Homology modeling and virtual screening of ubiquitin conjugation enzyme E2A for designing a novel selective antagonist against cancer

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Abstract
Cancer is a major health problem in the world. The initiation and progression of cancer is due to imbalance between the programmed cell growth and death. These processes are triggered by the ubiquitin family enzymes. The ubiquitin-like proteins are responsible for the cell metabolism. Ubiquitin-dependent proteolysis by the 26s proteasome plays a crucial role in cell cycle progression as well as in tumorigenesis. In the ubiquitin proteasomal degradation pathway, ubiquitin conjugation enzyme E2A (UBE2A) binds with ubiquitin ligase RAD18, results in polyubiquitination reaction and cell cycle progression. UBE2A is an important contributing factor for the control of tumorigenesis. In the present work, the 3D model of the protein UBE2A was generated by homology modeling technique. The generated 3D structure of the UBE2A was validated, and active site was identified using standard computational protocols. The active site was subjected to structure-based virtual screening using small molecule data banks, and new molecules were identified. The ADME properties of the new ligand molecules were predicted, and the new ligands are identified as potent UBE2A antagonists for cancer therapy.

Keywords
ADME, cancer therapy, RAD18, tumorigenesis, ubiquitin ligase

Introduction
Cancer control, since decades, remains a global problem, despite advances made in medicinal chemistry research (1). Cancer involves uncontrolled cell proliferation, invades nearby tissues and spreads through the blood stream, lymphatic system to other parts of the body (2). Cancer is a leading death cause worldwide and accounts for 13 million victims per year, and is expected to rise to 22 million annually within the next two decades (3–5). The ubiquitin proteins act as signaling messengers and control the cell cycle progression, including the post-DNA transcriptional modification, apoptosis and cell proliferation (6–10). The ubiquitin-mediated proteasomal degradation pathway is one of the novel drug targets of cancer therapy (11,12). The ubiquitin-mediated proteasome degradation pathway mainly involves three enzymatic reactions (Figure 1). Initially, the ubiquitin binds to ubiquitin-activating enzyme (E1) in the presence of ATP to form a thio-ester bond, and the activated ubiquitin is transferred to the ubiquitin conjugation enzyme (UBE2A). This reaction is followed by the covalent interaction of ubiquitin to the ε-amino group of one or more lysine residues of the target proteins which are in conjugation with ubiquitin protein ligase (RAD18). The process of ubiquitination is repeated by the attachment of another ubiquitin to form a polyubiquitin chain, which is essential for an effective recognition by the 26s proteasome leading to cell cycle progression (13,14). The negative regulation of ubiquitin-mediated proteasomal pathways leads to the development of different human diseases (15). In this pathway, ubiquitin ligase proteins act as carrier proteins, which control the cell cycle progression and tumor formation (16). In eukaryotes, the cell cycle progression is mainly dependent on the cyclin-dependent kinases (CDKs), phosphorylation and ubiquitin proteasomal degradation pathway (17,18). The negative regulation of the CDKs effects the ubiquitin proteasomal degradation process and forms tumors (19). The UBE2A protein is a RAD6 yeast homolog HR6A protein, which interacts with the ubiquitin ligase RAD18, involved in polyubiquitination reaction and cell cycle progression (20–22). Nowadays, different types of proteasomal inhibitors are available (23), nevertheless to inhibit the initial stage of cancer, the interaction between the UBE2A protein with its natural substrate ubiquitin ligase RAD18 is a novel drug designing target.

Materials and methods
The protein structure availability is essential for understanding the biological processes and functions at molecular level.
Protein structures can be determined at high resolution by either experimental methods such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy or by computational methodology (24).

**Homology modeling**

The X-ray crystal and NMR structures are not reported for ubiquitin conjugation enzyme E2A (UBE2A). The 3D structure of UBE2A was generated using comparative modeling...
techniques. The FASTA sequence of UBE2A protein sequences with accession code (P49459) was retrieved from the ExPASy proteomic server (UniProt) (25). The sequence was subjected to the Basic Alignment Tool (BLASTp) program (26,27), the PHYRE (28) and the JPred3 servers (29) by setting each server to search for the protein template. The template was selected based on the criteria of sequence identity and a statistical measure of e-value obtained from the sequences. Pairwise alignment of protein UBE2A with selected template protein (PDB ID: 1JAS) was performed using ClustalW (30) server to define the structurally conserved regions. The Gonnet matrix algorithm is used in ClustalW server, for the automated pairwise alignment to ensure the presence of conserved motifs and to minimize the atomic gaps (31). The 3D model of UBE2A protein was generated using homology modeling software MODELLER 9v7 (MODELLER 9v7, San Francisco, CA). Thirty initial models were generated, and the model with the least modeler objective function value was selected for structure refinement in the structurally variable regions (32,33). The RMSD value for the UBE2A protein and its template were calculated in the Swiss-Pdb Viewer (SPDVB_4.1.0) (34).

**Protein preparation and model validation**

The energy minimization for the 3D model of UBE2A protein was carried out using protein preparation wizard in Maestro 9.1 (Maestro v 9.1 Schrodinger, LLC, New York, NY). The energy minimization of UBE2A protein was performed using an all-atom Impact Refinement module (Impref) (Impact v 5.0, Schrodinger LLC, New York, NY), to adjust steric clashes, bond orders were assigned, water molecules were removed, hydrogen bonds were added and the default root mean square deviation (RMSD) value of 0.30 Å was assigned using OPLS_2005 (Optimized potentials for liquid simulations) force field (35–37). The refined and energy minimized 3D model of UBE2A protein was evaluated using validation programs PROCHECK (38,39), ERRAT (40), VERIFY_3D (41), available from the Structural Analysis and Verification Server (SAVES) and ProSA server (42). In PROCHECK server, Ramachandran plot elucidates the stereochemical quality of the resulting protein structure. The ERRAT program describes the overall quality factor of UBE2A protein, and the acceptable range of ERRAT program is above 50%. The Verify_3D gives the 3D structure quality of the
protein. The ProSA server calculates the native fold, pairwise energy and Z-score of the modeled 3D structure of UBE2A.

Active site prediction

Active site is a ‘‘specific binding site’’ in the protein, which is involved in the catalytic activity. The active site region of the protein UBE2A was identified using CASTp (Computed Atlas of Surface Topography of Proteins) server (43) and Q-site server (44). The protein–protein docking was carried out using ZDOCK 3.0.1 software (45), to identify the specific binding residues within the binding domain of UBE2A protein. Based on the active site and important binding residue predictions, the active site grid was generated using the receptor grid generation module of Glide module (Glide, version 5.6, Schro¨dinger, LLC, New York, NY, 2010).

Virtual screening

Virtual screening was carried out with a set of small molecules at the active site region of UBE2A. LifeChem and CB_NovaCare small molecule structural database ligands were prepared to perform the virtual screening work flow in the Schrödinger suite. The ligand 3D coordinates were generated using LigPrep (ligand preparation) module in the Schrödinger suite using OPLS_2005 force field. The process of ligand preparation involves conserving the specified chiralities to generate minimum five stereoisomers per ligand, using default conditions at pH 7.0 ± 2.0. Several conformers were obtained after the completion of ligand preparation. The virtual screening program of protein UBE2A using LifeChem and CB_Nova Care structural databases with active site grid was performed by three scoring protocols of docking with Glide (Glide, version 5.6, Schrödinger, LLC, New York, NY, 2010). The ADME properties of ligand molecules were calculated using QikProp module (47) of Schrödinger suite (QikProp, version 3.3, Schrödinger, LLC, New York, NY, 2010). The solvent accessible surface area (SASA) for the protein–ligand complexes, before docking and after docking values, is calculated using Accelrys Discovery Studio Visualizer (Accelrys Software Inc., 2012 Accelrys Discovery Studio Visualiser v 3.5. Accelrys Software Inc., San Diego, CA). The

Table 2. Secondary structure details of the UBE2A protein; (a) the α-helices and (b) the β-strands.

| S.No. | Start | End | No. of residues | Sequence of α-helices |
|-------|-------|-----|-----------------|-----------------------|
| 1     | Pro4  | Glu18 | 15               | PARRRLMRDFKRLQE       |
| 2     | Val102| Asp114 | 13               | VSSILTSQSLLD          |
| 3     | Ser124| Gln131 | 9                | SQQAQLYQE            |
| 4     | Lys134| Glu146 | 13               | KREYERKVAIVE         |

| S.No. | Start | End | No. of residues | Sequence of β-strands |
|-------|-------|-----|-----------------|-----------------------|
| 1     | Val24 | Pro28 | 5               | VSGAP                |
| 2     | Val35 | Phe41 | 7               | VWNAVIF              |
| 3     | Thr52 | Glu58 | 7               | TFKLTIE              |
| 4     | Thr69 | Phe72 | 4               | TVRF                 |

The protein has 4α-helices and 4β-strands identified using PDBsum server.

Figure 5. Conformational analysis of UBE2A protein using PROCHECK. Ramachandran plot statistics (summary) for UBE2A protein. The UBE2A protein stereochemical quality analysis obtained from PROCHECK. The red color in the plot indicates the most favored region. Yellow represents additionally allowed region, light yellow represents the generously allowed and white field indicates the disallowed region.
docked complexes were analyzed for their intermolecular interactions with the help of Accelrys Discovery Studio Visualizer (Accelrys Software Inc., 2012 Accelrys Discovery Studio Visualiser v 3.5. Accelrys Software Inc., San Diego, CA) and PyMOL 1.3 (48).

Results and discussion

Structural analysis of UBE2A protein

The experimental structure of UBE2A protein is not available in the Protein Data Bank, to study the structural properties. Comparative modeling techniques were applied to generate the 3D structure of UBE2A protein. The UBE2A protein has overall quality factor of 73.61% explained by ERRAT program. On the axis, two lines are drawn to indicate the confidence with which it is possible to reject regions that exceed the error value. Expressed as the percentage of the protein for which the calculated error value falls below the 95% rejection limit. Good high-resolution structures generally produce values around 95% or higher. For lower resolutions (2.5–3.5), the average overall quality factor is found to be 91%.

Figure 6. The Verify_3D value of UBE2A protein. Verify3D analyzed the compatibility of an atomic UBE2A model (3D) with its own amino acid sequence (1D). The scores of a sliding 21-residue window (from –10 to +10) are added and plotted for individual 152 residues. 100.00% of the residues having an averaged 3D-1D score >0.2.

Figure 7. The overall quality value of UBE2A protein using ERRAT program. The UBE2A protein has overall quality factor of 0.23 Å, which is calculated using the Swiss-Pdb Viewer (SPDBV_4.1.0, the permissible range of RMSD for any protein being ≤2 Å). The energy minimization of the protein was carried out using protein preparation wizard in Schrödinger suite. The total energy of the protein after optimization was 2.809 × 10^2 kcal/mol, and after protein minimization the value was 2.642 × 10^2 kcal/mol. The 3D model of the UBE2A protein evaluated is shown in Figure 4 and is used for further studies. The UBE2A protein has 4α-helices and 4β-strands (Table 2). The stereochemical analysis of the protein UBE2A was carried out using the Ramachandran plot, which is represented as a contour diagram of the backbone dihedral angles [Phi (φ) versus Psi (Ψ)] as shown in Figure 5. The plot statistics shows 95.4% of amino acid residues (124 AA) in most favored regions, 4.6% of amino acid (6 AA) in additionally allowed regions, and none in the generously allowed region and disallowed regions (more than 90% of amino residues are in the most favored region; therefore the generated 3D model is a good quality model). The UBE2A protein structural compatibility as explained by the Verify_3D plot (Figure 6) gives a value of 100%, infers this model as a good structure. The overall quality factor value of protein UBE2A is 73.61% calculated using ERRAT program.

The structure does not show any bad contacts, and the
model quality details are shown in Figure 7. ProSA energy plot explains the local quality of the modeled structure of the protein. Amino-acid residues with negative ProSA energies are more reliable, and most of the amino-acid residues in the UBE2A protein have negative energies as shown in Figure 8(a). The overall Z-score of UBE2A protein is $-7.3$ as shown by the Dark spot in Figure 8(b). It falls in the range of the Z-score of the existing protein structures deposited in PDB, determined either by NMR or X-ray techniques. The evaluated 3D structure of the UBE2A protein reveals a good quality model.

Table 3. UBE2A protein binding cavities were identified using the CASTp and Q-site servers.

| S.No. | CASTp Volume ($\text{Å}^3$) | Residues | Q-site Volume ($\text{Å}^3$) | Residues |
|-------|-----------------------------|----------|-----------------------------|----------|
| 1     | 253.8                       | Gly51, Thr52, Phe53, Val73, Ser74, Lys75, Ser148, Asp151 | 116      | Phe13, Leu16, Gln17, Pro20, Pro21, Val24, Ser25, Gly26 |
| 2     | 50.2                        | Leu16, Val24, Ser25, Gly26, Val102, Leu106             | 103      | Thr52, Phe72, Val73, Ser74, Lys75, Met76, Phe77, Val81, Tyr82, Ala83, Asp84, Gly85 |
| 3     | 11.1                        | Pro20, Pro21, Val24, Ser25                         | 82       | Gly23, Val24, Ser25, Val39, Ile40, Phe41, Thr52 |

The Ser25, GLU44, GLY 45 and ASP 50 residues in UBE2A protein are binding with its natural substrate ubiquitin ligase RAD18 identified in ZDOCK 3.0.1 software.

Table 4. Intermolecular interactions in the docked complex of the UBE2A protein with ubiquitin ligase RAD18.

| Hydrogen bonds | Residue name and atom name | Bond distance in $\text{Å}$ |
|----------------|-----------------------------|----------------------------|
| 1              | GLY45-ALA357                | 1.894                      |
| 2              | TYR342-SER25                | 1.628                      |
| 3              | GLN356-GLU44                | 2.471                      |
| 4              | ARG358-ASP50                | 2.247                      |

The Ser25, GLU44, GLY 45 and ASP 50 residues in UBE2A protein are binding with its natural substrate ubiquitin ligase RAD18 identified in ZDOCK 3.0.1 software.

Figure 8. (a) ProSA energy plot of UBE2A protein. The protein UBE2A overall model quality with its amino-acid residues shown in the graph with negative ProSA energies indicates high reliability and good quality of the protein. The ProSA analysis of the model showed more residues with negative energy region. (b) The local model quality of the protein UBE2A. The Z-score spot in the graph of UBE2A protein obtained from ProSA gives a value of 7.3, that reveals good quality of the model which falls in the range of the protein data bank as shown in the NMR (dark blue) and X-ray (light blue) based structures.

Figure 9. The protein-protein docking of UBE2A protein and Ubiquitin ligase RAD18. The protein-protein docking was carried out using ZDOCK 3.0.1 software. The specific binding residues are shown in sticks namely SER 25, GLU 44, GLY 45, ASP 50, TYR 342, GLN 356, ALA 357 and ARG 358.
Table 5. The structures and docked complex intermolecular interactions between the ligand molecules and UBE2A protein.

| S.No. | Structure of the molecule and IUPAC name | Glide energy (kcal/mol) | XP glide score | Docked complex (protein–ligand) interactions Bond distance (Å) |
|-------|-----------------------------------------|------------------------|----------------|---------------------------------------------------------------|
| D1    | (2S)-2-[(5-hydroxymethyl)furan-2-yl]methyl]-7-{3-(2-methoxyphenyl)propanamido}-1,2,3,4-tetrahydroisoquinolin-2-ium | -50.002 | -8.501 | Hydrogen bonds D1-ASP50 2.427 D1-ASP50 2.321 D1-SER74 1.932 D1-SER148 4.913 Pi–Pi interactions PHE41-D1 |
| D2    | (2R)-2-[(2R)-2-hydroxy-3-[2-((3-(pyridin-3-yloxy)propyl)azaniumyl)methyl]phenoxypropyl]-1,2,3,4-tetrahydroisoquinolin-2-ium | -48.657 | -8.398 | Hydrogen bonds D2-SER148 2.232 D2-ASP151 2.342 D2-SER148 2.425 |
| D3    | 4-{|6-[(2-(2-chlorophenyl)formamido)ethyl]amino|pyridazin-3-yl|amino|pyridin-1-ium | -41.678 | -8.383 | Hydrogen bonds D3-Pro21 2.407 D3-Thr52 1.914 D3-Phe41 5.022 Pi–Pi interactions D3-Phe41 |
| D4    | 4-{|6-[(2-[(4-(trifluoromethyl)phenyl]formamido)ethyl]amino|pyridazin-3-yl|amino|pyridin-1-ium | -43.376 | -8.303 | Hydrogen bonds D4-Ser25 1.858 D4-Val24 2.229 |

(continued)
| S.No. | Structure of the molecule and IUPAC name | Glide energy (kcal/mol) | XP glide score | Docked complex (protein–ligand) interactions Bond distance (Å) |
|-------|------------------------------------------|------------------------|----------------|-------------------------------------------------------------|
| D5    | N-(4-methylpyridin-2-yl)-6-oxo-1-\{[3-(trifluoromethyl)phenyl]methyl\}-1,6-dihydropyridine-3-carboxamide | -39.017               | -8.230         | Hydrogen bond D5-Ser25 2.104                                 |
| D6    | 4-\{[(S)-1-(1-carbamoylcyclopropanecarbonyl)piperidin-3-yl]ethylazaniumyl\}-methylpyridin-1-ium | -43.413               | -8.188         | Hydrogen bonds D6-THR52 D6-SER148 1.953 2.171               |
| D7    | 6-\{[(1R,3R)-3-(2-hydroxyethyl)-4-(2-methylpropyl)piperazin-1-ium-1-yl]methyl\}quinolin-1-ium | -43.401               | -8.090         | Hydrogen bonds D7-SER74 D7-SER148 2.105 1.912               |
| D8    | N-\{(2S,3R,4R,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-2-phenoxyoxan-3-yl\}acetamide | -32.917               | -7.944         | Hydrogen bonds D8-Asp151 D8-Ser148 D8-Gln147 1.788 1.920 2.137 |

(continued)
Active site prediction

The active site is unique in defining the activity of a protein in biochemistry. The active site residues were identified using CASTp, Q-site servers and protein–protein docking studies. The UBE2A protein binding sites and corresponding binding pockets’ volumes were identified based on the hydrophobicity of residues using CASTp server. Q-site server explains the interaction energy between the protein and a simple van der Waals probe to locate energetically favorable binding sites of the UBE2A protein. Similar protocols were reported for identification of the active site (50–52). The ligand binding site prediction for UBE2A from the CASTp and Q-site tools is shown in Table 3.

The protein–protein docking of UBE2A with its natural ligand, namely the ubiquitin ligase RAD18 protein was carried out using ZDOCK 3.0.1 software to identify the binding residues (Figure 9). The inter molecular interactions of UBE2A protein docked complex with the ubiquitin ligase RAD18 are shown in Table 4, visualized in Accelrys Discovery Visualizer (Accelrys Software Inc., 2012 Accelrys Discovery Studio Visualiser v 3.5 Accelrys Software Inc., San
Diego, CA). The intermolecular interactions gave the information regarding binding residues of UBE2A protein. In the docked complex of the UBE2A protein with the RAD18, the residues S-25, E-44, G-45 and D-50 are significant for ubiquitination process and its activity.

The UBE2A protein and template belong to the same phylogenic family. All the members of ubiquitin-conjugating enzyme family contain conserved motifs in similar binding domains (Figure 2), including UBE2A and its template 1JAS proteins. The active site prediction results of UBE2A protein obtained from CASTp, Q-site servers and protein–protein docking studies were corroborated and compared with that of the template active site region (Supplementary Figure 1). The UBE2A protein active site domain is predicted to be L16 to G26; V39 to F53; F72 to F77 and S148-D151. Keeping these results in view, the active site of UBE2A for the next step of virtual screening process was considered.

**Virtual screening and ADME properties of UBE2A protein**

A grid was generated considering the centroid of the residues (as discussed in the previous section) in the active site region with 80 Å × 80 Å × 80 Å dimensions for further virtual screening studies. The structure-based virtual screening was performed with a library of molecules prepared from the ligands of LifeChem and CB_Nova Care structural data banks. LifeChem databank containing 12 000 molecules was subjected to ligand preparation. The stereoisomers with specific chiralities were retained to generate five conformers per ligand. The stable conformers were retained and output of 20 658 stereoisomers generated. The generated conformers were subjected to the HTVS mode with output of 6790 structures. The output structures of 6790 were further screened successively in SP and XP modes with a filtration ratio of 10% at each stage of docking. This process gives an output of 67 docked structures. A similar protocol followed for LifeChem database was applied to the virtual screening of the CB_Nova Care. The CB_Nova Care molecular structural database contains 10 000 molecules and gave an output of 36 935 conformers after ligand preparation and further virtual screening through HTVS, SP and XP modes gave an output of 92 docked structures. These structures can be considered for the identification of potent ligands as antagonists for UBE2A protein. A total of 159 docked complexes obtained show a Glide score in the range –8.5 to –6.4. A sample of 12 best docked ligand molecules are shown in Table 5 based on the glide energy and glide score.

Of the 159 ligands, a sample of 12 ligand molecules are shown obtained from virtual screening against the active site of UBE2A with CB_Nova Care and LifeChem structural databases. The molecular interactions are prioritized based on the glide energy and glide score.
Figure 10. Docking interactions between the UBE2A proteins with the ligands. The protein is represented in pale violet, red solid ribbon form and the ligand in blue stick form. The hydrogen bond and PiPi interactions in Å are represented in black color. The UBE2A protein amino acid residues are represented in maroon-colored sticks.
Table 6. Pharmacokinetic properties of the docked molecules with QikProp module.

| S.No. | CNS | Mol wt. | DHB | AHB | Qplog P<sub>o/w</sub> | Qplog BB | % Human oral absorption | Rule of three | Rule of five |
|-------|-----|---------|-----|-----|-----------------------|----------|------------------------|--------------|-------------|
| D1    | 1   | 420.507 | 2.0 | 7.45| 4.006                 | −0.673   | 94.831                 | 1            | 0           |
| D2    | 1   | 447.576 | 2.0 | 8.20| 3.949                 | −0.020   | 90.558                 | 1            | 0           |
| D3    | −2  | 368.825 | 3.0 | 7.0 | 2.981                 | −1.429   | 88.423                 | 0            | 0           |
| D4    | 0   | 402.378 | 3.0 | 7.0 | 3.791                 | −0.953   | 100                    | 1            | 0           |
| D5    | 1   | 387.361 | 1.0 | 6.5 | 3.874                 | −0.587   | 100                    | 1            | 0           |
| D6    | 1   | 330.429 | 1.0 | 8.00| 0.010                 | −0.609   | 54.452                 | 0            | 0           |
| D7    | 1   | 327.469 | 1.0 | 6.70| 2.381                 | −0.298   | 79.577                 | 0            | 0           |
| D8    | −2  | 297.307 | 4.0 | 10.05| −0.653           | −1.276   | 65.051                 | 0            | 0           |
| D9    | 1   | 311.356 | 1.0 | 6.50| 2.480                 | −0.301   | 87.379                 | 0            | 0           |
| D10   | 0   | 370.456 | 2.0 | 9.00| 1.328                 | −0.991   | 66.486                 | 0            | 0           |
| D11   | 1   | 308.354 | 2.5 | 6.25| 1.993                 | −0.408   | 80.311                 | 0            | 0           |
| D12   | 2   | 287.404 | 2.0 | 5.2 | 1.975                 | 0.497    | 77.998                 | 0            | 0           |

The permissible ranges are as follows: central nervous system (CNS): −2 (inactive) +2 (active); molecular weight (Mol wt.): (130–725); hydrogen bond donors (DHB): (0.0–6.0); hydrogen bond acceptors (AHB): (2.0–20.0); octanol/water partition coefficient QPlog Po/w: (−2.0 to 6.5); brain–blood barrier partition coefficient QPlog BB: (−3.0 to −1.2); % Human oral absorption: >80% high, <25% low; Rule of three (3): Rule of five (4).

Figure 11. Solvent-accessible surface area (SASA) in the UBE2A protein before and after docking. The decreased SASA values of the protein active site residues are SER25, GLU44, GLY45, ASP50, THR52 and ASP151 after docking is shown in light and the residues before docking are in dark lines.
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Declaration of interest

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Supplementary material available online

Supplementary Figure 1.