

Isoform-specific phosphoinositide 3-kinase inhibitors from an arylmorpholine scaffold

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Abstract—Phosphoinositide 3-kinases (PI3-Ks) are an ubiquitous class of signaling enzymes that regulate diverse cellular processes including growth, differentiation, and motility. Physiological roles of PI3-Ks have traditionally been assigned using two pharmacological inhibitors, LY294002 and wortmannin. Although these compounds are broadly specific for the PI3-K family, they show little selectivity among family members, and the development of isoform-specific inhibitors of these enzymes has been long anticipated. Herein, we prepare compounds from two classes of arylmorpholine PI3-K inhibitors and characterize their specificity against a comprehensive panel of targets within the PI3-K family. We identify multiplex inhibitors that potently inhibit distinct subsets of PI3-K isoforms, including the first selective inhibitor of p110 β /p110 δ (IC₅₀ p110 β = 0.13 μ M, p110 δ = 0.63 μ M). We also identify trends that suggest certain PI3-K isoforms may be more sensitive to potent inhibition by arylmorpholines, thereby guiding future drug design based on this pharmacophore.

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1. Introduction

Phosphatidylinositol 3-kinases are activated by a wide range of cell surface receptors to generate the lipid second messengers phosphatidylinositol 3,4-bisphosphate (PIP₂) and phosphatidylinositol 3,4,5-trisphosphate (PIP₃). In the appropriate cellular context, these two lipids can regulate a remarkably diverse array of physiological processes, including glucose homeostasis, cell growth, differentiation, and motility.¹ These distinct functions are carried out by a family of eight related PI3-Ks in vertebrates that possess unique substrate specificities, localization, and modes of regulation.¹ These include the class IA PI3-Ks (p110 α , p110 β , p110 δ), which are activated by receptor tyrosine kinases, the class IB PI3-K (p110 γ), which is activated by heterotrimeric G-proteins, and the class II PI3-Ks (PI3KC2 α , PI3KC2 β , PI3KC2 γ) whose regulation remains poorly understood. Despite these known differences in up-

stream activation, the physiological roles of individual PI3-K isoforms remain largely unassigned, and dissecting the unique functions of members of this family is a major focus of ongoing research.²

In addition to sequence homology within their catalytic domain, PI3-Ks share sensitivity to two small molecule inhibitors, wortmannin and LY294002. Wortmannin is a fungal natural product that irreversibly inhibits PI3-Ks at low nanomolar concentrations,^{3,4} whereas LY294002 is a synthetic chromone that reversibly inhibits most PI3-Ks at low micromolar concentrations.⁵ Together, these two compounds have served as powerful probes for implicating PI3-Ks in a wide range of physiological processes, and much of our understanding of PI3-K action in cells derives from the use of these two reagents.

Although wortmannin and LY294002 are broadly active against the PI3-K family, they show little specificity among PI3-K family members. Since these compounds do not pharmacologically discriminate between PI3-K isoforms, comparatively little is known about the specific signaling functions of individual PI3-Ks. Knockout

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mice have defined essential roles for p110 γ and p110 δ in leukocyte function, including signaling from the B and T cell receptors^{6,7} (p110 δ) as well as chemotaxis of neutrophils and macrophages^{8,9} (p110 γ). Furthermore, microinjection of isoform-specific inhibitory antibodies has demonstrated that individual class I PI3-Ks can relay unique downstream signals following activation of a common upstream receptor. For example, colony-stimulating factor-1 treatment of macrophages induces DNA synthesis through p110 α , whereas actin cytoskeleton rearrangement and cell migration require p110 β and p110 δ .¹⁰ These studies suggest that PI3-K isoforms likely possess nonredundant functions downstream of the wide range of receptors that are known to activate PI3-Ks. In general, however, the systematic analysis of PI3-K isoform action in cells awaits the discovery of small molecule inhibitors that can selectively target PI3-K family members. For this reason, the development of isoform-specific inhibitors of PI3-Ks has been long anticipated.^{2,11–13}

Recently, patent disclosures have described new inhibitors based on the LY294002 arylmorpholine pharmacophore,^{11,12,14} although the detailed isoform selectivity of these compounds has not been reported. Since these compounds are potentially useful probes for PI3-K isoform activity in cells, we sought to determine their target specificity by characterizing their activity against a comprehensive panel of PI3-Ks. In this regard, a recent characterization of 28 common protein kinase inhibitors against a panel of 24 protein kinases has significantly challenged many preconceptions about the true selectivity of protein kinase inhibitors.¹⁵ The analysis we report here identifies several compounds that inhibit distinct subsets of the PI3-K family, including the first selective inhibitors of the p110 δ /p110 β isoforms.

We also sought to explore the potential of the morpholino chromone scaffold as a starting point for PI3-K inhibitor optimization. LY294002 has been shown to

be quite selective for lipid kinases relative to protein kinases¹⁵ and to possess broad activity within the PI3-K family. Indeed, until very recently, LY294002 was the only reversible inhibitor that had been reported to target the PI3-K family—even though this compound was originally described 10 years ago.⁵ Given the importance of PI3-Ks in signal transduction, it is remarkable that there has not been a single subsequent structure–activity study published investigating compounds of this class. Our comprehensive selectivity analysis of a panel of arylmorpholines identifies PI3-K isoforms that tend to be sensitive to this pharmacophore, as well as others that are more resistant. This information should prove useful in guiding future drug design based on this core structure.

2. Results

2.1. LY294002 analogs

Thrombogenix has disclosed a series of LY294002 analogs that differ in the substitution pattern of heteroatoms within the chromone core,¹⁴ as well as through replacement of the phenyl group at the eight position of LY294002 with more extended aromatic substituents (Fig. 1). Comparison of the co-crystal structures of p110 γ bound to LY294002¹⁶ and ATP¹⁷ suggests that the adenine ring of ATP and the morpholino-chromone core of LY294002 occupy essentially the same space in the interior of the ATP binding pocket, with both rings anchored by a hydrogen bond to the backbone amide of V882 (Fig. 1A). Likewise, the 8-phenyl moiety of LY294002 and the ribose sugar of ATP occupy a similar space at the entrance to the pocket and project outward toward solvent. To understand how LY294002 analogs with extended substituents at C8 might interact differentially with PI3-K isoforms, we identified all of the residues in p110 γ whose side chains possess a rotamer that can extend within 4 Å of either the adenine/LY294002 chromone

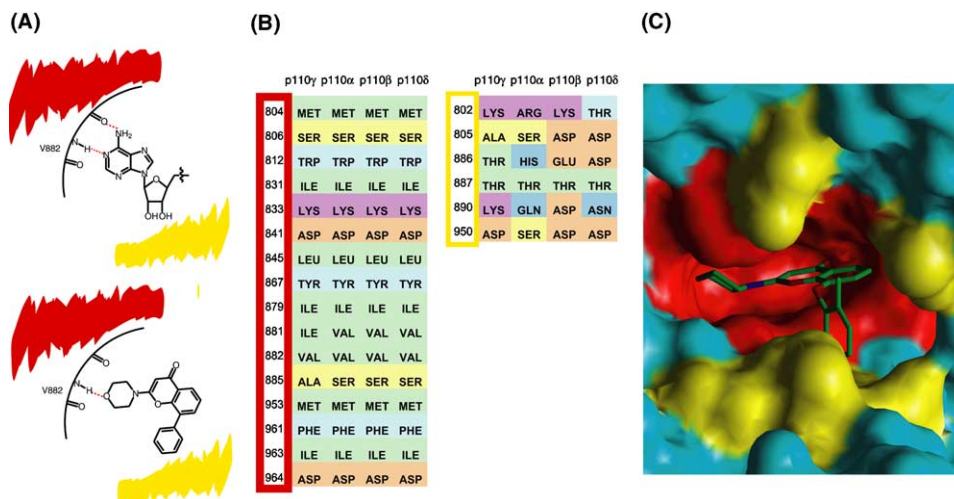


Figure 1. ATP binding site conservation among the class I PI3-Ks. (A) Schematic of the hydrogen bonds made by ATP (top) and LY294002 (bottom) in the active site of p110 γ . Pocket interior (red) and entrance (yellow) are marked for reference. (B) Sequence of alignment residues in the interior (left) and exterior (right) of the ATP binding pocket. Residue coloring: hydrophobic aliphatic (green), hydrophobic aromatic (light blue), small (yellow), polar uncharged (dark blue), basic (purple), and acidic (orange). Residue number is for porcine p110 γ . (C) Model of TGX115 bound to p110 γ , based on the LY294002–p110 γ co-crystal structure.¹⁶

core (Fig. 1, red) or the ATP ribose/8-phenyl of LY294002 (Fig. 1, yellow). This first set of residues defines the interior of the ATP binding pocket, while the second set mark the entrance to that pocket (Fig. 1C). We then aligned these residues with the corresponding residues from each of the class I PI3-Ks, and shaded them according to their physiochemical properties (hydrophobicity, size, and charge) (Fig. 1B). This alignment reveals that the interior of the ATP binding pocket is highly conserved within the class I PI3-Ks; the only differences are two conservative substitutions that distinguish p110 γ from the class IA PI3-Ks (Fig. 1B, left). By contrast, the residues that line the entrance to the ATP binding pocket are divergent, with major differences between isoforms in their size and charge (Fig. 1B, right). LY294002 analogs with larger substituents at C8 would be expected to extend outside the ATP binding pocket and potentially make extensive contacts with these less conserved residues (Fig. 1C). This suggests that it may be possible for such extended analogs to target specific PI3-K isoforms, although it is not immediately apparent how to design compounds that would exploit these differences.

We initially prepared (Supplementary Schemes 1 and 2) and tested two compounds from this series (TGX115 and TGX126) as well as a synthetic intermediate lacking the aromatic substituent at C8 (TGX066) and compared these compounds to LY294002 and the related LY292223 (which lacks the C8 phenyl group). To obtain a detailed picture of the target specificity of these compounds, we determined IC₅₀ values in vitro against a panel of 14 enzymes selected to span the most relevant targets for these molecules. These include seven mammalian PI3-Ks (p110 α , p110 β , p110 δ , p110 γ , PI3KC2 α , PI3KC2 β , and PI3KC2 γ), four protein kinases in the PI3-K family (DNA-PK, ATM, ATR, mTOR), PI4-kinase β (a PI3-K family member reported to be weakly inhibited by LY294002¹⁸), casein kinase II (the only protein kinase family member known to be inhibited by LY294002¹⁵), and the unrelated serine-threonine kinase G-protein coupled receptor kinase 2 (GRK2). These 14 proteins include all of the known targets of LY294002 and most of the proteins that contain sequence homology to the PI3-K catalytic domain. To facilitate comparison of IC₅₀ values across kinases with different substrate affinities, all assays were carried out in the presence of 100 μ M ATP.

As has been previously reported, LY29002 exhibits a very broad specificity profile, inhibiting the class I PI3-Ks, PI3KC2 β , PI3KC2 γ , mTOR, casein kinase 2, and DNA-PK all with IC₅₀ values in the low micromolar range. Within the class I PI3-Ks, LY294002 exhibits a maximum of approximately 10-fold selectivity between any two isoforms, with p110 β being most sensitive (2.9 μ M) and p110 γ the least sensitive (38 μ M). Interestingly, LY294002 did not show activity at 100 μ M against the PI3-K related protein kinases ATM and ATR. Although we are not aware of any reports that LY294002 inhibits these two enzymes at micromolar concentrations,¹⁹ the effects of LY294002 treatment of cells has been interpreted to imply a requirement for ATM or ATR kinase activity in several studies.^{20,21}

In contrast to LY294002, TGX115 and TGX126 were significantly more potent and selective. Surprisingly, we find that TGX115 inhibits p110 β and p110 δ at nanomolar concentrations, but p110 α and p110 γ only at concentrations more than 100-fold higher (Fig. 2). TGX126 showed a more modest specificity profile, although this compound was still $\sim$ 50-fold more potent against p110 β /p110 δ than against p110 α /p110 γ . Against the other 10 enzymes in this panel, TGX115 and TGX126 showed significant activity only against DNA-PK, with high nanomolar IC₅₀ values. Thus, within the lipid kinases, these two compounds represent the first selective inhibitors of the p110 β /p110 δ isoforms. By contrast, the unsubstituted analogs TGX066 and LY292223 were significantly less potent against all PI3-Ks, although they retained strong activity against DNA-PK (Fig. 2). This suggests that presence of an aromatic substituent at C8 plays an essential role in achieving binding affinity for PI3-Ks, but may be dispensable for DNA-PK inhibition.

On the basis of this initial data, seven additional compounds were prepared by palladium catalyzed coupling of different aromatic substituents to TGX066 (Supplementary Scheme 1) to explore the chemical space surrounding TGX115 and TGX126. These compounds, termed morpholino chromone analogs 49–55 (MCA049–MCA055), all contain the pyrimidone heterocycle scaffold of TGX126 and differ in their aromatic substitution at the position analogous to C8 in LY294002. They include compounds that substitute the pyrimidone core with anilines (MCA50–MCA52, MCA54), benzylamines (MCA53 and MCA55), and a biaryl linkage (MCA49) that mimics LY294002 in this new scaffold, and were prepared.

The specificity profile of these compounds was determined, and several structure–activity trends were identified (Fig. 2). Compounds containing a benzylamine substitution to the pyrimidone core exhibit potent activity against the class IA PI3-Ks p110 α / β / δ (IC₅₀=0.1–2 μ M), but display comparatively less activity against the class IB PI3-K p110 γ (IC₅₀=25–100 μ M). Compared to the closely related TGX126, these methyl-substituted compounds possess modestly enhanced activity toward p110 α and diminished activity against p110 γ . MCA55 showed the largest selectivity between p110 α and p110 γ of any compound in our panel ($\sim$ 50-fold) and this compound can be regarded as a multiplex inhibitor of the growth factor regulated class IA PI3-Ks with little activity against the G-protein regulated class IB enzyme, p110 γ .

In contrast, compounds containing an aniline substitution on the pyrimidone scaffold all showed a 10–100-fold loss of activity against the PI3-K isoforms p110 δ and p110 β relative to the parent compounds TGX115 and TGX126, while retaining potent DNA-PK inhibition. This trend includes MCA51, a compound that is isosteric to TGX115 within the pyrimidone scaffold. Compared to TGX115, this compound is $\sim$ 20-fold less potent against p110 δ /p110 β and $\sim$ 10-fold more potent against DNA-PK. This effect appears to be a

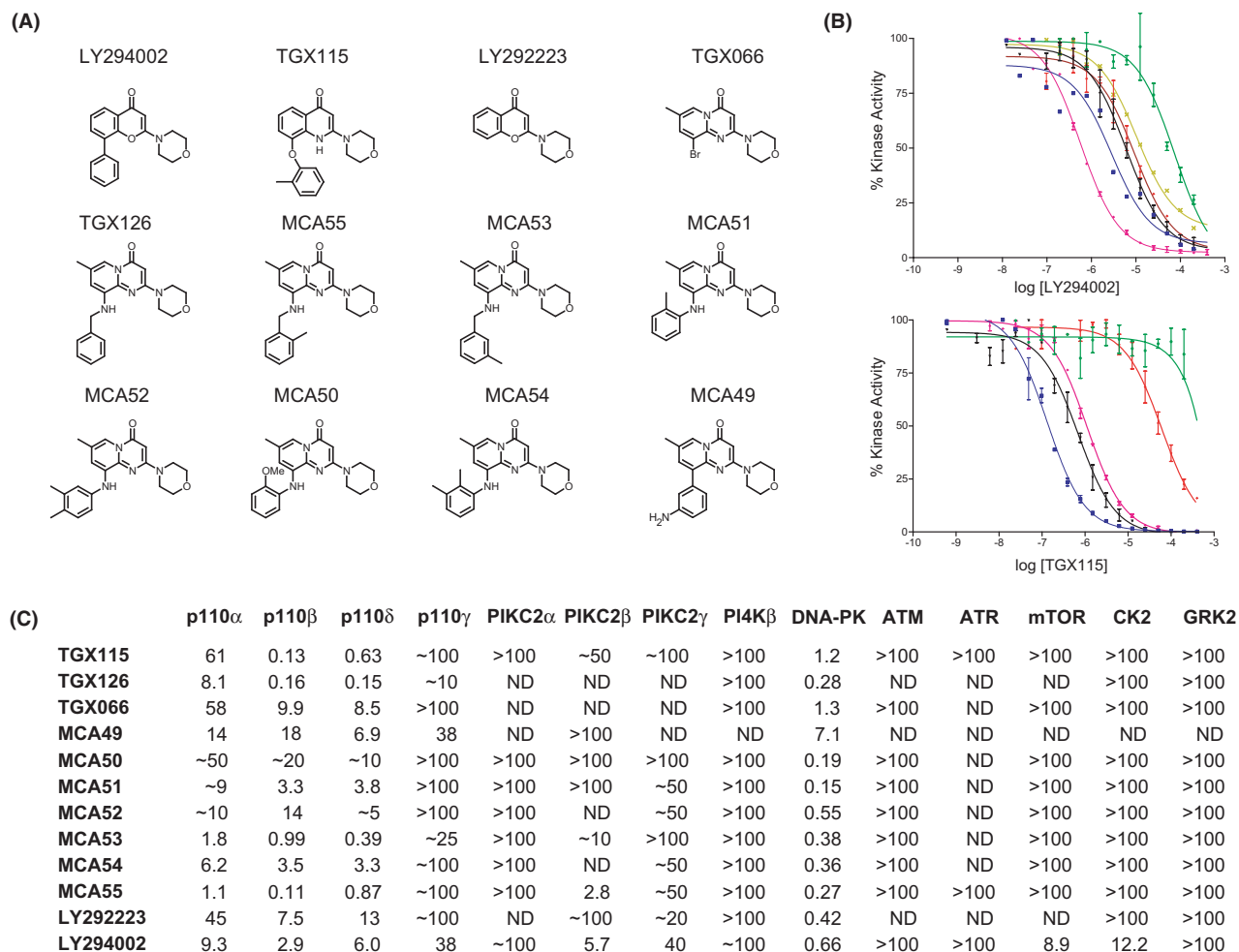


Figure 2. IC₅₀ values of LY294002 analogs against protein and lipid kinases. (A) Structures of LY294002 analogs. (B) Dose response curves for LY294002 (top) and TGX115 (bottom) against different PI3-K family members. DNA-PK (purple), p110 α (red), p110 β (blue), p110 γ (green), p110 δ (black), and CK2 (yellow). (C) IC₅₀ values (μ M) measured in the presence of 100 μ M ATP.

consequence of the aniline substitution, rather than the pyrimidone core itself, because TGX126 and related compounds containing a benzylamine substitution remain highly potent against p110 δ /p110 β . This suggests that very subtle changes to the aromatic substituent can significantly shift inhibitor specificity among PI3-K family members. Indeed, we find that several anilino-pyrimidones inhibit DNA-PK at nanomolar concentrations and display significant selectivity (10–100-fold) relative to all other members of the PI3-K family. These compounds may prove useful as selective inhibitors of DNA-PK, or as lead structures for the development of more highly specific compounds.

2.2. Arylmorpholine DNA-PK inhibitors

ICOS has recently disclosed a set of arylmorpholines^{22,23} that are potent inhibitors of DNA-PK (Fig. 3). These compounds are trisubstituted benzene derivatives with hydroxy and carbonyl moieties *meta* and *para* to the morpholine ring, respectively. As these molecules are structurally reminiscent of LY294002, we prepared a small set of eight arylmorpholines (Supplementary Scheme 3) and determined their specificity profile against our enzyme panel.

We find that IC60211 and IC86621 are high nanomolar inhibitors of DNA-PK, with IC₅₀ values against class I PI3-Ks in the low to mid-micromolar range, consistent with an earlier report.²² In this regard, these compounds exhibit a specificity profile that mirrors LY294002, with modestly enhanced DNA-PK activity (1.5–5-fold). Nonetheless, we find that these compounds are more selective than LY294002 in that they show no activity against the secondary targets mTOR, casein kinase II or any of the class II PI3-Ks. Thus, within the lipid kinases, this structural class is selective for the class I PI3-Ks. Arylmorpholine analog 37 (AMA37) is the most DNA-PK selective of these compounds, with ~10-fold specificity relative to p110 β and ~100-fold specificity relative to the other class I PI3Ks. This potency and selectivity is comparable to the most DNA-PK selective compounds identified from our panel of anilino pyrimidones.

We tested a series of analogs of these compounds to probe the requirements for DNA-PK inhibition, and confirm that both the hydrogen bond acceptor at the 1-position and the donor at the 2-position are required for potent DNA-PK inhibition (Fig. 3). For example, AMA57, which differs from AMA37 by the substitution

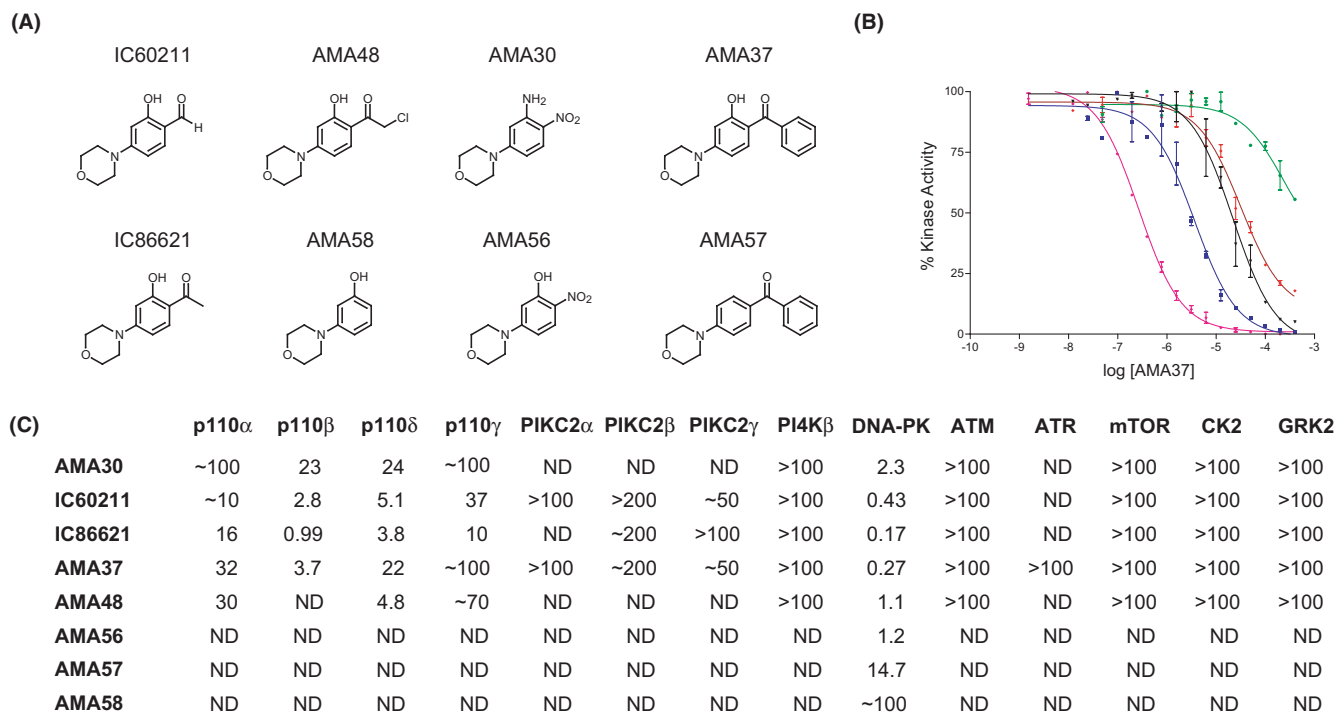


Figure 3. IC₅₀ values of arylmorpholines against protein and lipid kinases. (A) Structures of arylmorpholine analogs. (B) Dose response curves for AMA37 against different PI3-K family members (color coded as in Fig. 2). (C) IC₅₀ values (μ M) measured in the presence of 100 μ M ATP.

of a hydrogen for the 2-hydroxyl, is ~80-fold less potent, whereas AMA58, which differs from IC60211 potent. However, the specific identity of hydrogen bond donor and acceptor is not a requirement for potent DNA-PK inhibition. For example, AMA56, which differs from IC60211 by the replacement of the aldehyde with a nitro group, is only ~3-fold less potent. Likewise, AMA30, which differs from AMA56 by the further substitution of the 2-hydroxyl group with an amine, is only ~2-fold less potent.

3. Discussion

We have measured the activity of a panel of compounds that share the arylmorpholine pharmacophore first identified in LY294002 against a comprehensive set of targets within the PI3-K family. Analysis of this data suggests several trends in selectivity among different PI3-K family members. First, while we confirm that LY294002 inhibits three protein kinases at low micromolar concentrations (DNA-PK, mTOR, CK2), we find that two of these kinases (mTOR and CK2) are resistant to all other arylmorpholines in our panel. DNA-PK, by contrast, is potently inhibited by almost every compound tested, and shows very broad SAR with respect different analogs in the same series. In part, we believe these differences reflect the fact that DNA-PK binds with high affinity to the core arylmorpholine pharmacophore, whereas the other protein kinases (and to a lesser extent, the PI3-Ks) require more extensive interactions with other regions of the molecule for high affinity binding. Consistent with this view, the most structurally simple compounds in our panel (TGX066, LY292223, and IC60211) all exhibit potent inhibition of DNA-PK, even though their PI3-K

activity is significantly reduced compared to more substituted analogs. Moreover, a third class of DNA-PK inhibitors based on a limited arylmorpholine scaffold have recently been reported,²⁴ suggesting that diverse compounds containing this core structure can exhibit potent and selective DNA-PK inhibition.

Selectivity trends were also observed within the class I PI3-Ks. To visualize these differences, we plotted the IC₅₀ values of all of the compounds for inhibition of p110 β (x-axis) against the IC₅₀ values for inhibition of the other class I PI3-Ks and DNA-PK (y-axis), such that each data point represents the intersection of IC₅₀ values for the same compound against two different enzymes (Fig. 4). In this representation, compounds that lie along

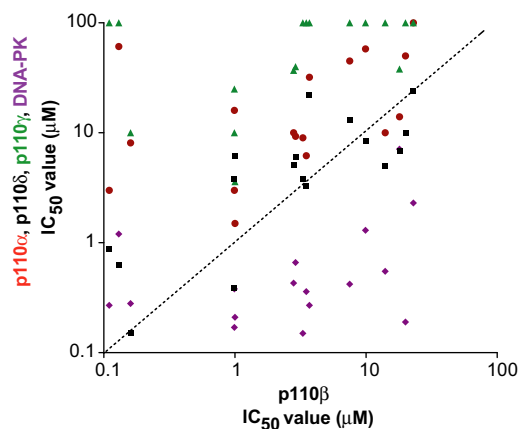


Figure 4. Correlation plot for inhibition of different PI3-K isoforms. IC₅₀ values (μ M) for p110 β (x-axis) plotted against IC₅₀ values for DNA-PK (purple), p110 α (red), p110 γ (green), and p110 δ (black).

the diagonal show equipotent inhibition of p110 β and the second kinase, whereas those that lie above or below the diagonal are more or less potent, respectively, against the second enzyme than p110 β . Virtually all of the DNA-PK IC₅₀ values (purple) occupy the lower section of the plot, reflecting the fact that DNA-PK is more sensitive to arylmorpholines than the class I PI3Ks. p110 δ IC₅₀ values (black) cluster around the diagonal as few compounds within this series show differential activity against p110 δ and p110 β , whereas p110 α (red) tends to be inhibited less potently. p110 γ (green) was unexpectedly resistant to inhibition by a large number of arylmorpholines, although this insensitivity is consistent with p110 γ 's underlying poor sensitivity to LY294002 relative to the other class I isoforms.

While we have not been able to rationalize these differences in inhibitor sensitivity in terms of specific residues that differ between PI3-K isoforms, several observations are consistent with these IC₅₀ trends. First, the similarity in the sensitivity of p110 δ and p110 β to diverse compounds is likely a reflection of the fact that these two kinases are more closely related in primary sequence than any other members of the PI3-K family. For this reason, it may be challenging to find analogs of potent p110 β /p110 δ inhibitors such as TGX115 containing the arylmorpholine core that can distinguish between these two isoforms. Second, we find that the overall trend in inhibitor sensitivity among these targets (p110 γ < p110 α < p110 β , p110 δ < DNA-PK) roughly mirrors the K_M for ATP of these proteins. That is, we have found that p110 γ has the lowest K_M for ATP among these enzymes (7.4 μ M), whereas DNA-PK has the highest (192 μ M), and the class IA PI3-Ks exhibit intermediate values. While this alone cannot explain

the potency and isoform selectivity of compounds such as TGX115, it does suggest that smaller differences between the class I isoforms (e.g., as observed for LY294002) likely reflect differential competition from ATP substrate between isoforms. This suggests that the PI3-K family members for which selective inhibitors have been identified to date (DNA-PK, and, to a lesser extent, p110 β and p110 δ) may be inherently easier to target with ATP competitive small molecules than other isoforms.

It has long been anticipated that the development of isoform-specific PI3-K inhibitors would make it possible to dissect the unique contributions of individual PI3-K isoforms in well-characterized signaling pathways.^{2,11–13} Recently, Sadhu et al. described the first isoform selective PI3-K inhibitor,²⁵ IC87114, which targets p110 δ , and this compound has found rapid use in identifying essential roles for p110 δ in chemotaxis and inflammatory responses of neutrophils,^{25–27} spontaneous tone of arteries²⁸ and EGF driven migration of cancer cell lines.²⁹ TGX115, when used either in conjunction with IC87114 or in cells that do not express p110 δ , should prove equally effective in elucidating physiological roles for p110 β , and we have begun to use this compound to explore p110 β mediated signaling in several systems (Z.A.K. and K.M.S., unpublished results). As other multiplex inhibitors we describe in this report also possess activity against unique combinations of PI3-K family members (Fig. 5), it may be possible to use these compounds in combination in order to interrogate additional PI3-K isoforms. In this way, we envision a route to begin to pharmacologically dissect the distinct contributions of members of this important family of signaling enzymes.

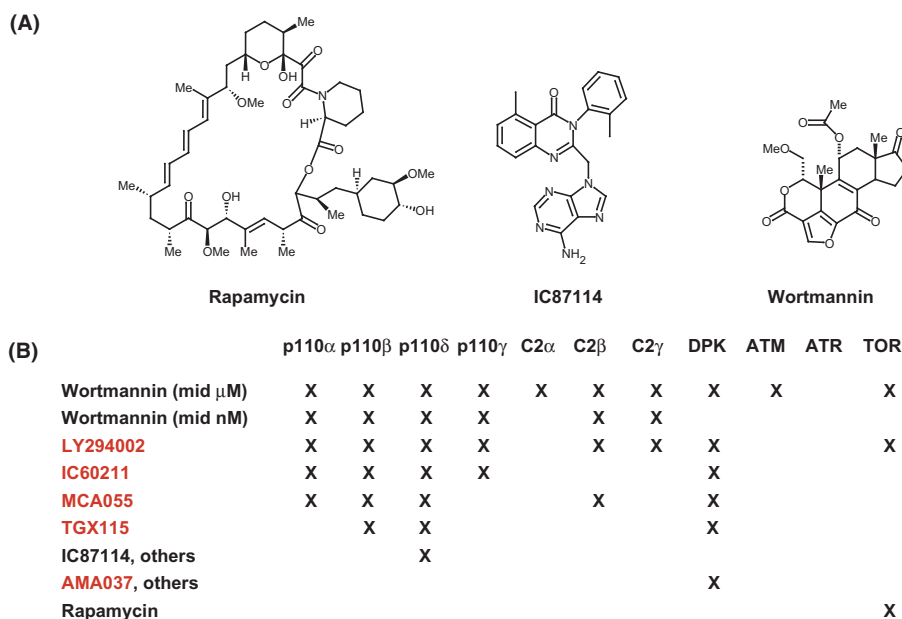


Figure 5. Spectrum of characterized PI3-K inhibitor selectivities. (A) Structures of reported inhibitors of PI3-K family members. (B) Selectivity profile of well-characterized PI3-K inhibitors. Data for wortmannin, IC87114, and rapamycin is drawn from published reports. Compounds characterized in this study are highlighted in red.

4. Experimental

4.1. Lipid kinase expression

Epitope tagged p110 α , p110 β , p110 δ , PI3KC2 α , PI3KC2 β , and PI3KC2 γ were expressed by transient transfection of cos-1 cells. Cells were lysed in lysis buffer (50mM Tris (pH 7.4), 300mM NaCl, 5mM EDTA, 0.02% NaN₃, 1% Triton X-100, and protease inhibitors) and the kinase immunoprecipitated with the appropriate antibody-protein G complex. Immunoprecipitates were washed twice with buffer A (PBS, 1mM EDTA, 1% Triton X-100), twice with buffer B (100mM Tris (pH 7.4), 500mM LiCl, 1mM EDTA), and twice with buffer C (50mM Tris (pH 7.4), 100mM NaCl). GST-PI4K β was expressed in BL21 *E. coli* and purified by glutathione chromatography essentially as described.¹⁸ Recombinant p110 γ was obtained from Sigma. In control experiments, no differences in inhibitor sensitivity were observed between p110 α immunoprecipitated from cos-1 cells and protein expressed in Sf9 cells using a baculovirus system.

4.2. Lipid kinase assays

All kinase assays were conducted at a final concentration of 100 μ M ATP and 2% DMSO. PI3-K and PI4-K assays were carried out essentially as described.¹⁸ Briefly, a reaction mixture was prepared containing kinase, inhibitor, buffer (25mM HEPES (pH 7.4), 10mM MgCl₂), and freshly sonicated phosphatidylinositol (200 μ g/mL). Reactions were initiated by the addition of ATP containing 10 μ Ci of γ -³²P-ATP to a final concentration 100 μ M. Reactions were incubated 15min at rt and then terminated by the addition of 105 μ L 1N HCl followed by 160 μ L CHCl₃-MeOH (1:1). The biphasic mixture was then vortexed, briefly centrifuged, and the organic phase transferred to a new tube using a gel loading pipette tip precoated with CHCl₃. This extract was spotted on TLC plates and developed for 3–4h in a 65:35 solution of *n*-propanol–2M AcOH. The TLC plates were then dried and quantitated using a PhosphorImager (Molecular Dynamics). For each compound, kinase activity was measured at 12–15 inhibitor concentrations representing twofold dilutions from the highest concentration tested (typically, 200 μ M). For compounds showing significant activity, IC₅₀ determinations were repeated two to six times, and the reported value is the average of these independent measurements.

4.3. Protein kinase expression

HA-ATM, FLAG-ATR, and AU-1 mTOR were expressed by transient transfection of HEK293 cells. The cells were lysed in lysis buffer (50mM Tris–Cl (pH 7.4), 100mM NaCl, 50mM β -glycerophosphate, 10% glycerol (w/v), 1% Tween-20, 1mM EDTA, 25mM NaF, and protease inhibitors), and lysates were subjected to immunoprecipitation with the appropriate epitope-tag antibody. Immune complexes were collected on rabbit anti-mouse Sepharose (Sigma) and washed three times in lysis buffer, once in high salt buffer (100mM Tris–Cl (pH 7.4), 500mM LiCl), and once in kinase wash buf-

fer (10mM HEPES (pH 7.4), 50mM NaCl, 50mM β -glycerophosphate, 10% glycerol). N-terminal His6-tagged GRK2 was expressed in Sf9 insect cells and purified using Ni-NTA beads (Qiagen) as described.³⁰ Casein kinase 2 was obtained from Upstate Biotechnology, and DNA-PK was obtained from Promega.

4.4. Protein kinase assays

ATM, ATR, and mTOR immunoprecipitates were resuspended in kinase assay buffer (10mM HEPES (pH 7.4), 50mM NaCl, 50mM β -glycerophosphate, 10% glycerol, 10mM MnCl₂, 1mM DTT) and incubated with inhibitor for 30min prior to the kinase reaction. Kinase reactions were initiated by the addition of 100 μ M ATP, 10 μ Ci (γ -³²P)-ATP and 1 μ g of either a GST-p70S6K fragment (amino acids 332–414) for mTOR, or 1 μ g of a GST-p53 fragment (amino acids 1–70) for ATM or ATR. Reactions were incubated at 30°C for 20min and terminated by the addition of 2 $\times$ SDS-PAGE sample buffer. Samples were resolved by SDS-PAGE and transferred to PVDF membranes. The portion of the membrane containing the ³²P-labeled substrate was quantitated by PhosphorImager, while the portion of the membrane containing ATM, ATR, or mTOR was subjected to Western blotting with anti-HA, anti-FLAG, or anti-AU1 antibody, respectively.

The amount of ³²P incorporation in the substrate was normalized to the amount of ATM or mTOR present. GRK2 assays were carried out using tubulin as substrate in 20mM HEPES, pH 7.4, 2mM EDTA, 10mM MgCl₂ containing 100 μ M ATP essentially as described.³⁰ DNA-PK assays were carried out using the DNA-PK assay system (Promega) as directed by the manufacturer. Casein kinase 2 assays were carried out using the casein kinase 2 assay kit (Upstate Biotechnology) as directed by the manufacturer.

4.5. Synthesis

4.5.1. General. The following reagents were obtained commercially: 2-amino-2'-methyldiphenyl ether (TCI America), 3-morpholinophenol (Avacado Research), 4-morpholinobenzophenone (Sigma), 5-morpholino-2-nitrophenol (Alfa Aesar), LY294002 (Calbiochem), and MnO₂ and PdCl₂ (dppf) (Strem Chemicals). All other reagents were from Aldrich, were of the highest grade commercially available, and were used as supplied by the manufacturer without further purification. Reversed phase high performance liquid chromatography (RP-HPLC) was performed on a Ranin SD-200 solvent delivery system equipped with a Zorbax 300-SB C18 column using a MeCN/H₂O/0.1% TFA gradient (0–100%) as the mobile phase.

4.5.2. 5-(Bis-methylsulfanyl-methylene)-2,2-dimethyl-(1,3)dioxane-4, 6-dione (1). Triethylamine (9.6mL, 69mmol) and CS₂ (2.05mL, 34.7mmol) were added consecutively to a stirred solution of Meldrum's acid (5.0g, 35mmol) in DMSO (50mL) at rt.³¹ After 1h, the reaction was cooled on ice, MeI (4.3mL, 69mmol) was added, and the reaction was allowed to proceed

overnight at rt. After 18h, the reaction was terminated by adding ice water, and the precipitate was chromatographed on silica gel (50% EtOAc/hexanes) to yield 1.962g (23%) of a yellow solid. ^1H NMR (400MHz, CDCl_3) δ 2.45 (6H, s), 1.53 (6H, s).

4.5.3. 2,2-Dimethyl-5-(1-methylsulfanyl-2-(2-o-tolylloxy-phenyl)-ethylidene)-(1,3)dioxane-4,6-dione (2). 2-Amino-2'-methylidiphenyl ether (802mg, 4.03mmol) was added to a solution of **2** (1.0g, 4.0mmol) in EtOH (9mL) and stirred at reflux for 9h essentially as described.^{14,32} When the reaction was complete, the solvent was removed in vacuo and the product chromatographed twice on silica (10% EtOAc/hexanes followed by 50% EtOAc/hexanes) to yield 1.54g (95.8%). ^1H NMR (400MHz, CDCl_3) δ 7.39 (1H, d, $J=8\text{Hz}$), 7.00–7.24 (5H, m), 6.78–6.81 (2H, m), 5.24 (1H, s), 2.27 (3H, s), 2.21 (3H, s), 1.66 (6H, s); ^{13}C NMR (100MHz, CDCl_3) δ 178.5, 153.8, 151.3, 131.8, 129.8, 129.5, 128.0, 127.7, 127.3, 124.7, 123.1, 118.9, 117.9, 117.1, 103.2, 86.8, 26.5, 19.0, 16.3.

4.5.4. 2-Morpholin-4-yl-8-o-tolylloxy-1H-quinolin-4-one (TGX115). Morpholine (0.44mL, 5.0mmol) was added to a solution of **2** (900mg, 2.25mmol) in THF (12mL).¹⁴ The reaction was heated to reflux for 24h. After cooling to rt, the solvent was removed in vacuo, the solid washed with Et_2O , and then dissolved in Ph_2O (10mL). The reaction was heated to 265°C for 15min, and then cooled to rt. The product was purified by chromatography on silica gel twice (50% EtOAc/hexanes followed by 10% MeOH/EtOAc) to yield 227mg (33%) of a white solid. ^1H NMR (400MHz, CDCl_3) δ 8.51 (1H, s), 7.88 (1H, d, $J=8\text{Hz}$), 7.11–7.27 (3H, m), 7.05 (1H, t, $J=16\text{Hz}$), 6.94 (1H, d, $J=8\text{Hz}$), 6.67 (1H, d, $J=8\text{Hz}$), 5.73 (1H, s), 3.81 (4H, s), 3.29 (4H, t, $J=4\text{Hz}$), 2.18 (3H, s); ^{13}C NMR (100MHz, $\text{DMSO}-d_6$) δ 161.6, 157.7, 157.0, 149.7, 140.9, 130.6, 126.8, 126.6, 121.7, 120.6, 119.6, 118.9, 117.6, 115.9, 91.1, 65.8, 44.8, 16.1; HR-EI MS (M)⁺ m/z calcd for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_3$ 336.1474, found 336.1457.

4.5.5. 9-Bromo-2-hydroxy-7-methyl-pyrido(1,2-a)pyrimidin-4-one (3). A stirred solution of 2-amino-3-bromo-5-methylpyridine (9.85g, 52.7mmol) in diethylmalonate (20mL, 130mmol) was heated to 200°C for 3.5h.¹⁴ The heat was then removed and the diethylmalonate was removed under a stream of argon as the reaction was allowed to cool to rt. The solid product was purified by chromatography on silica gel twice (50% EtOAc/hexanes, followed by 5% MeOH/ CH_2Cl_2) to yield 1.7g (12.7%) of a yellow solid. ^1H NMR (400MHz, $\text{DMSO}-d_6$) δ 11.71 (1H, br), 8.69 (1H, s), 8.25 (1H, s), 5.46 (1H, s), 2.30 (3H, s); HR-EI MS (M)⁺ m/z calcd for $\text{C}_9\text{H}_7\text{BrN}_2\text{O}_2$ 253.9691, found 253.9674.

4.5.6. 9-Bromo-7-methyl-2-morpholin-4-yl-pyrido(1,2-a)-pyrimidin-4-one (TGX66). A stirred solution of **3** (1.7g, 6.7mmol) in POCl_3 (20.0mL, 215mmol) was heated to reflux overnight.¹⁴ After 18h, the reaction was quenched by pouring onto ice. The aqueous phase was extracted three times with CH_2Cl_2 , concentrated to dryness in vacuo, and dissolved in EtOH (25mL). Morpholine

(1.5mL, 17mmol) was added to this solution and the reaction was heated to reflux for 1h. After 1h, the reaction was allowed to cool to rt, yielding a white precipitate. The precipitate was collected by filtration and rinsed with cold EtOH to yield 737mg (38.8%) of a white solid. ^1H NMR (400MHz, $\text{DMSO}-d_6$) δ 8.56 (1H, s), 8.14 (1H, s), 5.57 (1H, s), 3.63 (4H, s), 3.60 (4H, s), 2.26 (3H, s); ^{13}C NMR (100MHz, CDCl_3) 160.6, 159.0, 142.4, 125.2, 122.3, 119.2, 118.9, 81.1, 65.8, 65.7, 44.8, 44.7, 18.0; HR-EI MS (M)⁺ m/z calcd for $\text{C}_{13}\text{H}_{14}\text{BrN}_3\text{O}_2$ 323.0269, found 323.0259.

4.5.7. 9-Benzylamino-7-methyl-2-morpholin-4-yl-pyrido(1,2-a)pyrimidin-4-one (TGX126). A stirred solution of TGX66 (15mg, 0.046mmol), PdCl_2 (dppf) (1.9mg, 0.0023mmol), potassium *tert*-butoxide (10.4mg, 0.0928mmol), and benzylamine (4.97mg, 0.0464) in THF (1mL) was heated to reflux for 24h.¹⁴ The product was purified by chromatography on silica gel (5% MeOH/ CH_2Cl_2), followed by RP-HPLC, to yield 10mg (61%) of a white solid. ^1H NMR (400MHz, CDCl_3) δ 8.10 (1H, s), 7.22–7.31 (m), 6.35 (1H, s), 5.73 (1H, s), 4.47 (2H, s), 3.78 (4H, t, $J=4\text{Hz}$), 3.59 (4H, t, $J=4\text{Hz}$), 2.22 (3H, s); HR-EI MS (M)⁺ m/z calcd for $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_2$ 350.1743, found 350.1749.

4.5.8. 9-(3-Amino-phenyl)-7-methyl-2-morpholin-4-yl-pyrido(1,2-a)pyrimidin-4-one (MCA49). Prepared following the general procedure for TGX126, using TGX66 (50mg, 0.16mmol), 3-aminophenylboronic acid (96mg, 0.62mmol), and Na_2CO_3 (164mg, 1.55mmol) in place of potassium *tert*-butoxide. The product was purified by chromatography on silica gel (5% MeOH/EtOAc) to yield 30mg (58%) of a white solid. LR-ESI MS ($\text{M}+\text{H}$)⁺ m/z calcd for $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_3$ 337.2, found 337.0.

4.5.9. 9-(2-Methoxy-phenylamino)-7-methyl-2-morpholin-4-yl-pyrido(1,2-a)pyrimidin-4-one (MCA50). Prepared following the general procedure for TGX126, using TGX66 (50mg, 0.16mmol) and *o*-anisidine (0.017mL, 0.155mmol). The product was purified by chromatography on silica gel (2% MeOH/hexanes) followed by RP-HPLC to yield 3.8mg (6.7%) of a white solid. ^1H NMR (400MHz, $\text{DMSO}-d_6$) δ 8.37 (1H, s), 8.04 (1H, s), 7.52 (1H, d, $J=8\text{Hz}$), 7.17 (1H, s), 6.98–7.09 (4H, m), 3.83 (3H, s), 3.70 (4H, t, $J=4\text{Hz}$), 3.62 (4H, t, $J=4\text{Hz}$), 2.23 (3H, s); HR-EI MS (M)⁺ m/z calcd for $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_3$ 366.1692, found 366.1701.

4.5.10. 7-Methyl-2-morpholin-4-yl-9-o-tolylamino-pyrido(1,2-a)pyrimidin-4-one (MCA51). Prepared following the general procedure for TGX126, using TGX66 (50mg, 0.16mmol) and *o*-toluidine (0.017mL, 0.16mmol). The product was purified by chromatography on silica gel (2% MeOH/hexanes) followed by RP-HPLC to yield 6.8mg (12.6%) of an off-white solid. ^1H NMR (400MHz, $\text{DMSO}-d_6$) δ 7.99 (1H, s), 7.82 (1H, s), 7.23–7.34 (3H, m), 7.12 (1H, t, $J=7.2\text{Hz}$), 6.48 (1H, s), 5.59 (1H, s), 3.64 (8H, br), 2.18 (3H, s), 2.15 (3H, s); HR-EI MS (M)⁺ m/z calcd for $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_2$ 350.1743, found 350.1754.

4.5.11. 9-(3,4-Dimethyl-phenylamino)-7-methyl-2-morpholin-4-yl-pyrido(1,2-a)pyrimidin-4-one (MCA52). Prepared following the general procedure for TGX126, using TGX66 (50mg, 0.16mmol) and 3,4-dimethylaniline (18.8mg, 0.155mmol). The product was purified by chromatography on silica gel (2% MeOH/hexanes) followed by RP-HPLC to yield 13.4mg (23.9%) of a yellow solid. ^1H NMR (400MHz, DMSO- d_6) δ 8.00 (1H, s), 7.85 (1H, br), 7.05–7.13 (3H, m), 6.94 (1H, s), 5.58 (1H, s), 3.64 (8H, br), 2.18–2.21 (9H, m); HR-EI MS (M) $^+$ m/z calcd for $\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}_2$ 364.1899, found 364.1909.

4.5.12. 7-Methyl-9-(3-methyl-benzylamino)-2-morpholin-4-yl-pyrido(1,2-a)pyrimidin-4-one (MCA53). Prepared following the general procedure for TGX126, using TGX66 (50mg, 0.16mmol) and 3-methylbenzylamine (0.019mL, 0.16mmol). The product was purified by chromatography on silica gel (2% MeOH/hexanes) followed by RP-HPLC to yield 12.3mg (22%) of an off-white solid. ^1H NMR (400MHz, DMSO- d_6) δ 7.85 (1H, s), 7.09–7.19 (3H, m), 7.01 (1H, d, $J=8\text{Hz}$), 6.92 (1H, br), 6.32 (1H, s), 5.54 (1H, s), 4.43 (2H, s), 3.65 (4H, t, $J=5\text{Hz}$), 3.61 (4H, t, $J=5\text{Hz}$), 2.25 (3H, s), 2.11 (3H, s); HR-EI MS (M) $^+$ m/z calcd for $\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}_2$ 364.1899, found 364.1904.

4.5.13. 9-(2,3-Dimethyl-phenylamino)-7-methyl-2-morpholin-4-yl-pyrido(1,2-a)pyrimidin-4-one (MCA54). Prepared following the general procedure for TGX126, using TGX66 (50mg, 0.16mmol) and 2,3-dimethylaniline (19mg, 0.16mmol). The product was purified by chromatography on silica gel (2% MeOH/hexanes) followed by RP-HPLC to yield 12.3mg (22%). ^1H NMR (400MHz, DMSO- d_6) δ 7.96 (1H, s), 7.83 (1H, s), 7.05–7.14 (3H, m), 6.30 (1H, s), 5.58 (1H, s), 3.64 (8H, br), 2.27 (3H, s), 2.13 (3H, s), 2.06 (3H, s); HR-EI MS (M) $^+$ m/z calcd for $\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}_2$ 364.1899, found 364.1910.

4.5.14. 7-Methyl-9-(2-methyl-benzylamino)-2-morpholin-4-yl-pyrido(1,2-a)pyrimidin-4-one (MCA55). Prepared following the general procedure for TGX126, using TGX66 (50mg, 0.16mmol) and 2-methylbenzylamine (0.019mL, 0.16mmol). The product was purified by chromatography on silica gel (2% MeOH/hexanes) followed by RP-HPLC to yield 12.9mg (23%) of an off-white solid. ^1H NMR (400MHz, DMSO- d_6) δ 7.87 (1H, s), 7.09–7.17 (4H, m), 6.76 (1H, br), 6.31 (1H, s), 5.55 (1H, s), 4.44 (2H, s), 3.64 (4H, t, $J=5\text{Hz}$), 3.60 (4H, t, $J=5\text{Hz}$), 2.32 (3H, s), 2.11 (3H, s); HR-EI MS (M) $^+$ m/z calcd for $\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}_2$ 364.1899, found 364.1905.

4.5.15. 5-Morpholin-4-yl-2-nitro-phenylamine (AMA30). Morpholine (2.03mL, 23.2mmol) was added to a stirred solution of 5-chloro-2-nitroaniline (2.0g, 12mmol) in DMSO (10mL) and heated to 90°C for 18h.²³ When the reaction was complete, the product was precipitated by pouring into H_2O (150mL), and then purified by chromatography on silica gel (50% EtOAc/hexanes) to yield 660mg (25.4%) of a yellow solid. ^1H NMR (400MHz, DMSO- d_6) δ 7.79 (1H, d, $J=8\text{Hz}$), 7.25

(2H, s), 6.34 (1H, d, $J=10\text{Hz}$), 6.18 (1H, s), 3.67 (4H, t, $J=5\text{Hz}$), 3.23 (4H, t, $J=5\text{Hz}$); ^{13}C NMR (100MHz, DMSO- d_6) δ 155.9, 148.9, 127.8, 123.9, 105.8, 98.3, 66.4, 44.1; HR-EI MS (M) $^+$ m/z calcd for $\text{C}_{10}\text{H}_{13}\text{N}_3\text{O}_3$ 223.0957, found 223.0954.

4.5.16. 2-Hydroxy-4-morpholin-4-yl-benzaldehyde (IC60-211). DMF (10mL) was cooled on ice and POCl_3 (1.4mL, 15mmol) was added dropwise. 3-Morpholinophenol (2.5g, 14mmol) was slowly added to the stirred solution at rt, incubated at rt for an additional 40min, and then heated to 100°C for 9h essentially as described.²³ The reaction was terminated by dumping into 1M NaOAc (40mL). The solid was filtered, rinsed three times with H_2O , and purified by chromatography on silica gel (25% EtOAc/hexanes) to yield 802mg (28%) of an off-white solid. ^1H NMR (400MHz, DMSO- d_6) δ 11.07 (1H, s), 9.72 (1H, s), 7.46 (1H, d, $J=8\text{Hz}$), 6.55 (1H, dd, $J=9\text{Hz}$, 2.4Hz), 6.28 (1H, d, $J=2.4\text{Hz}$), 3.66 (4H, t, $J=5\text{Hz}$), 3.30 (4H, t, $J=5\text{Hz}$); ^{13}C NMR (100MHz, DMSO- d_6) δ 192.0, 163.7, 157.2, 133.9, 113.8, 106.8, 99.5, 66.4, 47.1; HR-EI MS (M) $^+$ m/z calcd for $\text{C}_{11}\text{H}_{13}\text{NO}_3$ 207.0895, found 207.0893.

4.5.17. 1-(2-Hydroxy-4-morpholin-4-yl-phenyl)-ethanone (IC86621). MeLi (2.4mL of 1.6M solution in Et_2O , 3.9mmol) was added dropwise to a solution of IC60211 (200mg, 0.996mmol) in THF (4mL) at -78°C .²³ The reaction was allowed to warm to rt and proceed overnight, at which point the reagent was quenched with satd NH_4Cl (1mL), extracted six times with Et_2O , and concentrated in vacuo. The product was chromatographed on silica gel (25% EtOAc/hexanes) to yield a white solid. The solid was dissolved in 10mL MeCN, MnO_2 (391mg, 4.5mmol) was added, and the reaction was allowed to proceed for three days at rt under an inert atmosphere. The product was chromatographed on silica gel (50% EtOAc/hexanes) to yield 17.8mg (18%) of a white solid. ^1H NMR (400MHz, CDCl_3) δ 12.71 (1H, s), 7.55 (1H, d, $J=9\text{Hz}$), 6.35 (1H, d, $J=9\text{Hz}$), 6.26 (1H, s), 3.79 (4H, t, $J=5\text{Hz}$), 3.30 (4H, t, $J=5\text{Hz}$), 2.50 (3H, s); ^{13}C NMR (100MHz, CDCl_3) δ 201.7, 165.0, 156.7, 132.5, 105.5, 100.6, 66.6, 47.2; HR-EI MS (M) $^+$ m/z calcd for $\text{C}_{12}\text{H}_{15}\text{NO}_3$ 221.1052, found 221.1049.

4.5.18. 1-(2-Hydroxy-4-morpholin-4-yl-phenyl)-phenyl-methanone (AMA37). Prepared according to the general procedure of IC86621 using PhMgBr (1.1mL, 3.4mmol) and IC60211 (150mg, 0.724mmol), except that the product of PhMgBr addition was taken on for oxidation by MnO_2 (249mg, 3.38mmol) without intervening chromatographic purification. Chromatography of the final product on silica gel (50% EtOAc/hexanes) yielded (28.9mg, 14.1%) of a yellow solid. ^1H NMR (400MHz, CDCl_3) δ 12.73 (1H, s), 7.41–7.61 (5H, m), 6.35 (1H, d, $J=2.4\text{Hz}$), 6.31 (1H, dd, $J=9\text{Hz}$, 2.8Hz), 3.81 (4H, t, $J=8\text{Hz}$), 3.31 (4H, t, $J=8\text{Hz}$); ^{13}C NMR (100MHz, CDCl_3) δ 199.1, 166.1, 156.6, 138.8, 135.5, 131.4, 129.0, 128.4, 119.2, 111.3, 105.3, 100.6, 66.7, 47.1; HR-EI MS (M) $^+$ m/z calcd for $\text{C}_{17}\text{H}_{17}\text{NO}_3$ 283.1208, found 283.1195.

4.5.19. 2-Chloro-1-(2-hydroxy-4-morpholin-4-yl-phenyl)-ethanone (AMA48). A solution of 3-morpholinophenol (5.0 g, 28 mmol) and chloroacetonitrile (2.1 mL, 34 mmol) in chloroethane (150 mL) in a 3-neck flask was cooled in an ice bath, and BCl_3 (100 mL, 1 M in CH_2Cl_2) was slowly added by an addition funnel essentially as described.²³ AlCl_3 (1.9 g, 14 mmol) was then added to this solution, the flask was equipped with a reflux condenser, and the reaction was heated to 60 °C for 24 h. The reaction was then cooled to 0 °C and 100 mL 2 N HCl was added by addition funnel. Following acidification, the organic phase was collected and rinsed twice with 2 N HCl (100 mL) and once with H_2O (100 mL). The organic phase was concentrated in vacuo and the product purified by chromatography on silica gel (30% EtOAc/hexanes) to yield 775 mg (10.9%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.91 (1H, s), 7.66 (1H, d, $J=9$ Hz), 6.53 (1H, dd, $J=9$ Hz, 2.4 Hz), 4.93 (2H, s), 3.66 (4H, t, $J=5$ Hz), 3.31 (4H, t, $J=5$ Hz); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 193.0, 163.6, 156.5, 132.1, 109.1, 105.8, 99.1, 65.7, 46.4, 46.3; HR-EI MS (M^+) m/z calcd for $\text{C}_{12}\text{H}_{14}\text{ClNO}_3$ 255.0662, found 255.0670.

4.5.20. LY292223. Prepared according to the general procedure described for the synthesis of 2-aminochromones.³³ 2'-Hydroxyacetophenone (0.50 mL, 4.2 mmol) was dissolved in 11 mL CH_2Cl_2 , cooled to –78 °C and TiCl_4 (6.5 mL, 1 M solution in THF, 6.5 mmol) was added. After 1 h, DIPEA (2.7 mL, 16 mmol) was added at –78 °C and the reaction was allowed to proceed for an additional 1 h. 4-Dichloromethylenemorpholin-4-ium³⁴ (1.2 g, 5.0 mmol) was then added and the reaction was allowed to proceed at –78 °C for 20 min. The reaction was warmed to 0 °C, MeOH (30 mL) was added, and the reaction was allowed to warm to rt overnight. The reaction was then concentrated in vacuo, washed once with satd NaHCO_3 , and the NaHCO_3 was extracted three times with CH_2Cl_2 . The combined organic phase was dried over MgSO_4 , filtered, and solvent removed in vacuo. The product was purified by chromatography on silica gel (10% MeOH/EtOAc) to yield 344 mg of a white powder (36%). ^1H NMR (400 MHz, CDCl_3) δ 7.95 (1H, s), 7.35 (1H, s), 7.14 (1H, s), 7.08 (1H, s), 5.27 (1H, s), 3.63 (4H, t, $J=5$ Hz), 3.30 (4H, t, $J=5$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 176.8, 162.5, 153.5, 132.2, 125.2, 124.6, 122.8, 116.3, 87.0, 65.8, 44.5; IR: 1616, 1555, 1418, 1300, 1251, 1117, 985, 766; HR-EI MS (M^+) m/z calcd for $\text{C}_{13}\text{H}_{13}\text{NO}_3$ 231.0895, found 231.0887.

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bmc.2004.06.022](https://doi.org/10.1016/j.bmc.2004.06.022).

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