Bioorganic & Medicinal Chemistry Letters 20 (2010) 636–639

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel purine and pyrazolo[3,4-*d*]pyrimidine inhibitors of PI3 kinase-α: Hit to lead studies

Adam M. Gilbert [a,*], Pawel Nowak [a], Natasja Brooijmans [b], Matthew G. Bursavich [a], Christoph Dehnhardt [a], Efren Delos Santos [a], Larry R. Feldberg [c], Irwin Hollander [c], Stephen Kim [c], Sabrina Lombardi [a], Kaapjoo Park [a], Aranapakam M. Venkatesan [a], Robert Mallon [c]

[a] *Medicinal Chemistry, Chemical Sciences, Wyeth Research, Pearl River, NY 10965, USA*
[b] *Computational Chemistry, Chemical Sciences, Wyeth Research, Pearl River, NY 10965, USA*
[c] *Discovery Oncology, Wyeth Research, Pearl River, NY 10965, USA*

ARTICLE INFO

Article history:
Received 15 October 2009
Revised 9 November 2009
Accepted 16 November 2009
Available online 5 December 2009

Keywords:
Phosphatidylinositol-3-kinase
Phosphatidylinositol-3-kinases alpha
Mammalian target of rapamycin
Cellular proliferation
Oncology

ABSTRACT

Series of purine and pyrazolo[3,4-*d*]pyrimidine inhibitors of phosphatidylinositol-3-kinases (PI3K) have been prepared. The optimized purine inhibitors show good potency in a PI3K p110α (PI3K-α) fluorescence polarization assay with good selectivity versus PI3K p110γ (PI3K-γ) and the mammalian target of rapamycin (mTOR). The related pyrazolo[3,4-*d*]pyrimidines show potent PI3K-α and mTOR inhibition with good selectivity versus PI3K-γ. Representative compounds showed activity in a cellular proliferation assay against Caco-2 colorectal, LoVo colorectal and PC3MM2 prostate adenocarcinoma cancer cells. Signaling through the PI3K pathway was confirmed via inhibition of phospho-AKT in MDA-361 cells.

Phosphatidylinositol-3-kinases (PI3K) are lipid kinases that associate with Receptor Tyrosine Kinases (RTKs) and Ras. Eight PI3Ks have thus far been identified and they have been separated into 4 classes (IA, IB, II and III) based on substrate specificity and sequence homology. The class IA PI3Ks function by phosphorylating the 3-hydroxyl position of phosphatidylinositol 4,5-diphosphate (PIP2) to produce phosphatidylinositol 3,4,5-triphosphate (PIP3) which influences cell shape, motility, growth, differentiation, and survival via activation of Akt kinase.[1,2] Of the 4 subtypes of class Ia PI3Ks, p110α (PI3K-α) deregulation has been shown to stimulate cell growth and inhibit apoptosis in epithelial tumor cells. Additionally, PI3K-α deregulation is involved ~26% of breast cancers in all stages, as well as being associated with lymph node metastasis. Similarly, PTEN (the phosphatase that regulates PIP3 levels) deregulation is associated with resistance to chemotherapeutic agents that target the EGFR/Her2 signaling pathway.[3,4] Thus PI3K-α has emerged as one of the premier targets for cancer treatment.[5]

A high-throughput screen of our compound library was run and a series of purines were identified as having activity against PI3K-α, p110γ (PIK3-γ), a related PI3K subtype (class IB) which is a potential therapeutic target for inflammatory and autoimmune diseases,[6] and the mammalian target of rapamycin (mTOR), a related kinase of interest in cancer chemotherapy that signals downstream of Akt.[7] We herein describe the initial hit to lead optimization of these purines to potent PI3K-α inhibitors with good selectivity versus PI3K-γ and mTOR. Work on related PI3K-α scaffolds has been previously published by Astellas[8] and Piramed.[9] We also describe a related series of pyrazolo[3,4-*d*]pyrimidines which show potent PI3K-α and mTOR activity with good selectivity versus PI3K-γ. We have recently published additional work on the pyrazolo[3,4-*d*]pyrimidines as a series of selective inhibitors of mTOR.[10,11]

Purines (Tables 1 and 2) were prepared via the route shown in Scheme 1.[12] Thus commercially available **1** when heated with cyclic amines selectively produces the 6-amino analogs **2**.[13] Suzuki cross-coupling under standard conditions produces the corresponding 2-aryl compounds **3**.[14] Mitsunobu reaction of **2** with 1-benzylpiperidin-4-ol produces piperidine analogs **4**.[15] Debenzylation (R = Bn to R = H) is accomplished by standard hydrogenation.

Compounds were assayed using PI3K-α and PI3K-γ fluorescence polarization assays monitoring PIP3 production.[16] Compounds were assayed for mTOR potency using the DELFIA format assaying for phosphorylated His6-SK6.[17] Cellular proliferation activity was assayed by monitoring cell growth densities in Caco2, LoVo, and PC3MM2 cell cultures.[18] Inhibition of PI3K signaling in MDA-361 cells was detected by immunoblotting protein lysates for phosphorylated-Akt (p-Akt).

* Corresponding author.
E-mail address: gilbera@wyeth.com (A.M. Gilbert).

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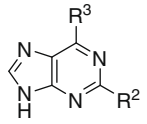
doi:10.1016/j.bmcl.2009.11.051

Table 1
Structure–activity relationships of purine **2**-(R^2) and **4**-(R^3) positions

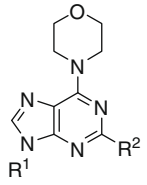


6-21

Compd	R^2	R^3	PI3K-α IC_{50} (nM)[a]	PI3K-γ IC_{50} (nM)[a]	mTOR IC_{50} (nM)[a]
6	(4-OH)Ph	*N*-Morpholine	2538	8558	450
7	(4-$HOCH_2$)Ph	*N*-Morpholine	2894	7972	4500
8	(4-$CONH_2$)Ph	*N*-Morpholine	6700	10,000	nt
9	(4-SO_2Me)Ph	*N*-Morpholine	1760	5830	nt
10	6-Indole	*N*-Morpholine	2330	7371	150
11	6-Dihydrobenzo-dioxane	*N*-Morpholine	3477	7582	nt
12	6-Benzodioxole	*N*-Morpholine	1211	6907	8900
13	3-Thiophene	*N*-Morpholine	2381	8000	3900
14	3-Furan	*N*-Morpholine	3787	11,000	nt
15	(3-OH)Ph	*N*-Morpholine	232	2343	2383
16	(3-OH)Ph	*N*-Piperidine	3769	10,322	nt
17	(3-CN)Ph	*N*-Morpholine	2915	8297	nt
18	(3-SO_2Me)Ph	*N*-Morpholine	3902	7907	nt
19	(3-$NHSO_2$Me)Ph	*N*-Morpholine	4379	7909	nt
20	(2-OH)Ph	*N*-Morpholine	667	5135	2500
21	2-Thiophene	*N*-Morpholine	640	6742	2800

[a] Values are means of two experiments, standard deviation is ±10%.

Table 2
Structure–activity relationships of purine **9**-(R_1) and **2**-(R_2) positions



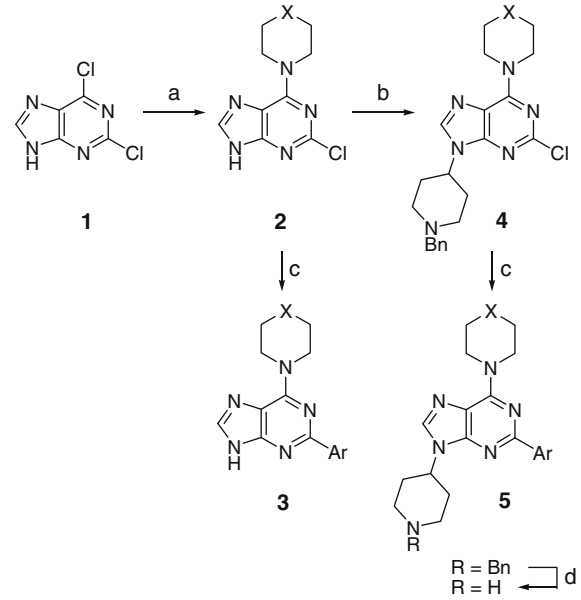
22-27

Compd	R^1	R^2	PI3K-α IC_{50} (nM)[a]	PI3K-γ IC_{50} (nM)[a]	mTOR IC_{50} (nM)[a]
22	4-(*N*-Bn-piperi-dine)-CH_2	(3-$HOCH_2$)Ph	248	1650	10,500
23	4-(*N*-Bn-piperi-dine)-CH_2	(3-OH)Ph	199	2378	600
24	4-(*N*-Bn-piperidine)	(3-$HOCH_2$)Ph	45	1134	691
25	4-(*N*-Bn-piperidine)	(3-OH)Ph	80	10,815	140
26	4-Piperidine	(3-$HOCH_2$)Ph	58	683	7200
27	4-Piperidine	(3-OH)Ph	65	131	870

[a] Values are means of two experiments, standard deviation is ±10%.

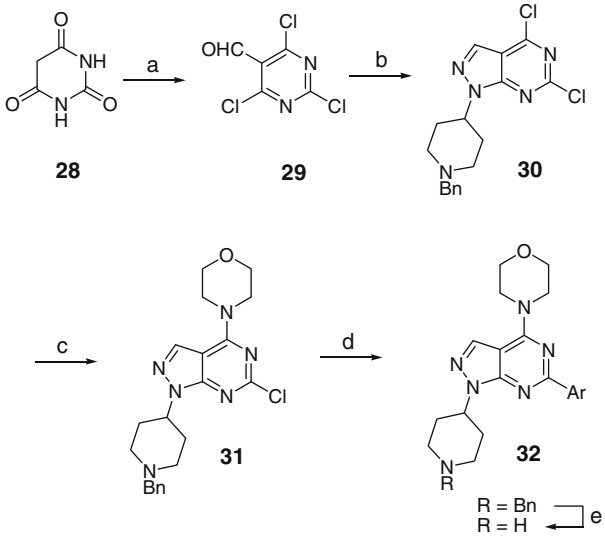
Initially the 2-aryl (R_2) moiety of the purine scaffold was varied (Table 1). The (4-OH)Ph compound **6** shows μM PI3K-α and PI3K-γ inhibition and sub-μM mTOR activity. Replacements for the (4-OH)Ph moiety (**7**–**14**) give compounds with similar PI3K-α potency. The R^2 = 6-indole moiety can be used as an isosteric replacement for the (4-OH)Ph group as shown in **10**. An improvement in PI3K-α potency as well as selectivity versus PI3K-γ and mTOR is seen with the R_2 = (3-OH)Ph analogs **15**. The importance of morpholine as the 4-purine substituent is seen in **16** which has reduced PI3K-α and PI3K-γ activity. Replacements of the (3-OH)Ph moiety in **15** give less potent compounds (**17**–**19**). The (2-OH)Ph and 2-thiophene compounds (**20**, **21**) show sub-μM PI3K-α potency, but are not as potent as the 3-OH analog **15**.

Additional PI3K-α potency can be obtained by functionalization of the 9-purine position R_1 (Table 2). While **22** and **23** show a similar profile to compounds in Table 1, analog **24** (R_1 = 4-(*N*-Bn-piperidine); R_2 = (3-$HOCH_2$)Ph) shows potent PI3K-α inhibition (IC_{50}: 45 nM), 25-fold selectivity versus PI3K-γ and 15-fold selectivity versus mTOR. The corresponding R_2 = (3-OH)Ph analog **25** shows even greater selectivity versus PI3K-γ and similar mTOR potency compared to **24**. The R_1 = 4-piperidine analog **26** also shows good PI3K-α potency, selectivity versus PI3K-γ (11-fold) and mTOR (>100-fold) while the corresponding R_2 = (3-OH)Ph analog **27** shows reduced selectivity over PI3K-γ and mTOR compared to **26**.



Scheme 1. Reagents and conditions: (a) Amine, EtOH, reflux; (b) 1-benzylpiperidin-4-ol, DEAD, PhP_3, 0–23 °C; (c) $ArB(OH)_2$, cat. $Pd(PPh_3)_4$, Na_2CO_3, DMF, H_2O, 100 °C; (d) H_2, Pd/C , MeOH, 23 °C.

Interested in how the corresponding pyrazolo[3,4-*d*]pyrimidine analogs of Table 2 would profile, these compounds were prepared according to the route in Scheme 2. Thus barbituic acid **28** is converted to pyrimidine **29** via a known procedure.[19] Formation of the corresponding 1-piperidinyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **30** is accomplished via reaction with 1-benzyl-4-hydrazinylpiperidine



Scheme 2. Reagents and conditions: (a) $POCl_3$, DMF, reflux, 60%; (b) 1-benzyl-4-hydrazinylpiperidine dihydrochloride, Et_3N, EtOH, −78 °C to 0 °C, 82%; (c) morpholine, EtOH, 61%; (d) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, dioxane, water, 100 °C; (e) H_2, $Pd(OH)_2$/C (10%), MeOH.

dihydrochloride.[10] Morpholine is then added selectively at the 4-position to produce **31**. The 6-aryl moiety is incorporated via standard Suzuki cross-coupling conditions to give **32**, (R = Bn to R = H) is accomplished by standard hydrogenation.

The pyrazolo[3,4-*d*]pyrimidines (Table 2) show potent PI3K-α inhibition, but a different selectivity profile compared to the purines. While the purine analogs tend to show modest mTOR potency, the pyrazolo[3,4-*d*]pyrimidines are more potent. Thus **33** (R_1 = 4-(*N*-Bn-piperidine); R_2 = (3-$HOCH_2$)Ph)) shows potent PI3K-α and mTOR potency (compare with **24**). The corresponding R_2 = (3-$HOCH_2$)Ph analog **34** shows improved selectivity versus PI3K-γ but maintains good mTOR potency (compare with **25**). The R_1 = 4-piperidine analogs **35** and **36** show improved PI3K-α selectivity versus mTOR compared to **33** and **34** but these compounds have more mTOR potency compared to purines **26** and **27**.

Rationalization of the potency and selectivity of the purine series of inhibitors is shown by analyzing a PI3K-α homology model, based on an X-ray structure of PI3K-γ, and compound **26** (Fig. 1). The critical H-bond between Val851 and the oxygen of the R_2 morpholine explains the reduction in potency seen for **16** (R_2 = piperidine) compared to the morpholine analogs. The R_2 = (3-$HOCH_2$)Ph moiety makes a hydrogen bond with Asp810. Selectivity of **26** for PI3-α versus PI3K-γ and mTOR is not readily apparent, but the observed selectivity profile of the compounds in Tables 1 and 2 leads us to hypothesize that the Asp810 H-bonding is more favorable in PI3K-α resulting in more potent compounds. The reduced selectivity versus PI3K-γ seen in **26** versus **24** may be rationalized by the protonatable piperidine nitrogen being better tolerated by Asp950 in PI3K-γ versus Ser919 and Ser2342 residues in PI3K-α and mTOR, respectively. It's also possible that large hydrophobic groups on the piperidine (R = –Bn) in **22**–**25** are better tolerated by hydrophobic residues in PI3K-α (Met858) and mTOR (Leu2264) versus the more hydrophilic Lys890 in PI3K-γ. In general, the purine inhibitors (Tables 1 and 2) show better PI3K-α selectivity versus mTOR than the corresponding pyrazolo[3,4-*d*]pyrimidines (Table 3), but as the nitrogens of the purine and pyrazolo[3,4-*d*]pyrimidine cores are not involved in direct interactions with the enzymes, it is not clear how the increased selectivity for the purines is achieved.

Several of the more potent purine and pyrazolo[3,4-*d*]pyrimidine analogs were tested for cellular proliferation activity against Caco-2 epithelial colorectal adenocarcinoma cells, LoVo human colon carcinoma cells, and PC3 human prostate cancer cells (Table 4).

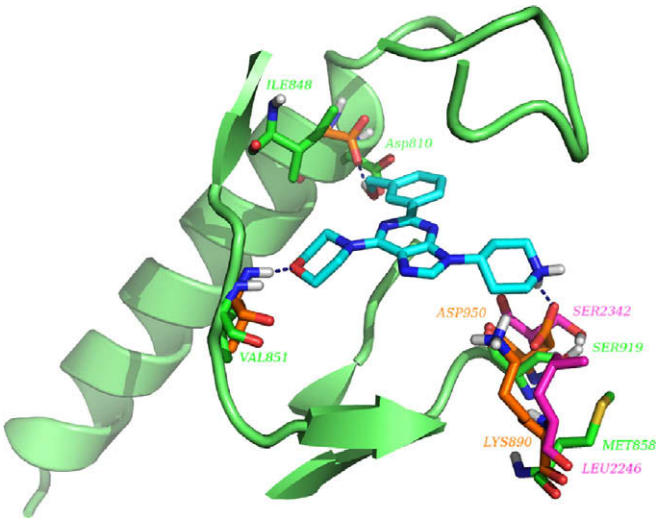
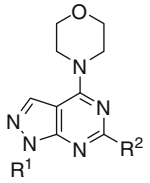


Figure 1. Binding mode of compound **26** in a PI3K-α homology model based on an X-ray structure of PI3K-γ. Residues of PI3K-α (green), PI3K-γ (orange) and mTOR (magenta) are included to help rationalize selectivity.

Table 3
Structure–activity relationships of pyrazolo[3,4-*d*]pyrimidine **1**-(R_1) and **6**-(R_2) positions



33-36

Compd	R^1	R^2	PI3K-α IC_{50} (nM)[a]	PI3K-γ IC_{50} (nM)[a]	mTOR IC_{50} (nM)[a]
33	4-(*N*-Bn-piperidine)	(3-$HOCH_2$)Ph	25	975	90
34	4-(*N*-Bn-piperidine)	(3-OH)Ph	32	2367	83
35	4-Piperidine	(3-$HOCH_2$)Ph	26	510	290
36	4-Piperidine	(3-OH)Ph	36	993	215

[a] Values are means of two experiments, standard deviation is ±10%.

Comparing this data with PI3K-α, mTOR and *p* log *D* (pH 7.4 data) reveals two possible trends. First, improved cellular activity appears to track with improving PI3K-α and mTOR potency. Secondly, the compound with the most potent cellular profile is pyrazolo[3,4-*d*]pyrimidine **33** which has a more desired *p* log *D* (1.46) for compounds with good cellular permeability and stability compared to other compounds with similar PI3K-α and mTOR potency profiles (compare with **25** (3.00) and **34** (1.95)).

To ensure that compounds were inhibiting PI3K signaling in cells, two analogs were tested for p-Akt (at threonine 308) inhibition in MDA-361 human breast adenocarcinoma cells. Immunostaining for cleaved-PARP (cPARP) was also conducted as a marker of apoptosis. Results for purine **24** and pyrazolo[3,4-*d*]pyrimidine **35** are shown in Figure 2. p-Akt inhibition is seen starting at 3 μM for **24** and is complete at 30 μM. Similarly **35** shows p-Akt reduction starting at 3 μM and is almost complete at 10 μM. Suppression of p-Akt by both compounds is indicative of inhibition of cellular PI3Ks which are upstream of Akt in the PI3K signaling pathway.

In conclusion, we have disclosed series of purine and pyrazolo[3,4-*d*]pyrimidines PI3K inhibitors which show activity in adenocarcinoma cellular proliferation assays and inhibit p-Akt formation in MDA-361 cells. Purine inhibitors have been identified which show potent PI3K-α activity, and good selectivity (>10-fold) versus PI3K-γ and mTOR. The related pyrazolo[3,4-*d*]pyrimidines maintain good PI3K-α potency and show good potency against mTOR

Table 4
Cellular proliferation inhibitory activity of selected purine and pyrazolo[3,4-*d*]pyrimidines

Compd	PI3K-α IC_{50} (nM)[a]	mTOR IC_{50} (nM)[a]	Caco IC_{50} (nM)[a]	LoVo IC_{50} (nM)[a]	PC3 IC_{50} (nM)[a]	*p* log *D* (pH 7.4)
20	667	2500	>10,000	7307	>10,000	2.42
21	640	2800	>10,000	>10,000	>10,000	2.72
22	248	10,500	5787	3077	9269	3.11
23	199	600	6489	3508	7347	3.52
24	45	691	5168	1047	1282	2.59
25	80	140	>10,000	1947	1111	3.00
26	58	7200	4496	1870	2364	−0.82
27	65	870	4037	2022	1521	−1.27
33	25	90	1342	511	345	1.46
34	32	83	5969	1608	940	1.95
35	26	290	5300	1433	690	−1.83
36	36	215	5584	1576	922	−1.27

[a] Values are means of two experiments, standard deviation is ±10%.

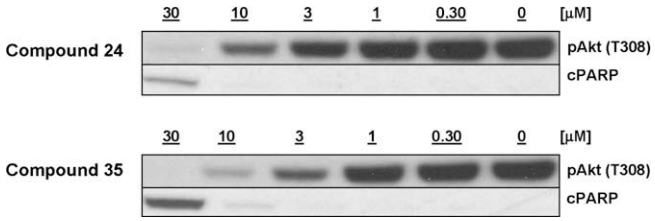


Figure 2. Inhibition of PI3K signaling in MDA-361 cells. MDA-361 cells were treated with DMSO or the indicated doses of **24** or **35** in growth media for 4 h. Protein lysates were prepared and immunoblotted for phospho-AKT T308 (p-Akt (T308)) and cleaved-PARP (cPARP).

as well. Analysis of a PI3K-α homology model derived from an X-ray structure of PI3K-γ allows one to rationalize the selectivity of the purines for PI3K-α versus PI3K-γ and mTOR. These leads provide an excellent foundation for the development of more potent inhibitors of the PI3K signaling pathway. Results will be disclosed in due course.

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