Emodin as an alternative to Pazopanib aided inhibition of tumor angiogenesis: an explicit molecular docking analysis

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Abstract: ***Fibroblast growth factor receptor-2 (FGFR-2) primarily regulating mitogenesis and differentiation, is considered as a major prognostic biomarker as well as a therapeutic target in human solid tumors. Most of the therapeutic drugs have been designed to target its catalytic domain which inhibit tyrosine phosphorylation of its kinase domain and hence block its downstream signaling. In this study, we analyzed selected drug-leads from western himalayan chemiome for their ability to bind and selectively inhibit FGFR-2 activation. Structure based bioinformatics were employed to select lead compound(s) targeting catalytic domain of FGFR-2. Out of 120 compounds from selective western himalayan chemiome library, Emodin showed best binding-affinity with catalytic domain of FGFR-2 blocking ATP binding. The binding efficiency of Emodin was comparable to Pazopanib, FDA approved Tyrosine Kinase Inhibitor which is a potent and selective inhibitor of FGFR-2 currently in clinical trials. To further corroborate these findings, we evaluated binding efficiency of Emodin in 2 constitutively active mutants of FGFR-2 viz N549T and E565A. Emodin efficiently blocked ATP pocket in their respective catalytic domain comparable to Pazopanib. The results in this study thus not only project Emodin as a lead molecule to overcome FGFR-2 mediated tumor mitogenesis but also demonstrates its potential as a tyrosine kinase inhibitor to overcome tumor angiogenesis.***

***IndexTerms*— FGFR-2, Tyrosine Kinase Inhibitors, Pazopanib, Emodin, Molecular Docking Simulation.**

1. Introduction:

Fibroblast growth factor receptor 2 (FGFR2) belongs to a family of receptor tyrosine kinases (RTKs) and mediates its signaling via tyrosine phosphorylation of its intra cellular kinase domain following activation by fibroblast growth factors (FGFs), which binds to its extracellular domain. FGFRs are key regulators of numerous developmental processes including induction of mitogenesis, pattern formation, cell growth and differentiation [1-4] besides growth and progression of tumors. Indeed, expression of FGFRs particularly FGFR-2 has been demonstrated to be associated with various types of cancers viz. breast, esophageal, gastric, prostate cancers etc. Mutations in FGFR-2 have been shown to accelerate tumor growth by facilitating proliferation and survival [5] of cancer cells possibly by allowing constitutively active conformation of FGFR-2. The association of these mutations in most of the cancers in addition to role of RTKs in cancer proliferation has lead to the development of Tyrosine Kinase Inhibitors (TIKs) that inhibit or block receptor tyrosine kinases and hence limit growth of tumors.

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TKIs were created out of modern genetics- the understanding of DNA, the cell cycle, and molecular signaling pathways- and thus represent a change from general to molecular methods of cancer treatment. This allows for targeted treatment of specific cancers, which has lessened the risk of damage to healthy cells and increased treatment success [6]. The western Himalayan region is considered as a major reservoir of medicinally important drugs and plethora of drugs have been discovered from the region with diverse pharmacological properties including anticancer, anti-malarial, antibacterial, anti- HIV activities etc [7]. The present study has been designed to screen Western Himalayan Chemiome for possible tyrosine kinase inhibitors that may bind to FGFR-2 and thus prevent tumor cell growth.

2. Materials and Methods

2.1 Data Preparation and Virtual drug library screening

A virtual in-house drug library consisting of a total of 120 selected bioactive compounds (Supplementary Table S1) from Western Himalayan Chemiome was used in the study. Each of the compound were screened for their ability to bind catalytic loop of FGFR-2 in the ATP-binding pocket after satisfying Lipinski Rule of five [8] predicted mutagenicity or carcinogenicity, and Absorption Distribution Metabolism Excretion (ADME) properties [9]. For structure based drug designing the Kinase Domain human fibroblast

growth factor receptor-2 (PDB ID: 1OEC) was used. Mutant were

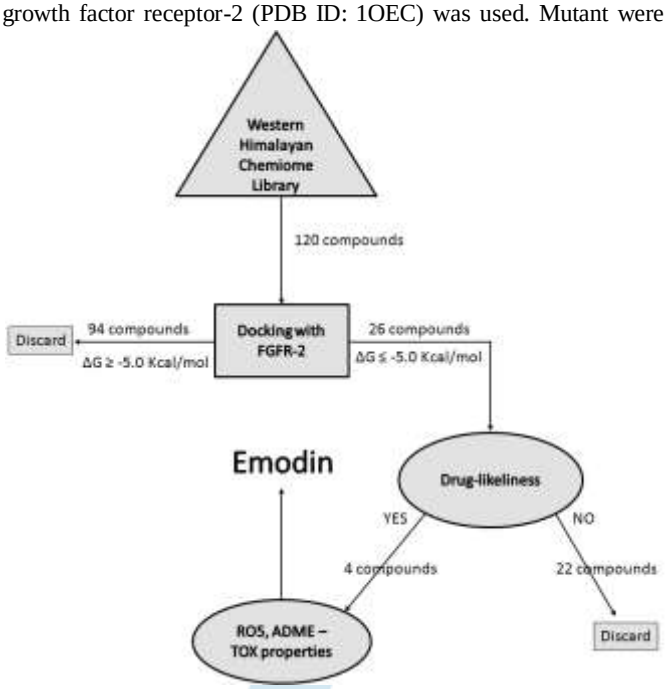

Figure 1. *Flowchart depicting the process for virtual screening of lead compounds targeting Fibroblast Growth Factor Receptor-2 (FGFR-2).*

generated from FGFR-2 (PDB ID: 1OEC) using Accelrys Discovery Studio 4.1 [10].

2.2 Molecular Docking Simulation

All the compounds were analyzed using AutoDock 4.2 [11] to confirm the binding mode with the catalytic loop of FGFR-2 as described by Amin et al 2016 [12]. Briefly, the docking energy was obtained from the summation of van der Walls energy and hydrogen bonding energy, while as binding energy was built up from van der Walls energy and desolvation energy. Lamarckian Genetic Algorithm (GA) was considered for the run and for each ligand 10 GA runs, with 27,000 maximum generations, 0.02 rate of gene mutation and 0.8 as rate of crossover were set. A grid of 40×40×40 points in X, Y, and Z direction was built centered around catalytic loop. Cutoff of -5Kcal/mole was set to limit the search for the lead compound showing binding with catalytic loop of FGFR-2.

3. Results:

3.1 *Emodin* shows best binding with FGFR-2:

The virtual screening of FGFR-2 binding drugs was carried out as depicted in Figure-1. Out of the 120 compounds initially screened, only 26 compounds qualified to showed binding with FGFR-2 with $\Delta G \leq -5.0$Kcal/mol. These compounds were then subjected to Drug-likeliness bias (carcinogenicity/ mutagenicity) which yielded only 4 compounds for next screening step. Finally, after RO5 (Lipinski Rule of five) and ADME (Absorption Distribution Metabolism Excretion) screening, only *Emodin* (Chem ID: 3220) was found to satisfying all the parametric biases. *Emodin* was found to bind the catalytic loop of FGFR-2 within the binding pocket consisting of ARG630, ASN631, CYS491, PHE492, ASP644, ASP626, GLY493, GLN494, LYS417, VAL495, GLY488, GLY430 and GLY489. The binding of *Emodin* with catalytic loop blocked the activation loop in such a way that may possibly prevent ATP from bind FGFR-2 and thereby may prevent its activation. Figure-2 shows the binding mode of *Emodin* with FGFR-2.

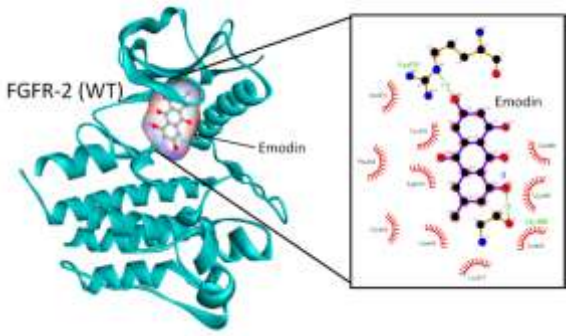

Figure 2. *Binding mode of Emodin with Fibroblast Growth Factor Receptor-2 (FGFR-2) showing ligand-binding interactions.*

3.2 Binding mode of Emodin mimics that of Pazopanib.

In order to evaluate the efficacy of *Emodin* to block FGFR-2 activation, we performed comparative docking analysis of *Emodin* with *Pazopanib* (ChemID: 10113978), a known Receptor Tyrosine Kinase Inhibitor (presently in clinical trials). The binding of *Emodin* (ΔG: -5.23Kcal/mol) to catalytic loop of FGFR-2 (ΔG: -6.52) was found comparable to that of *Pazopanib*. *Pazopanib* was found to bind the catalytic loop of FGFR-2 within the binding pocket consisting of ARG630, LEU633, ASP644, ASN631, ASP626, PRO666, LEU647, ARG664, CYS491, LEU487, VAL495, and GLY488 [Figure 3].

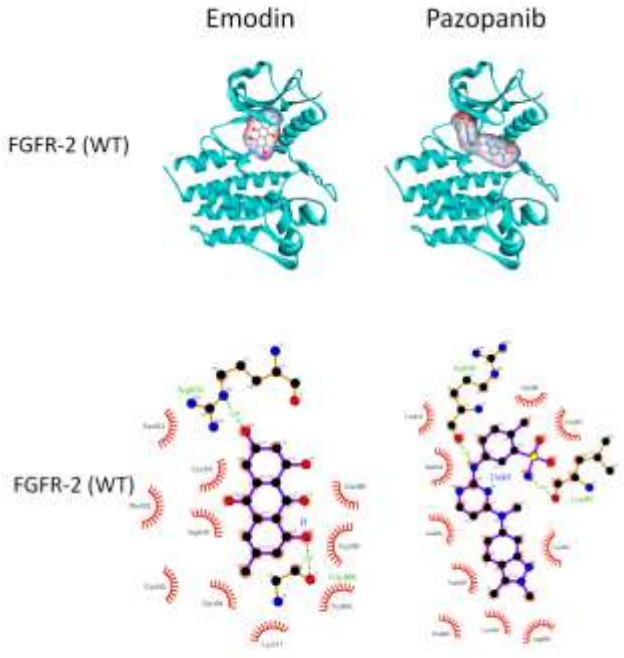

Figure 3. *Comparative binding of Emodin and Pazopanib with Fibroblast Growth Factor Receptor-2 (FGFR-2) showing ligand-binding interactions.*

3.3 Activating mutations do not affect catalytic blockage of FGFR-2 by Emodin.

Activating mutations, also called gain-of-function mutations alter the conformation of gene product in such a way so as to overcome any catalytic inhibition thus leading to its constitutive activation. In order to further analyze the inhibitory potential of *Emodin*, we generated FGFR-2 mutants harboring activating mutations (N549T and E565A) using Accelrys Discovery Studio (v 4.1). After energy minimization, the mutants FGFR-2 were subjected to molecular docking simulation using AutoDock (v 4.2). For comparative analysis, we also subjected the FGFR-2 mutants to molecular docking with *Pazopanib*. Despite activating nature of mutations, the *Emodin* showed satisfactory binding efficiency like *Pazopanib* (Figure-4). Table-1 summarizes the results of molecular docking simulation of mutants with *Emodin* and *Pazopanib*.

4. Discussion:

The structural mechanism by which this receptor tyrosine kinase (RTK) regulates catalytic activity has been implicated to subtle changes in its conformational dynamics [13]. This property of RTK's has been utilized for development of some RTK inhibitors (TKI's) that induce conformational changes in the receptor leading either to its inactivation (Allosteric TKI) or block its activation site (competitive TKI's) [14]. Molecular docking simulations have played a major role in virtual screening of prospective drugs including receptor tyrosine kinase inhibitors (TKI's).

In the present study, we utilized molecular docking simulations in virtual screening of in-house western himalyan drug library for screening of potential inhibitors blocking catalytic pocket of Fibroblast Growth factor Receptor-2 (FGFR-2) which is a major mitogenic and angiogenic determinant in various cancers. The inhouse target drug library consisted of 120 native compounds of Western Kashmir Himalaya origin with documented medicinal properties.

Fibroblast Growth factor Receptor-2 (FGFR-2) is a member of receptor tyrosine kinase family and is a receptor for fibroblast growth factor (FGF). The binding of ligand causes dimerization of receptor entities thus leading to trans phosphorylation of its specific tyrosine residues in its intracellular kinase domain commencing cascade of signaling processes [15]. The activation of receptor initiates by binding of ATP to nucleotide binding site (K517/ N571) that orients the incoming ATP to its catalytic site (ASP626) leading to trans-phosprorylation of the dimerized receptor protein which eventually induces autophosphorylation of its tyrosine kinase domain. We choose to target the catalytic loop (481-770) harboring the active site (ASP626) so as to block the active site from interacting with ATP. Virtual screening along the grid of 40×40×40 points in X, Y, and Z direction and centered around catalytic site (ASP626) was performed with the inhouse drug library of 120 compounds and only those complexes were extracted with binding energy less than -5.0 Kcal/mol. 26 out of 120 compounds showed efficient binding with the receptor and were further subjected to other parametric bias viz. Drug likeliness (carcinogenicity/ mutagenicity), RO5 and ADME properties. *Emodin* (1,3,8-trihydroxy-6-methylanthraquinone) showed the best binding and successfully passed all the parametric biases among the selected bioactive compound library. *Emodin* has been known for its anti-

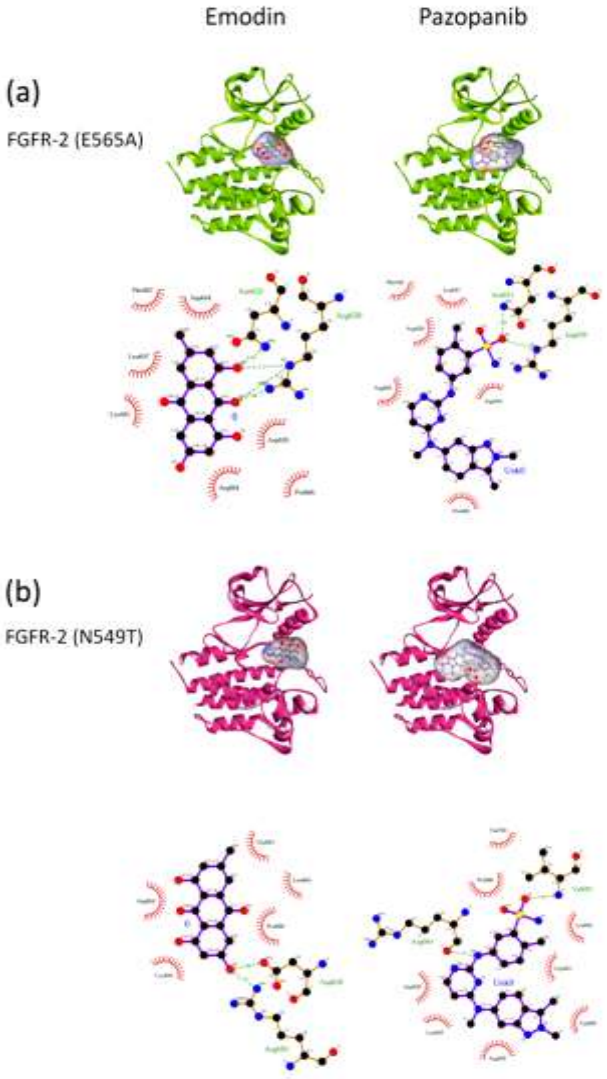

Figure 4. *Comparative binding of Emodin and Pazopanib with Fibroblast Growth Factor Receptor-2 (FGFR-2) mutants showing ligand-binding interactions a). E565A mutant and b) N549T mutant.*

inflammatory and anti-angiogenic capabilities in studies ranging from invitro cell assays to invivo mouse models [17-18]. *Emodin* showed binding interactions with catalytic loop of FGFR-2 comprising of ARG630, ASN631, CYS491, PHE492, ASP644, ASP626, GLY493, GLN494, LYS417, VAL495, GLY488, GLY430, GLY489. *Emodin* generated a Pi-Pi interaction with K517 and also formed 2 hydrogen bonds with each ARG630 and ASN631. *Pazopanib*, an FDA approved tyrosine kinase inhibitor showed an identical binding pattern with binding interactions involving ARG630, LEU633, ASP644, ASN631, ASP626, PRO666, LEU647, ARG664, CYS491, LEU487, VAL495 and GLY488. The identical binding pattern of the two compounds points towards a similar mode of action thus presenting *Emodin* as a new drug lead to inhibit FGFR-2 mediated tumor growth and angiogenesis. The observations were further corroborated by the analyzing binding pattern of *Emodin* in activating FGFR-2 mutants (E565A and N549T). These mutants commonly observed in various cancer viz breast cancer,

Table-1. Molecular Docking simulation of FGFR-2 and its mutants with *Emodin* and *Pazopanib*

Compound	Free Energy (Kcal/mol)	Pocket	Hydrogen Bond
Emodin-FGFR-2	-5.23	ARG630, ASN631, CYS491, PHE492, ASP644, ASP626, GLY493, GLN494, LYS417, VAL495, GLY488, GLY430, GLY489	2
Pazopanib-FGFR-2	-6.52	ARG630, LEU633, ASP644, ASN631, ASP626, PRO666, LEU647, ARG664, CYS491, LEU487, VAL495, GLY488	2
Emodin- E565A	-5.37	ARG630, ASN631, CYS491, PHE492, ASP644, ASP626, ARG664, PRO666, LEU647	4
Emodin-N549T	-4.95	ARG630, CYS491, ASP626, GLY663, ARG664, LEU665, PRO666	2
Pazopanib-E565A	-5.10	ARG630, ASN631, ASP644, LEU647, ASP626, PHE492, ARG664	2
Pazopanib- N549T	-5.60	ARG630, LEU647, ASP626, , TRP669, GLY663, ARG664, LEU665, PRO666, VAL667, VAL709	2

prostate cancer etc remain constitutive active and depict a high rate of tyrosine phosphorylation and are sensitive to feeble titer of ligands. *Emodin* successfully showed an efficient binding with both the FGFR-2 mutants forming 4 hydrogen bonds with E565A mutant (Binding energy: -5.37Kcal/mol) and 2 hydrogen bonds with N549T mutant (Binding energy: -4.95Kcal/mol). The binding mode *Emodin* with both the mutants was comparable to that *Pazopanib*. In conclusion, the present study not only projects *Emodin* as a lead molecule to overcome FGFR-2 mediated tumor mitogenesis but also demonstrates its potential to act as a tyrosine kinase inhibitor to overcome tumor angiogenesis.

Competing financial interests:
The authors declare no competing financial interests.

Supplementary Material: Supplementary material available in online version.

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References:

[1]. Touat, M., Ileana, E., Postel-Vinay, S., Andre, F., & Soria, J.-C. (2015). Targeting FGFR Signaling in Cancer. Clinical Cancer Research, 21(12), 2684–2694. doi:10.1158/1078-0432.ccr-14-2329

[2]. Zhao, Y., & Adjei, A. A. (2015). Targeting Angiogenesis in Cancer Therapy: Moving Beyond Vascular Endothelial Growth Factor. The Oncologist, 20(6), 660–673. doi:10.1634/theoncologist.2014-0465

[3]. Turner, N., & Grose, R. (2010). Fibroblast growth factor signalling: from development to cancer. Nature Reviews Cancer, 10(2), 116–129. doi:10.1038/nrc2780

[4]. Drafahl, K. A., McAndrew, C. W., & Donoghue, D. J. (2010). Signaling from Fibroblast Growth Factor Receptors in Development and Disease. Handbook of Cell Signaling, 1939–1947. doi:10.1016/b978-0-12-374145-5.00237-0

[5]. Marshall, C. . (1995). Specificity of receptor tyrosine kinase signaling: Transient versus sustained extracellular signal-regulated kinase activation. Cell, 80(2), 179–185. doi:10.1016/0092-8674(95)90401-8

[6]. Shawver, L. K., Slamon, D., & Ullrich, A. (2002). Smart drugs: Tyrosine kinase inhibitors in cancer therapy. Cancer Cell, 1(2), 117–123. doi:10.1016/s1535-6108(02)00039-9

[7]. Joshi, R., Satyal, P., & Setzer, W. (2016). Himalayan Aromatic Medicinal Plants: A Review of their Ethnopharmacology, Volatile Phytochemistry, and Biological Activities. Medicines, 3(1), 6. doi:10.3390/medicines3010006

[8]. Lipinski, C. A. Lead- and drug-like compounds: the rule-of-five revolution. Drug discovery today. Technologies 1, 337-341 (2004). doi:10.1016/j.ddtec.2004.11.007

[9]. Moroy, G., Martiny, V. Y., Vayer, P., Villoutreix, B. O. & Miteva, M. A. Toward in silico structure-based ADMET prediction in drug discovery. Drug discovery today 17, 44-55 (2012). doi:10.1016/j.drudis.2011.10.023

[10]. Accelrys Software Inc. (2012), Discovery Studio Modeling Environment, Version 3.5, San Diego, CA, USA. URL: http://www.accelrys.com

[11]. Morris, G. M. et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of computational chemistry 30, 2785-2791 (2009). doi:10.1002/jcc.21256

[12]. Amin, A., Chikan, N. A., Mokhdomi, T. A., Bukhari, S., Koul, A. M., Shah, B. A., … Qadri, R. A. (2016). Irigenin, a novel lead from Western Himalayan chemiome inhibits Fibronectin-Extra Domain A induced metastasis in Lung cancer cells. Scientific Reports, 6(1). doi:10.1038/srep37151

[13]. Karp, J. M., Sparks, S., & Cowburn, D. (2017). Effects of FGFR2 kinase activation loop dynamics on catalytic activity. PLOS Computational Biology, 13(2), e1005360. doi:10.1371/journal.pcbi.1005360

[14]. Hantschel, O., Grebien, F., & Superti-Furga, G. (2012). The Growing Arsenal of ATP-Competitive and Allosteric Inhibitors of BCR-ABL. Cancer Research, 72(19), 4890–4895. doi:10.1158/0008-5472.can-12-1276

[15]. Eswarakumar, V. P., Lax, I., & Schlessinger, J. (2005). Cellular signaling by fibroblast growth factor receptors. Cytokine & Growth Factor Reviews, 16(2), 139–149. doi:10.1016/j.cytogfr.2005.01.001

[16]. Shrimali, D., Shanmugam, M. K., Kumar, A. P., Zhang, J., Tan, B. K. H., Ahn, K. S., & Sethi, G. (2013). Targeted abrogation of diverse signal transduction cascades by emodin for the treatment of inflammatory disorders and cancer. Cancer Letters, 341(2), 139–149. doi:10.1016/j.canlet.2013.08.023

[17]. Srinivas, G., Babykutty, S., Sathiadevan, P. P., & Srinivas, P. (2007). Molecular Mechanism of Emodin Action: Transition from Laxative Ingredient to an Antitumor Agent. ChemInform, 38(45). doi:10.1002/chin.200745276