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Synthesis of *N*-acetyl and *N*-formyl pyrazoline derivatives from vanillin and their antigenotoxic activity

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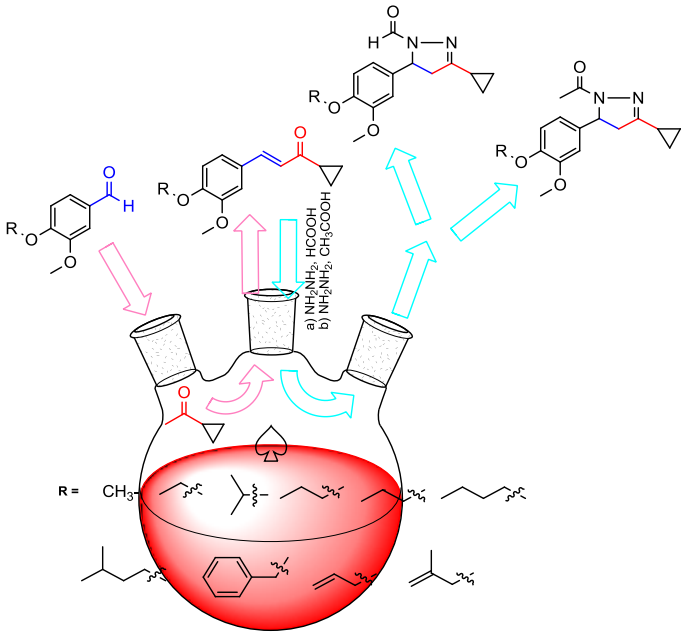
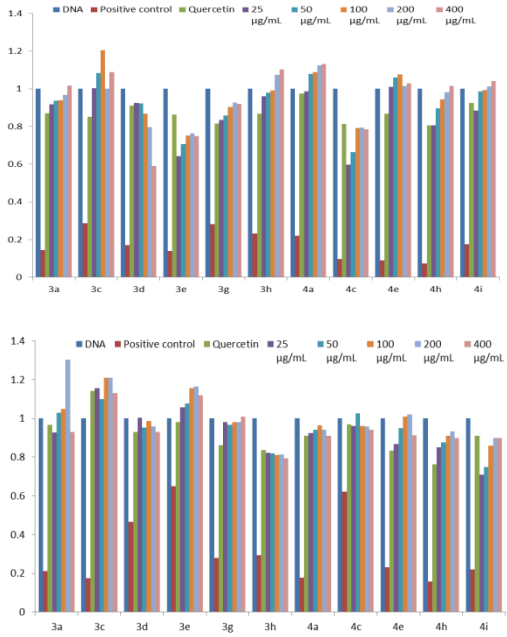




Abstract:

Vanillin is one of the most important natural products, used as a starting material in the new drug design procedures. Starting from vanillin, we can prepare different chalcones, which are known for their pronounced pharmacological and biological activities. For this reason some chalcone analogues have been synthesized from the corresponding vanillin derivatives. Further reaction with hydrazine in formic acid or acetic acid yielded 20 new pyrazoline compounds with *N*-formyl and *N*-acetyl groups, respectively. All new compounds were well characterized by IR, ^{1}H and ^{13}C NMR spectroscopy and physical data. *In vitro* DNA protective potential of selected compounds on hydroxyl and peroxyl radical-induced DNA damage was investigated. The results showed that the new synthesized compounds had expressed potential to prevent DNA damage.

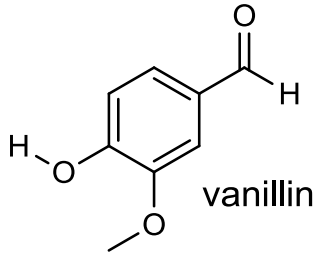
Keywords: dehydrozingerone analogues; cyclopropyl; pyrazoline; DNA

Introduction

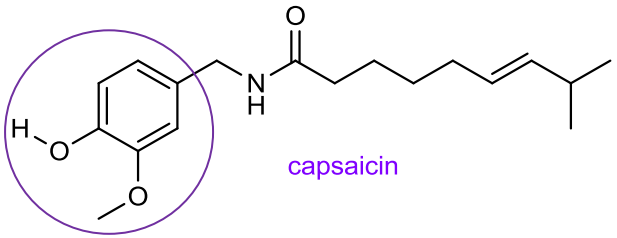
Many natural products were used as starting materials in the new drug design, and vanillin is one of them. Different kinds of compounds, which are isolated from some natural products, have vanillin fragment as part of their structure. Presence of those compounds in plants are usually responsible for very well expressed medicinal properties and they have been used in some forms of traditional medicine treatments.

Some of them are:

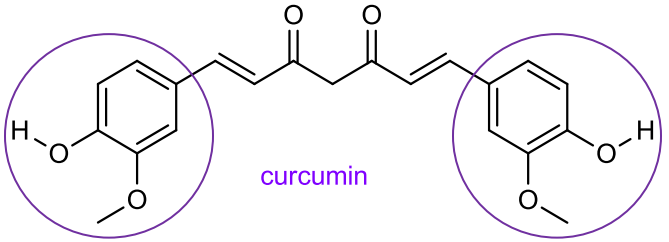
- **dehydrozingerone**-isolated from ginger,
- **capsaicin**-active component of chili peppers,
- **piperine**-isolated from black pepper,
- **curcumine**- produced by *Curcuma longa* plants



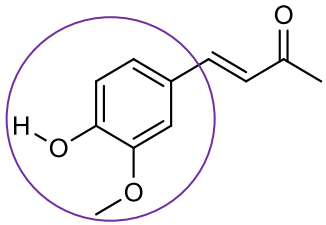
vanillin



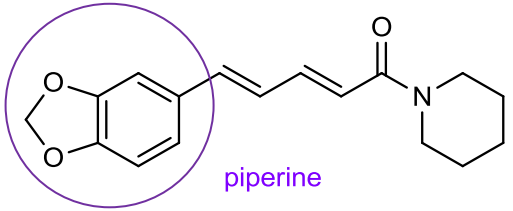
capsaicin



curcumin



dehydrozingerone

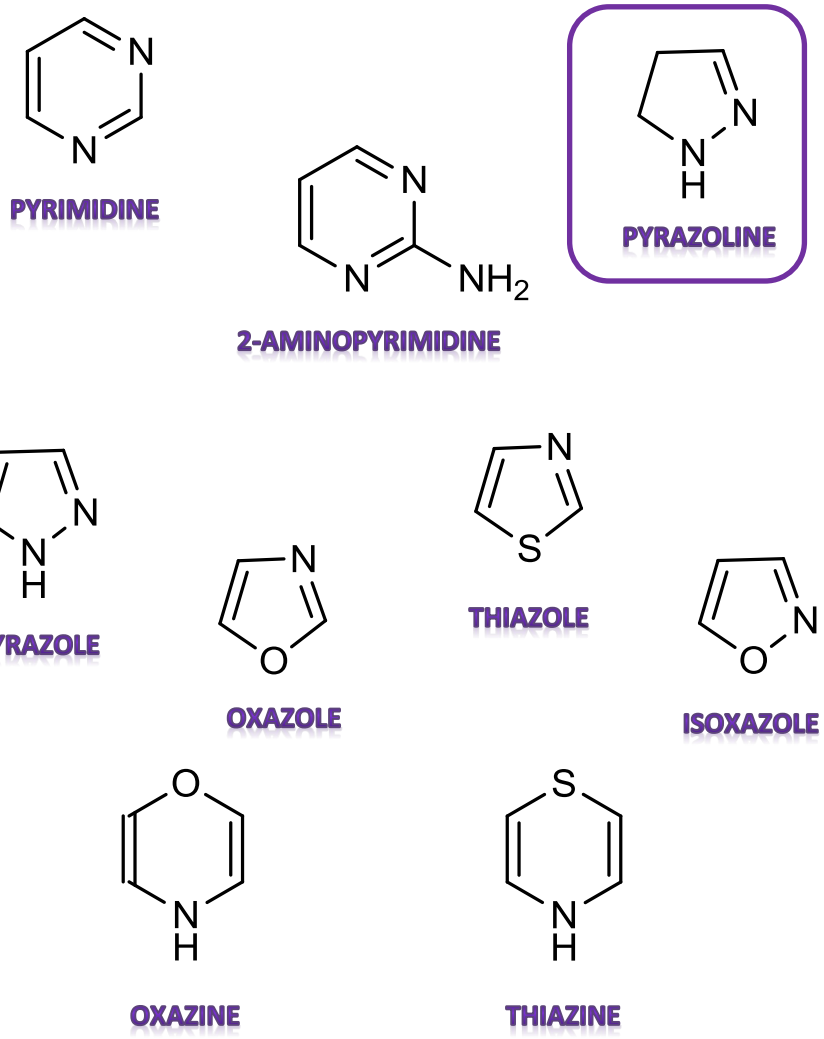


piperine

Dehydrozingerone is a well-known phenolic compound with a broad spectrum of biological activity. [1-3] It is the structural half analogue of curcumin, also exhibits an exhaustive range of activity such as antiinflammatory, antioxidative, antitumor, hypoglycaemic, hepatoprotective, anti-lipoperoxidation activity,…[4-7]

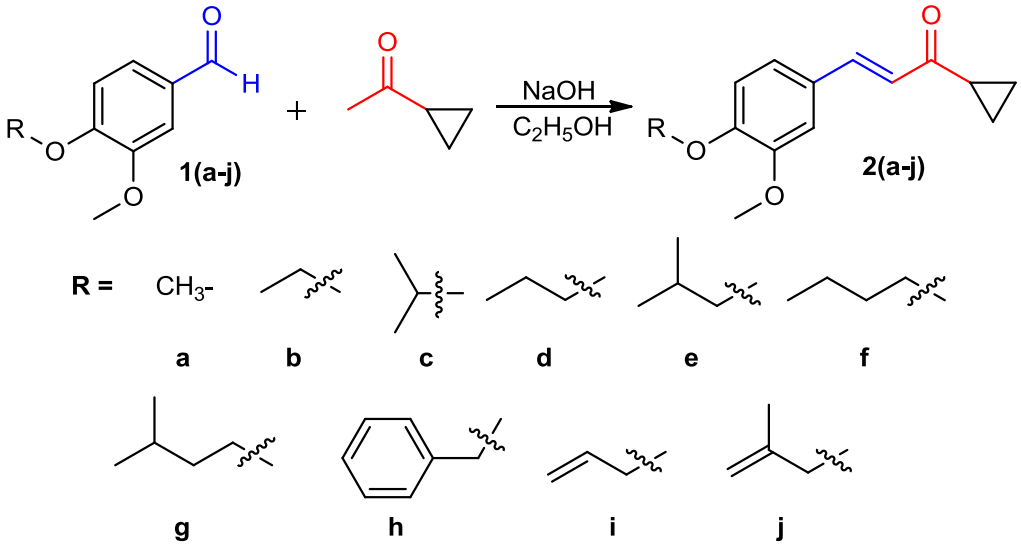
An interesting feature of these two compounds is that they serves as starting materials for the synthesis a large number of different kinds of compounds. Enone system presented in both of them is the key part of substrates and could be easily transformed into various usable heterocyclic derivatives such as pyrimidines, 2-aminopyrimidines, pyrazolines, pyrazoles, oxazoles, thiazoles, isoxazoles, oxazines, thiazines, …

Pyrazolines are extensive important synthons in the synthetic organic chemistry and drug designing.

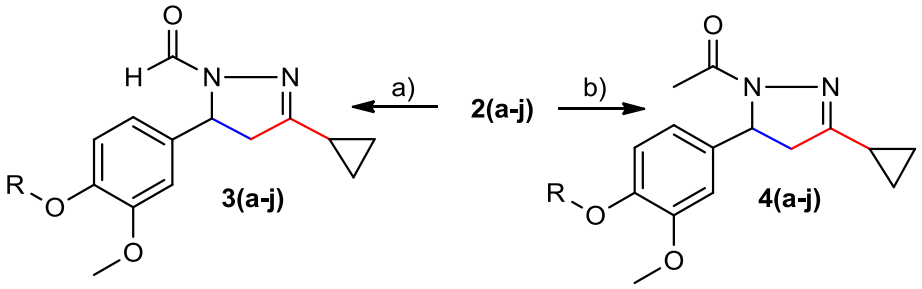


Results and discussion

In light of this, we supposed that vanillin is suitable substrate for further transformation. Alkylation/ allylation of the phenol group of the vanillin was achieved by a standard procedure[8-10] using an alkyl/allyl halide to yield the corresponding phenoxy compounds **1(a-j)**. Starting from our previous results in dehydrozingerone derivatives transformation[11,12] we decided to prepare some dehydrozingerone analogues, with rigid cyclopropane ring fragment instead of methyl one, in reaction *O*-alkyl vanillines and methyl cyclopropyl ketone. On this way, chalcone like compounds **2(a-j)**, (*E*)-1-cyclopropyl-3-(4-alkoxy-3-methoxyphenyl)prop-2-en-1-ones were synthesized.



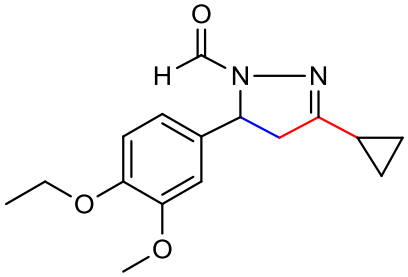
These enone compounds are very good starting point for different types of synthesis. In reaction with hydrazine hydrate in acidic solvent (boiling formic acid or acetic acid) obtained a series of novel *N*-formyl, **3(a-j)** and *N*-acetyl, **4(a-j)** pyrazoline derivatives in 69–98% yield.

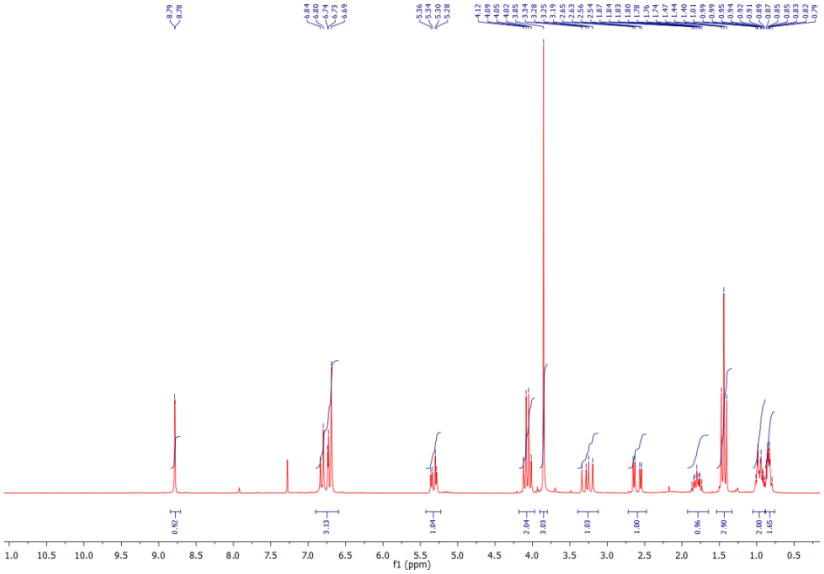


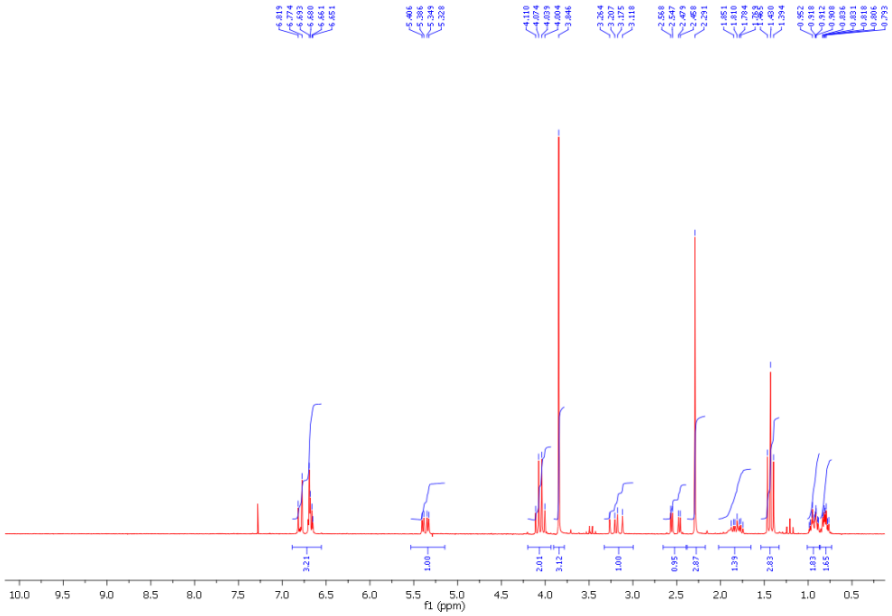
Reaction conditions: a) NH_2NH_2, HCOOH, reflux 5h
b) NH_2NH_2, CH_3COOH, reflux 5h

All new products were characterized by their spectral data (IR, ^{1}H NMR and ^{13}C NMR).

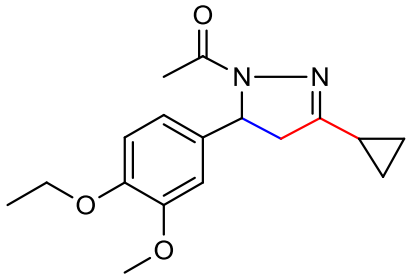
3-cyclopropyl-5-(4-ethoxy-3-methoxyphenyl)-4,5-dihydro--1*H*-pyrazole-1-carbaldehyde







1-(3-cyclopropyl-5-(4-ethoxy-3-methoxyphenyl)-4,5-dihydro--1*H*-pyrazol-1-yl)ethanone



In vitro DNA protective potential of compounds numbered from **3(a-j)** to **4(a-j)** were analysed using antioxidant assays. Whether selected compounds could protect against Fe^{2+}, H_2O_2 and AAPH-induced DNA damage Salmon sperm DNA was used as negative control while quercetin (100 µg/mL) was the reference compound.

The DNA protective activity of compounds in different concentrations (25, 50, 100, 200, and 400 µg/ml) against hydroxyl radical-induced DNA damage with Fe^{2+} and H_2O_2 was evaluated with Salmon sperm DNA.[13]

The DNA protective effect of derivatives (25, 50, 100, 200, and 400 µg/mL) against peroxyl radical-induced DNA damage with 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was assessed using herring sperm DNA. [14]

Table 1. DNA protective potential of selected compounds on hydroxyl radical–induced DNA damage

	Relative density[a]							
	DNA[b]	Positive control[c]	Quercetin 100 µg/mL[d]	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL
3a	1†‡	0.144*‡	0.870†	0.917†	0.936†	0.938†	0.968†	1.016†‡
3b	1†‡	0.158*‡	0.897*†	0.764*†	0.746*†	0.749*†	0.787*†	0.769*†
3c	1†‡	0.286*‡	0.850*†	1.002†‡	1.084†‡	1.205†‡	1.001†‡	1.089†‡
3d	1†‡	0.171*‡	0.911†	0.924†	0.921†	0.868†	0.796*†‡	0.591*†
3e	1†‡	0.139*‡	0.863*†	0.641*†‡	0.705*†‡	0.752*†‡	0.764*†‡	0.749*†‡
3f	1†‡	0.126*‡	0.830*†	0.789*†	0.820*†	0.874†	0.918†	0.930†
3g	1†‡	0.281*‡	0.816*†	0.834*†	0.857*†	0.904†	0.927†	0.919†
3h	1†‡	0.232*‡	0.867*†	0.960†	0.980*†	0.991†	1.073†‡	1.102†‡
3i	1†‡	0.221*‡	0.828*†	0.884†	0.932*†	0.960†	0.962†	0.960†
3j	1†‡	0.154*‡	0.821*†	0.930*	0.954*†	0.960†	0.909†	0.903†
4a	1†‡	0.219*‡	0.973†	0.987*	1.078†	1.087†	1.124†	1.131†
4b	1†‡	0.147*‡	0.795*†	0.510*†‡	0.568*†‡	0.655*†	0.698*†	0.760*†
4c	1†‡	0.097*‡	0.812*†	0.598*†‡	0.663*†‡	0.791*†	0.795*†	0.784*†
4d	1†‡	0.075*‡	0.710*†	0.644*†‡	0.585*†‡	0.491*†‡	0.390*†‡	0.043*†‡
4e	1†‡	0.089*‡	0.868*†	1.009†‡	1.060†‡	1.076†‡	1.014†‡	1.028†‡
4f	1†‡	0.111*‡	0.848*†	0.793*†	0.876*†	0.872*†	0.885*†	0.846*†
4g	1†‡	0.177*‡	0.866*†	0.749*†	0.780*†	0.834*†	0.798*†	0.812*†
4h	1†‡	0.074*‡	0.806*†	0.806*†	0.897*†	0.944†	0.982†	1.015†
4i	1†‡	0.175*‡	0.924*†	0.884*†	0.986*†	0.994†	1.011†	1.04†
4j	1†‡	0.126*‡	0.851*†	0.750*†	0.881*†	0.888†	0.897*†	0.857*†

- [a]The values are mean ± S.D. from three independent experiments
- [b]DNA: DNA control
- [c]Positive control: DNA damage control
- [d]Quercetin 100 µg/mL: standard drug quercetin
- *$p < 0.05$ when compared with the negative control group
- †$p < 0.05$ when compared with the positive control group
- ‡$p < 0.05$ when compared with the quercetin control group.

Table 1 indicates the protective effects of the selected compound from damage induced by Fe^{2+} and H_2O_2 in decreasing order: **3c**= **4a** = **4e** > **3h** = **4i** > **3a** = **4h** > **3j** > **3i** > **3g**> **3f**. DNA protective effect of **3a, 3c, 3f, 3g, 3h, 3i, 4a, 4h**, and **4i** was dependent upon concentrations compounds. The results indicated that compounds **4b** and **4d** cannot protect DNA against Fe^{2+} and H_2O_2 induced DNA damage and also compound **4b** is more effective than compound **4d**.

The decreasing order in the reduction of DNA damage were found to be **3c** = **3e** > **3a** = **4e** > **3d** = **3g = 4c** > **4a** > **3f** = **3i** = **4h** > **4g** = **4i** (Table 2). The protection against DNA damage induced with AAPH by **3g** and **4g** was dose-dependent, increasing with higher dosage. As well as in the previous assay the results indicated that the compound **4b** and **4d** possessed significantly less protective potential in relation to other compounds.

Table 2. DNA protective potential of selected compounds on peroxyl radical–induced DNA damage

	Relative density[a]							
	DNA[b]	Positive control[c]	Quercetin 100 µg/mL[d]	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL
3a	1†‡	0.211*‡	0.968†	0.928†	1.030†	1.050†	1.302†	0.93†
3b	1†‡	0.272*‡	0.801*†	0.659*†‡	0.731*†	0.787*†	0.817*†	0.843*†
3c	1†‡	0.175*‡	1.141†	1.156†	1.10†	1.211†	1.210†	1.132†
3d	1†‡	0.465*‡	0.930†	1.003†	0.952†	0.986†	0.959†	0.931†
3e	1†‡	0.649*‡	0.982†	1.057†	1.078†	1.157†	1.164†	1.120†
3f	1†‡	0.302*‡	0.781*†	0.839*†	0.866†	0.913†‡	0.942†‡	0.90†‡
3g	1†‡	0.28*‡	0.862*†	0.980†	0.967†	0.982†	0.980†‡	1.01†‡
3h	1†‡	0.295*‡	0.836*†	0.824*†	0.819†	0.810*†	0.813*†	0.793*†
3i	1†‡	0.228*‡	0.950†	0.968†	0.974†	0.885*†	0.92†	0.88*†
3j	1†‡	0.261*‡	0.712*†	0.815*†	0.869†	0.869*†	0.876*†	0.874*†
4a	1†‡	0.178*‡	0.91†	0.924†	0.941†	0.965†	0.942†	0.910†
4b	1†‡	0.171*‡	0.845*†	0.34*‡	0.42*†‡	0.40*†‡	0.49*†‡	0.42*†‡
4c	1†‡	0.623*‡	0.97†	0.96†	1.025†	0.961†	0.958†	0.941†
4d	1†‡	0.282*‡	0.970†	0.245*‡	0.215*‡	0.154*‡	0.124*‡	0.115*‡
4e	1†‡	0.232*‡	0.833*†	0.869*†	0.949†	1.01†‡	1.02†‡	0.914†
4f	1†‡	0.38*‡	0.723*†	0.712*†	0.803*†	0.822*†	0.83*†	0.78*†
4g	1†‡	0.325*‡	0.83*†	0.79*†	0.85†	0.88*†	0.913†	0.94†‡
4h	1†‡	0.157*‡	0.764*†	0.852*†	0.875*†	0.910†‡	0.934†‡	0.90†‡
4i	1†‡	0.22*‡	0.911†	0.71*†‡	0.75*†	0.86*†	0.90†	0.90†
4j	1†‡	0.351*‡	0.76*†	0.79*†	0.791*†	0.793*†	0.804*†	0.814*†

- [a]The values are mean ± S.D. from three independent experiments
- [b]DNA: DNA control
- [c]Positive control: DNA damage control
- [d]Quercetin 100 µg/mL: standard drug quercetin
- *$p < 0.05$ when compared with the negative control group
- †$p < 0.05$ when compared with the positive control group
- ‡$p < 0.05$ when compared with the quercetin control group.

Conclusions

- ✓ Vaniliin, as an easily accessible natural product, was modified by the simple synthetic procedure. The vanillic core give us a opportunity to tune their structure and properties by changing *O*-alkyl group in *p*-position. The new dehydrozingerone analogues were prepared by Claisen–Schmidt reaction and reacted with hydrazine in boiling formic or acetic acid. By this way new *N*-formyl and *N*-acetyl pyrazoline derivatives were prepared. All described compounds were synthesized in fairly good yields.

- ✓ All compounds were characterized by their spectral data (IR and ^{1}H- and ^{13}C-NMR). *In vitro* DNA protective potential of selected compounds for DNA damage caused by hydroxyl and peroxyl radicals, were performed.

- ✓ These results showed that the eleven compounds, namely **3a, 3c, 3d, 3e, 3g, 3h, 4a, 4c, 4e, 4h** and **4i,** could protect DNA against oxidative damage and that further studies might be beneficial. Selected compounds will be evaluated as *in vivo* genotoxic agents in Wistar rat livers and kidneys using the comet assay. Compounds without genotoxic activity well be applied prior to ethyl methane-sulfonate (EMS) to quantify potential antigenotoxic effect. Those compounds that will prevent EMS mutagenic effect can be applied in the cancer treatment to prevent the genotoxic effect of anticancer agents.

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