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Pyrazolines from dehydrozingerone analogues: Cytotoxicity and morphological changes on HeLa cells

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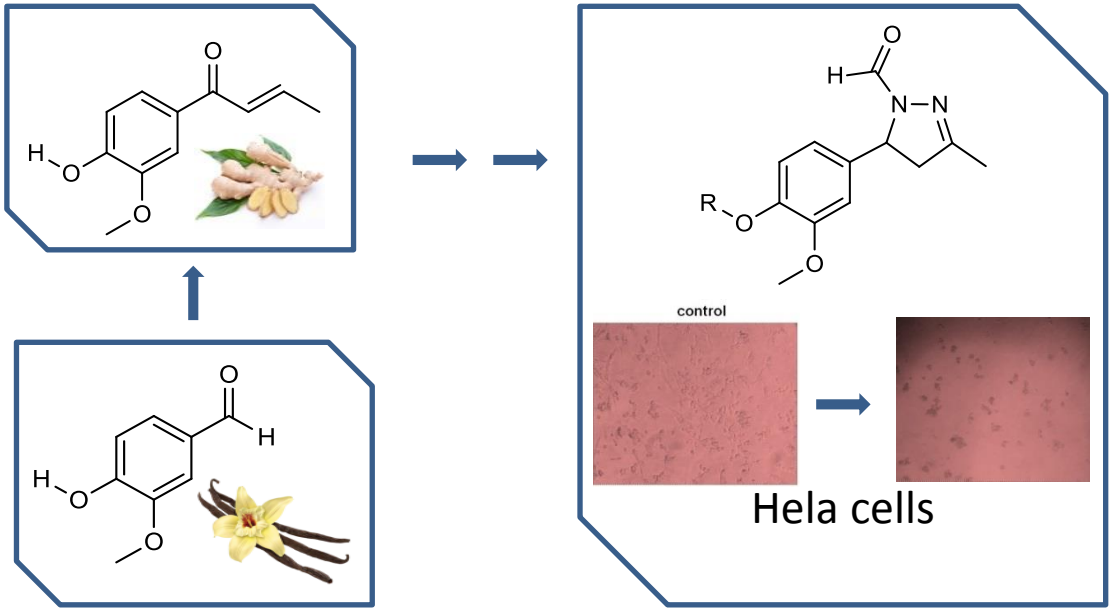
Pyrazolines from dehydrozingerone analogues: Cytotoxicity and morphological changes on HeLa cells



Abstract:

Ginger root is one of the most widely used natural product and is excellent source for many kinds of bioactive compounds. One of them is dehydrozingerone, also very attractive biological active compound. This compound served as a starting material in the synthesis of new derivatives. The synthesized products were used in reaction with formic acid and hydrazine hydrate in order to obtain *N*-formyl derivative of pyrazoline. All new compounds were identified and well characterized by IR, ^{1}H and ^{13}C NMR spectroscopy and physical data. *In vitro* cytotoxic activity of pyrazolines against human cervical cancer (*HeLa*) was determined using MTT method. Tested concentrations of substances were 100, 150 and 200 µM. Morphology changes of treated cells were visualized and compared to untreated cells using microscopy.

Keywords: ginger; dehydrozingerone; pyrazoline; HeLa

Introduction

Many natural materials have served as healing agents in order to treat various diseases. One of the most well-known and used plant drugs, for this purpose, is the root of the ginger plant. Ginger has been used for a wide array of unrelated aliments that include arthritis, rheumatism, sprains, muscular aches, pains, sore throats, indigestion, hypertension, infectious diseases,…[1]

Although the medicinal properties of ginger have been known for thousands of years, it is a very important fact that ginger root is also an excellent source of many types of active compounds. These compounds mostly have a broad spectrum of biological activities, such as antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, anti-allergic,…[2-8]

In ginger root many bioactive compounds have been identified as phenolic compounds. The most famous of them are dehydrozingerone **1**, zingerone **2**, gingerol **3**, shogaol **4**, paradol **5** and their derivatives (Figure 1).

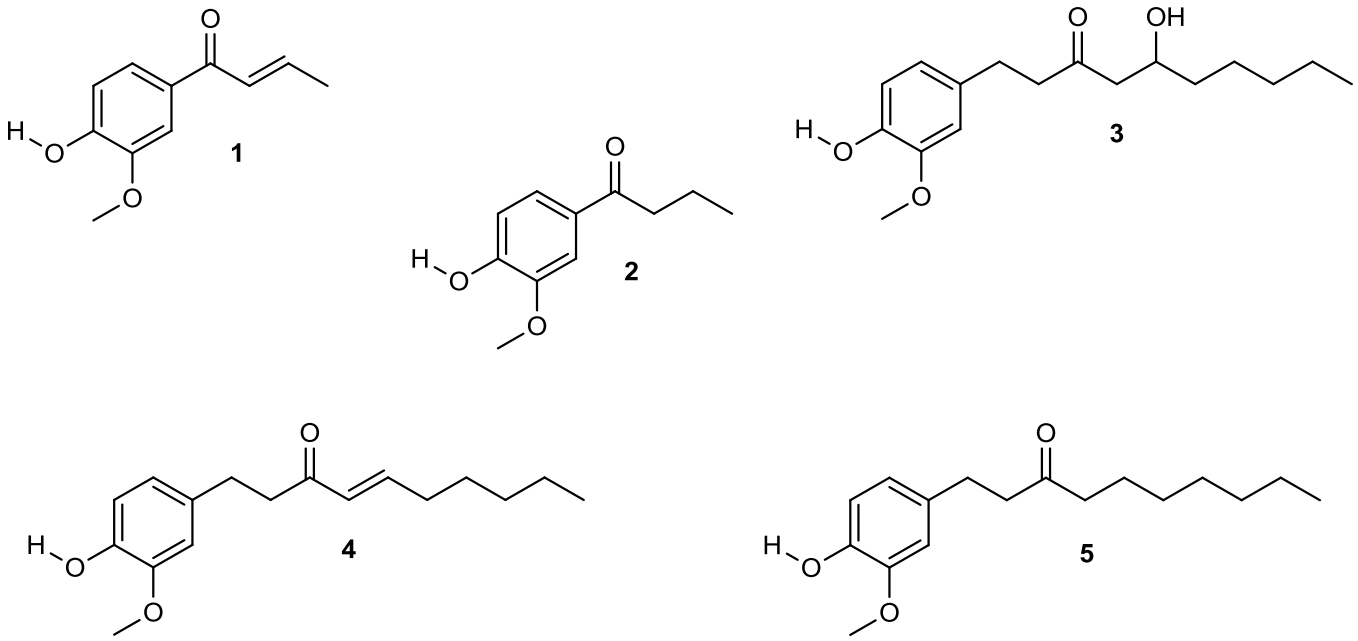


Figure 1. Structure of bioactive phenolic compounds in Ginger root

All these types of isolated compounds possess mutual property, they belong the vanilloid group of compounds, because they have a fragment of vanillin (also very significant natural product, Figure 2) in their structure.

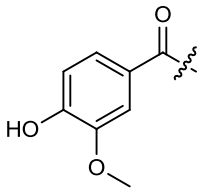
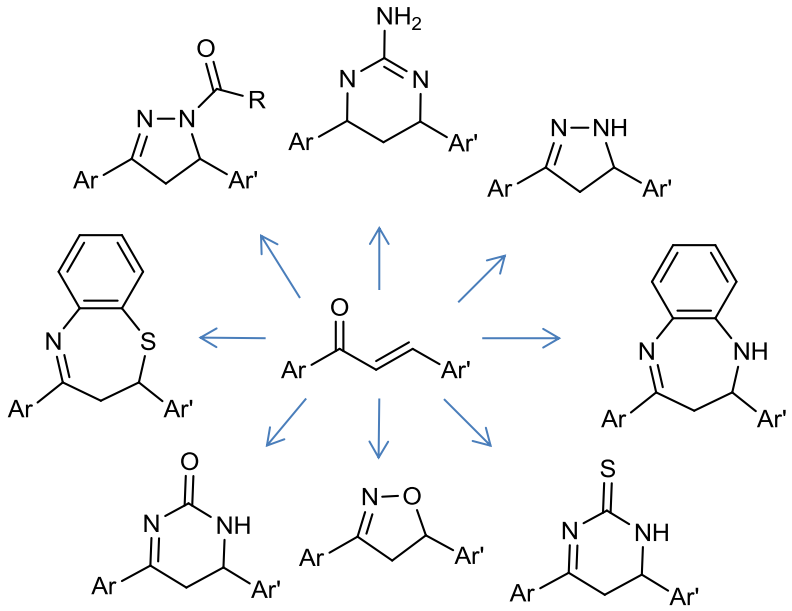


Figure 2. Structure of vanillin fragment

Another very important fact is that two of them have conjugate enone system in their molecule and on this way they can be easily transformed into some usable heterocyclic derivatives, similar to corresponding chalcones [9,10] (Scheme 1).

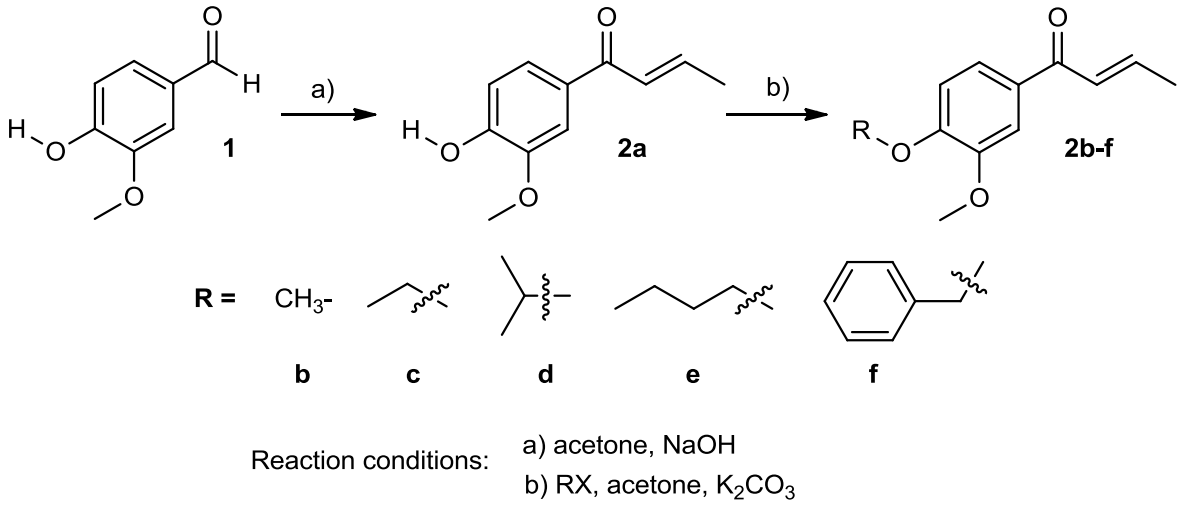


Scheme 1. Synthesis of different heterocycles starting from chalcone

These properties made a good starting point for further synthesis. The aim of present study was to synthesize new heterocyclic products, starting from dehydrozingerone, and investigate their antitumor activity.

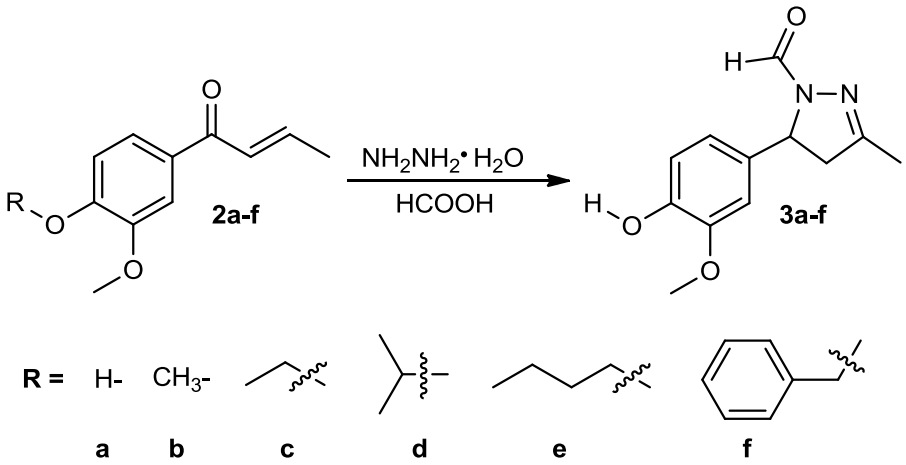
Results and discussion

Having in mind earlier described properties, we synthesized dehydrozingerone **2** in the large amount starting from vanillin **1**. The next step was synthesis of new derivative of dehydrozingerone. It is known that the presence of different alkyl groups on chalcones, can lead to a remarkable increase in bioactivity[11,12] and from this reason we wished to prepare series of dehydrozingerone *O*-alkyl derivatives. Alkylation of the phenolic group was achieved by standard procedure[13-15] using an alkyl halides in order to obtain 4-(4-alkoxy-3-methoxyphenyl)-but-3-en-2-ones, **2b-f**, Scheme 2.



Scheme 2. Synthesis of 4-(4-alkoxy-3-methoxyphenyl)-but-3-en-2-ones

In continuation of our interest in the synthesis of different heterocycles and starting from our previous results in dehydrozingerone derivatives transformation,[16] we decided to prepare some similar pyrazolines, but with formyl group in their molecule. Therefore, we expected that some changes in the structure of the products would lead to changes in antitumor activity. From this reason, a small series of a new *N*-formyl pyrazolines, 5-(4-alkoxy-3-methoxyphenyl)-3-methyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde, **3a-f** (Scheme 3), were synthesized in reaction of dehydrozingerones with hydrazine in boiling formic acid.



Scheme 3. Synthesis of 5-(4-alkoxy-3-methoxyphenyl)-3-methyl-4,5-dihydro-1H-pyrazole-1-carbaldehydes

All new products were characterized by their spectral data (IR, ^{1}H NMR and ^{13}C NMR). On Figures 3 and 4 are given NMR spectra of benzyl derivative of pyrazoline.

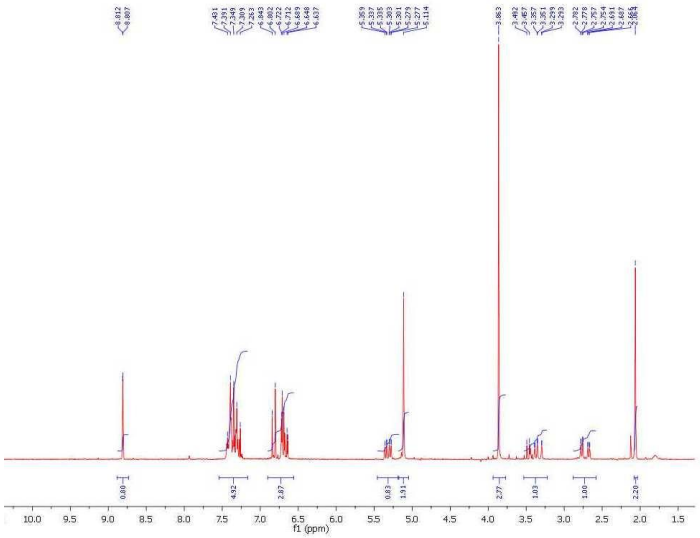
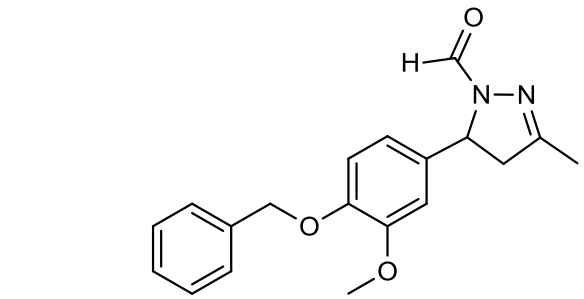


Figure 3. ^{1}H NMR spectrum of 5-(4-benzyloxy-3-methoxyphenyl)--3-methyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde



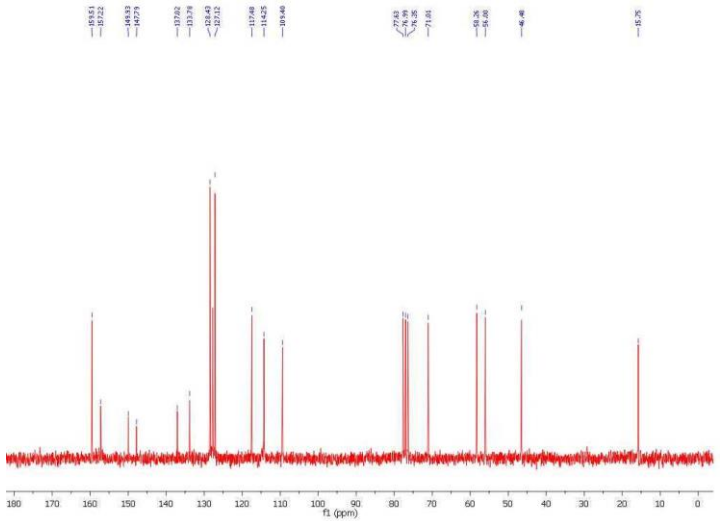


Figure 4. ^{13}C NMR spectrum of 5-(4-benzyloxy-3-methoxyphenyl)--3-methyl-4,5-dihydro-1H-pyrazole- 1-carbaldehyde

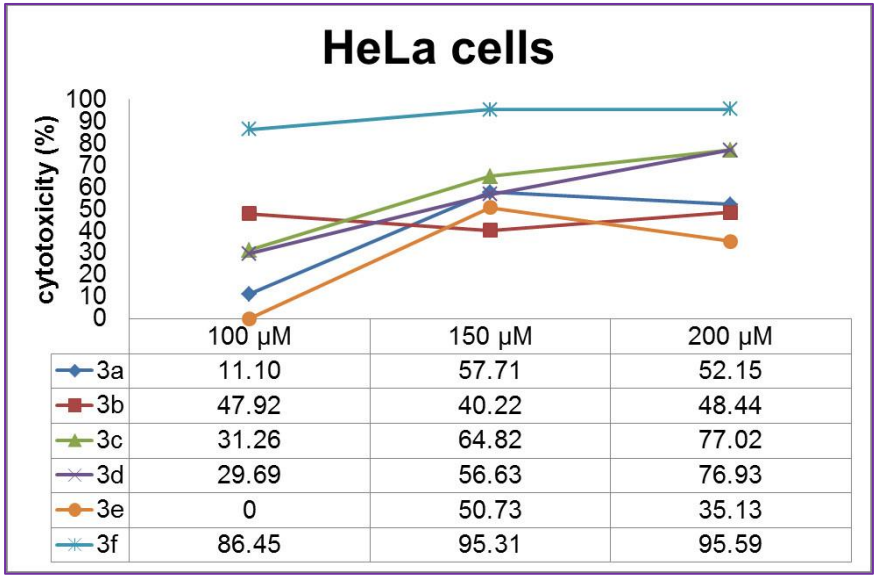
Cytotoxic effect of new pyrazolines had been tested on HeLa cells. Cells were seeded in a 96-well plate at $3x10^3$ cells/well. The cells were incubated at 37°C in 5% CO_2 atmosphere and absolute humidity for 48h with concentrations (100, 150 and 200 µM) in triplicate. Then, the cell culture media was removed, and 100µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 0.5 mg/mL) was added to each well. After 2h incubation at 37°C, MTT solution was removed and 150µl of DMSO was added to the wells.

The absorbance was measured at 595nm using a multiplate reader (Zenyth 3100, Anthos Labtec Instruments).

The percentage of cytotoxic cells was calculated using the formula:

Cytotoxicity (%) = (1 - (exp. group (ABS)) / (control group (ABS)) x 100).[17]

The cytotoxic effects of investigated pyrazolines are shown on Figure 5.



	100 µM	150 µM	200 µM
3a	11.10	57.71	52.15
3b	47.92	40.22	48.44
3c	31.26	64.82	77.02
3d	29.69	56.63	76.93
3e	0	50.73	35.13
3f	86.45	95.31	95.59

Figure 5. The cytotoxic effects of **3a-f** on HeLa cells after 48h treatment with various concentrations

All compounds have cytotoxic activity against HeLa cells line, except **3e** on 100µM concentration. Compounds **3c**, **3d** and **3f** showed better cytotoxic effects compared to other test substances, and **3f** exhibited the most effective cytotoxic effect on HeLa cells (100µM-86.45%; 150µM–95.31%; 200µM–95.59%).

In order to determine and compare the cytotoxic effects of the tested substances on the morphology of treated and untreated HeLa cells, we have used phase-contrast microscope. The HeLa cells were seeded in a 24-well plate and incubated for 48h with different concentrations pyrazolines (100, 150 and 200 µM). Morphological changes of both (Figure 6), experimental and control HeLa cells, were visualized with phase contrast microscopy under 100 X magnification on Olympus microscope (model BX51).

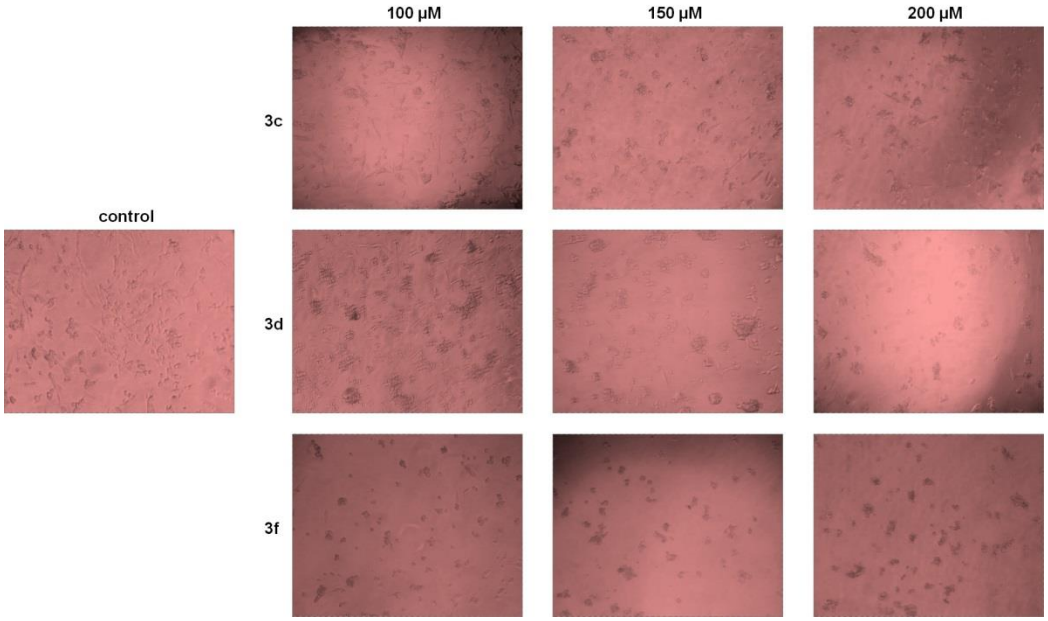


Figure 6. Changes of the HeLa cells morphology after 48h treatment with selected substances (**3c**, **3d** and **3f**).

Our results showed that compounds **3c**, **3d** and **3f** induced morphological changes of HeLa cells in dose dependent manner compared to the control group. Morphological changes (loss of shape cells and decrease in the number cells) were observed by phase-contrast microscopy**.**

Conclusions

Dehydrozingerone is present in different bioactive compounds and it is well known that he show a broad spectrum of biological activity. Conjugate enone system, which is presented in the dehydrozingerone, can be easily transformed. It is therefore, not surprising that many synthetic methods have been developed for the preparation of heterocycles. From this point of view the new derivative of pyrazolines were synthesized in very good yields starting from dehydrozingerone and its derivatives. All compounds were identified with IR and NMR spectra.

In vitro cytotoxic activity against HeLa cell line was performed in different concentration, and results showed that products have a very pronounced cytotoxicity, especially compounds **3c**, **3d** and **3f**. In order to compare cytotoxic effects the most active substances, morphology changes of HeLa cells were monitored.

The results showed that our compounds would be good candidates for further investigation of antitumor activity and more detailed investigation are in progress.

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Acknowledgments

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