

INVESTIGATION OF THE EFFECT OF STEROIDAL IMIDAZOHETEROCYCLES HORMONE DERIVATIVES ON JEG-3 TROPHOBLAST CELLS OF CHORIOCARCINOMA

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ABSTRACT. This study aimed to examine the biological activities of unique steroid hormone derivatives – steroidal imidazoheterocycles. The activity synthesized steroidal compounds were tested for the viability of JEG-3 trophoblast cells of choriocarcinoma. The results of the MTT cytotoxicity test showed that most of the examined steroidal imidazoheterocycles act proliferatively on the JEG-3 cell line, except (E, F, and J). Antiproliferative activity of these derivatives is probably influenced by different substituents at position 17-C atom in the chemical structure of the hormone and by different substituents on the oxygen atom of acetamide group in the hormone molecule. In order to better confirm the antiproliferative activity of these derivatives, it is necessary to perform complex research on a large panel of cancer cell lines and by different assay.

Keywords: JEG-3, steroidal imidazoheterocycles, choriocarcinoma, MTT.

INTRODUCTION

The placenta is one of the main tissues that synthesize steroid hormones and affect the normal maintenance of pregnancy and fetal development (KARAHODA *et al.*, 2021). Investigations of the mechanisms of synthesis, metabolism and secretion of steroid hormones are very important because disturbance of steroid hormones during pregnancy can lead to various disorders. The most common disorders are gestational diabetes, preeclampsia, Intrauterine Growth Restriction, and Gestational trophoblastic diseases (GTD) (SOLANO and ARCK, 2020).

Gestational trophoblastic diseases occur due to abnormal, proliferating transformation of placental cells (trophoblasts). Gestational trophoblastic neoplasia (GTN) is a malignant

formation of GTD and includes invasive moles, epithelioid trophoblastic tumor (ETT), placental site trophoblastic tumor (PSTT) and choriocarcinoma (LURAIN, 2010).

Choriocarcinoma is a rare, highly malignant germ cell carcinoma (BENTLEY, 2003). There are two types of choriocarcinoma, namely gestational (GC) and non-gestational (NGC) which differentiate in the genetic profile, morphology, pathophysiology and prognosis (MELLO *et al.*, 2017).

Choriocarcinoma research provides very important information on the etiology, pathogenesis and potential therapy. A very important alternative to research in patients with choriocarcinoma is immortalized trophoblast cells grown *in vitro*. Research methods on cell lines are very important because it is possible to repeat experiments several times and they can test the whole spectrum of bioactive substances of natural or artificial origin. The three most widely used cell lines for placental choriocarcinoma are BeWo, Jar, and Jeg-3 (ORENDI *et al.*, 2011). In this research, the model system for investigating the effects of substances obtained synthetically is the JEG-3 choriocarcinoma cell line.

Many natural and synthetic substances have been tested in the placental choriocarcinoma cell line. Some of them are daidzein, neuropeptide Y, fungicide ziram (CHEN *et al.*, 2017; MATIĆ *et al.*, 2017; ZHENG *et al.*, 2018). There is an even larger number of studies on the influence of steroid hormones and their derivatives on the JEG-3 cell line, but also on the modulation of their synthesis (LONG *et al.*, 1998; HONKISZ and WÓJTOWICZ, 2015; XU *et al.*, 2019).

Studies on steroid hormones and their derivatives have brought many important conclusions about the proliferation, migration, metastasis, and apoptosis of placental choriocarcinoma cells. Screening of 10 newly synthesized steroid hormones could lead to isolation of potential substances that would improve the treatment of patients with placental choriocarcinoma.

MATERIALS AND METHODS

Chemicals

In this study, the activity of ten new steroidal imidazoheterocycles on the placental choriocarcinoma cell line was examined. Tested compounds were synthesized on N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences. Steroidal imidazo[1,2-a]pyridines were synthesized from 2-aminopyridines and steroidal ketones in aerobic conditions with Fe (III) -Catalyzed synthesis as described in the previous paper. (SAMANTA *et al.*, 2020).

Cell preparation and culturing

In this study, the effect of steroidal imidazoheterocycles was tested on a human cytotrophoblastic choriocarcinoma cell line JEG-3 (American Type Culture Collection). Choriocarcinoma cells were grown in DMEM medium (Dulbecco's modified Eagle medium, GIBCO) in 75 cm² flasks up to 80% coverage. JEG-3 cells were seeded in 96-well plates, 10.000 cells per well. The study included ten different steroidal imidazoheterocycles (Fig. 1) at six different concentrations: 100 µM, 10 µM, 1 µM, 100 nM, 10 nM, and 1 nM.

The treated cells in microtiter plates were left in the Medline CO₂ incubator until the treatment time expired. Cells were exposed to the steroidal imidazoheterocycles for 24 h and 72 h. At the end of the treatment period, it was necessary to examine the effect of the tested substances.

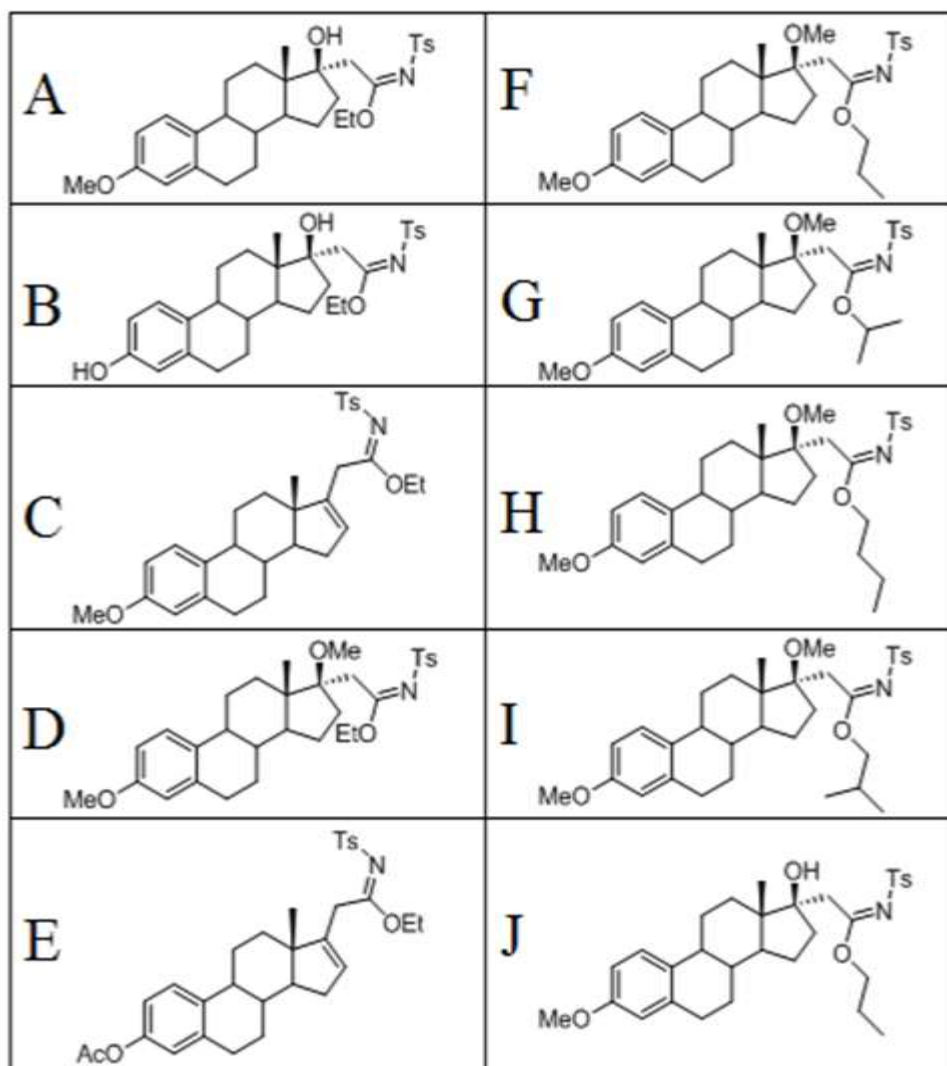


Figure 1. Steroid hormone derivatives.

MTT assay

The effect of steroidal imidazoheterocycles on the viability of the JEG-3 cell line was examined using the MTT assay (MOSMANN, 1983). MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is a substance that is absorbed by living cells and converted into a water-insoluble formazan with the help of mitochondrial reductase. To each of the wells was added 100 MTT solution (5 mg/ml) and the plates were left to incubate at 37°C in a Medline CO₂ incubator for 2 hours. After the incubation period, DMSO (Dimethyl Sulfoxide) was added to dissolve the formazan crystals. Absorbance (Abs) was measured on an ELISA microtiter plate reader (RT-21000C), and the percentage of cell viability was determined by the formula:

$$\text{Abs (treated cells)} / \text{Abs (untreated cells-control)} \times 100.$$

Statistical analysis

The biological activity of steroidal imidazoheterocycles was examined in two experiments, performed in triplicate for each dose. The obtained results are expressed as mean \pm SE. Correlation between variables was obtained using the statistical software package SPSS (Chicago, IL) (SPSS for Windows, ver. 17, 2008). Statistical significance * was determined

using one-way ANOVA test for multiple comparisons. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The results obtained by analyzing the cytotoxicity of newly synthesized steroidal imidazoheterocycles (A-J) using the MTT cell viability test are presented graphically. The effect of each of the ten substances was tested at six different concentrations (100 μM , 10 μM , 1 μM , 100 nM, 10 nM, and 1 nM) and at two exposure times (24 h and 72 h). Figure 2 shows the effect of the tested steroidal imidazoheterocycles in short-term treatment (24 h).

The results of the effect of steroidal imidazoheterocycles on the viability of the JEG-3 cell line after long-term treatment (72 h) are shown in Figure 3. The results of the effect of steroidal imidazoheterocycles on the viability of the JEG-3 cell line after long-term treatment (72 h) are shown in Figure 3.

After statistical processing, the results showed that synthetically obtained steroid imidazoheterocycles stimulated JEG-3 cells to increase proliferation, except the E, I, and J (Figs. 1 and 2).

Steroids containing various heterocycles such as imidazole, pyrazole and thiazole have a wide range of applications in medicine and their structural modifications give potentially new therapeutic agents (DEEP *et al.*, 2017). Synthesis and investigation of biological activities of imidazopyridine derivatives are used in medicine and oncology. Previous data have shown that these derivatives can inhibit cell growth and key signaling molecules such as EGFR, VEGFR or PI3K (GOEL *et al.*, 2016).

Biological activity has been demonstrated for a large number of compounds from the group of nitrogen imidazopyridine. These derivatives may have antiparasitic, antimicrobial, anti-inflammatory, anti-asthmatic, and anticancer activity (ENGUEHARD-GUEIFFIER *et al.*, 2007; BAVISKAR *et al.*, 2015; SCHERBAKOV *et al.*, 2018; SAMANTA *et al.*, 2020).

In this work, for the first time, as far as we know, the activity of ten newly synthesized imidazoheterocycles on the JEG-3 cell line was examined. Previous studies have examined a series of different derivatives but only in the breast cancer and prostate cancer cell line (RASSOKHINA *et al.*, 2016). The cytotoxic effect was demonstrated only on the MCF-7 breast cancer cell line at the micromolar level.

However, the results obtained in this study showed that the tested imidazopyridine derivatives on the JEG-3 cell line show an increase in proliferation and only derivatives labelled as E, I, and J have a statistically significant antiproliferative effect. These derivatives have C-17 substituted steroidal N- sulfonylimidates synthesized using Cu-catalyzed azidealkyne cycloaddition. The compound I at five of six concentrations showed an antiproliferative effect after 72h cultures, while substance J had an antiproliferative activity at all six concentrations at both exposure times.

The results of the tested imidazopyridine derivatives in the highest concentration of 100 μM after long-term treatment of 72 h show that under these conditions substances D, E, F and J show statistically significant antiproliferative activity. A comparative presentation of the effects of all ten tested derivatives in the highest concentration (100 μM) is shown in the graph (Fig. 4).

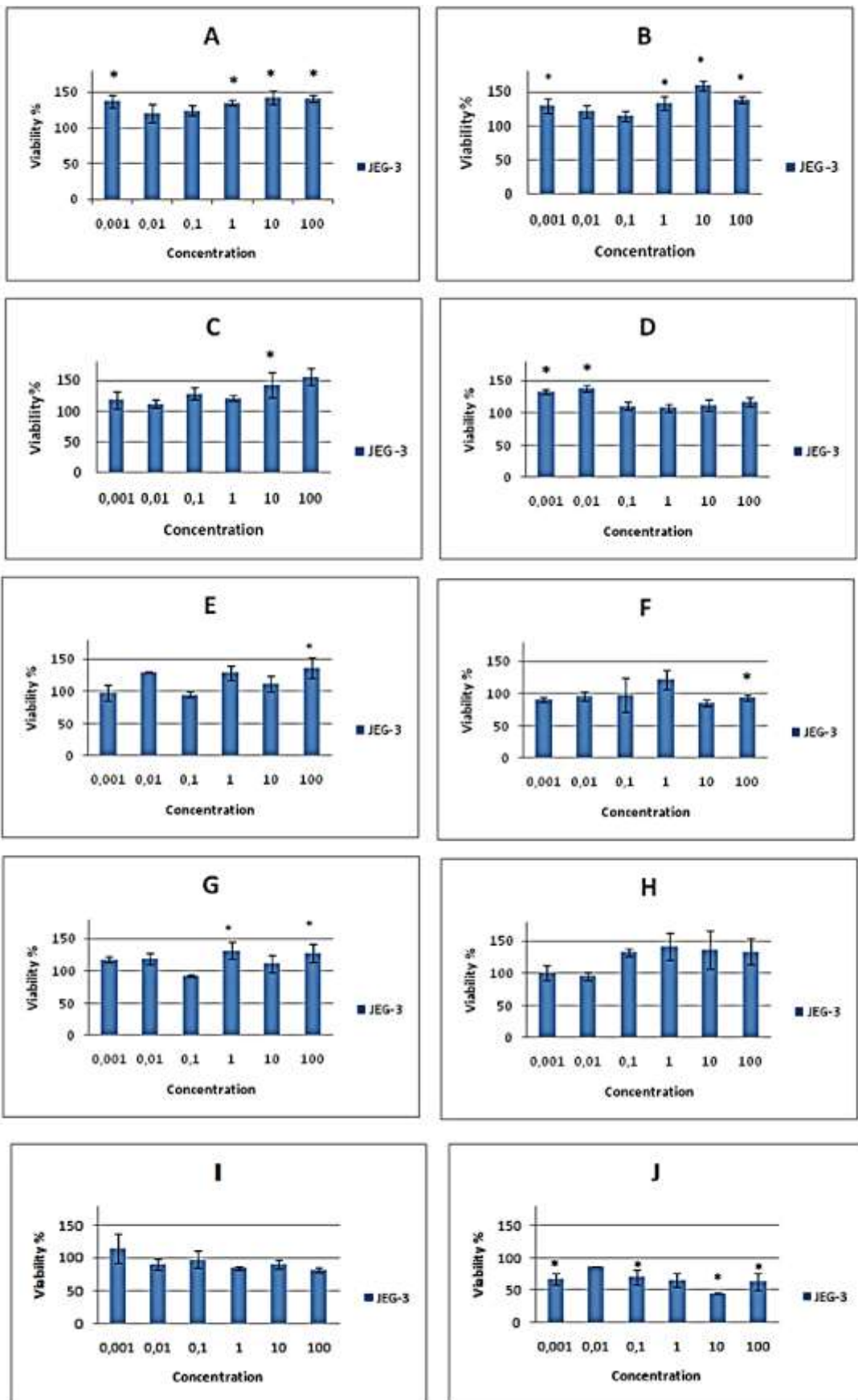


Figure 2. Effects of short-term (24 h) treatment of different concentrations of steroidal imidazoheterocycles on JEG-3 choriocarcinoma cell line.

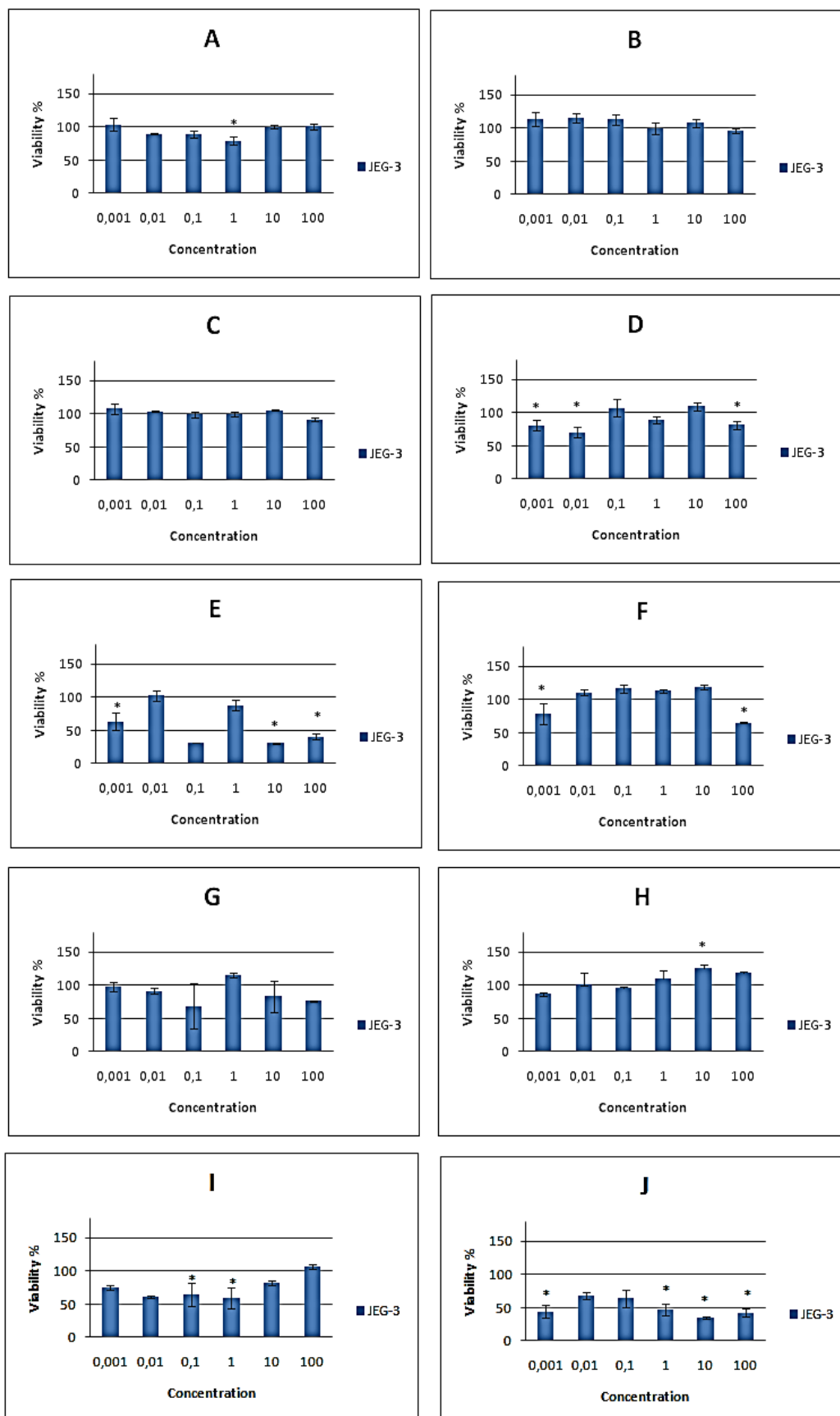


Figure 3. Effects of long-term (72 h) treatment of different concentrations of steroidal imidazoheterocycles on JEG-3 choriocarcinoma cell line.

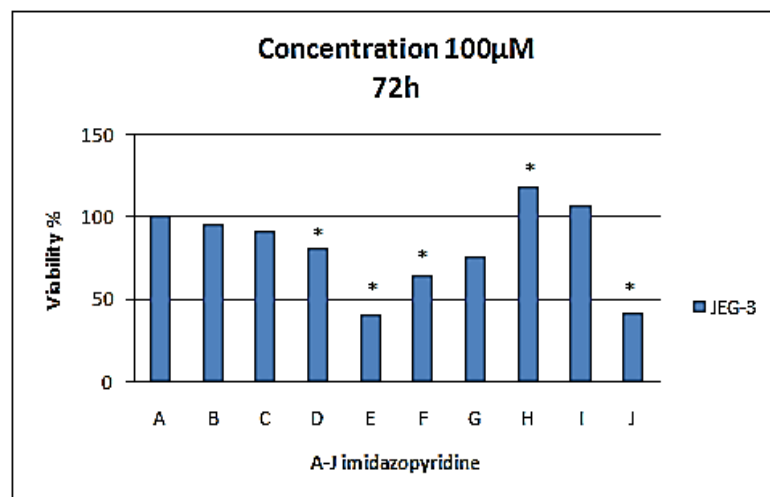


Figure 4. Effect of steroidal imidazopyridines A – J.

Under these experimental conditions, no trend between cytotoxicity and test substance concentration was demonstrated. For this reason, it would be good to repeat the research and better examine the mechanisms responsible for trophoblast's death, using diverse assay (JURISIC *et al.*, 2015).

CONCLUSION

The obtained results showed that the antiproliferative effect on the JEG-3 choriocarcinoma cell line is brought only by two derivatives of imidazopyridine. This could be mediated probably and mostly because of different substituents at position 17-C atom in the chemical structure of the hormone (hydroxy or methoxy substituent), and by different substituents on the oxygen atom of acetamide in the hormone molecule.

Compounds I and J are C-17 substituted steroidal N-sulfonylimidates synthesized using Cu-catalyzed azidealkyne cycloaddition. The synthesis and testing of new imidazopyridines and their derivatives can yield effective bioactive substances and potential drugs with an antiproliferative effect on the cancer cell lines.

In this study, the weak antiproliferative activity of newly synthesized imidazopyridine derivatives was demonstrated on the JEG-3 choriocarcinoma cell line of the placenta. In order to better confirm the antiproliferative activity of these derivatives on molecular level, it is necessary to perform research on a large cancer cell line panel.

Acknowledgments

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