

## Recent development of ATP-competitive small molecule phosphatidylinositol-3-kinase inhibitors as anticancer agents

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Keywords: phosphatidylinositol-3-kinase; PI3K; inhibitor; mTOR pathway; anticancer

Received: August 15, 2016

Accepted: October 04, 2016

Published: October 18, 2016

### ABSTRACT

**Phosphatidylinositol-3-kinase (PI3K) is the potential anticancer target in the PI3K/Akt/ mTOR pathway. Here we reviewed the ATP-competitive small molecule PI3K inhibitors in the past few years, including the pan Class I PI3K inhibitors, the isoform-specific PI3K inhibitors and/or the PI3K/mTOR dual inhibitors.**

### INTRODUCTION

The PI3K/AKT/mTOR signaling pathway (Figure 1) regulates diverse biological processes such as cell growth, cell proliferation, cell survival, protein synthesis, and glycolysis metabolism, which is frequently deregulated in human cancers [1-9]. PI3K (phosphatidylinositol-3-kinase) is the main anticancer target within this pathway, and its correlation with tumor-genesis, progression and maintenance has been validated by several extensive studies [10-20].

To date, a total of eight PI3Ks have been identified, which are divided into four classes (I, II, III and IV) based on their sequence homology. As the most relevant to the PI3K/Akt/mTOR pathway (Figure 1), Class I PI3Ks are always referred to as PI3Ks [21]. Generally, Class I PI3Ks are further divided into IA and IB based on their different regulatory subunits and upstream activators [22]. Class IA PI3Ks are activated by RTKs and GPCRs, which contains three isoforms (PI3K $\alpha$ , PI3K $\beta$  and PI3K $\delta$ ) with the respective p110 catalytic subunit (p110 $\alpha$ , p110 $\beta$ , and p110 $\delta$ ) bound to the p85 regulatory subunit [21]. Class IB PI3K consists of PI3K $\gamma$ , with the p110 $\gamma$  bound to p101 or p84, which is mainly activated by GPCRs such as chemokine receptors [23]. PI3K $\alpha$  is known to play an important role in tumor genesis, which has been detected with persistent mutations and amplification in most human cancers including breast, ovarian, colorectal, stomach and gastric cancers [17, 24, 25]. PI3K $\beta$  involves in the development of thrombotic diseases by activating platelets, while PI3K $\gamma$  and  $\delta$  are the therapeutic targets of inflammatory and auto-immune diseases [22]. Beside PI3K $\alpha$ , the other three isoforms ( $\beta$ ,  $\gamma$  and  $\delta$ ) are also

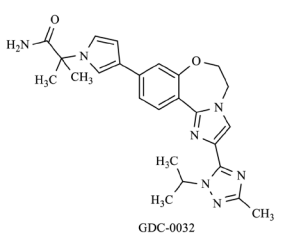
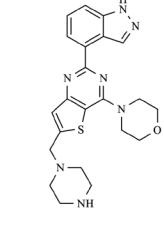
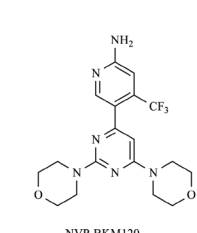
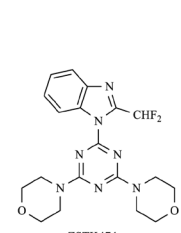
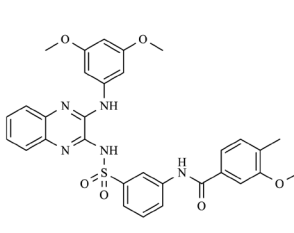
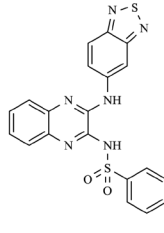
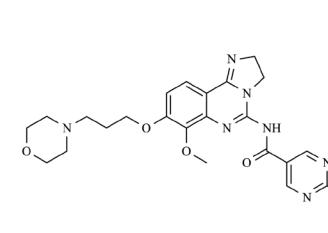
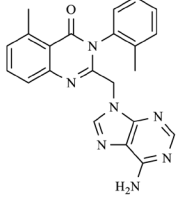
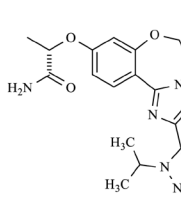
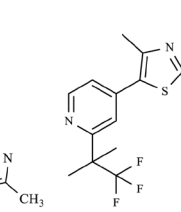
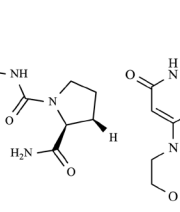
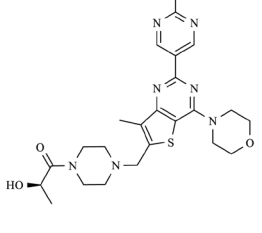
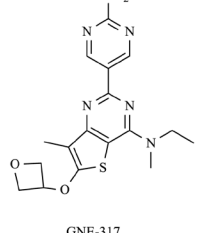
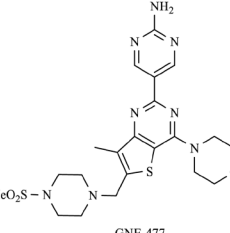
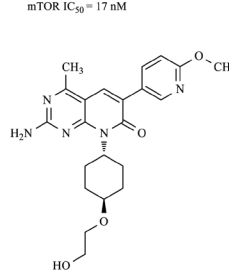
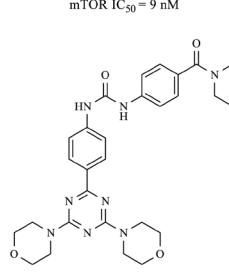
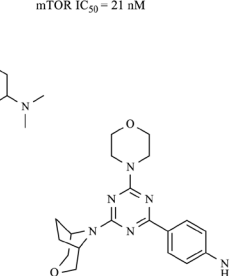
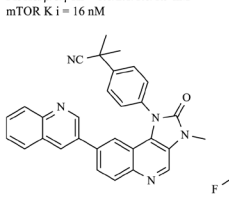
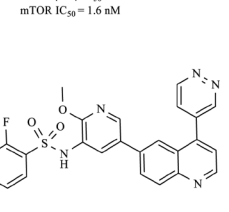
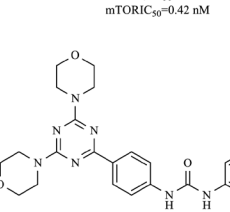
involved in tumor genesis, especially in the case of PTEN loss or inactivation. Moreover, as PI3K mutation and PTEN inactivation have been shown to be the causes of resistance to other targeted cancer therapies [26], the PI3K may even circumvent drug resistance to current chemotherapy in combination with other anticancer drugs [27].

### THE DEVELOPMENT OF PI3K INHIBITORS

The major PI3K inhibitors currently available are reversibly ATP-competitive. The X-ray crystal structure of PI3K [21] and those of its complexes with ATP, Wortmannin (1), LY294002 (3) [28, 29] and other diverse inhibitors facilitated and accelerated the development of PI3K inhibitors.

The binding models of inhibitors with the PI3K active site have also been generated in many recent studies. Overall, besides the solvent exposed area, the PI3K active site contains three key regions (PI3K $\gamma$ , Figure 2) [30]: the hinge region (Val882), the affinity pocket (Lys833, Asp841, Tyr867, Ala885, Ser806, Tyr867) or the back pocket (DFG-motif, gate keeper and catalytic lysine) and the ribose pocket (Met804, Ala805, Lys802, Met953, Asp964, Trp812, etc.). Accordingly, the ATP-competitive PI3K inhibitors mainly include (1) The hinge linker binder: substituents containing hydrogen donor/acceptor to interact with Val882 (morpholine, piperazine, indole, quinolone, amine, methoxy group, etc.) (2) The affinity pocket moiety: hydrophobic side chains or heterocycles, that may contain hydrogen donor/acceptor (sulfonamide, urea, pyridine, indazole, pyrazole, carbonyl

**Table 1: Major PI3K inhibitors that have entered into clinical trials**

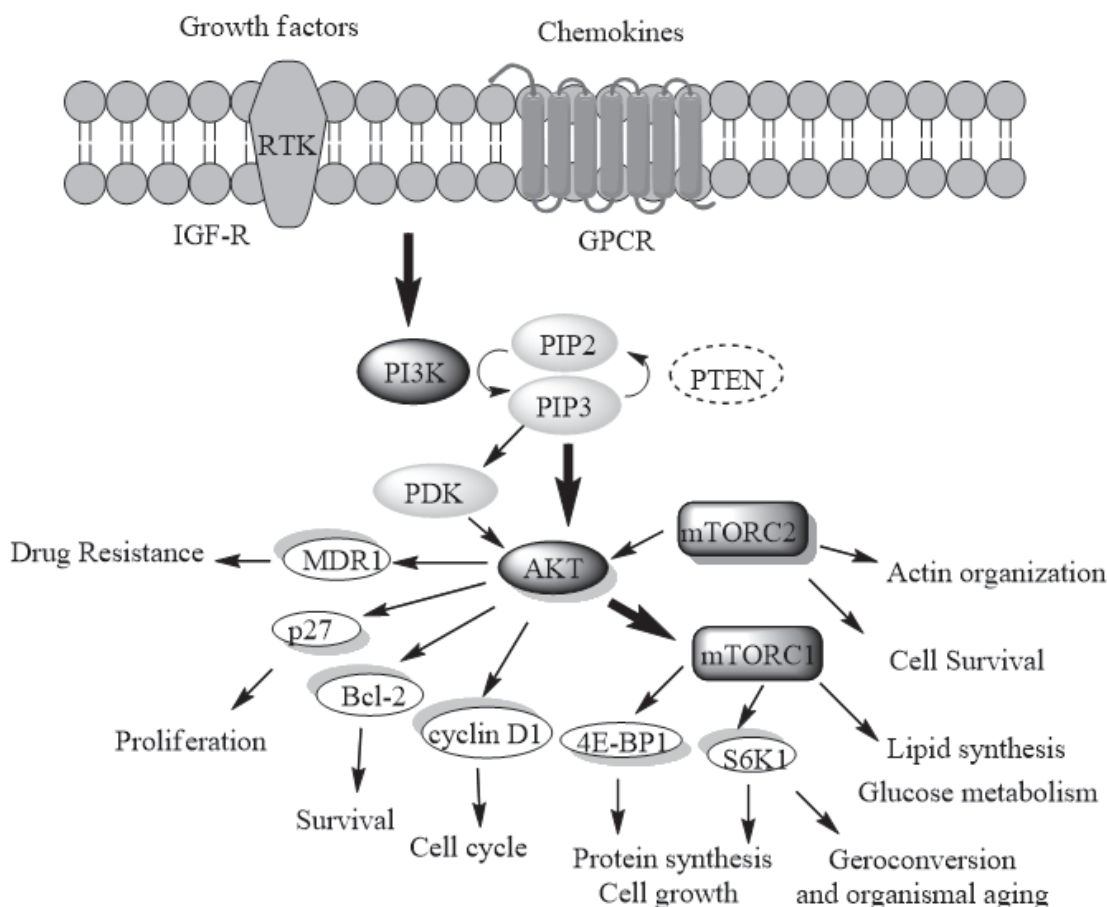
<p><b>Pan-Class I PI3K inhibitors</b></p>	<div style="display: flex; flex-wrap: wrap; justify-content: space-around;"> <div style="text-align: center; width: 20%;">  <p>GDC-0032</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 0.29/ 9.1/ 0.12/ 0.97 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>GDC-0941</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 3/33/3/75 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>NVP-BKM120</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 52/166/116/262 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>ZSTK474</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 8.6/44/5/49nM</p> </div> <div style="text-align: center; width: 20%;">  <p>XL-765</p> <p>PI3K <math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub>=39/113/43 /9 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>XL-147</p> <p>PI3K <math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 39/383/36 /23 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>BAY 80-6946</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 0.5/3.7/0.7/6.4 nM</p> </div> </div>
<p><b>Isoform-specific PI3K inhibitors</b></p>	<div style="display: flex; flex-wrap: wrap; justify-content: space-around;"> <div style="text-align: center; width: 20%;">  <p>CAL-101</p> <p>PI3K<math>\delta</math> IC<sub>50</sub> = 2.5 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>GDC-0326</p> <p>PI3K<math>\alpha</math> IC<sub>50</sub> = 0.2 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>BYL719</p> <p>PI3K<math>\alpha</math> IC<sub>50</sub> = 5 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>SAR260301</p> <p>PI3K<math>\beta</math> IC<sub>50</sub> = 23nM</p> </div> </div>
<p><b>PI3K/mTOR dual inhibitors</b></p>	<div style="display: flex; flex-wrap: wrap; justify-content: space-around;"> <div style="text-align: center; width: 20%;">  <p>GDC-0980</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 5/27/7/14 nM mTOR IC<sub>50</sub> = 17 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>GNE-317</p> <p>PI3K<math>\alpha</math> IC<sub>50</sub> = 2 nM mTOR IC<sub>50</sub> = 9 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>GNE-477</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 4/86/6/15 nM mTOR IC<sub>50</sub> = 21 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>PF-04691502</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> Ki = 1.8/2.1/1.6/1.9 nM mTOR Ki = 16 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>PKI-587/PF-05212384</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 0.4/6/6/8 nM mTOR IC<sub>50</sub> = 1.6 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>PKI-179</p> <p>PI3K<math>\alpha/\gamma</math> IC<sub>50</sub> = 8/74 nM mTORIC<sub>50</sub> = 0.42 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>NVP-BEZ2235</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 4/76/5/7 nM mTOR IC<sub>50</sub> = 21 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>GSK-2126458</p> <p>PI3K<math>\alpha/\beta/\delta/\gamma</math> IC<sub>50</sub> = 0.019/0.03/0.024/ 0.06 nM mTORC1/C2 IC<sub>50</sub> = 0.18/0.3 nM</p> </div> <div style="text-align: center; width: 20%;">  <p>PKI-587</p> <p>PI3K<math>\alpha/\gamma</math> IC<sub>50</sub> = 0.4/5.4 nM mTORIC<sub>50</sub> = 1.6 nM</p> </div> </div>

group, etc.) forming H-bonds with the residues directly or aided by a water molecule (3). The ribose pocket moiety: various substituted lipophilic ring systems and groups (pyrrolidine, pyrimidine, morpholine, thiomethyl, etc.) (4). The central core, cyclic or bicyclic, with diverse structure, having no obvious effects on the potency of the inhibitors (e.g., pyrimidine, quinazoline, pyridine, quinolone, indole, pyrazines, quinoxalines, triazoles, imidazoles, thiazoles, etc.). Of them, the hinge region binder is crucial for PI3K inhibitors, while the affinity pocket interaction could lead to improved potency and potential selectivity.

Driven by efforts in computer-based rational drug design and SAR (Structure-Activity Relationship) studies, numerous promising PI3K inhibitors have been developed and a dozen of them have entered clinical trials for treatment of cancer or other diseases (Table 1) [19]. The first approved PI3K inhibitor Idelalisib (Gilead Sciences, Inc., also known as CAL-101 and GS-1101), an orally bioavailable PI3K $\delta$  selective inhibitor with

high potency and selectivity (p110 $\delta$  IC<sub>50</sub> = 2.5 nM), was approved by the FDA in July 2014 for the treatment of several hematological malignancies, in combination with rituximab.

However, due to the high sequence homology of the catalytic domains and the conserved ATP-binding site, the key point for the development of PI3K inhibitors is to gain sufficient isoform- selectivity ( $\delta$  and/or  $\gamma$  vs.  $\alpha$  and  $\beta$ ) and cross-kinase selectivity. Although it's not an easy task, the discovery of isoform-specific PI3K inhibitors were facilitated by the elucidation of the X-ray crystal structure of PI3K isoforms and those of its complexes with diverse inhibitors [27, 29, 30]. Additionally, because mTOR is PI3K-related kinase that has similar ATP site with PI3K, a number of PI3K inhibitors could also exhibit inhibitory activity against mTOR (PI3K/mTOR dual inhibitors), which may be more effective by delivering a powerful two-spot inhibition of the pathway and have the advantage of being less susceptible to PI3K drug resistance and



**Figure 1: The PI3K/Akt/mTOR signaling pathway.** Stimulation of this pathway is commonly triggered by the growth factors (e.g. IGF) or chemokine. Subsequent activation of the lipid PI3K leads to the phosphorylation of PIP2 to PIP3, which activates AKT and PDK1. Besides direct activation by PIP3, Akt could also be activated by PDK1 and mTORC2 (Rictor-mTOR). Then mTORC1 (Raptor-mTOR) was finally activated, which regulates cell growth, glucose and lipid metabolism, autophagy as well as protein synthesis, while mTORC2 regulates cell survival and actin reorganization. Additionally, the pathway is negatively regulated by PTEN.

abrogating the compensatory effects of mTOR inhibitors [31]. In fact, most of the drug candidates in this area were PI3K/mTOR dual inhibitors (Table 1).

In this review we provide a recent view about the PI3K inhibitors including the pan PI3K inhibitors, the isoform-specific PI3K inhibitors and the PI3K/mTOR dual inhibitors, as anticancer drugs in the PI3K/Akt/mTOR pathway. As there are many well-written reviews in this field before, the novel PI3K inhibitors we emphasized here are those developed in the past eight years. Based on their core structures, the inhibitors were divided into five series: natural product derivatives; pyrimidines and quinazolines; pyridines, quinolines and indoles; pyrazines and quinoxalines; azoles and others.

## NATURAL PRODUCTS DERIVATIVES

Wortmannin (1), a steroidal furan derivative isolated from *Penicillium wortmanni*, was a pan PI3K inhibitor ( $IC_{50}$  at 50  $\mu$ M ATP~4.0, 0.7, 4.1, 9.0 nM for PI3K  $\alpha$ ,  $\beta$ ,  $\delta$  and  $\gamma$ , respectively). At higher concentrations, it could also inhibit irrelevant kinases and PI3K-related kinases, such as mTOR, DNA-PK, and ATM.

The quercetin (2), a flavonoid, is a moderate pan PI3K inhibitor (PI3K $\alpha$   $IC_{50}$ ~3.8  $\mu$ M). Diverse substitution of the chromone core of quercetin was studied and LY294002 (3), with a morpholine moiety replacement for the catechol ring, was identified by researchers at Lilly. Compound (3) was the first synthetic PI3K inhibitor (PI3K $\alpha/\beta/\delta/\gamma$   $IC_{50}$  = 0.55/ 16/1.6/12  $\mu$ M)[32], which demonstrated chemical stability and improved selectivity against irrelevant kinase, while the selectivity towards class I PI3Ks, PI3K-related kinases was not so good.

To overcome the disadvantage of toxicity due to lack of selectivity, poor solubility, and low stability [33], derivatives with good pharmacodynamics were studied and PX-866 (4), the stable, furan-ring-opened derivatives of Wortmannin was thus identified (PI3K $\alpha/\delta/\gamma$   $IC_{50}$  = 5.5/9.0/2.7 nM), which is currently being evaluated in phase I/II trials for the treatment of patients with advanced solid tumors.

Aller *et al* [34] discovered that (-)-epigallocatechin-3-gallate (5, EGCG,  $K_i$  = 0.38, 1.5, 0.81, 0.61 and 0.32  $\mu$ M for PI3K  $\alpha$ ,  $\beta$ ,  $\delta$ ,  $\gamma$  and mTOR, respectively), a major component of green tea, as well as its related catechins including CG (6,  $K_i$ =1.8/3./3.2/0.91/0.14  $\mu$ M, for PI3K  $\alpha/\beta/\delta/\gamma$  and mTOR, respectively), ECG (7,  $K_i$  = 4.2/5.4/5.2/3.0/0.28  $\mu$ M, for PI3K  $\alpha/\beta/\delta/\gamma$  and mTOR, respectively), GCG (8,  $K_i$  = 0.43/1.3/0.79/0.61/0.14  $\mu$ M for PI3K  $\alpha/\beta/\delta/\gamma$  and mTOR, respectively) were all pan-PI3K/mTOR inhibitors. Molecular docking studies showed that EGCG was an ATP-competitive inhibitor of PI3K by binding well to the PI3K kinase domain active site.

Besides, many recent studies have demonstrated that a variety of natural products (or nutraceuticals) isolated

from plants (e.g. fruits, vegetables, spices, nuts, legumes, herbs, etc.) also inhibit PI3K signaling, and exhibit potent anticancer activities. In 2013, Huang[35] briefly summarized that Apigenin, a family member of flavonoids, abundant in fruits (oranges, apples, cherries, grapes), vegetables (onions, parsley, broccoli, sweet green pepper, celery, barley, tomatoes) and beverages (tea, wine); Cryptotanshinone, one of the major tanshinones isolated from the roots of the plant *Salvia miltiorrhiza* Bunge (Danshen); Curcumin (diferuloylmethane), a polyphenol natural product of the plant *Curcuma longa*; Fisetin, a family member of flavonoids, occurring in fruits and vegetables (such as strawberries, apples, persimmons and onions); Indoles, natural compounds in cruciferous vegetables (such as broccoli, cauliflower, cabbage and brussels sprouts), especially indole-3-carbinol and its *in vivo* dimeric product 3,3-diindolylmethane (DIM); Isoflavones, a class of flavonoid phenolic compounds, rich in soybean; Quercetin, a polyphenolic compounds, mainly from consumption of tea, onions, red grapes, and apples; Resveratrol, a natural polyphenol rich in red grapes and red wine; Tocotrienols, members of vitamin E superfamily; and many other natural products (such as caffeine, epigallocatechin gallate (EGCG, in green tea), celastrol (in traditional Chinese medicine named “Thunder of God Vine”), butein (in the stems of *Rhus verniciflua*, used as a food additive and as an herbal medicine in Asia), capsaicin (in chili peppers) and  $\beta$ -elemene (from the traditional Chinese medicinal herb *Rhizoma zedoariae*), etc.), have been reported to act as anticancer agents at least partly by inhibiting PI3K, Akt or mTOR activity.

## PYRIMIDINES AND QUINAZOLINES

The pyrimidine containing PI3K inhibitors have always been the most interesting area, which include most of the clinical candidates, such as GDC-0941(13), PKI-402(55), GNE-477 (66), BKM-120(90), PI-103(102), GDC-0980(107), and PF-04691502 (122), PF-06465603 (124) PF-04979064 (127). Most of the compounds in this series are PI3K/mTOR dual inhibitors, while a few exhibited PI3K isoform or kinase selectivities, which is the major goal of the development of the PI3K inhibitors currently.

In 2008, series of thieno [3, 2-d] pyrimidine derivatives were prepared and evaluated as inhibitors of PI3K p110 $\alpha$  by Folkes *et al* [36]. The lead (9) was reported as a potent PI3K $\alpha$  inhibitor, but with poor pharmacokinetic profile. Derivatives with substitution of 6-positions (10) and the replacement of phenol group (11) were synthesized. Indazoles(12) as the replacements of phenols serve as a hydrogen bond donor with Tyr836, while reduced glucuronidation and resulted in acceptable oral bioavailability. This resulted in the discovery of GDC-0941(compound 13, PI3K $\alpha/\beta/\delta/\gamma$   $IC_{50}$  = 3/3.3/3/7.5 nM, mTOR  $IC_{50}$  = 0.58 $\mu$ M), a potent, selective, orally



bioavailable inhibitor of Class I PI3K including the p110 $\alpha$  mutant enzymes, and is currently being evaluated in human clinical trials for cancer treatment.

In 2009, series of morpholine-containing pyrazolopyrimidine analogues were synthesized and evaluated by Zask *et al* [37]. The optimization of compound 14 (PI3K $\alpha$  IC<sub>50</sub> = 47 nM, mTOR IC<sub>50</sub> = 9.6 nM) including the phenol group bioisosteres (15), piperidine ring substituents (16) and urea analogues (17), led to the discovery of potent mTOR kinase inhibitors (mTOR IC<sub>50</sub> ~ 0.08- 2450 nM) with great selectivity (~ 5-1500 fold) versus PI3K $\alpha$  ( PI3K $\alpha$  IC<sub>50</sub> ~ 6-2000 nM).

Then they [38] investigated the effects of morpholine substitution on the potency and selectivity of pyrazolopyrimidines (18-21) by incorporating chiral, achiral methyl substituted morpholines and bridged morpholines (22-29). The result showed that these chiral morpholines led to potent mTOR inhibitors (mTOR IC<sub>50</sub> ~ 0.1-100 nM) with great selectivity (~ 32-20000 fold) versus PI3K $\alpha$  (PI3K $\alpha$  IC<sub>50</sub> ~ 35-9000 nM). Molecular modeling [38] suggested that a leucine for phenylalanine substitution in mTOR versus PI3K in the hinge regions led to a deeper pocket in mTOR relative to PI3K that could better accommodate the steric bulk of the bridged morpholine.

Meanwhile, Verheijen *et al* [39] in the same team synthesized a series of 4-morpholino- 6-aryl -1H-pyrazolo [3, 4-d] pyrimidines (30-33) (mTOR IC<sub>50</sub> ~ 0.3-500 nM, PI3K $\alpha$  IC<sub>50</sub> ~ 14- 2000 nM) as mTOR inhibitors, while some 6-ureidophenyl substituents (34) led to potent dual inhibitors of mTOR and PI3K $\alpha$  (selectivity ~ 0.4-3000 fold).

Liu *et al* [40] in Pfizer discovered 4-methylpteridinones (36) as orally active and selective PI3K/mTOR dual inhibitors (PI3K $\alpha$  Ki ~ 2-82 nM, mTOR Ki ~ 0.85-3940 nM), with non-selective inhibitor 2-aminopyridopyrimidinone (35) as the lead. The 4-methylpteridinones were designed based on a small special pocket within PI3K and mTOR binding pocket to improve selectivity against other kinases, which was to be able to accommodate the methyl group of compound 37 and 38. Series of compounds (e.g. 39) with excellent selectivity for PI3K and mTOR were discovered. In addition, small changes in the C-6 aryl group will have profound effects on either PI3K or mTOR potency.

Nowak *et al* [41] reported the identification and optimization of pyrazolopyrimidines as mTOR kinase inhibitors (41-43, mTOR IC<sub>50</sub> ~ 4-7300 nM, PI3K $\alpha$  IC<sub>50</sub> ~ 31- 6000 nM). The lead (40, mTOR IC<sub>50</sub> = 215 nM, PI3K $\alpha$  IC<sub>50</sub> = 36 nM), a PI3K/ mTOR dual inhibitor, was identified by high throughput screening (HTS). Finally, a potent and selective mTOR inhibitor 44 (mTOR IC<sub>50</sub> = 9 nM, PI3K $\alpha$  IC<sub>50</sub> = 1962 nM) was discovered.

Malagu *et al* [42] discovered a novel series of mTOR kinase inhibitors(45, 46), but the PI3K inhibitory activity was not revealed, except that of compound 47,

which was 8.9 $\mu$ M against PI3K $\alpha$ , 1000- fold less potent than that against mTOR.

In 2010, Chen *et al* [10] reported a series of 4-morpholinopyrrolopyrimidine derivatives as PI3K inhibitors, by the modification of compound 48, an imidazolopyrimidine derivative with good PI3K $\alpha$  activity (PI3K $\alpha$  IC<sub>50</sub> = 63 nM). Followed by modification on the N5 of the imidazole ring, the 3-hydroxyl group on the phenyl ring and the N7 position (49-50), 4-ureidobenzamide derivatives with extended amino groups (51-53) were synthesized with excellent cell potency. As the most potent compound, 54 (PI3K $\alpha$  IC<sub>50</sub> = 0.9 nM, mTOR IC<sub>50</sub> = 0.6 nM) also demonstrated *in vivo* antitumor efficacy. The replacement of the 3-hydroxy methyl group with 4-arylurea is outstanding, which not only improved metabolic stability but also increased enzyme potency and cell potency [7] .

Dehnhardt *et al* [31] described the discovery of PKI-402 (55, PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  IC<sub>50</sub> = 1/7/14/16 nM, mTOR IC<sub>50</sub> = 1.7 nM), a novel dual PI3K/mTOR inhibitor. With imidazole- pyrimidine 56 (PI3K $\alpha$ / $\gamma$  IC<sub>50</sub> = 45/1134 nM, mTOR IC<sub>50</sub> = 634 nM) as the lead, triazolopyrimidine 57 (PI3K $\alpha$ / $\gamma$  IC<sub>50</sub> = 83/435 nM, mTOR IC<sub>50</sub> = 250 nM), arylureido and 4-benzamidoureido analogues (58-59) were synthesized and evaluated. Compound 56 was identified, with excellent potency *in vitro* and *in vivo*, good physical properties and pharmacokinetic parameters.

Pecchi *et al* [43] identified 2-morpholino 6-(3-hydroxyphenyl) pyrimidine (60, PI3K $\alpha$  IC<sub>50</sub> = 0.031  $\mu$ M) as a potent and selective PI3K inhibitor initially, then analogues of 2, 4, 6-trisubstituted pyrimidine (61-63) were prepared by solid phase synthesis and evaluated (PI3K $\alpha$  IC<sub>50</sub> = 0.031-16  $\mu$ M), which were approximately equipotent against  $\alpha$  and  $\delta$  isoforms, while about 10-fold less potent against the  $\beta$  and  $\gamma$  isoforms.

Heffron *et al* [44] (Genentech) discovered that, 2-aminopyrimidine derivatives replacing indazole moiety of GDC-0941 (13, PI3K $\alpha$ /mTOR IC<sub>50</sub> = 3/580 nM), exhibited improved mTOR inhibition and improved potency while maintaining PI3K $\alpha$  inhibition. This finding was an entry into the identification of many attractive PI3K/mTOR dual inhibitors. Modification in the 6- and 7- position (64-65)of the thienopyrimidine core resulted in comparable potency (IC<sub>50</sub> ~1-7 nM and 29-59 nM for PI3K and mTOR, respectively). The 7- methyl group was introduced to disrupt planarity and improve clearance *in vivo*. This led to the identification of GNE-477 (66, PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  IC<sub>50</sub> = 4/86/6/15 nM , mTOR IC<sub>50</sub> =4 /21 nM), a potent dual PI3K/mTOR inhibitor with desirable pharmacokinetic properties.

Zask and Verheijen *et al* (Wyeth) previously reported [38] that bridged morpholines on pyrazolopyrimidine(67) and thienopyrimidine (68) scaffolds with a para-ureidophenyl substituent led to potent mTOR inhibitors with greater selectivity for mTOR versus PI3K than the corresponding morpholine containing analogs.

They introduced the ethylene-bridged morpholine, and 4-ureidophenyl groups substituent in 4-morpholinothieno [3, 2-d] pyrimidines [45] (e.g., compound 69 and 70, moderate PI3K/mTOR dual inhibitors), which led to highly potent mTOR inhibitors ( $IC_{50} \sim 0.29-100$  nM) with good selectivity (up to >1000-fold) over PI3K $\alpha$  ( $IC_{50} \sim 8.3-10000$  nM).

In 2010, they extended these discoveries to other scaffolds, including thienopyrimidine (71-73) and triazine (74-76) scaffold. And then, bridged morpholines (26-29) were incorporated in monocyclic triazine PI3K/mTOR inhibitors [46], and compounds with ureidophenyl groups (76) gave highly potent and selective mTOR inhibitors ( $IC_{50} \sim 0.5-130$  nM) over PI3K $\alpha$  ( $IC_{50} \sim 24-3438$  nM), as expected.

Sutherlin *et al* [47] (Genentech) discovered (thienopyrimidin-2-yl) amino pyrimidines (77-78) as pan-PI3K and pan-PI3K/mTOR dual inhibitors. Structural modification of the GDC-0941 (13, a pan-PI3K inhibitor,  $IC_{50} = 3/33/3/66/580$  nM for PI3K  $\alpha/\beta/\delta/\gamma$  and mTOR, respectively) resulted in compound (79), a potent pan-PI3K/mTOR dual inhibitor ( $IC_{50} = 3.4/12/16/16/30$  nM for PI3K $\alpha/\beta/\delta/\gamma$  and mTOR, respectively). The increased mTOR potency was presumably caused by the aminopyrimidine group, which was adjacent to Asp836 (PI3K $\gamma$ / Glu2190 (mTOR), and the Glu2190 was more flexible than Asp836. Then compound (80) was designed, with 4-methyl group added on the aminopyrimidine, and lacked mTOR inhibitory activity ( $IC_{50} = 3.5/25/5.2/15/750$  nM for PI3K  $\alpha/\beta/\delta/\gamma$  and mTOR, respectively). The

crystal structure showed that [47] this selectivity was due to the 4-methyl group, which twisted the amino pyrimidine ring out of the plane of the thienopyrimidine, and pointed toward the upper surface of the binding pocket where differences in mTOR and PI3K exist.

Venkatesan *et al* [48] reported a series of novel 2-aryl or heteroaryl substituted-4-morpholino imidazolopyrimidine derivatives (81-82) as moderate to potent dual PI3K/mTOR inhibitors (PI3K $\alpha$   $IC_{50} \sim 11-189$  nM, PI3K $\gamma$   $IC_{50} \sim 47-10000$ nM, mTOR  $IC_{50} \sim 51-7200$  nM), which had good tumor cell growth inhibition and suppression of pathway specific biomarkers such as phosphorylation of Akt.

In 2011, Burger *et al* (Novartis) [49] discovered a series of 2-morpholino, 4-substituted, 6-heterocyclic morpholino pyrimidines (84-86, PI3K $\alpha$   $IC_{50} \sim 2-7740$  nM) as potent PI3K inhibitors. The lead compound 83 was a potent pan class I PI3K inhibitors (PI3K $\alpha$   $IC_{50} \sim 50$  nM) with poor pharmacokinetic properties due to the phenol group. Then the C6 phenol moiety was replaced by diverse heterocycles, and the aminopyrimidine turned to be the best choice, being equipotent to the phenol. After the C4 position was further optimized, pharmacokinetic and efficacy study conducted, compound 87 (PI3K $\alpha$   $IC_{50} < 2$  nM) was identified with efficacy and suitable *in vivo* pharmacokinetic properties.

Then in their continued study [50], C4' modified, C6 pyridyl or pyrimidyl substituted 2-morpholino 4-aminoquinolyl pyrimidines (88) were synthesized and evaluated, aiming to improve potency and reduce

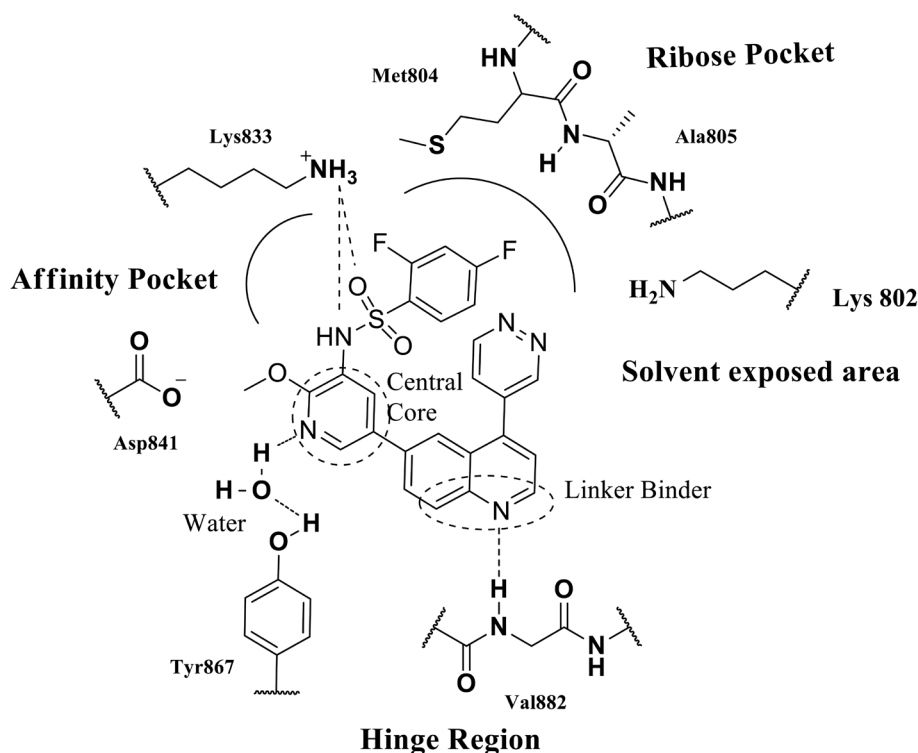


Figure 2: The potential interactions of PI3K  $\gamma$  with GSK2126458.

the *in vivo* CL values. “Incorporation of a morpholine group at the C4 position increased the aqueous solubility while maintaining potency, selectivity, and *in vivo* properties”. This led to the discovery of substituted 6-aminoheterocyclic 2, 4-bis morpholino pyrimidines (89), of which the highest soluble and the most potent compound was compound 90 (NVP-BKM120, PI3K $\alpha$  IC<sub>50</sub> ~ 30nM, mTOR IC<sub>50</sub> ~ 4600nM) that has entered into Phase II clinical trials for the treatment of cancer.

In the design of PI3K/mTOR inhibitors from pyrido[2,3-d]pyrimidin-7-one (91) and pteridinone (92), Liu *et al* (Pfizer) [51] introduced intra-molecular hydrogen bonding to the quinazoline motif to form a pseudo ring (intra-molecular H-bond scaffold, iMHBS), which was confirmed by the initial compound 94 (PI3K $\alpha$  Ki = 18 nM, mTOR Ki = 416 nM) and 95 (PI3K $\alpha$  Ki = 26 nM, mTOR Ki = 13 nM). This design resulted in potent PI3K/mTOR dual inhibitors (93, PI3K $\alpha$  Ki up to 0.3nM, mTOR Ki up to 3nM).

Heffron *et al* (Genentech) [52] described two chemical series achieving PI3K $\alpha$  selectivity versus PI3K $\beta$ , which could be explained using homology model of PI3K $\beta$ . In the thienopyrimidine series (96, PI3K $\alpha$  Ki ~ 0.4-47 nM, PI3K $\beta$  Ki ~ 7-1167 nM), the selectivity (e.g. 98 and 99) was derived from “a hydrogen bonding with Arg770 of PI3K $\alpha$  that is not attained with the corresponding Lys777 of PI3K $\beta$ ”. In the benzoxepine series (97), the selectivity (e.g. 100 and 101) was due to the “electrostatic potential differences between the two isoforms in a given region”.

Using PI-103 (102) as the lead, Large *et al* [53] designed two series of trisubstituted pyrimidines, 3-hydroxyphenol analogues (103-104) and bioisosteric replacements (105), as PI3K inhibitors. The 3-phenolic motif was replaced by three surrogate types (A, B and C), to avoid the glucuronidation *in vivo*. The most potent inhibitor was 6-aryl substitution compound 106 (PI3K $\alpha$  IC<sub>50</sub> = 62 nM), with similar activity against PI3K $\beta$  and  $\delta$ . All three surrogate types had metabolic stabilities and inhibitory activity similar to those of parent phenols.

Sutherlin *et al* (Genentech) [54] reported the discovery of GDC-0980 (107), a potent, selective, and orally available class I PI3K/ mTOR inhibitor (PI3K  $\alpha/\beta/\delta/\gamma$  IC<sub>50</sub> = 5/27/7/14 nM, mTOR Ki = 17 nM), modified from the class I PI3K inhibitor GDC-0941(13). The 2-aminopyrimidine substitution for the indazole (108) increased potency for mTOR by 20-fold. A methyl group was then added to the thienopyrimidine core (109) to lower the *in vivo* clearances and a lactic amide was used to replace sulfonamide to increase the solubility. This compound had entered clinical trials for cancer.

In 2012, Finlay *et al* (AstraZeneca) [55] described a high throughput screening approach to identify ATP competitive mTOR kinase inhibitors, starting with a modestly potent inhibitor sulfonyl morpholinopyrimidine 110 (mTOR IC<sub>50</sub> = 1.41  $\mu$ M, PI3K $\alpha$  IC<sub>50</sub> = 17.3  $\mu$ M).

Variation of substituents at the pyrimidine 2, 4, 6 position (111) provided compounds with higher mTOR potency (mTOR IC<sub>50</sub> = 0.02-100  $\mu$ M, PI3K $\alpha$  IC<sub>50</sub> = 0.56-300  $\mu$ M). The urea derivatives such as 112, exhibited the highest mTOR enzyme potency and also retained selectivity against PI3K $\alpha$  (mTOR IC<sub>50</sub> = 0.028  $\mu$ M, PI3K $\alpha$  IC<sub>50</sub> = 0.565  $\mu$ M), which confirmed that the [4-(4-morpholin-4-yl pyrimidin-2-yl) phenyl] urea motif was a privileged scaffold for mTOR and PI3K inhibition.

Koehler *et al* (Genentech) [56] designed and synthesized a novel class of potent, highly selective mTOR kinase inhibitors based upon saturated heterocycles fused to a pyrimidine core. The lead was morpholino thienopyrimidine (113), a potent mTOR inhibitor (mTOR Ki = 3 nM, 20-fold selective over PI3K). In an effort to improve the solubility and metabolic stability, by replacing the thiophene with a saturated ring, the pyrrolopyrimidines (115), 6-aza-tetrahydroquinazoline (116), 7-aza-tetrahydroquinazoline compound (117-118) were synthesized and evaluated (mTOR Ki = 1.3~1500 nM, PI3K $\alpha$  Ki = 6.5 ~ >1000 nM). The result showed that the compound (114), with the phenyl ethyl urea and s-methyl morpholine hinge binder, exhibited nanomolar potency and high selectivity against PI3K and other kinases.

A novel series of 4-methylpyrido pyrimidinone (MPP) were discovered as PI3K $\alpha$ /mTOR dual inhibitors by Le *et al* (Pfizer) [57]. The lead compound (119, PI3K $\alpha$  Ki = 1.56 nM, mTOR Ki = 142 nM) was potent, while had poor solubility and moderate lipophilic efficiency. Then through “integration of SBDD and physical properties based optimization”, a series of analogs were designed (120, PI3K $\alpha$  Ki = 12.5~138 nM, mTOR Ki = 10.6~663 nM). Notably, MPP derivative (121), with a pyrazole head piece and pyrrolidinyl, exhibited good potency (PI3K $\alpha$  Ki = 12.5 nM, mTOR Ki = 10.6 nM), desirable stability and oral bioavailability.

PF-04691502 (122), a 4-MPP derivative, exhibited potent activity against PI3K and mTOR (PI3K $\alpha$  Ki = 0.57 nM, mTOR Ki = 16 nM), while “modeling studies revealed that there was still space between the terminal alcohol and the polar residues of a solvent exposed region, and no H bond interaction between MeO-pyridine and Lys 833”. Then Cheng *et al* (Pfizer) [58] designed the MPP derivatives (123) with different heteroaryl groups in the 6 position, with cis or trans- cyclohexyl in the 8 position and with a terminal alcohol, a carboxylic acid or a carboxyl amide. These compounds (123) retained potent activity (PI3K $\alpha$  Ki ~ 0.31-26.4nM, mTOR Ki ~ 4.42-92.6 nM). PF-06465603 (124, PI3K $\alpha$  Ki = 0.35 nM, mTOR Ki = 8.63nM), a metabolite of PF-04691502 with a terminal carboxylic acid, was identified.

In their search for a structurally differentiated backup candidate to PF-04691502 (122, PI3K $\alpha$  Ki = 0.57 nM, mTOR Ki = 16 nM), compound (125), a tricyclic imidazo [1,5] naphthyridine, was identified as potent PI3K/mTOR inhibitor (PI3K $\alpha$ /mTOR Ki = 1.41/4.51



nM), while has poor solubility, and high metabolic clearance [59]. Then tricyclic derivatives (126) were synthesized through integration of SBDD and PPBO (Physical properties-based optimization), and the most suitable compound, PF-04979064 (127, PI3K $\alpha$ / $\gamma$ / $\delta$  Ki = 0.130/0.111/0.122 nM, mTOR Ki = 1.42 nM), was discovered as a backup candidate to PF-04691502.

Leahy *et al* [60] disclosed a novel series of potent and selective PI3K $\gamma$  inhibitors (131-136), based on hits 128 and 129, which were identified from HTS of ~4.6 million compounds. The sulfonyl piperazine series (131) of hit 128 had improved potency and selectivity, while with poor pharmacokinetic properties. The screening of hybrid sulfonamide derivatives (132-136) of hit 129, provided a series of promising leads with suitable pharmacokinetic properties (e.g., 130, PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  IC<sub>50</sub> = 435/2059/690/18 nM).

Lee *et al* [61] identified imidazolopyrimidine (137) as a modestly potent mTOR inhibitor (mTOR Ki = 72 nM, PI3K $\alpha$  Ki = 2 nM) by a HTS. To increase mTOR potency and selectivity over PI3Ks, they replaced the morpholine/aminopyrimidine of (137) with (S)-3-methyl-morpholine / ethyl phenyl urea to provide a more potent and selective mTOR inhibitor (138, mTOR Ki = 72 nM, PI3K $\alpha$  Ki = 190 nM). Using (138) as the lead, a variety of N-9-Me-imidazolopyrimidines (139, mTOR Ki = 7-12 nM, PI3K $\alpha$  Ki = 370-390 nM); N-7-Me-imidazolopyrimidines (140, mTOR Ki = 4-150 nM, PI3K $\alpha$  Ki > 5000 nM); N-5-Me-pyrrolo[3,2-d] pyrimidines (141, mTOR Ki = 6-19 nM, PI3K $\alpha$  Ki > 3000 nM), and N-1-Me-pyrazolo [4,3-d] pyrimidines (142, mTOR Ki = 1-28 nM, PI3K $\alpha$  Ki > 3000 nM) were synthesized and evaluated for mTOR inhibition and selectivity against PI3K.

In 2014, Han *et al* [62] identified structurally novel and potent PI3K/mTOR dual inhibitors from a series of 2-amino-4-methylpyrido [2, 3-d] pyrimidine derivatives (145-147). As indicated by the crystal structure of PF-04691502 (122, PI3K $\alpha$  /mTOR Ki = 0.57/16 nM) and GSK2126458 (143) docked into PI3K $\gamma$ , the amino-pyridopyrimidinone and quinoline formed critical hydrogen bonds with Val 882 in the hinge region. The aminopyrimidine (144) was initially synthesized and demonstrated modest inhibitory activity (PI3K $\alpha$ /mTOR IC<sub>50</sub> = 414/2790 nM). Then aiming to interact with Lys 833 in the affinity pocket, the methoxypyridine was modified by introducing diverse substituents in the 3-position. Compound 148 (PI3K $\alpha$ /mTOR IC<sub>50</sub> = 2.82 /45.8 nM) was discovered, which would be further optimized due to low permeability.

Consequently, Lin *et al* [63] from the same team reported the identification of novel 7-amino-5-methyl-1, 6-naphthyridin-2(1H)-one derivatives (149-156) as potent PI3K/mTOR dual inhibitors, by exploring the 4-methylpyridopyrimidinone (MPP), which was proven to be a potent scaffold for PI3K/mTOR dual inhibitors, such as PF-04691502 (122, PI3K $\alpha$ /mTOR Ki = 0.57/16 nM).

The identified representative compound (e.g. 157, PI3K $\alpha$ /mTOR IC<sub>50</sub> = 2.42/8.55 nM) demonstrated acceptable potency, cellular activity and pharmacokinetic profile.

To discover novel PI3K $\alpha$ /mTOR inhibitors, 2-amine-4- heterocyclic aryl-disubstituted pyrido[2,3-d]- and pyrido[3,2-d] pyrimidines with (158-161) were designed by Saurat *et al* [64]. Seven promising PI3K $\alpha$ /mTOR dual inhibitors (IC<sub>50</sub> values <100 nM) were discovered, and two urea derivatives 162 (PI3K $\alpha$ /mTOR IC<sub>50</sub> = 58/5 nM) and 163 (PI3K $\alpha$ /mTOR IC<sub>50</sub> = 40/1 nM) were further developed to enhance their efficacy.

Zhu *et al* [65] reported the discovery of 7, 8-dihydro-5H-thiopyrano [4, 3-d] pyrimidine derivatives (165-166) as mTOR inhibitors, using scaffold hopping of the lead compound (164, mTOR IC<sub>50</sub> = 1.37  $\mu$ M), a triazinehydrazone derivative. The selected compounds (mTOR IC<sub>50</sub> ~ 0.8-6.93  $\mu$ M) showing equal to more potency than the lead were further evaluated for the inhibitory activity against PI3K $\alpha$  (PI3K $\alpha$  IC<sub>50</sub> ~ 6.2-24.9  $\mu$ M). SARs and docking studies showed that “the thiopyrano [4, 3-d] pyrimidine scaffold had little effect on the antitumor activities, while variations in substitutions of the aryl moieties had a significant impact on the activities and 4-OH substitution produced the best potency”.

Shao *et al* [66] designed 2-substituted-3-sulfonamino- 5- (4-morpholinoquinazolin-6-yl) benzamides (167) as bioisostere of GSK2126458 (143). Compound 168 with potent antiproliferative activity *in vitro* was selected for PI3K /mTOR enzymatic activity assay (PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  IC<sub>50</sub> = 14/190/74/56 nM, mTOR IC<sub>50</sub> = 65 nM), Western blot assay and anticancer effects *in vivo*, and the result confirmed that these compounds could be potent PI3K inhibitors and anticancer agents. Furthermore, the docking result of 168 with PI3K $\gamma$  indicated that benzamide group could replace the complex of pyridine with water molecule in GSK2126458.

To discover dual pan-PI3K/mTOR inhibitors, Poulsen *et al* [67] generated a pharmacophore model and designed a series of novel compounds based on a purine scaffold. Three scaffolds (A-C) having a purine core substituted with a morpholine, a phenol headgroup, and a hydrophobic substituent were initially designed from three reference compounds PI-103(102), LY294002 (3) and ZSTK474 (169). Scaffold (A) was chosen for synthetic reason. The extensive SAR study of the headgroup, 8-position and 9-position substituents (170-177), utilizing the docking difference between PI3K $\alpha$  and mTOR, resulted in potent inhibitors with good pharmacokinetic properties, of which, a dual mTOR/PI3K inhibitor SB2343(178, VS-5584) and a selective mTOR inhibitor SB2602 (179) progressed into clinical trial.

Dihydropyrrolopyrimidine derivative (180, PI3K $\alpha$  IC<sub>50</sub> = 42 nM) was identified as a metabolically stable and potent PI3K inhibitor initially, while with poor oral bioavailability. To remove the H-bond acceptor and recover the water solubility, Kawada *et al* [68] designed



pyridine, benzylamine and benzamide derivatives (181, PI3K $\alpha$ IC<sub>50</sub> ~ 14-220 nM), by adding amine or amide (piperazine, morpholine) as a solubilizing group, replacing pyridine with a phenyl moiety and introducing an ortho-substituent in the phenyl group. Finally, compound 182 was identified with good pharmacokinetic profiles (oral bioavailability in monkey 8 times better than that of compound 180) and PI3K $\alpha$  inhibition (PI3K $\alpha$  IC<sub>50</sub> = 42 nM).

In 2016, to improve the low solubility of compound (183, PI3K $\alpha$  = 13 nM) by introducing a solubilizing group and ortho substituents to break molecular planarity, phenylurea derivative 184 (PI3K $\alpha$  ~ 10-260 nM) were designed by Kawada *et al* [69]. Finally, compound 185 (PI3K  $\alpha/\beta$  = 22/7 nM) with moderate solubility showed strong tumor growth inhibition *in vivo*.

Zhang *et al* [70] reported a series of N-(2-methoxy-5-(3-substituted quinazolin-4(3H)-one-6-yl) -pyridin-3-yl) phenyl sulfonamide (187-190) as PI3K inhibitors, using compound 186 as the lead. All compounds exhibited significant anti proliferative activities, of which, compounds 191 (PI3K  $\alpha/\beta/\delta/\gamma$  IC<sub>50</sub> = 7.3/209/106/116 nM, mTORIC<sub>50</sub> = 208 nM) and 192 (PI3K $\alpha/\beta/\delta/\gamma$ IC<sub>50</sub> = 6.7/24/21/ 181 nM, mTORI<sub>50</sub> = 11 4nM) displayed potent inhibitory activity against PI3K and mTOR.

## PYRIDINES, QUINOLINES, INDOLES AND INDAZOLES

Mostly, the few PI3K inhibitors based on pyridine, quinoline and indole structures reported since 2010 have been identified to be selective against PI3K $\alpha$  or mTOR, including GSK2126458 (143), the most potent PI3K inhibitor with low picomolar activity.

In 2010, Barile *et al* [16] identified a novel scaffold of the 3-ethynyl-1H-indazoles (193), as multiple PI3K/PDK1/mTOR inhibitors, and discovered a PI3K $\alpha$  isoform-specific compound (194), with 100-fold selectivity over the  $\beta$ - and  $\gamma$ -isoforms (IC<sub>50</sub> = 0.36, 40, 10.7, 39 and 3.87  $\mu$ M for PI3K $\alpha$ , PI3K $\beta$ , PI3K $\delta$ , PI3K $\gamma$ , mTOR and PDK, respectively). The binding mode revealed that “compound 194 was deeply inserted by forming hydrogen bonds with residues in the ATP-binding site: the N-2 with Tyr836 and Asp933, the NH-1 with Asp810, NH of the pyridine ring and Val851 of the hinge region”.

Zhang *et al* [71] (Wyeth) developed a series of 5-ureidobenzofuran-3-one indoles (195-197), as potent inhibitors of PI3K $\alpha$  (IC<sub>50</sub> ~ 0.2-1 nM) and mTOR (IC<sub>50</sub> ~ 0.3-1000 nM). The most potent compounds were 198 (PI3K $\alpha$ /mTOR IC<sub>50</sub> = 0.3/1 nM) and 199 (PI3K $\alpha$ /mTOR IC<sub>50</sub> = 0.2/0.3 nM), with a 4-[2-(dimethylamino) ethyl] methylamino amidophenyl group and 7-fluoro substituted on the indole ring. The predicted binding mode indicated that “the larger group (R1, R2) was more favorable for binding to mTOR, without affecting interactions with PI3K $\alpha$ ”.

Knight *et al* [72] (GlaxoSmithKline) reported the discovery of GSK2126458 (143), a highly potent, orally bioavailable PI3K/ mTOR inhibitor (Ki = 0.019/0.03/0.024/0.06/0.18/0.3 nM for PI3K $\alpha/\beta/\delta/\gamma$ , mTORC1 and mTORC2 respectively), as follow-up studies of GSK1059615 (200, PI3K $\alpha$  Ki = 2 nM), their first PI3K clinical compound. The crystal structure of 200 indicated that larger groups (e.g. pyridine, indazole, formazaindazole, pyridylsulfonamide, arylsulfonamides), instead of the thiazolidinedione (TZD) ring, could improve potency and selectivity. Then pyridine analogues (201) were designed and GSK2126458 (143) with low picomolar activity was identified. Cocrystal structure showed that the sulfonamide group made a strong charged interaction with Lys833, which may explain the superior potency of 143.

Hong *et al* [73] identified a series of [3, 5-d]-7-azaindole analogs (202-203) as PI3K $\alpha$  inhibitors, by varying groups on the 3, 5-positions of azaindole. In their pharmacophore- directed design, through fragment-based approach, 7-azaindole possessing both H-donor and H-acceptor was selected as a scaffold, and the pyridyl sulfonamide pharmacophore was introduced at C5-position to interact with the back pocket (DFG-motif, gate keeper and catalytic lysine). These 7-azaindole derivatives exhibited modest to good activity in cellular proliferation (PI3K $\alpha$ IC<sub>50</sub>=3~5200nM) and in apoptosis assays.

In 2011, Kim *et al* [74] designed and synthesized a new series of imidazo [1, 2-a] pyridine derivatives (204-205) as PI3K $\alpha$  inhibitors (PI3K $\alpha$  IC<sub>50</sub> ~ 0.2-720 nM). With the selected imidazopyridine as the hinge linker binder and pyridyl sulfonamide as the back pocket group, they explored the structures by attaching diverse groups at the C3 position to fill the ribose pocket. The SAR results showed that some moieties (e.g., ester, nitrile, oxadiazole, tetrazole, and pyridine) at the C3 position profoundly influenced PI3K $\alpha$  binding affinity (e.g. compound 206-208, PI3K $\alpha$  IC<sub>50</sub> ~ 1 nM).

In 2011, Liu *et al* [75] discovered benzonaphthyridinone analogs (210) as potent and selective small molecule mTOR inhibitors, by replacing the metabolically labile 4-amino-phenylpiperazine moiety of mTOR inhibitor Torin1 (209, PI3K $\gamma$ /mTOR EC<sub>50</sub> = 1800/2 nM) with a phenyl ring. Further modification resulted in a new mTOR inhibitor (231, PI3K $\gamma$ /mTOR EC<sub>50</sub> = 1000/5 nM) that had significantly improved stability, as well as retained potency and selectivity.

As their continued study, they [76] discovered Torin 2 (213, PI3K/mTOR EC<sub>50</sub> = 200/0.25 nM) as a potent, selective, and orally available mTOR inhibitor, utilizing a focused medicinal chemistry approach guided by cellular assays and pharmacokinetic and pharmacodynamic assays of compounds 212. The co-crystal structure revealed that the aminopyridine group formed three hydrogen bonds in the hydrophobic pocket.

Nishimura *et al* [77] reported the discovery of a series of substituted quinolines and quinoxalines

derivatives (215-217), as potent PI3K/mTOR dual inhibitors (e.g. 203, PI3K $\alpha$  Ki = 0.6 nM) with excellent pharmacokinetic properties and *in vivo* efficacies, using compound (214) as the lead. Initially, analogues with 6, 6-bicyclic heterocycles (quinoline, isoquinoline, quinoxaline, quinazoline, cinnoline, and naphthyridine) were designed, to replace the benzothiazole, as hinge linker binder. Then by incorporating suitable substituents at the 4-position of the quinoline or the 3-position of the quinoxaline rings, excellent cellular potencies were achieved, which indicated that the ribose pocket of the enzyme can be effectively utilized in optimizing both the potency and the physicochemical properties of PI3K inhibitors [77].

In 2013, Li *et al* [78] synthesized HS-106 (219, PI3K $\alpha$  IC<sub>50</sub>=11nM), by screening the above chemical library of imidazopyridine derivatives [74]. They found this compound “suppressed breast cancer cell proliferation and induced apoptosis by inducing apoptosis and suppressing angiogenesis”, which could be a potential drug for breast cancer treatment.

In order to design and optimize 3-pyridine heterocyclic derivatives as PI3K/mTOR dual inhibitors, molecular docking and 3D-quantitative structure–activity relationship (3D-QSAR) studies based on the ligand alignment and receptor alignment were applied using the comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were carried out by Yang *et al* [79]. Highly accurate and predictive 3D-QSAR models for designing new PI3K/mTOR inhibitors were constructed (Skelton 220-222), which would be useful for predicting activity and guiding the ligand modification of PI3K/mTOR inhibitors.

Tsou *et al* (Weyth) [80] discovered 2-(4-substituted-pyrrolo [2, 3-b] pyridin-3-yl) methylene-4-hydroxybenzofuran-3(2H)-ones (224-231) as potent and selective mTOR inhibitors. With the indole bearing 4, 6-dihydroxy benzofuranone (223) as the lead, a variety of 4-substituents, including 4-hydroxy phenyl, 4-benzamides and 4-piperidine amides were introduced on the indole. Also, since phenolic OH group was metabolically liable, one of the two hydroxyl groups was selectively removed. These optimizations generated subnanomolar, selective mTOR kinase inhibitors (mTOR IC<sub>50</sub> = 0.39-180 nM, PI3K $\alpha$  IC<sub>50</sub> = 16-5895 nM) with low nanomolar cellular activity.

In 2015, Lv *et al* [81] synthesized novel 4-alkynyl-quinoline derivatives (232-234) as PI3K/mTOR dual inhibitors (PI3K $\alpha$  IC<sub>50</sub>~1.63-300nM) by modification of GSK2126458 (143). “To improve the water solubility and explore potential interactions with residues in the ribose pocket (e.g. Lys802 and Ala805)”, the pyridazine of GSK2126458 was replaced with a hydrophilic substituent, while an alkyne was employed as a linkage between this hydrophilic group and the quinolone core. The target

compounds showed potent PI3K $\alpha$  inhibitory activities (PI3K $\alpha$  IC<sub>50</sub>~1.63-300nM) and good anti-proliferative effects. Compound 235, the 4-hydroxylpiperidine derivative, was further identified as a potent PI3K/mTOR dual inhibitor (PI3K $\alpha/\beta/\delta/\gamma$  IC<sub>50</sub> = 1.63/6.91/2.14/0.38 nM, mTORIC<sub>50</sub> = 3.26 nM).

## Pyrazines and quinoxalines

Given that the pan PI3K inhibitors XL-765 (236) and XL-147 (237) in clinical trials was quinoxaline derivatives, few PI3K inhibitors based on pyrazine and quinoxaline core have been developed, while with limited potency mostly.

In 2011, Wu *et al* [82] synthesized a series of novel 2-arylamino-3-(arylsulfonyl)quinoxalines (238) through a newly developed approach from XL-765 (236, PI3K  $\alpha/\beta/\delta/\gamma$  IC<sub>50</sub>=39/113/43 /9 nM ) and XL-147 (237, PI3K  $\alpha/\beta/\delta/\gamma$  IC<sub>50</sub> = 39/383/36 /23 nM).The most potent compound (239, PI3K $\alpha$  IC<sub>50</sub> = 0.07  $\mu$ M) validated the potential of 2-arylamino- 3- (arylsulfonyl) quinoxaline series for cancer treatment by targeting PI3K $\alpha$ .

Wu *et al* [83] identified novel piperidinylquinoxalines (242) and piperazinylquinoxalines (243-246) as PI3K $\alpha$  inhibitors, with previously identified morpholinoquinoxaline derivative 240 (PI3K $\alpha$  IC<sub>50</sub>=0.44  $\mu$ M) and piperidinylquinoxaline derivative 241 (PI3K $\alpha$  IC<sub>50</sub> = 0.025  $\mu$ M) as the lead. Modification at the 2-position of the quinoxaline scaffold and 4-substituent of phenylsulfonyl moiety led to novel PI3K $\alpha$  inhibitors with good to potent inhibitory activity (up to 24 nM).

In 2011, Mortensen *et al* [84] reported the discovery of the imidazo [4, 5-b] pyrazin-2-one series (247) as selective mTOR kinase inhibitors (e.g., 248 and 249). As the continued studies, through ring-expansion of the imidazo-ring by insertion of a methylene unit, they [85] discovered a new series of potent and selective mTOR kinase inhibitors (250) with exquisite kinase selectivity (mTOR IC<sub>50</sub> ~ 2-176 nM, PI3K $\alpha$  IC<sub>50</sub>> = 526 nM), which led to the identification of CC214-2 (251, mTOR IC<sub>50</sub>=0.002 nM, PI3K $\alpha$  IC<sub>50</sub>=1.38 nM), an orally available mTOR kinase inhibitor with demonstrated anti-tumor activity in mice.

In 2012, Martinez *et al* [86] described a novel series of 8-morpholinyl-imidazo [1,2-a]pyrazines (252-254) bearing an N atom in bridge head position as PI3K inhibitors, which showed good potency against PI3K $\delta$  and  $\alpha$ , with improved selectivity against mTOR kinase. The inhibitory activity of the most potent compound (255) was 2.8nM, 60nM and >10  $\mu$ M, against PI3K $\delta$ ,  $\alpha$  and mTOR, respectively. Then a variety of PI3K inhibitors (256-258) were explored, by replacing the 4-indazol moiety with heteroaryls, C2 position with additional amino alkyl substituents and C3 position with simple substituents such as bromine and methyl [87]. Finally, ETP-46321(259) has identified as a potent and orally available PI3K  $\alpha/\delta$

inhibitor (PI3K  $\alpha/\beta/\delta/\gamma$   $IC_{50}$  = 2.3/170/14.3/ 17 nM) with high selectivity over mTOR ( mTOR  $IC_{50}$  = 2.4  $\mu$ M) and 288 representative kinases.

## Azoles

Azoles (including diazole, triazole, thiazole, oxadiazole, etc.), five-member heteroaryls of pyrimidine isosteres, are also the proper scaffolds for PI3K inhibitors. Compounds in this series always achieved isoform selectivity, e.g. PI3K $\beta$  selective inhibitor SAR260301 (332, PI3K  $\alpha/\beta/\delta/\gamma$   $IC_{50}$  = 1539/23/469/10000 nM), PI3K $\alpha$  selective inhibitor BYL719 (341, PI3K $\alpha/\beta/\delta/\gamma$   $IC_{50}$  = 5/1200/290/250 nM) and GDC-0326 (310, PI3K $\alpha/\beta/\delta/\gamma$   $IC_{50}$  = 0.2/26.6/4/10.2 nM), as well as PI3K $\alpha/\delta/\gamma$  inhibitor GDC-0032 (309, PI3K $\alpha/\beta/\delta/\gamma$   $IC_{50}$  = 0.29/9.1/0.12/0.97 nM).

In 2008, Alexander [88] identified 4-(1, 3-thiazol-2-yl) morpholine derivatives (261) as potent and selective PI3K inhibitors by the modification of compound 260 (PI3K  $\alpha/\beta/\delta/\gamma$   $IC_{50}$  = 1333/ 693/701/3453nM). The most potent compounds lactam 263(PI3K  $\alpha/\beta/\delta/\gamma$   $IC_{50}$  = 59/1006/18/31 nM) showed similar potency to the ketone 262 (PI3K  $\alpha/\beta/\delta/\gamma$   $IC_{50}$  = 51/ 1157/35/49nM), while 263 exhibited better *in vivo* pharmacokinetic profiling based on its superior solubility.

In 2011, Bengtsson *et al* [89] patented the 5-heteroaryl thiazole derivatives (264) or a pharmaceutically acceptable salt and their use as PI3K and/or mTOR inhibitors. In general, compounds of the invention possessed PI3K inhibitory activity with PI3K $\gamma$   $IC_{50}$  ~ 0.1-40 $\mu$ M and PI3K $\alpha$ ~0.1-4.5 $\mu$ M.

Angelo *et al* [90] identified benzothiazole compound 265 (PI3K $\alpha$  Ki = 53 nM, mTOR Ki > 25  $\mu$ M) as an initial hit from HTS, and the crystal structure suggested that the ribose pocket might accommodate larger groups than pyrimidine. Extensive SAR studies, including the link atom (sulfur, oxygen and nitrogen) and the pyridine replacing pyrimidine (266-268), led to the sulfonamide 269 (PI3K $\alpha$  Ki = 38 nM, mTOR Ki = 269 nM) for as an early lead, with high *in vitro* and *in vivo* clearance. Subsequent modifications, including the central ring and the substitution (270-271), led to chloropyridine 272 (PI3K $\alpha$  Ki<1 nM, mTOR Ki = 2.1 nM). Further phenyl sulfonamide SAR studies (273) optimizing *in vitro* clearance led to the identification of as a potent PI3K/ mTOR dual inhibitor 274 (PI3K $\alpha$  Ki =1.2 nM, mTOR Ki = 2.0 nM), with low clearance and high oral bioavailability.

Liu *et al* (Pfizer) [91] discovered tetra-substituted thiophenes as highly selective PI3K inhibitors, with compound 275 (PI3K $\alpha$  Ki = 230 nM) as the lead, which was initially optimized by replacing the free carboxylic acid moiety with carboxylic amide (276, PI3K $\alpha$  Ki =13 nM). As nitrogen atom could form H-bond binding with a water molecule in the ATP binding site, different amide bioisosteres of compound 276 were designed (277-281),

of which compound 277 with a 1, 2, 4-triazole group stand out (PI3K $\alpha$  Ki = 1.7 nM, mTOR Ki = 434 nM). C-4 phenyl moiety was then replaced by diverse aryl and heteroaryl groups to maximize the mTOR selectivity. Finally, very potent compounds 282 (PI3K $\alpha$  Ki=0.35 nM, mTOR Ki=2470 nM) and 283 (PI3K $\alpha$  Ki=0.6 nM, mTOR Ki = 1440 nM) with excellent selectivity over mTOR (up to 7000-fold) was discovered, which demonstrated good potency *in vitro* and *in vivo*, as well as the desired pharmacokinetic properties.

“Compound (284), a potent PI3K $\alpha$ /mTOR inhibitor (PI3K $\alpha$   $IC_{50}$  = 1.2 nM, mTOR  $IC_{50}$  = 2.0 nM) *in vitro* and *in vivo*, was found to undergo deacetylation on the 2-amino substituent to yield compound 285 (PI3K $\alpha$  Ki = 5.6 nM, mTOR  $IC_{50}$  = 84 nM)”. To reduce or eliminate this metabolic deacetylation, Stec *et al* [92] examined a variety of 6, 5-hetero-cyclic analogues (286) as an alternative to the benzothiazole ring. Finally, imidazopyridazine (287, PI3K $\alpha$   $IC_{50}$ =1.4 nM, mTOR  $IC_{50}$  = 0.4 nM) was discovered, which exhibited similar *in vitro* potency and *in vivo* efficacy relative to the lead (284), while was more metabolically stable.

In 2012, Bruce (Novartis) *et al* [93] elaborated the progression from a pan-PI3K lead molecule (288, PI3K  $\alpha/\delta/\gamma$  / $\beta$  Ki= 0.11, 0.11, 0.21, 1.43  $\mu$ M) to  $\alpha$ ,  $\delta$  and  $\gamma$  isoform selective Class I PI3K inhibitors (289-293), which was urea derivatives using parallel synthesis to combine amines (RR'NH) with selected aminothiazole scaffolds. These inhibitors with good isoform selectivity and cellular activity “would be pharmacological tools for elucidating the relative contributions of individual isoforms in PI3K signaling pathways”.

Lin *et al* (GlaxoSmithKline) have reported [94, 95] the discovery of imidazopyrimidinone (294, PI3K $\alpha/\beta/\gamma/\delta$   $IC_{50}$  = 2.0/0.001/0.008/1  $\mu$ M) and triazolopyrimidinone (295, PI3K $\alpha/\beta/\gamma/\delta$   $IC_{50}$  = 0.32/0.0003/0.004/0.06  $\mu$ M), as novel potent PI3K $\beta$  selective inhibitors, while with poor pharmacokinetic profile. Then they designed [96] thiazolopyrimidinones (296), with a substituted benzyl group at the N1-position to induce the selectivity-pocket formed by Met-779 and Trp-787, a morpholine as the hinge binder and a carbonyl group to interact with the back-pocket. These compounds demonstrated potency (PI3K $\beta$   $IC_{50}$  = 0.05-790  $\mu$ M) and good selectivity ( PI3K $\beta$  selectivity >10 fold), and compound 297 (PI3K $\alpha/\beta/\gamma/\delta$   $IC_{50}$  = 2.5/0.0006/0.020/0.79  $\mu$ M) emerged as a potent, selective and orally bioavailable PI3K $\beta$  inhibitor.

Compound 298 was identified as an initial hit (PI3K $\gamma$   $IC_{50}$  = 5 nM) through HTS by Oka *et al* [97] and the docking mode indicated that nitrogen atoms in the acetylaminothiazole formed hydrogen bonds to the hinge Val882 ,while the benzoic acid moiety interacted with Lys807 and Lys833. Thus, they optimized the central heterocycles as the replacement of thiazole and identified oxazole derivative 299 (PI3K $\gamma$   $IC_{50}$  = 12 nM) as the lead for further optimization. A novel series of 2-aminothiazole-



oxazole derivatives (300) were synthesized and evaluated as PI3K $\gamma$  inhibitors (PI3K $\gamma$  IC<sub>50</sub> ~3-346 nM), of which the trifluoroethyl and tert-butyl derivatives displayed good enzymatic and cellular activities.

Peterson *et al* [98] identified new imidazopyridine and imidazopyridazine scaffolds (303) that demonstrated superior mTOR inhibition and selectivity, starting from the previously reported triazine-benzimidazole (301, mTOR IC<sub>50</sub> = 23 nM, PI3K $\alpha$  IC<sub>50</sub> = 798 nM) and triazine-imidazopyridine (302, mTOR IC<sub>50</sub> = 12 nM, PI3K $\alpha$  IC<sub>50</sub> = 590 nM). To capping sites of glucuronidation (the pyrazole NH) and introducing a ribose substituent to improve pharmacokinetics and potency, the imidazopyridazine core and substitution of the linker-binder pyrimidine were explored, which resulted in potent and selective mTOR inhibitor (303, mTOR IC<sub>50</sub> ~2-147 nM, PI3K $\alpha$  IC<sub>50</sub> ~47-12500 nM), with improved *in vivo* clearance.

In 2013, Morales *et al* [99] explored the 5-morpholino-7H thieno[3,2-b] pyran-7-ones (304) as potential PI3K inhibitors, with thiophene as the bioisostere of the phenyl ring of the classic pan-PI3K inhibitor LY294002. This series have improved potency with PI3K $\alpha$  selectivity (e.g., 305, PI3K $\alpha$ / $\beta$ / $\gamma$ / $\delta$  IC<sub>50</sub> = 34/214/960/158 nM), while in some cases, displayed an unexpected PI3K $\delta$  selectivity (e.g. 306, PI3K $\alpha$ / $\beta$ / $\gamma$ / $\delta$  IC<sub>50</sub> = 714/1750/27/1170 nM). The cell-based assay of these compounds also demonstrated potent cell growth inhibitory activity.

Then Ndubaku *et al* [100] (Genentech) derived a set of imidazobenzoxazepin compounds, by modification of compound 307, which displayed good *in vitro* potency (PI3K $\alpha$  IC<sub>50</sub> <0.5  $\mu$ M), while had low solubility and high *in vivo* unbound clearance. These imidazobenzoxazepin derivatives (308, PI3K $\alpha$  IC<sub>50</sub> ~0.1- 21.1 nM) had better tumor growth inhibition *in vivo*, and GDC-0032 (309, PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  IC<sub>50</sub> = 0.29/9.1/ 0.12/ 0.97 nM) was identified, which was undergoing clinical development for use in PI3K-related cancers. Then in 2016, they reported [101] the discovery of a series of PI3K $\alpha$ -specific inhibitors, which obtained PI3K $\alpha$ -selectivity through interactions with a non-conserved residue. Optimization of properties led to the identification of the clinical candidate GDC-0326 (310, PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  IC<sub>50</sub> = 0.2/26.6/4/10.2 nM).

Staben *et al* [102] (Genentech) had disclosed HTS derived thienobenzoxepin series (311) with aniline amide substituents as PI3K inhibitors, while the aniline amide was undesired and contributed to high clearance. To improve the clearance, stability and potency through interactions with the affinity pocket, they replaced the aniline amide with heterocyclic amide isosteres (312-320). Overall, simple branched alkyl substituted triazoles had better properties than those halo aryl substituted derivatives. The replacement of 'cis'-N-methyl aniline amides led to compound 321 (PI3K $\alpha$ / $\beta$ / $\gamma$ / $\delta$  IC<sub>50</sub> = 4.0/29/2.2/3.9 nM), a potent and selective PI3K inhibitor with high permeability, solubility and bioavailability.

To improve selectivity over PI3K $\beta$  and decrease the high clearance due to the amide hydrolysis of compound 322 (PI3K $\alpha$ / $\beta$ / $\gamma$ / $\delta$  IC<sub>50</sub> = 4.0/29/2.2/3.9 nM), they [103] further optimized the 2-triazolyl-benzoxepin 8-substitution, and replaced thiophene with other five-membered-heteroaryls (323-328, PI3K $\alpha$  IC<sub>50</sub> ~0.04- 1.9 nM, PI3K $\beta$  selectivity ~1.1-3160 fold). Finally, PI3K $\beta$ -sparing inhibitor, compound 329 (PI3K $\beta$  Ki/PI3K $\alpha$  Ki ~57 fold, PI3K $\alpha$ / $\beta$ / $\gamma$ / $\delta$  Ki ~0.27 /15 /0.55/ 0.61 nM) was discovered with a suitable pharmacokinetic profile. Furthermore, the binding mode revealed that "the selectivity might be due to difference in the conformation of a tryptophan residue present in all isoforms ( $\alpha$  TRP780,  $\beta$  TRP781,  $\delta$  TRP760,  $\gamma$  TRP812)", which presented a new structure-based hypothesis for reducing inhibition of the PI3K $\beta$  isoform while maintaining activity against  $\alpha$ ,  $\delta$  and  $\gamma$  isoforms.

The indoline amide (331), replacing the aniline of compound (330) with a secondary amine, displayed potent and selective PI3K $\beta$  inhibition (IC<sub>50</sub> = 460 nM, 4 nM, 28 nM and >10  $\mu$ M for PI3K $\alpha$ ,  $\beta$ ,  $\delta$  and  $\gamma$ , respectively), but had poor aqueous solubility due to a strong network of hydrogen bond and hydrophobic interactions. To improve the solubility, 2-methyl was introduced on the indoline to yield different substituted pyrimidone indoline amides (332, IC<sub>50</sub> ~85-10000 nM, 1-407 nM, 2-4135 nM, 1131-10000 nM for PI3K $\alpha$ ,  $\beta$ ,  $\delta$  and  $\gamma$ , respectively) by Certal *et al* [104] in 2014. Finally, compound 333 (SAR260301) was discovered as a potent and soluble PI3K $\beta$  inhibitor (PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  IC<sub>50</sub> = 1539/23 nM/469/10000 nM), which entered clinical trial in patients with advanced cancer.

Meanwhile, a novel thiazole carboxamide series was designed (e.g., compound 335, IC<sub>50</sub> = 8280, 45 nM, 227 nM and >10,000 nM on PI3K $\alpha$ ,  $\beta$ ,  $\delta$  and  $\gamma$ , respectively) by a fragment based rescaffolding [104], starting from pyrimidone 334 (PI3K $\beta$  selective inhibitor, IC<sub>50</sub> = 10000 nM, 42 nM, 118 nM and >10000 nM for PI3K $\alpha$ ,  $\beta$ ,  $\delta$  and  $\gamma$ , respectively). Then a series of morpholino thiazole derivatives were synthesized as PI3K $\beta$  selective inhibitors (336, IC<sub>50</sub> ~395-10000 nM, 8-340 nM, 58-2723 nM and >4203 nM for PI3K $\alpha$ ,  $\beta$ ,  $\delta$  and  $\gamma$ , respectively) with suitable properties.

Collier *et al* [105] discovered a series of C6 substituted benzothiazole and urea analogues (338-339, PI3K $\gamma$  Ki ~1-770 nM, PI3K $\alpha$  Ki ~7-4000 nM) as PI3K $\gamma$  selective inhibitors, evolved from a reported phenylthiazole pan-PI3K inhibitor PKI-93 (337). From the X-ray crystallography of compound 340 (PI3K $\gamma$  Ki = 7 nM, PI3K $\alpha$  Ki = 105 nM) bound to PI3K $\gamma$ , they found that the propylimidazole group occupied a previously unreported hydrophobic cleft adjacent to the ATP binding site and residue differences (Gly829 and Ala885 in PI3K $\gamma$ ) in this region caused the PI3K isoforms selectivity.

Gerspacher *et al* [106] (Novartis) converted the 5-(pyridin-4-yl)thiazol-2-amino bicyclic core scaffold of BYL719 (341) into novel 4H-thiazolo[5',4':4,5]



pyrano[2,3-c]pyridine tricyclic scaffold (342), via an oxygen as the linker. Activity result showed that 343 (PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  IC<sub>50</sub> = 5/670/220/200 nM) exhibited similar or better biochemical potency, selectivity against PI3K $\alpha$  and cellular activity, as well as favorable pharmacokinetic properties due to enhanced solubility, compared to noncyclized analogs BYL719 (341, PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  IC<sub>50</sub> = 5/1200/290/250 nM).

## Triazines

ZSTK474 (169), the first triazine derivatives as a potent pan class I PI3K inhibitor, was reported in 2006. With the liability of synthesis and suitable position of the substituent, PI3K inhibitors based on the triazine or triazine-benzimidazole core structure has been continued [107], such as PKI-587(348), PKI-179 (350).

In 2010, Richard *et al* [108] (Wyeth research) identified a variety of potent triazine mTOR inhibitors containing the (R)-3-methyl morpholine moiety and a pyridylureidophenyl group, which demonstrated good selectivity (greater than 500-fold) over the related PI3K $\alpha$  (344-346, mTOR IC<sub>50</sub> ~ 0.2-3.6 nM, PI3K $\alpha$  IC<sub>50</sub> ~ 41-1894nM). SAR studies revealed that “the addition of basic amines at the 4-position of the ureidophenyl ring was well-tolerated and offered the opportunity to develop inhibitors with improved physicochemical properties, while amide derivatives at the 4-position of the arylureidophenyl ring resulted in reduced selectivity over PI3K $\alpha$  but enhanced cellular activity”.

Venkatesan *et al* [48] (Pfizer) discovered that a series of bis(morpholino-1,3,5-triazine) derivatives bearing bis-aryl ureas (347) are potent dual PI3K/mTOR inhibitors. They also found that the amide bearing water solubilizing groups (eg, N (Me)<sub>2</sub>-piperidine, methylpiperazine, pyrrolidino-piperidine) enhanced potency, due to the increased H-bond accepting ability. Finally, compound 348 (PKI-587, IC<sub>50</sub> = 0.4, 5.4, 1.6 nM for PI3K $\alpha$ , PI3K $\gamma$  and mTOR, respectively) was identified as a highly efficacious PI3K/mTOR inhibitor *in vitro* and *in vivo*, which entered clinical trials as a single agent for i.v. administration.

However, PKI-587(348) could not be administered orally because of poor permeability, low clog P and high molecular weight. Hence, to obtain an orally efficacious PI3K/mTOR inhibitors by increasing the clog P and to lowering the molecular weight, a series of mono-morpholino 1, 3, 5-triazine derivatives bearing a 3-oxa-8-azabicyclo [3.2.1] octane were designed [109] (349, PI3K $\alpha$  IC<sub>50</sub> ~7-85nM, PI3K $\gamma$  IC<sub>50</sub> 44-717nM, mTOR IC<sub>50</sub> ~0.32-93.5nM). An orally efficacious dual PI3K/mTOR inhibitor, compound 350 (PKI-179, IC<sub>50</sub> = 8, 74, 0.42nM for PI3K $\alpha$ , PI3K $\gamma$  and mTOR, respectively) with lower molecular weight was discovered. Moreover, the active metabolite of PKI-587 was determined to be 351 (IC<sub>50</sub> = 4, 33, 0.8nM for PI3K $\alpha$ , PI3K $\gamma$  and mTOR, respectively).

In 2011, based on the triazolopyrimidine (55,

PKI-402) and triazine (348, PKI-587) scaffold as highly efficacious dual PI3K/mTOR inhibitors, Dehnhardt *et al* [110] (Pfizer) designed the novel 2-oxatriazines series (352-353), which also exhibited excellent potency and good metabolic stability. The most potent compound 354(PI3K $\alpha$  IC<sub>50</sub> = 0.2 nM, mTOR IC<sub>50</sub> = 0.7 nM) showed an *in vitro* profile comparable to PKI-587.

Peterson *et al* [111] discovered triazine-benzimidazoles as selective mTOR inhibitors, using compound 355 (PI3K $\alpha$  IC<sub>50</sub> = 0.32  $\mu$ M, mTOR IC<sub>50</sub> = 0.097  $\mu$ M) as the lead. The synthesized triazines provided broader kinase selectivity and improved potency (356-358), with diverse phenol bioisosteres to modify the affinity pocket binding moiety and the triazine linker-binder replacement to improve the pharmacokinetic properties. Compound 359 (PI3K $\alpha$  IC<sub>50</sub> = 2.2  $\mu$ M, mTOR IC<sub>50</sub> = 0.081  $\mu$ M) exhibited superior selectivity to other compounds, “with 200-fold selectivity over PI3K $\alpha$ , and greater than 100-fold selectivity over the other PI3K isoforms”.

As ZSTK474 (169, PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  IC<sub>50</sub> = 8.6/44/5/49 nM) had poor aqueous solubility which limited the development of an amorphous formulation [112], Rewcastle *et al* [113] explored the 2-substituted, 4-, 5- and 6-substituted and pyrimidine analogues of ZSTK474, to discover more soluble inhibitors (360-361). They found that substitution at the 4 and 6 positions of the benzimidazole ring generated highly potent PI3K inhibitors (e.g., compound 362, PI3K $\alpha$  IC<sub>50</sub> = 0.22 nM) with good pharmacokinetics and efficacy *in vivo*, while still had poor solubility properties.

In 2012, Smith *et al* [30] synthesized a series of highly selective class I PI3K inhibitors, starting from the potent PI3K/mTOR dual inhibitor 363 (PI3K $\alpha$  Ki = 350 nM, mTOR IC<sub>50</sub> = 93 nM) with poor pharmacokinetic properties due to the glucuronidation of the phenolic substituents and extensive metabolism of the benzimidazole *in vivo*. As “pyridylpyrimidine and pyridylpurine scaffolds had been demonstrated to have good *in vivo* properties”, scaffold 364 was explored, with additional substituents to interact with the ribose pocket (Ki values <10 nM against PI3K $\alpha$ , PI3K $\beta$ , PI3K $\gamma$ , PI3K $\delta$  and >1000-fold selectivity against mTOR). Finally, compound 365 (PI3K $\alpha$ / $\beta$ / $\delta$ / $\gamma$  Ki = 9/5/2/4 nM, mTOR IC<sub>50</sub> = 4800 nM) was discovered, with excellent selectivity over mTOR and related kinases.

In previous studies, Wurz *et al* [90] disclosed sulfonamide benzothiazole derivatives (e.g. 366, PI3K $\alpha$  Ki = 1.2 nM, mTOR IC<sub>50</sub> = 2.1 nM) as potent PI3K/mTOR dual inhibitor, while had low solubility. Meanwhile, another series of PI3K inhibitors (e.g. 367, PI3K $\alpha$  Ki = 9.1 nM, mTOR IC<sub>50</sub> = 4.7  $\mu$ M) with moderate *in vivo* pharmacokinetic properties, was identified. Then structure 368 was designed by a hybrid strategy [113], which underwent modification to the sulfonamide of the affinity pocket binding motif (R1) and the ribose pocket binding

motif (R2). A novel series of 4-amino-6-methyl- 1, 3, 5-triazine sulfonamides were discovered as PI3K inhibitors (PI3K $\alpha$  Ki~3.5-161 nM).

In 2013, Pinson *et al* [115] identified a series of amino acyl-triazine derivatives (369) as potent and isoform selective PI3K $\beta$  inhibitors, by modifying the pan-PI3K inhibitor ZSTK474. The selectivity based on the stereochemistry, with L-amino acyl derivatives preferring PI3K $\beta$ , while their D-congeners favored PI3K $\delta$  (PI3K $\alpha$  IC<sub>50</sub>~42-10000 nM, PI3K $\beta$  IC<sub>50</sub>~31-10000 nM, PI3K $\delta$  IC<sub>50</sub>~26-3600 nM,  $\beta/\delta$  selectivity~0.007-34). This could be explained by interaction with the non-conserved binding site residue ASP862 of PI3K $\beta$ , which provided an alternate mechanistic basis for selectivity.

## Others

To discover novel PI3K $\gamma$  inhibitors as anticancer agent, Taha *et al* [116] explored the pharmacophoric space of PI3K $\gamma$  via diverse inhibitors and used CATALYST-HYPOGEN to identify high quality binding model in 2014. Then QSAR model was assessed within training inhibitors (78 collected PI3K $\gamma$  inhibitors, scaffold 370-378) and two associated models were validated by screening for new PI3K $\gamma$  ligands. 19 NCI hits (379-397) exhibited good to moderate potencies against PI3K $\gamma$  (IC<sub>50</sub> =105-9157nM) *in vitro*, which suggested that “the combination of pharmacophoric exploration and QSAR analysis could be useful to find new and diverse PI3K $\gamma$  inhibitors”.

## CONCLUSION

Unregulated activation of the PI3K/Akt/mTOR pathway is a prominent feature of many human cancers and PI3K is activated or over-expressed in all major cancers. This makes PI3K as one of the most attractive anticancer targets, which may even circumvent drug resistance to current chemotherapies proved by preclinical and clinical evidences. The discovery of PI3K inhibitors brought a lot of promising compounds as drug candidates, a dozen of which have been advanced into preclinical or clinical trials for cancer treatment. Furthermore, the first approved PI3K inhibitor, Idelalisib (p110 $\delta$  selective) has already been used for the treatment of various hematological malignancies. However, there are many issues remained to be addressed.

Currently, the key point for the further development of PI3K inhibitors is selectivity. Much effort has been made to the development of class I PI3K inhibitors that exhibit sufficient isoform- selectivity and cross-kinase selectivity, with the help of the elucidation of the X-ray crystal structures of PI3K isoforms and those of their complexes with diverse inhibitors. As each PI3K isoform has its own function and is correspondingly involved

in various diseases, it was assumed that the isoform-specific PI3K inhibitors may obtain lower toxicity, better tolerability and safety, while the pan- PI3K inhibitors could offer enhanced therapeutic efficacy. Likewise, the PI3K/mTOR dual inhibitors was considered to be more effective by delivering a powerful two-spot inhibition of the pathway and have the advantage of being less susceptible to PI3K drug resistance. “Will the isoform-specific inhibitors be more tolerable than pan-PI3K inhibitors”, “Whether the dual inhibition of PI3K and mTOR is superior to inhibiting PI3K alone”, “How to find the proper balance between the safety (only through kinase selectivity) and the therapeutic efficacy” are still questions remained to be addressed. And the answer will not be known until the completion of ongoing clinical trials.

## Abbreviations

PI3K = phosphatidylinositol-3-kinase  
Akt = protein kinase B, PKB  
mTOR = mammalian target of rapamycin  
PTEN = phosphatase and tensin homologue deleted on chromosome ten  
RTK = receptor tyrosine kinases  
PIP2 = phosphatidylinositol 4, 5-bisphosphate  
PIP3 = phosphatidylinositol 3, 4, 5-triphosphate  
PDK1 = phosphatidyl inositol 3-dependent kinase 1  
mTORC1 = mTOR complex 1  
mTORC2 = mTOR complex 2  
4E-BP1 = 4E-binding protein 1  
MDR1= multidrug resistance protein-1  
PIKKs = PI3K-related kinases  
DNA-PK= DNA- dependent protein kinase  
GPCR = G-protein-coupled receptors  
SAR = Structure Activity Relationship  
SBDD = Structure-based drug design  
QSAR = Quantitative Structure-Activity Relationship  
HTS = high throughput screening

## ACKNOWLEDGMENTS

This work was supported by grants from Independent Innovation Foundation of Shandong University (No. 2012TS134), National Natural Science Foundation of China (No.81200344, 81470139).

## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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