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Synthesis of new compounds in the series of aryl-substituted ureas with cytotoxic and antioxidant activity

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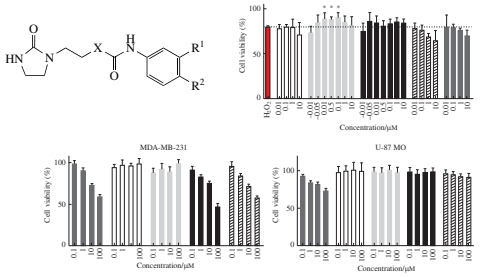
A series of aryl-substituted ureas and carbamates containing chlorinated aromatic and modified imidazolidinone moieties were synthesized. These compounds were found to be cytotoxic to breast cancer cell line MDA-MB-231, glioblastoma U-87 MG and neuroblastoma SH-SY5Y, but not to melanoma A-375.



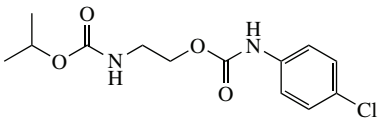
Keywords: synthetic cytokinins, substituted ureas, anti-stress effect, cytotoxicity, oxidative stress.

The search for anticancer drugs among existing cytokinins and cytokinin-like compounds is currently of considerable interest.[1] Cytokinins are small molecule biologically active compounds of phytohormone group that play an important role at all stages of plant growth and development.[2,3] In addition to the action exerted on plants, the biological activity of natural and synthetic cytokinins towards animals was reported.[4,5] The key aspect of this activity is a cytotoxic effect,[6] which is being studied on a number of malignant cells lines. Another interesting activity of cytokinin-like compounds is the ability to act as antioxidants, which can be of use in cosmetology.[7]

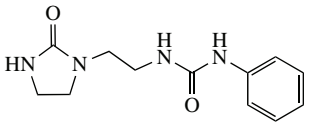
The antitumor activity of cytokinins is associated with regulation of cell proliferation and differentiation, similar to that occurring in plants. Thus, both N^6-substituted adenines and cytokinin-like arylureas showed positive *in vitro* and *in vivo* results against glioblastoma,[8,9] rhabdomyosarcoma, breast cancer, CNS tumors, colon cancer, lung cancer, leukemia, melanoma, prostate, ovaries and kidney cancers.[6,10,11] The mechanism of cytokinin action on tumor cells is thought to be based on stopping cell division, mainly by blocking various cyclin-dependent kinases,[12] inducing genotoxic stress in oncogenes, and activating the N-terminal kinase c-Jun.[2]

Arylureas like kartolin-2 and structurally related compound, *N*-[2-2-oxoimidazolidin-1-yl)ethyl]-*N′*-phenylurea (EDU analogue) (Figure 1) are similar in biological properties to natural adenine-type cytokinins, but are more synthetically available. Both of these compounds exhibit cytokinin-like activity; the ability of the latter to protect plants from the action of ozone by slowing down defoliation was also noted.[13,14] Similar antioxidant effects are observed in animal cells.[15]

Here we report the synthesis of aryl-substituted ureas and carbamates structurally close to EDU and kartolin-2 and the study of their biological activity.



Kartolin-2



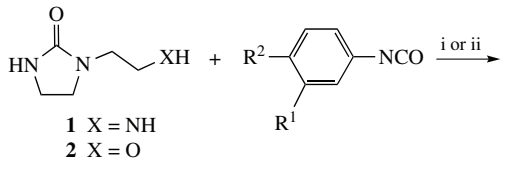
EDU analogue

Figure 1 Structure of cytokinin-like compounds.

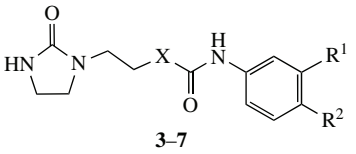
We suggested that the modification of EDU structure by introducing a chlorine atom into the aromatic ring may be of interest, because it reproduces a moiety similar to kartolin-2. Imidazolidinone **1** was obtained by the condensation of diethylene triamine with urea in 65% yield. Compound **2** is commercially available as a 75% aqueous solution. It was concentrated and dried by the azeotropic distillation of water with CCl_4 prior to synthesis.

Compounds **3**–**7** were produced by reaction of key compounds **1** and **2** with corresponding arylisocyanates in the presence of triethylamine in anhydrous toluene (for arylureas) or in acetonitrile (for arylcarbamates) in yields ranging from 35 to 68% (Scheme 1; for details, see Online Supplementary Materials).

The resulting compounds were tested for cytotoxicity against four human tumor cell lines: melanoma A-375, glioblastoma U-87 MG, breast cancer MDA-MB-231 and neuroblastoma SH-SY5Y. Cells were incubated with the compounds for 24 h, and the cell viability was determined by the MTT assay.



1 X = NH
2 X = O



3–7

	X	R^1	R^2	Yield (%)
3	NH	H	H	35
4	O	H	Cl	54
5	NH	H	Cl	53
6	O	Cl	Cl	39
7	NH	Cl	Cl	68

Scheme 1 *Reagents and conditions*: i, Et_3N, MeCN, room temperature, 24 h (**4**, **6**); ii, Et_3N, toluene, 5 °C, 24 h (**3**, **5**, **7**).

It was found that several compounds were cytotoxic for breast cancer and neuroblastoma cell lines (Figure 2), decreasing viability to 50–60% at 100 μM. Only parent compound **3** was cytotoxic for U-87 MG line, causing 40% viability decrease at 100 μM. We can conclude that introduction of chlorine into the benzene ring significantly reduced the cytotoxicity for glioblastoma. Beside that, the decrease of substituent size (replacing imidazolidinone with ethanol) and the introduction of chlorine into *para*-position of the benzene ring led to a loss of the activity.

Leaders in the cytotoxicity tests were compounds **3** and **7**. Their distinguishing feature is the presence of an imidazolidinone fragment or its analogue and this is consistent with the published cytotoxicity data.[16,17] Regardless of low cytotoxicity, a selectivity of action was present, which makes further search for analogous compounds with increased activity promising.

Since there are indications of a possible antioxidant effect of cytokinin analogues in literature, the protective effect of synthesized compounds was studied in three models of oxidative stress caused by reactive oxygen species. These models were protection against H_2O_2, chemical hypoxia induced by $CoCl_2$,[18] and low glucose conditions[19] (Figure 3). The toxic agent was added simultaneously with the tested compounds and incubated with the cells for 24 h, after that cell viability was determined using the MTT assay. Previously developed dose-response curves for H_2O_2 and $CoCl_2$ provided a basis on which the definite concentrations were selected for causing the death of 30–40% of cells to avoid nonspecific toxicity.[20]

The study of the protective effect showed that a significant increase in cell viability was observed only under oxidative stress conditions for the compound containing chlorine only in *para*-position (**4**). On the other hand, compound **5** with the same modification did not have a protective effect. The presence of such neuroprotective activity is consistent with previously reported data for halogen-containing 4-(cycloalkyl) piperidines.[21]

Thus, it has been found that the introduction of chlorine into the aromatic ring of cytokinin analogues significantly reduces cytotoxicity, however gives the molecule the capability of protecting cells from oxidative stress induced by H_2O_2.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.03.007.

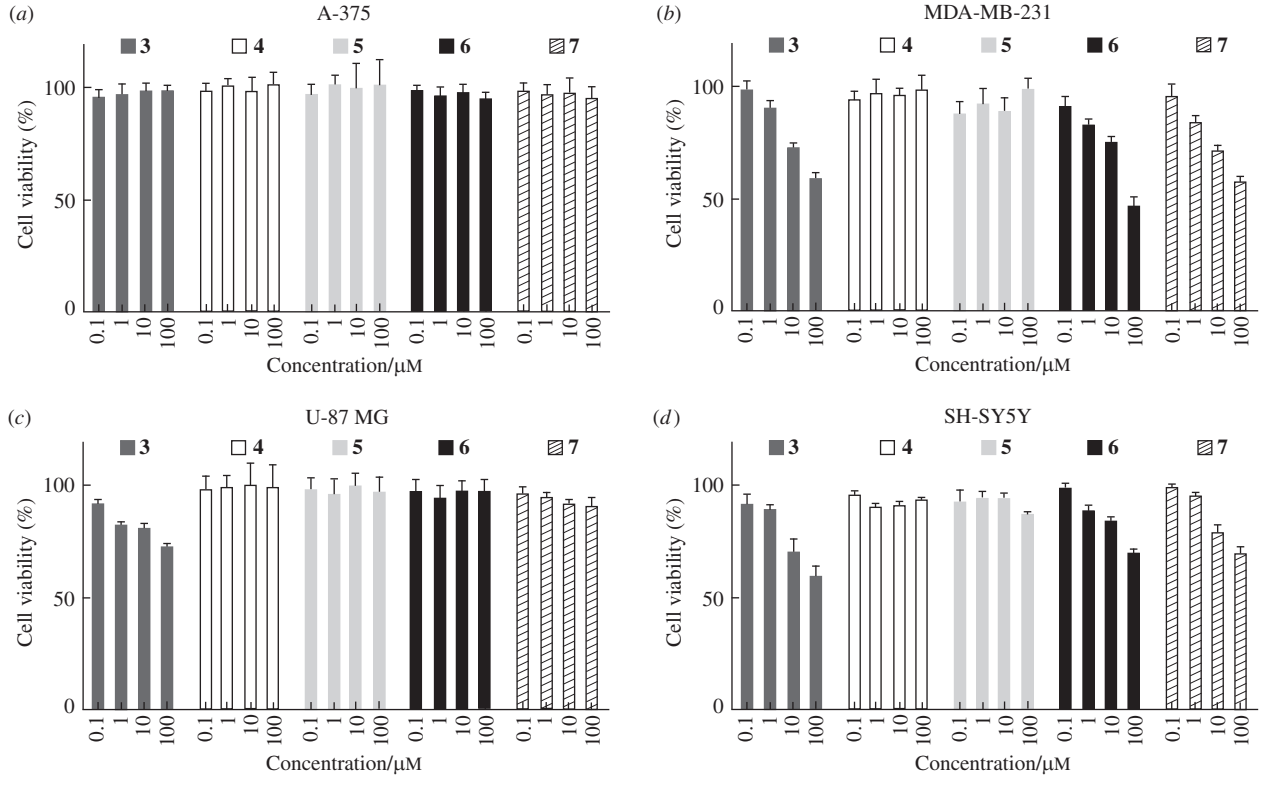

Figure 2 Cytotoxic effect of the synthesized compounds **3–7** against different lines of the tumor cells (*a*) A-375, (*b*) MDA-MB-231, (*c*) U-87 MG and (*d*) SH-SY5Y. Incubation time 24 h, MTT assay, mean ± SD ($N = 3$).

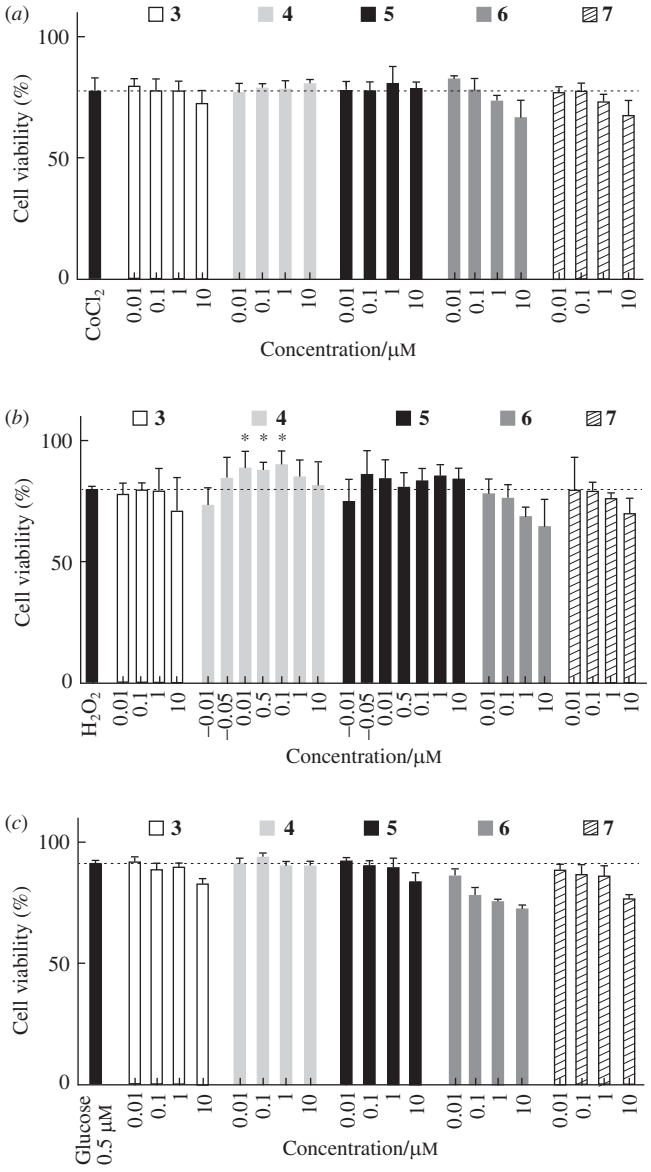

Figure 3 Protective effect of synthesized compounds **3**–**7** in stress models. (*a*) Chemical hypoxia (800 μM $CoCl_2$), (*b*) oxidative stress (1200 μM H_2O_2), (*c*) hypoglycemia (1 mM glucose concentration in cultural medium). Incubation time 24 h, MTT assay, mean ± SD (N = 3); * – statistically significant difference from control, $p < 0.05$, ANOVA with Dunnett's post-test.

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