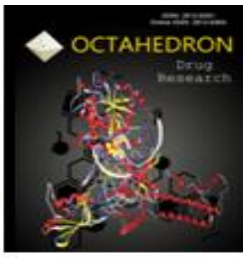
Octahedron Drug Research 4 (2024) 61-74

ISSN: 2812-6351
Online ISSN: 2812-636X

OCTAHEDRON
Drug Research
Vol. 4

Review Article



Hydantoin derivatives: A review on their anticancer activities

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ARTICLE INFO

Article history :
Received 28 August 2023
Received in revised form
3 December 2023
Accepted 5 December 2023
Available online 1 January 2024

Keywords: Hydantoin; anticancer; HDAC; EGFR; sirtuins.

ABSTRACT

Hydantoin, imidazolidine-2,4-dione, is an immensely valuable and highly favored heterocyclic scaffold in medicinal chemistry, as evidenced by its incorporation into several clinically approved drugs such as phenytoin, nitrofurantoin, and nilutamide. The hydantoin scaffold exhibits a wide range of pharmacological and biological properties, including antimicrobial, anticonvulsant, antidiabetic, anticancer, and anti-inflammatory activities. This comprehensive review primarily focuses on exploring the potential of hydantoin derivatives as anticancer agents, elucidating their various mechanisms of action such as histone deacetylase inhibition, modulation of B-cell lymphoma-2, interference with kinesin spindle proteins, inhibition of tubulin polymerization, and inhibition of epidermal growth factor receptor (EGFR). Moreover, this review underscores the importance of specific compounds and highlights the utility of pharmacophoric hybridization, wherein diverse bioactive groups are combined with hydantoin in a single molecule to achieve enhanced efficacy compared to individual scaffolds. Additionally, a concise analysis of the structure-activity relationships (SARs) is provided to offer insights into the correlation between the chemical structure of these compounds and their biological activity.

1. Introduction

Hydantoin, also known as imidazolidine-2,4-dione, is a five-membered heterocycle. The term "hydantoins" is commonly used to refer to a class of compounds that utilize the hydantoin substructure as a scaffold [1]. It is widely recognized that heterocyclic-based scaffolds are highly valuable for discovering bioactive compounds. These scaffolds contain at least one heteroatom, such as sulfur, oxygen, or nitrogen, and one carbon atom in the ring structure. This structural arrangement enables them to act as hydrogen bond donors or acceptors, effectively forming intermolecular hydrogen bonds with biological targets [2]. Therefore, these compounds have been extensively investigated due to their potential applications in medicine and industry as important pharmacophoric moieties or structural elements [2]. Despite the compact size of hydantoin, it possesses four hydrogen donors and

acceptors [3]. A few examples of clinically approved drugs containing the hydantoin moiety include the androgen receptor antagonists nilutamide **(1)** and enzalutamide **(2)**, the muscle relaxants nitrofurantoin **(3)** and dantrium **(4)**, and the anticonvulsants phenytoin **(5)**, mephenytoin **(6)**, ethotoin **(7)**, and fosphenytoin **(8)** [1](**Fig. 1**). Hydantoin-based compounds exhibit a broad spectrum of pharmacological and biological effects, including anticancer [4–6], anti-inflammatory [7,8], antidiabetic [9], antibacterial [10,11], adrenoceptor modulating [12–14], anticonvulsant [15,16], antiplatelet [17], and anti-HIV activity [18,19]. Also, there are two commonly employed synthetic methods for hydantoin synthesis. The first method involves the Bucherer Bergs reaction, which utilizes the corresponding cyclic ketones and involves a single-step reaction with potassium cyanide and ammonium carbonate. The second method consists of the synthesis from cyclic α, α-disubstituted α-amino esters through isocyanate coupling, followed by base-induced cyclization cleavage [20,21] (**Fig. 2**). Furthermore, cancer stands as the most formidable disease, accounting for the highest mortality rate globally, second only to cardiovascular diseases [22,23]. However, developing effective and tailored treatments for different cancer types remains a significant challenge for humanity. Additionally, chemotherapy often faces failures due to acquired resistance [24]. Consequently, researchers are compelled to invent novel anticancer drugs that offer enhanced efficacy, selectivity, and cost-effectiveness [25]. This review provides a comprehensive overview of the notable discoveries concerning the anticancer properties of hydantoin derivatives, highlighting their diverse biological targets. Moreover, the structure-activity relationships (SARs) between these derivatives and their respective targets are thoroughly discussed.

1. Anticancer activity of hydantoins

1.1. Hydantoins as HDAC inhibitors

Histone deacetylases (HDACs) and histone acetyltransferases (HATs) play a crucial role in maintaining the balance of histone acetylation. However, various pathologies, including cancer, have been associated with abnormal histone acetylation patterns caused by the overexpression of HDACs [26,27]. HDACs remove acetyl groups from lysine residues, producing a positively charged histone tail. This charge interacts significantly with the negatively charged DNA phosphate backbone, leading to distorted chromatin structure and transcriptional inhibition. In many malignancies, HDACs are overexpressed, resulting in histone hypoacetylation and the suppression of tumor suppressor genes (such as p53, p21, p27) [27,28]. HDACs have emerged as attractive targets for various human diseases, including cancer. Extensive studies have demonstrated the anti-cancer effects of inhibiting HDAC in different tumor cell lines [26,29]. The FDA and the China Food and Drug Administration (CFDA) have approved five drugs as histone deacetylase (HDAC) inhibitors: Vorinostat (SAHA), Romidepsin, Panobinostat, Belinostat, and Chidamide [30–35]. HDAC inhibitors typically consist of three essential pharmacophore groups: a Cap group that serves as a surface recognition moiety, a linker that effectively presents the ZBG (zinc-binding group) to the active site, and a Zn^{2+} binding group that acts as a chelating agent for Zn^{2+} at the HDAC active site **(Fig. 3)** [26,27,29,36,37].

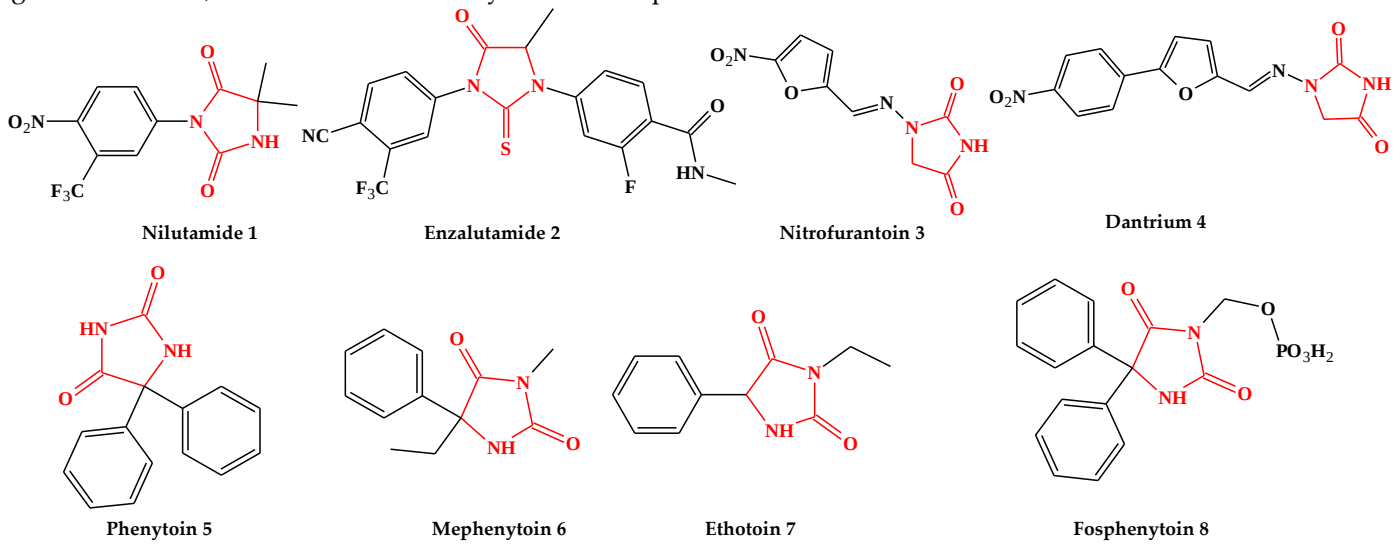
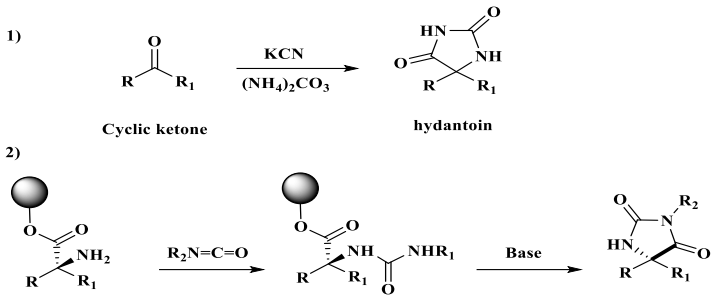


Fig. 1. Structure of hydantoin-based clinically approved drugs.



(1) Bucherer Bergs reaction. (2) From cyclic α, α-disubstituted α-amino esters.

Fig. 2. Representative synthetic methods for hydantoins.

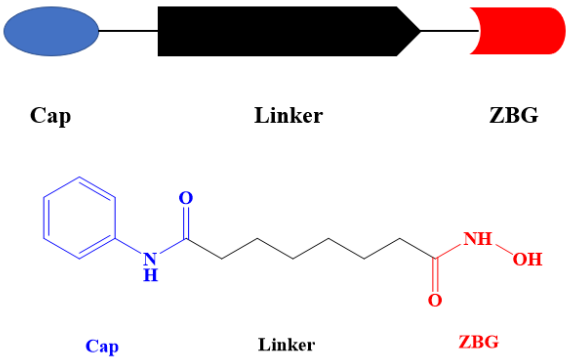
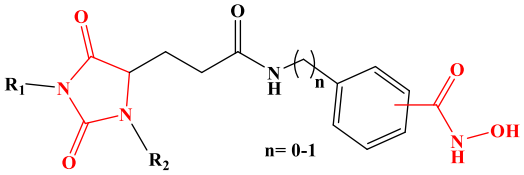


Fig. 3. Pharmacophore of HDACIs and the structure of SAHA.

In 2019, Liang and coworkers reported synthesizing and biological assessment of the 2,4-imidazolinedione *N*-hydroxybenzamide derivatives with disubstitutions (R1 and R2) **(9)** as HDAC6 selective inhibitors. HDAC6 overexpression has been observed in many tumor cell lines, and the presence of HDAC6 is crucial for efficient oncogenic cell transformation. A recent study indicates that the L1 loop pocket of HDAC6, located on the periphery of the lysine binding channel, serves as a conserved binding site that contributes to the selective binding of HDAC6 isoforms. Furthermore, the cap group of HDAC inhibitors plays a significant role in determining the isoform selectivity of these inhibitors [26,38,39]. In this study, all target compounds were evaluated for their ability to inhibit HDAC6, with vorinostat (SAHA) as the positive control. The data revealed that compound **(9a)** exhibited the most potent HDAC6 inhibitory action, surpassing the effectiveness of SAHA (IC_{50} = 4.4 nM and 39.9 nM for **9a** and **SAHA**, respectively) **(Fig. 4).** Suppression of HDAC6 has been linked to apoptosis promotion and inhibition of cancer cell growth. Consequently, the antiproliferative activity of the potent compound was assessed against various tumor cell lines [39]. Compound **(9a)** demonstrated superior antiproliferative properties against HL-60 and RPMI-8226 cells (IC_{50} = 0.25 μM and 0.23 μM, respectively). Furthermore, the antiproliferative effects of compound **(9a)** on K562, HCT-116, and A549 cell lines were nearly three times greater compared to the SAHA positive control **(Table 1).** These findings support the notion that compound **(9a)** exhibits remarkable selectivity for HDAC6 inhibition [39]. The SAR study on this compound series indicated that *N*-hydroxybenzamides with para-substitutions displayed superior HDAC6 inhibitory activity compared to meta-substitutions. Additionally, compounds lacking a spacer (n = 0) possessed suitable linkers that facilitated interaction between the cap groups and the L1 loop while enabling the ZBG to chelate effectively with zinc ions [39]. Molecular docking analysis of compound **(9a)** revealed that its hydroxamic acid group established essential hydrogen bonds with key residues (His 610, Tyr 782) and effectively chelated with the zinc ion. This observation provides insight into why para-substituted *N*-hydroxybenzamides exhibit greater potency than their meta-substituted counterparts [39].

Table 1: Antiproliferative activities of compound 9a and SAHA.

Comp	IC_{50} (mM)				
	K5622	HL-60	RPMI-8226	HCT-116	A549
9a	0.49	0.25	0.23	0.83	0.79
SAHA	1.45	0.52	0.57	1.81	2.42



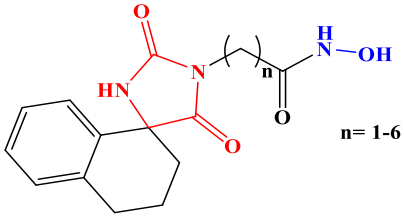
9a R_1= 4-Cl-Ph, R_2= 4-CH_3-Bn, n= 0, para position

HDAC6: IC_{50} = 4.4 nM
RPMI-8226 : IC_{50} = 0.23 μM
HL-60 : IC_{50} = 0.25 μM

Fig. 4. Design of hydantoin-based HDAC6 selective inhibitor, Series 9.

In 2018, Aboeldahab lab reported a series of 3',4'-dihydro-2'H-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione hydroxamic acid derivatives (**10**) **(Fig. 5)**. These hydantoin derivatives were evaluated for cytotoxicity against MCF-7 and HepG2 cell lines. According to the reported data, the hydroxamic acid derivatives were good growth inhibitors of MCF-7 cells and showed low activity against the HepG2 cell line. Moreover, as linker length **n** increased, the efficacy of the compounds increased as

well, making compound (**10a)** with spirohydantoin Cap and hexamethylene linker the most effective growth inhibitor of MCF-7 cells in this series with an IC_{50} value that is very similar to SAHA against this cancer cell line (IC_{50} = 2.56 mM and 2.18 mM, for **10a** and SAHA respectively) [27] **(Fig. 5)**. To gain insight into the mechanism of action of the synthesized compounds, Compounds' *in vitro* HDAC inhibitory activity was examined, their IC_{50} values against the HDAC1, HDAC2, HDAC4, and HDAC6 isoforms were calculated, and SAHA was used as a positive control. The findings demonstrated that the investigated substances significantly inhibited the four HDAC isoforms under investigation. However, it was clear that compound (**10a)** exhibited the most potent HDAC inhibitory action, with an IC_{50} value against HDAC4 that is equal to SAHA (IC_{50} = 0.0988 mM and 0.0911 mM, for **10a** and SAHA respectively) and an even lower IC_{50} value for HDAC1 (IC_{50} = 0.027 mM and 0.031 mM, for **10a** and SAHA, respectively) **(Fig. 5)** [27]. Through molecular docking simulations, compound **(10a)** was docked into the active site of HDLP, revealing interactions like SAHA, such as hydrogen bonding with Tyr297. Compound **(10a)** also formed hydrogen bonds with His132 and exhibited π-cation bonding between its aromatic ring and Lys267. Notably, compound **(10a)** displayed a higher CDocker energy, indicating a stronger binding affinity at the HDLP active site than SAHA [27].



Potency n= 6>>5>4>>3>2>>1
10a n= 6
MCF-7: IC_{50} = 2.56 μM
HDAC1: IC_{50} = 0.027 μM
HDAC4: IC_{50} = 0.0988 μM

Fig. 5. Design of hydantoin-based HDAC inhibitor, Series 10.

1.2. Hydantoins as sirtuin inhibitors

Recently, a growing interest has been in identifying new therapeutic targets involved in tumorigenesis. One such target is the family of NAD^+-dependent protein lysine deacetylases known as sirtuins (SIRTs) [40]. SIRTs belong to the class III histone deacetylases and rely on NAD^+ as a co-substrate for various enzymatic activities, including deacetylation and ADP-ribosylation [41]. These proteins are involved in multiple cellular pathways and play a role in preventing aging progression and age-related disorders such as cardiovascular diseases, diabetes, neurodegeneration, and various types of cancers [42]. Within the sirtuin family, SIRT1 and SIRT2 have garnered significant attention from researchers. Several studies have highlighted the prominent role of SIRT2 in regulating glucose and lipid metabolism through the deacetylation of various endogenous substrates.

Additionally, SIRT2 plays a crucial role in cancer development by influencing critical cellular processes such as metabolism, aging, inflammation, gene transcription, apoptosis, and even p53 deacetylation, which promotes cell growth. Consequently, the SIRT2 gene, located on human chromosome 19 and consisting of 18 exons in multiple organisms and invertebrate species, has emerged as an attractive therapeutic target in cancer research. Combined suppression of SIRT1 and SIRT2 isoforms has been shown to induce apoptosis in various tumor cell lines by increasing p53 acetylation [43–45].

In a study conducted by Sacconnay et al., novel 5-benzylidene hydantoins (**11**) were identified as inhibitors of SIRT enzymes (**Fig. 6**). Through cell-based assays and structure-based virtual screening, the researchers discovered these compounds and compared their inhibitory effectiveness to that of sirtinol, a pan-SIRT inhibitor. The derivatives featured scaffolds consisting of 3-benzyl-5-benzylidenehydantoin, with substitutions at the N3 and C3 positions of the hydantoin moiety [46]. Three compounds, **11a, 11b, and 11c**, exhibited promising inhibitory activities against SIRT1 and SIRT2, with IC_{50} values similar to or higher than sirtinol **(Table 2)**. Notably, compound **11b** demonstrated a significant increase in p53 acetylation ($P < 0.05$), comparable to the effects of sirtinol. Sacconnay and coworkers observed that the most potent inhibitors were those in which the R1 ring was substituted at the para or ortho position. Additionally, the R2 group was characterized by a halogen atom in the meta position and/or a hydroxyl group **(compound 11b**) or a tertiary amino group in the para position **(compounds 11a and 11c)** [46]**.** Molecular docking calculations were performed for compound **11b** within the catalytic pockets of SIRT1 (PDB code 4I5I) and SIRT2 (PDB code 3ZGV), both in the presence of co-crystallized NAD^+. In the case of SIRT1, the compound occupied the C-site sub-pocket rather than the lysine channel, like what was observed for EX-527, an uncompetitive SIRT1 inhibitor in relation to NAD^+. Interestingly, the position of the EX-527 derivative co-

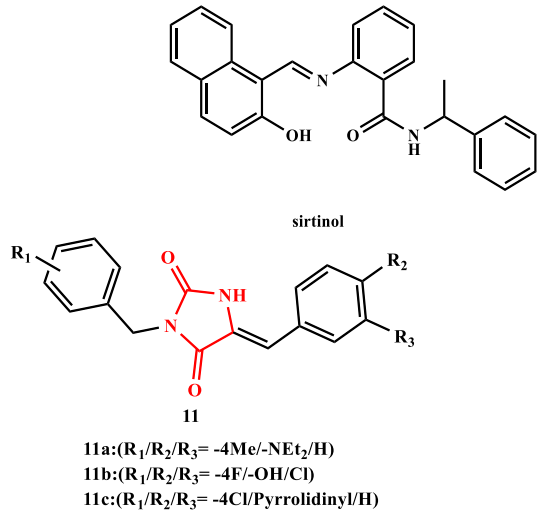


Fig. 6. Design of hydantoin-based sirtuin inhibitors, Series 11.

Table 2: Inhibitory activities of the selected 5-benzylidene-hydantoin derivatives against SIRT1 and SIRT2.

Comp	11a	11b	11c	Sirtinol
SIRT1 (IC_{50})	94.0	32.1	38.6	69.9
SIRT2 (IC_{50})	68.7	37.7	38.8	49.2

crystallized with SIRT1 aligned well with the docked structure of **11b**. The hydroxyl chlorophenyl ring of **11b** formed stacking interactions with the nicotinamide portion of NAD^+ and established van der Waals contacts with the Ile347 side chain. Additionally, the chlorine and hydroxyl groups of **11b** interacted with Phe297 and Val412, respectively, contributing to the stability of the complex. The hydantoin scaffold of **11b** fit well within a niche composed of Ala262, Phe273, Ile279, and Asn346 residues, while the chlorophenyl portion formed hydrophobic contacts with the Ile270 and Ile316 side chains. Notably, the docking results for **11b** demonstrated a similar interaction network in SIRT2, supporting its non-selective mechanism of action as observed *in vitro* [46].

1.3. Hydantoins as B-cell lymphoma-2 inhibitors

B-cell lymphoma-2 (Bcl-2) is a protein known for inhibiting apoptosis and its association with the development of various cancers [47]. Among the Bcl-2 protein family members, Bcl-2 was the first to be discovered [47,48]. The initial gene was found to promote prolonged cell survival and growth, emphasizing the significance of preventing cell death in tumor development [49]. Elevated expression of Bcl-2 has been observed in several cancer types, and it plays a crucial role in angiogenesis and cancer progression [50]. Apoptosis, triggered by tumor therapy, is a major mechanism of cell death. Targeting the Bcl-2 protein with chemotherapy drugs can potentially enhance apoptosis [51]. Therefore, Bcl-2 presents itself as a promising therapeutic target for malignancies, and Bcl-2 inhibitors have demonstrated promising results in various types of cancers, whether used alone or in combination with other medications [52].

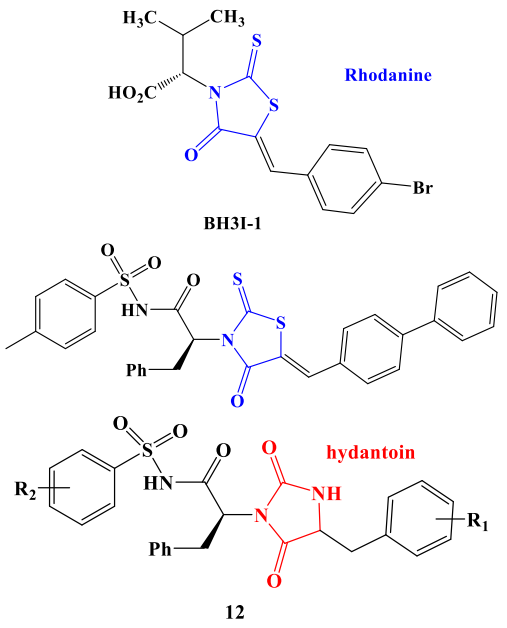
Rhodanine has undergone substantial research and has been considered a possible drug design scaffold for creating powerful Bcl-2 inhibitors. BH3I-1 and WL-276 are two examples of inhibitors with rhodanine scaffold [53]. By applying a bio-isosteric replacement strategy, Wang and coworkers succeeded in developing a new series of imidazolidine-2,4-dione derivatives **(12)** by replacing rhodanine core with a hydantoin moiety and testing their inhibitory activities against antiapoptotic Bcl-2 proteins **(Fig. 7)**. The results showed that the binding affinities to Bcl-2 were affected by various substituents in the aromatic ring of benzene-sulfonamide at the R2 position. For example, the methyl group in para position (**12a**, K_i >50 mM) is unfavorable for enhancing potency compared with the compound (**12b**, K_i = 19 mM) without substitution**.** On the other hand**,** the substitution of 3-NO2-4-Cl gave the most potent target compounds (**12c**, K_i = 3.7 mM and **12d**, K_i = 4.4 mM) comparable to the binding affinity of control WL-276 (K_i = 0.62 mM), which suggested that adding electron-withdrawing groups to the benzene-sulfonamide moiety would favorably increase the binding affinities with target protein [53]. Moreover, the researchers investigated whether the active target compounds exhibited affinities for other antiapoptotic Bcl-2 proteins, namely Bcl-X_L and Mcl-1, intending to determine if they could bind and inhibit these proteins. According to the data, the most potent compounds, **12c** and **12d**, displayed comparable binding affinities to all three antiapoptotic Bcl-2 proteins compared to the control compound WL-276 [53] **(Table 3)**. Furthermore, the selected compounds were evaluated for their antiproliferative activities against K562, PC-3, and MDA-MB-231 cell lines. Notably, compounds **12c** and **12d** exhibited superior antiproliferative activities compared to the other compounds. Specifically, compound **12d** demonstrated more potent inhibitory effects against the K562 and PC-3 cell lines than WL-276 **(Table 4)** [53].

Table 3: The binding affinities of the most active compounds to three Bcl-2 proteins.

Comp	K_i (mM)		
	Bcl-X_L	Bcl-2	Mcl-1
12c	6.1	3.7	2.9
12d	4.7	4.4	2.3
WL-276	0.66	0.62	0.25

Table 4: Antiproliferative activities of the most active compounds.

Comp	IC_{50} (mM)		
	K562	PC-3	MDA-MB-231
12c	39.4	51.7	70.8
12d	35.1	28.4	57.8
WL-276	44.9	39.8	35.2



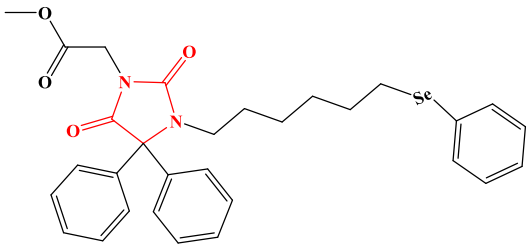
12a: R_1= 4-Cl-Ph / R_2= 4-CH_3
12b: R_1= 4-Cl-Ph / R_2= H
12c: R_1= 4-Cl-Ph / R_2= 3-NO_2,4-Cl
12d: R_1= 3-CH_3-Ph / R_2= 3-NO_2,4-Cl

Fig. 7. Design of hydantoin-based Bcl-2 inhibitors, Series 12.

1.4. Hydantoins as efflux pump P-glycoprotein inhibitors

Chemotherapy is considered one of the primary treatment options for cancer, and extensive research has been dedicated to discovering potent anticancer drugs. However, the emergence of multidrug resistance (MDR), wherein cancer cells reduce their sensitivity to drugs, poses a significant challenge in effectively treating malignancies with chemotherapy [54,55]. The development of drug resistance in cancer cells, resulting from previous exposure to the same or different chemotherapeutic agents, is the primary reason for the failure of cancer chemotherapy. One mechanism by which the MDR phenotype develops is the upregulation of cell transporters, which actively pump out anticancer drugs before reaching their intended therapeutic targets. Most transporters that confer resistance in cancer cells belong to the ATP-binding cassette (ABC) family, including the ABCB1 transporter, also known as P-glycoprotein (Pgp-1). This transporter functions as an efflux pump for various anticancer treatments. The MDR1 (ABCB1) gene, encoding P-gp, has been targeted as a therapeutic approach to overcome multidrug resistance, considering that cancer cells often overexpress P-gp [56,57]. In 2020, Wesam et al. discussed a novel chemical family of potent selenium-containing compounds that served as hybrids of phenylselenoethers with either aryl hydantoin or phenylpiperazine moieties **(Fig. 8)**. These hybrid compounds were assessed for their ability to modulate efflux in a mouse T-lymphoma cell line transfected with the human MDR1 gene, which encodes the ABC transporter ABCB1 [58]. The evaluation involved measuring the accumulation of rhodamine 123, a substrate for ABCB1. The percentage of mean fluorescence intensity was calculated for the treated MDR cells compared to the untreated cells, and a fluorescence activity ratio (FAR) was determined. Verapamil, a reference drug, was tested at the commonly used concentration of 20 μM, while all hybrid compounds were investigated at a 10-fold lower concentration of 2 μM [58]. Among the tested derivatives, the hydantoin derivatives (tested at 2 mM) exhibited strong inhibitory potency, surpassing the reference inhibitor verapamil by up to 2.6-fold (at 20 mM). Compound **13**, a hydantoin derivative, displayed the most potent activity with a FAR of 2.77 at 0.2 mM.

In comparison, verapamil had a FAR of 17.59 at 20 mM **(Fig. 8)**. Furthermore, cytotoxicity assays conducted on both sensitive (PAR) and resistant (MDR) mouse T-lymphoma cell lines revealed that the compound **13**, a 5,5-diphenylhydantoin derivative, exhibited the highest potency among the entire series, with IC_{50} values of 0.67 and 0.90 for PAR and MDR, respectively. Mechanistic studies indicated compound **13** inhibited cell cycle progression by reducing cyclin D1 expression and suppressed cell proliferation by inducing p53 expression. Additionally, the SAR analysis demonstrated that compounds with lipophilic properties and a longer selenoether spacer exhibited the most potent activity [58].



13
FAR= 2.77 at 0.2 mM
IC_{50}= 0.67 for PAR
IC_{50}= 0.90 for MDR

Fig. 8. Design of phenylselenoether-hydantoin hybrid-based Pgp-1 inhibitors.

Hydantoin-based new inhibitors targeting the Pgp-1 transporter were described by Martins *et al.* They evaluated the ABCB1-inhibitory properties of fourteen hydantoin compounds, which were classified into three distinct structural groups: completely substituted hydantoins (Class I) **14**, phenytoin derivatives (Class II) **15**, and monosubstituted benzylidene hydantoins (Class III) **16** [59] **(Fig. 9).** The researchers assessed the impact of hydantoin compounds on the accumulation of Ethidium bromide (EB) in MDR mouse lymphoma cells that were genetically modified to express the human ABCB1 gene, which encodes the Pgp-1 transporter responsible for the multidrug resistance (MDR) phenotype. The methodology involved monitoring the progressive increase in EB fluorescence induced by the hydantoin compound, which indicated its ability to inhibit the efflux of EB mediated by the transporter [59]. A higher accumulation of EB demonstrated a more significant inhibition of the cell's efflux pump system by the hydantoin compound. To determine the specific activity (SA) of the efflux pump inhibition in the MDR mouse lymphoma cells, the relative final fluorescence index (RFI) was divided by the number of micromoles of the hydantoin compound used in the experiment. All fourteen hydantoin compounds were evaluated at a consistent concentration of 20 mg/l to assess their activity against the Pgp-1 transporter. The obtained data was then converted into specific activity (SA) values represented as RFI/μmol, enabling a direct comparison of the effects of each hydantoin on the transporter. The results revealed that hydantoins **16a**, **16b**, **14b**, and **14e** exhibited the highest SA against ABCB1, while hydantoins **14a**, **14c**, **14d**, and **14f** demonstrated the lowest SA. Hydantoins **15**, **14g**, and **14h** were found to be inactive against the ABCB1 transporter [59] **(Fig. 10).** The structure-activity relationship (SAR) analysis for these compounds revealed that in Class I, the position and number of lipophilic substituents on both phenyl rings played a crucial role in determining their ABCB1-inhibitory properties. Specifically, methoxy or fluoride substitutions at the o-position on the phenylpiperazine ring led to the most effective structures, as observed in compounds **14b** and **14e**. Comparing the activities of compounds **14a**, **14b**, and **14c**, it was evident that both the m-position of the methoxy substituent (**14c**) and the absence of any lipophilic substituent in the aromatic rings (**14a**) resulted in a decrease in ABCB1 inhibition by these hydantoin derivatives [59]. The compounds **14g** and **14h** exhibited very low ABCB1-inhibitory properties, indicating a nonbeneficial impact of m-substitution at the phenylpiperazine phenyl ring for these structures. The phenytoin derivative, a Class II drug, displayed the least inhibitory action, and the authors hypothesized that the lower activity of this Class II compound was attributed to the hydrophilicity of its hydroxyethyl piperazine end fragment. On the other hand, the Class III derivatives (**16a** and **16b**) demonstrated the most potent activity, attributed to a lipophilic benzyloxy arylidene substituent at position 5 of the hydantoin structure [59].

1.5. Hydantoins as kinesin spindle protein inhibitors

Kinesins are ATPases that utilize ATP hydrolysis to generate energy for microtubule activity (MTs). The kinesin spindle enzyme (KSP), also known as Homo sapiens Eg5 (HsEg5), plays a significant role in the growth and function of the mitotic spindle, contributing to the regulation of mitosis by facilitating the formation of bipolar spindles and the separation of chromosomes. Due to its involvement in these crucial processes, targeting this protein, specifically inhibiting it, holds promise for cancer treatment. Inhibition of KSP leads to the formation of mono-astral spindles, resulting in cell cycle arrest and cell death while leaving other microtubule-dependent activities undisturbed [60,61]. In a previous study, a medium-sized chemical library consisting of 16,000 compounds underwent high-throughput screening to identify a selective inhibitor of Eg5, namely hexahydro-imidazo[1,5-β]-β-carboline-1,3-dione, also referred to as HR22C16 **(Fig. 11)** [62]. Shankaraiah *et al.* reported a series of new hydantoin-fused tetrahydro-b-carboline hybrids **(17)**, which were designed based on the structure of HR22C16 and evaluated for their *in vitro* cytotoxicity. The anticancer activity of these compounds was assessed using an MTT assay against five cancer cell lines. Interestingly, significant cytotoxicity was observed in the prostate cancer cell line (PC-3) for these compounds [61]. Among the tested compounds, compound **17a** exhibited

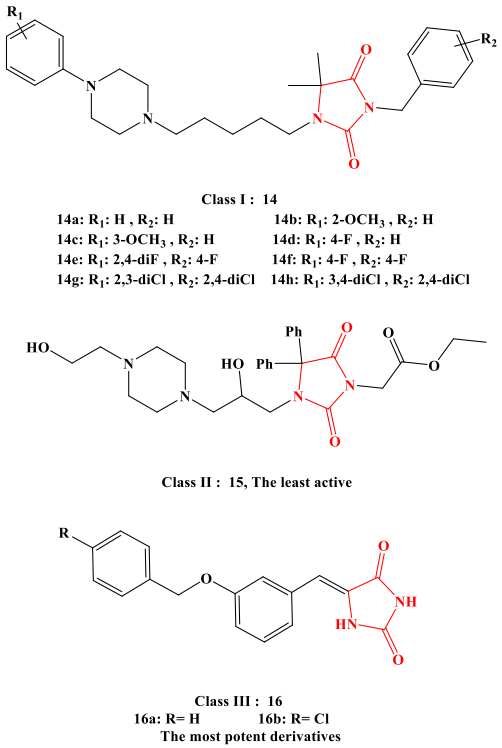


Fig. 9. Design of three classes of hydantoin-based Pgp-1 inhibitors.

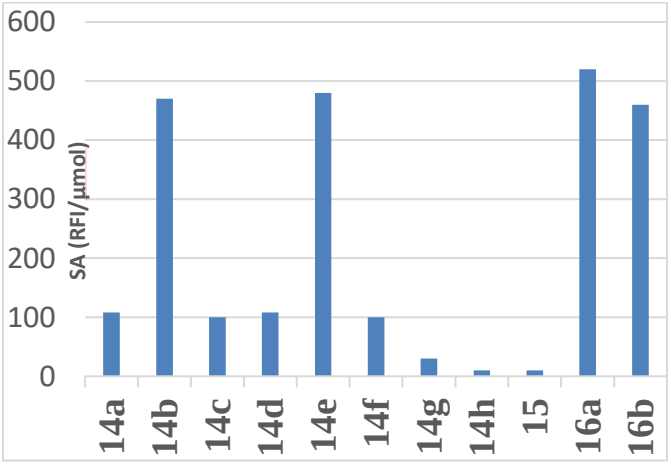


Fig. 10. The specific activity (SA) of hydantoin compounds on the Pgp-1 transporter of mouse lymphoma cells transfected with the human ABCB1 gene that codes for the ABCB1 transporter.

the highest cytotoxicity with an IC_{50} value lower than that of etoposide (IC_{50} = 6.08 µM and 14.4 µM for **17a** and etoposide, respectively) **(Fig. 11).** Flow cytometric analysis of compound **17a** in the PC-3 cell line indicated G2/M cell cycle arrest. To assess selectivity towards cancer cells, the compounds were also tested for cytotoxicity on normal prostate epithelial cells (RWPE). Compound **17a** showed high selectivity for prostate cancer cells compared to healthy prostate epithelial cells (IC_{50} values were five times higher in RWPE cells). In contrast, etoposide exhibited a 2-fold higher IC_{50} value in RWPE cells than PC-3 cells. Therefore, it was discovered that compound **17a** displayed more excellent selectivity for cancer cells than etoposide [61]. Molecular docking studies were employed to investigate the interaction of the targeted compounds (**17**) with the residues of the Kinesin spindle protein. The docking results revealed that all the ligands bound to the allosteric site of Eg5, consisting of the amino acids Tyr211, Leu214, Glu215, Gly117, Ala133, and Trp127. Similarly, to HR22C16, the aromatic ring of the phenol group in the compounds formed π-π stacking interactions with Tyr211, and the hydroxy group of this phenol group established hydrogen bond interactions with Gly117. Among the compounds, compound **17a** exhibited the most favorable interactions with the Kinesin spindle protein [61].

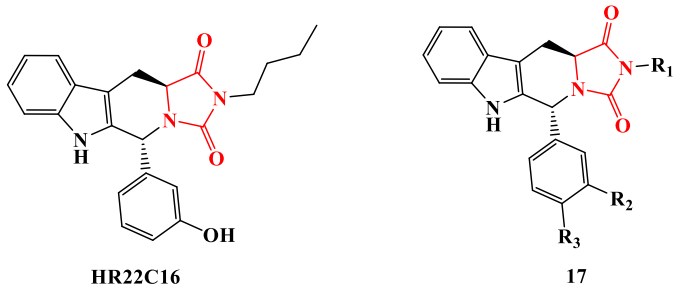


Fig. 11. Design of hydantoin-based kinesin spindle protein inhibitors, Series 17.

1.6. Hydantoins as tubulin polymerization inhibitors

Microtubules are vital components of the eukaryotic cytoskeleton, comprising α- and β-tubulin heterodimers. The dynamic equilibrium of polymerization and depolymerization is the physiological foundation of microtubule activity, playing essential roles in processes such as mitosis, cell shape maintenance, cell division, signal processing, organelle assembly, and transportation. Disrupting the dynamic equilibrium of the microtubule-tubulin system offers an effective antimitotic approach to halt the rapid proliferation of tumor cells [63,64]. Combretastatin A4 (CA-4) is a highly potent anticancer compound that interferes with tubulin polymerization. It is undergoing Phase III clinical trials as a phosphate prodrug for the treatment of various tumors. Extensive studies on the structure-activity relationship (SAR) of CA-4 and its analogs have revealed that the presence of a 3,4,5-trimethoxyphenyl moiety (Ring A) and the cis-orientation of the olefinic bond is crucial for their anticancer activity. Various cis-restricted CA-4 analogs,

incorporating rigid bridges, particularly heterocycles, have been identified to prevent isomerization to the trans-form. This isomerization would diminish both the anti-tubulin activity and cytotoxicity of the compounds [64].

A series of novel hydantoin-bridged analogs of combretastatin A-4 (CA-4) were designed, synthesized, and evaluated for antiproliferative activities by Zhang *et al.* **(Fig. 12)**. These compounds (**18**) were tested against four cancer cell lines, namely HeLa, A2780, HCT-116, and MDA-MB-231, with CA-4 used as a positive control. The results demonstrated that compound **18a** exhibited the most potent activity, with IC_{50} values ranging from 0.186 to 0.279 μM, while CA-4 displayed IC_{50} values ranging from 0.002 to 0.007 μM. Additionally, the cytotoxicity of compound **18a** was evaluated in a normal ovarian epithelial cell line (HOSE), with paclitaxel used as a positive control to assess selectivity between cancer cells and normal cells [64]. The findings revealed that at 10 and 50 μM, compound **18a** only reduced HOSE cell growth by up to 33%, whereas paclitaxel at the same concentrations caused 51% and 94% inhibition. This result demonstrated the high selectivity of compound **18a** towards tumor cells compared to normal cells. Although compound **18a** was less potent than CA-4, it exhibited non-toxicity towards normal cells (HOSE cells), unlike paclitaxel. Further investigations confirmed that compound **18a** effectively inhibited tubulin polymerization (IC_{50} = 16.2 μM for **18a** and 6.6 μM for CA-4), disrupted tumor vascularization, induced cell cycle arrest in the G2/M phase, and triggered cell apoptosis [64]. Moreover, nude mice xenograft tumor models were established to assess the in vivo anti-tumor efficacy of compound **18a**. The results demonstrated that at a dose of 20 mg/kg, **18a** significantly suppressed tumor growth with an inhibition ratio of 46.9% compared to the control group. Importantly, no observable body weight loss was observed at the end of the observation period (22.35 g in the control group and 22.05 g in the **18a** treated group), indicating the safety of compound **18a** at the therapeutic dosage. These findings confirmed the potent anti-tumor capabilities of compound **18a** in vivo [64].

1.7. Hydantoins as EGFR inhibitors

The epidermal growth factor receptor (EGFR) family plays a crucial role in the normal development of organs by regulating processes such as cell proliferation, differentiation, apoptosis, invasion, and angiogenesis [65–67]. In tumor cells, EGFR signaling is often dysregulated, allowing them to proliferate in unfavorable conditions, invade neighboring tissues, and promote angiogenesis

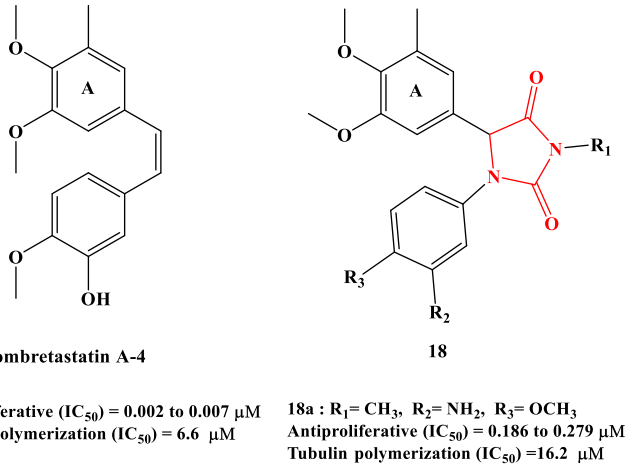
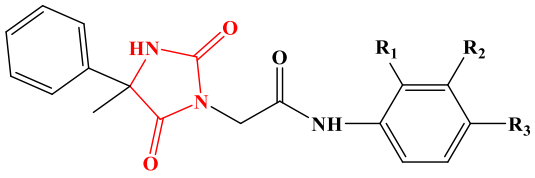


Fig. 12. Design of hydantoin-based tubulin polymerization inhibitors, Series 18.

This contrasts with normal cells with strict regulatory systems that govern EGFR pathways [65,68]. Unfortunately, the overexpression of the EGFR gene leads to the activation of various downstream signaling pathways, contributing to cancer aggressiveness and invasiveness [69]. Consequently, EGFR has become an important therapeutic target in anticancer therapy, supported by clinical evidence [70,71]. 2022 Hassanin and coworkers reported a series of hydantoin-acetanilide derivatives **19 (Fig. 13)** targeting $EGFR^{WT}$ and its mutations $EGFR^{L858R/T790M}$. Then, these derivatives were evaluated for their anti-NSCLC activity in A549, H1975, and PC9 cell lines, and erlotinib was used as a positive control. The findings demonstrated that the synthesized hydantoin acetanilide derivatives exhibited significant anticancer potency. Notably, compounds **19a** and **19b** displayed the highest potency, with average IC_{50} values lower than erlotinib (IC_{50} = 2.57 μM, 4.79 μM, and 11.26 μM for **19a**, **19b**, and erlotinib, respectively). In vitro, EGFR inhibition assays revealed that **19b** effectively inhibited both wild-type and mutant forms of EGFR. Specifically, **19b** demonstrated potent inhibitory activity against $EGFR^{WT}$, with approximately 3-fold lower IC_{50} values than the reference compound erlotinib. Moreover, it exhibited similar inhibitory activities against $EGFR^{L858R}$ and $EGFR^{T790M}$ mutations (IC_{50} = 0.05 μM and 0.09 μM, respectively) as compared to erlotinib (IC_{50} = 0.03 μM and 0.07 μM, respectively) [72]. The active hydantoin derivative **19b** induced significant cell cycle arrest at the sub-G1 and S phases and triggered apoptosis in A549 cells. To assess the safety of compounds **19a** and **19b**, an in vitro cytotoxic assay was performed on normal fibroblast WI-38 cells. The IC_{50} values indicated that hydantoin derivatives exhibited low cytotoxicity towards the normal

fetal lung WI-38 cells. Specifically, **19a** and **19b** were 2.3-fold and 2.6-fold more effective than the reference compound erlotinib, respectively (IC_{50} values of 42.45 μM and 49.30 μM for **19a** and **19b**, compared to 18.62 μM for erlotinib). These results suggest that **19a** and **19b** were minimally cytotoxic to the normal WI-38 cell line [72]. In the docking study conducted on EGFR (PDB: 5GTY), it was observed that all the target compounds **19** could interact with the active sites of the co-crystallized ligand binding sites, particularly with Met790 through hydrogen bonds. Additionally, all the compounds formed hydrogen bonding interactions with Thr854 and exhibited pi-H interactions with Leu777, along with hydrogen bonding with Lys745. The most potent derivative, **19b**, formed two hydrogen bonds with the gatekeeper mutant Met790, while the p-methoxy group was closer to Cys797 of the kinase hinge. In contrast, erlotinib did not show any binding interaction with Met790, which may explain the superior in vitro inhibition of mutated EGFR growth exhibited by the target compound. These results highlight the potential of **19b** as a promising lead compound for further development as an active agent in anticancer therapy [72].



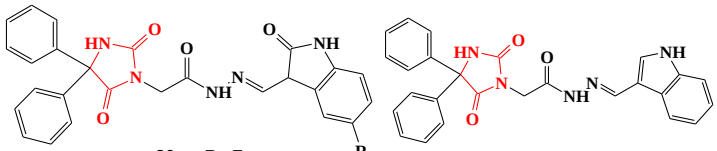
19

19a: R_1= F / R_2,R_3 = H

19b: R_1= H / R_2,R_3 = OCH_3

Fig. 13. Design of hydantoin-acetanilide derivatives as EGFR inhibitors, series 19.

In 2019, Alkahtani lab reported a series of novel hybrid derivatives of 5,5 diphenylimidazolidine-2,4-dione conjugated to 5-substituted isatin **(20)** and evaluated them for anticancer activity and EGFR and VEGFR2 inhibitory activity **(Fig. 14)**. The cytotoxic activity of the 2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl) acetamide derivatives was assessed against three cancer cell lines: HeLa, human lung adenocarcinoma (A549), and human breast adenocarcinoma (MDA-MB-231). Docetaxel was used as a reference compound. Overall, the results demonstrated that most compounds exhibited promising antiproliferative activity, with compound **20a** being the most effective [73]. It displayed an average IC_{50} value of 59 μM against the tested cell lines, compared to an IC_{50} of 83 μM for docetaxel. Notably, many of the tested compounds showed significant antiproliferative activity, specifically against HeLa cells, which were found to be the most sensitive cell line (average IC_{50} = 93 μM). In comparison to docetaxel (IC_{50} = 100 μM), compounds **20a**, **20b**, and **20c** exhibited higher cytotoxic effects on HeLa cells, with IC_{50} values of 18.5 μM, 10 μM, and 30 μM, respectively. Additionally, the inhibitory activity of the compounds against EGFR and VEGFR2 was evaluated using erlotinib as a reference. The results indicated that compounds **20b** and **20c** demonstrated potent inhibitory activity against EGFR, with lower or comparable IC_{50} values to erlotinib (IC_{50} = 0.10 μM, 0.37 μM, and 0.10 μM for **20b**, **20c**, and erlotinib, respectively) [73]. Notably, replacing the nitro group at position 5 in compound **20c** with a fluorine group in compound **20a** led to a significant reduction in inhibitory activity against EGFR (IC_{50} = 6.17 μM for **20a**). Interestingly, compound **20a** exhibited potent inhibitory activity against VEGFR2, surpassing the other compounds and showing an IC_{50} value comparable to erlotinib (IC_{50} = 0.09 μM and 0.004 μM for **20a** and erlotinib, respectively) [55]. Moreover, the docking study revealed that the hydantoin component of compound **20c** occupies the hydrophobic pocket of EGFR, establishing hydrophobic interactions with LEU 714, LEU 844, ALA 743, and VAL 726. On the other hand, the isatin moiety forms hydrogen bonds with SER 720, GLY 721, GLY 719, and LYS 745. It is observed that the presence of the nitro group at position 5 in compound **20c** diminishes the affinity of the isatin moiety towards the hydrophobic pocket. Consequently, these docking simulations provide insight into the selective nature of compound **20c** towards EGFR, while compound **20a** exhibits selectivity for VEGFR2 [73].



20a: R= F

20c: R= NO_2

20b

Fig. 14. Design of hydantoin-isatin conjugates as EGFR inhibitors, Series 20.

Zuliani and colleagues conducted a study on a series of 1,5-disubstituted hydantoins designed to interact with the ATP binding site of EGFR. They compared them to the compound **UPR1024**, which exhibited potent activity with 1-phenethyl and (E)-5-p-OH-benzylidene substituents. Pharmacological characterization of **UPR1024** revealed its dual mechanism of action, inhibiting EGFR autophosphorylation and inducing DNA damage in A549 cells. The researchers aimed to determine whether this dual mechanism was unique to **UPR1024** or a common characteristic among other compounds in the series [66].

Thus, they evaluated various derivatives with different substituents at positions 1, 3, and 5 on the hydantoin nucleus for their antiproliferative activity on the A549 cell line, known to respond to EGFR tyrosine kinase inhibitors. Four different classes of compounds were designed, and their inhibitory activity was assessed **(Fig. 15)**. The **21** series (**type A**), carrying a phenethyl side chain at position 1, showed growth inhibition ranging from 9.4% to 53% at a concentration of 20 μM. At the same time, the reference drug gefitinib exhibited 63% growth inhibition. No difference in activity was observed between E and Z isomers in the **type A** derivatives, and introducing a substituent on the 5-benzylidene ring improved the antiproliferative activity in the case of E-isomers [66]. The **22** series (**type B**) was designed by introducing different lipophilic side chains at the N1 position, with growth inhibition percentages ranging from 0% to 49%. N1-benzyl hydantoin derivatives exhibited increased activity in the E-isomers when a substituent was added to the 5-benzylidene ring. In contrast, compounds with N1-phenyl and N1-alkyl side chains displayed weak antiproliferative effects, indicating the significance of a phenethyl or benzyl group at position 1 for suppressing cell growth [66]. Methylation at the N3 position in the **23** series (**type C**) increased the activity of the E-isomer (growth inhibition percentage increased from 9.4% to 31%) without affecting the activity of the Z-isomer (growth inhibition percentage ranged from 42% to 45%). Compound **24** (**type D**) did not exhibit noticeable antiproliferative effects (growth inhibition percentage = 7.2%), highlighting the importance of the exocyclic double bond at position 5 on the hydantoin nucleus for both EGFR kinase inhibition and growth suppression in A549 cells [66]. Overall, none of the newly designed compounds surpassed the effectiveness of **UPR1024** (growth inhibition percentage = 53%). Still, most of them demonstrated growth suppression in human A549 cells at concentrations of 20 μM with inhibitions exceeding 20%. Elevated levels of the p53 protein are associated with cell cycle arrest and apoptosis, serving as biological indicators of DNA damage. To confirm the compounds' activity as DNA-damaging agents, selected derivatives were evaluated for their ability to increase p53 protein levels. Some of the chosen compounds exhibited a fold increase in p53 levels greater than 1.5 after 48 hours of treatment at a concentration of 20 μM. In conclusion, **UPR1024** and other 5-benzylidene hydantoin derivatives elevated p53 levels, indicating that the dual mode of action was a shared characteristic among **UPR1024** and other compounds in the series [66].

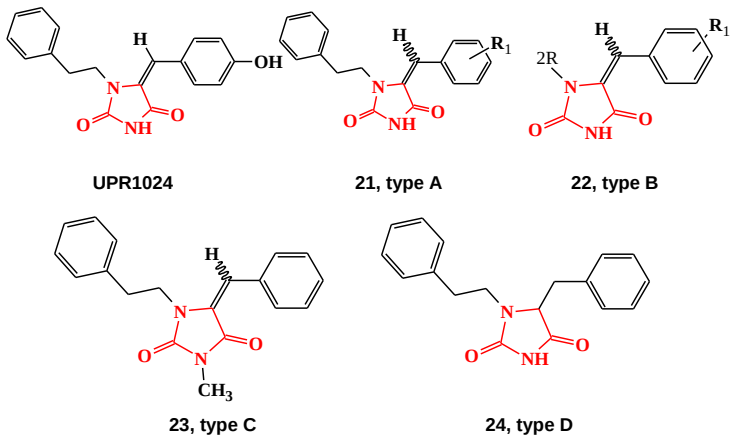


Fig. 15. Design of hydantoin-based EGFR inhibitors, Series 21-24.

2. Conclusion

Cancer represents the second major cause of mortality after cardiovascular disease. Hydantoin is a heterocyclic-based scaffold, which is highly valuable for discovering bioactive compounds. These scaffolds contain heteroatoms along with carbon atoms in the ring structure. This structural arrangement enables them to act as hydrogen bond donors or acceptors, effectively forming intermolecular hydrogen bonds with biological targets. Hydantoin derivatives can be synthesized by Bucherer Bergs reaction and from cyclic α, α-disubstituted α-amino esters through isocyanate coupling. This review highlighted the significance of hydantoin scaffolds and their recent applications in medicinal chemistry, showcasing their diverse pharmacological properties. Hydantoin-based compounds have demonstrated a range of pharmacological activities, including anticancer, antibacterial, anticonvulsant, and antidiabetic effects. Also, it focuses on the anticancer potential of hydantoin-containing compounds, elucidating their various targets such as HDAC, sirtuin, B-cell lymphoma-2, tubulin polymerization, kinesin spindle protein, and EGFR inhibitors. Despite the potential of hydantoin derivatives as targeted therapies for cancer treatment, several challenges and limitations must be addressed to maximize their effectiveness and safety. A significant challenge is the development of drug resistance, where cancer cells acquire mechanisms that reduce the efficacy of hydantoin-based agents over time. To overcome this, combination therapies that target multiple pathways simultaneously may offer a promising approach to combat resistance and improve treatment outcomes. Additionally, further investigation is required to elucidate the molecular

mechanisms of action for hydantoin derivatives fully. A better understanding of their precise mode of action would facilitate the rational design of more potent and specific compounds with enhanced efficacy.

Ethical consideration: All the participants in this study gave their informed permission.

Conflicts of Interest

No conflicts of interest are disclosed

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