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Synthesis of some new *N*-acyl derivatives of ferrocenyl pyrazolines and investigation of their cytotoxic effect

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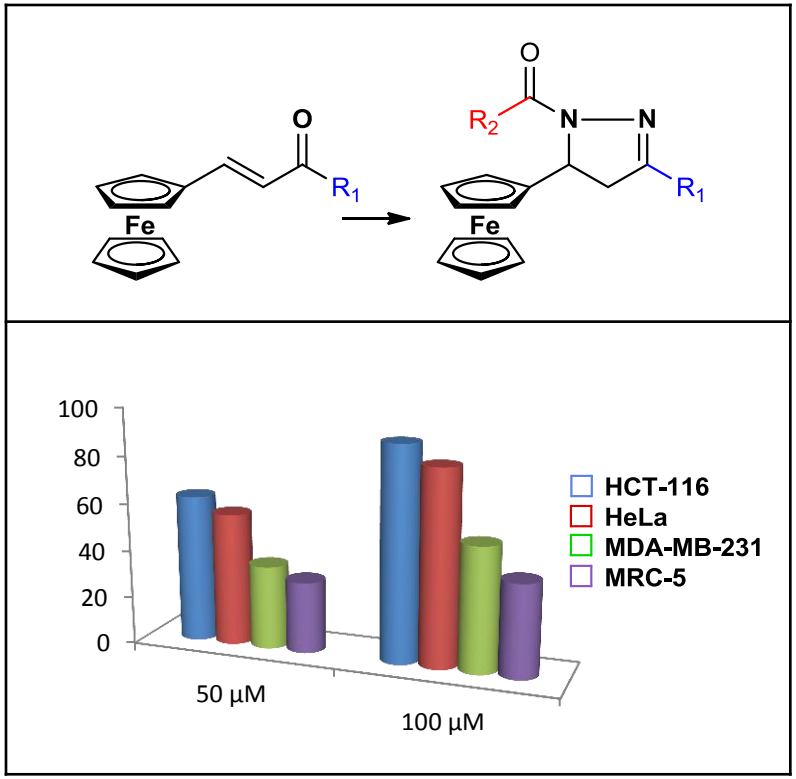


Abstract:

For several years, ferrocene moiety represents a significant part of the structure of different biologically active compounds. Ferrocene is stable and non-toxic compound and as such very useful for various syntheses. The combination of pharmacologically active *N*-heterocycles with ferrocene mostly leads to a favorable change in biological properties in a huge number of drugs. Considering the importance of these properties, this article describes the synthesis and characterization of a new series of ferrocenyl pyrazolines in good yields. Screening of the new products *in vitro* against human cervical cancer cells (HeLa), human breast cancer cells (MDA-MB-231), human colon cancer cells (HCT-116), and human fibroblast (MRC-5, as control cells) by the MTT method was performed.

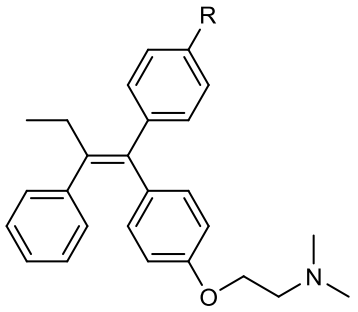
Keywords: cytotoxicity; ferrocene; pyrazolines

Introduction

In the past decade has been significant growth in demand and sales of pharmaceutical drugs worldwide. For this reason, the interest of many scientists in the synthesis of different types of compounds that could be used for the treatment of many diseases has greatly increased. One of the very useful achievements in this field is incorporating ferrocene moiety into the structure of some well-known biologically active molecules.[1-3] In these cases, the new ferrocenyl products are dramatically more effective than the original organic analog.[4]

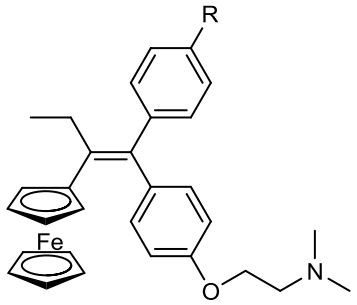
One of the examples that support the facts given above is the increase in the effectiveness of the well-known drugs Tamoxifen (the drug used most often to treat breast cancer) and its active metabolite Hydroxytamoxifen (Figure 1) when the aromatic part in their structure is replaced by a ferrocene part.[5]

Ferrocifen and Hydroxyferrocifen were obtained in this way (Figure 2).



Tamoxifen; R=H
Hydroxytamoxifen; R=OH

Figure 1. Structure of Tamoxifen and Hydroxytamoxifen



Ferrocifen; R=H
Hydroxyferrocifen; R=OH

Figure 2. Structure of Ferrocifen and Hydroxyferrocifen

After the development of these molecules, many researchers are designing and studying ferrocene hybrids with various other moieties including heterocycles.[6] The combination of pharmacologically active *N*-heterocycles with ferrocene core leads to favorable changes in the biological properties of a large number of drugs, often associated with reduced toxicity.[7-10]

Pyrazoline derivatives played a crucial role in the development of heterocyclic chemistry and drug design, because they mostly have very expressed activities such as antiinflammatory,[11] antimicrobial,[12,13] antiviral,[14] antimalarial,[15] insecticidal,[16] anticancer,[17] antidepressant,[18] antitubercular.[19]

Keeping in view the importance of this biological application, we presumed that the incorporation of two active and different pharmacophores into one molecule can lead to useful changes in the structure that may affect the improvement of the activity. The aim of this study was to synthesize some new ferrocenyl compounds as a continuation of our work in the field of ferrocene (Figure 3 and Figure 4).[21,22] In this way, we prepared ferrocenyl derivatives of *N*-formyl and *N*-acetyl pyrazolines.

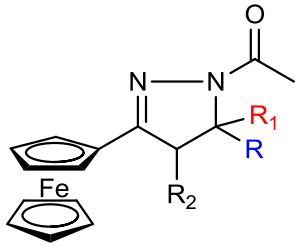


Figure 3. Structure of 1-(3-ferrocenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethanones

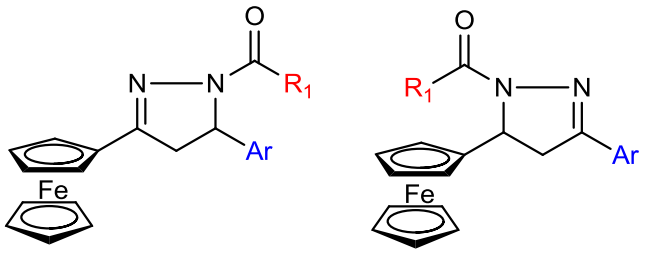
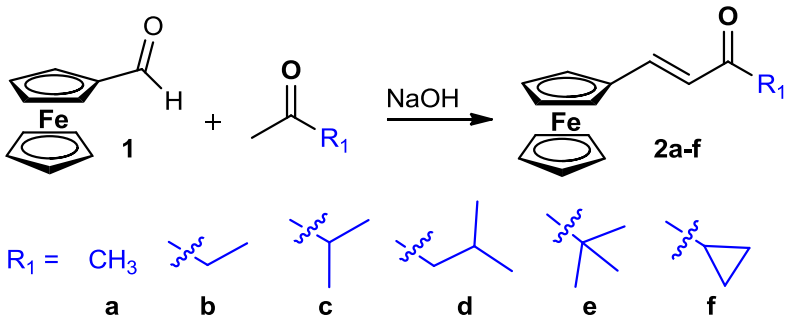


Figure 4. Structures of 3-ferrocenyl or 5-ferrocenyl-(4-alkoxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazoles)

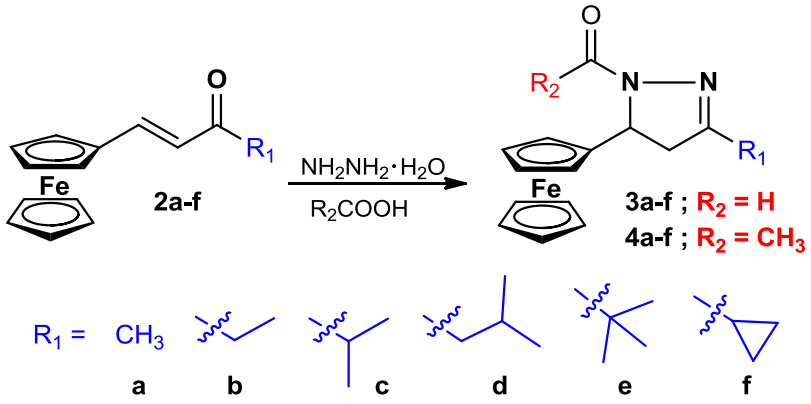
Results and discussion

In the first step of the synthesis, we prepared ferrocenyl chalcone analogs, **2a-f** by simple Claisen-Schmidt condensation of ferrocenyl aldehyde, **1** and corresponding ketone according to the literature procedure (Scheme 1).[21,22] Some of the products are new compounds, but in this case, they were used only as a substrate for the next step of the reaction.



Scheme 1. Synthesis of ferrocenyl chalcone analogs **2a-f**

Synthesized products, **2a-f** possess a conjugated enone system, which is suitable for further transformations. A series of novel *N*-acyl ferrocenyl pyrazolines (3-alkyl-5-ferrocenyl-4,5-dihydro-1*H*-pyrazole-1-carbaldehydes, **3a-f**, and 3-alkyl-5-ferrocenyl-4,5-dihydro-1*H*-pyrazolyl ethanones, **4a-f**) were prepared in the reaction of ferrocenyl compounds **2a-f** with hydrazine hydrate in the presence of corresponding boiling acid (formic or acetic acid, Scheme 2).



Scheme 2. Synthesis of *N*-acyl derivatives of ferrocenyl pyrazolines **3** and **4**

All new products were well characterized by spectral and physical data. ^{1}H NMR spectra of the ethyl derivatives of *N*-formyl and *N*-acetyl ferrocenyl pyrazolines are shown on Figure 5 and Figure 6.

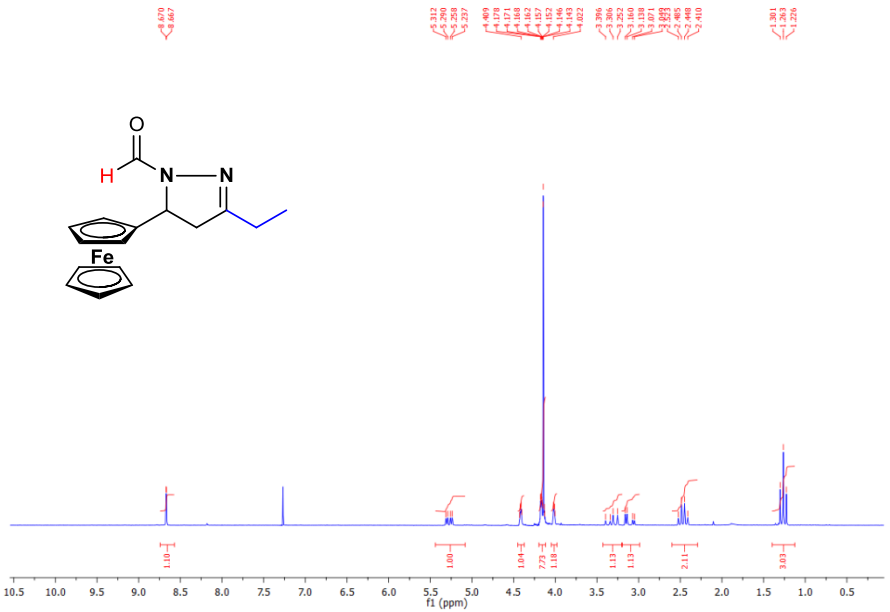


Figure 5. ^{1}H NMR spectrum of 3-ethyl-5-ferrocenyl-4,5-dihydro-1*H*-pyrazole-1-carbaldehyde

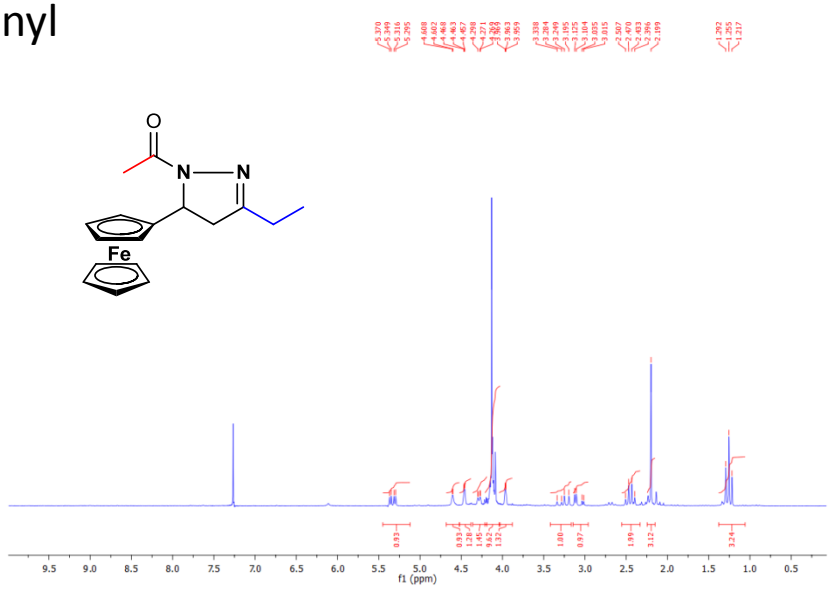


Figure 6. ^{1}H NMR spectrum of 1-(3-ethyl-5-ferrocenyl -4,5-dihydro-1*H*-pyrazol-1-yl)ethanone

Cytotoxic effect of new ferrocenyl pyrazolines had been tested on HCT-116, HeLa, MDA-MB-231 and MRC-5 cells. Cells were seeded in a 96-well plate and incubated overnight for adherence. After 24h medium was replaced with a medium containing a concentration of substances of 50 µM and 100 µM and with the fresh medium as a control. Cells were incubated at 37°C in an atmosphere of 5% CO_2 and absolute humidity for 48h. After incubation, MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 0.5 mg/ml) was added to each well and cells were incubated for 4h under culture conditions.

After dissolving formazan crystals in DMSO, absorbance was measured at 550 nm with a multiplate reader. Experiments were performed in triplicates and repeated in three independent series.

The cytotoxic activities of the new compounds (each at concentrations 50 and 100 µM) against HCT-116, HeLa, MDA-MB-231 cancer cells, and MRC-5 non-cancerous cells are shown in Table 1.

MTT results showed that the investigated substances exhibit the most effective cytotoxic effect against HCT-116 cells (in the range from 59.12 to 76.01% at a concentration of 50 µM, and 83.44 to 93.23% for 100 µM).

Table 1. Cytotoxicity of 12 new ferrocenyl pyrazolines at 50 and 100 µM concentrations against HCT-116, HeLa, MDA-MB-231 and MRC-5 cells after 48h.

		3a	3b	3c	3d	3e	3f
HCT-116	50 µM	66.87	61.65	68.02	68.97	72.09	62.13
	100 µM	83.44	86.16	91.73	90.78	92.28	89.99
		4a	4b	4c	4d	4e	4f
	50 µM	69.56	61.74	60.25	68.02	59.12	76.01
	100 µM	93.23	87.89	91.41	85.62	84.57	89.21
		3a	3b	3c	3d	3e	3f
HeLa	50 µM	45.24	41.18	35.22	51.66	48.08	55.75
	100 µM	72.98	73.25	64.03	77.31	66.40	81.87
		4a	4b	4c	4d	4e	4f
	50 µM	39.74	46.13	50.82	34.64	45.63	44.23
	100 µM	71.91	69.26	78.19	67.21	80.04	67.22
		3a	3b	3c	3d	3e	3f
MDA-MB-231	50 µM	35.10	29.22	41.88	31.05	47.28	35.11
	100 µM	51.27	54.16	74.70	57.36	59.55	52.31
		4a	4b	4c	4d	4e	4f
	50 µM	38.33	34.17	33.15	34.68	29.41	35.77
	100 µM	61.09	67.44	56.49	57.07	51.01	63.11
		3a	3b	3c	3d	3e	3f
MRC-5	50 µM	34.22	31.45	29.84	32.11	37.31	30.05
	100 µM	45.87	53.33	51.22	41.18	48.65	39.07
		4a	4b	4c	4d	4e	4f
	50 µM	34.44	35.15	35.88	32.57	36.31	34.43
	100 µM	43.66	49.22	45.46	46.06	50.20	51.21

In accordance with previous results, compound **4a** showed the strongest cytotoxic effect, which resulted in 93.23% of cytotoxic HCT-116 cells during 48h treatment. Also, compound **3f** exhibits the most effective cytotoxic activity (81.87% of cytotoxic cells) against HeLa cells, while **3c** has a similar effect (74.70% of cytotoxic cells) on MDA-MB-231 cells. A graphic representation of the results for the mentioned most active compounds is given in Figures 7, 8 and 9.

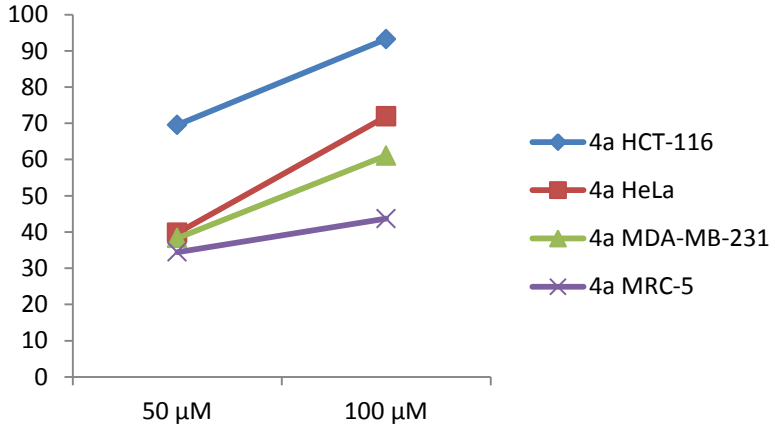


Figure 7. Cytotoxicity of **4a** against cancer cell lines and non-cancerous cell in two different concentrations

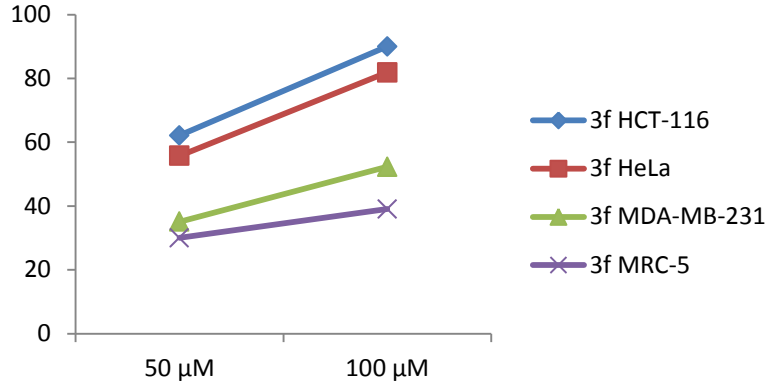


Figure 8. Cytotoxicity of **3f** against cancer cell lines and non-cancerous cell in two different concentrations

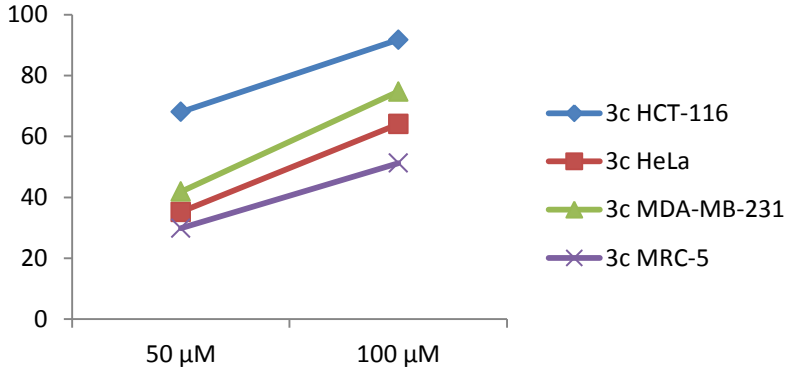


Figure 9. Cytotoxicity of **3c** against cancer cell lines and non-cancerous cell in two different concentrations

Conclusions

- Due to our research interest in the synthesis of different heterocyclic compounds with biological activity, we decided to synthesize new heterocyclic derivatives and investigate their cytotoxic activity. The present work describes simple methods for the preparation of new ferrocenyl pyrazolines by the reaction of ferrocenyl chalcones analogs **2a-f** with hydrazine hydrate in the presence of formic or acetic acid.
- All new compounds were well characterized by spectral and physical data.
- *In vitro* cytotoxic activity against HCT-116, HeLa, MDA-MB-231 cell lines, and non-cancerous MRC-5 cells was performed in different concentration, and results showed that products have a very pronounced cytotoxicity toward mentioned cancerous cell lines, especially compounds **3c**, **3f** and **4a**.
- Unfortunately, the cytotoxic activity towards non-cancerous MRC-5 cells is also higher, and for this reason investigation of some other biological activities on these compounds are in progress. These results will serve as a starting point for some further research.

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