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Graphical Abstract

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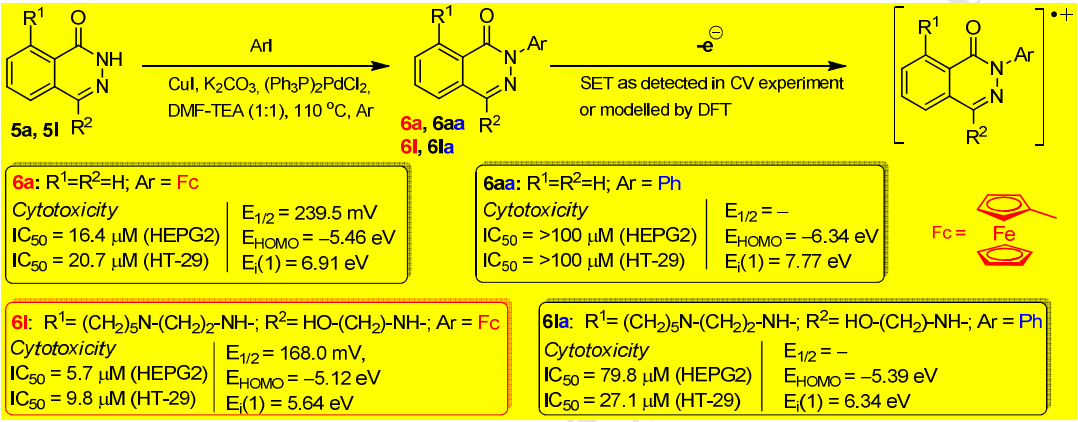

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N-Ferrocenylpyridazinones and new organic analogues: synthesis, Cyclic Voltammetry, DFT analysis and *in vitro* antiproliferative activity associated with ROS-generation

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ABSTRACT

Employing an optimized Pd-catalyzed cross-coupling reaction promoted by CuI, novel *N*-ferrocenylpyridazinones along with *N*-phenyl- and *N*-(2-pyridyl) analogues were synthesized from readily available heterocyclic precursors, iodoferrocene, iodobenzene and 2-bromopyridine. With exception of the ferrocenylation of 6-ferrocenylpyridazin-3(2*H*)-one yielding both *N*- and *O*-substituted products, the studied reactions exclusively afforded *N*-aryl lactams. The novel compounds exhibited cytotoxicity towards HEPG2 and HT-29 human malignant cells under *in vitro* conditions. The measured IC_{50} values supplemented with the results of cyclic voltammetry and DFT calculations suggest that the cytotoxic activity of the *N*- and *O*-ferrocenyl-substituted derivatives and the decreased effect of the *N*-phenyl analogues seem to be at least partly associated with the potential to generate reactive oxygen species (ROS). This interpretation, allowing the prediction of characteristic substituent-dependent SAR, was supported by the results of related studies on the practically inactive *N*-(2-pyridyl)pyridazinones assumed to be present in protonated chelate forms with highly a decreased propensity to undergo ionization.

1. Introduction

It is a generally accepted view that chemotherapy is one of the essential tools for treatment of malignancies. During the last decades, important advances have been made leading to the approval of well-known therapeutic agents, such as cisplatin, carboplatin and oxaliplatin[1], along with a variety of emblematic organic compounds which can be represented by eg. daunomycin, doxorubicin,[2] vinblastine and vincristine.[3] However, in order to overcome the frequent toxic limitations and to broaden the scope of treatable malignancies, an intense search was initiated for alternative therapeutic agents with enhanced activity and bioavailability. As a result, besides the afore mentioned classical metal complexes and organic molecules, organometallics have also emerged as potential anticancer agents. Among them, ferrocene derivatives with diverse molecular architectures are of pronounced importance. It is well documented that the replacement of an aromatic nucleus in certain organic compounds for a ferrocene unit can lead to compounds with enhanced biological activity.[4] Accordingly, a plethora of organic ferrocene derivatives were found to exhibit promising antitumor activities.[5] In most cases their effect is based on the generation of radicals through Fenton pathway that causes DNA damage and consequent apoptosis of cells.[6] In this regard, a variety of *N*-acyl derivatives of aminoferrocene emerged as an intriguing class of cytotoxic agents with considerable selectivity toward cancer cells, capable of playing a highly important role in ROS-production in tumorous cells,.[7] It was also demonstrated that aminoferrocene-based carbamates can be activated by intracellular redox processes which finally increase the oxidative stress in cancer cells leading to their death.[8] On the other hand, although the majority of pyridazinone-based heterocycles of biological relevance are known for anti-HIV,[9] antiviral,[10] antibacterial,[11] antihypertensive[12] and anti-inflammatory[13] activities, a number of them have been identified as anti-cancer drug candidates,[14] cyclooxygenase[15] and PFKFB3 disphosphatase inhibitors with simultaneous anti-tumouractivity,[16] showing their promising potential in fighting cancer. Since the chemistry and biological applications of ferrocene- and pyridazine derivatives are the focus of our interest, we have demonstrated that the

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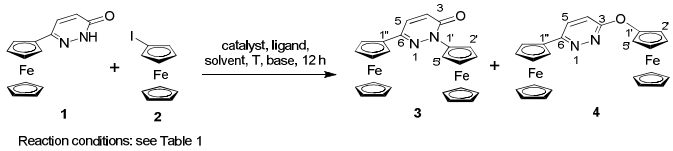
combination of ferrocene and pyridazinone moieties might result in highly promising hybrids exhibiting marked antiproliferative activity against human malignant cell lines.[17,18] All of this precedent prompted us to envisage the synthesis and preliminary *in vitro* evaluation of a small library of *N*-ferrocenylpyridazinones, the cyclic derivatives of *N*-acylhydrazinoferrocene, expected to have significant potential in ROS-generation associated with antiproliferative activity.

2. Results and Discussion

Since the synthesis and characterization of hydrazinoferrocene, a reagent with expected enhanced tendency to undergo oxidative degradation, have not been reported in the literature so far, we envisaged accessing the target *N*-ferrocenylpyridazinone derivatives through the cross-coupling of selected pyridazinone components with the easily available iodoferrocene (**2**). In order to optimize reaction conditions, a series of experiments were carried out employing 6-ferrocenylpyridazin-3(2*H*)-one (**1**)[19] as substrate (Scheme 1). First, we attempted to utilize copper-mediated reactions conducted in different solvents using CuI as metal source in catalytic or stoichiometric amount (Table 1). It is of interest that contrary to the general expectations regarding the regioselectivity of Ullmann-Goldberg type amide-*N*-arylations[20] independently on the conditions, the majority of the reactions afforded ca. 2:11 mixtures of *N*- and *O*-ferrocenyl-substituted products **3** and **4**, respectively, in low isolated yields. When the reactions were performed in dioxane, **3** and **4** could be exclusively or partly isolated as DMEDA-coordinated copper complexes (Table 1: entries 3 and 5), as indicated by ^{1}H-NMR spectroscopy measurements. In this series of experiments, the coupling reaction conducted in DMF containing potassium phosphate and a stoichiometric amount of CuI along with DMEDA seemed to be the best choice to effect ferrocenylation of **1** (Table 1: entry 9).

The simultaneous formation of **3** and **4** is closely related to copper(I)-mediated *O*-arylation of 2-hydroxypyridinesbwith sterically demanding aryl halides.[21] Considering the steric bulk of the three-dimensional iodoferrocene (**2**), the observed regioselectivity is in good agreement with the well-established view that Cu-catalyzed C-heteroatom bond-forming reactions are strongly influenced by the steric hindrance on the aryl halide component.[22]



Reaction conditions: see Table 1

Scheme 1

Since the copper-mediated transformations provided low isolated yields with a small share of the targeted *N*-ferrocenylpyridazinone **3**, we hoped to enhance the efficiency and *N*-selectivity of the model coupling reaction through the application of Pd-complexes $(PPh_3)_2PdCl_2$ and $(dppf)PdCl_2$ as single metal source or components in mixed metal systems (Table 2).

The Pd-catalysed reactions carried out in the absence of CuI led again to the formation of mixtures of **3** and **4** in low isolated yields with ca. 1:8 ratio which proved to be practically invariant to the reaction conditions (Table 2: entries 2-4). Changing to a Sonogashira type protocol (entry 5), a minimal increase was observed in the yield of the regioisomeric products (38%), with a doubled ratio of **3** in the isolated mixture. The third series of Pd-catalyzed reactions, carried out in the presence of a stoichiometric amount of CuI and K_2CO_3 in DMF:TEA (1:1 V/V), afforded the mixture of regioisomeric products in mediocre yields (59-65%: entries 7-9) with substantially increased share of **3**. It must be pointed out here that DMF seems essential to effect the desired conversions, as indicated by the fact that no any coupling reaction took place when toluene or TEA was used as single solvent (entries 1 and 6).

Table 1: Yields of the mixture of **3** and **4** isolated after the reactions conducted under different conditions employing catalytic or stoichiometric amounts of CuI

Entry	reagent or catalyst	ligand[a]	solvent	base	T [°C]	isolated yield (%)[b]
1	10% CuI	-	DMF	K_2CO_3	110	5
2	10% CuI	DMEDA	toluene	K_2CO_3	100	0
3	10% CuI	DMEDA	dioxane	K_2CO_3	90	(8[c])
4	10% CuI	DMEDA	DMF	K_2CO_3	110	7
5	100% CuI	DMEDA	dioxane	K_2CO_3	90	18 (9[c])
6	100% CuI	DMEDA	DMF	K_2CO_3	110	25
7	100% CuI	-	TEA	K_2CO_3	80	0
8	100% CuI	DMEDA	DMF	K_2CO_3	90	15
9	100% CuI	DMEDA	DMF	K_3PO_4	110	33
10	100% CuI	DMEDA	DMF	KO^{t}Bu	110	0
11	100% CuI	TEA	DMF	K_2CO_3	80	5
12	100% CuI	DIPA	DMF	K_2CO_3	80	3
13	100% CuI	-	DMF:TEA 1:1	K_2CO_3	110	30
14	100% CuI	-	DMF:DIPA 1:1	K_2CO_3	110	16

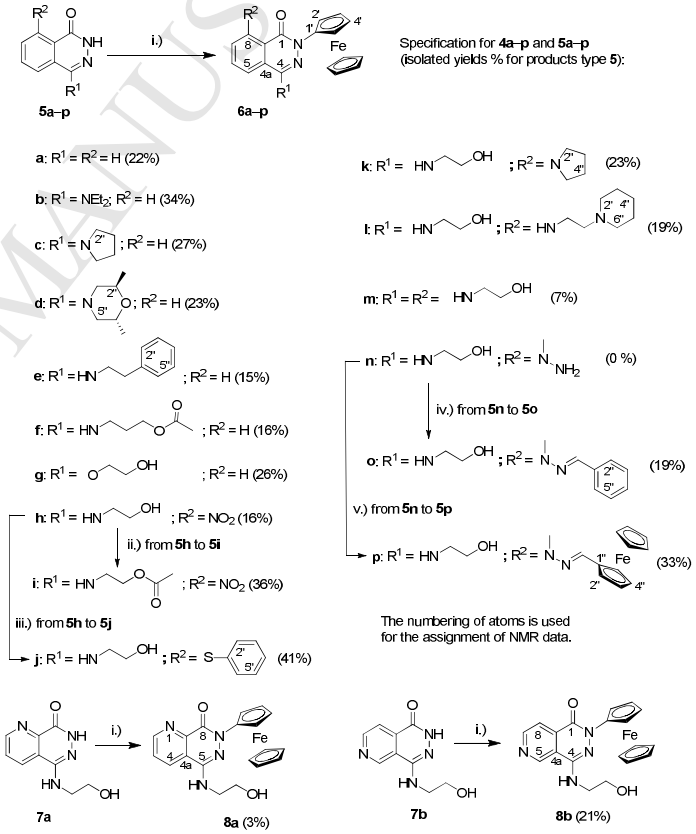
[a]Performed with 2 eq. of DMEDA or 4 eq. of TEA or 4 eq. of DIPA as ligand.
[b]The product ratio **3**:**4** was ca. 2:11 as determined by ^{1}H-NMR spectroscopy.
[c]Isolated mixture of stoichiometric mono-copper complexes of **3** and **4** as indicated by the CH_2- and CH_3 signals of DMEDA presumably coordinated to the Cu(I)-centre.

Table 2: Yields of the mixture of **3** and **4** isolated after the reactions conducted under different conditions employing Pd-complexes and a catalytic or stoichiometric amount of CuI.

Entry	reagent or catalyst	solvent	base	T [°C]	isolated yield (%)
1	5% $(PPh_3)_2PdCl_2$	toluene	K_2CO_3	100	0
2	5% $(PPh_3)_2PdCl_2$	DMF	K_2CO_3	110	27[a]
3	5% (dppf)$PdCl_2$	DMF	K_2CO_3	110	33[a]
4	5% $(PPh_3)_2PdCl_2$	DMF:TEA 1:1	K_2CO_3	110	34[a]
5	10%. CuI, 2.5% $(PPh_3)_2PdCl_2$	DMF:TEA 1:1	K_2CO_3	110	38[b]
6	100%CuI, 5% (dppf)$PdCl_2$	TEA	K_2CO_3	80	0
7	100%CuI, 5% $(PPh_3)_2PdCl_2$	DMF:TEA 1:1	K_2CO_3	110	61[c]
8	100%CuI, 5% (dppf)$PdCl_2$	DMF:TEA 1:1	K_2CO_3	110	65[c]
9	100%CuI, 2.5% $(PPh_3)_2PdCl_2$	DMF:TEA 1:1	K_2CO_3	110	59[c] (applied for further reactions)

[a]The product ratio **3:4** was ca.1:8 as determined by ^{1}H-NMR spectroscopy.
[b]The product ratio **3:4** was ca. 1:4 as determined by ^{1}H-NMR spectroscopy.
[c]The product ratio **3:4** was ca. 5:9 as determined by ^{1}H-NMR spectroscopy.

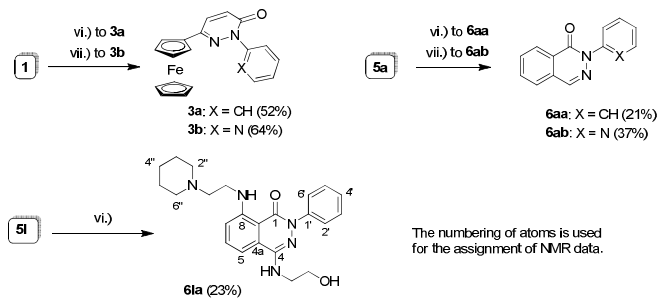
The conditions featuring a satisfactory overall yield (59%) for the mixture of **3** and **4** and a reasonable loading (2.5%) of the cheaper Pd-catalyst [$(PPh_3)_2PdCl_2$] (entry 9) was our choice to accomplish coupling reactions of condensed pyridazinones **5a-p** and **7a**,**b** (Scheme 1). These targets were equipped with such substituents that might enable diversity-oriented synthesis by a variety of further functionalizations (**5a-h**,**k-n** and **7a**,**b** have been reported,[23] while the procedures affording **5i**,**j** and **5o**,**p** are described in this contribution). It is worth pointing out that on the bicyclic substrates the coupling reactions took place on the sterically more accessible lactam nitrogen allowing the exclusive isolation of *N*-ferrocenyl derivatives **6a-p** and **8a**,**b** in low-to-mediocre yields as presented in Scheme 2. In order to investigate the effect of the free OH-group in the hydroxyethylamino chain attached to the phthalazinone scaffold in position 4 (a potential donor site in **5h)**, a representative substrate was protected by simple acetylation (**5h→5i**). This modification increased the efficiency of the coupling protocol (cf. the isolated yields 16% and 36% obtained for conversions **5h→6h** and **5i→6i**, respectively). On the other hand, in the presence of the intact 4-(2-hydroxyethyl) chain, the replacement of the 8-nitro group to phenylthio substituent (**5h→5j**) obviously makes *N*-ferrocenylation more feasible (cf. isolated yield 41% obtained for conversion **5j→6j**). It must also be pointed out that – probably due to the participation of the basic free amino group in the cross-coupling process affording highly unstable hydrazinoferrocene type intermediate inclined to undergo decomposition – a complex mixture of components was isolated when 4-((2-hydroxyethyl)amino)-8-(1-methylhydrazinyl) phthalazin-1(2*H*)-one (**5n**) was used as substrate under the studied conditions. However, hydrazones **5o** and **5p**, obtained by condensations with benzaldehyde and formylferrocene, underwent *N*-ferrocenylation affording **5o** and **6p** in low yields (19% and 33%, respectively). The extremely low yield of **8a** can probably be attributed to the chelation-induced deactivation of the metal-containing catalyst species by the carbonyl-oxygen and the adjacent pyridine nitrogen in precursor **7a**. In general, the unreacted components were partially recovered from the reaction mixture.



Scheme 2: *N*-Ferrocenylation of polyfunctional fused pyridazinones using reaction conditions presented in entry 9 of Table 2

In order to establish structure-activity relationships regarding the contribution of the *N*-aryl group to the cytostatic effect, the analogues of a representative selection of *N*-ferrocenylpyridazinones were also prepared to serve as references in the biological assays. Thus, under the conditions employed for the ferrocenylation reactions, **1** and **5a** were coupled with iodobenzene and 2-bromopyridine affording *N*-phenyl- and *N*-(2-

pyridyl)pyridazinones (**3a**/**6aa** and **3b**/**6ab**, respectively: Scheme 3), as exclusively isolable products which were not contaminated by the corresponding aryloxy-substituted pyridazines. Employing iodobenzene as coupling partner, phthalazinone **5l** was converted into **6la** serving as a further reference in the *in vitro* assays. However, the reaction conducted under the same conditions in the presence of 2-bromopyridine afforded a complex mixture of products of which separation has been unsuccessful so far.



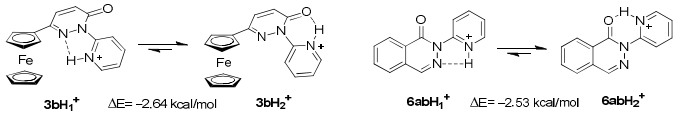
Scheme 3: Synthesis of *N*-arylated pyridazinones to serve as reference organic compounds in the *in vitro* tests.

The IR- ^{1}H- and ^{13}C-NMR spectroscopic data of the novel compounds listed in the Experimental part are consistent with their structure, but the following remarks about the unambiguous discrimination between the alternative sites of the amide-arylation reactions are necessary. The characteristic amide-I band discernible in the range 1620-1640 cm^{-1} of the IR spectra of pyridazinones **3**, **6a-p**, 8a,**b**, **3a**,**b**, **6aa**, **6ab** and **6la** refers to their *N*-arylated structure. Accordingly, the C1‘ resonance was identified around 100 ppm in the ^{13}C-NMR spectra registered for **3**, **6a-p** and **8a**,**b**, while in the ^{13}C-NMR spectrum of ferrocenyloxypyridazine **4** the same signal was detected at 119.9 ppm. The assignment of the C1‘-signal of pronounced diagnostic importance was confirmed by comparative GIAO analysis[24] carried out for the optimized structures of **3** and **4**, respectively, by B3LYP hybrid functional[25] using the extended 6-311++G(2d,p) basis set,[26] a well-established protocol for the calculation of NMR data (the measured/calculated shifts: 99.6 ppm/107.2 ppm and 119.9 ppm/126.2 ppm for **3** and **4**, respectively). The optimized structures of **3** and **4** were generated by the B3PW91 hybrid functional[27] employing the DGTZVP basis set.[28] Note that B3PW91 has been demonstrated to provide an improvement over B3LYP regarding the bonding characterization of metal-containing fragments.[29]

We have studied the cytostatic activity *in vitro* using human HepG2 hepatoma and HT-29 colorectal adenocarcinoma cell cultures for a reasonable selection of the novel ferrocene-containing- and the corresponding organic reference compounds. The cells were treated at 10^{-4} to 10^{2} μM concentration range and the viability of the cells was determined by MTT-assay. The activity was characterized by the IC_{50} values and compared with Tamoxifen as positive control.

The data summarized in Table 3 show that each representative compound with an *N*- or *O*-ferrocenyl substituent produces a characteristic *in vitro* cytostatic effect, in average comparable to that exhibited by Tamoxifen, on both cell cultures investigated. Highlighting the essential contribution of the heteroatom-bonded ferrocene unit to cytostatic activity, significantly decreased effects could be detected for *N*-phenylpyridazinones **3a**, **6aa** and **6la** relative to those measured for their *N*-ferrocenyl-substituted analogues **3**, **6a** and **6l**. These results related to a preliminary structure-activity relationship (SAR) suggesting that the experienced effects might be associated with the production of reactive oxygen species (ROS) possibly playing a crucial role in the observed cytotoxicity.[30] Accordingly, the compounds evaluated in MTT tests were also subjected to comparative cyclic voltammetry (CV) affording half-wave potentials $E_{1/2}(1)$ (Table 3). However, in the case of **3**, **3a**,**b**, **6g**, **6la** and Tamoxifen, the measurements could not be accomplished due to unrevealed irreversible reactions producing solid hardly removable undefined coatings on the electrode surface. It is also worth noting that two consecutive electron transfers characterized by half-wave potentials $E_{1/2}(1)$ and $E_{1/2}(2)$ (235.0 mV and 404.5 mV, respectively) could be detected only for **3** with spatially separated ferrocene redox centers linked to the aromatic pyridazine core. Nevertheless, taking into account that besides ROS-generation the cytotoxic activity of the investigated compounds might also be controlled by diverse factors including e.g. bioavailability and binding affinity to specific receptors, a satisfactory correlation can be established between the relevant $E_{1/2}(1)$ potentials and the measured IC_{50} values. For instance, **6l** and **6m**, displaying the most pronounced cytotoxic effects unambiguously emerged as the most efficient reductants in the series of the investigated model compounds. On the other hand, since only limited experimental data could be collected, and it is reasonable to assume that the measured half-wave potentials (particularly $E_{1/2}(2)$ obtained for **4**) are also affected by solvation, diffusion (a non-thermodynamic effect), ionic strength, chemical and physical interactions with the electrode surface and – under the employed experimental conditions – an enhanced degree of coordination of the solvent acetonitrile molecules with highly structure-dependent modes to the cationic species.[31] To eliminate the effects of previously listed, not ROS-generation dependent properties, which have significant effect on the measured cytotoxic activity or on the measured redox potential, we undertook complementary DFT studies on the redox properties of the tested compounds at B3PW91 level of theory using DGTZVP basis set on the neutral compounds and the corresponding radical cationic species with doublet electron configuration generated by removing one electron from the HOMO of the parent molecules. The first adiabatic ionization energy [$E_i(1)$] values,[32] presumably relevant under biological conditions were obtained as the difference in the total electron energy values calculated for the optimized structures of the analyzed compounds and the appropriate radical cationic species, respectively. Supporting our view regarding the above-discussed cytotoxic activity, at least particularly associated with ROS-generation, the measured $E_{1/2}(1)$ potentials, the calculated HOMO levels and $E_i(1)$ values (Table 3) seem to be in acceptable correlation with the IC_{50} data derived from *in vitro* assay. For instance, *N*-ferrocenylpyridazinones **3** and **6l** with higher HOMO levels undergo ionization more readily [$E_{HOMO}(1)/E_i(1)$= -5.47 eV/6.15 eV and = -5.12 eV/5.64 eV, resp.] than their *N*-phenyl-substituted counterparts **3a** and **6la** [$E_{HOMO}(1)/E_i(1)$= -5.80 eV/7.21 eV and -5.37 eV/6.34 eV, resp.]. However, it must be commented that *N*-(2-pyridyl)pyridazinone **3b** exhibiting no detectable effect in the test can be characterized by somewhat higher HOMO-level and lower ionization energy [$E_{HOMO}(1)$= -5.73 eV and $E_i(1)$=7.02 eV] than its *N*-phenyl-substituted counterpart **3a** [$E_{HOMO}(1)$= -5.80 eV and $E_i(1)$=7.21 eV]. This finding can be explained by the facile alternative chelate-forming modes of protonation of **3b** probably taking place under biological conditions to afford cationic pair $\mathbf{3bH_1}^+$ and $\mathbf{3bH_2}^+$ (Scheme 4) of which DFT analysis revealed their relative stability and highly decreased propensity to undergo ionization probably associated with ROS-generation [cf. enhanced HOMO levels and $E_i(A)$ data in Table 3.]. According to the relative energetics $\mathbf{3bH_2}^+$ stabilized by six-membered chelate ring is expected to be overpopulated relative to its isomeric pair

3bH$_1^+$ with a five-membered chelate residue. It must be noted here that contrary to *N*-ferrocenylphthalazinone **6a** with a relatively high $E_{HOMO}(1)$ level (-5.46 eV) and somewhat increased first adiabatic ionization energy ($E_i(1)$=6.42 eV) enabling to exert remarkable activity against both cell lines investigated, neither *N*-phenyl-substituted analogue **6aa** nor *N*-(2-pyridyl)phthalazinone **6ab** with substantially lowered $E_{HOMO}(1)$ levels (-6.34 eV and -6.54 eV, respectively) and increased $E_i(1)$ (-7.98 eV and -7.74 eV, respectively) values proved to be practically inactive in the *in vitro* tests. Moreover, **6ab** is also expected to readily bind proton in alternative chelated fashions to afford expectably highly deactivated cations **6abH$_1^+$** and **6abH$_2^+$** [cf. lowered $E_{HOMO}(1)$ and enhanced $E_i(1)$ values in Table 3] with a dominance of the latter one as suggested by their calculated relative energetics (Scheme 4).



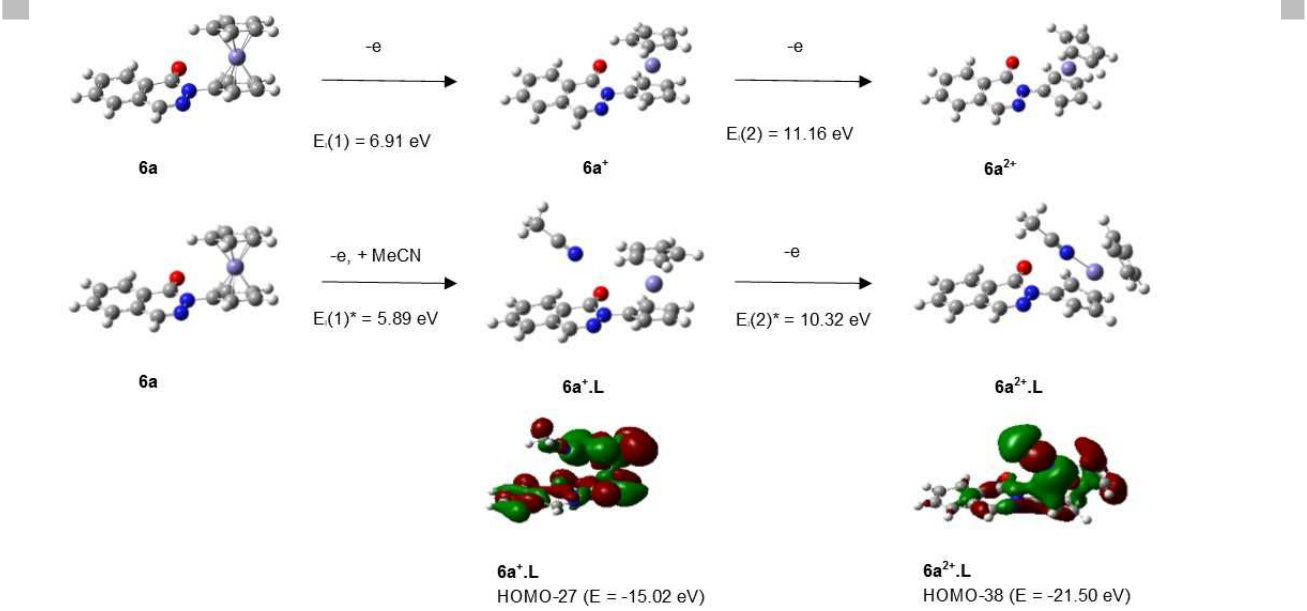
Scheme 4: Relative energy values calculated for isomeric pairs of protonated *N*-(2-pyridyl)pyridazinones, the species assumed to be present under biological conditions, with high ionization energy associated with significantly decreased tendency to generate ROS.

In order to obtain additional information about the structural characteristics that might provide plausible interpretation for redox properties of the prepared compounds and, in particular, for the unique propensity of ferrocenyloxypyridazin **4** to undergo sequentional SET processes under the CV conditions employed, a reasonable selection of diamagnetic dications were also modelled by B3PW91/DGTZVP method (Table 3). However, the second adiabatic ionization energy values [$E_i(2)$] obtained as the relative energetics of the optimized structures of dications and the appropriate radical cations (Table 3) do not suggest an outstanding propensity of **4$^+$** to undergo oxidation. This apparent contradiction might be attributed to an enhanced degree of coordination of the solvent acetonitrile molecules to the cationic- and particularly to dicationic species[31] which interaction was not taken into account for theoretical studies as a highly demanding uncertain explicit solvent model. Nevertheless, we attempted to support this concept by a highly preliminar theoretical study on acetonitrile-promoted stepwise ionization of **6a**, a characteristic representative of the studied compounds with reasonable structural complexity (Scheme 5). The modified ionization energies $E_i(1)$* and $E_i(2)$* unambiguosly referring to solvent-promoted SET processes were obtained as the appropriate differences of the energetics of the optimized structures of non-coordinated **6a** and cationic mono-acetonitrile complexes **6a$^+$.L** and **6a^{2+}.L** featuring slightly and substantially distorted metallocene structure with negligible and strong Fe-N interactions (interatomic distances: 4.547 Å and 1.944 Å, respectively). Selected bonding interactions inside the modeled acetonitrile complexes are visualized by representative delocalized MO's with energy levels referring to a much stronger coordination of the ligand in dication **6a^{2+}.L** than in radical cation **6a$^+$.L** (Scheme 5). The method used (B3PW91/DGTZVP) could not disclose any interaction between **6a** and MeCN.

Table 3. *In vitro* cytostatic effects on two human malignant cell lines expressed as IC_{50} values, measured for the selected compounds and Tamoxifen (serving as reference) along with experimental [$E_{1/2}(1)^a$ and $E_{1/2}(2)^b$] and theoretical parameters [$E_{HOMO}(1)^c$ / $E_{HOMO}(2)^d$ and $E_i(1)^e$ / $E_i(2)^f$]^g characterizing their redox properties of potential relevance in ROS-generation, one of the possible mechanisms responsible for the observed antiproliferative effects.

Compound	IC_{50}± SD [μM]		$E_{1/2}(1)$ [mV]^a	$E_{1/2}(2)$ [mV]^b	$E_{HOMO}(1)$ [eV]^{c,g}	$E_{HOMO}(2)$ [eV]^{d,g}	$E_i(1)$ [eV]^{e,g}	$E_i(2)$ [eV]^{f,g}
	HepG2	HT-29						
3	19.6 ± 2.4	11.0 ± 3.2	-	-	-5.47	-8.16	6.15	10.79
3a	43.8 ± 2.0	41.7 ± 3.5	-	-	-5.80	-9.20	7.21	10.72
3b	>100	>100	-	-	-5.73	-9.38	7.02	11.00
[3bH$_1^+$]^h	-	-	-	-	-8.61	-	9.89	-
[3bH$_2^+$]^h	-	-	-	-	-8.47	-	9.86	-
4	19.6 ± 0.5	18.6 ± 7.9	235.0	404.5	-5.43	-7.84	6.10	10.74
6a	16.4 ± 0.5	20.7 ± 7.0	239.5	-	-5.46	-8.22	6.91	11.16
6aa	>100	>100	-	-	-6.34	-10.76	7.74	12.16
6ab	>100	>100	265.5	-	-6.54	-10.94	7.97	12.34
[6abH$_1^+$]^h	-	-	-	-	-10.47	-	11.90	-
[6abH$_2^+$]^h	-	-	-	-	-10.54	-	11.53	-
6b	16.5 ± 4.3	25.2 ± 5.0	256.5	-	-5.35	-8.93	6.03	10.74
6g	19.9 ± 10.0	54.8 ± 11.1	-	-	-5.40	-9.53	6.05	11.44
6l	5.7 ± 0.1	9.8 ± 1.2	168.0	-	-5.12	-8.09	5.64	10.03
6la	79.8 ± 18.9	27.1 ± 8.0	-	-	-5.39	-8.56	6.34	10.01
6m	11.3 ± 4.0	15.5 ± 5.4	172.5	-	-5.16	-8.33	5.84	10.18
8b	24.1 ± 23.2	35.9 ± 7.5	207.0	-	-5.45	-9.15	6.09	11.05
[8bH$^+$]^h	-	-	-	-	-8.10	-	8.46	-
Tamoxifen	20.0 ± 6.5	15.6 ± 5.7	-	-	-5.55	-8.39	6.51	9.77

[a] First half-wave potential obtained in CV-measurements.
[b] Second half-wave potential obtained in CV-measurements.
[c] HOMO level of the parent compounds and the presented protonated forms.
[d] HOMO level of the corresponding radical cations.
[e] Adiabatic ionization energy of the parent compounds and the presented protonated forms
[f] Adiabatic ionization energy of the corresponding radical cations.
[g] Resulted from B3PW91/DGTZVP calculations.
[h] Assumed to be at least partially present under the conditions of *in vitro* assays.



Scheme 5. DFT-modelled stepwise oxidations of **6a** taking place in the absence and in the presence of 1 equivalent of acetonitrile as represented by the optimized structures of the parent molecule and the resulting non-coordinated and coordinated cationic intermediates supplemented with representative bonding MO's identified for cationic complexes. The calculated ionization energies refer to the promoter role of the solvent stabilizing the resulting cations.

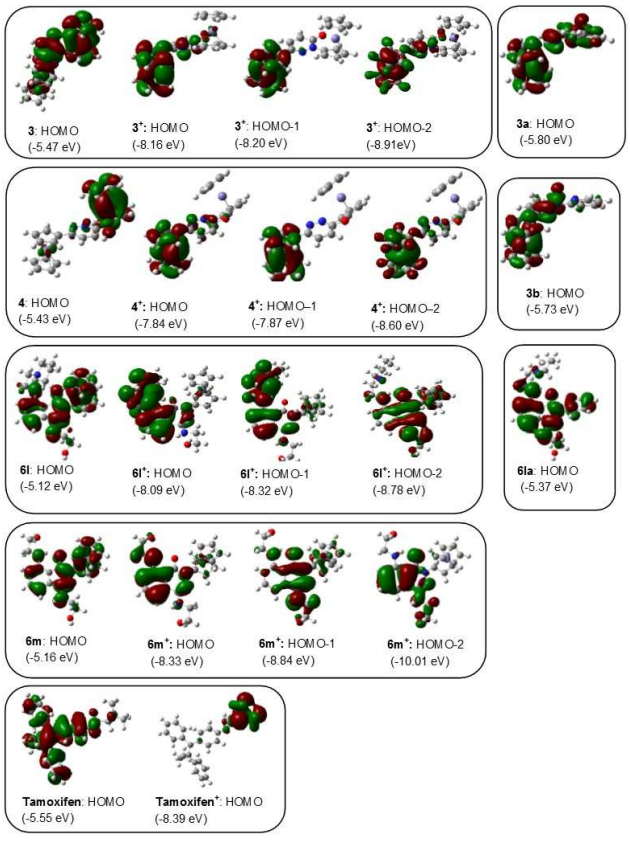


Figure 1. Optimized structures and relevant high-energy occupied MO's of selected pyridazine-based products and Tamoxifen along with their appropriate radical cations.

Besides relative ionization energies HOMO levels of the radical cations can also be considered as the measure of their propensity to undergo ionization affording dications. Thus, in accordance with the experimental findings, radical cation $\mathbf{4}^+$ features a HOMO lying substantially higher in energy [E_{HOMO}(2)=-7.84 eV] than the HOMO's of other studied radical cations with *N*-ferrocenyl/aryl s keleton [note as characteristic examples: E_{HOMO}(2)=-8.16 eV for $\mathbf{3}^+$, -8.09 eV for $\mathbf{6l}^+$ and -8.33 eV for $\mathbf{6m}^+$].

As indicated by the delocalization of the HOMO's of the selection of the modelled products (Figure 1), compound **4** undergoes an *O*-ferrocenyl-centered primary SET, while substantially extended parts of the whole molecular skeletons are involved in the first ionization of *N*-ferrocenyl/arylpyridazinones. The delocalization of the HOMO of radical cation $\mathbf{4}^+$ indicates that the second electron transfer detectable in CV experiments carried out with **4** is centered on the *C*-ferrocenyl group. Moreover, in this radical cation the HOMO-1 and HOMO-2, with energy levels higher to those identified for the isomeric radical cation $\mathbf{3}^+$, are also concentrated on the same metallocene fragment assumably also increasing the chance of the second ionization.

In this context the redox chemistry of reference Tamoxifen must also be commented on. Recent CV studies conducted under non-aqueous conditions disclosed that its irreversible primary oxidation generates a very short-lived amine radical cation which readily abstracts a H-atom from the solvent or an adventitious reactant finally affording the corresponding ammonium ion.[33] This finding is in accordance with the mainly amine-centered HOMO we identified for the radical cation generated from Tamoxifen (Figure 1). On the other hand, the reletively high-energy HOMO of Tamoxifen is delocalized over the whole phenylstyrene skeleton with slight extension toward the basic side-chain.

Besides the obvious electronic- and chelate-forming properties of the *N/O*-ferrocenyl/aryl group, the relative tendency of the tested fused pyridazinones type **5** to undergo ionization is controlled by the electronic effects of the substituents bonded in positions 4 and 8 which can significantly contribute to the stabilization of the radical cationic species, as clearly indicated by the markedly increased cytotoxic activity of **6l** relative to that of **6a**. The HOMO-raising activation of **6l** to behave as a potential reductant can be ascribed to the donor-type C-substituents significantly contributing to the stabilization of ground-state $\mathbf{6l}^+$, as

represented by HOMO, HOMO-1 and HOMO-2 with pronounced delocalization involving the side chains. This view gains support from oxidation-induced shortening of the C4-N and C8-N bond lengths discernible in the optimized structures of this redox pair (C4-N/C8-N: 1.381 Å/1.358 Å and 1.361 Å/1.350 Å in **6l** and **6l**$^+$, respectively) pointing to intensified lone-pair delocalization from the directly attached nitrogen atoms towards the heterocyclic skeleton in **6l**$^+$.

The important contribution of the amine-based side-chains to the ROS-induced cytotoxic effect is reflected in the comparison of IC_{50}-, $E_{HOMO}(1)$- and $E_i(1)$ values found for inactive **6aa** and active **6la**, respectively (Table 3). It is also worth mentioning the IC_{50}-, $E_{1/2}(1)$-, $E_{HOMO}(1)$- and $E_i(1)$ values obtained for **6l** and **6m** with an almost negligible structural difference in the terminal region of their C8-substituents. The lowered cytotoxic activity of **6m** relative to that of **6l**, assumed at least partly associated with the slightly decreased level of ROS-generation, might be the consequence of the decreased basicity of the hydroxyl group in **6m** relative to that of the piperidinyl group in **6l** which is involved in a five-membered chelate ring of a bifurcated H-bond system (with the carbonyl-oxygen as the second donor site), capable of strengthening the abovementioned lone pair delocalization from the directly attached nitrogen towards the electron-deficient heterocyclic skeleton in **6l**$^+$. This view gains support from the spectacularly different extensions and energies of the two highest-level occupied orbitals (HOMO and HOMO-1) visualized for **6l**$^+$ and **6m**$^+$, respectively, consistent with the small but definite difference in the C8-N bond lengths found in their optimized structures (1.349 Å and 1.353 Å in **6l**$^+$ and **6m**$^+$, respectively). Accordingly, the same C4-N atomic distance (1.361 Å) is discernible in both of these cations carrying a 2-hydroxyethylamino group in position 4.

3. Conclusions

Starting from easily available pyridazinone-based precursors and iodoferrocene (**2**), a Cu-mediated cross-coupling protocol employing Pd-catalysis provided 3-ferrocenyl-6-ferrocenyloxypyridazine and a series of novel *N*-ferrocenyl-substituted lactams, the first representatives of novel classes of functionalized organometallics with promising antitumor actions at micromolar concentrations on HepG2 and HT-29 tumor cell cultures. The use of a diverse range of pyridazinones and related lactams is expected to result in a library of related ferrocene-containing cytostatic heterocycles of which activity seems to be – at least partly – associated with ROS-generation. This view was supported by the well-correlated results of comparative biological tests, cyclic voltammetry (CV) experiments and DFT studies carried out on selected organometallic derivatives and on their *N*-phenyl- and *N*-(2-pyridyl)-substituted analogues. Besides the feasibility of further coupling reactions including conjugation to biomolecules enabled by the functional groups attached to the heterocyclic scaffolds, the established structure-activity relationships, disclosing the importance of highly substituent-dependent fine-tunable redox properties, might be taken into account for a rational fragment-based design of related organometallic- and purely organic drug candidates.

4. Experimental section

All chemicals (expect iodoferrocene (**2**), which was prepared according to the method of Fish and Rosenblum[34]) were obtained from commercially available sources (Aldrich, Fluka) and used without further purification, expect solvents. Methanol, dioxane and triethylamine (TEA) were distilled from sodium, *N*,*N*-dimethylformamide was distilled from 4A Molecular sieves under reduced pressure. Melting points (uncorrected) were determined with a Boethius microstage. Merck Kieselgel (230-400 mesh, 60 Å) was used for flash column chromatography. The IR spectra were run by the ATR (Attenuated Total Reflectance) method on a Bruker IFS-55 FT-spectrometer controlled by Opus 3.0 software. The exact mass measurements were performed using a Q-TOF Premier mass spectrometer (Waters Corporation, 34 Maple St, Milford, MA, USA) in positive electrospray mode. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ solution in 5 mm tubes at RT, on a Bruker DRX-500 spectrometer at 500 (^{1}H) and 125 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS as internal standard (^{1}H, ^{13}C). The 2D-COSY, HSQC and HMBC spectra were obtained by using the standard Bruker pulse programs. Optimized structures are available from the authors.

*Preparation of 4-((2-acetoxyethyl)amino)-8-nitrophthalazin-1(2H)-one (**5i**) from **5h***

Phthalazinone **5h** (2.50 g, 10mmol) was dissolved in glacial acetic acid (20 mL) and the solution was refluxed for 2 h. The red colored reaction mixture was concentrated in vacuum to half of its volume then diluted with water. The precipitated solid was filtered off and recrystallized from water (ca. 130-150 mL).

Yellow solid; Yield: 2.12 g (73%); mp.: 231.1-232.6 °C (from H_2O); IR (ATR, cm^{-1}): 3385, 3352, 1748, 1730, 1658, 1589, 1529, 1455, 1431, 1372, 1339, 1309, 1248, 1233, 1219, 1179, 1154, 1139, 1032, 981, 951, 851, 822, 746, 712, 663, 605, 574, 387, 370; ^{1}H-NMR (DMSO-d_6): 11.95 (1H, s, H2), 8.31 (1H, dd, *J* = 6.5 Hz, 2.9 Hz, H5), 8.08 (1H, d, *J* = 7.7 Hz, H7, overlapped by H6), 8.07 (1H, t, *J* = 7.6 Hz, H6, overlapped by H7), 6.98 (1H, t, *J* = 5.4 Hz, $CH_2N\underline{H}$), 4.21 (2H, t, *J* = 5.8 Hz, $C\underline{H}_2O$), 3.48 (2H, q, *J* = 5.7 Hz, $C\underline{H}_2NH$), 1.99 (3H, s, $COC\underline{H}_3$); ^{13}C-NMR (DMSO-d_6): 171.0 ($\underline{C}OCH_3$), 154.6 (C1), 149.2 (C8), 144.3 (C4), 134.5 (C6), 126.7 (C4a), 126.2 (C5), 125.7 (C7), 119.0 (C8), 62.3 ($\underline{C}H_2O$), 40.6 ($\underline{C}H_2NH$). HRMS exact mass calcd. for $C_{12}H_{13}N_4O_5$ [MH]$^+$, requires m/z 293.0886, found m/z 293.0879.

*Preparation of 4-((2-hydroxyethyl)amino)-8-(phenylthio)phthalazin-1(2H)-one (**5j**) from **5h***

Phthalazinone **5h** (2.50 g, 10 mmol), thiophenol (1.32 g, 12 mmol) and K_2CO_3 (2.21 g, 1.6 mmol) were dissolved in DMF (15 mL) and the solution was refluxed for 5 h. The yellow reaction mixture was diluted with water and the precipitated solid was filtered off and recrystallized from EtOH (ca. 80-100 mL).

White solid; Yield: 1.92 g (61%) ; mp.: 307.4-310.7 °C (from EtOH); IR (ATR, cm^{-1}): 3330, 2997, 2863, 1625, 1578, 1534, 1509, 1460, 1438, 1333, 1320, 1229, 1175, 1136, 1068, 1022, 998, 915, 810, 779, 757, 705, 693, 666, 606, 540, 476, 428, 415, 401, 362; ^{1}H-NMR (DMSO-d_6): 11.61 (1H, s, H2), 7.73 (1H, d, *J* = 8.0 Hz, H5), 7.58-7.51 (6H, m, H6 and H2”-H6”), 6.76 (1H, d, *J* = 8.0 Hz, H7), 6.45 (1H, t, *J* = 5.3 Hz, $CH_2N\underline{H}$), 4.69 (1H, t, *J* = 5.5 Hz, $CH_2O\underline{H}$), 3.59 (2H, q, *J* = 5.8 Hz, $C\underline{H}_2OH$), 3.29 (2H, q, *J* = 5.8 Hz, $C\underline{H}_2NH$); ^{13}C-NMR (DMSO-d_6): 159.8 (C1), 145.2 (C4), 144.0 (C8), 136.4 (C3” and C5”), 132.8 (C6), 132.4 (C1”), 130.8 (C2” and C6”), 130.2 (C4”), 127.4 (C7), 127.0 (C4a), 123.5 (C8a), 59.1 ($\underline{C}H_2OH$), 44.6 ($\underline{C}H_2NH$). HRMS exact mass calcd. for $C_{16}H_{16}N_3O_2S$ [MH]$^+$, requires m/z 314.0963, found m/z 314.0970.

*Preparation of imines **5o** and **5p** from 4-((2-hydroxyethyl)amino)-8-(1-methylhydrazinyl)phthalazin-1(2H)-one (**5n**)*

Benzaldehyde (0.531 g, 5 mmol) or formylferrocene (1.070 g, 5 mmol) in MeOH (1 mL) was added to **5n** (1.250 g, 5 mmol) suspended in MeOH (5 mL) and the resulting suspension was stirred at 65 °C for 12 h. The reaction mixture was cooled down to room temperature, and the solid precipitate formed was filtered off washed with water and dried over P_2O_5 in vacuum.

*(E)-8-(2-Benzylidene-1-methylhydrazinyl)-4-((2-hydroxyethyl)amino)phthalazin-1(2H)-one (**5o**)*
Light yellow solid; Yield: 1.459 g (86%); mp.: 274.2-276.9 °C (from MeOH); IR (ATR, cm^{-1}): 3266, 1628, 1589, 1562, 1533, 1477, 1445, 1418, 1393, 1377, 1309, 1291, 1252, 1200, 1162, 1130, 1100, 1089, 1067, 995, 922, 882, 873, 853, 821, 811, 790, 769, 757, 700, 691, 674, 653, 615, 581, 552, 520, 460; ^{1}H-NMR (DMSO-d_6): 11.31 (1H, s, CONH), 7.78 (1H, t, *J* = 7.8 Hz, H6), 7.76 (1H, d, *J* = 7.5 Hz, H5), 7.72 (1H, dd, *J* = 7.0 Hz, 2.0 Hz, H7), 7.68 (1H, s, CHPh), 7.67 (2H, d, *J* = 7.7 Hz, H2' and H6'), 7.38 (2H, t, *J* = 7.6 Hz, H3' and H5'), 7.27 (1H, t, *J* = 7.3 Hz, H4'), 6.33 (1H, t, *J* = 5.4 Hz, NH), 4.66 (1H, t, *J* = 5.7 Hz, OH), 3.64 (2H, q, *J* = 6.0 Hz, CH_2OH), 3.33 (2H, q, *J* = 5.8 Hz, CH_2NH, overlapped by HDO signal of the solvent), 3.28 (3H, s, CH_3); ^{13}C-NMR (DMSO-d_6): 157.0 (C1), 149.6 (C8), 145.2 (C4), 137.0 (C1'), 134.2 (CHPh), 132.9 (C6), 129.0 (C3' and C5'), 128.0 (C4'), 127.8 (C4a), 126.2 (C7 and C2' and C6'), 119.2 (C5), 118.2 (C8a), 59.8 (CH_2OH), 44.7 (CH_2NH), 42.2 (CH_3). HRMS exact mass calcd. for $C_{18}H_{20}N_5O_2$ $[MH]^+$, requires m/z 293.0886, found m/z 293.0892.

*(E)-8-(2-Ferrocenylidene-1-methylhydrazinyl)-4-((2-hydroxyethyl)amino)phthalazin-1(2H)-one (**5p**)*
Reddish orange solid; Yield: 1.93 g (87%); mp.: 235.2-238.6 (degr.) °C (from MeOH); IR (ATR, cm^{-1}): 3210, 1618, 1582, 1540, 1466, 1387, 1293, 1249, 1215, 1177, 1154, 1124, 1104, 1050, 1039, 990, 939, 875, 850, 822, 801, 765, 703, 682, 644, 621, 569, 520, 493, 482, 450, 440, 419; ^{1}H-NMR (DMSO-d_6): 11.29 (1H, s, CONH), 7.77 (1H, t, *J* = 7.9 Hz, H6), 7.70 (1H, d, *J* = 7.9 Hz, H5), 7.67 (1H, d, *J* = 7.9 Hz, H7), 7.49 (1H, s, FcCH), 6.34 (1H, t, *J* = 5.2 Hz, NH), 4.70 (1H, t, *J* = 5.4 Hz, OH), 4.62 (2H, br s, H2' and H5'), 4.33 (2H, br s, H3' and H4'), 4.19 (5H, s, η^5-C_5H_5), 3.63 (2H, q, *J* = 5.3 Hz, CH_2OH), 3.33 (2H, q, *J* = 5.3 Hz, CH_2NH), 3.18 (3H, s, CH_3); ^{13}C-NMR (DMSO-d_6): 157.0 (C1), 150.0 (C8), 145.2 (C4), 133.0 (C6), 127.8 (C4a), 125.3 (C7), 118.5 (C8a), 117.3 (C5), 134.7 (FcCH), 83.0 (C1'), 69.4 (η^5-C_5H_5), 69.3 (C3' and C4'), 66.9 (C2' and C5'), 59.8 (CH_2OH), 44.6 (CH_2NH), 42.4 (CH_3). HRMS exact mass calcd. for $C_{22}H_{24}N_5O_2{}^{56}Fe$ $[MH]^+$, requires m/z 446.1279, found m/z 446.1282.

*General method for the coupling reactions of pyrydazin-based lactams with aryl halides iodoferrocene (**2**), iodobenzene and 2-bromopyridine*

The precursor lactam (1 mmol) was dissolved in DMF-TEA 1:1 (2 mL) ,then K_2CO_3 (0.410 g, 3 mmol), CuI (190 mg, 1 mmol), $PdCl_2(PPh_3)_2$ (17.5 mg, 0.025 mmol) and aryl halide (1.1 mmol) were added to the solution. The reaction mixture was stirred under argon, at 110 °C for overnight. The reaction mixture was filtered and brine (30 mL) was added to the solution. The precipitated solid was filtered off then washed with brine and water. The crude product was purified by column chromatography (on silica, using DCM or DCM-MeOH 100-5:1 mixtures as eluent) and subsequent crystallization from MeOH, EtOH, ^{i}PrOH, ^{t}BuOH, MeOH-water or EtOH-water.

*2,6-Diferrocenylpyridazin-3(2H)-one (**3**)*
Red solid, Yield: 72.1 mg (16%); mp.: 197.3-199.2 °C (from EtOH); IR (ATR, cm^{-1}): 3095, 1654, 1597, 1551, 1488, 1411, 1394, 1368, 1304, 1270, 1230, 1120, 1105, 1078, 1058, 1026, 1000, 932, 890, 843, 817, 676, 579, 545, 503, 484, 443, 391, 381; ^{1}H-NMR (DMSO-d_6): 7.74 (1H, d, *J* = 9.6 Hz, H5), 6.94 (1H, d, *J* = 9.6 Hz, H4), 5.16 (2H, s, H2' and H5'), 4.97 (2H, t, *J* = 1.8 Hz, H2'' and H5''), 4.52 (2H, t, *J* = 1.8 Hz, H3'' and H4''), 4.26 (2H, s, H3' and H4'), 4.21 (5H, s, 2-η^5-C_5H_5), 4.18 (5H, s, 6-η^5-C_5H_5); ^{13}C-NMR (DMSO-d_6): 158.8 (C3), 146.3 (C6), 130.9 (C5), 129.7 (C4), 99.6 (C1'), 80.4 (C1''), 70.9 (C3'' and C4''), 70.0 (2-η^5-C_5H_5 and 6-η^5-C_5H_5), 67.3 (C2'' and C5''), 66.3 (C3' and C4'), 64.4 (C2' and C5'). HRMS exact mass calcd. for $C_{24}H_{21}N_2O^{56}Fe_2$ $[MH]^+$, requires m/z 465.0353, found m/z 465.0345.

*3-Ferrocenyl-6-ferrocenyloxypyridazine (**4**)*
Dark red solid, Yield: 201.9 mg (43%); mp.: 185.4-186.7 °C (from EtOH); IR (ATR, cm^{-1}): 1660, 1592, 1522, 1457, 1392, 1302, 1282, 1236, 1219, 1173, 1130, 1104, 1096, 1070, 1026, 998, 916, 882, 849, 820, 801, 687, 625, 503, 483, 454, 440, 398; ^{1}H-NMR (DMSO-d_6): 7.92 (1H, d, *J* = 9.2 Hz, H4), 7.29 (1H, d, *J* = 9.2 Hz, H5), 5.03 (2H, t, *J* = 1.8 Hz, H2' and H5'), 4.56 (2H, s, H2'' and H5''), 4.49 (2H, t, *J* = 1.8 Hz, H3'' and H4''), 4.27 (5H, s, 6-η^5-C_5H_5), 4.07 (7H, s, H3'' and H4'' and 3-η^5-C_5H_5); ^{13}C-NMR (DMSO-d_6): 165.0 (C6), 158.3 (C3), 128.8 (C4), 119.9 (C1'), 117.7 (C5), 80.9 (C1''), 70.8 (C3' and C4'), 70.0 (3-η^5-C_5H_5), 69.7 (6-η^5-C_5H_5), 67.7 (C2' and C5'), 63.6 (C3'' and C4''), 61.7 (C2'' and C5''). HRMS exact mass calcd. for $C_{24}H_{21}N_2O^{56}Fe_2$ $[MH]^+$, requires m/z 465.0353, found m/z 465.0348.

*2-Ferrocenylphthalazin-1(2H)-one (**6a**)*
Reddish orange solid; Yield: 73.0 mg (22%); mp.: 133.6-135.8 °C (from EtOH); IR (ATR, cm^{-1}): 1656, 1593, 1483, 1458, 1449, 1399, 1360, 1344, 1324, 1309, 1275, 1240, 1227, 1157, 1134, 1117, 1102, 1080, 1034, 1024, 1014, 996, 966, 929, 907, 989, 979, 844, 834, 815, 805, 763, 726, 686, 598, 537, 503; ^{1}H-NMR (DMSO-d_6): 8.57 (1H, br s, H4), 8.33 (1H, d, *J* = 7.9 Hz, H8), 7.97 (1H, d, *J* = 7.9 Hz, H5, overlapped by H5), 7.96 (1H, td, *J* = 7.9 Hz, 1.3 Hz, H6, overlapped by H6), 7.90 (1H, td, *J* = 7.9 Hz, 1.8 Hz, H7), 5.13 (2H, t, *J* = 2.0 Hz, H2' and H5'), 4.24 (2H, t, *J* = 2.0 Hz, H3' and H4'), 4.16 (5H, s, η^5-C_5H_5); ^{13}C-NMR (DMSO-d_6): 158.4 (C1), 139.2 (C4), 134.1 (C6), 133.0 (C7), 129.1 (C4a), 128.2 (C8a), 127.6 (C5), 126.6 (C8), 100.3 (C1'), 69.8 (η^5-C_5H_5), 66.0 (C3' and C4'), 64.0 (C2' and C5'). HRMS exact mass calcd. for $C_{18}H_{15}N_2O^{56}Fe$ $[MH]^+$, requires m/z 331.0534, found m/z 331.0540.

*4-(Diethylamino)-2-ferrocenylphthalazin-1(2H)-one (**6b**)*
Orange solid; Yield: 137.3 mg (34%); mp.: 131.5-132.5 °C (from EtOH); IR (ATR, cm^{-1}): 2973, 1656, 1584, 1551, 1484, 1464, 1448, 1407, 1397, 1382, 1370, 1331, 1316, 1290, 1234, 1204, 1171, 1140, 1117, 1105, 1085, 1074, 1062, 1021, 999, 967, 888, 856, 827, 809, 780, 745, 719, 695, 678, 600, 555, 502, 492, 461, 429, 401; ^{1}H-NMR (DMSO-d_6): 8.33 (1H, br d, *J* = 7.8 Hz, H8), 7.98-7.92 (2H, m, H5 and H6), 7.87 (1H, ddd, *J* = 7.8 Hz, 6.5 Hz, 1.9 Hz, H7), 5.14 (2H, t, *J* = 1.9 Hz, H2' and H5'), 4.21 (2H, t, *J* = 1.9 Hz, H3' and H4'), 4.14 (5H, s, η^5-C_5H_5), 3.32 (4H, q, *J* = 7.0 Hz, NCH_2), 1.19 (6H, t, *J* = 7.0 Hz, CH_3); ^{13}C-NMR (DMSO-d_6): 157.4 (C1), 147.9 (C4), 133.6 (C6), 132.2 (C7), 129.2 (C8a), 127.4 (C8), 126.8 (C4a), 125.4 (C5), 100.3 (C1'), 69.6 (η^5-C_5H_5), 65.7 (C3' and C4'), 63.6 (C2' and C5'), 45.6 (NCH_2), 12.6

(CH_3). HRMS exact mass calcd. for $C_{22}H_{24}N_3O^{56}Fe$ $[MH]^+$, requires m/z 402.1269, found m/z 402.1260.

*2-Ferrocenyl-4-(pyrrolidin-1-yl)phthalazin-1(2H)-one (**6c**)*
Orange solid; Yield: 107.6 mg (27%); mp: 140.2-142.5 °C (from EtOH); IR (ATR, cm^{-1}): 2960, 2930, 2861, 1641, 1609, 1575, 1526, 1492, 1466, 1438, 1407, 1363, 1330, 1241, 1231, 1161, 1148, 1104, 1076, 1030, 1019, 997, 949, 928, 912, 893, 856, 817, 788, 778, 756, 718, 686, 655, 643, 628, 615, 595, 535, 498, 485, 459, 446, 408; ^{1}H-NMR (DMSO-d_6): 8.34 (1H, dd, *J* = 7.8 Hz, 1.5 Hz, H8), 8.15 (1H, d, *J* = 8.0 Hz, H5), 7.91 (1H, td, *J* = 7.6 Hz, 1.5 Hz, H6), 7.85 (1H, td, *J* = 7.5 Hz, 1.2 Hz, H7), 5.13 (2H, t, *J* = 2.0 Hz, H2' and H5'), 4.18 (2H, t, *J* = 2.0 Hz, H3' and H4'), 4.14 (5H, s, η^5-C$_5$H$_5$), 3.61 (4H, t, *J* = 6.5 Hz, H2'' and H5''), 1.96 (4H, tt, *J* = 6.5 Hz, 2.0 Hz, H3''and H4''); ^{13}C-NMR (DMSO-d_6): 157.0 (C1), 147.2 (C4), 133.2 (C6), 132.0 (C7), 129.2 (C8a), 127.7 (C8), 126.1 (C4a), 125.9, (C5), 69.7 (η^5-C$_5$H$_5$), 65.5 (C3' and C4'), 63.6 (C2' and C5'), 51.0 (C2'' and C5''), 25.5 (C3'' and C4''). HRMS exact mass calcd. for $C_{22}H_{24}N_3O^{56}Fe$ $[MH]^+$, requires m/z 400.1112, found m/z 400.1107.

*4-(2,6-Dimethylmorpholino)-2-ferrocenylphthalazin-1(2H)-one (**6d**)*
Yellowish orange solid; Yield: 100.0 mg (23%); mp.: 160.6-162.8 °C (from EtOH); IR (ATR, cm^{-1}): 2977, 2853, 1656, 1587, 1547, 1487, 1468, 1452, 1422, 1398, 1379, 1363, 1329, 1317, 1269, 1234, 1123, 1187, 1174, 1144, 1104, 1084, 1072, 1054, 1038, 1024, 1000, 968, 930, 895, 860, 817, 790, 778, 767, 724, 698, 678, 658, 648, 599, 503, 489, 462, 428, 406; ^{1}H-NMR ($CDCl_3$): 8.45 (1H, br s, H8), 7.88 (1H, br d, *J* = 4.1 Hz, H5), 7.80 (1H, br s, H6), 7.76 (1H, br s, H7), 5.46 (2H, s, H2' and H5'), 4.45 (2H, s, H3' and H4'), 4.41 (5H, s, η^5-C$_5$H$_5$), 4.04 (2H, br s, CHCH$_3$O), 3.44 (2H, br d, *J* = 8.2 Hz, H3''a and H5''a), 2.79 (2H, br t, H3''b and H5''b), 1.31 (6H, s, CH_3, overlapped by HDO signal of the solvent); ^{13}C-NMR ($CDCl_3$): 157.8 (C1), 148.3 (C4), 132.5 (C6), 131.4 (C7), 129.6 (C4a), 127.8 (C8), 125.6 (C8a), 124.5 (C5), 71.7 (η^5-C$_5$H$_5$), 71.6 (C2'' and C6''), 66.9 (C3' and C4'), 64.3 (C2' and C5'), 57.0 (C3'' and C5''), 19.2 (CH_3). HRMS exact mass calcd. for $C_{24}H_{26}N_3O_2{}^{56}Fe$ $[MH]^+$, requires m/z 444.1374, found m/z 444.1379.

*2-Ferrocenyl-4-(phenethylamino)phthalazin-1(2H)-one (**6e**)*
Orange solid; Yield: 67.4 mg (15%); mp: 170.9-173.3 °C (from EtOH); IR (ATR, cm^{-1}): 3345, 1632, 1579, 1537, 1497, 1478, 1452, 1428, 1381, 1328, 1313, 1229, 1185, 1167, 1151, 1119, 1108, 1048, 1026, 1003, 925, 801, 771, 747, 719, 701, 687, 646, 613, 586, 474, 455, 406; ^{1}H-NMR (DMSO-d_6): 8.33 (1H, d, *J* = 7.5 Hz, H5), 8.14 (1H, d, *J* = 8.1 Hz, H8), 7.92 (1H, t, *J* = 7.3 Hz, H6), 7.85 (1H, t, *J* = 7.5 Hz, H7), 7.39-7.33 (4H, m, H2'' and H3'' and H5'' and H6''), 7.24 (1H, tt, *J* = 7.3 Hz, 2.0 Hz, H4''), 7.11 (1H, t, *J* = 5.0 Hz, NH), 5.20 (2H, br s, H2' and H5'), 4.19 (2H, br s, H3' and H4') 4.13 (5H, s, η^5-C$_5$H$_5$), 3.64 (2H, td, *J* = 7.4 Hz, 5.0 Hz, CH_2NH), 3.11 (2H, t, *J* = 7.4 Hz, CH_2Ph); ^{13}C-NMR (DMSO-d_6): 156.5 (C1), 144.6 (C4), 140.6 (C4a), 133.3 (C6), 132.1 (C7), 129.1 (C2'' and C6''), 128.9 (C3'' and C5''), 128.6 (C8a), 127.3 (C5), 126.5 (C4'), 124.4 (C1''), 123.4 (C8), 100.7 (C1'), 69.6 (η^5-C$_5$H$_5$), 65.5 (C3' and C4'), 63.5 (C2' and C5'), 43.8 (CH_2NH), 34.7 (CH_2Ph). HRMS exact mass calcd. for $C_{26}H_{24}N_3O^{56}Fe$ $[MH]^+$, requires m/z 450.1269, found m/z 450.1277.

*4-((2-Acetoxypropyl)amino)-2-ferrocenyl-phthalazin-1(2H)-one (**6f**)*
Yellow solid; Yield: 71.6 mg (16%); mp.: 133.9-135.2 °C (from EtOH); IR (ATR, cm^{-1}): 3420, 1715, 1651, 1590, 1544, 1483, 1463, 1443, 1412, 1398, 1366, 1340, 1329, 1309, 1287, 1260, 1195, 1173, 1154, 1134, 1118, 1105, 1089, 1035, 1026, 1016, 982, 929, 858, 815, 795, 768, 720, 680, 643, 629, 605, 505, 496, 481, 457, 441, 422, 401; ^{1}H-NMR (DMSO-d_6): 8.32 (1H, d, *J* = 7.9 Hz, H8), 8.14 (1H, d, *J* = 5.1 Hz, H5), 7.92 (1H, t, *J* = 7.6 Hz, H6), 7.85 (1H, t, *J* = 7.6 Hz, H7), 7.03 (1H, t, *J* = 4.9 Hz, NH), 5.19 (2H, br s, H2' and H5'), 4.20 (2H, t, *J* = 6.3 Hz, CH_2O), 4.17 (2H, br s, H3' and H4'), 4.13 (5H, s, η^5-C$_5$H$_5$), 3.49 (2H, q, *J* = 6.0 Hz, CH_2NH), 2.10 (2H, p, *J* = 6.3 Hz, CH$_2$CH$_2$CH$_2$), 2.04 (3H, s, CH_3); ^{13}C-NMR (DMSO-d_6): 171.0 (CH$_3$COO), 156.4 (C1), 144.6 (C4), 133.2 (C7), 132.0 (C6), 128.6 (C8a), 127.4 (C8), 124.4 (C4a), 123.4 (C5), 100.7 (C1'), 69.4 (η^5-C$_5$H$_5$), 65.4 (C3' and C4'), 63.5 (C2' and C5'), 62.8 (CH_2O), 38.7 (CH_2NH), 27.9 (CH$_2$CH$_2$CH$_2$), 21.3 (CH_3). HRMS exact mass calcd. for $C_{23}H_{24}N_3O_3{}^{56}Fe$ $[MH]^+$, requires m/z 446.1167, found m/z 446.1174.

*2-Ferrocenyl-4-(2-hydroxyethoxy)phthalazin-1(2H)-one (**6g**)*
Orange solid; Yield: 103.1 mg (26%); mp.: 152.6-153.5 °C (from EtOH); IR (ATR, cm^{-1}): 3428, 3087, 2944, 1636, 1619, 1587, 1495, 1454, 1410, 1384, 1348, 1316, 1240, 1187, 1102, 1079, 1027, 1017, 1000, 967, 930, 899, 858, 825, 809, 794, 778, 728, 690, 644, 574, 485, 457, 442; ^{1}H-NMR (DMSO-d_6): 8.31 (1H, d, *J* = 7.7 Hz, H8), 8.09 (1H, d, *J* = 7.6 Hz, H5), 7.97 (1H, td, *J* = 7.5 Hz, 1.5 Hz, H6), 7.93 (1H, td, *J* = 7.5 Hz, 1.5 Hz, H7), 5.14 (2H, t, *J* = 2.0 Hz, H2' and H5'), 5.03 (1H, t, *J* = 5.7 Hz, OH), 4.46 (2H, t, *J* = 4.9 Hz, COCH_2), 4.21 (2H, t, *J* = 2.0 Hz, H3' and H4'), 4.18 (5H, s, η^5-C$_5$H$_5$), 3.90 (2H, q, *J* = 5.2 Hz, CH_2OH); ^{13}C-NMR (DMSO-d_6): 157. (C1), 149.5 (C4), 134.0 (C6), 133.0 (C7), 127.2 (C8), 129.3 (C8a), 124.1 (C5), 123.9 (C4a), 69.8 (η^5-C$_5$H$_5$), 69.2 (COCH_2), 65.0 (C3' and C4'), 63.8 (C2' and C5'), 60.0 (CH_2OH). HRMS exact mass calcd. for $C_{20}H_{19}N_2O_3{}^{56}Fe$ $[MH]^+$, requires m/z 391.0745, found m/z 391.0752.

*2-Ferrocenyl-4-((2-hydroxyethyl)amino)-8-nitrophthalazin-1(2H)-one (**6h**)*
Dark red solid; Yield: 70.4 mg (16%); mp.: 199.9-201.4 °C (from EtOH); IR (ATR, cm^{-1}): 3574, 3336, 1643, 1582, 1538, 1521, 1455, 1399, 1376, 1343, 1331, 1299, 1238, 1168, 1122, 1106, 1076, 1045, 1032, 1002, 951, 876, 864, 849, 824, 812, 803, 779, 761, 725, 705, 676, 642, 540, 496, 486, 456, 434, 387, 375; ^{1}H-NMR (DMSO-d_6): 8.39 (1H, d, *J* = 7.4 Hz, H5), 8.12 (1H, d, H7, overlapped by H6), 8.09 (1H, t, H6, overlapped by H7), 7.14 (1H, br t, NH), 5.06 (2H, s, H2' and H5'), 4.78 (1H, t, *J* = 5.3 Hz, OH), 4.18 (2H, s, H3' and H4'), 4.15 (5H, s, η^5-C$_5$H$_5$), 3.76 (2H, q, *J* = 5.6 Hz, CH_2OH), 3.49 (2H, q, *J* = 5.5 Hz, CH_2NH); ^{13}C-NMR (DMSO-d_6): 152.6 (C1), 149.4 (C8), 143.9 (C4), 134.3 (C6), 131.4 (C4a), 126.3 (C7), 126.0 (C5), 118.9 (C8a), 99.9 (C1'), 69.7 (η^5-C$_5$H$_5$), 65.7 (C3' and C4' and CH_2OH), 63.8 (C2' and C5'), 44.8 (CH_2NH); HRMS exact mass calcd. for $C_{20}H_{19}N_4O_4{}^{56}Fe$ $[MH]^+$, requires m/z 435.0756, found m/z 435.0765.

*4-((2-Acetoxyethyl)amino)-2-ferrocenyl-8-nitrophthalazin-1(2H)-one (**6i**)*
Dark red solid; Yield: 172.6 mg (36%); mp.: ~220 (degr.) °C (from EtOH); IR (ATR, cm^{-1}): 3418, 1723, 1641, 1588, 1534, 1463, 1454, 1444, 1410, 1380, 1363, 1333, 1308, 1230, 1174, 1156, 1139, 1105, 1027, 1000, 958, 931, 914, 886, 851, 812, 801, 775, 761, 725, 699, 676, 643, 610, 570, 494, 459, 441, 405; ^{1}H-NMR (DMSO-d_6): 8.36 (1H, d, *J* = 7.5 Hz, H5), 8.14 (1H, br d, *J* = 8.4 Hz, H7), 8.10 (1H, t, *J* = 8.0 Hz, H6), 7.34 (1H, t, *J* = 5.4 Hz, NH), 5.08 (2H, s, H2' and H5'), 4.18 (2H, s, H3' and H4'), 4.16 (5H, s, η^5-C$_5$H$_5$), 4.40 (2H, t, *J* = 5.7 Hz, CH_2OH), 3.67 (2H, q, *J* = 5.5 Hz, CH_2NH), 2.04 (3H, s, CH_3); ^{13}C-NMR (DMSO-d_6): 171.0 (CH$_3$COO), 154.7 (C1), 149.4 (C8), 143.8 (C4), 134.4

(C6), 126.2 (C5), 126.1 (C7), 125.8 (C4a), 119.0 (C8a), 99.9 (C1'), 69.6 (η^5-$\underline{C}_5H_5$), 65.8 (C3' and C4'), 63.8 (C2' and C5'), 62.0 ($\underline{C}H_2O$), 41.0 ($\underline{C}H_2NH$), 21.4 ($\underline{C}H_3$). HRMS exact mass calcd. for $C_{22}H_{21}N_4O_5{}^{56}Fe$ $[MH]^+$, requires m/z 477.0861, found m/z 477.0856.

*2-Ferrocenyl-4-((2-hydroxyethyl)amino)-8-(phenylthio)phthalazin-1(2H)-one (**6j**)*

Orange solid; Yield: 203.1 mg (41%); mp.: 165.3-167.9 °C (from EtOH); IR (ATR, cm^{-1}): 3297, 1625, 1595, 1570, 1534, 1458, 1438, 1307, 1237, 1194, 1166, 1135, 1104, 1070, 1053, 1024, 998, 944, 910, 837, 818, 805, 778, 751, 698, 690, 647, 567, 534, 493, 459, 433, 411; ^{1}H-NMR (DMSO-d_6): 7.80 (1H, d, *J* = 5.6 Hz, H5), 7.65-7.51 (6H, m, H6 and H2''– H6''), 6.84 (1H, d, *J* = 6.4 Hz, H7), 6.79 (1H, br t, N$\underline{H}$), 5.13 (2H, s, H2' and H5'), 4.74 (1H, br t, O$\underline{H}$), 4.17 (7H, s, H3' and H4' and η^5-$\underline{C}_5H_5$), 3.75 (2H, br s, C$\underline{H}_2$OH), 3.48 (2H, br s, C$\underline{H}_2$NH); ^{13}C-NMR (DMSO-d_6): 156.8 (C1), 144.6 (C4 and C8), 136.4 (C3'' and C5''), 132.7 (C6), 132.6 (C1''), 130.9 (C2'' and C6''), 130.3 (C4''), 128.1 (C7), 126.0 (C4a), 123.5 (C8a), 119.0 (C5), 100.5 (C1'), 69.5 (η^5-$\underline{C}_5H_5$), 65.4 (C3' and C4'), 63.7 (C2' and C5'), 59.4 ($\underline{C}H_2$OH), 44.8 ($\underline{C}H_2$NH); HRMS exact mass calcd. for $C_{26}H_{24}N_3O_2S^{56}Fe$ $[MH]^+$, requires m/z 498.0939, found m/z 498.0946.

*2-Ferrocenyl-4-((2-hydroxyethyl)amino)-8-(pyrrolidin-1-yl)phthalazin-1(2H)-one (**6k**)*

Yellowish orange solid; Yield: 104.4 mg (23%); mp.: 198.1-199.5 °C (from EtOH); IR (ATR, cm^{-1}): 3320, 2871, 2819, 1618, 1528, 1470, 1459, 1436, 1392, 1376, 1356, 1338, 1319, 1298, 1290, 1247, 1224, 1194, 1161, 1147, 1119, 1105, 1027, 997, 867, 848, 814, 797, 787, 757, 711, 702, 665, 634, 566, 532, 494, 841, 449, 420; ^{1}H-NMR (DMSO-d_6): 7.57 (1H, t, *J* = 8.0 Hz, H6), 7.26 (1H, d, *J* = 7.7 Hz, H5), 7.07 (1H, d, *J* = 8.5 Hz, H7), 6.44 (1H, br t, *J* = 5.5 Hz, N$\underline{H}$), 5.09 (2H, s, H2' and H5'), 4.73 (1H, br t, *J* = 5.6 Hz, O$\underline{H}$), 4.11 (7H, s, H3' and H4' and η^5-$\underline{C}_5\underline{H}_5$), 3.75 (2H, br q, *J* = 6.1 Hz, C$\underline{H}_2$OH), 3.45 (2H, br q, *J* = 5.9 Hz, C$\underline{H}_2$NH), 3.34 (4H, s, H2'' and H5''), 1.90 (4H, br s, H3'' and H4''); ^{13}C-NMR (DMSO-d_6): 156.1 (C1), 149.5 (C8), 144.9 (C4), 132.6 (C6), 127.2 (C4a), 115.6 (C7), 113.9 (C8a), 109.9 (C5), 101.5 (C1'), 69.2 (η^5-$\underline{C}_5H_5$), 65.0 (C3' and C4'), 63.1 (C2' and C5'), 59.7 ($\underline{C}H_2$OH), 52.0 (C2'' and C5''), 44.8 ($\underline{C}H_2$NH), 25.9 (C3'' and C4''); HRMS exactmass$C_{24}H_{27}N_4O_2{}^{56}Fe$ $[MH]^+$, requires m/z 459.1483, found m/z 459.1489.

*2-Ferrocenyl-4-((2-hydroxyethyl)amino)-8-((2-(piperidin-1-yl)ethyl)amino)phthalazin-1(2H)-one (**6l**)*

Yellow solid; Yield: 100.1 mg (19%); mp.: 180.5-182.0 °C (from EtOH); IR (ATR, cm^{-1}): 3361, 2924, 2853, 1640, 1595, 1570, 1537, 1467, 1404, 1363, 1324, 1297, 1269, 1255, 1220, 1209, 1189, 1160, 1129, 1117, 1105, 1092, 1072, 1036, 1020, 1000, 860, 837, 812, 802; ^{1}H-NMR (DMSO-d_6): 9.50 (1H, t, *J* = 4.6 Hz, 8-N$\underline{H}$), 7.59 (1H, t, *J* = 7.9 Hz, H6), 7.09 (1H, d, *J* = 7.7 Hz, H5), 6.86 (1H, d, *J* = 8.7 Hz, H7), 6.55 (1H, t, *J* = 5.4 Hz, 4-N$\underline{H}$), 5.09 (2H, t, *J* = 2.0 Hz, H2' and H5'), 4.75 (1H, t, *J* = 5.0 Hz, O$\underline{H}$), 4.14 (7H, m, H3' and H4' and η^5-$\underline{C}_5\underline{H}_5$), 3.74 (2H, q, *J* = 5.5 Hz, C$\underline{H}_2$OH), 3.46 (2H, q, *J* = 5.7 Hz, C$\underline{H}_2$4-NH), 3.30 (2H, q, *J* = 5.5 Hz, C$\underline{H}_2$8-NH), 2.58 (2H, t, *J* = 6.4 Hz, C$\underline{H}_2$Pip), 2.43 (4H, br s, H2'' and H6''), 1.55 (4H, p, *J* = 5.6 Hz, H3'' and H5''), 1.42 (2H, q, *J* = 5.6 Hz, H4''); ^{13}C-NMR (DMSO-d_6): 159.5 (C1), 150.9 (C8), 145.1 (C4), 134.6 (C6), 125.9 (C4a), 111.8 (C7), 110.4 (C8a), 107.6 (C5), 100.7 (C1'), 69.4 (η^5-$\underline{C}_5H_5$), 65.2 (C3' and C4'), 63.7 (C2' and C5'), 59.6 ($\underline{C}H_2$OH), 57.1 ($\underline{C}H_2$Pip), 54.4 (C2'' and C6''), 44.7 ($\underline{C}H_2$4-NH), 40.3 ($\underline{C}H_2$8-NH, overlapped by solvent signal), 26.1 (C3'' and C5''), 24.6 (C4''); HRMS exact mass calcd. for $C_{27}H_{34}N_5O_2{}^{56}Fe$ $[MH]^+$, requires m/z 516.2062, found m/z 516.2056.

*2-Ferrocenyl-4,8-bis((2-hydroxyethyl)amino)phthalazin-1(2H)-one (**6m**)*

Yellow solid; Yield: 31.2 mg (7%); mp.: 222.3-224.1 (degr.) °C (from EtOH); IR (ATR, cm^{-1}): 3374, 2841, 1634, 1588, 1570, 1528, 1470, 1450, 1404, 1377, 1350, 1323, 1299, 1248, 1234, 1214, 1187, 1165, 1134, 1105, 1092, 1063, 1024, 1000, 980, 893, 874, 837, 812, 802, 762, 701, 686, 672, 633, 574, 539, 498, 491, 462, 448, 418; ^{1}H-NMR (DMSO-d_6): 9.48 (1H, t, *J* = 4.4 Hz, 8-N$\underline{H}$), 7.57 (1H, t, *J* = 7.8 Hz, H6), 7.09 (1H, d, *J* = 7.8 Hz, H5), 6.86 (1H, d, *J* = 8.3 Hz, H7), 6.56 (1H, t, *J* = 4.4 Hz, 4-N$\underline{H}$), 5.08 (2H, br s, H2' and H5'), 4.88 (1H, t, *J* = 4.9 Hz, 8-O$\underline{H}$), 4.74 (1H, t, *J* = 5.3 Hz, 4-O$\underline{H}$), 4.13 (5H, s, η^5-$\underline{C}_5\underline{H}_5$), 3.74 (2H, q, *J* = 5.8 Hz, 4-C$\underline{H}_2$OH), 3.66 (2H, q, *J* = 5.2 Hz, 8-C$\underline{H}_2$OH), 3.45 (2H, q, *J* = 5.5 Hz, 4-C$\underline{H}_2$NH), 3.27 (2H, q, *J* = 5.0 Hz, 8-C$\underline{H}_2$NH); ^{13}C-NMR (DMSO-d_6): 159.6 (C1), 151.1 (C8), 145.1 (C4), 134.7 (C6), 125.9 (C4a), 111.7 (C7), 110.3 (C8a), 107.6 (C5), 100.6 (C1'), 69.4 (η^5-$\underline{C}_5H_5$), 65.2 (C3' and C4'), 63.7 (C2' and C5'), 59.7 (8-$\underline{C}H_2$OH), 59.6 (4-$\underline{C}H_2$OH), 45.3 (4-$\underline{C}H_2$NH), 44.7 (4-$\underline{C}H_2$NH); HRMS exact mass calcd. for $C_{22}H_{25}N_4O_3{}^{56}Fe$ $[MH]^+$, requires m/z 449.1276, found m/z 449.1270.

*(E)-8-(2-Benzylidene-1-methylhydrazinyl)-2-ferrocenyl-4-((2-hydroxyethyl)amino) phthalazin-1(2H)-one (**6o**)*

Reddish orange solid; Yield: 96.6 mg (19%); mp.: 185.9-187.5 °C (from EtOH); IR (ATR, cm^{-1}): 3311, 1629, 1593, 1569, 1543, 1468, 1448, 1431, 1382, 1360, 1318, 1228, 1192, 1178, 1155, 1125, 1105, 1087, 1059, 1028, 992, 936, 920, 888, 811, 804, 790, 768, 752, 703, 692, 669, 642, 620, 586, 542, 498, 475, 451; ^{1}H-NMR (DMSO-d_6): 7.83-7.82 (2H, m, H5 and H7), 7.78 (1H, q, *J* = 4.6 Hz, H6), 7.74 (1H, s, C$\underline{H}$Ph), 7.71 (2H, d, *J* = 7.4 Hz, H2'' and H6''), 7.40 (2H, t, *J* = 7.5 Hz, H3'' and H5''), 7.29 (1H, t, *J* = 7.3 Hz, H4''), 6.75 (1H, t, *J* = 5.4 Hz, N$\underline{H}$), 5.12 (2H, s, *J* = 2.0 Hz, H2' and H5'), 4.80 (1H, br s, O$\underline{H}$), 4.15 (2H, t, H3' and H4', overlapped byη^5-C_5H_5 signal), 4.14 (5H, s, η^5-$\underline{C}_5\underline{H}_5$, overlapped by H3' and H4'), 3.78 (2H, t, *J* = 7.8 Hz, C$\underline{H}_2$OH), 3.47 (2H, q, *J* = 6.0 Hz, C$\underline{H}_2$NH), 3.31 (3H, s, C$\underline{H}_3$, overlapped by solvent signal); ^{13}C-NMR (DMSO-d_6): 155.0 (C1), 149.8 (C8), 144.5 (C4), 137.0 (C1''), 134.4 (Ph$\underline{C}$H), 133.0 (C6), 129.1 (C3'' and C5''), 128.1 (C4''), 126.8 (C5), 126.7 (C4a), 126.3 (C2'' and C6''), 118.9 (C8a), 118.2 (C7), 101.2 (C1'), 69.4 (η^5-$\underline{C}_5H_5$), 65.3 (C3' and C4'), 63.7 (C2' and C5'), 59.6 ($\underline{C}H_2$OH), 44.8 ($\underline{C}H_2$NH), 41.9 ($\underline{C}H_3$); HRMS exact mass calcd for $C_{28}H_{28}N_5O_2{}^{56}Fe$ $[MH]^+$, requires m/z 522.1592, found m/z 522.1599.

*(E)-8-(2-Ferrocenylidene-1-methylhydrazinyl)-2-ferrocenyl-4-((2-hydroxyethyl)amino) phthalazin-1(2H)-one (**6p**)*

Orange solid; Yield: 204.5 mg (33%); mp.: 189.7-192.8 °C (from EtOH); IR (ATR, cm^{-1}): 3358, 2918, 1626, 1567, 1550, 1463, 1434, 1413, 1383, 1363, 1321, 1250, 1224, 1209, 1175, 1153, 1121, 1106, 1090, 1068, 1022, 994, 935, 880, 810, 793, 772, 700, 669, 581, 497, 483, 457, 420; ^{1}H-NMR (DMSO-d_6): 7.81-7.68 (3H, m, H5 and H6 and H7), 7.52 (1H, s, C$\underline{H}$Fc), 6.68 (1H, br t, N$\underline{H}$), 5.11 (2H, s, H2' and H5'), 4.75 (1H, br t, O$\underline{H}$), 4.62 (2H, s, H2'' and H5''), 4.32 (2H, s, H3'' and H4''), 4.19 (5H, s, 8-η^5-$\underline{C}_5\underline{H}_5$), 4.13 (7H, s, H3' and H4' and 2-η^5-$\underline{C}_5\underline{H}_5$), 3.77 (2H, br q, *J* = 5.1 Hz, C$\underline{H}_2$OH), 3.47 (2H, br q, *J* = 4.6 Hz, C$\underline{H}_2$NH), 3.21 (3H, s, C$\underline{H}_3$); ^{13}C-NMR (DMSO-d_6): 155.2 (C1), 150.2 (C8), 144.6 (C4), 134.7 (Fc$\underline{C}$H) 133.0 (C6), 126.7 (C4a), 125.9 (C5), 118.2 (C8a), 117.2 (C7), 101.2 (C1'), 83.1 (C1''), 69.5 (1-η^5-$\underline{C}_5H_5$), 69.4 (8-η^5-$\underline{C}_5H_5$) 69.3 (C3'' and C4''), 67.0 (C2'' and C5''), 65.2 (C3' and C4'), 63.7 (C2' and C5'), 59.6 ($\underline{C}H_2$OH), 44.8 ($\underline{C}H_2$NH), 42.1 ($\underline{C}H_3$); HRMS exact mass calcd.for

$C_{32}H_{32}N_5O_2{}^{56}Fe_2$ $[MH]^+$, requires m/z 630.1255, found m/z 630.1247.

*7-Ferrocenyl-5-((2-hydroxyethyl)amino)pyrido[2,3-d]pyridazin-8(7H)-one (**8a**)*

Orange solid; Yield: 13.3 mg (3%); ^{1}H-NMR (DMSO-d_6): 9.06 (1H, br d, *J* = 4.3 Hz, H2), 8.84 (1H, br d, *J* = 8.3 Hz, H4), 7.92 (1H, dd, *J* = 8.3 Hz, 4.3 Hz, H3), 7.33 (1H, br t, NH), 5.17 (2H, t, *J* = 1.9 Hz, H2' and H5'), 4.98 (1H, br t, *J* = 6.0 Hz, OH), 4.19 (2H, t, *J* = 1.9 Hz, H3' and H4'), 4.16 (5H, s, η^5-C_5H_5), 3.77 (2H, q, *J* = 5.6 Hz, CH_2OH, overlapped), 3.48 (2H, q, *J* = 5.6 Hz, CH_2NH); ^{13}C-NMR (DMSO-d_6): 154.6 (C8), 154.1 (C2), 144.4 (C5), 143.6 (C4a), 132.8 (C4), 127.5 (C3), 121.7 (C8a), 101.0 (C1'), 69.7 (η^5-C_5H_5), 65.6 (C3' and C4'), 64.0 (C2' and C5'), 60.0 (CH_2OH), 45.0 (CH_2NH); HRMS exact mass calcd. for $C_{19}H_{19}N_4O_2{}^{56}Fe$ $[MH]^+$, requires m/z 391.0857, found m/z 391.0864.

*2-Ferrocenyl-4-((2-hydroxyethyl)amino)pyrido[3,4-d]pyridazin-1(2H)-one (**8b**)*

Red solid; Yield: 83.0 mg (21%); mp.: 209.2-210.3 °C (from EtOH); IR (ATR, cm^{-1}): 3311, 1633, 1608, 1569, 1528, 1461, 1410, 1394, 1346, 1321, 1300, 1241, 1219, 1175, 1146, 1117, 1107, 1081, 1065, 1046, 1025, 999, 929, 901, 847, 804, 793, 741, 693, 685, 669, 624, 563, 487, 464, 379; ^{1}H-NMR (DMSO-d_6): 9.53 (1H, br s, H5), 9.00 (1H, br s, H7), 8.13 (1H, d, *J* = 4.6 Hz, H8), 7.24 (1H, t, *J* = 5.4 Hz, NH), 5.16 (2H, s, H2' and H5'), 4.80 (1H, t, *J* = 5.4 Hz, OH), 4.20 (2H, s, H3' and H4'), 4.16 (5H, s, η^5-C_5H_5), 3.79 (2H, q, *J* = 5.4 Hz, CH_2OH), 3.51 (2H, q, *J* = 5.4 Hz, CH_2NH);^{13}C-NMR (DMSO-d_6): 155.1 (C1), 151.7 (C7), 147.1 (C5), 143.9 (C4), 134.0 (C8a), 119.5 (C8), 119.0 (C4a), 100.8 (C1'), 69.6 (η^5-C_5H_5), 65.7 (C3' and C4'), 63.7 (C2' and C5'), 59.3 (CH_2OH), 44.6 (CH_2NH); HRMS exact mass calcd.for $C_{19}H_{19}N_4O_2{}^{56}Fe$ $[MH]^+$, requires m/z 391.0857, found m/z 391.0850.

*6-Ferrocenyl-2-phenylpyridazin-3(2H)-one (**3a**)*

Orange solid, Yield: 184.6 mg (52%); mp.: 156.0-157.9 °C (from EtOH); IR (ATR, cm^{-1}): 1664, 1601, 1489, 1454, 1388, 1303, 1280, 1262, 1207, 1170, 1124, 1106, 1092, 1022, 1007, 912, 897, 839, 817, 764, 727, 689, 655, 642, 623, 610, 581, 564, 513, 491, 479, 443, 429, 391, 377; ^{1}H-NMR (DMSO-d_6): 7.81 (1H, d, *J* = 9.6 Hz, H5), 7.60 (2H, d, *J* = 7.8 Hz, H2' and H6'), 7.51 (2H, t, *J* = 7.8 Hz, H3' and H5'), 7.42 (1H, t, *J* = 7.4 Hz, H4'), 7.05 (1H, d, *J* = 9.6 Hz, H4), 4.83 (2H, t, *J* = 1.9 Hz, H2'' and H5''), 4.46 (2H, t, *J* = 1.9 Hz, H3'' and H4''), 4.18 (5H, s, η^5-C_5H_5); ^{13}C-NMR (DMSO-d_6): 159.1 (C1), 146.8 (C4), 142.1 (C1'), 132.4 (C5), 130.6 (C4), 129.0 (C3' and C5'), 128.2 (C4'), 126.0 (C1' and C6'), 80.9 (C1''), 70.7 (C3'' and C4''), 69.9 (η^5-C_5H_5), 67.2 (C2'' and C5''); HRMS exact mass calcd. for $C_{20}H_{17}N_2O^{56}Fe$ $[MH]^+$, requires m/z 357.0690, found m/z 357.0682.

*6-Ferrocenyl-2-(pyridin-2-yl)pyridazin-3(2H)-one (**3b**)*

Red solid, Yield: 228.8 mg (64%); mp.: 156.0-157.9 °C (from EtOH); IR (ATR, cm^{-1}): 1662, 1597, 1584, 1570, 1508, 1492, 1462, 1431, 1407, 1387, 1310, 1297, 1285, 1251, 1214, 1178, 1149, 1131, 1106, 1097, 1056, 1038, 1026, 1013, 999, 992, 967, 897, 856, 848, 838, 828, 819, 793, 751, 739, 650, 629, 610, 590, 582, 571, 523, 498, 484, 449, 434, 414, 389, 379; ^{1}H-NMR (DMSO-d_6): 8.62 (1H, ddd, *J* = 4.8 Hz, 1.9 Hz, 0.9 Hz, H6'), 8.02 (1H, td, *J* = 7.7 Hz, 1.9 Hz, H4') 7.86 (1H, d, *J* = 9.7 Hz, H5), 7.61 (1H, dt, *J* = 8.0 Hz, 1.0 Hz, H3'), 7.53 (1H, ddd, *J* = 4.8 Hz, 1.9 Hz, 0.9 Hz, H5'), 7.08 (1H, d, *J* = 9.7 Hz, H4), 4.79 (2H, t, *J* = 1.9 Hz, H2'' and H5''), 4.46 (2H, t, *J* = 1.9 Hz, H3'' and H4''), 4.19 (5H, s, η^5-C_5H_5); ^{13}C-NMR (DMSO-d_6): 159.2 (C1), 153.9 (C2'), 149.5 (C6'), 146.5 (C4), 139.1 (C4'), 133.2 (C5), 130.6 (C4), 124.7 (C5'), 122.3 (C3'), 80.0 (C1''), 70.7 (C3'' and C4''), 69.9 (η^5-C_5H_5), 67.3 (C2'' and C5''); HRMS exact mass calcd. for $C_{19}H_{16}N_3O^{56}Fe$ $[MH]^+$, requires m/z 358.0643, found m/z 358.0651.

*2-Phenylphthalazin-1(2H)-one (**6aa**)*

White solid, Yield: 46.2 mg (21%); mp.: 107.6-109.0 °C (from EtOH); IR (ATR, cm^{-1}): 3360, 2917, 2848, 1654, 1584, 1488, 1452, 1363, 1326, 1309, 1291, 1241, 1200, 1151, 1135, 1121, 1075, 1059, 1017, 971, 903, 871, 863, 844, 820, 793, 764, 734, 706, 686, 617, 598, 583, 537, 483, 466, 431, 421, 405, 391, 380; ^{1}H-NMR (DMSO-d_6): 8.59 (1H, s, H4), 8.33 (1H, d, *J* = 8.0 Hz, H8), 8.03 (1H, dd, *J* = 8.4 Hz, 1.4 Hz, H5, overlapped by H6), 8.00 (H6, td, *J* = 8.3 Hz, 1.2 Hz, 1H, overlapped by H5), 7.92 (1H, ddd, *J* = 7.9 Hz, 6.8 Hz, 1.8 Hz, H7), 7.62 (2H, dt, *J* = 7.4 Hz, 1.3 Hz, H2' and H6'), 7.53 (2H, tt, *J* = 8.2 Hz, 1.3 Hz, H3' and H5'), 7.43 (1H, tt, *J* = 7.4 Hz, 1.7 Hz, H4'); ^{13}C-NMR (DMSO-d_6): 158.8 (C1), 142.3 (C1'), 139.2 (C4), 134.4 (C6), 132.8 (C7), 129.7 (C4a), 129.0 (C3' and C5'), 128.2 (C8a), 128.0 (C4'), 127.5 (C5), 126.7 (C8), 126.5 (C2' and C6'); HRMS exact mass calcd for $C_{14}H_{11}N_2O$ $[MH]^+$, requires m/z 223.0871, found m/z 223.0867.

*2-(Pyridin-2-yl)phthalazin-1(2H)-one (**6ab**)*

Beige solid, Yield: 245.5 mg (37%); mp.: 120.2-123.7 °C (from EtOH); IR (ATR, cm^{-1}): 3040, 1666, 1587, 1482, 1465, 1450, 1442, 1365, 1333, 1295, 1241, 1163, 1151, 1074, 1043, 992, 915, 876, 802, 787, 761, 739, 711, 682, 623, 598, 589, 541, 486, 469, 412, 404; ^{1}H-NMR ($CDCl_3$): 8.71 (1H, br s, H6'), 8.52 (1H, d, *J* = 7.9 Hz, H8), 8.37 (1H, d, *J* = 6.7 Hz, H5), 7.90 (1H, td, *J* = 7.1 Hz, 1.6 Hz, H5', overlapped by H6), 7.88 (1H, td, *J* = 7.1 Hz, 1.1 Hz, H6, overlapped by H5'), 7.83 (1H, td, *J* = 7.4 Hz, 1.1 Hz, H7), 7.80 (1H, d, *J* = 7.1 Hz, H3', overlapped by H5), 7.78 (1H, d, *J* = 6.7 Hz, H5, overlapped by H3'), 7.37 (1H, dd, *J* = 7.4 Hz, 1.8 Hz, H4'); ^{13}C-NMR ($CDCl_3$): 159.3 (C1), 153.3 (C2'), 149.3 (C6'), 139.0 (C4), 137.8 (C5'), 133.8 (C6), 132.1 (C7), 129.6 (C4a), 128.6 (C8a), 127.3 (C8), 126.2 (C5), 123.2 (C4'), 121.3 (C3'); HRMS exact mass calcd for $C_{13}H_{10}N_3O$ $[MH]^+$, requires m/z 224.0824, found m/z 224.0818.

*4-((2-Hydroxyethyl)amino)-2-phenyl-8-((2-(piperidin-1-yl)ethyl)amino)phthalazin-1(2H)-one (**6la**)*

Bright yellow solid; Yield: 95.1 mg (23%); mp.: 77.4-80.7 °C (from EtOH); IR (ATR, cm^{-1}):3277, 2929, 2851, 1634, 1601, 1565, 1524, 1487, 1455. 1408, 1292, 1236, 1203, 1155, 1114. 1087, 1049, 962, 909, 857, 805, 755, 693, 665, 617, 518, 480, 460, 423, 410, 389, 375; ^{1}H-NMR (DMSO-d_6): 9.32 (1H, t, *J* = 5.0 Hz, 8-NH), 7.63 (2H, d, *J* = 7.9 Hz, H2' and H6', overlapped by H6), 7.62 (1H, t, *J* = 8.1 Hz, H6, overlapped by H2' and H6'), 7.46 (2H, t, *J* = 7.9 Hz, H3' and H5'), 7.32 (1H, tt, *J* = 7.4 Hz, 1.3 Hz, H4'), 7.13 (1H, d, *J* = 7.8 Hz, H5), 6.88 (1H, d, *J* = 8.4 Hz, H7), 6.48 (1H, t, *J* = 5.4 Hz, 4-NH), 4.66 (1H, t, *J* = 5.6 Hz, OH), 3.63 (2H, q, *J* = 5.5 Hz, CH_2OH), 3.33 (2H, q, *J* = 5.8 Hz, $CH_2$4-NH), 3.26 (2H, q, *J* = 5.0 Hz, $CH_2$8-NH), 2.54 (2H, t, *J* = 6.4 Hz, CH_2Pip), 2.39 (4H, br s, H2'' and H6''), 1.49 (4H, p ,*J* = 5.5 Hz, H3'' and H5''), 1.38 (2H, q ,*J* = 5.9 Hz, H4''); ^{13}C-NMR (DMSO-d_6): 159.9 (C1), 151.2 (C8), 145.6 (C4), 143.1 (C1'), 135.0 (C6), 128.7 (C3' and C5'), 126.7 (C4'), 126.6 (C4a), 126.2 (C2' and C6'), 111.8 (C7), 110.4 (C8a), 107.7 (C5), 59.7 (CH_2OH), 57.3 (CH_2Pip), 54.5 (C2'' and C6''), 44.5 ($CH_2$4-NH), 40.3 ($CH_2$8-NH, overlapped by solvent signal), 28.0 (C3'' and C5''), 24.5 (C4''); HRMS exact mass calcd for $C_{23}H_{30}N_5O_2$ $[MH]^+$, requires m/z 408.2399, found m/z 408.2391.

Cytotoxicity Assay

The adherent HepG2 human hepatoma cells[35] and HT-29 human colorectal adenocarcinoma cells[36] were cultured in RPMI-1640 medium supplemented with 10% FCS (fetal calf serum, Sigma Ltd.), 2 mM l-glutamine, and 160 μg/mL gentamicin. Cell cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

The cells were grown to confluency and were distributed into 96-well plate with initial cell number of $5.0x10^3$ per well. After 24 h incubation at 37 ^oC, the cells were treated with the compounds in 200 μL final volume containing 1.0 v/v% DMSO. The cells were incubated with the compounds at 10^{-4} -10^{2} μM concentration range for overnight. Control cells were treated with serum free medium (RPMI-1640) only or with DMSO (c=1.0 v/v %) at 37 ^oC for overnight. After incubation the cells were washed twice with serum free (RPMI-1640) medium. To determine the *in vitro* cytostatic effect, the cells were cultured for a further 72 h in 10% serum containing medium. MTT-solution (45 μL, 2 mg/mL, final concentration: 0.37 μg/mL) was added to each well. The respiratory chain[37] and other electron transport systems[38] reduce MTT and thereby form non-water-soluble violet formazane crystals within the cell.[39] The amount of these crystals can be determined spectrophotometrically and serves as an estimate for the number of mitochondria and hence the number of living cells in the well.[40] After 4 h of incubation the cells were centrifuged for 5 min (900 g) and the supernatant was removed. The obtained formazane crystals were dissolved in DMSO (100 μL) and optical density (OD) of the samples was measured at λ=540 and 620 nm, respectively, using ELISA Reader (iEMS Reader, Labsystems, Finland). OD_{620} values was substracted from OD_{540} values. The percent of cytostasis was calculated by using the following equation:

Cytostatic effect (%) = $[1 – (OD_{treated}/OD_{control})] x100$

Values $OD_{treated}$ and $OD_{control}$ correspond to the optical densities of the treated and the control cells, respectively. In each case two independent experiments were carried out with 4 parallel measurements. The 50% inhibitory concentration (IC_{50}) values were determined from the dose-response curves. The curves were defined using Microcal TM Origin1 (version 7.5) software: cytostasis was plotted as a function of concentration, fitted to a sigmoidal curve, and based on this curve, the half maximal inhibitory concentration (IC_{50}) value was determined. IC_{50} represents the concentration of a compound that is required for 50% inhibition *in vitro* and expressed in micromolar units.

Cyclic voltammetry assay

The samples were investigated by CV in a conventional three-electrode electrochemical glass cell with a Biologic SP150 potentiostat and the EC-LAB software package. The three-electrode system comprised a glassy carbon working electrode (d = 0.3 cm, geometric surface area A = 0.0707 cm^2), a Pt wire counter electrode and an Ag/AgCl reference electrode, but all potentials are given on the reversible hydrogen electrode (RHE) scale. Before each measurement, the glassy carbon working electrode was polished with 0.05 μm alumina to obtain a mirror finish and then cleaned ultrasonically and dried to remove any traces of organic impurities. Each sample was dissolved in the electrolyte solution [0.1 mm tetrabutylammonium hexafluorophosphate in acetonitrile] at a concentration of 1 mm; the solution was then bubbled with Ar for 15 min. The CV measurements were performed in the potential range -0.15–0.75 V (vs. RHE) at a scan rate of 10 mV s^{-1} at room temperature.

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