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Review Article

Phosphatidylinositol 3-kinase (PI3K) inhibitors as anticancer drugs

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CONTENTS

Abstract 537
Introduction 537
Wortmannin and derivatives 538
Quercetin derivatives 539
Pyridofuropyrimidines and related structures 540
Imidazopyridines 541
Imidazoquinolines and related structures 541
Thiazoles and related structures 541
Liphagal and derivatives 543
Other structures 543
Conclusions 544
References 545

Abstract

The phosphatidylinositol 3-kinases (PI3Ks) are members of a family of lipid kinases that regulate cellular metabolism and growth by phosphorylation of the 3-position of phosphatidylinositol to generate phosphatidylinositol triphosphate (PIP_3). The PI3K pathway is one of the most frequently mutated in human cancer, leading to amplification of signaling, and as such is a promising target for small-molecule inhibition, with exciting therapeutic opportunities for the treatment of cancer. The last several years have seen an explosive increase in publications and patents concerning inhibition of PI3K. This paper summarizes recent developments in the discovery of PI3K inhibitors as anticancer drugs. We describe representative examples of seven structurally diverse classes of PI3K inhibitors. These compounds represent a large arsenal of potent enzyme inhibitors (single-digit nanomolar IC_{50} values) with a variety of isoform specificity profiles. Currently, the first PI3K inhibitors are making their way into the clinic and we eagerly await data that will help address questions regarding the contribution of inhibition of various PI3K isoforms to tumor suppression and the associated side effect profiles.

Introduction

The phosphatidylinositol 3-kinases (PI3Ks) are members of a family of lipid kinases that regulate cellular metabolism and growth by phosphorylation of the 3-position of phosphatidylinositol to generate phosphatidylinositol triphosphate (PIP_3) (1-3). The lipid second messenger PIP_3 in turn couples the PI3Ks to downstream effectors, especially Akt, where binding leads to membrane recruitment and phosphorylation. The PI3K pathway is one of the most frequently mutated in human cancer, leading to amplification of signaling, and as such is a promising target for small-molecule inhibition, with exciting therapeutic opportunities for the treatment of cancer.

PI3Ks are divided into class I and class II kinases. The class I PI3Ks in turn are divided into two groups based on their structure and the receptors that activate them. The class IA PI3Ks (PI3Kα, PI3Kβ and PI3Kδ) are coupled to a wide range of receptor tyrosine kinases, whereas the only member of class IB PI3K, PI3Kγ, is activated by G-protein-coupled receptors. Recent studies with isoform-specific small-molecule inhibitors have helped to clarify the distinct cellular functions of the different class I PI3Ks. These studies have shown that inhibition of PI3Kα is essential to effect growth suppression in malignant cell lines (4). The other class I isoforms have therapeutic potential in a host of other disease areas, such as the treatment of inflammatory and autoimmune disease (PI3Kδ, PI3Kγ) and thrombosis (PI3Kβ) (1-3, 5-8). PI3Kα is also the key PI3K downstream of the insulin receptor and its inhibition has been linked to hyperglycemia, pointing to a potential side effect of this type of therapeutic approach (4). However, it has recently been reported that the insulin sensitivity enhancer pioglitazone can reverse hyperglycemia in mice treated with a derivative of the PI3K inhibitor wortmannin (9).

Although it seems clear that inhibition of the α isoform is essential for the antitumor activity of PI3K inhibitors, it is not clear whether a more selective inhibitor of a particular PI3K isoform may lead to fewer unwanted biological effects. It has recently been reported that non-PI3Kα class I isoforms (PI3Kβ, δ and γ) have the ability to induce oncogenic transformation of cells, suggesting that non-isoform-specific inhibitors may offer enhanced therapeutic potential over specific inhibitors (10). In this respect, it is also of interest to note that a selective inhibitor of PI3Kδ was found to enhance the effects of radiation on tumor growth (11).

Selectivity *versus* other related kinases is also an important consideration for the development of PI3K inhibitors. While selective inhibitors may be preferred in

order to avoid unwanted side effects, there have been reports that inhibition of multiple targets in the PI3K/Akt pathway (*e.g.*, PI3Kα and mTOR [mammalian target of rapamycin]) may lead to greater efficacy (12). It is possible that lipid kinase inhibitors may parallel protein kinase inhibitors in that nonselective inhibitors may also be brought forward to the clinic (13).

The development of selective inhibitors is challenging due to the structural similarity of the ATP-binding pockets of the PI3K isoforms and other PI3K kinase-related kinases (PIKKs). X-Ray crystal structures of inhibitors bound to PI3Kγ and homology modeling have shown that binding to the backbone amide nitrogen of valine-882 is a conserved interaction among all known PI3K inhibitors (4, 14). This review will focus on small-molecule inhibitors of PI3Kα for cancer, as well as surveying other lipid kinase ATP-binding site scaffolds for other therapeutic indications.

Wortmannin and derivatives

The natural product wortmannin and its analogue 17-hydroxywortmannin (Fig. 1) are potent, nonselective inhibitors of PI3Ks (wortmannin IC_{50} = 4.2 nM against PI3Kα) that bind irreversibly to lysine-833 in the ATP-binding pocket of PI3K via opening of the electrophilic furan ring at its C-20 position, as seen in the X-ray crystal structure of wortmannin bound to PI3Kγ (15). Wortmannin has played a major role in elucidating the role of PI3K in signal transduction pathways, although its lack of isoform specificity precludes its utility in identifying the isoform responsible for a particular biological process. Wortmannin has demonstrated potent cytotoxic activity against human tumor cell lines and in xenograft models in mice, although its toxicity (exemplified by a low therapeutic index), insolubility and aqueous instability (through hydrolytic ring opening of its reactive furan ring) have hampered its development as a viable anticancer agent. Despite the extensive structure-activity relationship (SAR) studies with wortmannin derivatives, there are few examples of compounds with improved properties, which are discussed below.

Recently, application of pegylation technology to wortmannin and 17-hydroxywortmannin gave conjugates (*e.g.,* PWT-458) with greatly improved properties (15, 16). PWT-458 is completely miscible with water and has improved stability in plasma relative to wortmannin. It was active in xenograft models in athymic mice with PTEN-deficient U-87 glioma, with 50% inhibition of tumor growth on day 7 at a minimally effective dose of 0.5 mg/kg (daily x 5 i.v.). Tumor growth inhibition increased in a dose-dependent manner up to a maximally tolerated dose of 15 mg/kg. In contrast, 17-hydroxywortmannin, although effective at the same minimally effective dose of 0.5 mg/kg, was not tolerated at doses above 1.5 mg/kg. Thus, pegylation enhanced by at least 10-fold the therapeutic index of 17-hydroxywortmannin.

Opening of the wortmannin furan ring with nucleophiles such as secondary amines leads to analogues that retain significant PI3K inhibition. For instance, PX-866 (ProlX Pharmaceuticals) is reported to have comparable potency to wortmannin in inhibiting PI3Kα (IC_{50} = 5.5 nM) (17). Interestingly, in contrast to wortmannin, it is selective *versus* one of the PI3Ks (PI3Kβ IC_{50} > 300 nM). PX-866 is active in human tumor xenograft models in

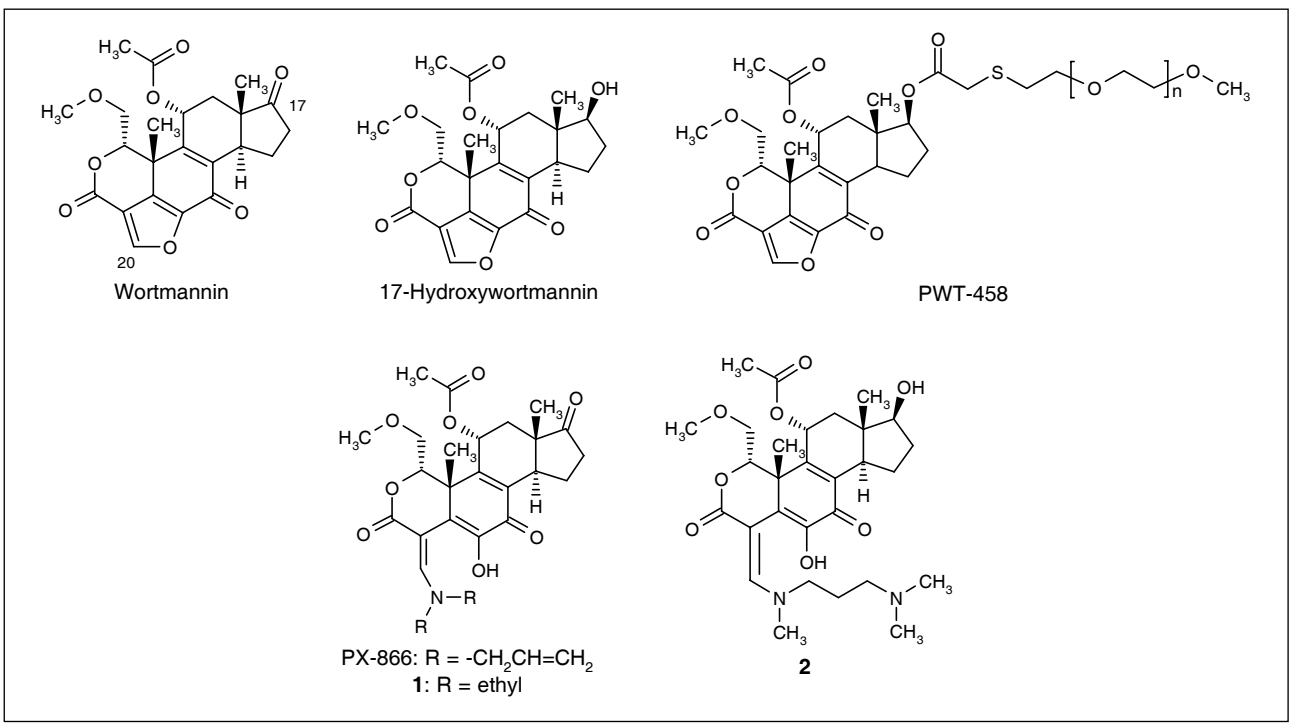


Fig. 1. Wortmannin and derivatives.

mice after i.p., i.v. and p.o. dosing (9, 18). The major toxicity was a target-related hyperglycemia with decreased glucose tolerance, which was reversible upon cessation of dosing. The hyperglycemia could also be reversed by treatment with insulin or the peroxisome proliferator-activated receptor γ (PPARγ) activator pioglitazone. PX-866 is also reported to have reduced liver toxicity relative to wortmannin. A related compound (**1**) has a reported IC_{50} against PI3Kα of 80 nM (19). Although the change from *N,N*-diallyl (PX-866) to *N,N*-diethyl (**1**) appears to result in a significant decrease in potency, caution should be exercised when comparing IC_{50} values reported by different groups, since many factors can contribute to variability in reported values (*e.g.,* ATP concentration, kinase construct homogeneity, etc.).

Ring-opened analogues of 17-hydroxywortmannin have also been reported. For example, compound **2** is a potent PI3K inhibitor (IC_{50} = 7 nM against PI3Kα) with efficacy in human tumor xenograft models in mice following i.v. dosing (20).

Quercetin derivatives

Over a decade ago, the first known noncovalent inhibitor of PI3K was described by Lilly (21). LY-294002 (Fig. 2), derived from the naturally occurring flavonoid quercetin, is a nonselective PI3K inhibitor with moderate potency (low micromolar IC_{50} values). The crystal structure of LY-294002 bound to PI3Kγ was solved, revealing that the carbonyl group binds to Lys-833, whereas the morpholine oxygen binds to the backbone amide nitrogen of Val-882 (14). The latter critical hydrogen bond to the hinge region of the kinase is conserved among all known noncovalent binders of PI3K. Although the relatively low potency and poor pharmacokinetic properties have prevented the clinical development of LY-294002, this inhibitor has proven valuable in revealing the biological role of PI3K and has functioned as a lead for the design of other PI3K inhibitors with various isoform selectivity profiles.

Semafore Pharmaceuticals has disclosed a series of prodrugs of LY-294002 (*e.g.,* structure **3**) (22). Their most advanced compound, SF-1126, reportedly has antitumor activity in xenograft models (www.semaforepharma.com). SF-1126 is scheduled to commence two phase I clinical trials in 2007 for the treatment of multiple myeloma and solid tumors.

Changes to the core of LY-294002 can result in compounds with isoform selectivity. For instance, the former Thrombogenix (which changed its name to Kinacia and was later purchased by the Australian bioscience company Cerylid) is investigating inhibitors of PI3Kβ as antithrombotics (23). They replaced the oxygen in the chromenone core of LY-294002 with a nitrogen to give TGX-115 and TGX-155 after optimization of the 8-substituent (Fig. 3) (24). Further derivatization of the core led to pyridopyrimidinones such as TGX-126 (24), TGX-221 (25) and TGX-286 (25). The most advanced compound, KN-309/CBL-1309 (structure not disclosed), successfully completed formal preclinical toxicology studies. Knight *et al*. reported additional SAR around pyridopyrimidinones (26). All compounds also have significant DNA-dependent protein kinase (DNA-PK) activity and the 8-substituent is essential for PI3K activity but not DNA-PK activity (removal leads to DNA-PK-selective compounds) (27-29).

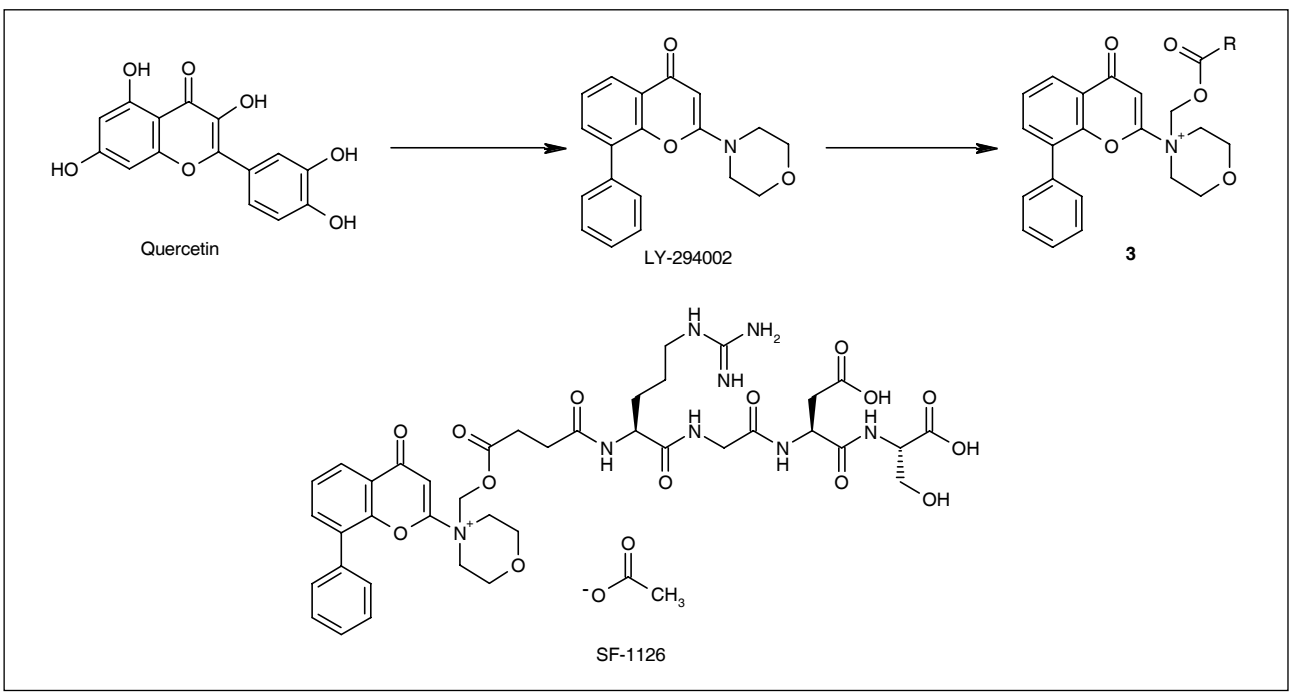


Fig. 2. Quercetin and derivatives.

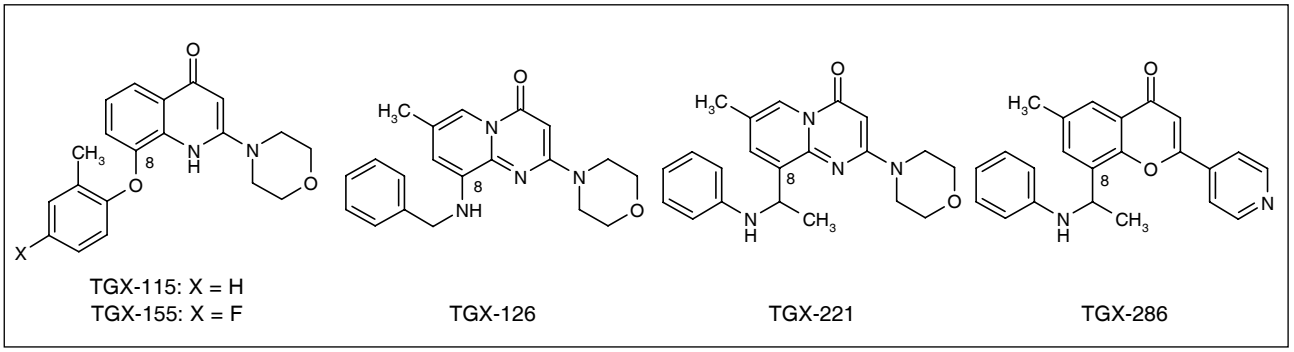


Fig. 3. Isoform-selective PI3K inhibitors derived from LY-294002.

Icos converted the chromenone core to quinazolinones (30, 31) to design selective inhibitors of PI3Kδ, such as IC-87114 (Fig. 4) (32-34). Although PI3Kδ inhibitors are mostly of interest as antiinflammatory compounds, another selective PI3Kδ inhibitor, IC-486068, was shown to enhance the effects of radiation on tumor suppression *in vivo* (11). Knight *et al.* further explored the SAR around quinazolinones and reported very potent and selective inhibitors of PI3Kδ, such as compound **4** (4).

Although none of the compounds described above is a selective inhibitor of PI3Kα, the available SAR data show that it is possible to bestow isoform selectivity upon quercetin-derived inhibitors. It is therefore possible that PI3Kα-selective inhibitors may be designed from this platform. Moreover, some of the literature cited above reveals compounds that retain significant inhibitory activity against PI3Kα, and as such may be of interest as antitumor compounds.

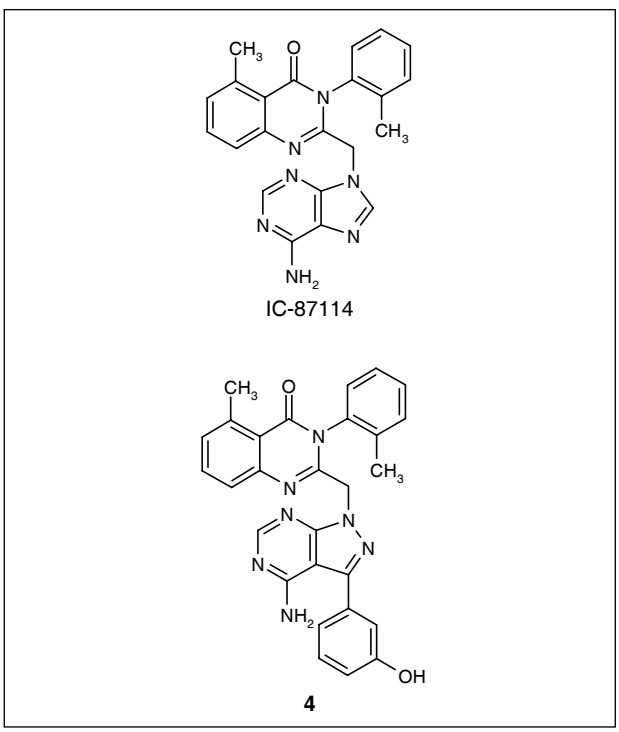


Fig. 4. Quinazolinone PI3K inhibitors.

Pyridofuropyrimidines and related structures

Yamanouchi and Piramed have reported pyridofuropyrimidines and related condensed heteroaryls as inhibitors of PI3K (35, 36). PI-103 (Fig. 5) is a nonselective inhibitor with nanomolar IC_{50} values for the various isoforms of PI3K (8, 88, 150 and 48 nM, respectively, against PI3Kα, β, γ and δ) (4, 36). A homology model suggests that the arylmorpholine in PI-103 binds to the ATP-binding pocket in a similar manner as LY-294002, *i.e.,* the morpholine oxygen binds to the backbone amide nitrogen of Val-882 (4). PI-103 inhibited the growth of human xenografts *in vivo* (12). Optimization of the pharmacokinetic properties of PI-103 led to several related compounds, such as PI-509, PI-516 and PI-540 (structures not disclosed). PI-540 was reported to have potent antitumor effects in several different human tumor xenograft models (37, 38).

Fan *et al.* (12) further explored the anticancer properties of PI-103 and a number of other PI3K inhibitors with various selectivity profiles. Although inhibition of either PI3Kα or PI3Kβ resulted in blockade of the phosphorylation of Akt, only inhibition of PI3Kα inhibited glioma cell proliferation (the glioma cell lines used in this study did not express PI3Kγ or PI3Kδ). PI-103 was also found to inhibit mTOR. PI-103 inhibits both complexes of mTOR (TORC1 and TORC2) with similar IC_{50} values (20-80 nM), consistent with direct inhibition of the TOR kinase and, unlike rapamycin, it does not require the formation of a ternary complex. PI-103 was more effective *in vivo* in glioma xenograft models than the other selective PI3Kα inhibitors investigated in this paper, which was ascribed to its additional effects on mTOR.

The 3-phenol group in PI-103 was replaced with other aromatic groups, such as acetamide **5**, which inhibited the α and δ isoforms with IC_{50} values of approximately 300 nM (34). Replacement of the pyridofuran group with other aromatic functionalities was explored by Hayakawa *et al.* (39, 40). The thienopyrimidine **6** displayed very strong inhibition of PI3Ks (IC_{50} PI3Kα: 2 nM, PI3Kβ: 16 nM, A-375 cells: 580 nM). The 3-phenol moiety in **6** could be replaced with other aryl groups, such as 4-indoles (41) or 4-indazoles (42). Interestingly, 4-indoles (*e.g.*, **7**) are reportedly selective inhibitors of PI3Kδ, whereas 4-indazoles (*e.g.,* **8**) show selectivity for PI3Kα. According to the

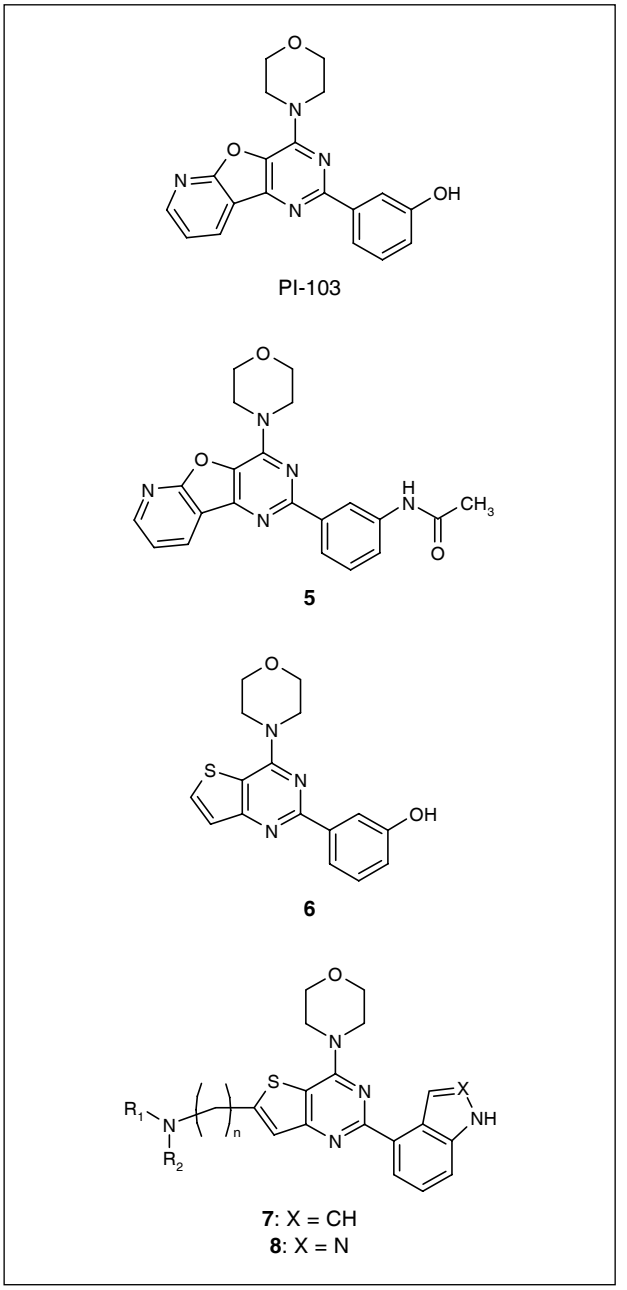


Fig. 5. Pyridofuropyrimidines and derivatives.

Yamanouchi/Piramed patent applications, the thienopyrimidines have good pharmaceutical properties (low clearance, no efflux, weak cytochrome P-450 inhibition). However, the 3-phenol thienopyrimidine **6** has a half-life of < 10 min following i.p. dosing in mice (43).

Other groups have reported similar compounds as well. Yaguchi and co-workers from Zenyaku Kogyo disclosed that the triazine ZSTK-474 (Fig. 6) inhibits PI3K with an IC_{50} of 37 nM and shows strong antitumor effects against human tumor xenografts (44). A molecular model for PI3K suggests that ZSTK-474 forms three hydrogen bonds to the active site of PI3K: the benzimidazole nitrogen binds the backbone amide nitrogen of Val-882, one of the morpholine oxygens binds the side-chain NH of Lys-802, whereas the other morpholine oxygen binds to the OH of Ser-806. AstraZeneca recently claimed several related structures (45-47). For example, compound **9** had an IC_{50} of 100 nM for PI3Kα.

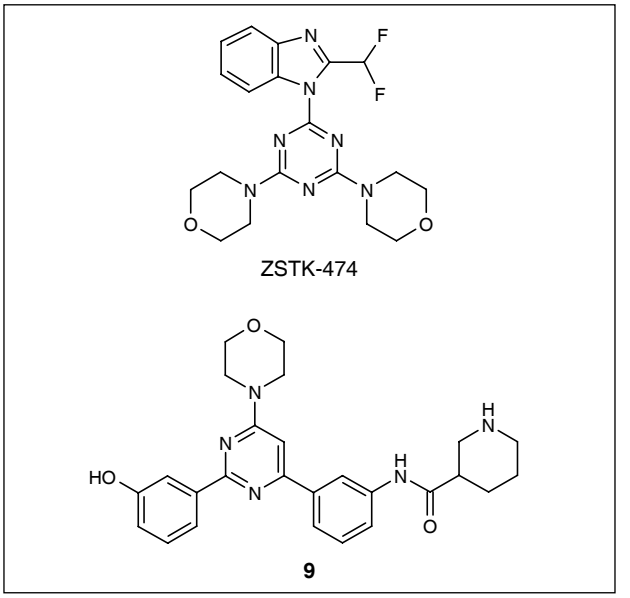


Fig. 6. ZSTK-474 and a related structure.

Additional related structures have been reported in patents by Chiron (48) and Pfizer (49). These patents do not provide any specific data related to the antitumor properties of the compounds.

Imidazopyridines

Piramed and Yamanouchi reported imidazopyridines (*e.g.,* **10** in Fig. 7) as PI3K inhibitors (50). Hayakawa *et al.* revealed several compounds with low single-digit IC_{50} values against PI3Kα and submicromolar cellular IC_{50} values (43). The most potent compound (**11**; IC_{50} = 2.8 nM against PI3Kα; IC_{50} = 140 nM against A-375 cell proliferation) was administered i.p. to nude mice bearing HeLa xenografts and resulted in 37% suppression of tumor growth after 2 weeks of dosing (25 mg/kg once daily). Opening of the pyrazole ring resulted in **12**, an inhibitor of PI3Kα (5.8 nM) and PI3Kγ (76 nM) (4).

Imidazoquinolines and related structures

Novartis revealed imidazoquinolines (*e.g.,* compound **13**; Fig. 8) as inhibitors of PI3K (51). Compound **13** was reported to inhibit PI3Kα with an IC_{50} of 72 nM (52). It was 32-fold selective *versus* PI3Kβ (IC_{50} = 2663 nM) and inhibited PI3Kγ and δ with respective IC_{50} values of 382 and 201 nM. Further optimization led to NVP-BEZ-235, which has IC_{50} values of 4, 76, 7 and 5 nM, respectively, *versus* PI3Kα, β, γ and δ (53). NVP-BEZ-235 is also a nanomolar inhibitor of mTOR (54). NVP-BEZ-235 was

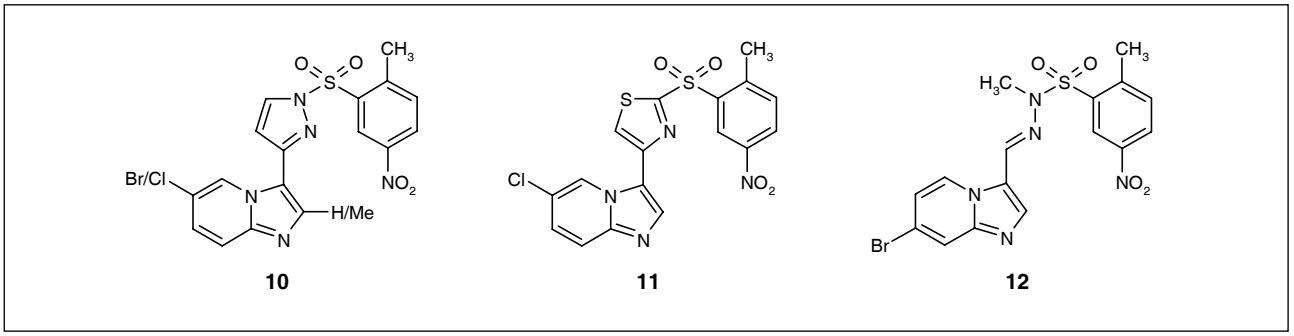


Fig. 7. Imidazopyridine PI3K inhibitors.

active in xenograft models of human cancer (PC-3 and U-87) upon oral dosing (52, 53) and entered clinical trials in December 2006 (53).

Related structures have been disclosed by Iconix (*e.g.,* structure **14**) (55) and Bayer (56). Although the Bayer patent does not give any quantitative data for inhibition of PI3Kα, Knight *et al.* published detailed data on a representative compound (compound **15**) (4). They reported that **15** is a potent, nonselective PI3K inhibitor (IC_{50} = 11, 350, 18 and 58 nM, respectively, against PI3Kα, β, γ and δ). A crystal structure of **15** bound to PI3Kγ shows interactions between the Val-882 backbone NH and the imidazolo nitrogen, as well as a hydrogen bond between the side-chain NH of Lys-833 and the pyridine nitrogen (4).

Thiazoles and related structures

Serono has reported several highly potent benzylidenethiazolidinediones as inhibitors of PI3Kγ, with varying degrees of selectivity over PI3Kα (7.5-18-fold), PI3Kβ (> 30-fold) and PI3Kδ (> 30-fold) (57). For example, AS-605240 (Fig. 9) had an IC_{50} of 8 nM for inhibition of PI3Kγ. According to a patent (58), the same compound inhibits PI3Kα with an IC_{50} of 64 nM and PI3Kγ with an IC_{50} of 8 nM. Crystallographic data revealed that AS-605240 binds in the ATP-binding pocket of PI3Kγ by formation of a salt bridge between the thiazolidinedione and the side-chain of Lys-833, while the amide nitrogen of Val-882 forms a hydrogen bond with the nitrogen of the quinoxaline ring (57). Knight *et al.* reported that a related compound (**16**) inhibited PI3Ks with IC_{50} values of 23 and 54 nM, respectively, for the α and γ subtypes (4).

Later papers by Serono investigators describe the discovery and activity of AS-252424 (34, 59), which has reported IC_{50} values of 12-33 nM for PI3Kγ and 6-30-fold selectivity over PI3Kα. Co-crystallization of AS-252424 with PI3Kγ also revealed a salt bridge between the positively charged Lys-833 side-chain and the thiazolidinedione nitrogen (59). The 2-hydroxyphenyl group functions as a hydrogen bond acceptor by interacting with the backbone NH of Val-882. Additional lipophilic interactions between the phenyl and furan rings and several hydrophobic residues in the active site complete the binding model.

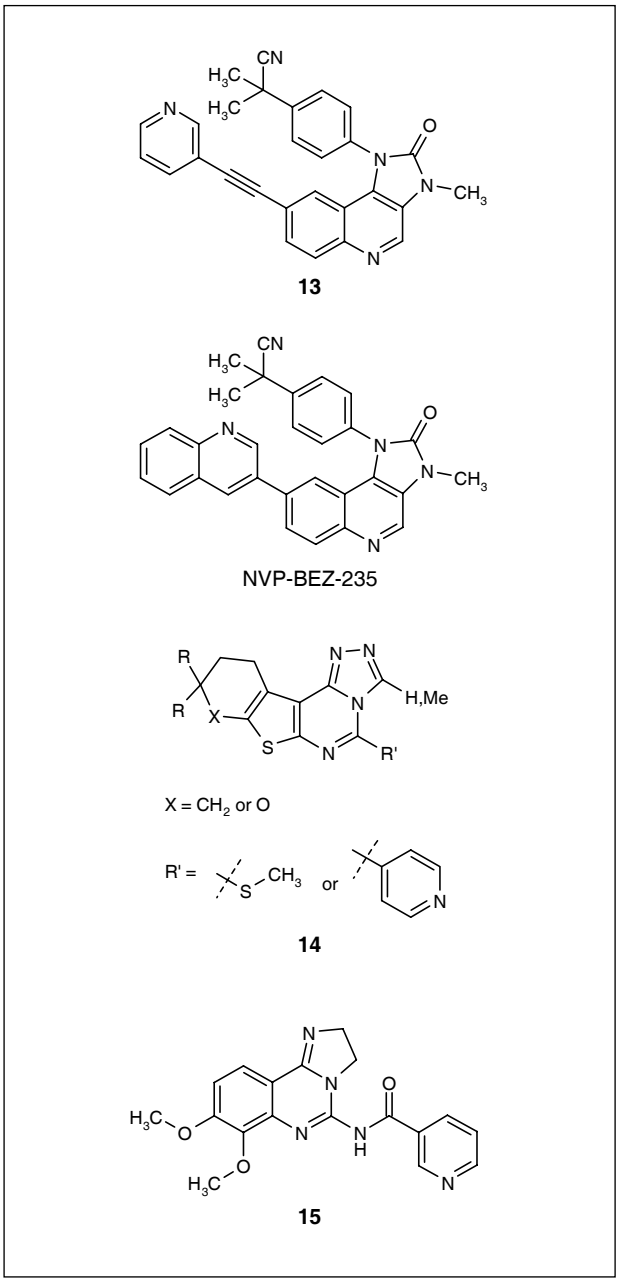


Fig. 8. Imidazopyridine and related structures.

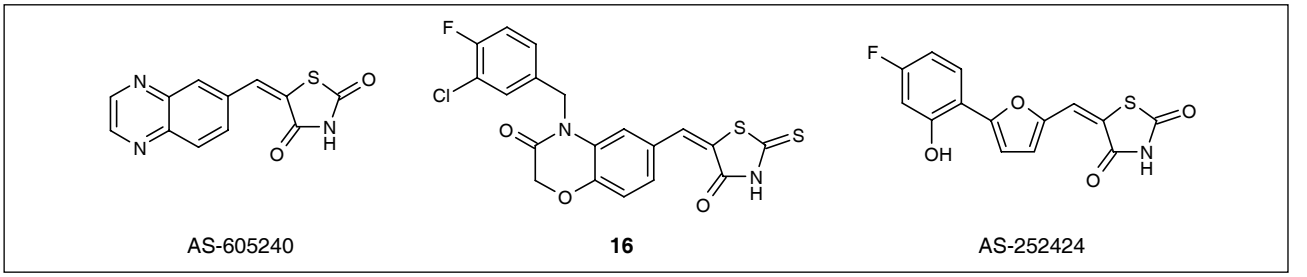


Fig. 9. Benzothiazolidinediones and related structures.

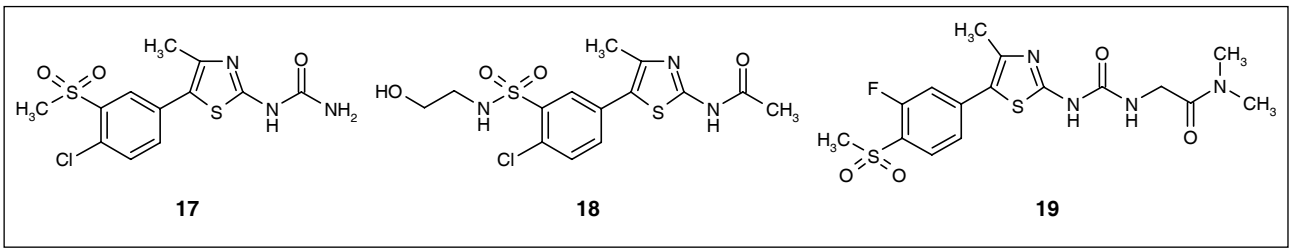


Fig. 10. Novartis' acylated 2-aminothiazolyls.

The recent patent literature contains several additional applications for benzylidenethiazolidinediones and related compounds by Serono (60-62) and Pfizer (63-65), including several single-digit nanomolar inhibitors of PI3Kγ. Such compounds may be of interest as antitumor agents, since potent inhibitors of PI3Kγ often also have high affinity for PI3Kα (4).

Acylated 2-aminothiazolyls have been reported by Novartis (66, 67). The patents describe several single-digit nanomolar inhibitors of PI3Kγ. For example, compound **17** (Fig. 10) has an IC_{50} of 5 nM and **18** has an IC_{50} of 2 nM (66). Compound **19** had IC_{50} values of 9, 12 and 28 nM, respectively, for PI3Kα, γ and δ (67). Knight *et al.* have also tested **18** and found that this compound inhibited PI3Kγ with an IC_{50} of 16 nM, while it inhibited PI3Kα with an IC_{50} of 39 nM (4). A crystal structure of **18** bound to PI3Kγ showed that binding to the ATP pocket involves a hydrogen bond between the backbone amide of Val-882 and the thiazole nitrogen, as well as hydrogen bonds between the acetamide NH and the backbone carbonyl of Val-882 and a third hydrogen bond between the sulfonamide and the side-chain of Asp-964 (4).

AstraZeneca also described acylated aminothiazolyls as inhibitors of PI3Kα (68). Compound **20** (Fig. 11) is a nonselective inhibitor of PI3Ks (IC_{50} = 10, 3, 4 and 1 nM, respectively, for α, β, γ and δ subtypes; IC_{50} mTOR = 10 nM). Reversal of the sulfonamide (to give compound **21**) had a small effect on the inhibitory activity against PI3Kα (IC_{50} = 30 nM), while the activity against mTOR was significantly decreased (IC_{50} = 700 nM). Compound **20** gave 20% tumor regression following a dose of 0.5 mg/kg p.o. in nude mice bearing human prostate cancer PC-3 xenografts (69).

The recent patent literature contains several other applications for acylated aminothiazoles and related compounds by Novartis (70, 71) and Serono (72).

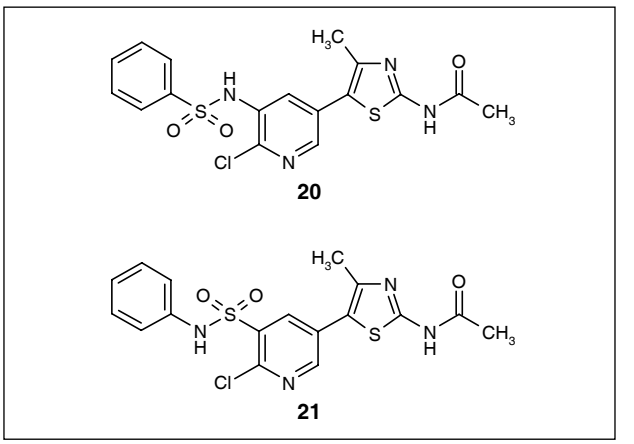


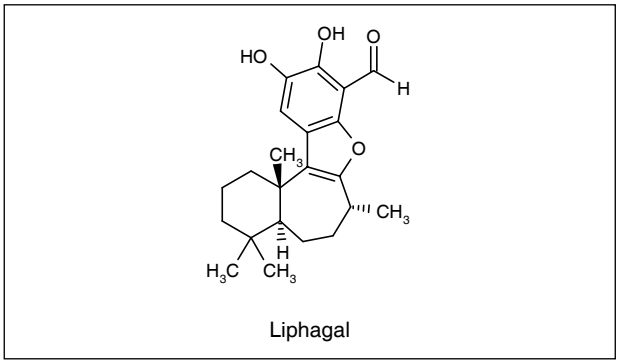
Fig. 11. AstraZeneca's acylated 2-aminothiazolyls.

Liphagal and derivatives

Marion *et al.* reported the discovery and identification of liphagal, a novel natural product that inhibits PI3K (Fig. 12) (73). Liphagal was isolated from the marine sponge *Aka coralliphaga*. Liphagal has an IC_{50} against PI3Kα of ~100 nM, with ~10-fold selectivity over PI3Kγ. Liphagal inhibited the growth of human colon carcinoma LoVo, human colon adenocarcinoma Caco-2 and human breast adenocarcinoma MDA-MB-468 cell lines with IC_{50} values of 600-1600 nM. The University of British Columbia and Wyeth have disclosed several liphagal derivatives as PI3K inhibitors (58).

Other structures

In addition to the compounds described in the preceding paragraphs, several other PI3K inhibitors have



Liphagal

Fig. 12. Liphagal.

appeared in recent literature. Of particular interest are the benzo[*b*]thiophene-2-carboxylic acid (2*H*-tetrazol-5-yl)amides described by Warner-Lambert/Pfizer (*e.g.,* structure **22**, Fig. 13) (74-79). Several single-digit nanomolar inhibitors of PI3Kγ are reported. As mentioned before, compounds with affinity for PI3Kγ often also inhibit PI3Kα and may be useful leads for the design of anticancer compounds. Boehringer Ingelheim also disclosed PI3K inhibitors (80, 81). Thus, compounds of general structure **23** are claimed to be antiinflammatory, whereas compounds like **24** are reported to have anticancer properties. Finally, additional PI3K inhibitors were disclosed by Iconix (structure **25**) (82), Echelon (compounds **26** and **27**) (83, 84) and Xinxiang Medical College (structure **28**) (85), although no specific data are provided regarding the anticancer properties of these inhibitors.

Conclusions

We have arrived at a crucial crossroads in the development of PI3K inhibitors. The last several years have witnessed an explosive increase in publications and patents concerning inhibition of PI3K. Recently, the first PI3K inhibitors entered clinical trials. Novartis started a phase I trial with its anticancer pan-PI3K/mTOR inhibitor NVP-BEZ-235 in December 2006. TargeGen's short-acting mixed inhibitor of PI3Kγ and δ, TG-100115 (86), is in phase I/II trials for treatment of infarct following myocardial ischemia-reperfusion injury (87). Several other compounds are poised to enter the clinic. Semafore is planning two clinical trials with SF-1126 in 2007 for the treatment of multiple myeloma and solid tumors. Exelixis recently filed INDs for XL-147 (a selective pan-PI3K inhibitor) and XL-765 (a mixed inhibitor of mTOR and PI3K) as anticancer agents. Cerylid's antithrombotic PI3Kβ inhibitor CBL-1309 (KN-309) has completed preclinical toxicology studies. ProlX, Wyeth, Novartis, Piramed/Yamanouchi, AstraZeneca, Serono, Pfizer, ICOS, Boehringer Ingelheim and several others also have preclinical programs.

The Semafore study and the Exelixis XL-147 study should provide important information regarding clinical efficacy of pan-PI3K inhibitors for the treatment of cancer, while the Novartis study and the Exelixis XL-765 study should clarify whether inhibition of mTOR in addition to PI3K translates into an additional clinical benefit. On the other hand, the more specific inhibitors currently being developed by TargeGen and Cerylid should furnish data

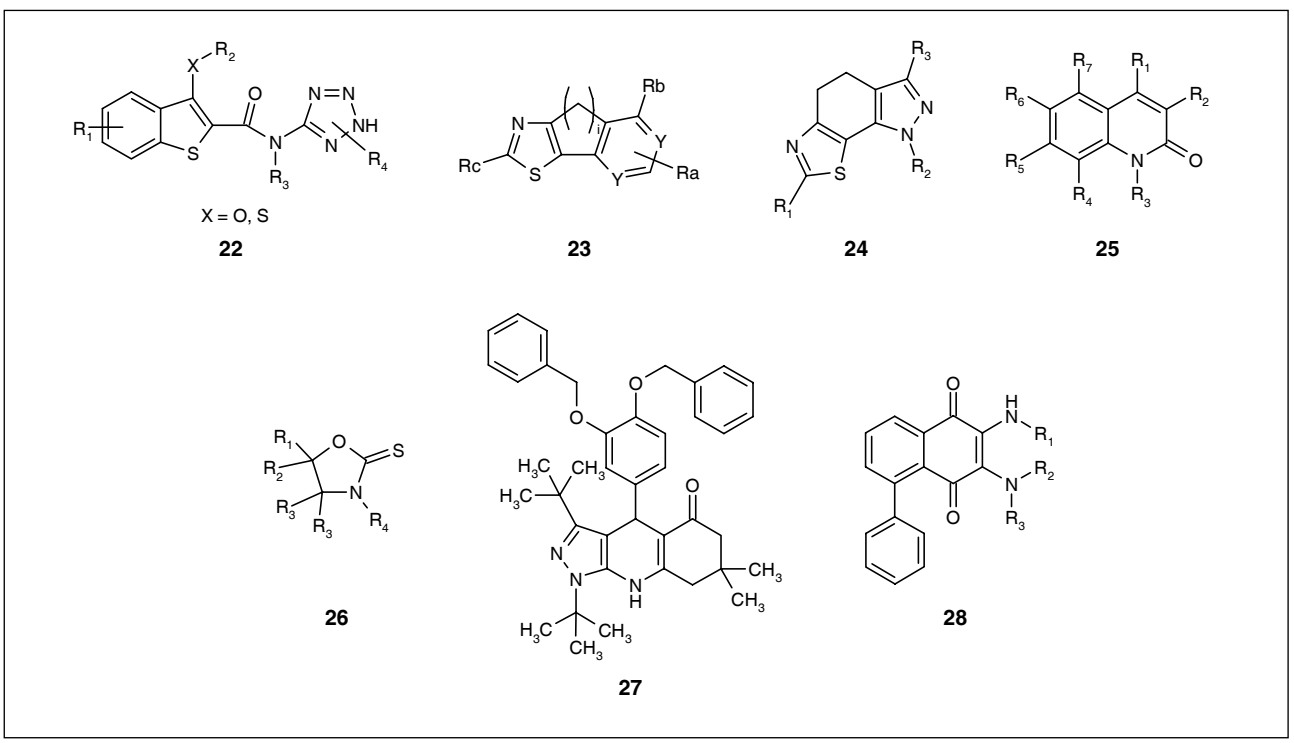


Fig. 13. Additional PI3K inhibitors.

on the relative contributions of the β, γ and δ isoforms to the side effect profile of PI3K inhibitors. A real breakthrough in our understanding of the clinical biology of PI3K inhibition can be expected when additional compounds move through the pipeline. As inhibitors with various isoform specificity profiles enter the clinic in the near future, our insight into the relative contributions of the various PI3K isoforms to clinical efficacy and side effects should expand exponentially.

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