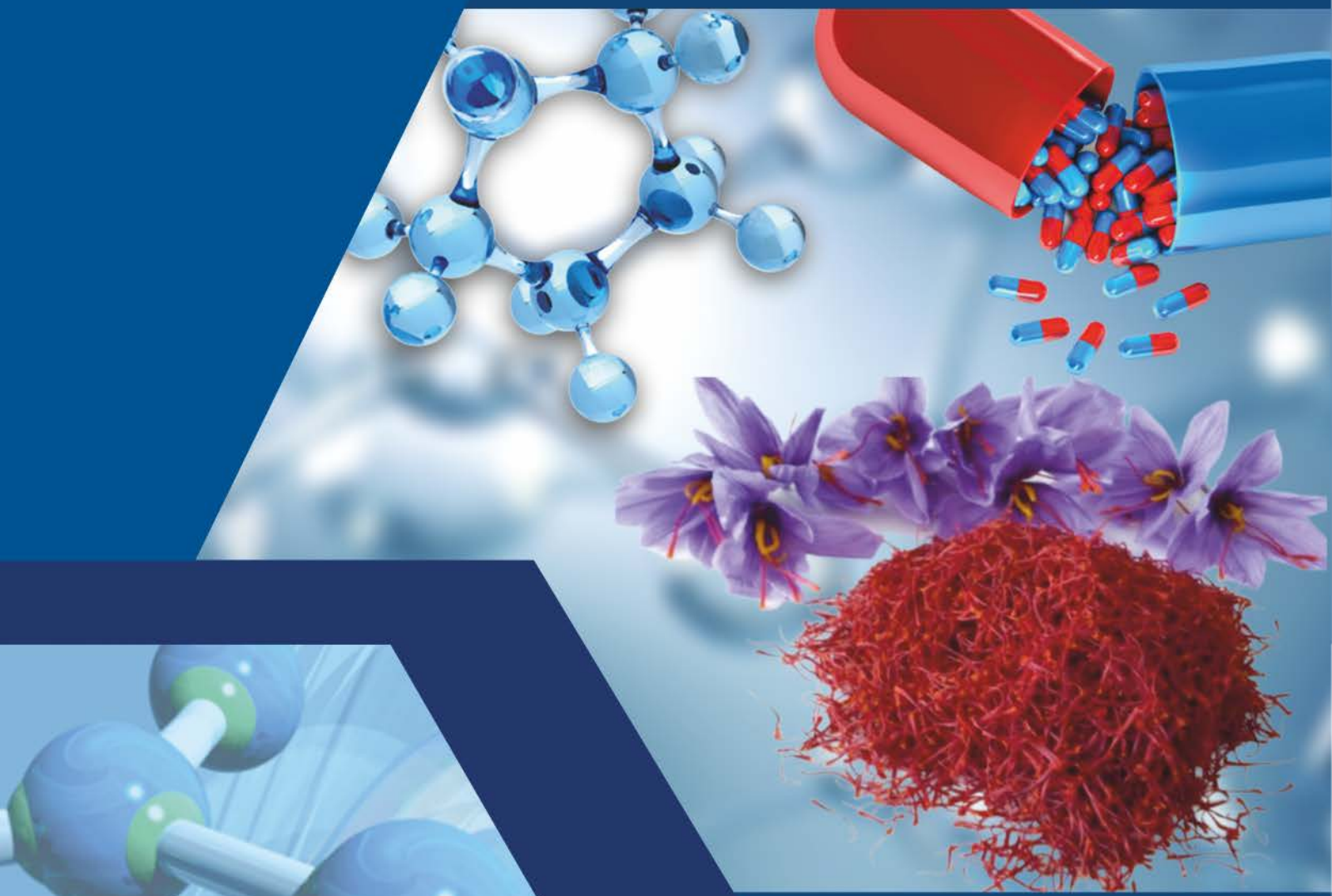
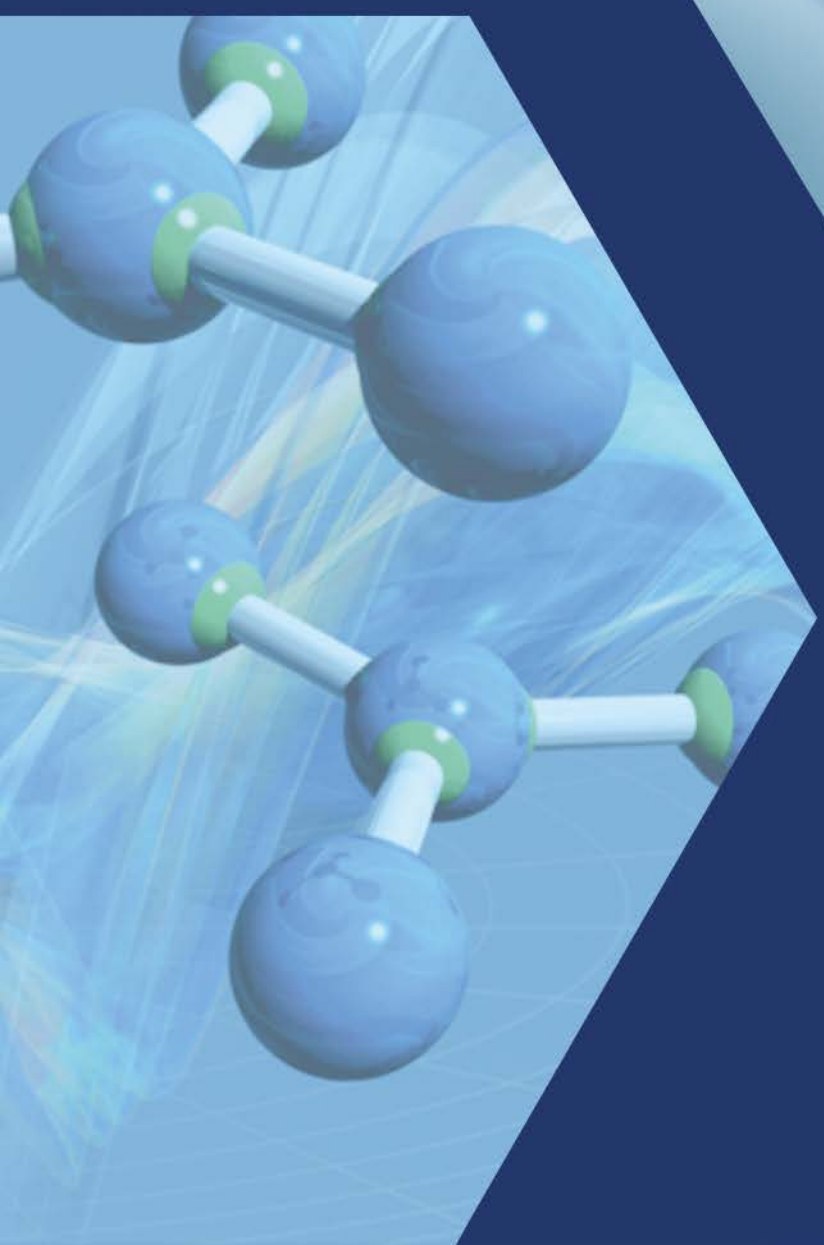
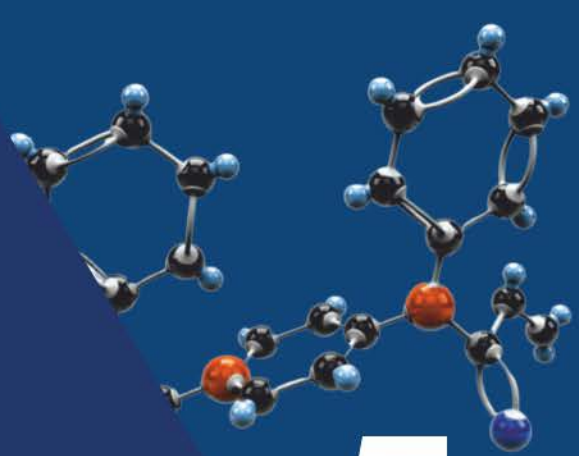
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CYTOTOXICITY OF ARENOBUFAGIN AND ITS DERIVATIVES AGAINST H9c2 CELLS

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Arenobufagin is a naturally occurring bufadienolide with diverse biological activities; however, its potential cardiotoxicity may limit its pharmacological application. In the present study, the cytotoxic effects of arenobufagin and two newly synthesized derivatives (Ar-004 and Ar-005) were evaluated in H9c2 rat cardiomyoblast cells using the MTT assay after 24 h of exposure. The tested compounds did not significantly affect cell viability at concentrations of 1 and 10 μM. In contrast, arenobufagin markedly reduced H9c2 cell viability at 100 μM, whereas both derivatives exhibited substantially lower cytotoxicity under the same experimental conditions. These findings suggest that structural modification of arenobufagin may reduce its cytotoxic effects on normal cardiac cells while preserving the need for further pharmacological investigation. Additional studies are warranted to elucidate the mechanisms underlying these observations and to comprehensively evaluate the safety profile of arenobufagin derivatives.

Keywords: *arenobufagin; bufadienolides; H9c2 cells; cardiomyoblasts; cytotoxicity; MTT assay.*

Introduction. Bufadienolides are a class of naturally occurring cardiotonic steroids predominantly isolated from toad venom and several plant species [1]. These compounds have attracted considerable scientific interest because of their diverse pharmacological activities, including cardiotonic, anti-inflammatory, antiviral, and antitumor effects [2]. Among them, arenobufagin, a bufadienolide isolated from the venom of *Bufo viridis*, has been extensively investigated owing to its potent biological activity and its ability to modulate multiple intracellular signaling pathways [3].

Chemical modification of naturally occurring compounds is a widely employed strategy for improving biological activity,

modifying pharmacokinetic properties, and reducing undesirable toxicity. Structural modification of arenobufagin may alter its interaction with biological targets and consequently influence its cytotoxic profile. Therefore, newly synthesized derivatives should be evaluated not only for their desired pharmacological activity but also for their effects on normal cells to obtain preliminary information regarding their biological safety.

The H9c2 rat cardiomyoblast cell line is one of the most commonly used in vitro models for investigating cardiac toxicity and evaluating the safety of biologically active compounds. Although H9c2 cells do not completely reproduce the characteristics of mature cardiomyocytes, they retain several morphological and biochemical properties of cardiac muscle cells and provide a convenient model for preliminary cytotoxicity screening.

Aim of the study. To evaluate the cytotoxic effects of arenobufagin and two newly synthesized derivatives on H9c2 rat cardiomyoblast cells using the MTT cell viability assay. The obtained results provide preliminary information regarding the effects of these compounds on normal cardiac cells and may contribute to the further biological evaluation of arenobufagin derivatives.

Materials and methods. Arenobufagin and two structurally modified derivatives were evaluated in the present study. The derivatives named Ar-004 and Ar005 were synthesized in our laboratory, and their chemical structures are shown in Figure 1. The compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions and diluted with culture medium to the desired concentrations immediately before use.

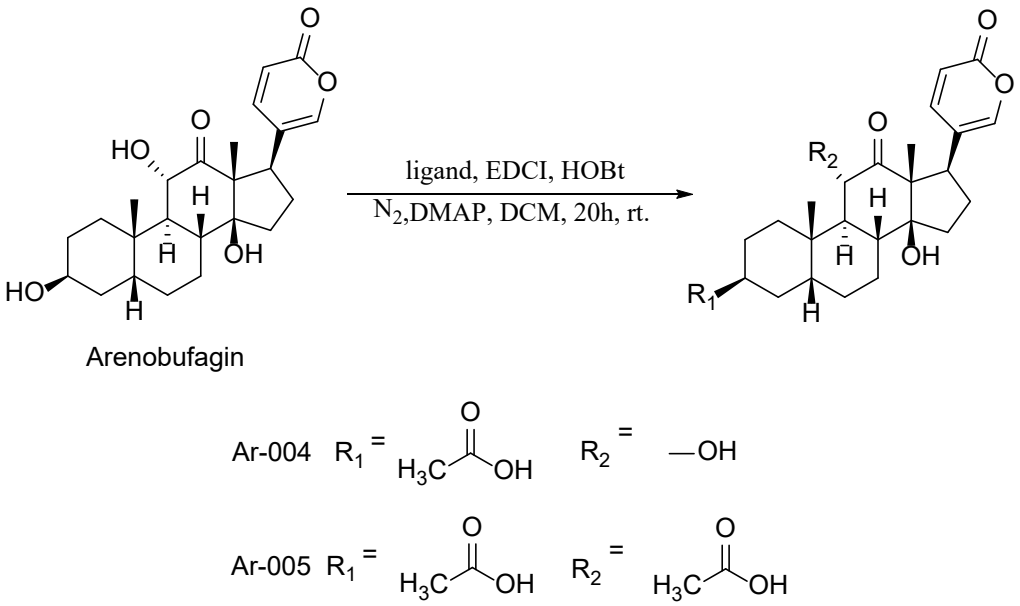


Figure 1. **Structures of arenobufagin and its derivatives.**

The MTT dye 2-4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide, Dulbecco's Modified Eagle's Medium (DMEM), phosphate-buffered saline (PBS), trypsin from porcine pancreas were purchased from Sigma-Aldrich (Budapest, Hungary). Penicillin-streptomycin mix, 96-well plates, and cell culture flasks were obtained from Thermo-Fisher (Darmstadt, Germany, CAS number: 156499).

The MTT assay was carried out according to the method described in [4]. The culture medium was Dulbecco's Modified Eagle's Medium (DMEM) (Sigma Aldrich, Budapest, Hungary) supplemented with 1200 mM L-glutamine (Glutamax, Thermo-Fisher, Darmstadt, Germany, CAS: 35050061), 10% fetal bovine serum (FBS), 4.5 g/L glucose, and penicillin–streptomycin (0.1 mg/mL) (Sigma-Aldrich, Budapest, Hungary). Cells were incubated at 37 °C in 5% CO2 humidified air and maintained by continuous passaging.

Cells were seeded at a 10,000 cells/well density on 96-well plates to achieve complete confluence. After draining the medium, the cells were washed with PBS and incubated with the test solutions for 24 h at 37 °C in 5% CO_2. After removing the test solutions, MTT solution (5 mg/mL) was added to the cells and incubated for 3 h in a light-free chamber. In the presence of living cells, the yellow tetrazolium dye was converted into purple formazan crystals by the action of oxidoreductase enzymes. The purple crystals were dissolved in a 25:1 ratio of isopropanol/hydrochloric acid. The absorbance of the samples was measured by a Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer (USA). All tests were carried out in triplicate.

Results and discussion. The MTT assay was used to evaluate the effects of arenobufagin and its derivatives on the viability of H9c2 cardiomyoblast cells after 24 h of exposure. Cell viability was determined at compound concentrations of 1, 10, and 100 µM. The results are presented in Figure 2. The results demonstrated that arenobufagin and its derivatives, Ar-004 and Ar-005, did not significantly affect the viability of H9c2 cells at concentrations of 1 and 10 µM. In contrast, exposure to arenobufagin at 100 µM markedly decreased cell viability by approximately 70% compared with the untreated control, indicating pronounced cytotoxicity at this concentration. Under the same experimental conditions, neither Ar-004 nor Ar-005 significantly reduced the viability of H9c2 cells at 100 µM.

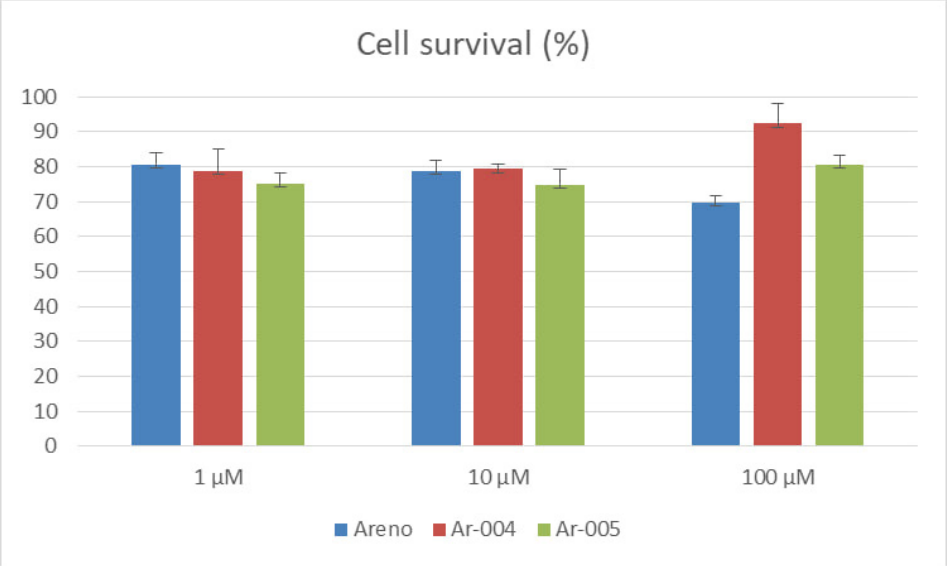


Figure 2. **Results of the study of the effect of arenobufagin and its derivatives Ar-004 and Ar-005 on H9c2 cardiomyoblast cells using the MTT test.**

These findings suggest that the structural modification of arenobufagin altered its cytotoxic profile in normal cardiac cells. While the parent compound exhibited substantial cytotoxicity at the highest tested concentration, both derivatives maintained significantly higher cell viability. This observation indicates that the chemical modifications introduced into arenobufagin may reduce its cytotoxic effects on H9c2 cardiomyoblasts. Nevertheless, additional studies involving a broader concentration range, mechanistic investigations, and *in vivo* evaluation are required to further characterize the cardiac safety of these derivatives.

Bufadienolides exert their biological effects primarily through inhibition of Na^+/K^+-ATPase [5]. However, structural modification of the parent compound may alter its interaction with the enzyme or affect cellular uptake, thereby influencing cytotoxicity. The higher viability of H9c2 cells observed after treatment with Ar-004 and Ar-005 suggests that these structural modifications reduced the toxic effects of arenobufagin toward normal cardiac cells. Although the precise mechanisms remain to be elucidated, the present findings indicate that chemical modification may improve the preliminary safety profile of arenobufagin derivatives.

Conclusion. The present study evaluated the effects of arenobufagin and two newly synthesized derivatives on the viability of H9c2 rat cardiomyoblast cells using the MTT assay. Arenobufagin and its derivatives did not affect cell viability at concentrations of 1 and 10 μM. In contrast, arenobufagin exhibited pronounced cytotoxicity at 100 μM, whereas the derivatives Ar-004 and Ar-005 maintained substantially higher cell viability under the same experimental conditions. These findings suggest that structural modification of arenobufagin may reduce its cytotoxic effects on normal cardiac cells. Although the results are preliminary, they provide a basis for further biological and pharmacological investigations, including detailed mechanistic studies, broader dose-response analyses, and in vivo evaluation of the safety profile of arenobufagin derivatives.

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References

1. Hernández-Parra, H., et al., *Bufadienolides as bioorganic anticancer agents: Mechanistic insights into apoptosis, cell cycle arrest and signal pathway modulation.* Bioorganic Chemistry, 2025. **164**: p. 108801.

2. El-Seedi, H.R., et al., *Chemistry and the Potential Antiviral, Anticancer, and Anti-Inflammatory Activities of Cardiotonic Steroids Derived from Toads.* Molecules, 2022. **27**(19).

3. Wang, S.-J., et al., *Cellular and molecular mechanisms of arenobufagin in cancer therapy: a systematic review.* Discover Oncology, 2025. **16**(1): p. 1207.

4. Papp, B., et al. *Preparation and Investigation of Artemisia annua L.-Loaded Alginate Hydrogels with Excipients*. Pharmaceuticals, 2026. **19**, 424 DOI: 10.3390/ph19030424.

5. Asrorov, A.M., et al., *Toad venom bufadienolides and bufotoxins: An updated review.* Drug Development Research, 2023. **84**(5): p. 815-838.

ARENOBUFAGIN VA UNI HOSILALARINING H9c2 HUJAYRALARIGA QARSHI SITOTOKSIKLIGI

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Annotatsiya. *Arenobufagin turli xil biologik faollikka ega bo‘lgan tabiiy bufadienolitdir; ammo, uning potentsial kardiotoksikligi uning farmakologik qo‘llanilishini cheklashi mumkin. Ushbu tadqiqotda arenobufagin va ikkita yangi sintezlangan hosilalarning (Ar-004 va Ar-005) sitotoksik ta’siri H9c2 kalamush kardiomioblast hujayralarida 24 soatlik ta’sirdan so‘ng MTT tahlili yordamida baholandi. Sinovdan o‘tgan birikmalar 1 va 10 μM konsentratsiyalarda hujayralarning yashovchanligiga sezilarli ta’sir ko‘rsatmadi. Aksincha, arenobufagin 100 μM da H9c2 hujayralarining yashovchanligini sezilarli darajada kamaytirdi, ikkala hosila ham bir xil eksperimental sharoitlarda ancha past sitotoksiklikni ko‘rsatdi. Ushbu topilmalar arenobufaginning strukturaviy modifikatsiyasi uning normal yurak hujayralariga sitotoksik ta’sirini kamaytirishi mumkinligini va shu bilan birga keyingi farmakologik tadqiqotlar zarurligini ko‘rsatadi. Ushbu kuzatuvlarning asosiy mexanizmlarini aniqlash va arenobufagin hosilalarining xavfsizlik profilini har tomonlama baholash uchun qo‘shimcha tadqiqotlar talab etiladi.*

Kalit so‘zlar: *arenobufagin; bufadienolitlar; H9c2 hujayralari; kardiomioblastlar; sitotoksiklik; MTT tahlili.*

ЦИТОТОКСИЧНОСТЬ АРЕНОБУФАГИНА И ЕГО ПРОИЗВОДНЫХ НА КЛЕТКИ H9c2

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Аннотация. *Аренобуфагин – это встречающийся в природе буфадиенолид с разнообразной биологической активностью; однако его потенциальная кардиотоксичность может ограничивать его фармакологическое применение. В настоящем исследовании цитотоксическое действие аренобуфагина и двух недавно синтезированных производных (Ar-004 и Ar-005) оценивали на клетках кардиомиобластов крысы H9c2 с использованием анализа MTT после 24 часов воздействия. Исследованные соединения не оказывали существенного влияния на жизнеспособность клеток при концентрациях 1 и 10 мкМ. В отличие от этого, аренобуфагин заметно снижал жизнеспособность клеток H9c2 при концентрации 100 мкМ, тогда как оба производных демонстрировали существенно меньшую цитотоксичность в тех же экспериментальных условиях. Эти результаты позволяют предположить, что структурная модификация аренобуфагина может снизить его цитотоксическое действие на нормальные клетки сердца, сохраняя при этом необходимость дальнейших фармакологических исследований. Необходимы дополнительные исследования для выяснения механизмов, лежащих в основе этих наблюдений, и для всесторонней оценки профиля безопасности производных аренобуфагина.*

Ключевые слова: *аренобуфагин; буфадиенолиды; клетки H9c2; кардиомиобласты; цитотоксичность; MTT-анализ.*