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Mini review

Hydantoin derivatives: Harnessing antitumor and immunomodulation potential

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Summary. Hydantoin and its newly synthesized derivatives have been the focus of research due to their numerous biological activities and emerging beneficial effects in various pathological conditions, including cancer. Their primary clinical use is for the treatment of epilepsy and cardiac arrhythmias, but hydantoin derivatives have also shown significant anti-inflammatory and antitumor potential. One of its most prominent antitumor properties is high antiproliferative potential against various cancer cell lines. Through various studies over the past decades, different series of hydantoin derivatives have shown antiproliferative activity with varying degrees of apoptosis in cancer cells. Different series of derivatives significantly decreased cell survival and caused a reduction in oxidative stress parameters in treated cells, indicating their significant antioxidant effects. The cell migration index was significantly decreased after treatment with different hydantoin derivatives, suggesting an inhibitory role in various processes of cancer cell motility and invasion, which are crucial for tumor invasion. An important feature of tumor progression is chronic inflammatory response, propagated by immune cells that activate pro-inflammatory genes and cytokines. Certain hydantoin derivatives reduce the expression of pro-inflammatory genes and pro-inflammatory cytokines related to carcinogenesis and tumor growth, suggesting that some hydantoin compounds possess significant anti-inflammatory effects, which could be an additional mechanism behind their antitumor effects. The results of various studies show that some of the investigated compounds from different synthetic series have the potential to be used as new chemotherapeutic agents against the growth and progression of cancers in different tumor types.

Keywords: anti-inflammatory potential, antitumor potential, hydantoin derivatives.

INTRODUCTION

Hydantoin, or imidazolidine-2,4-dione, is a five-membered heterocycle that is one of the oxidized forms of imidazolidine with a cyclic urea core. In generally, the term "hydantoins" refers to specific groups of compounds, and implies a class of compounds containing the hydantoin substructure as a scaffold. The term "hydantoin" is derived from a combination of the chemical reaction and substrate, as it was originally synthesized by the Bayer group by hydrogenation of allantoin. Since Bayer synthesized the first hydantoin, a large number of hydantoin derivatives have been synthesized, of which several 5,5-disubstituted derivatives have found use in medicine as commercially available drugs (Cho et al. 2019).

One of the most important biological functions of hydantoin derivatives today is their antitumor effect on various types of cancer cells (Trišović et al. 2011; Jintao et al. 2013; Obradović et al. 2013, 2019, 2020; Marinova et al. 2016), in addition to their use in the treatment of epilepsy, cardiac arrhythmias and inflammation (Herrera et al. 2015; Kumar et al. 2017).

Hydantoin derivatives are attracting the attention of researchers due to their increasing use in various areas of

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medicine. Numerous studies have shown a significant correlation between groups containing the basic structure of the hydantoin ring and their biological effects, while a number of research studies have focused on the synthesis of novel modified derivatives with the aim of extending their existing biological activities (Jansen et al. 2003). The unique physicochemical properties of hydantoin derivatives, especially their increased molecular surface area for participation in molecular interactions, make them promising agents for new biomedical applications. The aim of modifying existing hydantoin derivatives is to develop compounds with more potent or altered pharmacological profiles than existing forms (Cho et al. 2019).

The cytotoxicity of non-specific chemotherapeutics agents has limited their use in chemotherapy, and since most human malignant tumors develop drug resistance, the development of less toxic antitumor agents with more selectivity is essential. Accordingly, there is still a need to find less toxic drugs with broad antitumor activity. In accordance with the latest molecular immunological methods and a deeper understanding of cellular metabolism, more advanced chemotherapeutic agents are being developed today (Basak et al. 2021; Kotb et al. 2022).

Several studies suggest that certain classes of hydantoin derivatives have significant anti-inflammatory effects. Some of the synthesized compounds display stronger anti-inflammatory effects than the drug celecoxib, a frequently used NSAID (Nonsteroidal Anti-Inflammatory Drug). Investigation of the anti-inflammatory mode of action of some representative compounds from different derivative classes and the most potent analogues, shows that they display ability to significantly reduce the expression of pro-inflammatory cytokines (IL-1β IL-6 and TNF-α). In addition, a detailed *in silico* molecular docking study predicts that 1,3-disubstituted-2-thiohydantoins have significant binding affinity for the COX-2 binding site, suggesting that these compounds may have an inhibitory effect against the COX-2 enzyme (Khirallah et al. 2022).

HYDANTOIN DERIVATIVES WITH ANTITUMOR ACTIVITY

Cancer is one of the most serious health problems and the second leading cause of death in the world. Hallmarks of cancer include self-sufficient growth signaling, insensitivity to growth-inhibitory signals, evasion of apoptosis, unlimited replication potential, persistent angiogenesis, tissue invasion and metastasis, and inactivation of systems that regulate cellular response to DNA damage. One of the most striking antitumor properties of various hydantoin derivatives is their high antiproliferative potential against various cancer cell lines. Treatment options for cancer include surgery, radiotherapy, chemotherapy, or immunotherapy (Zagórska et al. 2021).

One of the best-known antitumor mechanisms of hydantoin derivatives is their significant antiproliferative potential, which has been tested on various types of tumor cells (Cavazzoni et al. 2008; Zuliani et al. 2009; Djaković Sekulić et al. 2015). Various studies have indicated the significant cytotoxic activity of hydantoin derivatives against different types of tumor cells: spirohydantoin derivatives show antiproliferative effects against cervical cancer cells and breast tumor cells and induce growth inhibition of leukemic cells (Rajić et al. 2006; Kavitha et al. 2009). A study by Kumar and coworkers revealed the significant inhibitory effects of certain diazaspiro-bicyclohydantoin derivatives with substituted cycloalkyl, phenyl and benzhydryl groups, on the proliferation of breast tumor cells (MCF-7 cell line), hepatocellular carcinoma cells (HepG-2 cell line), cervical cancer cells (HeLa cell line) and colon tumor cells (HT-29 cell line) (Ananda Kumar et al. 2009). It has been shown that aromatic units at the C5 position are important for interactions with biological structures. Studies have shown that 5-benzylidene-hydantoins, as bioisosters of 4-anilinoquinazoline, inhibit the expression of EGFR (Epidermal Growth Factor Receptor) and are already approved for the treatment of lung tumors (Carmi et al. 2006; Cavazzoni et al. 2008). Reducing the proliferation potential of tumor cells is one of the most important goals in the development of antitumor therapies.

Apoptosis plays a key role in the development of organisms, tissue homeostasis, immune response, and the development of numerous diseases, including tumors (Matsuura et al. 2016; Fuchs and Steller 2015). Apoptosis-inducing agents are often effective antitumor drugs because apoptosis is a tissue defense mechanism against tumor development that eliminates genetically damaged cells from the tissue before they spread clonally (Hassan et al. 2014). Spirohydantoin derivatives have been found to exhibit pro-apoptotic activity in ovarian and breast tumor cell lines (Gomez-Monterrey et al. 2005). Based on results obtained in proliferation assays, the pro-apoptotic potential of hydantoin derivatives was tested in one of our previous studies. Treatment with these compounds led to an increase in the number of cells undergoing apoptosis depending on the dose and period of exposure. The main type of cell death in tumor cells, induced by selected hydantoin derivatives, was apoptosis, while an insignificant percentage of necrotic cells was detected. Given that one goal of antitumor therapy is the induction of tumor cell apoptosis (Shin et al. 2009), the obtained results indicate that these hydantoin derivatives could be of great interest for further research in the development of more effective chemotherapies.

In one of our previous studies, the effects of newly synthesized hydantoin derivatives, with variable benzyl-substit-

uents at the N-3 atom of the hydantoin ring also showed the strongest antiproliferative effects (Trišović et al. 2011). In a subsequent study, we investigated the antitumor activity of the derivatives with the strongest effects from the previous study: 3-benzyl- 5,5-diphenylhydantoin and series of 3-(4-substituted benzyl)-5-isopropyl-5-phenylhydantoin derivatives. The possible antitumor mechanisms of the investigated hydantoin derivatives (3-(4-substituted benzyl)-5-isopropyl-5-phenylhydantoin) as potential antiproliferative agents were studied in human breast cancer MDA-MB-231, colon cancer HCT-116 and myeloid leukemia K562 cell lines. The derivatives, 3-(4-methoxybenzyl)-5-isopropyl-5-phenylhydantoin and 3-(4-chlorobenzyl)-5-isopropyl-5-phenylhydantoin showed the most pronounced effects. In addition, diazaspiro-bicyclo hydantoins with either an alkene, ester or ether substituent at the N-3 position showed antiproliferative effects on human leukemia cell lines, K562 and CEM. Moreover, spirohydantoin derivatives containing different side chains at the N-3 (acetic acid propyl ester, methoxyethane, pentene) and N-8 (phenyl ring with electronegative atoms) positions have been shown to inhibit the growth of leukemia cells (Ananda Kumar et al. 2009; Zagórska et al. 2021).

HYDANTOIN DERIVATIVES WITH ANTIOXIDANT ACTIVITY

Radical reactive species are one of the most important mediators of intracellular signaling cascades and the regulation of gene expression. They regulate the cell cycle and apoptosis, control the activation of the immune system, and are involved in the defense against infectious agents (Sena and Chandel 2012). However, excessive production of reactive oxygen and nitrogen species (ROS and RNS) or reduced activity of cell antioxidant systems leads to a disturbance of redox homeostasis in the cell, resulting in the occurrence and accumulation of cellular damage (Liou and Storz 2010). The consequences of the action of free radicals can be epigenetic and genetic changes, which in turn can lead to genomic instability and trigger the formation and development of tumors. As an antioxidant molecule, GSH prevents damage to cellular components that can be caused by reactive oxygen species such as free radicals and peroxides (Pompella et al. 2003). The investigated hydantoin derivatives affect the intracellular levels of glutathione, one of the most important non-enzymatic, antioxidant components of the cell. Glutathione and its oxidized form, GSSG, represent an important marker for the redox status of cells (Traverso et al. 2013).

Increased ROS production is one of the main features of malignant cells, and different antioxidants reduce the proliferation potential of tumor cells, alleviating tumor burden and also protecting non-malignant cells from oxidative damage, especially when administered in combination with chemotherapeutic agents. Different hydantoin derivatives exhibit significant antioxidative capacity. For example, selenohydantoins, derivatives of hydantoins in which one of the oxygen atoms is replaced by selenium, exhibit strong antitumor activity and high antioxidative potential (Ivanenkov et al. 2016). In addition, variants of the hydantoin nucleus can be found as components of many drugs used in chemotherapy. Nilutamide is an antineoplastic hormonal agent primarily used in the treatment of prostate cancer (Hassanin et al. 2024).

In our experimental studies, an increased level of glutathione was found in cancer cells treated with 3-(4-substituted benzyl)-5-isopropyl-5-phenylhydantoin derivatives, where the compounds 3-(4-methoxybenzyl)-5-isopropyl-5-phenylhydantoin and 3-(4-chlorobenzyl)-5-isopropyl-5-phenylhydantoin showed the strongest effects among all tested derivatives (Obradović et al. 2019, 2020). Since, the level of reduced glutathione (GSH) was also increased, these hydantoin derivatives stimulate the potential of tumor cells for *de novo* synthesis of GSH, indicating the activation of their intrinsic antioxidant capacity. These data show that the investigated hydantoin derivatives can stimulate antioxidant cell capacities and that *de novo* synthesis of GSH may represent one of the mechanisms of antitumor activity of this series of hydantoin derivatives.

HYDANTOIN DERIVATIVES AND MIGRATORY CAPACITY

Cell migration is essential for establishment and maintenance of the proper organization in an organism, as well as immune response, hematopoiesis, tissues reparation and wound healing. In addition to a crucial role in tissue repair, migration plays an important role in tumor progression and metastasis, representing the most serious problem in the entire process of tumor management in clinical treatments. Although the invasiveness of different tumor cells varies, they all express high levels of proteolytic enzymes, such as matrix metalloproteinases (MMPs) that degrade the extracellular matrix and facilitate tumor cell migration into the surrounding tissue (Clark and Matić Vignjević 2015).

The cell migration index was significantly reduced after treatment with the tested hydantoin derivatives, indicating that they inhibit the motility of the three cancer cell lines (human breast cancer MDA-MB-231, colon cancer HCT-116 and myeloid leukemia K562) and influence the invasion processes migration capacity in the process of tumor invasion. In our study, treatment with the tested hydantoin derivatives resulted in decreased migratory capacity. One possible mechanism by which these compounds decrease the migratory potential of human breast tumor cells is through increased NO bioavailability. NO is involved in the regulation of cell ad-

hesion molecules, mainly integrins, which are important for cell adhesion and motility (Roberts et al. 2008; Obradović et al. 2019, 2020). Other studies have identified some hydantoin compounds with antimigratory potential. Marine-derived phenylmethylene hydantoin (PMH) derivatives, which exert a multifold decrease in migratory and invasive capacities, were discovered using wound-healing and Cultrex invasion assays. Replacement of the benzene ring with other heterocyclic rings did not lead to a significant improvement in these activities of methylene hydantoins. PMH is a new anti-metastatic lead compound with potential therapeutic activity against prostate cancer (Khanfar and El Sayed 2010).

HYDANTOIN DERIVATIVES AS MATRIX METALLOPROTEINASE INHIBITORS

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases involved in diverse physiological processes, such as reproduction, extracellular matrix metabolism, cell migration, tissue remodeling and repair, as well as the regulation of inflammatory processes. However, they are also highly upregulated in cancers, helping cancer cells to evade the primary tumor tissue and undergo metastatic process (Herszényi et al. 2014). Accordingly, these enzymes represent useful biomarkers for cancer diagnosis, prognosis, monitoring of disease progression, predicting treatment efficacy, and prevention. MMP-9 degrades collagens, playing a crucial role in basement membrane degradation and promoting migration, invasion and metastasis of cancer cells (Taguchi et al. 2014). Moreover, the expression of MMP-9 positively correlates with cancer stage, severity and prognosis, making it a suitable marker, and a potential therapeutic target to prevent cancer invasion and metastasis (Roy et al. 2009; Alaseem et al. 2019).

In a study by De Savi and collaborators from AstraZeneca, three novel series of 4-phenoxypiperidines and 4-alkoxypyrimidinopiperazines, in which the hydroxyamide moiety was replaced by hydantoin as the central pharmacophore to bind zinc, were shown to exert stronger inhibition of MMP-13 activity at nanomolar concentrations (De Savi et al. 2013).

The results of our study show that prolonged treatment of tumor cells with the selected hydantoin derivatives 3-benzyl- 5,5-diphenylhydantoin, 3-(4-methoxybenzyl)-5-isopropyl-5-phenylhydantoin and 3-(4-chlorobenzyl)-5-isopropyl-5-phenylhydantoin leads to a statistically significant decrease in MMP-9 mRNA expression compared to control cells, while the compounds 3-(4-methoxybenzyl)-5-isopropyl-5-phenylhydantoin and 3-(4-chlorobenzyl)-5-isopropyl-5-phenylhydantoin showed a greater reduction in MMP-9 expression. These data suggest that these derivatives have a significant potential to reduce the invasiveness of tumor cells and that this novel series is superior to previous modifications (Obradović et al. 2019, 2020).

HYDANTOIN DERIVATIVES IMMUNOMODULATORY ACTIVITY

Inflammation represents an important aspect of immune response. The major mediators of inflammation are pro-inflammatory cytokines, such as IFN-γ, TNF-α, IL-1β, IL-6, IL-12, and IL-18. To date, NSAIDs are the first choice for the treatment of numerous inflammatory diseases. Most NSAIDs have common side effects, especially gastrointestinal toxicity, due to their free -COOH group. NSAIDs work by inhibiting the activity of Cox-enzymes and decreasing levels prostaglandin in the body, which leads to a decrease in inflammation (Khirallah et al. 2022).

Although the use of NSAIDs has been associated with reducing inflammation in tumors, particularly breast tumors (Bowers et al. 2014), adverse side effects have been noted due to their poor selectivity. Thus, new anti-inflammatory agents must be found that are safer, with lower toxicity, and greater efficacy (Khirallah et al. 2022). The use of new selective cyclooxygenase-2 (COX-2) inhibitors, such as Coxibs and NO-releasing NSAIDs can achieve satisfactory anti-inflammatory effects and are free of the side effects associated with classical, non-selective NSAIDs (Tołoczko-Iwaniuk et al. 2019; Rodrigues et al. 2024). Some recent studies suggest that selective COX-2 inhibitors play an important role in the prevention of various tumors, including breast tumors (Takkouche et al. 2008; Regulski et al. 2016).

Hydantoin compounds have been shown to exert anti-inflammatory properties. In a study performed by Khirallah and co-workers, the effects of novel derivatives of 1,3-disubstituted-2-thiohydantoin, which have been shown previously to exert anti-inflammatory effects, were investigated, and a number of new compounds showed stronger ability to reduce the expression of anti-inflammatory cytokines compared with the parent compound (Khirallah et al. 2022). Certain hydantoin derivatives such as 5,5-diarylhydantoin derivatives, have shown selective inhibition of the COX-2 enzyme (Zarghi et al. 2011). In our study, a series of synthesized 3-(4-substituted benzyl)-5-isopropyl-5-phenylhydantoin derivatives were investigated as potential anti-inflammatory agents. The mRNA level of the COX-2 gene as a pro-inflammatory gene associated with tumor progression was reduced compared to levels in non-treated cells, indicating the significant anti-inflammatory effect of these compounds (Obradović et al. 2019, 2020).

The various roles of NO in apoptosis, immune response, cell cycle, tumor progression, angiogenesis, and metastasis are currently being considered at the host tissue/ tumor interface, since NO has been found to be strongly associated

with both the tumor parenchyma and the tumor microenvironment (Vannini et al. 2015). NO also modulates the immune response via the regulation of apoptosis and upregulation of cytokine expressions (Navasardyan and Bonavida 2021). The tumor microenvironment includes cells of the immune system and vascular tissue, and NO appears to be one of the key regulators of their activity (Artacho-Cordón et al. 2012). Antitumor effects of NO have also been found in certain human and animal tumors (Choudhari et al. 2013; Vahora et al. 2016). Nevertheless, overexpression of iNOS has stimulatory or inhibitory effects on tumor growth, depending on the microenvironment and tumor type. Although the effect of NO depends on the expression level of iNOS, the duration and timing of NO delivery, the contents of the microenvironment, the genetic background and the type of cell; NO is undoubtedly involved in tumor progression (Vannini et al. 2015). In our study, the aforementioned hydantoin derivatives stimulate the production of NO in three cancer cell lines (MDA-MB-231, HCT-116, K562). Different studies indicate that certain concentrations of NO are required in order to inhibit tumor growth. Moreover, treatment of TNFR1 (Tumor necrosis factor receptor type I) knockout mice with the L-NAME (L-NG-Nitro arginine methyl ester) NOS (Nitric oxide synthase) inhibitor resulted in elimination of TNF-induced tumor suppression, suggesting that NO production is necessary for TNFR2 (Tumor Necrosis Factor receptor type II)-mediated suppression of tumor growth; likely due to mediation of regulatory and effector T cells (Zhao et al. 2007). Furthermore, it has been shown that treatment of cells with NO inhibits NF-κB activity. TNF-α gene expression is regulated by NF-κB, therefore, NO-mediated inhibition of NF-κB will result in the inhibition of TNF-α expression in T cells, reducing their antitumor potential (Nam 2006). Agents that promote the production of NO in tumor tissue may be of therapeutic benefit, since the presence of NO in the tumor microenvironment has been implicated in immune regulation systems of tumor tissue. Accordingly, hydantoin compounds that exert NO producing capacity could have therapeutic potential.

CONCLUSION

In summary, because of their wide range of pharmacological properties, here we have described important recent applications of hydantoin derivatives in medicinal chemistry. The data presented here demonstrates that many drug discovery projects could benefit from chemical diversification and inclusion of hydantoin synthesis. The hydantoin ring is present in compounds with diverse pharmacological actions, and hydantoin derivatives show promise for their potential antitumor activity and provide diverse antitumor mechanisms. This review presents results of recent studies, some of which have yielded promising data that can be considered as a basis for future developments. Despite various new therapeutic approaches, chemotherapy remains the most common form of cancer treatment, despite significant limitations because of the toxicity of commonly used chemotherapeutic agents and the development of chemotherapeutic resistance. The synthesized hydantoin derivatives showed significant antitumor activity against various tumor cell lines, as assessed by different biomarkers. The compounds whose antitumor properties are presented in this review showed significant antiproliferative, pro-apoptotic, anti-inflammatory and antioxidant effects in the tested cell lines, enabling development of hypotheses regarding their antitumor mechanisms. These compounds induced a decrease in cell migration and invasion capacity and showed significant selectivity in inhibiting proliferation of tumor cells versus non-malignant (healthy) cells. The novel hydantoin derivatives that we recently synthesized showed stronger antitumor effects compared to derivative 3-benzyl- 5,5-diphenylhydantoin from the previous study, suggesting that replacement of the phenyl group with an isopropyl group in the 5-position of the hydantoin ring may enhance the tumor inhibitory effects of this class of compounds. Hydantoin derivatives showed a similar effect on various tumor cells. Although comparative studies with selective cyclooxygenase inhibitors of NSAIDs have yet to be performed, these new hydantoin derivatives could be promising agents for reducing prostaglandin production in breast tumor cells, possibly with fewer side effects. The properties of these hydantoin derivatives against tumor cell lines suggests that they could be promising candidates for further research in *in vivo* animal models, with the aim of developing more effective antitumor agents. The presented features of these hydantoin compounds confirm and clarify their significance as novel important chemical compounds with potential for use in antitumor and immunomodulatory therapies.

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