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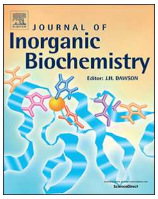

Bis-cations with two 2,3-diferrocenylcyclopropenium fragments stabilized with diamino-alkanes: Synthesis and cytotoxic activity

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ABSTRACT

Bis-cations with two 2,3-diferrocenylcyclopropenium fragments **3a–d,** and the *cis*-2-(1,2-diferrocenylvinyl)-2-imidazolinium tetrafluoroborates **4a, d** or the *cis*-2-(1,2-diferrocenylvinyl)-3,4,5,6-tetrahydropyrimidin-2-ium tetrafluoroborates **4b, c** were obtained by interactions of 2,3-diferrocenyl-1-ethoxycyclopropenium tetrafluoroborate **1** with *bis*-1,4-*N*,*N*-(**2a**, **d**) or *bis*-1,5-*N*,*N*-(**2b**, **c**) nucleophiles. The reactions of **3a–d** with sodium azide proceed with high regioselectivity, forming tetraferrocenyl-substituted compounds: *N*,*N*′-*bis*-(4′,6′-diferrocenyl-1′,2′,3′-triazin-5′-yl)-piperazine **5a**, *N*,*N*′-*bis*-(4′,6′-diferrocenyl-1′,2′,3′-triazin-5′-yl)-*N*,*N*′-dialkyl-1,3- or 1,2-alkanediamines **5b–d**. Sodium hydrogencyanamide reacts with **3a–d** to form *N*,*N*′-*bis*-(1′-aza-1′-cyano-3′,4′-diferrocenyl-1′,3′-butadien-2′-yl)-piperazine **6a**, *N*,*N*′-dialkyl-1,3- or 1,2-alkanediamines **6b–d** and *N*-(1′-cyano-3′,4′-diferrocenyl-1′-aza-1′,3′-butadien-2′-yl)-*N*,*N*′-dialkyl-alkanediamines **7a–d**. The characterization of new compounds was done by IR, ^{1}H and ^{13}C NMR spectroscopy, mass-spectrometry, elemental analysis, and X-ray diffraction analysis only for the compounds **4b**, **4d**, and **7a**. The biological activity of compounds **5a**, **6a**, **6b**, **6c** was assessed regarding anticancer activity against U-251, K-562, SKLU-1, HCT-15, and MCF-7 cell lines. All tested compounds showed good activity but compounds **6a** and **6b** had the best anticancer activity against U-251 (human glioblastoma) and SKLU-1 (human lung adenocarcinoma) cultures.

1. Introduction

Studies of the chemical properties of diferrocenylcyclopropenium salts revealed their ability to react with *C*-, and *N*-nucleophiles with retention of the small ring in the products, which are difficult to obtain through other synthetic methodologies [1–3]. Intramolecular transformations of the tetrasubstituted 2,3-diferrocenyl-1,1-dialkylamino-cyclopropenes [2,3], generated in the reactions of 1-dialkylamino-2,3-diferrocenylcyclopropenium tetrafluoroborates with *C*- and *N*- nucleophiles, served as the basis for the development of new synthetic procedures. Introduction of the 1,2-diferrocenylcyclopropene fragment, as the three-carbon building block, to prepare long-chain conjugated systems [4,5] as carbo- and heterocyclic compounds [5,6] opened an important category of new materials. These diferrocenyl-substituted compounds are characterized to present magnetic behavior, superconductivity, nonlinear optical effects, biological activity and so on [7–13]. So far, synthesis of *poly*-(1-amino-2,3-diferrocenyl-cyclopropenium) carbocations and their reactions with *C*- and *N*- nucleophiles have not been studied. [14] At the same time, the introduction of *poly*ferrocenyl- and *poly*nitrogen-containing fragments into unsaturated organic compounds or heterocyclic systems is of particular interest given their applications in pharmacology, electrochemistry, nonlinear optics, macromolecular chemistry, and more.

Especially, the *poly*ferrocenyl systems with conjugated spacer groups exhibit multielectron redox chemistry and are of particular interest owing to their unpaired characteristics. The biological potential of ferrocene (Fc) compounds has been demonstrated in a variety of applications. The antitumor (anti-cancer) effects of ferrocenyl derivatives, published in the 1980s [15]. Ferrocene compounds are a promising scaffold for new medicinal therapies due to advantages that include cost, stability, cell membrane permeability, and electrochemical behavior. The therapeutic application of ferrocene is currently an active area of research with many reports showing its activity, *in vivo* and *in vitro*, as antitumor [16], antimalarial and antifungal agent [17]. The mechanism of the antiproliferative effect of ferrocenyl derivatives is not fully understood. Since they generate reactive oxygen species (ROS), one of the possible explanations for their activity is based on the reversible cellular oxidation of the ferrocene moiety to ferrocenium ion accompanied by the formation of hydroxyl radicals that damage of DNA [18].

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In this work, we focus on the synthesis of a new *bis*-(1-amino-2,3-diferrocenylcyclopropenium)-carbocation as the first example of *poly*-cations of the diarylcyclopropeniums, and its evaluation in the reactions with *N*,*N*-nucleophiles. Also, we present the results of the antitumoral activity of the compounds **5a**, **6a–c** and the electrochemical behavior of the *poly*amino-tetraferrocenyl-substituted compounds **3a**, **5a**, **b**, **d**, **6a–c**, and **7a** is presented.

2. Results and discussion

2.1. Chemistry

The methodology of the synthesis of 1-dialkylamino-2,3-diferrocenylcyclopropenium tetrafluoroborates [19] has been published. In reactions with secondary *bis*-amines **2a–d**, an excess of compound **1** produce *bis*-tetrafluoroborates of 1,4- or 1,5-*N*,*N′*-dialkyl-*N*,*N′*-*bis*-(2′,3′-diferrocenylcyclopropenium-1′-yl)-alkanediamines **3a–d** (60–68%) (the precipitated salts were filtrated, washed with anhydrous ether, and dried *in vacuo*). The products 1,3-dialkyl-2-(1,2-diferrocenylvinyl)-2-imidazolinium **4a, d** (17, 21%, respectively), and 3,4,5,6-tetrahydropyrimidin-2-ium tetrafluoroborates **4b, c** (20, 18%, respectively) were filtrated and were separated by column chromatography (Al_2O_3, Brockmann activity III) (Scheme 1). The salts **3a–d** and **4a–d** are sedimented as red or violet substances. The dried compounds **3a–d** are rather stable under standard conditions.

The *bis*-cations **3a–d** are sparingly soluble in $CHCl_3$, CH_2Cl_2, $(CH_3)_2CO$. These ferrocenyl compounds rapidly decompose in solvents as CH_3CN, $(CH_3)_2SO$, that precludes their characterization by NMR spectroscopy. However, the structures of the *bis*-cations **3a–d** and the products of their transformations (**4a–d**, **5a–d**, **6a–d**, **7a–d**, and **8**) were confirmed by IR, ^1H and ^{13}C NMR, spectroscopy, elemental analysis, and mass spectrometry.

The parameters of the ^1H NMR spectra (number of proton signals, values of chemical shifts, and spin-spin interaction constants) of the aliphatic protons in compounds **3a–d** confirm the suggested symmetrical chemical structure of these compounds. Additional information on the structure of salts **3c** and **3d** is obtained by the ^{13}C NMR spectra. The presence of signals from quaternary carbon atoms in the ferrocenyl fragments of the compounds **3c, d**, together with the signals from four C_5H_5 groups unambiguously prove the formation of symmetrical *bis*-cations **3c** and **3d**.

The ^1H NMR spectra of the compounds **4a–d** contain the following characteristic signals which confirm its monomeric structure: one singlet for HC = groups; multiplets for CH_2, and CH fragments, signals for the CH_3 groups, and two singlets for C_5H_5 groups of the ferrocenyl substituents. Additional information is obtained from the ^{13}C NMR spectra of these compounds. The presence of two C_{ipso} atoms of the ferrocene substituents together with the presence of signals for two monosubstituted ferrocenes of the cyclopentadienyl rings unambiguously proves their monomeric character. The presence of signals for HC=, C, CH, CH_2 and CH_3 groups in compounds **4a–d** fully corresponds to their structures.

The independent structural determination for tetrafluoroborates **4b** and **4d** was performed by X-ray analysis. Single crystals of these compounds were grown from ether solution. The general view of the molecules **4b** and **4d** are shown in Figs. 1 and 2, respectively. The pivotal element of the molecule **4b** is the six-membered framework of a 2-(*cis*-1,2-diferrocenylvinyl)-1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-2-ium tetrafluoroborate, meanwhile for the molecule **4d** is the five-membered framework of a 2-(*cis*-1,2-diferrocenylvinyl)-1,3-diisopropyl-2-imidazolinium tetrafluoroborate.

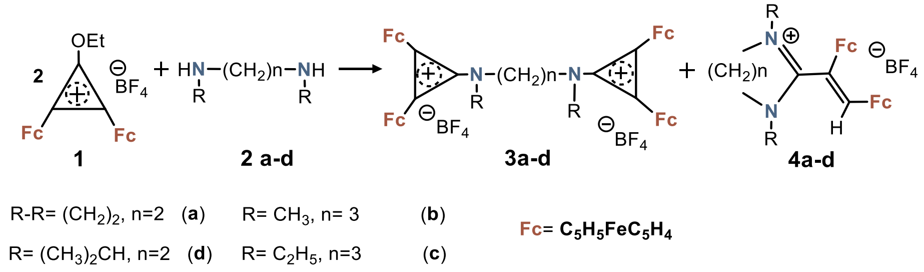
The ferrocenyl substituents of the 1,2-diferrocenylvinyl fragments are *cis* oriented relative to the C(6) = C(13) and C(6) = C(16) bonds. The packing of molecules **4b** and **4d** in crystals are shown in Figs. 1a and 2a. The geometrical parameters are indicated in the Supplementary material. Data from the X-ray analysis shown that the C(7)-N(1) [d = 1.313(3) Å] and C(7)-N(2) [d = 1.331(3) Å] bonds in the tetrahydropyrimidine **4b** and C(7)-N(1) [d = 1.325(3) Å] and C(7)-N(2) [d = 1.321(3) Å] bonds in the imidazoline **4d** are somewhat longer than the standard value of 1.29 Å [20].

We found that tetrafluoroborates of the *bis*-cations **3a–d** react with sodium azide in the presence of triethylamine in boiling acetonitrile to get stereospecific compounds *bis*-triazines **5a–d** (53–65%), were formed as the only reaction products (Scheme 2).

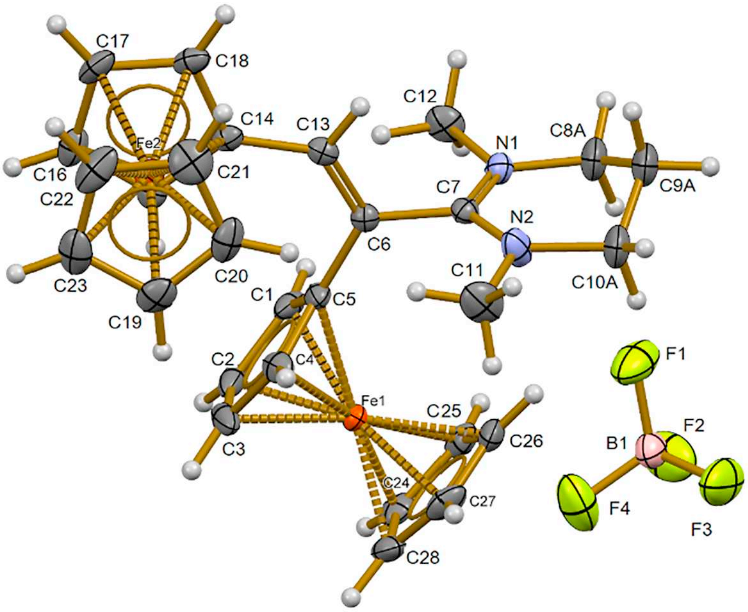
The structures of the compounds **5a** to **5d** were established based on the data from IR, ^1H and ^{13}C NMR spectroscopy, elemental analysis, and mass spectrometry. The data from the ^1H and ^{13}C NMR spectra of the compound **5a** completely corroborate this symmetrical chemical structure as an *N*,*N′*-*bis*-(4′,6′-diferrocenyl-1′,2′,3′-triazin-5′-yl)piperazine (see Experimental section). According to ^1H and ^{13}C NMR findings, compounds **5b–d** are generated in the form of an only isomer with two 4,6-diferrocenyl-5-triazine substituents and with signals of the proton and carbon, two signals from CH_3, CH_2CH_3 or $CH(CH_3)_2$ groups and signals from the CH_2 fragments, and two signals for C_5H_5 groups of the four ferrocenyl substituents.

Bis-tetrafluoroborates of the *N*,*N′*- *bis*-(2′,3′-diferrocenylcyclopropenium-1′-yl)-*N*,*N′*- dialkyl-1,2- and 1,3-alkanediamines **3a–d** also reacted with sodium hydrogencyanamide in the presence of triethylamine to produce **6a–d**, **7a–d**, and **8** (Scheme 3).

Compounds **6a–d** (~54–60%), **7a–d** (~17–21%) and **8** (~11–13%) were isolated using column chromatography (Al_2O_3), these are bright red powders, which are soluble in polar solvents. The structure of each compound was determined based on IR, ^1H and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis (see Experimental section). The IR spectra of compounds **6a–d** and **7a–d** contain bands at 2180–2253 cm^{-1}, which are characteristic of the cyano groups. The IR spectra of compounds **7a–d** also contain bands at 3300–3350 cm^{-1}, which are characteristic of the NH groups. The ^1H NMR spectra of **6a–d** and **7a–d** compounds contain characteristic signals for four or two ferrocenyl substituents, signals for two or one –CH= groups, signals of the –CH_3, –C_2H_5, –$CH(CH_3)_2$, and –CH_2– fragments. Data from ^{13}C NMR spectra of **6a–d** and **7a–d** corroborate the presence in each compound of four or two ferrocenyl fragments, two (–CH_3, –C_2H_5, –$CH(CH_3)_2$, and –CH_2–) groups, two or one −CN substituents, and they contain signals of four or two C_{ipso}Fc carbon atoms, respectively. Eluted last from the chromatographic column was 3-cyanoimino-1,2-diferrocenylcyclopropene **8**. It is possibly the pseudoaromatic



Scheme 1. Reaction of the 2,3-diferrocenyl-1-ethoxycyclopropenium tetrafluoroborate **1** with *bis*-1,4-*N*,*N*-(**2a**, **d**) or *bis*-1,5-*N*,*N*-(**2b**, **c**).



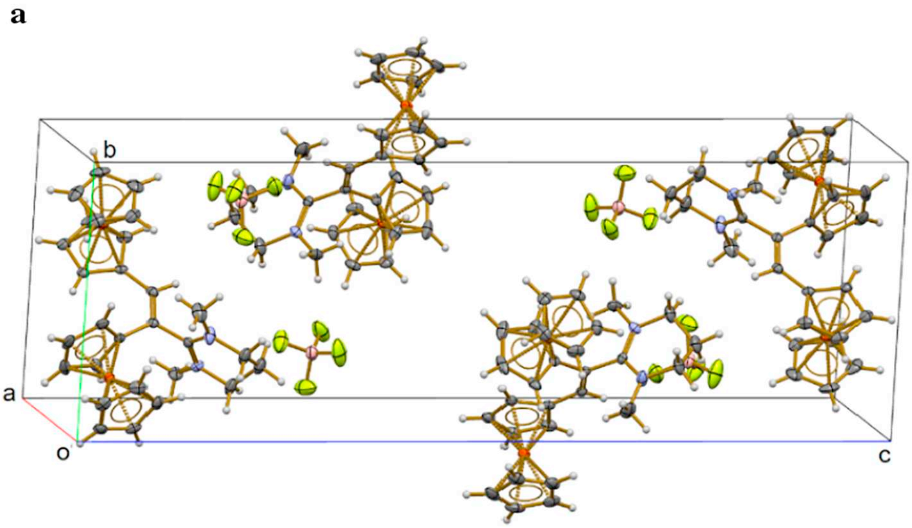


Fig. 1. X-ray crystal structure of **4b**.
Fig. 1a Crystal packing of **4b**.

character in the structure of the compound **8**, the one that determines the elution order [21].

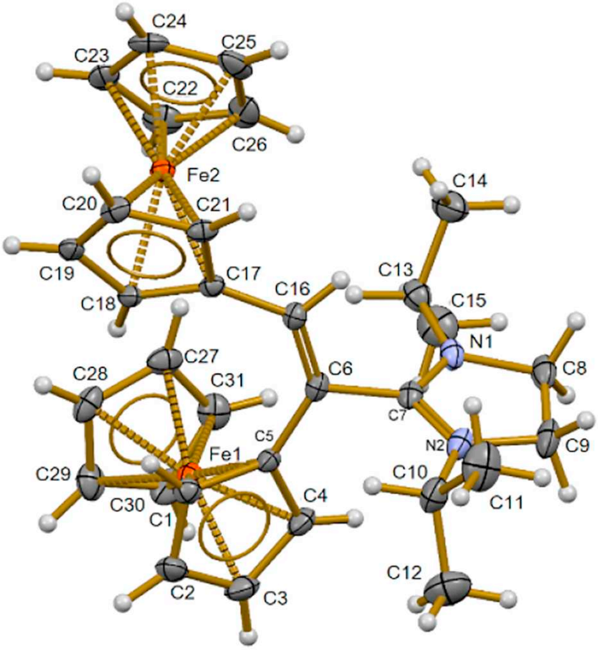
The molecular structure of compound **7a** has been elucidated by X-ray diffraction analysis of a single crystal prepared by crystallization from $CHCl_3$. The overall view of the molecule is shown in Fig. 3, the crystal packing, in Fig. 3a, while the main geometric parameters are listed in the Supplementary material. According to the data from X-ray diffraction analysis **7a** is *N*-(1′-cyano-3′,4′-diferrocenyl-1′-aza-1′,3′-butadien-2′-yl)piperazine with two ferrocenyl substituents in position *cis* at 1,2-diferrocenylvinyl fragments.

Further, we found that compounds **5a–d**, **6a–d, 7a–d**, and **8** were obtained by one-step synthesis based on the reaction of 2,3-diferrocenylcyclopropenone plus triethyloxonium tetrafluoroborate, plus *bis*-1,4-*N*,*N*- (**2a**, **d**) or *bis*-1,5-*N*,*N*- (**2b**, **c**) nucleophiles with sodium azide or sodium hydrogencyanamide in the presence triethylamine in CH_2Cl_2 (35 °C, 8 h). With only this reaction it is possible to get the following products: *viz.* **4a–d** (68%), **6a–d** and **7a–d** in the ratio 2:1 (46% + 23% respectively), and **8** (7%). These compounds are purified and separated by column chromatography (Al_2O_3, Brockmann activity III) (Scheme 4). The physical characteristics and ^{1}H NMR spectroscopic data of compounds **4a–d**, **6a–d, 7a–d** and **8** confirm their suggested chemical structure.

Fig. 3a Crystal packing of **7a**.

The formation of tetraferrocenyl-*bis*-triazines **5a–d** (Scheme 5) proceeds apparently with the intermediary of tetraferrocenylcyclopropenylium azides **9a–d** as ion pairs [22,23], which undergo reversible transformation to 3,3′-diazide(tetraferrocenyl)-*bis*-cyclopropenes **10a–d**, they represent the *bis*-allylazides. The dicyclopropenyl azides **10a–d** transform into 1,2,3- the mechanism of the [3,3]-sigmatropic rearrangement of allyl azides [24] *via* intermediates **10a–d**, **11a–d**, **12a–d**, and products **5a–d.**

The proposed mechanism of the formation of compounds **6a–d**, **7a–d** and **8** is shown in Scheme 6. As is seen from Scheme 6 the nitrogen of the hydrogencyanamide attack the 2C(1) carbon atoms of the cyclopropenium **3a–d** with the formation of intermediary addition-products **13a–d**, which subsequently undergo an intramolecular transformation (*via* the opening of the three-carbon ring) into *bis*-vinylcarbenes **14a–d** and *mono*-vinylcarbenes **15a–d** then into polyenes **6a–d**, **7a–d**.



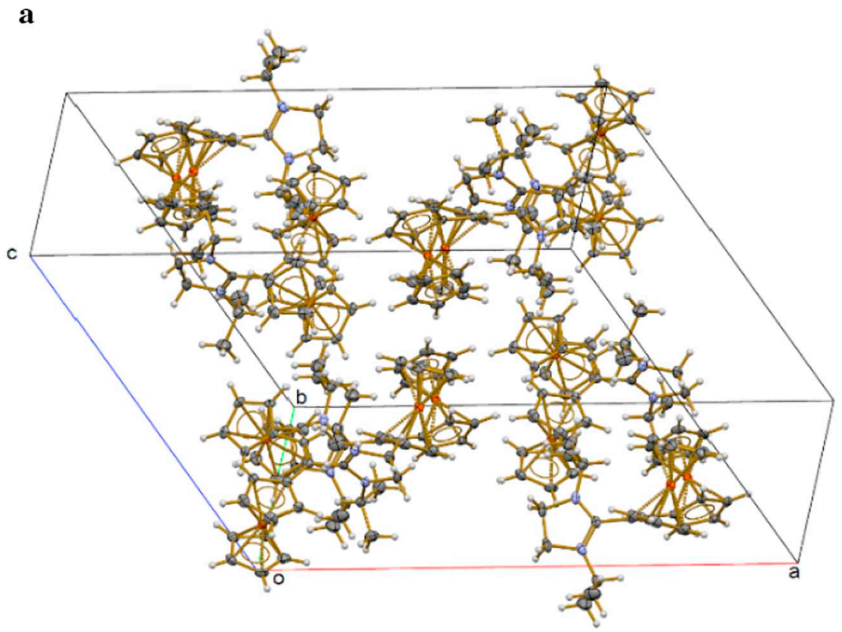
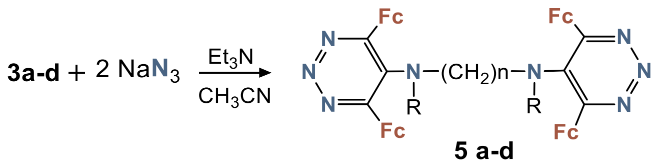


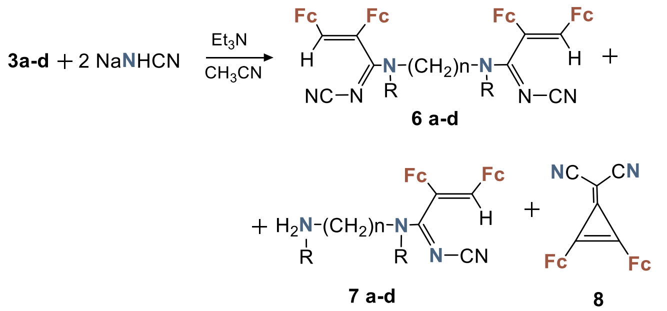
Fig. 2. X-ray crystal structure of **4d**.
Fig. 2a Crystal packing of **4d**.



Scheme 2. The reaction of *bis*-diferrocenycyclopropenium salts **3a–d** with sodium azide.

2.2. Electrochemistry

*Poly*ferrocenyl *PFc* (*bi-* and *tetra-*) compounds show multi-step electron transfer processes according to the next scheme:



Scheme 3. The reaction of *bis*-diferrocenylcyclopropenium salts **3a–d** with sodium hydrogencyanamide.

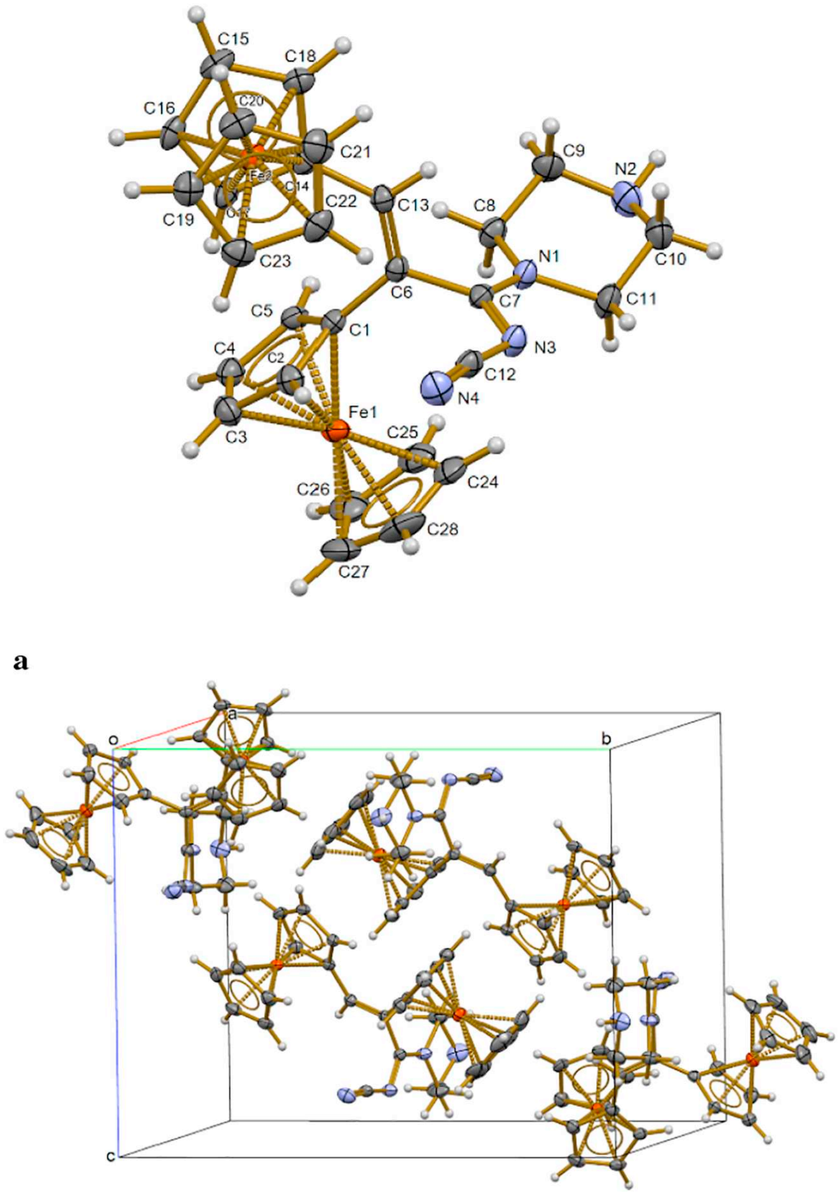
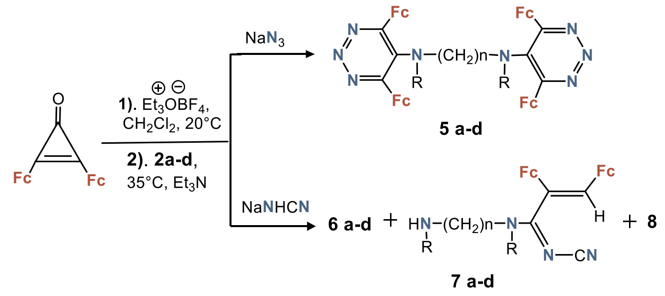


Fig. 3. X-ray crystal structure of **7a**.

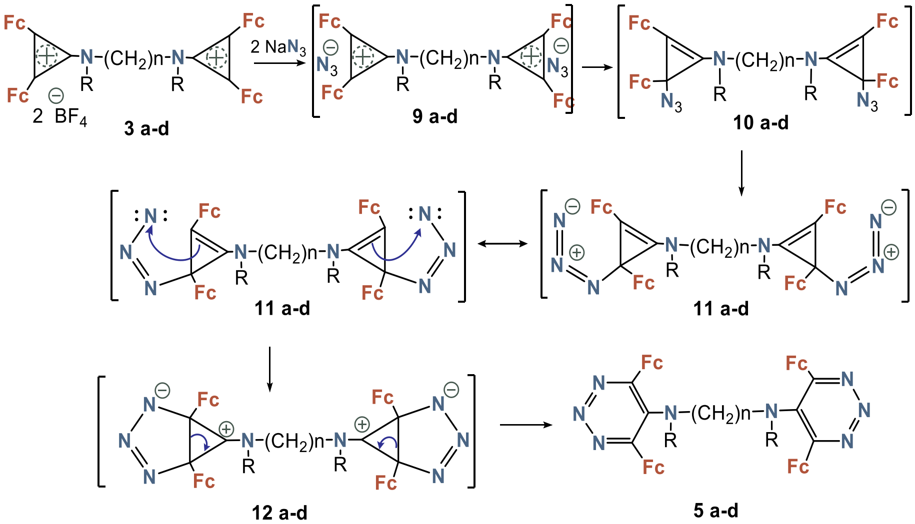


Scheme 4. One-step synthesis of **5a–d**, **6a–d** + **7a–d**, **8**.

$$PFc - e \overset{Ox_1}{\rightarrow} PFc^{+} - e \overset{Ox_2}{\rightarrow} PFc^{2+} - e \overset{Ox_3}{\rightarrow} PFc^{3+} - e \overset{Ox_4}{\rightarrow} PFc^{4+}$$

When *poly*ferrocenyl is bi-ferrocenyl, two consecutive oxidations are represented by $PFc \rightarrow PFc^{+} \rightarrow PFc^{2+}$, when it is tetra-ferrocenyl four oxidations occur $PFc \rightarrow PFc^{+} \rightarrow PFc^{2+} \rightarrow PFc^{3+} \rightarrow PFc^{4+}$. The difference between sequential oxidation steps and closely spaced values of $E_{1/2(1)}$, $E_{1/2(2)}$, $E_{1/2(3)}$, and $E_{1/2(4)}$ may strongly depend on the nature of supporting electrolyte [25,26]. Consecutive $\Delta E_{1/2}$ values of electron exchange depend on the formation of ion-pairs (that involves the "inert" salt) and the donor or acceptor number, besides the polarity, of the solvent [27]. In this preliminary electrochemical study, acetonitrile/Bu_4NPF_6 combination was used as electrolyte support. CH_3CN was used as a solvent since they dissolve most of the *poly*ferrocenyl compounds rather than to be the best option to maximize the separation of progressive $E_{1/2}$ values. The $[PF_6]^-$ ion, from the Bu_4NPF_6 salt, is a "traditional anion" [27] and it is recommended as a starting point for organometallic electrochemistry [28]. We expect to go deeper in the next study.

Traced Voltammetries (cyclic, square wave, and differential pulse) for selected *poly*ferrocenyl compounds **3a**, **5a**, **5b**, **5d**, **6a**, **6b**, **6c,** and **7a**, showed that all systems are reversible. The only one bi-ferrocenyl compound **7a** showed two well-resolved oxidation currents (Ox_1 and Ox_2) and their corresponding reduction currents (Red_1 and Red_2) in Cyclic Voltammetry (CV). It is an electrochemical system with 1- and 1-electron exchanges and later confirmed by Square Wave Voltammetry (SWV) and Differential Pulse Voltammetry (DPV) traces (shown in the Supplementary material).



Scheme 5. A possible mechanism of the formation of tetraferrocenyltriazines **5a–d**.

The tetra-ferrocenyl compounds **3a**, **5a**, **5b**, **5d**, **6a**, **6b**, and **6c,** were also analyzed by the three mentioned techniques (see Fig. 4 and Supplementary material). Compounds **3a**, **5b**, **6a**, and **6b** showed two well-resolved oxidation signals (**3a** shows a small pre-peak). Electrochemical parameters are shown in the Table 1. Compounds **5a**, **5d**, and **6c** did not display well-resolved peaks, although SWV traces improve the separation of three peaks for **5d** and **6c** (being 1, 1, and 2 electron exchanges respectively). By another hand, compound **5a** exhibited only one broad peak by CV, DPV, and SWV traces showed that the system could be formed by two not well-resolved peaks (2 and 2-electron exchanges). In the case of compounds **5a**, **5d**, and **6c** the SWV experimental voltammograms were fitted (see Supplementary material) using the DigiElch software to get the corresponding $E_{1/2}$ and K_{com} values (see Table 2).

Fig. 4 shows typical cyclic (top), square wave (middle), and differential pulse (bottom) voltammograms of two of the most bioactive **6a** and **6b** compounds. Both compounds have two well-separated peaks and the electrochemical parameters are shown in the Table 1. Here the cyclic voltammogram parameters are summarized by for each compound at 100 mV/s scan rate [8,29]. The ΔEp values obtained from 1 to 2 and 3–4 oxidation processes range from 0.045 V to 0.130 V; these values are slightly different at higher scan rates 200 mV/s (not shown). So, this suggests two-step reversible oxidations (two-electron or one-electron) of ferrocenyl moieties except for **5a**.

The comproportionation reaction 1 K_{com1} occurs between PFc^{2+}, produced after the second oxidation reaction, and PFc species that meet close to the surface of the working electrode. The compounds **3a**, **5b**, and **6a** show only two peaks, each one with two-electron exchange. Here we consider $E_{1/2(1)} = E_{1/2(2)}$ and $E_{1/2(3)} = E_{1/2(4)}$ so, there is only one global comproportionation constant $K_{com,global}$ (see Table 1).

$$2PFc + 2PFc^{2+} \overset{K_{com2PFc,2PFc^{2+}}=K_{com,global}}{\Leftrightarrow} 2PFc^{+}$$

When tetra-ferrocenyl compounds have four steps of 1-electron exchange as could be for **5a**, **5d**, and **6c** compounds, there are three comproportionation equilibria: between PFc with PFc^{2+}; PFc^{+} with PFc^{3+}; and PFc^{2+} with PFc^{4+}, giving the K_{com1} K_{com2} and K_{com3} constants, respectively [29].

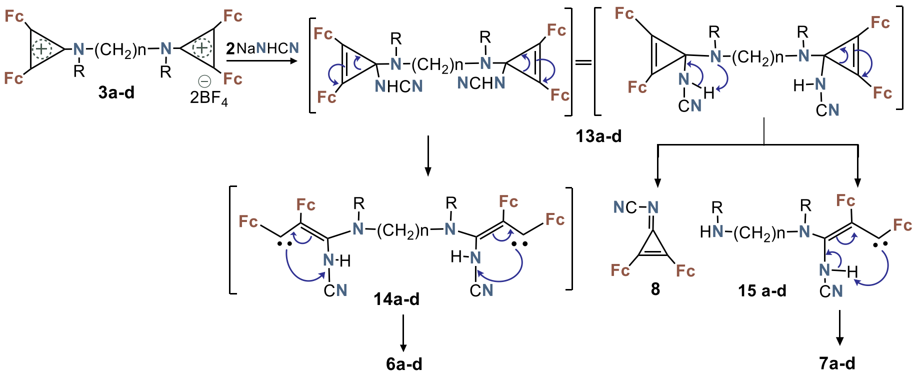
$$PFc + PFc^{2+} \overset{K_{comPFc,PFc^{2+}}=K_{com1}}{\Leftrightarrow} 2PFc^{+}$$

$$PFc^{+} + PFc^{3+} \overset{K_{comPFc^{+},PFc^{3+}}=K_{com2}}{\Leftrightarrow} 2PFc^{2+}$$

$$PFc^{2+} + PFc^{4+} \overset{K_{comPFc^{2+},PFc^{4+}}=K_{com3}}{\Leftrightarrow} 2PFc^{3+}$$

Here, $E_{1/2(1)}$, $E_{1/2(2)}$, $E_{1/2(3)}$, and $E_{1/2(4)}$ values are needed to calculate the comproportionation constants K_{com1}, K_{com2}, and K_{com3}, respectively. The four individual $E_{1/2}$ and three individual K_{com} values for compounds **5a**, **5d**, and **6c** were obtained by the best fitting of experimental SWV voltammograms using Digielch (see Table 2 and Supplementary material).

According to the separation of two-oxidation consecutive signals and by the K_{com} values found here, the *poly*ferrocenyl compounds were evaluated according to the Robin–Day classification. All compounds evaluated by cyclic, square wave and differential pulse voltammetries are type II since $10^4 > K_{com} > 10^2$ (partially delocalized electronic charge in the mixed valence state generated electrochemically, see



Scheme 6. A possible mechanism of the formation of tetra- and diferrocenyl-aza butadienes **6a–d**, **7a–d** and **8**.

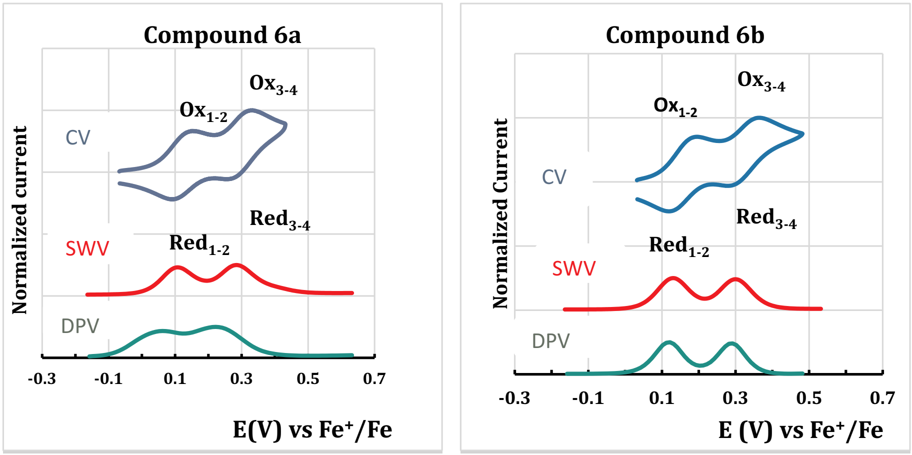


Fig. 4. Representative voltammograms of compound **6a** (3.3×10^{-4} mol/L) and **6b** (1.4×10^{-3} mol/L) in 0.1 M NBu_4PF_6 in acetonitrile. All traces were to anodic direction from starting potential. The scan rate for CV (blue line) was 0.1 V/s, the frequency for SWV (red line) was 10 Hz, and the pulse amplitude for DPV (green line) was 10 mV. The working electrode, Glassy Carbon Electrode (GCE); counter-electrode Pt; reference electrode AgCl/Ag^0. Normalized Current = $I/Ip_{(anodic)}$.

Table 1
Electrochemical data for compounds **3a, 5–b, d, 6a–c**, and **7a**.

Compound	Concentration (mmol/L)	E_{pa}(1–2) (V)	E_{pc}(1–2) (V)	$E_{1/2}$(1,2) (V)	E_{pa}(3–4) (V)	E_{pc}(3–4) (V)	$E_{1/2}$(3,4) (V)	$\Delta E_{1/2}$ (V)	$K_{com,global}$
3a	3.49	0.411	0.346	0.379	0.526	0.481	0.504	0.125	121.2
5a	1.31	0.181	0.064	0.123	ND	ND	–	–	–
5b	1.89	0.265	0.197	0.231	0.397	0.325	0.361	0.130	146.8
5d	0.68	0.248	0.118	0.183	0.378	0.288	0.333	0.150	316.2
6a	0.33	0.103	0.018	0.061	0.273	0.183	0.228	0.168	619.2
6b	1.43	0.154	0.087	0.121	0.322	0.257	0.290	0.169	655.6
6c	1.34	0.169	0.106	0.138	0.339	0.271	0.305	0.168	619.0
7a*	0.69	0.432	0.348	0.390	0.556	0.486	0.521	0.131	152.5

ND: only one broad peak is detected, the second one almost overlaps with the first one. The $E_{1/2}$(1, 2) and $E_{1/2}$(2, 3) values represent the potential at which there are the two electron exchange simultaneously from 2PFc to 2PFc^{2+} and from 2PFc^{2+} to 2PFc^{4+}. (*) For compound **7a**, there are two sequential 1-electron exchanges.

Table 2
Electrochemical parameters obtained by fitting of the SWV voltammograms for compounds **5a, 5d,** and **6c.**

Compound	Concentration (mmol/L)	$E_{1/2}$(1) (V)	$E_{1/2}$(2) (V)	$E_{1/2}$(3) (V)	$E_{1/2}$(4) (V)	K_{com1}	K_{Com2}	K_{Com3}
5a	0.59	0.161	0.215	0.279	0.298	8.1	12.0	2.1
5d	0.68	0.088	0.165	0.269	0.325	19.7	56.2	8.8
6c	1.37	0.194	0.281	0.269	0.417	29.7	36.6	5.3

Table 1). It is important to notice that we consider compounds **3a**, **5b**, **6a**, and **6b** have two oxidation peaks of two-electron transferences from 2*PFc* to 2*PFc*$^{2+}$ and 2*PFc*$^{2+}$ to 2*PFc*$^{4+}$, respectively. Compound **7a** has two steps of 1-electron transferences from *PFc* to *PFc*$^{+}$ to *PFc*$^{2+}$. The additional evaluation of the compounds **5a**, **5d**, and **6c**, by fitting experimental SWV voltammograms consider four oxidation steps from *PFc* to *PFc*$^{4+}$. The three K_{com} values are smaller than 10^2 and with this criteria, all of them are type I (electronic charge in the mixed valence state is localized, see Table 2) [27].

2.3. Cytotoxicity

The cytotoxic activity of the compounds **5a**, **6a**, **6b**, and **6c** was evaluated to know their *in vitro* activity against five human cancer cell lines: U-251 (human glioblastoma), K-562 (human chronic myelogenous leukemia cells), HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma) and SKLU-1 (human lung adenocarcinoma). As a noncancerous control, we also tested on COS-7 (African Green monkey kidney fibroblast-like) cell culture. This screening, at 10 μM concentration, showed that all compounds have differential cytotoxicity activity against the five human tumor cell lines tested (see Table 3). The Cisplatin, at the same concentration, was the positive control.

Compounds **5a**, **6a**, **6b**, and **6c** showed better antitumor activity on K-562 cell line than *cisplatin*; 46.5–75.9% inhibition *versus* 43%, respectively (see Fig. 5).

Besides, the IC_{50} values for these compounds were determined and the results are summarized in Table 4. The cytotoxic *in vitro* activity of the ferrocene derivatives **5a**, **6a–c** varied from $IC_{50} = 33.8$ μM (SKLU-1 cancer cell line) for **6c** to $IC_{50} = 8.2$ μM and $IC_{50} = 10.5$ μM (SKLU-1), $IC_{50} = 9.0$ μM and $IC_{50} = 10.2$ μM (U-251) for **6b** and **6a**, respectively. These values, lower than those for *cisplatin* ($IC_{50} = 10.8$ μM for U-251and $IC_{50} = 17.4$ μM for SKLU-1), make compounds **6a** and **6b** as promising antiproliferative agents against the human tumor cell lines U-251 and SKLU-1 (Table 5 and Fig. 6). The other compounds, do not exhibit significant activity compared to *cisplatin*.

3. Experimental

3.1. Materials

All reactions were implemented under an N_2 atmosphere and using solvents which were dried by routine procedures and were freshly distilled before use [21]. The reagents, purity in parenthesis, were purchased from Aldrich: ferrocene (98), aluminum chloride (99.99), tetrachlorocyclopropene (98), triethylamine (99.5), *N,N′*-dimethylpropanediamine (99), *N,N′*-diethyl-1,3-propanediamine (97), *N,N′*-diisopropyl-ethylenediamine (99), sodium azide (99), sodium hydrogencyanamide (98), and piperazine (99); and triethyloxonium tetrafluoroborate (1.0 M solution in dichloromethane).

Table 3
Percentage of the growth inhibition on the human and COS-7 cell lines by compounds **5a**, **6a–c** at 10 μM in DMSO.

Cell lines, % growth inhibition						
Compounds	U-251	K-562	HCT-15	MCF-7	SKLU-1	COS-7
5a	18.02 ± 1.5	46.5 ± 2.9	10.7 ± 1.7	17.7 ± 3.7	23.3 ± 0.1	19.6 ± 7.0
6a	33.0 ± 1.0	53.2 ± 3.1	34.4 ± 7.2	32.5 ± 4.5	39.8 ± 3.0	18.7 ± 0.3
6b	37.2 ± 1.2	75.2 ± 5.3	44.6 ± 3.6	49.0 ± 6.7	52.4 ± 3.2	40.6 ± 3.1
6c	19.9 ± 1.6	75.9 ± 5.8	35.7 ± 4.8	24.9 ± 1.5	18.7 ± 2.4	21.3 ± 4.2
Cisplatin	87.49 ± 8.1	42.65 ± 4.1	79.15 ± 7.1	52.58 ± 2.5	81.35 ± 2.1	22.9 ± 5.9

Data were expressed as average ± standard deviation (S.D.).

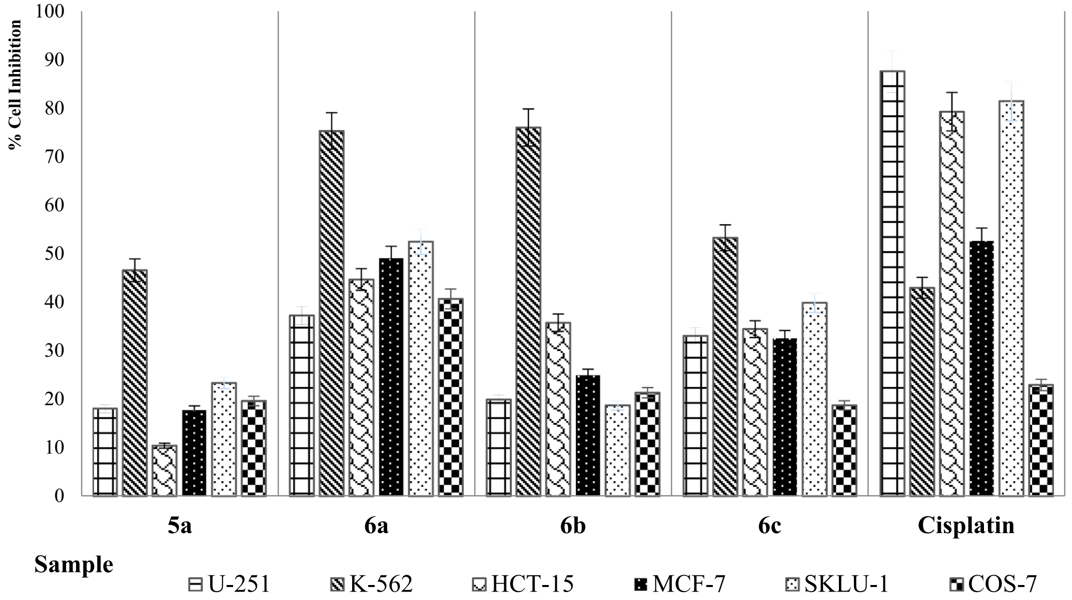


Fig. 5. Growth inhibition percentage (%) of human cell lines and COS-7 by compounds **5a**, **6a–c** at 10 μM in DMSO.

Table 4
In vitro results of compounds **5a**, **6a–c** against different tumor cell lines and normal COS-7 fibroblasts expressed as IC_{50} in μM.

Cell lines (μM ± SD)					
Compound	U-251	K-562	HCT-15	SKLU-1	COS-7
5a	13.8 ± 0.6	8.5 ± 0.3	20.7 ± 2.0	21.0 ± 2.0	16.1 ± 0.9
6a	9.0 ± 0.3	6.4 ± 0.5	14.8 ± 1.3	10.5 ± 0.6	14.6 ± 0.8
6b	10.2 ± 0.9	6.0 ± 0.3	12.5 ± 0.4	8.2 ± 0.6	13.1 ± 1.0
6c	22.5 ± 1.6	8.4 ± 1.0	21.8 ± 1.4	33.8 ± 0.4	24.8 ± 1.0
Cisplatin	10.8 ± 0.7	1.2 ± 0.08	12.3 ± 0.8	17.4 ± 0.8	12.2 ± 0.9

IC_{50} values are presented as the mean ± (standard deviation) from five separate experiments.

3.2. Physical measurements

A Varian Unity Inova spectrometer was used to record all ^1H and ^{13}C NMR spectra from solutions in $CDCl_3$, $(CD_3)_2CO$, DMSO-d_6, and CD_3CN. Chemical shifts (δ ppm) are relative to tetramethylsilane (TMS) as the internal standard. ^1H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet), coupling constants (Hz), integration and assignment. Data for ^{13}C NMR spectra presented regarding chemical shift and assignment. The infrared spectra were acquired with an FT-IR Spectrophotometer (Spectrum RXI Perkin Elmer) using KBr pellets. The mass spectra were acquired at 70 eV (MAT CH-6 Varian instrument). The elemental analysis was carried out on Elementar Analysensysteme LECO CHNS-900. Column chromatography was performed on alumina (Brockmann activity III) as the stationary phase. X-ray diffraction measurements were performed on Oxford Diffraction Gemini-Atlas diffractometer.

3.3. Preparation of compounds

3.3.1. Obtention of starting compounds

The 2,3-diferrocenyl 1- ethoxycyclopropenium tetrafluoroborate **1** was obtained from 2,3-diferrocenylcyclopropenone and triethyloxonium tetrafluoroborate (1.0 M solution in dichloromethane) [19]. 2,3-Diferrocenylcyclopropenone was prepared from the ferrocene and tetrachlorocyclopropene in the presence of $AlCl_3$ according to the standard procedure [30] The physical and ^1H NMR spectroscopic characteristics of compound **1** were in accord with the literature data [4,19,30].

3.3.2. Reactions of 2,3-diferrocenyl-1-ethoxycyclopropenium tetrafluoroborate with 1,2- and 1,3-diamines

A solution of 2,3-diferrocenyl-1- ethoxycyclopropenium tetrafluoroborate **1** (1.35 g, 2,5 mmol) in anhydrous CH_2Cl_2 (50 ml) was stirred vigorously at 10–15 °C under nitrogen atmosphere for 20 min. The compound **2a** (or **2b**, **2c**, **2d**, 1.25 mmol) dissolved in anhydrous CH_2Cl_2 (10 ml) was added dropwise and the reaction was prolonged for 4 h. The mixture was cooled and the precipitate of the *bis*-cation with two 2,3-diferrocenylcyclopropenium fragments **3a** (or **3b, 3c, 3d**) was filtered. The filtrate was dried (under reduced pressure) and the remnant was chromatographed on column with Al_2O_3 (Brockmann activity III) to afford the *cis*-2-(1,2-diferrocenylvinyl)-1,3-diazabicyclo[1,2,2] heptan-2-ium tetrafluoroborate **4a** (0.81 g, 59%) (or *cis*-2-(1,2-diferrocenylvinyl)-1,3-dimethyl- and 1,3-diethyl-3,4,5,6-tetrahydropyrimidin-

Table 5
Selected bond lengths and bond angles for compounds **4b, 4d, 7a.**

Selected bond lengths (Å)		Selected bond angles (°)	
4b			
C(6)-C(7)	1.496(3)	N(1)-C(7)-N(2)	121.4(2)
C(8)-C(9)	1.537(6)	N(1)-C(7)-C(6)	120.1(2)
C(9)-C(10)	1.490(6)	N(2)-C(7)-C(6)	118.5(2)
C(7)-N(1)	1.313(3)	C(13)-C(6)-C(7)	117.8(2)
C(7)-N(2)	1.331(3)	C(7)-N(1)-C(8)	123.7(3)
C(11)-N(2)	1.459(3)	C(7)-N(1)-C(12)	123.7(2)
C(12)-N(1)	1.463(3)	C(12)-N(1)-C(8)	112.6(3)
C(8)-N(1)	1.448(5)	C(7)-N(2)-C(10)	115.4(2)
C(10)-N(2)	1.507(4)	N(1)-C(78-C(9)	110.4(4)
C(10A)-N(2)	1.507(4)	C(10)-C(9)-C(8)	108.8(4)
C(6)-C(13)	1.342(39	N(2)-C(10)-C(9)	109.5(3)
4d			
C(6)-C(5)	1.473(3)	C(16)-C(6)-C(7)	114.05(18)
C(6)-C(16)	1.357(3)	C(5)-C(6)-C(7)	113.32(17)
C(6)-C(7)	1.495(3)	C(16)-C(6)-C(5)	132.44(19)
N(2)-C(7)	1321(3)	N(2)-C(7)-N(1)	112.59(19)
N(1)-C(7)	1.325(3)	N(2)-C(7)-C(6)	123.41(19)
N(1)-C(8)	1.488(3)	N(1)-C(7)-C(6)	123.99(19)
N(2)-C(9)	1.478(3)	N(1)-C(8)-C(9)	102.60(17)
N(2)-C(10)	1.471(3)	N(2)-C(9)-C(8)	103.50(18)
C(8)-C(9)	1.529(4)	N(2)-C(10)-C(11)	110.20(2)
C(10)-C(12)	1.527(4)	N(2)-C(10)-C(12)	110.00(2)
C(6)-C(5)	1.457(3)	N(1)-C(13)-C(15)	109.81(19)
		N(1)-C(13)-C(14)	110.42(19)
7a			
N(1)-C(23)	1.323(3)	C(5)-C(1)-C(2)	107.30(19)
N(3)-C(23)	1.325(3)	C(5)-C(1)-C(22)	126.02(19)
C(24)-N(4)	1.156(3)	C(2)-C(1)-C(22)	126.50(2)
N(2)-C(26)	1.471(3)	C(22)-C(21)-C(11)	129.50(2)
N(3)-C(24)	1.317(3)	C(21)-C(22)-C(1)	124.83(19)
N(1)-C(25)	1.466(3)	C(21)-C(22)-C(23)	117.32(19)
N(2)-C(27)	1.467(3)	C(1)-C(22)-C(23)	117.79(18)
Fe(1)-C(5)	2.040(2)	N(1)-C(23)-N(3)	118.20(19)
Fe(2)-C(11)	2.039(2)	N(1)-C(23)-C(22)	119.75(18)
C(21)-H(21)	0.9500	N(4)-C(24)-N(3)	172.40(2)
C(11)-C(21)	1.456(3)	C(23)-N(1)-C(25)	124.36(18)
C(21)-C(22)	1.334(3)	O(3)-N(3)-O(4)	119.61(19)
C(22)-C(23)	1.498(3)	C(24)-N(3)-C(23)	122.05(19)

2-ium tetrafluoroborates **4b** and **4c**, respectively, and *cis*-2-(1,2-diferrocenylvinyl)-1,3-diisopropyl-2-imidazolinium tetrafluoroborate **4d.**

***Bis*-tetrafluoroborates of *N,N′*–*bis*–(2′,3′-diferrocenylcyclopropenium-1′-yl)-piperazine 3a**: yield 1.43 g (67%), m.p. dec. *ca* 190–195 °C. IR (KBr): ν (cm^{-1}) 482, 591, 674, 764, 795, 823, 901, 920, 985, 1020, 1065, 1106 1139, 1241, 1285,1372, 1398, 1413, 1455, 1480, 1507, 1556, 1600, 1626, 1855, 1919, 2457, 2634, 2743, 2984, 3041, 3111, 3180. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.19 (m, 8H, 4CH$_2$), 4.44 (s, 20H, 4C$_5$H$_5$), 4.18 (s, 4H, C$_5$H$_4$), 4.28 (s, 4H, C$_5$H$_4$), 4.95 (s, 4H, C$_5$H$_4$), 5.08 (s, 4H, C$_5$H$_4$). Anal. calcd. for $C_{50}H_{44}B_2F_8Fe_4N_2$ (1069.85): C, 56.13; H, 4.15; N, 2.62. Found: C, 56.43; H, 4.30; N, 2.47%. MS (El, 70 eV): *m/z* 172, 897.85 [M]$^+$.

***Bis*-Tetrafluoroborates of *N,N′-bis*-(2′,3′-diferrocenylcyclopropenium-1′-yl)-*N,N′*–dimethyl-1,3-propanediamine 3b:** yield 1.48 g (68%), m.p. dec. *ca* 208–209 °C. IR (KBr): ν (cm^{-1}) 470, 487, 522, 603, 672, 690, 783, 822, 855, 981, 1002, 1029, 1051, 1107, 1144, 1170, 1202, 1324, 1363, 1387, 1397, 1410, 1493, 1630, 1893, 2862, 3005, 3121, 3218, 3598. ^{1}H NMR (400 MHz, CD$_3$CN): δ 2.04 (m, 2H, CH$_2$), 3.05 (s, 6H, 2CH$_3$), 3.14 (m, 4H, 2CH$_2$), 4.31 (s, 20H, s, 4C$_5$H$_5$), 4.35 (m, 4H, C$_5$H$_4$), 4.69 (m, 4H, C$_5$H$_4$), 4.84 (m, 4H, C$_5$H$_4$), 4.88 (m, 4H, C$_5$H$_4$). Anal. calcd. for $C_{51}H_{48}$ B_2F_8 Fe_4N_2 (1084.29): C, 56.41; H, 4.46; N, 2.59. Found: C, 56.27; H, 4.33; N, 2.25%. MS (El, 70 eV): *m/z* 172, 913[M]$^+$.

***Bis*-Tetrafluoroborates of *N,N′-bis*-(2′,3′-diferrocenylcyclopropenium-1′-yl)-*N,N′*-diethyl-1,3-propandiamine 3c**: yield 1.40 g (63%), m.p. dec. *ca* 217 °C. IR (KBr): ν (cm^{-1}) 475, 487, 518, 628, 680, 761, 817, 898, 932, 961, 1001, 1031, 1047, 1106, 1178, 1216, 1249, 1299, 1332, 1351, 1386, 1411, 1452, 1514, 1604, 1853, 2878, 2937, 2977, 3078, 3097. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.90 (bs, 6H, 2CH$_3$), 4.24 (bs, 20H, 4C$_5$H$_5$), 4.38 (m, 10H, 5CH$_2$), 4.64 (bs, 8H, 2C$_5$H$_4$), 4.85 (bs, 8H, 2C$_5$H$_4$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.10 (2CH$_3$), 43.88 (CH$_2$), 65.46 (4CH$_2$), 71.02 (4C$_5$H$_5$), 71.13 (2C$_5$H$_4$), 72.60 (2C$_5$H$_4$), 79.64 (4C$_{ipso}$Fc), 118.61, 145.28 (6C) Anal. calcd. for $C_{53}H_{52}$ B_2F_8 Fe_4N_2(1113.95): C, 57.14; H, 4.71; N, 2.52. Found: C, 56.98; H, 4.63; N, 2.20%. MS (El, 70 eV): *m/z* 172, 938 [M]$^+$.

***Bis*-Tetrafluoroborates of *N,N′-bis*-(2′,3′-diferrocenylcyclopropenium-1′-yl)-*N,N′*- diisopropyl-1,2-ethylenediamine 3d**: yield 1.34 g (60%), m.p. dec. *ca* 218–221 °C. IR (KBr): ν (cm^{-1}) 479, 621, 761, 817, 895, 961, 1001, 1031,1048, 1093, 1106, 1215, 1299, 1333, 1354, 1393, 1453, 1476, 1514, 1604, 1849, 1895, 1909, 2878, 2936, 2978, 3072, 3098. ^{1}H NMR (400 MHz, CD$_3$CN): δ 2.04 (m, 2H, CH$_2$), 3.05 (s, 6H, 2CH$_3$), 3.14 (m, 4H, 2CH$_2$), 4.31 (s, 20H, 4C$_5$H$_5$), 4.35 (m, 4H, C$_5$H$_4$), 4.69 (m, 4H, C$_5$H$_4$), 4.84 (m, 4H, C$_5$H$_4$), 4.88 (m, 4H, C$_5$H$_4$). Anal. calcd. for $C_{54}H_{50}$ B_2F_8 Fe_4N_2 (1123.34): C, 57.78; H, 4.45; N, 2.49. Found: C, 57.63; H, 4.67; N, 2.60%. MS (El, 70 eV): *m/z* 173, 949 [M]$^+$.

***Cis*-2-(1,2-diferrocenylvinyl)-1,3-diazabicyclo [1,2,2] heptan-2-ium tetrafluorobo-rate 4a:** yield 0.25 g (17%), m.p. dec. *ca* 196 °C.[14]. IR (KBr): ν (cm^{-1}) 476, 612, 754, 815, 905, 928, 1001, 1029, 1051, 1089, 1105, 1223, 1287, 1330, 1363, 1393, 1455, 1461, 1534, 1615, 1687, 2898, 2962, 2989, 3078, 3098. ^{1}H NMR (400 MHz, CDCl$_3$): δ 2.74 (m, 4H, 2CH$_2$), 3.54 (m, 4H, 2CH$_2$), 4.23 (s, 10H, 2C$_5$H$_5$), 4.30 (m, 4H, C$_5$H$_4$), 4.36 m, 4H, C$_5$H$_4$). Anal. calcd. for $C_{27}H_{27}BF_4$ Fe_2N_2 (577.99): C, 56.11; H, 4.71; N, 4.84. Found: C, 56.28; H, 4.90; N, 4.84%. MS (El, 70 eV): *m/z* 492, 578 [M]$^+$.

***Cis*-2-(1,2-diferrocenylvinyl)-1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-2-ium tetrafluoroborate 4b:** yield 0.30 g (20%), m.p. dec. *ca* 189–193 °C. IR (KBr): ν (cm^{-1}) 474, 494, 519, 655, 728, 761, 815, 827, 886, 924, 966, 1001, 1027, 1044, 1060, 1105, 1231, 1254, 1284, 1325, 1388, 1408, 1446, 1470, 1532, 1621, 2883, 2951, 2988, 3081, 3100, 3120. ^{1}H NMR (400 MHz, CDCl$_3$): δ 2.19 (m, 1H, CH$_2$), 2.33 (m, 1H, CH$_2$), 3.18 (s, 6H, 2CH$_3$), 3.54 (t, 1H, J = 6.0 Hz, CH$_2$), 3.58 (t, 1H, J = 6.0 Hz, CH$_2$), 3.82 (m, 2H CH$_2$), 4.24 (s, 10H, 2C$_5$H$_5$), 4.33 (m, 2H, C$_5$H$_4$), 4.37 (m, 2H, C$_5$H$_4$), 4.40 (m, 2H, C$_5$H$_4$), 4.66 (m, 2H, C$_5$H$_4$), 6.84 (s, 1H, CH=). ^{13}C NMR (100 MHz, CDCl$_3$): δ 19.15 (CH$_2$), 41.90 (2CH$_3$), 47.53 (2CH$_2$), 69.33, 69.57 (2C$_5$H$_5$), 68.17, 69.22, 70.12, 70.62 (2C$_5$H$_4$), 78.13, 79.23 (2C$_{ipso}$Fc), 134.93 (CH=), 121.26, 163.49 (2C). Anal. calcd. for $C_{28}H_{31}BF_4$ Fe_2N_2 (594.00): C, 56.61; H, 5.26; N, 4.71. Found: C, 56.70; H, 5.25; N, 4.35%. MS (El, 70 eV): *m/z* 87, 570 [M]$^+$.

***Cis*-2-(1,2-diferrocenylvinyl)-1,3-diethyl-3,4,5,6-tetrahydropyrimidin-2-ium tetrafluoroborate 4c:** yield 0.28 g (18%), m.p. dec. *ca* 204–206 °C. IR (KBr): ν (cm^{-1}) 474, 519, 654, 680, 761, 814, 827, 886, 924, 966, 1001, 1026, 1045, 1060, 1104, 1230, 1254, 1325, 1374, 1389, 1408, 1420, 1446, 1471, 1532, 1621, 2882, 2951, 3081, 3100, 3120. ^{1}H NMR (400 MHz, CDCl$_3$): δ 1,26 (t, 6H, J = 6.3 Hz, 2CH$_3$), 2.17 (m, 1H, CH$_2$), 2.31 (m, 1H, CH$_2$), 3.50 (m, 4H, 2CH$_2$), 3.81 (m, 4H, 2CH$_2$), 4.25 (s, 10H, 2C$_5$H$_5$), 4.39 (m, 2H, C$_5$H$_4$), 4.42 (m, 2H, C$_5$H$_4$), 4.43 (m, 2H, C$_5$H$_4$), 4.82 (m, 2H, C$_5$H$_4$), 6.80 (s, 1H, CH=). ^{13}C NMR (100 MHz, CDCl$_3$): δ 12.99 (2CH$_3$), 18.96 (CH$_2$), 44.55 (2CH$_2$), 49.19 (2CH$_2$), 69.38, 69.73 (2C$_5$H$_5$), 68.28, 69.33, 70.17, 70.69 (2C$_5$H$_4$), 78.21, 79.24 (2C$_{ipso}$Fc), 133.43 (CH=), 121.82, 162.87 (2C). Anal. calcd. for $C_{30}H_{35}BF_4$ Fe_2N_2 (622.10): C, 57.92; H, 5.67; N, 4.50. Found: C, 58.18; H, 5.48; N, 4.38%. MS (El, 70 eV): *m/z* 87, 534 [M]$^+$.

***Cis*-2-(1,2-diferrocenylvinyl)-1,3-diisopropyl-2-imidazolinium tetrafluoroborate 4d:** yield 0.33 g (21%), m.p. 204–206 °C. IR (KBr): ν (cm^{-1}) 475, 519, 631, 698, 731, 822, 911, 964, 1001, 1029, 1045, 1106, 1176, 1188, 1235, 1253, 1299, 1372, 1394, 1412, 1462, 1572, 2876, 2936, 2977, 3097. ^{1}H NMR (400 MHz, CDCl$_3$): δ 1,26 (d, 6H, J = 5.1 Hz, 2CH$_3$), 1.37 (d, 6H, J = 5.1 Hz, 2CH$_3$), 4.07 (m, 2H, J = 5.1 Hz, 2CH), 3.95 (t, 2H, J = 7.5 Hz, CH$_2$), 4.52 (t, 2H, J = 7.5 Hz, CH$_2$), 4.23 (s, 5H, C$_5$H$_5$), 4.27 (s, 5H, C$_5$H$_5$), 4.22 (m, 2H, C$_5$H$_4$),

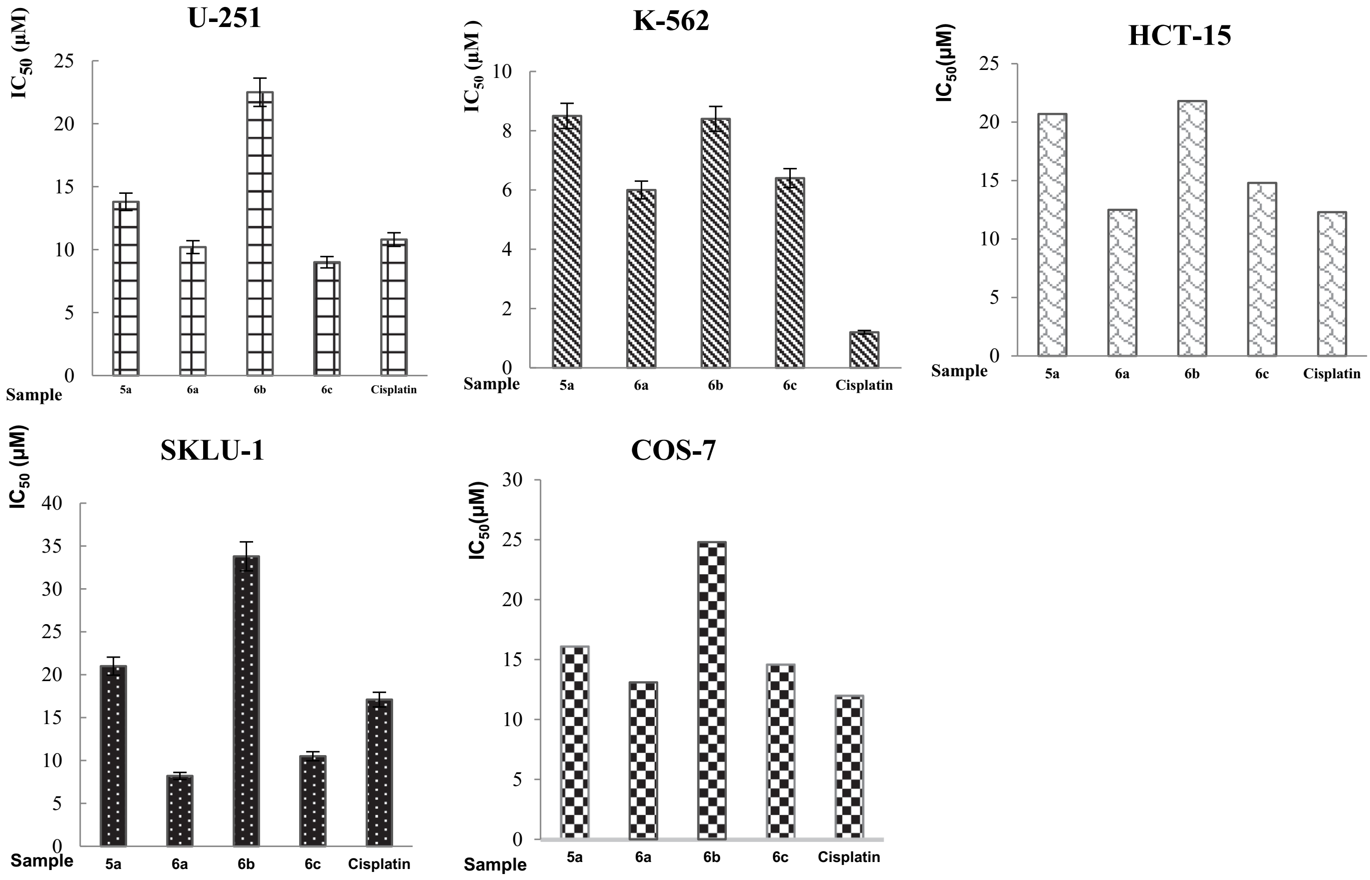


Fig. 6. Graphic of IC_{50} values (µM) of compounds **5a, 6a–6c**, and *cisplatin* towards the U-251, K-562, HCT-15, and SKLU cell lines.

4.25(m, 2H, C_5H_4), 4.47(m, 2H, C_5H_4), 4.91 (m, 2H, C_5H_4), 6.75 (s, 1H, CH=). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.38, 18.24 ($4CH_3$), 46.13, 50.10 ($2CH_2$), 48.73 (CH), 69.50, 69.54 ($2C_5H_5$), 68.37, 69.26, 69.89, 70.39 ($2C_5H_4$), 77.85, 78.21 ($2C_{ipso}Fc$), 134.84 (CH=), 116.14, 159.19 (2C). Anal. calcd. For $C_{31}H_{37}BF_4Fe_2N_2$ (636.13): C, 58.53; H, 5.86; N, 4.40. Found: C, 58.52; H, 5.61; N, 4.45%. MS (El, 70 eV): *m/z* 87, 546 $[M]^+$.

3.3.3. *Reactions of bis-tetrafluoroborates of N,N′- bis-(2′,3′-diferrocenylcyclopropenium-1′-yl)- N,N′- dialkyl-1,2- and 1,3-alkenylendiamines **3a–d** with NaN_3 and NaNHCN*

A mixture of the tetrafluoroborate of *bis*-cation **3a** (or **3b**, **3c**, **3d**) (2.0 mmol), 0.15 g of NaN_3 or 0.32 g NaNHCN, Et_3N (1.5 ml) in anhydrous CH_3CN (50 ml) in a nitrogen atmosphere was heated to refluxed (8 h). The organic layer was concentrated, and the residue was chromatographed on alumina (Brockmann activity III, hexane-ether, 3:1, hexane-dichloromethane, 2:1) to give compounds **5a** (or **5b–d**), **6a** (or **6b–d**), **7a** (or **7b–d**) and **8**.

***N,N′-Bis*-(4′,6′-diferrocenyl-1′,2′,3′-triazine-5′-)piperazine 5a:** orange powder, yield 0.64 g (65%), m.p. 185–187 °C. IR (KBr): ν (cm^{-1}) 486, 504, 543, 595, 603, 665, 694, 735, 819, 840, 889, 910, 944, 1000, 1021, 1034, 1068, 1106, 1161, 1186, 1207, 1255, 1262, 1276, 1360, 1375, 1385, 1431, 1445, 1467, 1506, 1668, 2812, 2852, 2898, 2925, 2963, 3093. ^1H NMR (400 MHz, $CDCl_3$): δ 2.80 (8H, m, $4CH_2$), 4.22 (20H, s, $4C_5H_5$), 4.45 (8H, m, $2C_5H_4$), 4.96 (8H, m, $2C_5H_4$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 45.34, 50.73 ($4CH_2$), 70.42 ($4C_5$ H_5), 69.39, 71.08 ($4C_5$ H_4), 81.71 ($4C_{ipso}Fc$), 138.89 (2C), 155.86 (4C). Anal. calcd. for $C_{50}H_{44}Fe_4N_8$ (980.25): C, 61.26; H, 4.52; N, 11.43. Found: C, 61.40; H, 4.60; N, 11.58%. MS (El, 70 eV): *m/z* 980 $[M]^+$.

***N,N′-Bis*-(4′,6′-diferrocenyl-1′,2′,3′-triazine-5′)-*N,N′*-dimethyl-1,3-propandiamine 5b:** orange powder, yield 0.53 g (53%), m.p. 193–194 °C. IR (KBr): ν (cm^{-1}) 474, 519, 680, 760, 815, 827, 886, 908, 924, 966, 1000, 1026, 1044, 1060, 1104, 1240, 1254, 1283, 1325, 1361, 1388, 1408, 1420, 1446, 1531, 1621, 1707, 2854, 2922, 2989, 3100, 3120. ^1H NMR (400 MHz, $CDCl_3$) δ 2.21 (m, 1H, CH_2), 2.42 (m, 1H, CH_2), 3.33 (s, 6H, $2CH_3$), 3.63 (m, 2H, CH_2), 4.09 (m, 2H, CH_2), 4.22 (s, 10H, $2C_5H_5$), 4.24 (s, 10H, $2C_5H_5$), 4.35 (m, 4H, C_5H_4), 4.37 (m, 4H, C_5H_4), 4.40 (m, 4H, C_5H_4), 4.65 (m, 4H, C_5H_4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.15 (CH_2), 41.92 ($2CH_3$), 47.60 ($2CH_2$), 69.39, 69.67 ($4C_5H_5$), 68.28, 69.33, 70.17, 70.69 ($4C_5H_4$), 78.29, 79.22 ($4C_{ipso}Fc$), 121.32, 134.93, 163.50 (6C). Anal. calcd. For $C_{51}H_{48}Fe_4N_8$ (996.29): C, 61.48; H, 4.86; N, 11.24. Found: C, 61.75; H, 4.70; N, 10.90%. MS (El, 70 eV): *m/z* 995 $[M]^+$.

***N,N′-Bis*-(4′,6′-diferrocenyl-1′,2′,3′-triazine-5′)-*N,N′*-diethyl-1,3-propanediamine 5c:** orange powder, yield 0.59 g (58%), m.p. 190–192 °C. IR (KBr): ν (cm^{-1}) 474, 519, 655, 761, 815, 827, 886, 924, 966, 1001, 1026, 1044, 1060, 1105, 1240, 1254, 1325, 1374, 1389, 1408, 1420, 1446, 1532, 1621, 2882, 2951, 2988, 3081, 3121. ^1H NMR (400 MHz, $CDCl_3$): δ 1.23 (t, 3H, $J = 6.0$ Hz, CH_3), 1.29 (t, 3H, $J = 6.0$ Hz, CH_3), 2.24 (m, 1H, CH_2), 2.36 (m, 1H, CH_2), 3.73 (m, 4H, $2CH_2$), 3.78 (m, 4H, $2CH_2$), 4.22 (s, 5H, C_5H_5), 4.23 (s, 5H, C_5H_5), 4.26 (s, 10H, $2C_5H_5$), 4.41 (bs, 2H, C_5H_4), 4.42 (bs, 2H, C_5H_4), 4.51 (m, 2H, C_5H_4), 4.87 (bs, 4H, C_5H_4), 4.87 (m, 2H, C_5H_4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.72, 13.31 ($2CH_3$), 19.40 (CH_2), 45.02 ($2CH_2$), 49.29 ($2CH_2$), 69.43, 69.83 ($4C_5$ H_5), 68.57, 69.51, 70.28, 70.72 ($4C_5$ H_4), 71.26, 78.23 ($4C_{ipso}Fc$), 121.84, 133.58, 162.97 (6C). Anal. calcd. for $C_{53}H_{52}Fe_4N_8$ (1024.35): C, 62.14; H, 5.12; N, 10.93. Found: C, 62.27; H, 5.28; N, 11.00%. MS (El, 70 eV): *m/z* 1024 $[M]^+$.

***N,N′-Bis*-(4′,6′-diferrocenyl-1′,2′,3′-triazine-5′)-*N,N′*-diisopropylethylenediamine 5d:** orange powder, yield 0.61 g (59%), m.p. 98–99 °C. IR (KBr): ν (cm^{-1}) 474, 519, 655, 680, 761, 815, 828, 886, 924, 966, 1001, 1028, 1045, 1060, 1105, 1231, 1254, 1283, 1326, 1374, 1388, 1408, 1447, 1471, 1535, 1621, 2882, 2950, 3081, 310, 3120. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (d, 6H, $2CH_3$, J = 6.0 Hz), 1.38 (d, 6H, $2CH_3$, J = 6.0 Hz), 4.01 (t, 2H, CH_2, J = 6.0 Hz), 4.08 (m, 2H, 2CH), 4.16 (t, 2H, CH_2, J = 6.0 Hz), 4.24 (s, 10H, $2C_5H_5$), 4.28 (s, 10H, $2C_5H_5$), 4.43 (s, 8H, $2C_5H_4$), 4.48, 4.91 (m, 8H, $2C_5H_4$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.03 ($4CH_3$), 41.55 (2CH), 48.79 ($2CH_2$), 69.53, 69.63 ($4C_5H_5$), 68.40, 69.34, 70.55, 71.10 ($4C_5H_4$), 78.00, 78.11 ($2C_{ipso}$Fc), 134.51 (4C), 165.52 (2C). Anal. calcd. for $C_{55}H_{56}Fe_4N_8$ (1052.40): C, 62.77; H, 5.36; N, 10.64. Found: C, 62.50; H, 5.42; N, 10.43%. MS (El, 70 eV): *m/z* 1051 $[M]^{+}$.

N,N′-Bis-(1′-aza-1′-cyano-3′,4′-diferrocenyl-1′,3′-butadien-2′-yl)piperazine 6a: orange powder, yield 0.53 g (54%), m.p. dec. *ca* 330–333 °C. IR (KBr): ν (cm^{-1}) 475, 534, 617, 688, 730, 815, 896, 925, 1000, 1024, 1047, 1105, 1179, 1209, 1257, 1293, 1311, 1343, 1377, 410, 1460, 1496, 1592, 1645, 2253, 2853, 2921, 2957, 3090. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.59 (m, 2H, $2CH_2$), 3.04 (m, 2H, $2CH_2$), 3.42 (m, 2H, $2CH_2$), 3.54 (m, 2H, CH_2), 4.05 (s, 5H, C_5H_5), 4.24 (s, 5H, C_5 H_5), 4.26 (s, 5H, C_5H_5), 4.32 (s, 5H, C_5H_5), 4.12 (m, 2H, C_5H_4), 4.17 (m, 2H, C_5H_4), 4.19 (m, 2H, C_5H_4), 4.22(m, 2H, C_5H_4), 4.31(m, 2H, C_5H_4), 4.37(m, 2H, C_5H_4), 4.41(m, 2H, C_5H_4), 4.45 (m, 2H, $4C_5H_4$), 6.84 (s, 2H, 2CH =). ^{13}C NMR (100 MHz, $CDCl_3$): δ 45.58, 46.23, 49.82, 53.45 ($4CH_2$), 69.53 ($2C_5$ H_5), 69.73, 70.04 ($2C_5H_5$), 68.04, 68.83, 69.02, 69.09, 70.52, 70.03, 71.14, 71.99 ($4C_5H_4$), 78.33, 80.70 ($4C_{ipso}$Fc), 118.34 (2CN), 133.48 (2CH =), 125.74, 173.00 (4C). Anal. calcd. for $C_{52}H_{46}Fe_4N_6$ (978.29): C, 63.84; H, 4.74; N, 8.59. Found: C, 64.00; H, 4.63; N, 8.80%. MS (El, 70 eV): *m/z* 978 $[M]^{+}$.

N,N′-Bis-(1′-cyano-3′,4′-diferrocenyl-1′-aza-1′,3′-butadien-2′-yl)-_N,N′_-dimethyl-1,3-propa-nediamine 6b: orange powder, yield 0.55 g (56%), m.p. 209–211 °C. IR (KBr): ν (cm^{-1}) 474, 491, 530, 554, 649, 664, 670, 726, 810, 882, 908, 967, 998, 1040, 1050, 1105, 1126, 1170, 1203, 1222, 1247, 1268, 1328, 1361, 1392, 1408, 1426, 1444, 1480, 1535, 1595, 1650, 2250, 2902, 2954, 3094. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.17 (m, 1H, CH_2), 2.37 (m, 1H, CH_2), 3.31 (s, 6H, $2CH_3$), 3.52 (dt, 2H, CH_2, J = 3.9, 10.2 Hz), 3.90 (m, 2H, CH_2), 4.23 (s, 10H, $2C_5H_5$), 4.24 (s, 10H, $2C_5H_5$), 4.33(m, 4H, C_5H_4), 4.37(m, 4H, C_5H_4), 4.41(m, 4H, C_5H_4), 4.66 (m, 4H, C_5H_4), 6.90 (s, 2H, 2CH =). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.13 (CH_2), 41.81 ($2CH_3$), 47.51 ($2CH_2$), 69.29, 69.61 ($4C_5H_5$), 68.22, 70.10, 70.61, 71.15, 70.61 ($4C_5H_4$), 78.23, 79.16 ($4C_{ipso}$Fc), 116.12 (2CN), 134.69 (2CH =), 121.32, 163.37 (4C). Anal. calcd. for $C_{53}H_{50}Fe_4N_6$ (994.33): C, 64.02; H, 5.07; N, 8.45. Found: C, 63.80; H, 5.17; N, 8.57%. MS (El, 70 eV): *m/z* 994 $[M]^{+}$.

N,N′-Bis-(1′-cyano-3′,4′-diferrocenyl-1′-aza-1′,3′-butadien-2′-yl)-_N,N′_-diethyl-1,3-propandiamine 6c: orange powder, yield 0.62 g (60%), m.p. 251–253 °C. IR (KBr): ν (cm^{-1}) 472, 733, 817, 889, 930, 1001, 1030, 1046, 1107, 1179, 1216, 1249, 1299, 1332, 1351, 1388, 1411, 1452, 1514, 1604, 1625, 2200, 2879, 2934, 2978, 3022, 3077, 3099. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24 (t, H, J = 5.4 Hz, $2CH_3$), 2.18 (m, 1H, CH_2), 2.32 (m, 1H, CH_2), 3.47 (q, 4H, J = 5.4 Hz, $2CH_2$), 3.79 (m, 4H, $2CH_2$), 4.23 (s, 10H, $2C_5$ H_5), 4.24 (s, 10H, $2C_5$ H_5), 4.38 (m, 4H, C_5H_4), 4.41 (m, 4H, C_5H_4), 4.43 (m, 4H, C_5H_4), 4.81 (m, 4H, C_5H_4), 6.74 (s, 2CH =). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.60, 13.70 ($2CH_3$), 19.06 (CH_2), 44.68 ($2CH_2$), 48.92 (CH_2), 49.26 (CH_2), 69.45, 69.80, 70.16, 70.32 ($4C_5H_5$), 68.50, 69.93, 70.18 70.80 71.27 ($4C_5H_4$), 78.33, 79.11, 79.31, 80.96 ($4C_{ipso}$Fc), 126.96 (2CN), 133.48, 134.57 (2CH =), 123.46 (2C), 160.09, 162.93 (2C = N). Anal. calcd. for $C_{55}H_{54}Fe_4N_6$ (1022.38): C, 64.61; H, 5.32; N, 8.22. Found: C, 64.63; H, 5.47; N, 8.32%. MS (El, 70 eV): *m/z* 994 $[M]^{+}$.

N,N′-Bis-(1′-cyano-3′,4′-diferrocenyl-1′-aza-1′,3′-butadien-2′-yl)-_N,N′_-diisopropyl-1,2-ethylendiamine 6d: orange powder, yield 0.58 g (56%), m.p. 198–201 °C. IR (KBr): ν (cm^{-1}) 483, 504, 587, 607, 648, 744, 783, 810, 825, 836, 873, 896, 931, 944, 1001, 1042, 1053, 1104, 1139, 1222, 1264, 1323, 1359, 1380, 1410, 1451, 1573, 1623, 1676, 2219, 2892, 2924, 3039, 3103, 3143. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (d, 3H, CH_3, J = 6.0 Hz), 1.31 (d, 3H, CH_3, J = 6.0 Hz), 1.36 (d, 3H, CH_3, J = 4.2 Hz), 1.38 (d, 3H, CH_3, J = 4.2 Hz), 3.93 (m, 4H, $2CH_2$), 4.05 (m, 1H, CH), 4.13 (m, 1H, CH), 4.22 (s, 5H, C_5H_5), 4.26 (s, 5H, C_5H_5), 4.28 (s, 5H, C_5H_5), 4.29 (s, 5H, C_5H_5), 4.31 (m, 2H, C_5H_4), 4.36 (m, 2H, C_5H_4), 4.40 (m, 4H, C_5H_4), 4.44 (m, 2H, C_5H_4), 4.45 (m, 2H, C_5H_4), 4.54 (m, 2H, C_5H_4), 4.89 (m, 2H, C_5H_4), 5.11 (m, 2H, C_5H_4), 6.73 (s, 2H, 2CH =). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.98, 20.01, 20.03, 20.12 ($4CH_3$), 41.54, 41.87 ($2CH_2$), 48.82, 49.16 (2CH), 69.51, 69.59, 69.89, 70.39 ($4C_5H_5$), 68.36, 68.95, 69.25, 69.84, 70.40, 70.54, 71.09, 71.54 ($4C_5H_4$), 77.20, 77.86, 78.23, 80.66 ($4C_{ipso}$Fc), 116.07, 118.54 (2CN), 134.88 (2CH =), 123,14 (2C), 162.39, 165.63 (C = N). Anal. calcd. For $C_{57}H_{58}Fe_4N_6$ (1050.43): C, 65.17; H, 5.57; N, 8.00. Found: C, 65.24; H, 5.52; N, 8.13%. MS (El, 70 eV): *m/z* 1034 $[M]^{+}$.

_N_-(1′-Aza-1′-cyano-3′,4′-diferrocenyl-1′,3′-butadien-2′-yl)piperazine 7a: orange powder, yield 0.10 g (18%), m.p. 215–217 °C. IR (KBr): ν (cm^{-1}) 473, 483, 533, 592, 723, 776, 819, 905, 980, 1001, 1028, 1039, 1048, 1106, 1148, 1219, 1256, 1274, 1293, 1322, 1363, 1413, 1439, 1481, 1542, 1626, 2180, 2747, 2796, 2813, 2853, 2914, 2940, 3108, 3183, 3314. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.97 (m, 4H, $2CH_2$), 3.63 (m, 4H, $2CH_2$), 4.24 (s, 5H, C_5 H_5), 4.25 (s, 5H, C_5H_5), 4.04 (m, 2H, C_5H_4), 4.16 (m, 2H, C_5H_4), 4.31(m, 2H, C_5H_4), 4.38 (m, 2H, C_5H_4), 4.80 (bs, 1H, NH), 6.41 (s, 1H, CH =). ^{13}C NMR (100 MHz, $CDCl_3$): δ 45.34, 45.58, 46.23, 49.83 ($4CH_2$), 69.53, 69.73 ($2C_5H_5$), 68.05, 68.11, 68.82, 69.03, 69.10, 69.95, 70.52, 71.14 ($2C_5H_4$), 78.33, 80.70 ($2C_{ipso}$Fc), 118.34 (CN), 133.48 (CH =), 125.74, 173.01 (2C).Anal. calcd. for $C_{28}H_{28}Fe_2N_4$ (532.20): C, 63.19; H, 5.30; N, 10.52. Found: C, 63.33; H, 5.52; N, 10.50%. MS (El, 70 eV): *m/z* 532 $[M]^{+}$.

_N_-(1′-Cyano-3′,4′-diferrocenyl-1′-aza-1′,3′-butadien-2′-yl)-_N,N′_-dimethyl-1,3-propanediamine 7b: orange powder, yield 0.09 g (17%), m.p. 178–179 °C. IR (KBr): ν (cm^{-1}) 476, 521, 611, 698, 736, 754, 818, 876, 912, 941, 1001, 1023, 1104, 1161, 1210, 1251, 1267, 1335, 1378, 1408, 1432, 1520, 1554, 1621, 1657, 1671, 2234, 2932, 3088, 3125, 3345. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.23 (m, 1H, CH_2), 2.37 (m, 1H, CH_2), 3.30 (s, 6H, $2CH_3$), 3.56 (m, 2H, CH_2), 3.88 (m, 2H, CH_2), 4.15 (s, 1H, NH), 4.22 (s, 5H, C_5H_5), 4.23 (s, 5H, C_5H_5), 4.32 (m, 2H, C_5H_4), 4.37 (m, 2H, C_5H_4), 4.40 (m, 2H, C_5H_4), 4.65 (m, 2H, C_5H_4), 6.87 (s, 1H, CH =). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.07 (CH_2), 41.78 ($2CH_3$), 47.50 ($2CH_2$), 69.39, 69.61 ($2C_5H_5$), 68.24, 69.41, 70.10, 70.68 ($2C_5H_4$), 78.28, 79.21 ($2C_{ipso}$Fc), 115.27 (CN), 134.46 (CH =), 121.30, 163.47 (2C). Anal. calcd. for $C_{29}H_{32}Fe_2N_4$ (548.25): C, 63.53; H, 5.89; N, 10.21. Found: C, 63.37; H, 5.68; N, 10.30%. MS (El, 70 eV): *m/z* 548 $[M]^{+}$.

_N_-(1′-Cyano-3′,4′-diferrocenyl-1′-aza-1′,3′-butadien-2′-yl)-_N,N′_-diethyl-1,3-propanediamine 7c: orange powder, yield 0.12 g (21%), m.p. 178–180 °C. IR (KBr): ν (cm^{-1}) 478, 526, 602, 699, 730, 774, 820, 884, 915, 938, 1001, 1019, 1106, 1127, 1158, 1209, 1243, 1292, 1341, 1383, 1412, 1437, 1518, 1552, 1623, 1650, 1678, 2215, 2852, 2925, 2994, 3093, 3243, 3340. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (t, 6H, $2CH_3$ J = 6.0 Hz,), 2.20 (m, 1H, CH_2), 2.35 (m, 1H, CH_2), 3.47 (m, 2H, CH_2), 3.57 (m, 2H, CH_2), 3.83 (m, 4H, $2CH_2$), 4.24 (s, 10H, $2C_5H_5$), 4.09 (bs, 1H, NH), 4.39 (m, 2H, C_5H_4), 4.42 (m, 2H, C_5H_4), 4.44 (m, 2H, C_5H_4), 4.81 (m, 2H, C_5H_4), 6.80 (CH =). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.99 ($2CH_3$), 18.96 (CH_2), 44.55 ($2CH_2$), 49.19 ($2CH_2$), 69.38, 69.73 ($2C_5$ H_5), 68.39, 69.35, 70.17, 70.74 ($2C_5H_4$), 78.21, 79.24 ($2C_{ipso}$Fc), 113.2 (CN), 133.43 (CH =), 121.82 (C), 162.87 (C = N). Anal. calcd. for $C_{31}H_{36}Fe_2N_4$ (576.30): C, 64.60; H, 6.30; N, 9.72. Found: C, 64.53; H, 6.45; N, 9.90%. MS (El, 70 eV): *m/z* 576 $[M]^{+}$.

_N_-(1′-cyano-3′,4′-diferrocenyl-1′-aza-1′,3′-butadien-2′-yl)-_N,N′_-diisopropyl-1,2-ethylendiamine 7d: orange powder, yield 0.11 g (18%), m.p. 182–183 °C. IR (KBr): ν (cm^{-1}) 482, 579, 823, 1001, 1047, 1107, 1129, 1171, 1189, 1257, 1300, 1372, 1464, 1452, 1595, 1655, 2151, 2190, 2876, 2936, 2969, 3209, 3321. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (d, 6H, $2CH_3$, J = 5.1 Hz), 1.38 (d, 6H, $2CH_3$, J = 5.1 Hz), 3.94 (t, 2H, CH_2, J = 7.5 Hz), 4.07 (m, 2H, 2CH, J = 5.1 Hz), 4.16 (t, 2H, CH_2, J = 7.5 Hz), 4.25 (s, 5H, C_5H_5), 4.27 (s, 5H, C_5H_5), 4.30 (bs, 1H, NH), 4.42 (m, 4H, C_5H_4), 4.48 (m, 2H, C_5H_4), 4.91 (m, 2H, C_5H_4), 6.75 (s, 1H, 1CH =). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.01, 21.24 ($4CH_3$), 44.53, 46.13 ($2CH_2$), 48.77, 49.16 (2CH), 69.51, 69.60 ($2C_5H_5$), 68.38, 69.32, 69.89, 71.39 ($2C_5H_4$), 77.98, 78.09 (C_{ipso}Fc), 116.36 (CN), 134.49 (CH=), 159.14 (C=N). Anal. calcd. for $C_{32}H_{38}Fe_2N_4$ (590.32): C, 65.10; H, 6.49; N, 9.49. Found: C, 65.33; H, 6.38; N, 9.38%. MS (EI, 70 eV): *m/z* 590 $[M]^+$.

3.3.4. Reaction of 2,3-diferrocenylcyclopropenone with 1,2- and 1,3-diamines in the presence of NaN_3 or NaNHCN

A mixture of 1.06 g (2.5 mmol) of 2,3-diferrocenylcyclopropenone, CH_2Cl_2 (70 ml), 5.0 ml of the triethyloxonium tetrafluoroborate (1.0 M solution in dichloromethane), Et_3N (1 ml), 1,2- and 1,3-diamines **2a–d** (1,25 mmol) refluxed for 6 h. The solvents were removed *in vacuo* and the remnant was dissolved in dichloromethane (~30 ml). The solution was mixed with Al_2O_3 (15 g) and the solvent was evaporated in air. This sorbent was applied into a column (loaded with Al_2O_3, the height of alumina is *ca.* 15 cm) and the reaction products were eluted first with petroleum ether, then with a 2:1 hexane-dichloromethane to give compounds **5a–d** (~56–64%), **6a–d** (~45–56%), **7a–d** (~23–27%) and **8** (~12–13%).

3-Cyanoimino-1,2-diferrocenylcyclopropene 8: yield 0.13–0.14 g (12–13%), orange crystals, m.p. 214–215 °C (lit. [21]m.p. 214–216 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 4.28 (10H, s, $2C_5H_5$), 4.71 (m, 4H, C_5H_4), 4.91 (m, 4H, C_5H_4).

3.4. Crystal structure determination

The crystal suitable for X-ray analysis of **4b**, **4d** was obtained by crystallization from ether, crystals of **7a** were obtained by crystallization from chloroform. X-ray analysis was performed on a diffractometer (Oxford Diffraction Gemini-Atlas). Data collection routine and data reduction were carried out with CrystAlisPro and CrysAlis Pro, (Oxford Diffraction Ltd) [31]. The structures of all molecules were solved using SHELXS-97 [32,33], and refined using SHELXL-97. All non-hydrogen atoms were polished anisotropically and the hydrogen atoms were found by difference. Fourier maps, placed at geometrically calculated positions and refined by using the rinding model. Crystal data and refinement details are presented in Table 5 while selected bond distances and angles are listed in the Supplementary material.

3.5. Electrochemical measurements

Cyclic (CV), differential pulse (DPV), and square wave (SWV) voltammetries were carried out in a conventional three-electrode cell shielded by a Faraday cage (BAS). The working electrode was a 1 mm diameter glassy carbon electrode (GCE). The reference electrode was an $AgNO_3/Ag$ electrode (a silver wire immersed in 0.01 mol/L silver nitrate + 0.1 mol/L tetrabutylammonium hexafluorophosphate / acetonitrile). All the cyclic voltammograms were traced using the CHI660C or EDAQ 161 potentiostats commanded by CHI660C or EChem software, respectively. All potentials reported here are referred to as Ferrocenium/Ferrocene (Fc^+/Fc) couple [37]. The working electrode was cleaned before each voltammogram using CH_2Cl_2 or CH_3CN; when necessary the electrode surface was polished with 0.5 μm alumina slurry (Buehler), deeply rinsed with water, sonicated for 8 min in deionized water rinsed deeply with water and/o dichloromethane and dried with a tissue (Kimwipes). Acetonitrile was anhydrous quality, was kept in 4A molecular sieve, and manipulated using syringes. Solutions were bubbled with ultra-high purity argon (Infra, previously saturated with CH_3CN) during 5 min before starting the recordings. Voltammograms were recorded in Bu_4NPF_6 0.1 M/CH_3CN at 0.06 to 0.2 V/s. Consecutive voltammograms (four cycles at 100 mV/s) were also recorded for each compound and scanning rate (see Supplementary material). The CV scans were from 60 to 200 mV/s each 20 mV. In DPV pulse amplitude was selected from 10 mV to 25 each 5 mV. In SWV the frequency was varied from 5 Hz to 15 Hz each 5 Hz. Recordings initiated from different initial potential E_i to anodic direction. DigiElch (Elchsoft, version 8 Professional) was used to fit SWV voltammograms. The half-wave potential $E_{1/2}$ was evaluated using experimental anodic and cathodic peak potentials with the equation $E_{1/2} = (E_{pa} + E_{pc})/2$ [8], for VC. In the case of DPV the formal potential $E^0 \approx E_{1/2}$ was evaluated by the $E_{1/2} = E_p + \Delta E/2$; for SWV, $E_{1/2} = E_p$ or by fitting the experimental data using DigiElch.

3.6. Biology

3.6.1. Viability assay in vitro

The compounds were screened *in vitro* against U-251, K-562, HCT-15, SKLU-1 (human), and COS-7 (monkey) cell lines.

Cell lines were supplied by The National Cancer Institute (NCI, U.S.A). The cytotoxicity of compounds on tumor cells was determined using the protein-binding dye sulforhodamine B protocol (SBR) in microculture assay and was according to the National Cancer Institute protocol [34]. The SRB assay varies linearly with cell number and cellular protein [35,36].

Cells were exposed to 50 μg/ ml for 48 h. All experiments were conducted by quintuplicate. Data are indicated as the average ± standard deviation.

The cell lines were cultured in RPMI-1640 (Sigma Chemical) supplemented with 10% fetal bovine serum (Invitrogen), 2 mM L-glutamine, 10,000 units/ml of penicillin G, 10,000 μg/ml streptomycin sulfate and 0.25 μg/ml Fungiozone (Gibco) and 1% non-essential amino acids (Gibco). They were incubated at 37 °C in 5% CO_2 atmosphere with 95% humidity. For the assay, 5104 cell/ml (K-562, MCF-7), 7510 cell/ml (U-251) and 10,104 cell/ml (SKLU-1, HCT-15) and 100 ml/well of the cell suspension was seeded in 96-well microtiter plates and incubated to allow for cell attachment. After 24 h, 100 μl of each test compounds and positive substances were added to each well. After 48 h, adherent cell cultures were fixed *in situ* by adding 50 ml of cold 50% (*w/v*) trichloroacetic acid (TCA) and incubated for 60 min at 4 °C. The supernatant was dismissed and the plates were washed three times with water and air dried. Cultures fixed with TCA were stained for 30 min with 100 μl of 0.4% SRB solution. Protein-bounded dye was extracted with 10 mM unbuffered Tris base and the optical densities were read on a Microplate Reader Synergy HT (Elx 808, BIOTEK Instruments) at the wavelength of 515 nm. Results were expressed as IC_{50} values, they were calculated according to the protocol of Monks [34], where a dose-response curve was traced for each compound, and the concentration giving 50% inhibition (IC_{50}) was estimated from non-linear regression equations.

4. Conclusions

Molecules with four ferrocenyl substituents can be obtained from the reaction of 2,3-diferrocenyl-1-ethoxycyclopropenium tetrafluoroborate with *bis*-1,4- and *bis*-1,5-*N,N*-nucleophiles **2a–d,** to obtain the corresponding *bis*-cations. These molecules can serve as building blocks of macromolecules with *poly*-ferrocenyl substituents.

The results show that the redox-properties of the ferrocenyl-fragments in compounds **5a**, **6a–c** of six or eight nitrogen-atoms conferred their biological activities. The correlation of the anticancer activity of these compounds with the relative ease of oxidation of the Fc center suggests that the Fc center acts as a "redox antenna".

These results also identified, that synthesized compounds are new leads in antitumor chemotherapy. The study suggests that the potential of these candidates needs to be further explored to discover and develop better and safer therapeutic antitumor agents.

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Appendix A. Supplementary data

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, numbers CCDC-1882218 (for **4b**), CCDC-188220 (for **4d**) and CCDC 1882219 (for **7a**) Copies of this information may be obtained free of charge at www.ccdc.cam.ac.uk/const/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge DB2 1EZ, UK. E-mail: deposit@ccdc.cam.ac.uk]. Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinorgbio.2019.04.003.

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