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Synthesis, characterization, electrochemical studies and antitumor activity of some new chalcone analogues containing ferrocenyl pyrazole moiety

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ABSTRACT

A series of new α,β-unsaturated conjugated ketones containing ferrocenyl pyrazole unit were synthesized and fully characterized by IR and NMR spectroscopy. Electrochemical characterization of subject compounds was performed by means of cyclic voltametry. The *in vitro* cytotoxic activity of all the synthesized compounds was studied against cervix adenocarcinoma HeLa, melanoma Fem-x and myelogenous leukemia K562 cell lines by the MTT method. Derivative **1l** containing 3-pyridyl moiety exhibited a better cytotoxic activity in the cell growth inhibition of K562 cell lines in comparison with cisplatin as a reference compound.

1. Introduction

Research into the antitumor properties of ferrocene compounds has received significant attention over the last few years, particularly with discovery of ferrocenyl derivatives of hydroxytamoxyfen, an active tamoxifen metabolite in inhibition of cancer cell proliferation by competitive binding to the estrogen receptors in endocrine therapy [1]. Ferrocifen and its analogue which bears an additional hydroxyl group are examples of the most important application of ferrocene in bio-organometallic chemistry presenting a new and promising class of hormone-dependent breast cancer [2,3] and melanoma [4] drug candidates.

Some derivatives of the chalcone class of compounds have been described in literature as inhibitors of ovarian cancer cell proliferation [5] and pulmonary carcinogenesis [6]. The cytotoxic activity against B16 murine melanoma, HCT 116 human colon cancer and A31 human epidermoid carcinoma was reported for a series dihydroxychalcones [7]. Chalcones with carboxylic acid substituents were shown to inhibit the oncoprotein MDM 2 binding to a tumor suppressor protein p53, inactivated in many human tumors either by mutations or by binding to oncogenic proteins [8]. A large number of methoxylated chalcones was found to show an antimitotic activity against HeLa cell lines [9]. Investigations on α-substituted chalcones identified some of them as the very potent compounds against K562 human leukemia cell lines [10]. Structural properties of chalcone derivatives influenced on mechanism of cytotoxicity against tumor cell lines by disruption of the cell cycle, inhibition of angiogenesis, interaction with p53 gene, mitochondrial uncoupling or induction of apoptosis [11].

Introduction of ferrocene scaffold into chalcone compounds can bring about significant changes in biological effects. For example, modification of structural profile of ferrocenyl chalcones caused by nature of substituents resulted in enhanced antimalarial activity [12]. Moreover, some ferrocenyl chalcones containing glycoside units have been observed to show *in vitro* antitumor activity on HL-60 human leukemia cells [13]. Linking of ferrocene and pyrazole with a structurally diverse side chain is an effective way to obtain new organometallic heterocyclic derivatives with high biological potential. We assumed that incorporation of pyrazole pharmacophore into the ferrocene scaffold should have an attracting structural result for development of novel antitumor agents, expecting the interesting features due to the coexistence of two kinds of promising pharmacophores with different action mechanism. In continuation of our research program of the preparation of novel ferrocene compounds [14,15], we describe here the synthesis, electrochemical properties and antitumor activity of a series of new chalcone analogues.

2. Materials and methods

2.1. Physical measurement

Melting points were determined on a Mel-Temp capillary melting points apparatus, model 1001 and are uncorrected. FT-IR mea-

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surements: IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR Spectrometer. The ^{1}H and ^{13}C NMR spectra were collected on a Varian Geminy 200 instrument operating at 200 MHz, in $CDCl_3$ solution, with the TMS as internal standard. Assignment of all the reported ^{1}H and ^{13}C NMR signals were deduced on the bases two-dimensional NMR experiments. Electrochemical measurements were performed by using a CH Instruments (Austin, TX) potentiostat CHI760b Electrochemical Workstation. A standard three-electrode cell (5 mL) equipped with a platinum wire and a silver wire immersed in 0.1 M $LiClO_4$ solution in CH_3CN as the counter and reference electrode, respectively. A gold disk (d = 2 mm) was used as the working electrode.

2.2. General procedure for the preparation of chalcone analogues **1a–l**

Chalcone analogues were prepared by condensation of 1H-3-ferrocenyl-1-phenylpyrazole-4-carboxaldehyde and corresponding methyl-ketones in ethanol solution, applying 30% aqueous NaOH (0.8 cm^3) as a catalyst.

To a stirred solution of 0.356 g 1H-3-ferrocenyl-1-phenylpyrazole-4-carboxaldehyde (1 mmol) and corresponding methyl-ketone (1.05 mmol) in ethanol (5 ml), an aqueous solution of 30% NaOH (0.8 cm^3) was added. The resulting solution was heated to 60 °C for 4 h and then allowed to stand overnight at room temperature with continuous stirring. The reaction mixture was poured into water and precipitate was collected by filtration, washed with 50% ethanol, dried, and recrystallized from ethanol to give **1a–l** in 55–94% yields.

Compound **1i** was obtained by pouring of the reaction mixture into water previously acidified with diluted HCl, extracting with methylene chloride, drying and evaporating solvent to give pure product.

2.2.1. (E)-3-(3-ferrocenyl-1-phenylpyrazol-4-yl)-1-ferrocenyl-2-propen-1-one (**1a**)

Yield: 0.43 g (75%); mp: 86–87 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 4.23 (s, 5H, Fc); 4.25 (s, 5H, Fc); 4.39 (t, 2H, J = 1.84 Hz, Fc); 4.58 (t, 2H, J = 1.88 Hz, Fc); 4.77 (t, 2H, J = 1.84 Hz, Fc); 4.92 (t, 2H, J = 1.88 Hz, Fc); 6.95 (d, 1H, J = 15.58 Hz, H_α); 7.28–7.36 (m, 1H, p-phenyl); 7.45–7.53 (m, 2H, m-phenyl); 7.78 (dd, 2H, J = 7.62 and 1.30 Hz, o-phenyl); 8.29 (s, 1H, Pz); 8.35 (d, 1H, J = 15.58 Hz, H_β); ^{13}C NMR (50 MHz, $CDCl_3$): 68.7 (Fc), 69.1 (Fc), 69.6 (Fc), 69.7 (Fc), 70.0 (Fc), 72.6 (Fc), 76.9 (Fc), 80.7 (Fc), 118.5 (C-4, Pz), 119.1 (o-phenyl), 121.4 (C_α), 125.6 (C-5, Pz), 126.8 (p-phenyl), 129.5 (m-phenyl), 131.9 (C_β), 139.5 (C-3, Pz), 152.7 (N-substituted phenyl), 192.8 (CO). IR (KBr, cm^{-1}): 499, 754, 1237, 1290, 1452, 1504, 1543, 1586, 1644, 3092. Anal. Calcd for $C_{32}H_{26}N_2OFe_2$ (566.25 g/mol): C, 67.87; H, 4.63; N, 4.95. Found: C, 67.60; H, 4.63; N, 4.93.

2.2.2. (E)-3-(3-ferrocenyl-1-phenylpyrazol-4-yl)-1-phenyl-2-propen-1-one (**1b**)

Yield: 0.43 g (93%); mp: 162–163 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 4.18 (s, 5H, Fc); 4.38 (t, 2H, J = 1.80 Hz, Fc); 4.74 (t, 2H, J = 1.80 Hz, Fc); 7.26–7.35 (m, 1H, p-phenyl); 7.39 (d, 1H, J = 15.72 Hz, H_α); 7.45–7.61 (m, 5H, 2H at m-phenyl, 2H at 3a and 1H at 4a); 7.70 (dd, 2H, J = 7.96 and 1.74 Hz, o-phenyl); 8.05 (dd, 2H, J = 7.88 and 1.84 Hz, 2H at 2a); 8.32 (s, 1H, Pz); 8.40 (d, 1H, J = 15.72 Hz, H_β); ^{13}C NMR (50 MHz, $CDCl_3$): 68.6 (Fc), 69.2 (Fc), 69.7 (Fc), 76.7 (Fc), 118.3 (C-4, Pz), 119.1 (o-phenyl), 120.4 (C_α), 125.7 (C-5, Pz), 126.9 (p-phenyl), 128.4 (2a), 128.6 (3a), 129.5 (m-phenyl), 132.6 (4a), 136.0 (C_β), 138.4 (1a), 139.4 (C-3, Pz), 152.9 (N-substituted phenyl), 190.3 (CO). IR (KBr, cm^{-1}): 506, 685, 751, 1014, 1215, 1292, 1502, 1553, 1573, 1591, 1662, 3098. Anal. Calcd for $C_{28}H_{22}N_2OFe$ (458.33 g/mol): C, 73.37; H, 4.84; N, 6.11. Found: C, 73.09; H, 4.84; N, 6.08.

2.2.3. (E)-3-(3-ferrocenyl-1-phenylpyrazol-4-yl)-1-(4-methylphenyl)-2-propen-1-one (**1c**)

Yield: 0.44 g (92%); mp: 158 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 2.45 (s, 3H, CH_3); 4.20 (s, 5H, Fc); 4.38 (t, 2H, J = 1.86 Hz, Fc); 4.75 (t, 2H, J = 1.86 Hz, Fc); 7.29–7.37 (m, 3H, 2H at 3a and 1H at p-phenyl); 7.40 (d, 1H, J = 15.64 Hz, H_α); 7.44–7.53 (m, 2H, m-phenyl); 7.77 (dd, 2H, J = 8.32 and 1.54 Hz, o-phenyl); 7.97 (d, 2H, J = 8.24 Hz, 2H at 2a); 8.31 (d, 1H, J = 0.64 Hz, Pz); 8.39 (dd, 1H, J = 15.64 and 0.64 Hz, H_β); ^{13}C NMR (50 MHz, $CDCl_3$): 21.7 (CH_3), 68.6 (Fc), 69.2 (Fc), 69.7 (Fc), 77.2 (Fc), 118.4 (C-4, Pz), 119.1 (o-phenyl), 120.4 (C_α), 125.7 (p-phenyl), 126.9 (C-5, Pz), 128.5 (2a), 129.3 (3a), 129.5 (m-phenyl), 135.5 (C_β), 135.8 (1a), 139.4 (C-3, Pz), 143.4 (4a), 152.9 (N-substituted phenyl), 189.7 (CO). IR (KBr, cm^{-1}): 508, 759, 822, 1221, 1289, 1409, 1502, 1552, 1590, 1607, 1658, 3081. Anal. Calcd for $C_{29}H_{24}N_2OFe$ (472.36 g/mol): C, 73.74; H, 5.12; N, 5.93. Found: C, 73.61; H, 5.13; N, 5.90.

2.2.4. (E)-3-(3-ferrocenyl-1-phenylpyrazol-4-yl)-1-(2-thiophenyl)-2-propen-1-one (**1d**)

Yield: 0.44 g (94%); mp: 168 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 4.23 (s, 5H, Fc); 4.39 (t, 2H, J = 1.84 Hz, Fc); 4.75 (t, 2H, J = 1.84 Hz, Fc); 7.19 and 7.21 (two doublets, 1H, J = 3.78 Hz, 4a); 7.27 (d, 1H, J = 15.54 Hz, H_α); 7.29–7.37 (m, 1H, p-phenyl); 7.45–7.53 (m, 2H, m-phenyl); 7.69 (dd, 1H, J = 4.96 and 1.14 Hz, 1H at 3a), 7.77 (dd, 2H, J = 8.40 and 1.44 Hz, o-phenyl); 7.86 (dd, 1H, J = 3.86 and 1.14 Hz, 1H at 5a); 8.31 (d, 1H, J = 0.68 Hz, Pz); 8.44 (dd, 1H, J = 15.54 and 0.68 Hz, H_β); ^{13}C NMR (50 MHz, $CDCl_3$): 68.7 (Fc), 69.2 (Fc), 69.7 (Fc), 76.7 (Fc), 118.2 (C-4, Pz), 119.1 (o-phenyl), 119.9 (C_α), 125.8 (C-5, Pz), 126.9 (p-phenyl), 128.2 (4a), 129.5 (m-phenyl), 131.3 (5a), 133.5 (3a), 135.2 (C_β), 139.4 (C-3, Pz), 145.7 (2a), 153.1 (N-substituted phenyl), 181.8 (CO). IR (KBr, cm^{-1}): 506, 723, 1217, 1291, 1415, 1503, 1552, 1589, 1650, 3097. Anal. Calcd for $C_{26}H_{20}N_2OSFe$ (464.36 g/mol): C, 67.25; H, 4.34; N, 6.03; S, 6.91. Found: C, 67.18; H, 4.33; N, 6.00; S, 6.90.

2.2.5. (E)-3-(3-ferrocenyl-1-phenylpyrazol-4-yl)-1-(2-pyridyl)-2-propen-1-one (**1e**)

Yield: 0.34 g (75%); mp: 100–101 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 4.22 (s, 5H, Fc); 4.39 (t, 2H, J = 1.82 Hz, Fc); 4.79 (t, 2H, J = 1.82 Hz, Fc); 7.27–7.35 (m, 1H, p-phenyl); 7.43–7.52 (m, 3H, 2H at m-phenyl and 1H at 5a); 7.76 (dd, 2H, J = 8.54 and 1.30 Hz, o-phenyl); 7.89 (td, 1H, J = 7.62 and 1.68 Hz, 4a); 8.11 (d, 1H, J = 16.02 Hz, H_α); 8.24 (dt, 1H, J = 7.86 and 1.06 Hz, 3a); 8.43 (d, 1H, J = 0.66 Hz, Pz); 8.57 (dd, 1H, J = 16.02 and 0.66 Hz, H_β); 8.75 (dt, 1H, J = 4.70 and 0.92 Hz, 6a). ^{13}C NMR (50 MHz, $CDCl_3$): 68.6 (Fc), 69.2 (Fc), 69.7 (Fc), 76.6 (Fc), 118.6 (C-4, Pz), 118.9 (C_α), 119.1 (o-phenyl), 122.9 (3a, Py), 126.2 (C-5, Pz), 126.8 (5a, Py), 126.9 (p-phenyl), 129.5 (m-phenyl), 136.1 (C_β), 137.1 (4a, Py), 139.3 (C-3, Pz), 148.7 (6a, Py), 153.2 (N-substituted phenyl), 154.4 (2a,Py), 189.1 (CO). IR (KBr, cm^{-1}): 507, 683, 756, 1024, 1218, 1503, 1549, 1577, 1594, 1663, 3092. Anal. Calcd for $C_{27}H_{21}N_3OFe$ (459.32 g/mol): C, 70.60; H, 4.61; N, 9.15. Found: C, 70.35; H, 4.59; N, 9.18.

2.2.6. (E)-3-(3-ferrocenyl-1-phenylpyrazol-4-yl)-1-(2-aminophenyl)-2-propen-1-one (**1f**)

Yield: 0.26 g (55%); mp: 194 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 4.22 (s, 5H, Fc); 4.39 (t, 2H, J = 1.72 Hz, Fc); 4.77 (t, 2H, J = 1.72 Hz, Fc); 6.36 (bs, 2H, NH_2); 6.67–6.75 (m, 2H, 1H at 5a and 1H at 3a); 7.26–7.36 (m, 2H, 1H at 4a and 1H at p-phenyl); 7.45–7.52 (m, 2H, m-phenyl); 7.47 (d, 1H, J = 15.36 Hz, H_α); 7.77 (dd, 2H, J = 8.24 and 1.40 Hz, o-phenyl); 7.87 (dd, 1H, J = 8.34 and 1.58 Hz, 1H at 6a); 8.29 (s, 1H, Pz); 8.32 (d, 1H, J = 15.68, H_β); ^{13}C NMR (50 MHz, $CDCl_3$): 68.6 (Fc), 69.2 (Fc), 69.7 (Fc), 77.2 (Fc), 115.8 (3a), 117.3 (5a), 118.6 (C-4, Pz), 119.1 (o-phenyl), 119.2

(1a), 121.4 (C_α), 125.5 (C-5, Pz), 126.8 (p-phenyl), 129.5 (m-phenyl), 130.8 (6a), 134.1 (C_β), 134.1 (4a), 139.5 (C-3, Pz), 151.0 (2a), 152.6 (N-substituted phenyl), 191.4 (CO). IR (KBr, cm^{-1}): 504, 745, 1162, 1211, 1286, 1503, 1552, 1569, 1584, 1596, 1611, 1641, 3081, 3357. Anal. Calcd for $C_{28}H_{23}N_3OFe$ (473.35 g/mol): C, 71.05; H, 4.90; N, 8.88. Found: C, 71.00; H, 4.88; N, 8.85.

*2.2.7. (E)-3-(3-ferrocenyl-1-phenylpyrazol-4-yl)-1-(3-aminophenyl)-2-propen-1-one (**1g**)*

Yield: 0.31 g (65%); mp: 142–143 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 3.83 (s, 2H, NH_2); 4.19 (s, 5H, Fc); 4.39 (t, 2H, *J* = 1.88 Hz, Fc); 4.75 (t, 2H, *J* = 1.88 Hz, Fc); 6.92 (ddd, 1H, *J* = 7.68, 2.52 and 1.16 Hz, 1H at 4a); 7.30–7.37 (m, 2H, 1H at p-phenyl and 1H at 5a); 7.33 (d, 1H, *J* = 15.68 Hz, H_α); 7.40 (t, 1H, *J* = 1.24 Hz, 1H at 2a); 7.44 (m, 1H at 6a); 7.45–7.53 (m, 2H, m-phenyl); 7.77 (dd, 2H, *J* = 8.66 and 1.32 Hz, o-phenyl); 8.30 (d, 1H, *J* = 0.64 Hz, Pz); 8.37 (dd, 1H, *J* = 15.68 and 0.64 Hz, H_β); ^{13}C NMR (50 MHz, $CDCl_3$): 68.6 (Fc), 69.2 (Fc), 69.7 (Fc), 76.7 (Fc), 114.4 (2a), 118.4 (C-4, Pz), 118.7 (6a), 119.1 (o-phenyl), 119.2 (4a), 120.8 (C_α), 125.7 (C-5, Pz), 126.9 (p-phenyl), 129.4 (5a), 129.5 (m-phenyl), 135.7 (C_β), 139.3 (1a), 139.5 (C-3, Pz), 146.8 (3a), 152.9 (N-substituted phenyl), 190.5 (CO). IR (KBr, cm^{-1}): 506, 737, 1199, 1293, 1501, 1548, 1582, 1592, 1617, 1659, 3084, 3375. Anal. Calcd for $C_{28}H_{23}N_3OFe$ (473.35 g/mol): C, 71.05; H, 4.90; N, 8.88. Found: C, 71.01; H, 4.87; N, 8.85.

*2.2.8. (E)-3-(3-ferrocenyl-1-phenylpyrazol-4-yl)-1-(4-aminophenyl)-2-propen-1-one (**1h**)*

Yield: 0.26 g (55%); mp: 208 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 4.16 (s, 2H, NH_2); 4.21 (s, 5H, Fc); 4.37 (t, 2H, *J* = 1.76 Hz, Fc); 4.76 (t, 2H, *J* = 1.76 Hz, Fc); 6.72 (d, 2H, *J* = 8.50 Hz, 2H at 3a); 7.27–7.35 (m, 1H, p-phenyl); 7.40 (d, 1H, *J* = 15.58 Hz, H_α); 7.44–7.52 (m, 2H, m-phenyl); 7.76 (dd, 2H, *J* = 8.06 and 1.48 Hz, o-phenyl); 7.96 (d, 2H, *J* = 8.74 Hz, 2H at 2a); 8.28 (s, 1H, Pz); 8.36 (d, 1H, *J* = 15.58 Hz, H_β); ^{13}C NMR (50 MHz, $CDCl_3$): 68.7 (Fc), 69.1 (Fc), 69.7 (Fc), 77.3 (Fc), 114.0 (3a), 118.6 (C-4, Pz), 119.1 (o-phenyl), 120.4 (C_α), 125.5 (C-5, Pz), 126.8 (p-phenyl), 128.7 (1a), 129.5 (m-phenyl), 130.9 (2a), 134.2 (C_β), 139.5 (C-3, Pz), 150.9 (4a), 152.7 (N-substituted phenyl), 187.8 (CO). IR (KBr, cm^{-1}): 508, 755, 1174, 1220, 1289, 1503, 1552, 1588, 1597, 1621, 1654, 3090, 3380. Anal. Calcd for $C_{28}H_{23}N_3OFe$ (473.35 g/mol): C, 71.05; H, 4.90; N, 8.88. Found: C, 69.97; H, 4.88; N, 8.86.

*2.2.9. (E)-3-(3-ferrocenyl-1-phenylpyrazol-4-yl)-1-(2-hydroxyphenyl)-2-propen-1-one (**1i**)*

Yield: 0.40 g (85%); mp: 135–136 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 4.24 (s, 5H, Fc); 4.42 (t, 2H, *J* = 1.90 Hz, Fc); 4.76 (t, 2H, *J* = 1.90 Hz, Fc); 6.95 (td, 1H, *J* = 6.98 and 1.10 Hz, 1H at 5a); 7.03 (dd, 1H, *J* = 8.50 and 1.12 Hz, 1H at 3a); 7.31–7.38 (m, 1H, p-phenyl); 7.46–7.55 (m, 3H, 2H at m-phenyl and 1H at 4a); 7.51 (d, 1H, *J* = 15.54 Hz, H_α); 7.78 (dd, 2H, *J* = 8.40 and 1.44 Hz, o-phenyl); 7.92 (dd, 1H, *J* = 8.40 and 1.24 Hz, 1H at 6a); 8.35 (s, 1H, Pz); 8.55 (d, 1H, *J* = 15.54 Hz, H_β); 13.08 (s, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$): 68.7 (Fc), 69.3 (Fc), 69.77 (Fc), 76.5 (Fc), 118.0 (C_α), 118.2 (C-4, Pz), 118.6 (3a), 118.7 (5a), 119.2 (o-phenyl), 120.0 (1a), 126.0 (C-5, Pz), 127.1 (p-phenyl), 129.4 (6a), 129.5 (m-phenyl), 136.1 (4a), 136.7 (C_β), 139.3 (C-3, Pz), 153.3 (N-substituted phenyl), 163.6 (2a), 193.3 (CO). IR (KBr, cm^{-1}): 650, 754, 1208, 1305, 1505, 1544, 1562, 1578, 1597, 1633, 3083, 3450. Anal. Calcd for $C_{28}H_{22}N_2O_2Fe$ (474.33 g/mol): C, 70.90; H, 4.67; N, 5.91. Found: C, 70.73; H, 4.66; N, 5.93.

*2.2.10. (E)-4-(3-ferrocenyl-1-phenylpyrazol-4-yl)-3-buten-2-one (**1j**)*

Yield: 0.35 g (87%); mp: 167 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 2.42 (s, 3H, CH_3); 4.19 (s, 5H, Fc); 4.39 (t, 2H, *J* = 1.82 Hz, Fc); 4.73 (t, 2H, *J* = 1.82 Hz, Fc); 6.60 (d, 1H, *J* = 16.20 Hz, H_α); 7.28–7.36 (m, 1H, p-phenyl); 7.44–7.52 (m, 2H, m-phenyl); 7.74 (dd, 2H, *J* = 8.22 and 1.40 Hz, o-phenyl); 8.04 (d, 1H, *J* = 16.20 Hz, H_β); 8.19 (s, 1H, Pz); ^{13}C NMR (50 MHz, $CDCl_3$): 26.4 (CH_3), 68.4 (Fc), 69.2 (Fc), 69.6 (Fc), 76.7 (Fc), 117.6 (C-4, Pz), 119.1 (o-phenyl), 125.5 (C-5, Pz), 125.6 (C_α), 127.0 (p-phenyl), 129.5 (m-phenyl), 134.2 (C_β), 139.3 (C-3, Pz), 152.4 (N-substituted phenyl), 197.8 (CO). IR (KBr, cm^{-1}): 513, 684, 757, 1240, 1262, 1402, 1503, 1544, 1597, 1606, 1649, 3128. Anal. Calcd for $C_{23}H_{20}N_2OFe$ (396.26 g/mol): C, 69.71; H, 5.09; N, 7.07. Found: C, 69.64; H, 5.08; N, 7.09.

*2.2.11. (E)-1-(3-ferrocenyl-1-phenylpyrazol-4-yl)-4,4-dimethyl-1-penten-3-one (**1k**)*

Yield: 0.33 g (75%); mp: 67–68 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 1.25 (s, 9H, CH_3); 4.20 (s, 5H, Fc); 4.36 (t, 2H, *J* = 1.82 Hz, Fc); 4.72 (t, 2H, *J* = 1.82 Hz, Fc); 6.95 (d, 1H, *J* = 16.18 Hz, H_α); 7.25–7.33 (m, 1H, p-phenyl); 7.41–7.49 (m, 2H, m-phenyl); 7.74 (dd, 2H, *J* = 8.44 and 1.38 Hz, o-phenyl); 8.23 (d, 1H, *J* = 0.62 Hz, Pz); 8.27 (d, 1H, *J* = 16.18 and 0.62 Hz, H_β); ^{13}C NMR (50 MHz, $CDCl_3$): 26.4 (CH_3), 43.0 (C), 68.5 (Fc), 69.0 (Fc), 69.6 (Fc), 76.7 (Fc), 118.1 (C-4, Pz), 119.0 (o-phenyl), 119.1 (C_α), 125.5 (C-5, Pz), 126.7 (p-phenyl), 129.4 (m-phenyl), 133.9 (C_β), 139.3 (C-3, Pz), 152.7 (N-substituted phenyl), 204.0 (CO). IR (KBr, cm^{-1}): 506, 688, 756, 1074, 1407, 1504, 1549, 1594, 1675, 2965, 3093. Anal. Calcd for $C_{26}H_{26}N_2OFe$ (438.34 g/mol): C, 71.24; H, 5.98; N, 6.39. Found: C, 71.14; H, 5.96; N, 6.40.

*2.2.12. (E)-3-(3-ferrocenyl-1-phenylpyrazol-4-yl)-1-(3-pyridyl)-2-propen-1-one (**1l**)*

Yield: 0.28 g (60%); mp: 215 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): 4.20 (s, 5H, Fc); 4.40 (t, 2H, *J* = 1.88 Hz, Fc); 4.74 (t, 2H, *J* = 1.88 Hz, Fc); 7.30–7.38 (m, 1H, p-phenyl); 7.36 (d, 1H, *J* = 15.58 Hz, H_α); 7.44–7.54 (m, 3H, 2H at m-phenyl and 1H at 5a); 7.77 (dd, 2H, *J* = 8.46 and 1.38 Hz, o-phenyl); 8.33 (dt, 1H, *J* = 8.02 and 1.84 Hz, 4a); 8.35 (d, 1H, *J* = 0.62 Hz, Pz); 8.46 (d, 1H, *J* = 15.58 Hz, H_β); 8.82 (m, 1H, Py at 2a); 9.27 (m, 1H, 6a). ^{13}C NMR (50 MHz, $CDCl_3$): 68.7 (Fc), 69.3 (Fc), 69.7 (Fc), 76.5 (Fc), 118.1 (C-4, Pz), 119.2 (o-phenyl), 119.3 (C_α), 123.7 (5a, Py), 126.0 (C-5, Pz), 127.1 (p-phenyl), 129.5 (m-phenyl), 133.6 (3a, Py), 135.8 (4a, Py), 137.1 (C_β), 139.3 (C-3, Pz), 149.6 (6a, Py), 153.0 (2a, Py), 153.3 (N-substituted phenyl), 188.5 (CO). IR (KBr, cm^{-1}): 513, 693, 765, 1020, 1232, 1292, 1503, 1544, 1583, 1588, 1658, 3106. Anal. Calcd for $C_{27}H_{21}N_3OFe$ (459.32 g/mol): C, 70.60; H, 4.61; N, 9.15. Found: C, 70.32; H, 4.61; N, 9.16.

2.3. In vitro studies

2.3.1. Drugs and solutions

The MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was dissolved (5 mg/ml) in phosphate buffer saline pH 7.2 and filtered (0.22 μm) before use. The RPMI 1640 cell culture medium, fetal bovine serum (*FBS*), and MTT, were purchased from Sigma Chemical Company, USA.

2.3.2. Cell lines

Cervix adenocarcinoma HeLa and melanoma Fem-x cell lines were maintained in monolayer culture, and myelogenous leukemia K562 cells in suspension culture, in nutrient medium RPMI 1640, with 10% (inactivated at 56 °C) FBS, 3 mM of L-glutamine, and antibiotics.

2.3.3. Treatment of cell lines

Stock solutions (10 mM) of compounds were made in dimethyl sulfoxide (DMSO), and were dissolved in corresponding medium to

the required working concentrations. Target cells HeLa, (2000 cells per well), Fem-x (2000 cells per well), or K562 cells (3000 cells per well) were seeded into wells of a 96-well flat-bottomed microliter plate. Twenty hours later, after the cell adherence, 50 μl of the investigated compound was added to cells in final concentrations (6.25, 12.5, 25, 50, and 100 μM), except in the control wells, where only nutrient medium was added to the cells. Exceptionally compounds were applied to the suspension of leukemia K562 cells 2 h after the cell seeding. The intensity of agents action on cancer cell survival was determined 72 h later by MTT test [16], modified by Ohno and Abe [17]. Briefly, 20 μl of MTT (5 mg/ml) dye was added to each well. After incubation for further 4 h, 100 μl of 10% SDS were added to extract the insoluble product formazan, resulting from conversion of the MTT dye by viable cells. The number of viable cells in each well is proportional to the intensity of the absorbance of light, which was then read in an ELISA plate reader at 570 nm. To achieve cell survival (%), absorbance at 570 nm of a sample with cells grown in the presence of various concentrations of agent was divided with absorbance of control sample (the absorbance of cells grown only in nutrient medium), having subtracted from absorbance of a corresponding sample with target cells the absorbance of the blank. IC_{50} is used as the measure of the toxic agents action and is determined from the graph $S(\%) = f(c)$, as the concentration of the agent which induces decrease in cell survival to 50%.

3. Results and discussion

3.1. Synthesis and spectral characterization

Synthesis of all compounds was achieved in good to high chemical yields using Claisen–Schmidt condensation of 1H-3-ferrocenyl-1-phenylpyrazole-4-carboxaldehyde [18] with different methyl-ketones (Scheme 1).

In order to investigate the role of ferrocenyl pyrazole unit, two classes of α,β-unsaturated conjugated compounds have been synthesized in this study. The first class is characterized by the presence ferrocenyl pyrazole as Ring B and various substituted phenyl, heterocyclic and ferrocenyl moieties as Ring A. The second class has ferrocenyl pyrazole as Ring B and two alkyl groups (methyl and t-butyl) attached to the carbonyl group. The substituents on phenyl ring were selected to provide a reasonable coverage of polar, steric and electron donating or withdrawing properties.

The IR stretching frequencies for carbonyl absorption of s-cis and s-trans isomers appeared as a consequence of free rotation along the single bond between carbonyl group an olefinic bond. The equilibrium between the two isomers is dependent on their structural properties, solvent and temperature [19,20]. Comparing the carbonyl stretching frequencies in this study with those reported for similar compounds [21,22] we can conclude that s-cis conformers exhibit higher absorption than their s-trans analogues. The absorption band at lower frequencies was observed in the IR spectra of several compounds indicating the presence of s-trans conformers in the solid state (Table 1). The significant difference in carbonyl frequencies between s-cis and s-trans conformers may be attributed to the greater steric requirements of voluminous alkyl group or aromatic ring and its substituents.

Another evidence for predominate existence of s-cis conformation of all compounds was observed from their ^{1}H NMR spectra in the solution state. The relatively high δ-values of chemical shifts for ethylenic β-protons (Table 1) led us to conclude that s-cis conformation gives the possibility of an intramolecular interaction between the free electron pair of the oxygen atom and H_β ethylenic proton resulting in the downfield shift for this proton. These observations are in accordance with similar findings for ferrocenyl chalcones containing anthracenyl group [23].

The ^{1}H NMR spectra of all investigated compounds showed a pair of AB doublets at δ = 6.60–8.57 ppm consistent with olefinic protons of chalcone moieties with large coupling constants (J = 15.54–16.20 Hz) indicating expected (E)-configuration [24]. In the HETCOR experiment it was found that these protons correlated with the carbon atoms resonating at 118.04–125.63 and 131.96–137.09 respectively, supporting the assignment of C_α and C_β carbon atoms. Even at 200 MHz, in several examples we observed additional splittings of singlets attributed to the pyrazole proton into doublets with small coupling constants ($J \sim 0.7$ Hz) as well as splitting of doublets of H_β proton into dd with the same value of coupling constants indicating their spatial couplings, only possible in (E)-configuration. The assignment of the carbonyl group was unambiguous in all compounds having in mind its resonance position in the lowest field (181.83–204.03 ppm).

3.2. Electrochemical studies

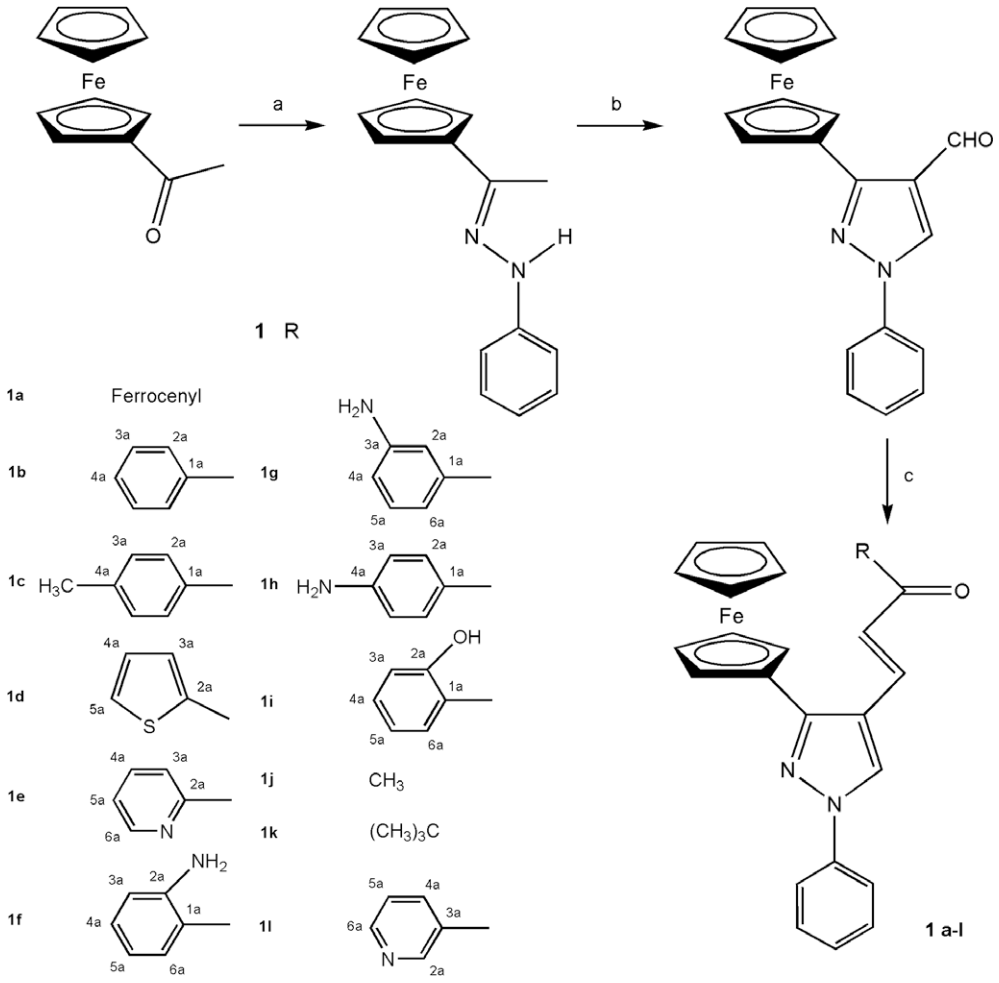
The electrochemical properties of compounds **1a–l** were investigated by cyclic voltammetry in acetonitrile containing 0.1 mol/L lithium perchlorate as a supporting electrolyte. The anodic and cathodic peak potentials of compounds **1a–l** obtained by a 0.1 V s^{-1} scan rate are listed in Table 2., whereas Fig. 1 shows cyclovoltammograms of compounds **1a** and **1l**, as the representative ones. As it could be seen, all tested compounds exhibit o reversible one-electron redox couple attributed to the ferrocene unit connected to the pyrazole ring at very similar potential. More positive values of oxidation peaks in comparison with unsubstituted ferrocene could be explained by the electron-withdrawing properties of the rest of molecule. On the other hand, it is evident that the structure of the group connected to the carbonyl group of the conjugated enone system does not affect this potential considerably. The difference between anodic and cathodic peak potentials is close to the theoretical value and independent of the scan rate v.

The anodic and cathodic peak currents are proportional to the square root of the scan rate. As it is depicted at Fig. 2 (data of compound **1l**, as a representative example), their ratio is independent of the scan rate, indicating a diffusion-controlled process.

The additional redox couple in the case of compound **1a** corresponds to the second ferrocene moiety. The better electron-withdrawing properties of a conjugated enone system in comparison with the pyrazole ring are responsible for more positive oxidation potential of this couple.

3.3. Biological studies

The antiproliferative action of compounds **1a–l** was tested against malignant cell lines (human cervix carcinoma HeLa cells, human myelogenous leukemia K562 and human Fem-x cells) with cisplatin as a positive control (Table 3). Compounds **1e**, **1g**, **1h**, **1k** and **1l** exhibited significant cytotoxic effects against all tested human cancer cells, especially to Fem-x and K562. The most active compound (**1l**) and the two less active **1d** and **1f** (Fig. 3.) exerted a dose dependent antiproliferative action at micromolar concentrations towards investigated tumor cell lines, as detected by the MTT test. The cytotoxic *in vitro* activity of these ferrocene derivatives varied from IC_{50} > 100 μg/ml (in the case of all cancer cell lines) for **1c** to IC_{50} = 5.42 μg/ml for **1l**. The last value, being lower than that for cisplatin (IC_{50} = 5.90 μg/ml), makes compound **1l** as promising antiproliferative agent against human myelogenous leukemia. Moreover, the pronounced cytotoxicity was manifested in other ferrocene derivatives (**1g**, **1h** and **1e**) possessing a basic nitrogen atom in the moiety (designated R in Scheme 1) originating from the methyl-ketones, of the pyridine or aniline type. The only exception is more moderate activity of compound **1f**, with a sterically hindered *ortho* amino group. It is evident that the most active



Scheme 1. Reagents and conditions: (a) $PhNHNH_2$, EtOH, reflux; (b) DMF, $POCl_3$ (3 equiv), r.t.; (c) methyl-ketones, 30% NaOH, EtOH, 4 h; 60 °C, then rt, overnight.

Table 1
Characteristic IR and NMR spectral data for compounds **1a–l**.

Compound	s-cis (cm^{-1})	s-trans (cm^{-1})	C_α (ppm)	C_β (ppm)	H_α (ppm)	H_β (ppm)	$J_{H\alpha,H\beta}$ (Hz)	C=O (ppm)
1a	1644	–	121.43	131.96	6.95	8.35	15.58	192.80
1b	1662	–	120.43	136.04	7.39	8.40	15.72	190.30
1c	1658	1607	120.38	135.52	7.40	8.39	15.64	189.66
1d	1650	–	119.88	135.23	7.27	8.44	15.54	181.83
1e	1663	–	118.98	136.06	8.11	8.57	16.02	189.06
1f	1658	–	121.39	134.08	7.47	8.32	15.68	191.37
1g	1641	1611	120.76	135.73	7.33	8.37	15.68	190.49
1h	1659	1617	120.39	134.25	7.40	8.36	15.58	187.80
1i	1654	1621	118.04	136.68	7.51	8.55	15.54	193.35
1j	1633	–	125.63	134.24	6.60	8.04	16.20	197.81
1k	1649	1606	119.12	133.93	6.95	8.27	16.18	204.03
1l	1675	–	119.33	137.09	7.36	8.46	15.58	188.55

compounds **1l** and **1g** possess a *meta* relationship of the basic center with the rest of the molecule. The nonexistence of an aromatic moiety in the starting methyl-ketones does not preclude the activity as exemplified by the "aliphatic R" compounds **1j** and **1k**. The presence of an additional ferrocene unit, in compound **1a**, does not seriously affect the activity compared to the phenyl analogue **1b**. The inclusion of a *para* methyl group on the phenyl core of R seems to notably reduce the cytotoxic activity.

In order to make an easier discussion to follow the statistically supported conclusions, we performed agglomerative hierarchical clustering (AHC) on the mentioned samples (Tables 3 and 1), using the Excel program plug-in XLSTAT version 2008.6.07. The method was applied utilizing the values of IC_{50} of the compounds (Table 2) or the spectral data presented in Table 1 (the IR stretching frequencies of the C=O groups and/or the corresponding chemical shifts of this carbon or the other two of the conjugated enone of the chalcone moiety) as original variables without any recalculation. We decided to use the spectral data as variables in order to test whether the well known Michael acceptor property of chalcones (all of the spectral data should be, if steric effects are

Table 2
Peak potentials of compounds **1a–l** at a 0.1 V s^{-1} scan rate.

Compound	$E_{ox.}$ (mV)	$E_{ox.2}$ (mV)	$E_{red.}$ (mV)	$E_{red.2}$ (mV)	ΔE (mV)	ΔE_2 (mV)
1a	228	398	147	309	81	89
1b	224		152		72	
1c	227		150		77	
1d	250		134		116	
1e	223		154		69	
1f	230		147		81	
1g	231		156		75	
1h	232		156		76	
1i	231		143		88	
1j	231		159		72	
1k	231		152		79	
1l	225		160		65	

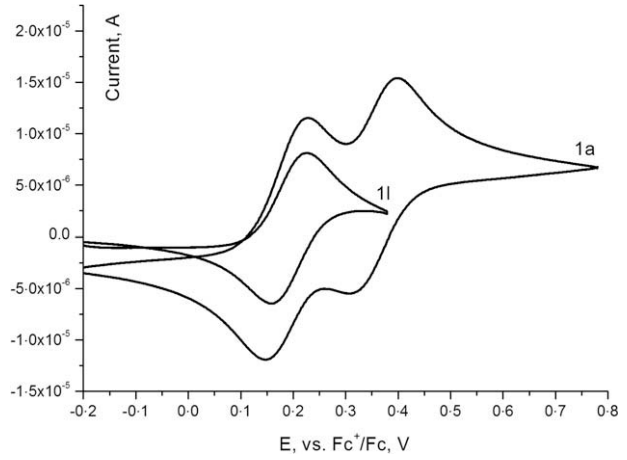


Fig. 1. Cyclovoltammograms of compounds **1a** and **ll** at Au disc in 0.1 M $LiClO_4$ in acetonitrile at $v = 0.1$ V s^{-1}.

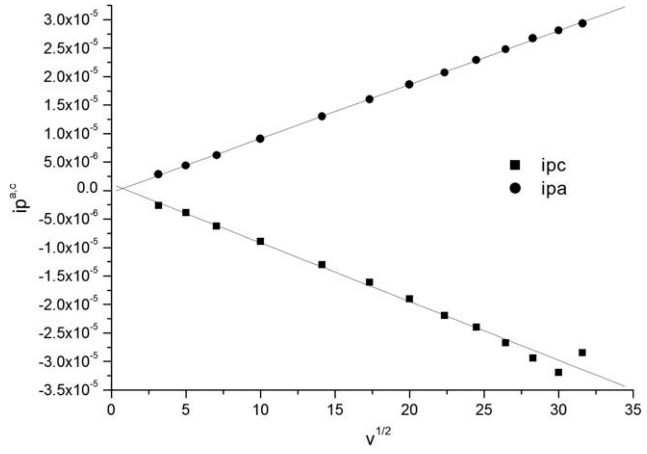


Fig. 2. Anodic and cathodic peak currents of **1l** obtained at different scan rates in acetonitrile.

neglected, in direct connection to the electrophilicity of the carbons and the general Michael acceptor reactivity of the compounds) has any correlation with the observed activities.

Some of the results of AHC are presented in Fig. 4. AHC was performed using Pearson dissimilarity (an aggregation criteria simple linkage, unweighted pair-group average and complete linkage were used) and Euclidean distance (aggregation criterion: weighted pair-group average, unweighted pair-group average and Ward's method).

Table 3
IC_{50} (μg/ml) for the 72 h of action of investigated compounds and cisplatin on the HeLa, Fem-x and K562 cells determined by MTT test.

Compound	HeLa	Fem-x	K562
	IC_{50} (μg/ml)		
1a	95.62 ± 6.19	41.4 ± 6.16	42.15 ± 5.41
1b	>100	36.20 ± 1.85	33.78 ± 3.77
1c	>100	>100	>100
1d	139.13	87.14 ± 12.85	58.37 ± 9.50
1e	113.22 ± 17.18	9.15 ± 1.29	9.00 ± 1.22
1f	78.16 ± 14.24	34.67 ± 5.12	39.32 ± 7.47
1g	24.24 ± 0.54	9.27 ± 0.06	5.92 ± 0.67
1h	47.71 ± 5.42	9.96 ± 0.34	10.31 ± 2.32
1i	>100	75.69 ± 0.28	46.16 ± 7.38
1j	54.95 ± 7.46	41.28 ± 0.15	46.94 ± 12.03
1k	>100	30.13 ± 4.96	10.19 ± 1.36
1l	11.28 ± 6.17	8.08 ± 2.28	5.42 ± 0.53
Cisplatin	2.10 ± 0.20	4.70 ± 0.20	5.90 ± 0.20

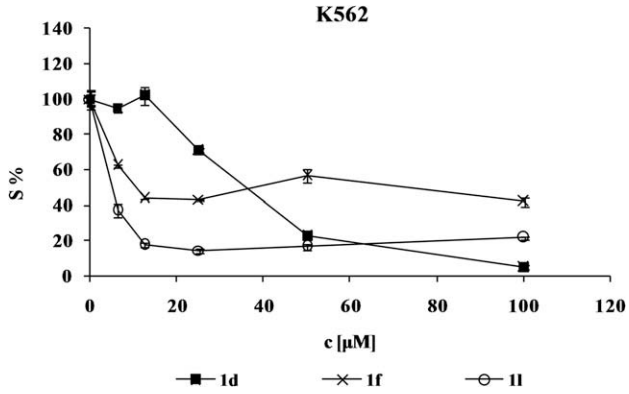


Fig. 3. Representative graph showing survival of K562 cell grown for 72 h in the presence of increasing concentrations of **1d**, **1f** and **1l**.

The definition of the groups was based on Pearson correlation, using complete linkage and unweighted pair-group average method. All AHC analyses have clearly indicated the existence of three groups of compounds under study (designations of the compounds were given in Scheme 1).

Dendrogram A, representing the grouping according to the observed cytotoxic activity, shows clearly the separation of the most active compounds (including cisplatin) as a separate subclade of class C1, as well as the least active one (**1c**) from the rest of the tested compounds. It is interesting to note that all of the basic nitrogen containing Rs belong to the clade of class C1. Possibly, this segregation of compounds **1l**, **1g** and **1h** with cisplatin from the rest of the samples indicates their similar *modus operandi*.

The most striking feature of dendrogram B (spectral data as variables) is the complete separation of compound **1l** with the highest values of C=O IR frequencies and the chemical shift of the β-carbon of the enone among the synthesized compounds. This spectral property-biological activity correlation suggests that the Michael acceptor ability of compound **1l** might convey at least one part of the observed activity. This might be a simplified picture of a general trend. In this context it is worthy to consider the example of ferrocifen (ferrocene derivative of tamoxifen). The activity of ferrocifen arises from the peculiar redox properties of the Fe^{II} complex that initiates the chain of reactions leading to the formation of a quinone methide susceptible to nucleophilic attack from biomolecules [25,26].

The closely related compounds **1d**, **1i**, **1b** (except for **1k**) and **1c** partially, with respect to the shown activity, remain a part of the

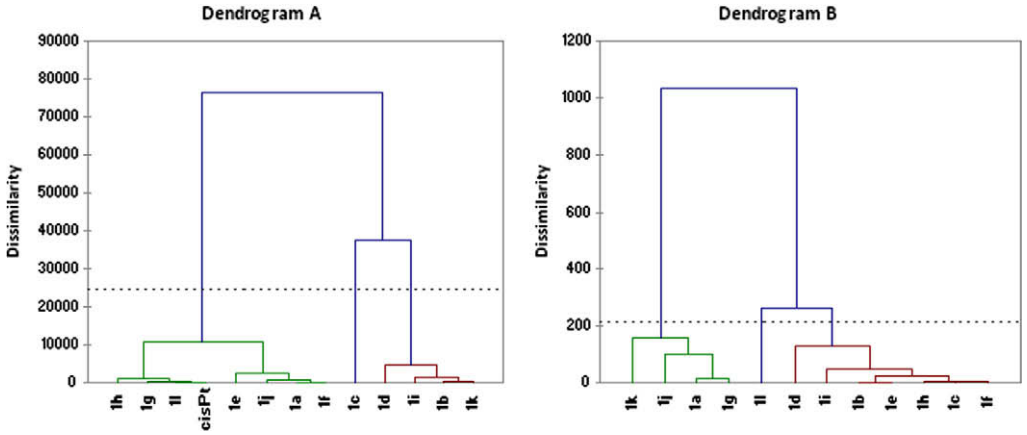


Fig. 4. Dendrograms (AHC analysis) represent cytotoxic activity (IC_{50} values, dendrogram A, the original variables) and spectral data from Table 1 (the IR stretching frequencies of the C=O groups and the corresponding chemical shifts of this carbon and the other two of the conjugated enone of the chalcone moiety, dendrogram B). Dissimilarity relationship of 12 synthesized compounds (observations) (including cisplatin (cisPt) in dendrogram A) was obtained by Euclidian distance dissimilarity (dissimilarity within the intervals [0, 8000] and [0, 1100], dendrograms A and B, respectively), using aggregation criterion-Ward's method. Three groups of compounds were found (from left to right) C1, C2 and C3.

same clade/class in the spectral data dendrogram as revealed by further subdivision of class C3. The unexpected lower activity of compound **1k**, according to the assumed electrophilicity from the AHC analysis, could be explained keeping in mind that the *tert*-butyl group may block any approach of the nucleophilic center of biomolecule to the enone carbonyl group.

The presented results on the antiproliferal properties of these ferrocene containing molecules, especially **1l** as a promising compound in combat against human cancer cells, encourage further investigations to throw more light on the role of iron in cancer therapy.

4. Conclusion

Twelve novel α,β-unsaturated conjugated ketones containing ferrocenyl pyrazole unit were prepared and structurally characterized using spectroscopic techniques. Cyclovoltammetric studies showed a reversible one-electron redox couple attributed to the ferrocene unit connected to the pyrazole ring at very similar potential. Compounds containing 3-pyridyl moiety appeared to be the most active against myelogenous leukemia K562 cell lines with a cytotoxic potential better than cisplatin in inhibition of K562 cell lines.

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