Targeting dysregulation signatures of p53-MDM2 interactions to identify newer small molecule inhibitors for cancer therapy: Insight from computational direction

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Abstract

Dysregulation of the p53-MDM2 interactions has been implicated in majority of human tumors presenting a target for finding small molecule inhibitors. In this study, a training set of 17 experimentally tested inhibitors of MDM2 was used to develop series of pharmacophore models among which a four-featured (AHRR_1) model with one hydrogen bond acceptor, one hydrophobic group and two aromatic ring features and characterized by a survival score of 4.176 was considered significant among the top ranked generated hypothesis. Further, the model was validated by an external set of actives and decoy molecules and was found to exhibit encouraging statistical attributes (such as AUC > 0.7, BEDROC > 0.5 and EF > 1.0 etc). The model was used to screen the ZINC compound database, from the database, the top best 1375 hits satisfying the pharmacophore model were docked to MDM2 protein to identify the likely interactions of the compounds as well as their binding affinity with MDM2. Further, druglikeness and pharmacokinetic properties screening on top-ranked compounds with higher binding affinity than reference inhibitors revealed four compounds (ZINC02639178, ZINC38933175, ZINC77969611, and ZINC06752762) with suitable pharmacological properties including low ligand toxicity. Investigation of the dynamic behaviour of each candidate inhibitors in complex with MDM2 via molecular dynamic simulation suggested ZINC02639178 and ZINC06752762 as the most potential inhibitors. Thus, these compounds may emerged as therapeutic option for cancer treatment after extensive in vitro and in vivo studies.

1.0 Introduction

Regulating cellular activities for normal tissue homeostasis is a complex process dependent on numerous signalling cascade involving proteins. However, a number of this proteins have been compromised at expressional level resulting in several cellular anomalies. Ostensibly, in most types of human cancer the expression of the p53 tumor suppressor protein is diminished affecting cells that rely on its pathway for modulating tumor growth, proliferation, transition and progression. The tumor suppressor protein p53 is a requisite modulator of most delicate cellular activities including cell proliferation, death and reprogramming [1, 2], and is negatively regulated by the oncogene MDM2 in a self-orchestrated feedback loop activating the proteasome pathway, as revealed by models. Mdm2 possesses E3 ubiquitin ligase activity that allows it to prime p53 through its hydrophobic cleft to polyubiquitate p53 and tagging it for degradation by cytoplasmic proteases [3]. Ostensibly, studies have shown that the rate of cellular proliferation under conditions such as stress, genetic aberration and DNA injury is often dysregulated in cells with the genetic lesions resulting in supernumerary masses [4]. As such, p53 is upregulated to induce apoptosis-mediated cell cycle arrest to ultimately abrogate tumor initiation. In contrast, its repression is a signature for MDM2 hyper-activation and so far, has been the most widely contemplated hallmark of human tumors.

Disrupting the p53-MDM2 interactions using small molecule inhibitors has gained recognition in oncology. To date, a number of small molecule ligands generally the analogues of cis-imidazole (e.g. nutlins) [5], spiro-oxindoles (such as MI-63 and MI-219) [6], chalcones [7], sulfonamides [8], quinolins [9], isoquinolines [10], terphenyls [11], pyrrolidones [12], indoles [13], piperidinones [14], morpholinones [15], imidazothiazoles [16] and benzodiazepinedione [17] have been reported with inhibitory activities against p53-MDM2. These compounds could subvert hyper-activation of MDM2 oncogenes in malignant tumors serving as a rational to safe treatments for cancer. Unfortunately, none of this compounds have been approved worthy as anti-cancer drugs, although a number of them are currently in preclinical and clinical studies. The inability of the majority of these compounds to establish desirable pharmacokinetic profiles in spite exhibiting interesting binding affinity with MDM2 as target in bioassays is a major problem. Furthermore, p53 is highly susceptible and could be easily compromised thus, its reactivation might fail. Considering the high rate of mutation of the TP53 gene and its influence as a mechanistic for tumor survival, there is need for more treatment option. So far, computational method have been helpful to identify potential and promising inhibitors of MDM2 from diverse chemical scaffolds [18], some of which have been substantiated to display sufficiently high binding affinity, desirable pharmacokinetic and medicinal chemistry properties based on pharmacological predictions. Computational based-approaches involving virtual high throughput screening has proved to be an intrinsic component of drug discovery, and has enabled accelerated screening of chemical repositories to find newer hit compounds. Besides being a quicker approach for developing drugs, this technique takes the benefit of cost compared to traditional drug development. Moreover, identified hits compounds are already approved therefore, the need to re-evaluate their safety profiles such as toxicity and carcinogenicity may not necessarily apply and thus, labour is explicitly minimized. Realistically, the appropriate use of this method in drug discovery/development

process, would continually improve the ability to not only identify hits with likeliness to serve as potential scaffolds for producing new therapeutics but also optimize these compounds for better selectivity and specificity.

In this study, we applied pharmacophore based virtual screening, molecular docking techniques, and molecular dynamic simulations to establish newer small molecular ligands against the generally recognised regulator of the p53 pathways (i.e. MDM2).

2.0 Materials And Methods

2.1 pharmacophore modelling

The modelling of pharmacophores is an integral aspect in drug discovery and drug development, since it can aid in identifying selective hits against various biological targets implicated in diseases. In this study, a chemically diverse set of 17 experimentally tested MDM2 inhibitors with pIC50 values spanning between 5.91nM and 10.0nM were taken for 3D pharmacophore model development using the PHarmacophore Search Engine (PHASE) of Schrodinger. The list of compounds, SMILES, and activity in pIC50 are summarized in Table 1.

Dataset preparation: 3D structures of the compounds were obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) repository of small molecule ligands and was prepared using the Ligprep module of Schrodinger software. During dataset preparation, the OPLS-2005 force field implemented in the mentioned software was used with default settings for geometry optimization and energy minimization.

Generation of pharmacophore hypothesis: The selected compounds for pharmacophore generation were bifurcated into categories comprising of active and inactive molecule sets with activity threshold value of pIC50 $\geq$ 7.50 nM taken for active and pIC50 $<$ 7.50 nM for inactive compounds category which resulted in 8 actives whereas the rest were considered as inactive dataset. Further, the dataset was used for generating a series of pharmacophore models with different descriptor combinations among which the model AHRR_1 was selected among the top best ranked pharmacophore hypothesis based on the initial statistical attribute characterized by the corresponding survival score.

2.2 Pharmacophore validation and virtual screening

Especially noteworthy in pharmacophore modelling is the model's robustness and capability to distinguish between active from inactive or less active compounds. Since the selected pharmacophore model would be employed for virtual screening, it is essential to validate this model. Here, the validation metrics considered for the selected model among the presently employed statistical criteria for evaluating the performances of a prevailing pharmacophore hypothesis are: the area under the receiver operating characteristics curve (AUC-ROC), the area under the accumulation curve (AUAC), the enrichment factor (EF), the robustness initial enhancement (RIE) and the Boltzmann-enhanced discrimination of receiver operating characteristics (BEDROC). Subsequently, the model was used to screen the ZINC database of commercially available compounds through the ZincPharmer server (<https://zincpharmer.cbs.pitt.edu/>) [19]. Noteworthy, to obtain the best similarity hits from the ZINC database, we applied the following constraint which include a maximum root mean square deviation of 0.2 (RMSD = 0.2), number of rotatable bond cut-off between 1 and 10 (i.e. $1 \leq \text{nrB} \leq 10$), and a molecular weight range of 200-500 ($200 \leq \text{MW} \leq 500$) Daltons. The potential hit compounds obtained were regarded as candidate inhibitors against the MDM2 protein.

2.3 Protein structure and ligand dataset preparation

The crystal structure of MDM2 (PDB: 3JZK) [20] was obtained from the protein data bank accessible from (<https://www.rcsb.org/>) [21]. Prior to molecular docking, hetatoms co-crystallized with the protein were removed. The protein was further subjected to a preparation step using the Dock prep module implemented in the UCSF chimera software program (University of California, USA) [22]. In the Dock prep preparation protocol, all co-crystallized solvent molecules were deleted and the 3D protonation module implemented in chimera was used to add hydrogen atoms. Further, incomplete side chains were replaced using Dunbrack 2010 rotamer library [23] and AM1-BCC was used to define charges. Next to the preparation protocol, energy minimization was performed on the protein structure using AMBERff14SB force field. The minimization was done with

default parameters which include: 100 steepest descent steps at a step size of 0.02 Å, conjugate gradient steps at 10 and an update interval of 10.

In the ligand preparation step, the top 1375 hits obtained from the ZINC database were imported into PyRx through the open babel module implemented in the software. An energy minimized step was carried out on all the ligands using the universal force field (UFF) geometry optimization with default settings.

2.4 Molecular docking simulation

Having prepared the working structures which included the ligand library and the target (receptor), the binding hotspot of the target was defined using the native ligand. The hotspot residues are located in the hydrophobic cavity on the protein structures composed of 14 key amino acids that could actively facilitate the interaction of the protein with small molecule compounds. The docking simulation was performed using the vina module [24], integrated in PyRx v 0.8 software [25] and ran at an exhaustiveness of 8. Native ligand, as well as two known experimentally tested inhibitors of the protein were used as standard inhibitors throughout the docking simulation. To define the search space, a grid box resolution was centred at 7.6748, -9.7283, 25.8077 along the x, y and z axes respectively with a grid dimension of 19.7982 Å × 20.0006 Å × 34.6640 Å, thereby allowing the entire coverage of the hotspot residues. These residues included Leu54, Leu57, Ile61, Met62, Tyr67, Gln72, Val75, Phe86, Phe86, Phe91, Val93, His96, Ile99, Tyr100, and Ile101 [26]. At the end of the docking study, the crystallographic pose of each ligand were examined for on the basis of their binding affinity and those with the highest binding affinities were selected for further analysis. The 2D interactions of the protein-ligand complexes were evaluated using the LigPlot⁺ 2.2 software [27], and the visualization of the binding mode performed in PyMOL (<https://www.pymol.org/>) [28]. The docking protocol was validated by re-docking of co-crystallized MDM2 (3jzk) ligand and the resulted pose superimposed to the binding mode of un-processed ligand x-ray structure. The docking validation resulted in a root mean square deviation (RMSD) of 0.485 Å which is way below the maximum recommend value (i.e. 2 Å) thus, indicating the reliability of the docking criteria.

2.5 Drug-likeness prediction and pharmacokinetic properties evaluation

In the early stage of drug discovery, risk assessment is important to determine the safety profile and behavioural mechanism of small molecule ligands on biological systems. To date numerous servers and software are available to evaluate the safety profile of drugs using pre-defined experimental models. Here, those compounds that exhibited higher binding affinity compared to the standard inhibitors were screened for drug-likeness and ADMET properties respectively. Drug-likeness and ADMET properties have been the most used methods to evaluate the pharmacokinetic properties of drugs, and have been helpful to speed up the approval of drugs.

In this study, drug-likeness based on Lipinski rule was used to evaluate the ligands using the swissADME server (<https://www.swissadme.ch/index.php/>) [29]. Further, the absorption, distribution, metabolism, excretion and toxicity properties of the compounds were analysed through the admetSAR server (<https://lmmd.ecust.edu.cn/admetSar2/>) [30].

2.6 Molecular dynamics simulations

Molecular dynamics simulations were carried out on energy minimized receptor-ligand complexes using the Desmond package [31]. The OPLS3e force field was used in the system builder to model the protein interactions. The complexes were immersed in solvation molecules that have been predefined using the Simple Point Charge (SPC) water model [32], in a rectangular periodic box allowing for an equidistance of 10 Å buffer region between protein atoms and box sides. The systems were neutralized with appropriate number of sodium (Na⁺) and chloride (Cl⁻) counter ions for stability. Particle-mesh Ewald (PME) method [33], with a grid spacing of 0.8 Å was applied for calculating long-range electrostatic interactions. The simulation temperature and pressure were controlled using Nose-Hoover thermostats and Martyna-Tobias-Klein barostats respectively [34, 35]. A multiple time step RESPA integration algorithm was used throughout the simulations with an inner time step of 2.0 fs for bonded and non-bonded interactions within the short-range cut-off, and an outer time step of 6.0 fs for non-bonded interactions beyond the cut-off. Periodic boundary conditions (PBC) were applied on all the systems. Thereafter, a 50 ns production run in the NPT ensemble was performed for all the complexes.

3.0 Results And Discussion

It has been demonstrated that the activity of the MDM2 oncogenes determines the expressional status of p53. MDM2 interacts with p53 through its hydrophobic cleft to modulate p53 hyper-activation. Nonetheless, upregulation of MDM2 could be lethal and is a hallmark of human tumor. Therefore, targeting MDM2 hyper-activity has been an option in cancer therapy.

3.1 Pharmacophore model generation and validation

Series of pharmacophore model with different combination of physiological features have been developed with 8 actives ($\text{pIC}_{50} \geq 7.50$) and 10 inactive compounds ($\text{pIC}_{50} < 7.50$) from selective and experimentally tested MDM2 inhibitors using the PHarmacophore Search Engine (PHASE) module of Schrodinger. The model AHRR_1 which was characterized by one hydrogen bond acceptor, one hydrophobic group and two aromatic centroids was selected for use in further investigation on the basis of adjusted survival score. The statistical details of this pharmacophore hypothesis is shown in Table 2. Figure 1 depicts the spatial arrangement of pharmacophore features, along with the alignment of most active compound to the pharmacophore model. Although adjusted survival score could be a reliable criterion to select significant pharmacophore hypothesis nonetheless, useful models can also have low adjusted survival scores which is consistent with the observation by Bahera et al [36]. To circumvent this limitation, we employed the techniques of discriminating active compounds from false binders (decoys). As such, we could truly extrapolate the robustness and sensitivity of the model to selective MDM2 inhibitors rather than naïve molecules lacking activity. To do achieve this, a dataset containing 40 MDM2 inhibitors and 1200 false positive molecules obtained from DEKOIS database [37], was employed in ROC curve generation (see Figure 2) and to analyse the calculated area under curve (AUC). Further, the Boltzmann-Enhanced discrimination of ROC curve, the enrichment factor (EF), the area under accumulation curve (AUAC) and the robust initial enhancement (RIE) was used to appraise the predictive power of the model to active compounds among the loch molecules in the dataset. Table 3 contains the statistical validation criteria and the minimum recommended value of the parameters in view [38, 39]. According to Table 3, the early enrichment factor ($\text{EF}_{10\%}$) at 8.5 and AUC-ROC value of 0.85 indicated that the selected pharmacophore hypothesis was rational for virtual screening as it was able to retrieve 34 active molecules from 40 active compounds in the dataset which approximate to about 85 % actives at 10 % of the dataset. Besides, the model exhibited BEDROC, AUAC, and RIE value well above the critical values as evidenced in Table 3, suggesting the usefulness of model to earlier and ordered recovery of active compounds [38]. Based on this statistical merits it can be inferred that the model was better than a randomly generated model and has not been generated by chance, thus adequate for virtual screening of external molecules

3.2 Pharmacophore-based virtual screening

Once the AHRR_1 model have been substantiated to be reliable and adequate for use, the model was used to screen the ZINC compound database through the ZINCPharmer server. Among the 206, 433, 075 compounds from the screened database, 563, 879 compounds had showed molecular group that fit the pharmacophore hypothesis. The top ranked 1375 hit compounds within the specified search tolerance of the model as earlier described in the method section, were selected to molecular docking studies.

3.3 Molecular docking simulation based virtual screening

Virtual screening presents a strategic approach to identify druggable compounds that could be use as treatment option against various diseases. The screening method could be either ligand-based or structure-based [40–42], nonetheless, both of this methods have been deemed suitable for finding potential lead compounds against disease signatures (target) and have been synergistically employed to improve screening precisions and the success rate of drug discovery and development. Ligand-based virtual screening uses ensemble of the molecular characteristics in multiple ligands to identify potential hit compounds from combinatorial scaffolds while the latter select hits based on likely interactions of the compounds with receptors as well as their binding affinity against these target, by selectively docking the ligands into a confined region within the target called hotspot. In this study, a constraint-specific pharmacophore screening was employed to identify compounds that exhibited similar features with the selected pharmacophore hypothesis yielding a total top hit list of 1375 compounds based on RMSD preferences. Interestingly, molecular docking of all the 1375 hit compounds retrieved from the ZINC database against MDM2 (PDB: 3JZK)

resulted in binding affinities ranging from -5.3 to -9.9 Kcal/mol. The validation of the docking methodology which was performed by re-docking of cognate ligand to its receptor (MDM2) resulted in a conformation with binding energy of -8.9 Kcal/mol and RMSD value of 0.485 Å upon alignment to x-ray structure (Figure 3). The docked ligands could be categorized as highly selective, moderately selective, and selective on the basis of their binding affinities compared to three control inhibitors of MDM2 (i.e. Nutlin-3a, NVP-CGM097 and co-crystallized ligand) having binding affinities of -7.5, -8.6 and -8.9 Kcal/mol respectively. According to our observation, a total of 18 compounds exhibited higher binding affinities compared to that of the native ligand. 49 compounds showed higher binding affinities than NVP-CGM097, while a total of 541 of the ligands had binding affinities surpassing that of Nutlin-3a. The ligands with binding energies $\geq$ -8.9 Kcal/mol were considered for further investigations among which 25 ligands had met the criterion. (Table 4) presents the results of the 25 selected hit compounds, their binding energies, and interactions with MDM2 (PDB: 3JZK) after the docking calculations. According to the result, it is apparent that these compounds adapted similar binding mode as the reported standard inhibitors within the active site of MDM2 receptor. More also, the docked ligands form extensive hydrophobic interactions with the hydrophobic residues embedded within the catalytic domain which is consistent with the hydrophobic interactions made control inhibitors. Importantly, the aromatic ring of almost all candidate molecules could interact with Leu54, Leu57, Gly58, Ile61, Val93, His96, and Tyr100 amino acid residues which supports the observation by Atatreh et al [43]. Especially noteworthy is ZINC06670015 (-9.9 Kcal/mol) and ZINC02639178 (-9.6 Kcal/mol) which made hydrogen bond with the -COO termini and -NH side chain of Leu54 and Gln24 amino acids of MDM2 (see figure 4), which are absent in the reported control inhibitors and are uncommon interactions exhibited by most native and experimentally tested MDM2 inhibitors. This hydrogen bonding interaction to either of the mentioned residues may have contributed to their increased binding specificity as evidenced in (Table 4), and hence enhanced their selectivity against MDM2 over others except in the case of ZINC24394575 (-9.8 Kcal/mol) which is poorly understood. Nonetheless, ZINC24394575 was found to interact through hydrophobic interactions with both amino acids which is believed to be the reason for the observed high binding affinity against MDM2 (Figure 4).

3.4 Drug-likeness prediction and pharmacokinetic properties evaluation

Reportedly, several molecules have showed interesting biological properties that tentatively render them druggable at the preliminary stage of drug establishment process, some even shows desirable binding affinities against their biological targets, increasing the precision for their approval. Majority of these molecules could also abscond rejection until the penultimate phase of clinical trials and later fail. So far, the most significant reasons for these failures are due to late identification of side effects. Therefore, practicing the predictions of pharmacokinetic and toxicological profiling of any potential lead molecule at the initial stage of drug discovery process is an effective paradigm against futuristic menaces. Here, we investigated the likeliness of the top 25 hits from the virtual screening process to establish desirable pharmacokinetic profile. In this regard, we employ the admetSAR and SwissADME web servers to help with these predictions which include the generally recognized ADMET (i.e. absorption, distribution, metabolism, excretion, and toxicity) and drug-likeness properties in order to screen out potential nuisance and meticulously decrease drug redundancy before further investigating's. The results of the predictions are presented in (Supplementary Table 1 and 2). All the 25 potentially selective drug candidates obeyed the Lipinski rule of five. According to this rule, a compound is considered druggable if it fits in the following constraints; $\text{LogP} \leq 5$, $\text{MW} \leq 500$, no of rotatable bond (nRB) ≤ 10 , Hydrogen bond donor (HBD) ≤ 5 and Hydrogen bond acceptor (HBA) ≤ 10 . In contrast, compounds with more than a single violation of the mentioned molecular properties is likely to exhibit poor absorption and oral bioavailability [44]. In spite obeying the aforementioned rule, majority of the compounds showed unfavourable ADMET status. However, the compound ZINC06752762, ZINC02639178, ZINC38933175, and ZINC77969611 exhibited the most desirable pharmacological properties. (Table 5) shows the result of the drug-likeness screening of ZINC06752762, ZINC02639178, ZINC38933175, and ZINC77969611. The result of the predicted pharmacokinetic properties of these compounds are reported in (Table 6). According to Table 6, it can be inferred that all four compounds indicated satisfactory toxicity status due to their non-carcinogenic and non-mutagenic properties as well as their inability to inhibit the hERG gene encoding the potassium ion channels necessary for the normal electric activity in the heart. Interestingly these compounds exhibited acceptable LD₅₀ (lethal dose at 50) value with rat models ranging from (2.1785- 3.0322) mol/kg and were found to be in class III on the basis of their oral toxicity which is quite desirable. Furthermore, the compounds showed good metabolism profile with the CYP450 iso-enzymes (the key enzymes in drug metabolism) which is characterized by the non-inhibition of the CYP450 2D6, CYP450 3A4 and CYP450 2C19 enzymes. The CYP450 2C9 also show non-inhibitory property for ZINC06752762 and ZINC38933175 whereas it was the enzyme was inhibited

by ZINC02639178 and ZINC77969611. Similarly, the CYP450 1A2 enzyme was also inhibited by ZINC38933175, and ZINC77969611. In contrast, the ZINC06752762 and ZINC02639178 had shown non-inhibitory property for these drug metabolizing enzyme. Additionally, all the four selected ZINC compounds also exhibited non-substrate alert for CYP450 2C9 and CYP450 2D6. All except ZINC38933175 considered substrate for CYP450 3A4. Oral bioavailability is the hallmark of any potential drug candidate. Conversely, for a compound to be orally bioavailable, it must possess several unique functions that enables it to pass through cellular membrane. Otherwise, they may be trapped within these barriers and might constitute serious health threat. These functions are desolvation, diffusion, resolvation and physicochemical properties including lipophilicity [45]. In the context of absorption, distribution and excretion of drugs, admetSAR provides several ways to identify this pharmacokinetic models including Human Intestinal Absorption (HIA), blood-brain barrier permeability, cell permeability based on caco-2, aqueous solubility (Absorption); p-glycoprotein inhibition (distribution); and renal organic cationic transporters inhibition (excretion). It is important to identify the aforementioned pharmacokinetic parameters because they provide additional knowledge for drug acceptability or rejection in any drug development process. Apparent in Table 6, all the four hit molecule are permeable to the human intestinal membrane and Blood-Brain Barrier. All except ZINC38933175 lacks the Caco-2 cell permeability. The four ZINC compounds also showed good aqueous solubility. The p-glycoprotein serves as a relevant drug transporter [46], as such investigating the possibility of the lead compounds to act as a substrate or inhibitor of p-glycoprotein is clinically relevant. The p-glycoprotein showed inhibitory alert for all the lead compounds. Regardless of this, the inability of ZINC02639178, ZINC38933175 and ZINC77969611 to act as substrate of the p-glycoprotein provided the likelihood for insignificant inhibition. Moreover, the four hits are non-inhibitor of the renal organic cationic transporter 2 which implies the overall clearance of the compounds. From our analysis, we can infer that the selected hits could be a safe to prescribe treatment option against cancer. (Figure 5) shows the 2-Dimensional structures of the four potentially selective inhibitors

3.5 Molecular dynamics simulations

3.5.1 Rmsd and Rmsf analysis

In order to take account into the behavioural mechanism of the candidate inhibitors on protein stability and flexibility, the docked complexes of ZINC02639178, ZINC06752762, ZINC38933175, and ZINC77969611 bound MDM2 protein was studied in a molecular dynamics simulation in an explicit water solution for 50 ns using the Desmond package. The trajectory plots representing the protein-ligand root mean square deviation (RMSD) and the protein root mean square fluctuation (RMSF) is illustrated in (Figure 6). Figure 6 clearly indicated that ZINC02639178, ZINC06752762, and ZINC77969611, each complexed with MDM2 receptor, are more stable than the ZINC38933175 bound MDM2 complex. Importantly noteworthy, the complexes have comparable stability pattern and convergence time as the cognate ligand-MDM2 complex. From our observation of the trajectory plot, it was evident that major fluctuation of each complexes was till about 18 ns after which they attain plateau around 20 ns of the simulation time and showed no major deviation till the end of the simulation whereas, the ZINC38933175-MDM2 complex fluctuated significantly between 0.8 and 1.7 Å till 25 ns and then comes to 1.2 Å round 28 ns, beyond which the complex experienced only slight deviations after 30 ns of the simulation time.

To investigate the structural changes occurring in each complexes due to ligand binding, the dynamic mobility of the residues of each docked structures were analysed by calculating the root mean square fluctuations (RMSF) (Figure 7). RMSF characterizes the local changes along a protein chain. An inspection of the RMSF trajectory plot showed that ZINC06752762, ZINC77969611, ZINC38933175 bound MDM2 complexes were somewhat similar fluctuation pattern to co-crystallized ligand-MDM2 complex. The Gln18 residues in the loop region of MDM2 had the highest fluctuation in all complexes with RMSF value between 2.0 and 2.75 nm. In contrast, major fluctuation in the ZINC02639178-MDM2 complex were observed between position 50-55 of the α -alphas atom of MDM2 residues. Especially noteworthy, is the ZINC77969611-MDM2 complex which showed the highest fluctuation pattern suggesting less stability during the simulation (Figure 7).

4.0 Conclusion

Considering the high rate of mutation resulting in several forms of malignancy, there is need for consistent approach for remedy. The noteworthy tumor suppressor protein p53 modulates majority of the activities undergoing in cells and are regulated by the oncogenic protein MDM2 to maintain the normal tissue functions. Dysregulation in the expression of either p53 or the cellular

regulators could be lethal resulting in the overall tissues detriment. Conversely, MDM2-p53 interaction is compromised in relatively all types of human tumors. The overexpression of MDM2 is a hallmark of tumor, and a mechanistic by which cancerous cells abscond cell division regulation, allowing their indefinite proliferation into malevolent cells capable of invading and reprogramming normal tissues to cancerous tissues (in a process called metastasis). Therefore, there is an urgent need to develop potent arsenals to combat both current and futuristic threats [47]. The upregulation of the p53 tumor suppressor auto-regulators MDM2 serves as an effective signature to find potent anticancer drugs.

Consequently, we have identified in this present study four small molecule inhibitors (ZINC06752762, ZINC02639178, ZINC38933175, and ZINC77969611) against the p53-MDM2 interactions using a pharmacophore model of clinically tested MDM2 inhibitors. Apparently from our studies, these compounds exhibited interesting binding affinities of -9.2, -9.6, -8.9 and - 8.9 Kcal/mol indicating their high selectivity for MDM2 protein. It is noteworthy to mention that the candidate hits had satisfactory pharmacokinetic properties and do not violate the Lipinski rule of five indicating a safe treatment option in cancer management. Further, molecular dynamics simulation studies on all four ZINC ligands revealed the relative stability in complex with MDM2 oncoprotein. In conclusion, we believe that the garnered result from the current study would trigger and facilitate the development of other active compounds against MDM2 overexpression thereby providing the premium and availability of more promising anti-cancer therapeutic candidates.

Abbreviations

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity; MD: Molecular dynamics; MDM2: Mouse double Minute-2 homolog; PME: Particle-mesh Ewald; RMSD: Root mean square deviation; RMSF: Root mean square fluctuation; SPC: Simple Point Charge; AUC-ROC: Area Under Curve-Receiver Operating Characteristics; AUAC: Area Under the Accumulation Curve; EF: the enrichment factor; RIE: robustness initial enhancement; BEDROC: Boltzmann-enhanced discrimination of receiver operating characteristics.

Declarations

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Competing interests

No potential conflict of interest was reported by the authors

Availability of data and materials

The data generated in this study are reported in the manuscript and supplementary file

Code availability

Not applicable

Authors' contributions

The study in this manuscript was collaboratively executed by all aforementioned authors. POC conceptualize the idea and design the experiment described in this study. POC, HIU, and OI were involved in the investigation process of this study by conducting the experiment, analysing and evaluating the results of the molecular docking and molecular dynamics simulation. CBO and FOI were involved in the validation and visualization of the molecular interactions of the reported complexes. OJO, MOE and JOE drafted the manuscript and were involved in the methodology. FJA and OMO edited the manuscript and analysed the results of the druglikeness and pharmacokinetic properties of the selected lead compounds. The overall study was carried out under the supervision of OI and HIU. All the authors read and edited the manuscript prior to submission.

Ethical approval

Not applicable

Consent to participate

Not applicable

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Tables

Table. 1: Selective MDM2 inhibitors used for pharmacophore model building, with their PubChem CID, SMILES and corresponding pIC50 values

Compounds	PubChem CID	Smiles	pIC50 value
Nutlin-3a	11433190	<chem>Clc1ccc([C@H]2N(C(=N[C@H]2c2ccc(Cl)cc2)c2c(OC(C)C)cc(OC)cc2)C(=O)N2CCNC(=O)C2)cc1</chem>	7.0458
Nutlin-2	100078330	<chem>BrC1ccc([C@H]2N(C(=O)N3CCN(CC3)CCO)C(=N[C@@H]2c2ccc(Br)cc2)c2c(OCC)cc(OC)cc2)cc1</chem>	6.8539
RG7112	57406853	<chem>Clc1ccc([C@]2(N(C(=N[C@]2(c2ccc(Cl)cc2)C)c2c(OCC)cc(C)(C)C)cc2)C(=O)N2CCN(CC2)CCCS(=O)(=O)C)C)cc1</chem>	7.7447
Nutlin-1	10008863	<chem>Clc1ccc([C@H]2N(C(=N[C@@H]2c2ccc(Cl)cc2)c2c(OC(C)C)cc(OC)cc2)C(=O)N2CCN(CC2)C(=O)C)cc1</chem>	6.5850
RG7388	53358942	<chem>Clc1c(F)c([C@@H]2[C@]([C@@H](N[C@H]2C(=O)Nc2c(OC)cc(cc2)C(=O)O)CC(C)(C)C)(c2c(F)cc(Cl)cc2)C#N)ccc1</chem>	8.2219
MI-63	11512578	<chem>Clc1cc2NC(=O)[C@]3([C@H](N[C@H]([C@@H]3c3c(F)c(Cl)ccc3)C(=O)NCCN3CCOCC3)CC(C)(C)C)c2cc1</chem>	8.0458
MI-219	24877503	<chem>Clc1cc2NC(=O)[C@@]3([C@H](N[C@H]([C@@H]3c3cc(Cl)ccc3)C(=O)NCC[C@H](O)CO)C(C)(C)C)c2cc1F</chem>	6.7447
MI-888	53477213	<chem>O=C([C@@H]1N[C@H]([C@]2([C@H]1c1cccc(c1F)Cl)C(=O)Nc1c2ccc(c1)Cl)CC(C)(C)C)NC1CC(C1)(C)O</chem>	8.1675
RO-2443	136683437	<chem>Clc1c(c2N=CC(=Cc3[nH]c(=O)n(Cc4cc(F)c(F)cc4)c3O)c2cc1)C</chem>	7.4815
SAR-405838 (MI-773)	53476877	<chem>Clc1cc2NC(=O)[C@@]3([C@H](N[C@H]([C@@H]3c3c(F)c(Cl)ccc3)C(=O)N[C@@H]3CC[C@@H](O)CC3)CC(C)(C)C)c2cc1</chem>	7.0000
RO-5963	136683438	<chem>Clc1c(c2N=CC(=Cc3[nH]c(=O)n([C@@H](c4cc(F)c(F)cc4)C(=O)NC(CO)CO)c3O)c2cc1)C</chem>	7.7620
RO-8994	53238217	<chem>Clc1cc2NC(=O)[C@@]3([C@H](N[C@H]([C@@H]3c3c(F)c(Cl)ccc3)C(=O)Nc3c(OC)cc(cc3)C(=O)N)CC(C)(C)C)c2cc1</chem>	8.3010
AM-8553	56965957	<chem>Clc1cc([C@@H]2[C@H](N(C(=O)[C@](C2)(CC(=O)O)C)[C@H]([C@@H](O)C)CC)c2ccc(Cl)cc2)ccc1</chem>	8.9586
AM-6761	73386675	<chem>Clc1cc([C@H]2[C@@H](N([C@H](C3CC3)CS(=O)(=O)C(C)(C)C)C(=O)[C@@](C2)(Cc2scn2)CC(=O)O)C)c2cc(F)c(Cl)cc2)ccc1</chem>	10.0000
AM-8735	382146102	<chem>Clc1ccc([C@@H]2N([C@H](C3CC3)CS(=O)(=O)C(C)(C)C)C(=O)[C@@H](O[C@H]2c2cc(Cl)ccc2)CC(=O)O)cc1</chem>	7.6021
NVP-CGM097	53240420	<chem>Clc1ccc([C@@H]2N(c3ccc(N(C[C@H]4CC[C@H](N5CCN(C(=O)C5)C)CC4)C)cc3)C(=O)Cc3c2cc(OC(C)C)c(OC)c3)cc1</chem>	8.7696
YIN*	-	<chem>c1(ccc(cc1)Br)[C@@H]1n2ncnc2N=[C]2=C1[C@H](Oc1cccc21)c1ccc(cc1)Br</chem>	5.9101

*Co-crystalized ligand of (PDB: 3JZK)

Table. 2: Statistical details of selected pharmacophore hypothesis

SN	Parameters	Scores
1	Survival	4.176
2	Site	0.534
3	Vector	0.8043
4	Volume	0.4901
5	Selectivity	1.4445
6	Matches	8
7	Inactive	1.6857
8	Adjusted	2.4903
9	BEDROC	0.6144

Table. 3: Statistical validation parameters obtained from decoy set for the selected pharmacophore model AHRR_1.

SN	Parameters used for screening validation	Values	Threshold
1	% of actives (1%; 2%; 5%; 10%; 20%)	2;15; 31; 34; 35	–
2	EF (1%; 2%; 5%; 10%; 20%)	5.2; 6.2; 11; 8.5; 4.4	≥ 0.1
3	AUC–ROC	0.85	> 0.5
4	RIE	8.18	High
5	AUAC	0.88	> 0.7
6	BEDROC (alpha=8.0; 20.0; 160.9	0.709; 0.555; 0.283	> 0.5

Table. 4: Interactions between MDM2 (PDB: 3JZK) and the selected top hits compounds with desirable binding affinity, after docking calculations with AutoDock vina

SN	Compounds	Binding energy (kcal/mol)	Interacting residues	
			H- bonding	Hydrophobic interactions
1	ZINC06752762	-9.2	None	Val93, Gly58, Gln72, Ile61, Leu54, Phe86, Ile99, Phe91, Leu57, His96, Tyr100
2	ZINC06670015	-9.9	Leu54	Val93, Gly58, Gln24, Ile61, Ile99, Phe91, Leu57, Lys51, Tyr100, Phe55 Tyr100, His96, Phe55, Leu54, Val93, Ile61, Ile99, Gly58, Met62
3	ZINC04984893	-9.1	None	Tyr100, Leu54, Phe91, Gly58, His96, Ile61, Ile99, Val93, Gln72, Tyr67, Met62
4	ZINC01745609	-8.9	None	Ile19, Tyr100, His96, Ile99, Gly58, Ile61, Met62, Leu54, Lys51 Gln72, Tyr67, Met62, Val93, Gly58, Phe91, Ile61, Leu54, Leu57, Ile99, His96, Tyr100
5	ZINC02639178	-9.6	Gln24	Gln72, Ile61, Gly58, Phe91, Leu57, Val93, Ile99, His96, Leu54, Tyr100
6	ZINC02216169	-9.3	None	His96, Leu54, Ile99, Ile61, Phe55, Gly58, Val93, Met62
7	ZINC15292831	-9.0	None	Lys51, Tyr100, His96, Gln24, Phe86, Ile99, Leu54, Phe91, Val93, Leu57, Gly58, Ile61, Gln72
8	ZINC14549384	-9.2	None	Tyr67, Ile61, Met62, Phe91, Gln72, Gly58, Leu57, Leu54, Ile99, Val93, Tyr100, His96 Tyr100, His96, Leu54, Leu57, Ile99, Gly58, Phe91, Val93, Ile61, Met62, Tyr67, Gln72
9	ZINC78607261	-8.9	None	Tyr100, His96, Gln24, Ile19, Phe91, Leu54, Ile99, Val93, Leu57, Ile61, Gly58, Gln72, Met62
10	ZINC41546116	-9.4	None	Met62, Gln72, Tyr67, Val93, Ile61, Ile99, Phe91, Tyr100, His96, Gly58, Leu54, Leu57
11	ZINC45942161	-9.0	None	Tyr100, His96, Ile99, Phe91, Leu54, Leu57, Gly58, Ile61, Val75, Met62, Val93, Gln72
12	ZINC63530260	-9.1	None	
13	ZINC38933175	-9.1	None	
14	ZINC58196782	-9.2	None	

Table. 4 (continued): Interactions between MDM2 (PDB: 3JZK) and the selected top hits compounds with desirable binding affinity, after docking calculations with AutoDock vina

SN	Compounds	Binding energy (kcal/mol)	Interacting residues	
			H- bonding	Hydrophobic interactions
15	ZINC24394575	-9.8	None	Ile99, Ile61, Phe91, Leu57, Val93, Gly58, Leu54, Gln24, Ile19, Phe55, Lys51, His96, Tyr100
16	ZINC07738703	-9.4	None	His96, Tyr100, Leu54, Ile99, Leu57, Ile61, Gly58, Phe91, Val93 Ile19, Tyr100, Gln24, His96, Val93, Ile61, Leu54, Gly58, Ile99, Leu57, Phe91
17	ZINC67435163	-9.4	None	Tyr67, Gln72, Met62, Ile61, Gly58, Val93, Ile99, His96, Phe55, Tyr100, Leu54, Lys51
18	ZINC03202479	-8.9	None	Gln24, Tyr100, Ile19, His96, Leu54, Ile99, Leu57, Gly58, Phe91, Ile61, Val93 Ile61, Val93, Leu57, Gly58, Ile99, Leu54, His96, Tyr100, Gln24
19	ZINC06053031	-9.1	None	Leu57, Gly58, Phe91, Val93, Ile61, Ile99, Leu54, His96, Tyr100 Ile19, His96, Leu57, Leu54, Tyr100, Phe55, Val93, Ile61, Gly58, Ile99, Phe91, Phe86
20	ZINC12251570	-8.9	None	Tyr100, His96, Phe91, Ile99, Ile61, Ile19, Leu54, Gly58, Leu57, Val93, Gln72, Tyr67, Met62
21	ZINC84525615	-9.0	None	Leu54, Ile61, Gly58, Leu57, Tyr100, Ile99, Val93, Phe91, Met62, Tyr67, Gln72
22	ZINC68659402	-8.9	None	Gln24, Tyr100, Ile19, His96, Leu54, Leu57, Ile99, Gly58, Val93, Ile61, Phe91 Gln72, His73, Ile61, Val93, Phe91, Gly58, Leu57, Phe86, Leu54, Ile99, Tyr100, Lys51, His96, Gln24, Ile19
23	ZINC15913486	-9.0	None	Ile103, Ile99, Leu54, Phe86, Ile61, Lys94, Leu57, His96, Phe91, Gly58, Val93, Gln72, Met62
24	ZINC58027350	-8.9	None	Phe86, Tyr100, Ile99, His96, Leu54, Ile61, Val93, Gly58, Met62, Gln72
25	ZINC77969611	-8.9	None	
26	NVP-CGM097*	-8.6	None	
27	Nutlin-3a#	-7.5	None	
28	YIN##	-8.9	None	

Table. 5. Drug-likeness properties of top twenty-five potentially selective ligand small molecule inhibitors of Mdm2-p53 interaction predicted using SwissADME server.

SN	Compounds	Models							
		Molecular Weight	MlogP	Number of HBA	Number of HBD	(tPSA) A ²	RB	Rule of 5 violation	Lipinski Alert
1	ZINC06752762	403.47	2.45	4	2	78.45	5	0	Accepted
2	ZINC02639178	413.40	2.73	6	1	95.98	3	0	Accepted
3	ZINC77969611	412.46	3.02	4	1	104.6	5	0	Accepted
4	ZINC38933175	391.37	4.59	6	1	68.02	6	1	Accepted

Table. 6. ADMET predicted profile of top twenty-five potentially selective ligand small molecule inhibitors of Mdm2-p53 interaction predicted by admetSAR v2.0 server.

Models	ZINC06752762	ZINC02639178	ZINC38933175	ZINC77969611
Absorption and Distribution				
Human intestinal absorption	HIA ⁺	HIA ⁺	HIA ⁺	HIA ⁺
Blood- brain barrier	BBB ⁺	BBB ⁺	BBB ⁺	BBB ⁺
Caco-2 permeability	Caco2 ⁻	Caco2 ⁻	Caco2 ⁺	Caco2 ⁻
P-glycoprotein (substrate)	Substrate	Non-substrate	Non-substrate	Non-substrate
P-glycoprotein (inhibitor)	Inhibitor	Inhibitor	Inhibitor	Inhibitor
LogS (aqueous solubility)	-3.4412	-3.2275	-4.3869	-3.7802
Renal organic cation transporter 2 (OCT2)	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
Metabolism				
CYP450 2C9 (substrate)				
CYP450 2C9 (inhibitor)	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6 (substrate)	Non-inhibitor	Inhibitor	Non-inhibitor	Inhibitor
CYP450 2D6 (inhibitor)	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 (substrate)	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 3A4 (inhibitor)	Substrate	Substrate	Non- Substrate	Substrate
CYP450 1A2	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19	Non-inhibitor	Non-inhibitor	Inhibitor	Inhibitor
Toxicity				
Ames toxicity	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
hERG inhibition	Non-toxic	Non-toxic	Non-toxic	Non-toxic
Carcinogenicity (binary)	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
Acute oral toxicity	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic
Rat LD50	III	III	III	III
	2.1785	3.0322	2.7912	2.3459

hERG: Human ether a-go-go gene

Figures

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Figure 1

Pharmacophore hypothesis AHRR_1: Red ball depict hydrogen bond acceptor, Green ball represent hydrophobic group, and brown rings show aromatic features

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Figure 2

Superposition of re-docked and x-ray pose with RMSD value of 0.485 Å (Cyan depicts x-ray pose, blue represent re-docked pose).

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Figure 3

Percentage screen and ROC curve plot for model AHRR_1.

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Figure 4

2D interactions plot of MDM2 amino acid residues with the top 25 potentially selective leads after molecular docking simulation by AutoDock vina.

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Figure 5

2-Dimensional structures of the four potentially selective inhibitors, and control inhibitors

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Figure 6

RMSD of Protein-ligand complexes throughout molecular dynamics simulations. Backbone RMSD of MDM2 in complex with: A. Co-crystalized ligand B. ZINC02639178 C. ZINC06752762 D. ZINC38933175 E. ZINC77969611

Image not available with this version

Figure 7

RMSF of complexes throughout molecular dynamics simulations. Backbone RMSF of: A. Co-crystallized ligand B. ZINC02639178 C. ZINC06752762 D. ZINC38933175 E. ZINC77969611 bound MDM2 (PDB: 3JZK).

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