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Synthesis, Structure, and in vitro Antitumor Activity of Some Glycoside Derivatives of Ferrocenyl-Chalcones and Ferrocenyl-Pyrazolines**

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In memory of Professor László von Vargha

*Some new glycosides of 3-ferrocenyl-1-(4′-hydroxyphenyl)-prop-2-en-1-one were prepared and transformed into the corresponding pyrazoline and pyrazole derivatives. Using methyl-hydrazine, formation of regioisomers was observed. DDQ was found to be a mild and efficient reagent for the pyrazoline-pyrazole dehydroaromatization process. The structure of the new compounds was proved by IR and NMR spectroscopy. The in vitro antitumor activity of the substances was investigated against human leukemia (HL-60) cells by the MTT method. Among these new compounds some chalcone derivatives (**3a**, **3b**, **5a**, and **5b**) showed attractive in vitro antitumor effects on human leukemia cells. These molecules contained ferrocenyl moieties and a p-hydroxy-phenolic ring or a size-independent apolar substitution of that.*

Introduction

In the frame of our systematic study on ferrocene-heterocycles we describe herein the synthesis, structure, and biological activity of some new glycosides of chalcones and their pyrazoline and pyrazole derivatives bearing ferrocenyl and *p*-hydroxyphenyl substituents, respectively.

The strategy of this project is based on an old idea of László von Vargha (1903–1971), the distinguished Hungarian carbohydrate chemist, who proposed[1,2] incorporation of potential biologically active moieties into body-friendly type compounds to avoid toxic or other disadvantageous side-effects and thus, obtain molecules with better chances for pharmacological applications.

The parent chalcones (1,3-diaryl-prop-2-en-1-ones) have been thoroughly investigated because of their valuable biological properties, such as anti-inflammatory,[3] antimalarial[4,5] immunomodulatory,[6] cytotoxic,[7,8] and anticancer[9] activities. 1,3-diaryl-prop-2-en-1-ones bind MDM2 human oncoprotein and disrupt its complex with tumor suppressor protein p53, resulting in the lowering of the signal threshold of p53 induced tumor cell apoptosis.[9] Another chalcone, herbal flavonoid isoliquiritigenin (4,2′,4′-trihydroxychalcone) has been observed to affect several intracellular proteins including cyclooxygenase, p53, and p21.[10]

Incorporation of a ferrocene unit into such compounds can result in favorable changes in biological efficiency, for example, enhanced activity or decreased toxicity.[5,11,12] Previously we synthesized a number of chalcone analogous ferrocenes[13–15] and cyclic ferrocenyl-enones[16,17] and then investigated their spectroscopic and electronic characteristics. Transformation of chalcone derivatives into pyrazole-containing heterocycles can be carried out by reacting the former compounds with hydrazines.[18–19] To our knowledge, ferrocenyl-chalcones and ferrocenyl-pyrazolines containing a carbohydrate moiety have not been described, therefore their synthesis and biological study seemed to be an attractive task. Synthetic methods and biochemistry of several other sugar containing ferrocene derivatives have been reviewed recently.[20]

In the present work we studied the antitumor activity of the new glycosylated ferrocenyl-aryl-chalcones, and their pyrazoline and pyrazole derivatives, against human leukemia (HL-60) cells. To investigate the role of the ferrocenyl group and the

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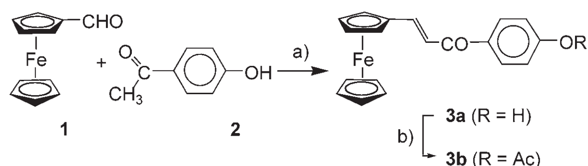
carbohydrate moiety on biological activity, the cytotoxicity of the parent 1,3-diphenyl-chalcone and some other simple analogous chalcones as model compounds was also tested.

Results and Discussion

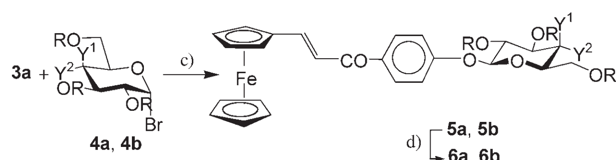
Synthesis

The key compound for our synthetic route was 3-ferrocenyl-1-(*p*-hydroxyphenyl)-chalcone **3a** (Scheme 1). Recently this compound was prepared[5] in a three-step procedure, using the tetrahydropyranyl ether of *p*-hydroxy-acetophenone (**2**). However, according to our new method, chalcone **3a** can be prepared in a single step in high yield, by base-catalyzed Claisen-Schmidt condensation of ferrocene-carboxaldehyde **1** with **2**. A larger than normal amount (~5 equiv) of potassium hydroxide was needed because of the presence of the acidic phenolic group.[18,19,21]



Scheme 1. Synthesis of compounds **3a** and **3b**. Reagents: a) KOH/H_2O in EtOH; b) Ac_2O, pyridine.

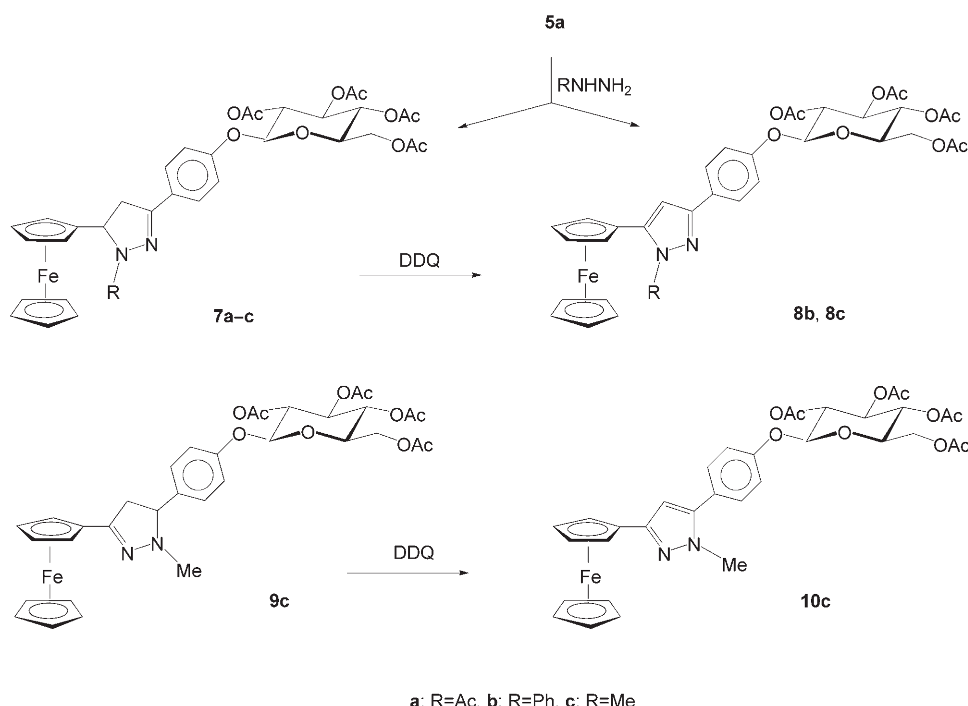
For glycosylation of **3a** we applied a classical method frequently used for the synthesis of aryl glycosides.[22] The ferrocenyl-(*p*-hydroxyphenyl)-chalcone was reacted with tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4a**), or tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4b**) in acetone solution, using aqueous potassium hydroxide as base. The acetylated glycosyloxy-phenyl-chalcones (**5a** and **5b**) were formed in high yield (~75%). Slight partial deacetylation occured during the reaction, and the yield was further elevated by reacetylation of the by-products. Deacetylation of **5a** and **5b** was performed by the Zemplén transesterification method, resulting in the corresponding chalcone glycosides (**6a** and **6b**) with free hydroxyl groups. The presence of the polar groups increased solubility in water, but within a few hours partial decomposition of **6a** and **6b** was observed in aqueous solution. (Scheme 2)



Y^1: H, Y^2: OR for **4a**, **5a** and **6a**; (**a**-type compd.)
Y^1: OR, Y^2: H for **4b**, **5b** and **6b**; (**b**-type compd.)
R = Ac for **4a**, **4b**, **5a**, and **5b**; R = H for **6a**, and **6b**

Scheme 2. Synthesis of ferrocenyl-(*p*-glycosyloxyphenyl)-chalcones **5a**, **5b**, **6a**, and **6b**. Reagents: c) KOH/H_2O in acetone; d) NaOMe in MeOH-$CHCl_3$.

To synthesize ferrocene derivatives containing both *N*-heterocycle and sugar moieties, the chalcone glycoside **5a** was reacted with hydrazine hydrate in boiling glacial acetic acid. To avoid the presence of partially *N*- or *O*-deacetylated by-products, the crude reaction mixture was treated with acetic anhydride in pyridine solution. The ring-closure can be followed by a change in color: the starting chalcone derivative is purple because of its conjugated structure, whereas the *N*-acetyl-pyrazoline derivative (**7a**) is yellow. Reaction of **5a** with phenyl-hydrazine yielded the glycosylated *N*-phenyl-pyrazoline (**7b**) as the major product, but the corresponding pyrazole derivative (**8b**), was also present in the reaction mixture. The pyrazoline/pyrazole ratio was ~3:1. In the case of aryl-pyrazolines, similar dehydroaromatization reactions are described in the literature, and the mechanism of the reaction was interpreted.[23,24] The isolated pyrazoline (**7b**) can be transformed to pyrazole derivative **8b** with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) under mild conditions and in high yield. (Scheme 3)



Scheme 3. Synthesis of ferrocenyl-(*p*-glycosyloxyphenyl) pyrazolines (**7a–c**, **9c**) and -pyrazoles (**8b**, **8c**, **10c**).

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Reaction of **5a** with methyl-hydrazine under similar conditions resulted in a more complicated mixture. In addition to the main product (**7c**) the regioisomeric 5-aryl-3-ferrocenyl-1-methyl-pyrazoline (**9c**) and the regioisomeric pair of the corresponding pyrazoles (**8c** and **10c**) were also formed. In our experiences with lengthening reaction times and methyl-hydrazine excess, the ratio of the aromatic pyrazoles increases, as previously observed.[24] The regiomeric pyrazolines (**7c** and **9c**) were transformed into the corresponding pyrazole compounds (**8c** and **10c**) with DDQ in good yield. This oxidation was also carried out spontaneously to some extent in solution.

Structure and stereochemistry

The NMR (^{1}H and ^{13}C) and IR data proving the structure of our new compounds are given in Tables 1–3. The following additional remarks are necessary:

The *E*-configuration of the olefinic group in **5a**, **5b**, **6a**, and **6b** follows from the large ^3J(H,H) coupling (15.3 Hz) expected for *trans* 1,2-disubstituted ethenes.[25a]

In accordance with the presence of two chiral centers (H-5 in the pyrazoline ring and H-1 of the glucose part) in **7a–c** and **9**, these solutions are 1:1 mixtures of two diastereoisomers

Table 1. ^{1}H NMR data[a] of compounds **5a**, **5b**, **6a**, **6b**, **7a–c**, **8b**, **8c**, **9c**, and **10c**.[b]

Compd	CH or CH$_2$[c]	H-5[d]	H-2′,5′	H-3′,4′	H-1-5	H-2,6	H-3,5	H-1	H-2	H-3	H-4	H-5	CH$_2$
	enone or pyrazole/ine		substituted *Cp* ring[e]		*Cp*[f]	4-OR-benzoyl[g]		R (glucose/galactose) group[h]					
5a	7.02	7.66	4.52	4.40	4.10	7.90	6.99	5.13	5.23	5.24	5.11	3.84	4.11, 4.22
5b	7.33	7.55	4.75	4.45	4.10	8.00	7.03	5.57	5.16	5.22	5.28	4.41	4.03
6a	7.43	7.61	4.83	4.51	4.17	8.05	7.13	5.00	3.28	3.30	3.17	3.38	3.46, 3.69
6b	7.44	7.63	4.84	4.53	4.18	8.06	7.15	4.98	3.62[i]	3.45	3.75	3.62[i]	3.52, 3.57
7a	3.54, 3.78	5.35	4.00, 4.38	4.10, 4.13	4.17	7.85	7.10	5.68	5.10	5.44	5.02	4.29	4.08, 4.22
7b	3.72, 3.77	5.05	4.11, 4.15	4.20, 4.21	4.12	7.76	7.05	5.15	5.30	5.32	5.19	3.89	~4.19
7c	3.20, 3.42	3.95	4.26	~4.2	4.17	7.62	6.99	5.12	5.28	5.31	5.18	3.88	~4.2
8b	6.75	–	4.21[i]	4.23[i]	4.11	7.85	7.06	5.15	5.31	5.34	5.20	3.91	~4.21,[i] 4.32
8c	6.52	–	4.52	4.36	4.18	7.74	7.03	5.11	5.29	5.32	5.18	3.89	4.19, 4.30
9c	2.91, 3.25	3.60	4.67	4.31, 4.33	4.16	7.41	7.01	5.10	5.28	5.31	5.18	3.88	4.19, 4.29
10c	6.29	–	4.68	4.28	4.11	7.39	7.08	5.16	5.31	5.32	5.20	3.90	4.21, 4.31

[a] In CDCl$_3$ (**5a**, **7b**, **7c**, **8b**, **8c**, **10c**) and (**5b**, **9c**)/or (CD$_3$)$_2$SO (**6a**, **6b**, **7a**) solution at 500 MHz. Chemical shifts in ppm ($\delta_{Me_4Si}=0$ ppm), coupling constants in Hz. Other signals: CH$_3$(OAc), 4×*s* (4×3H): 1.87–2.11 (**5a**, **5b**, **7a–c**, **8b**, 8c, **9c**, and **10c**), CH$_3$(NAc, **7a**), *s* (3H): 2.175, 2.178, OH (Pos. 2-4), *d* or broad (1-1H): 5.04, 5.11, 5.36 (**6a**), 4.55, ~4.9, 5.24 (**6b**), (CH$_2$)OH, *t* ($J\approx4$, 1H): 4.56 (**6a**), 4.70 (**6b**), *N*-phenyl: 7.16 *d* (2H), 7.24 *t* (2H), 6.81 *t* (1H) for **7b**, for **8b** ~7.43 *m* (5H), NCH$_3$, s (3H): 2.81 (**7c**), 4.00 (**8c**), 2.56 (**9c**), 3.84 (**10c**). [b] Assignments were supported by HMQC, HMBC (except for **5b**, **6a**, **7a**), COSY (except for **7c**, **8c**, **10c**) and for **5a**, **7c**, **9c**, **10c** by DIFFNOE measurements. [c] Enone H$_\alpha$ (**5a**, **5b**, **6a**, and **6b**), *d* (*J*: 15.3), H-4 (pyrazole in **8a**, **8b**, and **10c**), s (1H), CH$_2$ (Pos. 4, pyrazoline), two *dd*'s (2×1H), ^2J: 18.0 (**7a**), 16.6 (**7b**), 15.9 (**7c**), ^3J (upfield *dd*): 3.5 (**7a**), 5.8 (**7b**), 2.5 (**7c**), ^3J (downfield *dd*): 11.3 (**7a**, **7b**), 9.8 (**7c**), two *t*'s (2×1H) with coalesced lines for **9c**. [d] Enone H$_\beta$, (**5a**, **5b**, **6a**, and **6b**), *d*, H-5 (pyrazoline), *dd* (2×1H, **7a-c**), *m* (1H, **9c**). [e] ~*s* (2H, *t* with coalesced lines). Due to molecular asymmetry separated signals (2×1H) for **7a**, **7b**, and **9c** (H-3′,4′), in overlap for **7c** and **9c** (H-2′,5′). [f] Unsubstituted ring. [g] *AA′BB′*-type multiplet: 2×~*d* (2H), *J*: 8.7±0.1. [h] H-1, *d* (1H), *J*: 7.6 (**5b**, **6b** and **9c**), 7.3 (**6a**, **8b**, **8c**, and **10c**), 5.8 (**7b**), 6.7 (**7c**), in overlap with H-4 (**5a**), doubled (**7a**), H-2,3,4: 3×*t* (3×1H), *J*: 9.4±0.1 (**7c**, **8b**, **8c**, **9c**, and **10c**), *J*: 8.5 (H-4, **6a**), *t*-like signal with coalesced lines (**5a** and **7b**), unresolved (**6b**), hidden by the H$_2$O signal of the solvent: H-2 (**6a**), H-3 (**6a**, **6b**), doubled signals in overlap (**7a**), *dd*, *J*: 10.3, 7.7 (H-2, **5b**), 10.3, 3.3 (H-3, **5b**), *d*, *J*: 3.1 (H-4, **5b**), H-5: *m* (1H), CH$_2$: 2×*dd* (2×1H), *J*: 12.3, 2.2 and 12.3, 5.4 (**5a**, **8c**, **9c** and **10c**), *dd* (downfield signal, 1H), *J*: 12.3, 5.4 (**7c** and **8b**), *d* (2H), *J*: 6.3 (**5b**), 2×*m* (2×1H) for **6a**, **6b**, and **7a**,**b**, upfield signal (1H) for **7c** and **8b**. One of the *m*'s in overlap with the signals of the substituted Cp ring (**7b**, **7c**, and **8b**). [i] Overlapping signals.

Table 2. ^{13}C NMR chemical shifts (in ppm, $\delta_{Me_4Si}=0$ ppm)[a] of compounds **5a**,**b**, **6a**,**b**, **7a–c**, **8b**,**c**, **9c**, and **10c**.[b]

Compound	C-3	C-4	C-5	C-1′	C-2′,5′	C-3′,4′	C-1-5	C-1	C-2,6	C-3,5	C-4	C-1	C-2	C-3	C-4	C-5	CH$_2$
	enone or pyrazole/ine[c]			substituted *Cp* ring			*Cp*[d]	4-OR-benzoyl				R (glucose/galactose) group					
5a	188.5	119.1	147.0	79.6	69.4, 69.5	71.8	70.2	134.2	130.8	116.8	160.2	98.7	71.5	73.0	68.6	72.7	62.3
5b	187.6	119.8	146.7	80.0	70.1, 70.2	72.1	70.4	133.6	131.4	116.9	160.5	97.9	69.1	71.0	68.1	71.4	62.2
6a	187.5	119.8	146.3	80.1	70.0, 70.1	72.0	70.4	132.6	131.2	116.8	161.8	100.8	74.1	77.5	70.5	78.1	61.5
6b	187.6	119.7	146.4	80.1	70.0, 70.1	72.0	70.4	132.5	131.3	116.8	161.8	101.3	71.1	74.1	69.0	76.5	61.2
7a	155.0	40.0	55.8	88.3	66.6, 68.3	68.7, 70.7	69.4	126.9	129.2	117.4	158.6	97.6	71.6	72.8	68.9	71.8	62.5
7b	147.2	42.6	59.8	91.0	67.4, 68.2	68.65, 68.68	69.1	128.7	127.5	117.5	157.5	99.4	71.6	73.1	68.5	72.6	62.4
7c	149.4	41.2	68.7	86.0	66.3, 68.3	69.1, 69.9	69.0	128.9	127.6	117.3	157.5	99.4	71.6	73.1	68.7	72.6	62.4
8b	151.4	103.9	143.6	75.3	69.2[e]	69.2[e]	70.3	129.1	127.5	117.5	157.1	99.6	71.7	73.2	68.8	72.5	62.4
8c	149.9	102.9	142.9	75.4	68.7	69.4	70.0	129.5	127.1	117.6	156.8	99.7	71.7	73.2	68.8	72.5	62.4
9c	151.0[f]	44.9[f]	72.8[f,g]	78.3[f]	66.9, 67.5	69.9, 70.2	69.7	135.9[f]	129.0	117.5	156.8	99.5	71.6	73.2	68.8	72.5	62.4
10c	144.1	104.0	150.2	79.2	67.0	68.9	69.9	126.4	130.5	117.5	157.2	99.3	71.6	73.1	68.7	72.6	62.4

[a] In CDCl$_3$ (**5a**, **7b**, **7c**, **8b**, **8c**, **10c**) and (**5b**, **9c**)/or (CD$_3$)$_2$SO (**6a**, **6b**, **7a**) solution at 125 MHz. Chemical shifts in ppm ($\delta_{Me_4Si}=0$ ppm), coupling constants in Hz. Other signals: CH$_3$(OAc), 4 lines: 20.99–21.40 (**5a**, **5b**, **7a–c**, **8b**, **8c**, **9c**, and **10c**), CH$_3$(NAc, **7a**): 22.6, NCH$_3$: 41.5 (**7c**), 38.3 (**8c**), 42.3 (**9c**), 37.7 (**10c**), *N*-phenyl C-1, C-2,6, C-3,5, C-4: 145.9, 114.5, 129.2, 119.6 (**7b**), 140.8, 126.7, 129.3, 128.6 (**8b**), C=O(OAc), 4 lines: 169.6–171.0 (**5a**, **5b**, **7a–c**, **8b**, **8c**, **9c**, and **10c**), C=O(OAc): 168.1 (**7a**). [b] Assignments were supported by DEPT, HMQC and HMBC (except for **5b**, **6a**, **7a**) measurements. [c] For enones: C-3→C=O, C-4→C$_\alpha$, C-5→C$_\beta$. [d] Unsubstituted ring. [e] Two overlapping lines. [f] In (CD$_3$)$_2$SO measured data. [g] In overlap with glucose C-3 line (at 72.8 ppm in (CD$_3$)$_2$SO).

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Table 3. Characteristic IR frequencies [cm^{-1}] of compounds **5a**, **5b**, **6a**, **6b**, **7a–c**, **8b**, **8c**, **9c**, and **10c** (in KBr discs).

Compound	νOH band[a]	νC=O band[b]	$\gamma C_{Ar}H$ band[c]	νC=O band[d]	ν_{as}C-O band[d]	ν_sC-O band[d]	ν_{as}Cp-Fe-Cp and tilt of Cp
5a	–	1657	810	1746	1222	~1050	~490
5b	–	1656	837	1749	1226	1081, 1055	494
6a	~3400	1650	832	–	–	1075	481
6b	~3380	1652	833	–	–		480
7a	–	1655[e]	836	1754	1232	1046	442, 467, 484
7b[f]	–	–	832	1750	1228	1060	491
7c	–	–	840	1754	1231	1042	480
8b	–	–	838	1750	1226	1043	501
8c	–	–	839	1755	1230	1045	499
9c	–	–	820	1755	1229	1068	512, 485
10c	–	–	840	1754	1232	1046	506, 489

[a] Broad. [b] Chalcone (enone) group. [c] *p*-Disubstituted benzene ring. [d] Ester group. [e] Amide-I band. [f] $\gamma C_{Ar}H$ and $\gamma C_{Ar}C_{Ar}$ bands (monosubstituted benzene ring): 748 and 694.

(the other chiral atoms in the carbohydrate part should not be considered in this respect because their configuration is fixed in this moiety). This is confirmed by the *ABX*-type multiplets of the CH–CH_2 part of the five-membered hetero ring which are doubled in the ^{1}H NMR spectra of the compounds in question. The separations of the signals of the diastereomers are very small (<4 Hz) because of the distant chiral centers (isolated by the phenyl ring).

Of course, the C/H-2′,5′ and C/H-3′,4′ pairs of the substituted Cp ring are chemically unequal and give separated signals. However, this kind of signal separation was also observed for C-2′,5′ lines of **5a**, **5b**, **6a**, and **6b** chalcones, where there is no possibility of formation of diastereomers. Here, the molecular asymmetry leads to chemical nonequivalence. The C/H-3′,4′ pairs of chalcones and compounds **8b**, **8c**, and **10c**, and similarly, the H-2′,5′ pairs of chalcones and C-2′,5′ pairs of **8b**, **8c**, and **10c** are chemically equivalent. This means that the free and fast rotations of the ferrocenyl group, enone or five-membered hetero ring, the *para*-disubstituted benzene ring, and the carbohydrate moiety around single bonds equalize the difference in chemical environment sufficiently for accidental equivalence.[25b]

It should be noted that the more crowded structure of galactose derivatives (**5b**, **6b**) as compared to their glucose analogues (**5a**, **6a**) reveals itself in upfield shifts of the carbon lines of this moiety (field effect[25c,26]). Accordingly, the sum of ^{13}C NMR shifts[25d] for **5a** and **6a** is larger by 7.1 and 9.3 ppm than for **5b** and **6b**.

The unsubstituted Cp ring in ferrocene, isolated by the Fe-atom from the rest of the molecule, is not sensitive to structural changes. Nevertheless, a well identifiable upfield shift of the ^{13}C NMR line of this ring can be observed for **7a–c**, where the ferrocenyl group is attached to an *sp*^{3} carbon. This means that double bonds can transfer electron flow between the two parts of the molecules being investigated.

The position of the *N*-methyl group in **c**-type derivatives was proved by DIFFNOE measurements[25e,27] for **7c**, **9c**, and **10c**. Irradiating the NCH_3 signal intensity enhancements of the H's in Cp ring (at 4.17 and ~4.2 ppm) were observed for **7c**, whereas the *ortho*-H's of the benzene ring gave no response. For **9c** and **10c** we observed the reverse results: the signals of the *ortho*-H's of the benzene ring did not respond, but the H's in the Cp ring gave stronger signals, demonstrating the steric close arrangement of the *N*-methyl group and the benzene ring in **9c** and **10c**. The conjugation of the C=N double bond with the benzene ring in **7c** and with the ferrocene moiety in **9c** (and **10c**), respectively, resulted in a downfield shift of the H-2,6 (*ortho*-H's) signal (**7c**) or the H-2′,5′ (Cp ring) (**9c**) by 0.21 (0.23) and 0.41 (0.42) ppm, respectively, supporting the NOE results.

For the pair **8c** and **10c**, the anisotropic neighboring effect of the *sp*^{2} nitrogen[25f] can be utilized to prove the regioisomeric structures. The downfield shifted position (by 0.35 ppm) of the ^{1}H NMR signal of the *ortho*-H's in **8c** (relative to **10c**) and of the H-2′,5′ signal of **10c** (by 0.36 ppm) demonstrated the correctness of the supposed structures, position 5 and position 3 of the ferrocenyl substituent on the pyrazole ring in **8c** and **10c**, respectively.

The H-2′,5′ Cp-signal is upfield shifted as compared to the other compounds (by approximately 0.5 ppm) for **7a–c** and **8b**, because of the saturated carbon substituent (instead of *sp*^{2}-type C) on C-1′ in **7a–c** and as a consequence of anisotropic shielding[25g] of the *N*-phenyl ring in **8b**.

In vitro antitumor activity

The antitumor effect of ferrocene derivatives **3–12** was tested and compared with those of 1,3-diphenyl-chalcone (**13**) and its *p*-methoxy derivative (**14**) at a concentration range of 10^{-9}–10^{-3} M, on HL-60 human leukemia cells. The percentage cytotoxicity level caused by treatment with the compounds was studied as a function of concentration. Based on these curves, the IC_{50} values were determined. Results summarizing the inhibitory effect of ferrocene derivatives and the aryl analogue control substances are presented in Table 4 and in Figure 1 and 2 (for structures of compounds **11–14** see the footnote of Table 4).

Low IC_{50} values were obtained for chalcones **3a** and **11** indicating that these compounds had pronounced antiproliferative effects on HL-60 cells (IC_{50} = 1.75, and 3.00 μM, respectively).

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Table 4. Antiproliferative effect of ferrocene derivatives on HL-60 cells[a]

Compound	IC_{50} [μM]	Compound	IC_{50} [μM]
3a	1.75	**8b**	>200
3b	2.26	**8c**	>200
5a	2.97	**9c**	>200
5b	3.87	**10c**	>200
6a	>200	**11**[b]	3.00
6b	>200	**12**[c]	2.89
7a	21.04	**13**[d]	10.19
7b	>200	**14**[e]	12.43
7c	>200		

[a] IC_{50} was determined by MTT assay. [b] Fc-CH=CH-CO-Ph (**11**, 3-ferrocenyl-1-phenyl-chalcone).[18] [c] Fc-CH=CH-CO-C_6H_4(*p*-OCH_3) (**12**, 3-ferrocenyl-1-(*p*-methoxyphenyl)-chalcone).[18] [d] Ph-CH=CH-CO-Ph (**13**, 1,3-diphenyl-chalcone).[f] [e] Ph-CH=CH-CO-C_6H_4(*p*-OCH_3) (**14**, 1-(*p*-methoxyphenyl)-3-phenyl-chalcone).[f] [f] **13** and **14** used as control substances.

The acetyl (**3b**), or methyl (**12**) substitution of the phenolic hydroxyl group of **3a** did not alter the antitumor effect significantly (IC_{50} = 2.26, and 2.89 μM, respectively). Also, the chalcones without the ferrocenyl moiety (**13** and **14**) had somewhat higher IC_{50} values (IC_{50} = 10.19, and 12.43 μM, respectively). These data indicate that the replacement of the phenyl group by ferrocenyl substituent resulted in more efficient compounds against HL-60 cells. (Figure 1)

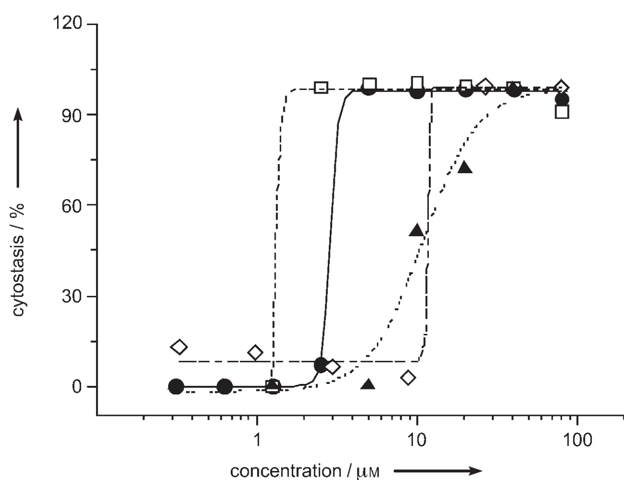

Figure 1. Cytostatic effect of compounds **3a**, **11**, **13**, and **14** on HL-60 cells. **3a**: □; **11**: ● **13**: ▲; **14**: ◇

It is interesting to note that incorporation of the bulky acetylated glucose (**5a**) or galactose (**5b**) moiety, instead of a CH_3 group, on the phenolic OH caused similarly high in vitro antitumor effects (IC_{50} = 2.97 μM for **5a**, and 3.87 μM for **5b**, respectively). Also, the presence of free alcoholic OH groups on the sugar part is not favorable in the context of the antiproliferative properties of these compounds (IC_{50} > 200 μM for **6a** and **6b**, respectively). (Figure 2).

The new glycosylated ferrocenyl-heterocycles **7**–**10** failed to be active. Neither the pyrazoline derivatives (**7a**–**c**, **9c**) nor the pyrazole derivatives (**8b**, **8c**, **10c**), possessing different substituents on the N-1 atom of the heterocyclic ring, elicited significant cytotoxic effects with the HL-60 cells, almost all IC_{50} values were higher than c = 200 μM. We observed a slight inhibitory effect only in the case of compound **7a** (IC_{50} = 21.04 μM).

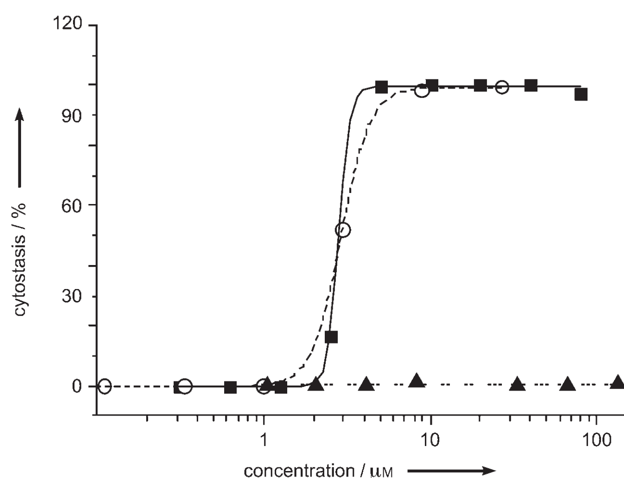

Figure 2. Cytostatic effect of compounds **5a**, **6a**, and **12** on HL-60 cells. **12**: ■; **5a**: ○ **6a**: ▲

The parent compound, 1,3-diphenyl chalcone (**13**) exhibited toxic effects on HL-60 cells (LD_{50} = 50 μM). One ferrocenyl derivative, 3-ferrocenyl-1-phenyl-chalcone (**11**) also possessed some toxicity (LD_{50} = 20 μM), but no other compounds showed a cytotoxic effect on the HL-60 cell line.

Conclusions

New glycosides (**5**, **6**) of 3-ferrocenyl-1-(*p*-hydroxyphenyl)-prop-2-en-1-one (**3a**) were synthetized and transformed with hydrazines into *p*-(glycosyloxy-phenyl)-ferrocenyl-*N*-heterocycles (**7**–**10**). Using methyl-hydrazine, regioisomeric pairs of the corresponding *N*-methyl-pyrazolines (**7c**, **9c**) and pyrazoles (**8c**, **10c**) were prepared. The new pyrazolines were converted to the corresponding pyrazole derivatives by DDQ under mild conditions with high yield. Structures were elucidated by complex IR and NMR studies including 2D techniques and DIFFNOE measurements.

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Among these new compounds some chalcone derivatives (**3a**, **3b**, **5a**, and **5b**) showed attractive in vitro antitumor effects on human leukemia cells. These molecules contained ferrocenyl moieties and a *p*-hydroxy-phenolic ring or a size-independent apolar substitution of that. Based on data presented herein, these ferrocenyl chalcones could be classified as a new group of compounds with promising antitumor properties.

Experimental Section

General methods:
TLC (thin layer chromatography) was performed on aluminum plates precoated with Silica Gel 60 F_{254} developed with solvent mixtures A) 9:1 $CHCl_3$/acetone; B) 9:1 EtOAc/MeOH; C) 92:8 CH_2Cl_2/EtOAc; and D) 3:2 EtOAc/*n*-hexane. The spots were detected visually and the plates were also checked by exposure to UV light, R_f data were determined on 10 cm long plates. Column chromatography was performed on silica gel (0.020–0.043 mesh).
IR spectra were recorded in KBr pellets with a Bruker IFS-55 FT-spectrometer. The ^{1}H- and ^{13}C NMR spectra were recorded in $CDCl_3$ solution in 5 mm tubes at room temperature, on a Bruker DRX 500 spectrometer at 500.13 (^{1}H) and 125.76 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and Me_4Si as internal standard. The standard Bruker microprogram NOEMULT.AU to generate NOE was used. DEPT spectra were run in a standard manner, using only the $\Theta = 135°$ pulse to separate CH/CH_3 and CH_2 lines phased "up" and "down", respectively. The 2D-HSC spectra were obtained by using the standard Bruker pulse program HXCO.AU.
Formylferrocene (**1**), *p*-hydroxyacetophenone (**2**), phenyl-hydrazine, methyl-hydrazine and DDQ were purchased from Sigma–Aldrich. Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4a**) and tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4b**) were prepared by known procedures.[28]

Syntheses:
Details for the preparation of compounds **3a**, acetyl- (**3b**) and glycosyloxy derivatives **5a**, **5b**, **6a**, and **6b** are available in the Supporting Information, together with the synthesis of the new ferrocenyl-pyrazoline (**7a–c, 9c**) and pyrazole (**8b–c, 10c**) arylglycosides.

Cell culture:
HL-60 human leukemia cell line was cultured at 37 °C and 5% CO_2 in RPMI-1640 medium containing 10% FBS, 2 mmol mL^{-1} L-glutamine and 0.16 mg mL^{-1} gentamycin.[29] Cells were placed into 96 well tissue-culture plate in 100 μL culture medium (RPMI 1640, 10% fetal bovine serum (FBS), 2 mmol mL^{-1} L-glutamine, and 0.16 mg mL^{-1} gentamycin) with initial cell number of 5×10^3 cells/well 24 h prior to the experiment. Before treating the cells, culture medium was replaced with serum-free RPMI.

In vitro cytostatic effect of ferrocene derivatives:
The ferrocene derivatives were dissolved in $(CH_3)_2SO$ and 5 μL of the samples were added to each well containing 195 μL serum-free medium. Cells were incubated with each compound at 37 °C in 5% CO_2 atmosphere for 3 h. In control experiments, cells were treated with 5 μL $(CH_3)_2SO$ only at 37 °C. Plates were washed twice with serum-free RPMI. Between the washing steps, cells were centrifuged at 1000 rpm for 5 min. Following the last washing step, serum-free medium was replaced with culture medium containing 10% FBS and cells were incubated at 37 °C in 5% CO_2 atmosphere for further 72 h. The number of the living cells was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)-assay.[30] The yellow solution of MTT at concentration of 0.45 mg mL^{-1} was added to each well. After 3.5 h the purple crystal obtained was dissolved in 100 μL $(CH_3)_2SO$ and the optical density (OD) of the samples was measured at $\lambda = 540$ nm and 620 nm, as reference wavelength using ELISA Reader. We have calculated the % of cytotoxicity using the following equation: Cytotoxicity % = $(1 - OD_{treated}/OD_{control})\,100$, where $OD_{treated}$ and $OD_{control}$ correspond to the optical density of the samples from treated cells and the control cells at $\lambda = 540$ nm, respectively. The IC_{50} value was determined by fitting a sigmoid curve on the data points and the calculating X values at Y = 50.

In vitro cytotoxicity of ferrocene derivatives:
Cells were treated with ferrocene derivatives dissolved in $(CH_3)_2SO$ 5 μL of the samples were added to each well containing 195 μL serum-free medium. Cells were incubated with the compounds at 37 °C in 5% CO_2 atmosphere for 3 h. In control experiments cells were treated only with 5 μL $(CH_3)_2SO$ at 37 °C. Plates were washed once with serum-free RPMI. The number of living cells was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)-assay as described above. A LD_{50} value for each compound was determined by fitting a sigmoid curve to the data points and calculating X values at $Y = 50$.

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