In silico analysis of indole-3-carbinol and its metabolite DIM as EGFR tyrosine kinase inhibitors in platinum resistant ovarian cancer vis a vis ADME/T property analysis

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ABSTRACT

Epidermal Growth Factor Receptor (EGFR) is one of the four members of the Human Epidermal Receptor (HER) family, which is deregulated and over expressed in platinum resistant ovarian cancer. Thus, targeting EGFR receptor along with platinum drugs is one of the major strategies to increase the platinum drug sensitivity. That’s why, in this study, we aimed to investigate the inhibitory activity and binding site analysis of indole-3-carbinol and its active metabolite 3,3'-diindolylmethane by using molecular simulation studies, also metabolic profile had been investigated by SOM prediction. The 3,3'-diindolylmethane showed significant inhibitory activity and binding energy comparing to indole-3-carbinol, also it processed lower toxicity and will undergo aromatic hydroxylation due its high intrinsic activity and Fe accessibility. Though our research study supports previous reports of EGFR inhibition, further in vivo study is necessary for validation of toxicological and pharmacokinetic study. However, the current work tries to address most of the variables in the dynamic drug design process by In silico study in order to boost the potentiality of the selected molecule to serve as good leads in terms of optimum pharmacokinetic and toxicological attributes.

INTRODUCTION

Until recently, ovarian cancer is one of the most lethal of all gynecologic malignancies, causing more than about 140000 deaths each year worldwide (Jemal et al., 2011). Most women with epithelial ovarian cancer (EOC) are diagnosed at advanced stages, where it is the fact that high mortality rate of this disease is caused by its poor prognosis (Slatnik and Duff, 2015). The clinical response rate is initially high and the platinum based compounds are standard first-line agents for ovarian cancer (Herzog, 2004). However, the ensuing reversion and recurring challenges of chemotherapeutical agents leads to the development of acquired chemoresistance, which is recurrent and closely linked to the poor survival associated with this cancer (Agarwal and Kaye, 2003; Rabik and Dolan, 2007). Therefore, developing novel and effective therapeutic strategies against ovarian cancer is urgently needed, and chemotherapy in combination with other inhibitors, is one of the standard chemotherapy standard initial management for ovarian cancer patients at advanced stages (Hennessy et al., 2009; Bible et al., 2012). Epidermal Growth Factor Receptor (EGFR) is one of the four members of the Human Epidermal Receptor (HER) family, which is over expressed in multiple malignancies, especially in ovarian cancer with poor prognosis (Bull Phelps et al., 2008).

It is one of the tyrosine kinases that can be activated by extracellular ligands, which leads to receptor autophosphorylation and subsequent activation of downstream pathways involved in proliferation, survival, angiogenesis, and invasion (Scaltriti and Baselga, 2006; Lemmon and Schlessinger, 2010).

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The EGFR pathway is recurrently activated in cancer cell, and its targeted inhibition with a small molecule kinase inhibitor has been successful for lung, breast and ovarian cancer with EGFR mutations (Yarden and Sliwkowski, 2001; Cataldo et al., 2011). 3,3-diindolylmethane (DIM), an active metabolite of indole-3-carbinol, is present in cruciferous vegetables (Ciska et al., 2009). Several studies have indicated an inverse relationship between intake of cruciferous vegetables and the risk of ovarian cancer and also concluded that DIM possesses chemopreventive and therapeutic properties (Rahman and Sarkar, 2005; Kandala and Srivastava, 2010).

Therefore, the present study was aimed to investigate the therapeutic properties of indole-3-carbinol and its metabolite DIM as EGFR tyrosine kinase inhibitor through various computational analyses, including molecular docking (Dash et al., 2014; Dash et al., 2015; Raju Dash et al., 2015) and ADME and Toxicity analysis.

MATERIALS AND METHODS

Protein Preparation

Three dimensional crystal structure of epidermal growth factor receptor kinase domain (PDB id: 1M17) was downloaded in pdb format from the protein data bank (Berman et al., 2000). After that, structure was prepared and refined using the Protein Preparation Wizard of Schrödinger-Maestro v9.4. Charges and bond orders were assigned, hydrogens were added to the heavy atoms, selenomethionines were converted to methionines, and all waters were deleted. Reorientation of certain hydroxyl and thiol groups, amide groups of asparagines, glutamines and imidazole ring of histidines, protonation states of histidines, aspartic acids, and glutamic acids was optimized at neutral pH. Using force field OPLS_2005, minimization was carried out setting maximum heavy atom RMSD to 0.30 Å.

Ligand Preparation

Compounds were retrieved from Pubchem databases, i.e. indole-3-carbinol (CID 3712) and 3, 3'-diindolylmethane (CID 3071). The 3D structures for these were built by using Ligprep2.5 in Schrödinger Suite 2013 with an OPLS_2005 force field. Their ionization states were generated at pH7.0±2.0 using Epik2.2 in Schrödinger Suite. Up to 32 possible stereoisomers per ligand were retained.

Receptor grid generation

Receptor grids were calculated for prepared proteins such that various ligand poses bind within the predicted active site during docking. In Glide, grids were generated keeping the default parameters of van der Waals scaling factor 1.00 and charge cutoff 0.25 subjected to OPLS 2001 force field.

A cubic box of specific dimensions centred around the centroid of the active site residues (Reference ligand active site) was generated for receptor. The bounding box was set to 14 Å x 14 Å x 14 Å for docking experiments.

Glide Standard Precision (SP) ligand docking

SP flexible ligand docking was carried out in Glide of Schrödinger-Maestro v9.4 (Friesner et al., 2004; Friesner et al., 2006) within which penalties were applied to non-cis/trans amide bonds. Van der Waals scaling factor and partial charge cutoff was selected to be 0.80 and 0.15, respectively for ligand atoms. Final scoring was performed on energy-minimized poses and displayed as Glide score. The best docked pose with lowest Glide score value was recorded for each ligand.

Prime MM-GBSA

Prime MM-GBSA approach was used to calculate ligand binding energies and ligand strain energies for a ligand and a single receptor. MM-GBSA is a method that combines OPLSAA molecular mechanics energies (EMM), an SGB solvation model for polar solvation (GSG), and a non-polar solvation term (GNP) composed of the non-polar solvent accessible surface area and van der Waals interactions. Here, the Glide pose viewer file of the best conformation chosen was given as the source in Prime MM-GBSA simulation. The total free energy of binding:
\[
\Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{protein}} + G_{\text{ligand}}), \text{ where } G = \text{EMM} + \text{GSG} + \text{GNP}
\]

ADME/T property analysis

Ligand based ADME/Toxicity prediction

The QikProp module of Schrodinger is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program design to produce certain descriptors related to ADME. It predicts both physicochemical significant descriptors and pharmacokinetically relevant properties. ADME properties determine drug-like activity of ligand molecules based on Lipinski’s rule of five. ADME/T properties of the compound (DIM) was analysed using Qikprop 3.2 module (Natarajan et al., 2015).

Structure-based P450 site of metabolism

The P450 Site of Metabolism (SOM) of selected compound was determined by ‘induced fit docking’ on CYP2D6 isofrom using the ‘P450 Site of Metabolism’ module of Schrodinger Suite v9.3. For a given atom in a molecule to be a significant site of metabolism by a CYP450 isofrom, it must have some degree of ‘reactivity’ in the absence of the enzyme and also be accessible to the reactive heme iron center. To address both these requirements, the ‘P450 Site of Metabolism’ workflow which combines induced-fit docking (IFD) for the determination of accessibility to the reactive center, with a rule-based approach to intrinsic reactivity, was used.

RESULTS

Molecular docking and binding energy analysis

In order to study the interaction of indole-3-carbinol and its active metabolite DIM with EGFR kinase, we performed Glide docking analysis by Schrodinger suite, where DIM processed
higher docking score than indole-3-carbinol, shown in Table 1. The negative and low value of free energy of binding demonstrates a strong favorable bond between EGFR kinase and DIM in most favourable conformations. Post docking analysis, shown in Figure 1 & 2, described the binding mode of indole-3-carbinol and DIM, in the active site of EGFR kinase domain. Though major residues like ASP831, THR830, MET742, PHE832, GLU738, LYS721, LEU764, THR766, ILE765, ALA719, ILE720, VAL702, LEU820 were found to be complimentary in both ligand’s binding sites, significant differences in the hydrogen bonding were also observed. Here, hydrogen of hydroxyl group of indole-3-carbinol was seen to have hydrogen bonding with ASP831 residue, while hydrogen of amino group was involved with the same residue, in case of DIM. GLU 738 residue was also occupied with hydrogen of amino group indole-3-carbinole. Again, in order to describe the overall clarity of docking studies, we introduced Prime MM-GBSA (Adasme-Carreno et al., 2014) approach to calculate ligand binding energies. As the MM-GBSA binding energies are approximate free energies of binding, a more negative value indicates stronger binding. In our study, highest binding energy was observed in DIM, comparing to indole-3-carbinol, indicating the accuracy of ligand protein binding (Table 1).

Table 1: Docking results of selected hits in kcal/mol.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>ΔGbind</th>
<th>Docking score</th>
<th>Glide energy</th>
<th>Glide ligand efficiency</th>
<th>Strain penalty</th>
<th>Glide Emodel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole-3-carbinol</td>
<td>-32.46</td>
<td>-6.442</td>
<td>-27.542</td>
<td>-0.585</td>
<td>0</td>
<td>-39.026</td>
</tr>
<tr>
<td>3,3'-Diindolylmethane</td>
<td>-49.00</td>
<td>-6.875</td>
<td>-37.112</td>
<td>-0.361</td>
<td>0</td>
<td>-52.481</td>
</tr>
</tbody>
</table>
Table 2: ADME and drug likeness properties of the selected ligands by QikProp.

<table>
<thead>
<tr>
<th>Name</th>
<th>MW</th>
<th>HB donor</th>
<th>HB acceptor</th>
<th>SASA</th>
<th>QP log HERG</th>
<th>QP log S</th>
<th>QP log Po/w</th>
<th>% Human Oral Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3-Diindolylmethane</td>
<td>246.311</td>
<td>2</td>
<td>0</td>
<td>489.494</td>
<td>-5.425</td>
<td>-4.364</td>
<td>4.215</td>
<td>100</td>
</tr>
</tbody>
</table>

*Subscripts: *aMolecular weight (acceptable range: <500).
*Subscripts: *bHydrogen bond donor (acceptable range: ≤5).
*Subscripts: *cHydrogen bond acceptor (acceptable range: ≤10).
*Subscripts: *dTotal Solvent Accessible Surface Area in using a probe with a 1.4 radius (acceptable range: 300–1000).
*Subscripts: *ePredicted IC50 value for blockage of HERG K+ channels (concern: below ≤5).
*Subscripts: *fPredicted aqueous solubility, S in mol/dm^3^ (acceptable range: −6.5–0.5).
*Subscripts: *gPredicted octanol/water partition coefficient (acceptable range: −2–6.5).
*Subscripts: *hPredicted human oral absorption on 0 to 100% scale (<25% is poor and >80% is high).

Fig. 3: Metabolic maps. (i) Overall SOM, (ii) Fe accessibility and its (iii) Intrinsic reactivity for DIM. Overall SOM score is the linear combination of the accessibility and the intrinsic reactivity larger scores mean higher reactivity. Fe accessibility (>): values indicate accessibility of the atoms to the iron center of CYP, full ray represents a unit of accessibility, with the length of the final ray representing the decimal portion. Intrinsic reactivity ('): Labels the intrinsic reactivity of atoms, positive values are more reactive. The size of the circle indicates the overall SOM score, color of the circle indicates the atomic intrinsic reactivity and a blue ring around the circle indicates that the atom passed through the CYP filtering stage.

ADME and Toxicity analysis

**Ligand based ADME/Toxicity prediction**

The drug-like activity of the ligand molecule was categorized using ADME properties by QikProp module of Schrodinger. The ADME property of the indole-3-carbinol and DIM were evaluated with QikProp module of Schrodinger, shown in Table 2. The selected properties are known to influence metabolism, cell permeation, and bioavailability. All the predicted properties of the isolated compound was in the range for 95% of known oral drugs and also satisfy the Lipinski’s rule of five to be considered as drug like potential.

**Structure-based P450 site of metabolism**

Cytochrome P450 enzymes are heme-containing enzymes, play an integral role in the metabolism pathway of drugs by undergoing a variety of reactions, such as hydroxylation, dealkylation, and double-bond oxidation that result in the degradation of small molecules (Liu et al., 2013). A metabolic reaction can ensue at a highly reactive site of a molecule, on condition that it is properly oriented within the CYP enzyme to interact with the reaction center. Therefore, accessibility and intrinsic reactivity are the crucial aspects for a metabolic reaction to take place. For this reason metabolism of indole-3-carbinol and DIM were studied by induced fit docking with CYP2D6 and the results were analyzed on the basis of “Fe accessibility”, “intrinsic reactivity” and “overall SoM (Site of Metabolism”). Details of these parameters, the metabolic maps of are illustrated in Figure 3. In Fe accessibility, labeled atoms are considered as the accessibility of the atoms to the iron. This is defined as the natural logarithm of the number of poses for the atom in which the atom was within 5 Å of Fe. Larger values of DIM indicate greater accessibility. The reactivity is predicted with a linear free energy approach based on the Hammett and Taft scheme, where the reactivity of a given atom is the sum of a baseline reactivity rate and a series of perturbations determined by the connectivity (Ryberg and Olsen, 2012). The intrinsic reactivity of DIM is quite high, according to the result, where positive values are more reactive, negative values are less reactive.

**DISCUSSION**

In few last decades, the implication of EGFR pathway in cancer progression has been suggested by various studies (Blume-Jensen and Hunter, 2001; Chan et al., 2005). This consequence is often serious to the development of platinum drug resistance, in case of ovarian cancer treatment. Briefly, over expression of EGFR is linked with constitutive activation of STAT3 (signal transducers and activators of transcription 3) that confers resistance to chemotherapy-induced apoptosis in epithelial malignancies (Coley et al., 2006; Duan et al., 2006). The STAT3 protein, a member of the STAT family from latent cytoplasmic transcription factors, is phosphorylated by EGFR and other tyrosine kinases, which abnormally activated with a high frequency in many other human tumors, especially in ovarian cancer (Huang et al., 2000; Burke et al., 2001). The main fact is that the activation of STAT3 at Tyr-705 leads to accumulation of anti-apoptotic events, such as Bcl-2, Bcl-xL, and PI 3-kinase/Akt activity in the recurrent and resistant ovarian cancer (Richardson and Kaye, 2005) and granted constitutively active Stat3-mediated deregulation of gene expression is a well recognized mechanism to
promote malignant tumorigenesis, and tumor progression (Bowman et al., 2000; Turkson, 2004), also it may be suggested that combination therapy like platinum drug with EGFR blocker may enhance the platinum chemosensitivity in human ovarian cancer. Regarding this, we selected indole-3-carbinol and its active metabolite 3, 3′-diindolylmethane (DIM) to investigate the interactions and binding modes in active site of EGFR kinase domains. As described previously in result section, it may be concluded that the DIM has greater affinity towards the EGFR binding than the indole-3-carbinol, and will formed a stable protein ligand complex and may result induced inhibition. Moreover, numerous research had already established the mechanism of DIM, for inducing apoptosis, by inhibiting EGFR signaling in ovarian cancer (Kandala et al., 2012), breast cancer and lung cancer cell (Rahimi et al., 2010). Additionally, DIM has been exposed to decrease carcinogen-induced breast and lung tumor formation in rodent models of carcinogenesis with little toxicity (Chen et al., 1998). Considering that, in our study, we tried to explore out the ADME and toxicity profiles both of two compounds, i.e., DIM and Indole-3-carbinol. As described in table 2, the ADME profile of DIM was quite low, though the concerning value of hERG K+ channel (which indicates the potential of a compound for cardiac toxicity) is high. This compound also processes high oral adsorption value. The metabolic map of DIM indicates the probability of deamination, as the amino group shows moderate Fe accessibility (Shown in Figure 3). This profile also suggested that DIM has the probability of aromatic hydroxylation due to their proximity to Fe also their intrinsic reactivity is high.

CONCLUSION

The current study is an attempt to explore out the therapeutic potentiality of 3,3′-diindolylmethane compound regarding the perspectives in platinum resistant ovarian cancer. In parallel, our experimental data supports the existing evidence in literature. Although, we explored out the pharmacokinetics profile of 3, 3′-diindolylmethane, further in vivo experiment is necessary to validate its biotransformation profile. However, the current work tries to address most of the variables in the dynamic drug design process right at the outset with the convenience of In silico tools in order to boost the potentiality of the selected molecule to serve as good leads in terms of optimum pharmacodynamic and toxicological attributes.

COMPETING INTERESTS

All authors declare that they have no competing interests.

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