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Molecular Docking studies of FKBP12-mTOR inhibitors using binding predictions

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Abstract:
Mammalian target of rapamycin (mTOR) is a key regulator of cell growth, proliferation and angiogenesis. mTOR signaling is frequently hyper activated in a broad spectrum of human cancers thereby making it a potential drug target. The current drugs available have been successful in inhibiting the mTOR signaling, nevertheless, show low oral bioavailability and suboptimal solubility. Considering the narrow therapeutic window of the available inhibitors, through computational approaches, the present study pursues to identify a compound with optimal oral bioavailability and better solubility properties in addition ensuing high affinity between FKBP12 and FRB domain of mTOR. Current mTOR inhibitors; Everolimus, Temsirolimus Deforolimus and Echinomycin served as parent molecules for similarity search with a threshold of 95%. The query molecules and respective similar molecules were docked at the binding cleft of FKBP12 protein. Aided by MolDock algorithm, high affinity compounds against FKBP12 were retrieved. Patch Dock supervised protein-protein interactions were established between FRB domain of mTOR and ligand (query and similar) bound and free states of FKBP12. All the similar compounds thus retrieved showed better solubility properties and enabled better complex formation of mTOR and FKBP12. In particular Everolimus similar compound PubChem ID: 57284959 showed appreciable drugs like properties bestowed with better solubility higher oral bioavailability. In addition this compound brought about enhanced interaction between FKBP12 and FRB domain of mTOR. In the study, we report Everolimus similar compound PubChem ID: 57284959 to be potential inhibitor for mTOR pathway which can overcome the affinity and solubility concerns of current mTOR drugs.

Key Words: mTOR, FRB domain, FKBP12, Solubility, Human oral absorption, virtual screening, Protein-protein interactions.

Abbreviations: mTOR: Mammalian Target of Rapamycin; **FRB domain:** FKBP12-rapamycin associated protein; **FKBP12:** FK506-binding protein 12; **OPLS:** Optimized Potentials for Liquid Simulations; **Akt:** RAC-alpha serine/threonine-protein kinase; **PI3K**: phosphatidylinositide 3-kinases

Background:
The mammalian target of rapamycin (mTOR) - an atypical serine/threonine (S/T) protein kinase, is a central controller of cell growth, proliferation and metabolism **[1, 2].** mTOR is regarded as the "master switch" of cellular metabolic processes owing to its unique ability to sense nutrient

availability, cellular energy levels, oxygen levels, and mitogenic signals **[3,4]** that regulates cell signaling process. Dysregulation of mTOR and its associated proteins in the signaling pathway often hallmarks tumor development angiogenesis and metastasis **[5].** For example, abnormal activation of the mTOR pathway was detected in squamous cancers **[6],** adenocarcinomas **[7],** bronchioloalveolar carcinomas **[8],** colorectal cancers **[9],** astrocytomas **[10]** and glioblastomas **[11].**

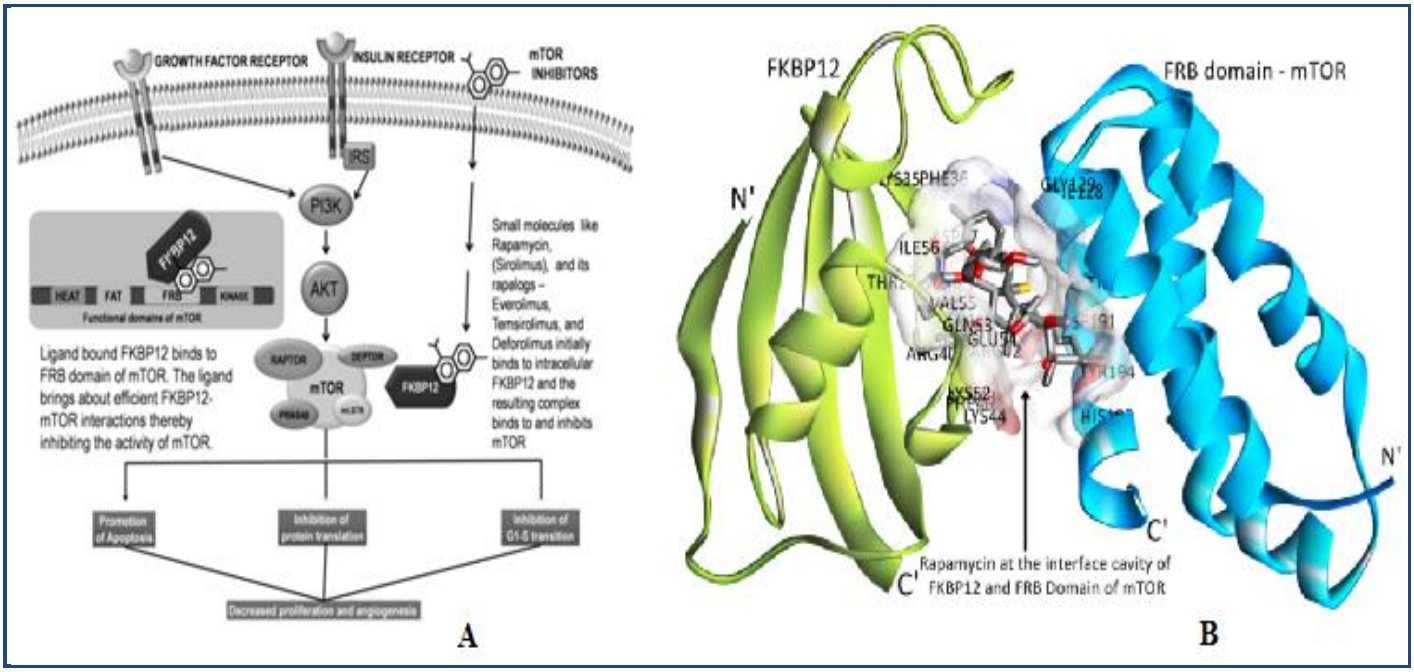

Figure 1: A) Ligand stimulation of growth receptors (like VEGFR, HER etc) and insulin receptors activates the mTOR complex through a series of upstream signaling proteins like PI3K and AKT. Over-activation of mTOR signaling significantly contributes to abnormal cellular proliferation and development of tumors through deregulation of upstream PI3K/AKT signaling through a variety of mechanisms, including overexpression or activation of growth factor receptors, and IGFR (insulin-like growth factor receptor) or mutations in PI3K and mutations/amplifications of AKT. Rapamycin and rapalogs crosslink the immunophilin FKBP-12 protein then rapamycin-FKBP12 complex interferes with FRB domain of mTOR and inhibits the mTOR activity. The inhibition of mTOR blocks the binding of the accessory protein raptor (regulatory-associated protein of mTOR) to mTOR, As a consequence, the synergistic binding reduces protein synthesis which leads to late blockage of G1/S cell cycle and induces cancer cell death by stimulating autophagy or apoptosis. Inset: Domain structure of mTOR. The N-terminus of mTOR contains tandem repeated HEAT motifs (protein interaction domains found in Huntington, Elongation factor 3, PR65/A and TOR), a FAT (domain shared by FRAP, Ataxia telangiectasia mutated, and TRRAP, all of which are PIKK family members) domain, a FRB (FKBP12-rapamycin-binding site, found in all eukaryotic TOR orthologs) domain. TheFRB domain forms a deep hydrophobic cleft that serves as the high-affinity binding site for the inhibitory complex FKBP12-rapamycin; **B)** Protein complex (PDB ID: 3FAP) of FKBP12 (green helices) and FRB domain of mTOR (blue helices). Ligand-receptor complex is first established between Rapamycin (bound at the interface) and FKBP12. The complex thereafter binds to FRB domain of mTOR. The synergistic binding of rapamycin bound FKBP to mTOR results in inhibition of mTORC1 downstream signaling pathways leading to translational suppression of oncogenes.

Given the ubiquitous role in carcinomas, mTOR surfaced as an interesting anti-metastatic target in the clinical treatment of broad range of carcinomas. A recent immune-histochemical study performed in tissue arrays containing 124 tumors from 8 common human tumor types revealed that approximately 26% of tumors (32/124) are predicted to be sensitive to mTOR inhibition **[12].** These findings indicates potential role of dysregulated mTOR signaling in tumorigenesis and support the currently ongoing clinical development of mTOR inhibitors as a potential tumor-selective therapeutic strategy. The first ever drug targeting mTOR pathway was Rapamycin isolated from the bacterium *Streptomyces hygroscopicus* discovered to have potent immunosuppressive and anti-tumour properties **[13–15].** As an immunosuppressive drug, rapamycin (rapamune or sirolimus) was approved by FDA (USA Food and Drug Administration) in 1999 for prevention of renal allograft rejection **[16].** Subsequent studies described that rapamycin can also act as a cytostatic agent, slowing or arresting growth of cell lines derived from different tumour types. Rapamycin forms complex with the intracellular receptor FKBP12, this complex binds to mTOR and inhibits mTORC1 downstream signaling **[17, 18]** thereby preventing translations of the proteins involved in cancer progression **(Figure 1a & b).**

However, being potent - rapamycin suffers solvent solubility concerns. In order to overcome issues with the "conventional" rapamycin, several derivatives of rapamycin called "rapalogs" with more favourable pharmacokinetic and solubility properties have been synthesized, such as RAD001 (Everolimus, Novartis, Novartis, Basel, Switzerland), CCI-779 (Temsirolimus, Wyeth, Madison, NJ, USA) and AP23573 (Deforolimus, ARIAD, Cambridge, MA, USA), which have overcome the drawbacks of rapamycin.

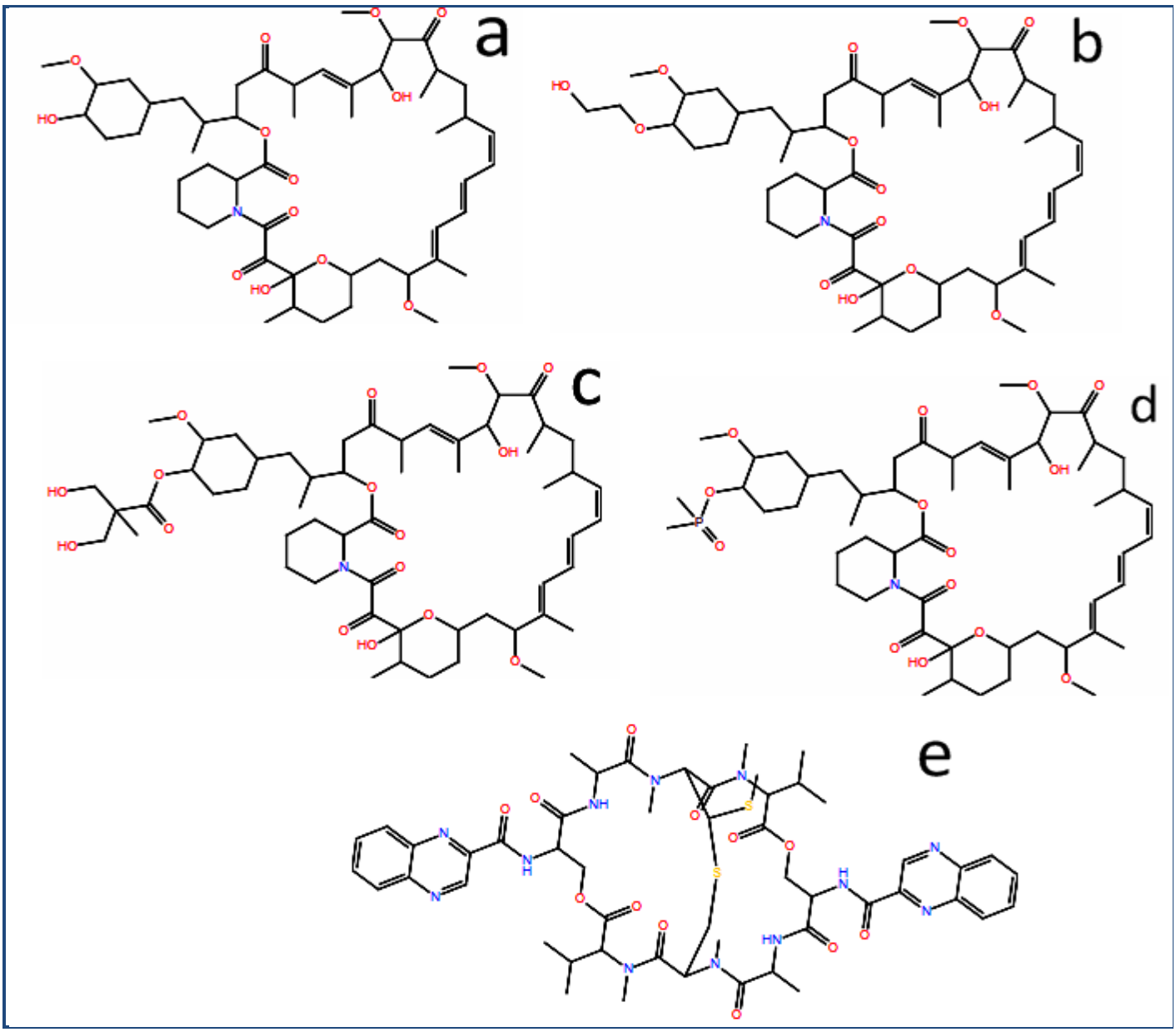

Figure 2: Structures of established mTOR inhibitors **a)** Rapamycin; **b)** Everolimus; **c)** Temsirolimus; **d)** Deforolimus; **e)** Echinomycin.

Although, the rapalogs have been efficient for tumor reversal, at clinical grounds, however, these drugs produce numerous side effects including decrease in lymphocytes and hemoglobin that could be serious and/or debilitating and often unpredictable. In addition, the oral bioavailability of these drugs is still a concern owing to its low aqueous solubility **[19, 20].** In the view of above given concerns, the present study endeavors to identify mTOR inhibitor with optimal aqueous solubility bestowed with superior inhibitory potential against mTOR anticipated to have safety profile over the established rapalogs

Methodology:

Selection of inhibitors

Potent compounds mTOR inhibitors like Rapamycin, (Sirolimus), and its rapalogs – Everolimus, Temsirolimus, Deforolimus and Echinomycin served as parent molecules for similarity search (**Figure 2**).

Similarity search, preparation of protein and compounds

The selected inhibitors served as query molecules for shape similarity search. Similarity search was supervised by binary finger print based tanimoto similarity equation to retrieve compounds with similarity threshold of 95 % against NCBI's PubChem compound database. All the structures were optimized through OPLS 2005 force field algorithm **[21]** embedded in the LigPrep module of Schrödinger suite, 2013 (Schrodinger. LLC, New York, NY) **[22].** Structural complex of Human FKBP12 and FRB domain of mTOR was retrieved from Protein Data Bank (PDB ID: 3FAP) **[23]** which was processed by removing all bound crystal water molecules and adding hydrogen bonds. Explicit hydrogen, bond orders, disulphide bonds, hybridizations and charges were assigned wherever

missing. The resulting structure was energy minimized at protonation state of 7.4 using OPLS-2005 force field by protein preparation wizard of Schrödinger suite 2013.

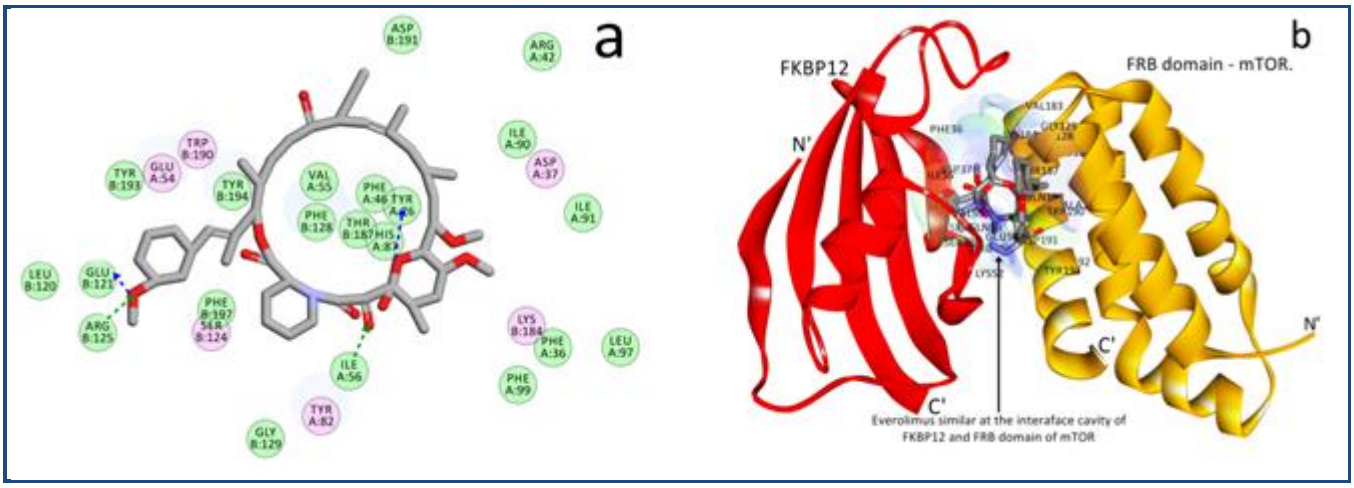

Figure 3: a) Interactions of PubChem ID: 57284959 –Everolimus similar with FKBP12 and FRB domain of mTOR. Residues (residues prefixed with 'A' belong to FKBP12 and 'B' belong to FRB domain of mTOR). Residues circled in green participate in van der Waals interaction while residues in pink forms electrostatic interactions. Hydrogen bond donors and acceptors are shown in blue and green color respectively; **b)** Evorilumus similar bound at the interface of FKBP12 (red helices) and FRB domain of mTOR (golden helices).

Solubility prediction and ADMET prediction of compounds

Solubility parameters like QP log S for aqueous solubility , QP log P for hexadecane/gas, QP log P for octanol/gas, QP log P for water/gas, QP log P for octanol/water were calculated by QikProp module of Schrödinger suite 2013 **[24].** All the similar compounds retrieved were screened for its ADMET by admetSAR web server **[25].**

Ligand receptor docking

Molecular docking program- Molegro Virtual Docker (MVD) **[26]** which incorporates highly efficient PLP (Piece wise Linear Potential) and MolDock scoring function provided a flexible docking platform. The leads (Rapamycin (Sirolimus), Temsirolimus, Everolimus, Deforolimus) and similar chemical structures were docked in predicted cavity of FKBP12. Docking parameters were set to 0.20Å as grid resolution, maximum iteration of 1500 and maximum population size of 50. Simplex evolution was set at maximum steps of 300 with neighborhood distance factor of 1. Binding affinity and interactions of compounds with protein were evaluated on the basis of the internal ES (Internal electrostatic Interaction), internal hydrogen bond interactions and sp2-sp2 torsions. Post docking energy of the ligand-receptor complex was minimized using Nelder Mead Simplex Minimization (using non-grid force field and H bond directionality) **[27].** On the basis of MolDock - rerank score best interacting high affinity compound was selected respective to each parent compound.

Protein-protein docking studies

Structural complex of Human FKBP12 and FRB domain of mTOR was retrieved from Protein Data Bank (PDB ID: 3FAP). The FKBP12 and FRB domain of mTOR domain were separated and saved in two different pdb files. The free and ligand bound FKBP12 was further docked with FRB domain of mTOR. Protein - Protein docking was executed through object recognition and image segmentation algorithm embedded in Patchdock server **[28].** Default parameter was set as clustering RMSD at 4.0.

Solvent Accessible Surface Area (SASA) and Interface Property Calculation

Solvent accessible surface area of the protein complexes (FKBP12 drug bound/free and FRB domain of mTOR) was calculated by GETAREA server **[29],** protein interfaces was calculated by Aquaprot **[30]** and interface properties were calculated by 2P2I inspector **[31]** online server.

Results & Discussion:

Evident from docking (rerank) scores, it was interesting to note that all the similar compounds identified against parent compound had higher binding affinity against FKBP12 protein in comparison to their respective parent compounds. Further, compound (PubCid: 57254959) akin to Everolimus showed highest affinity against the FKBP 12 amongst all the compounds (parent and similars) undertaken in this study. Everolimus similar (PubCid: 57254959) showed 1.50 folds higher affinity than its parent compound and 1.73 folds better affinity than conventional 'rapamycin'. The docking scores of parent and their respective similars are provided in **Table 1 (see supplementary material)**.

In the further analysis we investigated the rationale behind the high affinity of Evorilumus similar against FKBP12. Molecular insights revealed that the internal ligand interactions of Evorilumus similar with FKBP12 was 2.6 folds higher than rapamycin- FKBP12 interactions and approximately 1.2 folds higher than Evorilumus- FKBP12 interactions. As shown in **Table 1**, the higher binding affinity of Evorilumus similar can further be attributed to higher hydrogen bonding potential along with long and short range electrostatic interaction. We later investigate that, in the cavity of FKBP12 34 amino acid residues interacted with Evorilumus similar while only 26 and 28 residues of FKBP12 interacted with rapamycin and

Evorilumus which further testifies the better binding affinity of Evorilumus similar than its parent compound Evorilumus and rapamycin. The similar compounds retrieved against each parent were further tested for their *in silico* ADMET profile and solubility properties **Table 2 (see supplementary material)**.

Except for Temsirolimus similar (PubChem ID: 10167669) the entire similar compounds retrieved, demonstrated appreciable pharmacological profile. In particular, Everolimus similar (PubChem ID: 57284959) exhibited better pharmacological profile than any of the similar compounds retrieved.

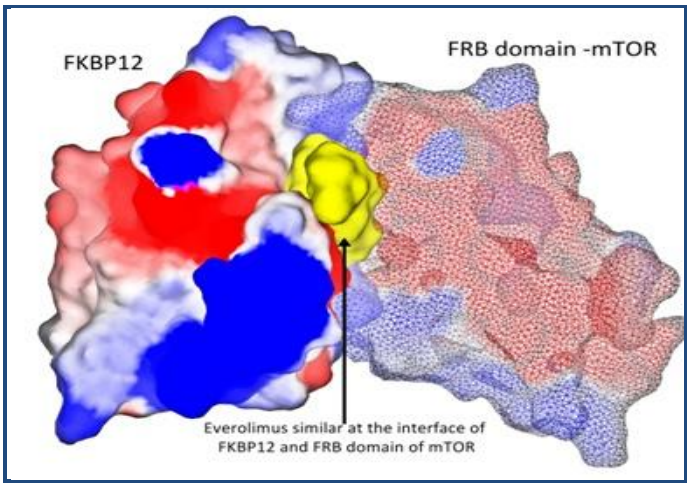

Figure 4: Electrostatic surfaces of FKBP 2(Solid) and FRB domain of mTOR (mesh) in FKBP12-mTOR complex. Everolimus similar (PubChem ID: 57284959) (solid yellow) is bound between the interfaces

The key issue that rapamycin fails to form an ideal mTOR inhibitors can be attributed to its poor oral absorption and lower solubility. As shown in **Table 3 (see supplementary material)**, rapamycin falls short in demonstrating acceptable aqueous solubility and oral absorption in the gastro-intestinal tract. In addition, rapamycin also shows poor solubility coefficients for hexadecane/gas partition and octanol/gas partition. Owing to these serious drawbacks, rapalogs were discovered bestowed with better absorption and solubility properties. Rapalogs- Everolimus, Temsirolimus and Deforolimus which are derivatives of rapamycin were anticipated to overcome the solubility issues. However, in our *in silico* analysis, although rapalogs although had good pharmacological profile, nevertheless suffered solubility concerns like sub optimal hexadecane/gas and octanol/gas partition coefficients. The structures screened, similar to parent compounds were successful in overcoming the given solubility concerns. Interestingly, Everolimus similar compound (PubChem ID: 57284959) even showed better solubility properties than the compounds similar to Temsirolimus and Deforolimus. Evident from protein-protein docking studies, the FKBP12-mTOR interactions are efficiently increased in presence of inhibitors as compared to ligand free FKBP12, thus implying there occurs a strong FKBP12-mTOR interactions in presence of inhibitors. It is interesting to note that, all the similar compounds had superior inhibitory potential than their parent compounds. In particular, the FKBP12-mTOR interactions enhanced in presence of Evorilumus similar compared to remaining the compounds (parent and their respective similar) undertaken in the study. Everolimus similar was efficient to enhance FKBP12-mTOR interactions by 1.92 folds than conventional "rapamycin" and by 1.72 folds than its parent - Everolimus. The patch dock scores (protein-protein docking scores) of FKBP12-mTOR interaction in presence of inhibitors and their respective akin is shown in **Table 4 (see supplementary material)**.

Table 5 (see supplementary material) shows the various molecular interactions of compounds against both FKBP12 and FRB domain of mTOR. In terms of van der Waals contacts, electrostatic interactions and hydrogen bond interactions in a both FKBP12 and FRB domain, Evorilumus similar stands as a high affinity compound against these two proteins implying Evorilumus similar brings about best interactions between FKBP12 and mTOR

In order to figure out the molecular rationales of enhanced affinity of FKBP12 to mTOR in presence of Everolimus similar as revealed from protein-protein docking results we further performed interface property calculations. Interface properties of FKBP12-mTOR complex in presence and absence of inhibitors is shown in **Table 6 (see supplementary material)**. From extensive interface properties calculations it is quite apparent that all the similar compounds brought about enhanced FKBP12-mTOR interactions than their respective parent compound. It is interesting to note that the concurrence between patch dock results and FKBP12-mTOR interface property calculations is supported by the fact that the total interface area in FKBP12-mTOR complex was 2.1 folds elevated in presence of compound Everolimus similar compared to the complex harboring its parent compound Everolimus. Likewise, it is also imperative to note that gap index and SASA was declined between FKBP12-mTOR in presence of Everolimus, indicating it to be the powerful and potential inhibitor undertaken in the existing study. The ratio of interface atoms to buried atoms was highest which in addition indicates that Evorilumus similar brings about far better interaction of FKBP12-mTOR than any other compound undertaken in the study.

Owing to superior inhibitory potential of Everolimus similar it was further mapped for its pharmacophoric properties. At the mTOR-FKBP12 interface and specifically in FKBP12, compound shows van der waals interaction with Val 55, Phe 46, Tyr 26, His 87, Ile 56, Ile 90, Ile 91, Leu 97 and Phe 36 and electrostatic interactions with Asp 37,Glu 54,Tyr 82, Ile 56, and Val55, hydrogen bonding interactions with Glu 121, Arg 125 and in the FRB domain of mTOR the compound interacts through van der waals with Thr 187, Arg 125, Gly 129, Tyr 194,Trp 190, Asp 191, Ser 124, Phe 128, Phe 197 and Glu 121 and electrostatic interactions with Ser 124, Trp 190 and Lys 184 . In addition, in the FKBP12 cavity, the compound forms H bonds with Ile 56 and Tyr 26 (**Figure 3a**). The solvent accessible surface area of Everolimus similar at the FKBP 12 and FRB is shown in **Figure 3b**. Electrostatic interactions of FKBP12 and mTOR in presence of Everolimus similar is shown in **Figure 4.**

In conclusion, together with molecular docking analysis, protein-protein patch docking, solubility analysis, ADMET predictions and interface property calculations has put forth Everolimus akin compound (PubChem ID: 57284959) to demonstrate and brings about strongest interaction between FKBP12 and FRB domain of mTOR.

Conclusion:
The current drugs that facilitate FKBP12 and mTOR interactions have been successful, nevertheless have known to demonstrate serious concerns like declined oral absorption property and suboptimal solubility. To overcome the narrow therapeutic window of the current drugs, we identified Everolimus similar compound PubChem ID: 57284959 to show appreciable drug like properties bestowed with better solubility higher oral bioavailability. In addition, this compound brought about enhanced interaction between FKBP12 and FRB domain of mTOR. Extensive investigations using molecular docking, ADMET predictions, solubility analysis, protein-protein docking and interface property calculations testifies Evorolimus similar to be superior inhibitor of mTOR pathway; however *in vitro* and *in vivo* experimental correlates are required to complement our observations.

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Supplementary material:

Table 1: MolDock algorithm aided docking of compounds in FKBP12 protein. PubChem ID: 57284959 akin to Everolimus shows highest affinity against the FKBP12.

Compound	MolDock Score	Rerank Score	Internal	H Bond	Electrostatic range	
					Long	short
Rapamycin	-110.209	-72.9045	-10.8754	-1.0106	0.252	0.185
Evorilimus	-142.95	-84.1997	-34.8749	-2.45121	0.356	0.291
Temsirolimus	-121.4	-82.5204	8.99076	0	0.485	0.174
Deforolimus	-117.551	-70.9914	5.64802	-1.37174	0.577	0.362
Echinomycin Similar	-171.56	-107.8956	-18.3279	-1.8975	0.46	0.265
Everolimus similar (PubChem ID: 57284959)	-174.95	-126.647	-28.9939	-2.63359	0.642	0.554
Temsirolimus similar (Compound 10167669)	-168.829	-114.109	6.92253	-2.5	0.436	0.622
Deforolimus similar (Compound 22770627)	-132.099	-98.7467	44.023	0	0.552	0.451
Echinomycin Similar (PubCd:6917949)	-171.25	-95.238	-12.325	-1.278	0.295	0.652

Table 2: ADMET prediction of virtually screened compounds by ADMETSAR server. All the virtually screened compounds predicted to be safe except for Temsirolimus similar compound (PubChem ID: 10167669) which showed Ames toxicity

	Everolimus similar (PubChem ID: 57284959)	Temsirolimus similar (PubChem ID: 10167669)	Deforolimus similar (Pub Cid: 22770627)	Echinomycin Similar (PubCd:6917949)
Absorption				
Blood-Brain Barrier	BBB-	BBB-	BBB-	BBB-
Caco-2 Permeability	Caco2-	Caco2+	Caco2+	Caco2+
P-glycoprotein Substrate	Substrate	Substrate	Substrate	Substrate
Renal Organic Cation Transporter	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
Distribution & Metabolism				
CYP450 2C9 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 Substrate	Substrate	Substrate	Substrate	Substrate
Excretion & Toxicity				
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	Weak inhibitor	Weak inhibitor	Weak inhibitor
AMES Toxicity	Non AMES toxic	AMES toxic	Non AMES toxic	Non AMES toxic
Carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens
Acute Oral Toxicity	III	III	III	III

Table 3: Predicted solubility properties of parent compounds and respective similars.

Solubility properties (Range 95% of Drugs)	Rapamycin	Everolimus	Deforolimus	Temsirolimus	Echinomycin	Everolimus similar (PubChem ID: 57284959)	Temsirolimus similar (PubChem ID: 10167669)	Deforolimus similar (Pub Cid: 22770627)	Echinomycin PubChem ID: 6917949
QP log P for hexadecane/gas (4.0 / 18.0)	21.52 *	25.58*	23.01 *	24.55 *	20.65*	15.03	15.61	17.02	19.85*
QP log P for octanol/gas (8.0 / 35.0)	38.05 *	42.24*	40.80 *	41.10 *	33.56	30.92	29.16	28.56	30.47
QP log P for	16.91	19.28	18.63	16.58	15.54	12.26	16.07	15.81	18.39

water/gas (4.0 / 45.0)									
QP log P for octanol/water (-2.0 / 6.5)	6.25	6.38	5.59	7.05 *	3.4	6.42	3.11	3.49	5.4
QP log S for aqueous solubility (-6.5 / 0.5)	-6.74*	-5.01	-1.58	-2.93	-5.88	-4.51	-3.98	-3.22	-4.6
% Human Oral Absorption in GI (<25% is poor)	16% *	48%	41%	44%	52%	62%	51%	54%	58%

* indicates does not fall in the range for the soluble properties as evaluated in 95% of available drugs.

Table 4: mTOR and FKBP12 protein docking scores evaluated by Surface Patch Matching (Patch Dock Server)

PROTEIN 1 FKBP12 bound to :	PROTEIN 2	Score	Transformation
Rapamycin	mTOR FRB -DOMAIN	5490	-0.36 -0.56 -3.11 40.78 -50.82 11.99
Everolimus		6081	3.13 -0.72 -1.54 -4.20 -5.68 4.29
Temsirolimus		5842	1.35 0.45 -1.09 8.77 0.92 1.44
Deforolimus		5021	-0.52 -1.20 -1.68 43.65 -12.35 21.52
Echinomycin		9854	-0.16 -1.22 -1.72 38.55 -15.25 28.45
Everolimus similar (PubChem ID: 57284959)		10534	-0.18 -1.14 -2.61 23.66 -54.55 12.43
Temsirolimus similar (PubChem ID: 10167669)		8546	-2.79 -0.16 2.45 51.31 -25.60 -15.44
Deforolimus similar (Pub Cid: 22770627)		7758	-3.11 -0.64 -1.18 -5.16 -16.32 -3.05
Echinomycin similar (Pub Cid: 6917949)		9656	-2.34 -0.56 -3.79 -8.65 -15.99 -7.5
FKBP12 unbound		4456	2.43 0.75 2.88 60.62 -11.85 -21.74

Table 5: Interaction profile of compounds in the binding pockets of FKBP12 and FRB domain of mTOR

	Interactions with FKBP12				Interactions with mTOR- FRB domain			
	Van der Waals Contacts (n)	**Electrostatic Contacts (n)**	**H Bonds (n)**	**σ/π-π interactions (n)**	**Van der Waals Contacts (n)**	**Electrostatic Contacts (n)**	**H Bonds (n)**	**σ/π-π interactions (n)**
Rapamycin	3 Trp 59, Phe 46, Ser 38	3 Val55, Ile 56, Asp 37	1 Ile 56	0	4 Ser 124, Tyr 127, Tyr 26, Gln188	2 Asp 191, Thr187	0	0
Everolimus	5 Phe 46, Phe 36, Ile 90, Ile 91,Gly 129	5 Lys 184, Asp 191, Tyr 26, Arg 42, Tyr 82	1 Ser 124	0	4 Gly 129, Tyr 193, Trp 190, Tyr 193	3 Lys 184, Ser 124, Asp 194	1 Tyr 26	0
Temsirolimus	3 Phe 46, Ile 91,Asp 37	3 Glu 54, Tyr 82, Ile 56	1 Ser 124	0	4 Phe 128, Tyr 193, Tyr 194, Gly 129	3 Glu 121, Ser 124, Trp 190	0	0
Deforolimus	4 Ile 91, His 87, Ile 90, Phe 99	3 Tyr 82, Ile 56, Val 55	0	0	4 Phe 197, Phe 128, Thr 187, Leu 120	2 Tyr 194, Trp 190,	0	0
Echinomycin	5 Phe 46, His 87, Tyr 26, Ile 90, Ile 91	4 Arg 131, Tyr 82, Asp 37, Tyr 26	1 Glu 121,	0	4 Gly 129, Phe 128, Tyr 127, Asp 191	2 Arg 131, Tyr 194	1 Tyr 26	0

PubChem ID: 57284959 (Everolimus similar)	9 Val 55, Phe 46, Tyr 26, His 87, Ile 56, Ile 90, Ile 91, Leu 97, Phe 36	5 Asp 37,Glu 54,Tyr 82, Ile 56, Val55	2 Glu 121, Arg 125	0	10 Thr 187, Arg 125, Gly 129, Tyr 194,Trp 190, Asp 191, Ser 124, Phe 128, Phe 197, Glu 121	3 Ser 124, Trp 190, Lys 184	2 Ile 56, Tyr 26	0
Temsirolimus similar (PubChem ID: 10167669)	4 His 87, Ile 91, Asp 37,Phe 36	3 Val 55, Asp 191, Asp 37	0	0	3 Tyr 193, Trp 190, Ser 191	2 Ser 124, Trp 190,	1 Ile 56	0
Deforolimus similar (Pub Cid: 22770627)	4 His87, Tyr 26, Phe 46, Ile 90	3 Tyr 82, Ile 56, Arg 131	1 Arg 57	0	3 Phe 128, Tyr 194, Trp 190	2 Arg 131, Ser 124	0	0
Echinomycin Similar (Pub Cid: 6917949)	4 His 87, Ile 90, Ile 91, Phe 36	4 Arg 131, Asp 37, Tyr 82, Glu 54	1 Arg 125	0	3 Tyr 127, Ser 191, Thr 187	2 Arg 131,Ser 124	1 Tyr 26	0

Table 6: Interface property calculations in ligand free and ligand bound states of FKBP12 and FRB domain of mTOR

	Total Interface Area (Å²)	**Gap volume (Å³)**	**Gap Index (Å)**	**SASA (Å²)**	**Interface atoms**	**Buried atoms**	**Ratio interface/buried residues**
FKBP12 –mTOR UNBOUND STATE	421.1	1666.2	3.95678	6051	962	666	1.444
FKBP12-mTOR BOUND TO:							
Rapamycin	532	1432.2	2.69211	6016	1044	584	1.788
Everolimus	733.2	1132.6	1.54474	5981	1114	514	2.167
Temsirolimus	709.2	1221	1.72166	5941	1100	528	2.083
Deforolimus	639.7	1253.6	1.95967	6016	1118	510	2.192
Echinomycin	1156.2	685	1.68788	5882	1124	504	2.230
PubChem ID: 57284959 (Everolimus similar)	1492.8	728	0.48767	5028	1227	401	3.060
Temsirolimus similar (PubChem ID: 10167669)	1351.6	732.2	0.54173	5834	1150	478	2.406
Deforolimus similar (Pub Cid: 22770627)	1277.5	794	0.62153	5805	1190	438	2.717
Echinomycin Similar (Pub Cid: 6917949)	1121.2	710	1.57	5887	1129	499	2.26