**In Silico Anti-Cancer Activity Prediction of Secondary Metabolites from Salinispora spp.**

Dinesh Kumar K.
Research Scholar, Department of Bioinformatics
School of Bioengineering,
SRM University,
Kattankulathur, Tamil Nadu, India
e-mail: dineshkumark@ktr.srmuniv.ac.in

Waheeta Hopper
Professor & Head, Department of Bioinformatics
School of Bioengineering,
SRM University,
Kattankulathur, Tamil Nadu, India
e-mail: hod.bi@ktr.srmuniv.ac.in

**Abstract**—A molecular docking investigation was carried out on secondary metabolites from the marine actinomycete, Salinispora to identify their bioactive targets. Over-expression of epidermal growth factor receptor (EGFR) is an essential feature in the Basal-like breast cancer, for which there are currently no therapies. Hence a docking study was carried out to check for the anti-cancer activity of the compounds from S. tropica targeting the EGFR. The compounds were subjected to virtual screening and Extra precision (XP) docking using Schrödinger protocol. To preliminarily investigate the potential molecular target, the docking was performed using EGFR. The docking result revealed that the compounds, areniclide A, arenamycin A and arenamycin B exhibited good binding interaction to EGFR. The identified interactions could be a prelude to perform experimental assays.

**Keywords**—Salinispora; secondary metabolites; Epidermal Growth Factor Receptor; virtual screening; molecular docking

I. INTRODUCTION

Actinomycetes are a prolific source of structurally diverse secondary metabolites, many of these possess pharmacologically relevant biological activities [1]. Around 23,000 bioactive secondary metabolites from microorganisms have been reported and over 10,000 of these are from actinomycetes, representing 45% of all bioactive microbial metabolites [2]. These organisms have evolved with greatest genomic and metabolic diversity and hence efforts have been directed towards exploring marine actinomycetes as a source for the discovery of novel secondary metabolites [3]. *Salinispora* are marine actinomycetes, widespread in tropical and subtropical marine mud. There are three species of the genus are *S. tropica, S. arenicola* and *S. pacifica*. The secondary metabolites from *Salinispora* strains have varied biological activities however, they have been tested only in limited, biomedical assays, and therefore many of their activities remain unknown. Salinosporamide A, a potent proteasome inhibitor from *Salinispora* has entered phase I clinical trials as an anticancer agent [4].

Receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) and three related proteins (the ERBB family) play crucial roles in both normal physiological and cancerous conditions [5]. Many human tumors express EGFR in lung, head, neck, colon, pancreas, breast, ovary, bladder, kidneys and in gliomas. Expression of EGFR and its role in cancer prognosis have been investigated in many human cancers [6]. The epidermal growth factor (EGF) is the prototype of a family of peptide ligands that bind to cell membrane receptors and activate intracellular signaling pathways to control tumor cell growth, proliferation, survival, metastasis, and angiogenesis. The EGF receptor (EGFR, ErbB1, or HER1) is one of a four-membered family of transmembrane receptors that, similar to HER2, is often overexpressed in cancer cells, correlating with poor prognosis. EGFR therefore represents a reasonable target for the development of novel anticancer therapies [7].

EGFR are single chain transmembrane polypeptide proteins possessing three different domains: (a) the extracellular domain, which binds to ligands that activate the receptor, (b) the transmembrane domain that is involved in dimerization interaction between receptors, and (c) the intracellular tyrosine kinase domain that phosphorylates tyrosine residues on substrate proteins. The crystal structures of extracellular domains of EGFR, HER-2 and HER-3 show that they consist of four subdomains (I – IV) [8-9].

Endogenous ligands for EGFR known are: epidermal growth factor (EGF), transforming growth factor- α, amphiregulin, betacellulin, heparin-binding EGF, and epiregulin. Upon ligand binding to the extracellular domain, EGFR undergoes a conformational change that allows activation either by homodimerization or heterodimerization with other members of the erbB family and phosphorylation of several tyrosine residues [10]. The formation of EGF receptor homodimers is induced by the binding of EGF to its receptor, resulting in autophosphorylation of the EGF receptor, stimulation of down-stream signaling pathways, and enhanced cell proliferation. This complex network represents a significant factor for the observed phenotypic heterogeneity and variable drug responses among cancer types [11].

The crystal structure of EGFR (PDB 1IVO) contains 2:2 EGF: EGFR complex per asymmetric unit. In the 2:2 EGF: EGFR complex, each EGF molecule is bound to only one of the two EGFR molecules, two 1:1 EGF: EGFR complexes are dimerized (Fig.1). The receptor dimerization occurs mostly through interactions between each domain II. A 20
residue region ("dimerization arm"), forming a hairpin in the middle, protrudes from the domain II globule of each receptor. Dimerization is a prerequisite for EGF receptor activation and is driven by interactions between the extracellular domains of the two monomeric partners. Mutation of either Tyr-246 or Tyr-251 within this arm abolished EGF receptor homodimer formation [12]. Some of the secondary metabolites of *Salinispora* show moderate anticancer activity in cancer cell lines in *in vitro* studies. Hence this work attempts to identify biological targets for the secondary metabolites of *Salinispora* spp. by using *in silico* methods.

II. METHODS

A. Ligand Preparation

A compound database of the 23 metabolites of *Salinispora* were created and energy minimized using LigPrep module of Schrödinger suite version 9 [13] keeping one conformer per ligand, and the rest of the parameters were kept as default.

B. Protein Preparation

The crystal structure of EGFR was retrieved from Protein Data Bank [14] that contains 2:2 EGF: EGFR complex per asymmetric unit (PDB ID: 1IVO) (Fig.1). All water molecules were removed, and multimeric complexes were simplified from the PDB structure. Prior to molecular docking, receptor structure was preprocessed and prepared by adding the missing hydrogen, correcting the bond orders followed by optimization and energy minimization with force field: OPLS ("optimized potential for liquid simulations") using the protein preparation wizard of the Schrödinger’s suite. The grid generation was carried out using three separate sets of regions, the EGF binding site and Arenamycin A (-5.083) interacted with dimerization site and Arenamycin B (-5.686) interacted with domain II residues (Table 1). The Hydroxyl group of arenicolide A interacted with oxygen of Tyr64, carbonyl group of Ser11 and carbonyl group of Asn12 (Fig. 3) in the EGF binding region. Carbonyl group of arenamycin B interacted with carbonyl group of Gly410 and carbonyl group of Gln408 (Fig. 4) in the domain II region. Hydroxyl group of arenamycin A interacted with oxygen of Gln258 (Fig. 5) in the dimerization site.

*Salinispora* species shows structurally diverse secondary metabolites. *In silico* activity prediction of these compounds were performed using virtual screening and molecular docking protocol. The molecular docking results presented in this study revealed that the three secondary metabolites of *Salinispora* showed strong binding affinities for the EGFR.

In the receptor-dimerization interface, Tyr251 of one monomer forms hydrogen bonds with Arg285 and Phe263 hydrophobically interacts with other monomer. In the crystal structure 1IVO the replacement of Arg285 to the Ser285 in receptor-dimerization interface reduce the bioactivity of EGFR (12). Studies show that Arg285 play an important role for structure stability. Here we report that the compounds bind to the Arg285, EGF binding site and Domain II residues which inhibit the dimerization and prevent the binding of EGF to the EGFR receptor.

IV. CONCLUSION

Screening methods are extensively used to reduce cost and time of drug discovery. It has been clearly demonstrated that the approach utilized in this study is successful in finding novel anticancer inhibitors from marine actinomycetes. Some of the compounds from *Salinispora* species showed high binding affinity against EGFR. The conformation of the docked compounds fits exactly into the active site region of the receptor. This study indicates the importance of small molecules from *Salinispora* species and their use as bioactive molecules. Further, work can be extended towards experimental studies and evaluation of their biological activity. The findings suggest that these compounds could be developed as lead compounds for designing of anti-cancer drugs.

ACKNOWLEDGMENT

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REFERENCES


[14] Protein Data Bank (http://www.pdb.org/pdb/home/home.do)

[15] PDBSum Database (http://www.ebi.ac.uk/pdbsum/)

### Table 1.

<table>
<thead>
<tr>
<th>Targeted site of EGFR receptor</th>
<th>Compound</th>
<th>Glide score (Kcal/mol)</th>
<th>Interaction</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF binding Site</td>
<td>Arenicolide A</td>
<td>-8.398</td>
<td>OH---O (SER 11) OH---O (TYR 64) OH---O (ASN 12)</td>
<td>2.230 2.427 1.823</td>
</tr>
<tr>
<td>Domain II</td>
<td>Arenimycin B</td>
<td>-8.886</td>
<td>OH---O (GLY 410) (ARG285)NH---O OH---O (GLN408)</td>
<td>1.676 1.797</td>
</tr>
<tr>
<td>Dimerization site</td>
<td>Arenimycin A</td>
<td>-5.083</td>
<td>(GLU238)HO---O HO---H (ASN210)</td>
<td>2.494 2.321</td>
</tr>
</tbody>
</table>

**Figure 1.** Structure of EGFR receptor and their domains

**Figure 2.** Residue interactions of EGFR (chain A) with EGF (chain C)
Figure 3. Docking interaction of Arenicolide A with EGFR at the EGF binding site

Figure 4. Docking interaction of Arenamycin A with EGFR at the dimerization site

Figure 5. Docking interaction of Arenamycin B with EGFR at domain II