Journal of Drug Discovery, Development and Delivery

Open Access

Austin Publishing Group

Research Article

Inhibition of γ-H2AX Protein by Molecular Modeling in Radiation-Resistant Cancer Cells

Dali-Sahi M[1]*, Nafuye G[1], Dennouni-Medjati N[1], Merad M[2] and Harek Y[1]

[1]Department of Biology, University of Tlemcen, Algeria

[2]Department of Chemistry, University of Tlemcen, Algeria

***Corresponding author:** Dali-Sahi M, Department of Biology, University of Tlemcen, Laboratory of Analytical Chemistry and Electrochemistry, Algeria BP119 13000, Tlemcen, Algeria

Received: March 03, 2020; **Accepted:** April 28, 2020; **Published:** May 05, 2020

Abstract

The objective of this study is to propose therapeutic targets to inhibit *in silico* the activity of γ-H2AX with MDC1 responsible for recruiting DNA repair proteins to make cancer cells radiosensitive.

The protein complex to be studied was retrieved from a protein database (PDB ID - 2AZM) and the constraints were removed using Biova Discovery Studio Visualizer. The docking ligands were selected from the PubChem database and modifications were made using ChemDraw ultra 12.0 and molecular docking was performed with Autodock 4.2. After docking, the ADME analysis and toxicity were performed against possible inhibitors using the admetSAR web server.

The molecular docking results indicated that ligand 6 ($C_{20}H_{14}N_2O_3S_2$) and R6 ($C_{19}H_{14}N_2O_3S_2$) had a minimal binding energy (-6.7Kcal/mol) and a positive ADMET analysis prediction profile. After modification of ligand 6, the results also showed that R6 had the minimum binding energy (-7.3Kcal/mol) and a convincing ADMET prediction profile.

We therefore conclude that the ligands used in this study, in particular ligand 6 and its modified derivatives R1 ($C_{21}H_{16}N_2O_3S_2$), R2 ($C_{21}H_{16}N_2O_2S_2$), R3 ($C_{20}H_{16}N_2OS_2$), and R6 ($C_{19}H_{14}N_2O_3S_2$) are considered as potential radio-sensitizers to improve the effectiveness of radiotherapy and can also be used for further studies.

Keywords: DNA repair; γ-H2AX; ADMET; Molecular docking; Radio-sensitizer

Introduction

Since the discovery of ionizing radiation in 1895, radiation therapy has become the treatment of choice for many types of cancer and has been applied as a first-line treatment for many malignant tumors in humans [1].

However, many cancer cells have a standard resistance to radiotherapy, and in many cases, resistance to radiotherapy is an adaptive response to the hyperactive repair mechanisms of Double-Strand Breaks (DSB) [2].

Phosphorylated H2AX, called gamma-H2AX (γ-H2AX), is one of the first proteins involved in DNA damage response pathways (DDRs). It is necessary for amplification of DNA damage signal and subsequent accumulation of many DDR proteins at DSB sites to form ionizing radiation-induced foci (IRIFs) [3-6].

In response to DSB, the conserved C-terminal tail of H2AX rapidly becomes phosphorylated on the serine-139 by Phosphoinositide Kinase 3-kinase (PI3-K) kinases, including Ataxia Telangiectasia Mutated protein kinase (ATM), Ataxia Telangiectasia and Rad3-related protein (ATR) and a DNA-dependent protein kinase, catalytic sub unit (DNA-PKcs).

ATMs and DNA-PKcs show functional redundancy in H2AX phosphorylation after ionizing irradiation, whereas ATRs are more important for phosphorylation of H2AX in response to DNA damage that would slow or block replication [7].

The mediator of DNA damage check point protein 1 (MDC1) works closely with γH2AX in DDR, as it is necessary for almost all foci formation events induced by ionizing radiation dependent on γ-H2AX as a result of DNA damage. In response to DSB, MDC1 binds directly to γ-H2AX through its C-terminal BRCT protein domains [8,9].

The objective of this study is to propose a therapeutic target, use *insilico* methods to inhibit γ-H2AX activity with the MDC1 responsible for the recruitment of DNA repair proteins to make cancer cells radiosensitive.

Materiel and Methods

Software

Discovery Studio v17.2.0.16349 [10], AutoDock tools and vina 4.2 [11] and ChemDraw Ultra 12.0 were used for three dimension structure preparation, binding site defining, molecular docking and derivatives generating.

Protein structure and ligand presentation

The crystal structure of the BRCT domain of MDC1--H2AX complex was downloaded from the Protein Data Bank (PDB code: 2AZM). According to the residues of the BRCT domain of MDC1 revealing its hydrogen bond interaction with γ-H2AX, the involved residues were defined as its binding site.

Citation: Dali-Sahi M, Nafuye G, Dennouni-Medjati N, Merad M and Harek Y. Inhibition of γ-H2AX Protein by Molecular Modeling in Radiation-Resistant Cancer Cells. J Drug Discov Develop and Deliv. 2020; 6(1): 1034.

We screened a chemical library (PubChem database) to find potential inhibitors of the MDC1-H2AX interaction. The search was based on the chemical similarity of the functional groups of the phosphoserine of -H2AX. Six potential inhibitors (Lig 1, Lig 2, Lig 3, Lig 4, Lig 4 and Lig 6) were identified.

Molecular docking

Here we used Autodock 4.2 for molecular docking. Molecular docking fits two molecules in favorable configuration using their topographical features. Practically molecular docking has been an important technique for the modeling protein-ligand interactions and has been used in studies of the structural basis of biological functions. Essential parameters like hydrogen atoms, and kollman charges were added to the modeled protein structure using Autodock tool. Grid box was then generated using Autogrid program so that it cover entire protein binding sites and make ligand to move freely in that site. For the inhibitor, charges of the Gasteiger type were assigned using Autodock Tool. Other docking parameters were set to the software's default values. After docking completion the docked model was ranked according to their docked energy as implemented in the AutoDock program.

Molecular docking of ligands at the MDC1 binding site was performed using Autodock Vina software. The docking tests were carried out with a radius of 0.375Åwith the coordinates x: 47.35, y: 77.28 and z: 85.487. The best ranked docking pose of each ligand in complex with MDC1 was obtained based on the scores and the binding energy value. The docked complex was then analyzed using BIOVIA Discovery Studio Visualizer to show the type of interactions between the ligands and MDC1, to determine the distance of the ligands from the binding site on MDC1 and to generate the 2D structures of the complexes.

The ADMET Analysis

The Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of drug candidates or environmental chemicals play a key role in drug discovery and environmental risk assessment. The ADMET structure-activity relations server, called admetSAR, is a comprehensive tool for predicting the ADMET properties of drug candidates and environmental chemicals [12]. This web server allowed us to calculate the penetration of the Blood-Brain Barrier (BBB), Human Intestinal Absorption (HIA), permeability of human colon adenocarcinoma cell lines ($Caco_2$), plasma glycoprotein binding substrate and inhibition, CYP inhibitory promiscuity, human ether-a-go-go gene inhibition (hERG), AMES toxicity and carcinogenicity. Pre-ADMET is useful for high throughput screening and combinatorial chemistry library design considering the Lipinski's rule or lead-like rule, drug absorption and water solubility.

Results and Discussion

Molecular docking allowed us to evaluate the interaction energies of the complexes; first between the BRCT domain of the MDC1 protein (NFBD1) and the γ-H2AX tail (Ref) and then between the BRCT domain of the MDC1 protein and the different prospective inhibitors (ligands) that were downloaded from the PubChem databases. Table 1 includes the results of calculations made in the search for the best possible conformation.

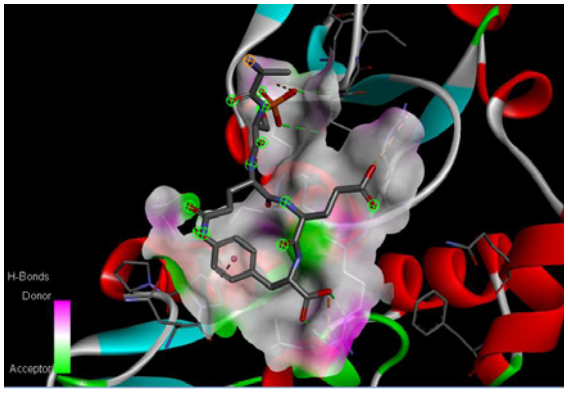


Figure 1: 3D structure of the interaction between the BRCT domain of MDC1 protein and the γ-H2AX tail.

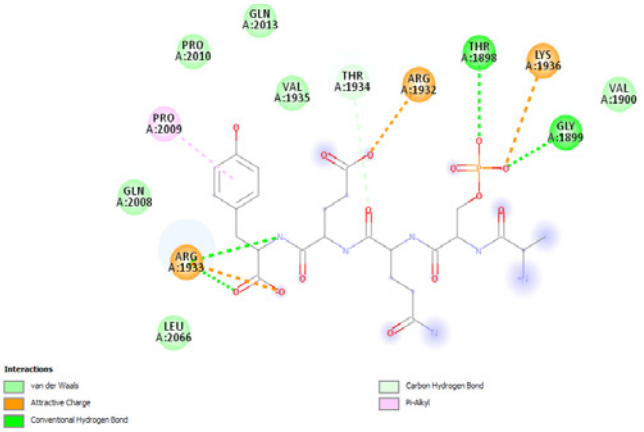


Figure 2: 2D structure of the interaction between the BRCT domain of MDC1 protein and the γ-H2AX tail.

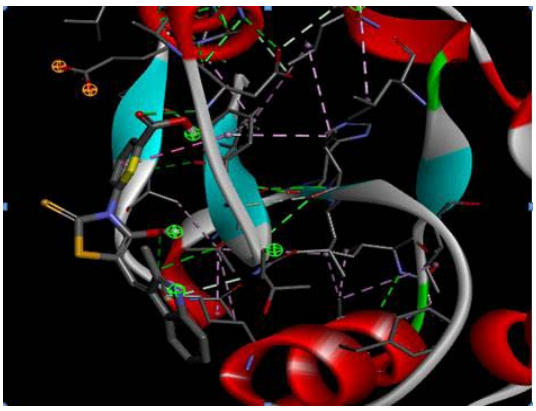


Figure 3: 3D structure of the interaction between the BRCT domain of MDC1 protein and ligand 6.

The results in Table 1 show that the energy of interaction obtained after docking between the ligands and MDC1. From these results, we can attest that ligand 6 presents the minimum energy of interaction (-6.7Kcal/mol) and ligand 4 ($C_{16}H_{16}BrNO_3S_2$) presents the maximum energy of interaction (-5.3Kcal/mol). As it is in molecular docking, the smaller the energy of interaction the more stable the complex formed between the ligand and the receptor.

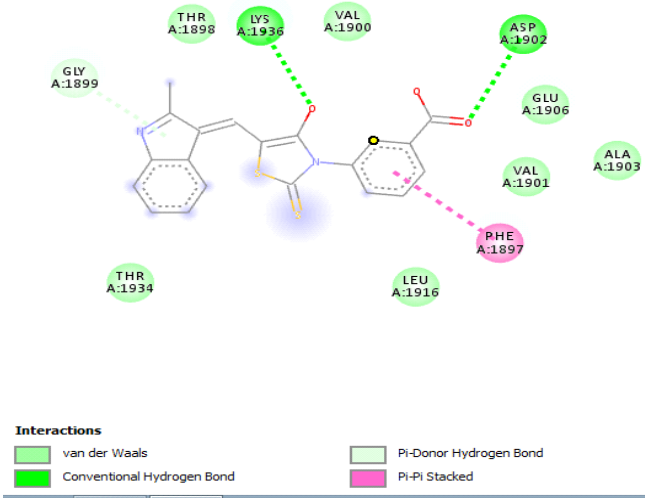


Figure 4: 3D structure of the interaction between the BRCT domain of MDC1 protein and ligand 6.

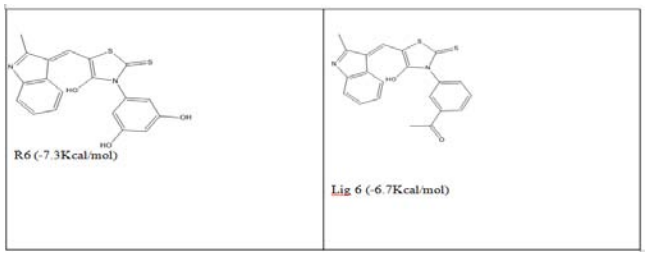


Figure 5: Structure of ligand 6 and R6 with their respective binding energy.

Analysis of the MDC1 BRCT-H2AX co-crystal structure revealed that 3 residues of BRCT1 engage in direct hydrogen-bond interactions with γ-H2AX: Thr1898 and Lys1936 contact the phosphoserine, and Arg1933 contacts both the peptide backbone and the C-terminal carboxylate group [9]. However this almost corroborated in our results as Thr1898 engaged in direct hydrogen bond with the phosphoserine, whereas Lys 1936 engaged in salt bridge interaction with an attractive charge with the phosphoserine (Figure 1&2). Meanwhile Arg1933 contacts both the peptide backbone and the C-terminal carboxylate group as documented elsewhere [9].

More to that, from the docking results all the ligands engaged in a direct interaction with at least one of the three functional amino acids of the binding site on the BRCT 1 domain of MDC1. Lig 1, Lig 2, Lig 4 and Lig 6 (Figure 3,4 &5) engaged in direct hydrogen-bond interaction with Lys 1936, whereas ligand 3 engaged in a pi-cation interaction with Lys 1936 and ligand 5 engaged in a pi-alkyl interaction with Lys1936 (results not shown).

The Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) analysis results and their Probabilities (Prob) are summarized in table 2. These Results (Res) are categorical for instance, blood brain barrier penetration (BBB+/BBB-), human intestinal absorption (HIA+/-), human adenocarcinoma cell lines permeability ($Caco_2$-/$Caco_2$+), p-glycoprotein substrate and inhibitor (yes/no), CYP inhibitory promiscuity (low/high), human Ether-a-go-go-Related Gene inhibition (yes/no), AMES toxicity (toxic/no) and carcinogenicity (carcinogen/no) Table 2.

Table 1: Molecular docking results of -H2AX and inhibitors with MDC1.

Ligand	PubChem CID	Binding energy (Kcal/mol)	H-bond	Binding residue
Ref	γ-H2AX	-5.6	4	Thr 1898
				Gly 1899
				Arg 1933
Lig 1	565699	-5.9	3	Lys 1936
				Gly 1918
				Asp 1902
Lig 2	44429173	-5.8	1	Lys 1936
Lig 3	44429172	-6.4	2	Gly 1899
				Val 1900
Lig 4	4515070	-5.3	2	Lys 1936
Lig 5	1576659	-5.5	-	
Lig 6	1391580	-6.7	2	Lys 1936
				Asp 1902

Lig: Ligand

Using Chemdraw Ultra 12.0, we drew and modified Lig 6 by substituting the carboxylic group on the benzene ring with different chemical groups (CH_3C00- to form R1, CH_3CO- to form R2, CH_3 to form R3, $(CH_3)_2CH_2$ to form R4, OH to form R5and $(OH)_2$ to form R6) and Chem3D Pro were used to change their structures from two-dimension to three-dimension. The purpose of these modifications was to see if there could be variation in the energy of interaction. We determined the physicochemical properties of this ligand and docked them into the binding site of the BRCT domain of MDC1 to determine their energy of interaction Table 3. These modifications further decreased the energy of interaction with ligands R1, R2, R3, and R6 showing improvement Table 3.

The Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) predicted profile also showed improved results Table 4, there was improvement in the blood-brain barrier penetration, human intestinal absorption, decreased hERG inhibition, non-AMES toxic, and non-carcinogenic. However, the predictions showed that there could be inhibition of the plasma glycoproteins and also a high CYP inhibitory promiscuity as compared to the ADMET predicted profile of ligand 6.

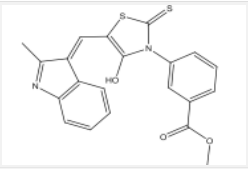
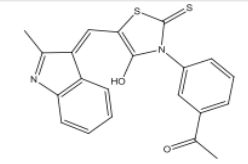
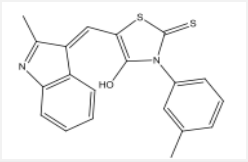
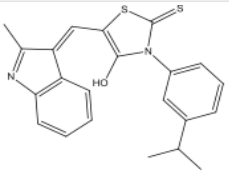
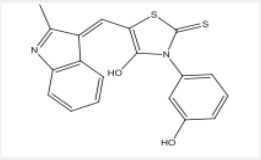
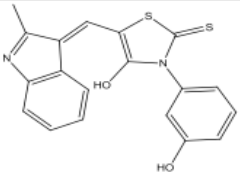
In other studies conducted elsewhere in search for radio-sensitizing agents, success has been registered. An antimetabolite designed by Taiho Pharmaceuticals is currently used in conjunction with radiotherapy in Japan [13] and it's under phase III trials in Europe and USA [14]. Another radio-sensitizing agent AZ0156 which targets ATM kinase has demonstrated potential to hypersensitize cancer cells to ionizing radiation [15] and is currently in phase one trials. Another radio-sensitizing agent veliparib which targets Poly(ADP-ribose) Polymerase (PARP) has shown promising results in sensitizing Melanoma, pancreatic cancer, glioma, non-small cell lung cancer, breast cancer to ionizing radiation and is currently under phase III/clinical trials [16]. Though most radio-sensitizers are of chemical nature, few natural compounds have also been identified to sensitize cancer cells to ionizing radiations. These include curcumin [16-18], genistein [19,20] and quercetin [21].

Table 2: Results of the ADMET predicted profile with admetSAR.

	Lig 1		Lig 2		Lig 3		Lig 4		Lig 5		Lig 6	
	Res	Prob	Res	Prob	Res	Prob	Res	Prob	Res	Prob	Res	Prob
Blood-brain barrier	BBB-	0.533	BBB-	0.628	BBB+	0.569	BBB+	0.67	BBB+	0.826	BBB+	0.612
Human intestinal absorption	HIA-	0.731	HIA+	0.978	HIA+	0.984	HIA+	0.963	HIA+	0.918	HIA+	0.958
$Caco_2$ permeability	Cac_2-	0.565	$Caco_2$-	0.54	$Caco_2$-	0.52	$Caco_2$-	0.535	$Caco_2$-	0.522	$Caco_2$-	0.526
P-glycoprotein substrate	Non	0.778	Non	0.656	Non	0.628	Non	0.742	Non	0.797	Non	0.685
P-glycoprotein inhibitor	Non	0.958	Non	0.523	Non	0.565	Non	0.762	Non	0.759	Non	0.623
CYP inhibitory promiscuity	Low	0.887	high	0.766	High	0.825	high	0.746	high	0.771	high	0.895
hERG inhibition	Non	0.911	Non	0.914	Non	0.884	Non	0.911	Non	0.86	Non	0.632
AMES toxicity	Toxic	0.912	Non toxic	0.727	Non	0.731	Non	0.682	Non	0.672	Non	0.595
Carcinogens	Non	0.768	Non	0.876	Non	0.901	Non	0.832	Non	0.855	Non	0.663

Lig: Ligand; Res: Result; Prob: Probability; BBB: Blood Brain Barrier

Table 3: Summary of the physicochemical properties and interaction energy between the modified ligands and MDC1.

	Structure	Name	Molecular mass (g/mol)	Binding energy (Kcal/Mol)	H-bond	Binding residue
R1		Methyl 3-{4-hydroxy-5-[(E)-(2-methyl-3H-indol-3-ylidene)methyl]-2-thioxo-1,3-thiazol-3(2H)-yl}benzoate	408.50	-7.0	2	Gly1899 Thr1898
R2		1-(3-{4-hydroxy-5-[(E)-(2-methyl-3H-indol-3-ylidene)methyl]-2-thioxo-1,3-thiazol-3(2H)-yl}phenyl)ethanone	392.51	-7.2	3	Thr1898 Gly1899 Val1900
R3		4-hydroxy-5-[(E)-(2-methyl-3H-indol-3-ylidene)methyl]-3-(3-methylphenyl)-1,3-thiazole-2(3H)-thione	364.5	-6.8	1	Gly1899
R4		4-hydroxy-5-[(E)-(2-methyl-3H-indol-3-ylidene)methyl]-3-[3-(propan-2-yl) phenyl]-1,3-thiazole-2(3H)-thione	392.55	-6.7	1	Gly1899
R5		4-hydroxy-3-(3-hydroxyphenyl)-5-[(E)-(2-methyl-3H-indol-3-ylidene)methyl]-1,3-thiazole-2(3H)-thione	366.47	-6.5	3	Thr1898 Gly1899 Val1900
R6		3-(3,5-dihydroxyphenyl)-4-hydroxy-5-[(E)-(2-methyl-3H-indol-3-ylidene) methyl]-1,3-thiazole-2(3H)-thione	382.47	-7.3	2	Lys1936 Gly1899

Conclusion

Ionizing Radiation (IR) as the basis of radiotherapy is one of the three standard treatment modalities used against cancer and is indicated for approximately 60% of cancer patients [20]. Certain cancers such as glioblastoma, cancer of the bladder, breast cancer, advanced non-small cell lung cancer, soft tissue carcinoma show high survival rates after treatment with radiotherapy due to radio-resistance. Targeting pathways such as the DNA Damage Repair (DDR) which induce radio-resistance could improve on the

Table 4: Results of the ADMET predicted profile with admetSAR.

Model	R1		R2		R3		R4		R5		R6	
	Res	Prob	Res	Prob	Res	Prob	Res	Prob	Res	Prob	Res	Prob
Blood-brain barrier	BBB+	0.97	BBB+	0.857	BBB+	0.881	BBB+	0.818	BBB+	0.772	BBB+	0.627
Human intestinal absorption	HIA+	0.954	HIA+	0.992	HIA+	0.971	HIA+	0.976	HIA+	0.98	HIA+	0.969
$Caco_2$ permeability	$Caco_2$-	0.528	$Caco_2$	0.52	$Caco_2$+	0.513	$Caco_2$+	0.5	$Caco_2$-	0.507	$Caco_2$-	0.52
P-glycoprotein substrate	Non	0.712	Non	0.749	Non	0.753	Non	0.733	Non	0.745	Non	0.708
P-glycoprotein inhibitor	Yes	0.59	Yes	0.707	Yes	0.673	Yes	0.781	Yes	0.637	Yes	0.678
CYP inhibitory promiscuity	high	0.93	high	0.966	High	0.973	high	0.973	high	0.974	high	0.962
hERG inhibition	Non	0.509	Non	0.515	Non	0.518	Non	0.53	Non	0.585	Non	0.509
AMES toxicity	Non	0.561	Non	0.555	Non	0.513	Non	0.543	Non	0.557	Non	0.591
Carcinogens	Non	0.725	Non	0.684	Non	0.709	Non	0.677	Non	0.68	Non	0.621

effectiveness of radiotherapy.

In our study we analyzed the protein-protein interaction between the gamma-H2AX and the BRCT domain of MDC1 using molecular docking tools and further anticipated inhibitors which could prevent this interaction. As the interaction between these two proteins leads to the recruitment of DNA Damage Repair (DDR) proteins and thus enhances radio-resistance in cancerous cells [9].

The results obtained after molecular docking of the BRCT domain of MDC1 and various ligands showed that ligand 6($C_{20}H_{14}N_2O_3S_2$) presented the minimum energy of interaction (-6.7Kcal/mol) and a positive ADMET predicted profile. Modification of ligand 6 by substitution of its carboxylic group with several chemical groups again showed better results with the modified ligand R6($C_{19}H_{14}N_2O_3S_2$) presenting the minimum energy of interaction (-7.3Kcal/mol) and a positive ADMET predicted profile.

Virtual screening methods are regularly used for the cost and time of new drug discovery. It has been clearly demonstrated that the approach used in this study proves that the new inhibitors to be modified (R1, R2, R3, and R6) have shown a high binding energy affinity with a score of (-7.0, -7.2, -6.8 and -7.3) Kcal/mol, respectively. According to Lipinski's rules, all compounds could be good candidates for the development and could improve on the effectiveness of radiotherapy.

To conclude, given the results obtained in this work, which consists in elucidating the inhibition of the gamma-H2AX protein by molecular modeling methods, it seems that R6 probably has a better contribution to inhibition for prevent recurrence after treatment. The modification of ligand 6 by addition of the radical probably increased the stability of the complex formed. Subsequently the synthesis of compound is proposed as well as the study of the biological activity.

References

1. Lewanski CR, Gullick WJ. Radiotherapy and cellular signalling. Lancet Oncol. 2001; 26: 366-370.
2. Begg AC, Stewart FA, Vens C. Strategies to improve radiotherapy with targeted drugs. Nat Rev Cancer. 2011; 11: 239-253.
3. Bassing CH, Suh H, Ferguson DO, Chua KF, Manis J, Eckersdorff M, et al. Histone H2AX: a dosage-dependent suppressor of oncogenic translocations and tumors. Cell. 2003; 114: 359-370.
4. Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, et al. Genomic instability in mice lacking histone H2AX. Science. 2002; 296: 922-927.
5. Huen MSY, Chen J. Assembly of checkpoint and repair machineries at DNA damage sites. Trends Biochem Sci. 2010; 35: 101-108.
6. Paull TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr Biol CB. 2000; 10: 886-895.
7. Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S, et al. γH2AX and cancer. Nat Rev Cancer. 2008; 8: 957-967.
8. Lou Z, Minter-Dykhouse K, Franco S, Gostissa M, Rivera MA, Celeste A, et al. MDC1 Maintains Genomic Stability by Participating in the Amplification of ATM-Dependent DNA Damage Signals. Mol Cell. 2006; 21: 187-200.
9. Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 Directly Binds Phosphorylated Histone H2AX to Regulate Cellular Responses to DNA Double-Strand Breaks. Cell. 2005; 123: 1213-1226.
10. Discovery Studio Visualization.
11. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem. 2010; 31: 455-461.
12. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, et al. AdmetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. J Chem Inf Model. 2012; 52: 3099-3105.
13. Tsujinaka T, Fujitani K, Hirao M, Kurokawa Y. Current status of chemoradiotherapy for gastric cancer in Japan. Int J Clin Oncol. 2008; 13: 117-120.
14. Home - ClinicalTrials.gov.
15. Pike KG, Barlaam B, Cadogan E, Campbell A, Chen Y, Colclough N, et al. The Identification of Potent, Selective, and Orally Available Inhibitors of Ataxia Telangiectasia Mutated (ATM) Kinase: The Discovery of AZD0156 (8-{6-[3-(Dimethylamino)propoxy]pyridin-3-yl}-3-methyl-1-(tetrahydro-2 H-pyran-4-yl)-1,3-dihydro-2 H-imidazo[4,5-c]quinolin-2-one). J Med Chem. 2018; 61: 3823-3841.
16. Werner TL, Sachdev J, Swisher EM, Gutierrez M, Kittaneh M, Stein MN, et al. Safety and pharmacokinetics of veliparib extended-release in patients with advanced solid tumors: a phase I study. Cancer Med. 2018; 7: 2360-2369.
17. Kuttan R, Bhanumathy P, Nirmala K, George MC. Potential anticancer activity of turmeric (Curcuma longa). Cancer Lett. 1985; 29: 197-202.
18. Rodrigues FC, Anil Kumar NV, Thakur G. Developments in the anticancer activity of structurally modified curcumin: An up-to-date review. Eur J Med Chem. 2019; 177: 76-104.
19. Davis TA, Mungunsukh O, Zins S, Day RM, Landauer MR. Genistein induces radioprotection by hematopoietic stem cell quiescence. Int J Radiat Biol. 2008; 84: 713-726.

20. Papazisis KT, Zambouli D, Kimoundri OT, Papadakis ES, Vala V, Geromichalos GD, et al. Protein tyrosine kinase inhibitor, genistein, enhances apoptosis and cell cycle arrest in K562 cells treated with gamma-irradiation. Cancer Lett. 2000; 160: 107-113.

21. Perez C. Principles and practice of radiation oncology. Philadelphia: Lippincott Williams & Wilkins. 2004.