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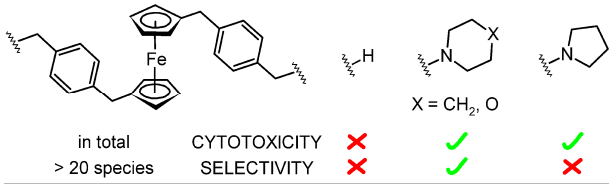


Improving cytotoxic properties of ferrocenes by incorporation of saturated N-heterocycles

Tomáš Hodík,[a,l] Martin Lamač,[a] Lucie Červenková Šťastná,[b] Petra Cuřínová,[b] Jindřich Karban,[b] Hana Skoupilová,[c] Roman Hrstka,[c] Ivana Císařová,[d] Róbert Gyepes,[a,d] Jiří Pinkas[a,*]

[a] J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, v.v.i., Dolejškova 2155/3, 182 23 Prague 8, Czech Republic

[b] Institute of Chemical Process Fundamentals, Academy of Sciences of the Czech Republic, v.v.i., Rozvojová 135, 165 02 Prague 6, Czech Republic

[c] Regional Centre for Applied and Molecular Oncology, Masaryk Memorial Cancer Institute, Žlutý kopec 7, 65653 Brno, Czech Republic

[d] Department of Inorganic Chemistry, Charles University, Hlavova 2030, 128 43, Prague 2, Czech Republic

* Corresponding Author: tel. (+420) 266053735; e-mail, jiri.pinkas@jh-inst.cas.cz.

‖ Present address: Institut für Organische Chemie, Universität Leipzig, Johannisallee 29 D-04103 Leipzig, Deutschland

† Electronic supplementary information (ESI) available: experimental conditions for preparation of commercially unavailable starting materials, NMR spectra of new prepared compounds, crystallographic data for structures **4a** and **4a·2HCl**.

Abstract

A family of ferrocene derivatives of the general formula $[Fe(\eta^5\text{-}C_5H_4CH_2(p\text{-}C_6H_4)CH_2(N\text{-het}))_2]$ bearing saturated six- and five-membered N-heterocycles (N-het) was prepared. Reactions of the selected complexes with acids (HCl, acetic acid) afforded either the corresponding hydrochlorides or led to deprotection of the functionalized pendant N-heterocycles. The reaction of $[\{Ru(\eta^6\text{-}p\text{-cymene})Cl_2\}_2]$ with the corresponding cyclopentadienide derivatives afforded cationic ruthenium complexes $[Ru(\eta^6\text{-}p\text{-cymene})(\eta^5\text{-}C_5H_4CH_2(p\text{-}C_6H_4)CH_2(N\text{-het}))]Cl$ while ruthenocenes $[Ru(\eta^5\text{-}C_5H_4CH_2(p\text{-}C_6H_4)CH_2(N\text{-het}))_2]$ were formed as minor byproducts. The prepared complexes (20 examples) were characterized by elemental analysis, melting point, NMR and ESI-MS and the molecular structures of selected ferrocene derivatives were determined by X-ray diffraction analysis. The ferrocene derivatives and the ruthenium complexes were tested *in vitro* for their cytotoxic properties against three cell lines derived from ovarian cancer (A2780, A2780cis, and SK-OV-3) and against non-tumour embryonic cell line HEK293

(human kidney cells). The most active ferrocene derivatives displayed cytotoxicity in submicromolar and low micromolar concentration against both cisplatin (CisPt) sensitive and resistant cells. The results showed a significant effect of the pendant N-heterocycle on the ferrocene derivative toxicity and selectivity against cancer cells. Ultimately, ferrocene derivatives bearing either piperidine or morpholine groups were proposed to be the most promising substitutes for platinum drugs, as they exhibited comparable or even higher activity (in comparison to CisPt) against cancer cells, whereas these compounds were found to exhibit lower toxicity against embryonic HEK293 cells.

Keywords: Metallodrugs; Ferrocene derivatives; N-heterocycles; Cytotoxicity; Ovarian cancer.

Introduction

Cancer is a family of diseases characterized by aggressive, uncontrolled growth of cells, representing one of the greatest causes of death, leading to an estimated 8 million of deaths occurring in 2012 worldwide.[1] Ovarian cancer is the seventh most common malignancy among women, and it represents the eighth leading cause of cancer-related death among women, being the deadliest among gynaecologic tumours.[2] It is a heterogeneous disease and even though chemotherapy and surgery have improved the outcome for patients with gynaecologic malignancies over the last 20 years, "women's cancers" still account annually for over 10% of cancer related deaths. The current treatment protocol consists in delivering platinum-drug chemotherapy (e.g. cisplatin(CisPt), oxaliplatin, carboplatin) which is however frequently accompanied by a number of side-effects. In addition, the therapy is often hampered by acquired resistance of cancer cells towards platinum-drugs.[3-4] Therefore, the introduction of new types of active metallodrugs with a different mode of action in comparison with platinum drugs is strongly desirable.

Among non-platinum coordination (organometallic) compounds, ferrocene derivatives play a prominent role due to their unique properties, such as thermal and hydrolytic stability, inclusion of the reversibly redox active $Fe^{2+/3+}$ center, their simple preparation and straightforward modification. These properties render ferrocene derivatives very popular for biological applications, especially as potential cytostatic drugs in cancer treatment, which has been documented by several reviews.[5-8]

In our previous work, we described the new ferrocene derivative containing 1-piperidinyl moiety [Fe(η^5-$C_5H_4CH_2$(*p*-C_6H_4)CH_2($N(CH_2)_2CH_2$))$_2$]·2HCl (**4a·2HCl**, Scheme 2) which possessed superior cytotoxicity in comparison with CisPt against ovarian (A2780 and A2780cis) and breast (MCF7) cancer cell lines in 24 h assay.[9] In addition, a newly developed electrochemical measurement allowed us to determine an efficient intracellular transport of the complex, and to establish that its accumulation occurs predominantly in cytoplasm.

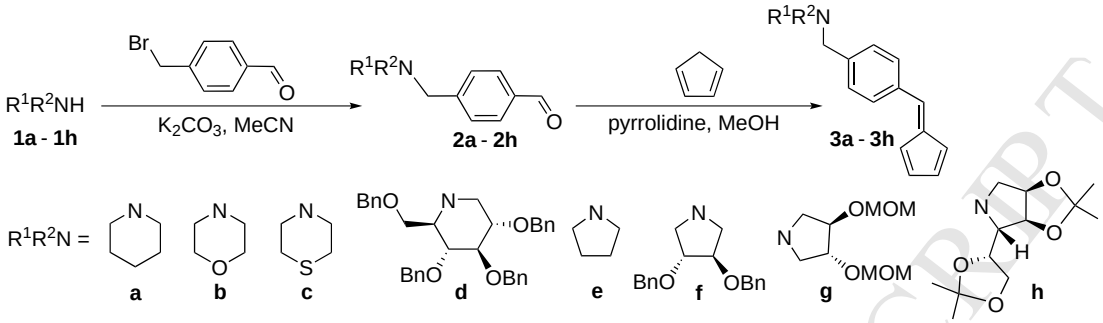
In this article, we report the continuation of investigation of the promising concept of ferrocenes bearing saturated N-heterocycles at their periphery. A series of ferrocene derivatives bearing either substituted or unsubstituted saturated five- and six-membered nitrogen-containing heterocycles was synthesized. In addition, two nitrogen-free ferrocenes were prepared to compare their efficacy. We also investigated the possibility of preparing cationic ruthenium cyclopentadienyl-arene complexes accommodating a saturated N-heterocycle, while ruthenocene complexes were formed as byproducts. To understand the role of the particular N-heterocycle on the organometallic complex activity towards tumour cells and to compare the anticancer activity of our compounds with cisplatin representing the treatment of choice for ovarian cancer patients, the cytotoxic effects of these newly prepared complexes as well as of two structurally related organic species against three human ovarian cancer cell lines (A2780, A2780cis, and SK-OV-3) were investigated. Human embryonic kidney cell line HEK293 was included in the study to determine tumour selectivity of our compounds and to exclude incidental nephrotoxicity, which is frequently observed in patients treated with cisplatin.

Results and discussion

*Synthesis of 6-substituted fulvenes **3a** − **3h***

Fulvenes **3a** − **3h** were prepared according to the general synthetic Scheme 1 adapted from literature.[9-10] As starting secondary amines **1** we used simple saturated 5- and 6-membered nitrogen heterocycles: piperidine (**1a**), morpholine (**1b**), thiomorpholine (**1c**) and pyrrolidine (**1e**). To probe the influence of hydroxyl substituents, we also employed the readily available O-protected iminoalditols: O-benzyl- (OBn) and O-methoxymethyl- (OMOM) protected (3*S*,4*S*)-pyrrolidine-3,4-diol (1,4-dideoxy-1,4-iminothreitol) **1f** and **1g**, 1,4-dideoxy-2,3:5,6-di-*O*-isopropylidene-1,4-imino-D-talitol **1h**,[11] and 2,3,4,6-tetra-*O*-

benzyl-1,5-dideoxy-1,5-imino-D-glucitol (O-benzylated deoxynojirimycin) **1d**.[12-13] In choosing iminoalditols for ferrocene functionalization we were also inspired by a recent discovery that some iminocyclitol-ferrocene conjugates show cytotoxic properties.[14-15]

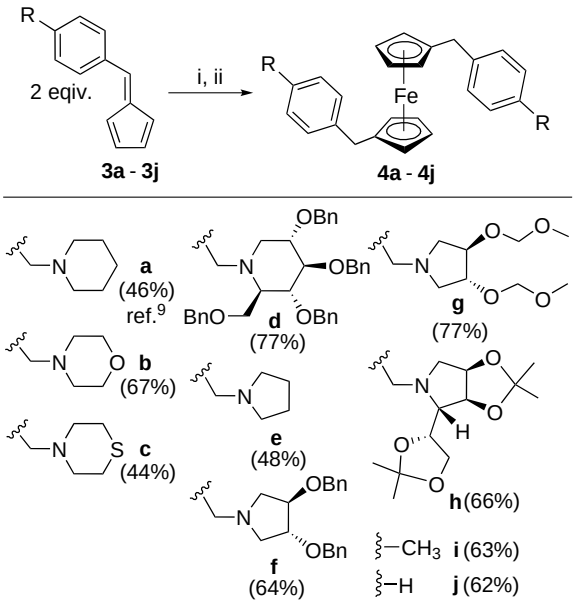


Scheme 1. Synthesis of 6-substituted fulvenes **3a** − **3h**

With the set of the required heterocycles in hand, we subjected them to reaction with 4-(bromomethyl)benzaldehyde[16] in acetonitrile in the presence of potassium carbonate to obtain *N*-(4-formylphenyl)methyl derivatives **2a** − **2h**. The pyrrolidine-promoted condensation with freshly cracked cyclopentadiene according to the procedures by Stone[17] or Erden[18] afforded 6-substituted fulvenes **3a** − **3h**. Detailed experimental procedures for the new fulvenes **3c**, **3d**, and **3f** − **3h** are given in Supporting Information.

A cytostatic effect of 6-aryl fulvenes against a wide family of cancer cells was recently published.[19] Therefore, we prepared a fulvene hydrochloride **3a·HCl** by the reaction of **3a** with HCl in THF. In comparison with **3a** the hydrochloride showed an increased solubility both in DMSO and in water, which was advantageous for biological evaluations. We also prepared the corresponding cyclopentadienyl hydrochloride **3aa·HCl** (as a mixture of isomers) by the reaction of **3a** with $LiBEt_3H$, followed by protolysis with an excess of HCl. Formally, **3aa·HCl** represents a free ligand released from **4a·2HCl** and the concept was used for determination of the ligand cytotoxicity and its potential contribution to the cytotoxicity of ferrocene **4a·2HCl**.

*Syntheses and characterization of ferrocenes **4a − 4j***



Scheme 2. Preparation of ferrocene derivatives **4a** − **4j**. Reagents and conditions: (i) $LiBEt_3H$, THF, rt; (ii) $FeCl_2$, THF, rt. Values in parentheses correspond to isolated yields of **4a** − **4j**.

Ferrocenes **4b** − **4j** were prepared by a procedure similar as reported for **4a**.[9] In addition to fulvenes **3b** − **3h**, we also used heterocycle-free fulvenes **3i** and **3j** as starting materials. The reaction of fulvenes **3b** − **3j** with $LiBEt_3H$ in THF afforded the corresponding lithium cyclopentadienide. This was reacted *in situ* with solid anhydrous $FeCl_2$ in the same solvent for 10 − 24 h at room temperature as schematically depicted in Scheme 2. The crude products were purified by chromatography on silica gel and in selected examples recrystallized from an appropriate solvent (alcohols, THF) or a mixture of solvents (THF/alcohol). Pure ferrocene derivatives **4b** − **4j** were obtained in moderate yields (44 − 77%) as yellow to orange solids or orange waxes depending on the nature of the substituent. It should be noted, that two alternative routes to ferrocene **4j** were already published: either by reaction of thallium benzylcyclopentadienide with $FeCl_2$, or by reduction of 1,1´-dibenzoylferrocene with borane-dimethyl sulfide complex.[20-21]

Ferrocenes **4a** − **4i** showed good solubility in low polar organic solvents (toluene, $CHCl_3$, THF) and low solubility in organic polar solvents such as DMSO (as an exception, complex **4c** was totally insoluble in DMSO, which hampered its biological evaluation), and in organic protic solvents (EtOH). The solubility of all complexes in water was negligible.

All new prepared ferrocene complexes **4b** − **4i** were characterized by ^{1}H and ^{13}C NMR, ESI-MS spectrometry and elemental analysis. In addition, melting points of solid samples were determined.

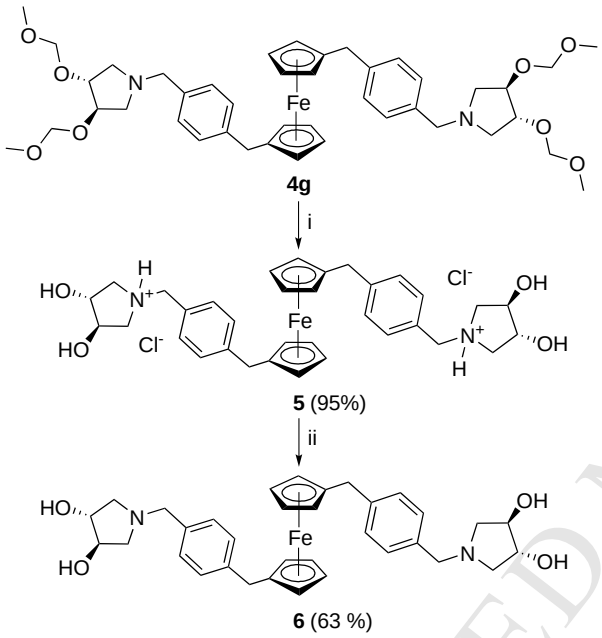
^{1}H NMR spectra of ferrocenes **4b** − **4i** show the characteristic presence of two doublets ($^3J_{HH}$ ~ 8 Hz) for aromatic phenylene C_6H_4 protons positioned in the region 7.1−7.3 ppm. Cyclopentadienide protons C_5H_4 appeared as a singlet in the region 4.03−4.07 ppm (with an exception of **4g** where two multiplets centered at 4.03 and 4.07 ppm were found). A signal for methylene group $C_5H_4CH_2C_6H_4$ connecting cyclopentadienyl and phenylene appeared as a singlet in a narrow region for both ^{1}H and ^{13}C NMR spectra (δ_H/δ_C = 3.59 − 3.68 ppm/34.82 − 35.73 ppm). A methylene group connecting the phenylene group with a nitrogen atom displayed as a singlet signal (in the region 3.45 − 3.57 ppm) in ^{1}H NMR spectra only for achiral ferrocenes **4b**, **4c**, and **4e**. On the other hand, the presence of chiral centers in ferrocenes **4d** and **4f** − **4h** led to splitting of the methylene group signal into two doublets ($^2J_{HH}$ = 12.9 − 13.8 Hz) centered at 3.37 and 4.01 for **4d**, 3.52 and 3.60 ppm for **4f**, 3.43 and 3.51 ppm for **4g**, and 3.55 and 4.18 ppm for **4h**. ESI-MS spectrometry proved a molecular composition of all prepared compounds with $[M + H]^+$ and $[M + Na]^+$ ions as the most abundant within the spectra of **4b** − **4h**. Complex **4i** lacking any electronegative atom (such as O, N) represented the only exception showing just the molecular ion M^+ at *m/z* = 394 as the only peak of relevance.

Reaction of selected ferrocenes with acids (quaternization, deprotection)

4x + excess HCl → **4x·2HCl** (where **4x** = **4b**, **4c**, **4e**, **4f**) eq. 1.

Reaction (equation 1) of selected ferrocene amines **4b, 4c, 4e, 4f** with a slight excess of anhydrous HCl solution in ether (either 3M in methylcyclopentyl ether or 1M in Et_2O) gave the corresponding amine hydrochlorides **4b·2HCl, 4c·2HCl, 4e·2HCl, 4f·2HCl** in medium to excellent yields (55 − 97%), similarly as reported for **4a·2HCl**.[9] The reaction was advantageously conducted in ether solvents (THF, Et_2O), where the starting materials showed high solubility, whereas the products were almost insoluble and could be simply isolated by filtration. The amine hydrochlorides were well soluble in polar organic solvents (DMSO, EtOH, MeOH) and in water. They remained intact in aqueous environment for several days as was proved by ^{1}H NMR. The formation of hydrochlorides **4·2HCl** was supported by ^{1}H NMR spectroscopy, where the signal of the ammonium proton appeared as a broad singlet (at 11.37 ppm for **4b·2HCl**, 10.93 ppm for **4c·2HCl**, 11.27 ppm for **4e·2HCl**, and 11.67 ppm for **4f·2HCl**).

In the case of **4g**, we observed a different reactivity with HCl due to lability of the protecting methoxymethyl group under acidic conditions. The reaction of **4g** with HCl (either anhydrous or aqueous) gave ferrocene bishydrochloride **5** (Scheme 3) having four free hydroxyl groups in excellent yields (89 and 95% in two independent experiments). Both ammonium protons in **5** were easily removed by treatment with anion-exchange resin maintained in -OH^- cycle to give neutral ferrocene alcohol **6** in 63% yield (Scheme 3). As expected, **5** and **6** were well soluble in alcoholic solvents, while the former one exhibited a considerably higher solubility in water in comparison with the latter one.

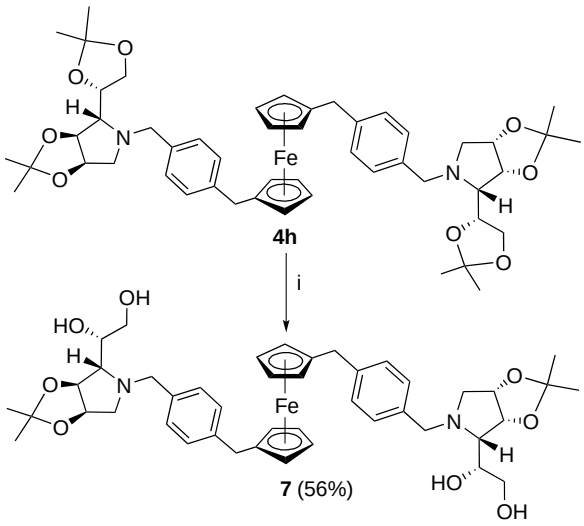


Scheme 3. Reaction of **4g** with an excess of HCl and formation of **5** and **6**. Reagents and conditions: (i) HCl, THF, rt; (ii) anion exchange resin AG-1-X8 (in OH^- cycle), H_2O, rt.

^{1}H NMR spectra displayed signals attributable to hydroxyl groups as a broad singlet positioned at 5.8 ppm and 4.8 ppm for **5** and **6**, respectively. In IR spectra, valence O-H vibration was found at 3286 and 3337 cm^{-1} for **5** and **6**, respectively. The signal of ammonium protons in **5** appeared at 11.05 ppm in ^{1}H NMR spectrum.

A selective removal of the exocyclic isopropylidene groups in **4h** was conducted similarly as described in literature.[11] A reaction of **4h** with aqueous 80% acetic acid resulted in selective hydrolysis of both exocyclic isopropylidene groups and formation of **7** (Scheme 4) in satisfactory yield (56%). ^{1}H and ^{13}C NMR spectra of the product were in accordance with the proposed structure of **7**, although the hydroxyl protons could not be localized in ^{1}H NMR spectra. However, the presence of hydroxyl groups was supported by the presence of a vibration band at 3364 cm^{-1} in IR spectra. The attempted total deprotection

of all hydroxyls in **4h** with aqueous trifluoroacetic acid led to the decomposition of the ferrocene scaffold and no product was isolated.



Scheme 4. Selective removal of two isopropylidene groups resulting in formation of **7**. Reagents and conditions: (i) 80% AcOH, 50 °C.

*Preparation of cationic ruthenium complexes **8** and ruthenocenes **9***

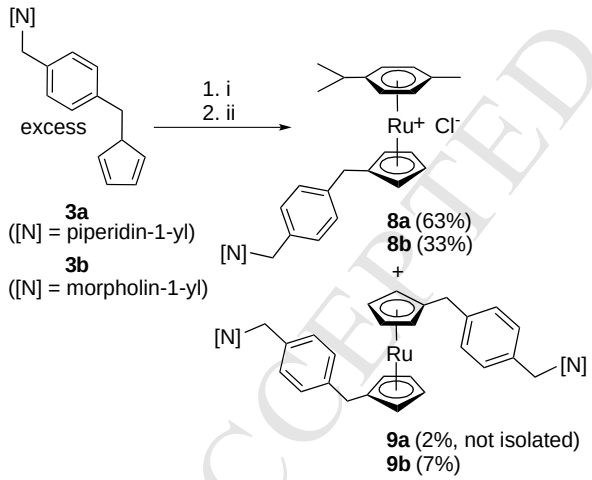
The high cytotoxic potency of **4a** led us to the idea to prepare analogous ruthenium species. A reaction of an excess of lithium cyclopentadienides generated *in situ* from **3a** or **3b**, with [{Ru(η^6-*p*-cymene)Cl_2}$_2$] in THF (Scheme 5) gave cationic arene-cyclopentadienyl complexes **8a** (yield 63%) or **8b** (yield 33%), respectively. Formation of the corresponding ruthenocenes **9a** and **9b** as by-products in crude reaction mixtures was observed by NMR spectroscopy. Indeed, the similar formation of ruthenocene from a reaction of [{Ru(η^6-*p*-cymene)Cl_2}$_2$] with sodium cyclopentadienide was mentioned in the literature.[22] The complex **9a** was generated only in 2% yield (as calculated from ^{1}H NMR of crude reaction mixture) and was not isolated. A high amount of **3b** and increased reaction temperature led to a slightly higher yield (7%) of **9b** in comparison to **9a**. A different polarity of **8b** and **9b** allows their efficient separation by column chromatography on neutral alumina. In an independent experiment an excess of lithium cyclopentadienide derivative (prepared from **3b** and $LiBEt_3H$) reacted with **8b** in boiling toluene for 63h. After workup, **9b** was isolated in 87% yield. We propose that a reaction of [{Ru(η^6-*p*-cymene)Cl_2}$_2$] with lithium cyclopentadienide Cp^1Li (equation 2.) in THF at room temperature, followed by reaction of generated (and eventually purified) [(η^6-*p*-cymene)(η^5-Cp^1)Ru]Cl with Cp^2Li (equation 3.) in

boiling toluene would be a feasible general pathway for generation of ruthenocenes [(η^5-Cp^1)(η^5-Cp^2)Ru] bearing differently substituted cyclopentadienyl rings.

$[\{Ru(\eta^6\text{-}p\text{-cymene})Cl_2\}_2] + 2\ Cp^1Li \rightarrow 2\ [(\eta^6\text{-}p\text{-cymene})(\eta^5\text{-}Cp^1)Ru]Cl$ eq. 2.

$[(\eta^6\text{-}p\text{-cymene})(\eta^5\text{-}Cp^1)Ru]Cl + Cp^2Li \rightarrow [(\eta^5\text{-}Cp^1)(\eta^5\text{-}Cp^2)Ru]$ eq. 3.

Cationic complexes **8a** and **8b** were well soluble in polar solvents and in water, while the considerably less polar **9b** showed a reasonable solubility only in less polar organic solvents, such as chloroform. ^{1}H and ^{13}C NMR spectra of **8a**, **8b** and **9b** were in accordance with their proposed structures. The signals of cyclopentadienyl protons in **8** (pseudotriplets centered at 5.31 and 5.43 ppm for **8a**; 5.31 and 5.42 ppm for **8b**) were considerably downfield shifted in comparison to **9** (pseudotriplets centered at 4.38 and 4.44 ppm for **9a**; multiplet 4.38 − 4.40 ppm for **9b**) in ^{1}H NMR spectra as a result of decreased electron density in the former species. ESI-MS spectra of **8a** and **8b** showed a peak corresponding to arene cyclopentadienyl ruthenium cation at *m/z* 488 and 490, respectively. The molecular composition of **9b** was supported by the presence of $[M + H]^+$ ion at *m/z* 611.



Scheme 5. Formation of cyclopentadienyl arene ruthenium complexes **8** and ruthenocenes **9**. Reagents and conditions: (i) $LiBEt_3H$, THF, rt; (ii) $[\{Ru(\eta^6\text{-}p\text{-cymene})Cl_2\}_2]$, THF, rt or reflux.

Molecular structure determination

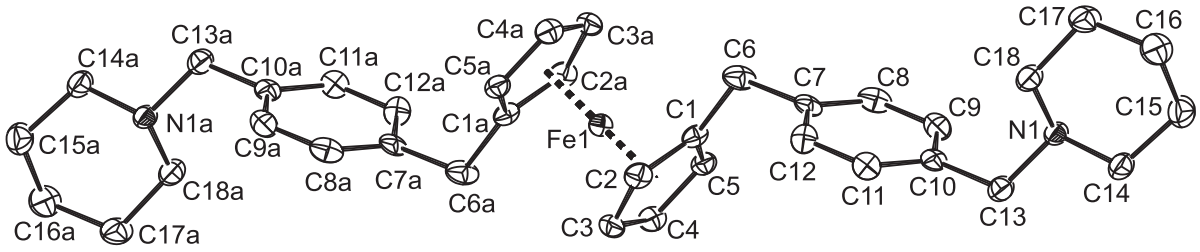


Fig. 1 An ORTEP view of molecular structure of **4a** at 50% probability level. Hydrogen atoms are omitted for clarity. Selected distances [Å] and angles [°]: Fe1-Cg1 1.6510(17), C-$C_{(ring\ C1-C5)}$ 1.410(6) − 1.423(5), C-$C_{(ring\ C7-C12)}$ 1.378(6) − 1.399(5), C-$C_{(C14-C18)}$ 1.511(5) − 1.526(5), C1-C6 1.504(5), C6-C7 1.525(5), C10-C13 1.509(5), N1-$C_{(C13,\ C14,\ C18)}$ 1.462(4) − 1.467(5), C1-C6-C7 112.8(3), N1-C13-C10 112.8(3), Fe1-Cg1-C6-C7 178.11.

Crystals of **4a** suitable for X-ray analysis were grown by diffusion of methanol into a solution of **4a** in THF. **4a** crystallizes in a monoclinic lattice with space group $P2_1/c$. The molecule view and the most important structural parameters are given in Fig. 1. Crystallographic parameters for **4a** and its molecular packing view are given in Supporting Information. The molecule of **4a** possessed an ideal staggered conformation of its cyclopentadienyl rings with the substituents oriented anti-periplanarly within the ferrocene scaffold. The phenylene rings are positioned outwards from the ferrocene core (torsion angle Fe1-Cg1-C6-C7 178.11°) and their orientation towards the cyclopentadienyl rings is close to perpendicular (dihedral angle 110.26°). The piperidine rings adopted chair conformations (θ = 4.8(4)°).

Crystals of **4a·2HCl** suitable for X-ray analysis were obtained from saturated aqueous solution upon storing several days at room temperature. The molecule view as well as structural parameters are given in Supporting Information.

Cytotoxicity studies

The cytotoxicity of complexes **4** − **9** was tested *in vitro* against selected ovarian cancer lines: A2780 (sensitive to cisPt), A2780cis (acquired resistance to cisPt), and SK-OV-3 (intrinsic resistance to cisPt) using MTT test. As nephrotoxicity remains the main side effect in cisPt treatment, the cytotoxicity of selected (most active) complexes against human embryonic kidney cells (HEK293) was evaluated as well. Results (IC_{50}) for 24h and 72h treatments are collected in Table 1.

In comparison to CisPt (IC_{50} = 13±2 and 1.7±0.3 μM for 24h and 72h assay, respectively), several complexes possessed similar activity in both 24 h assay and 72 h assay

against A2780 cells. The high activities below 10 µM were achieved with ferrocene derivatives **4a**, **4b**, **4e**, **4a·2HCl**, **4c·2HCl**, **4e·2HCl**, **5**, **6**, **7** and ruthenocene **9b** in both 24 and 72 h treatment. The moderate activities below 50 µM were found for ferrocenes **4g**, **4h** and organic hydrochlorides **3a·HCl** and **3aa·HCl**. Benzyloxy-substituted ferrocenes **4d**, **4f**, ferrocenes lacking N-heterocycle **4i**, **4j** and cationic ruthenium complexes **8** were found inactive (for all studied cell lines) and will not be discussed further.

Interestingly, exposure of cisplatin resistant cell line A2780cis to CisPt revealed ca five-fold decrease in cytotoxic activity (IC_{50} = 50±9 and 10±2 µM for 24h and 72h assay, respectively), while the most of the active ferrocene derivatives (**4b**, **4g**, **4b·2HCl**, **4c·2HCl**, **5**, **6**, **7**) and ruthenocene **9b** retained their potency or dropped in their cytotoxicity only 2-3 times (**4a**, **4e**).

Similarly SK-OV-3 cell line showed low response to CisPt treatment (IC_{50} > 100 and 5.6±1.0 µM for 24h and 72 h assay, respectively). However, ferrocenes **4a**, **4b**, **4g**, **4b·2HCl** and ruthenocene **9b** maintained cytotoxicity below 50 µM, while ferrocenes **4e·2HCl**, (the most potent one showed IC_{50} = 1.76±0.08 µM in 72h assay), **4e**, **4a·2HCl**, **4c·2HCl**, **5**, and **6** possessed activity below 10 µM in 72h assay. These results together with the results obtained for A2780 imply a different mechanism of action of ferrocene and ruthenocene derivatives in comparison to CisPt.

Unfortunately, most of the derivatives (**4c·2HCl**, **4e·2HCl**, **4e**, **4g**, **5**, **6**, **7**) having high cytotoxic activity against all three cancer cell lines displayed a similar or even higher toxicity against HEK293 cell line and therefore lack of selectivity necessary for application in cancer treatment. Nonetheless, another potent ferrocene derivatives **4a**, **4b**, **4a·2HCl**, and **4b·2HCl** bearing the six-membered piperidine or morpholine rings displayed an enhanced selectivity against cancer cell lines with selectivity factor S_f in the range from 2 to 6 (where S_f = IC_{50}(HEK293) / IC_{50}(cancer cell line); for details see Table 1).

In addition to transition metal complexes, hydrochlorides of fulvene **3a·HCl** and cyclopentadiene **3aa·HCl** (a presumed product of ferrocene **4a·2HCl** degradation) were evaluated for their cytotoxic properties. Both species revealed a quite remarkable cytotoxicity in micromolar range against all cell lines, although their potency did not reach the level of **4a·2HCl** and lacked its high selectivity. Interestingly, the cytotoxicity of fulvene **3a·HCl** was found ca twice higher in comparison to **3aa·HCl** for all studied lines, which is in accordance with known cytotoxicity of different 6-arylfulvenes in low to medium micromolar range.[19]

All the above given results let us draw several conclusions concerning this type of compounds: (i) The studied complexes showed a reasonable stability in biological

environment as the prolonged assay usually led to increased efficiency against the studied human cell lines. (ii) The presence of a saturated aza-heterocycle is essential for achieving acceptable activity of the complexes, as the nitrogen-free ferrocenes (**4i** and **4j**) displayed no activity. (iii) The nature of the substituents attached to the N-heterocycle also plays a significant role. The complexes bearing benzyloxy groups (**4d**, **4f** and even the polar and well soluble **4f·2HCl**) were completely inactive in comparison to their unsubstituted, highly active parent compounds (**4a** and **4e**). However, the presence of hydroxyl substituents protected by easily hydrolysable methoxymethyl acetals in **4g** resulted in a species exerting an activity comparable to analogous unprotected ferrocenes **5** and **6** bearing (3*S*,4*S*)-pyrrolidine-3,4-diol, or even to **4e** bearing pendant unsubstituted pyrrolidine. Similarly, ca 5-fold increase in activity was also observed after hydrolysis of the exocyclic isopropylidene acetal in **4h** to **7**. (iv) The cationic ruthenium complexes **8** showed little promise as anticancer agents despite their high stability and solubility in aqueous environment. This is in accordance with the known inactivity of a range of cationic cyclopentadienyl-arene ruthenium complexes recently reviewed by Morais *et al.*[23] (v) The cytotoxicity of complexes roughly relates to the toxicity of the pendant heterocycle as could be seen when comparing of the cytotoxicity of ferrocenes (following the order **4a** ~ **4b** < **4e**) with the toxicity of the corresponding cyclic amines which increases in the order morpholine << piperidine < pyrrolidine (in other words, six-membered heterocycles are less toxic than five-membered ones).[24] However, ferrocenes with six-membered aza-heterocycles (particularly **4a**, **4b**, **4a·2HCl**, and **4b·2HCl**) seem to be more promising as potential drugs in comparison to ferrocenes with a five-membered heterocycle (e.g. **4e** bearing pyrrolidine functionality) as they showed higher selectivity against cancer cell lines compared to embryonic kidney cells HEK293.

Present, mostly preliminary results indicate a potential mechanism of action in an increased formation of reactive oxygen species (ROS) by selected ferrocene derivatives. An ability of ferrocene/ferrocenium pair to induce ROS production, which subsequently induces DNA damage is well known.[25] However, a detailed mechanism of action of the most active complexes remains to be elucidated. Predominantly cytostatic activity in terms of cell cycle blockage without killing the cells or clonogenic survival assay to test long-term activity of our complexes should accompany determination of cytotoxic activity that should also be extended to determination of the mechanism of cell death.

Table 1. Cytotoxicity[a] of ferrocenes **4** − **7**, cationic ruthenium complexes **8**, ruthenocene **9b**, and organic hydrochlorides (**3a·HCl**, **3aa·HCl**) against selected ovarian cancer cell lines (A2780, A2780cis, SK-OV-3) and human embryonic kidney cells (HEK293)

cmd.	**Cell line (treatment time)**							
	A2780 (24h)	**A2780 (72h)**	**A2780cis (24h)**	**A2780cis (72h)**	**SK-OV-3 (24h)**	**SK-OV-3 (72h)**	**HEK293 (24h)**	**HEK293 (72h)**
4a	6.9±0.9	3.4±0.1 S_f [b] = 6	18±7	6.6±1.5 S_f [b] = 3	91±11	37±1	>100	19±3
4b	6.6±0.9	5.4±2.3 S_f [b] = 2	9±4	5.5±0.2 S_f [b] = 2	>100	32±10	>100	9.4±0.1
4d	>100	>100	>100	>100	>100	>100	n.d.	n.d.
4e	3.7±0.5	2.8±0.4	8.9±1.3	4.6±1.9	6±3	2.2±0.2	6.2±1.6	2.2±0.6
4f	>100	>100	>100	>100	>100	>100	n.d.	n.d.
4g	30±5	7±2	23±2	8±2	>100	34±11	>100	7±2
4h	29±9	37±7	53±29	33±20	>100	95±18	n.d.	n.d.
4i	>100	>100	>100	>100	>100	>100	n.d.	n.d.
4j	>100	>100	>100	>100	>100	>100	n.d.	n.d.
4a·2HCl	2.9±0.3	0.7±0.6 S_f [b] = 6	5.4±0.6	5.9±0.8	7.8±1.0	6.2±0.3	29±9	4.3±0.8
4b·2HCl	60±5	11±3 S_f [b] = 2	65±12	6.5±1.4 S_f [b] = 4	>100	20.1±0.3	>100	23±4

4c·2HCl	6.8±1.6	5.3±1.4	6±3	7.4±0.6	15±2	9.4±1.0	3.0±0.3	2.8±0.7
4e·2HCl	4.7±0.2	2.7±0.3	10.5±1.0	3.3±0.4	6.2±0.6	1.76±0.08	5.4±0.5	1.46±0.04
4f·2HCl	>100	>100	>100	>100	>100	>100	n.d.	n.d.
5	12±5	6±4	8.4±0.5	5.4±1.1	19±1	9±3	12±4	3.6±0.9
6	9±3	4.3±0.8	12±1	5.3±0.1	8.1±0.3	4.8±0.4	10±1	3.6±0.3
7	5.9 ±0.3	5.6±0.5	12±2	7.3±1.6	55±11	24±8	20±5	5.7±0.5
8a	>100	39±21	>100	37±8	>100	>100	n.d.	n.d.
8b	58±10	28±4	39±3	37±14	>100	>100	n.d.	n.d.
9b	10±2	9±5	50±15	10±4	>100	49±20	n.d.	n.d.
3a·HCl	31 ± 5	12±2	44±10	14±4	38±3	22±7	>100	12±3
3aa·HCl	53 ± 3	29 ± 3	62±8	34±6	>100	43±5	n.d.	n.d.
CisPt	13±2	1.7±0.3 S_f [b] = 2	50±9	10±2	>100	5.6±1.0	35±5	3.8±0.5

[a] Cytotoxicity was expressed as IC_{50} [μM] i.e. concentration leading to 50% inhibition of cells; given as the mean of at least three experiments±standard deviation (SD); $SD = [1/N(\Sigma(x_i - x_{av})^2]^{½}$ (N is number of measurements, x_{av} is average).

[b] Selectivity factor S_f is defined for a given compound as the ratio between its activity (IC_{50}) in normal cells compared to cancer cells.[26] For our purposes $S_f = IC_{50}$(HEK293) / IC_{50}(cancer cell line). Only values where $S_f > 1$ are displayed.

n.d. = not determined

Conclusions

A series of 8 ferrocene derivatives **4a** − **4h** of the general formula [Fe(η^5-$C_5H_4CH_2$(*p*-C_6H_4)CH_2(N-het))$_2$] were prepared by a straightforward way starting from N-heterocycle substituted 6-phenylenefulvenes, $LiBEt_3H$ and $FeCl_2$. The obtained ferrocenes bearing a pendant saturated N-heterocycle were further modified either by nitrogen quaternization or by deprotection. The reaction of [{Ru(η^6-*p*-cymene)Cl_2}$_2$] with the corresponding cyclopentadienide derivatives afforded cationic ruthenium complexes [Ru(η^6-*p*-cymene)(η^5-$C_5H_4CH_2$(*p*-C_6H_4)CH_2(N-het))]Cl **8**, while ruthenocenes [Ru(η^5-$C_5H_4CH_2$(*p*-C_6H_4)CH_2(N-het))$_2$] **9** were found as minor byproducts.

An evaluation of the cytotoxic properties of the prepared complexes against 3 ovarian cancer cell lines showed that the presence of a pendant saturated N-heterocycle linked to the ferrocene moiety is an essential feature for high cytotoxicity. A careful choice of the pendant N-heterocycle allowed achieving not only a high activity, but also a sufficient selectivity of a particular ferrocene. Hydrochloride **4a·2HCl** (bearing pendant piperidinium cation) with a cytotoxicity in submicromolar region and high selectivity (S_f ~ 6) would be suggested as the most promising CisPt substitute for the treatment of ovarian cancer. Interestingly, ferrocenes **4a**, **4b**, and **4b·2HCl** (bearing pendant piperidine or morpholine groups) also showed remarkable cytotoxicity against the cisplatin resistant cell line A2780cis (ca twice more efficient than CisPt) while still maintaining selectivity against the cancer line (S_f = 2 − 4) compared to human embryonic HEK293 cell line. The ferrocene **4e** and the corresponding hydrochloride **4e·2HCl** were found to be the most cytotoxic species towards CisPt intrinsically resistant SK-OV-3 cell line, however they lack any selectivity and possessed a comparable or even higher cytotoxicity against embryonic HEK293 cells as well.

Experimental

All manipulations with air sensitive compounds were carried out under argon atmosphere using standard Schlenk techniques. NMR spectra were measured either on a Varian Mercury 300 (^{1}H at 300 MHz; ^{13}C at 75 MHz), Varian Inova 500 (^{1}H at 500MHz, ^{13}C at 125 MHz) or Bruker Avance 400 (^{1}H at 400 MHz; ^{13}C at 100 MHz) at 25 ºC. ^{1}H and ^{13}C chemical shifts (δ/ppm) are given relative to solvent signals (δ_H/δ_C: $CDCl_3$ 7.26/77.16; DMSO-d_6 2.50/39.52). Electrospray mass spectra (ESI-MS) were measured with a Bruker Esquire 3000 instrument on dichloromethane/acetonitrile solutions. IR spectra were measured in Nujol suspensions on

a Nicolet Avatar FTIR spectrometer in the range of 400−4000 cm^{-1}. Melting points were determined on a Kofler block and were uncorrected. Elemental analyses were carried out on a FLASH EA1112 CHN-O Automatic Elemental Analyzer (Thermo Scientific).

Chemicals. Solvents were appropriately dried by refluxing over either Na/benzophenone (THF, diethyl ether, toluene), or CaH_2 (dichloromethane), distilled and stored over 4Å molecular sieves. $LiBEt_3H$ (1.0 M solution in THF), HCl (1M solution in Et_2O, 3M in methylcyclopentyl ether), [{Ru(η^6-*p*-cymene)Cl_2}$_2$], were purchased from Aldrich and used as received. 6-(4-(piperid-1-ylmethyl)-phenyl)fulvene (**3a**), 6-(4-(morpholin-4-ylmethyl)-phenyl)fulvene (**3b**),[10] 6-(4-(pyrrolidin-1-yl)methyl)-phenyl)fulvene (**3e**),[10] 6-(4-methyl-phenyl)fulvene (**3i**),[18], 6-phenylfulvene (**3j**);[17] **4a**, **4a·2HCl**[9] were prepared as described in literature.

Anion exchange resin AG-1-X8 (Bio-Rad) was converted into OH^- cycle by washing with 1M NaOH solution followed by water washing to neutral reaction.

*Preparation of 1-[4-(cyclopenta-2,4-dien-1-ylidenemethyl)benzyl]piperidinium chloride (**3a·HCl**)*

A solution of HCl in Et_2O (1.00 ml, 1.00 mmol, 1M) was added dropwise into a solution of **3a** (0.112 g, 0.45 mmol) in THF (5 ml). The mixture was stirred for 15 min, a formed precipitate was isolated, washed with THF (3 × 3 ml), Et_2O (3 × 2 ml) and dried in vacuum to obtain **3a·HCl** as an orange powder. Yield 0.087 g (67%).

M.p. 160 ºC (decomp). ^{1}H NMR (300 MHz, DMSO-d_6): 1.24 − 1.43 (m, 1H, $NCH_2CH_2CH_2$); 1.62 − 1.93 (m, 5H, $NCH_2CH_2CH_2$); 2.76 − 2.93 (m, 2H, $NCH_2CH_2CH_2$); 3.27 (d, $^2J_{HH}$ = 12.0 Hz, 2H, $NCH_2CH_2CH_2$); 4.28 (d, $^3J_{HH}$ = 5.1 Hz, 2H, $NCH_2C_6H_4$); 6.35 − 6.39 (m, 1H, C_5H_4); 6.48 − 6.53 (m, 1H, C_5H_4); 6.67 − 6.75 (m, 2H, C_5H_4); 7.37 (s, 1H, =C*H*); 7.72 (s, 4H, C_6H_4); 11.00 (bs, 1H, N*H*). ^{13}C{^{1}H} NMR (300 MHz, DMSO-d_6): 21.41 ($NCH_2CH_2CH_2$); 22.07 ($NCH_2CH_2CH_2$); 51.55 ($NCH_2CH_2CH_2$); 58.30 ($NCH_2C_6H_4$); 119.85 (C_5H_4, *C*H); 127.53 (C_5H_4, *C*H); 130.67 (C_6H_4, *C*H); 130.81 (C_5H_4, *C*H); 130.96 (C_6H_4, C_qCH_2N); 132.00 (C_6H_4, *C*H); 136.06 (C_5H_4, *C*H); 137.00 (C_6H_4, $C_qCHC_5H_4$); 137.48 (=*C*H); 145.22 (*C*=CH). ESI-MS, *m/z* (ESI$^+$): 539 ([2M − Cl]$^+$), 503 ([2M − Cl − HCl]$^+$), 252 ([M − Cl]$^+$), 167. Elemental analysis for $C_{18}H_{22}ClN$, calculated C, 75.11; H, 7.70; N, 4.87%, found C, 75.26; H 7.75; N, 4.81%.

*Preparation of 1-[4-(cyclopentadienylmethyl)benzyl]piperidinium chloride (a mixture of isomers) (**3aa·HCl**)*

A slight excess of $LiBEt_3H$ solution in THF (0.70 ml, 0.70 mmol, 1M) was added slowly dropwise into an orange solution of **3a** (0.163 g, 0.65 mmol) in THF (6 ml), which caused a gradual decolorization of the mixture. The mixture was stirred for 4h and then a solution of HCl in Et_2O (2.1 ml, 2.1 mmol, 1M) was added in several portions. The mixture was stirred for 30 min and volatiles were evaporated in vacuum. A solid residue was extracted in chloroform and filtered. Solvent evaporation in vacuum gave **3aa·HCl** as a mixture of regioisomers. Yield 0.138 g (73%).
^{1}H NMR (300 MHz, DMSO-d_6): 1.20 − 2.00 (m, overall 6H, $NCH_2CH_2CH_2$); 2.45 − 3.25 (m, overall 4H, $NCH_2CH_2CH_2$); 3.50, 3.67, 3.72 (3 × s, overall 2H, $C_5H_4CH_2C_6H_4$); 4.15 − 4.25 (m, overall 2H, $NCH_2C_6H_4$); 6.90 − 6.86 (m, overall 5H, C_5H_5); 7.10 − 7.70 (m, overall 4H, C_6H_4); 11.38 (bs, 1H, N*H*). ESI-MS, *m/z* (ESI^+): 543 ($[2M - Cl]^+$), 507 ($[2M - Cl - HCl]^+$), 254 ($[M - Cl]^+$), 169. Elemental analysis for $C_{18}H_{24}ClN$, calculated C, 74.59; H, 8.35; N, 4.83%, found C, 74.71; H 8.38; N, 4.80%.

*General procedure for preparation of ferrocenes **4***

A 1M solution of $LiBEt_3H$ (1.00 ml, 1.00 mmol) in THF was slowly added dropwise into a solution of the fulvene **3** (1.00 mmol) in the same solvent (10 ml). As the reaction proceeded the reaction mixture changed colour from intense yellow-orange to almost colourless within minutes. The reaction mixture was stirred for 4 h, a solid $FeCl_2$ (0.064 g, 0.50 mmol) was added at once and the reaction mixture was stirred for 15 h. Solvents were evaporated in vacuum and the solid residue was purified as described below for individual compounds (either by column chromatography on silica or by crystallization).

*Preparation of [{η^5-$C_5H_4CH_2(p\text{-}C_6H_4)CH_2$(N-het)}$_2$Fe] where N-het = morpholin-1-yl (**4b**)*

Prepared by the general procedure mentioned above from **3b** (0.840 g, 3.32 mmol), $LiBEt_3H$ (3.40 ml, 1 M solution in THF, 3.40 mmol) and $FeCl_2$ (0.212 g, 1.67 mmol). Purified by recrystallization from hot EtOH and obtained as a yellow powder. Yield 0.633 g (67%). M.p. 115 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): 2.38 − 2.45 (m, 8H, NCH_2CH_2O); 3.45 (s, 4H, $NCH_2C_6H_4$); 3.64 (s, 4H, $C_5H_4CH_2C_6H_4$); 3.66 − 3.72 (m, 8H, NCH_2CH_2O); 4.04 (s, 8H,

C_5H_4); 7.12, 7.22 (2 × d, $^3J_{HH}$ = 8.2 Hz, 2 × 4H, C_6H_4). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): 35.65 ($C_5H_4CH_2C_6H_4$); 53.71 (NCH_2CH_2O); 63.29 ($NCH_2C_6H_4$); 67.11 (NCH_2CH_2O); 68.42, 69.44 (C_5H_4, *C*H); 88.11 (C_5H_4, C_{ipso}); 128.33, 129.27 (C_6H_4, *C*H); 135.36, 140.70 (C_6H_4, C_q). ESI-MS, *m/z* (ESI^+): 587 $[M + Na]^+$); 565 ($[M + H]^+$); 490. Elemental analysis for $C_{34}H_{40}FeN_2O_2$, calculated C, 72.33; H, 7.14; N, 4.96%, found C, 72. 53; H 7.18; N, 4.91%.

Preparation of [{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(N-het)}$_2$Fe] where N-het = thiomorpholin-1-yl ***(4c)***

Prepared by the general procedure mentioned above from **3c** (1.000 g, 3.71 mmol), $LiBEt_3H$ (3.70 ml, 1 M solution in THF, 3.70 mmol) and $FeCl_2$ (0.232 g, 1.83 mmol). Purified by recrystallization from hot THF and obtained as yellow microcrystals. Yield 0.485 g (44%). M.p. 177 ºC. ^1H NMR (300 MHz, $CDCl_3$): 2.53 − 2.58 (m, 16H, NCH_2CH_2S); 3.46 (s, 4H, $NCH_2C_6H_4$); 3.63 (s, 4H, $C_5H_4CH_2C_6H_4$); 4.04 (s, 8H, C_5H_4); 7.11, 7.19 (2 × d, $^3J_{HH}$ = 8.0 Hz, 2 × 4H, C_6H_4). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): 28.14 (NCH_2CH_2S); 35.67 ($C_5H_4CH_2C_6H_4$); 55.01 (NCH_2CH_2S); 63.54 ($NCH_2C_6H_4$); 68.45, 69.47 (C_5H_4, *C*H); 88.13 (C_5H_4, C_{ipso}); 128.34, 129.14 (C_6H_4, *C*H); 135.66, 140.68 (C_6H_4, C_q). ESI-MS, *m/z* (ESI^+): 597 ($[M + H]^+$). Elemental analysis for $C_{34}H_{40}FeN_2S_2$, calculated C, 68.44; H, 6.76; N, 4.70%, found C, 68.48; H 6.73; N, 4.71%.

Preparation of [{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(N-het)}$_2$Fe] where N-het = 2,3,4,6-tetra-O-benzyl-1,5-dideoxy-1,5-imino-D-glucitol-N-yl ***(4d)***

Prepared by the general procedure mentioned above from **3d** (0.550 g, 797 μmol), $LiBEt_3H$ (0.80 ml, 1 M solution in THF, 800 μmol) and $FeCl_2$ (0.050 g, 398 μmol). Purified by chromatography on silica using heptane/ethyl acetate (5/1, v/v) and obtained as an orange wax. Yield 0.438 g (77%).

R_f = 0.2 heptane/ethyl acetate (5/1, v/v). ^1H NMR (300 MHz, $CDCl_3$): 1.99 (t, $^2J_{HH} \sim {}^3J_{HH}$ ~ 11.2 Hz, 2H, C(1)H_2); 2.37 (d, $^3J_{HH}$ = 9.8 Hz, 2H, C(5)*H*); 3.02 (dd, $^2J_{HH}$ = 11.2 Hz, $^3J_{HH}$ = 4.6 Hz, 2H, C(1)H_2); 3.37 (d, $^2J_{HH}$ = 13.7 Hz, 2H, $NCH_2C_6H_4$); 3.41 − 3.66 (m, 6H, C(2)*H*, C(3)*H*, C(4)*H*); 3.68 (s, 4H, $C_5H_4CH_2C_6H_4$); 3.71 (dd, $^2J_{HH}$ = 10.6 Hz, $^3J_{HH}$ = 2.6 Hz, 2H, C(6)H_2); 3.79 (dd, $^2J_{HH}$ = 10.6 Hz, $^3J_{HH}$ = 1.2 Hz, 2H, C(6)H_2); 4.01 (d, $^2J_{HH}$ = 13.7 Hz, 2H, $NCH_2C_6H_4$); 4.03 − 4.07 (m, 8H, C_5H_4); 4.40 − 4.61 (m, 9H, CH_2, CH_2Ph); 4.75 − 4.97 (m, 7H, CH_2, CH_2Ph); 7.01 − 7.40 (m, 48H, C_6H_4 and *Ph*). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$):

35.73 ($C_5H_4CH_2C_6H_4$); 54.31 (*C*(1)); 56.35 ($NCH_2C_6H_4$); 65.01 (*C*(5)); 66.67 (*C*(6)); 68.55, 69.50 (C_5H_4, *C*H); 72.65, 73.42, 75.36(2C) (CH_2Ph); 78.38 (*C*(2)); 78.84 (*C*(4)); 87.43 (*C*(3)); 88.14 (C_5H_4, C_{ipso}); 127.52 − 128.50 (*Ph* and C_6H_4, *C*H); 129.12 (C_6H_4, *C*H); 135.78 (C_6H_4, C_q); 138.12, 138.59, 138.68, 139.17 (*Ph*, C_{ipso}); 140.54 (C_6H_4, C_q). ESI-MS, *m/z* (ESI^+): 1486, 1460 $[M + Na]^+$); 1444 ($[M + Li]^+$). Elemental analysis for $C_{94}H_{96}FeN_2O_8$, calculated C, 78.53; H, 6.73; N, 1.95% found C, 78.56; H 6.90; N, 1.93 %.

*Preparation of [{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(N-het)}$_2$Fe] where N-het = pyrrolidin-1-yl (**4e**)*

Prepared by the general procedure mentioned above from **3e** (0.833 g, 3.51 mmol), $LiBEt_3H$ (3.5 ml, 1 M solution in THF, 3.5 mmol) and $FeCl_2$ (0.222 g, 1.75 mmol). Purified by chromatography on silica using THF/hexane (1/1, v/v). Recrystallization from methanol gave product as a yellow solid. Yield 0.446 g (48%).

M.p. 75 − 78 ºC. ^{1}H (300 MHz, $CDCl_3$): 1.73-1.80 (m, 8H, NCH_2CH_2); 2.46-2.52 (m, 8H, NCH_2CH_2); 3.57 (s, 4H, $NCH_2C_6H_4$); 3.64 (s, 4H, $C_5H_4CH_2C_6H_4$); 4.03 (s, 8H, C_5H_4); 7.11, 7.22 (2 × d, 2 × $^3J_{HH}$ = 8.1 Hz, 2 × 4H, C_6H_4). ^{13}C{^{1}H} (75 MHz, $CDCl_3$): 23.57 (NCH_2CH_2); 35.67 ($C_5H_4CH_2C_6H_4$); 54.28 (NCH_2CH_2); 60.56 ($NCH_2C_6H_4$); 68.41, 69.47 (C_5H_4, *C*H); 88.23 (C_5H_4, C_{ipso}); 128.35, 128.98 (C_6H_4, *C*H); 137.01 (C_6H_4, C_qCH_2N); 140.39 (C_6H_4, $C_qCH_2C_5H_4$). ESI-MS, *m/z* (ESI^+): 533 ($[M + H]^+$). Elemental analysis for $C_{34}H_{40}FeN_2$, calculated C, 76.68; H, 7.57; N, 5.26%, found C, 76.42; H 7.61; N, 5.32%.

*Preparation of [{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(N-het)}$_2$Fe] where N-het = (3S,4S)-3,4-bis(benzyloxy)pyrrolidin-1-yl (**4f**)*

Prepared by the general procedure mentioned above from **3f** (1.142 g, 2.54 mmol), $LiBEt_3H$ (2.50 ml, 1 M solution in THF, 2.50 mmol) and $FeCl_2$ (0.157 g, 1.24 mmol). Purified by chromatography on silica using hexane/THF (2/1, v/v) and obtained as a yellow-orange oil. Yield 0.760 g (64%).

R_f = 0.4 hexane/THF (2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): 2.57 (dd, $^2J_{HH}$ = 10.0 Hz, $^3J_{HH}$ = 4.1 Hz, 4H, NCH_2CH); 2.88 (dd, $^2J_{HH}$ = 10.0 Hz, $^3J_{HH}$ = 6.3 Hz, 4H, NCH_2CH); 3.52, 3.60 (2 × d, 2 × $^2J_{HH}$ = 12.9 Hz, 2 × 2H, $NCH_2C_6H_4$); 3.64 (s, 4H, $C_5H_4CH_2C_6H_4$); 4.04 (bs, 12H, C*H*OBn, C_5H_4);); 4.45, 4.49 (2 × d, 2 × $^2J_{HH}$ = 11.9 Hz, 2 × 4H, CH_2Ph); 7.11 (d, $^3J_{HH}$ = 7.9 Hz, 4H, C_6H_4); 7.21 (d, $^3J_{HH}$ = 7.9 Hz, 4H, C_6H_4); 7.31 (m, 20H, *Ph*). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): 35.67 ($C_5H_4CH_2C_6H_4$); 58.48 (NCH_2CH); 60.15 ($NCH_2C_6H_4$); 68.45, 69.47

(C_5H_4, *C*H); 71.52 (*C*H_2Ph); 83.73 (*C*HOBn); 88.13 (C_5H_4, C_{ipso}); 127.75, 127.93 (*Ph*, *C*H); 128.30, (C_6H_4, *C*H); 128.47 (*Ph*, *C*H); 128.93 (C_6H_4, *C*H); 135.94 (C_6H_4, $C_q$$CH_2$N,), 138.93 (*Ph*, C_{ipso}); 140.57 (C_6H_4, $C_q$$CH_2C_5H_4$). ESI-MS (*m/z*; ESI^+): 957 ($[M + H]^+$), 674. Elemental analysis for $C_{62}H_{64}FeN_2O_4$, calculated C, 77.81; H, 6.74; N, 2.93%, found C, 78.03; H 6.77; N, 2.90%.

*Preparation of [{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(N-het)}$_2$Fe] where N-het = (3S,4S)-3,4-bis(methoxymethyl)pyrrolidin-1-yl (**4g**)*

Prepared by the general procedure mentioned above from **3g** (1.170 g, 3.26 mmol), $LiBEt_3H$ (3.20 ml, 1 M solution in THF, 3.20 mmol) and $FeCl_2$ (0.203 g, 1.60 mmol). Purified by chromatography on silica using hexane/THF (3/1, v/v) and obtained as a yellow-orange oil. Yield 0.957 g (77%).

R_f = 0.2 hexane/THF (3/1, v/v). ^{1}H NMR (300 MHz, DMSO-d_6): 2.39 (dd, $^2J_{HH}$ = 10.2 Hz, $^3J_{HH}$ = 4.5 Hz, 4H, NCH_2CH); 2.76 (dd, $^2J_{HH}$ = 10.2 Hz, $^3J_{HH}$ = 6.0 Hz, 4H, NCH_2CH); 3.21 (s, 12H, O*Me*); 3.43, 3.51 (2 × d, 2 × $^2J_{HH}$ = 12.9 Hz, 2 × 2H, C_6H_4CH_2N); 3.59 (s, 4H, C_5H_4C$H_2C_6H_4$); 3.98 (pseudo t, $^3J_{HH}$ = 6.0 Hz, $^3J_{HH}$ = 4.5 Hz, 4H, NCH_2C*H*); 4.01-4.04, 4.05-4.08 (2 × m, 2 × 4H, C_5H_4); 4.54, 4.58 (2 × d, 2 × $^2J_{HH}$ = 6.9 Hz, 2 × 4H, OCH_2O); 7.12, 7.17 (2 × d, 2 × $^3J_{HH}$ = 8.4 Hz, 2 × 4H, C_6H_4). ^{13}C{^{1}H} NMR (75 MHz, DMSO-d_6): 34.82 (C_5H_4C$H_2C_6H_4$); 54.80 (O*Me*); 58.16 (N*C*H_2CH); 59.07 (C_6H_4CH_2N); 67.93, 68.97 (C_5H_4, *C*H); 81.13 (NCH_2C*H*); 88.16 (C_5H_4, C_{ipso}); 94.98 (OCH_2O); 128.07, 128.33 (C_6H_4, *C*H); 135.89, 140.42 (C_6H_4, C_q). ESI-MS, *m/z* (ESI^+): 773 $[M + H]^+$. Elemental analysis for $C_{42}H_{56}FeN_2O_8$, calculated C, 65.28; H, 7.30; N, 3.63%, found C, 65.49; H 7.35; N, 3.60%.

*Preparation of [{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(N-het)}$_2$Fe] where N-het = 1,4-dideoxy-2,3:5,6-di-O-isopropylidene-1,4-imino-D-talitol-N-yl (**4h**)*

Prepared by the general procedure mentioned above from **3h** (0.336 g, 0.82 mmol), $LiBEt_3H$ (0.82 ml, 1 M solution in THF, 0.82 mmol) and $FeCl_2$ (0.051 g, 0.40 mmol). Purified by chromatography on silica using hexane/ethyl acetate (5/1 → 1/1, v/v) and obtained as a yellow powder. Yield 0.233 g (66%).

M.p. 121 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): 1.30, 1.34, 1.43, 1.51 (4 × s, 4 × 6H, CMe_2); 2.49 (dd, $^2J_{HH}$ = 9.9 Hz, $^3J_{HH}$ = 3.9 Hz, 2H, C(1)H_2); 2.84 − 2.99 (m, 2H, C(4)*H*); 3.17 (dd, $^2J_{HH}$ = 9.9 Hz, $^3J_{HH}$ = 6.0 Hz, 2H, C(1)H_2); 3.55 (d, $^2J_{HH}$ = 13.8 Hz, 2H, NC$H_2C_6H_4$); 3.64 (s, 4H,

$C_5H_4CH_2C_6H_4$); 3.83 (t, $^2J_{HH}$ = $^3J_{HH}$ = 7.8 Hz, 2H, C(6)H_2); 3.96 − 4.06 partly overlapped (m, 2H, C(6)H_2); 4.03 (s, 8H, C_5H_4); 4.11 − 4.24 (m, 4H, $NCH_2C_6H_4$ and C(5)*H*); 4.33 − 4.44 (m, 2H, C(3)*H*); 4.51 − 4.63 (m, 2H, C(2)*H*); 7.10, 7.19 (2 × d, 2 × $^3J_{HH}$ = 7.9 Hz, 2 × 4H, C_6H_4). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): 25.11, 25.46, 26.69, 27.44 (CMe_2); 35.69 ($C_5H_4CH_2C_6H_4$); 58.94 (*C*(1)); 59.29 ($NCH_2C_6H_4$); 66.54 (*C*(6)); 68.50, 69.45 (C_5H_4, *C*H); 70.71 (*C*(5)); 77.32 (*C*(4)); 78.87 (*C*(2)); 82.38 (*C*(3)); 88.19 (C_5H_4, C_{ipso}); 109.61, 113.03 (CMe_2); 128.38, 128.87 (C_6H_4, *C*H); 136.78 (C_6H_4, C_qCH_2N,); 140.56 (C_6H_4, $C_qCH_2C_5H_4$). ESI-MS, *m/z* (ESI^+): 915 $[M + K]^+$, 899 $[M + Na]^+$, 883 $[M + Li]^+$, 877 $[M + H]^+$. Elemental analysis for $C_{50}H_{64}FeN_2O_8$, calculated C, 68.48; H, 7.36; N, 3.20%, found C, 68.11; H 7.31; N, 3.15%.

*Preparation of [{η^5-C_5H_4(4-methylbenzyl)}$_2$Fe] (**4i**)*

Prepared by the general procedure mentioned above from **3i** (2.76 g, 16.4 mmol), $LiBEt_3H$ (16.4 ml, 1 M solution in THF, 16.4 mmol) and $FeCl_2$ (1.04 g, 8.2 mmol). Crude product was purified by filtration through a short silica column and crystallization from EtOH to give **2i** as a yellow powder. Yield 2.04 g (63%).
M.p. 113 ºC. ^1H NMR (300 MHz, $CDCl_3$): 2.34 (s, 6H, C_6H_4Me); 3.66 (s, 4H, CH_2); 4.07 (s, 8H, C_5H_4); 7.10 (s, 8H, C_6H_4). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): 21.13 (C_6H_4Me); 35.55 ($C_5H_4CH_2C_6H_4$); 68.40, 69.41 (C_5H_4, *C*H); 88.38 (C_5H_4, C_{ipso}); 128.37, 129.05 (C_6H_4, *C*H); 135.42, 138.80 (C_6H_4, C_q). ESI-MS, *m/z* (ESI^+): 394 ($[M]^+$). Elemental analysis for $C_{26}H_{26}Fe$, calculated C, 79.19; H, 6.65%, found C, 79.15; H 6.68%.

*Preparation of [{η^5-C_5H_4(benzyl)}$_2$Fe] (**4j**)*

Prepared by the general procedure mentioned above from 6-phenylfulvene (0.100 g, 0.65 mmol), $LiBEt_3H$ (0.65 ml, 1 M solution in THF, 0.65 mmol) and $FeCl_2$ (0.041 g, 0.32 mmol). Purified by chromatography on silica using Et_2O as an eluent and obtained as a yellow solid. Yield 0.074 g (62%). NMR spectra were consistent with literature.[20-21]
^1H NMR (300 MHz, $CDCl_3$): 3.69 (s, 4H, CH_2), 4.08 (s, 8H, C_5H_4), 7.20 − 7.33 (m, 10H, *Ph*). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): 35.98 (CH_2), 68.47, 69.47 (C_5H_4, *C*H), 88.11 (C_5H_4, C_{ipso}), 126.02, 128.37, 128.49 (*Ph*, *C*H), 141.77 (*Ph*, C_{ipso}).

*Preparation of **4b·2HCl***

A HCl solution in methylcyclopropylether (0.75 ml, 2.25 mmol, 3M solution) was added dropwise into solution of **4b** (0.244 g, 0.43 mmol) in THF (5 ml), which caused an immediate precipitate formation. The mixture was stirred for 30 min and the precipitate was collected on frit, washed with THF (3 × 1 ml) and dried in vacuum. The product was obtained as a yellow-orange solid. Yield 0.155 g (56%).

M.p. 165 ºC (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6): 3.01 − 3.17 (m, 8H, NCH_2CH$_2$); 3.67 (bs, 4H, C$_5$H$_4$CH_2C$_6$H$_4$); 3.82 − 3.86 (m, 8H, CH_2O); 4.06, 4.10 (2 x bs, 2 x 4H, C$_5H_4$); 4.24 (bs, 4H, NCH_2C$_6$H$_4$); 7.25 (bd, $^{3}J_{HH}$ = 6.7 Hz, 4H, C$_6H_4$), 7.52 (bs, 4H, C$_6H_4$), 11.37 (bs, 2H, N*H*). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6): 34.88 (C$_5$H$_4$*C*H$_2$C$_6$H$_4$); 50.46 (N*C*H$_2$CH$_2$); 58.63 (N*C*H$_2$C$_6$H$_4$); 62.95 (*C*H$_2$O); 68.09, 69.02 (*C*$_5$H$_4$, *C*H); 87.67 (*C*$_5$H$_4$, *C*$_{ipso}$); 126.66 (*C*$_6$H$_4$, *C*$_q$CH$_2$N); 128.50, 131.39 (*C*$_6$H$_4$, *C*H); 143.27 (*C*$_6$H$_4$, *C*$_q$CH$_2$C$_5$H$_4$).

Elemental analysis for C$_{34}$H$_{42}$Cl$_2$FeN$_2$O$_2$, calculated C, 64.06; H, 6.64; N, 4.40%, found C, 64.13; H 6.72; N, 4.35%.

*Preparation of **4c·2HCl***

A HCl solution in methylcyclopropylether (0.70 ml, 2.10 mmol, 3M solution) was added dropwise into solution of **4c** (0.311 g, 0.52 mmol) in a THF/CH$_2$Cl$_2$ (10 ml/17 ml) solvent mixture. The mixture was stirred for 1h, while formation of precipitate was gradually observed. The volume of the mixture was reduced to a half and the precipitate was isolated. The precipitate was washed with THF (3 × 4 ml), Et$_2$O (3 × 4 ml) and dried in vacuum to give a product as a yellow powder. Yield 0.190 g (55%).

M.p. 176 ºC (decomp.). ^{1}H NMR (500 MHz, DMSO-d_6): 2.76 (bd, $^{2}J_{HH}$ = 14.3 Hz, 4H, CH_2S); 3.03 (bq, $^{2}J_{HH}$ ~ $^{3}J_{HH}$ ~ 12.0 Hz, 4H, NCH_2CH$_2$); 3.15 (bt, $^{2}J_{HH}$ ~ $^{3}J_{HH}$ ~ 12.7 Hz, 4H, CH_2S); 3.52 (bd, 4H, $^{2}J_{HH}$ = 12.3 Hz, NCH_2CH$_2$); 3.66 (bs, 4H, C$_5$H$_4$CH_2C$_6$H$_4$); 4.06, 4.12 (2 × bs, 2 × 4H, C$_5H_4$); 4.27 (bs, 4H, NCH_2C$_6$H$_4$); 7.27, 7.48 (2 × d, $^{3}J_{HH}$ = 7.7 Hz, 2 × 4H, C$_6H_4$), 10.93 (bs, 2H, N*H*). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6): 23.70 (*C*H$_2$S); 34.90 (C$_5$H$_4$*C*H$_2$C$_6$H$_4$); 52.41 (N*C*H$_2$CH$_2$S); 59.01 (N*C*H$_2$C$_6$H$_4$); 68.14, 69.05 (*C*$_5$H$_4$, *C*H); 87.66 (*C*$_5$H$_4$, *C*$_{ipso}$); 126.64 (*C*$_6$H$_4$, *C*$_q$CH$_2$N); 128.59, 131.47 (*C*$_6$H$_4$, *C*H); 143.39 (*C*$_6$H$_4$, *C*$_q$CH$_2$C$_5$H$_4$). Elemental analysis for C$_{34}$H$_{42}$Cl$_2$FeN$_2$S$_2$, calculated C, 60.99; H, 6.32; N, 4.18%, found C, 61.20; H 6.38; N, 4.22%.

*Preparation of **4e·2HCl***

A HCl solution in methylcyclopropylether (0.90 ml, 2.70 mmol, 3M solution) was added dropwise into solution of **4e** (0.231 g, 0.43 mmol) in THF (8 ml) and the mixture was stirred overnight. A formed solid was isolated, washed with Et_2O (2 × 5 ml) and dried in vacuum to give a product as a yellow powder. Yield 0.206 g (79%).
M.p. 155 ºC (decomp.). ^{1}H (400 MHz, DMSO-d_6): 1.89 (bs, 8H, NCH_2CH_2); 2.87-3.37 (m, 8, NCH_2CH_2); 3.65 (s, 4H, $C_5H_4CH_2C_6H_4$); 4.05, 4.10 (2 × pseudo t, 2 × $^3J_{HH}$ = 1.8 Hz, 2 × 4H, C_5H_4); 4.22 (s, 4H, $NCH_2C_6H_4$); 7.24, 7.50 (2 × d, 2 × $^3J_{HH}$ = 7.9 Hz, 2 × 4H, C_6H_4); 11.27 (bs, 2H, N*H*). ^{13}C{^{1}H} (100 MHz, DMSO-d_6): 22.55 (NCH_2CH_2); 34.85 ($C_5H_4CH_2C_6H_4$); 52.35 (NCH_2CH_2); 56.33 ($NCH_2C_6H_4$); 68.09, 69.03 (C_5H_4, *C*H); 87.76 (C_5H_4, C_{ipso}); 128.50 (C_6H_4, *C*H); 129.31 (C_6H_4, C_qCH_2N); 130.34 (C_6H_4, *C*H); 142.77 (C_6H_4, $C_qCH_2C_5H_4$).
Elemental analysis for $C_{34}H_{42}Cl_2N_2FeS_2$, calculated C, 60.99; H, 6.32; N, 4.18%, found C, 61.20; H 6.38; N, 4.22%.

*Preparation of **4f·2HCl***

The ferrocene **4f** (0.088 g, 0.09 mmol) was dissolved in Et_2O (3 ml) and an excess of solution of HCl in Et_2O (0.25 ml, 1 M solution, 0.25 mmol) was added gradually dropwise, which caused an immediate formation of yellow precipitate. The mixture was stirred for 2 min and the precipitate was isolated, washed with Et_2O (3 × 2 ml) and dried in vacuum. The product was obtained as a yellow powder. Yield 0.093 g (97%).
M.p. 72 ºC. ^{1}H (300 MHz, DMSO-d_6): 3.20−3.30 (m, 2H, NCH_2CH); 3.32−3.48 (m, 4H, NCH_2CH); 3.58−3.67 partly overlapped (m, 2H, NCH_2CH); 3.66 (s, 4H, $C_5H_4CH_2C_6H_4$); 4.06, 4.12 (2 × pseudo t, 2 × 4H, C_5H_4); 4.20−4.38 (m, 8H, $NCH_2C_6H_4$ and C*H*OBn); 4.48, 4.55 partly overlapped (2 × d, 2 × $^2J_{HH}$ = 11.8 Hz, 2 × 2H, CH_2Ph); 4.57 (s, 4H, CH_2Ph); 7.25 (d, $^3J_{HH}$ = 7.9 Hz, 4H, C_6H_4); 7.27−7.41 (m, 20H, *Ph*); 7.51 (d, $^3J_{HH}$ = 7.9 Hz, 4H, C_6H_4); 11.67 (bs, 2H, N*H*). ^{13}C{^{1}H} (75 MHz, DMSO-d_6): 34.89 ($C_5H_4CH_2C_6H_4$); 55.38, 55.76 (NCH_2CH); 58.85 ($NCH_2C_6H_4$); 68.14, 69.06 (C_5H_4, *C*H); 70.42, 70.82 (CH_2Ph); 79.30, 79.92 (*C*HOBn); 87.68 (C_5H_4, C_{ipso}); 127.67−128.36 (*Ph*, *C*H); 128.59, 130.79 (C_6H_4, *C*H); 137.45 (*Ph*, C_{ipso}); 137.47 (C_6H_4, C_qCH_2N); 143.17 (C_6H_4, $C_qCH_2C_5H_4$). ESI-MS (*m/z*; ESI$^+$): 957 ([M − HCl − Cl]$^+$). Elemental analysis for $C_{62}H_{66}Cl_2FeN_2O_4$, calculated C, 72.30; H, 6.46; N, 2.72%, found C, 72.12; H 6.44; N, 2.78%.

Preparation of [{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(N-het)}$_2$Fe] where
*N-het = (3S,4S)-3,4-dihydroxypyrrolidin-1-yl hydrochloride (**5**)*

The ferrocene **4g** (0.950 g, 1.23 mmol) was dissolved in THF (20 ml) and a slight excess of concentrated hydrochloric acid (0.4 ml, 4.8 mmol) was added in one portion. The mixture was stirred for 4h, which led to separation of a brown wax on the flask bottom. Solvents were decanted off and the wax was washed with THF (3 × 15 ml), acetone (10 ml), Et_2O (2 × 10 ml) and dried in vacuum. Dissolving of the residue in MeOH and evaporation of the formed solution in vacuum gave **5** as a brown solid. Yield 0.783 g (95%).
Note: The same product was obtained when anhydrous HCl (0.54 ml, 0.54 mmol, 1M) in Et_2O was used for reaction with **4g** (0.125 g, 0.16 mmol) in THF (5 ml). Yield of **5** was 0.96 g (89%).
M.p. 80 ºC (decomp.). ^{1}H (300 MHz, DMSO-d_6): 2.96−3.17 (m, 2H, NCH_2CH); 3.32−3.62 partly overlapped (m, 6H, NCH_2CH); 3.66 (s, 4H, $C_5H_4CH_2C_6H_4$); 4.05 (s, 4H, C*H*OH); 4.10 (s, 8H, C_5H_4); 4.25, 4.33 (2 × d, 2 × $^2J_{HH}$ = 12.8 Hz, 2 × 2H, NC$H_2C_6H_4$); 5.8 (bs, 4H, CHO*H*); 7.25, 7.51 (2 × d, 2 × $^3J_{HH}$ = 7.9 Hz, 8H, C_6H_4); 11.05 (bs, 2H, N*H*). ^{13}C{^{1}H} (75 MHz, DMSO-d_6): 34.82 ($C_5H_4CH_2C_6H_4$); 58.35 (NCH_2CH); 59.35 (NC$H_2C_6H_4$); 68.09, 69.02 (C_5H_4, *C*H); 74.41 (*C*HOH); 87.68 (C_5H_4, C_{ipso}); 128.54 (C_6H_4, *C*H); 128.75 (C_6H_4, $C_q$$CH_2$N); 130.56 ($C_6H_4$, *C*H); 142.98 ($C_6H_4$, $C_q$$CH_2C_5H_4$). ESI-MS (*m/z*; ESI$^-$): 631 ([M − HCl − H]$^-$). IR (nujol, cm^{-1}): 3286 (br, $\nu_{O\text{-}H}$). Elemental analysis for $C_{34}H_{42}Cl_2FeN_2O_4$, calculated C, 61.00; H, 6.32; N, 4.19%, found C, 61.43; H 6.42; N, 4.11%.

*Preparation of [{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(N-het)}$_2$Fe] where N-het = (3S,4S)-3,4-dihydroxypyrrolidin-1-yl (**6**)*

Ferrocene bishydrochloride **5** (0.259 g, 0.39 mmol) solution in water (2 ml) was transferred to the top of a small column (2 × 8 cm) packed with anion-exchange resin AG-1-X8 in OH^- form. The column was washed with 200 ml of water and the crude product was eluted with acetone (70 ml) as an orange band. The acetone solution was adsorbed on the top of a silica column and the column was washed with an additional acetone (total amount 300 ml). A product was eluted with ethanol (total amount 250 ml) as a yellow band. Evaporation of the solvent gave **6** as a yellow-orange wax, which spontaneously solidified at room temperature. Yield 0.146 g (63%).
M.p. 128 ºC. ^{1}H NMR (500 MHz, DMSO-d_6): 2.27 (dd, $^2J_{HH}$ = 9.6 Hz, $^3J_{HH}$ = 4.2 Hz, 4H, NCH_2CH); 2.71 (dd, $^2J_{HH}$ = 9.6 Hz, $^3J_{HH}$ = 6.0 Hz, 4H, NCH_2CH); 3.40 (d, $^2J_{HH}$ = 13.0 Hz, 2H, NC$H_2C_6H_4$); 3.51 (d, $^2J_{HH}$ = 13.0 Hz, 2H, NC$H_2C_6H_4$); 3.60 (s, 4H, $C_5H_4CH_2C_6H_4$); 3.82

(dd, $^{3}J_{HH}$ = 6.0 Hz, $^{3}J_{HH}$ = 4.2 Hz, 4H, C*H*OH); 4.02, 4.07 (2 × pseudo t, 2 × $^{3}J_{HH}$ = 1.8 Hz, 2 × 4H, C_5H_4); 4.80 (bs, 4H, O*H*); 7.11, 7.16 (2 × d, $^{3}J_{HH}$ = 8.1 Hz, 2 × 4H, C_6H_4). $^{13}C\{^{1}H\}$ NMR (125 MHz, DMSO-d_6): 34.86 ($C_5H_4CH_2C_6H_4$); 59.64 ($NCH_2C_6H_4$); 60.74 (NCH_2CH); 67.95, 68.98 (C_5H_4, *C*H); 77.60 (*C*HOH); 88.18 (C_5H_4, C_{ipso}); 127.94, 128.35 (C_6H_4, *C*H); 136.40 (C_6H_4, C_qCH_2N); 140.26 (C_6H_4, $C_qCH_2C_5H_4$). ESI-MS (*m/z*; ESI^+): 597 ($[M + H]^+$). IR (nujol, cm^{-1}): 3337 (br, $\nu_{O\text{-}H}$). Elemental analysis for $C_{34}H_{40}FeN_2O_4$, calculated C, 68.45; H, 6.76; N, 4.70%, found C, 68.44; H 6.79; N, 4.70%.

*Preparation of [{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(N-het)}$_2$Fe] where N-het = 1,4-dideoxy-2,3-O-isopropylidene-1,4-imino-D-talitol-N-yl (**7**)*

A ferrocene **4h** (0.248 g, 0.28 mmol) was dissolved in 80% acetic acid (5 ml) and the mixture was saturated with argon (stripping with argon for 5 min). The mixture was stirred at 50 °C until the starting material no longer remained in the mixture (ca 40 h) as shown by TLC. Volatiles were evaporated in vacuum under slight heating (up to 50 °C) leaving the crude product as an orange oil. The crude product was purified by chromatography on silica using dichloromethane/methanol mixture (30/1 → 10/1, v/v) as an eluent. After evaporation of solvents and trituration in heptane, pure **7** was obtained as an orange solid. Yield 0.125 g (56%).

M.p. 61 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): 1.22, 1.43 (2 × s, 2 × 6H, CMe_2); 2.48 partly overlapped by solvent signal (dd, $^{2}J_{HH}$ = 11.4 Hz, $^{3}J_{HH}$ = 3.2 Hz, 2H, C(1)H_2); 2.95 (dd, $^{2}J_{HH}$ = 11.4 Hz, $^{3}J_{HH}$ = 5.3 Hz, 2H, C(1)H_2); 3.00 (dd, $^{2}J_{HH}$ = 4.8 Hz, $^{3}J_{HH}$ = 1.5 Hz, 2H, C(6)H_2); 3.32 − 3.40 (m, 2H, C(5)*H*); 3.44 − 3.56 (m, 4H, C(4)*H* and C(6)H_2); 3.59 (s, 4H, $C_5H_4CH_2C_6H_4$); 3.72, 4.00 (2 × d, 2 × $^{2}J_{HH}$ = 13.3 Hz, 2 × 2H, $NCH_2C_6H_4$); 4.02, 4.07 (2 × pseudo t, 2 × 4H, C_5H_4); 4.49 − 4.56 (m, 2H, C(2)*H*); 4.60 (dd, $^{3}J_{HH}$ = 6.2 Hz, $^{3}J_{HH}$ = 1.5 Hz, 2H, C(3)*H*); 7.12, 7.17 (2 × d, 2 × $^{3}J_{HH}$ = 8.2 Hz, 2 × 4H, C_6H_4). ^{13}C NMR (75 MHz, DMSO-d_6): 24.39, 27.15 (CMe_2); 34.87 ($C_5H_4CH_2C_6H_4$); 58.20 (*C*(1)); 59.52 ($NCH_2C_6H_4$); 63.19 (*C*(6)); 67.96, 69.00 (C_5H_4, *C*H); 71.04 (*C*(4)); 71.55 (*C*(5)); 79.94 (*C*(3)); 82.98 (*C*(2)); 88.23 (C_5H_4, C_{ipso}); 110.81 (CMe_2); 128.08, 128.35 (C_6H_4, *C*H); 137.26 (C_6H_4, C_qCH_2N,); 140.29 (C_6H_4, $C_qCH_2C_5H_4$). ESI-MS (*m/z*; ESI^+): 819 ($[M + Na]^+$), 803 ($[M + Li]^+$), 797 ($[M + H]^+$). IR (nujol, cm^{-1}): 3364 (br, $\nu_{O\text{-}H}$). Elemental analysis for $C_{44}H_{56}FeN_2O_8$, calculated C, 66.32; H, 7.08; N, 3.52%, found C, 66.45; H 7.12; N, 3.47%.

*Reaction of [{Ru(η^6-p-cymene)Cl_2}$_2$] with a lithium cyclopentadienide generated from **3a***

3a (0.308 g, 1.23 mmol) was dissolved in THF (15 ml) and $LiBEt_3H$ (1.20 ml, 1.20 mmol, 1M solution in THF) was added dropwise. The mixture was stirred for 4h and then solid [{Ru(η^6-*p*-cymene)Cl_2}$_2$] (0.355 g, 0.58 mmol) was added. The resulting orange mixture was stirred for 7 days and the volatiles were evaporated in vacuum. ^{1}H NMR spectrum of the mixture showed **8a** as a main product (molar ratio 96%), while the corresponding ruthenocene **9a** was detected as a minor product (molar ratio 4%, total yield ca 2%). The crude **8a** was purified by chromatography on alumina (neutral, Brockmann II). The column was first eluted with CH_2Cl_2 (giving a fraction enriched with **9a**). Then elution of the column with ethanol, followed by solvent evaporation gave a brown waxy solid. The solid was recrystallized from acetone/heptane mixture to give **8a** as a light grey wax. Yield 0.385 g (63%).

*[{η^6-p-cymene}{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(piperidin-1-yl)}Ru]Cl (**8a**)*

^{1}H NMR (300 MHz, DMSO-d_6): 1.16 (d, 6H, $^3J_{HH}$ = 6.9 Hz, CH*Me*$_2$); 1.31 − 1.40 (m, 2H, $NCH_2CH_2CH_2$); 1.40 − 1.51 (m, 4H, $NCH_2CH_2CH_2$); 2.24 (s, 3H, *Me*, p-cymene); 2.22 − 2.32 (m, 4H, $NCH_2CH_2CH_2$); 2.63 (septuplet, 1H, $^3J_{HH}$ = 6.9 Hz, C*H*Me$_2$); 3.36 (s, 2H, $NCH_2C_6H_4$); 3.54 (s, 2H, $C_5H_4CH_2C_6H_4$); 5.31, 5.43 (2 × pseudo t, 2 × 2H, C_5H_4); 6.13, 6.16 (2 × d, 2 × $^3J_{HH}$ = 6.8 Hz, 2 × 2H, C_6H_4, p-cymene); 7.22 (s, 4H, C_6H_4). ^{13}C{^{1}H} NMR (75 MHz, DMSO-d_6): 19.12 (*Me*, p-cymene); 23.07 (CH*Me*$_2$); 23.98 ($NCH_2CH_2CH_2$); 25.52 ($NCH_2CH_2CH_2$); 31.09 (*C*HMe$_2$); 32.65 ($C_5H_4CH_2C_6H_4$); 53.83 ($NCH_2CH_2CH_2$); 62.47 ($NCH_2C_6H_4$); 79.81, 80.80 (C_5H_4, *C*H); 84.37, 86.62 (p-cymene, *C*H), 100.74 (p-cymene, C_q); 102.32 (C_5H_4, C_{ipso}); 111.46 (p-cymene, C_q); 128.19, 128.96 (C_6H_4, *C*H); 137.01, 138.16 (C_6H_4, C_q). ESI-MS (*m/z*; ESI$^+$): 488 ([M − Cl]$^+$). Elemental analysis for $C_{28}H_{36}ClNRu$, calculated C, 64.29; H, 6.94; N, 2.68%, found C, 64.35; H 6.96; N, 2.65%.

*Reaction of [{Ru(η^6-p-cymene)Cl_2}$_2$] with a lithium cyclopentadienide generated from **3b***

An excess of **3b** (0.498 g, 1.97 mmol) was dissolved in THF (20 ml) and $LiBEt_3H$ (2.00 ml, 2.00 mmol, 1M solution in THF) was added dropwise. The mixture was stirred for 4h and then solid [{Ru(η^6-*p*-cymene)Cl_2}$_2$] (0.294 g, 0.48 mmol) was added. The resulting red mixture was stirred overnight at room temperature and then refluxed for 8 h. Volatiles were evaporated in vacuum, resulting red solid was redissolved in toluene (20 ml) and refluxed for additional 7h. Evaporation of toluene gave dark brown solid which was purified

by chromatography on alumina (neutral, Brockmann II). The first elution with dichloromethane gave crude **9b** (0.182 g), second elution with THF was discarded and final elution with ethanol gave pure **8b** (0.173 g) as a brownish wax.

*[{η^6-p-cymene}{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(morpholin-1-yl)}Ru]Cl (**8b**)*

Yield 0.173 g (33%). ^{1}H NMR (300 MHz, DMSO-d_6): 1.17 (d, 6H, $^3J_{HH}$ = 6.9 Hz, CH*Me*$_2$); 2.24 (s, 3H, *Me*, p-cymene); 2.28 − 2.34 (m, 4H, NC*H*$_2$CH$_2$); 2.63 (septuplet, 1H, $^3J_{HH}$ = 6.9 Hz, C*H*Me$_2$); 3.42 (s, 2H, NC*H*$_2$C$_6$H$_4$); 3.53 (s, 2H, C$_5$H$_4$C*H*$_2$C$_6$H$_4$); 3.51 − 3.58 partly overlapped (m, 4H, NCH$_2$C*H*$_2$); 5.31, 5.42 (2 × pseudo t, 2 × 2H, C$_5$*H*$_4$); 6.14 (s, 4H, C$_6$*H*$_4$, p-cymene); 7.22, 7.26 (2 × d, 2 × $^3J_{HH}$ = 8.2 Hz, 2 × 2H, C$_6$*H*$_4$,). ^{13}C{^{1}H} NMR (75 MHz, DMSO-d_6): 19.15 (*Me*, p-cymene); 23.07 (CH*Me*$_2$); 31.13 (*C*HMe$_2$); 32.71 (C$_5$H$_4$*C*H$_2$C$_6$H$_4$); 53.11 (N*C*H$_2$CH$_2$); 62.05 (N*C*H$_2$C$_6$H$_4$); 66.17 (NCH$_2$*C*H$_2$); 79.84, 80.78 (*C*$_5$H$_4$, *C*H); 84.36, 86.60 (p-cymene, *C*H), 100.76 (p-cymene, *C*$_q$); 102.18 (*C*$_5$H$_4$, *C*$_{ipso}$); 111.50 (p-cymene, *C*$_q$); 128.24, 129.19 (*C*$_6$H$_4$, *C*H); 136.20, 138.36 (*C*$_6$H$_4$, *C*$_q$). ESI-MS (*m/z*; ESI$^+$): 490 ([M − Cl]$^+$). Elemental analysis for $C_{27}H_{34}ClNORu$, calculated C, 61.76; H, 6.53; N, 2.67%, found C, 61.68; H 6.55; N, 2.70%.

*[{η^5-$C_5H_4CH_2$(p-C_6H_4)CH_2(morpholin-1-yl)}$_2$Ru] (**9b**)*

Crude **9b** was purified by trituration with ethanol. Pure **9b** was obtained as a beige powder. Yield 0.041 g (7%).
^{1}H NMR (300 MHz, $CDCl_3$): 2.33 − 2.53 (m, 8H, NC*H*$_2$CH$_2$O); 3.46 (s, 8H, NC*H*$_2$C$_6$H$_4$ and C$_5$H$_4$C*H*$_2$C$_6$H$_4$); 3.62 − 3.79 (m, 8H, NCH$_2$C*H*$_2$O); 4.38 − 4.40 (m, 4H, C$_5$*H*$_4$); 4.42 − 4.45 (m, 4H, C$_5$*H*$_4$); 7.12, 7.22 (2 × d, 2 × $^3J_{HH}$ = 8.0 Hz, 2 × 4H, C$_6$*H*$_4$). ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): 35.48 (C$_5$H$_4$*C*H$_2$C$_6$H$_4$); 53.73 (N*C*H$_2$CH$_2$O); 63.31 (N*C*H$_2$C$_6$H$_4$); 67.12 (NCH$_2$*C*H$_2$O); 70.49; 71.96 (*C*$_5$H$_4$, *C*H); 91.70 (*C*$_5$H$_4$, *C*$_{ipso}$); 128.47, 129.20 (*C*$_6$H$_4$, *C*H); 135.41, 140.79 (*C*$_6$H$_4$, *C*$_q$). ESI-MS, *m/z* (ESI$^+$): 611 ([M + H]$^+$), 490. Elemental analysis for $C_{34}H_{40}N_2O_2Ru$, calculated C, 66.97; H, 6.61; N, 4.60%, found C, 67.12; H 6.67; N, 4.58%.

An alternative preparation of **9b** could be performed by reaction of **8b** with excess of **3a** in boiling toluene as follows. A suspension of an excess of lithium cyclopentadienide (0.066 g, 0.25 mmol; prepared from $LiBEt_3H$ and **3b**) and **8b** (0.052 g, 0.08 mmol) was stirred in boiling toluene for 63 h. After cooling to room temperature, the suspension was passed through short column (2 × 10 cm) of neutral alumina (Brockmann II). The column was

washed with toluene (total volume 30ml) and the product was eluted with dichloromethane. Evaporation of solvents from the eluent gave **9b** as an almost white solid. Yield 0.045 g (87%). NMR spectra of the product were identical as for the above prepared complex.

X-ray Crystallography

Single-crystal X-ray diffraction data for **4a** and **4a·2HCl** were obtained using Nonius KappaCCD difractometer equipped with Bruker ApexII detector. Data were collected using monochromatized MoKα radiation (λ=0.71073 Å) at 150(2)K. The phase problem was solved by direct methods (SHELXS)[27] and refined by full-matrix least squares based on F^2 (SHELXL97).[28] The hydrogen atoms were fixed into idealized positions (riding model) and assigned temperature factors H_{iso}(H) = 1.5 U_{eq}(pivot atom) for the methyl groups and H_{iso}(H) = 1.2 U_{eq}(pivot atom) for the rest. The graphic depiction of molecular structure was carried out with the PLATON program.[29] Selected crystal data for **4a** and **4a·2HCl** are presented in Supporting information. Crystallographic data for the structure of **4a** and **4a·2HCl** have been deposited with the Cambridge Crystallographic Data Centre with CCDC numbers 1508819 and 1508820. Copies of the data can be obtained, free of charge, on application to Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.Uk).

Cytotoxicity Tests

Due to the growth rate and size, the A2780, A2780cis cells were seeded in density 10000 cells/well for 24h and 5000 cells/well for 72h treatment. SK-OV-3 cells were seeded in density 5000 cells/well for 24h and 2000 cells/well for 72h treatment. The next day the cells were exposed to all tested compounds diluted in DMSO in concentration range 0 − 100 μM (each in pentaplicates) for mentioned time points. The cell viability was measured using colorimetric MTT assay as described previously.[30] All experiments were made independently in triplicates. Data from cytotoxicity assay were analysed in GraphPadPrism software and expressed as IC_{50} values (compound concentrations that produce 50% of cell metabolic inhibition). Errors were calculated as standard deviations (SD) and confidence interval is expressed as 1SD. A cytotoxicity of complex **4c** was not tested due to its insolubility in DMSO as well as in biological medium.

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Appendix A. Supplementary data

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Highlights

- ferrocene derivatives bearing five- and six- membered N-heterocycles were prepared
- cytotoxicity of complexes towards ovarian cancer cells and non-tumor cells were evaluated
- presence of both N-heterocyle and ferrocene moieties is essential for complex cytotoxicity
- piperidine and morpholine derivatives possessed higher selectivity towards cancer cells