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SEARCH FOR A NATURAL ANTAGONIST OF HEPATOCYTE GROWTH FACTOR RECEPTOR (HGFR) USING VIRTUAL SCREENING APPROACH

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ABSTRACT: MET receptor is a complex protein with extracellular SEMA domain and the intracellular tyrosine kinase domain. The binding of HGF to MET induces MET dimerization and transactivate by phosphorylation selected tyrosine residues. HGF-MET pathway being a critical driver in cancer development/progression selective natural product derived inhibitors at extracellular domain may be of clinical use either alone or in combination with classical therapy. In the present study, we included 94 potential phytopharmaceuticals with reported anticancer activity in-vitro as ligand against MET SEMA domain to antagonize the HGF binding. Obtained Results indicated Theaflavin and Ginkgetin as the probable antagonist by hindering the binding of the natural ligand in a competitive manner. However further *in-vitro* and *in-vivo* validations are required to substantiate the *in-silico* claim.

KEYWORDS: HGF, MET, receptor tyrosine kinase, drug target, virtual screening, Docking.

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1. INTRODUCTION

Hepatocyte growth factor receptor (HGFR/MET), is a protein with tyrosine kinase activity, encoded by the MET gene in human. The protein produced as a single-chain precursor is further processed and proteolytically cleaved to generate two subunits, a highly glycosylated extracellular α-subunit, and a transmembrane β-subunit, which are linked through a disulfide linkage to form the mature receptor [1]. MET receptor plays an important role in organogenesis during embryonic development and wound healing in the adults. Hepatocyte growth factor (HGF) and its two splice isoforms NK1

& NK2 act as a ligand for HGFR/MET receptor [2]. MET is normally expressed on the surface of cells with an epithelial origin, while expression of HGF/SF is from cells with mesenchymal origin. Binding of HGF to MET receptor induces receptor dimerization leading to its activation and initiates a signaling cascade to facilitate several biological responses e.g. cell proliferation, migration, morphogenesis, and survival. This ligand-mediated activation of MET is a key contributor for invasion, metastasis, and resistance to therapy in various cancers [3]. overexpression of c-MET/HGFR and had been detected in cancerous cells of classical Hodgkin's lymphoma, gastric cancer, lung cancer, non-small cell lung cancer (NSCLC), breast cancer, hepatocellular carcinoma, renal cell carcinoma and malignant melanomas [4,-15]. HGFR is composed of an extracellular α chain (145 kDa) and a β chain(50 kDa). The extracellular part is composed of the SEMA domain, plexin–semaphorin–integrin (PSI), and four immunoglobulin like fold–plexin–transcription factor (IPT1–4) domains. The intracellular region contains JM and Tyrosine Kinase domains. The binding of HGF to MET induces MET dimerization and phosphorylation of Y1234 and Y1235, followed by phosphorylation of Y1349 and Y1356 in the carboxyl-terminal region, to which adaptor molecules associate and transmit signals downstream. The Hepatocyte growth factor is secreted as a pro HGF, a single chain precursor protein and extracellular processing into a two chain mature HGF is coupled to the activation of HGF. HGF binds to its receptor through two interfaces, one through the high- affinity binding with N terminal and first kringle domainsNK1 (NK1) and with lower affinity with β chain binds. The activation of MET receptor by bivalent MET binding macrocyclic peptides indicate that stable dimerization of MET with ligands of appropriate length provides a fundamental structural basis for activation of MET [16]. The intracellular region contains JM and Tyrosine Kinase domains. The JM domain is composed of 47 amino acids having two phosphorylation sites namely Y1003 and other S985 and it also acts as a negative regulator of MET dependent signal transduction. The CBL ubiquitin ligase binds phosphorylated Y1003, leading to MET ubiquitination, endocytosis, and degradation. The CBL mediated degradation of activated MET provides a clue for developing antagonist that either attenuates or terminates MET mediated signaling. However, phosphorylation of Ser985 suppresses HGF induced MET activation which in turn suppresses subsequent biological responses [17,18]. Most of the agents currently under development include either mAbs directed at HGF or low molecular weight compounds that competitively antagonize ATP binding to MET. Few of these selective MET inhibitors have received regulatory approval in several indications, however, none of them have shown any remarkable efficacy in phase II or III clinical trials [19]. some of the c-MET/HGFR inhibitors in clinical phase I trials include CM-118, Boxitinib, Altiratinib, bozitinib, kanitenib etc. whereas, the c-MET inhibitors in clinical phase 2 trials includes GM-604, emibetuzumab, AMG-337, Tepotinib etc [20]. Certain selective c-MET/HGFR inhibitors which are small molecule inhibitors includes Tivantinib,INC280 and MSC2156119. c-Met inhibitors that are non selective inhibitors includes

Cabozantinib, foretinib and, Golvantinib [21]. This still keeps open the possibility of further validation of the HGF-MET pathway as a critical driver in cancer development/progression using novel biomolecules as HGF-MET inhibitors for clinical use.

2. MATERIALS AND METHODS

Selection of ligand

A dataset consisting of 94 small molecule ligands (phytochemicals) have been prepared. These ligands were taken from PubChem database [21] and were cleaned in using Marvin Suite. All the ligands were exported as the library in a single .sdf file format for virtual screening.

Selection of target

Protein target selection was done from the Protein Databank (PDB), a reservoir of diverse protein structures. The target protein with PDBID of 1SHY had been selected. It consists of the X-Ray crystal structure (resolution 3.22 A°) of HGF beta Chain in complex with the SEMA domain of the HGFR extracellular protein region [22].

Target ligand interaction by Hex Software

The Protein–Protein docking/interaction of the ligand (HGF, 1SHY, chain A) and the SEMA domain of HGFR C-Met receptor (HGFR, 1SHY, Chain B) were carried using the HEX tool (version:8.0.0)[23, 24]. Before the docking process water molecules were removed from the receptor and ligand molecules and were cleaned for missing bonds as well. Energy minimization of both the HGF ligand (chain A) and HGFR receptor (Chain B) was done using the software YASARA Structure (Version 17.8.15) [25].

Target ligand library docking using YASARA Structure

Protein-ligand docking was carried out using YASARA Structure. The HGFR (C-Met)Sema domain(1SHY, chain B) had been chosen as the receptor and the ligand library as ligands for the docking process. Prior to Docking, it had been ensured that the water molecules were deleted and the protein molecules were cleaned and energy minimized for the Protein-Ligand docking in YASARA structure. It utilizes the Auto Dock Vina algorithm for calculation of energy which is based on the following formula.

$$\Delta G = \Delta G_{(vdw)} + \Delta G_{(HBond)} + \Delta G_{(elec)} + \Delta G_{(tor)} + \Delta G_{(desolv).}$$

Here, $\Delta G_{(vdw)}$ = component energy terms related to vanderwalls bond, $\Delta G_{(HBond)}$ = the component energy term related to Hydrogen bonds, $\Delta G_{(elec)}$ =component energy terms related to electrostatics, $\Delta G_{(tor)}$ = component energy term related to the ligand's torsional free energy and $\Delta G_{(desolv)}$= component energy term related to the desolvation for the empirical calculation of the docking/binding energy for a protein-ligand complex. The Higher docking score in YASARA Structure represents the better protein-ligand binding whereas, the negative score represents no binding. The protein-ligand interactions were further visualised in 3d and 2d using the Accelrys Discovery Studio visualiser.

3. RESULTS AND DISCUSSION

Protein – Protein Docking

Docking of Protein (HGFR (PDB ID: 1SHY, chain B)) and natural ligand HGF (PDB ID : 1SHY, chain A) was carried out using Hex tool. The 2D interaction map was generated as shown in Figure-1. Interaction map revealed the ligand (HGF) binding sites on the receptor HGFR at ASN 38, LEU 57, TYR 125, VAL 188, ASP 190, SER 255, ASN 256, ASN 257, SER 283, ILE 284, SER 286, VAL 322, ARG 331, ASP 340, HIS 484, GLU 493. The Protein (HGF) - Protein (HGFR) docking in hex revealed E total value= -742.60 and EShape value = -742.60 after docking.

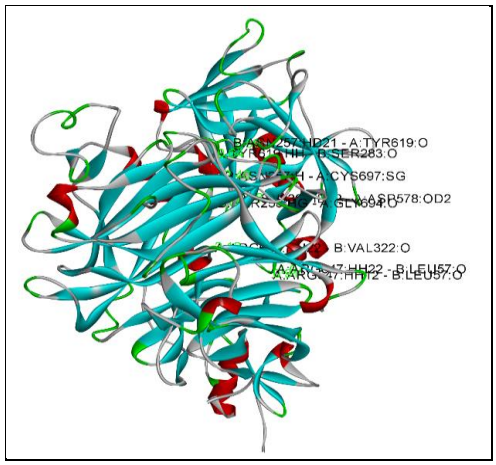


Figure 1: Protein-Protein interaction of HGF-beta chain with SEMA domain of c-MET receptor

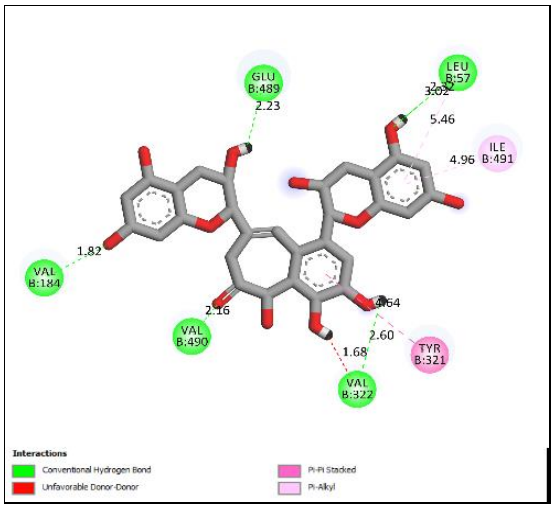
Virtual Screening

Protein-Ligand (phytochemical library) docking done on YASARA and post-processing of docking score resulted in top five phytochemicals ranked on the basis of their efficacy, binding free energy, dissociation constant (Table-1). Contacting residues indicated in bold suggestive of matched residue as per Hex interaction with natural ligand HGF. Common residues confirm some overlapping with natural ligand.

Table1: Docking result of top five phytochemicals showing interacting residues with receptor

Sr. no.	Ligand Name	Binding.Energy [kcal/mol]	Dissociation constant [pM]	Contacting receptor residues on the Receptor (1SHY, chain B)
1	Theaflavin	9.96	49860.56	ASN 54, VAL 55,ILE 56, **LEU 57**,ALA 119,LEU 120 LYS 183, VAL 184, HIS 251, ALA 320,TYR 321, **VAL 322**, PRO 488, GLU 489, VAL 490, ILE 491, VAL 492, **GLU 493**, HIS 494.
2	Ginkgetin	9.60	91546.27	VAL 55, ILE 56, **LEU 57**, LEU 120, LYS 183, VAL 184, LEU 185, HIS 251, ALA 252, PHE 253, GLU 254, TYR 321, **VAL 322**, SER 323, LYS 324, ASP 340, PRO 488, GLU 489, VAL 490, ILE 491.
3	Iso-Tetrandrine	9.50	108378.13	GLN 53, ASN 54, ASN 117, MET 118, LEU 180, GLY 181, ALA 182, LYS 183, LYS 248, TYR 249, VAL 250,VAL 264, ILE 316, LEU 317, GLN 318, PRO 356, THR 440, PRO 485, VAL 486, SER 487, PRO 488, GLU 489, GLY 507
4	Fangchinoline	9.31	149352.84	GLN 53, ASN 54, ASN 117, MET 118, LEU 180, GLY 181, ALA 182, LYS 183, LYS 248, TYR 249, VAL 250, VAL 264, ILE 316, LEU 317, GLN 318, PRO 356, THR 440, HIS 484, PRO 485, VAL 486, SER 487, PRO 488, GLU 489, ILE 505, GLY 507.
5	Spirosolane	9.12	207213.19	PHE 96, PRO 97, CYS 98, GLN 99, CYS 160, PHE 162, PRO 164, PRO 169, PRO 207, ASP 208, HIS 209.

The 2D and 3D representations of the top three protein- ligand interactions have been further described.

A. 

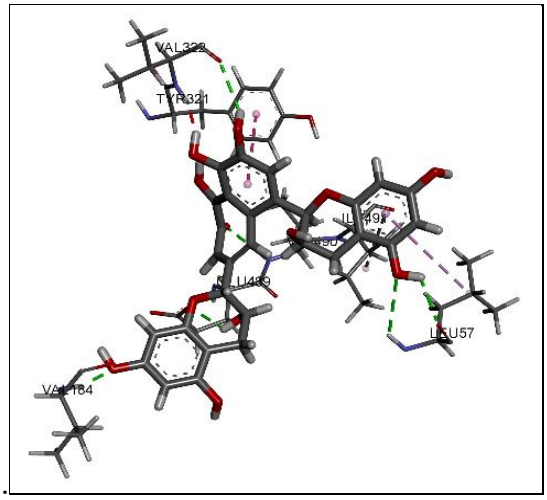
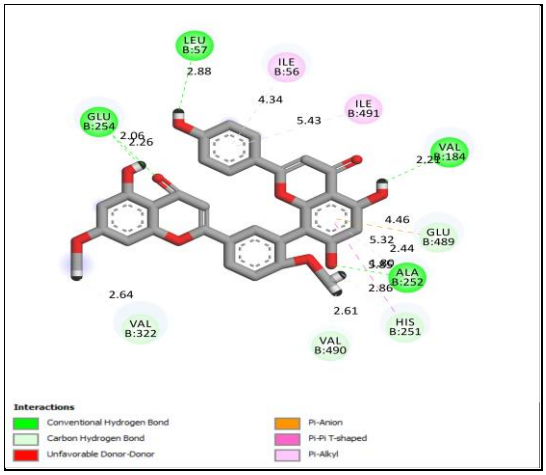
B. 

Figure 2 (A) 2D interaction map of theaflavin with extracellular SEMA domain of receptor (B) 3D interaction map of theaflavin with extracellular SEMA domain of receptor

2Dand 3D representations of the ligandtheaflavin with extracellular SEMA domain of receptor with binding energy of 9.96 kcal/mol have been shown. Theaflavin forms H-bonds with the receptor residues GLU 489, lEU 57,VAL 322, VAL 490 AND VAL 184 at the distances of 2.23 Å, 3.02 Å,2.60 Å, 2.16 Å and 1.82 Å respectively.

A. 

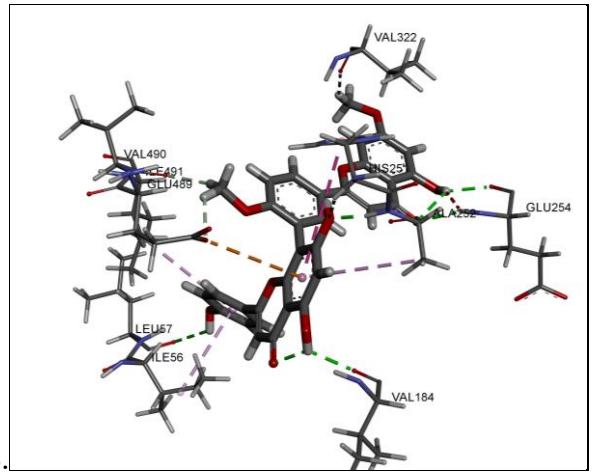
B. 

Figure3(A) 2D interaction map of Ginkgetin with extracellular SEMA domain of receptor (B) 3D interaction map of Ginkgetin with extracellular SEMA domain of receptor

2Dand 3D representations of the ligandGinkgetin with extracellular SEMA domain of receptor with binding energy of 9.60 kcal/mol have been shown in figure 3. Ginkgetin forms Hydrogen bonds with receptors GLU 254, LEU 57,VAL 184 and VAL 252 at the distances of 2.26 Å, 2.88 Å ,2.21 Å and 5.85 Å respectively.

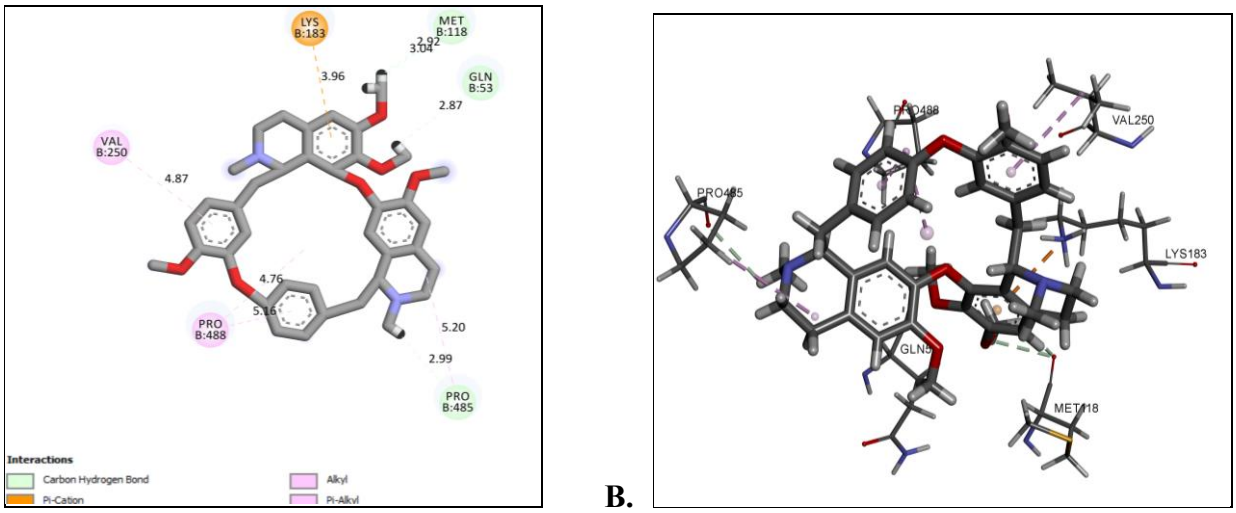


Figure 4(A)2D interaction map of Iso-Tetrandrine with extracellular SEMA domain of receptor. (B) 3D interaction map of Iso-Tetrandrine with extracellular SEMA domain of receptor

2Dand 3D representations of the ligandIso-Tetrandrine with extracellular SEMA domain of receptor with binding energy of 9.50 kcal/mol have been shown in figure 4. the ligand Iso-Tetrandrine forms hydrogen bonds with the receptors MET 118, GLN 53 and PRO 485 at the distances of 2.92 Å, 2.87 Å and 2.99 Å respectively. The Above computational results shows the binding of the top three phytochemicals viz. Theaflavin, Ginkgetin and Iso-Tetrandrine with the extracellular SEMA domain of protein receptor C-Met.

4. CONCLUSION

A paradigm shift has been observed in search of novel therapeutics from natural products for cancer therapy in the last decade. Aberrant activation of receptor tyrosine kinases (RTKs) plays a critical role in tumor formation, invasion, and metastasis. Hence, they are considered as pharmaceutically attractive targets for Tyrosine Kinase Inhibitors. Binding of HGF to its receptor c-met is a normal process of growth and development in many tissues. Acceptance and approval of natural product-derived anticancer drugs are increasing day by day. Consensus docking approach has been adopted to increase docking accuracy and decrease false positives hits during virtual screening. The present study also involved virtual screening approach against MET receptor for multiple sites i.e. ligand binding site, tyrosine kinase site and c-terminal domain using a 94 natural compound library shortlisted from published literature with proven anticancer activity in-vitro. HGF being the sole ligand of MET, the formation of HGF: MET complex leads to MET activation. Lots of work have been done using truncated HGF, anti-HGF neutralizing antibodies, and an uncleavable form of HGF to antagonize the MET's biological activity. However, the major limitation of such an approach is that they block only HGF-dependent MET activation. A complete antagonist e.g. NK4 have been found to compete with HGF for MET binding without inducing receptor activation. It has homology

with HGF having the N-terminal hairpin and the four kringle domains and with angiostatins as well [26-28]. Amongst the five two-hit molecules, namely Theaflavin and Ginkgetin showed some overlapping binding residue with the natural ligand HGF. Theaflavin, the major black tea polyphenols, have been reported to have anti-inflammatory and anticancer activity in-vitro and in-vivo models [29]. It suppresses constitutive and inducible STAT3 phosphorylation with concomitant downregulation of anti-apoptotic proteins (Bcl-2 and Survivin) and the invasion-related proteins (MMP-2, MMP-9). Jianping et al., have reported that theaflavins suppress the growth and metastasis of human Hepato Cellular Carcinoma through the blockage of the STAT3 pathway [30]. Ginkgetin, a natural biflavonoid, had been also reported to have promising anticancer activity in non-small cell lung cancer cell lines and in a xenograft nude mouse model. It is shown to have better IC50 value than cisplatin alone. Earlier reports have claimed autophagic cell death in A549 cells induced by Ginkgetin with potential binding affinity to p62. Cell cycle analysis by flow cytometry has also shown Ginkgetin induced G2/M phase arrest in Daoy cells [31]. As the phytochemicals Theaflavin and Ginkgetin binding showed partial overlapping of interacting residue at the ligand binding site on the MET receptor, they probably will act as antagonist by hindering the binding of the natural ligand in a competitive manner. Natural compounds being larger in size and lesser cell membrane penetrating ability can better bind to extracellular targets. However, the efficacy of both the natural phytochemicals may be tested in-vitro in multiple cell line expressing the MET gene and its downstream signal transducers to establish its antagonistic ability towards MET receptor.

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CONFLICT OF INTEREST

The Authors declare that there are no conflicts of Interest.

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