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Inhibition of FGFR2 signal transduction in *Acne Vulgaris* using Bioactive Flavonoids: An *Insilico* approach

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Abstract

Acne vulgaris is a common, chronic inflammatory disease, which was androgen-induced sebum production, altered keratinisation, inflammation and bacterial colonization of the sebaceous follicle that primarily affects the face, chest, and back by *Propionibacterium acnes*. The study aims at finding leads from phytochemicals to target the protein involved in the growth factor of acne vulgaris. The fibroblast growth factor receptor 2 (FGFR2) is a receptor for fibroblast growth factor which involved in the signaling of skin appendage formation, pilosebaceous follicle homeostasis, comedogenesis, sebaceous gland proliferation and lipogenesis. The FGFR2-gain-of-function mutations in Apert syndrome and unilateral acrofacial nevus are most helpful model diseases pointing the way to androgen-dependent dermal-epithelial FGFR2-signaling in acne. Docking analysis was carried out for the above target with the selected bioactive flavonoids and the efficiency was compared with the synthetic drug 'PD 1373074' using Molegro docking software. The flavonoid compound Hesperidin showed higher docking scores with the FGFR2 receptor

Keywords: fibroblast growth factor receptor 2, Hesperidin, Molegro, flavonoids

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1. Introduction

Flavonoids are the polyphenolic compounds that contain most abundant dietary natural products. The flavonoids are present in fruits, vegetables, beverages and various dietary supplements (A. Scalbert *et al.*, 2000). Extensive investigation shows that the potential of flavonoids to prevent various diseases, including cardiovascular disease, viral infections, diabetes, neurological conditions and inflammatory disorders (P. Ratheet *et al.*, 2009). Flavonoids are the key products which provide important link between diet and prevention of chronic disorder such as Acne Vulgaris (P Lampila *et al.*, 2009). Acne vulgaris is a chronic inflammatory skin disease, which mostly affecting the sebaceous follicles (AM Kligman *et al.*, 1958). In United States, Acne affects between 40 million and 50 million individuals (GM White, 1998). In most cases, Acne is finding between 14-17 years old in girls and 16-19 years old in boys (AE Rivera, 2008). Acne not only affects the adolescence, it is also present in children and adults. The pathogenesis of acne is abnormal follicular differentiation, enhanced sebaceous gland activity, bacterial



hypercolonization, inflammation, also the immunological host was induced by proinflammatory cytokine IL-1 (G Plewig, 1974).

The sebaceous gland function was directly related to acne and the secreted sebum normally contains mixture of lipids, squalene, wax and cholesterol both in free and ester forms and triglycerides that naturally provide a skin barrier function (GS Pilgram *et. al.*, 2001). The keratinocyte proliferation, sebum production and the size of sebocytes are depends on the Androgens (PE Pochiet. *al.*, 1969). The resulting skin condition ie., enhanced sebum activity is prone to the anaerobic growth of main causative organism in acne *Propionibacterium acnes*. In addition *Staphylococcus epidermidis* and *Pityrosporumovaleare* also present in acne lesions (NH Shehadeh *et. al.*, 1963). The proliferation of these micro-organisms mainly *P.acnes* leads to inflammatory lesions and severe acne.

Acne in Apert syndrome is associated with increased fibroblast growth factor receptor-2 (FGFR2)-signaling and has been suspected to be of pathophysiological importance in acne vulgaris. The protein structure of FGFRs is similar with a cytoplasmic tyrosine kinase with an ATP-binding domain, three immunoglobulin like domains (D1-D3) in the extracellular region and a single member spanning segment (VP Eswarakumar *et. al.*, 2005). The signal via one or more receptor tyrosine kinases encoded by fur FGF receptor genes (FGFR 1-4) (DMOrnitz *et. al.*, 2001). Fibroblast Growth Factor (FGF) signaling is required for epidermal growth, skin barrier formation and hair-cycle activation (V Greco *et. al.*, 2009).

There are 22 FGF family members in mammals. Ser252Trp or Pro253Arg, predicted to lie in the linker region between D2 and D3 immunoglobulin like regions of FGFR2 ligand binding domain (AOM Wilkie *et. al.*, 1995). The mutations increase the FGF-binding affinity and are thus gain of mutations. Both mutations have been found to introduce additional interactions between FGFR2 and FGF2, thereby augmenting FGF2-FGFR2 affinity (OA Ibrahimiet. *al.*, 2001). FGF2 is find in cytoplasm of BCA cells as wel as in tumourstroma and shown, similarity to other members of FGF2 family, to generate signal by activating FGFR2 either in autocrine or paracrine manner. Upregulation of ligands FGF2, FGF7, FGF10 and FGF22 and their activation of FGFR2 signaling, has been shown to initiate keratinocyte proliferation in diseased states such as acne, psoriasis and wound healing (S Braun *et. al.*, 2004). The standard drug used to inhibit FGFR2 is PD173074 (A Sara Byronet. *al.*, 2013). In this study, we investigated the binding interaction of flavonoid such as hesperidin, quercetin, rutin, naringenin and naringin using ligand based approach to propose the inhibitory activity of FGFR2 receptor.

2. Materials and Method

2.1 Ligand Preparation:

The structure of flavonoid compounds were retrived from Pubchem (<https://pubchem.ncbi.nlm.nih.gov/>) and Chemspider database (<http://www.chemspider.com/>). The compounds were converted into compatible format (.sdf) using Open Babel tool. The root mean square gradient value was selected less than 0.001 kcal/mol. The energy minimized structure was used for docking studies.

2.2 Protein preparation:

The three dimensional crystal structures for the target Fibroblast Growth Factor Receptor-2 (FGFR2) was retrieved from Protein Data Bank (<http://www.pdb.org/>). The PDB ID for the selected target was 1DJS. The bonds, bond orders, explicit hydrogen, charges (calculated by MVD), flexible torsion and Tripos atom types were assigned if they were missing by using 'Protein Preparation' module of Molegro Virtual Docker for the protein Fibroblast Growth Factor Receptor-2.

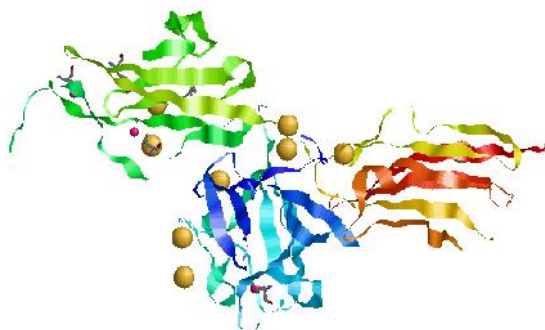


Figure 1: Crystal Structure of FGFR2

The analysis of side chain flexibility affords insights valuable to improve docking algorithms and can provide an index of amino acid side chain flexibility, potentiality useful in molecular docking. The atoms are ignored away

from the binding site by using the ignored distant atom. The bonding between hydrogen bond donor and acceptor can be checked by the hydrogen bond directionality option. The ideal bonding angle deviations is the basis for the docking score. Based on the Evolutionary Algorithms (EAs), the docking was performed by the Moldock function (Abhik Seal *et. al.*, 2011). The active sites on the Fibroblast Growth Factor Receptor-2 are predicted using Molegro Virtual Docker.

2.3. Molecular docking:

Moldock SE is the search algorithm taken. 2000 as maximum interaction, number of runs are taken. The population size was set at 50 with energy threshold of 100 also at each step least 'min' torsions/translations/rotations are tested and the one giving lower energies is chosen. The bonds flexibility of the ligands was set and the side chain flexibility of the amino acids in the binding cavity was set with a tolerance of 1.10 and strength of 0.90 for docking simulations. RMSD threshold for multiple cluster poses was set at 2.00\AA. The docking algorithm was set at a maximum iteration of 1500 with a simple evolution size of 50 and minimum of 20 runs. (Preetom Regonet. *al.*, 2014).

The reranking score function is computationally more expensive than the scoring function used during the docking simulation but it is generally better than the docking score function at determining the best pose among several poses originating from the same ligand (R Thomsen *et. al.*, 2006). Ligand efficiency is most commonly defined as the ratio of the free energy of binding over the number of heavy atoms in a molecule (C Abad-Zapatero *et. al.*, 2005). Binding affinities were calculated using Molegro data modeler and MVD used to find Ligand Efficiency 1 (LE 1) as Moldock score divided by Heavy Atoms count, ligand efficiency 2 (LE2) as a result of binding Affinity divided by Heavy Atoms count and Ligand Efficiency 3 (LE 3) as rerank Score divided by Heavy Atoms count. The scoring function used by MolDock is derived from the piecewise linear potential (PLP) scoring functions. The scoring function used by MolDock further improves these scoring functions with a new hydrogen bonding term and new charge schemes (R Thomsen *et. al.*, 2006).

3. Results and Discussion

3.1 Prediction of Binding site:

The cavities present in the protein Fibroblast Growth Factor Receptor-2 is detected based on the cavity detection algorithm in MVD tool. The volume of the cavities are listed in Table 1. In most cases, the cavities with the largest size and volume is associated with the binding site. Cavity 2 and Cavity 3 have the larger volume of 76.288\AA^3 (fig 2.1) and 51.2\AA^3 (fig 2.2) respectively. The cavity with larger size has been selected as the binding site for the protein enoyl- ACP reductase during docking with Molgro Virtual Docker (fig 3).

Table 1: Volume of cavities

S.No	Cavities	Volume of cavities (\AA^3)	Surface Area of cavities (\AA^2)
1.	Cavity 1	29.696	87.04
2.	Cavity 2	76.288	266.24
3.	Cavity 3	51.2	180.48
4.	Cavity 4	37.376	149.76
5.	Cavity 5	31.744	83.2

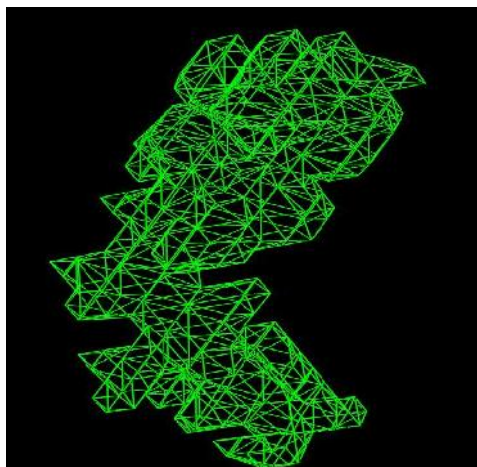


Figure 2.1: Binding site for FGFR-2: Cavity 2

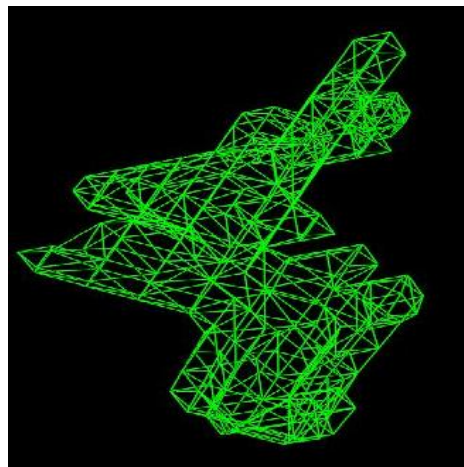


Figure 2.2. Binding site for FGFR-2: Cavity 3

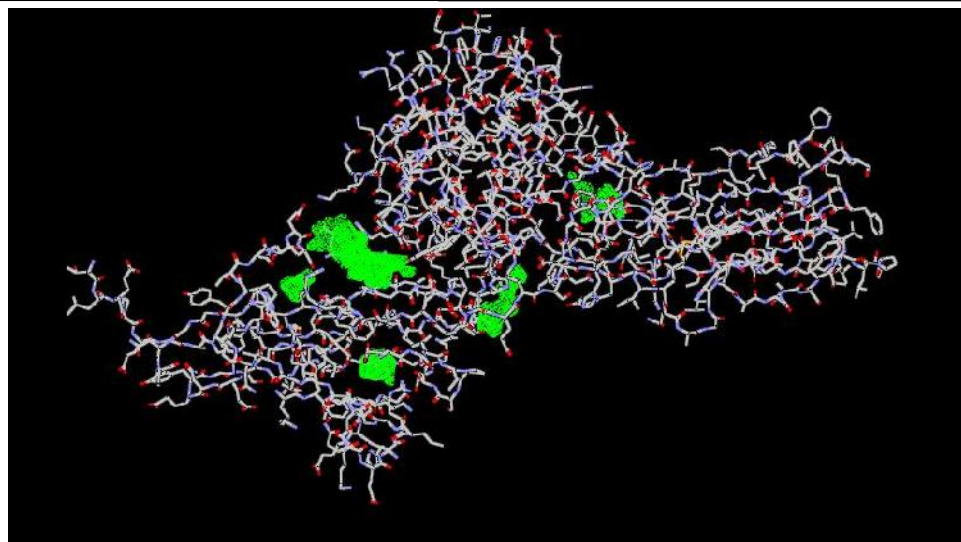


Figure 3: Cavities Detected In FGFR-2

Molecular Docking:

Docking analysis was carried out for the above target with the selected Flavonoids and compared with the synthetic drug 'PD 173074' to evaluate the efficiency as bacterial infection using Molegro docking software. The flavanone compound Hesperidin shows the higher docking scores for the FGFR2 receptor than the synthetic drug which was analyzed using Molegro Viewer. The Hesperidin is abundant in the citrus plants. The hesperidin is the natural compound and shows the higher activity than the standard drug 'PD173074'.

Table 2: Moledock score for various flavonoids

S.NO	LIGAND	MOLDOCK SCORE	RERANK SCORE	HBOND SCORE	DOCKING SCORE
1	Hesperidin	-181.803	-151.715	-13.0201	-193.721
2	Theaflavins	-143.261	-94.887	-9.67089	-152.233
3	rosinidin	-135.764	-108.625	-7.1558	-136.487
4	Rutin	-135.282	-119.621	-12.1188	-139.301
5	Naringin	-132.312	-97.6652	-12.6695	-144.422
6	Peonidin	-127.487	-104.751	-6.97345	-129.853
7	aurantinidin	-125.913	-105.953	-11.8214	-130.04
8	Isorhamnetin	-125.732	-108.488	-6.5037	-126.768
9	Proanthocyanidins	-125.572	-89.3075	-10.2865	-128.797
10	Europinidin	-125.292	-69.0709	-5.80871	-130.974
11	Hirsutidin	-125.056	-45.0929	-3.98661	-123.699
12	Delphinidin	-122.419	-101.656	-9.31466	-128.686
13	Capsaicin	-122.355	-68.6751	-3.17876	-128.085
14	Myricetin	-122.342	-106.983	-8.93081	-126.619
15	Pulchellidin	-121.922	-96.2275	-12.6072	-125.248
16	luteolin	-121.874	-104.617	-9.34132	-125.279
17	Cyanidin	-120.931	-99.4887	-9.61754	-123.513
18	Apigenin	-120.338	-102.643	-7.87195	-121.367
19	Quercetin	-120.3	-104.505	-8.96786	-124.607
20	Catechin	-120.075	-99.7765	-7.69218	-121.168
21	Capensinidin	-120.055	-99.7132	-6.93845	-121.314



22	Luteolinidin	-119.44	-99.6154	-9.64692	-123.672
23	Pelargonidin	-119.117	-97.3088	-7.04455	-121.773
24	Eriodictyol	-118.553	-103.66	-9.89008	-123.693
25	Kaempferol	-117.296	-100.178	-6.93589	-119.621
26	Malvidin	-115.908	-83.8835	-8.27285	-117.568
27	Canthaxanthin	-112.526	363.338	-0.64996	-111.033
28	Epicatechin	-107.849	-93.578	-5.63381	-115.57
29	Tangeretin	-107.512	-66.9198	-3.84936	-106.528
30	Daidzein	-107.122	-90.9486	-4.42153	-109.177
31	Genistein	-105.111	-88.5779	-3.43941	-110.133
32	Nobiletin	-102.303	-75.4009	-1.25715	-102.402
33	Gallic Acid	-73.6236	-65.4398	-10.8685	-80.665
34	Allicin	-62.9486	-51.5098	-0.14744	-64.3777

The receptor bound ligand was docked deeply in binding pocket region by strong interaction. The active compound Hesperidin binds with the receptor with a moldock score of -181.803. It binds to 9 different amino acids in the FGFR-2 (fig 4.1). And has 13 Hydrogen bond interactions. The standard Drug PD173074 have the lower moldock score of and it bind to 8 different amino acid and has 9 Hydrogen bond interaction (fig 4.2).Table 4. Indicated that the amino acids, Gly 6, Val 248 and Lys 12 are found common in most compounds.

Table 3: Amino acid residue of FGFR-2 involved in binding with bioactive components of Flavonoids

S.No	Ligand	No. of H bond	Hydrogen bond interaction with Amino acids
1	Hesperidin	13	Gly 6, Asn 7, Lys 10, Asp 140, Glu 250, Arg 251, Val 248, Asp 283
2	Theaflavins	11	Gly 6, Pro 11, Lys 12, Asp 140, Asp 247, Val 248, Ser 252, Tyr 281
3	Rosinidin	4	Gly 6, Lys 12, Asp 247, Val 248
4	Rutin	9	Tyr 3, Gly 6, Pro 11, Pro 134, Pro 136, Val 137, Val 248, Tyr 281
5	Naringin	14	Lys 10, Lys 12, Asn 7, Val 248, Glu 250, Arg 251, Asp 283
6	Peonidin	6	Gly 6, Pro 11, Lys 12, Pro 134, Asp 247, Val 248
7	aurantinidin	9	Gly 6, Asn7, Lys 10, Pro 11, Lys 12, Pro 134, Asp 247, Val 248
8	Isorhamnetin	6	Gly 6, Pro 11, Lys 12, Pro 134, Asp 247, Val 248
9	Proanthocyanidins	8	Pro 134, Asp 140, Asp 247, Val 248, Glu 250
10	PD173074	9	Tyr 94, Ser 17, Lys 128, Ala 168, Arg 178, Lys 176, Thr 174, His 164

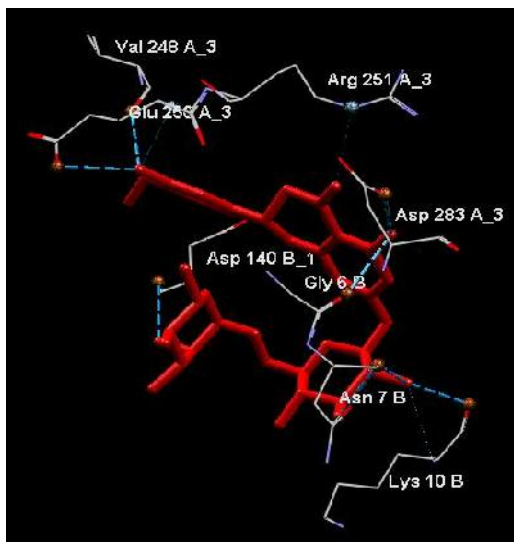


Figure 4.1: Interaction of Hesperidin

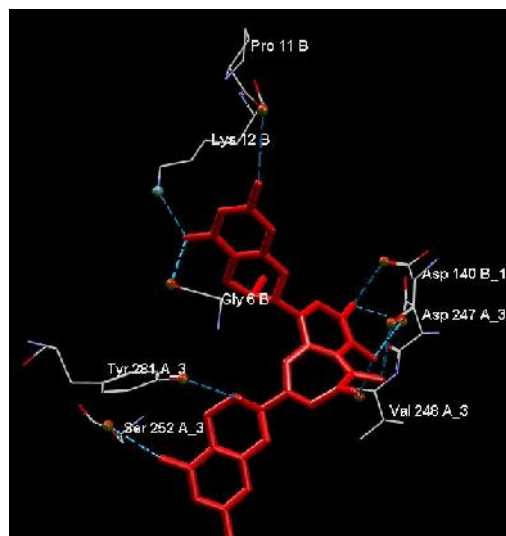


Figure 4.2: Interaction of Theaflavin

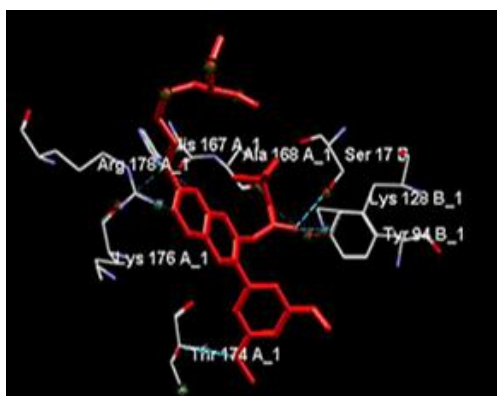


Figure 4.3: Interaction of PD173074

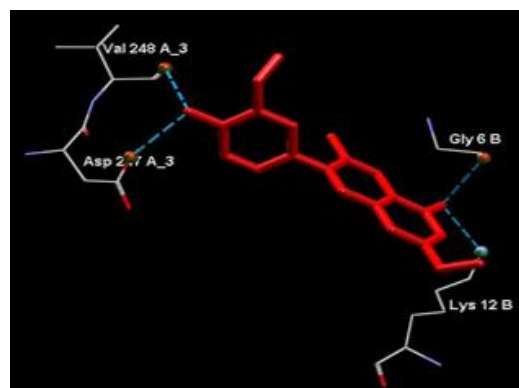


Figure 4.4: Interaction of Rosinidin

4. Conclusion

The present molecular docking studies provide insights into inhibition of FGFR-2 by various bioactive flavonoids. Docking study propose that Hesperidin has a high binding affinity for FGFR-2 protein, which is much higher than the known inhibitor PD173074. This study has led to the development of novel lead molecules for the treatment of *Acne vulgaris*. Further these lead compounds can be used for designing more effective inhibition of FGFR-2

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