Original article

*In-silico* screening for DNA-dependent protein kinase (DNA-PK) inhibitors: Combined homology modeling, docking, molecular dynamic study followed by biological investigation

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**A R T I C L E  I N F O**

Article history:  
Received 12 June 2016  
Received in revised form 17 July 2016  
Accepted 19 July 2016

**Keywords:**  
DNA-PK  
Homology modeling  
Virtual screening  
Molecular dynamic

**A B S T R A C T**

DNA-dependent protein kinase (DNA-PK) is a key enzyme in non-homologous DNA end joining (NHEJ) repair pathway. The targeted inhibition of such enzyme would furnish a valuable option for cancer treatment. In this study we report the development of validation of enzyme homology model, and the subsequent use of this model to perform docking-based virtual screening against a database of FDA-approved drugs. The nominated highest ranking hits (Praziquantel and Dutasteride) were subjected to biological investigation. Additionally, molecular dynamic study was carried-out for binding mode exploration. Results of the biological evaluation revealed that both compounds inhibit the DNA-PK enzymatic activity at relatively high concentration levels with an IC\(_{50}\) of 17.3 μM for praziquantel and >20 μM for dutasteride. Furthermore, both agents enhanced the anti-proliferative effects of doxorubicin and cisplatin on breast cancer (MCF7) and lung cancer (A549) cell lines. This result indicates that these two hits are good candidate as DNA-PK inhibitors and worth further structural modifications to enhance their enzyme inhibitory effects.

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1. Introduction

DNA-PK is a serine/threonine nuclear kinase machinery consists of a 70/80-kDa regulatory heterodimer protein called Ku, and a 470-kDa catalytic subunit, termed as DNA-PKcs [1]. The carboxy-terminal of the catalytic subunit (~500 residues) belongs to the phosphatidylinositol-3 (PI-3) kinase-like kinase (PIKK) family [2,3].

One of the most severe lesions that may affect the human genome is the DNA double strand break (DSB) that emerges as a consequence of exposure to different clastogens [4,5]. The eukaryotic cells respond to such kind of lesions by initiating one of two major repair mechanisms namely, homologous recombination (HR) and non-homologous DNA end joining (NHEJ). It is estimated that around ten double-strand breaks occur per each cell per day, where some of them (such as replication across the neck, chromatid breaks during the S-phase) are fixed by the action of homologous recombination mechanism [6]. On the other hand, in the absence of nearby DNA homology donor, the NHEJ DNA-repair mechanism will be in effect. Moreover, improper response to DSBs can lead to genomic instability and carcinogenic events, while, unrepaired breaks usually induce apoptosis and cell death [7].

DNA-PK plays an essential role in protecting genome stability during the events of double-strand break (DSB), and considered crucial component in NHEJ repair pathway. Where, in such pathway, DNA-PKcs is recruited at the site of DSB by the Ku protein, which in turn recruits and phosphorylates a nuclease called Artemis that cleans the DNA ends before ligation with XRCC4/ligase IV complex [8,9].

Anticancer agents such as ionizing radiation and chemotherapy eradicate cancer cells by inducing different types of DNA lesions. Among these lesions, DSB is the most important in terms of cell death. Experimental evidences demonstrated that elevated DSB repair activity is correlated with the resistance shown by different cancer cells toward these agents [10–12]. On the other hand, hypersensitivity to ionizing radiation and enhancement of chemotherapeutics effect were seen in mutant cell lines known to be DNA-PKcs deficient [13].
A number of small molecule inhibitors of the DNA-PK were identified over the years (Fig. 1). The potent sterol fungal metabolite wortmannin was found to exhibit irreversible inhibition pattern against members of the PIKK family including; DNA-PK, ATM and ATR [14,15]. The naturally-inspired chromosome derivative LY294002 and the product of three-substituted indolin-2-one library screening SU11752 were found to have higher selectivity toward the DNA-PK than other members of the family showing reversible competitive inhibition pattern within the ATP-binding site [16,17]. Structural investigations based on the earlier compounds led to discovery of a chromone derivative named NU7026, which is considered as the first selective/reversible inhibitor of DNA-PK with an IC\textsubscript{50} of 0.23 μM (Fig. 2). Cancer cells exposed to the effect of NU7026 showed G2/M arrest and impairment in their double-strand break repair mechanism [18].

Tumor cells exposed to the effect of these compounds were shown to become chemo and radio-sensitive. Based on that, it was possible to anticipate the selectivity requirement for DNA-PK inhibition and the best route for their development. Accordingly, prompted by the clinical significance and the therapeutic value offered by inhibiting such crucial enzyme, we designed this study to utilize a hybrid of computational techniques such as; homology modeling, docking-based virtual screening and molecular dynamic for capturing DNA-PKcs inhibitory candidates employing an FDA-approved drugs as screening database. Herein, we report the results of in-silico study and the biological investigations carried-out on the top candidates.

2. Materials and methods

2.1. Molecular modeling

2.1.1. Homology modeling and model quality evaluation

The 259 amino acid sequence of the human DNA-PK catalytic subunit from 3728 to 3986 was obtained from the National Center for Biological Information (NCBI) database under accession number P78527.3. The sequence was then blasted [19] using the Blossum62 scoring metric and the Protein Data Bank [20] (PDB) as search database. Templates were identified and the best template was selected based on the E-value, sequence identity and coverage. The template and the target sequences were aligned using ClustalW [21] setting up the gap opening and extension penalties to 10 and 0.05, respectively. Generation of the human DNA-PK catalytic subunit homology model was carried out employing the latest version of Phyre server (Protein Homology/analogy Recognition Engine V 2.0) [22]. Phyre2 employs hidden Markov fingerprints to match the target/template sequences; furthermore it utilizes an ab-initio technology called “Poing” to dynamically generate the protein folds. The Poing proved to be successful in modeling protein regions having no homology match.

The quality characteristics of the modeled structure were evaluated by means of WHAT-CHECK subset within the WHATIF [23,24] as well as the ProSA [25,26] (Protein Structure Analysis) web servers.

2.1.2. Database selection and virtual screening

The modeled enzyme structure was used as template to perform docking-based virtual screening against compound databases. A database of FDA-approved drugs called eDrug3D was selected for this purpose. The database consisted of 1774 annotated 3D chemical structures of standardized stereochirality and tautomeric forms [27]. The docking study was performed employing the program Autodock Vina [28]. The homology model was prepared by adding polar hydrogens and Gasteiger charges followed by generating the corresponding charge file format using Autodock Tools (MGL Tools 1.5.6rc2). A grid box of the size 28 × 28 × 28 was established to cover the active site of the macromolecule, with a spacing of 1.0 Å between the grid points, and centered towards the coordinates of 50.36 (x), −3.75 (y) and −46.81 (z). The exhaustiveness and the number of poses were set to 12 and 10 respectively.

2.1.3. Assessment of docking performance

The docking protocol performance was examined by means of decoy test. For this purpose a virtual decoy set was prepared to include subsets of actives (inhibitors) and non-actives (decays). Thirty potent DNA-PK inhibitors were selected from the literature [29–34] to serve as an actives subset (Fig. 2). On average 72 decoys were generated for each active compound utilizing the directory of useful decoys (DUD-E) web server [35,36]. The final decoy set was consisted from 2173 decay molecules.

The actives were drawn using ChemDraw Ultra 8.0 software and were optimised for the energy and geometry using MMFF94 force field to provide a reasonable initial seed for the later conformational space exploration. All the compounds were treated employing the previous docking procedure mentioned earlier. The docking results were visualized utilizing Discovery Studio client [37].

For the decoy test docking experiment, a number of performance metrics were calculated which include; sensitivity, specificity, enrichment factor at different cut-off points (Table 2). The sensitivity and specificity were calculated employing Eqs. (1) and (2), respectively.

\begin{equation}
\text{Sensitivity} = \frac{TP}{TP + FN}
\end{equation}

\begin{equation}
\text{Specificity} = \frac{TN}{TN + FP}
\end{equation}

where, TP is the number of actives retrieved (true positives), FN is the number of actives discarded (false negative), TN is the number of decay compounds identified as inactive (true negative), while FP is the number of decay compounds identified as actives. On the other hand, the enrichment factor was calculated using Eq. (3).

\begin{equation}
EFx = \frac{na/Nx}{n/N}
\end{equation}

where, n represents the total number of actives included within the database, N is the total number of molecules in the database, na is
the number of actives captured within x% of the database, while Nx % is the number of molecules within x% of the database.

2.1.4. Molecular dynamic simulation

All-atoms molecular dynamic simulations were carried-out employing Desmond software v3.8 [38,39] embedded within Maestro interface v9.8 [40]. OPLS-2005 force field parameters were used during all calculations. The following dynamic protocol was adapted, in brief; the protein-ligand complex was solvated using TIP3P explicit water molecules as solvent model within an orthorhombic periodic boundary box of the size 10 Å^3, then, the system was neutralized by adding appropriate counter-ions followed by adding 0.15 M of salt to resemble the physiological conditions. Before applying the actual dynamic production run, the system was relaxed by performing a series of short (2000 iterations) restrained and non-restrained solute minimizations steps followed by short 12 ps simulation steps using NVT and NPT ensembles. Subsequently, the production run was carried-out for 50 ns using the NPT ensemble class integrating the equation of motion every 2 fs and setting the temperature and pressure to

Fig. 2. The chemical structure of DNA-PK inhibitors included in the decoy database with their corresponding IC_{50} values in μM. (NU7026 inhibitor in bold).
300 K and 1 atmosphere, respectively. The short range interactions (van-der Waals) cut-off was set to 9 Å, while the long range electrostatic interactions were calculated employing the particle mesh Ewald (PME) method. Trajectories were visualized within maestro environment and the results were analyzed using Desmond interaction diagram panel.

2.2. Biological evaluation

2.2.1. Drugs and chemicals

Nu7026, praziquantel, dutasteride, doxorubicin, cis-platin and bleomycin were purchased from Sigma, Germany and prepared as a stock solution at 5 mg/ml in dimethyl sulfoxide (DMSO). The stock solution was serially diluted in 1X reaction buffer (RB) and used at concentrations from 0.0195 to 40 μM.

5X reaction buffer was prepared by adding 250 mM Hepes (pH 7.5), 500 mM KCl, 50 mM MgCl₂, 0.5 mM EDTA and 5 mM DTT.

DNA-dependent protein kinase (DNA-PK) enzyme system (Cat # V5811), DNA-dependent protein kinase peptide substrate (Cat # V5671) and ADP-GloTM Kinase assay kit (Cat # V6930), were purchased from Promega, USA.

2.2.2. In-vitro DNA-PK enzymatic inhibition assay

DNA-PK enzymatic activity was measured by ADP-GloTM Kinase assay according to the manufacturer instructions. It is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra GloTM Luciferase. The luminescence was measured using Varioskan Flash (Thermoscientific, USA).

Solid white 96-multipwell plates were used to measure the inhibitory effect of NU7026 (positive control), Praziquantel or Dutasteride compounds on the activity of DNA-PK enzyme. According to the manufacturer instructions, serial dilutions of each inhibitor were loaded in the wells. To each well 140 U of DNA-PK Kinase Enzyme were added followed by 10X ATP/0.05 mg DNA-dependent protein kinase peptide substrate. The components were mixed well and incubated at RT for 60 min. After incubation, ADP-Glo reagent was added to each well and incubated for 40 min. The Kinase detection reagent was loaded to all the wells and then incubated for 40 min. The luminescence in each well was measured using Varioskan Flash (Thermoscientific, USA).

2.3. Synergistic effect study

The ability of two potential DNA-PK inhibitors (praziquantel and dutasteride) to enhance the anti-proliferative effects of three anticancer drugs (doxorubicin, cisplatin and bleomycin) was investigated by sulfonohamide-B assay as previously described [41]. Three human cancer cell lines; breast adenocarcinoma (MCF7), lung adenocarcinoma (A549) and cervix adenocarcinoma (HeLa) were used in this assay. Cells were seeded in 96 multi-well plates at a cell density of 5 × 10³ cells/well. After overnight incubation, the DNA-PK inhibitors were added at a final concentration of 1 μM. After two hours, the anticancer drug(s) were added at different concentration (0.001–10 μM). After 48 h of incubation, cells were fixed by trichloroacetic acid and were stained with sulfonohamide-B and the stain was solubilized in 200 μl/well TE buffer and the colour intensity was measured in an ELISA reader at 495 nm. The base line absorbance was deduced from all readings and the absorbance at each drug concentration was divided by the absorbance of control cells to give the fraction of cells remaining survival (surviving fraction) at each drug concentration.

2.3.1. Statistical analysis

Each experiment was repeated at least 3 times and the data are presented as the mean ±SEM. Multiple comparisons were carried out using a one-way analysis of variance (ANOVA) followed by LSD (least significant difference) for post hoc analysis. Statistical significance was acceptable to a level of P < 0.05. Statistical analysis, data fitting and graphics were performed by the Prism computer program (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1. Homology modeling

The approach of constructing 3D model for our protein based on known homologous experimental templates was adapted in this study due to the non-availability of sufficiently resolved X-ray crystal structure for the human DNA-PK catalytic subunit. The search for suitable template considered vital for constructing robust model, hence we have started the search by performing PSI-BLAST for the catalytic site amino acid sequence against PDB database. The results identified a recently published co-crystal structures of truncated mTOR with mLST8 subunit (4JSP) [42] as the best template candidate with E-value of 2 × 10⁻³¹. Subsequently, the target sequence was submitted to the Phyre2 server for 3D model generation and the model based on 4JSP crystal structure was considered for further evaluation. The target/sequence alignment (Fig. 3) showed a percentage sequence identity of 31% and sequence similarity of 45%. The percentage identity and similarity are considered sufficient for producing reliable model.

3.2. Evaluation of the homology model

The proper virtual screening protocol is heavily influenced by the quality of the homology model being generated; hence checking model quality and establishing its validity is a prerequisite for the later computational modeling. The WHAT IF server computes a number of quality check parameters for the modeled structure, and subsequently compares them to average scores of

Fig. 3. Sequence alignment of DNA-PK catalytic subunit and the template (PDB code: 4JSP). Dashed lines showing gaps, identical residues highlighted in green, similar residues highlighted in blue. Key amino acid residues within the binding site were shown as black arrows.
Table 1
Quality parameters for the modeled structure calculated by the WHATIF server.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st generation packing quality (a)</td>
<td>-1.701</td>
</tr>
<tr>
<td>2nd generation packing quality (a)</td>
<td>-2.340</td>
</tr>
<tr>
<td>Ramachandran plot appearance (a)</td>
<td>-0.062</td>
</tr>
<tr>
<td>chi1/chi2 rotamer normality (a)</td>
<td>0.664</td>
</tr>
<tr>
<td>Backbone conformation (a)</td>
<td>-0.974</td>
</tr>
<tr>
<td>Bond lengths (b)</td>
<td>0.951</td>
</tr>
<tr>
<td>Bond angles (b)</td>
<td>1.268</td>
</tr>
<tr>
<td>Omega angle restraints (b)</td>
<td>0.778</td>
</tr>
<tr>
<td>Side chain planarity (b)</td>
<td>0.294</td>
</tr>
<tr>
<td>Improper dihedral distribution (b)</td>
<td>1.021</td>
</tr>
<tr>
<td>Inside/Outside distribution (b)</td>
<td>1.137</td>
</tr>
</tbody>
</table>

\(a\) Structure Z-scores, positive is better than average.
\(b\) RMS Z-scores, should be close to 1.

reliable-protein structures within its internal database. The stereochemical quality indicators of our model were given in (Table 1). The packing quality parameters estimate the model amino acids local environment compared to other similar residues in well-refined structures. For the first and second generation-packing an average Z-score values higher than -1 and -2, respectively should be achieved to furnish a good model. However, values of -1.701 for the 1st-generation packing and -2.34 for the 2nd-generation packing not surprisingly seen in models containing an active site residues as our model.

The quality of the protein backbone conformation could be estimated by analyzing the phi/psi torsion angles plot (Ramachandran plot), where the analysis for our model revealed that 86.5% of residues are located in the most-favored regions, 11.4% in the additional allowed regions, 1.3% in generously allowed regions and only 0.9% in the disallowed regions (Fig. 4b). Furthermore, the normalized backbone conformation Z-score (-0.974), which illustrates super-positioning of \(\alpha\)-carbon while restraining backbone oxygens indicates an average value usually seen in well-refined structures. The model deviation from optimal cis/trans conformation is evaluated based on (chi-1/chi-2) angles correlation, where a Z-value of (0.664) achieved by our model clearly indicating a normally populated structure.

The RMS Z-scores for the bond lengths and angles achieved by our model compared to Engh and Huber standard distance parameters [43] revealed no pathological patterns. On the other hand, the model chirality indicated by the dihedral distribution appeared healthy. The relative locations of hydrophobic/
hydrophilic residues within the modeled structure are in consistent to native-like proteins. Restained omega angles at 180° appeared normal. The side chain planarity RMS Z-score of our model (0.294) is quite low indicating a strained structure; however such score remained within normal range and usually expected when modeling active site residues.

Another test for quality assessment is to examine the ProSA profile which describes the energy of residues as a function of sequence position; in which positive values indicate a problematic or erroneous part of the structure and vice versa. The energy analysis for our model (Fig. 4d) demonstrated favorable residues energy as indicated by the negative values achieved along the amino acids sequence. On the other hand, the overall model quality is assessed based on the ProSA Z-score plot which describes the score of the modeled structure in comparison to all experimentally determined protein chains. The score achieved by our model is within the range of scores found in native-protein structures of similar size (Fig. 4c). The overall quality parameters (stereochemical, geometrical as well as energetic) achieved by our models suggested that it can be trusted. The model proved to be well-validated, reliable and good enough for the subsequent modeling steps such as docking-based virtual screening.

### 3.3. Assessment of docking protocol performance

Benchmarking the docking protocol and evaluating its ability in discriminating actives from inactive compounds remained fundamental for any successful virtual screening workflow. For this purpose we have adapted the decoy test approach, where in such approach a number of decoy structures being generated for each known-active ligand. The emerged pooled-data set (containing subsets of actives and decoys) was utilized to perform virtual docking experiment. The obtained Autodock vina scores were quantitatively assessed by calculating a number of performance metrics such as: sensitivity, specificity, ROC-AUC and enrichment factor. The directory of useful decoys-enhanced (DUD-E) was employed for the automatic generation of decoys set. The DUD-E server generates decoy compounds having similar physical properties (i.e., M.Wt, LogP, HBD, HBA, number of rotatable bonds and molecular net charge), but rather expressing different chemical structures. During the process of decoys generation, the chemotypic diversity is guaranteed using combination of Bemis-Murcko atomic framework clustering procedure [44] as well as the extended connectivity fingerprint (ECPF) similarity measure. Only the most 25% dissimilar decoy structures were considered for each active ligand.

The receiver-operating characteristic (ROC) curve is an informative performance indicator commonly used to validate any modeling procedure. ROC curve is basically a relationship between true-positive rates (sensitivity) against false positive rates (1-specificity) at different thresholds. The graph gives an overview about study protocol performance in successfully ranking actives earlier than decoys during the sequence of virtual screen. One important metric furnished by ROC is the area under the curve (AUC). Ideally, AUC should be equal to 1, however the closer the AUC to 1, the better is the classification performance. Our model achieved an AUC of 0.838 (Fig. 5) which indicates a very good discrimination power.

Enrichment factor is another classical metric describing the protocol power in capturing actives within a pre-defined fraction of the scanned database, where higher number indicates better performance.

In order to reduce the rate of false positive and maximizing the rate of true positive, we decided to rationally select an appropriate classification threshold. For this purpose we carefully calculated a number of performance metrics at different docking score cut-offs

![Table 2. ROC curve analyses at various docking score cut-off levels.](image)

<table>
<thead>
<tr>
<th>Docking Score (kcal/mol)</th>
<th>E\textsubscript{Enrichment}</th>
<th>Sensitivity</th>
<th>1-Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>-11</td>
<td>24.47</td>
<td>3.33%</td>
<td>0.092%</td>
</tr>
<tr>
<td>-10.5</td>
<td>20.02</td>
<td>10.0%</td>
<td>0.368%</td>
</tr>
<tr>
<td>-10.0</td>
<td>19.44</td>
<td>30.0%</td>
<td>1.150%</td>
</tr>
<tr>
<td>-9.5</td>
<td>7.67</td>
<td>46.67%</td>
<td>5.520%</td>
</tr>
<tr>
<td>-9.0</td>
<td>3.99</td>
<td>66.67%</td>
<td>16.013%</td>
</tr>
</tbody>
</table>

(E\textsubscript{Enrichment}: Enrichment factor at E\textsubscript{Enrichment} of the database. Sensitivity: True positive rate, expressed as percentage. 1-Specificity: False positive rate, expressed as percentage.

(Table 2), At cut-off (−11 kcal/mol), the protocol achieved a 24.47 enrichment, with ability to capture 3.33% of actives and excluding 99.908% of the decoys, however at (−10.5 kcal/mol) cut-off, 10% of actives were captured and 99.632% of decoys being excluded. These two cut-offs appeared superior, but failed in capturing considerable percentage of actives. On the other hand, the next in raw cut-off value (−10 kcal/mol) was found to be the most appropriate, since at this level, the protocol achieved 19.44 enrichment and was able to capture 30% of actives and excluding 98.85% of decoys.

### 3.4. Results of virtual screening

In-silico virtual screening has become an exceedingly useful technique in the field of drug discovery, aiding scientists in identifying new promising lead compounds for certain macromolecular target of interest. In recent years, VS has been utilized in another field of application known as drug-repositioning “drug-repurposing” which is based on attempts of exploring new indications for already approved drugs.

The two commonly used approaches for virtual screening are: ligand-based approach (i.e., pharmacophore) and structure-based approach (i.e., docking). The availability of either an experimentally resolved structure for the target protein or well-validated homology model would permit the employment of structure-based virtual screening (SBVS) approach. In our case, we have employed our validated homology model to perform docking-

![Fig. 5. Receiver-operating characteristic (ROC) plot for the decoy testing experiment carried-out using zinc-decoy database. Area under the curve (AUC) is shown in the white box.](image)
based virtual screening of compound database consisted of FDA-approved drugs.

Based on the performance metrics achieved by our model (Table 2), hits scoring ≥ –10 kcal/mol were identified as potential inhibitors. The highest ranking hits retrieved along with their docking energy scores were presented in Fig. 6.

Interestingly, the captured hit praziquantel was noticed to share structural similarity with the fungal metabolite Wortmannin as well as the chromone derivative LY294002 (Fig. 7a and b). On the other hand, common structural framework was observed between dutasteride and Wortmannin (Fig. 7c). Furthermore, the initial investigation of praziquantel and dutasteride binding modes revealed their abilities to form an important hydrogen bonding with the key amino acid residue (Arg-3737). Additionally, both of them were able to establish hydrophobic contacts with the key Met-3729 and Trp-3805 residues within the catalytic site (Fig. 8a and b and Table 3).

Based on these earlier findings; the high docking scores, the structural similarity with known inhibitors, as well as binding mode analysis, we decided to nominate praziquantel and dutasteride for further biological exploration.

3.5. Biological investigation

3.5.1. Effect of praziquantel and dutasteride on the DNA-PK enzymatic activity

The results of in-vitro DNA-PK cell-free enzyme assay showed that praziquantel and dutasteride inhibit DNA-PK kinase activity at a concentration level of more than 10 μM with an IC\textsubscript{50} of 17.3 μM for praziquantel and >20 μM for dutasteride compared to an IC\textsubscript{50} of 0.21 μM for the positive control NU7026 (Fig. 9). These results indicate that praziquantel and dutasteride are weak inhibitors of DNA-PK and structural modifications are needed to enhance their DNA-PK inhibitory activity.

3.5.2. Effects of praziquantel and dutasteride on the anti-proliferative activity of anticancer drugs

DNA-PK is a central protein for non-homologous end-joining repair of DNA double-strand breaks [9,10]. This is the predominant pathway for repairing DNA double strand breaks induced by ionizing radiation and radiomimetic anticancer drugs such as bleomycin and doxorubicin. We, therefore, studied the effect of praziquantel, dutasteride on the anti-proliferative effects of bleomycin, doxorubicin and cis-platin in three human cancer cell lines (breast, cervix and lung adenocarcinoma). Cis-platin was included because DNA damage induced by this drug is repaired mainly by homologous recombination in which DNA-PK does not play an important role. Results of the survival assay showed that both praziquantel and dutasteride have weak anti-proliferative activity on all cell lines. Combination of 1 μM of praziquantel or dutasteride with doxorubicin enhanced the anti-proliferative effects of doxorubicin on MCF7 cells by decreasing its IC\textsubscript{50} from 0.1(doxorubicin alone) to 0.05 μM (doxorubicin + praziquantel or dutasteride) with a 2X sensitizing factor (Fig. 10a). The same results were also obtained for the lung adenocarcinoma A549 cell line where Cisplatin, praziquantel or dutasteride alone showed very weak anti-proliferative effects with an IC\textsubscript{50} > 10 μM. Addition of 1 μM of praziquantel or dutasteride 2h before cisplatin

Fig. 6. Top hits retrieved by virtual screening. The values between brackets represent the docking scores in kcal/mol. Chemical structures and numbering: Eltrombopag (1), Solifenacin (2), Praziquantel (3), Bexarotene (4), Dutasteride (5).

Fig. 7. 2D-structural overlay of captured compounds; (A) Praziquantel (blue) over Wortmannin (black); (B) Praziquantel (blue) over LY294002 (magenta) and (C) Dutasteride (red) over Wortmannin (black).
increased the cytotoxic effects of cisplatin on A549 cells by reducing its IC₅₀ to 7 μM with dutasteride and to 8 μM with praziquantel (Fig. 10b). On the other hand, pre-treatment of HeLa cells with praziquantel or dutasteride did not enhance the anti-proliferative effects of bleomycin on this cervix adenocarcinoma cells (Fig. 10c). This result shows that both praziquantel and dutasteride sensitize two cancer cell lines to the cytotoxic effects of doxorubicin and cisplatin at concentration that does not inhibit the DNA-PK activity (1 μM). This indicates that both compounds at this concentration level may have effects other than inhibiting DNA-PK such as induction of apoptosis or modification of cell cycle progression, which are responsible for augmenting the anti-proliferative effects of doxorubicin and cisplatin.

### 3.6. Molecular dynamic simulation

Given that praziquantel has achieved an IC₅₀ of 17.3 μM, which indicate its promising potential as lead compound for future structural modifications. We decided to carry-out 50 ns molecular dynamic simulation experiment for gaining more detailed and rational idea about praziquantel binding when providing physiological-like conditions and taking the enzyme flexibility into account.
This might help in revealing interactions not seen using traditional rigid-body docking technique.

The RMSD values give us an insight about the conformational changes taking place over the simulation period. Accordingly, it is clearly evident upon examining the system of Praziquantel/DNA-PKcs (Fig. 11a) and that the systems has reached equilibration toward the end of the simulation period as indicated by the minor fluctuations (i.e., less than 3 Å) in the protein Ca-atoms RMSD values.

An analysis of praziquantel interaction with the amino acid residues (Fig. 11b) illustrated its ability for form a number of important interactions within the DNA-PKcs binding site not observed earlier. Praziquantel showed ability to interact with Ala-3730, Lys-3753, Thr-3809 and Asn-3926 by direct hydrogen bonding 71%, 3%, 5% and 6% of the simulation time respectively, while the amino acid Phe-3928 was shown to be able to water-bridge praziquantel 40% of the time. Furthermore, praziquantel...
interacts with the key amino acids Leu-3751 (16% hydrophobic) and Lys-3753 (4% ionic) which in agreement with the binding mode of NU7026 (Fig. 8c). Furthermore, the short interaction time with Lys-3753 (3% HB and 4% ionic) could to some extent explain the modest inhibitory pattern of praziquantel.

4. Conclusion

In this study, we described the utilization of computational techniques such as; homology modeling, docking, virtual screening and molecular dynamic simulation for repurposing FDA-approved drugs toward capturing inhibitors for the DNA-PK catalytic activity. Our in-silico efforts nominated Praziquantel and Dutasteride as inhibitory candidates. The two candidates showed average DNA-PK inhibitory activity compared to the positive control NU7026 and they enhanced the anti-proliferative effects of two anticancer drugs on human cancer cell lines. We think that, the two drugs worth further structural modification to enhance their DNA-PK inhibitory activity.

Conflict of interest

Declared none.

Acknowledgments

The authors acknowledge the technical assistance of Ms Varsha Menon. This work is financially supported by Aljalila foundation, project number AJF201409.

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