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Identification and structure–activity relationship of 2-morpholino 6-(3-hydroxyphenyl) pyrimidines, a class of potent and selective PI3 kinase inhibitors

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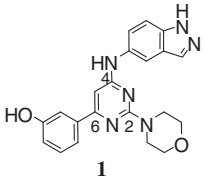
ABSTRACT

PI3 Kinases are a family of lipid kinases mediating numerous cell processes such as proliferation, migration, and differentiation. The PI3 kinase pathway is often de-regulated in cancer through PI3Kα overexpression, gene amplification, mutations, and PTEN phosphatase deletion. PI3K inhibitors represent therefore an attractive therapeutic modality for cancer treatment. Herein we describe a novel series of PI3K inhibitors sharing a pyrimidine core and showing significant potency against class I PI3 kinases in the biochemical assay and in cells. The discovery, synthesis and SAR of this chemotype are described.

Phosphatidylinositol 3-kinases (PI3Ks) comprise a family of lipid and serine/threonine kinases that catalyze the transfer of phosphate to the D-3′ position of inositol lipids to produce phosphoinositol-3-phosphate (PIP), phosphoinositol-3,4-diphosphate (PIP_2), and phosphoinositol-3,4,5-triphosphate (PIP_3) that, in turn, act as second messengers in numerous signaling cascades.[1] Class IA PI3Ks are heterodimers composed of a catalytic p110 subunit (α, β, and δ isoforms) constitutively associated with a regulatory subunit that can be p85α, p55α, p50α, p85β, or p55γ. The Class IB has one family member, a heterodimer composed of a catalytic p110γ subunit associated with one of two regulatory subunits, p101 or p84. Class IA PI3Ks are activated by tyrosine kinases while Class IB is activated directly by G protein-coupled receptors, therefore linking upstream receptors with downstream cellular activities including proliferation, survival, chemotaxis, cellular trafficking, motility, metabolism, inflammatory and allergic responses, transcription, and translation.[2,3] In many cases, PIP_2 and PIP_3 recruit AKT to the plasma membrane where it acts as a nodal point for many intracellular signaling pathways important for growth and survival.[4,5] Aberrant regulation of PI3K, which often increases survival through AKT activation (phosphorylation on S473), is one of the most prevalent events in human cancer.[6] This can occur at multiple levels such as *PTEN* deletion[7], gene amplification (*PIK3CA* and *Akt*)[8], p85α mutations (and translocation) and *PIK3CA* somatic missense mutations.[9] These observations support inhibition of Class IA PI-3 kinases as a potential treatment for a variety of tumor types and other proliferative diseases[10,11,12]

In the course of our efforts toward the identification of PI3K inhibitors we discovered that a pool from a solid phase combinatorial library of 2,4,6-trisubstituted pyrimidines showed sub



1

Figure 1. Initial hit in the 2-morpholino-4-amino-6-phenylpyrimidine scaffold, PI3Kα IC_{50} 0.031 μM.

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micromolar activity against PI3Kα. Pool deconvolution and screening of the singletons led to the identification of compound **1** (Fig. 1), a hit with the remarkable potency of 0.031 μM. The compound was also quite selective against protein kinases. Out of 31 tyrosine and serine threonine kinases screened, 24 had IC_{50} >10 μM, 4 were between 5 and 10 μM and 3 were between 1 and 5 μM. In particular, PDK1 and AKT, important effectors in the PI3 kinase pathway, were not significantly inhibited, with IC_{50} >25 and >10 μM, respectively. Further analoging in the series was initially performed through solid phase synthesis (Scheme 1, top).[13] Resin bound methyl malonate **2** was reacted with aldehydes under Knoevenagel conditions and the resulting α,β-unsaturated ester **3** was cyclized to the dihydropyrimidinone **4** with pyrazolo guanidine in basic conditions. Oxidation to the pyrimidinone **5** and reaction with amines in the presence of PyBOP led to the formation of 2-pyrazolo-4-alkylamino-6-substituted pyrimidines **6**. The 2-pyrazole group could then be displaced by a different set of amines under SnAr conditions (**7**) and after cleavage from the resin and decarboxylation the desired trisubstituted pyrimidines **8** were obtained. Later on, a solution phase route was devised for 2-morpholino substituted pyrimidines, allowing the synthesis of larger amounts of material (Scheme 1, bottom).

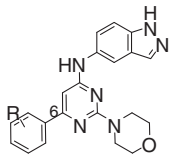
Substituted ethyl 3-oxo-3-aryl propanoates **9**, prepared via a modification of a literature route[14] were cyclized to the pyrimidinones[15] with commercially available morpholino carboxamidine hydrobromide. Conversion to the 4-position triflate **10** and reaction with a variety of primary or secondary amines yielded the desired 2-morpholino 4-amino 6-substituted pyrimidines **11**.[16]

We initially undertook the exploration of the substitution around the phenyl group in the 6-position of the pyrimidine ring. Some of the results are shown in Table 1. It was immediately apparent that a phenol in the 6-position was a key binding feature. *m*-Hydroxy seemed optimal, as shown by the *o*- and *p*-hydroxyphenyl analogs **12** and **13**, with a decrease in potency of 30- and 200-fold, respectively, compared to **1**. Removal of the hydroxyl group (e.g., **14**) or its replacement with F (e.g., **15**) caused 300-fold potency loss. A series of *m*-position substituents did not improve potency with every compound (e.g., **16**–**20**) being at least 300-fold less potent than **1**.

In the 4-position of the pyrimidine ring numerous substituents were tolerated (Table 2), with the most favorable being heteroaromatic groups with hydrogen bond acceptors (e.g., **1**, **25–27**).

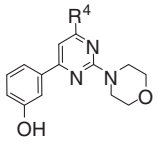
Benzylic (e.g., **28**) or aliphatic amines, (e.g., **29**–**30**) reduced activity about 100-fold or more. The NH in the 4-position was not necessary for binding, as apparent comparing example **25** with its aryloxy analog **27**. Data also suggested that most of the affinity was achieved through position 2- and 6-substituents, as the 4-unsubstituted example **21**, was only 10-fold less potent than

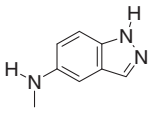
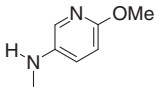
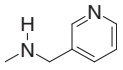
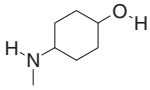
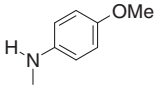
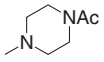
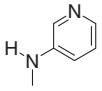
Table 1
Position 6- SAR in the 2-morpholino pyrimidine scaffold (IC_{50} in μM)

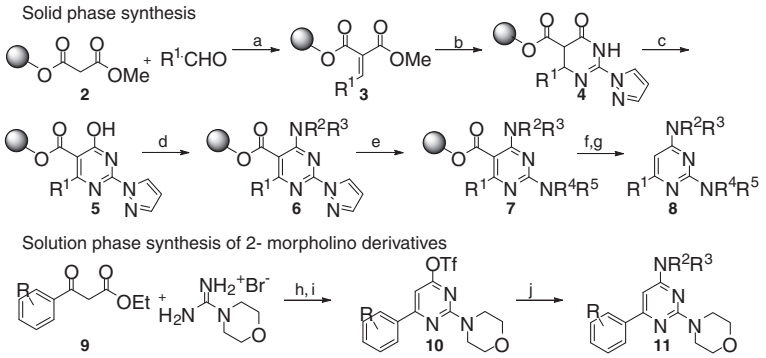


Ex #	R	PI3Kα IC_{50} (*n*)	Ex #	R	PI3K α IC_{50} (*n*)
1	*m*-OH	0.031 (11)	**16**	*m*-Cl	13 (2)
12	*o*-OH	0.91 (6)	**17**	*m*-OMe	15 (6)
13	*p*-OH	6.5 (2)	**18**	*m*-CN	14 (3)
14	H	9.4 (3)	**19**	*m*-COOMe	16 (2)
15	*m*-F	9.5 (3)	**20**	*m*-O$(CH_2)_2$OH	11 (3)

Table 2
Position 4- SAR in the 2-morpholino-6-(3′-hydroxyphenyl) pyrimidine series (IC_{50} in μM)

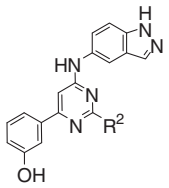


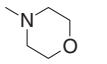
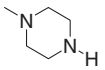
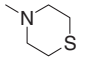
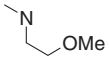
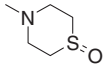
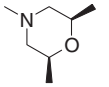
Ex #	R^4	PI3Kα IC_{50} (*n*)	Ex #	R^4	PI3Kα IC_{50} (*n*)
1		0.031 (11)	**26**		0.017 (9)
21	H	0.26 (6)	**27**		0.05 (6)
22	NHMe	1.1 (6)	**28**		2.0 (6)
23	NHPh	1.3 (3)	**29**		0.34 (6)
24		0.19 (6)	**30**		0.57 (6)
25		0.056 (14)	**31**		0.4 (7)



Scheme 1. Reagents and conditions: (a) piperidine, AcOH, rt; (b) pyrazologuanidine, $NaHCO_3$, NMP, 50 °C; (c) DDQ, toluene 50 °C; (d) R^2R^3NH, PyBOP, NMP, rt; (e) R^4R^5NH, AcOH, NMP; (f) 95% TFA/H_2O; (g) CH_3CN/H_2O 1:1, 60 °C; (h) Cs_2CO_3, DMF, 150 °C; (i) *N*-phenyl bis (trifluoromethane sulfonimide), Et_3N, DMAP, CH_2Cl_2; (j) R^2R^3NH, Cs_2CO_3, BINAP, THF, 60 °C.

Table 3
Position 2- SAR in the 2-morpholino-6-(3′-hydroxyphenyl) pyrimidine series (IC_{50} in μM)



Ex #	R^2	PI3Kα IC_{50} (*n*)	Ex #	R^2	PI3Kα IC_{50} (*n*)
1		0.031 (11)	**34**		16 (4)
32		5.6 (5)	**35**		15 (7)
33		7.8 (3)	**36**		11 (10)

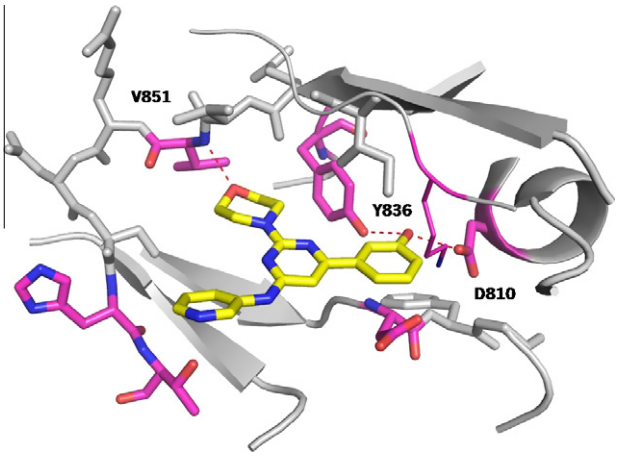


Figure 2. The proposed binding mode of compound **25** in the class 1A PI3K-α isoform is shown. Key binding interactions with V851, D810, and Y836 are shown by dotted red lines.

Table 4
Biochemical selectivity profile, cell mechanism modulation and functional effects of selected PI3K inhibitors from the morpholino pyrimidine scaffold

Ex#	PI3Kα IC_{50}	PI3Kβ IC_{50}	PI3Kδ IC_{50}	PI3Kγ IC_{50}	pAKT EC_{50}	Prolif. EC_{50}
1	0.031 (11)	0.24 (18)	0.029 (5)	0.57 (2)	0.44 (1)	0.94 (4)
26	0.017 (9)	0.24 (23)	0.006 (1)	nd	0.073 (1)	0.37 (2)

IC_{50} and EC_{50} in μM. Cell line: A2780 ovarian carcinoma, PTEN deleted.

compound **1**. Although, in general, this series suffered from poor solubility, 4-position variations allowed some modulation of the physicochemical properties. As an example, compared to compound **1** (<0.1 μM), compounds **26**, **29**, and **31** were at least 50-fold more soluble.

SAR around position 2- (Table 3) underlined the unique role of morpholine in binding.

The replacement with S, SO, or NH (e.g., **32–34**) or removal of the cyclic constraint (e.g., **35**) were, in fact, not tolerated and steric bulk on both sides of the morpholine oxygen also caused a significant (>300-fold, e.g., **36**) potency loss.

The proposed binding mode of compound **25** in p110α is shown in Figure 2. The model was built based on literature data and a co-structure of the compound in p110γ.[17] Given the high homology between the α and γ isoforms a similar binding mode was expected and this assumption was supported by SAR data. In fact, biochemical assay data showed only about a 10-fold potency difference of **25** against the two isoforms.[18] The model of **25** in the p110α ATP binding site provides a rationale for the observed SAR trends and especially for the key role of the 2-position substituent.

The morpholine oxygen forms a key productive hydrogen bond interaction with V851 in the hinge domain of the ATP binding site. This interaction has been described previously.[19,20] The pyrimidine core provides the appropriate scaffolding to place the phenolic moiety near the catalytic region, where the OH binds tightly between D810 and Y836. These interactions achieve maximum efficiency through a nearly coplanar conformation of the central pyrimidine ring and the position 4- and 6-substituents. Substituents in position 4- reach toward a partially solvent exposed area, hence the higher tolerability.

Compounds in this series inhibit class I PI3 kinases and are in general approximately equipotent against the α and δ isoforms, while they are about 10-fold less potent against the β and γ isoforms.

Modulation of the PI3K pathway is confirmed by inhibition of AKT phosphorylation on S473 in the A2780 cell line (ovarian carcinoma, PTEN deleted) and by the resulting inhibition of cell proliferation in the same cell line, as shown in Table 4.

In conclusion, we have identified a series of 2-morpholino-6-hydroxyphenyl pyrimidines which are potent and selective class I PI3K kinase inhibitors, showing cellular activity both as mechanism modulation (inhibition of AKT^{S473} phosphorylation) and inhibition of cell proliferation in a cell line with PI3K pathway deregulation. Additional SAR, especially focused on identifying heterocyclic alternatives to the phenol in the 6-position will be reported in due course.

Acknowledgments

The authors wish to acknowledge Yasuki Yamada for the synthesis of the original solid phase library, John Nuss and Alex Harris for guidance and advice during the early stage of the program.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.021.

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16. Spectral data for compound **1**: 3-(6-(1*H*-indazol-5-ylamino)-2-morpholinopyrimidin-4-yl)phenol hydrochloride. ^{1}H NMR (acetonitrile-d_3/D_2O, 300 MHz): δ ppm 8.09 (s, 1H), 8.03 (br s, 1H), 7.61 (d, 1H, J = 7.8 Hz), 7.55 (br m, 1H), 7.38 (app. t, 1H, J = 7.8 Hz), 7.17 (br d, 1H, J = 7.8 Hz), 7.10 (br s, 1H), 7.06 (d, 1H, J = 8.7 Hz), 6.42 (br s, 1H), 3.75 (br s, 8H); LCMS m/z (%): 389.2 (M+H).
17. The model for compound in p110α was built in Maestro 8.0.308 after merging the compound **25** coordinates from a Novartis structure of the compound bound to PI3K p110γ (PDB accession code: 3P2B) with the aligned coordinates of the public domain p110α apo-structure (PDB deposition code: 2RD0). All key protein-compound interactions are retained between the p110γ structure and the p110α model and no new interactions are expected with the other PI3K Class I isoforms.
18. PI3Kγ IC_{50} is 0.55 μM for **25**.
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