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Comparative modeling, comparative molecular docking analyses, and revealing of potential binding pockets of MDM-2: A candidate cancer gene

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Abstract

MDM-2 is also known as E3 ubiquitin-protein ligase encoded by *Mdm-2*. MDM-2 is an important negative regulator of *p53* tumor suppressor and performs key function as an inhibitor of *p53* transcriptional activation and E3 ubiquitin ligase. MDM-2 also plays significant role in human cancers and therapeutic target. Hundred different structures were predicted through comparative modeling, threading and *ab initio* approaches followed by the evaluation of predicted structures through various evaluation tools including ERRAT, ProSa-web, Rampage, molprobidity, verify3D and Anolea. The selected 3D structure of MDM-2 showed 13 α- helix chains, 2 β-pleated sheets along with 97.4468% overall quality factor of the predicted structure. Interestingly, it was observed that only 4.5% residues were present in outlier region and the observed errors were fixed. Moreover, 91.1% residues of the selected structure were present in favored region and 8.9% in allowed region having -6.0 Z-score. High throughput virtual screening and comparative molecular docking studies was performed. Four novel compounds have been reported that showed minimum binding energy (-8.1 Kcal/mol) and maximum binding affinity against MDM-2. Molecular docking analyses revealed that Ser154, Arg155, Pro156, Ser157, Lys185, Ser186, Ser188, Ser190, Ile189, Val247, Glu257, Asp173, Glu174, Glu178, Arg161, Ard181, Lys182, Arg183 and His184 residues are significant residues for therapeutic drug targets. The reported compounds showed effective energy scores. In addition, the site-directed mutagenesis may be helpful for further analyses. The reported compounds may act like potent drug compounds against MDM-2.

Introduction

MDM-2 is also known as E3 ubiquitin-protein ligase encoded by Murine Double Minute (*MDM-2*). It is an important negative regulator of *p53* [1-4] and a major tumor suppressor gene [2, 3, 5]. MDM-2 performs a key function as an inhibitor of p53 transcriptional activation and E3 ubiquitin ligase [2, 3, 6]. The inhibition of transcriptional activation leads to favors the nuclear export and stimulates the proteasome-based degradation [2, 6]. It is also an important positive regulator of E2F-1 involved in cell cycle [7]. MDM-2 plays significant role in human cancers as well as in various cancer therapeutic targets [2, 8].

George and co-workers discovered *MDM-2* located on double minute chromosomes which was overexpressed in spontaneously arising tumorigenic murine Balb/c 3T3 fibroblast cell line (3T3DM) with 50 fold amplification [9, 10]. In human, *mdm-2* is located on chromosome 12q13-14 [4], Oliner, Kinzler, Meltzer, GeorgeVogelstein (11),. Whereas, *MDM-2* is found on chromosome 10 C1-C3 in mice [9]. MDM-2 has 491 amino acids in human and 489 amino acids in mouse [[10],. *MDM-2* consists of 12 exons and two p53 responsive elements (*p53 RE*) in intron 1 and two promoter regions [6, 12]. MDM-2 is divided into three major domains including central domain containing an acidic region, a C4 zinc finger domain consists of the N-terminal region and the C-terminal RING finger domain for the regulation of p53 ubiquitination [2, 12, 13]. The RING family of E3 ubiquitin ligases consists of MDM-2 and is responsible for its E3 ligase activity [6, 12, 14, 15].

MDM-2 performs the ubiquitination of proteins which is an enzymatic cascade system and occurs in complex series of steps [6, 12]. Number of steps is involved including ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). E1 enzyme binds and activates the ubiquitination [6, 13]. Ubiquitin is 76 amino-acid protein which further activates E2 conjugating enzyme and is transferred to E3 enzyme [14, 16]. E3 enzyme is a ligase covalently bonds the ubiquitin to the substrate. MDM-2 has the ability of self-ubiquitination and it functions as E3 ligase to ubiquitination of p53 at several lysine residues (**Fig. 1**) [8, 15].

MDM-2 was identified as highly amplified gene involved in the inactivation of p53 which is activated in response to physiological stress. The activation of p53 results into G1 arrest of cells leads to apoptosis [8, 16]. The inactivation of p53 by *MDM-2* held by the by direct blockage of the p53 domain and also by the degradation of protein through polyubiquitin-proteasome pathway [6, 13, 17]. Thus, these two proteins form an auto-regulatory feedback loop in which p53 positively regulates the MDM-2 expression levels [1, 6] and MDM-2 negatively regulates the p53 [2, 6, 16].

MDM-2 exerts major role in oncogenic activities in human cancers including breast cancer and gastric cancer [7, 18, 19]. MDM-2 inhibitors perform anticancer activity inhibiting the MDM-2 and also through down-regulating the MDM2 [8, 18-20]. MDM-2 is also involved in numerous chronic diseases including autoimmune diseases, dementia and neurodegenerative diseases, heart failure and cardiovascular diseases, nephropathy, diabetes, obesity, sterility and inflammation [1, 6, 18]. MDM-2 has multiple non-carcinogenic roles. The pathway of p53-MDM-2 is the significant approach for neuroblastoma therapy [20]. Therefore, the study of MDM-2 and its drug designing will prove promising for treating and preventing malignant as well as nonmalignant diseases.

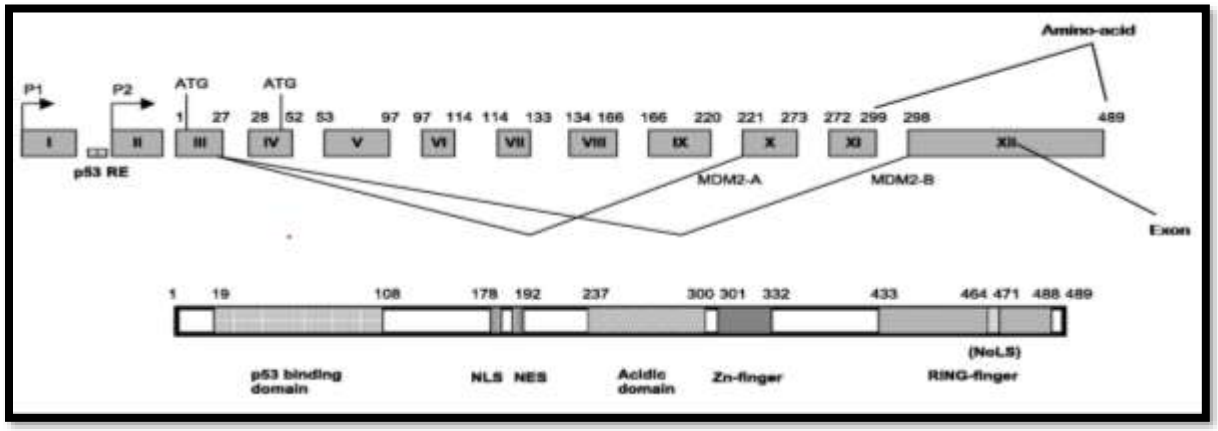


Fig. 1: 2D structure of MDM-2 protein and gene. MDM-2 consists of Zn-finger, Zn-finger domain, nuclear localization signal NLS and NoLS, nucleolar localization signal.

Bioinformatics techniques have resolved number of biological problems [21-23]. It has reported various novel compounds against cancer and other biological disorders [24]. The aim of current work was to use bioinformatics approaches to predict 3D structure of MDM-2. The primary approaches of this research are computational analyses, 3D structural prediction of MDM-2 and comparative molecular docking analyses. Extensive literature review evidenced that no compound is reported against the direct inhibition of MDM-2. Therefore, the aim of this work was to design small molecules to target MDM-2. To accomplish the goals of work, sequence collection, comparative modeling, threading and *ab initio* approaches were utilized followed by the comparative molecular docking analyses. From the observed findings, it was concluded that the binding interactional residues may have the ability to use for further wet lab experiments.

Materials and Methods

In the current studies, 3D structure prediction, sequence analyses, and comparative molecular docking analyses were done on an advance DELL workstation. The amino acid sequence of MDM-2 was retrieved for 3D structure prediction of the target protein, as the selected gene (*MDM-2*) is considered as a suspected candidate of cancer. UniProt Knowledgebase (KB) [25] was utilized to retrieve the amino acid sequence of MDM-2 in FASTA format having accession number A7UKY0. The amino acid sequence of the selected target protein MDM-2 was subjected to BLASTp search and Protein Data Bank (PDB [26]) was utilized for the search and identification of a suitable template. The x-ray crystallographic structure of p53 epitope-scaffold based on inhibitor of cysteine proteases in complex with human MDM2 having resolution of 1.92 Å was selected for homology modeling of MDM-2 as a suitable template, with max score of 288, 58% query coverage, 100% identity and E-value of 1e-99. Modeller 9.14 [27, 28], the reliable protein-modeling automated program was employed for 3D structure prediction of MDM-2 through satisfying the spatial restraints. Computational *ab initio* and threading approaches were used to predict the 3D structures of MDM-2. The energy minimization of the predicted MDM-2 structures was performed followed by the geometry optimization by employing the UCSC Chimera 1.9. The energy minimization of the selected 3D structure of MDM-2 was performed for 1000 steps, performed the conjugate-gradient method followed by protonation of wild-type histidines using the Amber ff98 method. Various 3D structure evaluation tools including Errat [28], ProCheck [29], Anolea [30], Rampage [31] and WhatCheck were employed to evaluate the reliability and quality of the 3D predicted structures of MDM-2. All the predicted 3D structures of the selected target protein were further evaluated by the MolProbity. The final selected structure of MDM-2 having poor rotamers and ramachandran outliers were corrected through employing the WinCoot.

Various bioinformatics tools (I-Tasser, Modeller, Swiss-model, RaptorX [32], Robetta [33], Rosetta [34], Quark, ChemDraw [35], AutoDock Tools, PyMol [36], Discovery Studio [37] and UCSF Chimera [38]) were used for *in silico* studies of MDM-2 followed to design novel compounds that may act as potent inhibitors against MDM-2. Molecular docking studies were done by using the AutoDock and AutoDock Vina. For molecular docking analyses, the receptor molecule was prepared by adding the hydrogen polar atoms. Hundred molecular docking runs were performed for each molecular docking experiment. The utilized grid size for molecular docking studies was set at 56×56×56 Å in the x-, y, and z-axis, respectively, having the grid spacing of 0.648 Å to cover the complete receptor protein. ZINC database was utilized for high throughput library screening for molecular docking analyses (**Fig. 2**).

Results and Discussion

The aim of the current effort under consideration was depends on the relation of MDM-2 with cancer. Extensive *in silico* studies were performed to design, identify, and evaluate the novel compounds against cancer by targeting MDM-2. The five top ranked optimally aligned suitable templates having maximum score, maximum identity, query coverage and E-values are presented in **Table 1**. All the selected templates showed reliable predicted structure however 5SWK selected template showed better evaluation results for homology modeling of MDM-2. The query coverage of the target sequence and template sequence was satisfactory for the prediction of 3D structures of MDM-2.

For more reliable structures and to lower down the error chances, threading and *ab intio* approaches were also used to predict the 3D structures of MDM-2. For 3D structure prediction of MDM-2, numerous tools (Robetta [33], I-TASSER [40], Rosetta [34], Quark, RaptorX [32], and Swiss Model [41]) and Modeller [42] were utilized. Numerous 3D evaluation tools

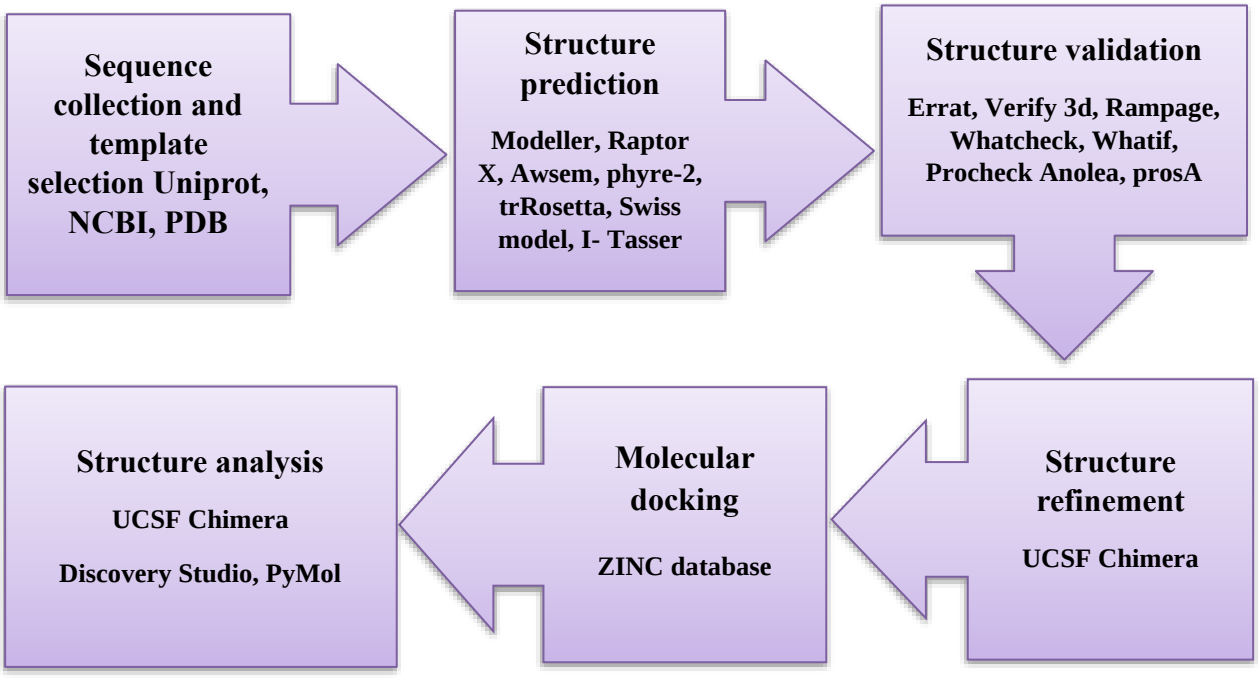


Fig. 2: The followed methodology for predicting 3D structure prediction and molecular docking analyses [39].

Table 1: The most suitable aligned template observed from BLASTp against MDM-2 having E-value, Maximum identity, query coverage and maximum score

Accession ID	Max. score	Total score	Query coverage	Max. Identity	E-value
5SWK	288	288	58%	100%	1e-99
2LZG	238	238	48%	100%	3e-80
4HBM	226	226	46%	100%	2e-75
5WTS	225	225	46%	100%	3e-75
1Z1M	225	225	45%	100%	3e-75

were employed to compare all the 3D predicted structures and the most reliable and suitable optimally predicted 3D structure of MDM-2 was selected for further experiments. The 3D selected structure of MDM-2 was subjected to molecular docking studies. The energy minimization of the selected model was performed to remove and fix the steric constraints by relaxing the system (**Fig. 3**). The selected 3D model of MDM-2 was analyzed and visualized through UCSF Chimera (**Fig. 4**).

Various evaluation tools were selected to assess the predicted 3D structure of MDM-2 and the observed results indicate the efficacy and reliability of the predicted structure of MDM-2. The observed ramachandran plot [43] of the predicted structure showed the presence of 95.5% residues in the allowed and the favored region of the plot, and 4.5% of the residues were observed in the outlier region of the plot. The overall quality factor of 97.4468% was observed for the final selected structure of MDM-2 by ERRAT evaluation tool (**Fig. 3**).

ZINC database selected library was screened by molecular docking analyses and the top ranked forty compounds were selected for further studies. AutoDock 4 and AutoDock Vina molecular docking tools were used for molecular docking analyses and the top ranked two complexes having least binding energy were selected (Figure 6). The observed results showed satisfactory analyses and conclusion were deduced from docked complexes of the selected molecules against MDM-2. The effort to elucidate the novel hits, top ranked four scrutinized compounds from the selected library was revealed (**Table 2**). Interestingly, it was observed that the majority of the compounds showed the binding interactions at similar binding site of the selected target protein.

The top ranked scrutinized compounds through molecular docking analyses having least binding

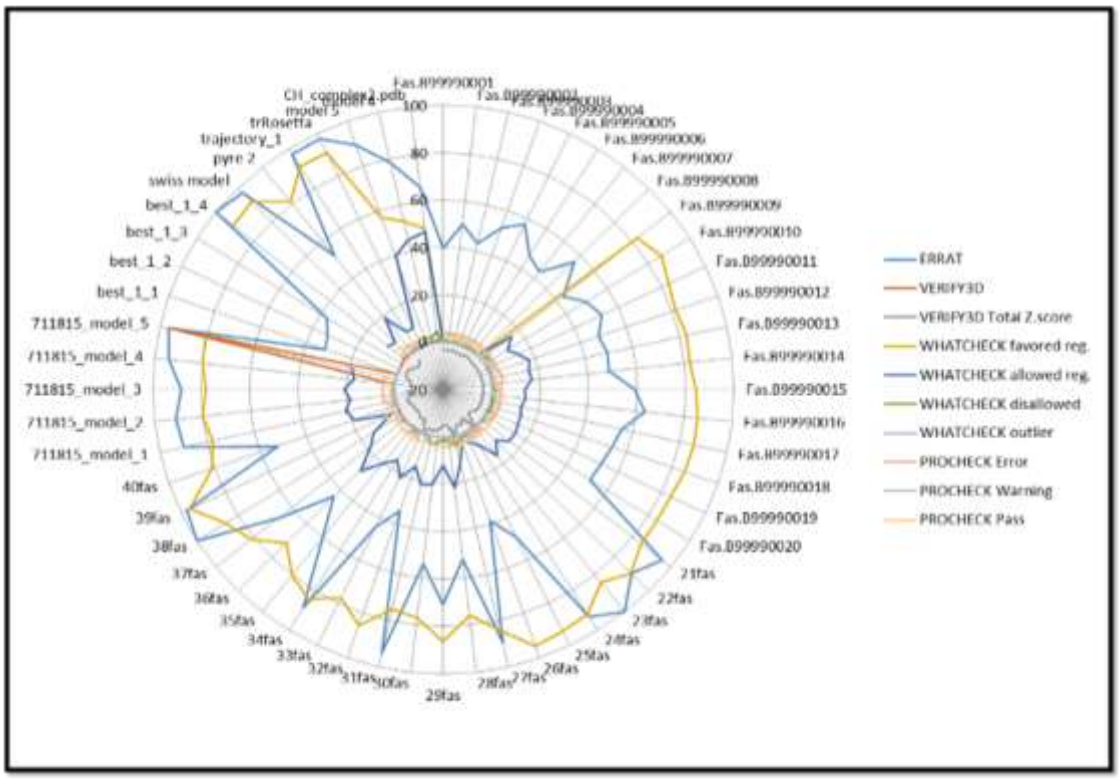


Fig. 3: Comparative evaluation analyses of the predicted 3D structures of MDM-2 target protein based on favored region, ERRAT quality factor, outliers and allowed region

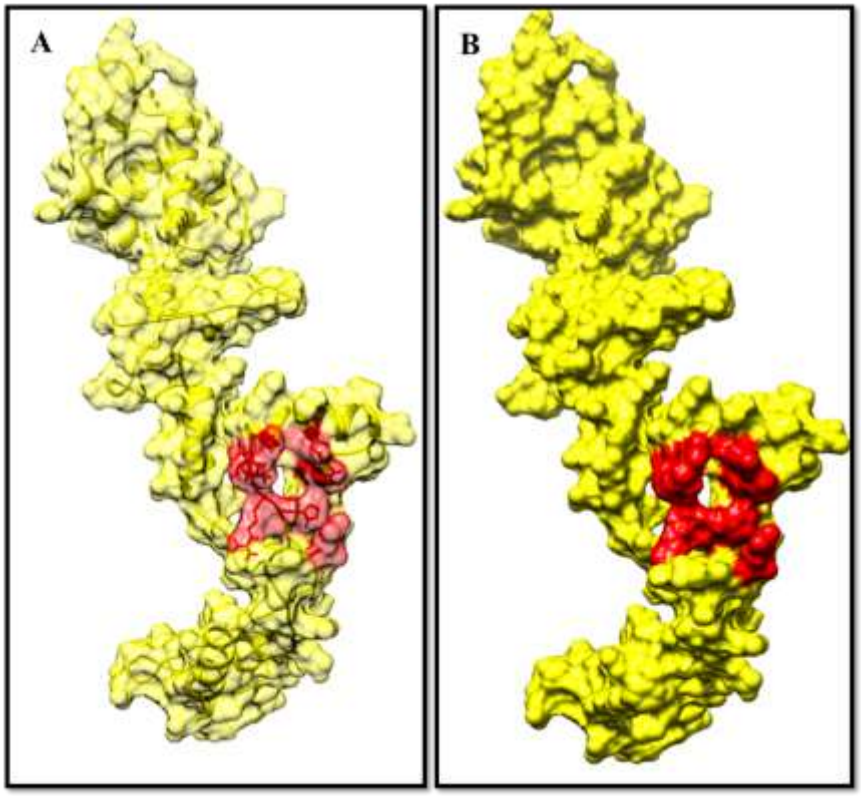
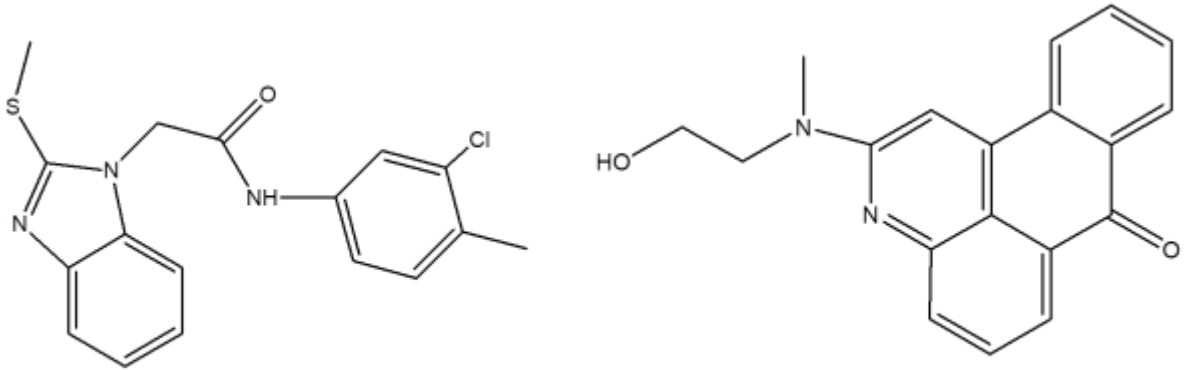


Fig. 4: 3D predicted structure of MDM-2. Red color represents the binding pocket **A)** surface having transparency **B)** surface of the MDM-2 with no transparency.

energies were revealed, namely ZINC8765218 and ZINC5186521 (**Fig. 5**). It was observed that Ser154, Arg155, Pro156, Ser157, Lys185, Ser186, Ser188, Ser190, Ile189, Val247, Glu257, Asp173, Glu174, Glu178, Arg161, Ard181, Lys182, Arg183 and His184 interacting residues of MDM-2 exhibited maximum binding affinity (**Table 2**). The interactional studies of the scrutinized compounds through molecular docking analyses against MDM-2 were analyzed and interactional plots of the selected compounds were visualized by employing UCSF Chimera [38] (**Fig. 6**).



ZINC8765218 **ZINC5186521**

Fig. 5: 2D structures of the scrutinized top ranked two compounds

Table 2: Molecular docking studies of the selected top ranked 2 novel compounds

Ligand	Binding affinity	rmsd/ub	rmsd/lb	Interacting residues
ZINC8765218	-8.1 kcal/mol	0	0	Ser154, Arg155, Pro156, Ser157, Lys185, Ser186, Ser188, Ser190, Ile189, Val247, Glu257
ZINC5186521	-7.6 kcal/mol	0	0	Ser154, Arg155, Asp173, Glu174, Glu178, Arg161, Ard181, Lys182, Arg183, His184, Lys185, Ser186

Bioinformatics used the computational analyses to solve the biological problems by employing the statistical and mathematical methods [21, 23, 24]. The structural bioinformatics helped to solve numerous biological problems [23, 44] and also contributes to design the vaccines against viral diseases including SARS-CoV-II through immunoinformatics approaches [44]. The drug design is a time consuming and costly procedure. Therefore, various computational methodologies and approaches were applied in current project.

In present study, computational analyses were carried out followed by the 3D structure prediction of MDM-2. The predicted 3D structure the selected target protein showed good degree of accuracy and the active site of the selected target protein MDM-2 were focused. Molecular docking analyses were performed by AutoDock Vina and AutoDock 4 to reveal the interactions between the selected receptor protein and the scrutinized compounds. The scrutinized compounds showed efficient binding at similar binding positions and critical interactional binding residues (Ser154, Arg155, Pro156, Ser157, Lys185, Ser186, Ser188, Ser190, Ile189, Val247, Glu257, Asp173, Glu174, Glu178, Arg161, Ard181, Lys182, Arg183 and His184) were observed through comparative molecular docking analyses (Figure 6) and the binding region was revealed. The scrutinized compounds showed least binding energy score. Comparative molecular docking analyses suggested that the scrutinized compounds may satisfy the drug properties. Based on extensive *in silico* analyses, it is suggested that the reported compounds (ZINC8765218 and ZINC5186521) have potential.

Conclusion

In conclusion, the current extensive computational analyses suggested that the reported compounds are effective against cancer by targeting MDM-2. Although numerous divergences usually exist among the computational analyses however the *in silico*-based analyses seem to be sufficient to conclude that the reported compounds may be a good option. Further experimental analyses and synthesis of the reported compounds considering the observed results may expect similar response rates.

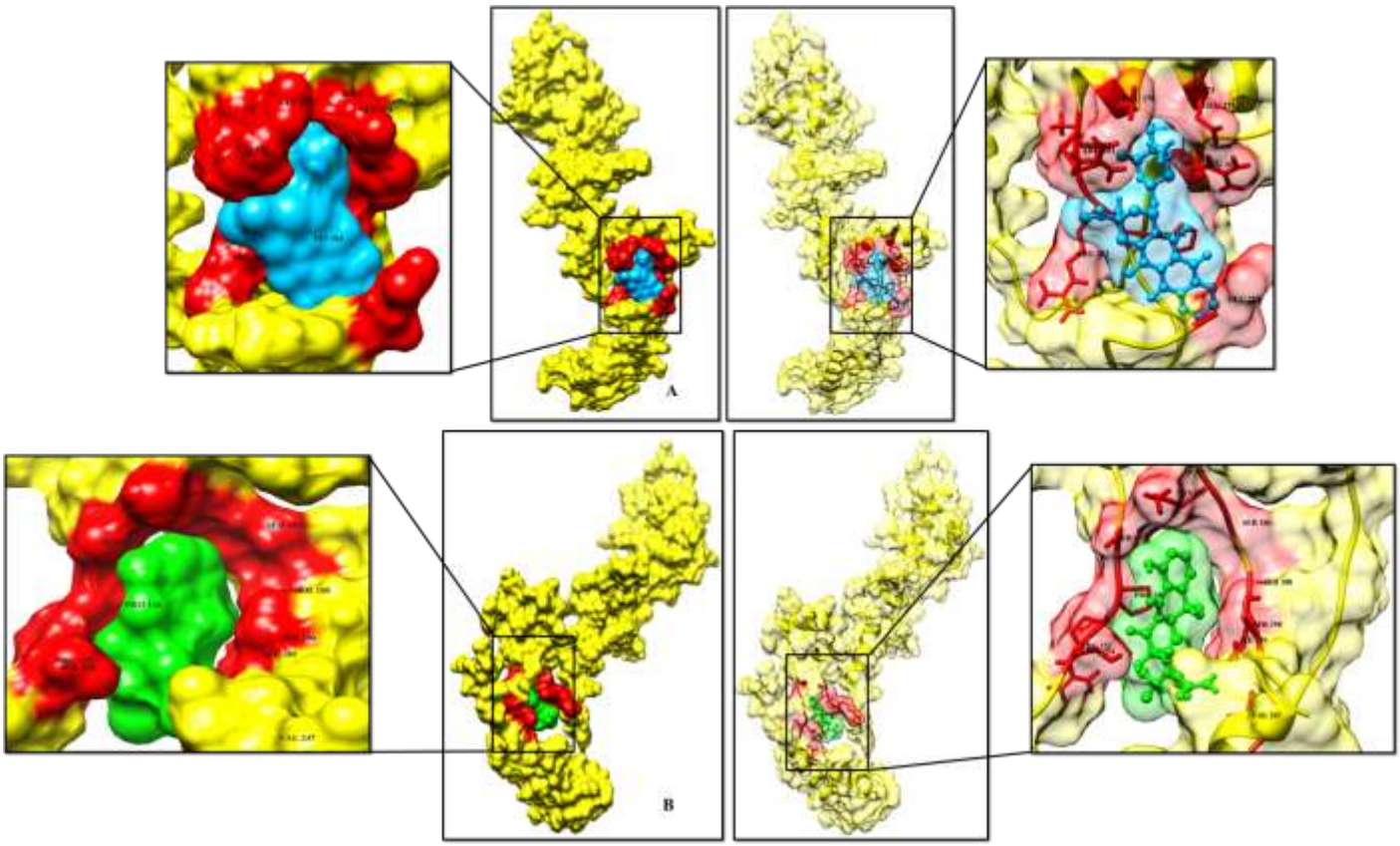


Figure 6: The observed potential binding interactions of the top ranked 2 scrutinized compounds through molecular docking analyses **A)** ZINC8765218 **B)** ZINC8765218

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Conflict of interest

The authors declare no conflict of interest.

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