IN-SILICO STUDY OF MAPK INHIBITION BASED LEAD IDENTIFICATION FROM THE ISOLATED COMPOUNDS OF CROTON OBLONGIFOLIUS ROXB FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA

Sajon, S.R.*

1Department of Pharmacy, Faculty of Biological Science and Technology, Jashore University of Science and Technology, Jashore-7408, Bangladesh

sadiur.sajon@yahoo.com

Abstract

The aim of this study was to identify potential lead compounds from Croton oblongifolius Roxb for treatment of hepatocellular carcinoma by inhibition of MAPK by insilico strategy. We used Schrödinger Maestro v 10.1 for processing of retrieved ligands and protein molecule along with grid generation, docking analysis and physicochemical property analysis. In our study, we found that Oblongionoside B (-8.90 kcal/mol), Oblongionoside C (-9.54 kcal/mol), Oblongionoside D (-9.61 kcal/mol), Isohamnetin (-8.57 kcal/mol), Hyperoside (-8.53 kcal/mol) and clerspide A (-8.63 kcal/mol) are the six potential lead compounds that can be used in the MAPK inhibition mediated hepatocellular carcinoma treatment. We hope that further study will be conducted to evaluate the potentiality of these compound in both in-vitro and in-vivo method.

Keywords: Croton oblongifolius; MAPK; Hepatocellular carcinoma; Molecular docking
Introduction

Cancer is defined as the abnormal cells division without control and is able to produce immature cells. These cells can spread to other parts of the body through the blood and lymphatic systems. Cancer is not one disease but it is a very complex form of many diseases. The cancer agents (carcinogens) can be present in air, water, food, chemicals and sunlight that are exposed to the people [1]. Hepatocellular carcinoma is the fifth most common cancer in men (5,54,000 cases, 7.5% of the total) and the ninth in women (2,28,000 cases, 3.4% of the total), with 7,82,000 new cases occurring in 2012 and approximately 7,46,000 persons die each year from this [2,3]. Whereas, in Bangladesh, the prevalence of HCC in male is 1.5% and prevalence of HCC in female is 0.6% [4]. Several experiments suggests that HCC is a possibility of factors such as inactivation of tumour suppressor gene, activation of multiple oncogene and over production of growth factors. More than 20 genes are identified to play a role in HCC which includes Ras, c-myc, c-fos and c-jun, rho, transforming growth factor-a, hepatocyte growth factor and c-met, c-ErbB-2, u-plasminogen activator, MXR7, MDM2, MAGE, matrix metalloproteinase and many more [5, 6]. MEK-MAPK signal transduction pathway is the most frequently targeted in the downstream of receptor and non-receptor tyrosine kinase and ras family of GTP-binding protein and enhancement of MEK-MAPK is notably observed in carcinogenic cell lines [7-10]. Studies with small molecule inhibitors of MEK activity [19, 20] demonstrate a role for MEK in mediating expression of proteinases implicated in invasion and metastasis [11, 12], and disruption of normal epithelial morphology [13, 14]. Increased MAPK (ERK1/2) and MEK1/2 expression and p42 MAPK in 5 HCC samples has been reported [15]. Treatment of cells with various growth factors produces activation of MEK1/2 and its downstream target, MAPK, resulting in proliferation, differentiation and survival [16]. Another study conducted by Huynh et al suggests that MAPK is activated in all the experimental HCC samples which suggests that targeting MAPK for treatment of HCC is a great initiative in this field [17].

Croton oblongifolius Roxb belongs to the Euphorbiaceae family mostly found in Central, Western and Southern India, also eastwards to Bengal [18]. Seeds of Croton oblongifolius Roxb are reported to be used in diarrhea and oedema, whereas root bark and seeds are used as purgative, in liver disease and also in reducing high blood pressure. Moreover, it is used to treat gastric ulcer, dyspepsia and dysentery [19]. There are many compounds which are isolated from different parts of Croton oblongifolius Roxb which exhibits a variety of pharmacological activities such as nasmiluim A isolated from leaves was reported to show cytotoxicity against MOLT-3 cell line and also showed anti-microbial effect against some microbes [19]. Furanocembranoids from stem bark exhibited to have cytotoxic effect against BT474, CHAGO, Hep-G2, KATO-3, SW-620 cell lines in MTT colometry [20], (-)-ent-kuar-16-endo-19-oic acid isolated from stem bark showed inhibition of Na+,K+-ATPase activity with cytotoxicity [21], croblongifolin from stem bark exhibited cytotoxicity against HEP-G2, KATO-3, BT-474, CHAGO cell lines [22], neocrotocembranal isolated from stem bark showed cytotoxicity against P-388 cell lines etc. There are also many compounds which were isolated from different parts of Croton oblongifolius Roxb such as (+)-Cyclosativene, (e)-Caryophyllene, gamma-Muurolene, gamma-Terpinene, Germacrene D [23], (-)-hardwickic acid, 11-dehydro (-)-hardwickic acid [24], Clerspide A, Canthoside A, Glochidionioside D, Oblongionioside A_F [25], hyperoside, isohamnetin [26] etc. Among all the isolated compounds many were reported to be cytotoxic against hep-G2 cell lines which is cancerous immortal liver cell line [20, 22] and aerial part of the plant exhibited hepatoprotective activity in-vivo [26]. And we can see that this plant has a potential to be a good source of medicine for the treatment of hepatocellular carcinoma.

However, this in-silico study was performed to identify potential compounds isolated from Croton oblongifolius Roxb which can be used in-vitro and if proven to be useful then in-vivo study of hepatocarcinoma treatment by inhibiting the enzyme mitogen activated protein kinase (MAPK).

Methods

Retrieval and preparation of compounds

A total of 83 compounds which were reported in different articles [20, 21, 22, 23, 24, 25, 26, 28-39]
were retrieved from PubChem. Doxorubicin (InchKey: AOJJSUZBOXQNZ-TZSSRYMLSA-N; Pubchem CID: 31703) used as standard and was also retrieved from pubchem. All the compounds were downloaded in 3 dimensional state in .sdf format and assigned to LigPrep 3.1, (Schrödinger, LLC, New York, NY, 2015) for energy minimization by applying OPLS 2005 force field and where pH was maintained at 7.0±2 using Epic 3.1 module of Schrödinger-Maestro (Version 10.1). The output file was also saved in .sdf format. LigPrep assists to convert 2D to 3D structures efficiently and accurately for computer analysis. LigPrep optionally expands tautomeric and ionization states, ring conformations and removal of problematic structure, optimization of geometric structure and conversion of output file for varied types of application of this software.

Retrieval and preparation of protein

Crystal structure of mitogen activated protein kinase (MAPK) was retrieved from RCSB Protein Data Bank in pdb format. The retrieval id of MAPK crystal structure is 4lmm. Before processing of the protein unwanted molecules such as ANP and MG were removed from the 3D structure using Discovery studio version 4.5 and the refined structure was saved in .pdb format. Protein Preparation Wizard of Schrödinger-Maestro v 10.1 was used to make the protein compatible for Glide module. In this process, in primary stage, bond order was assigned, hydrogen molecules were added, zero-order bonds with metal were created, disulfide bonds were created and selenomethionines were converted to methionines. In protein refinement stage, hydrogen bonds were assigned using the PROPKA module at pH 7, water molecules with less than three hydrogen bonds to non-water molecules were removed and at last energy minimization was carried out using OPLS 2005 force field setting the maximum heavy atom RMSD to 30 Å.

Receptor Grid Generation

Glide version 6.6 was used for receptor grid generation. Glide is a module which is used for excluding the ligand from ligand-protein complex and defining a box where the desired ligands can be added as a complex. In this study, van der waals radius scaling was set to default of scaling factor 1.00 Å and charge cut-off 0.25 Å. A cubic box of specific dimensions centred on the centroid of the active site residues was generated for the receptor. The bounding box was set to 15 Å x 15 Å x 15 Å and x, y, z axis are set at 31.7, 30.02, -13.06 sequentially.

Glide molecular docking

After completing the preliminary steps, the glide 6.6 module of Schrödinger-Maestro v 10.1 was used for determining the docking scores of desired complex. Glide is designed to assist in high-throughput screening of potential ligands based on binding mode and affinity for a given receptor molecule. We can compare ligand scores with those of other test ligands, or compare ligand geometries with those of a reference ligand. Additionally, we can use Glide to generate one or more plausible binding modes for a newly designed ligand. In this study, Flexible ligand docking was performed with Glide of Schrödinger-Maestro (version 10.1) [40, 41] within which penalties were applied to non-cis/trans amide bonds. Glide standard precision docking was performed with these molecules, and hits above 4 kcal/mol based on docking score with MAPK enzyme in XP mode, keeping all docking parameters as default. No bonding constraints were given during docking calculations. Using Monte Carlo random search algorithm, ligand poses were generated for each input molecule, and binding affinity of these molecules to the MAPK enzyme was predicted regarding Glide docking score. Post-docking minimization was performed with OPLS 2005 force field, and one pose per ligand was saved.

Molecular Property and ADME/T analysis

QikProp 4.3 module Schrödinger-Maestro (version 10.1) is used to determine the physical property of the moecules under study. We used Lipinsky’s rule of five [42] and Jorgensen’s rule of three [43, 44] to describe the acceptability of the compounds. Also, admet SAR web server was used to identify selected pharmacokinetic parameters and toxicity of the best docked compounds.

Results

In our study, out of all the 83 compounds, six compounds expressed better docking score compared to standard drug doxorubicin (−8.31
kcal/mol). They are Oblongionoside B (-8.90 kcal/mol), Oblongionoside C (-9.54 kcal/mol), Oblongionoside D (-9.61 kcal/mol), Isohamnetin (-8.57 kcal/mol), Hyperoside (-8.53 kcal/mol) and clerspide A (-8.63 kcal/mol). They were further docked in extra precision mode to exclude false positive and in this mode Oblongionoside B showed best docking score which is -13.55 kcal/mol and Oblongionoside D showed docking score of -12.59 kcal/mol. Interacting amino acids were also identified using 2D mode of ligand-protein interaction. All these results are included in table-1.

Physical properties of the compounds are displayed in table 2 and 3. In table 2, physical properties according to Lipinsky’s rule of five are expressed. And in table 3, physical properties of the molecules according to Jorgensen’s rule of three are expressed.

Selected pharmacokinetic and toxicological parameters such as absorption through blood-brain barrier and human intestine, inhibition of P-glycoprotein, inhibition of CYP450 2C9, modification of human ether-a-go-go gene, acute oral toxicity and rat acute toxicity of the selected best docked compounds are generated in table-4.

Discussion

With rapid development of modern biological and chemical technologies, computer aided drug design is rapidly changing the nature of novel drug like candidate identification and also, this method widely accepted due to its ease of application and reduction of process cost [45]. And in the field of computer aided drug design, molecular docking is a competent tool for identification of novel drug like molecules [46]. Molecular docking is a computation based study which considers covalent association between a macromolecule such as receptor or enzyme and a micro molecule such as ligand efficiently. Prediction of binding energy of small molecules with receptors or enzyme have a huge impact on lead identification as it screens a huge database of drug like molecules to find the best fit for the receptor and further modify it for using it as a drug candidate [47]. Molecular docking study of protein structures involves different association between protein and ligand and the associations are verified on the basis of binding energy. The association with the least energy is considered the best pose and the complex is then further analyzed. One of the areas of molecular docking is protein-ligand docking which is gaining popularity due to its role in structure based drug design [47-52].

In our study, we docked 83 compounds isolated from different parts of Croton oblongifolius Roxb and docked it against mitogen activated protein kinase (4lmn) to identify potent compounds that can inhibit the protein more efficiently than the standard drug doxorubicin. Doxorubicin a anthracycline based chemotherapeutic agent which is isolated from Streptomyces peucetius and clinical studies showed that systemic administration of doxorubicin showed limited clinical benefits however, administration of doxorubicin through hepatic artery route, showed significant tumour shrinkage and partial responses were in 30-70% patients [53]. During our study, we found that some compounds such as Oblongionoside B (-8.90 kcal/mol), Oblongionoside C (-9.54 kcal/mol), Oblongionoside D (-9.61 kcal/mol), Isohamnetin (-8.57 kcal/mol), Hyperoside (-8.53 kcal/mol) and clerspide A (-8.63 kcal/mol) showed better docking score than the standard drug doxorubicin (-8.31 kcal/mol). By further docking these compounds in glide XP mode, we found that Oblongionoside B and Oblongionoside D showed best docking score which is -13.55 kcal/mol and -12.59 kcal/mol sequentially. Glide XP is a harder function that exacts severe penalties such as poses that violates established physical chemistry principles and useful in lead optimization and other studies where a limited number of compounds are considered [40]. 2D and 3D binding mode of Oblongionoside B and D along with their hydrophobicity of binding pocket is shown in figure 1-6. And figure 7-9 demonstrates the 2D and 3D binding mode of standard drug doxorubicin and the hydrophobicity of binding pocket.

In table 2, descriptors used in Lipinsky's rule of five are molecular weight of the molecule (mol_MW), estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (donorHB), estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (acceptHB), predicted
octanol/water partition coefficient (QP\textsubscript{logPo/w}) and number of non-hindered rotatable bonds (#rotor). On the other hand, in Jorgensen’s rule of three, the denoted descriptors are predicted aqueous solubility, log S (QP\textsubscript{logS}), predicted apparent Caco-2 cell permeability in nm/sec (QPPCaco) and number of likely metabolic reactions (#metab) which is expressed in table 3. All the acceptable values are crosschecked with software manual for reliability. And, we found that all these compounds are within the acceptable range to be used as potential lead compound.

In table 4, we considered the server based selected pharmacokinetic and toxicological parameters. Here, we can see that all six compounds except oblongioside B and Clerspide A showed negative result in blood-brain barrier absorption. In case of human intestinal absorption, oblongioside C and D showed negative results. Moreover, oblongionoside C and D has only inhibitory potential of P-glycoprotein whereas other four has both inhibitory and non-inhibitory potential. Among the six compounds only isohamnetin has inhibitory potential for CYP450 2C9. All the six compounds are weak inhibitors of human ether-a-go-go gene. Also, human oral toxicity and rat acute toxicity is also shown in table 4.

**Conclusion**

Computer aided drug design is a process where we can shorten the time for library search for finding out our desired compound for specific drug target. In our study, we used this method to identify lead compounds for MAPK inhibition based hepatocellular treatment among the 83 compounds isolated from *Croton oblongifolius* Roxb and identified six compounds which can act as potential lead compounds for the treatment of hepatocellular carcinoma. I hope to extend my experiment in future by evaluating these compounds for their desired activity by both in-vitro and in-vivo method and if possible we would like to modify the leads for better activity and selectivity.

**Abbreviations**

HCC: Hepato Cellular Carcinoma; MAPK: Mitogen Activated Protein Kinase; ERK: Extracellular signal-Regulated Kinases; mol\_MW: molecular weight of the molecule; donorHB: estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution; acceptHB: estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution; QP\textsubscript{logPo/w}: predicted octanol/water partition coefficient; #rotor: number of non-hindered rotatable bonds; QP\textsubscript{logS}: predicted aqueous solubility, log S; QPPCaco: predicted apparent Caco-2 cell permeability in nm/sec; #metab: number of likely metabolic reactions; SP: Standard Precision; XP: Extra Precision; LD: Lethal Dose.

**References**


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Table 1: Docking score of best docked compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Protein</th>
<th>Interacting amino acids</th>
<th>Docking score (kcal/mol) (SP)</th>
<th>Docking score (kcal/mol) (XP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>4lmn</td>
<td>ASN78, GLY77, LYS192</td>
<td>-8.31</td>
<td>-8.32</td>
</tr>
<tr>
<td>Hyperoside</td>
<td>4lmn</td>
<td>GLY77, ARG189, ARG234, ASN221</td>
<td>-8.53</td>
<td>-11.67</td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>4lmn</td>
<td>PHE209, VAL211, SER212</td>
<td>-8.57</td>
<td>-9.81</td>
</tr>
<tr>
<td>Oblongionoside B</td>
<td>4lmn</td>
<td>GLY77, ASP190, LYS192, ASN195, ASP208, ARG234</td>
<td>-8.90</td>
<td>-13.55</td>
</tr>
<tr>
<td>Oblongionoside C</td>
<td>4lmn</td>
<td>GLY77, ASP190, LYS192, ASN195, ASP208, PHE209</td>
<td>-9.54</td>
<td>-12.46</td>
</tr>
<tr>
<td>Oblongionoside D</td>
<td>4lmn</td>
<td>GLY77, ASP190, LYS192, ASN195, ASP208, PHE209, ARG234</td>
<td>-9.61</td>
<td>-12.59</td>
</tr>
<tr>
<td>Clerspide A</td>
<td>4lmn</td>
<td>GLY77, ASN78, ASP190, LYS192, ASP208</td>
<td>-8.63</td>
<td>-11.68</td>
</tr>
</tbody>
</table>
### Table 2: Molecular property according to Lipinsky’s Rule of five of the best docked compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>mol MW</th>
<th>DonorHB</th>
<th>acptHB</th>
<th>QPlogPo/w</th>
<th>#rotor</th>
<th>Number of violations</th>
<th>Acceptable number of violations [42]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubucin</td>
<td>543.526</td>
<td>5</td>
<td>14.8</td>
<td>-0.411</td>
<td>11</td>
<td>3</td>
<td>Maximum 4</td>
</tr>
<tr>
<td>Hyperoside</td>
<td>464.382</td>
<td>7</td>
<td>13.75</td>
<td>-1.381</td>
<td>11</td>
<td>2</td>
<td>Maximum 4</td>
</tr>
<tr>
<td>Isohamnetin</td>
<td>316.267</td>
<td>3</td>
<td>5.25</td>
<td>1.208</td>
<td>5</td>
<td>0</td>
<td>Maximum 4</td>
</tr>
<tr>
<td>Oblongionoside B</td>
<td>522.589</td>
<td>8</td>
<td>18.5</td>
<td>-1.021</td>
<td>16</td>
<td>3</td>
<td>Maximum 4</td>
</tr>
<tr>
<td>Oblongionoside C</td>
<td>508.605</td>
<td>7</td>
<td>17.75</td>
<td>-0.761</td>
<td>16</td>
<td>3</td>
<td>Maximum 4</td>
</tr>
<tr>
<td>Oblongionoside D</td>
<td>508.605</td>
<td>7</td>
<td>17.75</td>
<td>-0.773</td>
<td>16</td>
<td>3</td>
<td>Maximum 4</td>
</tr>
<tr>
<td>Clerspide A</td>
<td>446.407</td>
<td>6</td>
<td>17.1</td>
<td>-1.255</td>
<td>13</td>
<td>2</td>
<td>Maximum 4</td>
</tr>
</tbody>
</table>

Acceptable values- mol MW: 130.0 – 725.0, donorHB: 0.0 – 6.0, acptHB: 2.0 – 20.0, QPlogPo/w: -2.0 – 6.5, #rotor: 0-15 [Qikprop 4.3 User manual]
Table 3: Molecular property according to Jorgensens’s Rule of three of the best docked compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>QPlogS</th>
<th>QPPCaco</th>
<th>#metab</th>
<th>Number of violations</th>
<th>Acceptable number of violations [43, 44]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>-2.353</td>
<td>2.466</td>
<td>9</td>
<td>2</td>
<td>Maximum 3</td>
</tr>
<tr>
<td>Hyperoside</td>
<td>-2.596</td>
<td>2.605</td>
<td>8</td>
<td>2</td>
<td>Maximum 3</td>
</tr>
<tr>
<td>Isohamnetin</td>
<td>-3.344</td>
<td>60.509</td>
<td>5</td>
<td>0</td>
<td>Maximum 3</td>
</tr>
<tr>
<td>Oblongionoside B</td>
<td>-2.617</td>
<td>28.149</td>
<td>8</td>
<td>1</td>
<td>Maximum 3</td>
</tr>
<tr>
<td>Oblongionoside C</td>
<td>-2.383</td>
<td>18.171</td>
<td>7</td>
<td>2</td>
<td>Maximum 3</td>
</tr>
<tr>
<td>Oblongionoside D</td>
<td>-2.355</td>
<td>17.874</td>
<td>7</td>
<td>2</td>
<td>Maximum 3</td>
</tr>
<tr>
<td>Clerspide A</td>
<td>-1.637</td>
<td>104.185</td>
<td>6</td>
<td>0</td>
<td>Maximum 3</td>
</tr>
</tbody>
</table>

Acceptable value: QPlogS: -6.5 – 0.5; QPPCaco: <25 poor, >500 great; #metab: 1-8 [Qikprop 4.3 user manual]
**Table 4:** Selected pharmacokinetic parameters of best docked compounds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Oblongionoside B</th>
<th>Oblongionoside C</th>
<th>Oblongionoside D</th>
<th>Hyperoside</th>
<th>Isohamnetin</th>
<th>Clerspide A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood-Brain Barrier</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Human Intestinal absorption</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>P-glycoprotein Inhibitor</td>
<td>I/NI</td>
<td>I</td>
<td>I</td>
<td>NI</td>
<td>NI/I</td>
<td>I/NI</td>
</tr>
<tr>
<td>CYP450 2C9 Inhibitor</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>I</td>
<td>NI</td>
</tr>
<tr>
<td>Human Ether-a-go-go-Related Gene</td>
<td>WI</td>
<td>WI</td>
<td>WI</td>
<td>WI</td>
<td>WI</td>
<td>WI</td>
</tr>
<tr>
<td>Acute Oral Toxicity</td>
<td>iii</td>
<td>i</td>
<td>i</td>
<td>iii</td>
<td>iii</td>
<td>iii</td>
</tr>
<tr>
<td>Rat Acute Toxicity (LD50, mol/kg)</td>
<td>2.88</td>
<td>3.41</td>
<td>3.41</td>
<td>2.39</td>
<td>2.72</td>
<td>2.64</td>
</tr>
</tbody>
</table>

+= Positive, - = Negative, I = Inhibitor, NI = Non-Inhibitor, i = Category I contains compounds with LD50 values less than or equal to 50mg/kg, iii = Category III includes compounds with LD50 values greater than 500mg/kg but less than 5000mg/kg.
Figure 1: 2D presentation of interacting amino-acids of 4LMN with Oblongioside B.
**Figure 2:** 3D presentation of Oblongionoside B in its binding site of 4LMN.

**Figure 3:** Hydrophobicity of the binding pocket of 4LMN with Oblongionoside B.
Figure 4: 2D presentation of interacting amino-acids of 4LMN with Oblongionoside D.
**Figure 5:** 3D presentation of Oblongionoside D in its binding site of 4LMN.

**Figure 6:** Hydrophobicity of the binding pocket of 4LMN with Oblongionoside D.
Figure 7: 2D presentation of interacting amino-acids of 4LMN with Doxorubicin.
**Figure 8**: 3D presentation of Doxorubicin in its binding site of 4LMN.

**Figure 9**: Hydrophobicity of the binding pocket of 4LMN with Doxorubicin.