The analysis of NQO1 isoforms as an Off-target to anti-cancer drug RH1 using molecular modeling and structure-based drug discovery approaches

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**ABSTRACT**

NQO1 is already evaluated for its high-level expression in numerous human cancers as compared with normal tissues. RH1 acts as an indigenous prodrug to NQO1. In our preceding work Off-targeting of (RH1) drug to protein kinases is well reported. Numerous protein isoforms have reported a potential drug target and biomarkers in cancer and related diseases. In the present study, the 3D structure of all the three NQO1 isoforms is modelled using the homology-based concept, evaluated for their conformational stability and energy forms by MD simulation. MSA and related method used for binding site prediction. Protein-ligand interactions were studied using molecular docking approach. The 3D modelled structure of NQO1 isoform 2 and 3 exhibited a conformational change in the protein FAD and RH1 binding region due to the absence of a few key amino acids. Docking results revealed a good degree of binding energy and interaction between the selected NQO1 isoforms, FAD and RH1. As FAD acts as a floor surface to RH1, a similar trend observed in the NQO1 isoform 2 and 3. Hence the NQO1 isoforms 2 and 3 could be a drug target to anticancer prodrug RH1 and can be further investigated in the lab.

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**INTRODUCTION**

The fear of drug failure and time-consuming process doubles the average cost of introducing a new drug in every 09 years (Eder et al., 2014; Paris, 2018). However, the early detection of false-positive facts will impact low-cost failure. A substantial investment in the computational techniques for the purpose of simulation, data generation and warehousing is essential for the life science projects (Mandal et al., 2009; Paul, 2010).

In the last few decades, the new computational methods and techniques have been a value addition to the drug discovery projects (Kuntz, 1992; Kapetanovic, 2008). From sequence analysis to advance 3D modeling and MD simulation have immensely supported in understanding structural phenomenon (Alonso et al., 2006). Molecular docking technique is widely used in pharmaceutical design as structure-based virtual screening, is con-
sidered economical than lab-based methods (Meng, 2011; Chen, 2012).

Our earlier work, reports multiple protein kinases as Off-target to prodrug RH1 using the bioinformatics approach (Gupta, 2017). However, it has been observed that some off-target act positive to desire activity; in many cases, no off-target activity (Simon, 2006), is preferred as it may limit the activity of molecule as in RSK inhibitor with RSK isoforms (Casalvieri et al., 2017). This paper postulates that as the kinases are predicted as off-target to RH1, in a similar manner the NQO1 isoforms could be a plausible off-target to RH1 unless analyzed and studied.

Generally, NQO1 activity is elevated in the cancer tumor than in normal cells. In comparison to normal tissue the NQO1 expression is reported in highly elevated form in the breast, colon, liver tumor and non-small cell lung cancer (NSCLC). Moreover, expression levels are similarly higher in developing tumors, suggesting a role for NQO1 in the prevention of tumor development (Begleiter et al., 2004). NQO1 is a foremost activating enzyme for MMC and other bioreductive agents including, RH1 and MeDZQ (Begleiter et al., 2004). RH1 treatment turn on c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK), together are involved in mitochondrial apoptotic cell death in NAD(P)H quinone oxidoreductase 1 (NQO1)+-MDA-MB-231 cells (Park, 2011).

Three more isof orm of NQO1 were referred from Uniprot database. NQO1-Isoform 1, isoform 2 and isoform 3 with a length of 274 aa, 240 aa and 236 aa. The study aims to understand the structural conformation of these isoforms, its binding site and analyze the effect of RH1 binding using in-silico based analysis. The paper includes a series of in-silico modeling and analysis and compared with the native form of NQO1 to provide us useful insights for the binding mode.

The solved crystallized structure of the NQO1 PDB-id: 1H66, with prodrug RH1 and FAD is already reported. The knowledge for the binding pocket is lined from both the monomers, where Phe-178, Tyr 126 and Tyr-128 from one monomer makes the roof, the isoalloxazine ring FAD makes up the floor for the RH1 and supports the RH1 optimum binding conformation, including His-161, Glycerine 149 and 150. A hydrogen bond between Tyr-128 from one monomer and His-161 from another monomer is well-reported on ligand interaction in PDB website.

MATERIALS AND METHODS

Data collection
The sequences of human NQO1-Isoform 1, 2, 3 were retrieved from the UniProt/KB database (P15559-1,2,3). The NQO1 isoform 1 with a sequence length of 274 amino acid residues. Whereas, Isoform 2 and Isoform 3 with a sequence length of 240 and 237 amino acid residues, the missing amino acid residue for Isoform 2 is from residue 140 – 173 and for Isoform 3 from residue 102 – 139. Crystallized 3D structure for NQO1 is already solved and available in a complex form with FAD, RH1 and other inhibitors in Protein data bank (1H66.pdb). A detail methodology in flow chart is represented in Figure 1.

Sequence analysis
Multiple Sequence Alignment (MSA) is the alignment process of two or more biological sequences (protein or nucleic acid) of a similar length to infer the homology and the evolutionary relationship. In our case, we have subjected 04 sequences i.e. 1H66.pdb solved crystallized data, NQO1-Isoform 1, 2 and 3 for MSA using the Clustal Omega online tool (http://www.ebi.ac.uk/Tools/msa/clustalo/) (Li, 2015) in Figure 2.

Homology modeling of NQO1 Isoforms
As our protocol follows comparative molecular interaction studies between all the Isoforms of NQO1 and hence requires 3D structure of all the isoforms. The principle governing approach follows, evolutionarily linked proteins share similar three-dimensional structures (Xiang, 2006). The homology modeling is carried out using the Expasy Swiss-model server (Waterhouse, 2018). The sequence identity of NQO1 Isoform -1, 2 and 3 are 100% identical to PDB-1d 5EA1 and 5EA2 is a tetrameric complex of NQO1 with an anticancer drug and FAD (Pidugu, 2016) and 99.96% identical to PDB-id: 1H66 is a tetrameric complex structure with RH1 and FAD (Faig, 2001).

Finally, we selected 1H66.pdb as a suitable template for 3D modeling of our NQO1 isoform 1, 2 and 3. Isoform 2 and 3 has the missing residue with a length of 34 and 38. Swiss-Model server follows an automated homology modeling server pipeline followed by similarity search (template identification) using BLAST and HHBlits algorithm, refinement of target and template alignment, Cyclic coordinate descent (Ccd) a robotic algorithm is used to fix the distantly placed residues (Canutescu and Dunbrack, 2003; Bienert, 2017).

Structure validation and assessment
The structure assessment and validation is carried
Figure 1: Workflow of molecular modeling methodology

Figure 2: Multiple sequence alignment of NQO1-isoform 1, 2, 3 and 1H66.pdb. The red color underline and box figure indicates the amino acid residue involved in making interactions with FAD and green color with RH1

Table 1: NQO1-isoforms simulation NAMD and Gromacs based, before and after simulation RMSD value

|       | Isoform 1 | Isoform 2 | Isoform 3 |
|-------|-----------|-----------|-----------|
| NAMD  | 1.864     | 1.106     | 1.130     |
| Gromacs | 1.072     | 2.315     | 2.436     |
Table 2: RMSD value for NQO1 isoform 1 vs NQO1 isoform 2 and 3

| Parameters | RMSD of NQO1 isoform Vs isoform 2 and 3. |
|------------|-----------------------------------------|
| Isoform 2  | Isoform 3                                |
| NAMD model | 1.252                                    |
| Gromacs Model | 2.294                                |

Table 3: Binding site region

| S. no | Protein   | Active site for ligand | Cavity center | Cavity dimension |
|-------|-----------|------------------------|---------------|-----------------|
|       |           |                        | Center_X     | Center_Y       | Center_Z | Size_X | Size_Y | Size_Z |
| 1     | NQO1_1    | FAD_A                  | 15.791       | -9.546         | -4.409   | 40      | 40     | 40     |
|       |           | FAD_B                  | 5.209        | 24.479         | 6.597    | 40      | 40     | 40     |
|       |           | RH1_A                  | -2.117       | 15.174         | 6.628    | 40      | 40     | 40     |
|       |           | RH1_B                  | 19.002       | -8.11          | 7.478    | 40      | 40     | 40     |
| 2     | NQO1_2    | FAD_A                  | 10.591       | -3.918         | -0.92    | 60      | 94     | 60     |
|       |           | FAD_B                  | 5.928        | 18.181         | 9.873    | 64      | 78     | 60     |
|       |           | RH1_A                  | -5.936       | 15.6           | 3.319    | 40      | 40     | 40     |
|       |           | RH1_B                  | 22.987       | -5.843         | 8.531    | 40      | 40     | 40     |
| 3     | NQO1_3    | FAD_A                  | 17.749       | -11.28         | -1.878   | 40      | 58     | 82     |
|       |           | FAD_B                  | 6.021        | 19.223         | 11.672   | 60      | 40     | 50     |
|       |           | RH1_A                  | -0.488       | 7.286          | 10.739   | 20      | 20     | 20     |
|       |           | RH1_B                  | 8.497        | -6.532         | 10.61    | 20      | 20     | 20     |

Table 4: Binding energy

| S. no | Protein   | FAD A | FAD B | RH1 A | RH1 B |
|-------|-----------|-------|-------|-------|-------|
| 1     | NQO1_1    | -14.7 | -15.0 | -7.6  | -7.9  |
| 2     | NQO1_2    | -9.9  | -8.9  | -6.3  | -6.4  |
| 3     | NQO1_3    | -8.4  | -9.2  | -4.5  | -4.0  |

Figure 3: Di-sulphide bond in NQO1-isoform 2 and 3

Figure 4: NQO1 isoform-1 under acceptable range

Molecular dynamics simulation

NAMD

The modeled 3D protein NQO1- isoform 1, 2 and 3 structures were considered as the starting point for the MD simulation. MD simulations is performed...
using the NAMD program version 2.9 (Phillips, 2005) and all input files were generated using VMD (Humphrey et al., 1996). The protein is solvated under water using TIP3P water box with 5 Å³ of water. The protein preliminary energy was minimized with 1000 steps at temperature (310 K), followed by simulation of 50,000,000 steps with 2 fs at each run equals to 10 nano seconds using with CHARMM 22 parameter files for proteins and lipids (Ryckaert et al., 1977; Miyamoto and Kollman, 1992).

**GROMACS**

The second MD simulation is carried out under GROMACS version 5.1 using OPLS method (Jorgensen et al., 1996; Kaminski, 2001). The PDB file format was analyzed for Molecular dynamics including simple water model, followed by ionization and neutralization of simulation cube with positive ions Na and negative ions Cl ions to neutralize the system. Geometry optimization was carried out for 1000 iteration. Final unconstrained based molecular dynamics performed with coupled temperature (310 K) and pressure (1 bar) for 10 nanoseconds with 50,000,000 iterations.

**Binding site region study**

The cavity / binding site was predicted using Computed Atlas of Surface Topography of proteins 3.0 (CASTp 3.0) with a probe size of 1.4 Å as a default
Figure 9: NQO1 isoform-3 under low energy

Figure 10: NQO1 isoform-1 bond (blue), bond angle (red), dihedral (green) and improper (yellow) value plot

Figure 11: NQO1 isoform-2 bond, bond angle, dihedral and improper value plot

Figure 12: NQO1 isoform-3 bond, bond angle, dihedral and improper value plot

Figure 13: NQO1 isoform-1 RMSD graph

Figure 14: NQO1 isoform-2 RMSD graph

Figure 15: NQO1 isoform-3 RMSD graph
Figure 16: Structural changes in NQO1-isoform-2 and NQO1-isoform-3

Figure 17: NQO1-isoform-1 with two distinct cavity region

Figure 18: NQO1-isoform-2 with collated cavity region

Figure 19: NQO1-isoform-3 with collated cavity region

value (Tian, 2018) and predicted cavity regions are correlated with MSA results Figure 2.

Molecular docking

The NQO1-isoform 1 is 99.66% identity to 1H66.pdb structure, the FAD and RH1 binding cavity with 100% identity. The binding site residual information for FAD and RH1 were considered default from the pdb 1H66 and 5EAI. We have used Autodock tool 4.2 to select the amino acid residue and grid size space (cavity region). Each grid space is included with pharmacological important amino acid residue as per listed in crystallographic data pdb file 1H66. The receptor and ligand were prepared and saved in .pdbqt file format and docking is carried out using Autodock Vina tool.

Here we initially docked the FAD within the cavity region of NQO1-isoforms in both the chain; later RH1 docking is carried out with NQO1-isoforms within both the chains. As pharmacologically the FAD is an important factor to RH1 by providing a support at one end to occupy the cavity of NQO1, hence in all cases we have kept the standard procedure of docking FAD initially and later RH1 to check the variability of FAD and RH1 presence and their interactions. For NQO1-isoform 2 and 3 where few important amino acid were missing and cavity space is increased for FAD and RH1, as represented in MSA output Figure 2. The binding site residue for FAD and RH1 is selected from MSA result. In each case of molecular the top most ligand pose
Figure 20: A: NQO1-Isoform 1, chain A FAD interaction pose 1: -14.7 kcal/mol. B: NQO1-Isoform 1, chain B FAD interaction pose 1: -15.0 kcal/mol. C: NQO1 isoform-1 chain A, RH1 interaction pose 1: -7.6 Kcal/mol. D: NQO1 isoform-1 chain B, RH1 interaction, pose 1: -7.9 Kcal/mol

Figure 21: A: NQO1 isoform-2, chain A FAD interaction pose 1: -9.9 kcal/mol. B: NQO1 isoform-2, chain B FAD interaction pose 1: -8.9 kcal/mol. C: NQO1 isoform-2 chain A, RH1 interaction pose 1: -6.3 Kcal/mol. D: NQO1 isoform-2 chain B, RH1 interaction, pose 1: -6.4 Kcal/mol

Figure 22: A: NQO1 isoform-3, chain A FAD interaction pose 1: -8.4 kcal/mol. B: NQO1 isoform-3, chain B FAD interaction pose 1: -9.2 kcal/mol. C: NQO1 isoform-3 chain A, RH1 interaction pose 1: -6.5 Kcal/mol. D: NQO1 isoform-3 chain B, RH1 interaction, pose 1: -4.0 Kcal/mol

Figure 23: Isoform 1, 2 and 3 superimposition with cyan color NQO1 isoform 1, orange color NQO1 isoform 2 and red color NQO1 isoform 3, wireframe structure are FAD and RH1 binded in their respective binding region, purple color indicates Chain A and yellow color indicates chain B region
with the minimum binding energy score is considered, receptor and ligand complex is generated using UCSF Chimera. The 2D and 3D intermolecular interactions between receptor and ligand analyzed and studied using Discovery Studio Visualizer.

RESULTS AND DISCUSSION

The sequence alignment between NQO1-isoform 1, 2 and 3 revealed a high percentage identity. It also located the missing region in NQO1 isoform-2 from amino acid no, 139-172 with a total length of 34 amino acid and in NQO1 isoform-3, from amino acid residue no, 102-139 with a total length of 38 amino acid as compared to NQO1 isoform-1, explained in Figure 2.

Homology model result

The modeled structure of NQO1 isoforms 1, 2 and 3 using template 1H66.pdb revealed better structural data. As the isoforms are modeled in the dimer form we have evaluated the structural data using P db-sum. Isoform-2 revealed an association of 2 disulfide bond interactions between isoform-2 chain A and B (residue Glu 14 (A) with Arg 53 (B) and Arg 53(A) with Glu 14 (B) and identical interactions were obtained in isoform-3, Figure 3.

The structure assessment and validation result carried out using ProSA resulted in most positive way. The ProSA score for crystallized data 1H66.pdb is -8.56, 5EA1, 5EA2 is -8.61 and -8.62 with respect to our modeled 3D structure of NQO1 isoform 1, 2 and 3 scores are as follows -8.56, -7.66 and -5.85 which falls under acceptable zone of high-resolution structural data and low energy conformation Figure 4, Figure 5, Figure 6, Figure 7, Figure 8, Figure 9.

After Comparing the energetically allowed regions for backbone dihedral angles \( \psi \) against \( \phi \) of amino acid residues in protein structure, Ramachandran analysis exhibited an optimum outcome, where the amino acid residue from NQO1 isoform 1 falls 95.4% under favored region and 4.1% in allowed region with 0.5% as outlier in NQO1 isoform 2 it is 95.0%, 4.2% and 0.8% and in NQO1 isoform 3 it is 96.6%, 2.8%and 0.6%.

Quantitative wise the total favored and allowed regions counts more than 99% in each of the NQO1 isoforms, outliers are very less not making more than 0.8%. NQO1 isoforms 3D structure can be found at PMDB (Protein Model Database):

http://srv00.recas.ba.infn.it/PMDB/main.php: 1. NQO1 Isoform-1, PMDB-id: PM0082685.pdb; 2. NQO1 Isoform-2, PMDB-id: PM0082218.pdb; 3. NQO1 Isoform-3, PMDB-id: PM0082219.pdb

Molecular dynamic study

NAMD

The molecular dynamic study revealed that the bond, bond angle, dihedral and improper in the model, all exhibited a dramatic change in the initial time steps, later gained a linear form of energy level and followed a stability path Figure 10, Figure 11, Figure 12. The outcomes exhibited the RMSD values below to 2 Å among all the three isoforms Table 1. The standard deviation for all the models is listed in the Table 2. However, the structural alignment illustrated that they are structurally highly identical in the entire aligned residues with a root-mean-square deviation (RMSD) of 1.252 and 0.986 Å between the backbones of NQO1 isoform-1 Vs isoform-2 and NQO1 isoform-1 Vs isoform-3 Table 2. The final energy after the simulation are as follows, NQO1-isoform-1 = -76671.6753 KJ/mol, isoform-2 = -73691.2529KJ/mol and isoform 3 = -73544.6880KJ/mol.

GROMACS

The results demonstrate an overall conformation of the protein RMSD values ranging between 1.0 - 2.5 Å Table 1, Figure 13, Figure 14, Figure 15. However, the structural alignment illustrated that they are highly identical in the entire aligned residues with a root-mean-square deviation (rmsd) of 2.294 and 2.074 Å between the backbones of NQO1 isoform-1 Vs isoform-2 and NQO1 isoform-1 Vs isoform-3 Table 2. The final energy after the simulation are as follows, NQO1-isoform-1 = -76671.6753 KJ/mol, isoform-2 = -73691.2529KJ/mol and isoform 3 = -73544.6880KJ/mol.

Structural changes in NQO1-isoform 2 and NQO1-isoform 3

The NQO1-isoform 2 dimer structure is devoid of a long chain of amino acid with a total number of residues 34 from each of its chain, the region is consisting of helix, loop and a sheet region. The peptide region is led by a loop region of “FRSGLH” and connects a sheet and a helix region Figure 16. Similarly, in the NQO1-isoform 3, a long amino acid residue from 102 to 139 with a total length of 38 amino acid residues are absent. The amino acid SKKAV makes a short loop region and connects two small sheet regions. Whereas the missing part of the amino acids from the Isoform 2 and 3 are mainly responsible to holds the quinone ring of the FAD molecule and RH1. This creates a void in the protein conformation, leading to a structural change in the FAD-binding region as well as to RH1 Figure 16. A: Isoform 1 and 2 superimposition with cyan color NQO1-isoform 1, orange color NQO1 isoform 2 and
red color region is the missing part from NQO1-isoform 2 and blue color is the connecting loop part in the isoform 2. B: Isoform 1 and 3 superimposition with cyan color NQO1-isoform 1, orange color NQO1 isoform 3 and red color region is the missing part from NQO1-isoform 3 and blue color is the connecting loop part in the isoform 3 (Figure 16).

**Binding site**

In NQO1 – isoform 1 two cavities can be observed, dimer form of protein separated by a center part of amino acid residue from each monomer. In NQO1 – isoform 2 and 3, where missing region removes the barrier and collates both the cavity region from each of the monomer and merges into one. Missing amino acid residues in each monomer of isoform 2 and 3, removes the borderline and also a change in the conformation of the cavity for binding FAD and RH1 with partial presence of pharmacophoric residues in the region stated in the Figure 2. MSA results Figure 17, Figure 18, Figure 19. Isoform 2 loses 05 amino acids involved in interactions with FAD and RH1, isoform 3 loses 05 amino acids involved in interactions with FAD and RH1.

**Molecular docking output**

3D structure from NAMD simulations was considered for the docking experiment. The selected binding site region are listed in Table 3. To verify the docking results were adequately precise for studying protein-ligand interactions in the NQO1 enzyme system, the NQO1 isoform 1 stands as a reference in the study, as the binding region is 100% identical to 1H66 and 5EAI.pdb structure. We calculated the docking of NQO1 – isoform 1 initially and identical pharmacophoric interactions were generated in comparison to the crystallized structure of NQO1 with PDB-id 1H66.

In NQO1 isoform 1, we identified a pharmacophoric interaction of FAD in chain A and B, RH1 in chain A and B are as follows: Chain A-FAD A (Figure 21A) : H-bond: Leu A 104, Gln A 105, Trp A 106, Ser A 158, Tyr B 127, His B 224.; Pi-interaction: Tyr A 157, Phe A 199, Phe B 145, Phe B 203.; Chain B – FAD B (Figure 21B): H-bond: Phe A 100, Gln B 105, Trp B 106, Ile B 150, Ser B 158, Tyr A 127. Pi-interaction: Tyr B 157, Pro A 69. Chain A – RH1 (Figure 21C): H-bond: Tyr A 127, Trp B 106, Ser B 158, FAD-B.; Pi-interaction: Tyr A 127, Phe 145, Trp B 106.; Chain B – RH1 (Figure 21D): H-bond: Tyr B 127, Tyr B 129, Ile A 159, Gly A 160, His A 161., FAD – A.

In NQO1 isoform-3 where part of the amino acid region is absent depicts a change in active site region has resulted in a following binding pattern, here we identified a pharmacophoric interaction of FAD in chain A and B, RH1 in chain A and B are as follows: Chain A – Fad A (Figure 22A): H-bond: Gln A 101, Thr A 110, Thr A 111, Tyr A 118, Gly B 137.; Pi-interaction: Ile A 130, Pro A 133, Ile A 134, Pro B 69, Pro B 133.; Chain B – Fad B (Figure 22B): H-bond: Asn B 19, Tyr B 43, Thr 110.; Pi-interaction: Ala B 11, Ile B 51, Leu B 107, Ile B 109, Leu A 42, Pro A 49.; Chain A-RH1 (Figure 22C): H-bond: Pro A 133, Gly A 137, Tyr B 118. Pi-interaction: Ile A 138, Ile B 130.; Chain B- RH1 (Figure 22D): H-bond: Tyr 118, Fad A. p-interaction: Pro B 133, Ile A 130 (Figure 22)

Comparing the binding energies and interaction in NQO1- isoforms 2 and 3 with FAD and RH1, exhibits a differences. In NQO1-isoform 2 due to the missing 05 amino acid residues from a peptide of length 34 i.e. TGG involved in interactions with FAD, Y and H involved with RH1Figure 2. FAD interactions in chain A is retained with Gln 105, Trp 106, Phe 145 and chain B is retained with Gln 105 and Trp 106. RH1 interactions in NQO1 isoform 2 are retained with Tyr 127 in chain A and Tyr 127 and Tyr 129 with chain B and supported by the FAD co-factor in both the chain.

In case of Isoform 3 where, a conserved pattern of QWF is missing in the structure responsible for making hydrogen bond with FAD and Y and Y with RH1 (Figure 2), exhibited a changes in the binding affinity and in acquired conformational spaces too. Binding interactions of FAD with NQO1- isoform 3 chain A is retain with Thr 110, Tyr 118 and Asn 19, Thr 110 in Chain B with FAD. Whereas due to missing TYR residue (Figure 2) from the binding site region, RH1 interaction with Tyr 118, Ile 130 is maintained in both the chain A and B. In chain A the RH1 is close to FAD but in chain B makes hydrogen bond with FAD.

These common interactions with respect to crystallized datasets helps us to understand the keen involvement of pharmacophoric interactions and...
acquired conformational space of FAD and RH1 in NQO1- isoform 1, 2 and 3. The change in the conformational space in the NQO1-isoform 2 and 3 depicted an intermolecular interaction differences by making a distant from the standard binding region as reported in pdb file 1H66 and 5EAI.

After superimposition all the three NQO1-isoforms docked structures one can easily observe the conformational space acquired by the FAD and RH1 within the NQO1-isoform 1, 2 and 3 are in close vicinity to each other and supports the binding of the RH1 within the NQO1-isoforms 1, 2 and 3 close to the known crystallized data Figure 23.

Several studies have found the enzyme or drug target isoforms to act as a potential off-target in the therapeutic application. Sabbah et al. (2010) has explored the avenues for the design of isoform- and/or mutant-specific PI3K inhibitors against the PI3Kα, PI3Kγ and the PI3Kα H1047R mutant, Gohlke (2015) has shown that the VEGFR inhibitor Vatalanib exhibits off-target activity as a PARP inhibitor using *in silico* docking simulation and confirmed by *in vitro* HT universal colorimetric PARP assay confirmed, broadening its mode of action. The off-target effect of BX795, inhibitor of TANK-binding kinase 1 (TBK1), strongly suppressed infection by multiple strains of HSV-1 (Jaishankar, 2018). Tyrosine kinase inhibitors (TKIs) Imatinib and Nilotinib are used in the clinical management of hematological neoplasms exhibited an Off-Target Effects on Human NK Cells, Monocytes and M2 Macrophages (Bellora, 2017).

The modeled isoforms 2 and 3 of NQO1 are stable and exhibit conserveness in most of the region in comparison to Isoform 1 of NQO1. As per the stability of modeled protein 3D structure is concerned, the final model of NQO1 isoform 2 exhibited a 94.9% and 4.2% of residue lying in favored and allowed region makes a total of 99.1% whereas the isoform 3, amino acid residues lies within a 96.6% in favored and 2.8% in allowed region makes a 99.4% in all. Supported by MD simulations a stable conformation of modeled structures were obtained. Due to the missing amino acids in both the isoforms of NQO1, 2 and 3 led to the conformational change in the structure and a huge void can be clearly observed in the modeled structure. In isoform-2 where due to the absence of five amino acids led to a change in the binding site of FAD and RH1, and made a partial conserveness in the cavity region. In the Isoform-3 where an absence of five amino acids leads to change in the conformation of the binding site of FAD and RH1 with a partial binding site residues. As the binding of FAD is highly facilitated by the binding site region and RH1 is supported by a floor of FAD from another monomer and roof of binding site residue and making a hydrogen bonds as in the native form 1H66.Pdb and NQO1 isoform 1. Similar interactions could be observed in isoforms 2 and 3.

CONCLUSIONS

By implementing numerous modeling approaches, modeled, assessed and analyzed the binding interaction of the NQO1 isoforms with co-factor FAD and anticancer prodrug RH1. The outcomes of the 3D protein structure modeling procedure revealed an optimum protein structure with respect to well-known assessment techniques. The ranked poses of the docking experiment revealed good interactive features and exhibited similar pharmacophoric interactions and conformational space in the protein binding site in comparison to crystallized data. The overall in-silico based analysis suggests that the NQO1 reported isoforms could be a drug target to anticancer prodrug RH1 and could possibly further study for the same.

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Compliance with ethical standards

Yes

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Abbreviations Used

DMF: dimethyl fumarate; RH1: 2,5-bis(aziridin-1-yl)-3-(hydroxymethyl)-6-methylcyclohexa-2,5-diene-1,4-dione; NAMD: Nanoscale Molecular Dynamics; VMD: Visual Molecular Dynamics; GROMACS: GROningen MAchine for Chemical Simulations; FAD: Flavin adenine dinucleotide; RSK: Ribosomal s6 Kinase; NQO1: NAD(P)H Quinone Dehydrogenase 1; NSCLC: non-small cell lung cancer; MMC: Mitomycin C; MeDZQ: 2,5-diaziridinyl-1,4-benzoquinone; ERK: extracellular signal-regulated kinase; CHARMM: Chemistry at HARvard Macromolecular Mechanics; OPLS: Optimized Potential for Liquid Simulations; PDB: The Protein Data Bank; MSA: Multiple Sequence
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