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Novel imidazolopyrimidines as dual PI3-Kinase/mTOR inhibitors

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ABSTRACT

This article describes the syntheses and SAR of a series of imidazolopyrimidine derivatives, which are evaluated as inhibitors of PI3-Kinase (PI3 K) and mTOR. These compounds were found to be ATP competitive with good tumor cell growth inhibition, and suppression of pathway specific biomakers such as phosphorylation of Akt at T308.

In recent years, the phosphatidylinositide-3-kinase (PI3 K/Akt) signaling pathway has been recognized as a key pathway in cell proliferation, growth, survival, protein synthesis and glucose metabolism.[1–6] Phosphatidylinositol-3-kinases (PI3 K) are lipid kinases, that phosphorylate the phosphatidylinositol-diphosphate (PIP-2) to its corresponding phosphatidylinositol-triphosphate (PIP-3). PI3 Ks belong to the super family of PI3K related Kinases (PIKKs) which includes mTOR, ATM, ATR and DNA-PK. The product of PI3 K activity, PIP-3, acts as a second messenger that is responsible for the activation of the downstream kinase Akt.[1]

Eight PI3-kinases have been identified and categorized as class IA, class IB, class II, and class III enzymes. This classification is based on the sequence homology and substrate preference(s). The class IA subgroup consists of p110α, p110β and p110δ isoforms. Among these three isoforms, the gene encoding the p110α subunit, PIK3CA, is over expressed in ovarian, colorectal, brain (glioblastoma), and gastric cancers.[6,9–11] Additionally, mutations in PIKC3A have been documented to cause elevated PI3 K-α kinase activity in breast, endometrial, colon, and other cancers.[1b] There is significant evidence suggesting that the PI3 K/Akt pathway is deregulated in many human cancers.[1–6] PI3 K overexpression or mutation causes activation of Akt kinase, which leads to tumor progression, proliferation, survival, growth, invasion, angiogenesis and metastasis. Additionally, the PIP3 lipid phosphatase PTEN (Phosphatase and tensin homologue deleted on chromosome ten) is frequently mutated in numerous late stage tumors causing elevated levels of PIP-3 which might contribute to oncogenesis.[7,8]

Although only the p110α isoform is mutated in human cancers, the other PI3 K class I isoforms also have oncogenic potential. p110β PIK3CB isoform gene overexpression has been observed in breast and ovarian cancers.[1b,12] Hence, inhibiting PI3 Kα is an attractive target for cancer therapy. Additionally, it has been demonstrated that the PI3 K pathway is activated following mTOR inhibition, thus providing a strong rationale for developing dual PI3 K and mTOR kinase inhibitors.[13,14]

Towards this end, several pharmaceutical companies and academic institutions have been concentrating their efforts on development of small molecule PI3 K/mTOR inhibitors. The first generation of pan-PI3 K inhibitors, LY294002 **1** and Wortmannin **2** (Fig. 1), which inhibit the catalytic activity of all class I PI3 Ks, were not developed clinically because of toxicity and poor pharmaceutical properties.[2,15] More recently, a bismorpholino-triazine based compound, ZSTK-474 **3,** was reported by the Japanese cancer foundation.[16,17] ZSTK-474 is an ATP competitive pan-PI3 K class I enzyme inhibitor. Another compound that inhibits all class I PI3 K isoforms, but has significantly less potency versus mTOR is GDC-0941. This compound **4** was reported by Genentech, and recently entered phase I clinical studies.[18]

Among the various small molecule PI3 K inhibitors, BEZ-235 (Novartis) **6** is the most advanced clinical candidate. It inhibits PI3 K and mTOR kinases in an ATP competitive manner. BEZ235 also reported to inhibit cancer cell proliferation, causing cell cycle arrest at the GI stage.[19,20] Other novel small molecules that inhibit

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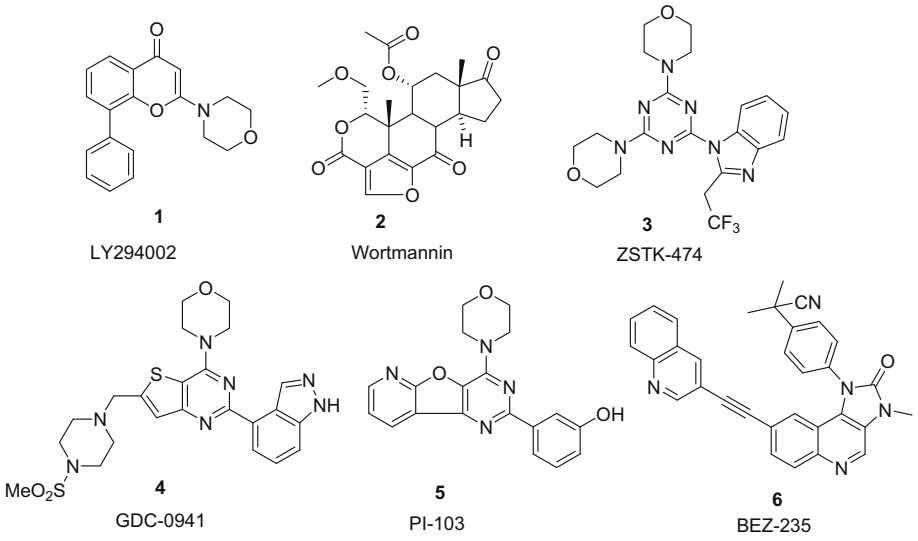
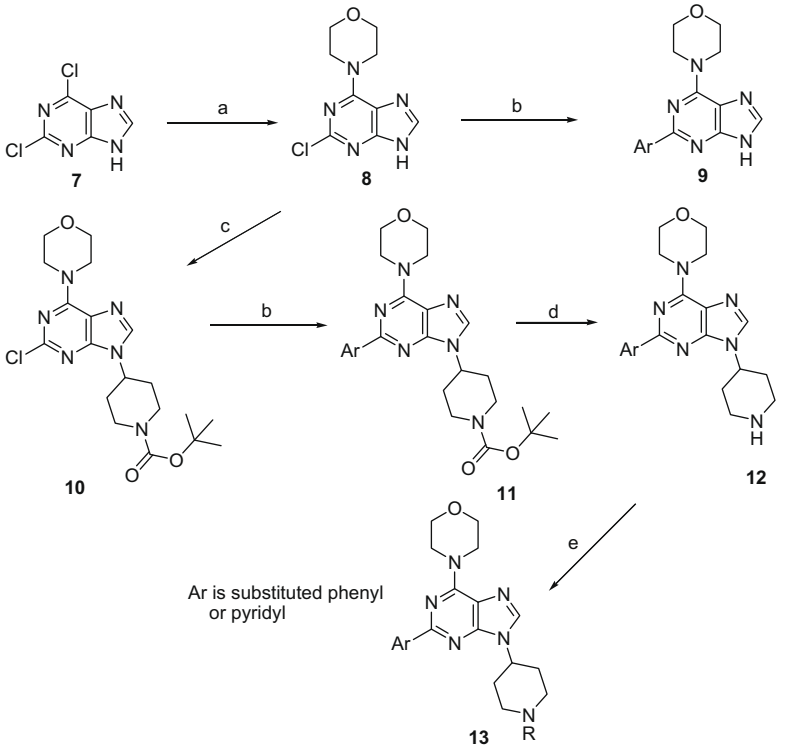


Figure 1. Known PI3-K inhibitors.

PI3 K/mTOR are on the horizon, but their structures have not yet been revealed.[21] Here in, we describe the syntheses and biological evaluation of a series of 4-morpholino-2-aryl-purine derivatives which are potent PI3 Kα/mTOR inhibitors.

The required 2-aryl-substituted-4-morpholino-imidazolopyrimidine derivatives **9** and **13** were conveniently prepared from the commercially available 2,4-dichloropurine **7** as indicated in Scheme 1. The 2,4-dichloropurine **7** was reacted with 2 equiv of morpholine in ethanol at room temperature to yield the monomorpholino derivative **8** in good yield. The 2nd chlorine in intermediate **8** was substituted with aryl or heteroaryl groups using their respective arylboronic acids under thermal or microwave assisted Suzuki reaction condition. The piperidino group was introduced in the 9-position of compound **8** using the *N*-BOC protected piperidine-4-ol, Ph_3P and DEAD at room temperature to yield **10**. Different, R groups on the piperidine nitrogen in compound **12** were introduced by a two step process. Initially the BOC-group in compound **11** was removed using trifluoro acetic acid in dichloromethane to yield **12,** in almost quantitative yields and the R groups were introduced by a $ZnCl_2$ mediated reductive amination protocol.

It was reported from several sources[18,22] as well confirmed by Wyeth workers[23](based on PI3 K-γ homology model,[23] Fig. 2) that



Scheme 1. General method to synthesize **14**–**34**. Reagents and conditions: (a) 2 equiv of morpholine, ethanol, rt/2 h; (b) $ArB(OH)_2$, DMF, $Pd(PPh_3)_4$, μ Wave, 175 °C, 15 min; (c) *tert*-butyl 4-hydroxy-1-piperidinecarboxylate, PPh_3, DEAD, THF, rt; (d) dioxane, TFA; (e) R–CHO, $ZnCl_2$, $NaBH_3CN$, MeOH, rt, 12 h.

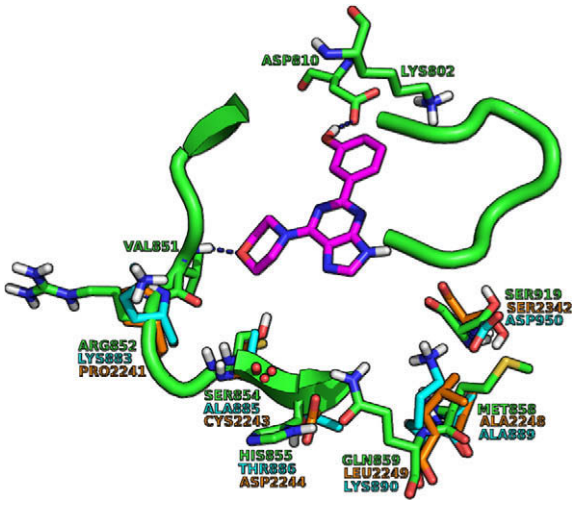


Figure 2. Docking of **14** in a PI3Kγ homology model.

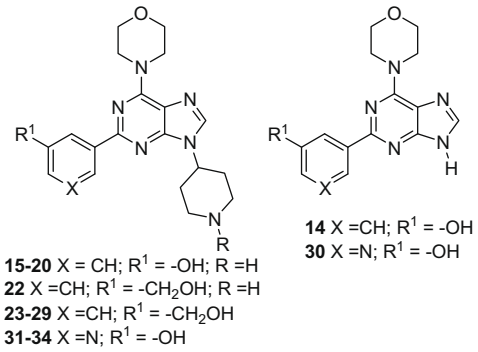


Figure 3. Novel imidazolopyrimidines.

the morpholino oxygen of **14** forms a hinge region H-bond interaction with Val851, that is crucial for the inhibition of PI3 Kα enzyme activity. The phenolic–OH group in the aryl entity formed an H-bond interaction with the Asp810. The initial compound **14** prepared in this series, exhibited an IC_{50} value of 150 nM against PI3 Kα, and molecular modeling of **14** (Fig. 2) suggested that by introducing polar groups at N-9 position of **14**, potency against PI3 K and mTOR could be increased, through interaction of the N-9 substituent with Ser919 in PI3 Kα, Ser2342 in mTOR and Asp950 in PI3 Kγ. In the present study, modifications were not only made at the N-9 position as of the purine scaffold of compound **14**, but also on the aryl moiety to improve potency.

When a piperidine ring was introduced at the N-9 position of the purine core of **14**, the resulting compound **15** was two-fold more potent than the N-9 unsubstituted derivative **14.** Compound **15** also had an improved solubility profile over **14**. Substitution of the piperidine nitrogen with various groups yielded analogues **16–20**. Among these five compounds, analogues **18** and **19** displayed a better PI3 Kα potency compared to **15** (twofold)**.** However, the microsomal stability of these phenolic derivatives was very poor. In vitro (phase II) metabolism studies in microsomes indicated that glucuronidation of the parent compound was common to all the phenol derivatives (data not shown). This indicated that the phenolic group is a metabolic liability. Therefore, the phenolic –OH was replaced by a $–CH_2OH$ group. This modification, led to examples **21–29**. As can be seen from Table 1, replacing the phenolic –OH group with $–CH_2OH$ did not dramatically alter the potency against PI3 Kα, but potency against mTOR decreased significantly for derivatives **22** and **23**. Comparison of compound **16** with **21** illustrates that potency for PI3 Kα remains nearly the same for the both compounds; but compound **21** was 15-fold more selective for PI3 Kα than mTOR. The most potent compound in this series was **29**, which had growth inhibition IC_{50} values of 0.685 μM versus MDA-361 (breast) and 0.512 μM versus PC3MM3 (prostate) human tumor cell lines. This compound also had good microsomal stabilities.

Next, the phenyl moiety in the 2-position of the purine was replaced with a 5-hydroxy-3-pyridyl group (e.g., **30–34**). This modification gave potent analogues such as **30, 31** and **32**.

The pyridyl substituted compound **30** was found to be 10-fold more potent than the corresponding phenyl substituted derivative **14**. This could be due to an extra hydrogen bond interaction that could possibly occur with Lys 802 with the 3-pyridyl nitrogen atom (see Fig. 3).

Compound **29** was assayed in vitro for its ability to suppress appropriate cellular biomarkers. PI3 Kα inhibition should result

Table 1
Biological data for imidazolopyrimidines **14–34**

Compd #	X	R^1	R	IC_{50} values in nM		
				PI3 Kα	PI3 Kγ	mTOR
14	CH	–OH		150	2343	1650
15	CH	–OH	H	64	133	950
16	CH	–OH	Benzyl	75	10,000	140
17	CH	–OH	$–CH_2$-3-pyridyl	66	583	170
18	CH	–OH	$–CH_2$-(6-morpholino)-3-pyridyl	40	368	152
19	CH	–OH	$–CH_2$-(5-chloro)-3-pyridyl	37	341	200
20	CH	–OH	$–CH_2$-(6-methoxy)-3-pyridyl	55	306	51
21	CH	CH_2OH	Benzyl	63	1134	1000
22	CH	CH_2OH		57	683	7200
23	CH	CH_2OH	$CH_3CO–$	189	1378	2100
24	CH	CH_2OH	$–CH_2$-2-pyridyl	65	776	310
25	CH	CH_2OH	2-Flourobenzyl	42	1102	160
26	CH	CH_2OH	4-Fluorobenzyl	70	594	1700
27	CH	CH_2OH	$–CH_2$-{4-(4-pyridyl)-phenyl}	23	223	720
28	CH	CH_2OH	2,4-Difluoro-phenyl	44	640	2050
29	CH	CH_2OH	$–CH_2$-(4-$O(CH_2)_3NMe_2$)phenyl	16	265	>4000
30	N	–OH		11	47	620
31	N	–OH	H	14	44	970
32	N	–OH	$–CH_2$-4-chlorophenyl	16	119	160
33	N	–OH	$–CH_2$-4-tolyl	42	204	590
34	N	–OH	$–CH_2$-6-flouoro-3-pyridyl	94	61	570

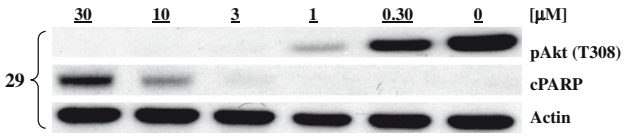


Figure 4. Inhibition of pAkt at T308, and induction of cleaved PARP by **29**.

in suppression of the phosphorylation of Akt particularly at the threonine 308 site (T308, Fig. 4). As can be seen in Fig. 4, compound **29** inhibits Akt T308 phosphorylation in MDA361 breast tumor cells, with an IC_{50} value of about ~300 nM, after a 4 h exposure time. Compound **29** also induced cleaved PARP at 10 and 30 μM. Cleaved PARP is a marker for cell apoptosis (programmed cell death). Actin (control protein) signal was unaffected by **29**.

In this Letter, we have disclosed a series of novel 2-aryl or heteroaryl substituted-4-morpholino imidazolopyrimidine derivatives **14–34** possessing moderate to excellent PI3K/mTOR inhibitory activity. Most interestingly, from the selectivity point of view (against mTor) analogues such as **21**, **22** and **29** are all of great interest. Introduction of the 5-hydroxy-3-pyridyl appendage at the 2-position of the imidazolopyrimidine scaffold yielded potent analogues such as **30** and **32**. This could be due to an extra hydrogen bond interaction that could possibly occur between the pyridyl nitrogen and Lys802. Analog **29** showed a good correlation between cell growth inhibition and biomarker (pAkt at T308) suppression. Further studies concerning agents that target the PI3-K and mTor will be reported in due course.

Cell growth inhibition assay: The MDA361 and PC3mm2 cell lines were obtained from ATCC. Cell lines were grown at 37 °C in 5% CO_2 incubators in growth media supplemented with penicillin/streptomycin and 10% fetal calf serum. Cell growth inhibition was determined using the CellTiter 96 aqueous non-radioactive cell proliferation assay from Promega. This homogeneous colorimetric method determined the number of viable cells in proliferation assays. The assay was carried out in 96 well format following manufacturer's instructions, with cell number per well being adjusted based on growth characteristics of the various cell lines used. Assay endpoint data was quantitated after 72 h compound exposure using a Victor^2 V (Wallac) model 1420 multilabel HTS counter.

Cell lysis and Western blotting: Cell lysis enabled biochemical analysis of PI3 K/mTOR signaling pathway proteins after exposure of cells to compounds. Cells (3×10^5) were seeded onto 6-well microtiter plates (Nunc) 24 h prior to being exposed to compound in complete growth media. Cells were exposed to these compounds for 4 h. After exposure to compounds cell growth media was removed and cells were washed twice with cold (4 °C) PBS. Cell lysis buffer (0.2 ml) was then added to each microtiter plate well with sufficient mixing to insure complete cell lysis. Cell lysis buffer consisted of: 20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM b-glycerophosphate, 1 mM Na_3VO_4, and 1 μg/ml leupeptin. Cell lysates were then spun for 30 s at 14,000 rpm. Supernatant (75 μl) was combined with 30 μl of 3× protein gel loading buffer [187.5 mM Tris–HCl (pH 6.8), 6% (w/v) SDS, 30% glycerol, 0.03% (w/v) bromophenol blue, and 125 mM DTT]. Samples were boiled (5 min) separated by SDS–PAGE, transferred to nitrocellulose and probed with antibodies (Ab) specific to the protein and phosphoprotein components of the PI3 K/Akt/mTOR signaling pathway. Abs obtained from Cell Signaling Technology were: anti(α)-Akt, α-phospho(p)-Akt at T308, α-Akt, α-cleaved PARP, and α-actin. Specific antigen/antibody interactions were identified by a horseradish peroxidase (HRP) conjugate secondary Ab that enabled chemiluminescent signal detection.

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