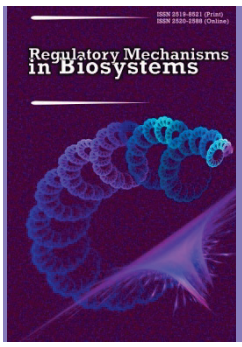

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Non-nucleoside O^6-methylguanine-DNA methyltransferase inhibitors in murine spontaneous tumor experimental chemotherapy *in vivo*

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Alkylating chemotherapy agents are well-established for inducing DNA lesions that result in apoptosis in cancer cells. However, the efficacy of these agents is often diminished due to the activity of the repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT), which confers resistance to chemotherapy by catalyzing dealkylation reactions. Recent studies have identified novel non-nucleoside MGMT inhibitors with promising properties. In this study, we evaluated the effectiveness of these novel non-nucleoside MGMT inhibitors in combination with alkylating chemotherapy *in vivo*. Our experimental model involved ICR female mice that spontaneously developed malignant tumors. These mice were treated with a combination of the alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) and new MGMT inhibitors. We analyzed tumor growth dynamics and observed the levels of MGMT and other proteins using western blot analysis. Our findings demonstrated that the addition of MGMT inhibitors significantly improved the tumor growth-inhibiting effects of the alkylating chemotherapy. Tumor growth was more effectively suppressed in the mice receiving the combination therapy compared to those receiving the alkylating agents alone. Additionally, MGMT levels were significantly reduced following the combined treatment. Furthermore, the active form of caspase 3 was detected in treated tumors, suggesting that the reduction in tumor growth may be mediated through an apoptotic pathway. These results underscore the potential for these novel MGMT inhibitors to enhance the efficacy of alkylating agents in cancer therapy, holding substantial promise for improving therapeutic outcomes against tumors that exhibit high MGMT activity.

Keywords: MGMT; non-nucleoside inhibitors; alkylating agent; combined chemotherapy; MNNG; tumor; ICR mice; caspase 3.

Introduction

O^6-methylguanine-DNA methyltransferase (MGMT) is an inducible DNA repair enzyme crucial for the maintenance of genomic integrity in mammalian cells. MGMT functions by specifically removing alkyl groups, particularly methyl groups, from the O^6 position of guanine residues within DNA, thereby preventing the formation of mutations and preserving the sequence fidelity of the DNA molecule (Christmann et al., 2011).

The repair activity of MGMT assumes critical importance in the context of cancer chemotherapy, where alkylating agents are widely utilized for their ability to damage DNA of rapidly dividing tumor cells. These chemotherapeutic agents achieve their anticancer effects by causing alkylation of DNA bases, which can lead to DNA strand breaks and subsequent cell death. However, the effectiveness of alkylating agents is limited by the rapid repair capabilities of MGMT in tumor cells, which efficiently reverse the DNA damage caused by these agents (Maki et al., 2005; Kaina et al., 2010; Pegg, 2011).

To overcome this challenge, modern therapeutic strategies are exploring the combined use of alkylating agents with MGMT inhibitors. By inhibiting MGMT activity alongside chemotherapy, these inhibiting compounds aim to prolong the presence of alkylated DNA lesions in tumor cells, thereby enhancing the cytotoxic effects of alkylating agents and potentially improving treatment outcomes for cancer patients (Verbeek et al., 2008; Kaina et al., 2010; Pegg, 2011). This approach represents a promising avenue in cancer therapy research, offering the potential to maximize the efficacy of existing chemotherapeutic agents. Another potential strategy to attenuate MGMT-mediated DNA repair involves the administration of high doses of simple methylating agents, such as temozolomide or streptozotocin, followed by treatment with chloroethylating drugs. Methylators create numerous targets for MGMT within the DNA of cells, where MGMT binds to and removes methyl groups from O^6 positions of guanine, depleting the cellular pool of active MGMT temporarily. However, this approach has proven unsuitable due to its significant toxicity in healthy tissues (Verbeek et al., 2011; Ortiz et al., 2021; Petrenko et al., 2022).

Consequently, the application of MGMT inhibitors appears more promising. For instance, the administration of guanine derivatives, such as O^6-benzylguanine (O^6-BG), alongside alkylating chemotherapy is currently undergoing clinical trials and shows greater potential as part of comprehensive cancer treatment strategies. Unfortunately, this therapeutic approach also induces significant toxic effects on hematopoietic stem cells (Ranson, 2006; Kaina et al., 2010).

One more category of potential MGMT inhibitors includes non-nucleoside compounds, which are being explored to identify new, low-toxicity inhibitors suitable for use in combined cancer chemotherapy regimens. In our previous studies, new MGMT inhibitors were designed and created with molecular docking methodology. The compounds have been tested *in vitro*. Based on these results, compounds showing the most promising outcomes were selected for further investigation in *in vivo* studies, aimed at validating their potential as MGMT inhibitors for therapeutic applications.

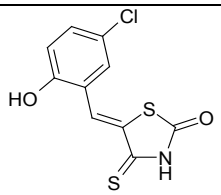
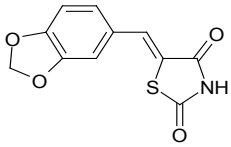
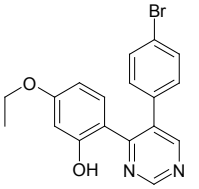
Materials and methods

Inhibitors. In our previous investigations (Zhuvaka et al., 2023), we utilized a semi-flexible molecular docking methodology to identify poten-

tial inhibitors of human MGMT. The OTAVA compound collection, which encompasses approximately 100,000 ligands, was subjected to molecular docking simulations targeting the active site of MGMT (PDB ID: 1EH6) using AutoDock 4.2 software (Scripps Research Institute, USA, 2009). Before docking, water molecules and ligands from the crystal structure were removed, and receptor mapping was facilitated using MGL Tools software (Molecular Graphics Laboratory, USA, 2002) (Morris et al., 2009). Selection criteria for compounds included compliance with Lipinski's Rule of Five for drug-likeness (Lipinski, 2004), and chemical structures were prepared using Vega ZZ 2.4 software (Vega Software, Italy, 2012). High-scoring compounds from the docking simulations were visually analyzed with a Discovery Studio Visualizer (Biovia, USA, 2006) to evaluate their interactions within the MGMT active site. The most promising compounds were subsequently synthesized and tested *in vitro*. Initial evaluations assessed cytotoxicity, with compounds exhibiting higher cytotoxicity than O^6-BG being excluded from further analysis. Compounds that demonstrated potential in reducing MGMT activity in a cell-free system were further evaluated *in vitro* to determine their efficacy in live cells and their ability to enhance the cytotoxic effects of alkylating agents (Zhuvaka et al., 2023; Zhuvaka et al., 2024). Based on these evaluations, three compounds showing the most promising results were selected for *in vivo* studies.

In this study, three novel non-nucleoside MGMT inhibitors were employed, each assigned a unique serial number for convenience due to the complexity of their chemical names. The compounds used were as follows: inhibitor 41 (5-(5-chloro-2-hydroxy-benzylidene)-4-thioxo-thiazolidin-2-one), inhibitor 41b (benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), and inhibitor 89 (2-[5-(4-bromo-phenyl)-pyrimidin-4-yl]-5-ethoxy-phenol) (Table 1). All inhibitors were dissolved in DMSO (Thermo Scientific, USA) at a concentration of 10 μM.

Table 1
Novel MGMT inhibitors analyzed in this study

CAS number	Serial number	Chemical structure	Chemical name
496020-86-1	41		5-(5-Chloro-2-hydroxybenzylidene)-4-thioxothiazolidin-2-one
6318-41-8	41b		5-Benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione
903183-98-2	89		2-[5-(4-Bromophenyl)pyrimidin-4-yl]-5-ethoxyphenol

Experimental model and treatments. ICR mice were used as the experimental model. The animals were maintained under conditions that ensured their comfort and minimized physical and psychological distress, following the guidelines for the treatment of laboratory animals. The research protocol was approved by the ethics committee (protocol number 39 dated 18.06.2024). To develop the experimental murine spontaneous tumor model, ICR female mice were housed with males and allowed to breed. After six months, all female mice were examined twice weekly, and those that developed tumors were removed from breeding and grouped into cohorts of 3–8 animals. Tumors at the time of discovery typically measured approximately 3–4 mm in diameter. Tumor dimensions were recorded every 3–4 days in three dimensions using calipers. Tumor size in each successive measurement was normalized to the initial tumor dimensions to study the dynamics of tumor growth.

Four groups of mice were included in the experiment:

Group 1 – untreated control (intact control);

Group 2 – treated with MGMT inhibitors only;

Group 3 – treated with the alkylating agent MNNG (N-methyl-N′-nitro-N-nitrosoguanidine);

Group 4 – treated with both MGMT inhibitors and MNNG.

Each group consisted of 6 female mice. The experimental therapy spanned 17 days. MNNG was administered at a concentration of 0.5 μg/kg body weight per day, while MGMT inhibitors were administered at 10 μM/kg body weight per day. Drugs were injected locally into the mammary gland or tumor site.

After 9 days of treatment, tumors and healthy breast tissue samples were isolated from ICR mice. Proteins were extracted using standard methods (Ahmed, 2004), and protein concentration was determined using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA, 2009) in Protein A280 mode.

Western-blot analysis. The isolated proteins were separated by polyacrylamide gel electrophoresis (PAGE) using the Mini-PROTEAN Tetra system (Bio-Rad, Great Britain, 2010) and transferred to polyvinyldifluoride membranes (Millipore, USA) via wet transfer method. Western blotting was performed using primary antibodies against MGMT (Santa Cruz Biotechnology, USA), β-catenin (Thermo Fisher Scientific, USA), APC (Thermo Fisher Scientific, USA), TCF (Thermo Fisher Scientific, USA), Parkin52 (Thermo Fisher Scientific, USA), LC3B (Thermo Fisher Scientific, USA), and caspases 9 and 3 (Novus Biologicals, USA) and secondary antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology, USA; Thermo Fisher Scientific, USA). Chemiluminescence signals were detected using a ChemiDoc Imaging System (Bio-Rad, USA, 2010), with exposure times ranging from 10 to 200 seconds based on signal intensity.

The membrane was stained with Coomassie Brilliant Blue R-350 (Sigma-Aldrich, USA) as a loading control, and densitometric analysis of protein bands was conducted using ImageJ1.53t (National Institutes of Health, USA, 2021), to ensure uniform protein loading on the membrane.

Statistical analysis. The results are presented as the mean (x) ± standard deviation (SD) and median (M). Differences between the means of the studied groups were analyzed using analysis of variance (ANOVA) followed by the Bonferroni post hoc test, performed with Origin 8.1 software (OriginLab Corporation, USA, 2008). Statistical significance was defined as $P < 0.05$. Each variant of the Western blot analysis was performed in triplicate. The most representative images were selected for presentation, along with the corresponding densitometric analysis. An amendment was made to the loading control.

Results

We developed an experimental model involving mice ICR with spontaneous mammary gland tumors. The dynamics of tumor growth in untreated mice was measured. Tumor size in each successive measurement was normalized to initial tumor dimensions. It was revealed that in our model animals tumor size increased relatively slowly in first week after discovery, changing by the 2–3-week exponential growth phase, and then began metastasis growth with simultaneous decreasing of main tumor growth rate (Fig. 1a). Due to metastasis growth and the beginning of tumor necrosis processes, animals were euthanized at this stage.

The sizes of the tumors of the intact control group and the group of mice treated with MNNG were compared on the 9th day of the experiment (Fig. 1b). The control group displayed a broad range of tumor sizes, with some tumors expanding to over 1200% of their original size. The median value in this group is significantly higher, indicating that tumors tend to grow substantially without treatment. Conversely, the therapy group exhibited a markedly narrower range of tumor sizes, with most tumors remaining below 400% of their original size. The median tumor size in the therapy group is considerably lower than in the control group.

The data suggest that chemotherapy has a substantial impact on reducing tumor growth. In the absence of treatment, tumors exhibited uncontrolled growth, as shown by the control group. However, with chemotherapy, the increase in tumor size was significantly restrained, highlighting the efficacy of MNNG therapy in managing tumor progression.

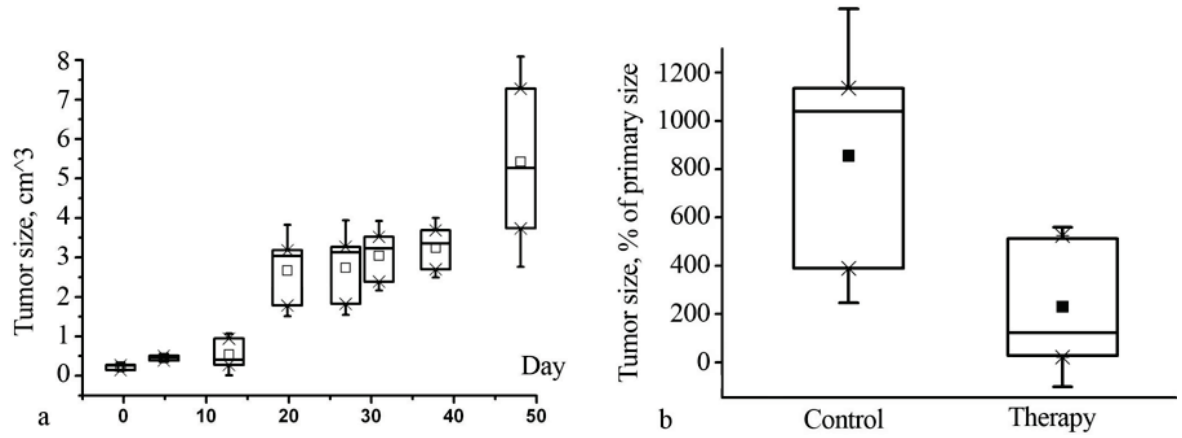


Fig. 1. Experimental ICR murine spontaneous tumor model: tumor growth rate without any treatments (*a*), where abscissa shows the day of the experiment and y-axis is tumor size in cm^3; circles represent mean tumor size at each time point, while bars indicate standard deviation (SD); the graph labeled (*b*) compares the tumor size (as a percentage of the primary size) between the control group (untreated) and a therapy group (treated with MNNG (N-methyl-N′-nitro-N-nitrosoguanidine), 0.5 μg/kg body weight) at the 9th day of the experiment; boxes indicate interquartile range; horizontal lines inside boxes – median; black squares – mean; asterisk (*) suggests a statistically significant difference ($P < 0.05$) between control and therapy groups; n = 6

Thus, our model is suitable for studying the therapeutic effects of MGMT inhibitors used as complex chemotherapy compounds. Due to the significant slowdown of tumor growth in the MNNG therapy group, we excluded an untreated control group in further tumor dynamic studies. This decision was made to address the limitations of experimental duration associated with the necessity for early euthanasia in untreated animals.

All three non-nucleoside inhibitors studied significantly enhanced the efficacy of MNNG tumor therapy (Fig. 2a). When used independently, without MNNG therapy, the inhibitors demonstrated no effect (data not shown). Importantly, the group treated with inhibitors did not differ significantly from the intact control group. This suggests that the new inhibitors are non-toxic and safe for use in mice.

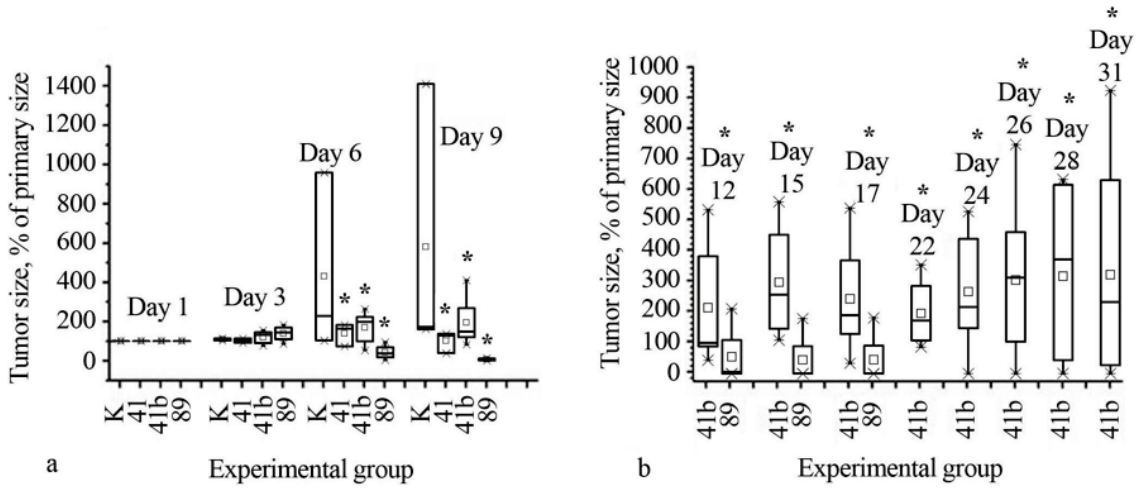


Fig. 2. Tumor growth rate in mice treated with MNNG (N-methyl-N′-nitro-N-nitrosoguanidine) alone (*K*) and with combined treatments with inhibitors 41, 41b, and 89 over several days at early (*a*) and longer (*b*) period: daily administration of MNNG and the inhibitors were 0.5 μg/kg and 10 μM/kg body weight, respectively; control group K disappeared after the 9th day of the experiment, as it was euthanized for ethical reasons; abscissa shows the day of the experiment and y-axis is tumor size as a percentage of the primary size; boxes indicate the interquartile range; horizontal lines inside boxes – median; small squares – mean; bars – standard deviation (SD); asterisks (*) suggest a statistically significant difference ($P < 0.05$) between K group and combined therapy groups; n = 6

The most effective one was inhibitor 89: tumor size in 5 from 6 animals of this experimental group began to decrease after 6 days of experiment; all these animals had initial tumor sizes near 4-6 mm Ø. But in animals with initial tumor size >9 mm Ø the therapy was far less effective. The 41b inhibitor has the mildest effect; nevertheless, tumors in 41b+MNNG-treated group had significantly slowed down growth rate comparing MNNG-only-treated ones.

After the 9th day of the experiment, animals in the control group were euthanized because some of them had excessively large tumors with signs of massive tissue necrosis. In 89+MNNG- and 41b+MNNG-treated groups further tumor therapy and observations were performed (Fig. 2b). After day 17 only 41b+MNNG-treated animals remained in the experiment due to permanent remission in 89+MNNG-treated ones. In the 41b inhibitor-treated group slow tumor growth took place in most animals; in some cases, minor tumor regress was observed when the initial tumor size was relatively small (3–5 mm Ø). Thus, all three inhibitors demonstrated an ability to reduce tumor growth rates when administered in combination with chemotherapy. We subsequently investigated the levels of MGMT protein, various caspases, and regulatory proteins in vivo under different therapeutic regimens.

Western blot analysis of post-treatment tumor samples from mice revealed bands at 24 and 50 kDa following incubation with anti-MGMT antibodies, corresponding to MGMT and MARP (anti-methyltransferase antibody recognizable protein), respectively (Fig. 3). Thus, in MNNG-only-treated tumors, a sufficient increase of MGMT expression was observed: in intact animals, the level of this protein expression was quite low or even non-detectable by western blot analysis – but after MNNG therapy this indicator multiplied (Fig. 3b, 3c). In tumors after combined therapy, MGMT level was low or undetectable in all samples studied in the case of inhibitor 41 (Fig. 3a); and under inhibitors 41b (Fig. 3b) and 89 (Fig. 3c) treatment – only in samples from animals with treatment begun on early tumor growth stage, but not from those at the pre-metastatic stage.

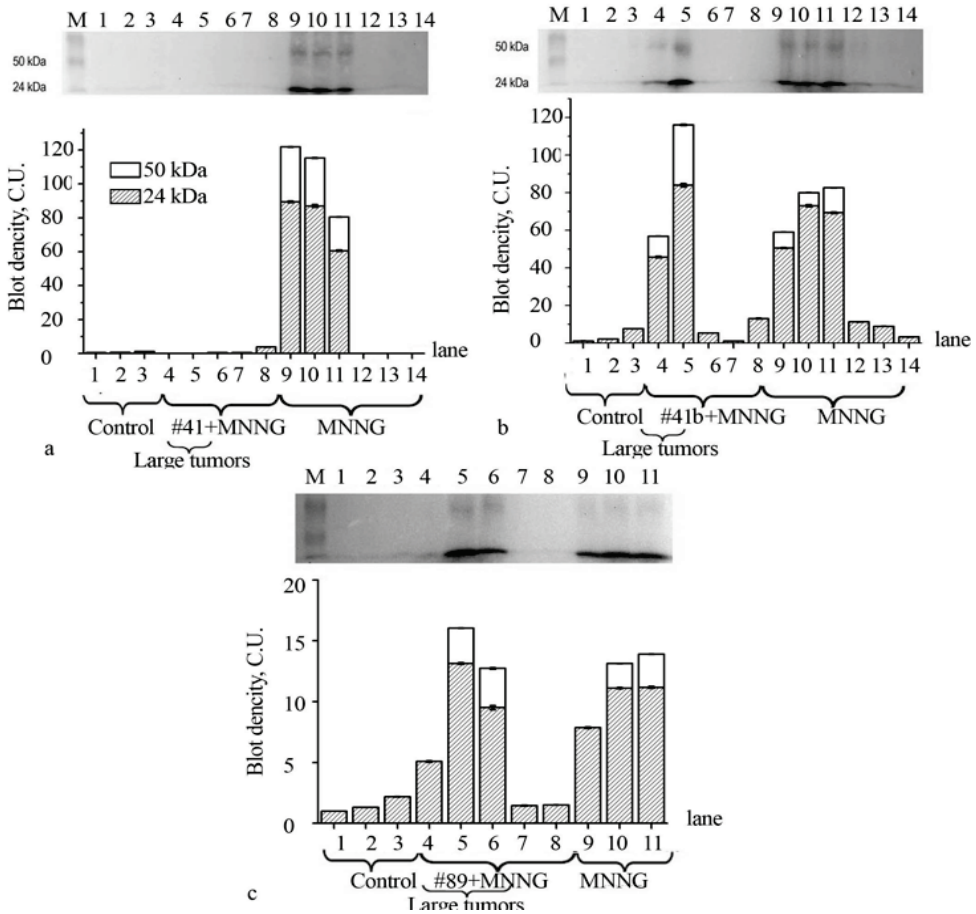


Fig. 3. MGMT protein level in malignant tissues in ICR mice under combined treatment of MNNG (daily administration was 0.5 μg/kg body weight) and inhibitors 41 (*a*), 41b (*b*) and 89 (*c*) (daily administration were 10 μM/kg body weight) at 9th day of the experiment: the top panel shows a western blot image with MGMT bands at 24 kDa and MARP bands at 50 kDa; the lower panel represents the quantification of blot density for the MGMT protein level (an amendment was made to the loading control), where abscissa shows the lane number corresponding to different experimental groups; control (untreated), inhibitor 41/41b/89 + MNNG, and MNNG alone; y-axis represents blot density in C.U. (arbitrary units); bars – standard deviation (SD)

Thus, all three inhibitors were shown to decrease MGMT expression in tumor tissue *in vivo* and to improve the therapeutic effect of MNNG in our experimental *in vivo* model.

Some preliminary investigations into the potential anti-tumor mechanisms of inhibitor 41 were conducted. The expression levels of proteins associated with apoptosis, autophagy, and β-catenin signaling pathways were examined, specifically β-catenin, APC, TCF, Parkin52, LC3B, and caspases 9 (initiating) and 3 (executioner).

Despite significant individual variability in the expression of these regulatory proteins, no substantial differences were observed between untreated and treated tissues, both malignant and healthy, with the exception of caspase 3. In both untreated and MNNG-treated tumors, caspase 3 was present only in its inactive 31 kDa form. However, following combined treatment, the cleaved 12 and 17 kDa forms of caspase 3 were detected in some samples (Fig. 4). In healthy tissues, neither MNNG nor combined treatment induced the expression of cleaved caspase 3.

When tumor degradation took place, combined therapy with inhibitor 41b caused massive tissue necrosis, whilst therapy with 41 and 89 inhibitors didn't lead to severe ulceration nor even was wound formation observed.

Discussion

The repair enzyme MGMT plays a crucial role in mediating chemoresistance to alkylating agents by removing alkyl lesions from guanine in DNA (Pegg, 2011). Under treatment with alkylating agents, cells increase the production of MGMT, confirming it as an inducible enzyme (Yu et al., 2020). Our data (Fig. 3) demonstrated that MGMT levels significantly increased under MNNG treatment compared to intact control. This effect was also observed *in vivo* with other alkylating agents, such as streptozocin (Hijioka et al., 2019) and temozolomide (Jiang et al., 2014).

Inhibiting MGMT has been shown to enhance the efficacy of cancer therapies (Kaina et al., 2010), a finding confirmed by our study. We observed that novel MGMT inhibitors positively influenced the cytotoxic effects of MNNG *in vivo*. Specifically, inhibitor 41 exhibited high efficacy but caused significant tissue necrosis. Conversely, inhibitors 41b and 89 were more effective during the early stages of tumor growth, with initial tumor sizes of 4–6 mm in diameter.

These findings are consistent with our western blot results, which showed low MGMT protein levels in all tumors following treatment with inhibitor 41. In contrast, treatments with inhibitors 41b and 89 resulted in low protein levels only in small tumors, while pre-metastatic tumors exhibited high MGMT protein levels. Therefore, low MGMT protein levels were correlated with successful outcomes in combined therapy.

Also according to western blot data, anti-methyltransferase antibody recognizable protein (MARP) (Fig. 3) was detected, which was detectable at 48 kDa approximately (Kotsarenko et al., 2018). This protein may be a part of the family of MGMT homologs – ATLs (alkyltransferase-like proteins) that has a similar function (Latypov et al., 2012; Kotsarenko et al., 2018).

Common MGMT inhibitors, such as O^6-BG and its analogs like O^6-(4-bromothenyl)-guanine (lomeguatrib) (O^6-BTG), are associated with myelosuppression (Ranson, 2006). The high glucose consumption characteristic of tumor cells (Quail & Joyce, 2017) provides a potential strategy for developing selective inhibitors. To address this, inhibitors conjugated with glucose, such as O^6-BG-Glu and O^6-BTG-Glu, have been developped. These inhibitors showed promising inhibitory properties across different cell lines and enhanced the cytotoxic effect of TMZ *in vitro* (Tomaszowski et al., 2015). However, their MGMT inhibition was 3–5 times weaker in cultured cells compared to non-conjugated inhibitors, likely due to the action of multidrug efflux pumps (Tomaszowski et al., 2015, 2017).

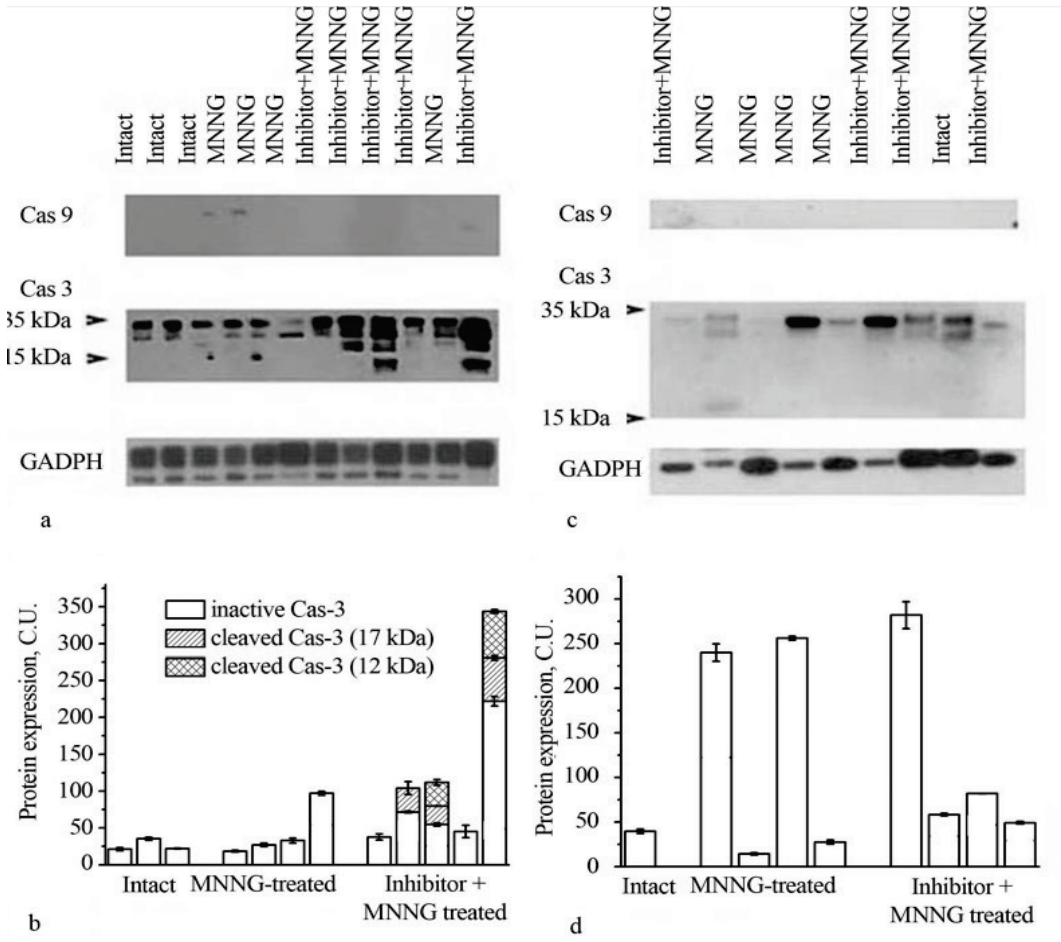


Fig. 4. Caspase-3 (Cas-3) and caspase-9 (Cas-9) protein levels in malignant (*a*, *b*) and healthy (*c*, *d*) mammary gland tissues under various treatments, comparing Intact (untreated), MNNG-treated, and Inhibitor + MNNG-treated groups: panels *a* and *c* show western blot images displaying bands for Cas-3 near 35 kDa, its cleaved forms at 17 and 12 kDa (near 15kDa), and Cas-9, with GAPDH as a loading control; panels *b* and *d* quantify the protein expression levels in C.U. (arbitrary units) for inactive and cleaved Cas-3 forms; caspase expression was assessed under MNNG alone and combined inhibitor 41 + MNNG treatment (10 μM/kg and 0.5 μg/kg body weight, respectively) in a murine ICR spontaneous tumor model; densitometry data are corrected by the loading control; bars represent standard deviation (SD)

In this study, we utilized a novel class of potential MGMT inhibitors – non-nucleoside low-weight inhibitors named by their serial number 41, 41b, and 89 (Table 1). Unlike nucleoside inhibitors, these compounds do not integrate into DNA, potentially reducing off-target effects and toxicity (Pan et al., 2017). Consequently, these inhibitors are hypothesized to exhibit lower cytotoxicity towards hematopoietic cells compared to their nucleoside counterparts. Further investigation into this aspect is of significant importance. In our previous studies (Zhuvaka et al., 2023; Zhuvaka et al., 2024), we observed that all tested inhibitors reduced MGMT protein levels in the malignant HEp-2 cell line *in vitro*. This study confirms their inhibitory properties. It is important to note that MGMT is a proteasomal degradative protein that is eliminated following an irreversible enzymatic reaction (Li et al., 2022). Therefore, observing a reduction in protein levels allows us to conclude that the inhibitors successfully inhibit the enzyme.

Additionally, preliminary studies investigating the potential anti-tumor mechanisms of non-nucleoside inhibitors were conducted. To understand the processes activated following combined treatment in cancer cells, key proteins involved in apoptosis, autophagy, and β-catenin signaling were analyzed. These processes are known to be interconnected and influence each other (Eisenberg-Lerner et al., 2009; Su et al., 2013). In our previous study, administration of inhibitors 41 and 41b in combination with alkylating treatment increased autophagy levels and resulted in high mortality in glioma cells *in vitro* (Zhuvaka et al., 2024). However, in an *in vivo* model, the levels of key autophagy proteins in tumors did not differ from those in healthy tissue after combined therapy. This discrepancy may be attributed to differing mechanisms of action in various tissue types or experimental models. The signaling pathways and regulatory mechanisms involved in autophagy could be modulated differently in a living organism compared to cultured cells (Jensen & Teng, 2013). Also, the pharmacokinetics (absorption, distribution, metabolism, and excretion) of the inhibitors *in vivo* could be different from *in vitro* conditions. Factors such as drug metabolism, bioavailability, and tissue penetration can affect the efficacy and mechanism of action of the inhibitors, leading to different outcomes in autophagy regulation (Blanco et al., 2014). Moreover, tumors *in vivo* may activate compensatory pathways that alter autophagy, which might not be present *in vitro* (Sharma et al., 2017).

Preliminary studies investigating the potential anti-tumor mechanisms of non-nucleoside inhibitors demonstrated changes in the levels of analyzed proteins under combined therapy, specifically in the case of Caspase 3 (Fig. 4). Caspases categorized into initiator caspases (e.g., caspase-8 and -9) and effector caspases (e.g., caspase-3 and -7), which orchestrate the cell death process. Initiator caspases respond to apoptotic signals and activate effector caspases, leading to the systematic dismantling of cellular components (Galluzzi et al., 2016; Sahoo et al., 2023). Caspases are involved in apoptosis, which is a known component of the antitumor action of alkylating drugs in MGMT-deficient cells, but not in cells with high MGMT activity (Nyskohus et al., 2013). In our experiment, the level of MGMT in treated tumor cells was significantly reduced, as confirmed by western blot results (Fig. 3). Consequently, we observed a reduction in tumor size (Fig. 2).

Interestingly, we observed significant mortality in T98G glioma cells *in vitro* following combined treatment with the novel non-nucleoside MGMT inhibitors and alkylating agents (Zhuvaka et al., 2024). This finding suggests that the induction of cancer cell death in both *in vivo* and *in vitro* models treated with these inhibitors may be associated with apoptotic pathways. However, to conclusively validate the involvement of

apoptosis, additional studies are required, including detailed molecular analyses and confirmation in different cancer types and models.

The novel non-nucleoside MGMT inhibitors investigated in this study show considerable potential in enhancing the effectiveness of alkylating agents in cancer treatment while potentially reducing associated toxicities. Future research should aim to further elucidate their mechanisms of action, optimize their pharmacological properties, and validate their efficacy and safety in clinical settings. This study lays a strong foundation for developing more effective and safer cancer therapies through targeted MGMT inhibition.

Conclusions

An in vivo murine ICR model can be used to study the efficacy of complex anti-tumor therapies, including O^6-methylguanine-DNA methyltransferase (MGMT) inhibitors and alkylating agents. In this study, three new non-nucleoside MGMT inhibitors were tested and demonstrated an enhanced therapeutic effect of the alkylator N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) by reducing MGMT-mediated DNA repair. Preliminary data suggests that the tumor degradation observed under the combined treatment operates through apoptotic mechanisms. Further studies are required to confirm these findings and elucidate the underlying pathways. The promising results of this study underscore the potential of these novel MGMT inhibitors to significantly enhance the efficacy of alkylating agents in cancer therapy. Future research will aim to optimize the dosage and administration schedule of these inhibitors to maximize therapeutic benefits while minimizing side effects. Clinical trials will be essential to investigate their safety and efficacy in human patients.

The authors declare no conflicts of interest.

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