Pt($NH_2C_5H_4$)$_2Cl_2$	480		
	Cyclophosphane	182		
	5-Fluorouracile	288		

LD_{50} was found by the V. B. Prozorovsky's express method.[23]
MTD, maximum tolerated dose (for those preparations where the determination of LD_{50} turned out to be impossible due to the small solubility of the complexes in water).
LD_{50} values for cyclophosphane and 5-fluorouracil were determined in experiments with rats (literature data).
Fc-C(O)-C_6H_4COO]$^-Na^+ \cdot 4H_2O$ (ferroceronum) was used as an antianemic drug in hospitals in the Soviet Union in the 1970s until the early 1990s.

and its naphthatriazole analog **12** are presented in Figs 2 and 3). The phenomenon of migration of the ferrocenylalkyl unit from benzotriazole to adenine and the reverse one – from adenine to benzotriazole – was experimentally found. We believe that the low toxicity of ferrocene compounds can be connected with this fact.

Results and Discussion

A model

Over the period 1974–1978, V. N. Babin *et al*. elaborated a model of chemical carcinogenesis.[1] According to it, the neoplastic changes

Copyright © 2008 John Wiley & Sons, Ltd.

Table 2. Results of antitumor SCA upon human tumors (operating materials) of α-(1*N*-benzotriazolyl)ethyl ferrocene (**1**), *bis*-(α-ferrocenylethyl)benzotriazolium tetrafluoroborate (**8**), ferricenium triiodid (**9**) and 1,1′-diethylferricenium triiodid (**10**). Daily doses (total doses) were 0.5, 1.5, 3.0 and 4.5 mg kg^{-1} (2.0–18.0 mg kg^{-1})

		Treatment effectiveness		
Ferrocene Compound	Human tumor in SCA	Inhibition (%)	Regression (%)	Stimulation (%)
$FcCH(CH_3)BTr$(**1**)	NSLC		45	
	NSLC		20	
	Es.C		16	
$(FcCHCH_3)_2BTr]^+BF_4^-$(**8**)	NSLC	65		
	NSLC			13
	End.C	72		
	Es.C		36	
$Fc^+I_3^-$(**9**)	NSLC			70
	NSLC		16	
	End.C			108
	Es.C		30	
1,1′-$Et_2Fc^+I_3^-$ (**10**)	NSLC		15	
	NSLC		10	
	End.C			107
	Es.C	30		
Cisplatin	NSLC		23	

NSLC, non-small-cell lung cancer; End.C, endometrial cancer; Es.C, esophageal cancer.

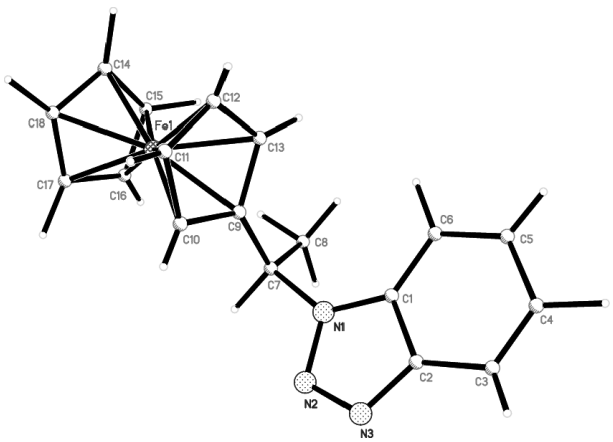


Figure 2. Molecular structure of α-(1-benzotriazolyl)ethylferrocene (**1**). Selected bond lengths (′Å) and bond angles (deg): C(7)–C(8) 1.503(6), C(7)–N(1) 1.488(6), C(7)–C(9) 1.495(6), N(1)–N(2) 1.359(5), N(2)–N(3) 1.323(6), C(9)–C(7)–N(1) 108.7(4), C(8)–C(7)–N(1) 111.2(4), C(9)–C(7)–C(8) 116.0(4), C(7)–N(1)–N(2) 118.1(4), C(7)–N(1)–C(1) 130.9(4), C(1)–N(1)–N(2) 110.8(4), N(1)–N(2)–N(3) 107.6(4).

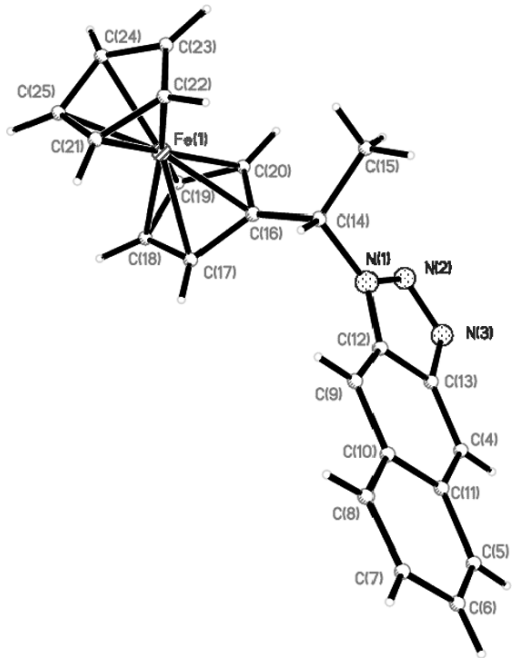


Figure 3. Molecular structure of α-(1-naphthatriazolyl)ethylferrocene (**12**). Selected bond lengths (′Å) and bond angles (deg): C(16)–C(14) 1.503(6), C(14)–C(15) 1.510(6), C(14)–N(1) 1.481(5), N(1)–N(2) 1.347(5), N(2)–N(3) 1.303(5), N(1)–C(12) 1.371(5), N(3)–C(13) 1.387(6), C(16)–C(14)–C(15) 114.7(4), C(16)–C(14)–N(1) 110.8(3), C(15)–C(14)–N(1) 109.2(4), C(14)–N(1)–N(2) 121.4(3), C(14)–N(1)–C(12) 128.3(3), N(1)–N(2)–N(3) 110.2(4), N(2)–N(3)–C(13) 107.0(4).

of cells caused by the action of various chemical carcinogens (polycyclic aromatic hydrocarbons, amino aromatic compounds, heavy metals) bring about DNA injuries and the decay of a great number of cells. At the same time, the mechanism of recombinational repair in some cells is induced. This occurs through migration and incorporation of large DNA fragments into injured segments of a genome.

The authors[1] suggested that this mechanism of reparation is inherent just in carcinogen-transformed cells and ensures not only the effective reparation of the genome, but also allows such tumor cells to overcome the so-called Hyflic threshold[24] of reproductive death. Thus the transformed cells gain immortality at a population level. The system of recombinational repair stimulates migration of mobile genetic elements and favors the heterogeneity of populations, being an important genetic marker of tumor cells. In accordance with the above model, the impeding such a 'migratory–recombination' activity in the cell genome is required

 Copyright © 2008 John Wiley & Sons, Ltd.

for the normalization of the cell behavior. It was suggested[1] that molecules are capable of being transferred into a cell nucleus and bonding to DNA just at the cleavage sites could impede the integration of free DNA elements into the genome. These molecules should have:

- bulky structural fragments able to prevent the contacts between the migrating DNA fragments and cleavage sites of the residential DNA;
- coordination centers for forming bonds at the DNA cleavage sites, the replication not being blocked.

The mono- and bi-nuclear functionalized ferrocene compounds including ferricenium salts were considered as potential antitumor drugs.[1]

We have developed a strategy for synthesis of novel metalloorganic compounds for chemotherapy of tumors directed towards the normalization of genotypic and phenotypic behavior of tumor cells.[1,2,6–8]

Ferrocenylethyl benzotriazole,[25,26] FcCH(Me)BTr (**1**), was chosen as a model of the antitumor compound. This molecule has the following fragments (Scheme 1):

(1) the hydrophilic (benzotriazolyl) group providing transport in aqueous media qne the lipophilic (ferrocenyl) moiety ensuring membrane permeability;
(2) the groups which are capable of forming ionic bonds (after oxidation to the ferricenium form) and hydrogen bonds (after protonation of the azolyl moiety) with phosphate groups at cleavage points of DNA, for example, the $\sim P-O^{-}\cdots Fc^{+}(Et)BTr\sim$ or $\sim P-O^{-}\cdots H-N^{+}\sim$ types;
(3) the plane heterocyclic ring which can intercalate between the planes of DNA nucleic bases;
(4) the bulky ferrocenyl fragment the size of which corresponds geometrically to the distance between the DNA plane nucleotides (0.34 nm);
(5) the swinging alkyl bridge for the formation of the ligand–receptor complexes.

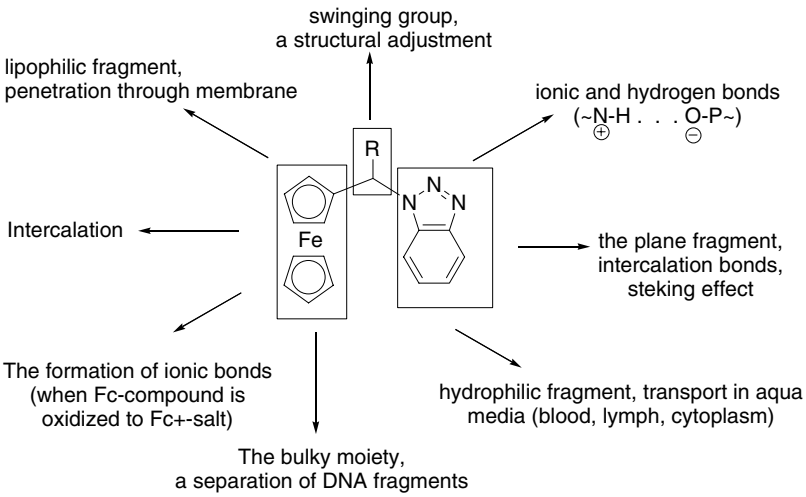
Synthesis

We used a version of the α-ferrocenylalkylation reaction of azoles (benzotriazoles, fluoro-containing benzimidazoles) in an aqueous–organic media in the presence of strong inorganic acids.[8,25,28,29] Using this method, we prepared a variety of neutral ferrocene-containing benzotriazoles, fluoro-containing benzimidazoles and diferrocenyl-substituted salt of benzotriazole. Some of these have been prepared for *in vivo* experiments (Fig. 1): 1*N*-ferrocenylethyl benzotriazole FcCH(Me)BTr (**1**), ferrocenylethyl naphthatriazole FcCH(Me)NaphthaTr (**12**) (it is known that naphthalene diimide carrying two ferrocenyl moieties at its ends forms a relatively stable complex with double-stranded DNA[30]), benzimidazole-containing ferrocenes $FcCH_2$-S-BimH (**4**) (**4** is prepared from 2-merkaptobenzimidazole; the C–S–bond is realized in **4**), polyfluoro benzimidazoles $FcCH_2Bim(CHFOCF_3)$ (**5**) and $FcCH_2Bim(CHFCF_3)$ (**6**) with fluoro-containing substituents at the 2-position of benzimidazole. Ferrocenylmethyl imidazole $FcCH_2Bim$ (**11**) and ferrocenylmethyl adenine $FcCH_2Ad$ (**7**) were prepared from heterocycle (benzimidazole, adenine) and (ferrocenylmethyl)threemethylammonium iodide $FcCH_2N(CH_3)_3I$ in boiling water. Ferricenium salts $FcCH(CH_3)BTr]^{+}FeCl_4^{-}$ (**2**), $Fc^{+}I_3^{-}$ (**9**), $Et_2Fc^{+}I_3^{-}$ (**10**) and $t\text{-}Bu_4Fc^{+}I_3^{-}$ (**3**) were obtained by one-electron oxidation of initial ferrocene compounds – **1**, ferrocene and alkylferrocenes (ferricenium salts showed DNA cleaving activity[31]). Ferrocene derivative $[FcCH(CH_3)\text{-}BTr\text{-}CH(CH_3)Fc]^{+}BF_4^{-}$ (**8**) was synthesized as compound **1** using 100% excesses of ferrocenylethanol and fluoroboric acid.

Structures

Compounds **1** (Fig. 2) and **12** (Fig. 3) are presented ferrocene fragments connected to heterocycles – benzotriazole and naphthabenzotriazole with the $-CH(CH_3)-$ bridge. Cyclopentadienyl rings in these compounds (**1, 12**) are almost parallel (the dihedral angles are 2.3 and 1.4°, respectively). Iron atoms are disposed between Cp-rings close to the unsubstituted ring in compound **1** and to the substituted one in compound **12**. Bond length Fe–C is 2.013–2.049 ´Å. The dihedral angles between the substituted Cp-rings and benzotriazole and naphthabenzotriazole planes are equal to 71.7 and 80.2°, respectively for **1** and **12**.

The exocyclic C–N(1) bonds in molecules **1** and **12** [1.488(6) and 1.481(5) ´Å] are somewhat longer than in ferrocenylmethyl imidazole (1.474 ´Å)[8] and ferrocenylmethyl benzimidazole (1.450 ´Å).[8]



Scheme 1. α-(1*N*-benzotriazolyl)ethylferrocene (R = CH_3) (**1**). Possible transportation routes and interactions in the cell[27] (reproduced with permission from *Russ. Chem. J.*).

Copyright © 2008 John Wiley & Sons, Ltd.

In vivo studies

Toxicity

By their acute toxicities, ferrocene and alkylferrocenes according to the Khodge and Sterner classification belong to either low toxicity or nontoxic compounds. Ferricenium salts belong to the medium toxicity series.[3] Preliminary *in vitro* investigations showed the low cytotoxicity of such types of ferrocene compounds as benzimidazolium and benzotriazolium bis-ferrocene-containing salts (**8**), ferricenium and *symm.*-diethylferricenium triiodides (**9,10**), and ferrocenylenemethylene oligomer.[2] As we have found, for ferrocenylalkyl azoles, a pronounced decrease in toxicity is observed as compared with the toxicity of ferrocene (even by 1.5–4 times). The lethal doses (LD_{50}) and the MTD values are given in Table 1. These data were defined for ferrocene compounds at a single intraperitoneal dose. A number of compounds have been studied comprising neutral $FcCH(CH_3)$–benzotriazole (**1**), $FcCH_2$–benzimidazole (**11**), $FcCH_2$–mercapto-benzimidazole (**4**), $FcCH_2$–polyfluoro-containing benzimidazoles (**5, 6**), adenine derivative $FcCH_2Ad$ (**7**) and salts of three types, namely, (1) ferricenium triiodide $Fc^+I_3^-$ (**9**) and symmetrically substituted alkylferricenium triiodides (**10, 3**); (2) the ferricenium salt of benzotriazole derivative $[Fc\text{-}CH(CH_3)BTr]^+FeCl_4^-$ (**2**); and, finally, the salt of benzotriazolyl cation with two ferrocenyl fragments $[Fc\text{-}CH(CH_3)\text{-}BTr\text{-}CH(CH_3)Fc]^+BF_4^-$ (**8**). The lethal doses (literature data) for some ferrocene compounds – ferrocene, (2-carboxybenzoyl)ferrocene sodium salt (ferrocerone) $FcC(O)C_6H_4CO_2Na^{\bullet}4H_2O$, ferricenium trichloroacetate $Fc^+CCl_3CO_2^-$ – and antitumor drugs – cisplatin, cyclophosphane, 5-fluorouracile – are also included in Table 1.

In vivo experiments showed that most of the investigated compounds displayed low toxicity. These ferrocene compounds were tolerated well by animals. During the examination period (14 days), they did not cause any noticeable alterations in both visual appearances of mice or the condition of their internal organs. All the compounds were found to have toxicities almost 1–2 orders of magnitude lower than those for clinically used drugs. For example, for the neutral ferrocenylalkyl azoles, the MTD values fall within the range from 630 mg kg^{-1} for $FcCH(CH_3)$-benzotriazole (**1**) to 1500 mg kg^{-1} for fluorobenzimidazole (**6**) and adenine (**7**) derivatives. The LD_{50} of cyclophosphane is 182 mg kg^{-1}; that for cisplatin is 12–15 mg kg^{-1} (Table 1).

The toxicities of ferricenium salts prepared by one-electron oxidation of the ferrocene nuclei increase approximately twofold, as compared with that of ferrocene. Ferricenium salts **2,3, 9** and **14** have LD_{50} 178–240 mg kg^{-1} excluding 1,1′-$Et_2Fc^+I_3^-$ (**10**) (MTD 800 mg kg^{-1}; cf. the LD_{50} of ferrocene – 420 mg kg^{-1}). The toxicity obviously depends on either the structure (substituent effects) of the compounds (see Table 1) or the character of the anion (see Table 1[3]). The same correlation, as we have found, takes place for azole-substituted ferrocenes. Thus, the maximum tolerated dose for neutral α-(1*N*-benzotriazolyl)ethylferrocene (**1**) is 630 mg kg^{-1}. At the same time the corresponding ferricenium salt, (1*N*-benzotriazolyl)ethylferricenium tetrafluoroborate (**2**), shows LD_{50} 178 mg kg^{-1}, i.e. it is by a factor of 3.5 more toxic than the neutral analog **1**. It should be noted that the toxicity levels of ferricenium triiodide (**9**) and $FcCH(CH_3)$–benzotriazolium tetrafluoroborate (**2**) are the same, 178 mg kg^{-1}.

In general, the modification of organic compounds by the ferrocenyl moiety, $(C_5H_5)Fe(C_5H_4)$, produces an ambiguous effect. In several cases, such modification rather significantly (even strongly) decreases their toxicity. These data were first found by Yashchenko *et al.*[21] in the investigation of antitumor drugs embichine and sarcolysine modified by introduction of the ferrocenyl group. On the other hand, the water-soluble compound *o*-carboxybenzoyl ferrocene sodium salt tetrahydrate, used for correction of pathological iron deficiently conditions (ferroceronum), is 7 times more toxic than ferrocene (LD_{50} for ferroceron 60 mg kg^{-1}[32]).

Thus the nature of both organometallic and organic moieties influences the toxicity of the whole compound. This is possibly comes from the change of lipophilic properties of the hydrophilic compounds caused by the modification with ferrocenyl moiety.

Subrenal capsular assay

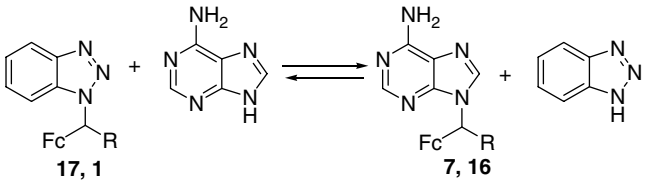
The activities of ferrocenylethyl benzotriazole $FcCH(CH_3)BTr$ (**1**), diferrocenyl benzotriazolium tetrafluoroborate $[Fc\text{-}CH(CH_3)\text{-}BTr\text{-}CH(CH_3)Fc]^+BF_4^-$ (**8**), ferricenium salts $Fc^+I_3^-$ (**9**) and 1,1′-$Et_2Fc^+I_3^-$ (**10**) in the so-called subrenal capsular assay on human tumors were studied. The groups of mice were preliminarily γ-irradiated (4.5 Gy dose), which resulted in a temporary immunodepressive effect. All groups comprised five to seven animals each. Two fragments of a human solid tumor (1×1 mm^2) were implanted under a kidney capsule of each mouse. The tested doses varied from 0.5 to 4.5. mg kg^{-1} for each mode of tumor, the total doses being between 2.0 and 18.0 mg kg^{-1}. Solutions (ethanol–physiological solution) of the ferrocene compounds were administered intraperitoneally to each group of treated animals four times every day beginning from the day after implantation of the tumors. Over six days a comparison was made between the dynamics of the tumor growth for the following groups of animals: treated, untreated (negative control) and treated with cisplatine (positive control) (Table 2). The effects were evaluated as percentages of the tumor growth inhibition or stimulation and the regression of the implantates. The index of tumor growth inhibition was calculated as $(C - T)/C$, %, where C and T are the average sizes of tumors in groups of control and treated animals, respectively.

The experiments showed that uncharged ferrocenylethyl benzotriazole (**1**) exhibited dose-dependent effectiveness with respect to two histological types of non-small-cell lung cancer (NSLC) and esophageal cancer (Es.C.) (Table 2). For NSLC, this effect increased to a marked degree with the dose enhancement in the investigated range and reached to 45% regression at a total dose of 18.0 mg kg^{-1}. Such an effect is comparable with the clinical use of cisplatin (23% regression in our experiments). Moreover, it should be noted that ferrocenylethyl benzotriazole (**1**) almost never stimulated the neoplasm growth.

All substances under investigation were ineffective towards the endometrial cancer. Ferricenium salts **9** and **10** stimulated the growth of implantates by more than 100%. Only benzotriazolium salt **8** inhibited this cancer by up to 72%.

The stomach and esophageal cancers are considered as most chemo-resistant tumors. Most of the known chemotherapeutic drugs do not have an essential antiblastomic effect on these tumors. Application of complex chemotherapy schemes leads to positive results, at best, for 30% of patients. In our experiments with esophageal cancer, when benzotriazolium salt $[Fc\text{-}CH(CH_3)]_2BTr$ (**8**), ferricenium salt $Fc^+I_3^-$ (**9**) and neutral ferrocenylethyl benzotriazole $Fc\text{-}CH(CH_3)]_2BTr$ (**1**) were used, 100% inhibition of the tumor growth was found and regression was 36, 30 and 16%, respectively.

Copyright © 2008 John Wiley & Sons, Ltd.



R = H (**7, 17**), CH_3 (**1, 16**); Fc = $(C_5H_5)Fe(C_5H_4)$

Scheme 2. Ferrocenylalkylation of adenine under the acidic conditions.

Alkylating effects of ferrocene derivatives

The exocyclic bond lengths N(1)–C(7) between the benzotriazole nitrogen atom 1N- and the bridge C-atom in $FcCH(CH_3)$-BTr (**1**) (1.488 ´Å, Fig. 2), the adenine nitrogen N(9)–C and ferrocenylalkyl bridge carbon atoms in $FcCH_2$-Ad (**7**) (1.486 ´Å)[38b] and in $FcCH(CH_3)$-Ad (**16**)[38] (1.490 ´Å) are somewhat longer than in ferrocenylmethyl benzimidazole (1.450 ´Å)[8] and than the average bond calculated for 52 N-9-substituted adenine molecules (1.459 ´Å; Cambridge Structural Database). These data are in good agreement with the increased lability of these bonds, making possible the transfer of the ferrocenylalkyl group from the benzotriazole to another substrate.[39] Moreover, benzotriazole is living group.[40] Indeed compound **1** reacts with adenine in CH_3OH–HCl mixture, affording the derivative **16**, yielding 12% (Scheme 2). Compounds **16** and **7** readily react in their turn with benzotriazole under similar conditions, affording compound **1** and its methyl analog **17,** $FcCH_2$-BTr, at 67 and 60% yields, respectively. Moreover, ferrocenylethyl adenine (**16**) reacts with benzotriazole to give ferrocenylmethyl benzotriazole (**1**) at 50% yield without acid.

It is noteworthy that 9*N*-methyladenine did not show any alkylating ability with respect to benzotriazole under acidic conditions. These facts may indicate the reversible character of the ferrocenylalkylation process.

The low toxicity of neutral ferrocene compounds can be connected with these experimental facts. We believe that the formation of labile covalent bonds between purine bases and ferrocenylalkyl fragments, such 'soft' ferrocenylalkylation, will not cause necrotic cell destruction, which normally accounts for the toxic effect of the alkylating type of antitumor cytostatics. Moreover, the ferrocenylalkylation process does not block replication completely but can hinder recombination. On the other hand, the introduction of the bulky ferrocene labels into DNA can trigger the activity of the Ca- and Mg-dependent endonucleases, the enzymes playing a crucial role at the early stages of apoptosis, i.e. the process of the pre-programmed cell destruction.

Experimental

^{1}H NMR spectra were obtained on Bruker WP-200SY and WM-250 instruments. EI mass spectra were taken on a Kratos MS-890 spectrometer at 70 eV, and IR spectra were recorded with an UR-20 spectrometer (Karl Zeiss).

Synthesis

The starting ferrocenylmethanol $FcCH_2OH$ was obtained from trimethylferrocenylmethylammonium iodide.[41] Acylation of ferrocene with acid chloride was carried out.[42] Ferrocenylethanol $FcCH(CH_3)OH$ was prepared by reduction of acyl ferrocene with lithium aluminum hydride in diethyl ether or THF.[43,29]

Naphthotriazole was synthesized from 2,3-diaminonaphthtalene and sodium nitrite in acetic acid using a modified published procedure for preparing benzotriazole.[44] EI MS, *m/z*: 169 $[M^+]$, $C_{10}H_7N_3$; 141 (relative intensity 100%) $[M - N_2]^+$; 114 $[M - N_2 - HCN]^+$.

9N-methyladenine

9*N*-methyladenine was prepared according to the literature;[45] m.p. 298–300 °C, m.p.[45] 298–300 °C.

*1N-(Ferrocenylethyl)benzotriazole (**1**)*[8,25]

$FcCH(CH_3)$BTr was obtained by the reaction of 1-ferrocenylethanol with benzotriazole in methylene dichloride in the presence of 45% fluoroboric acid at room temperature for 10 min. Yield: 93%. Yellow crystals, m.p. 131–132 °C. Anal.: C, 65.74; H, 5.24; Fe, 16.56; N, 12.06%. Calcd for $C_{18}H_{17}FeN_3$: C, 65.28; H, 5.17; Fe, 16.86; N, 12.69%. EI MS, *m/z*: 331 (relative intensity 100%) $[M^+]$; 303 $[M - N_2]^+$; 238 $[M - N_2 - Cp]^+$; 213 $[M - C_6H_4N_3]^+$; 165.5 $[M]^{2+}$. ^{1}H NMR (acetone-d_6, δ, ppm): 1.31 (d, J = 7.0 Hz, 3H, CH_3); 4.19 (s, 2H, C_5H_4); 4.23 (s, 5H, C_5H_5); 4.43 (s, 2H, C_5H_4); 5.60 (m, H, CH); 7.33–8.00 (m, 4H, Ph). IR (KBr, ν, cm^{-1}): 3090, 2985, 2950, 1500, 1460, 1381, 1320, 1276, 1240, 1170, 1150, 1109, 1008, 994, 825.

*1N-(Ferrocenylmethyl)benzotriazole (**17**)*[8,25]

$FcCH_2$BTr, was obtained in the same way from benzotriazole and ferrocenylmethanol. Yield: 96%. Yellow crystals, m.p. 134–135 °C. Anal.: C, 64.47; H, 4.90; Fe, 17.87; N, 12.89%. Calcd for $C_{17}H_{15}FeN_3$: C, 64.38; H, 4.77; Fe, 17.60; N, 13.25%. EI MS, *m/z*: 317 (relative intensity 100%) $[M^+]$; 289 $[M - N_2]^+$; 252 $[M - Cp]^+$; 158.5 $[M]^{2+}$. ^{1}H NMR (acetone-d_6, δ, ppm): 4.17 (s, 2H, C_5H_4); 4.24 (s, 5H, C_5H_5); 4.43 (s, 2H, C_5H_4); 5.72 (s, 2H, CH_2); 7.33–8.00 (m, 4H, Ph). IR (KBr, ν, cm^{-1}): 3093, 2996, 1458, 1337, 1240, 1218, 1162, 1104, 1002, 815.

*α-(1N-benzotriazolyl)ethylferricenium tetrachloroferrat (**2**)*

α-(1*N*-benzotriazolyl)ethylferricenium tetrachloroferrat (**2**) was prepared on the analogy of ferricenium tetracloroferrat.[46] The mixture of α-ferrocenylethyl benzotriazole (**1**) (1.0 mmol), hydrochloric acid (conc. 2.0 mmol), *p*-benzoquinone (0.55 mmol) and $FeCl_3$ (anhydrous; 1.1 mmol) were stirred in methylene dicloride at room temperature for 4 h. A green powder precipitate was washed with cold methylene dichloride, a mixture of diethyl ether–pentane 2 : 1 and pentane. The solvents were removed and the residue was dried *in vacuo*. Anal.: C, 34.25; H, 2.70; Cl, 33.28; Fe, 21.07%. Calcd for $C_{18}H_{17}Cl_4Fe_2N_3^{\bullet}1/3\ HCl^{\bullet}FeCl_3^{\bullet}2/3\ C_6H_4(OH)_2$: C, 33.99; H, 2.75; Cl, 33.50; Fe, 21.63%. IR (KBr, ν, cm^{-1}): 3113, 2952, 1620–1655, 1465, 1425, 1355, 1309, 1250, 1227, 1200, 1092–1041, 1013, 861, 759, 395.

Ferricenium salts

Ferricenium salts $Fc^+I_3^-$ (**9**), 1,1′-$Et_2Fc^+I_3^-$ (**10**) and 1,1′,3,3′-$^tBu_4Fc^+I_3^-$ (**3**) were synthesized by oxidation of the respective ferrocenes with benzene solution of iodine according to a standard method.[2,36]

*Ferricenium triiodide (**9**)*[2,36]

Dark-violet crystals, m.p. 169–171 °C (acetone) with decomposition. Anal.: Fe, 9.77; I, 67.11%. $C_{10}H_{10}FeI_3$. Calcd: Fe, 9.85; I, 67.17%.

Copyright © 2008 John Wiley & Sons, Ltd.

*1,1′-Diethylferricenium triiodide (**10**)*[47]

Dark-violet crystals, m.p. 49 °C with decomposition. Anal.: C, 27.03; H, 2.83; Fe, 9.05; I, 60.96%. $C_{14}H_{18}FeI_3$. Calcd: C, 26.99; H, 2.91; Fe, 8.99; I, 61.12%.

*1,1′,3,3′-Tetra(tert.buthyl)ferricenium triiodide (**3**)*

Dark green powder, m.p. 207 °C with decomposition. IR (KBr, ν, cm^{-1}): 3104, 2982, 2887, 1492, 1471, 1400, 1375, 1304, 1262, 1209, 1178, 1066, 1037, 939, 888.

*S-(Ferrocenylethyl)-2-thiobenzimidazole (**4**)*

$FcCH(CH_3)$-(2-S-BimH) was synthesized from ferrocenylethanol and 2-thiobenzimidazole as the benzotriazole analog **1**, crystallized from benzene. Yield 97%. Yellow powder, m.p. 150 °C. Anal.: H, 5.24; Fe, 14.10; N, 7.64%. $C_{19}H_{18}FeN_2S^{\bullet}0.5\,C_6H_6$. Calcd: H, 5.27; Fe, 13.92; N, 6.98%. ^{1}H NMR (acetone-d_6, δ, ppm): 1.84 (d, J = 8.3 Hz,3H,CH_3); 4.12–4.70 (m,9H,C_5H_5,C_5H_4), 6.68 (m,1H,CH), 6.90–7.28 (m,4H,Ph), 7.36 (c,1H,NH). EI MS, *m/z*: 362 [M^+]. $C_{19}H_{18}FeN_2S$.

*1-Ferrocenylmethyl-2-(trifluoromethoxyfluoromethyl)benzimidazole (**5**)*[29]

$FcCH_2$-2-(CHF-O-CF_3)Bim was prepared from ferrocenylmethanol and 2-(trifluoromethoxyfluoromethyl)benzimidazole according to the procedure for compound **1**. Yield 93%. Yellow powder, m.p. 125–127 °C. Anal.: C, 56.03; H, 3.72; F, 16.95; N, 6.16%. $C_{20}H_{16}F_4FeN_2O$. Calcd: C, 56.03; H, 3.73; F, 17.58; N, 6.48%. ^{1}H NMR (acetone-d_6, δ, ppm): 4.15 (s,2H,C_5H_4); 4.28 (s,5H,C_5H_5); 4.55 (s,2H,C_5H_4); 5.50 (s,2H,CH_2); 7.35 (m,2H,Ph), 7.60 (s,1H,CHF), 7.75 (m,2H,Ph). EI MS, *m/z*: 432 [M^+]. $C_{20}H_{16}F_4FeN_2O$.

*1-Ferrocenylmethyl-2-(α-hydrotetrafluoroethyl)benzimidazole (**6**)*[29]

$FcCH_2$-2-(CHF-CF_3)Bim was prepared from ferrocenylmethanol and 2-(α-hydrotetrafluoroethyl)benzimidazole according to the procedure for compound **1**. Yield 99%. Yellow powder, m.p. 125–127 °C. Anal.: C, 57.84; H, 4.12; Fe, 13.45; N, 6.37%. $C_{20}H_{16}F_4FeN_2$. Calcd: C, 57.72; H, 3.87; Fe, 13.42; N, 6.73%. ^{1}H NMR (acetone-d_6, δ, ppm): 4.16 (s,2H,C_5H_4); 4.23 (s,5H,C_5H_5); 4.51 (s,2H,C_5H_4); 5.56 (m,2H,CH_2); 6.78 (m,1H,CHF); 7.38 (m,2H,Ph), 7.80 (m,2H,Ph). EI MS, *m/z*: 416 [M^+]. $C_{20}H_{16}F_4FeN_2$.

*9N-(ferrocenylmethyl)adenine (**7**)*[48]

$FcCH_2$-Ad was prepared from adenine and (ferrocenylmethyl)threemethylammonium iodide $FcCH_2N(CH_3)_3I$ in boiling water over 5 h. After chromatographic resolution the yield was 40%. Yellow crystals, after crystallization from acetone m.p. 242–244 °C. ^{1}H NMR (benzene-d_6, δ, ppm): 1.95 (d, J = 6.3 Hz, 3H, CH_3); 3.92 (s, 5H, C_5H_5); 4.08–4.44 (m, 4H, C_5H_4); 5.67 (m, 1H, CH); 7.50 (s, 1H, C(8)H); 8.79 (s, 1H, C(2)H). EI MS, *m/z*: 333 [M^+]. $C_{16}H_{15}FeN_5$.

*9N-(Ferrocenylethyl)adenine (**16**)*[38]

$FcCH(CH_3)$-Ad was prepared from adenine and ferrocenylethanol in methylene dichloride in the presence of 45% fluoroboric acid as described above for ferrocenylethyl benzotriazole (**1**). Yield: 30%, yellow crystals, m.p. 194–196 °C. Anal.: C, 58.62; H, 4.96; N, 20.12%. $C_{17}H_{17}FeN_5$. Calcd: C, 58.79; H, 4.89; N, 20.17%. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.85 (d, J = 5.9 Hz,3H,CH_3) 4.08–4.37 (m, 9H, Fc); 5.54 (m, 1H, CH); 7.01 (s, 2H, NH_2); 7.98 [s, 1H, C(8)H]; 8.15 [s, 1H, C(2)H]. EI MS, *m/z*: 347 [M^+]. $C_{17}H_{17}FeN_5$.

*1,3-Bis(α-ferrocenylethyl)benzotriazolium tetrafluoroborate (**8**)*

1,3-Bis(α-ferrocenylethyl)benzotriazolium tetrafluoroborate (**8**)[2,35] was obtained as compound **1** using twofold excesses of ferrocenylethanol and fluoroboric acid, m.p. 117–119 °C (with dec.), m.p.[35] 117–119 °C. Anal.: H, 4.57; N, 7.27; Fe, 17.36%. $C_{30}H_{30}BF_4Fe_2N_3$. Calcd: H, 4.79; N, 6.66; Fe, 17.70%. ^{1}H NMR (acetone-d_6, δ, ppm): 2.26 (d, J = 7.1 Hz, 6H, $2CH_3$) 4.15 (c, 10H, $2C_5H_5$); 4.25–4.60 (m, 8H, $2C_5H_4$); 5.64 (m, 2H, CH); 8.00–8.38 (m, 4H, Ph).

*α-(1-Naphthatriazolyl)ethylferrocene (**12**)*

α-(1-Naphthatriazolyl)ethylferrocene (**12**) was obtained by the reaction of 1-ferrocenylethanol with naphthatriazol in methylene dichloride in the presence of 38% fluoroboric acid at room temperature for several minutes, as described above for ferrocenylethyl benzotriazole (**1**). The crude product was purified by column chromatography: Al_2O_3, Brockman II neutral, eluent CH_2Cl_2–benzene 1 : 3. The solution was concentrated and the product was precipitated by hexane. Yield 73%. Orange crystals, m.p. 154–155 °C. Anal.: C, 69.45; H, 4.87; N, 10.99%. Calcd for $C_{22}H_{19}FeN_3$: C, 69.29; H, 4.99; N, 11.02%. EI MS, *m/z*: 381 [M^+]; 353 [$M - N_2$]$^+$; 288 [$M - N_2 - Cp$]$^+$; 213 [$M - C_{10}H_6N_3$]$^+$; 212 (relative intensity 100%) [$M - C_{10}H_7N_3$]$^+$; 169 [$C_{10}H_7N_3$]$^+$. ^{1}H NMR ($CDCl_3$, δ, ppm): 2.10 (d, J = 7.1 Hz, 3H, CH_3); 4.15–4.42 (m, 9H, Fc); 6.21 (m, H, CH); 7.42 (m, 2H, Naphtr); 7.82 (s, 1H, Naphtr); 7.88 (m, 1H, Naphtr); 8.01 (m, 1H, Naphtr); 8.60 (s, 1H, Naphtr). IR (KBr, ν, cm^{-1}): 3100, 3005, 2960, 1642, 1590, 1514, 1469, 1408, 1387, 1324, 1270, 1248, 1111, 1080, 980, 858, 825, 685.

*Interaction of 9N-(ferrocenylethyl)benzotriazole (**1**) with adenine*

To a solution of 0.166 g (0.5 mmol) ferrocenylethyl benzotriazole (**1**) in 3 ml methanol were added a solution of 0.135 g (1.0 mmol) adenine in 6 ml H_2O and 0.22 ml hydrochloric acid (conc.). The resulting mixture was boiled for 2.5 h; after neutralization by 10 ml 50% KOH the reaction mixture was extracted with diethyl ether (3 × 20 ml), organic fraction was separated and a solvent was removed. The solid was chromatographied on column with $Al_2O_3^{\bullet}$ neutral, Brokman II. A mixture of starting ferrocenylethyl benzotriazole (**1**), vinylferrocene and its dimer was eluted by diethyl ether. EI MS, *m/z*: 331, 212, 424 accordingly.

The yellow zone was eluted by methanol. After removing a solvent the yellow solid was isolated, *9N-*(α-ferrocenylethyl)adenine (**16**). Yield 0.021 g (12%), m.p. 194–196 °C, EI MS, *m/z*: 347 [M^+]. $C_{17}H_{17}FeN_5$. ^{1}H NMR (benzene-d_6, δ, ppm): 1.95 (d, J = 6.3 Hz, 3H, CH_3); 3.92 (s, 5H, C_5H_5); 4.08–4.44 (m, 4H, C_5H_4); 5.67 (m, 1H, CH); 7.50 [s, 1H, C(8)H]; 8.79 [s, 1H, C(2)H].

*Variant A: interaction of benzotriazole with 9N-(ferrocenylmethyl)adenine (**7**)*

A 0.167 g (0.5 mmol) aliquot of *9N-*(ferrocenylmethyl)adenine (**7**) was dissolved in 5 ml CH_3OH under heating, then a solution of 0.06 g (0.5 mmol) benzotriazole in 3 ml H_2O and

Copyright © 2008 John Wiley & Sons, Ltd.

0.18 ml hydrochloric acid (conc.) was added. The resulting mixture was boiled for 2.5 h; after neutralization by 5 ml 10% water solution KOH the product was extracted with 10 ml Et_2O. The organic layer was separated, the solvent was removed, then the solid was washed with hexane (3 × 5 ml) and dried. 1*N*-(Ferrocenylmethyl)benzotriazole was prepared, yield 0.10 g (60%), m.p. 133–135 °C, EI MS, *m/z*: 317 $[M]^+$. $C_{17}H_{15}FeN_3$.

*Interaction of 9N-(ferrocenylethyl)adenine (**16**) with benzotriazole*

Interaction of 9*N*-(ferrocenylethyl)adenine (**16**) with benzotriazole was carried out as described for ferrocenylmethyl adenine (**7**) from 0.174 g (0.5 mmol) 9*N*-(α-ferrocenylethyl)adenine and 0.06 g (0.5 mmol) benzotriazole in the presence of 0.18 ml hydrochloric acid, obtaining 1*N*-(α-errocenylethyl)benzotriazole, yield 0.11 g (67%), m.p. 131.5–132 °C, EI MS, *m/z*: 331 $[M]^+$. $C_{18}H_{17}FeN_3$.

*Variant B: **i**nteraction benzotriazole with 9N-(ferrocenylethyl) adenine (**16**)*

Interaction benzotriazole with 9*N*-(ferrocenylethyl)adenine (**16**) was carried out as described above without hydrochloric acid. After boiling the mixture was treated with 10 ml methylene dichloride, the organic layer was separated, solvent was removed, then the solid was washed with hexane (3 × 5 ml) and dried. Ferrocenylethyl benzotriazole was prepared at yield 50%, m.p. 131.5–132 °C, EI MS, *m/z*: 331 $[M]^+$. $C_{18}H_{17}FeN_3$.

Interaction of 9N-methyladenine with benzotriazole under acidic conditions

Interaction of 9*N*-methyladenine with benzotriazole under acidic conditions was carried out as described in variant A. The crude product was analyzed by EI MS, *m/z*: 133 $[M]^+$. $C_7H_7N_3$ corresponding to 1*N*-methylbenzotriazole was not found in the solid.

Structure

Crystal data for compounds **1** and **12** are shown in Figs 2 and 3 and Table 3. Single-crystal X-ray diffraction experiments were carried out with a CAD4 Enraf–Nonius diffractometer, using graphite monochromated Mo-K_α radiation at 293 K; no absorption correction was applied.

The structure was solved by direct method and refined by the full-matrix last-squares technique for nonhydrogen atoms in the anisotropic approximation. All H atoms were placed in the geometrically calculated positions and included in the refinement using the riding model approximation with Uiso(H) = 1.2 Ueq(C) for the methyne and Uiso(H) = 1.5 Ueq(C) for methylene and methyl groups, where Ueq(C) is the equivalent isotropic temperature factor of the carbon atom bonded to the corresponding H atom. All calculations were carried out on IBM PC using SHELXTL program.[49]

Toxicity

For the assessment of toxicity intact DBA and C57/Bl mice were used. Ferrocene compounds were dissolved in DMSO or ethanol (10 mg cm^{-3}) and diluted with physiological solution to give a final range of concentrations. Compounds were administered intraperitoneally. The LD_{50} was found by V. B. Prozorovsky's express method.[23] Eight groups of animals were used in each experiment, three mice in each group on the dose. The experimental dose interval was 5.0-1500 mg kg^{-1}. MTD values were found for those compounds where the determination of LD_{50} turned out to be impossible due to the small solubility of the complexes in water or physiological solution.

Table 3. Crystallographic data for crystal structure determinations **1** and **12**

	FcCH(CH$_3$)BTr, **1**	FcCH(CH$_3$)NaphthaTr, **12**
Formula	$C_{18}H_{17}FeN_3$	$C_{22}H_{19}FeN_3$
Formula weight	331.20	381.25
Crystal appearance	Light yellow plate	Orange prism
Crystal size (mm^3)	0.50 × 0.40 × 0.25	0.50 × 0.40 × 0.20
Crystal system	Monoclinic	Monoclinic
Space group	$P4_32_12$	$P2_1/c$
a (´Å)	8.9107(8)	10.188(3)
b (´Å)	8.9107(8)	10.917(3)
c (´Å)	30.742(4)	14.489(4)
β (deg)	90	94.93(2)
V (´Å^3)	2440.9(4)	1605.5(8)
Z	4	4
D_{calc} (g cm^{-3})	1.497	1.416
μ (mm^{-1})	1.218	0.939
F (000)	1144	712
θ_{max} (deg)	58	50
Number of reflections	18 906	3031
Independent reflections	3218	2795
R_{int}	0.0557	0.0428
Number of parameters	163	208
*R*1 ($I > 2\sigma(I)$)	0.0515 (2517)	0.0493 (1969)
*wR*2	0.1298	0.1220
GOF	0.992	1.035
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e ´Å^{-3})	1.503/−0.317	0.414/−0.248

Supplementary material

Crystallographic data and refinement parameters for compounds **1** and **12** are presented in Table 3. Atomic coordinates are available from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, deposition numbers: CCDC-635 472 (compound **1**) and 635 473 (compound **12**).

Acknowledgments

This work has been partially supported by The Scientific Training Center of Biomedical Chemistry (grant no A0078, the Federal Target Program 'Integration'), the Russian Academy of Sciences Presidium Programs 'Fundamental Sciences – for Medicine' and 'Support for Young Scientists'. L.V.S. wishes to thank Dr Olga I. Skotnikova and Dr Irina V. Storozhenko for carrying out the subrenal capsular assay, the Scientific Research Firm 'Ultrasan and Mr Dmitriy G. Genzel for assistance, as well as Dr Tatiyana A. Belousova and Dr Yury I. Lyakhovetsky for their kind help in editing the manuscript.

 Copyright © 2008 John Wiley & Sons, Ltd.

References

[1] Babin VN, Dubinin AV, Raevskii PM, Sviridov AF, Sherman AL. Model of chemically-induced carcinogenesis. In *Modeli. Algoritmy. Prinyatie Reshenii*. Nauka: Moscow, 1979; pp. 153–167.

[2] Popova (Snegur) LV, Babin VN, Belousov YuA, Nekrasov YuS, Snegireva AE, Borodina NP, Shaposhnikova GM, Bychenko OB, Raevskii PM, Morozova NM, Ilyina AI, Shitkov KG. *Appl. Organometal. Chem.* 1993; **7**: 85.

[3] (a) Kopf-Maier P, Kopf H, Neuse EW *Angew. Chem.* 1984; **96**: 446; *Angew. Chem. Int. Edn* 1984; **23**: 456; (b) Kopf-Maier P, Kopf H, Neuse EW. *J. Cancer Res. Clin. Oncol.* 1984; **108**: 336; (c) Kopf-Maier P, Kopf H, Neuse EW, *Ger. Offen, DE*, 3403443, 1985.

[4] (a) Kopf-Maier P, Kopf H *Chem. Rev.* 1987; **87**: 1137; (b) Ward SG, Taylor RC, Kopf-Maier P, Kopf H, Balzarini J, De Clercq E *Appl. Organomet. Chem.* 1989; **3**: 491; (c) Neuse EW, Kanzawa F. *Appl. Organomet. Chem.*, 1990; **4**: 19.

[5] (a) Wenzel M, Wu J, Liss E, Neuse EW *Z.Naturforsch.*, 1988; **43c**: 963; (b) Neuse EW, Kanzawa F *Appl. Organomet. Chem.* 1990; **4**: 19; (c) Houlton A, Roberts RMG, Silver J. *J. Organomet. Chem.* 1991; **418**: 107.

[6] Babin VN, Raevskii PM, Shitkov KG, Snegur LV, Nekrasov YuS. *Mendeleev Chem. J.* 1995; **39**: 17.

[7] (a) Snegur LV, Babin VN, Polyakova NV, Boev VI, Kolomiets AF, Nekrasov YuS, Shitkov KG, Pankratov AA, Morozova NB, Ilyina AI, Iron complex compounds – ferrocenylalkyl polyfluoro benzimidazoles possessed of antitumor activity. Russian Federation Patent 2089507, 1996; (b) Shitkov KG, Snegur LV, Morozova NB, Ilyina AI, Babin VN, Borisov VI, Rusakov IG, Snegireva AE, Borodina NP, Shaposhnikova GM, Means suppressing the growth of cancer cells. Russian Federation Patent 2098096, 1993.

[8] Snegur LV, Simenel AA, Nekrasov YuS, Morozova EA, Starikova ZA, Peregudova SM, Kuzmenko YuV, Babin VN, Ostrovskaya LA, Bluchterova NV, Fomina MM. *J. Organomet. Chem.* 2004; **689**: 2473.

[9] (a) Caldwell G, Meirim MG, Neuse EW, van Rensburg CEJ *Appl. Organomet Chem.* 1998; **12**: 793; (b) Neuse EW. *Polym. Adv. Technol.* 1998; **9**: 786.

[10] (a) Neuse EW, Meirim MG, Bloom NF *Organometallics* 1988; **7**: 2562; (b) Al-Allaf TAK, Rashan LJ *Appl. Organometal. Chem.* 1999; **13**: 63; (c) Henderson W, Alley SR. *Inorg. Chim. Acta* 2001; **322**: 106.

[11] Zatsepin TS, Andreev SYu, Hianik T, Oretskaya TS. *Russ. Chem. Rev.* 2003; **72**: 17.

[12] van Staveren DR, Metzler-Nolte N. *Chem. Rev.* 2004; **104**: 5931.

[13] Neuse EW. *J. Inorg. Organomet. Polym. and Mater.* 2005; **15**: 3.

[14] Kraatz HB. *J. Inorg. Organomet. Polym. and Mater.* 2005; **15**: 83.

[15] Osella D, Ferrali M, Zanello P, Laschi F, Fontani M, Nervi C, Cavigiolio G. *Inorg. Chim. Acta* 2000; **306**: 42.

[16] Tamura H, Miwa M. *Chem. Lett.* 1997; 1177.

[17] Popova (Snegur) LV, Babin VN, Materikova RB, Boev VI, Snegireva AE, Borodina NP, Shaposhnikova GM, Bychenko OB, in *V IUPAC Symposium on OrganoMetallic Chemistry Directed Towards Organic Synthesis*, OMCOS-V, Florence, 1989; pp. S2–28.

[18] (a) Nesmeyanov AN, Bogomolova LG, Kochetkova NS, Vilchevskaya VD, Palitsyn NP, USSR Patent 263807, 1966;, (b) Nesmeyanov AN, Bogomolova LG, Kochetkova NS, Vil'chevskaya VD, Palitsyn NP, Gorelikova YuYu, Andrianova IG, Belozerova OP, Syundyukova VKh, (2-Carboxybenzoyl)ferrocene sodium salt. British Patent 1320046, 1973 [*Chem. Abstr.* **1973**, *79*, 78966].

[19] Nesmeyanov AN, Bogomolova LG, Andrianova IG, Vilchevskaya VD, Kochetkova NS. *Khim.-Farm. Zh.* 1972; **6**(4): 61.

[20] Ward RJ, Dexter D, Florence A, Aouad F, Hinder R, Jenner P, Crichton RR. *Biochem. Pharmac.* 1995; **49**: 1821.

[21] Yashchenko GN, Shashmurina AA, Gorelova GM, Evstigneeva NG, Alekseeva LV, Radina LB. *Khim.-Farm. Zh.*, 1978; **12**: 68.

[22] Oparin DA, Mahaev VD, Vilchevskaya VD, Zimatkina TI, Motilevich ZhV, Zimatkin SM, Zabrodskaya SV, Krilova AI, Gorelikova YuYu. *Khim.-Farm. Zh.* 1996; **30**(2): 11.

[23] Prozorovskiy VB, Prozorovskaya MP, Demchenko VM. *Pharmac. Toxicol.* 1978; **41**(4): 497.

[24] Hyflic E. *Genetics* 1965; **51**(1): 48.

[25] Kochetkova NS, Boev VI, Popova LV, Babin VN. *Bull. Acad. Sci. USSR, Div. Chem. Sci.* 1985; **34**: 1278.

[26] Simenel AA, Kuzmenko YuV, Morozova EA, Ilyin MM, Gun'ko IF, Snegur LV. *J. Organomet. Chem.* 2003; **688**: 138.

[27] Snegur LV, Nekrasov YuS, Gumenyuk VV, Zhilina ZhV, Morozova NB, Sviridova IK, Rodina IA, Sergeeva NS, Shitkov KG, Babin VN. *Russ. Chem. J.* 1998; **42**: 178.

[28] Kochetkova NS, Boev VI, Babin VN, Materikova RB, Popova (Snegur) LV, Bondarenko VM, USSR Patent 1320212, 1985.

[29] Snegur LV, Boev VI, Nekrasov YuS, Ilyin MM, Davankov VA, Starikova ZA, Yanovsky AI, Kolomiets AF, and Babin VN. *J. Organomet. Chem.* 1999; **580**: 26.

[30] Takenaka S, Uto Y, Saita H, Yokoyama M, Kondo H, Wilson D. *Chem. Commun.* 1998; 1111.

[31] Tamura H, Miwa M. *Chem. Lett.* 1997; 1177.

[32] (a) Ferroceron , *Prospect of Medical Industry of the USSR*, Ferroceron Moscow.; (b) Mashkovsky MD. *Medicine* 1993; **2**: 150 (in Russian).

[33] Morozova NB, Popova (Snegur) LV, Ilyina AI, Babin VN, Shitkov KG, Nekrasov YuS, Borisov VI, Rusakov IG, Kulabuhova TK, Snegireva AE, Boev VI, Sergeeva NS, Skotnikova OI, Borodina NP, Shaposhnikova GM, Means possessed of antitumor activity. Russian Federation Patent 2025125, 1994.

[34] Snegur LV, Candidate Thesis in Chemical Sciences, A.N. Nesmeyanov Institute of OrganoElement Compounds, Russian Academy of Sciences, Moscow, 1993.

[35] Snegur LV, Boev VI, Babin VN, Dzhafarov MKh, Batsanov AS, Nekrasov YuS, Struchkov YuT. *Russ. Chem. Bull.* 1995; **44**: 537.

[36] Nesmeyanov AN, Yurjeva LP, Materikova RB, Hetnarski B. *Izv. Akad. Nauk SSSR. Ser. Khim.* 1965; **4**: 731.

[37] Nesmeyanov AN, Bogomolova LG, Kochetkova NS, Vilchevskaya VD, Palitsyn NP, Gorelikova JJ, Andrianova IG, Belozerova OP, Sjundjukova VK, Process for preparing the sodium salt of *o*-carboxybenzoyl ferrocene. US Patent 3957841, 1976.

[38] (a) Zhilina ZhV, Gumenyuk VV, Nekrasov YuS, Babin VN, Snegur LV, Starikova ZA, Yanovsky AI. *Russ. Chem. Bull.* 1998; **47**: 1781; (b) Zhilina ZhV, Candidate Thesis in Chemical Sciences, A.N. Nesmeyanov Institute of OrganoElement Compounds, Russian Academy of Sciences, Moscow, 1999.

[39] Gumenyuk VV, Zhilina ZhV, Nekrasov YuS, Babin VN, Belousov YuA. *Russ. Chem. Bull.* 1997; **46**: 168.

[40] Katritzky AR, Lan X. *Chem. Soc. Rev.* 1994; **23**: 363.

[41] Lindsay JK, Hauser CR. *J. Org. Chem.* 1957; **22**: 355.

[42] (a) Weliky N, Gould ES *J. Amer. Chem. Soc.* 1957; **79**: 2742; (b) Rausch M, Vogel M, Rosenberg H. *J. Org. Chem.* 1957; **22**: 903.

[43] Arimoto FS, Haven AS. *J. Am. Chem. Soc.* 1955; **77**: 6295.

[44] *Organic Syntheses. An Annual Publication of Satisfactory Methods for the Preparation of Organic Chemicals*, Wiley: Chichester, 1940; Vol. 20, p. 16.

[45] Benoit RL, Boulet D, Seguin L, Frechette M. *Can. J. Chem.* 1985; **63**: 1228.

[46] Nesmeyanov AN, Perevalova EG, Jurjeva LP. *Chem. Ber.* 1960; **93**: 2729.

[47] Nesmeyanov AN, Kochetkova NS, Materikova RB, Palitsyn NP, Ksenzenko VI, Soboleva TS. *Zh. Org. Khim.* 1973; **9**: 378 (in Russian).

[48] Chen SC. *J. Organomet. Chem.* 1980; **202**: 183.

[49] Sheldrick GM, SHELXTL, version 5.10, Structure Determination Software Suite, Bruker AXS, Madison, WI, 1998.

 Copyright © 2008 John Wiley & Sons, Ltd.