CHAPTER FIFTEEN

3-Bromofascaplysin is a prospective chemical compound for developing new chemotherapy agents in glioblastoma treatment

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Abstract

Glioblastoma (GB) is one of the most aggressive human brain tumors. The prognosis is unfavorable, its treatment is relatively ineffective, and the median survival is about 15 months. Medication development with new chemical compounds is one of the ways to solve the problem of current treatment inefficiency. This study is focused on the group of chemical substances, based on pentacyclic system of 12*H*-pyrido[1,2-*a*:3,4-*b*] diindole, and the most well-known part of this group is fascaplysin, first extracted from the sponge *Fascaplysinopsis* spp. We have synthesized a series of the following fascaplysin derivatives: 7-phenylfascaplysin, 3-chlorofascaplysin, 3-bromofascaplysin, 9-bromofascaplysin. The paper is aimed at analyzing the cytotoxic effect of these compounds on GB cells.

Materials and methods. The study used rat glioma C6 cell line (ATCC®; cat no CCL-107), U-87MG cell line (ATCC; cat no. HTB-14™) and human glioblastoma T98-G cells (ATCC® CRL-1690™). Cell culture method, experimental pharmacological trials and γ-radiation in vitro, as well as flow cytofluorometry were used in the study.

Results: Cytotoxic effect of the tested compounds is stronger than the effect of unsubstituted fascaplysin, and appears to be dose-dependent and time-dependent. 3-bromofascaplysin is more efficient for cancer cells elimination, and by the end of the experiment the amount of living cancer cells in G_0 phase remained at its lowest. Cytotoxic effect of 3-bromofascaplysin on glioblastoma T98-G cells is inferior to that of TMZ, and in case of preliminary radiation treatment of cancer cells with 48 Gy the effect of the compound matches the TMZ treatment results.

Conclusion: 3-Bromofascaplysin is a prospective chemical compound for development of new anti-cancer chemotherapeutic agents.

1. Introduction

Glioblastoma (GB) is one of the most aggressive brain tumors, and is responsible for a half of all primary tumors in the central nervous system; it is characterized by fast invasive growth and unfavorable prognosis for patients (Omuro & DeAngelis, 2013). The modern treatment protocol (Stupp et al., 2015) involves a surgery, but radical removal of the tumor is impossible in the overwhelming majority of cases. Mostly, treatment method relies on high doses of radiation, reaching 60–70 Gy, and chemotherapy (Stupp et al., 2017) where temozolomide (TMZ)—the DNA alkylating agent—is a drug of choice. Patients usually undergo 6–12 cycles of TMZ chemotherapy, but despite all the efforts, the median survival does not exceed

15 months (Dittrich et al., 2016), and about a quarter of patients manage to live for 2 years since being diagnosed, while a 5-year survival rate is true for only 5% of cases.

The attempts of improving chemotherapy effect via combining TMZ with other cytostatics and targeted anti-tumor drugs resulted to be not very impressive after large-scale clinical trials (Touat, Idbaih, Sanson, & Ligon, 2017). Such results could be attributed to highly heterogeneous nature of GB cells (Friedmann-Morvinski, 2014; Patel et al., 2014) and their unique ability to repair DNA. That is why developing new prospective medication, based on molecules with genotoxic activity and ability to inhibit proliferation and invasion mechanisms of tumor cells, is a priority method for dealing with this issue.

Since 2014 our research has been focusing on the group of low-molecular compounds, based on pentacyclic system of pyrido[1,2-a:3,4-b′]diindol. The most well-known part of this group is a red pigment fascaplysin—a bis-indole alkaloid (Bharate et al., 2012; Segraves et al., 2004; Wang et al., 2019), first extracted from the sponge *Fascaplysinopsis* sp. Its complex anti-tumor effect is based on its ability for DNA intercalation, as well as creating active complexes with cyclin-dependent kinases of types 4 and, possibly, 6 (Soni et al., 2000), inhibition of PI3K/AKT/mTOR signaling pathway (Oh et al., 2017), anti-angiogenic effect, triggering mitochondrial pathway of apoptosis and autophagy induction (Kumar et al., 2015).

Fascaplysin has significant cytotoxic effect on glioma and carcinoma cells (Bryukhovetskiy et al., 2017), however, it is less pronounced than TMZ effect. Attempting to address this issue, we synthesized a series of fascaplysin derivatives, two of which have never been synthesized before.

This study is aimed at comparing the anti-tumor effects of synthetic derivatives of fascaplysin (7-phenylfascaplysin, 3-chlorofascaplysin, 3-bromofascaplysin, 9-bromofascaplysin) and temozolomide on experimental glioblastoma models in vitro.

2. Materials and methods

2.1 Cancer cells

The study used poorly differentiated invasive C6 glioma cells, U-87MG and human T98-G cell lines. The cell lines were tested for mycoplasma contamination with the Universal Mycoplasma Detection Kit (ATCC® 30-1012K™).

The rat glioma C6 cell line was obtained from the American Type Culture Collection (ATCC®; cat no CCL-107). This tumor is the most suitable and popular experimental animal GB model (Grobben, De Deyn, & Slegers, 2002).

The U-87MG GB cell line was obtained from the American Type Culture Collection (ATCC; cat no. HTB-14™). This cell line is not the original U-87 line established at the University of Uppsala, but derives from a human GB of unknown origin (Allen et al., 2016). This fact significantly increases the value of the experiment, since the wild type of glioblastoma accounts for more than 90% of cases of this tumor.

T98-GGB cells was obtained from the American Type Culture Collection (ATCC® CRL-1690™). This GB cell line is poorly responsive to TMZ (Lee et al., 2014; Paul-Samojedny et al., 2016; Valtorta et al., 2017) that is mainly associated with a low level of MGMT methylation and high content of O^6-methylguanin-DNA methyltransferase, allowing to recreate the tumor condition right after its complex treatment with radiation and chemotherapy.

All cells were cultured in 6-well plates with DMEM—Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) with Penicillin-Streptomycin (100 U/mL), Antibiotic-Antimitotic 100 × (cat. no. 15240062, Gibco, ThermoFisher Scientific, US) at 37 °C (5% CO_2). All chemicals were obtained from Gibco (Thermo Fisher Scientific, Inc.). Adhesive cells were cultured until 80% confluent and passaged at a 1:3 ratio. Cells were used in experiments after the third passage since the moment of being obtained from the manufacturer.

2.2 Tested substances

The study used synthetic fascaplysin and derivatives of pyrido[1,2-a:3,4-b′]diindol: 7-phenylfascaplysin, 3-chlorofascaplysin, 3-bromofascaplysin, 9-bromofascaplysin (Fig. 1). Fascaplysin, 3-bromofascaplysin were synthesized via the previously developed three-stage method (Zhidkov et al., 2007) from corresponding halogen derivatives of tryptamine and phenylacetic acid and 9-bromofascaplysin were obtained from fascaplysin by halogenation reaction (Fretz, Ucci-Stoll, Hug, Schoepfer, & Lang, 2000). Spectral properties of the synthesized substances are identical to those of the natural alkaloids.

3-Chlorofascaplysin was synthesized with the same method (Zhidkov et al., 2007), using tryptamine and 2,4-Dichlorophenylacetic acid. The structure was checked with mass spectrometry and NMR-spectroscopy

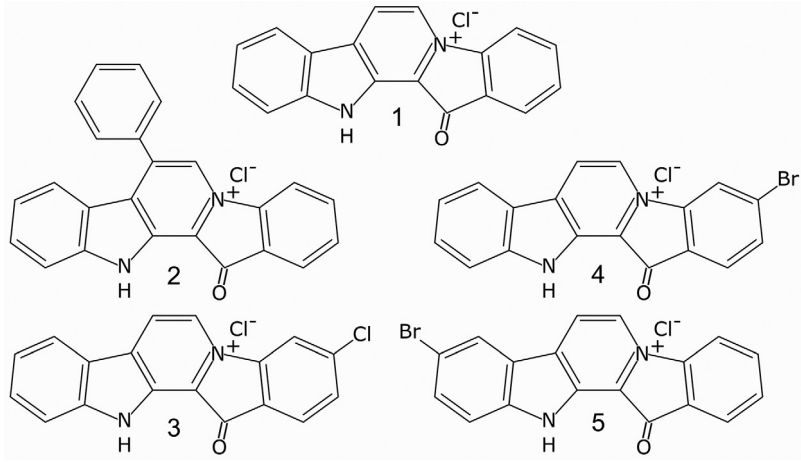

Fig. 1 The tested compounds: fascaplysin (Zhidkov et al., 2007); 7-phenylfascaplysin (Zhidkov, Kantemirov, Koisevnikov, Andin, & Kuzmich, 2018); 3-chlorofascaplysin (Zhidkov et al., 2007); 3-bromofascaplysin (Zhidkov et al., 2007); 9-bromofascaplysin (Fretz et al., 2000).

of 1H and 13C nuclei. Mass spectra (APCI), *m/z*: 409/411 (1:0.3) (M+). ^{1}H NMR spectra (400 MHz, MeOH-d4) d: 9.36 (d, J = 5.8, 1H, H-6), 8.97 (d, J = 5.8, 1H, H-7), 8.53 (d, J = 1.3, 1H, H-4), 8.50 (d, J = 7.8, 1H, H-8), 8.02 (d, J = 7.8, 1H, H-1), 7.90 (t, J = 7.6, 1H, H-10) 7.82 (d, 1H, J = 7.6, 1H, H-11), 7.77 (dd, J1 = 7.8, J2 = 1.3, 1H, H-2), 7.54 (t, J = 7.6, 1H, H-9). ^{13}C NMR spectra (100 MHz, MeOH-d4) d: 180.2, 149.5, 148.2, 142.9, 138.7, 138.6, 137.3, 135.4, 135.1, 132.8, 132.5, 131.5, 127.1, 126.9, 126.4, 122.7, 120.5, 116.3.

7-Phenylfascaplysin was synthesized with a two-stage method (Zhidkov et al., 2018) from indigo. Mass spectra (APCI), *m/z*: 347 (M^+). ^{1}H NMR (400 MHz, MeOH-d4) δ 7.25 (ddd, J = 8.2, 5.0, 3.1 Hz, 1H), 7.63 (d, J = 8.2 Hz, 1H), 7.72-7.76 (m, 4H), 7.79-7.85 (m, 4H), 7.94 (t, J = 7.4 Hz, 1H), 8.05 (d, J = 7.4 Hz, 1H), 8.38 (d, J = 8.0 Hz, 1H), 9.35 (s, 1H); ^{13}C NMR (100 MHz, MeOH-d4) δ 113.6, 115.6, 119.7, 122.9, 124.5, 124.6, 125.6, 126.5, 129.2, 129.5, 130.5, 131.6, 132.2, 133.8, 134.3, 137.0, 137.7, 138.2, 147.4, 147.7, 169.0, 181.9.

3. Research design

3.1 Stage one

The experiment used four derivatives of 12*H*-pyrido[1,2-*a*:3,4-*b*]diindole. Fascaplysin was used for comparing the results. Initially, different

concentrations (0.5 μmol; 0.05 μmol; 0.005 μmol) of the tested compounds interacted with C6 glioma cells in vitro -2×10^4 of GB cells were planted in a 24-well cell culture plate with DMEM medium, containing 10% FBS with 100 U/mL, Antibiotic-Antimitotic 100 × at 37 °C (5% CO_2). All chemicals were produced by Gibco (ThermoFisher scientific, US).

3.2 Stage two

The comparative study of leading chemicals (7-phenylfascaplysin, 3-bromofascaplysin and 9-bromofascaplysin) was conducted the concentration of 0.5 μmol, which it the most suitable one. TMZ cytotoxic effect on the U87 cell line of GB was evaluated, using the concentration of 500 μmol, corresponding to IC50 for this tumor.

3.3 Stage three

To develop radiation resistance in human T98-G cell line of GB with poor reaction to TMZ (Paul-Samojedny et al., 2016; Valtorta et al., 2017; Yang et al., 2016), they were treated with teleirradiation, using ROCUS-M distant gamma therapeutic unit (Russia, St. Petersburg), with ^{60}Co as a radionuclide source. One fraction equaled 6 Gy, and there were performed eight fractions with 72-h intervals to reach the total dose of 48 Gy. The amount of living cells in the culture was 70 ± 12.5%. Further increase of the dose up to 60 Gy did not change the amount of cells significantly.

4. Flow cytometry

At the stage one we used the method, based on two fluorescent dyes—TMRM and DRAQ7. Living cells have very bright TMRM fluorescence, but do not accumulate DRAQ7. The cells in early stages of apoptosis have weak TMRM fluorescence, while showing DRAQ7 stain.

Aliquots 100 μL of cell suspension were incubated with 20 × TMRM solution, having the final concentration of 150 nmol (Thermo, USA). The samples were incubated for 20 min at 37 °C in 5% CO_2 without light access, after the incubation they were washed with the excess saline, containing 2% FBS. 5 μM of DRAQ7 stock solution (Beckman Coulter, USA) was added to the obtained cell suspension, afterwards cytometric analysis was performed. At least 15,000 events were analyzed for each sample After the incubation 100 μL FBS was added to the samples and analyzed with flow cytometer BD Accuri™ C6 (BD Biosciences, USA).

To study the effect of fascaplysin derivatives on cancer cell cycle phases we used propidium iodide (PI, BioLegend, USA) and diamidino-2-phenylindole (DAPI, Biolegend, USA) dyes. During the staining the cell pellet was re-suspended in 100 μL of FBS, then, the obtained cell suspension was diluted with 900 μL ethanol solution (70%, −20 °C) at a 1:9 ratio. After that the samples were kept at −20 °C for 1 h. 10 DAPI solution (Biolegend, USA) was used for DNA staining (BioLegend, США) with final concentration of 10 μg/mL. The samples were incubated with dyes for 20 min at a room temperature in the dark. After incubation period was over, the samples received 200 μL of FBS and were analyzed with flow cytometer Navios™ (Beckman Coulter, USA). At least 10,000 events were analyzed for each sample. To differentiate between single cells and cell aggregates and discriminate them from the subsequent analysis, we combined peak and integrate signals of DAPI fluorescent stain. The results indicated the cell distribution in the G0/G1, S and G2/M phases, presented as a percentage of the total cell amount. Kaluza™ Software, 10 UserNetworkPack (FullVersion) and ModFit TL (Verity Software House, USA) were used for processing cytometry data.

The second and third stages of the experiment involved adding 20-fold diluted DiOC6 (Dittrich et al., 2016) (Invitrogen, USA) to 100 μL of cell suspension (2×10^6 cells/mL), obtaining the final dye concentration of 20 nmol. After introducing the dye, the samples were thoroughly stirred and incubated for 20 min at 37 °C in 5% CO_2 without light access. After completing the incubation, the samples were washed with excess saline, containing 2% FBS (8 min for 300 g). Then the supernatant was decanted, and the cell pellet was transferred into 100 μL of fresh PBS. The obtained cell suspension received 10 μL (DAPI), resulting in the final DAPI concentration of 1 μg/mL. Afterward the samples were incubated in the dark for 10 min at a room temperature and received 200 μL of PBS each.

At least 50,000 single cells were analyzed in each sample. To differentiate between single cells and cell aggregates and discriminate cell aggregates from the subsequent analysis, we combined the forward and side scatter signals—intensity of the peak signal against the intensity of the integrated FSC or SSC, as well as the time of flight against the intensity of integrated FSC or SSC. The results were analyzed with CytExpert™ software (Beckman Coulter, USA).

GraphPadPrism 4.00 was used for the statistical analysis of the obtained results. The data were analyzed with ANOVA, and mean differences were compared, using Tukey method, and presented as a mean value ± standard deviation. The differences were considered significant with $P < 0.05$.

5. Results

5.1 Stage one of the experiment

After being incubated for 6 h, all samples exhibited early stages of apoptosis, indicated by weaker TMRM fluorescence. 24 h later there was an increased number of fluorescent objects (apoptotic bodies) that accumulate DRAQ7 dye, being a sign of oligonucleosomal DNA degradation and later stages of apoptosis. 48 h later cytotoxic effect of the tested compounds on C6 glioma cells was stronger than that of the unsubstituted fascaplysin, while the samples with 0.5 μmol 3-bromofascaplysin contained less living cells of C6 glioma even after the 6-h incubation (Fig. 2A, 6 h). After 48 h this effect significantly intensified (Fig. 2A, 48 h). In turn, cytotoxic effect of 7-phenylfascaplysin was inferior of 3-bromofascaplysin effect, but significantly superior to other tested compounds (Fig. 2A, 48 h).

By the 12th hour of the experiment, the samples with 0.05 μmol of tested derivatives exhibited cytotoxic effect of 3-bomofascaplysin that was inferior to the unsubstituted fascaplysin results (Fig. 2B, 12 h). By the 24-h mark 7-phenylfascaplysin and 3-chlorofascaplysin had the strongest cytotoxic effect that remained by the 48th hour of the experiment (Fig. 2B, 48 h).

The unsubstituted fascaplysin samples with 0.005 μmol concentration showed weak cytostatic effect that turned into a cytotoxic one by the 12th hour of the experiment (Fig. 2C, 12 h). Similar dynamics was exhibited by other derivatives. 24-h exposure of glioma cells to 3-chlorofascaplysin led to more of them dying, as compared with other samples in the experiment. 48-h incubation resulted in 9-bromofascaplysin (0.005 μM) having the strongest cytotoxic effect on C6 glioma in vitro (Fig. 2C, 48 h).

Fascaplysin derivatives produced significant effect on the life cycle of tumor cells (Fig. 3). 6-h exposure resulted in all five substances having smaller amount of cells in G_0/G_1 phase and greater amount of cells in S-phase, as compared with the control numbers. At this point, proliferation rates decreased insignificantly and only in case of maximum concentration of the tested substances. 3-chlorofascaplysin demonstrated the strongest cytostatic effect (Fig. 3, 6 h).

After 12-h incubation of glioma cells in 0.5 μmol of fascaplysin derivatives there was an increase in the amount of cells that entered the S-phase (Fig. 3, 12 h). These data signify the primary cytostatic effect of all five compounds due to cell accumulation in the S-phase and mitotic arrest, but the decreasing amount of living cells brought about the increase of cells in

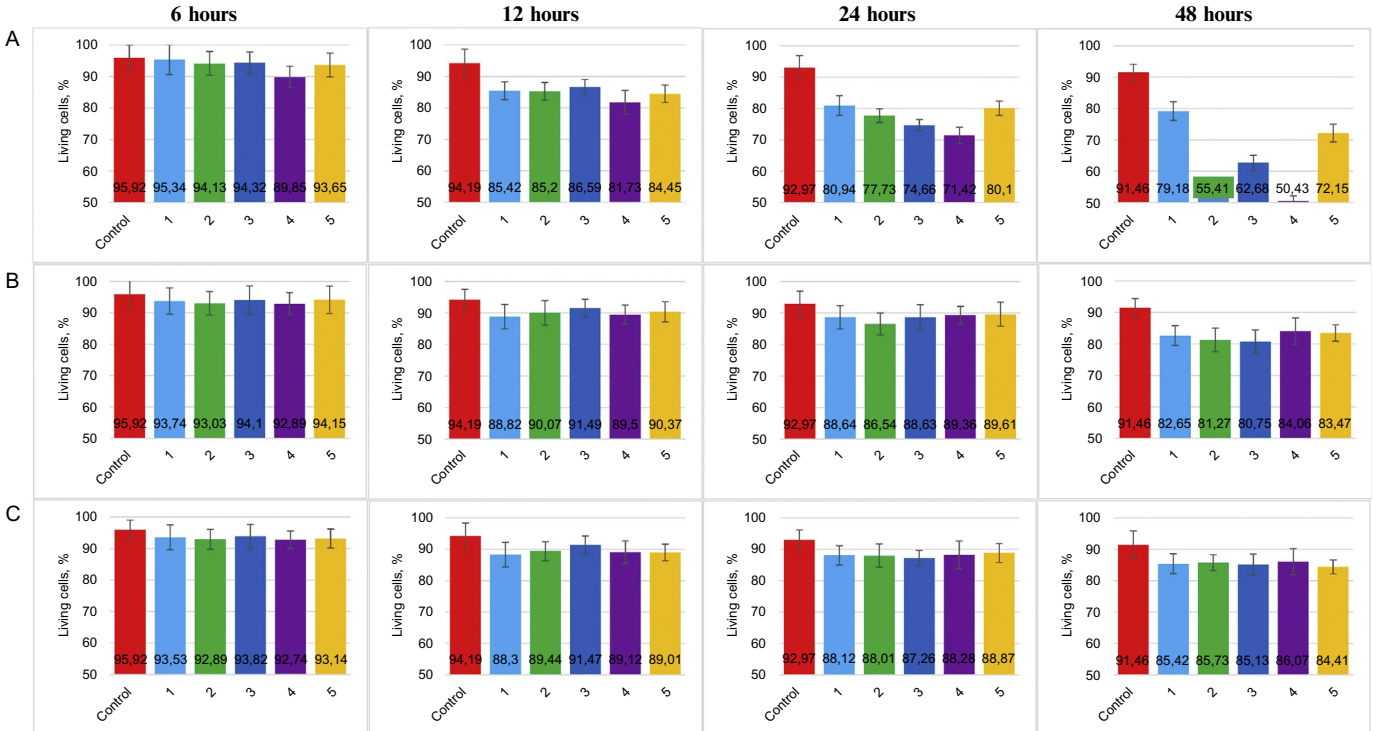

Fig. 2 Effect of fascaplysin—(Allen et al., 2016), 7-phenylfascaplysin (Bharate et al., 2012), 3-chlorofascaplysin (Dittrich et al., 2016), 3-bromofascaplysin (Friedmann-Morvinski, 2014), 9-bromofascaplysin (Grobben et al., 2002) on C6 glioma cells. Staining with cationic lipophilic dye (TMRM) and DNA-binding dye (DRAQ7). Concentration of the tested compounds: (A)—0.5 μmol; (B)—0.05 μmol; (C)—0.005 μmol. Control points—6, 12, 24, 48 h.

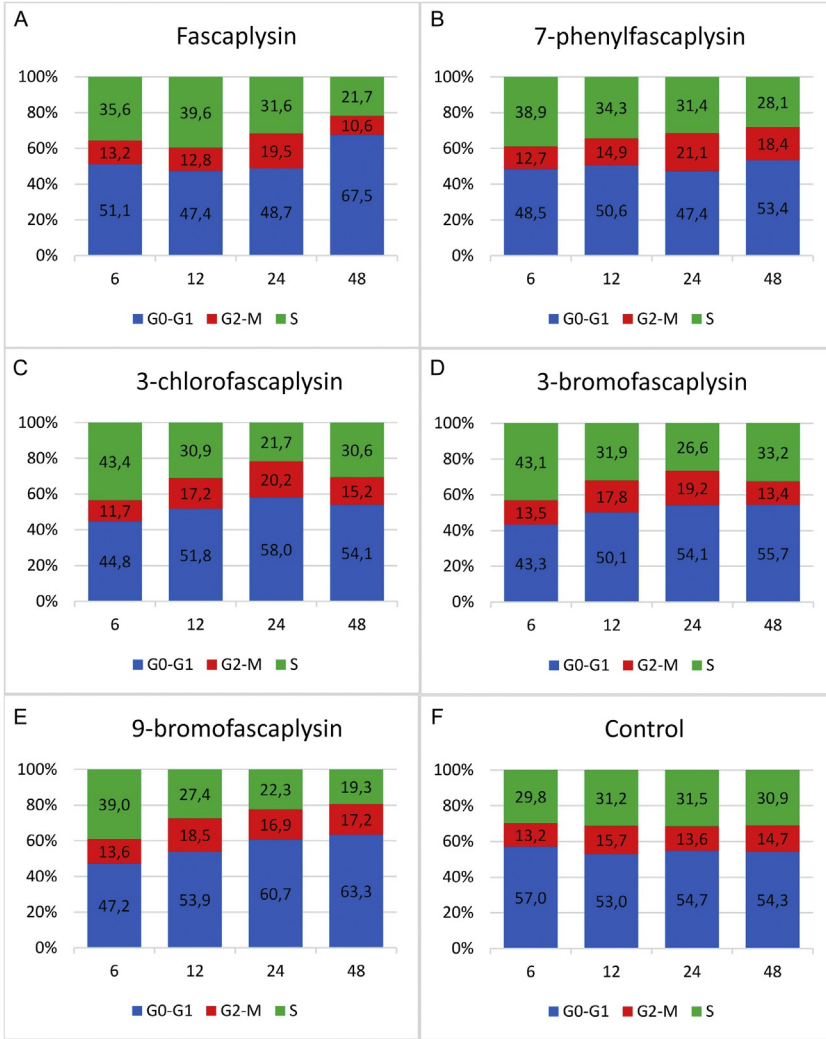

Fig. 3 Distribution of C6 glioma cells (percentage) in the cell cycle phases (G_0-G_1, S, G_2-M) under the tested compounds influence (0.5 μmol concentration).

G_0/G_1 phase in the samples with the tested substances. For instance, by the 48th hour of incubation the glioma cell cultures with 0.5 μmol of unsubstituted fascaplysin had the largest amount of cells in G_0/G_1 phase (Fig. 3, 48 h).

5.2 Stage two of the experiment

After 24-h observation the amount of living U-87MG cells of glioblastoma in the medium with the unsubstituted fascaplysin was similar to the control group (Fig. 4A), while the culture medium with fascaplysin derivatives also did not demonstrate significant differences in the amount of living cells. By the 48th hour of the experiment the plates with 3-bromofascaplysin showed a sharp decrease in the amount of living cells (Fig. 4B). Cytotoxic effect of 9-bromofascaplysin was less pronounced, nevertheless, the amount of living cells in the plates with this compound was significantly different from the control group. By the 72nd hour of the experiment the cytotoxic effect of 3-bromofascaplysin was at its highest (Fig. 4C), being significantly different from that of 9-bromofascaplysin, 7-phenylfascaplysin and unsubstituted fascaplysin.

5.3 Stage three of the experiment

TMZ had a pronounced cytotoxic effect on T98-G cells of glioblastoma without previous radiation treatment (Fig. 5A) that was evident even by the 24th hour of the observation, and after 48 h it culminated with the death of more than a half of GB cell population. Cytotoxic effect of fascaplysin derivatives was not that evident in the same timeframe. 9-Bromofascaplysin efficiency was similar to that in the control group. The cell culture with 3-bromofascaplysin showed a significant decrease in the amount of living GB cells (Fig. 5B), but the cytotoxic effect of TMZ was more evident and was at its peak by the 72nd hour of the observation (Fig. 5C).

On the contrary, the cytotoxic effect of 9-bromofascaplysin on radiation-treated GB cells was similar to TMZ influence (Fig. 6A). But by the 48th hour of the observation the amount of living GB cells in 9-bromofascaplysin medium stabilized (Fig. 6B), while TMZ and 3-bromofascaplysin media continued to show the decreasing amounts. By the 72nd hour of the experiment (Fig. 6C) the cytotoxic effect of 3-bromofascaplysin on radiation-treated GB cells was significantly different from the control group and similar to the TMZ treated culture.

6. Discussion

Cytotoxic effect of fascaplysin has been demonstrated on many types of malignant tumors, but there is almost no research of its influence on glial

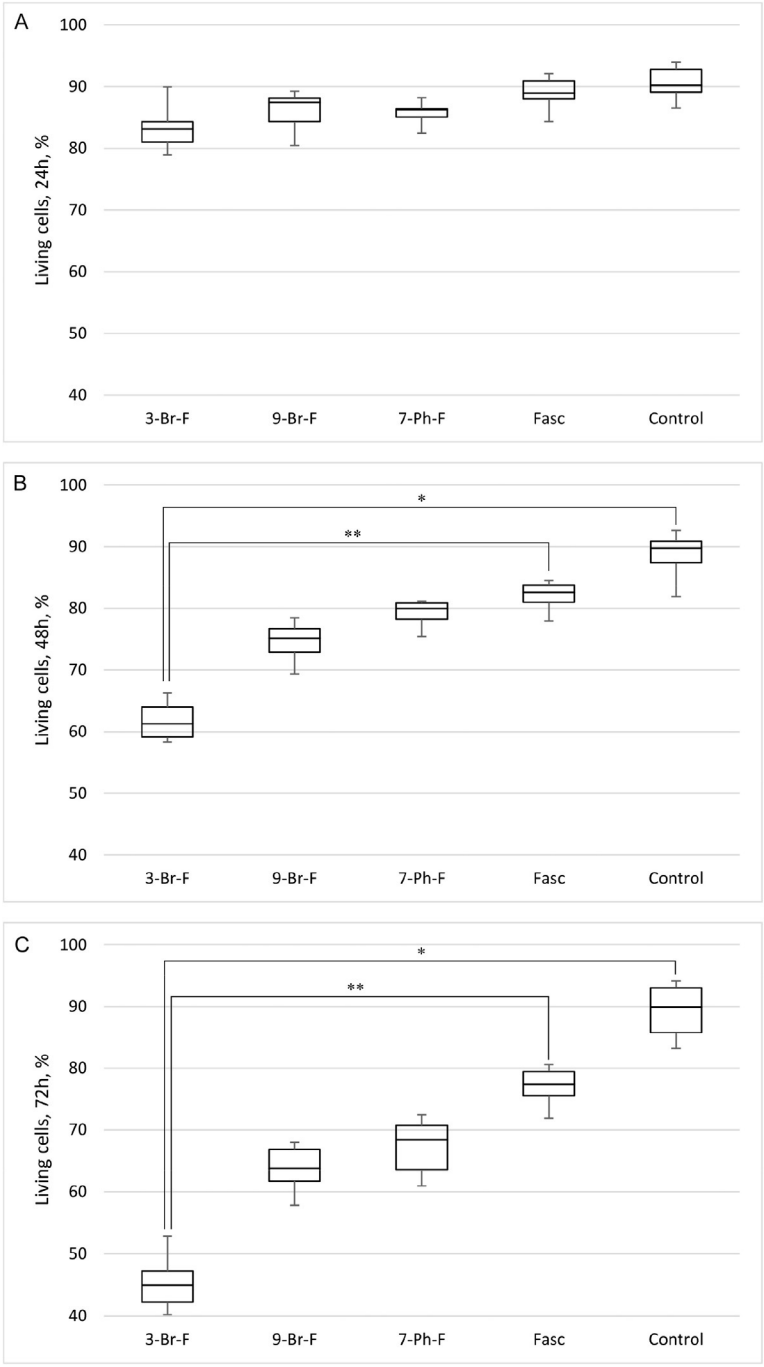

Fig. 4 Cytotoxic effect of fascaplysin derivatives on U-87MG cells of human glioblastoma. (A)—24 h, (B)—48 h, (C)—72 h.

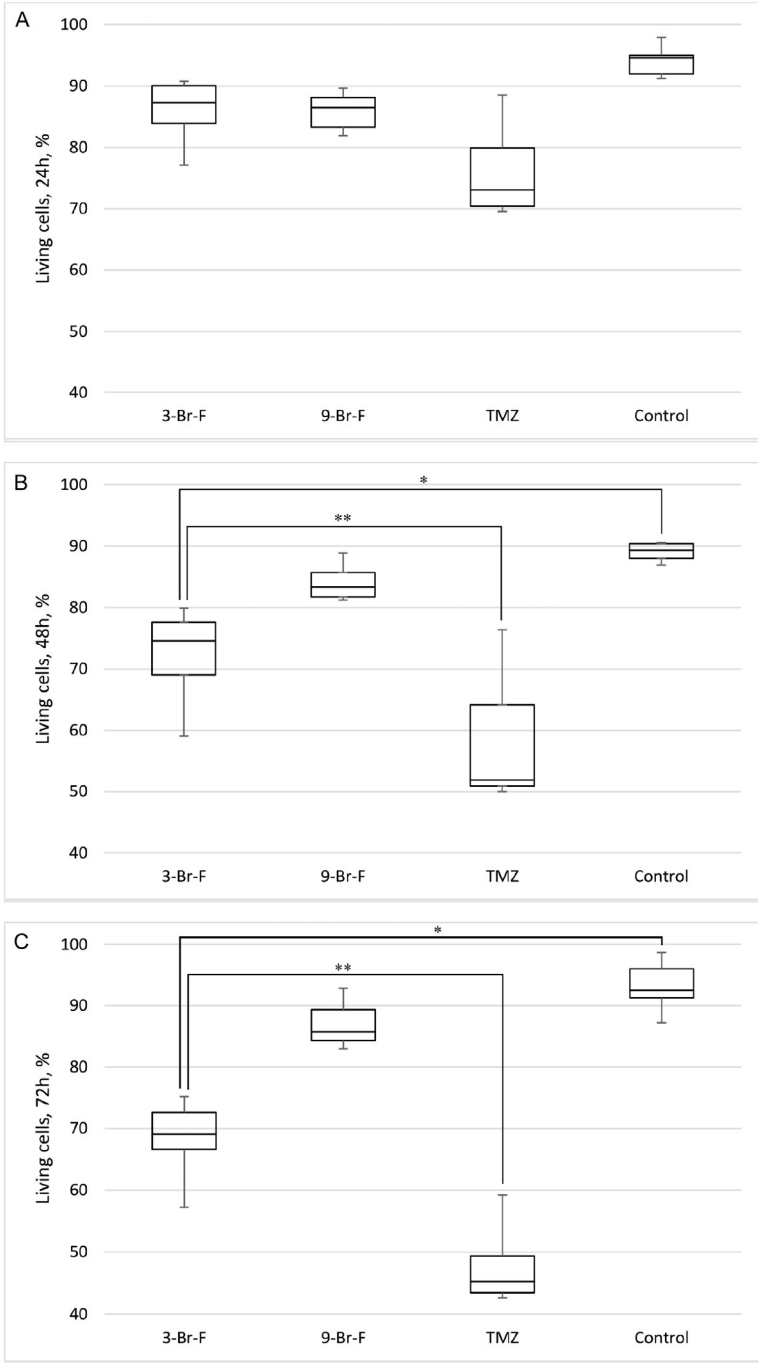

Fig. 5 Cytotoxic effect of fascaplysin derivatives on T98-G cells of human glioblastoma. (A)—24 h, (B)—48 h, (C)—72 h.

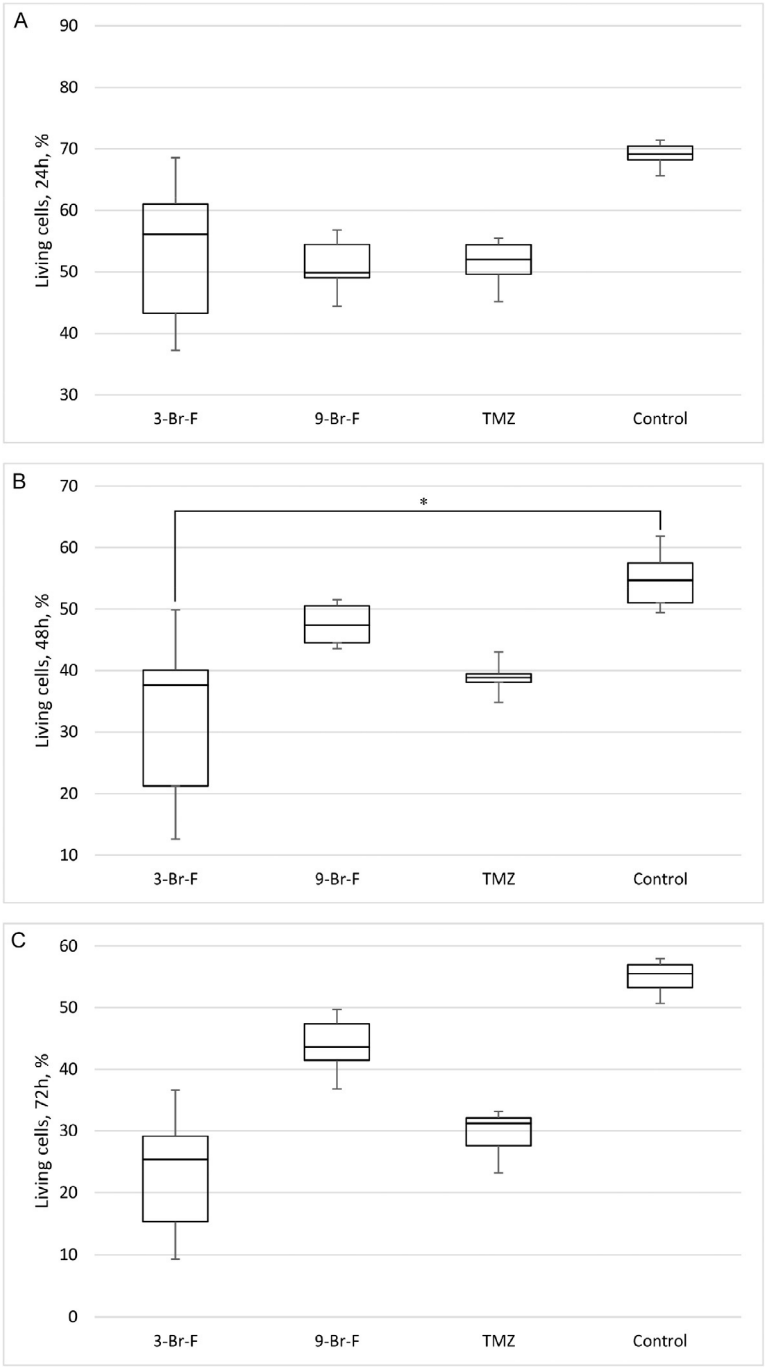

Fig. 6 Cytotoxic effect of fascaplysin derivatives on T98-G cells of human glioblastoma that have been previously treated with radiation of 68 Gy. (A)—24 h, (B)—48 h, (C)—72 h.

brain tumors. Therefore, the present study is not only researching more effective compounds, but also indicates the need for a more detailed investigation of interaction between this chemical and its molecular targets, responsible for proliferation and growth of cancer cells.

The obtained data suggest that fascaplysin and its derivatives have a significant cytotoxic effect on brain cancer cells. The efficiency of these alkaloids and derivatives proved to be different in their dependence on exposure time and concentration of the chemical agent.

The compounds, tested in this study, exhibited their strongest effect with concentration of 0.5 μmol, while with 0.005 μmol concentration fascaplysin and 9-bromofascaplysin showed the results that were not unlike the effect with 0.05 and 0.5 μmol after 48 h of incubation, indicating their dependence on the exposure time. It is highly possible that exposure time or time-dependent effect in this case is more important than the direct cytotoxic influence of the chemical. Obviously, cytotoxic effect of fascaplysin and its derivatives 3-bromofascaplysin and 9-bromofascaplysin is determined mostly by the time, spent in the glioma cell culture, since after 48-h incubation the amount of living cells significantly drops throughout the whole concentration range.

The earlier publications suggest that fascaplysin induces apoptosis via inhibition of CDK 4/6 and cyclin D1 complex (Kumar et al., 2015), resulting in the cell cycle arrest in G_1 phase and triggering apoptosis. However, this mechanism is not the only point of interest, since the interaction with other enzymes, involved in cancer cells life cycle, also is worth researching further.

As the experiment shows, fascaplysin derivatives extend the S-phase of C6 glioma cell cycle, and there is no cell division after 24 h of the experiment. This effect is the same for the samples with the unsubstituted fascaplysin and some of its derivatives (3-chlorofascaplysin, 7-phenylfascaplysin, 3-bromofascaplysin). Another important fact is that the first hours of incubation with the tested compounds show the decrease of cancer cells amount in the G_0 phase. However, the effect of the tested chemicals with weaker cytotoxic influence, compared to that of the leading drugs (3-bromofascaplysin and 7-phenylfascaplysin), increases the amount of cells in G_0/G_1 phase by the end of the experiment. This effect remains even in the samples with the unsubstituted fascaplysin where the amount of cells significantly drops in G_2-M and S phases. These observations indicate that smaller amount of living cells brings about the sharp increase in the G_0/G_1 phase by the 48th hour of the experiment. Obviously, the great increase of cell amount in G_0 phase is

one of the typical neoplastic cell responses to cytostatics, and this should be considered when planning patients' anti-tumor treatment.

The experiment results provide new opportunities for creating innovative chemotherapeutic agents, based on the tested chemical compounds. It should be noted that the efficiency of the studied marine alkaloids and its derivatives depends on the dose and exposure time. Using these parameters, the general toxicity level of chemotherapy could be decreased, and its efficiency could be improved, for instance, via combining several chemicals in one biodegradable capsule. Targeted delivery of such drug to a neoplastic lesion could effectively eliminate proliferating cancer cells, while also influencing on resting cells, this way preventing a possible relapse.

3-Bromofascaplysin is a leader among all the tested substances. All fascaplysin derivatives show the dependence of their cytotoxic effect on exposure time. The main mechanism, ensuring cell death, is apoptosis, as have been demonstrated in many previous studies (Bryukhovetskiy et al., 2017; Kuzmich et al., 2010; Lyakhova et al., 2018) and proven by cytometry data.

The results of this experiment show a strong cytotoxic effect of TMZ on GB cells. This drug is rightly considered to be the gold standard for brain tumors chemotherapy (Omuro & DeAngelis, 2013), and its cytotoxic effect remains both before, and after radiation treatment. It should be noted that T98-G line of human glioblastoma (Lee, 2016; Paul-Samojedny et al., 2016; Valtorta et al., 2017) is not highly receptive to TMZ and has significant amount of cells, expressing the CD133 antigen—the main immunohystochemical marker of cancer stem cells.

Unlike many others, this study does not concentrate on this cell type, since high plasticity and ability to quickly and effectively repair the genetic structure (Wenger et al., 2019) is typical for all GB cells. These properties are evident when radiation-treated GB cells become less responsive to TMZ. A crucially important fact is that 3-bromofascaplysin and 9-bromofascaplysin are significantly inferior in their cytotoxic effect to TMZ, when GB cells without radiation treatment are concerned, and they have a similar and even stronger cytotoxic effect on cancer cells that have been previously treated with 48 Gy radiation.

It should not be left unnoticed that radiation resistance of cancer cells that they develop after the first cycle of combined chemotherapy and radiation treatment of GB, is one of the main reasons for unsatisfactory treatment results. This phenomenon is based on activation of DNA repair mechanisms (Erasimus, Gobin, Niclou, & Van Dyck, 2016), as well as induction of strategically important enzymes synthesis, helping the cancer cell to pass

through the milestones of the cell cycle. Therefore, we could assume that it is multi-targeted influence of synthetic fascaplysin derivatives that lets these compounds inhibit TMZ-resistant cancer cells.

The experiment results present opportunities for developing new chemotherapeutic agents, based on the tested compounds. 3-Bromofascaplysin is a definite leader in the conducted experiment, however, 9-bromofascaplysin should not be discounted as a less effective one either. Theoretically speaking, GB cells eradication could be more effective, for example, if combining several compounds in one biodegradable capsule. Targeted delivery of this drug to a tumor lesion would effectively eliminate radiation-resistant cancer cells, thus, preventing a relapse and extending patients' life expectancy.

Therefore, the experiment results allow to draw the following conclusions. Cytotoxic effect of all tested compounds is superior to that of unsubstituted fascaplysin; 3-bromofascaplysin and 7-phenylfascaplysin are the most effective substances for elimination of C6 glioma cells. Cytotoxic effect of these compounds is dose-dependent and time-dependent. Fascaplysin derivatives affect all phases of neoplastic cell life cycle, and after treatment with 3-bromofascaplysin and 7-phenylfascaplysin the amount of living cells in the G_0 phase remains at its lowest. Cytotoxic effect of 3-bromofascaplysin on U-87 MG cell line is superior to that of unsubstituted fascaplysin. In turn, cytotoxic effect of 3-bromofascaplysin on T98-G cell line is inferior to TMZ effect, while the 3-bromofascaplysin is more effective for T98-G cell line with strong radiation resistance.

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References

Allen, M., Bjerke, M., Edlund, H., et al. (2016). Origin of the U87MG glioma cell line: Good news and bad news. *Science Translational Medicine*, *8*, 354re3.

Bharate SB, Manda S, Mupparapu N et al. Chemistry and biology of fascaplysin a potent marine derived CDK-4 inhibitor. Mini Reviews in Medicinal Chemistry 2012. Vol. 12, N 7. P. 650–664.

Bryukhovetskiy, I., Lyakhova, I., Mischenko, P., Milkina, E., Zaitsev, S., Khotimchenko, Y., et al. (2017). Alkaloids of fascaplysin are effective conventional chemotherapeutic drugs, inhibiting the proliferation of C6 glioma cells and causing their death *in vitro*. *Oncology Letters*, *13*(2), 738–746. https://doi.org/10.3892/ol.2016.5478.

Dittrich, C., Kosty, M., Jezdic, S., et al. (2016). ESMO/ASCO recommendations for a global curriculum in medical oncology edition 2016. *ESMO Open*, *1*(5), e000097.

Erasimus, H., Gobin, M., Niclou, S., & Van Dyck, E. (2016). DNA repair mechanisms and their clinical impact in glioblastoma. *Mutation Research/Reviews in Mutation Research*, *769*, 19–35. https://doi.org/10.1016/j.mrrev.2016.05.005.

Fretz, H., Ucci-Stoll, K., Hug, P., Schoepfer, J., & Lang, M. (2000). Investigations on the reactivity of fascaplysin. Part I. Aromatic electrophilic substitutions occur at position 9. *Helvetica Chimica Acta*, *83*, 3064–3068.

Friedmann-Morvinski, D. (2014). Glioblastoma heterogeneity and cancer cell plasticity. *Critical Reviews in Oncogenesis*, *19*(5), 327–336.

Grobben, B., De Deyn, P. P., & Slegers, H. (2002). Rat C6 glioma as experimental model system for the study of glioblastoma growth and invasion. *Cell and Tissue Research*, *310*(3), 257–270.

Kumar S, Guru SK, Pathania AS et al. Fascaplysin induces caspase mediated crosstalk between apoptosis and autophagy through the inhibition of PI3K/AKT/mTOR signaling cascade in human leukemia HL-60 cells. Journal of Cellular Biochemistry 2015. Vol. 116, N 6. P. 985–997.

Kuzmich AS, Fedorov SN, Shastina VV et al. The anticancer activity of 3- and 10-bromofascaplysins is mediated by caspase-8, -9, -3-dependent apoptosis. Bioorganic & Medicinal Chemistry 2010. Vol. 18, N 11. P. 3834–3840.

Lee, S. Y. (2016). Temozolomide resistance in glioblastoma multiforme. *Genes & Diseases*, *3*(3), 198–210. https://doi.org/10.1016/j.gendis.2016.04.007.

Lee, Y., Dominy, J. E., Choi, Y., et al. (2014). Cyclin D1-Cdk4 controls glucose metabolism independently of cell cycle progression. *Nature*, *510*, 547–551.

Lyakhova, I. A., Bryukhovetsky, I. S., Kudryavtsev, I. V., Khotimchenko, Y. S., Zhidkov, M. E., & Kantemirov, A. V. (2018). Antitumor activity of fascaplysin derivatives on glioblastoma model in vitro. *Bulletin of Experimental Biology and Medicine*, *164*, 666–672. https://doi.org/10.1007/s10517-018-4055-4.

Oh, T. I., Lee, J. H., Kim, S., Nam, T. J., Kim, Y. S., Kim, B. M., et al. (2017). Fascaplysin sensitizes anti-cancer effects of drugs targeting AKT and AMPK. *Molecules*, *23*(1), 42.

Omuro, A., & DeAngelis, LM. (2013). Glioblastoma and others malignant gliomas: A clinical review. *Journal of the American Medical Association*, Vol. 310, N 17. P. 1842–1850.

Patel, A. P., Tirosh, I., Trombetta, J. J., et al. (2014). Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science*, *344*(6190), 1396–1401.

Paul-Samojedny, M., Łasut, B., Pudełko, A., et al. (2016). Methylglyoxal (MGO) inhibits proliferation and induces cell death of human glioblastoma multiforme T98G and U87MG cells. *Biomedicine & Pharmacotherapy*, *80*, 236–243.

Segraves NL, Robinson SJ, Garcia D et al. Crews comparison of fascaplysin and related alkaloids: A study of structures, cytotoxicities, and sources. Journal of Natural Products 2004. Vol. 67, N 5. 783–792.

Soni R, Muller L, Furet P et al. Inhibition of cyclin-dependent kinase 4 (Cdk4) by fascaplysin, a marine natural product. Biochemical and Biophysical Research Communications 2000. Vol. 275, N 3. P. 877–884.

Stupp R, Taillibert S, Kanner AA et al. Maintenance therapy with tumor-treating fields plus temozolomide vs temozolomide alone for glioblastoma: A randomized clinical trial. Journal of the American Medical Association 2015. Vol. 314, N 23. P. 2535–2543.

Stupp, R., Taillibert, S., Kanner, A., et al. (2017). Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: A randomized clinical trial. *Journal of the American Medical Association*, *318*(23), 2306–2316.

Touat, M., Idbaih, A., Sanson, M., & Ligon, K. L. (2017). Glioblastoma targeted therapy: Updated approaches from recent biological insights. *Annals of Oncology*, *28*(7), 1457–1472. https://doi.org/10.1093/annonc/mdx106.

Valtorta, S., Lo Dico, A., Raccagni, I., et al. (2017). Metformin and temozolomide, a synergic option to overcome resistance in glioblastoma multiforme models. *Oncotarget*, *8*(68), 113090–113104.

Wang, Q., Tang, X. L., Luo, X. C., et al. (2019). Aplysinopsin-type and bromotyrosine-derived alkaloids from the South China sea sponge Fascaplysinopsis reticulata. *Scientific Reports*, *9*(1), 2248.

Wenger, A., Ferreyra Vega, S., Kling, T., Bontell, T. O., Jakola, A. S., & Carén, H. (2019). Intratumor DNA methylation heterogeneity in glioblastoma: Implications for DNA methylation-based classification. *Neuro-Oncology*, *21*(5), 616–627. https://doi.org/10.1093/neuonc/noz011.

Yang, S. H., Li, S., Lu, G., et al. (2016). Metformin treatment reduces temozolomide resistance of glioblastoma cells. *Oncotarget*, 7(48), 78787–78803.

Zhidkov ME, Baranova OV., Dubovitskii SV et al. The first syntheses of 3-bromofascaplysin, 10-bromofascaplysin and 3,10-dibromofascaplysin-marine alkaloids from Fascaplysinopsis reticulata and Didemnum sp. by application of a simple and effective approach to the pyrido[1,2-a,3,4-b']diindole system. Tetrahedron Letters 2007. Vol. 48, N 5. P. 7998–8000.

Zhidkov, M. E., Kantemirov, A. V., Koisevnikov, A. V., Andin, A. N., & Kuzmich, A. S. (2018). Syntheses of the marine alkaloids 6-oxofascaplysin, fascaplysin and their derivatives. *Tetrahedron Letters*, *59*(8), 708–711.