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Searching for potential mTOR inhibitors: Ligand-based drug design, docking and molecular dynamics studies of rapamycin binding site☆

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ABSTRACT

The PI3K/Akt/mTOR pathway is an important intracellular signaling pathway in cell cycle regulation and its dysregulation is associated with various types of diseases. mTOR (mechanistic or mammalian target of rapamycin) is the main enzyme that performs intermediate control of the signaling pathway through a phosphotransfer process. The classical inhibition of the mTOR pathway is effected by rapamycin and its analogous blocking allosterically the catalytic phosphorylation site, avoiding the deleterious side effects induced by ATP-competitive inhibitors. We employed ligand-based drug design strategies such as pharmacophore searching and analysis, molecular docking, absorption, distribution, metabolism, excretion and toxicity (ADMETox) properties filtering, and molecular dynamics to select potential molecules to become non-ATP competitive inhibitors of the mTOR complex. According to our findings, we propose eight novel potential mTOR inhibitors with similar or better properties than the classic inhibitor complex, rapamycin.

1. Introduction

The PI3K/Akt/mTOR pathway is an important intracellular signaling pathway in cell cycle responsible for regulating multiple nutritional and environmental factors, including growth factors, energy levels, cell stress and amino acids. However, its dysregulation has been implicated in major diseases such as cancer, metabolic disorders, neurological diseases and inflammation. The translation of these signals leads to the promotion of cell growth by substrates' phosphorylation triggering anabolic processes such as translation of messenger ribonucleic acid (mRNA) and lipid synthesis or by limiting the catabolic processes such as autophagy. Briefly, the dynamic of this pathway resides in the activation of phosphatidylinositol-3 kinase (PI3K) by extra/intracellular substrates that phosphorylates and activates protein kinase B (Akt), which is located on the plasma membrane. In addition, Akt is also able to activate the mTOR (mechanistic target of rapamycin) [1–5].

The mTOR – formerly called before being recognized as highly conserved among eukaryotes as a mammalian target of rapamycin or FK506-binding protein 12-rapamycin-associated protein 1 (FRAP1) – is a 289 kDa serine/threonine kinase (EC 2.7.11.1) belonging to the phosphatidylinositol-3 kinase (PI3K) family. It is the main enzyme responsible for performing an intermediate control of the signaling pathway through a phosphotransfer process using adenosine triphosphate (ATP) as a phosphate donor [1,6,7].

Structurally, mTOR kinase is composed of 2549 amino acid residues (289 kDa) and organized into five distinct functional domains, (i) 32 replicates called "Huntington, elongation factor 3, PR65/A, TOR" (HEAT) (from amino acid residue 16 to 1345); (ii) domain "FRAP, ATM, TRRAP" (FAT) (from amino acid residue 1382 to 1982); (iii) region "FKBP12 rapamycin-binding" (FRB) (from amino acid residue 2012 to 2144); (iv) catalytic domain "kinase domain" (KD) and regulator "regulatory domain" (RD) (from amino acid residue 2182 to 2516); and (v) domain "FRAP ATM TRRAP carboxy terminus" (FATC) (from amino acid residue 2517 to 2549) [8].

This atypical kinase is present in two functional and structurally distinct complexes, mTORC1 and mTORC2, located in different cell compartments, each containing distinct and common subunits nec-

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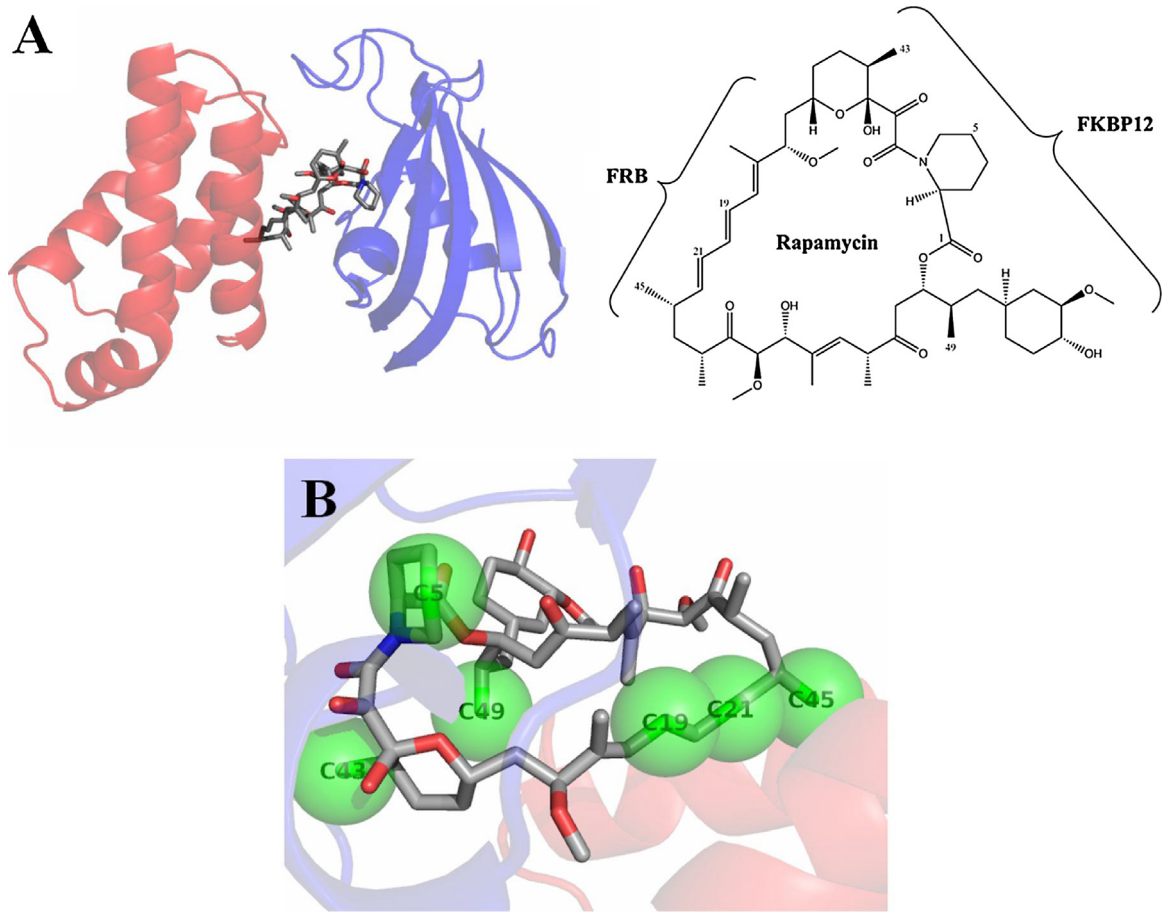

Fig. 1. Pharmacophore model employed for ZincPharmer search. (A) FKBP12-rapamycin-FRB complex. FKBP12 and FRB are drawn as cartoon and color in blue and red, respectively. The rapamycin molecule is shown as sticks and color by CPK. (B) The six hydrophobic pharmacophore points are colored in color in green and represented as sphere.
Elaborated using PyMol v.1.7.5.0 and ACD/ChemSketch Freeware.

essary for the catalytic activity and regulation of the main enzyme. The mTORC1 complex transmits signals of nutrient availability to control various cellular functions, while the mTORC2 complex regulates mainly the assembly of the cytoskeleton by actin [4,7–9].

The classical inhibition of the mTOR pathway is effected by rapamycin and its analogous, called rapalogs, which are considered the first-generation inhibitors [10–13]. The rapamycin, or sirolimus (Rapamune, Wyeth Pharmaceuticals), is a potent macrolide antibiotic produced by the microorganism *Streptomyces hygroscopicus* and it is widely used in therapy due to its immunosuppressive and antitumor properties [14]. However, because of their unfavorable properties, such as its low solubility in water and stability in solution, molecular analogues have been developed – the rapalogs – such as everolimo (RAD001, Afinitor, Novartis Pharmaceuticals), temsirolimus (CCI-779, Torisel, Wyeth Pharmaceuticals) and ridaforolimus (AP23573; formally called deforolimus, ARIAD Pharmaceuticals), which improved pharmacokinetic properties without altering the mechanism of action [12].

The inhibition mechanism of mTOR by rapamycin and its analogues occurs through the binding of the intracellular receptor of the FK506-binding protein 12 kDa (FKBP12), forming the rapamycin-FKBP12 complex. This association binds directly to the FKBP12-rapamycin binding (FRB) domain, blocking allosterically the catalytic phosphorylation site [7,15,16].

The rapalogs revealed efficacy, safety and tolerability of adverse events in inhibition of the mTOR pathway on treatment of many diseases [17,18]. However, the success of these first-generation inhibitors has shown flaws especially due to the feedback mechanism once that inhibition of the complex results in over activation of PI3K/Akt signaling and increases cell survival; a probable insensitivity of mTORC1 to rapalogs has recently been revealed, challenging the dogma that rapamycin completely inhibits the activity of mTORC1; alternative pathways and cross-pathways

Table 1
Pharmacophore features employed as query search on ZincPharmer.

Pharmacophore/class	X coordinates	Y coordinates	Z coordinates	Radius (Å)
C5 – Hydrophobic	−8.20	23.65	41.43	1.00
C19 – Hydrophobic	−12.38	26.13	34.19	1.00
C21 – Hydrophobic	−11.65	26.22	31.88	1.00
C43 – Hydrophobic	−10.86	29.29	43.53	1.00
C45 – Hydrophobic	−11.17	26.30	28.46	1.00
C49 – Hydrophobic	−8.74	29.35	38.66	1.00