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In silico molecular docking studies of squalene against gastric cancer related proteins: Prologue studies

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ABSTRACT

In the present study, squalene a terpenoid compound was isolated from *R. Mucronata* methanolic leaves extract. The isolated and identified squalene compound was fixed with molecular docking against gastric cancer proteins such as HpFabZ, bcl2 and VEGF. Molecular docking studies were performed using autodock 4.0 sever. The docking score were noticed for isolated squalene compound and compared with some of the natural inhibitors like apigenin, amoxillin and luteolin and standard anti gastric cancer drugs like. Among these, squalene exhibited higher values of (-13.579 against HpFabZ. But in the case of Bcl2 and VEGF proteins, squalene doesn't show better docking score compared to the standard antigastric cancer drugs. In this present study, can be useful to design and develop novel compound with better inhibitory activity against a type of cancer. This potential agent would be a promising candidate and can be further validated in wet lab to study its proper function.

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INTRODUCTION

Cancer, a malignant neoplasm, indicates a term for a large group of dissimilar diseases, all involving unregulated cell growth. Cancer cells divide and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body through the lymphatic system or bloodstream. Thus, the cancerous cells breaks from the tumor and enters into the bloodstream and in turn spreads the disease from place of origin to other organs by metastasis process. Even though the cancer cells get metastasized and affected other areas of the body, the disease is still referred to the organ of origination. In worldwide, cancer is one of the leading cause of death and likely continues with an estimate of 12 million deaths in 2030 (Jemal *et al.,* 2010). It is also identified that the Vascular Endothelial Growth Factors (VEGF) superfamily critically influences angiogenesis in solid tumours. Various published data recommended that distant metastases are more likely occurs in the presence of tumour angiogenesis-related factors (Yancopoulos *et al.,* 2000; Carmeliet and Jain 2000). Interestingly it has been also demonstrated that the angiogenic phenotype may differ between intestinal-type and diffuse-type gastric cancer. In different investigations, it seems that intestinal-type tumours are found to be more biologically dependent on angiogenesis rather than diffuse-type tumours (Takahashi *et al.,* 1996; Kitadai 2010).

BCl-2 proteins a product of bcl-2 gene is an anti-apoptotic protein plays important roles in regulating cell survival and apoptosis in response to a

wide variety of stimuli (Yong *et al.,* 1997; Jia *et al.,* 1999). Enforced bcl-2 expression delays apoptosis in cell lines (Rosse *et al.,* 1998).

Gastric cancer is the fourth most common cancer, associated with alterations in oxidant and antioxidant status, increased cell proliferation and angiogenesis, and dysregulation of apoptosis (Arivazhagan *et al.,* 1997; Crew and Neugut 2006). It is the second leading cause of cancer death worldwide. Adenocarcinoma is the most common form of gastric cancer (Lauren 1965; Lewin and Appelman 1995).

The major goal of molecular docking is to predict the prevalent binding interaction of a ligand with a protein of target molecule. Nowadays, in modern drug design molecular docking are routinely used to understand drug-receptor interaction. From the literature, it is known that computational techniques strongly support and helps to design novel inhibitors by enlightening the mechanism of drug –receptor interactions (Srivastava, 2008).

Squalene, a terpenoid compound acts as a precursor for steroids with biological activities against some of the cancers such as colon, lung and skin. Squalene also act as a potent cytoprotective agent against chemotherapeutic toxicities (Senthil Kumar *et al.,* 2006; Das *et al.,* 2008). According to Van Duuren and Goldschmidt (1976), benzo[*a*]pyrene (B[*a*]P)-induced skin carcinogenicity was inhibited on topical application of squalene to mouse. Similarly, squalene suppressed the tumor inducing effect of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) on 7,12- dimethylbenz[*a*]anthracene (DMBA) -initiated mouse skin a proposed by Murakoshi *et al.,* (1992). Several Japanese investigators (Ohkima *et al.,* 1983; Yamaguchi 1985; Ikikawa *et al.,* 1986) has also described the Anti-tumorigenic activity of squalene. Hence, the present study was designed to evaluate the interaction of squalene as ligand molecule with the target protein associated with gastric cancer.

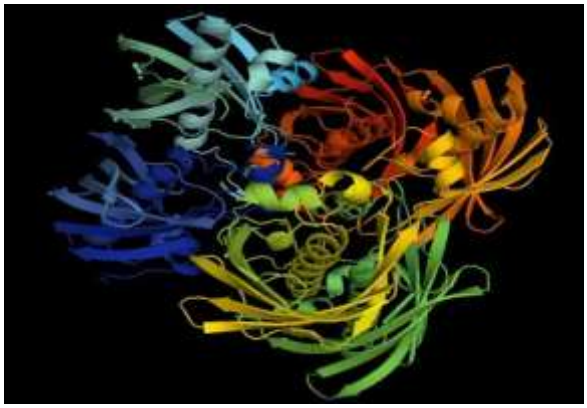

Figure 1: 3D structure of β-hydroxyacyl-acyl carrier protein dehydratase from *Helicobacter pylori*

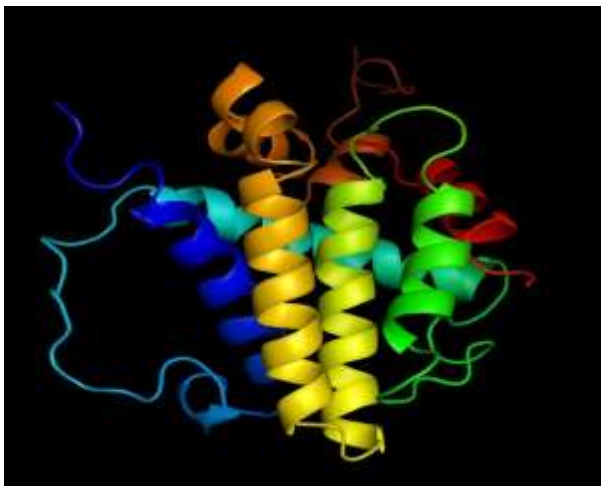

Figure 2: 3D structure of Bcl2 (B-cell lymphoma 2)

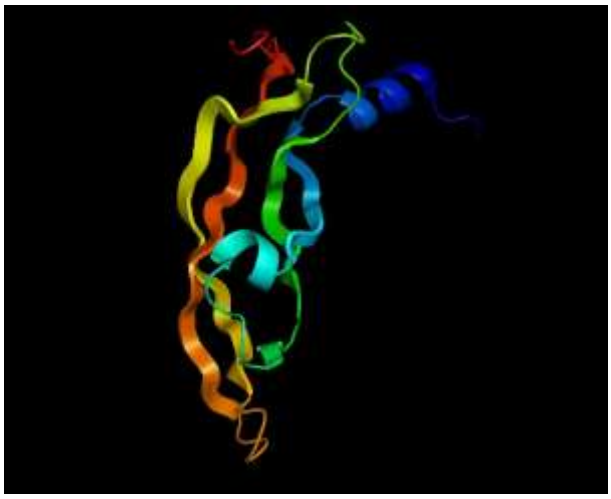

Figure 3: 3D structure of VEGF- (Vascular endothelial growth factor (VEGF)

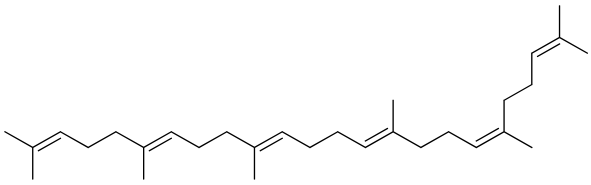

Figure 4: -2D structure of Squalene

MATERIALS AND METHODS

Preparation of Crude extracts

The air-dried leaves of *R.mucronata* plant (1 Kg) were extracted with various solvents like, ethanol, methanol and chloroform in Soxhlet apparatus for 24 h using 500 - 800 mL of solvent. The extracts were concentrated by rotary evaporator and stored in refrigerator for future use. Various extraction methods were summarized in flow chart (Fig-11)

Thin Layer Chromatography

Thin Layer Chromatography was carried out for the crude ethyl acetate and n-hexane to check the

compounds present in crude extract. The mobile phase used was methanol, ethyl acetate and hexane extract at 8:2 ratio. The plates were air dried at room temperature, viewed under UV light and upon spraying with sprayed DPPH• reagent. The crude extract initially shows two spots with various R_f values. It confirms that methanol extract contains two different products (Gu, *et al.,* 2009).

Column chromatography

Alumina (aluminum oxide) and silica gel (silicon dioxide) were the most common solid adsorbents. Acidic silica gel was used as a stationary phase. Mobile phase is a solvent, while stationary phase is a finely divided solid surface. At varying degrees, the components of the mixture get absorbed to the stationary phase. The sample (*R.mucronata* plant extract), was dissolved in a minimum amount of solvent and added to the top of the column. The samples were added carefully to the top of the stationary phase so that not to disrupt the silica top layer. Stopcock was opened and the solvent was collected at the bottom of the column until the level of the solvent is just 1cm above the level of the silica bed. Fractions of a standard volume were collected. The volume of solvent collected for each fraction should correspond to the amount of material being separated (*i.e.*) larger fractions for larger quantities. The various fractions were identified by TLC, using ethyl acetate and n-hexane as a solvent at different ratio (8:2). Once the desired fractions (squalene fraction) are identified, the solvent was removed by rotary evaporation and the compounds were isolated (Rajkumar *et al.,* 2012).

Molecular docking

The target receptor proteins 3D structure of β-hydroxyacyl-acyl carrier protein dehydratase (m) enzyme (HpFabZ) (PDB ID: 3CF9) from *Helicobacter Pylori*, Bcl2 (PDB ID: 2O2F) and VEGF (PDB ID: 1FLT) with the resolution of 2.6Å, not defined and 1.70Å respectively were retrieved from the Protein Data Bank (http://www.rcsb.org/pdb/). The chemical structure of natural inhibitors namely apigenin, amoxycillin, luteolin, omeprazole, quercetin, sakuranetin, 4-(4-Benzyl-4-Methoxypiperidin-1-Yl)-N-[(4-{[1,1-Dimethyl-2-(Phenylthio)Ethyl]Amino}-3 Nitrophenyl)Sulfonyl]Benzamide and pazopanib as standard and the identified compound (squalene) were drawn from SMILES notation (Simplified Molecular Input Line Entry Specification) using the Chemsketch Software (http://www.acdlabs.com/).The active site was predicted by using RCSB ligand explorer software. The list of amino acid residues selected for docking are listed in Table.4**.** To explore the protein ligand interactions docking analysis was carried out using Argus Lab 4.0.1 software. Hydrogen was added to both protein and ligand and was geometrically optimized prior to docking. Flexible docking of all target proteins used for the computational study was carryout on the active site of HpFabZ, Bcl2 and VEGF enzyme identified by RCSB ligand explorer software. Docking study was performed by using "GADock" as the docking engine and Grid resolution was set at 0.40A^o. The docked results were saved as "pdb" file and binding affinity and molecular interaction between standard, test compounds and the receptor protein were visualized using PyMol Molecular Graphic System (Ver. 1.0) and Discovery Studio (Ver 3.1) software, respectively.

RESULTS

The identified compound (squalene) was screened against HpFabZ protein, Bcl2 and VEGF using molecular docking analysis. Squalene showed best docking score of **-**13.579 Kcal/mol than the previously reported natural inhibitors ranging from -8.7939 to -9.0713 Kcal/mol against HpFabZ protein (Table 1 and Fig 9). Based on docking energy and good interaction with the active site residues the docked ligand molecules were selected. Lower the docking score will increase the binding efficiency. The docking result revealed that the squalene has the high specificity and efficiency towards the target HpFabZ protein (Fig-9). But in the case of Bcl2 and VEGF proteins, squalene doesn't show better docking score compared to the standards (Table 2 and Table 3). Fig-5 and 7 shows the molecular interaction of the ligand and protein as visualized in Discovery studio molecular visualization tools.

Table 1: Molecular docking results of squalene and natural inhibitors with the target protein HpFabZ

Compound	Docking Score
Squalene	-13.579
Apigenin	-8.7939
Amoxicillin	-8.2559
Luteolin	-9.0276
Omeprazole	-7.5552
Quercetin	-9.0443
Sakuranetin	-9.07139

Table 2: Molecular docking results of the squalene and natural inhibitors with the target protein Bcl2

Compound	Docking Score
Squalene	-7.871
4-(4-benzyl-4-methoxypiperidin-1-yl)-n-[(4- {[1,1-dimethyl-2-(phenylthio)ethyl]amino}- 3-nitrophenyl)sulfonyl]benzamide (Standard)	-8.820

Table 3: Molecular docking results of the squalene and natural inhibitor with the target protein VEGF

Compound	Docking Score
Squalene	-4.352
Pazopanib (Standard)	-5.273

Table 4: List of Active Amino acids selected in all of the target proteins

Protein	Amino acid residues in the active site of proteins
HpFabZ	Glu159, Ile98, Lys62, Tyr100, Phe52, Pro112, Ile111, Arg110, Phe109
Bcl2	Glu133, Leu134, Val130, Phe147, Met112, Phe150, Glu149, Phe195, Trp141, Phe109, Val145, Arg143, Asp108, Tyr105, Arg104, Asp100, Gln96, Ala97, Phe195
VEGF	Cys-57, Gly-59, Leu-32, Glu-30, Thr-31, Arg-56, Ile-29

The present findings clearly demonstrated the therapeutic importance of squalene based on the molecular docking analysis. However, further *in vitro* and *in vivo* experiments are required to determine the efficacy of squalene for inhibition of HpFabZ protein, Bcl2 and VEGF leading to control of gastric cancer.

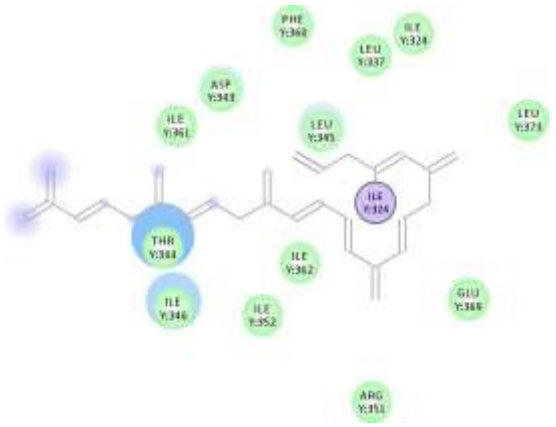

Figure 5: Molecular interaction of VEGF protein and squalene

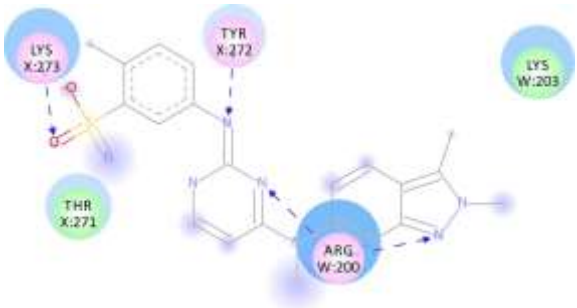

Figure 6: Molecular interaction of VEGF protein and squalene

DISCUSSION

Squalene is a triterpene compound and acts as an intermediate in the biosynthesis of cholesterol. It was named so because of its occurrence in large quantities in shark liver oil, which is considered to be its richest source. However, squalene is found to be widely distributed in nature, with a reasonable amount found in olive oil, palm oil, wheat-germ oil, amaranth oil, and rice bran oil (Bargossi *et al.,* 1994). Triterpenoids belongs to economically and medicinally important natural products with widespread usage for their attractive pharmacological medicinal activities (Meng *et al.,* 2011). In the present investigation, squalene was isolated from the mangrove species of *R.mucronata* and docked against some gastric cancer proteins such as HpFabZ, Bcl2 and VEGF. Nematollahi et al 2012 reported Autodock ranged from –3.95 kcal·mol−1 to – 5.47 kcal·mol−1 in HpFanZ. In the present study indicated moderated result at range of docking score 13.58. Kcal/mol.

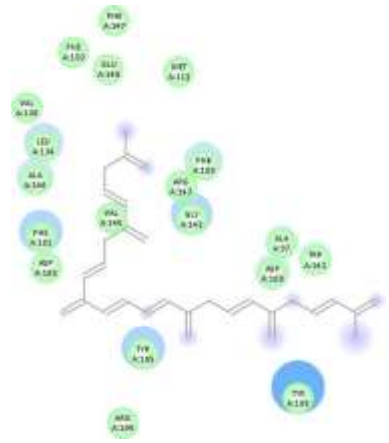

Figure 7: Molecular interaction of target Bcl2 protein and squalene

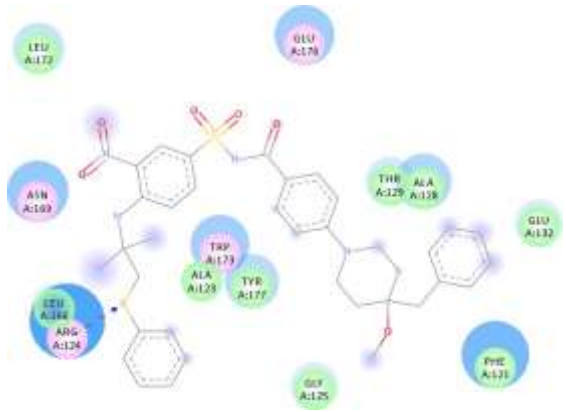

Figure 8: Molecular interaction of target Bcl2 protein and squalene

In the present study, squalene showed good interaction with HpFabZ protein. The interactions between protein and ligand increase the binding efficiency. The lower energy value indicates the higher binding efficiency. In this result, squalene exhibited the best docking score (13.58 Kcal/mol), where the other ligands showed low score. The bonding interaction between the residues of the

active site of HpFabZ protein and squalene was given in Fig.9.

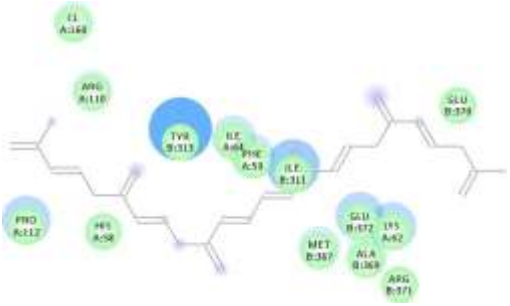

Figure 9: Molecular interaction of HpFabZ protein and squalene

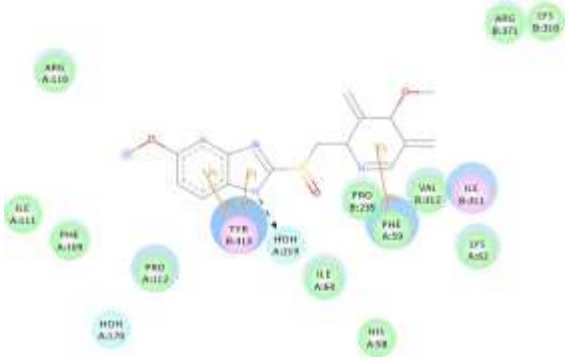

Figure 10: Molecular interaction of HpFabZ protein and squalene

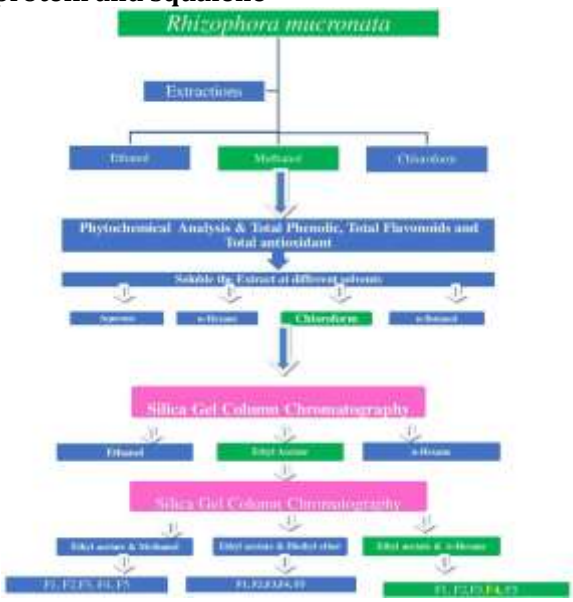

Figure 11: Flow chart of isolation methods for active compound from *R. mucronata*

Now computational methods have aroused as a dominant strategy to predict human pharmacokinetic properties (PK) which lead the candidate to reduce the failures in late stages of drug development. A drug proposed for use in humans should have a perfect balance of value and protection, as well as good PK properties. Therefore, further absorption and distribution along with QSAR studies, should be considered as important parameters to choose compounds as drug candidates (Tiago et al.,2007).

Helicobacter pylori infection is directly concerned with the peptic ulcer and gastric cancer. Hence, nowadays treatment and control of this infection is considered as the first line of therapy for patients with peptic ulcer disease (Kwon *et al.,* 2001). Different medical procedures have been administered for the treatment of *H. pylori* with numerous combinations of medicines including antibiotics, bismuth subcitrate, proton pump inhibitors and H2-blockers. (Hentschel *et al.,* 1993).

Similar to present results, Nematollahi *et al.,* 2012 also reported a good interaction of luteolin derivatives with HpFabZ to inhibit the Ki (inhibition constant). Thus, luteolin derivative can be a potent HpFabZ inhibitors as like squalene and act as novel anti-Helicobacter agents. The proto-oncogene bcl-2 is initially identified in human follicular and diffuse B-cell lymphomas characterized by the reciprocal translocations (Korsmeyer, 1992). The proto-oncogene bcl-2 produces a 26-kDa protein blocks apoptosis and is localized in the mitochondria, endoplasmic reticulum and nuclear envelope. Recently, bcl-2 gene gets expressed on several normal and malignant tissues other than hematolymphoid cells including the lung, breast, prostate, stomach, small bowel and colon (Higashiyama *et al.,* 1995; Lauwers *et al.,* 1995).

In the present study, bcl2 was well expressed in gastric cancer cell. The squalene compound had a significantly decreased capacity to block the bcl-2 and VEGF when compare to standard. From the literature studies, it is reported that bcl-2 protein expression was mainly associated with morphologic phenotype and different grades of gastric carcinomas differentiation. The difference in the bcl-2 protein expression in the intestinal and diffuse types demonstrated that aberrant bcl-2 protein expression was preferentially associated with development of intestinal-type gastric carcinoma, indicating again the different biologic mechanisms involved in the development of these two histologic subtypes.

Those cells that stimulates vasculogenesis and angiogenesis produces vascular endothelial growth factor (VEGF) as a signal protein. If the blood circulation is inadequate VEGF restores the oxygen supply to tissues as a part of the system. (Shin *et al.,* 2010). VEGF-C participates in lymphangiogenesis during embryogenesis and in the maintenance of differentiated lymphatic endothelium in adults (Lymboussaki *et al.,* 1999). Moreover, VEGF-C is expressed by a significant fraction of human tumors including those of breast, cervix, colon, lung, prostate (Siegfried *et al.,* 2003), and stomach

(Shida *et al.,* 2005). Thus, VEGF-C represents a potential anti-cancer target.

CONCLUSION

The interaction between protein and ligand plays an important role in structural based designing. In the present findings, the receptor HpFabZ protein was taken and identified a bioactive anticancer phytochemical when the gastric cancer genes (*H.pylori*) were docked with squalene. -13.579k cal/mol energy value was obtained using ArgusLab. When Bcl and VEGF proteins were docked against the same compound (squalene) was unable to get better docking score. From this we conclude that from squalene is better anticancer phytochemical than currently presented natural inhibiters.

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