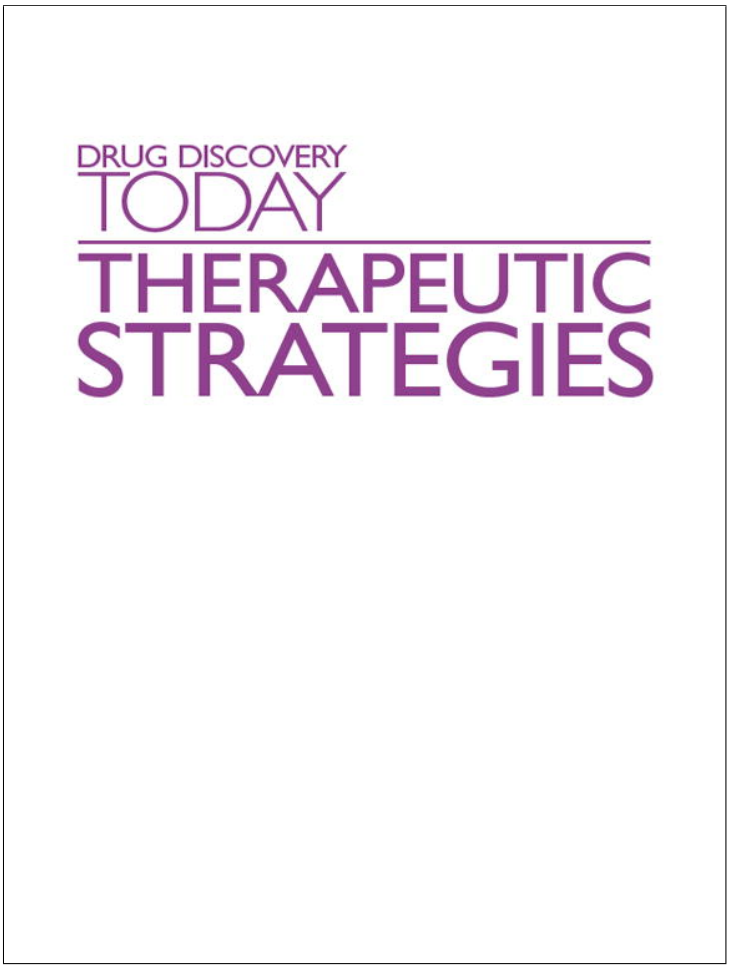

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Cancer

mTOR mediated anti-cancer drug discovery

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The mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase and the founding member of a signaling pathway that regulates many fundamental features of cell growth and division. In cells, mTOR acts as the catalytic subunit of two functionally distinct complexes, called mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2). Together, these complexes coordinate a variety of processes that include protein translation, autophagy, proliferation, survival and metabolism in response to nutrient, energy and growth factor signals. Consistent with its role as a growth-promoting pathway, numerous studies have found that mTOR signaling is hyper-activated in a broad spectrum of human cancers. In particular, mTORC2 is considered a primary effector of the phosphatidylinositol-3-kinase (PI3K) signaling pathway, which is mutated in a majority of human cancers, in part through its ability to phosphorylate and regulate the proto-oncogene Akt/PKB. Many biological functions of mTOR have been pharmacologically explored using the natural product rapamycin, an allosteric inhibitor that has been reviewed extensively elsewhere. This review will focus specifically on the development of small molecule ATP-competitive inhibitors of mTOR and their prospects as a targeted therapy.

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Introduction of mTOR signalling pathway

Rapamycin is a 31-membered macrocyclic lactone that was first developed as immunosuppressant by Wyeth in 1997 and more recently as an anti-cancer agent in the form of various analogs, often referred to as rapalogs. In complex with the small protein FKBP12, rapamycin binds to the FKBP12-rapamycin (FRB) domain of mTOR and inhibits its kinase activity through an allosteric mechanism that is still under investigation. Because of its exquisite selectivity, rapamycin has been an indispensable pharmacological probe for elucidating the biological functions of the mTOR serine/threonine kinase in governing cell growth and proliferation. However, a series of discoveries over the last decade have revealed a more complicated picture than initially suspected. Partly, this derives from the fact that mTOR serves as the catalytic subunit in two protein complexes, named mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2) with complementary but distinct roles [1]. Only mTORC1 is affected by acute rapamycin treatment, presumably because the FKBP12-rapamycin binding site is occluded in mTORC2. Both complexes are activated

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by growth factor signaling through the PI3K pathway, while mTORC1 is additionally regulated by nutrient and energy signals. Together, these complexes control a diverse array of processes that are required for basic cell growth, including protein translation, cell division, autophagy and cell survival.

In addition to mTOR, mTORC1 contains the large scaffolding protein Raptor (Regulatory associated protein of mTOR), the small WD-40 repeat protein mLST8, and two regulatory proteins named PRAS40 and DEPTOR [1,2]. In cells, this complex is regulated by a diverse collection of energy and nutrient signals. The majority of these are transmitted through three primary modes: the GTPase Rag (Ras related GTPase) proteins, which signal the availability of amino acids; the GTPase rheb (Ras homolog enriched in brain), which transmit signals from both growth factor and energy sensing pathways; and factors that signal to mTORC1 directly, through phosphorylation or protein–protein interactions [1,3,4]. In the absence of amino acids, mTORC1 is diffusely localized throughout the cytoplasm [3]. In their presence, the Rag proteins bind to Raptor and sequester mTORC1 on the surface of an endomembrane compartment. This re-localization is required, though insufficient to activate mTORC1, which additionally requires rheb. In its GTP-bound state, rheb binds to mTOR near its kinase domain and directly stimulates its activity [5]. Rheb, itself, is regulated by a large GTPase-activating TSC1/TSC2 heterodimer, which integrates inputs from many upstream signaling pathways that reflect PI3K signaling (Akt), MAPK signaling (Erk), energetic stress (AMPK) and hypoxic stress [4]. mTORC1 activity can also be modified by interactions with PRAS40 and DEPTOR, which provide additional inputs from PI3K, or by direct phosphorylation by kinases such as AMPK [1,2].

The best-understood mTORC1 substrates are the AGC-family S6-kinases (S6K1 and S6K2) and the 4E-BP1/2 proteins, both of which are involved in the regulation of protein translation. S6K phosphorylates the S6 ribosomal subunit, components of the eIF4F cap-binding complex and the kinase eEF2K [6]. 4EBPs, when dephosphorylated, bind to the eIF4E mRNA cap-binding protein and prevent its assembly into the eIF4F cap-binding complex, thereby blocking cap-dependent translation. In most cell types, complete inhibition of mTORC1 suppresses overall protein translation by approximately 50% [7,8]. However, different capped mRNAs have different dependencies on eIF4F for efficient translation. For instance, translation of cyclin D mRNA requires eIF4F, while mRNA encoding the cdk inhibitor p27/Kip1 does not [6,9]. This dependency is determined partly by the degree of secondary structure in the 5′ UTR, which eIF4F can untangle, and partly by the presence of internal ribosome entry sites (IRES) that offer an alternate method for directing mRNAs to ribosomes. Thus, the mTORC1-dependent decrease in overall translation does not simply affect all mRNAs equally, but rather leads to a fundamental shift in the translational program.

Inhibition of mTORC1 also activates the cellular recycling program known as autophagy (self-eating). Autophagy is induced under starvation conditions, and involves the formation double-membrane vesicles called autophagosomes that can engulf both cytoplasmic contents and damaged organelles. Autophagosomes then fuse with lysosomes, leading to degradation of their contents and providing the cell with an additional source of nutrients. mTORC1 normally suppresses autophagy by phosphorylating two proteins, the serine/threonine kinase ATG1 and the regulatory protein ATG13 [10]. In the absence of phosphorylation, ATG1 and ATG13 associate to form an activated complex that is capable of initiating autophagy. Prolonged starvation and activation of autophagy leads to progressive cellular atrophy that is sometimes referred to as autophagic cell death. However, it is debated whether autophagy promotes this process or simply reflects the cell's attempt to overcome a lack of sufficient nutrients.

Considerably less is known about mTORC2. Instead of Raptor, this complex contains the large scaffold protein Rictor (Rapamycin-insensitive companion of mTOR), as well as mLST8, mSIN1, DEPTOR and PROTOR. mTORC2 is activated by PI3K signaling through an unknown mechanism, and, in turn, phosphorylates the AGC kinases Akt/PKB and SGK1 at a C-terminal site known as the hydrophobic motif (HM) [11]. The effect of this phosphorylation event is different for the two kinases: Akt/PKB requires it for maximal, but not basal activity, while SGK1 requires it for all activity [12]. In both cases, HM phosphorylation influences the activity of the kinase by regulating the phosphorylation of the activation loop motif by the kinase PDK1. The finding that mTORC2 might be required for only hyperactive Akt/PKB activity has particularly interesting consequences in animals. In flies, deletion of the mTORC2 components Sin1 or Rictor has only minor effects on normal organismal growth [13]. By contrast, it was clearly required for phenotypes caused by hyperactive PI3K signaling. Expression of a constitutively active PI3K mutant or inactivation of PTEN in flies where mTORC2 is functional causes severe hyperplasia, but not in flies where mTORC2 had been inactivated [13]. The same situation holds true in mice. Conditional deletion of PTEN in the prostate causes the development of tumors in a manner that closely mimics the progression of the human disease [14]. Concurrent deletion of Rictor prevents tumor formation and while having little impact on normal tissue. The conclusion that mTORC2 might exclusively mediate hyperactive, and not basal, PI3K activity indicates that it might be uniquely required for the development of PI3K/PTEN driven tumors while being dispensable for other adult tissues.

Despite its original classification as rapamycin-insensitive, it is now known that mTORC2 is affected by the drug in two important ways. The first is that prolonged exposure to rapamycin can, after all, inhibit mTORC2 in some but not

all cell types, apparently by interfering with the assembly of the complex [15]. The factor(s) that determine this phenomenon are still unknown. The second is that rapamycin-dependent inhibition of mTORC1 suppresses a negative feedback loop that normally dampens growth factor signaling to PI3K [14]. As a result, rapamycin treatment frequently causes hyper-activation of PI3K and its downstream effectors, including mTORC2. The combination of these conflicting effects has complicated the interpretation of how rapamycin might work as an anti-cancer therapeutic, which have been modest for the majority of cancers that have been tested. It is unclear whether the rapamycin sensitivity of a particular cancer is primarily determined by the degree of mTORC2 inhibition, the degree of PI3K hyper-activation, or neither. Recently, work from our lab [7] and others [16,17] has introduced a third complicating factor. Using ATP-competitive mTOR inhibitors, we found that rapamycin is only a partial inhibitor of mTORC1 in most cell types, and suggest that this, too, might contribute to the varied response across different cancers (Fig. 1).

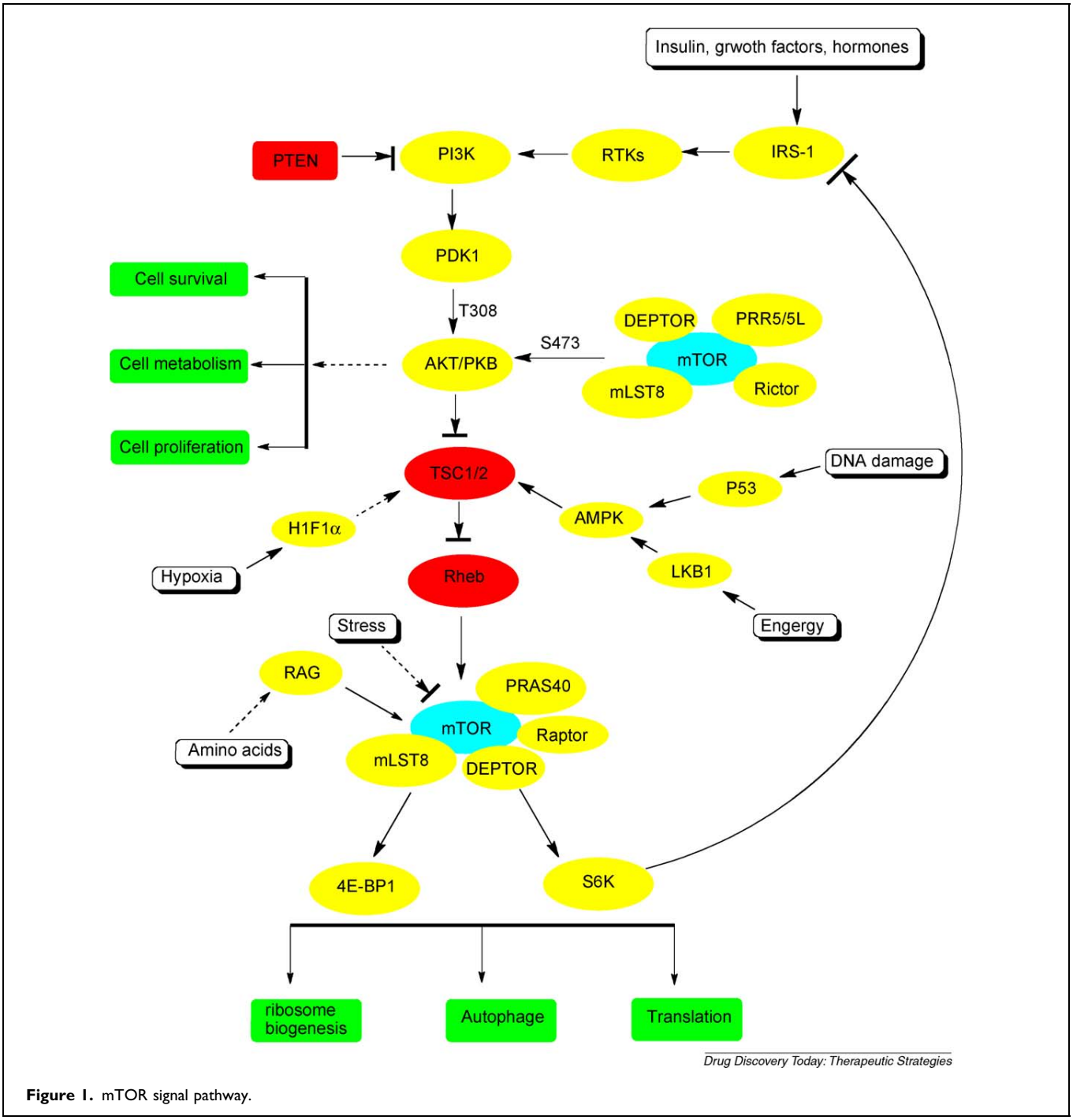

Figure 1. mTOR signal pathway.

Rapamycin and rapalogs

Rapamycin was originally identified as an antifungal compound in 1975, later discovered to have anti-tumor activity in 1984, and eventually commercialized as immunosuppressant by Wyeth in 1997. More recently, there has been a renewed interest in its anti-cancer properties, which has sparked the development of several analogs that are collectively referred to as 'rapalogs.' Rapamycin has very poor water solubility, severely limiting its bioavailability [18]. Thus, many of the rapalogs, such as Temsirolimus (CCI-779), Everolimus (RAD001) and Ridaforolimus (AP23573), were developed to improve the pharmacokinetics properties and to establish independent intellectual property positions. Temsirolimus is a prodrug of sirolimus that contains a dihydroxylmethyl propionic acid ester moiety at C-40-*O* position, which is hydrolyzed quickly after intravenous administration. In the case of Everolimus (Novartis), a hydroxylethyl group was introduced to C-40-*O* position to improve water solubility. Finally, Merck and Ariad are collaboratively developing Ridaforolimus, which bears a dimethyl phosphate group at C-40-*O* position. Many of these are currently undergoing clinical trials for anti-cancer efficacy. Ridaforolimus is currently undergoing phase III clinical trials for a variety of cancers that include advanced malignancies such as metastatic soft tissue and bone sarcoma and relapsed hematological malignancies. Everolimus, which was originally introduced as an immunosuppressant, was recently approved for the treatment of advanced renal cancer. The chemical modifications to each of these rapalogs preserve their interactions with both FKBP12 and mTOR, and therefore all act in a fundamentally similar mode [19].

Surprisingly, and in contrast to *in vitro* results, the clinical success of rapamycin has been limited to a few rare cancers, including mantle cell lymphoma, renal cell carcinoma and endometrial cancer. Efficacy against other major solid tumors has been limited. The most popular explanations for the limited efficacy of rapamycin are that rapamycin is only effective in tumors where it is also capable of inhibiting mTORC2, rapamycin concurrently causes hyper-activation of PI3K signaling by disabling a negative feedback loop thereby undermining its anti-proliferative effect on mTORC1, and that rapamycin is only a partial inhibitor of mTORC1 kinase activity. It is currently unclear whether any or all of these explanations account for this limited clinical efficacy. However, the recent development of potent and selective ATP-competitive mTOR kinase inhibitors that circumvent most of these limitations will allow pharmacological validation of the effect of complete mTOR inhibition in the clinic.

Under the expectation that additive or synergistic effects might induce a cytotoxic response, rapalogs have also been tested in combination with standard chemotherapies, receptor tyrosine kinase (RTK) targeted therapies and angiogenesis inhibitors [20]. Accumulating evidence suggests that combinations with chemotherapeutics such as paclitaxel and cisplatin can induce a stronger apoptotic response and enhanced anti-tumor efficacy compared with single-agent therapy [21]. Combinations of rapalogs with RTK inhibitors like sutent and sorafenib are currently under clinical investigation. Rapalogs have also been administered in combination with estrogen antagonists such as tamoxifen and raloxifene in resistant breast cancers and appear effective in preclinical models [22]. The conclusions from these preliminary investigations are that specific combinations can induce a potent cytotoxic effect and induce the tumor regression in contrast to single agent rapalog treatment which at best induces disease stabilization.

ATP-competitive inhibitors

The development of ATP-competitive mTOR inhibitors has historically been neglected because it was difficult to imagine an ATP-competitive inhibitor competing with the potency and selectivity of the rapalogs. Several factors converged to change this. First was the discovery of mTORC2 complex and its insensitivity to acute inhibition by rapamycin. Second was the discovery that combinations of rapamycin and ATP-competitive PI3K inhibitors or dual-mTOR/PI3K ATP-competitive compounds like PI-103 resulted in a substantially more anti-proliferative and anti-tumor response when compared to single agent [23]. Third was a greater awareness of rapamycin's ability to 'rev-up' the PI3K/Akt axis by suppressing the negative feedback loop between mTORC1 and PI3K/Akt. Pharmacologically the development of ATP-competitive mTOR inhibitors was facilitated because of the tremendous efforts expended to develop PI3K inhibitors, initially targeting PI3Kγ [24] for inflammatory conditions and later targeting PI3K, especially PI3Kα because of its central role in controlling cellular growth and the prevalence of PI3Kα mutations [25]. Through kinase selectivity profiling and cellular assays it was discovered that several of these PI3K inhibitors were in fact dual PI3K/mTOR inhibitors with founding examples being PI-103 [26] and NVP-BEZ235 [27].

PI3K and mTOR dual inhibitors

On the basis of amino acid sequence similarity, mTOR is classified as Class IV PI3K family member. This family is a subset of the 6-membered PIKK family of kinases, which is divided between the phosphatidyl inositol kinases including class 1, class 2 and class 3 and the protein kinase class IV which includes ATM, ATR, DNA-PK, SMG and mTOR. The first PI3K kinase inhibitor which was recognized to also inhibit mTOR in an ATP-competitive fashion was the morpholino quinazoline derivative PI-103 which was discovered using high-throughput screening followed by medicinal chemistry campaign by Astella's Pharmaceutical in Japan [26]. It was shown that PI-103 could inhibit biochemical

kinase activity of mTOR (IC_{50}: 20–83 nM), PI3K (IC_{50}: α, 3.6 nM; β, 3.0 nM) and DNA-PK (IC_{50}: 2.0 nM). PI-103 could exert anti-proliferative activity against a variety of transformed cell lines such as melanoma, cervix, lung and breast. In a combination with the ATP-competitive EGFR inhibitor erlotinib, PI-103 enhanced efficacy against an EGFR-dependent PTEN-mutant glioma tumor model [28]. Although PI-103 has not been evaluated in the clinical trial because of poor 'drug-like' properties, it has served as a lead compound for other PI3K and mTOR selective inhibitors such as GDC-0941 [29].

An understanding of the binding mode of ATP-competitive inhibitors has to a large extent been elucidated by the pioneering efforts to crystallize numerous inhibitors with PI3Kγ by Rodger William's research group. The binding mode observed crystallography was quite similar to the well established mode exploited by ATP-competitive inhibitors that target protein kinases (Fig. 2). The binding pocket can be roughly divided into three zones: a central part normally occupied by the adenine-ring of ATP with two potential hydrogen bonds to the kinase 'hinge' region (Val882 N–H and Glu880 C=O) [30], a hydrophobic back pocket that is typically exploited to obtain potency and selectivity, and a forward pocket that exits to solvent. The crystal structure of the morpholine-chromenone class of structures as exemplified by LY294002 demonstrates that the morpholine oxygen forms a hydrogen bond with the Val882 N–H, whereas the chromenone scaffold occupies the adenine-binding site and the phenyl ring is directed out towards solvent. Consistent with the weak potency of this inhibitor (cellular IC50: 1.4 μM) [31], the hydrophobic back pocket is not occupied. Molecular modeling in conjunction with the X-ray structure of GDC0941 suggests that PI-103 also exploits its morpholine oxygen as hinge binder but in contrast to LY294002 its phenol moiety extends to the hydrophobic back pocket with the phenol forming a hydrogen bond with residue Asp 841 where it greatly contributes to potency and selectivity [32]. As discussed further below, a variety of selective mTOR inhibitors including WAY-001, WYE-354, WAY-600, WYE-687, Wyeth-BMCL-200910075-16b, Wyeth-BMCL-200910096-27, KU0063794 and KU-BMCL-200908069-5 were developed using PI-103 as a lead compound demonstrating that it is possible to achieve selectivity for mTOR relative to PI3K. Interestingly GDC-0941, an inhibitor that exhibits selectivity for PI3K over mTOR, was also developed starting with PI-103 as a lead compound, which demonstrates that the reciprocal selectivity profile can also be achieved.

NVP-BEZ235, which contains a tricyclic imidizo[4,5,c]quinoline core scaffold, was developed by Novartis as a pan PI3K and mTOR inhibitor (IC_{50}: α, 4 nM; β, 75 nM; γ, 7 nM; δ, 5 nM; mTOR,20 nM) [27]. By analogy with other quinoline-derived kinase inhibitors[33], BEZ235 was proposed to form a hydrogen bond with the kinase hinge using the quinoline nitrogen atom [34]. Consistent with studies that demonstrate superior anti-proliferative and glioma xenograft efficacy from simultaneous inhibition of PI3K and mTOR [35], NVP-BEZ235 also displays enhanced activity relative to rapamycin-derived mTOR or PI3K-only inhibition. For example NVP-BEZ235 displays better anti-proliferative activity than the

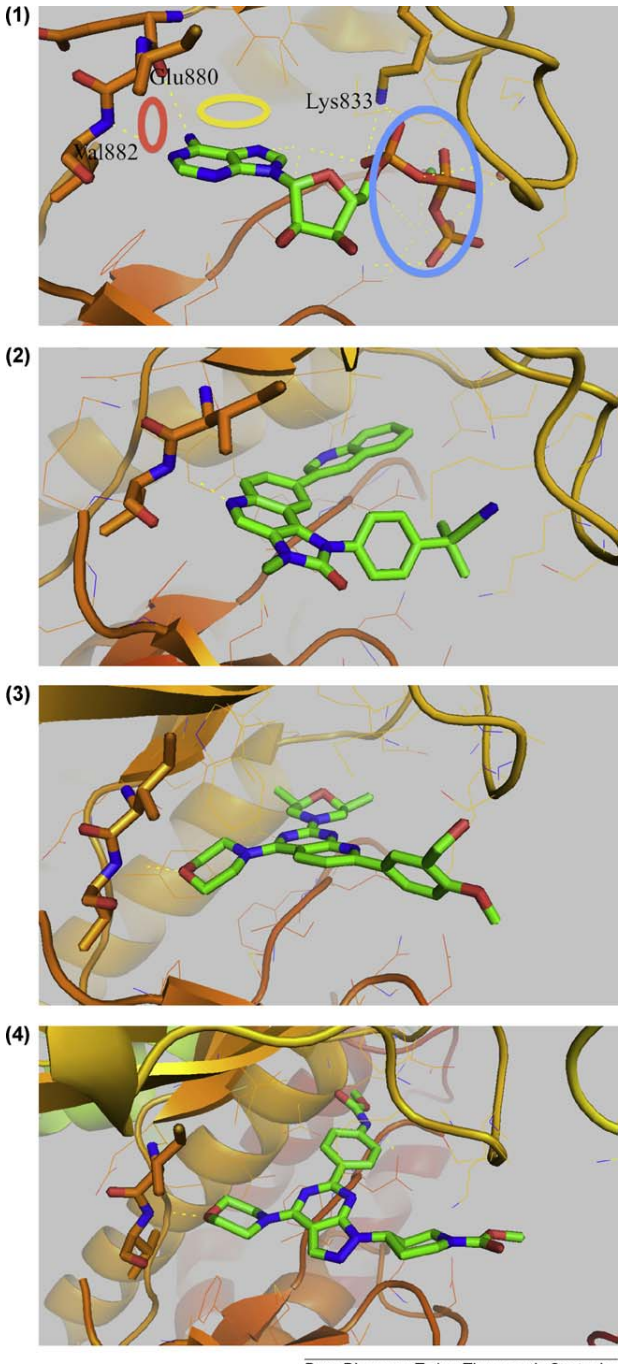

Figure 2. ATP co-crystal structure with PI3Kγ (shown in orange ribbons) (1) and modeling binding mode of BEZ-235 (2), Ku-0063794 (3), WYE-354 (4) (shown in green stick figure, carbon green, nitrogen blue and oxygen red) to PI3Kγ.

rapalog Everolimus against a panel of 21 different cancer lines [27]. BEZ235 is active in many tumor xenografts upon once daily dosing of 40 mg/kg [27]. NVP-BEZ235 is currently in phase I/II clinical trials for numerous cancers and there is a rapidly expanding literature using this compound as a pharmacological probe of Pi3K-dependent phenomena.

XL-765, a PI3K/mTOR dual inhibitor, bearing a quinoxaline scaffold was developed by Exelixis [36]. Detailed biological characterization for this compound is not yet released, it is currently undergoing phase I clinical trials now both as a single agent and in combination with chemotherapy such as temozolomide and ATP-competitive EGFR inhibitor erlotinib [37].

The pyridylsulfonamide GSK2126458 is a pan PI3K family kinase inhibitor with biochemical Ki's of 0.019 nM (PI3Kα), 0.13 nM (PI3Kβ), 0.024 nM (PI3Kδ), 0.06 nM (PI3Kγ) and 0.18; 0.3 nM (mTORC1/2). It also potently inhibits the PIKK family kinase DNA-PK (IC_{50} = 0.8 nM) and VPS34 (IC_{50} = 8 nM) but is selective relative to a panel of 240 protein kinases. GSK2126458 exhibits anti-proliferative effects against HCC1954 and T-47D cells that correlate with the induction of the caspase 3/7 activity in a time- and concentration-dependent manner. In human BT-474 tumors xenografts, a 0.3 mg/kg dose resulted in a profound and sustained pharmacodynamic response over 10 h. Currently the GSK2126458 is under phase I clinical trial in solid tumors and lymphoma [38].

mTOR selective inhibitors

Recently several selective ATP-competitive mTOR inhibitors have been reported. The pyridinonequinoline compound Torin1, which was discovered from high-throughput screen followed by medicinal chemistry optimization is as a highly potent and selective mTOR kinase inhibitor [7]. The biochemical kinase assay demonstrated that Torin1 was an ATP-competitive inhibitor and inhibited both mTORC1 and mTORC2 complexes equally with IC_{50} of 2–10 nM. Using mouse embryonic fibroblasts (MEF) Torin1 was demonstrated to potently inhibit the phosphorylation level of downstream mTORC1 and mTORC2 substrates S6K1 and S473 of Akt with IC_{50} of 2–10 nM. The selectivity among other structurally related kinases like PI3K, DNA-PK and ATM against mTOR was determined to be at least 200-fold. Broad spectrum screening against a panel of 353 kinase using the Ambit approach did not reveal any significant off-targets.

The pyrazolopyrimidine analogs PP242 and PP30 were discovered by Shokat and co-workers through their group's efforts to annotate the selectivity of numerous PI3K inhibitory scaffolds (Fig. 3) [16]. In biochemical assays, PP242 and PP30 inhibited mTOR with IC_{50}s of 8 nM and 80 nM, respectively, and were selective relative to other PIK kinases with the exception of P110γ (IC_{50} = 102 nM to PP242). Perhaps because of the kinase binding mode of the pyrazolopyrimidine core structure being very similar to that of the adenine-ring of ATP, PP242 also inhibits several protein kinases including PKC, RET and JAK2 kinases. Further medicinal chemistry effort on this scaffold series resulted in the identification of compounds such as PP487 and PP121, which are potent inhibitors of mTOR and several tyrosine kinases such as Abl, Hck, VEGFR2 and PDGFR [39]. This finding is interesting because therapeutically it maybe advantageous to be capable of simultaneously inhibiting receptor tyrosine kinase signaling and mTOR.

KU0063794 (AstraZeneca) that contains a morpholino pyridinopyrimidine core structure, and was presumably developed using PI-103 as a lead compound, is a highly potent and selective mTOR inhibitor with an IC_{50} of 10 nM [17]. Ku0063794 has excellent selectivity relative to all the PI3Ks and lipid kinases and a broader screen against a panel of 76 proteins kinases also did not reveal any significant cross activities. KU-BMCL-200908069-1, a bis-morpholino triazine has an IC_{50} of 270 nM against mTOR and is selectively relative to PI3K and ATM, was discovered via high-throughput screening. Further medicinal chemistry optimization to replace the metabolically undesirable aryl hydrazone with an imidazole, afforded KU-BMCL-200908069-5 as a potent and selective mTOR inhibitor with an enzymatic IC_{50} of 21 nM against mTOR without inhibiting PI3K ($IC_{50} > 10$ μM). Unfortunately, Ku-BMCL-200908069-5 failed to inhibit the proliferation of U87MG cells (GI_{50}: 24 μM), which maybe a consequence of poor cell permeability of the phenol [40].

Wyeth reported a series of selective mTOR inhibitors derived from a morpholino pyrazolopyrimidine scaffold that are also structurally related to PI-103 [41]. Starting from the lead compound WAY-001 which possessed an IC_{50} of 220 nM against mTOR, further derivatization resulted in the identification of WAY-600, WYE-687 and WYE354 which possessed biochemical IC_{50}s of 9, 7 and 5 nM, respectively, against mTOR. These compounds displayed impressive selectivity to PI3K families (>100-fold to PI3Kα and >500-fold to PI3Kγ) and did not exhibit significant activity when tested against a limited panel of 24 protein kinases. These inhibitors exhibit anti-proliferative activity against cancer cell lines like U87MG, MDA-MB-361 and MDA-MB-468 in 0.3–1 μM range. In a PTEN-null U87MG glioma xenograft model, WYE354 displayed a dose-dependent suppression of tumor growth at a 50 mg/kg with twice-daily dosing without any significant observed adverse effect. Unfortunately, the poor physical properties of these compounds prevented their further clinical development. Introducing isosteres of morpholine or phenol ring in PI-103 has been demonstrated to be beneficial to both the potency and selectivity towards mTOR [42]. Compared to the structure of GDC-0941, incorporation and 8-oxa-3-azabicyclo[3.2.1]octane at the 4-position and removal of methyl sulfonyl piperazine moiety from the thiophene 2-position resulted in Wyeth-BMCL-200910075-9b, which exhibited an IC_{50} of 0.7 nM against mTOR and 1856-fold selectivity relative

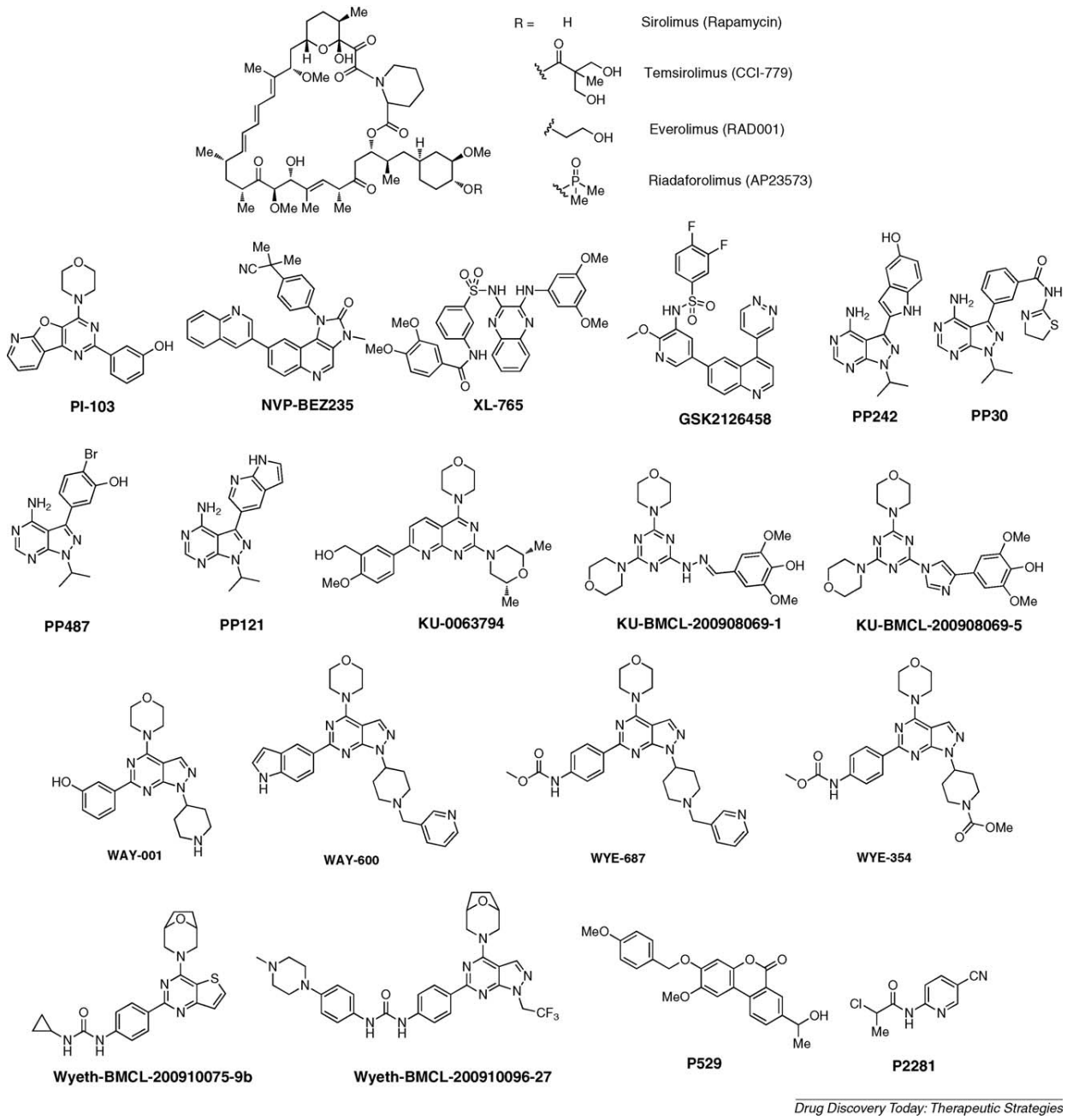

Figure 3. Structures of known mTOR inhibitors.

to PI3K, and an IC_{50} of 12 nM against LNCap cell proliferation. Further optimization to improve water solubility resulted in the identification of Wyeth-BMCL-200910096-27, which exhibits an enzymatic IC_{50} of 0.6 nM, a cellular IC_{50} of 25 nM and 230-fold selectivity to PI3Kα. Wyeth-BMCL-200910096-27 exhibited potent anti-proliferative activity against LNCaP (IC_{50} of 0.8 nM) and MDA468 (IC_{50} of 1.5 nM) cells. In addition, the pharmacological properties of the compound were improved [43].

INK-128 (structure not disclosed) is a potent and selective mTOR inhibitor developed by Intellikine, which exhibited an enzymatic IC_{50} of 1 nM against mTOR and more than 100-fold selectivity to PI3K kinases. In a ZR-75-1 breast cancer xenograft model, INK128 exhibited tumor growth inhibition efficacy at a dose of 0.3 mg/kg/day. When combined with other standard targeted therapy or chemotherapy such as sorafenib, sutent and paclitaxel, enhanced anti-tumor growth activity was observed. INK128 is reported to have

excellent physiochemical properties and is currently undergoing preclinical evaluation [44].

XL388 (structure not disclosed), developed by Exelixis, is reported to possess an IC_{50} of 9.8 nM against mTORC1 and an IC_{50} of 166 nM against mTORC2 in biochemical assays and was selective relative to a panel of 140 protein kinases ($IC_{50} > 3$ μM). In MCF-7 and Colo-205 tumor cell lines XL388 exhibited anti-proliferative IC_{50}s of 94 and 406 nM, respectively. Profiling anti-proliferative activity against a panel of 89 tumor cell lines revealed 14 cell lines that exhibited an IC_{50} of less than 1 μM, nine of which hematopoietic in origin. In a MCF-7 and colo-205 xenograft model, XL-388 demonstrated dose- and time-dependent inhibition of the mTOR signal pathway. Tumor growth was arrested nearly completely at 100 mg/kg PO bid dosage in both models. Synergistic effect has been observed when combining XL388 with carboplatin/paclitaxel and doxorubicin both in A2780 ovarian cancer cell lines and xenograft model [45].

Several other mTOR selective inhibitors from Astra Zeneca (AZD8055), and OSI pharmaceuticals (OSI-027) are currently in phase I or late preclinical testing but the biological profile of these inhibitors has not been disclosed [46].

Miscellaneous mTOR inhibitors

P2281, is a chloroacetamide-containing aminopyrimidine that was discovered by Piramal life science in 2008 in the course of searching anti-cancer compounds [47]. Although the molecular target of P2281 is unknown, it is capable of inhibiting cellular phosphorylation of S6K and 4E-BP1 without significantly inhibiting PI3K. P2281 has demonstrated efficacy in a murine colitis inflammation model.

P529 was obtained via a medicinal chemical effort focused on a dibenzo[*c*]chromen-6-one scaffold that possesses anti-estrogenic activity [48]. P529 is reported to be capable of inhibiting tumor cell growth, angiogenesis and vascular permeability through inhibition of S6K and Akt without binding to the estrogen receptor. The authors speculate that this compound maybe an allosteric mTOR inhibitor, the molecular mechanism of this compound is unclear.

Future direction

Accumulated evidence has demonstrated that mTOR is a key node in the PI3K/Akt/mTOR signalling pathway, which is arguably the most commonly activated pathway in human cancer. As such, there is a strong rationale for targeting mTOR therapeutically, especially in cancers that are known to carry alterations in PI3K signaling. Several hereditary cancer syndromes are reasonable candidates, such as Cowden's Syndrome (PTEN), Tuberous Sclerosis Complex (TSC1 or TSC2), and Peutz-Jeghers Syndrome (LKB1/STK11). Some sporadic cancers are also strongly associated with PI3K/mTOR pathway mutations as well. Among these, endometrial cancers, which often carry PTEN deletions, and mantle cell lymphoma, which is defined by over-expression of Cyclin D1/3, are already known to be clinically responsive to rapamycin therapy. However, these represent a relatively small proportion of cancers where PI3K/mTOR signaling is hyperactive. With the recent discoveries that mTOR-targeted ATP-competitive compounds are more complete inhibitors of both mTORC1 and mTORC2, there is reason to believe that they might be therapeutically effective in a broader range of cancers. Indeed, many major pharmaceutical companies are currently testing the hypothesis that ATP-competitive mTOR or dual-PI3K/mTOR inhibitors will be able to overcome the limited clinical responses that have been observed with rapalogs. Additionally, there are recent suggestions that mTOR inhibitors might act synergistically with RTK and MAPK inhibitors, potentially expanding their utility across a broader range of cancers.

An alternative strategy is to pursue the development of allosteric inhibitors that selectively target mTORC2 without directly affecting mTORC1. Genetic evidence indicates that disabling mTORC2 is sufficient to prevent the development of tumors driven by inactivation of PTEN. Although existing ATP-competitive mTOR inhibitors prevent mTORC2 activity, the concurrent inhibition of mTORC1 might introduce complications, including hyper-activation of PI3K signaling and possible deleterious effects to normal host tissue, that limit their therapeutic potential. Molecules that inhibit mTORC2 only would avoid these complications.

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