



## MOLECULAR DOCKING AND QSAR STUDIES ON BIOACTIVE COMPOUNDS ISOLATED FROM MARINE ORGANISMS INTO THE MUC1 ONCOPROTEIN

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### ABSTRACT

Mucins play critical roles and attractive targets for anticancer therapies. It is desirable to develop new types of small-molecule inhibitors of Mucins. In this study, the binding features of several inhibitors to mucins have been studied by molecular docking analyses. Bioactive compounds isolated from marine organisms which are very potent antioxidant, free radical scavenger have been extensively studied to explore their potential utilization in chemoprevention. The main objective of the present work is to perform a docking analysis of bioactive compounds derived from marine bacteria and fungi into the Mucin (MUC1) active site to determine the probable binding model. Docking studies of these compounds were performed using Arguslab. QSAR studies have been implemented to predict the biological properties of the bioactive compounds. The results thus implied that 14-hydroxyterezine could lead to the development of novel inhibitors against MUC1 oncoprotein. The current work has potential for application in structure based MUC1 inhibitor discovery.

**Keywords:** Anticancer, Bioactive compounds, Docking, Mucins.

### INTRODUCTION

Mucins are extensively O-glycosylated proteins that are predominantly expressed by epithelial cells. The secreted and membrane-bound mucins form a physical barrier that protects the apical borders of epithelial cells from damage induced by toxins and with the external environment<sup>1</sup>. MUC1 is translated as a single polypeptide that undergoes autoproteolysis into two subunits in the endoplasmic reticulum. The MUC1 N-terminal subunit is the mucin component with variable numbers of 20 amino acid tandem repeats that are modified by o-glycosylation<sup>2</sup>. In border of the normal epithelial cell, MUC1-N extends beyond the glycocalyx through a noncovalent complement with transmembrane MUC1 COOH-terminal subunit<sup>3</sup>. However, with transformation and loss of polarity, MUC1 is overexpressed throughout the entire cell membrane of carcinoma cells, allowing MUC1 to interact with receptors that are normally restricted to the lateral and basal border<sup>4</sup>.

Over expression of MUC1 is sufficient for the induction of anchorage independent growth and tumorigenicity. Over expression of MUC1 also confers resistance to stress-induced cell death, conferred by exposure to certain genotoxic anticancer agents<sup>5</sup>. MUC1 over expression is conferred, at least in part, by regulation of MUC1 mRNA levels at the transcriptional level. MUC1 interacts with ER and certain other transcription factors, and thereby contributes to the regulation of gene expression<sup>6</sup>. High expression of MUC1 is closely associated with cancer progression and metastasis, leading to poor prognosis<sup>7</sup>. Besides mucin's functioning in the mechanical and chemical protection of cells, it can also mediate signal transduction through out the  $\beta$ -catenin and MAP kinase pathways leading in some instances to more aggressive tumor behaviour<sup>8</sup>.

Natural compounds isolated from marine organisms have been found to be a very rich source of bioactive molecules. Reported biological effects of these compounds include anti-tumor, anti-inflammatory and anti-viral activities as well as immunomodulatory and analgesic properties<sup>9</sup>. Since the late 1980s, more than 5000 natural products have been discovered from marine microorganisms. More than 10000 had biological activity among them, 8000 had antibiotic and antitumor activities<sup>10</sup>.

Indeed, many candidates have proceeded to clinical trials, and some of them are now in clinical use<sup>11</sup>. A number of marine drugs have been developed in recent years. Marine natural products offer

challenging targets to synthetic chemists due to their complicated structure that exhibits remarkable biological activities<sup>12</sup>.

### MATERIALS AND METHODS

#### Preparation of protein structure

The 3D co-ordinates of the crystal structure of MUC1 (PDB ID: 2ACM) was downloaded from the Protein Data Bank (<http://www.rcsb.org/pdb/home.do>)<sup>13</sup>. MUC1 (chains A) were selected for the docking simulations. Before docking all water molecules are removed from protein file 2ACM. After removing the water molecules H-atom were added to protein for correct ionization and tautomeric states of amino acid residues such as Asp, Ser, Glu, Arg and His.

#### Preparation of ligand structures

A dataset of 15 bioactive compounds from marine bacteria and fungi were used as reference molecules<sup>14</sup>. The ChemSketch, chemically intelligent drawing interface freeware developed by Advanced Chemistry Development, Inc., (<http://www.acdlabs.com/download>). This can be used for generating chemical structure of bioactive compounds, 2D structure cleaning, 3D optimization and viewing, InChI generation and conversion, drawing of polymers, organometallics and Markush structures<sup>15</sup>.

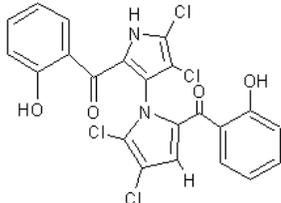
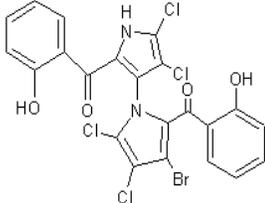
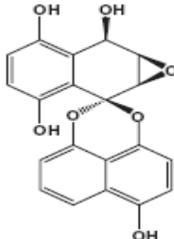
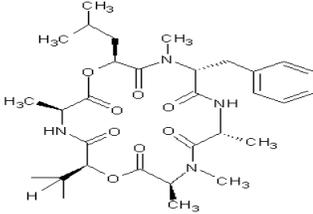
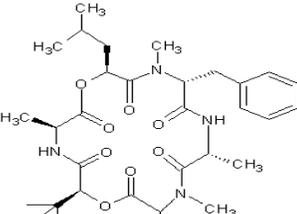
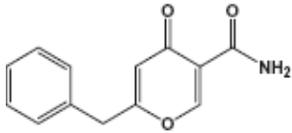
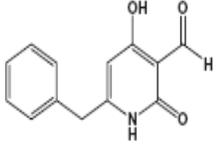
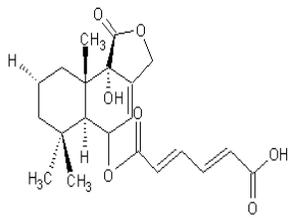
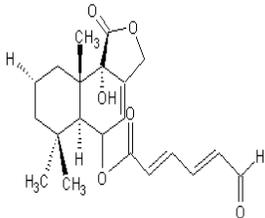
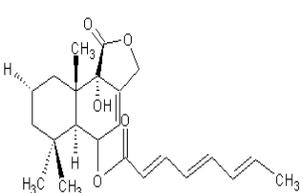
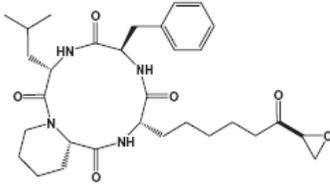
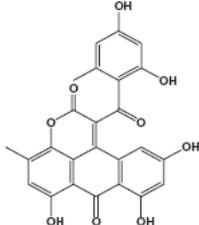
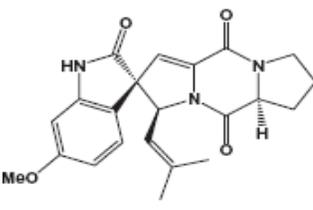
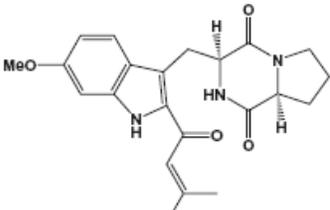
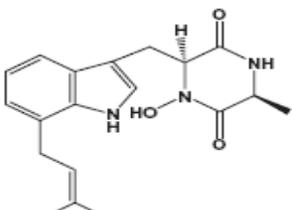
#### Determination of active site

Q site finder server was analyzed for the identification of the most potential active site where the ligand can bind and interact with the target protein MUC1. It uses the interaction energy between the protein and a simple Vander waals probe to locate energetically favourable binding sites. Energetically favourable probe sites are clustered according to their spatial proximity and clusters are then ranked according to the sum of interaction energies for sites within each cluster<sup>16</sup>.

#### Docking and descriptive analysis

The docking scores of the prepared ligands with MUC1 receptor were determined using ArgusLab 4.0.1 (Mark A Thompson, Planaria Software LLC, Seattle, WA, USA, <http://www.arguslab.com>) to identify active potential of drug<sup>17</sup>. The molecular descriptors such as Molecular weight, hydrogen donor, acceptors, LogP, Total Polar Surface Area (TSPA), were obtained using FAF drugs-ADME/Tox filtering<sup>18</sup>.

Table 1: Chemical structure of bioactive compounds isolated from bacteria and fungi

<p><b>Marinopyrroles A</b></p> 	<p><b>Marinopyrroles B</b></p> 	<p><b>Ascochyatin</b></p> 
<p><b>Spicellamide A</b></p> 	<p><b>Spicellamide B</b></p> 	<p><b>Carbonarones A</b></p> 
<p><b>Carbonarones B</b></p> 	<p><b>Drimane sesquiterpenoids A</b></p> 	<p><b>Drimane sesquiterpenoids B</b></p> 
<p><b>Drimane sesquiterpenoids C</b></p> 	<p><b>Cyclic tetrapeptide</b></p> 	<p><b>Aspergillide</b></p> 
<p><b>6-methoxySpirotryprostatin</b></p> 	<p><b>18-oxotryprostatin</b></p> 	<p><b>14-hydroxyterezine D</b></p> 

## RESULTS

Analyses of docking interaction with MUC1 crystal structure (2ACM) were performed to identify the original binding mode between the ligands and MUC1 oncoprotein. The structures of ligands used for docking were constructed using ChemSketch were shown in Table 1.

## Potential binding site in MUC 1

Q site finder server was used for the identification of the most potential active site where the ligand can bind and interact with the target protein MUC 1. Residues PHE 1043, LEU 1045, SER 1046, PHE 1047, HIS 1048, ILE 1049, SER 1050, LEU 1052, GLN 1053, PHE 1054, ASN 1055, LEU 1058, LEU 1069, GLN 1070, ILE 1073, SER 1074, PHE 1077, PHE 1086, GLY 1088, LEU 1089, SER 1090,

ASN 1091, ILE 1092, LYS 1093, PHE 1094, ARG 1095, PRO 1096 and GLY 1097 were predicted as active site in the target protein MUC1.

#### Docking analysis

The docking scores were obtained from the analogues with MUC1 as the receptor. The output of all the ligands were given by energy values in kcal/mol was shown in Table 2. The docking scores were highest for 14-hydroxyterezine with docking score -12.60 kcal/mol followed by cyclic tetrapeptide with -12.05 kcal/mol, 18-oxotryprostatin with -10.36 kcal/mol, carbonaroner B with -10.32 kcal/mol, carbonaroner A with -10.02 kcal/mol. Crucial interaction between the ligands (pink colour) and target protein

MUC1 (blue colour) were shown from figure 1 to figure 4. The MUC1 residues interacting with the ligands were shown in stick model.

Moreover, based on the results of molecular docking, the four bioactive compounds showed much better binding energy with MUC1 receptor, and is therefore considered as the most active compounds. Known anticancer agent Lapatinib showed binding energy in the range of -11.54 kcal/mol demonstrating efficacy of the bioactive compounds in the treatment of cancer with little or no cytotoxicity. The interactions were stronger (energetically lesser) for all the ligands which are used for docking simulation.

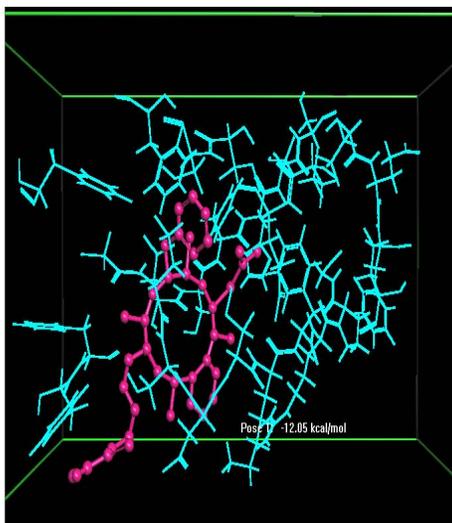


Fig. 1: 14-Oxotryprostatine with MUC1

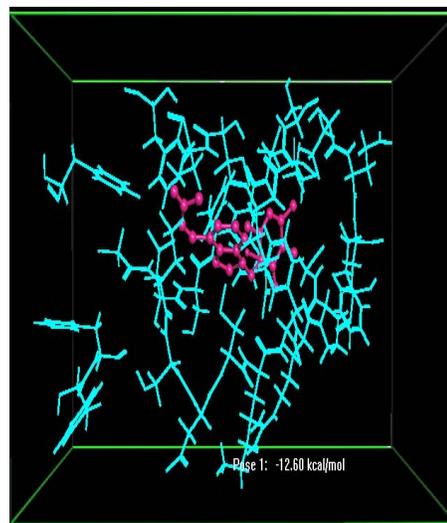


Fig. 2: Cyclic tetrapeptide with MUC1

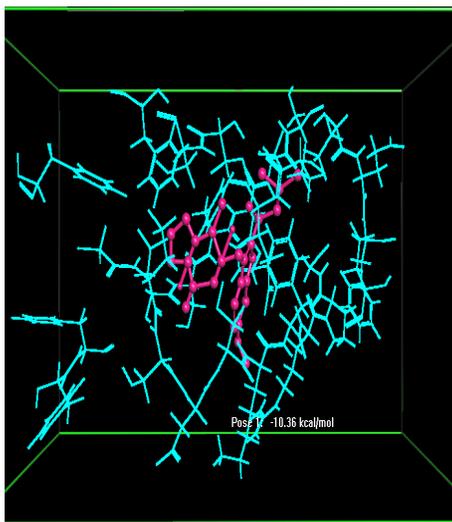


Fig. 3: 18-Oxotryprostatin with MUC1

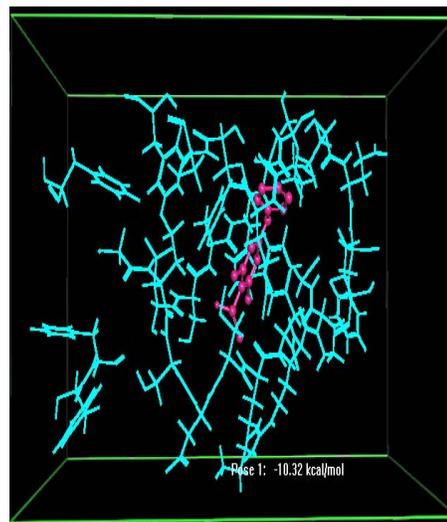


Fig. 4: Carbonarones B with MUC1

#### Validation of ligands

QSAR and toxicity studies were performed to obtain the molecular properties of ligands was shown in table 2. QSAR studies of the ligand reveals that 11 ligands out of 15 were passed, and acted as drug molecule by their adherence to the properties such as

Absorption, Distribution, Metabolism, and Excretion (ADME) as per the FAF drugs tool. From the inspection of ligand molecules, it was observed that the ligand molecule 14-hydroxyterezine showed better molecular properties than the other molecules. Thus it could lead to the development of drug discovery process.

Table 2: Docking score and molecular properties of the bioactive compounds

Compound	Energy value (kcal/mol)	MW	HD (OH+NH)	HA (O+N)	LogP	TPSA
Drug (Lapatinib)	-11.54	580.0	2	8	5.57	114.73
Marinopyrroles A	-10.93	509.1	2	6	6.38	92.42
Marinopyrroles B	-11.12	588.0	2	6	7.17	92.42
Ascochyatin	-5.59	338.2	4	6	2.77	99.38
Spicellamide A	-7.39	502.3	2	10	2.18	125.1
Spicellamide B	-7.43	590.3	3	13	1.28	171.6
Carbonarones A	-10.02	229.1	1	4	1.88	69.39
Carbonarones B	-10.32	229.1	2	4	1.98	66.40
Drimane sesquiterpenoids A	-3.47	366.2	1	6	3.34	89.90
Drimane sesquiterpenoids B	-2.95	378.2	1	6	3.54	89.90
Drimane sesquiterpenoids C	-5.45	392.2	1	5	6.14	72.82
Cyclic tetrapeptide	-12.05	568.4	3	10	2.66	137.2
Aspergiolide	-8.84	407.2	4	8	-0.04	135.5
66-methoxyspirotryprostatin	-8.62	393.3	1	7	1.75	78.95
18-Oxotryprostatin	-10.36	394.3	1	7	1.55	88.60
<b>14-hydroxyterezine D</b>	<b>-12.60</b>	<b>343.2</b>	<b>3</b>	<b>6</b>	<b>0.87</b>	<b>81.67</b>

## DISCUSSION

The identification of oncogenes involved in the initiation and progression of tumors has generated targets for the development of new anticancer drugs<sup>19</sup>. The field of molecular docking has emerged during the last three decades and now is becoming an integral aspect in drug discovery and development area<sup>20</sup>. In the present study docking simulation was performed between MUC1 oncoprotein with fifteen marine derived bioactive compounds using ArgusLab 4.0.1 to find out the binding orientation and binding affinities of the ligands.

Identifying the location of ligand binding sites on a protein is of fundamental importance for a range of applications including molecular docking, *de novo* drug design and structural identification and comparison of functional sites<sup>21</sup>. In our study, Q-site finder was used to predict the active site of the target protein MUC 1 with a higher average precision.

Docking has become one of the reputable methods for drug discovery and enhancing the efficiency in lead optimization<sup>22</sup>. One of the most critical steps involved in discovering a new drug molecule is to understand its ADME properties<sup>23</sup>. QSAR is a powerful lead-compound optimization technique, which quantitatively relates variations in biological activity to changes in molecular properties<sup>24</sup>.

TPSA descriptor is described as a polar part of the molecule associated with the oxygen, nitrogen, sulfur atoms and also hydrogen connected to these heteroatom. It has been reported to be one of the best predictivity descriptors to build a QSAR model for the drugs Medicinal agents with TPSA varying in the range from 61 Å to 140 Å and with LogP less than 5 are proposed to be well absorbed<sup>25</sup>. In the present study also, FAF Drugs predicted the molecular properties of the ligands with least TPSA, LogP less than 5, with a reasonable hydrogen bond interaction.

Lapatinib is an active and well-tolerated oral dual tyrosine kinase inhibitor for the treatment of breast cancer<sup>26</sup> has been used as a standard drug. All the docking score of the bioactive compounds and QSAR results were compared with the synthetic drug.

According to this study, the strong possible alkaloid 14-hydroxyterezine D were identified as a better inhibitor against MUC1 and follow most of the ADME properties, leading to a good drug candidate for anti-cancer activity. Thus with the least binding energy, least TPSA, with a reasonable hydrogen bond interaction and with no toxicity risk at all ensures this ligands is a better source for inhibiting the MUC1 oncoprotein and can be useful for further drug development studies.

## CONCLUSION

Flexible docking of ligand to receptor molecules is an emerging approach and is extensively used to reduce cost and time in drug discovery. The approach utilized in this study is successful in finding potent inhibitors against MUC1 oncoprotein. The compound 14-

hydroxyterezine show lowest docked energy and hydrogen bonding stabilizes the interactions. Thus the result demonstrates that 14-hydroxyterezine is a potential inhibitor for MUC1. The molecular weight, hydrogen donor, acceptor, TPSA, LogP are generated using FAF drugs ADME/Tox filtering to discover the essential features of ligands, which are invaluable to examine the potential lead of MUC1. Further the work can be evaluated experimentally to study the receptor-ligand interactions, which would help in designing of compounds based on virtual screening.

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