Dual Inhibitors of PI3K/mTOR or mTOR-Selective Inhibitors: Which Way Shall We Go?

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Abstract: The phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR signaling pathway is a central regulator in cell proliferation, growth, and angiogenesis. Inhibition of this pathway therefore is a major strategy for cancer chemotherapy. In order to induce the maximal therapeutic outcome in cancer treatment, vertical inhibition of the PI3K/AKT/mTOR pathway or horizontal inhibition of PI3K/AKT/mTOR and other kinases has been reported. In this review, we discuss the drug design and clinical development of dual inhibitors of PI3K and mTOR as well as the mTOR-selective inhibitors, classified based on the mechanism of action and the chemical structures. Structural determinants for increasing selectivity toward PI3Kα or mTOR are revealed from the structure-activity relationship of the reported inhibitors. Current clinical development in combination therapy of inhibitors involving in the PI3K/AKT/mTOR pathway is also discussed.

Keywords: PI3Kα, mTORC1, mTORC2, kinase inhibitors, selectivity, rapamycin, and cancer.

1. INTRODUCTION

Cancer is a public health threat with 7.6 million deaths (approximately 13% of all deaths) worldwide in 2008. The mortality rate of cancer in advanced countries such as U.S.A. is even higher: cancer-caused mortality rate in the U.S.A. was 23.1% of all deaths in 2006, ranking second only to cardiovascular diseases. Cancer is a generic term for a large group of diseases characterized by uncontrolled cell division and growth of abnormal cells. Cancerous cells can start in any organ or tissue: lung, stomach, liver, kidney, brain, colorectal, breast, skin, haematopoietic stem cells and inflammatory cells. The rapid proliferation of abnormal cells can also move beyond their organs of origin and spread to other organs, a process called metastasis.

The growth and metastasis of cancerous cells in many cases are regulated by more than one disease-modulating protein. The phosophoinositide-3-kinases (PI3K) and its downstream mammalian target of rapamycin (mTOR) are two proteins essential in regulating cancer growth and proliferation. PI3Ks phosphorylate the 3-hydroxy position of phosphatidylinositol 4,5-biphosphate (PIP_2), yielding phosphatidylinositol 3,4,5 triphosphates (PIP_3), an important second messenger coordinating the activity of PI3K downstream effectors AKT and mTOR. PIP_2 can be synthesized by phosphorylating the phosphatidylinositol-4-phosphate (PI4P) by PI4P 5-kinase. PI3Ks are divided into three classes (I, II, and III) based on their primary structures and downstream substrates. The activation of PI3K/AKT signaling triggers cell proliferation, growth, survival, tumor progression, invasion, angiogenesis, and metastasis. Aberrant PI3K/AKT pathway is an attribute for a panel of human cancers [1]. The PI3Kα coding gene (PIK3CA) is mutated, amplified and overexpressed in numerous human tumors. The activation of PI3K pathway is negatively regulated by the lipid phosphatase PTEN, and mutations in PTEN or loss of PTEN function or expression often occur in human cancers [2]. The oncogenic potential of PI3Kα makes it an attractive therapeutic target for cancer treatment. The PI3K/AKT/mTOR pathway (Fig. **1**) clearly shows that there is more than one upstream regulator that activates PI3K. Fig. (**1**) also indicates that there's more than one way to block cell growth and proliferation. The simultaneous inhibition of both PI3Kα and mTOR by two different drugs or a drug that dually inhibits both enzymes is called "vertical inhibition" because both enzymes are in the PI3K/AKT/mTOR pathway. The approach of blocking both VEGFR and PI3K to treat cancer, on the other hand, is called "horizontal inhibition."

Class IA PI3Ks comprise α, β, and δ isoforms encoded by their respective genes PIK3CA, PIK3CB, and PIK3CD. The only member of class IB PI3Ks is PI3Kγ encoded by gene PI3KCG. PI3Kα is the principal isoform in the regulation of tumor growth and proliferation. PI3Kβ promotes the activation and aggregation of platelets by regulating integrin α(IIb)β(3). Studies showed that PI3Kβ inhibitors such as TGX-221 suppress platelet aggregation, Erk phosphorylation and thromboxane A2 generation in human platelets [3]. Recent studies show that it is the down-regulation of the PIK3CB not the depletion of the PIK3CA that inactivates the PI3K pathway and subsequently inhibits the growth *in vivo* and *in vitro* in the PTEN-deficient models [4-5]. PI3Kδ plays an important role in regulating the inflammatory and overall immune response by controlling IL-1 Ra induction in monocytes and therefore it may be a target for multiple sclerosis (MS) [6], and for chronic lymphocytic leukemia [7]. PI3Kγ mediates inflammatory pathway and decreases the concentration of cAMP, negatively affecting cardiac contractility and therefore could be considered as a target for rheumatoid arthritis, psoriasis, asthma, thrombosis, atherosclerosis and cardiac hypertrophy [8]. Due to their important role in cancer progression, we will focus our discussion on PI3Kα and mTOR inhibitor design.

PI3Kα is comprised of catalytic p110α and regulatory p85α subunits. p110α contains 1,068 residues distributed in five domains: ABD (adaptor binding domain), RBD (Ras binding domain), C2 domain, a helical domain, and a catalytic kinase domain. The ATP binding site is located in the kinase domain, which shows a certain degree of homology among class I PI3Ks: the sequence identity between PI3Kα and γ is 35% [9]. Prior to December 2007 when the apoprotein of PI3Kα (2RD0) was deposited in the PDB, PI3Kγ was used as a template to build a homology model for PI3Kα for anticancer inhibitor design targeting the α isoform [10-11].

mTOR, the mammalian target of rapamycin is a serine/threonine kinase with 2,549 residues. It is also known as FK506-binding protein (FKBP) 12-rapamycin complex-associated (FRAP). The ATP-binding catalytic domain of mTOR ranges from residues 2,153 to 2,431 (catalytic loop: 2,335-2,343). The FKBP-rapamycin-binding (FRB) domain is at a helical region ranging from residues 1,980 to 2,150. Two structurally and functionally distinct mTOR complexes were identified in mammalian cells. The first mTOR complex (mTORC1) includes raptor (regulatory associated protein of mTOR), mLST8 (GβL), and PRAS40 (proline-rich AKT substrate 40 kDa). mTORC1 binds to and is

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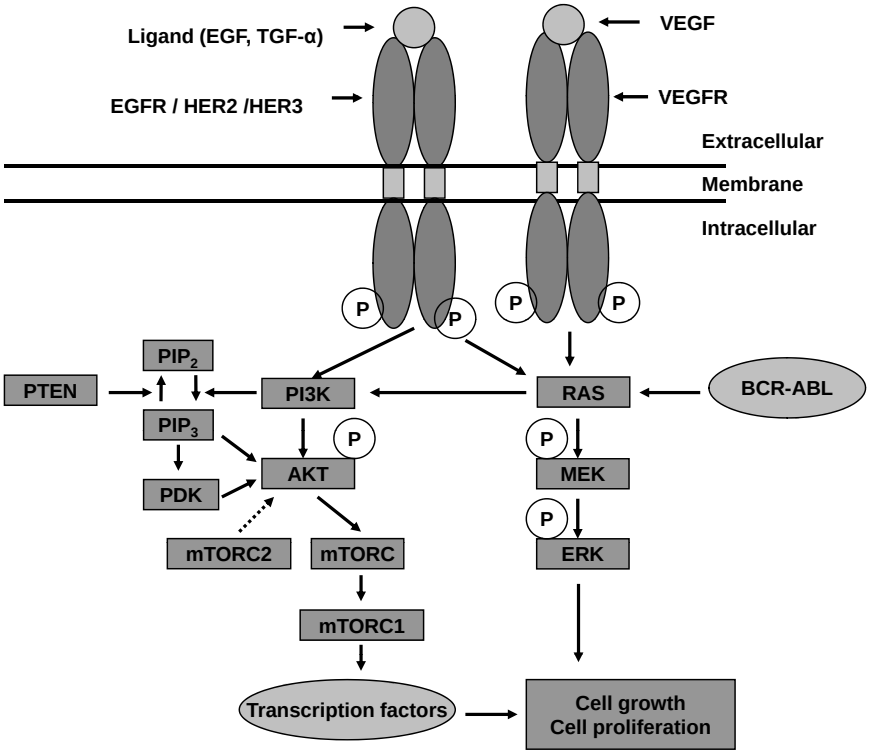


Fig. (1). Overview of the PI3K/AKT/mTOR pathway.

inhibited by FKBP12-rapamycin. Upon the activation of the PI3Kα/AKT pathway by EGFR, VEGFR, and/or BCR-ABL, phosphorylation is relayed from the TSC (tuberous sclerosis complex) to the RHEB (Ras homolog enriched in brain) GTPase and to the mTORC1. The phosphorylated mTORC1 phosphorylates 4EBP1 (eukaryotic initiation factor 4E binding protein-1) and $p70^{S6K}$ (ribosomal p70S6 kinase), two key regulators of mRNA translation and ribosome biogenesis, thereby inducing cell growth and proliferation (Fig. **1**). The binding of rapamycin to the FKBP-rapamycin-binding (FRB) domain of mTORC1 induces the dissociation of Raptor from mTOR. This uncoupling of mTOR from its substrate proteins inhibits the function and activities of mTORC1, leading to the suppression of the formation of phosphorylated $p70^{S6K}$ and hence suppressing protein translation from mRNA [12]. The activation of mTORC1 is regulated by AKT by phosphorylating $Ser^{2,448}$ of mTOR catalytic domain.

It is worthy to point out the limitation of efficacy of using single agent of mTORC1 inhibitor such as rapamycin in treating cancers. The inhibition of mTORC1 by RAD001 (everolimus, Norvartis AG) promotes the activation of the MAPK pathway in metastatic breast cancer patients, as measured by marked increase of ERK phosphorylation after RAD001 treatment [13]. The increase of pERK in turn leads to cell growth and proliferation. In addition, the chronic inhibition of mTORC1 by rapamycin or RNA interference reduced the inhibitory serine phosphorylation on the insulin receptor substrate (IRS-1), and increased its insulin-stimulated tyrosine phosphorylation and associated PI3K activation [14]. The mTORC1-MAPK/ERK feedback loop and the mTORC1-PI3K feedback loop provide a potential combined therapeutic approach targeting mTORC1 with MAPK or PI3K inhibitors. The tumor weight of combination therapy of MAPK inhibitor (PD0325901) and RAD001 was reduced to half of those caused by either monotherapy [13].

mTORC2, on the other hand, consists of mTOR, Rictor (rapamycin-insensitive companion of mTOR), Sin1 (stress-activated protein kinase-interacting protein), mLST8, and Protor1 proteins [15]. The phosphorylation of AKT at Ser473 by mTORC2, along with phosphorylation of Thr308 of AKT by PDK1, fully activates AKT1 [16]. In addition, mTORC2 modulates cytoskeleton organization via phosphorylation of PKC-S657 [17]. Therefore, whereas mTORC1 is a downstream effector of AKT, mTORC2 is an upstream regulator of AKT. The activity of mTORC2 appears to be unaffected by rapamycin; however, prolonged treatment of rapamycin can affect mTORC2 assembly [18].

AKT (also known as protein kinase B, PKB) is a downstream effector of PI3K (Fig. **1**). Fig. (**1**) also shows that AKT can be activated by PIP_3, PDK, RAS and/or HER_2. Once activated, AKT can interact with mTOR, IκB, and MDM2, leading to cell survival and growth. As a kinase, the modification on AKT generally can be observed in threonine, and serine. For instance, the above AKT is phosphorylated by mTORC2 at Ser473 and by PDK1 at Thr308, forming pAKT-Ser473, and pAKT-Thr308, respectively. Treating well-differentiated liposarcoma (WDLPS) with BEZ235, a PI3K/mTOR inhibitor effectively inhibited the formation of pAKT-S473 and hence impaired AKT activation and its resulting activities [19].

In addition to cell cycle arrest and inhibiting cell proliferation, the binding of rapamycin to mTOR can suppress angiogenesis, a fundamental process for both solid and hematologic malignancies [20]. Given the importance of PI3K/mTOR in cancer signal transduction, a number of reviews have been published in the past two years, each with different perspectives [21-24]. The remainder of our review focuses on drug design and clinical development of mTOR and PI3K inhibitors, from a mechanism- and structure-based perspective.

2. PI3K REGULATOR INHIBITORS

In the past three years, significant progress has been made in drug development targeting EGFR, the upstream regulator of the

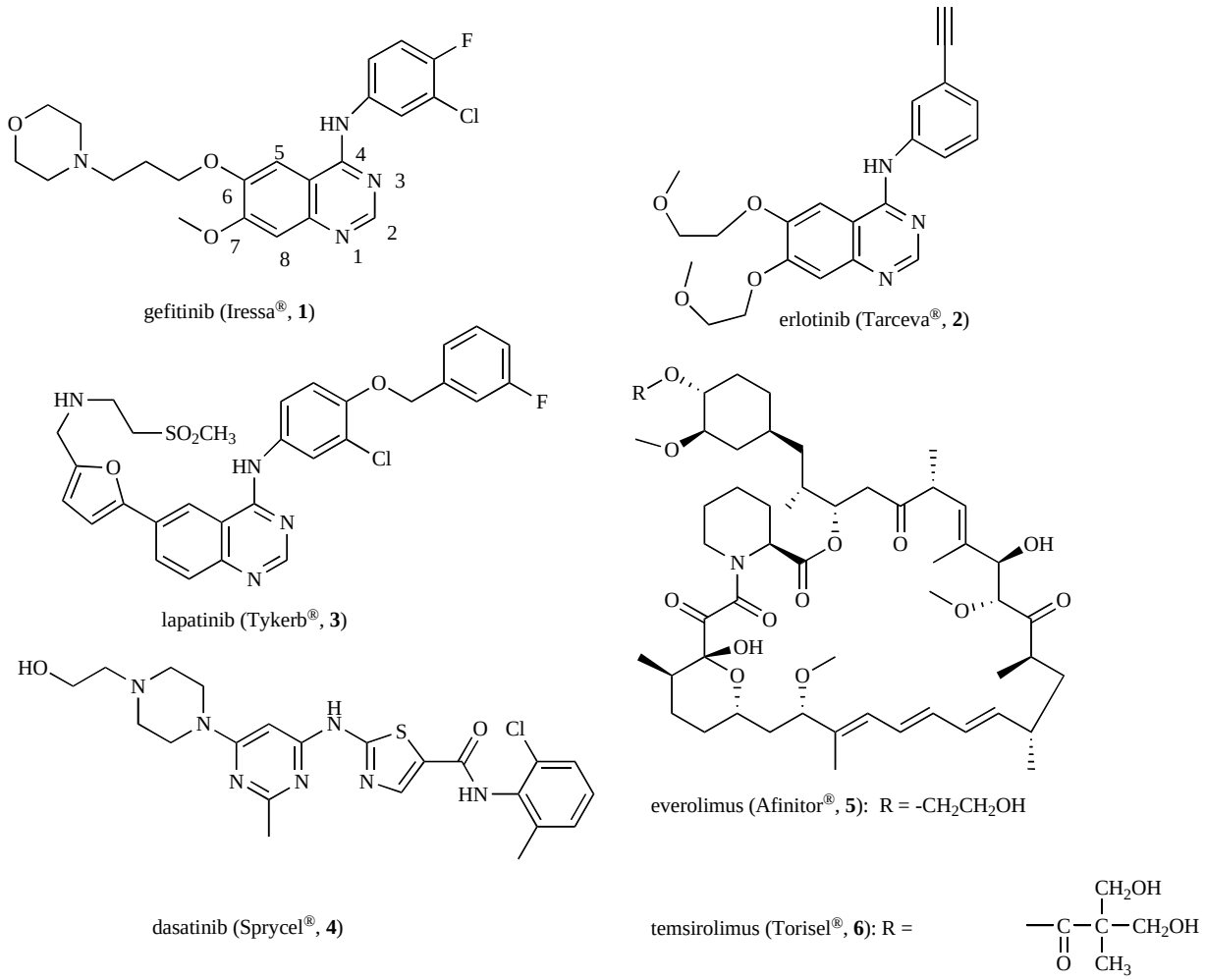


Fig. (2). Structures of approved anticancer VEGFR, EGFR, and/or mTOR inhibitors.

PI3K/mTOR pathway. In this period, gefitinib (**1**, Iressa®, AstraZeneca/Teva), erlotinib (**2**, Tarceca®, OSI), lapatinib (**3**, Tykerb®, GlaxoSmithKline), dasatinib (**4**, Sprycel®, Bristol-Myers-Squibb), and the monoclonal antibodies panitumumab (Vectibix®, Amgen) and Cetuximab (Erbitux®) received FDA approval. Gefitinib (**1**) and erlotinib (**2**) were among the first selective EGFR inhibitors for the treatment of advanced non-small lung cancer cells (NSCLC). Gefitinib, however, was removed from the U.S. market due to the lack of prolonging survival in patients with advanced NSCLC in the Iressa Survival Evaluation in Lung Cancer (ISEL) study [25]. Erlotinib, on the other hand, showed a survival benefit in patients resistant to gefitinib [26]. The enhanced sensitivity of erlotinib toward EGFR appears to be related to Cys797 [27]: docking studies showed that gefitinib forms H-bonds with Met793, Lys745, and Asp855 of EGFR whereas erlotinib interacts with EGFR via H-bonds with Cys797, Met793, Lys745 and Asp855. The importance of Cys797 was confirmed by the observations that HKI-272 [28], EKB-569 [29], BIBW2992 [30] and PF00299804 [31] irreversibly formed a covalent bond with Cys797 and therefore irreversibly inhibited EGFR T790 mutant. AST1306 irreversibly interacts with Cys797 and Cys805 in the catalytic domains of EGFR and ErbB2, respectively [32]. Lapatinib (**3**) is approved as the front-line therapy in ER+/EGFR+/HER2+ ("triple positive") breast cancer. EGFR resistance mutation screens showed that lapatinib was uniquely effective against EGFR with mutations located deep inside the binding pocket [33]. Dasatinib (**4**) is used to treat chronic myeloid leukemia (CML) and is effective in inhibiting the growth of breast cancer cells characterized with over-expressed EGFR [34]. The monoclonal antibody Cetuximab (Erbitux®) is approved by the USFDA for the treatment of metastatic colon cancer, and Head and Neck cancer [35-36].

Inhibiting mTOR, the downstream effector of PI3K, has found its application in treating patients with advanced renal cell carcinoma (RCC). Everolimus (**5**, Afinitor®, Novartis) and Temsirolimus (**6**, Torisel®, Pfizer) are mTOR inhibitors used to treat advanced RCC. Everolimus was approved by USFDA in May 2011 for the treatment of progressive or metastatic pancreatic neuroendocrine tumors not surgically removable, based on favorable Phase III studies which showed that the median progression-free survival in everolimus-treated patients was 11.0 months as compared with 4.6 months with placebo [37]. Everolimus was also approved in April 2010 for prevention of organ rejection after renal transplant. Rapamycin (Sirolimus) was approved by the USFDA in September 1999 as an immunosuppressant drug to prevent rejection in organ transplant, especially in kidney tranplants. In addition to providing effective immunosuppression, rapamycin inhibited the progression of dermal Kaposi's sarcoma in kidney-transplant patients [38]. Treatment of clear cell ovary adenocarcinoma with everolimus did not alter mTOR expression but sharply depressed expression of phosphorylated-mTOR (p-mTOR), HIF-1α and VEGF, suggesting that the key mechanism of action of everolimus is mediated through these three proteins [39]. Temsirolimus on the other hand inhibited tumor cell proliferation through cell cycle arrest and caused an antiangiogenic effect with decreased tumor microvessel density and

Table 1. Drugs Currently Approved in Cancer Therapy Targeting the EGFR/PI3K/mTOR Pathway

Agent Name (Manufacturer)	Target Enzymes	Indications
panitumumab (Vectibix, Amgen)	EGFR	metastatic colon cancer
Cetuximab (Erbitux)	EGFR	metastatic colon cancer, Head and Neck cancer
Gefitinib (Iressa, AstraZeneca/Teva)	EGFR	NSCLC
Erlotinib (Tarceva, OSI)	EGFR	NSCLC
Dasatinib (Sprycel, Bristol-Myers-Squibb)	BCR-ABL, EGFR	CML and ALL
Lapatinib (Tykerb, GlaxoSmithKlein)	EGFR	metastatic breast cancer
Everolimus (Afinitor, Novartis)	mTORC1	RCC, prevention of organ rejection, subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis (TS), and metastatic pancreatic neuroendocrine tumors
Temsirolimus (Torisel, Pfizer)	mTOR, and VEGF	RCC

lowered expression of VEGF [40]. The EGFR and/or PI3K/mTOR signaling pathway inhibitors are prescribed to patients with advanced NSCLC, metastatic colon cancer, metastatic breast cancer and advanced RCC (Table **1**).

EGFR inhibitors are effective in treating colon, lung, and breast cancer. However, resistance to dasatinib has been observed in ABL mutants (V299L, T315A/I, F317I/L) [41]. In order to overcome drug resistance to EGFR-targeted drugs, combination therapy or identification of new drug targets has been recommended as alternative approaches. The combination of vascular endothelial growth factor receptor (VEGFR) inhibitor vatalanib and mTOR inhibitor everolimus in an *in vivo* gastric cancer model reduced tumor size by about 50% relative to that observed with everolimus monotherapy [42]. A combination therapy phase I study of mTOR inhibitor temsirolimus and VEGFR inhibitor pazopanib for patients with advanced solid tumors [43] is ongoing. Similarly, a phase I study of temsirolimus and EGFR inhibitor erlotinib for patients with resistant solid malignancies has been carried out [43].

3. PI3K INHIBITORS

3.1. Pan-PI3K Inhibitors

PI3Kα, central to regulating cell growth, proliferation and survival, is considered to be an attractive anticancer drug target. The first generation PI3K inhibitors (Fig. **3**) generally lack selectivity, inhibiting PI3Kα, PI3Kγ and other PI3K isoforms. Wortmannin (**7**) is an anti-inflammatory fungal metabolite isolated from *Penicillium funiculosum Thom* and it forms a covalent bond with Lys802 in PI3Kα (or Lys833 in PI3Kγ) [44-45]. LY294002 (**8**) is a potent reversible PI3Ks inhibitor that is often used as a pharmacological reference compound [46]. Staurosporine (**9**) is an alkaloid isolated from *Steptomyces bacterium* that triggers apoptosis in various cancer cell lines, causes nuclear fragmentation and disrupts mitochondrial membranes and microtubules [47]. Quercetin (**10**) and myricetin (**11**) are polyphenolic flavonoids. The polyphenol intake (containing both **10** and **11**) in diet exhibited preventive effect in pancreatic cancer development, particularly for current smokers [48]. Myricetin inhibits TNF-α, MKK4, and MEK1 [49] whereas quercetin significantly reduced tumor volume over 6 weeks in HT-29 colon cancer cells via the mechanism of increasing cell cycle arrest in the G1 phase and up-regulating apoptosis-related proteins [50]. ZSTK474 (**12**) was identified as an PI3K inhibitor by screening a chemical library with a triazine scaffold. ZSTK474 inhibited cell growth in cell lines NSCLC (A549), prostate (PC-3), and colon cancer (WiDr human xenografts) with chronic administration of ZSTK474 (for 13 days) at a dose of 400 mg/kg. The growth inhibitory activity of ZSTK474 is approximately 10-fold stronger than that of LY294002 [51].

3.2. PI3Kα Selective Inhibitors

In order to minimize toxicity toward immune and cardiovascular systems, inhibitors selectively targeting PI3Kα rather than the PI3Kγ and/or PI3Kβ have been developed as potential anticancer agents. Various structural scaffolds were reported to show anticancer activities.

3.2.1. Morpholino-Based Fused Heterocyclic Derivatives

The morpholine ring at C2 position of LY294002 (**8**) is critical for its activity against PI3Kα binding. The substitution of the C2-morpholine oxygen atom with S, CH_2, NH, or CHOH caused a dramatic decrease in the efficacy against PI3Kα binding [46]. This suggests that the morpholine ring is important for PI3K binding. Docking studies of 33 PI3K inhibitors to PI3Kα showed that the morpholine forms H-bonds with residues Val851 of PI3Kα, an essential interaction for ligand binding to PI3K [52]. As will be seen below, LY294002 led to a number of other PI3K inhibitors containing the morpholine heteroycle (Fig. **4**).

4-morpholino-2-phenylquinazolin-6-ol (**13**) was discovered by high-throughput screening (HTS) and has an IC_{50} of 1.3μM against PI3Kα [53]. Although the quinazoline phenol at C6 was not required for activity, it was more active than its C5 and C7 regioisomers. The removal of 6-phenol in **13** and at the same time introducing a 3'-OH phenol functional group maintained inhibitory activity. The quinazoline core structure could be replaced with other heterocycles such as pyrido[3,2-*d*] pyrimidine, pyrido[4,3-*d*] pyrimidine, pyrido[3,4-*d*] pyrimidine, and thieno [3,2-*d*] pyrimidine (**14**). Compound **14** was 10-fold more selective for PI3Kα over PI3Kβ and 100-fold more selective for PI3Kα over PI3Kγ [53]. Despite its potency and selectivity, **14** had a poor pharmacokinetic profile and a short half-life which together attenuated its effectiveness *in vivo* [54].

The replacement of phenol functional group in **14** with an indazole combined with the addition of a piperazine methane sulfonamide substituent to the 6-position of the thienopyrimidine core led to GDC-0941 (**15**), a compound with increased potency and significantly improved metabolic stability and oral bioavailability relative to **14** [55]. Compound **15** has high selectivity against PI3K over mTOR. **15** forms H-bonds with Val882, Lys802, Asp841, and Tyr867 in the PI3Kγ hinge region. GDC-0941 exerted anti-proliferative effects against an array of human tumor cell lines and entered phase I clinical trials for cancer treatment [56]. ETP-45658 (Fig. **4**, **16**) was identified as a potent and selective PI3K inhibitor by screening a library of 33,992 small molecules using a cell-based imaging assay that monitored the translocation of the PI3K/AKT effector protein, Forkhead box O (FOXO). The IC_{50} values of **16** against PI3Kα, -δ, -β, and –γ were 22.0, 39.8, 129.0, and 717.3 nM respectively. Compound **16** was injected in MMTV-myr-p110α transgenic female mice and it led to

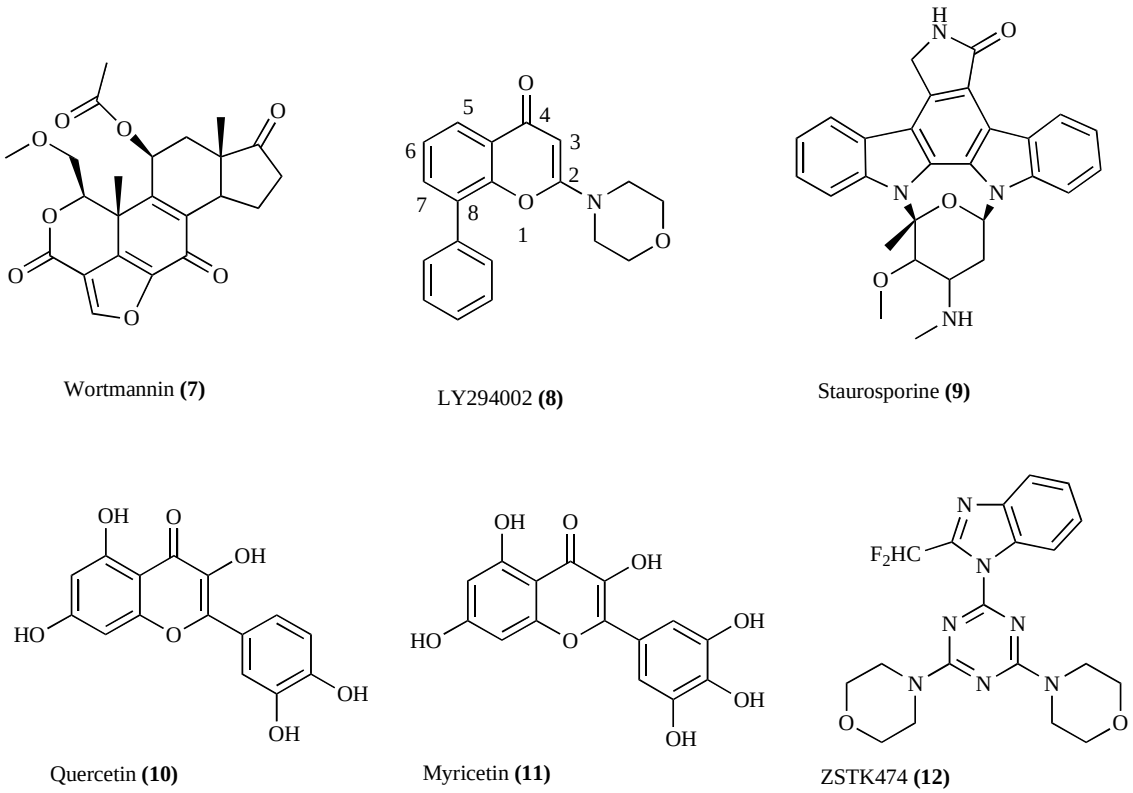


Fig. (3). Structures of the pan-PI3K inhibitors.

a lower level of phosphorylated AKT (pAKT) on Ser473 and a clear reduction of phosphorylation status of $p70^{S6K}$ on Thr389. Similar to compounds **13** and **14**, compound **16** contains both a morpholine and meta-phenol moiety [57]. It should be noted that P-FOXO assay not only detects compounds inhibiting mTOR and PI3K, but those compounds also showed direct inhibition of Akt as well [57].

Alteration of **14** by replacement of the phenol substructure with a 2-aminopyrimidine, and introduction of a tertiary carbinol at the 6-position generated GNE-493 (**17**) [58], a potent, non-selective PI3Kα inhibitor (IC_{50} 3.4 nM). The potency of **17** was attributed to the aminopyrimidine. An X-ray structure show that the amino group of the aminopyrimidine forms H-bonds with Asp836 and Asp841 of PI3Kγ, confirming the importance of this substituent group. The selectivity of **17**, however, is not impressive, inhibiting PI3Kα/β/δ/γ subtypes and mTOR in 3-32 nM [58]. Building on this observation, replacement of the indazole of **15** with a 2-aminopyrimidine led to the discovery of GNE-477 (**18**), a potent PI3Kα inhibitor with IC_{50} of 4 nM [59]. Pharmacokinetic investigation of **18** showed that it had high oral bioavailability in mouse (98%) and dog (90%) models. **18** inhibited PI3Kα and mTOR in 2 and 29 nM, respectively. No activities of **18** against other PI3K subtypes have been reported [59].

Replacement of the thieno[3,2,*d*]pyrimidine core of **14**-**15**, **17**, and **18** with a triazolopyrimidine and incorporation of a urea piperazinamide yielded PKI-402 (**19**) [60]. *In vivo* biomarker analysis of **19** showed that it suppressed phosphorylation of AKT at Thr308 (pAKT-T308), pAKT-S473, and $p70^{S6K}$. However, **19** is not PI3Kα-selective with IC_{50} values of 1.4, 7.0, 9.2, 14, and 1.7 nM against PI3Kα, β, γ, δ and mTOR, respectively [60]. *In vitro*, **19** inhibited the growth of an array of diverse cancer cell lines including breast, lung, colon, renal, epidermal, prostate, pancreas, and brain. When administered at a daily 100 mg/kg dose for 5 days**,** **19** reduced the tumor growth of glioblastoma xenograft (U87MG with deleted PTEN), and inhibited a human breast carcinoma cell line (MDA-MB-361) with a tumor volume reduction of 260 to 129 $mm^3$and suppression of tumor growth for 70 days [61].

Structural optimization of **14** afforded pyrido[3', 2':4,5]furo[3,2-*d*]pyrimidine PI-103 (**20**) with respective IC_{50} values of 3.6, 3.0, and 250 nM against PI3Kα, β, and γ as a potent PI3Kα inhibitor [62]. Compound **20** inhibited doxorubicin (ADR)-selected human breast cancer cell (MCF-7/ADR-RES), human cervical cell (HeLa), human lung tumor cell (A549), and melanoma (A375) tumor cell proliferation at sub μM concentrations [63]. Combined treatment with **20** and mTOR inhibitor rapamycin led to a synergistic suppression of pAKT and $p70^{S6K}$, induced apoptosis, and significantly reduced the tumor growth in comparison to either monotherapy. These data show that simultaneous inhibition of PI3K and mTOR (also called "vertical inhibition") leads to superior efficacy against malignant melanoma [63]. Experiments with two gefitinib-resistant NSCLC cell lines (A549 and H460) showed that H460 cells harboring PI3Kα mutations were more sensitive to **20** than were the wild-type A549 cells. It was proposed that **20** inhibits phosphorylation of $p70^{S6K}$ and AKT and therefore induces cell arrest in the G0-G1 phase of the cell cycle [64]. This indicates that PI-103 could be used to treat gefitinib-resistant NSCLC. PI-103 in combination with erlotinib is effective in treating erlotinib-resistant glioma that express with EGFR and PTEN mutant [65]. PI-103 showed more effectiveness than rapamycin in suppressing the wortmannin-resistant pre-B cell acute lymphoblastic leukemia (pre-B-ALL) and Philadelphia (Ph) chromosome-encoded human CD19(+)CD34(+) Ph+ ALL leukemia cells [66]. Compound **20** also inhibits constitutive and growth factor-induced PI3K/AKT and mTORC1 activation and induces G1 cell cycle arrest in human leukemic cell lines and in primary blast cells from acute myeloid leukemia (AML) patients [67]. Compound **20** had additive proapoptotic effects with topoisomerase II inhibitor etoposide in blast cells and in immature T-cell acute lymphoblastic leukemia (T-ALL). Compound **20** is more potent than wortmannin and LY294002 or the mTOR inhibitor rapamycin in inhibiting the

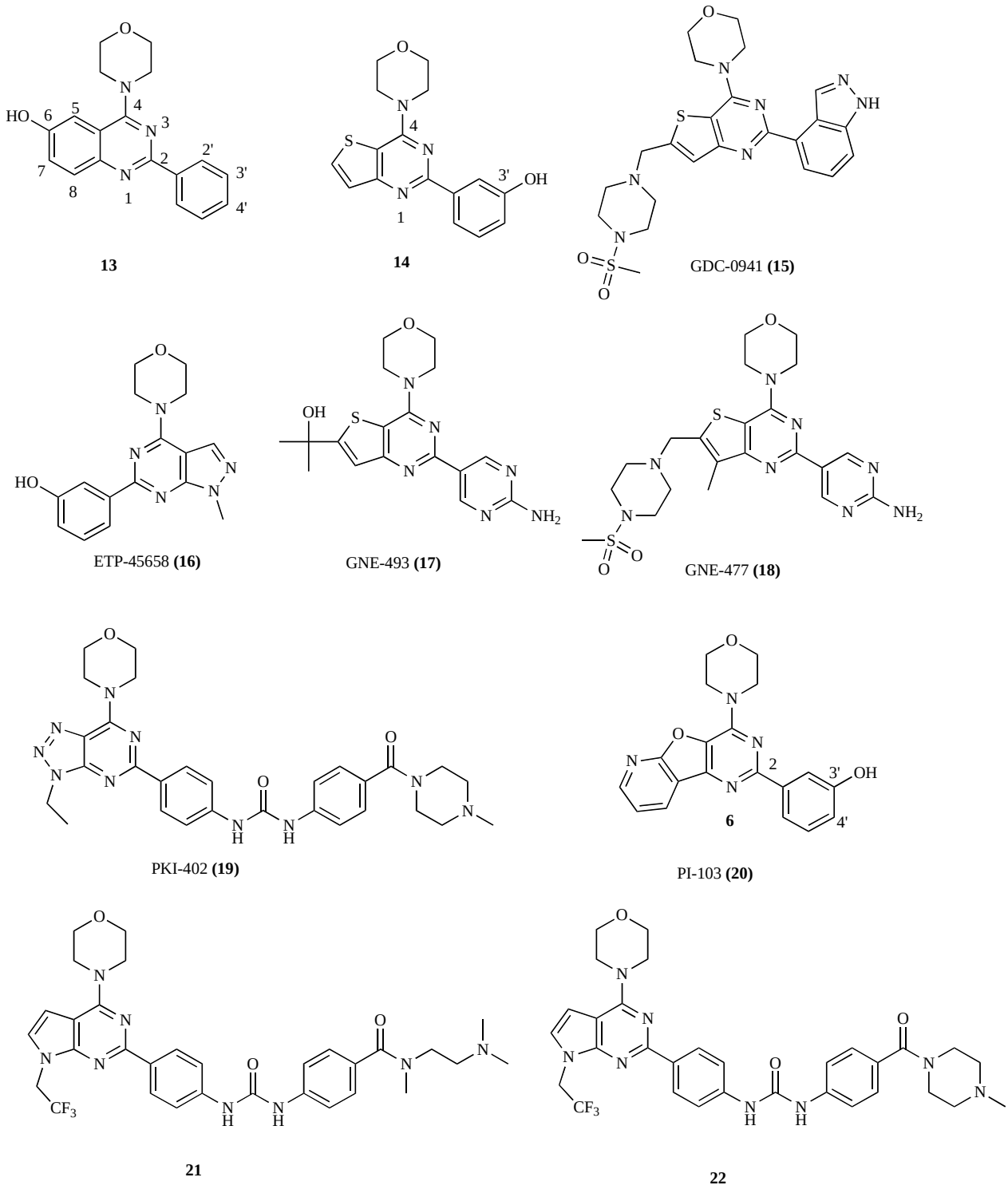


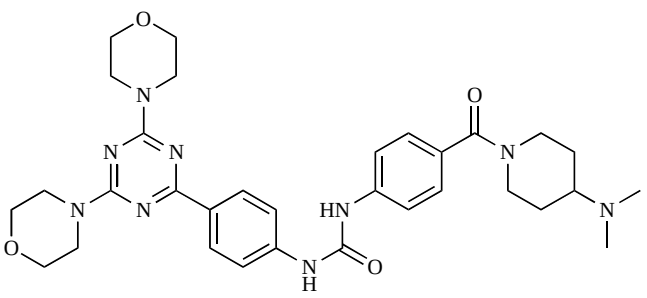
Fig. (4). Structures of 4-morpholino-2-arylpyrimidine PI3K inhibitors.

growth of T-ALL for which the PI3K/AKT/mTOR signaling pathway is constitutive activated. Compound **20** is strongly synergistic with the mitotic inhibitor vincristine, indicating that multi-targeted therapy of PI3K and/or mTOR inhibitors with existing drugs may be an efficient approach in treating T-ALL cells [68].

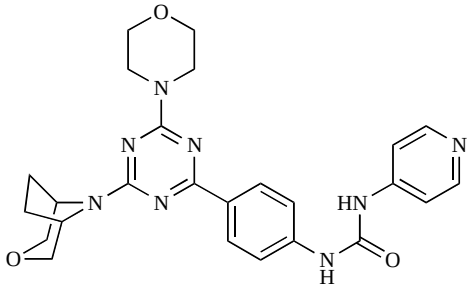
Finally, **21** and **22** (Fig. **4**), two 6-trifluoroethyl-pyrrolo[2,3-*d*]-pyrimidine analogues of **19** have IC_{50}s of 0.9 and 0.6 nM against PI3Kα and 2.4 and 1.7 nM against mTOR. Both compounds have aqueous solubility greater than 100 μg/mL (pH 3.0), and at doses of 25 mg/kg, **21** and **22** suppressed the formation of pAKT-T308, pAKT-S473, and pS6K for up to 8 hours. *In vivo* efficacy studies demonstrated that **21** and **22** inhibit tumor growth in the MDA361 xenograft model with respective IC_{50}s of <3.0 and 6.7 nM [69].

3.2.2. Morpholino-Based Mono-Heterocyclic Derivatives

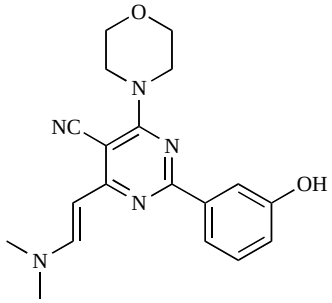
Important for PI3Kα binding, the morpholine substructure in the fused pyrimidines **13**-**22** also represents a liability in that the



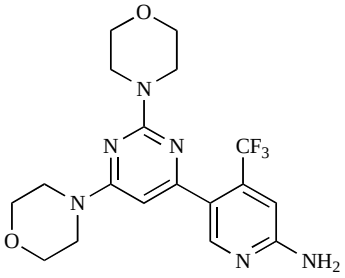
PKI-587 (**23**)



PKI-179 (**24**)



WJD-008 (**25**)



NVP-BKM120 (**26**)

Fig. (5). Structures of morpholino-based mono-heterocyclic PI3K inhibitors.

carbon α to the morpholine ring oxygen is prone to metabolic oxidation, leading to a decrease in potency. For instance, advanced studies with PKI-402 (**19**) were halted due to poor solubility.

To solve the solubility and metabolic oxidation liabilities of morpholino ring-fused pyrimidines **13**-**22**, several morpholino mono-heterocyclic pyrimidines and 1,3,5-triazines were synthesized (Fig. **5**). By replacing the ring-fused pyrimidine ring of **19** with a 1,3,5-triazine combined with the introduction of a second morpholine at the 4-position yielded PKI-587 (**23**). In **23**, the important urea moiety forms H-bonds with residues Asp810 and Asp805 of PI3Kγ and the morpholino oxygen atom provides H-bond interactions with Val851 of the protein [70]. The *in vitro* IC_{50} values of **23** against PI3Kα/γ/β/δ and mTOR are 0.4, 5.4, 6, 6, and 1.6 nM respectively. *In vitro* phosphoblot studies show that at 0.3 μM, **23** inhibits the phosphorylation of AKT at Thr308 and at Ser473 (i.e., inhibits pAKT-T308 and pAKT-S473). Phosphorylation of AKT kinase effector proteins such as GSK3 kinase (S9/21), endothelial nitric oxide synthase, eNOS (S1177) was also suppressed by **23** [70]. Modification of **23** by introduction of a bicyclic as 3-oxa-8-azabicyclo[3,2,1]-octane-morpholine heterocycle and truncation of the terminal aromatic moiety of the urea led to PKI-179 (**24**). Compound **24** had IC_{50} values of 8, 74, and 0.42 nM against PI3Kα, PI3Kγ, and mTOR, respectively. **24** suppressed the AKT phosphorylation at Thr308 and Ser473 positions in the breast cancer tumor xenograft model MDA-361 at a 50 mg/kg dose [71].

WJD008 **(25)** was identified as a dual PI3K/mTOR inhibitor from a series of 5-cyano-6-morpholino-4-substituted-pyrimidine analogs, and **25** inhibited the PI3K-AKT-mTOR signaling pathway and its downstream effectors, $p70^{S6K}$ and 4E-BPI, triggering G1-phase arrest with no apoptosis. Compound **25** has an IC_{50} of 1.7 nM against PI3Kα, blocks AKT activation by suppressing phosphorylation of AKT at Ser473 (p-AKT-S473), reverses the hyperactivation of the PI3K pathway caused by oncogenic p110α H1047R, and inhibits a panel of cancer cell line with IC_{50} values of 20 μM or less [72].

Elimination of the urea functional group and replacement of the triazine with a pyrimidine and simplification in **23** afforded the PI3Kα inhibitor NVP-BKM120 (**26**). The IC_{50} values of **26** against a panel of melanoma cell lines ranged from 1.06 to 2.08 μM. Cell viability assays showed that a combination of rapamycin and **26**, or LY294002 and **26**, significantly decreased the viability of tumor cells [73].

3.2.3. Non-Morpholino-Based Heterocyclic Derivatives

Other than the morpholine ring, there are many other scaffolds observed in PI3Kα inhibitors. NVP-BEZ235 (**27**) is such a non-morpholine-based dual inhibitor of PI3K and mTOR. Compound **27** is effective against rapamycin-resistant AML. It is believed that allosteric inhibition of mTORC1 by rapamycin does not block protein translation in AML cells due to the sustained high level of 4E-BP1 phosphorylation [74]. In addition, rapamycin generally does not inhibit mTORC2 activity. The activated mTORC2 activates the oncogenic kinase AKT, leading to cell growth and survival. **27** is able to inhibit both mTORC1 and mTORC2, inducing a complete dephosphorylation of 4E-BP1, suppressing the phosphorylation of AKT-S473 in mTORC1 and phosphorylation of Tyr118 of paxillin, a mTORC2 downstream effector protein [75]. In addition, compound **27** has been found to induce growth arrest and cell death in renal cell carcinoma [76], in breast tumor cell lines containing HER2 amplification and/or PIK3CA mutation [77], in NSCLC expressing oncogenic KRAS [78], in human gliomas [79], and in human multiple myeloma (MM) [80]. Targeting both PI3K and mTOR showed higher anti-proliferative activity than that of mTOR alone [81]. In addition, **27** blocked neovascularization and induced tumor necrosis in treated mice [82].

The replacement of 8-quinoline of **27** with a 8-pyridin-3-ylethynyl group yielded NVP-BAG956 (**28**), a dual PI3K/PDK-1 inhibitor. Potentiation of the inhibitory effects of the tyrosine kinase inhibitors such as imatinib and nilotinib or the mTOR inhibitors (such as rapamycin and RAD001) by **28** was demonstrated in the BCR-ABLE-positive CML and AML cells [83].

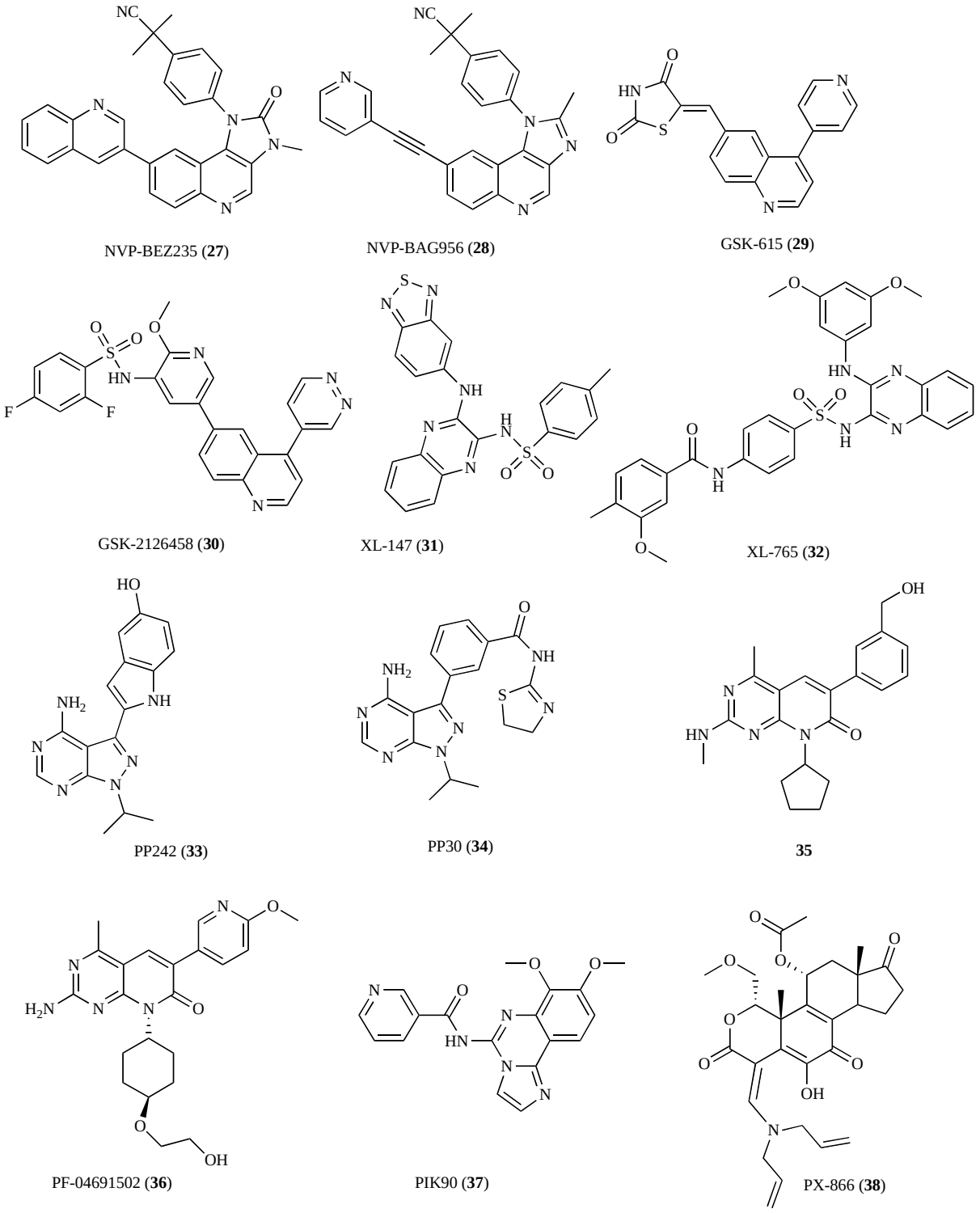


Fig. (6). Structures of non-morpholino-based heterocyclic PI3Kα inhibitors.

A structurally novel PI3K inhibitor GSK-615 (**29**) inhibited pAKT formation, induced apoptosis, and triggered cell death in an array of cancer cell lines with no apparent toxicity and body weight loss. It is currently in phase I clinical trials [84]. GSK-2126458 (**30**), a dual PI3Kα and mTOR inhibitor, inhibited PI3Kα in nM concentration. The apparent K_i values of **30** against PI3K-α/β/δ/γ, and mTORC1 and mTORC2 ranged from 0.019 nM to 0.30 nM. **30** reduced the level of pAKT-S473 and inhibited the phosphorylation of AKT-T308 and $p70^{S6k}$ at low nanomolar concentrations. It displayed a good pharmacokinetic profile in mouse, rat, dog, and monkey and exhibited a low blood clearance and good oral bioavailability. It is currently in the phase I clinical evaluations for the treatment of solid tumors and lymphoma. The crystal structure of PI3Kγ/**30** suggested that Lys833, Tyr867, and Val882 are important for the binding [85].

XL-147 (**31**) inhibited PI3K but not mTOR. Further optimization of XL-147 afforded XL-765 (**32**) which is a dual inhibitor of PI3K and mTOR. In preclinical cancer models, both

exerted a cytostatic effect and reduced the tumor volume when administered alone. It also augmented the efficacy of other anticancer compounds (such as erlotinib and letrozole) when used in combination. Both compounds are currently in phase I trials with or without combination (erlotinib, or letrozole, and/or radiation) in patients with breast cancer, NSCLC, glioblastoma, or other solid tumors [86]. Our docking studies showed that **31** and **32** formed H-bonds with Val851 and Tyr836 of PI3Kα, and Ser802, Val882, and Ala805 of PI3Kγ [52] .

The pyrazolopyrimidines PP242 (**33**) and PP30 (**34**) were discovered as selective mTOR inhibitors by high throughput screening of tyrosine kinase inhibitors against PI3Kα [87]. The *in vitro* IC_{50} values of **33** and **34** against mTOR were 0.008, and 0.080 μM, respectively. The IC_{50}s of **33** and **34** against PI3Kα/β/δ/γ ranged from 0.1 to 5.8 μM. Therefore, **33** and **34** are mTOR selective inhibitors. Compound **33** is more effective than rapamycin in suppressing the formation of p4E-BP1 and pAKT and therefore is active in inhibiting mTORC1 in rapamycin-resistant cells [87]. In models of acute leukemia harboring the Philadelphia chromosome (Ph+) translocation, **33** not rapamycin, caused death of mouse and human leukemia cells. *In vivo* compound **33** significantly delayed the onset of leukemia where rapamycin failed to delay at all. The combination of imatinib or dasatinib with **33** significantly augmented the apoptosis in human Ph+ B-ALL cells *in vitro* where rapamycin was less effective [88].

The lead compound **35** (**35**) was identified by HTS with potent inhibition against mouse PI3Kα. However, **35** showed poor metabolic stability. Modification of **35** afforded PF-04691502 (**36**) as a potent dual PI3K/mTOR inhibitor with satisfactory *in vivo* efficacy. **36** is currently in Phase I human clinical trials in combination with a MEK inhibitor (PD-0325901) or irinotecan in patients with advanced cancer [89]. PIK90 (**37**) at 10 μM induced apoptosis in chronic lymphocytic leukemia (CLL) B cells which contain consitutively activated PI3Ks [90]. At 1 μM **37** and **20** significantly reduced both phosphorylation of AKT-S473 and S6 at Ser235/235 in CLL cells. In combination with fludarabine **37** and **20** yieled a more than additive cytotoxic effect in CLL cells. The cell viability after monotherapy of **37** at 1 μM was 75.6% whereas the combination of **37** and fludarabine decreased the viability of the CLL cells to only 23% [90].

Modification of wortmannin (**7**) led to PX-866 (**38**) with much better pharmacokinetic properties (e.g., better water solubility and larger plasma AUC) than **7**. Addition of **38** to a platinum compound BBR3610-treated glioblastoma cells resulted in synergistic killing of cultured glioma cells with an extended survival, an increased level of apoptosis and a marked reduction in pAKT formation. Therefore the addition of **38** to platinum-based anticancer agent BBR3610 has a translational potential in glioblastoma therapy [91].

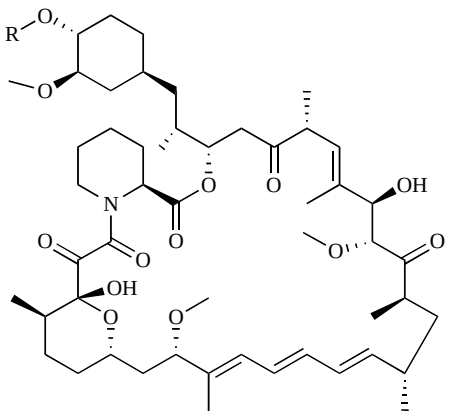
Many PI3Kα inhibitors discussed aforementioned have entered clinical trials to evaluate their efficacy and safety in treating a wide array of cancers. Table **2** lists some of the PI3Kα inhibitors that are currently in clinical trials [43]. Please note that Table **2** is not intended to be comprehensive because a particular inhibitor may have more than 10 clinical trials and therefore it is unnecessary to list all the clinical trials for each inhibitor. Readers are encouraged to go to the website (http://clinicaltrials.gov) to get the full list of clinical trials information for an interested compound [43].

4. mTOR-SELECTIVE INHIBITORS

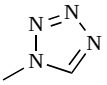
Many of the above PI3K inhibitors (for instance, compounds **15**, **19**, **20**-**25**, **27**, and **30**) are actually dual PI3K and mTOR inhibitors. These inhibitors are able to bind to the mTOR catalytic domain ranging from residues 2,153 to 2,431. This domain is part of a large superfamily that includes the catalytic domains of other kinases such as PI3Kα and PI3Kγ. This is why some of the PI3K inhibitors show mTOR inhibitory activities. A phylogenetic analyses of PI3K and type III phosphoinositide 4-kinase (PI4K) show that PIK3CA (the gene encoding PI3Kα) is evolutionarily closer to PIK3CG than PIK3CB or PIK3CD. The alignment of the kinase domains of PI3Ks, PI4K and mTOR and the mapping of inhibitors to respective kinases show that some potent PI3K

Table 2. PI3Kα Inhibitors Currently in Clinical Trials

Inhibitors	Combinational Drugs	Indications	Phases of Clinical Trials
GDC-0941 (**15**)	Erlotinib	Advanced solid tumors	Ib
GDC-0941 (**15**)	Paclitaxel and Bevacizumab	Locally recurrent or metastatic breast cancer; Advanced NSCLC	Ib/II
GDC-0941 (**15**)	GDC-0973	Advanced solid tumors	Ib
PKI-587 (**23**)	alone	Solid tumors	I
NVP-BKM120 (**26**)	Irinotecan	Advanced colorectal cancer	I
NVP-BKM120 (**26**)	Paclitaxel and Carboplatin	Advanced solid tumors	I
NVP-BKM120 (**26**)	Bevacizumab	Advanced renal cell carcinoma	I
NVP-BEZ235 (**27**)	alone	Advanced breast cancer, solid tumors	I/II
NVP-BEZ235 (**27**)	Endocrine treatment	Metastatic breast cancer	I
NVP-BEZ235 (**27**)	MEK162	Advanced solid tumors	I/II
GSK-2126458 (**30**)	GSK1120212	Advanced solid tumors	I
XL-147 (**31**)	Paclitaxel and Carboplatin	Solid tumors (ovarian cancer and NSCLC)	I
XL-147 (**31**)	Letrozole	Breast cancer	I/II
XL-147 (**31**)	Erlotinib	Solid tumors	I
XL-765 (**32**)	Letrozole	Breast cancer	I/II
XL-765 (**32**)	Erlotinib	Solid tumors	I
PF-04691502 (**36**)	Letrozole	Breast cancer	II
PF-04691502 (**36**)	MEK inhibitor or Irinotecan	Advanced cancer	I
PX-866 (**38**)	alone	Advanced solid tumors	I
PX-866 (**38**)	alone	metastatic prostate cancer	II



rapamycin (sirolimus, **39**) : R = -H



zotarolimus (Endeavour, **40**): R = (4S)

ridaforolimus (AP-23573, **41**): R = -P(CH$_3$)$_2$

Fig. (7). Structures of rapamycin and its analogs.

inhibitors possess high potency against mTOR as well [92]. To reduce toxicity due to multiple inhibitions of various kinases, efforts have been taken to develop mTOR-selective inhibitors. The development of ATP-competitive mTOR inhibitors was accelerated by the realization that rapamycin-based therapeutics only show limited efficacy.

4.1. Rapamycin and its Analogs

Rapamycin (**39**) also known as sirolimus is a macrolide found in *Streptomyces hygroscopicus* culture and was initially reported as an antifungal agent targeting *Candida albicans*, *Microsporum gypseum*, and *Trichophyton granulosum* [93]. Later, it was found to be an immunosuppressive drug with 100-fold more potent than cyclosporine [94]. **39** bound to FKBP12 protein and inhibited phosphorylation of p70^{S6K} and 4E-BP1, DNA synthesis, and basal cell growth in human pancreatic cancer cells [95], whereas cyclosporine binds to cyclophylline P, an intracellular receptor different from the FKBP12 [96]. Other than pancreatic cancer, **39** has found applications in metastatic RCC, NSCLC, breast, and prostate cancer [97]. It also potentiated the cytotoxic effects of cisplatin [98], and inhibited cancer vascularization through inhibiting angiogenesis and vascular proliferation [99]. In spite of these advantages, rapamycin's clinical applications are very limited due to its rapid and poor oral bioavailability.

Modifications of rapamycin afforded everolimus (**5**) and temsirolimus (**6**) which were approved by the USFDA for the treatment of patients with advanced renal RCC after failure of treatment with sunitinib or sorafenib. Other indications of **5** include clinical studies in clear cell ovary adenocarcinoma and gastric tumor [100]. In addition, **5** sensitized lung carcinoma cells (A549) to apoptosis induced by cisplatin [101]. Combination of **6** with radiotherapy showed superior anticancer activity to chemoradiotherapy with cisplatin [102].

Introduction of a tetrazole to rapamycin yielded Zotarolimus (Endeavor, **40**), a drug used to prevent coronary artery stenosis [103]. Ridaforolimus (AP-23573, also called Deforolimus, **41**) is a phosphorus-containing rapamycin derivative that is able to rapidly reduce the level of p4E-BP1. Phase I, II, and III clinical trials are currently ongoing and the phase I results showed that toxicity of **41** was well tolerated and that pharmacokinetic profiles of **41** were similar to those of other mTOR inhibitors [104]. Combination of **41** with cytotoxic docetaxel, doxorubicin, and cisplatin in breast, ovarian and endometrial cancer cell lines showed an additive effect [105].

Approved by the USFDA for the treatment of metastatic RCC, everolimus and temsirolimus treatments have not shown substantial tumor regression. Some attribute this limited efficacy to the inability of these rapamycins to inhibit mTORC2. Others maintain that failure to block 4E-BP1 phosphorylation attributes to limited tumor regression of rapamycins. Indeed, phosphorylation in both mTORC1 and mTORC2 was effectively inhibited by NVP-BEZ235 while rapamycin only selectively inhibited mTORC1 phosphorylation. Even in mTOCR1 rapamycin fails to lower the level of p4E-BP1 to a significant extent [106]. Due to the limited efficacy of rapamycin and its analogs in cancer treatment, the journey of drug design targeting mTOR other than the rapamycin binding site continues on.

4.2. Small Molecules of mTOR Inhibitors

The deficiencies in rapamycin-based drugs have spurred the development of mTOR inhibitors targeting binding sites other than the rapamycin binding site (residues ranging from 2015 to 2114 of mTOR proteins). The catalytic domain of mTOR ranging from 2153 to 2431 captured attentions. However, no crystal structure of the mTOR catalytic domain has been reported. This complicates the drug design efforts targeting catalytic/ATP binding domain. Liu *et al.* built a homolog model of mTOR based on the PI3Kγ crystal structure (PDB code: 3DBS) and docked the mTOR inhibitors to the mTOR homology model [107]. However, caution must be taken when using these homolog models in structure based drug design: the sequence identity between the mTOR catalytic domain (residues ranging 2101 to 2460) and that of PI3Kγ (residues between 721 and 1099) is only 16.3%.

High-throughput screening against a recombinant mTOR enzyme discovered WAY-001 (**41**) as a lead compound (IC_{50}, 0.22 μM) for mTOR inhibitors. Structural optimization yielded WAY-600 (**42**), WYE-687 (**43**), and WYE-354 (**44**) as potent and selective mTOR inhibitors [108]. At 5 μM these three compounds blocked the formation of pAKT-S473 and pS6K-T389, the phosphorylation products of the mTORC2 and mTORC1 respectively [108]. The replacement of the unstable phenol functional group in **41** with a carbamate or urea moiety greatly enhanced the selectivity, defined by IC_{50}(mTOR)/IC_{50}(PI3Kα), by more than 1,000 fold [109]. The mTOR selectivity index for the arylureido analogue WYE-125132 (also called WYE-132, **45**) was 1,410 [110]. Compound **45** was efficacious in shrinking the tumor size from approximately 540 mm^3 in the control case to 200 mm^3 in the **45**-treated MDA361 tumor cells. Biomarker inhibition analyses showed that **45** inhibited the formation of pS6K-T389, pAKT-S473, and pS6 (S240/244) [111]. **45** exerted high potency against MDA361 breast, U87MG glioma, A549 and H1975 lung, as well as A498 and 786-O renal tumors [112]. A homology modeling showed that the 3,5-ethylene bridged morpholine of **45** inserted deeply in mTOR binding site interacting with Leu961, whereas a bulky phenylalanine in PI3Kγ (the template for the mTOR homology model) at the same position would block the binding of **45** [110].

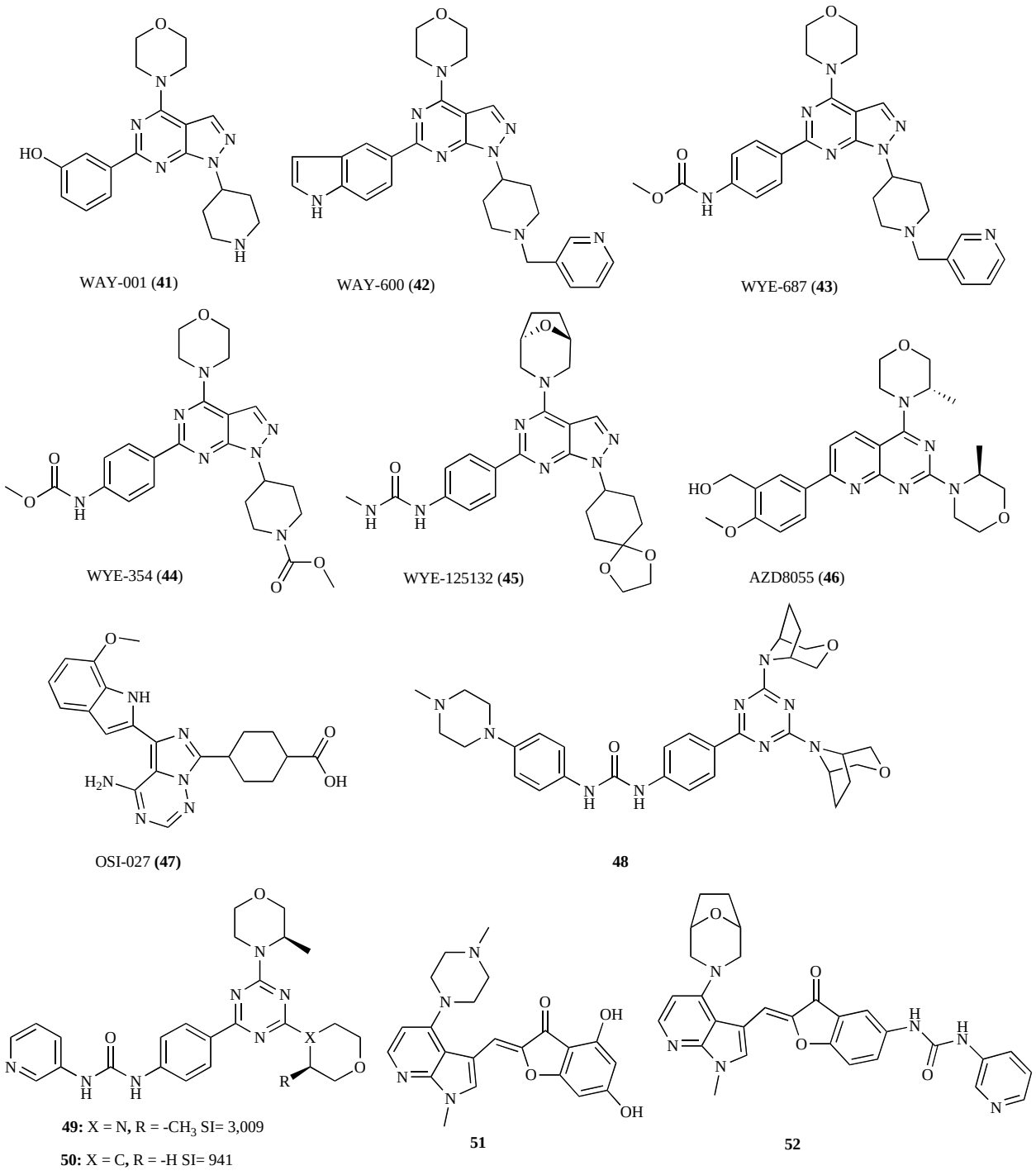


Fig. (8). Structures of mTOR-selective inhibitors.

AZD8055 (**46**) is a potent, selective, and orally bioavailable ATP-competitive mTOR inhibitor showing both *in vitro* and *in vivo* antitumor activity. It was discovered through screening of a library of pyridopyrimidine-based compounds. The IC_{50} of **46** against mTOR enzyme complexes extracted from HeLa cells was 0.8 nM. Enzymatic reactions indicated that **46** competed with ATP for the binding site. Compound **46** suppressed NSCLC *in vitro* and *in vivo* and is currently in phase I clinical trials [113].

OSI-027 (**47**) inhibits the mTOR catalytic sites of both mTORC1 and mTORC2 and elicits much more potent antileukemic responses in AML cells than rapamycin which targets only mTORC1 inhibitor [114]. **47** exhibits potent suppressive effects on primary leukemic progenitors in a dose-dependent manner and blocks phosphorylation of all key phosphorylation sites (Thr37/46, Ser65, and Thr70) of 4E-BP1. **47** also suppresses phosphorylation of these residues in primary CML cells that harbors the T315I-BCR-ABL mutation. This mutation is refractory to all BCR-ABL kinase inhibitors which are currently in clinical use [115]. This dual inhibition of **47** against mTORC1/2 stems from the observation that both enzymes share a common catalytic domain and may provide approach to overcome imatinib-resistant CML and AML [115]. The combination of mTOR inhibitor **47** with VEGFR inhibitor sunitinib shrank tumor sizes to half of those treated by either monotherapy [116].

Triazine-based morpholine derivatives **23**-**26** are dual PI3Kα and mTOR inhibitors. Similar to **45**, introduction of a 2-ureidophenyl group to the triazine ring afforded **48** and **49** with significant increase in mTOR selectivity. The selectivity indices of **48** and **49** were 899 [117] and 3,009 [118], respectively. Both **48** and **49** suppressed growth of prostate and breast cancer cells. Simplification of both bridged morpholines to a bis-(R)-3-methylmorpholine yielded **49** with much improved selectivity toward mTOR over PI3Kα. These two chiral centers on the bis-(R)-3-methylmorpholine appears to be critical. Removing one chirality afforded compound **50** with more than 3-fold less selectivity [119].

Compound **51** is a potent mTOR inhibitor with weak selectivity (SI toward mTOR: 46). The IC_{50} values against mTOR and PI3Kα are 0.94 and 43 nM, respectively [119]. The crystal structure of PI3Kγ/**51** (PDB id: 3LJ3) was employed to build an mTOR homology model. Modification of **51** based on this homology model and the following synthesis yielded **52** with a slightly better selectivity (SI: 145) toward mTOR inhibitor. However, this moderate increase in selectivity is at the expense of potency. The IC_{50} values of **52** against mTOR and PI3Kα are 14.3 and 2,080 nM, respectively [120].

Table **3** lists mTOR-selective inhibitors that are currently in clinical trials. For a complete set of clinical trials for a particular inhibitor, readers are encouraged to check it out on the clinical trials website (http://clinicaltrials.gov) [43]. In Table **3**, CC-223 is a new class of experimental drug with dual mTOR inhibitory activities (Celgene Corp., structurally undisclosed) [121]. AZD2014 (AstraZeneca) is another structurally undisclosed mTOR inhibitor that is currently recruiting participants to assess the safety, tolerability, pharmacokinetics and preliminary efficacy in patients with advanced solid tumors [122]. Combination of INK128 with paclitacel is expected to enhance anti-tumor activity and block both mTORC1 and mTORC2 signaling in solid tumors (clinical trials). For patients with HER2+ breast cancer, trastuzumab will be added to the combination of INK128 and paclitaxel to assess the safety and efficacy [123].

5. CONCLUSIONS AND FUTURE PERSPECTIVES

The drug design efforts targeting the PI3K/AKT/mTOR pathway has generated a number of candidates that are in various stages of clinical trials. Many current mTOR inhibitors were developed based on the homology modeling of mTOR using PI3Kγ as a template. The sequence identity between these two proteins, however, is very low. The development of the new generation of mTOR inhibitors will undoubtedly benefit from a crystal structure of the catalytic domain of mTOR/inhibitor complex should such a crystal be available in the near future.

Mutations in the helical, and kinase domains of PI3Kα were observed in various tumors. Recently Liu *et al*. reported that PI3Kα mutations were observed in a wide array of tumor cells (% of observations containing mutants): breast, 27% (468/1766); endometrial, 24% (102/429); colon, 15% (448/3024); upper digestive tract, 11% (38/352); stomach, 8% (29/362), pancreas, 8% (29/362); and ovarian, 8% (61/787) [124]. The most observed helical domain mutants E542K and E545K, and kinase domain mutant H1047R show enhanced kinase activities, inducing cancerous cell proliferation. Given the central role of PI3K in regulating cell proliferation, it might be wise to design ligands that inhibit p110α mutants only. However, designing such a mutant-specific inhibitor might prove to be challenging because the structural differences between the wild-type and the H1047R p110α mutant are not so significant. Besides, p110α wild-type is upregulated in cancer cells as well. Ligands targeting mutant-only protein would overlook the overexpressed wild-type protein. Most ligands reported nowadays exhibit inhibition against both the wild-type and the H1047R mutant.

We have reviewed the development of mTOR-selective inhibitors. The advantage of developing mTOR-selective compounds is that it might reduce the toxicity of inhibiting PI3K. It is shown that LY294002 interrupted T-cell proliferation by preventing the induction of both cyclin D2 and cyclin D3 mRNAs and proteins, the first cell cycle proteins that regulate T-cell proliferation [125]. As discussed earlier, the immunosuppressive effect of the PI3K inhibitors such as LY294002 probably results from the inhibition of PI3Kγ. Isoform-selective (PI3Kα-selective) inhibitors might help to alleviate the immunosuppressive effect. However, it is worthy to point out that rapamycin and its analogs such as everolimus are able to inhibit the induction of cyclin D2 and cyclin D3 proteins, although they don't block the formation of mRNA of these two proteins. This is why rapamycin and everolimus are also approved to prevent rejection in organ transplant. Another potential toxicity of PI3K/AKT/mTOR pathway inhibitors could be hyperglycemia and glucose intolerance: rector null mice were shown to exhibit these two symptoms due to a reduction in B-cell proliferation and a decreased glucose-stimulated insulin secretion [126]. The disadvantage of selectively inhibiting mTORC1 and/or mTORC2 is that the mTORC1-MAPK/ERK feedback loop and the mTORC1-PI3K feedback loop allow marked increase of ERK phosphorylation after RAD001 treatment [13-14]. To address the feedback loop problem, it would be better to use combination therapy to inhibit two proteins.

Dual-pathway inhibition by rapamycin and the Ras/Raf/mitogen-activated protein kinase (MAPK, MEK)/ERK inhibitor AZD6244 exhibited 60% more in growth inhibition than rapamycin monotherapy. This combination treatment caused an intense G1 arrest in cell culture and reversible cytostatic inhibition

Table 3. mTOR-Selective Inhibitors Currently in Clinical Trials

Inhibitors	Combinational Drugs	Indications	Phases of Clinical Trials
AZD8055 (**46**)	alone	Recurrent gliomas	I
AZD8055 (46)	alone	Advanced hepatocellular carcinoma	I/II
AZD8055 (**46**)	alone	Advanced solid tumors	I/II
OSI-027 (**47**)	alone	Advanced solid tumors or lymphoma	I
CC-223	alone	Advanced solid tumors or lymphoma or multiple myeloma	I/II
AZD2014	alone	Advanced solid tumors	I
Ridaforolimus (AP-23573, **41**)	alone	Refractory hematologic malignancies	II
Ridaforolimus (AP-23573, **41**)	alone	Recurrent endometrial cancer	II
Ridaforolimus (AP-23573, **41**)	alone	Androgen-independent prostate cancer	II
INK128	Paclitaxel and/or trastuzumab	Advance solid tumors	I

in a thyroid cancer xenograft model [127]. As reviewed earlier, the addition of the BCR-ABL kinase inhibitors imatinib or nilotinib to NVP-BAG956 (**28**) potentiated antileukemic therapy in animal models harboring drug-resistant leukemia [83]. The Pim 1 kinase inhibitor ETP-45299 synergized the suppression of cellular proliferation of PI3Kα inhibitor GDC-0941 (**15**) in MV-4-11 AML cells: the combination of ETP-45299 and **15** increased the anti-proliferative activity of **15** by 19-fold [128]. All these combination therapies can be classified as horizontal inhibition since two target proteins are from different pathways. The simultaneous inhibition of both PI3Kα and mTOR is called vertical inhibition because PI3Kα and mTOR are in the PI3K/AKT/mTOR pathway. This dual inhibition may be especially effective in cancers (such as melanoma) that harbor upregulated PI3Kα and mTOR. The combination of rapamycin and **26** significantly increased cell death in human melanoma tumor cells [73]. The benefit of dual inhibition of PI3Kα and mTOR has brought forth several candidate compounds that are currently in clinical trials. A recent trend of developing mTOR-selective small molecule inhibitors targeting the ATP-binding site may be the results of (1) mTOR allosteric inhibitors rapalog sometimes are ineffective in treating cancer, and (2) the concept of being mTOR-selective may be more effective and less toxic. As a result, many mTOR-selective inhibitors have been reported, as reviewed in this paper and in Schenone's recent review paper [123]. However, a recent report may suggest the opposite. The sensitivity of tumor cells to mTOR-inhibitors may be dependent upon the mutational status of PI3Kα. A recent study showed that breast cancer cells with wild-type PIK3CA were resistant to mTOR-selective PP242 (**33**) treatment, whereas those breast cancer cell lines with the PIK3CA mutants (E545K or H1047R) were sensitive to treatment of **33** [129]. The dual inhibition of PI3Kα and mTOR is further confirmed by the observation that the combination treatment of rapamycin and PI-103 (**20**) exhibited more inhibitory activity than single agents in human ovarian and prostate cancer cells [130]. All these and other combination therapy data have suggested that the vertical or horizontal pathway inhibition of the PI3K/AKT/mTOR pathway may bring better therapeutic outcome than mTOR-selective inhibitors in treating cancers with up-regulated PI3Kα and mTOR. Dual PI3K/mTOR inhibitors like NVP-BEZ235 (**27**) and GDC-0941 (**15**) were able to completely inhibit mTORC1 and mTORC2 as measured by blocking the formation of pS6K-T389 and pAkt-S473, respectively. However, the PI3K/mTOR inhibition induced HER receptor activation and the subsequent ERK activation (evidenced by marked increase of pERK) in HER2-overexpressing breast cancer [131]. The observed ERK activation with multiple PI3K/mTOR inhibitors suggests a class-effect. This compensatory ERK activation may weaken the efficacy of PI3K/mTOR inhibitors. An alternative strategy to maximize therapeutic outcome would be by combining ERK phosphorylation blockers (MEK inhibitors) with PI3K/mTOR inhibitors. In addition, cells with KRAS/BRAF mutation may be less sensitive to PI3K inhibitors [132]. The combination therapy of PI3K/mTOR inhibitors with VEGFR or EGFR inhibitors has shown additive effect to overcome resistance to monotherapies [133].

AKNOWLEDGEMENTS

The support from Research Corporations is gratefully acknowledged. DAS thanks the support from the Bukey Fellowship (University of Nebraska Medical Center).

ABBREVIATIONS

4EBP1 = 4E binding protein-1

AML = Acute myeloid leukemia

BCR-ABL = Breakpoint cluster region-Abelson tyrosine kinase

CML = Chronic myeloid leukemia

EGFR = Epidermal growth factor receptor

FDA = Food and Drug Administration

FKBP12 = FK506-binding protein 12 kD

FRAP = FKBP and rapamycin-associated protein

FRB = FKBP12-rapamycin binding

HER2 = Human epidermal growth factor receptor 2

mTOR = mammalian target of rapamycin

mTORC1/2 = mTOR complex 1/mTOR complex 2

NSCLC = Non-small cell lung cancer

$p70^{S6K}$ = p70 S6 ribosomal kinase

pAKT = phosphorylated AKT

PI3K = Phosphatidylinositol 3-kinase

PIP3 = phosphatidylinositol 3,4,5 triphosphates

PKB = Protein kinase B or AKT

Raptor = Regulatory associated protein of mTOR

RCC = Renal cell carcinoma

RHEB = Ras homolog enriched in brain

Rictor = Rapamycin-insensitive companion of mTOR

SAR = Structure-activity relationship

Sin1 = stress-activated protein kinase-interacting protein

T-ALL = T-cell acute lymphoblastic leukemia

VEGFR = Vascular endothelial growth factor receptor

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Received: August 12, 2011 **Revised: October 18, 2011** **Accepted: October 19, 2011**