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Bioorganic & Medicinal Chemistry 15 (2007) 1586–1605
Bioorganic & Medicinal Chemistry

4-Amino-5-aryl-6-arylethynylpyrimidines: Structure–activity relationships of non-nucleoside adenosine kinase inhibitors

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Received 3 November 2006; accepted 15 December 2006
Available online 20 December 2006

Abstract—A series of non-nucleoside adenosine kinase (AK) inhibitors is reported. These inhibitors originated from the modification of 5-(3-bromophenyl)-7-(6-morpholin-4-ylpyridin-3-yl)pyrido[2,3-*d*]pyrimidin-4-ylamine (**ABT-702**). The identification of a linker that would approximate the spatial arrangement found between the pyrimidine ring and the aryl group at C(7) in **ABT-702** was a key element in this modification. A search of potential linkers led to the discovery of an acetylene moiety as a suitable scaffold. It was hypothesized that the aryl acetylenes, **ABT-702**, and adenosine bound to the active site of AK (closed form) in a similar manner with respect to the orientation of the heterocyclic base. Although potent acetylene analogs were discovered based on this assumption, an X-ray crystal structure of 5-(4-dimethylaminophenyl)-6-(6-morpholin-4-ylpyridin-3-ylethynyl)pyrimidin-4-ylamine (**16a**) revealed a binding orientation contrary to adenosine. In addition, this compound bound tightly to a unique open conformation of AK. The structure–activity relationships and unique ligand orientation and protein conformation are discussed.

1. Introduction

During cellular stress,[1] local intracellular concentrations of adenosine (ADO) increase followed by the active transport of ADO into the extracellular space and subsequent activation of ADO receptor (P1) subtypes.[2,3] Upon activation of these receptor subtypes, a variety of homeostatic inhibitory cellular events contribute to anti-nociceptive and anti-inflammatory responses in vivo.[4] Attempts to identify selective ADO receptor agonists[5,6] for CNS therapeutic indications have suffered from cardiovascular side effects such as hypotension and bradycardia.[2]

Strategies to increase local concentrations of ADO have included inhibition of enzymes responsible for the metabolic transformation of ADO. Specifically, inhibitors of adenosine deaminase (ADA)[7] and adenosine kinase (AK)[7] have received considerable attention in an attempt to increase concentrations of endogenous ADO. AK is a ubiquitous intracellular enzyme responsible for the phosphorylation of ADO to adenosine monophosphate. Inhibition of AK increases local concentrations of ADO and displays neuroprotective potential[8–10] in such areas as pain[11–13] and inflammation.[14,15]

Previously, we reported[16–24] the identification and structure–activity relationships of non-nucleoside[25] pyridopyrimidine inhibitors of AK. Although the series was successful, some compounds occasionally suffered toxicological[23] and CNS side effects,[17] and also lacked appropriate aqueous solubility[19] and oral bioavailability.[21] This indicated that significant structural changes

Keywords: Adenosine kinase; Inhibitor; Acetylene; Analgesic.
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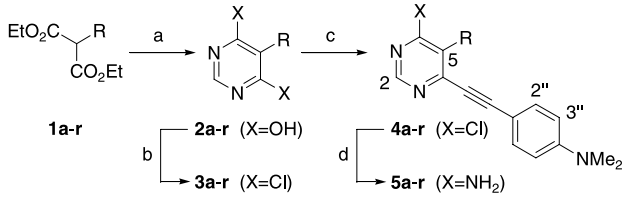
doi:10.1016/j.bmc.2006.12.029

to the core were necessary in order to expand on current achievements. The design of a new class of AK inhibitors based on the pyridopyrimidines required that key structural elements (for in vitro potency) be identified and retained. In the pyridopyrimidines, the 4-amino pyrimidine fragment and the aryl ring occupying the C(7) position were found to be critical pharmacophoric elements. Upon removal of the fused pyridine ring and replacement with an acetylene linker, we discovered that these pyrimidine analogs were equipotent with the pyridopyrimidines in their ability to inhibit AK in vitro.[26] However, an X-ray crystal structure of **16a** bound to the AK active site revealed a binding motif contrary to adenosine bound to the AK active site.[27] In addition, it was also discovered that the protein adopted a separate, active conformation to accommodate the unique ligand orientation. Although our overlap hypothesis was challenged, we were able to discover novel and potent inhibitors of AK based on the acetylene substitution. The present paper delineates the general structure–activity relationships of these novel AK inhibitors as well as a discussion relating to their unique binding orientation and accommodating AK protein conformation.

2. Results

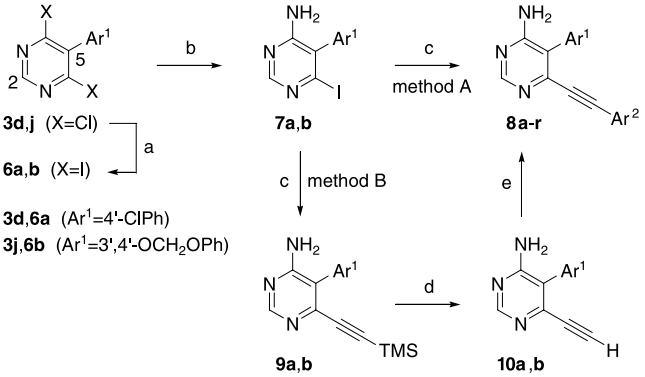
2.1. Chemistry

The initial strategy to assemble the pyrimidine ring relied on a formamidine acetate ring cyclization[28] (Scheme 1). Treatment of malonate ester **1** with formamidine acetate in the presence of an alkoxide base gave dihydroxypyrimidine, **2**. This intermediate was converted to the dichloropyrimidine, **3**, with $POCl_3$ followed by Sonogashira coupling[29–34] to provide the C(4) chloro acetylene, **4**. This particular reaction was found to be low yielding due to both C(4)/C(6) bis acetylene formation[35] and Glaser-type homocoupling[36] of the starting acetylene. Copper-free variations[37–43] to eliminate homocoupling were not attempted. Final aminolysis provided the desired amino pyrimidine, **5**, in moderate yields. Our initial investigation of this synthetic route serendipitously utilized an electron-releasing dimethylamino residue on the aryl acetylene. Electron withdrawing groups on the aryl acetylene were problematic. First, in the Sonogashira coupling, it was discovered these electron-poor acetylenes gave largely Glaser-type reaction products with little or no traces of desired chloro acetylene, **4**. Second, the attempted aminolysis of **4** resulted in significant amounts of hydroamination product. After aqueous workup, the corresponding ketone was isolated. Scheme 2 shows two alternative synthetic routes to overcome these issues. Initially, both routes convert the dichloropyrimidine, **3**, into the diiodo analog, **6**, followed by aminolysis to provide the amino pyrimidine **7**. In method A, the amino pyrimidine is reacted directly with the desired acetylene to provide **8**. In method B, the iodide is reacted with trimethylsilyl acetylene followed by removal of the silyl group to give terminal acetylene **10**. This intermediate was more versatile (compared to



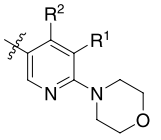
	R		R		R
a	Ph	**g**	4'-MeOPh	**m**	CH_2Ph
b	2'-ClPh	**h**	3',4'-di-MeOPh	**n**	$CHPh_2$
c	3'-ClPh	**i**	4'-Me_2NPh	**o**	Ph{C(2)-Me}
d	4'-ClPh	**j**	3',4'-OCH_2OPh	**p**	4'-ClPh{C(6)-4''-H}
e	4'-BrPh	**k**	4'-*i*-PrPh	**q**	4'-ClPh{C(6)-4''-Me}
f	4'-MePh	**l**	cyclohexyl	**r**	4'-ClPh{C(6)-4''-Pr-*i*}

Scheme 1. Reagents and conditions: (a) formamidine acetate, NaOEt, EtOH, 60 °C; (b) $POCl_3$, reflux; (c) acetylene, $PdCl_2(PPh_3)_2$, CH_3CN, Et_3N, 60 °C; (d) NH_4OH, EtOH, 100 °C, sealed tube.



	Ar^1=4'-ClPh Ar^2=		Ar^1=4'-ClPh Ar^2=
8a	5'-pyrimidinyl		
8b	2'-pyridyl	**8j**	NH_2
8c	2'-thienyl	**8k**	NMe_2
8d	4'-NO_2Ph	**8l**	$N(CH_2CH_2)_2O$
8e	4'-NH_2Ph	**8m**	$N(Me)CH_2CH_2OMe$
8f	4'-morpholinePh	**8n**	$N(CH_2CH_2)_2NSO_2NMe_2$
8g	2'-morpholin-4'-ylpyrimidin-5'-yl	**8o**	$N(CH_2CH_2)_2NBoc$
8h	6'-morpholin-4'-ylpyridazin-3'-yl	**8p**	$N(CH_2CH_2)_2NC(O)Me$
8i	5'-morpholin-4'-ylpyridin-3'-yl	**8q**	$N(CH_2CH_2)_2CH_2$

Ar^1=3',4'-OCH_2OPh
Ar^2=

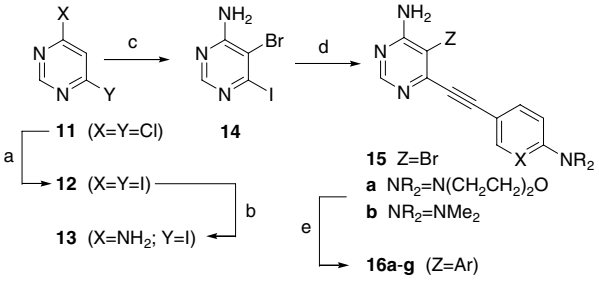


	R^1	R^2
8r	H	H
8s	NMe_2	H
8t	H	NMe_2
8u	NHC(O)NHPr-*i*	H
8v	H	$N(Me)CH_2CH_2OMe$

Scheme 2. Reagents and conditions: (a) HI, NaI, acetone, rt; (b) NH_4OH, EtOH, 100 °C, sealed tube; (c) acetylene, $PdCl_2(PPh_3)_2$, CH_3CN, Et_3N, 60 °C; (d) 1 M K_2CO_3, MeOH; (e) ArI, $PdCl_2(PPh_3)_2$, CH_3CN, Et_3N, 60 °C.

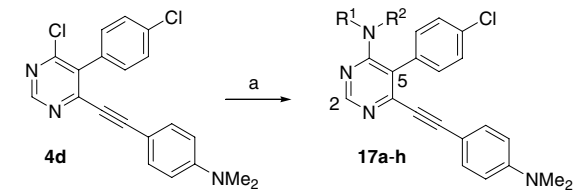
method A), in that, aryl iodides and bromides could be reacted directly to provide the desired compound **8**, thereby alleviating the need to synthesize each acetylene coupling partner. The shortcoming of the strategy outlined in Scheme 2 was purification of final product. Multiple chromatographic purifications were necessary to remove trace palladium impurities.[44]

To expand the scope of C(5)-substituted analogs, a more versatile and expedient route was required (Scheme 3). Dichloropyrimidine, **11**, was converted to the diiodide, **12**, with hydroiodic acid and sodium iodide. After aminolysis and treatment with bromine, iodopyrimidine **14** was isolated as a white solid. Palladium coupling with 4-ethynyl-*N*,*N*-dimethylaniline provided **15b** in good yield. Bromides **15a** or **15b** were reacted with various aryl boronic acids to afford **16a–g**. As before, the final products required multiple chromatographic purifications to remove palladium impurities. Of the synthetic routes utilized for the construction of these analogs, Scheme 1 was preferred for ease of purification but method B in Scheme 2 was preferred for versatility.



	R=N(CH$_2$CH$_2$)$_2$O; X=N Ar=		R=NMe$_2$; X=CH Ar=
16a	4'-Me$_2$NPh	**16f**	3-NO$_2$
16b	2'-thienyl	**16g**	3-NH$_2$
16c	3'-thienyl		
16d	2'-benzothienyl		
16e	2'-benzofuranyl		

Scheme 3. Reagents and conditions: (a) HI, NaI, acetone, rt; (b) NH_4OH, EtOH, 100 °C, sealed tube; (c) Br_2, AcOH, rt; (d) alkyne, $Pd(PPh_3)_4$, CH_3CN, Et_3N, 60 °C; (e) $ArB(OH)_2$, $Pd(PPh_3)_4$, 2 M Na_2CO_3, DME, reflux.



	R^1	R^2		R^1	R^2
17a	H	Me	**17f**	CH$_2$CH$_2$OCH$_2$CH$_2$	
17b	Me	Me	**17g**	CH$_2$CH$_2$NHCH$_2$CH$_2$	
17c	H	cyclohexyl	**17h**	CH$_2$CH$_2$N(SO$_2$NMe$_2$)CH$_2$CH$_2$	
17d	H	histamine			
17e	H	tryptamine			

Scheme 4. Reagents and conditions: (a) amine, EtOH, 100 °C, sealed tube.

Finally, C(4)-substituted amino analogs (Scheme 4) were accessed by aminolysis of **4d** with an assortment of amines in ethanol at elevated temperatures.

2.2. Biology

Compounds were evaluated (in vitro) for their ability to inhibit cytosolic AK (AK_{cyt}) as well as ADO phosphorylation in intact cells (AK_{cell}).[45] Analgesic activity (in vivo) was measured utilizing one of the following methods. Acute thermal nociception (hotplate test) was performed in mice (n = 6–8 per group) using the 55 °C hotplate test as previously described.[45,46] Nociceptive paw flinching (formalin test) in rats (n = 6 per group) was observed 30 min following an intraplantar injection of 5% formalin (50 μL) into the right hindpaw. Compounds were administered intraperitoneally 30 min before nociceptive testing as described earlier.[47] Analgesia in a neuropathic pain animal model (Chung assay) was carried out with male Sprague–Dawley rats after L5/L6 spinal nerve ligation as described by Chung et al.[48,49]

3. Discussion

3.1. General structure–activity relationships

From initial SAR studies to identify a linker replacement for the pyridopyrimidines (Fig. 1), a two-atom linker was believed important to represent the approximate spatial arrangement found in the pyridine ring and the C(5)-substituted aryl ring of **ABT-702**. Several functional groups were evaluated including amides, sulfonamides, alkenes, and alkyl chains but all were found to be inactive. The inactivity of these analogs was attributed to the linker and its inability to provide proper overlap between the aromatic ring at C(6) and the aromatic ring at C(7) in **ABT-702**. Based on simple models, it was concluded that the linearity of an acetylene functionality could provide the required distance to overlap the C(7) aryl group of **ABT-702**. Synthesis of acetylene **5d** was completed (Table 1) and found to be a potent AK inhibitor (AK_{cyt} IC_{50} = 3 nM). Further expansion of the SAR with the acetylene linker was necessary to define the scope of this new series of AK inhibitors. Addition of a methyl group at C(2) (**5o**) or removal of a nitrogen from the pyrimidine ring (data not shown) resulted in loss of AK inhibitory activity. If the aryl ring at C(5) was replaced by alkyl (cyclohexyl, **5l**), the result-

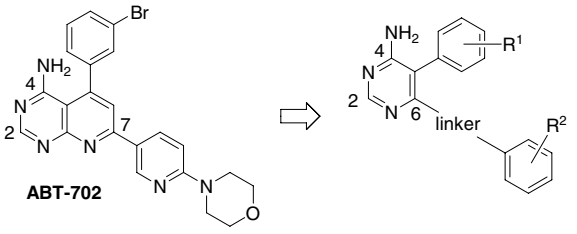
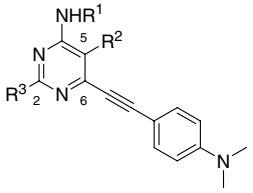
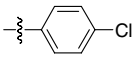
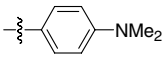
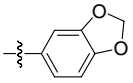
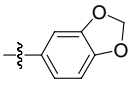
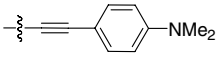


Figure 1. Linker strategy for identification of novel non-nucleoside AK inhibitors.

Table 1. General structure–activity relationship of **5d**



Compound	R^1	R^2	R^3	AK inhibition (IC_{50})[a]
5a	H	Ph	H	20
5d	H		H	3
5i	H		H	5
5j	H		H	9
5l	H	Cyclohexyl	H	300
5m	H	Bn	H	1000
5n	H	$CHPh_2$	H	600
5o	H	Ph	Me	>1000
10b[b]	H		H	>1000
18	H	H	H	>1000
19	Bn	H	H	>1000
20[c]	H		H	100

[a] Assay with cytosolic AK. Mean values for inhibitors (IC_{50} in nM) calculated from at least three determinations (standard error of the mean (SEM) $\leqslant$ 10%).
[b] Terminal acetylene at C(6).
[c] *p*-Dimethylaminophenyl at C(6).

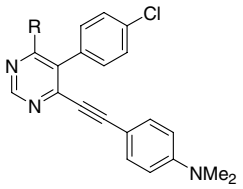
ing analogs were approximately 100 times less potent. Placement of a nitrogen between the pyrimidine ring and the C(5) aryl group provided potent AK inhibitors (data not shown).[26] When the aryl ring was removed from C(5) or the acetylene at C(6) (**18** and **10b**, respectively), AK inhibitory potency was lost. An attempt to fill the space at C(5) of **18** by substitution with an aminobenzyl (**19**) at C(4) was unsuccessful. If the phenyl ring at C(5) in **5d** was replaced with benzyl (**5m**), a decrease in activity (AK_{cyt} IC_{50} = 1000 nM) was observed. If an aromatic ring was added (benzhydryl **5n**, AK_{cyt} IC_{50} = 600 nM), some activity returned. This seemed to indicate that larger groups are tolerated at this position. In our previous report,[26] it was discovered that larger alkyl and aromatic groups at C(5) are potent inhibitors (AK_{cyt} IC_{50} < 50 nM). Based on these previous results, an exchange of the C(6) aryl acetylene with the C(5) aryl group was initiated. Acetylene **20** was completed (modification of **5i**) and found to have an AK_{cyt} IC_{50} of 100 nM. This result was intriguing. Clearly, there is no overlap between the aryl group at C(6) in **20** and the C(7) aryl group in **ABT-702**, yet AK inhibitory potency was retained. We began to question the binding orientation (relative to the adenosine heterocyclic base) of the acetylenes in the active site of AK. Furthermore, these findings prompted the question as to

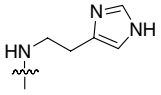
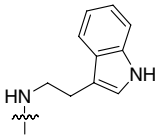
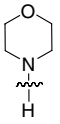
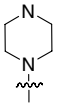
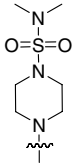
whether all the acetylene analogs of this series were bound in the same orientation or was there a possibility of multiple binding motifs within this series (e.g., **5d** vs **20**). In addition to ligand orientation within the active site, we had to consider the possibility of different protein conformations. The ability of structurally unique inhibitors to bind tightly to different protein conformations[50] of a single enzyme is not without precedence. The concept of either a lock and key[51] or induced fit[52] mechanism has evolved to the idea of receptor conformational ensembles.[53] In essence, this concept states that several distinctive enzyme conformations (different than the native substrate–protein interactions) exist of which any one could bind tightly to structurally different substrates.[54–56] Recent examples include both aspartic peptidase inhibitors[57] as well as HIV protease inhibitors.[58] The possibility of this particular series of acetylene inhibitors binding to different AK conformational ensembles had to be considered. Therefore, if the potency of **5d** validated an SAR expansion of the acetylene series, then the potency of **20** stressed the importance of obtaining an X-ray structure of an acetylene inhibitor in the active site of AK to probe such a question. Although our initial assumptions relating to ligand overlap with adenosine were unclear, the acetylene series was a potent class of non-nucleoside AK inhibitors. With the general scope of the acetylene series defined, research focus was turned to the C(4) amino group.

3.2. C(4) Structure–activity relationships

Table 2 shows the effect of amino substitution at C(4). The observed activity, by successive methyl substitution, decreased from amino (AK_{cyt} IC_{50} = 3 nM), to mono methyl amino, **17a** (AK_{cyt} IC_{50} = 14 nM) to dimethyl amino, **17b** (AK_{cyt} IC_{50} = 300 nM). Larger amino alkyl residues, such as cyclohexyl (**17c**), were inactive. If, however, an alkyl linker was placed between amine at C(4) and the 'large' group (**17d**, **17e**) or the dialkyl amine was tied up as a morpholine ring (**17f**, AK_{cyt} IC_{50} = 6 nM) or piperizine ring (**17g**, AK_{cyt} IC_{50} = 8 nM) activity returned. Even substituted piperazine **17h** retained activity (AK_{cyt} IC_{50} = 20 nM). In general, C(4) substitution retained AK_{cyt} inhibition (compared to **5d**) and, in some cases (**17a**, **17h**), improved the AK_{cell} inhibition.[59] However, the AK inhibitory activity in intact cells (AK_{cell}) of these analogs was too weak to be considered for any further in vivo studies in animal models of pain.[24] Therefore, subsequent investigation of C(4)-substituted analogs was suspended and the expansion of C(5) and C(6) substitution was pursued to improve biochemical potency.

Table 2. In vitro characterization at C(4) of AK inhibitors **17a–h** in cytosolic and intact cell assays



Compound	R	AK inhibition (IC_{50})[a]	
		Cytosolic	Intact cells
5d	NH_2	3	700
17a	NHMe	14	350
17b	NMe_2	300	>1000
17c	NHcyclohexyl	>1000	>1000
17d		80	>1000
17e		38	>1000
17f		6	970
17g		8	>1000
17h		13	330
ABT-702		1.7[b]	50.7[b]

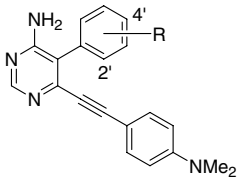
[a] Assay with cytosolic AK. Mean values for inhibitors (IC_{50} in nM) calculated from at least three determinations (standard error of the mean (SEM) ⩽ 10%).
[b] Jarvis et al.[45]

3.3. C(5) structure–activity relationships

Table 3 shows the effect of aromatic substitution at C(5). Evaluation of mono chloro substituted phenyl analogs revealed that potency decreased from the para substituted analog (**5d**; AK_{cyt} IC_{50} = 3 nM) to the meta position (**5c**; AK_{cyt} IC_{50} = 10 nM) to the ortho position (**5b**; AK_{cyt} IC_{50} = 75 nM). It is interesting to note that the 3,4-dimethoxy analog, **5h** (IC_{50} = 20 nM), had comparable potency to **5g**. This suggests that the meta position could be used to modulate physiochemical properties without detrimental effects on AK inhibition.

The electronic nature of the substituents on the aromatic ring appears to be important and favors electron-withdrawing groups. Compounds **5e**, **5d**, and**16f** were more potent than **5f**, **5g**, and **16g**. The notable exceptions were **5i** (AK_{cyt} IC_{50} = 5 nM; AK_{cell} IC_{50} = 240 nM) and **5j** (AK_{cyt} IC_{50} = 9 nM; AK_{cell} IC_{50} = 250 nM). In addition, these analogs showed significant inhibition in

Table 3. In vitro characterization of selected C(5)-substituted AK inhibitors **5a–k** and **16f,g** in cytosolic and intact cell assays



Compound	R	AK inhibition (IC_{50})[a]	
		Cytosolic	Intact cells
5a	H	20	>1000
5b	2′-Cl	75	700
5c	3′-Cl	10	550
5d	4′-Cl	3	700
5e	4′-BrPh	3	600
5f	4′-Me	13	330
5g	4′-OMe	18	730
5h	3′,4′-DiOMe	20	500
5i	4′-NMe_2	5	240
5j	3′,4′-OCH_2O	9	250
5k	4′-*i*-Pr	22	>1000
16f	3′-NO_2	16	>1000
16g	3′-NH_2	90	1000

[a] Assay with cytosolic AK. Mean values for inhibitors (IC_{50} in nM) calculated from at least three determinations (standard error of the mean (SEM) ⩽ 10%).

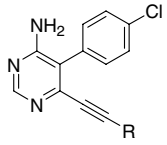
intact cell AK; believed to more closely resemble the physiological environment. Factors that influence the IC_{50} AK_{cell} were studied with both more lipophilic analogs, such as 4′-isopropyl, **5k**, as well as more polar compounds like 3′-amino phenyl, **16g**. These polarity extremes showed comparable cytosolic potency to **5i** but were inactive in the intact cell assay. These findings suggest a 'U-shaped' correlation between lipophilicity and cell permeability. Calculations related to log *P* (clog *P*)[60] for **5i** (clog *P* = 4.68), **5k** (clog *P* = 5.79), and **16g** (clog *P* = 3.38) suggested that values between 4 and 5 provided optimal AK inhibition in intact cells. Although **5i** and **5j** were potent inhibitors of AK_{cell}, both were inactive in a mouse hotplate model of analgesia.[46] Attempts to further optimize AK_{cell} inhibition at C(5) with dimethylaminophenyl acetylene were unsuccessful. Consequently, focus was turned to optimization of the aryl acetylene at C(6).

3.4. C(6) structure–activity relationships

Table 4 shows the optimization of substituted phenyl acetylene at C(6). In general, AK_{cyt} inhibition seems to show a substituent effect opposite that in the C(5) SAR. Electron-donating substituted phenyl analogs seemed to be more potent than those containing electron-withdrawing groups. For example, **8e** was more potent (AK_{cyt}) than **8d**. Additionally, unsubstituted heterocyclic replacements (pyrimidine **8a**, pyridine **8b**) seem to show less activity than phenyl, **5p**. The thiophene bioisostere, **8c**, displayed similar potency to **5p**. None showed activity in inhibiting intact cell AK.

Examination of AK_{cell} inhibition showed a polarity-dependent trend similar to the C(5) SAR. Analogs with

Table 4. In vitro characterization of selected C(6) aryl and heteroaryl acetylene AK inhibitors **5p–r** and **8a–f** in cytosolic and intact cell assays



Compound	R	AK inhibition (IC_{50})[a]	
		Cytosolic	Intact cells
5p	Ph	60	>1000
5q	4′-MePh	25	830
5r	4′-*i*-PrPh	50	>1000
8a	5′-pyrmidinyl	216	>1000
8b	2′-pyridyl	500	>1000
8c	2′-thienyl	43	>1000
8d	4′-NO_2Ph	43	>1000
8e	4′-NH_2Ph	10	230
8f	4′-morpholinePh	17	275

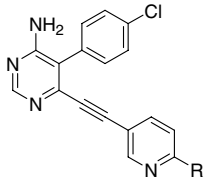
[a] Assay with cytosolic AK. Mean values for inhibitors (IC_{50} in nM) calculated from at least three determinations (standard error of the mean (SEM) ⩽ 10%).

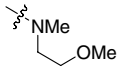
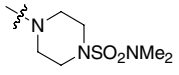
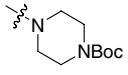
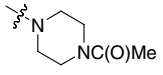
calculated log *P* values between 3 and 4 appeared to be the most potent. For example, **5d** (AK_{cell} IC_{50} = 700 nM, clog *P* = 5.09) and **5r** (AK_{cell} IC_{50} > 1000 nM, clog *P* = 6.35) were less potent than **8e** (AK_{cell} IC_{50} = 230 nM, clog *P* = 3.70) and **8f** (AK_{cell} IC_{50} = 275, clog *P* = 4.38). The most potent inhibitors from this group were screened in a mouse hotplate model of analgesia. None displayed significant activity, due most likely to relatively poor potency in inhibiting AK in intact cells. Previous observations from the pyridopyrimidines (like **ABT-702**) suggested that an acetylene at C(6) with a substituted heterocycle such as pyridine would increase cell penetration (and AK inhibition) and, perhaps, lead to activity in an animal model of pain.

Table 5 shows the C(6) 2-aminopyridyl replacements. Generally, this replacement (vs substituted aryl) had a positive effect on the AK_{cell} inhibition. The potent analogs have a clog *P* of 3.5–4.5. The most potent compound from this initial screen was the morpholine analog, **8l** (AK_{cell} IC_{50} = 58 nM; clog *P* = 3.43). More polar analogs (like **8j** and others not shown) with clog *P* < 3.5 and more lipophilic analogs **8o** and **8q** (clog *P* > 4.5) were less potent (AK_{cell}) than those analogs with a clog *P* between 3.5 and 4.5. There are notable exceptions, however. Pyrimidine **8g** (AK_{cell} IC_{50} = 200 nM, clog *P* = 2.67) and acetyl piperazine **8p** (AK_{cell} IC_{50} = 39 nM, clog *P* = 3.01) both showed better AK_{cell} inhibition than predicted, while the pyridazine, **8h** (AK_{cell} IC_{50} = 700 nM, clog *P* = 2.40), and morpholine regioisomer, **8i** (AK_{cell} IC_{50} = 110 nM, clog *P* = 3.43), behaved as predicted. The physiochemical properties and their relationship to cell penetration are not well understood. These examples illustrate the care that must be taken in attempted correlations between predicted lipophilicity (clog *P*) and expected potency in AK_{cell}.

The most potent analogs **8g**, **8i**, **8k**, **8l**, and **8m** were evaluated in animal models of pain. Mouse hotplate

Table 5. In vitro characterization of selected C(6) pyridyl acetylene AK inhibitors **8g–q** in cytosolic and intact cell assays



Compound	R	AK inhibition (IC_{50})[a]	
		Cytosolic	Intact cells
8g[b]		8	200
8h[c]		45	700
8i[d]		8	110
8j	NH_2	14	333
8k	NMe_2	6	83
8l		3	58
8m		1	80
8n		6	131
8o		62	>1000
8p		1	39
8q		9	733

[a] Assay with cytosolic AK. Mean values for inhibitors (IC_{50} in nM) calculated from at least three determinations (standard error of the mean (SEM) $\leqslant$ 10%).
[b] C(6) pyridine replacement: C(6)-[2′-morpholin-4′-ylpyrimidin-5′-yl].
[c] C(6) pyridine replacement: C(6)-[6′-morpholin-4′-ylpyridazin-3′-yl].
[d] C(6) pyridine replacement: C(6)-[5′-morpholin-4′-ylpyridin-3′-yl].

experiments using **8g**, **8k**, and **8l** at doses of 30 μmol/kg showed significant efficacy (% MPE[61] > 70). All of these compounds were assessed further in the formalin assay (Table 6). Although these analogs displayed lower dose activity in a formalin assay, none showed efficacy in a neuropathic animal model of pain (Chung assay).[49] Pyridopyrimidines, however, substituted with 2-aminopyridyl at C(7) were efficacious in several animal models of pain.[8,45,48,62] The conclusion from the heteroaryl

Table 6. In vivo characterization of selected AK inhibitors

Compound	Hotplate analgesia,[a] % MPE[b]	Formalin,[c] % RFN[d]
8f	67	20
8g	100	36
8i	nd[e]	49
8k	73	20
8l	100	29
8m	nd	48
8r	nd	47
16a	100	44
16b	nd	44
16d	nd	49
16e	nd	36
ABT-702	[f]	[g]

[a] Values represent compounds administered by ip injection at 30 μmol/kg dose.
[b] % MPE = % maximal protective effect ([postdrug latency] − [vehicle latency])/([maximum latency] − [vehicle latency]) × 100%, where maximum (cutoff) latency was 180 s. Values represent means within ±8% and $p < 0.05$ to vehicle treated mice.[46]
[c] Values represent compounds administered by ip injection at 10 μmol/kg dose.
[d] % RFN = % reduction in formalin-induced nociception ([vehicle flinches] − [postdrug flinches])/[vehicle flinches] × 100%. Values represent means within ±5% and $p < 0.05$ to vehicle treated rats.
[e] nd, not determined.
[f] ED_{50} = 8 μmol/kg from Jarvis et al.[45]
[g] ED_{50} = 15 μmol/kg from Kowaluk et al.[47]

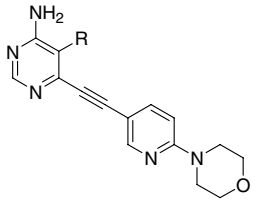
acetylene SAR was that the 2-morpholino pyridine acetylene at C(6) was adequate for modest in vivo activity but reoptimization of C(5) was required to further increase the AK_{cell} potency.

3.5. Analog reoptimization and in vivo evaluation

Although the in vivo efficacy of **8l** was encouraging, our expectation was to discover a more efficacious acetylene analog that displayed activity in several different animal models of pain including neuropathic pain. Despite the anomaly between clog*P* and AK_{cell} with **8g** and **8p**, we decided to target analogs with a clog*P* between 3 and 4.5. Substitution (Table 7) in the para position produced compounds that were less potent (AK_{cell}) than **8l** (data not shown). The one exception was **16a** (AK_{cell} IC_{50} = 68 nM) that was nearly equipotent to **8l**. Heterocyclic replacements at C(5) included thienyl analogs (**16b**, **16c**) and bicyclic heterocycles (**16d**, **16e**). These replacements showed good AK inhibitory potency in intact cells, however, these did not surpass the parent compound **8l**. Compounds **16a–e** all showed good efficacy in the formalin assay but, like in earlier analogs, were inactive in the Chung model of neuropathic pain.

It was previously reported[63] that the active site of AK possesses a hydrophilic binding pocket that accommodates a phosphate group needed for the conversion of ADO to AMP. To investigate this secondary binding site, a series of C(6) trisubstituted pyridyl acetylene analogs were synthesized and tested. Table 8 shows polar examples at both C(5)′ and C(6)′ of pyridyl acetylene analogs.[64] As indicated in Table 8, these trisubstituted

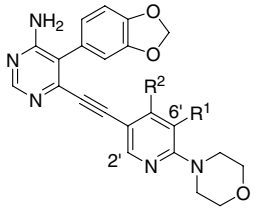
Table 7. Reoptimization of C(5) aryl acetylene AK inhibitors **8l**, **8r** and **16a–e** in cytosolic and intact cell assays

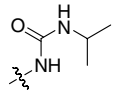
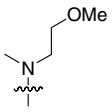


Compound	R	AK inhibition (IC_{50})[a]	
		Cytosolic	Intact cells
8l	4′-ClPh	3	58
8r	3′,4′-OCH_2OPh	10	120
16a	4′-Me_2NPh	2	68
16b	2′-Thienyl	13	167
16c	3′-Thienyl	38	212
16d	2′-Benzothienyl	0.4	100
16e	2′-Benzofuranyl	1	123

[a] Assay with cytosolic AK. Mean values for inhibitors (IC_{50} in nM) calculated from at least three determinations (standard error of the mean (SEM) ⩽ 10%).

Table 8. In vitro characterization of selected C(6) trisubstituted pyridylacetylene AK inhibitors **8s–v** in cytosolic and intact cell assays



Compound	R^1	R^2	AK inhibition (IC_{50})[a]	
			Cytosolic	Intact cells
8r	H	H	10	120
8s	NMe_2	H	26	161
8t	H	NMe_2	72	215
8u		H	18	88
8v	H		101	198

[a] Assay with cytosolic AK. Mean values for inhibitors (IC_{50} in nM) calculated from at least three determinations (standard error of the mean (SEM) ⩽ 10%).

analogs did not offer any potency advantage in vitro. Furthermore, in vivo evaluation of **8s** in an animal model (formalin) showed no efficacy up to 30 μmol/kg (ip). Because of the continued lack of in vivo efficacy in these analogs, the physiochemical properties of selected acetylene compounds were evaluated.

3.6. ADME considerations

The pharmacokinetic (PK) analysis of **16a** (Table 9) indicated that both the half-life and volume of distribution were slightly less than **ABT-702**; plasma clearance was slightly greater for **16a**. In vitro metabolism studies in both rat hepatocyte and human microsomes of **16a** indicated a significant decrease in parent after 24 h in rat and 1 h in human microsomes (compared to **ABT-702**). Although identification of metabolic products was not performed on **16a**, several possible sites include the morpholine ring,[65] the dimethyl amino group at C(5), and the acetylene itself. The increases in both clearance and in rat metabolism of **16a** (compared to **ABT-702**) may be a part of several factors contributing to lack of efficacy in animal models. Later reoptimization of C(5) to address some of these issues led to acetylene analogs that displayed good efficacy in both formalin and neuropathic pain animal models.[26] Key to the in vivo efficacy of these latter compounds was the placement of a nitrogen atom at C(5) and extending the alkyl chain several carbon atoms.

3.7. X-ray crystal structure analysis

As the biological evaluation of **8s–v** was concluding, an X-ray crystal structure[27] of **16a** bound to the AK active site was determined. Surprisingly, **16a** was not observed to bind in the same manner as adenosine (Fig. 2). A previously determined crystal structure reported[66] that two key hydrogen bonds and a π–π interaction with F170 were important for adenosine binding to the AK protein. First, the N(6) amino group forms a hydrogen bond with T173 through a water bridge. Second, the side chain of N14 donates a hydrogen bond to N(1) of adenosine. In addition, a 'flap' region closes down over the native substrate and the catalytic process occurs. Modeling this same binding motif to the acetylene series (Fig. 1) predicts a hydrogen bond between the C(4) amino group (presumably through a water bridge) and T173 as well as a hydrogen bond between N14 and N(3) of the pyrimidine ring. However, the crystal structure of **16a** shows hydrogen bonds between the C(4) amino group and D18 as well as between the N(3) pyrimidine and the S65 amide nitrogen. This orients the C(6) aryl acetylene group through a groove in the enzyme, formed by a hinge opening rearrangement of the protein, and out into solvent space thus stabilizing an open form of the enzyme.[27] In short, the binding orientation for the

Table 9. Pharmacokinetic data and metabolism for **ABT-702** and **16a**

Compound	Pharmacokinetics[a]					Metabolism[b]	
	$t_{1/2}$	V_β	CL_p	C_{max}	F	Rat[c]	Human[d]
ABT-702	0.9	1.0	0.8	0.55	23	61	78
16a	0.5	0.7	1.0	0.08	9	21	74

[a] iv dose in rat at 5 μmol/kg for $t_{1/2}$, V_β, and CL_p. po dose in rat at 5 μmol/kg for C_{max} and F. Units: $t_{1/2}$ in h; V_β in L/kg; CL_p in L/hkg; C_{max} in μg/mL; F as %.

[b] % parent.

[c] Assay run in hepatocytes. Analyzed after 24 h.

[d] Assay run in microsomes. Analyzed after 1 h.

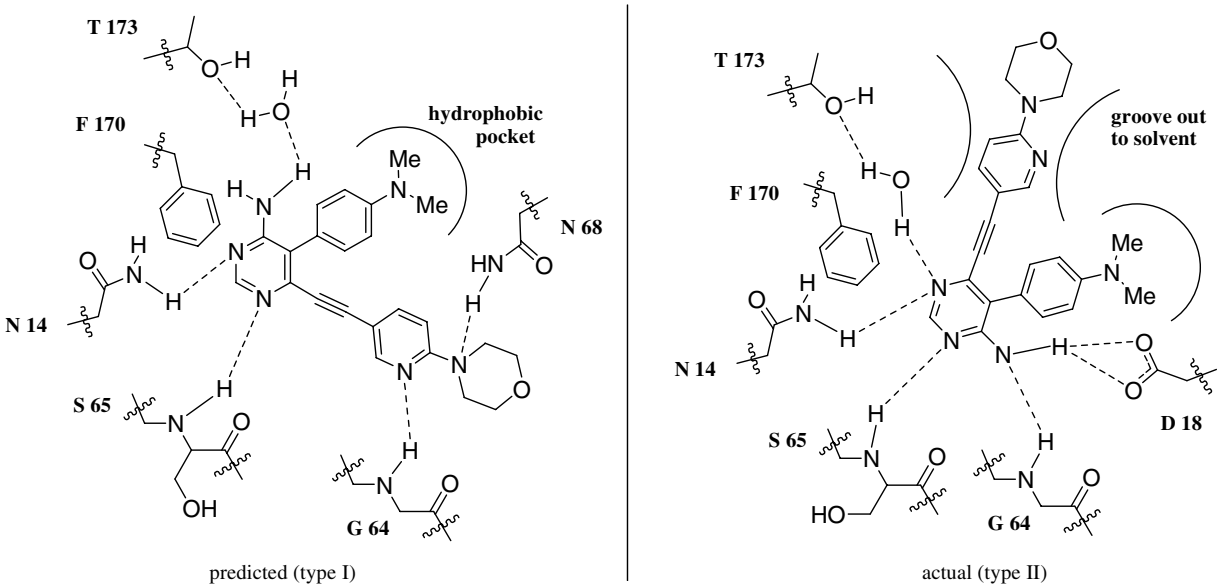


Figure 2. Predicted (type I) and actual (type II) binding of **16a** in the active site of adenosine kinase.

acetylene series (type II) is flipped approximately 180 degrees through the C(5) aryl group (compared to adenosine type I) in the active site of AK. In addition, the protein itself exists in an open state with **16a** compared to a closed state in the case of adenosine binding.

The type II binding observed in the **16a** crystal structure can now explain the SAR of other analogs in this series. For example, when a type I binding mode was assumed for the trisubstituted analogs, **8s**–**v**, one would predict increased potency by reaching into the hydrophilic pocket. No such increase in potency was observed. However, this result is consistent with the type II binding mode. The aryl acetylene fragment projects through a groove in the active site and out into solvent space. As a result, the earlier hypothesis of reaching a secondary phosphate binding site by using a trisubstituted aryl ring at C(6) proved false. To reach the phosphate binding site, placement of more polar side chains should originate from C(4). However, no further attempts were initiated at C(4) to confirm this redirected hypothesis.

Acetylene analog, **20**, showed moderate (AK_{cyt} $IC_{50} = 100$ nM) AK inhibition, which could not be rationalized using a type I binding orientation. Type II binding predicts that the C(5) aryl acetylene projects though the enzyme groove thus providing a potent analog. Several interesting questions concerning **20** can, therefore, be posed. How is the rest of this analog oriented in the active site? Does the C(6) aryl group reside near F170 (π–π interaction as in adenosine) or does the C(4) amino group form a hydrogen bond with T173? Even if acetylene **20** adopts one orientation, perhaps other analogs within the C(5) acetylene series prefer other orientations depending on specific substitutions. No further expansion of C(5) aryl acetylenes was explored. This same question may be posed to the C(6) acetylene analogs as well. Although **16a** was shown to prefer the orientation shown in Figure 1, could other orientations within the series be possible as long as the aryl acetylene projected into solvent space? Additional X-ray structures of C(6) acetylene analogs would be necessary to answer these questions.

4. Conclusion

In conclusion, we identified novel non-nucleoside inhibitors of AK with an acetylene functional group. The in vitro potency of these acetylene analogs compared favorably to **ABT-702**. The efficacy in animal models of pain, however, was diminished compared to **ABT-702**. This lack of in vivo efficacy is thought to result from lower plasma levels after systemic dosing of these aryl acetylene analogs relative to the pyridopyrimidines like **ABT-702**. During the course of this investigation, an X-ray structure was obtained of **16a** bound in the active site of AK.[27] This result showed that binding orientation of the acetylene series (type II binding) was flipped 180 degrees through the C(5) aryl group compared to adenosine (type I binding). The aryl acetylene portion projects through a groove in the enzyme out into solvent space thus stabilizing an open form of the enzyme. The anomalous SAR exhibited by some analogs, like **20**, may be explained if type II binding is considered. What is unclear, however, is the possibility of other aryl acetylenes adopting other binding orientations or additional AK enzyme conformations that could be stabilized similar to type II binding. These questions will require considerable SAR and X-ray crystallographic expansion of the current series.

5. Experimental

5.1. General procedures

General experimental procedures and techniques are reported in the supplemental section of this report. Preparation of **ABT-702** is reported[22] elsewhere.

5.2. Chemical procedures

5.2.1. General procedure for the formation of 4-amino-5-aryl-6-arylethynylpyrimidines (aminolysis-method A). To a slurry of **4** in EtOH (0.1 M) was added concentrated NH_4OH (0.2 M), the reaction mixure was sealed in a tube and heated to 100 °C until judged complete by TLC (typically 30–60 h). The mixture was cooled, the product filtered, and washed with EtOH. The solid was dried in a vacuum oven at 50 °C overnight. In an alternative work up (aqueous), the mixture was concentrated, the residue taken up in CH_2Cl_2, washed with saturated $NaHCO_3$, brine, and concentrated. The residue was purified by chromatography with the indicated solvent.

5.2.2. General procedure for the formation of 4-alkylamino-5-aryl-6-arylethynylpyrimidines (aminolysis-method B). A solution of **4d** (1.0 equiv) in EtOH (0.1 M) was treated with 2.2 equiv of the amine (or excess as indicated) sealed in a tube and heated to 100 °C overnight. The reaction mixture was cooled, concentrated, and the residue purified by chromatography (elution with the indicated solvent) to provide the desired amine addition product.

5.2.3. 6-(4-Dimethylaminophenylethynyl)-5-phenylpyrimidin-4-ylamine (5a). Starting with **4a**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (elution with 50% EtOAc–hexane then 10% MeOH–CH_2Cl_2) provided 70 mg (79%) of **5a**: yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (s, 1H, =N–C*H*=N), 7.50 (m, 5H, Ar*H*), 7.08 (AA′BB′, 2H, $J = 7.5$, Ar*H*), 6.53 (AA′BB′, 2H, $J = 7.5$, Ar*H*), 4.88 (br s, 2H, NH_2), 2.97 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.9 (s), 156.9 (d), 150.6 (s), 146.6 (s), 134.0 (s), 132.6 (d), 129.8 (d), 128.6 (d), 128.0 (d), 118.9 (s), 111.6 (d), 106.8 (s), 95.7 (s), 86.3 (s), 39.5 (q). MS (ESI) *m*/*z* 315 $(M+H)^+$. Anal. Calcd for $C_{20}H_{18}N_4 \cdot 0.2H_2O$: C, 75.54; H, 5.83; N, 17.62. Found: C, 75.38; H, 5.75; N, 17.33.

5.2.4. 5-(2-Chlorophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-ylamine (5b). Starting with **4b**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (elution with 5% MeOH–CH_2Cl_2 (300 mL) then 10% MeOH–CH_2Cl_2) provided 287 mg (71%) of **5b**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.33 (s, 1H, =N–C*H*=N), 7.63 (m, 1H, Ar*H*), 7.54-7.45 (m, 2H, Ar*H*), 7.38 (m, 1H, Ar*H*), 6.85 (AA′BB′, 2H, $J = 8.8$, Ar*H*), 6.61 (AA′BB′, 2H, $J = 9.2$, Ar*H*), 6.56 (br s, 2H, NH_2), 2.91 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.7 (s), 157.5 (d), 150.6 (s), 147.4 (s), 133.7 (s), 133.2 (s), 132.7 (d), 132.3 (d), 130.0 (d), 129.5 (d), 127.5 (d), 116.9 (s), 111.6 (d), 106.6 (s), 96.4 (s), 85.7 (s), 39.5 (q). MS (DCI/NH_3) *m*/*z* 349 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}ClN_4 \cdot 0.2H_2O$: C, 68.16; H, 4.98; N, 15.90. Found: C, 68.05; H, 4.78; N, 16.27.

5.2.5. 5-(3-Chlorophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-ylamine (5c). Starting with **4c**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and triturating with Et_2O provided 176 mg (65%) of **5c**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.31 (s, 1H, =N–C*H*=N), 7.53 (m, 2H, 4′- and 6′-Ar*H*), 7.47 (m, 1H, 2′-Ar*H*), 7.36 (m, 1H, 5′-Ar*H*), 6.99 (AA′BB′, 2H, $J = 8.8$, Ar*H*), 6.65 (AA′BB′, 2H, $J = 9.2$, Ar*H*), 6.60 (br s, 2H, NH_2), 2.93 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.8 (s), 157.2 (d), 150.7 (s), 146.7 (s), 136.3 (s), 133.1 (s), 132.7 (d), 130.5 (d), 129.8 (d), 128.7 (d), 128.0 (d), 117.6 (s), 111.7 (d), 106.6 (s), 96.3 (s), 86.1 (s), 39.5 (q). MS (DCI/NH_3) *m*/*z* 349 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}ClN_4 \cdot 0.2H_2O$: C, 68.16; H, 4.98; N, 15.90. Found: C, 68.21; H, 4.67; N, 15.72.

5.2.6. 5-(4-Chlorophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-ylamine (5d). Starting with **4d**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (elution with 50% EtOAc–hexane then 5% MeOH–$CHCl_3$) gave 75 mg (59%) of **5d**: yellow solid; R_f 0.29 (50% EtOAc–hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (s, 1H, =N–C*H*=N), 7.51 (AA′BB′, 2H, $J = 8.8$, Ar*H*), 7.44 (AA′BB′, 2H, $J = 8.4$, Ar*H*), 7.11 (AA′BB′, 2H, $J = 9.2$, Ar*H*), 6.56 (AA′BB′, 2H, $J = 9.2$, Ar*H*), 5.01 (br s, 2H, NH_2), 2.97 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6 (s), 157.5 (d), 150.9 (s), 148.4 (s), 134.6 (s), 133.5 (d), 132.4 (s), 131.4 (d), 129.3 (d), 118.1 (s), 111.6 (d), 107.8 (s), 98.2 (s), 85.7 (s), 40.0 (q). MS (DCI/NH_3) *m*/*z* 349 $(M+H)^+$. IR (KBr) 3291, 2951, 2206, 1608, 817 cm^{-1}. Anal. Calcd for $C_{20}H_{17}ClN_4$: C, 68.86; H, 4.91; N, 16.06. Found: C, 68.61; H, 4.85; N, 15.88.

5.2.7. 5-(4-Bromophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-ylamine (5e). Starting with **4e**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (50% EtOAc–hexane then EtOAc) afforded 130 mg (45%) of **5e**: light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.30 (s, 1H, =N–C*H*=N), 7.71 (AA′BB′, 2H, $J = 8.5$, Ar*H*), 7.37 (AA′BB′, 2H, $J = 8.5$, Ar*H*), 6.99 (AA′BB′, 2H, $J = 8.8$, Ar*H*), 6.65 (AA′BB′, 2H, $J = 9.2$, Ar*H*), 6.58 (br s, 2H, NH_2), 2.93 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.8 (s), 157.1 (d), 150.6 (s), 146.5 (s), 133.3 (s), 132.6 (d), 132.1 (d), 131.5 (d), 121.3 (s), 117.7 (s), 111.7 (d), 106.6 (s), 96.0 (s), 86.1 (s), 39.5 (q). MS (DCI/NH_3) *m*/*z* 393/395 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}BrN_4 \cdot 0.2$ EtOAc: C, 60.80; H, 4.56; N, 13.63. Found: C, 60.95; H, 4.45; N, 13.72.

5.2.8. 6-(4-Dimethylaminophenylethynyl)-5-*p*-tolylpyrimidin-4-ylamine (5f). Starting with **4f**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (5%

MeOH–CH_2Cl_2) provided 152 mg (69%) of **5f**: orange solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.28 (s, 1H, =N–C*H*=N), 7.34 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.30 (AA′BB′, 2H, J = 7.8, Ar*H*), 6.98 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.64 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.40 (br s, 2H, N*H*$_2$), 2.93 (s, 6H, N(C*H*$_3$)$_2$), 2.40 (s, 3H, ArC*H*$_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.1 (s), 156.7 (d), 150.5 (s), 146.5 (s), 137.2 (s), 132.6 (d), 130.9 (s), 129.6 (d), 129.1 (d), 118.8 (s), 111.6 (d), 106.9 (s), 95.5 (s), 86.3 (s), 39.5 (q), 20.9 (q). MS (DCI/NH_3) m/z 329 (M+H)$^+$. Anal. Calcd for $C_{21}H_{20}N_4 \cdot 0.6H_2O \cdot 0.1CH_2Cl_2$: C, 72.88; H, 6.20; N, 16.11. Found: C, 73.15; H, 5.81; N, 15.78.

5.2.9. 6-(4-Dimethylaminophenylethynyl)-5-(4-methoxyphenyl)pyrimidin-4-ylamine (5g). Starting with **4g**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (5% MeOH–CH_2Cl_2) gave 243 mg (70%) of **5g**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.27 (s, 1H, =N–C*H*=N), 7.34 (AA′BB′, 2H, J = 8.8, Ar*H*), 7.08 (AA′BB′, 2H, J = 8.8, Ar*H*), 7.01 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.65 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.42 (br s, 2H, N*H*$_2$), 3.83 (s, 3H, OC*H*$_3$), 2.93 (s, 6H, N(C*H*$_3$)$_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.3 (s), 159.0 (s), 156.6 (d), 150.6 (s), 146.6 (s), 132.6 (d), 131.1 (d), 125.9 (s), 118.7 (s), 114.1 (d), 111.7 (d), 106.9 (s), 95.5 (s), 86.4 (s), 55.2 (q), 39.5 (q). MS (DCI/NH_3) m/z 345 (M+H)$^+$. Anal. Calcd for $C_{21}H_{20}N_4O$: C, 73.23; H, 5.85; N, 16.27. Found: C, 72.88; H, 5.81; N, 15.89.

5.2.10. 5-(3,4-Dimethoxyphenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-ylamine (5h). Starting with **4h**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A) followed by filtration to provide 210 mg (62%) of **5h**: light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (s, 1H, =N–C*H*=N), 7.15 (AA′BB′, 2H, J = 9.2, Ar*H*), 7.04 (m, 3H, Ar*H*), 6.56 (AA′BB′, 2H, J = 9.2, Ar*H*), 5.04 (br s, 2H, N*H*$_2$), 3.97 (s, 3H, OC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 2.97 (s, 6H, N(C*H*$_3$)$_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.2 (s), 156.6 (d), 150.5 (s), 148.7 (s), 148.5 (s), 146.5 (s), 132.6 (d), 126.1 (s), 122.1 (d), 118.8 (s), 113.4 (d), 112.0 (d), 111.7 (d), 106.9 (s), 95.4 (s), 86.5 (s), 55.5 (q), 55.4 (q), 39.5 (q). MS (DCI/NH_3) m/z 375 (M+H)$^+$. Anal. Calcd for $C_{22}H_{22}N_4O_2$: C, 70.47; H, 6.03; N, 14.87. Found: C, 70.60; H, 5.92; N, 14.96.

5.2.11. 5-(4-Dimethylaminophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-ylamine (5i). Starting with **4i**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A) followed by aqueous work up and triturating with Et_2O to provide 150 mg (50%) of **5i**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.24 (s, 1H, =N–C*H*=N), 7.25 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.05 (AA′BB′, 2H, J = 8.9, Ar*H*), 6.86 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.64 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.39 (br s, 2H, N*H*$_2$), 2.97 (s, 6H, N(C*H*$_3$)$_2$), 2.93 (s, 6H, N(C*H*$_3$)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4 (s), 156.2 (d), 150.5 (s), 150.0 (s), 146.3 (s), 132.7 (d), 130.3 (d), 120.8 (s), 119.2 (s), 112.2 (d), 111.7 (d), 107.1 (s), 95.0 (s), 86.8 (s), 40.0 (q), 39.6 (q). MS (DCI/NH_3) m/z 358 (M+H)$^+$. Anal. Calcd for $C_{22}H_{23}N_5 \cdot 0.2H_2O$: C, 73.18; H, 6.53; N, 19.40. Found: C, 73.15; H, 6.37; N, 19.10.

5.2.12. 5-Benzo[1,3]dioxol-5-yl-6-(4-dimethylaminophenylethynyl)pyrimidin-4-ylamine (5j). Starting with **4j**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (elution with 50% EtOAc–hexane then 10% MeOH–CH_2Cl_2) provided 112 mg (51%) of **5j**: yellow solid; R_f 0.12 (50% EtOAc–hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H, =N–C*H*=N), 7.19 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.96 (m, 3H, Ar*H*), 6.57 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.06 (s, 2H, OC*H*$_2$O), 5.06 (br s, 2H, N*H*$_2$), 2.98 (s, 6H, N(C*H*$_3$)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.3 (s), 156.7 (d), 150.6 (s), 147.4 (s), 146.9 (s), 146.6 (s), 132.7 (d), 127.4 (s), 123.5 (d), 118.7 (s), 111.7 (d), 110.2 (d), 108.6 (d), 106.9 (s), 101.0 (t), 95.7 (s), 86.3 (s), 39.5 (q). MS (DCI/NH_3) m/z 359 (M+H)$^+$. Anal. Calcd for $C_{21}H_{18}N_4O_2 \cdot 0.20H_2O \cdot 0.30$EtOAc: C, 68.65; H, 5.40; N, 14.42. Found: C, 68.74; H, 5.21; N, 14.48.

5.2.13. 6-(4-Dimethylaminophenylethynyl)-5-(4-isopropylphenyl)pyrimidin-4-ylamine (5k). Starting with **4k**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A) followed by filtration to afford 225 mg (60%) of **5k**: orange solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (s, 1H, =N–C*H*=N), 7.40 (AA′BB′, 2H, J = 8.1, Ar*H*), 7.32 (AA′BB′, 2H, J = 8.1, Ar*H*), 6.92 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.59 (AA′BB′, 2H, J = 9.1, Ar*H*), 6.43 (br s, 2H, N*H*$_2$), 2.99 (m, 1H, C*H*(CH$_3$)$_2$), 2.92 (s, 6H, N(C*H*$_3$)$_2$), 1.29 (d, 6H, J = 6.8, CH(C*H*$_3$)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.0 (s), 156.7 (d), 150.5 (s), 148.1 (s), 146.8 (s), 132.6 (d), 131.4 (s), 129.8 (d), 126.5 (d), 119.0 (s), 111.5 (d), 106.9 (s), 95.8 (s), 86.5 (s), 39.5 (q), 33.3 (d), 23.8 (q). MS (DCI/NH_3) m/z 357 (M+H)$^+$. Anal. Calcd for $C_{23}H_{24}N_4 \cdot 0.4H_2O$: C, 75.96; H, 6.87; N, 15.41. Found: C, 76.16; H, 6.82; N, 15.55.

5.2.14. 5-Cyclohexyl-6-(4-dimethylaminophenylethynyl)-pyrimidin-4-ylamine (5l). Starting with **4l**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A) followed by filtration to afford 500 mg (59%) of **5l**: yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1H, =N–C*H*=N), 7.39 (AA′BB′, 2H, J = 8.9, Ar*H*), 6.77 (AA′BB′, 2H, J = 8.9, Ar*H*), 6.75 (br s, 2H, N*H*$_2$), 2.97 (s, 6H, N(C*H*$_3$)$_2$), 2.94 (m, 1H, cyclohexyl-*H*), 2.25 (m, 2H, cyclohexyl-*H*), 1.83–1.71 (m, 3H, cyclohexyl-*H*), 1.56 (m, 2H, cyclohexyl-*H*), 1.45–1.32 (m, 3H, cyclohexyl-*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.6 (s), 155.2 (d), 150.7 (s), 145.6 (s), 132.2 (d), 121.5 (s), 112.0 (d), 107.3 (s), 95.7 (s), 87.0 (s), 39.6 (q), 36.4 (br d), 28.6 (t), 26.4 (t), 25.4 (t). MS (DCI/NH_3) m/z 321 (M+H)$^+$. Anal. Calcd for $C_{20}H_{24}N_4 \cdot 0.6H_2O \cdot 0.5$ EtOH: C, 71.20; H, 8.02; N, 15.81. Found: C, 71.14; H, 7.66; N, 15.74.

5.2.15. 5-Benzyl-6-(4-dimethylaminophenylethynyl)pyrimidin-4-ylamine (5m). Starting with **4m**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A) followed by filtration to provide 101 mg (69%) of **5m**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.21 (s, 1H, =N–C*H*=N), 7.35–7.15 (m, 5H, Ar*H*), 7.32 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.87 (br s, 2H, NH_2), 6.71 (AA′BB′, 2H, J = 9.2, Ar*H*), 4.06 (s, 2H, CH_2Ph), 2.96 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.0 (s), 156.2 (d), 154.7 (s), 147.6 (s), 138.8 (s), 132.9 (d), 128.2 (d), 128.0 (d), 126.1 (d), 116.6 (s), 111.8 (d), 106.8 (s), 95.8 (s), 85.7 (s), 39.5 (q), 32.1 (t). MS (DCI/NH_3) m/z 329 (M+H)$^+$. Anal. Calcd for $C_{21}H_{20}N_4$·0.3H_2O: C, 75.56; H, 6.22; N, 16.78. Found: C, 75.59; H, 5.91; N, 16.85.

5.2.16. 5-Benzhydryl-6-(4-dimethylaminophenylethynyl)-pyrimidin-4-ylamine (5n). Starting with **4n**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (elution with 20% EtOAc–hexane then 5% MeOH–CH_2Cl_2) provided 95 mg (14%) of **5n**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H, =N–C*H*=N), 7.37–7.22 (m, 10H, Ar*H*), 7.11 (AA′BB′, 2H, J = 8.9, Ar*H*), 6.64 (AA′BB′, 2H, J = 8.9, Ar*H*), 6.34 (br s, 2H, NH_2), 6.02 (s, 1H, C*H*Ph$_2$), 2.92 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.9 (s), 156.2 (d), 150.6 (s), 148.2 (s), 140.5 (s), 132.9 (d), 128.5 (d), 126.6 (d), 118.8 (s), 111.6 (d), 106.7 (s), 97.4 (s), 86.6 (s), 48.2 (d), 39.5 (q), one quaternary carbon overlapped. MS (DCI/NH_3) m/z 405 (M+H)$^+$. Anal. Calcd for $C_{27}H_{24}N_4$·1.2EtOH: C, 76.80; H, 6.80; N, 12.19. Found: C, 76.80; H, 6.62; N, 12.33.

5.2.17. 6-(4-Dimethylaminophenylethynyl)-2-methyl-5-phenylpyrimidin-4-ylamine (5o). Starting with **4o**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (elution with 50% EtOAc–hexane then 5% MeOH–CH_2Cl_2) provided 440 mg (63%) of **5o**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.55–7.35 (m, 5H, Ar*H*), 6.95 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.62 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.38 (br s, 2H, NH_2), 2.92 (s, 6H, N(CH_3)$_2$), 2.34 (s, 3H, ArCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3 (s), 161.0 (s), 150.5 (s), 146.8 (s), 134.2 (s), 132.6 (d), 130.0 (d), 128.5 (d), 127.8 (d), 116.3 (s), 111.6 (d), 107.0 (s), 95.1 (s), 86.5 (s), 39.5 (q), 25.1 (q). MS (DCI/NH_3) m/z 329 (M+H)$^+$. Anal. Calcd for $C_{21}H_{20}N_4$·0.2H_2O: C, 75.97; H, 6.19; N, 16.87. Found: C, 76.00; H, 6.08; N, 16.94.

5.2.18. 5-(4-Chlorophenyl)-6-phenylethynylpyrimidin-4-ylamine (5p). Starting with **4p**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (elution with 3% MeOH–CH_2Cl_2) gave 204 mg (62%) of **5p**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.35 (s, 1H, =N–C*H*=N), 7.59 (AA′BB′, 2H, J = 8.8, Ar*H*), 7.45 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.43–7.35 (m, 3H, Ar*H*), 7.23–7.19 (m, 2H, Ar*H*), 6.72 (br s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2 (s), 157.3 (d), 145.7 (s), 132.9 (s), 132.6 (s), 131.8 (d), 131.3 (d), 129.6 (d), 128.8 (d), 120.9 (s), 119.0 (s), 93.1 (s), 87.3 (s), one methine overlapped. MS (DCI/NH_3) m/z 306 (M+H)$^+$. Anal. Calcd for $C_{18}H_{12}ClN_3$: C, 70.71; H, 3.96; N, 13.74. Found: C, 70.59; H, 3.79; N, 13.76.

5.2.19. 5-(4-Chlorophenyl)-6-*p*-tolylethynylpyrimidin-4-ylamine (5q). Starting with **4q**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method A). Aqueous work up and purification by chromatography (elution with 50% EtOAc–hexane the 10% MeOH–CH_2Cl_2) gave 71 mg (76%) of **5q**: white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.55 (s, 1H, =N–C*H*=N), 7.52 (AA′BB′, 2H, J = 8.8, Ar*H*), 7.44 (AA′BB′, 2H, J = 8.8, Ar*H*), 7.16 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.10 (AA′BB′, 2H, J = 7.8, Ar*H*), 4.96 (br s, 2H, NH_2), 2.33 (s, 3H, ArCH_3).

This material was converted to the HCl salt for final biological testing: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56 (s, 1H, =N–C*H*=N), 7.63 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.49 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.24 (AA′BB′, 2H, J = 8.1, Ar*H*), 7.15 (AA′BB′, 2H, J = 7.8, Ar*H*), 2.32 (s, 3H, ArCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.6 (s), 151.8 (d), 141.3 (s), 136.0 (s), 134.1 (s), 131.72 (d), 131.69 (d), 129.7 (s), 129.6 (d), 129.1 (d), 118.9 (s), 116.2 (s), 100.8 (s), 80.9 (s), 21.1 (q). MS (DCI/NH_3) m/z 320 (M+H)$^+$. Anal. Calcd for $C_{19}H_{14}ClN_3$·1.5HCl·0.5H_2O: C, 59.51; H, 4.34; N, 10.96. Found: C, 59.72; H, 4.71; N, 10.63.

5.2.20. 5-(4-Chlorophenyl)-6-(4-isopropylphenylethynyl)-pyrimidin-4-ylamine (5r). Starting with **7a**, the title compound was prepared according to the general procedure described for **8b** substituting 1-ethynyl-4-isopropylbenzene (**S12**) for 2-ethynylpyridine. Residue was purified by chromatography (1% gradient elution from 0% to 2% MeOH–CH_2Cl_2) to provide 430 mg (41%) of **5r**: light yellow solid; R_f 0.58 (5% MeOH–CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.35 (s, 1H, =N–C*H*=N), 7.58 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.44 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.26 (AA′BB′, 2H, J = 8.1, Ar*H*), 7.13 (AA′BB′, 2H, J = 8.5, Ar*H*), 6.69 (br s, 2H, NH_2), 2.88 (sept, 1H, J = 6.5, C*H*(CH$_3$)$_2$), 1.17 (d, 6H, J = 6.8, CH(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1 (s), 157.3 (d), 150.4 (s), 145.9 (s), 132.9 (s), 132.7 (s), 131.8 (d), 131.4 (d), 128.7 (d), 126.8 (d), 118.8 (s), 118.3 (s), 93.5 (s), 86.8 (s), 33.3 (d), 23.4 (q). MS (DCI/NH_3) m/z 348 (M+H)$^+$. Anal. Calcd for $C_{21}H_{18}ClN_3$·0.5H_2O: C, 70.68; H, 5.37; N, 11.78. Found: C, 70.74; H, 5.04; N, 12.01.

5.2.21. 5-(4-Chlorophenyl)-6-pyrimidin-5-ylethynylpyrimidin-4-ylamine (8a). A solution of **10a** (200 mg, 0.873 mmol) in CH_3CN (5 mL) and Et_3N (5 mL) was treated with 5-bromopyrimidine [4595-59-9] (277 mg, 1.74 mmol), Pd(PPh_3)$_2Cl_2$ (31 mg, 0.044 mmol), and CuI (7 mg, 0.037 mmol), and the reaction mixture heated to 60 °C for 2 h. The mixture was cooled, concentrated, and purified by chromatography (elution with 2%

MeOH–CH_2Cl_2) to provide 86 mg (32%) of **8a**: light tan solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.19 (s, 1H, =N–C*H*=N), 8.67 (s, 2H, 4′- and 6′-pyrimidine-*H*), 8.39 (s, 1H, =N–C*H*=N), 7.60 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.48 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.83 (br s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2 (s), 158.6 (d), 157.4 (d), 144.8 (s), 133.1 (s), 132.2 (s), 131.7 (d), 128.9 (d), 119.6 (s), 117.6 (s), 93.2 (s), 86.2 (s), one methine overlapped. MS (DCI/NH_3) m/z 308 $(M+H)^+$. Anal. Calcd for $C_{16}H_{10}ClN_5 \cdot 0.9H_2O \cdot 0.05$ PPh_3: C, 60.06; H, 3.77; N, 20.72. Found: C, 60.37; H, 3.53; N, 20.73.

5.2.22. 5-(4-Chlorophenyl)-6-pyridin-2-ylethynylpyrimidin-4-ylamine (8b). To a solution of **7a** (250 mg, 0.755 mmol) in CH_3CN (3 mL) and Et_3N (3 mL) were added 2-ethynylpyridine [1945-84-2] (187 mg, 1.81 mmol), $Pd(PPh_3)_2Cl_2$ (26 mg, 0.037 mmol), CuI (6 mg, 0.03 mmol), and the reaction mixture was heated to 50 °C for 1 h. The mixture was cooled, concentrated, and purified by chromatography (elution with 3% MeOH–CH_2Cl_2) to give 48 mg (21%) of **8b**: tan solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.57 (ddd, 1H, J = 4.8, 1.8, 0.7, 3′-pyridine-*H*), 8.38 (s, 1H, =N–C*H*=N), 7.81 (ddd, 1H, J = 7.8, 7.8, 1.7, 5′-pyridine-*H*), 7.57 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.46 (AA′BB′, 2H, J = 8.1, Ar*H*), 7.41 (ddd, 1H, J = 7.8, 5.8, 1.0, 4′-pyridine-*H*), 7.25 (ddd, 1H, J = 7.8. 0.7, 0.7, 6′-pyridine-*H*), 6.75 (br s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.3 (s), 157.4 (d), 150.3 (d), 145.0 (s), 141.1 (s), 136.8 (d), 133.1 (s), 132.2 (s), 131.7 (d), 128.9 (d), 127.6 (d), 124.2 (d), 119.5 (s), 91.9 (s), 85.8 (s). MS (DCI/NH_3) m/z 307 $(M+H)^+$. Anal. Calcd for $C_{17}H_{11}ClN_4 \cdot 0.1H_2O$: C, 66.18; H, 3.66; N, 18.16. Found: C, 65.86; H, 3.43; N, 18.08.

5.2.23. 5-(4-Chlorophenyl)-6-thiophen-2-ylethynylpyrimidin-4-ylamine (8c). Starting with **10a**, the title compound was prepared according to the procedure described for **8a** substituting 2-iodothiophene [3437-95-4] for 5-bromopyrimidine. Residue was purified by chromatography (elution with 3% MeOH–CH_2Cl_2) to afford 110 mg (40%) of **8c**: light tan solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.35 (s, 1H, =N–C*H*=N), 7.70 (dd, 1H, J = 5.1, 1.4, 3′-thienyl-*H*), 7.57 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.43 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.22 (dd, 1H, J = 3.7, 1.0, 5′-thienyl-*H*), 7.09 (dd, 1H, J = 5.1, 3.7, 4′-thienyl-*H*), 6.72 (br s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.1 (s), 157.3 (d), 145.6 (s), 133.7 (d), 133.0 (s), 132.4 (s), 131.7 (d), 130.6 (d), 128.8 (d), 127.8 (d), 120.3 (s), 118.9 (br s), 91.3 (br s), 86.9 (s). MS (DCI/NH_3) m/z 312 $(M+H)^+$. Anal. Calcd for $C_{16}H_{10}ClN_3S \cdot 0.5H_2O \cdot 0.4CH_2Cl_2$: C, 55.52; H, 3.35; N, 11.84. Found: C, 55.41; H, 3.19; N, 11.44.

5.2.24. 5-(4-Chlorophenyl)-6-(4-nitrophenylethynyl)pyrimidin-4-ylamine (8d). Starting with **10a**, the title compound was prepared according to the procedure described for **8a** substituting 1-iodo-4-nitrobenzene [636-98-6] for 5-bromopyrimidine. Upon completion of the reaction, the solution was cooled, filtered, and washed with cold CH_3CN. Solid was dried to give 201 mg (66%) of **8d**: light green solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39 (s, 1H, =N–C*H*=N), 8.23 (AA′BB′, 2H, J = 9.2, Ar*H*), 7.59 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.49 (AA′BB′, 2H, J = 8.8, Ar*H*), 7.48 (AA′BB′, 2H, J = 8.5, Ar*H*), 6.82 (br s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.3 (s), 157.4 (d), 147.4 (s), 144.9 (s), 133.1 (s), 132.6 (d), 132.2 (s), 131.7 (d), 128.9 (d), 127.5 (s), 123.9 (d), 119.6 (s), 91.4 (s), 90.6 (s). MS (DCI/NH_3) m/z 351 $(M+H)^+$. Anal. Calcd for $C_{18}H_{11}ClN_4O_2 \cdot 1.2H_2O$: C, 58.06; H, 3.63; N, 15.05. Found: C, 57.89; H, 3.36; N, 14.86.

5.2.25. 6-(4-Aminophenylethynyl)-5-(4-chlorophenyl)pyrimidin-4-ylamine (8e). Starting with **7a**, the title compound was prepared according to the procedure described for **8b** substituting 4-aminophenylacetylene[67] [14235-81-5] for 2-ethynylpyridine to provide 410 mg (42%) of **8e**. light yellow solid; R_f 0.14 (5% MeOH–CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (s, 1H, =N–C*H*=N), 7.57 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.41 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.83 (AA′BB′, 2H, J = 8.5, Ar*H*), 6.54 (br s, 2H, NH_2), 6.48 (AA′BB′, 2H, J = 8.8, Ar*H*), 5.70 (br s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.0 (s), 157.1 (d), 150.4 (s), 146.8 (s), 133.1 (s), 132.9 (d), 132.7 (s), 131.9 (d), 128.7 (d), 117.7 (br s), 113.5 (d), 106.4 (s), 96.6 (s), 85.6 (br s). MS (DCI/NH_3) m/z 321 $(M+H)^+$. Anal. Calcd for $C_{18}H_{13}ClN_4 \cdot 0.5H_2O$: C, 65.56; H, 4.28; N, 16.99. Found: C, 65.80; H, 3.95; N, 16.96.

5.2.26. 5-(4-Chlorophenyl)-6-(4-morpholin-4-ylphenylethynyl)pyrimidin-4-ylamine (8f). Starting with **10a**, the title compound was prepared according to the procedure described for **8a** substituting 4-(4-iodophenyl)morpholine[68] [87350-77-4] for 5-bromopyrimidine. Residue was purified by chromatography (elution with 5% MeOH–CH_2Cl_2) to provide 182 mg (43%) of **8f**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (s, 1H, =N–C*H*=N), 7.57 (AA′BB′, 2H, J = 8.8, Ar*H*), 7.43 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.04 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.90 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.61 (br s, 2H, NH_2), 3.70 (app t, 4H, J = 4.7, NCH_2CH_2O), 3.17 (app t, 4H, J = 4.7, NCH_2CH_2O). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.0 (s), 157.1 (d), 151.4 (s), 146.3 (s), 132.85 (s), 132.78 (s), 132.6 (d), 131.8 (d), 128.7 (d), 118.1 (s), 114.2 (d), 109.8 (s), 95.1 (s), 86.2 (s), 65.8 (t), 47.1 (t). MS (DCI/NH_3) m/z 391 $(M+H)^+$. Anal. Calcd for $C_{22}H_{19}ClN_4O$: C, 67.60; H, 4.90; N, 14.33. Found: C, 67.38; H, 5.04; N, 14.26.

5.2.27. 5-(4-Chlorophenyl)-6-(2-morpholin-4-ylpyrimidin-5-ylethynyl)pyrimidin-4-ylamine (8g). A slurry of 4-{5-[5-(4-chlorophenyl)-6-iodopyrimidin-4-ylethynyl]pyrimidin-2- yl}morpholine (**S17**; 255 mg, 0.506 mmol) in EtOH (3 mL) and concentrated NH_4OH (1 mL) was sealed in a tube and heated to 100 °C for 28 h. The reaction mixure was concentrated and the residue purified by chromatography (elution with 50% EtOAc–hexane to remove non-polar impurities then 10% MeOH–CH_2Cl_2) to give 56 mg (28%) of **8g**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.33 (s, 1H, =N–C*H*=N), 8.19 (s, 2H, pyrimidinyl-*H*), 7.58 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.44 (AA′BB′, 2H, J = 8.5, Ar*H*), 6.71 (br s, 2H, NH_2), 3.74 (app t, 4H, J = 5.1, OCH_2CH_2N), 3.63 (app t, 4H, J = 5.1, OCH_2CH_2N).

^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1 (s), 160.1 (d), 159.2 (s), 157.2(d), 145.7 (s), 132.9 (s), 132.6 (s), 131.8 (d), 128.8 (d), 118.4 (s), 104.5 (s), 90.5 (s), 89.4 (s), 65.8 (t), 43.8 (t). MS (DCI/NH_3) m/z 393 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}ClN_6O{\cdot}0.8H_2O$: C, 58.98; H, 4.60; N, 20.64. Found: C, 58.96; H, 4.30; N, 20.36.

5.2.28. 5-(4-Chlorophenyl)-6-(6-morpholin-4-ylpyridazin-3-ylethynyl)pyrimidin-4-ylamine (8h). Starting with **7a**, the title compound was prepared according to the procedure described for **8b** substituting 4-(6-ethynylpyridazin-3-yl)morpholine (**S20**) for 2-ethynylpyridine to provide 38 mg (11%) of **8h**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.38 (s, 1H, =N–C*H*=N), 7.56 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.46 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.22 (d, 1H, J = 9.8, pyridazinyl-*H*), 7.16 (d, 1H, J = 9.5, pyridazinyl-*H*), 6.78 (br s, 2H, N*H*$_2$), 3.70 (app t, 4H, J = 4.4, OC*H*$_2$CH$_2$N), 3.61 (app t, 4H, J = 4.5, OCH$_2$C*H*$_2$N). MS (DCI/NH_3) m/z 393 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}ClN_6O{\cdot}1.6H_2O$: C, 56.97; H, 4.83; N, 19.93. Found: C, 56.90; H, 4.38; N, 19.53.

5.2.29. 5-(4-Chlorophenyl)-6-(5-morpholin-4-ylpyridin-3-ylethynyl)pyrimidin-4-ylamine (8i). The silyl group of 4-(5-trimethylsilanylethynylpyridin-3-yl)morpholine (**S22**; 1.33 mmol) was removed as previously described for 4-(5-ethynylpyrimidin-2-yl)morpholine (**S16**) to provide 4-(5-ethynyl-pyridin-3-yl)-morpholine (**S23**): light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (d, 1H, J = 3.1), 8.09 (d, 1H, J = 1.4), 7.38 (dd, 1H, J = 3.1, 1.7), 4.34 (s, 1H), 3.73 (app t, 4H, J = 4.7), 3.19 (app t, 4H, J = 4.8). ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.0 (s), 141.9 (d), 137.3 (d), 123.4 (d), 118.4 (s), 83.1 (d), 80.9 (s), 65.8 (t), 47.2 (t). MS (DCI/NH_3) m/z 189 $(M+H)^+$.

Starting with **7a**, the title compound was prepared according to the procedure described for **8b** substituting **S23** for 2-ethynylpyridine to provide 60 mg (16%) of **8i**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.37 (s, 1H, =N–C*H*=N), 8.34 (d, 1H, J = 2.7, 2′- or 6′-pyridyl-*H*), 7.80 (d, 1H, J = 1.7, 2′- or 6′-pyridyl-*H*), 7.61 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.45 (AA′BB′, 2H, J = 8.5, Ar*H*), 6.93 (dd, 1H, J = 2.7, 1.7, 4′-pyridyl-H), 6.75 (br s, 2H, N*H*$_2$), 3.74 (app t, 4H, J = 4.8, OC*H*$_2$CH$_2$N), 3.15 (app t, 4H, J = 4.7, OCH$_2$C*H*$_2$N). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1 (s), 157.4 (d), 146.0 (s), 145.6 (s), 141.2 (d), 138.0 (d), 133.0 (s), 132.7 (s), 131.9 (d), 128.8 (d), 122.7 (d), 119.5 (s), 117.7 (s), 90.9 (s), 89.5 (s), 65.7 (t), 47.1 (t). MS (DCI/NH_3) m/z 392 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}ClN_5O{\cdot}0.6H_2O$: C, 62.64; H, 4.81; N, 17.39. Found: C, 62.86; H, 4.84; N, 17.02.

5.2.30. 6-(6-Aminopyridin-3-ylethynyl)-5-(4-chlorophenyl)pyrimidin-4-ylamine (8j). The *N*-formyl compound was prepared according to **8b** substituting *N*-(5-ethynylpyridin-2-yl)formamide (**S26**) for 2-ethynylpyridine to provide 125 mg (17%) of *N*-{5-[6-amino-5-(4-chlorophenyl)pyrimidin-4-ylethynyl]pyridin-2-yl}formamide: light yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) at 90 °C δ 10.51 (br s, 1H), 8.86 (br s, 1H), 8.38 (s, 1H), 8.07 (d, 1H, J = 2.3), 7.57 (m, 1H), 7.56 (AA′BB′, 2H, J = 8.4), 7.44 (AA′BB′, 2H, J = 8.4), 7.43 (m, 1H), 6.37 (br s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) at 90 °C δ 160.8 (s), 156.8 (d), 151.0 (s), 150.3 (d), 145.4 (s), 140.3 (d), 132.7 (s), 132.2 (s), 131.2 (d), 128.4 (d), 118.6 (s), 112.8 (s), 111.6 (br d), 90.0 (s), 89.1 (s), one methine broad or overlapped. MS (DCI/NH_3) m/z 350 $(M+H)^+$. Anal. Calcd for $C_{17}H_{12}ClN_5 0.3H_2O 0.8$ MeOH: C, 59.30; H, 4.18; N, 18.39. Found: C, 59.39; H, 4.08; N, 18.22. The light tan solid was dissolved in MeOH (10 mL) and treated with 1 M NaOH (2 mL). The mixture was heated to reflux for 10 min, cooled, concentrated, and the residue purified by chromatography (elution with 3% MeOH–CH_2Cl_2) to afford 74 mg (64%) of **8j**: light tan solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.33 (s, 1H, =N–C*H*=N), 7.72 (dd, 1H, J = 2.2, 0.7, 2′-pyridyl-*H*), 7.58 (AA′BB′, 2H, J = 8.1, Ar*H*), 7.44 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.17 (dd, 1H, J = 8.5, 2.2, 4′-pyridyl-*H*), 6.42 (dd, 1H, J = 8.5, 0.7, 5′-pyridyl-*H*). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.0 (s), 159.7 (s), 157.2 (d), 151.9 (d), 146.4 (s), 139.2 (d), 132.9 (s), 132.8 (s), 131.9 (d), 128.7 (d), 118.0 (s), 107.7 (d), 104.6 (s), 93.4 (s), 87.8 (s). MS (DCI/NH_3) m/z 322 $(M+H)^+$. Anal. Calcd for $C_{17}H_{12}ClN_5{\cdot}0.2H_2O$: C, 62.75; H, 3.84; N, 21.52. Found: C, 62.73; H, 3.88; N, 21.43.

5.2.31. 5-(4-Chlorophenyl)-6-(6-dimethylaminopyridin-3-ylethynyl)pyrimidin-4-ylamine (8k). Starting with **7a**, the title compound was prepared according to the procedure described for **8b** substituting (5-ethynylpyridin-2-yl)dimethylamine (**S29**) for 2-ethynylpyridine to provide 112 mg (47%) of **8k**: tan solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (s, 1H, =N–C*H*=N), 7.88 (d, 1H, J = 2.0, 2′-pyridine-*H*), 7.58 (AA′BB′, 2H, J = 8.1, Ar*H*), 7.43 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.25 (dd, 1H, J = 8.8, 2.4, 4′-pyridine-*H*), 6.62 (d, 1H, J = 8.8, 5′-pyridine-*H*), 6.62 (br s, 2H, N*H*$_2$), 3.04 (s, 6H, N(C*H*$_3$)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.3 (s), 158.4 (s), 157.5 (d), 151.5 (d), 146.7 (s), 139.5 (d), 133.15 (s), 133.10 (s), 132.2 (d), 129.0 (d), 118.3 (s), 105.8 (d), 104.3 (s), 93.4 (s), 88.5 (s), 37.7 (q). MS (DCI/NH_3) m/z 350 $(M+H)^+$. Anal. Calcd for $C_{19}H_{16}ClN_5$: C, 65.24; H, 4.61; N, 20.02. Found: C, 64.98; H, 4.47; N, 20.04.

5.2.32. 5-(4-Chlorophenyl)-6-(6-morpholin-4-ylpyridin-3-ylethynyl)pyrimidin-4-ylamine (8l). Starting with **7a**, the title compound was prepared according to the procedure described for **8b** substituting 4-(5-ethynylpyridin-2-yl)morpholine (**S32**) for 2-ethynylpyridine to provide 220 mg (61%) of **8l**: light tan solid, R_f 0.34 (5% MeOH–CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.33 (br s, 1H, =N–C*H*=N), 7.92 (d, 1H, J = 2.4, 5′-pyridyl-*H*), 7.58 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.44 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.31 (dd, 1H, J = 8.8, 2.4, 4′-pyridyl-*H*), 6.82 (d, 1H, J = 9.2, 3′-pyridyl-*H*), 6.63 (br s, 2H, N*H*$_2$), 3.66 (app t, 4H, J = 4.4, OC*H*$_2$CH$_2$N), 3.51 (app t, 4H, J = 4.4, OCH$_2$C*H*$_2$N). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.0 (s), 158.0 (s), 157.1 (br s), 151.0 (d), 146.1 (br s), 139.6 (d), 132.8 (s), 131.8 (d), 128.7 (d), 106.4 (d), 105.8 (s), 92.5 (s), 65.7 (t), 44.5 (t), 2 quaternary carbons and one methine carbon overlapped or broad. MS (DCI/NH_3) m/z 392 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}ClN_5O{\cdot}0.4H_2O$: C,

63.21; H, 4.75; N, 17.55. Found: C, 63.21; H, 4.65; N, 17.55.

5.2.33. 5-(4-Chlorophenyl)-6-{6-[(2-methoxyethyl)methylamino]pyridin-3-ylethynyl}pyrimidin-4-ylamine (8m). Starting with **7a**, the title compound was prepared according to the procedure described for **8b** substituting (5-ethynylpyridin-2-yl)-(2-methoxyethyl)methylamine (**S35**) for 2-ethynylpyridine. Residue was purified by chromatography (1% gradient elution from 0% to 4% MeOH–CH_2Cl_2) to provide 72 mg (24%) of **8m**: light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (s, 1H, =N–C*H*=N), 7.88 (d, 1H, J = 2.2, 2′-pyridine-*H*), 7.59 (AA′BB′, 2H, J = 8.8, Ar*H*), 7.43 (AA′BB′, 2H, J = 8.9, Ar*H*), 7.25 (dd, 1H, J = 8.8, 2.2, 4′-pyridine-*H*), 6.63 (d, 1H, J = 8.8, 5′-pyridine-*H*), 6.63 (br s, 2H, NH_2), 3.69 (app t, 2H, J = 5.5, NCH_2CH_2OMe), 3.47 (app t, 2H, J = 5.5, NCH_2CH$_2$OMe), 3.23 (s, 3H, OCH_3), 3.03 (s, 3H, NCH_3). IR (KBr) 3409, 2207. MS (DCI/NH_3) m/z 394 $(M+H)^+$. HRMS calcd for $C_{21}H_{20}ClN_5O$ $(M+H)^+$ 394.1435. Found 394.1428. Anal. Calcd for $C_{21}H_{20}ClN_5O{\cdot}0.35H_2O$: C, 63.03; H, 5.21; N, 17.50. Found: C, 63.07; H, 5.42; N, 17.23.

5.2.34. 4-{5-[6-Amino-5-(4-chlorophenyl)pyrimidin-4-ylethynyl]pyridin-2-yl}piperazine-1-sulfonic acid dimethylamide (8n). Starting with **10a**, the title compound was prepared according to the procedure described for **8a** substituting 4-(5-iodopyridin-2-yl)piperazine-1-sulfonic acid dimethylamide (**S38**) for 5-bromopyrimidine. The residue was purified by chromatography (elution with 5% MeOH–CH_2Cl_2) to provide 285 mg (49%) of **8n**: light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.83 (s, 1H, =N–C*H*=N), 7.92 (d, 1H, J = 1.7, 6′-pyridine-*H*), 7.58 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.44 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.33 (dd, 1H, J = 8.8, 2.4, 4′-pyridine-*H*), 6.86 (d, 1H, J = 9.2, 3′-pyridine-*H*), 6.64 (br s, 2H, NH_2), 3.64 (app t, 4H, J = 4.7, NCH_2CH$_2$NSO$_2$), 3.22 (app t, 4H, J = 5.1, NCH$_2$CH_2NSO$_2$), 2.78 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.0 (s), 157.5 (s), 157.2 (d), 151.0 (d), 146.1 (s), 139.8 (d), 132.82 (s), 132.77 (s), 131.8 (d), 128.7 (d), 118.2 (br s), 106.7 (d), 106.0 (s), 92.2 (s), 88.4 (br s), 45.6 (t), 43.9 (t), 37.8 (q). MS (DCI/NH_3) m/z 498 $(M+H)^+$. Anal. Calcd for $C_{23}H_{24}ClN_7O_2S$: C, 55.47; H, 4.86; N, 19.69. Found: C, 55.27; H, 4.73; N, 19.49.

5.2.35. 4-{5-[6-Amino-5-(4-chlorophenyl)pyrimidin-4-ylethynyl]pyridin-2-yl}piperazine-1-carboxylic acid tert-butyl ester (8o). Starting with **10a**, the title compound was prepared according to the procedure described for **8a** substituting 4-(5-iodopyridin-2-yl)piperazine-1-carboxylic acid *tert*-butyl ester (**S36**) for 5-bromopyrimidine. The residue was purified by chromatography (1% gradient elution from 1% to 5% MeOH–CH_2Cl_2) to afford 117 mg (29%) of **8o**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (s, 1H, =N–C*H*=N), 7.91 (d, 1H, J = 2.4, 6′-pyridine-*H*), 7.58 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.43 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.31 (dd, 1H, J = 8.8, 2.4, 4′-pyridine-*H*), 6.82 (dd, 1H, J = 9.2, 3′-pyridine-*H*), 6.64 (br s, 2H, NH_2), 3.56 (app t, 4H, J = 4.7, NCH_2CH$_2$NBoc), 3.39 (app t, 4H, J = 4.7, NCH$_2$CH_2NBoc), 1.42 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.0 (s), 157.7 (s), 157.2 (d), 153.8 (s), 151.0 (d), 146.1 (s), 139.1 (d), 132.8 (s), 132.7 (s), 131.8 (d), 128.7 (d), 118.2 (s), 106.6 (d), 105.6 (s), 92.5 (s), 88.3 (s), 79.1 (s), 43.8 (t), 42.9 (br t), 28.0 (q). MS (DCI/NH_3) m/z 491 $(M+H)^+$. Anal. Calcd for $C_{26}H_{27}ClN_6O_2{\cdot}0.8H_2O{\cdot}0.6$MeOH: C, 60.90; H, 5.96; N, 16.02. Found: C, 60.62; H, 5.68; N, 15.81.

5.2.36. 1-(4-{5-[6-Amino-5-(4-chlorophenyl)pyrimidin-4-ylethynyl]pyridin-2-yl}piperazin-1-yl)ethanone (8p). Starting with **7a**, the title compound was prepared according to the procedure described for **8b** substituting 1-[4-(5-ethynylpyridin-2-yl)piperazin-1-yl]ethanone (**S41**) for 2-ethynylpyridine to provide 16 mg (3%) of **8p**: off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (s, 1H, =N–C*H*=N), 7.91 (d, 1H, J = 2.4, 2′-pyridyl-*H*), 7.58 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.44 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.31 (dd, 1H, J = 8.8, 2.4, 4′-pyridyl-*H*), 6.84 (d, 1H, J = 8.5, 5′-pyridyl-*H*), 6.65 (br s, 2H, NH_2), 3.64-3.50 (m, 8H, NCH_2CH_2N x 2), 2.03 (s, 3H, C(O)CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.4 (s), 161.0 (s), 157.6 (s), 157.2 (d), 151.0 (d), 146.2 (s), 139.7 (d), 132.81 (s), 132.77 (s), 131.8 (d), 128.7 (d), 118.2 (s), 106.5 (d), 105.6 (s), 92.4 (s), 88.3 (s), 45.0 (t), 44.1 (t), 43.8 (t), 40.3 (t), 21.1 (q). MS (DCI/NH_3) m/z 433 $(M+H)^+$. Anal. Calcd for $C_{23}H_{21}ClN_6O$: C, 63.81; H, 4.89; N, 19.41. Found: C, 63.55; H, 5.10; N, 19.19.

5.2.37. 5-(4-Chlorophenyl)-6-(3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-5′-ylethynyl)pyrimidin-4-ylamine (8q). Starting with **7a**, the title compound was prepared according to the procedure described for **8b** substituting 5′-ethynyl-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl (**S44**) for 2-ethynylpyridine. This material was converted to the HCl salt to provide 135 mg (27%) of **8q**: orange solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.31 (s, 1H, =N–C*H*=N), 7.87 (d, 1H, J = 2.4, 5′-pyridyl-*H*), 7.58 (AA′BB′, 2H, J = 8.1, Ar*H*), 7.43 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.24 (dd, 1H, J = 9.2, 2.4, 4′-pyridyl-*H*), 6.79 (d, 1H, J = 9.2, 2′-pyridyl-*H*), 6.65 (br s, 2H, NH_2), 3.57 (app t, 4H, J = 5.1, piperidinyl-*H*), 1.60 (m, 2H, piperidinyl-*H*), 1.49 (m, 4H, piperidinyl-*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8 (s), 154.8 (s), 150.8 (d), 148.0 (d), 141.2 (d), 134.5 (s), 134.3 (s), 131.8 (d), 129.3 (d), 118.1 (s), 109.3 (d), 102.6 (s), 100.3 (s), 82.1 (s), 46.4 (t), 25.1 (t), 23.7 (t), one quaternary carbon overlapped. MS (DCI/NH_3) m/z 390 $(M+H)^+$. Anal. Calcd for $C_{22}H_{20}ClN_5{\cdot}3.0HCl{\cdot}1.0H_2O$: C, 51.08; H, 4.87; N, 13.54. Found: C, 50.96; H, 4.60; N, 13.50.

5.2.38. 5-Benzo[1,3]dioxol-5-yl-6-(6-morpholin-4-ylpyridin-3-ylethynyl)pyrimidin-4-ylamine (8r). Starting with **7b**, the title compound was prepared according to the procedure described for **8b** substituting 4-(5-ethynylpyridin-2-yl)morpholine (**S32**) for 2-ethynylpyridine to give 121 mg (33%) of **8r**: yellow solid; R_f 0.38 (5% MeOH–CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (s, 1H, =N–C*H*=N), 7.97 (d, 1H, J = 2.2, 2′-pyridyl-*H*), 7.35 (dd, 1H, J = 8.8, 2.2, 4′-pyridyl-*H*), 7.07 (d, 1H, J = 8.1, 3′-Ar*H*), 6.95 (d, 1H, J = 1.8, 6′-Ar*H*), 6.87 (dd, 1H, J = 8.1, 1.8, 4′-Ar*H*), 6.84 (d, 1H, J = 8.8, 3′-pyridyl-*H*), 6.09 (br s, 2H, NH_2), 3.66 (app t, 4H,

J = 5.2, OCH_2CH_2N), 3.51 (app t, 4H, J = 5.2, OCH_2CH_2N). MS (DCI/NH_3) m/z 402 $(M+H)^+$. Anal. Calcd for $C_{22}H_{19}N_5O_3 \cdot 0.15H_2O$: C, 65.39; H, 4.81; N, 17.33. Found: C, 65.21; H, 4.78; N, 17.00.

5.2.39. 5-Benzo[1,3]dioxol-5-yl-6-(5-dimethylamino-6-morpholin-4-ylpyridin-3-ylethynyl)pyrimidin-4-ylamine (8s). To a solution of (5-iodo-2-morpholin-4-ylpyridin-3-yl)dimethylamine (**S48**; 478 mg, 1.43 mmol) in DMF (5 mL) and Et_3N (5 mL) were added $Pd(PPh_3)_2Cl_2$ (49 mg, 0.070 mmol) and CuI (27 mg, 0.14 mmol). To this mixture was added **10b** (690 mg, 2.88 mmol) in DMF (3 mL) and Et_3N (3 mL). After 15 min, the reaction mixture was concentrated and the residue purified by chromatography (1% gradient elution from 0% to 4% (5% NH_4OH–EtOH)–$CHCl_3$) to provide 636 mg (64%) of **8s**: yellow solid. ^1H NMR (300 MHz, DMSO-d_6) δ 8.30 (br s, 1H, =N–C*H*=N), 7.72 (d, 1H, J = 1.7, 6′-Ar*H* or pyridyl-*H*), 7.08 (d, 1H, J = 7.8, 3′-Ar*H*), 6.96 (d, 1H, J = 1.7, 6′-Ar*H* or pyridyl-*H*), 6.88 (dd, 1H, J = 7.8, 1.7, 4′-Ar*H*), 6.82 (d, 1H, J = 1.7, 6′-Ar*H* or pyridyl-*H*), 6.60 (br s, 2H, NH_2), 6.07 (s, 2H, OCH_2O), 3.72 (app t, 4H, J = 4.8, NCH_2CH_2O), 3.37 (app t, 4H, J = 4.4, NCH_2CH_2O), 2.67 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.3 (s), 156.8 (br d), 152.5 (s), 147.4 (s), 147.1 (s), 146.0 (br s), 142.0 (d), 137.7 (s), 127.2 (s), 126.2 (d), 123.7 (d), 110.3 (s), 110.1 (d), 108.6 (d), 101.1 (t), 91.9 (s), 89.1 (br s), 66.1 (t), 46.7 (t), 39.9 (q), one quaternary carbon too broad or overlapped. MS (DCI/NH_3) m/z 445 $(M+H)^+$. Anal. Calcd for $C_{24}H_{24}N_6O_3 \cdot 0.2H_2O$: C, 64.33; H, 5.49; N, 18.75. Found: C, 64.01; H, 5.68; N, 18.45.

5.2.40. 5-Benzo[1,3]dioxol-5-yl-6-(4-dimethylamino-6-morpholin-4-ylpyridin-3-ylethynyl)pyrimidin-4-ylamine (8t). Starting with **10b**, the title compound was prepared according to the procedure described for **8a** substituting (5-iodo-2-morpholin-4-ylpyridin-4-yl)dimethylamine (**S54**) for 5-bromopyrimidine to afford 144 mg (15%) of **8t**: yellow solid. ^1H NMR (300 MHz, DMSO-d_6) δ 8.27 (s, 1H, =N–C*H*=N), 7.65 (s, 1H, pyridyl-*H*), 7.03 (d, 1H, J = 7.8, 3′-Ar*H*), 6.91 (d, 1H, J = 1.7, 6′-Ar*H*), 6.82 (dd, 1H, J = 8.1, 1.7, 4′-Ar*H*), 6.45 (br s, 2H, NH_2), 6.07 (s, 2H, OCH_2O), 5.83 (s, 1H, pyridyl-*H*), 3.65 (app t, 4H, J = 4.4, NCH_2CH_2O), 3.44 (app t, 4H, J = 4.4, NCH_2CH_2O), 2.86 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.4 (s), 159.5 (s), 157.9 (s), 156.8 (d), 154.6 (d), 147.5 (s), 147.0 (s), 146.7 (s), 127.4 (s), 123.5 (d), 118.0 (s), 110.1 (d), 108.7 (d), 101.1 (t), 96.2 (s), 93.4 (s), 92.2 (s), 89.9 (d), 65.8 (t), 44.8 (t), 41.0 (q). MS (DCI/NH_3) m/z 445 $(M+H)^+$. Anal. Calcd for $C_{24}H_{24}N_6O_3 \cdot 0.1H_2O$: C, 64.59; H, 5.47; N, 18.83. Found: C, 64.65; H, 5.54; N, 18.48.

5.2.41. 1-[5-(6-Amino-5-benzo[1,3]dioxol-5-ylpyrimidin-4-ylethynyl)-2-morpholin-4-ylpyridin-3-yl]-3-isopropylurea (8u). To a solution of 1-(5-iodo-2-morpholin-4-ylpyridin-3-yl)-3-isopropylurea (**S49**; 565 mg, 1.45 mmol) in DMF (5 mL) and Et_3N (5 mL) were added $Pd(PPh_3)_2Cl_2$ (50 mg, 0.071 mmol) and CuI (26 mg, 0.14 mmol). To this mixture was added **10b** (695 mg, 2.91 mmol) in DMF (3 mL) and Et_3N (3 mL). After 2 h, the reaction was concentrated and the residue purified by chromatography (1% gradient elution from 0% to 4% (5% NH_4OH–EtOH)–$CHCl_3$) to provide 315 mg (43%) of **8u**: light tan solid. ^1H NMR (300 MHz, DMSO-d_6) δ 8.31 (s, 1H, =N–C*H*=N), 8.18 (d, 1H, J = 2.0, pyridine-*H*), 7.77 (d, 1H, J = 2.0, pyridine-*H*), 7.54 (br s, 1H, N*H*), 7.06 (d, 1H, J = 8.1, 3′-Ar*H*), 7.02 (m, 1H), 6.94 (d, 1H, J = 1.4, 6′-Ar*H*), 6.86 (dd, 1H, J = 7.8, 1.7, 4′-Ar*H*), 6.63 (br s, 2H, NH_2), 6.11 (s, 2H, OCH_2O), 3.79 (app t, 4H, J = 5.1, NCH_2CH_2O), 3.75 (m, 1H, $CH(CH_3)_2$), 2.99 (app t, 4H, J = 4.8, NCH_2CH_2O), 1.12 (d, 6H, J = 6.4, $CH(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4 (s), 156.9 (d), 154.1 (s), 152.1 (s), 147.5 (s), 147.2 (s), 145.7 (s), 142.4 (d), 128.1 (d), 128.0 (s), 126.9 (s), 123.4 (d), 119.8 (s), 112.8 (s), 109.9 (d), 108.7 (d), 101.1 (t), 90.7 (s), 89.0 (s), 65.7 (t), 49.3 (t), 41.2 (d), 22.8 (q). MS (DCI/NH_3) m/z 502 $(M+H)^+$, 443 $(M-NPr\text{-}i)^+$. Anal. Calcd for $C_{26}H_{27}N_7O_4 \cdot 0.3H_2O$: C, 61.60; H, 5.49; N, 19.34. Found: C, 61.39; H, 5.53; N, 19.13.

5.2.42. 5-Benzo[1,3]dioxol-5-yl-6-{4-[(2-methoxyethyl)-methylamino]-6-morpholin-4-ylpyridin-3-ylethynyl}pyrimidin-4-ylamine (8v). Starting with **10b**, the title compound was prepared according to the procedure described for **8a** substituting (5-iodo-2-morpholin-4-ylpyridin-4-yl)-(2-methoxyethyl)methylamine (**S57**) for 5-bromopyrimidine to afford 113 mg (11%) of **8v**: brown solid. ^1H NMR (300 MHz, DMSO-d_6) δ 8.27 (s, 1H, =N–C*H*=N), 7.59 (s, 1H, pyridine-*H*), 7.05 (d, 1H, J = 7.8, 3′-Ar*H*), 6.91 (d, 1H, J = 1.4, 6′-Ar*H*), 6.83 (dd, 1H, J = 8.1, 1.7, 5′-Ar*H*), 6.50 (br s, 2H, NH_2), 6.07 (s, 2H, OCH_2O), 5.84 (s, 1H, pyridine-*H*), 3.65–3.59 (m, 6H, OCH_2CH_2N), 3.44–3.40 (m, 6H, OCH_2CH_2N), 3.19 (s, 3H, OCH_3), 2.90 (s, 3H, NCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4 (s), 159.6 (s), 156.8 (d), 156.6 (s), 154.9 (d), 147.5 (s), 147.0 (s), 146.7 (s), 127.4 (s), 123.5 (d), 118.0 (s), 110.1 (d), 108.8 (d), 101.1 (t), 95.6 (s), 93.5 (s), 92.1 (s), 90.0 (d), 70.4 (t), 65.9 (t), 58.0 (q), 51.9 (t), 44.8 (t), 39.3 (q). MS (ESI) m/z 489 $(M+H)^+$. Anal. Calcd for $C_{26}H_{28}N_6O_4 \cdot 0.5H_2O$: C, 62.76; H, 5.87; N, 16.89. Found: C, 62.55; H, 5.78; N, 16.69.

5.2.43. 5-(4-Dimethylaminophenyl)-6-(6-morpholin-4-ylpyridin-3-ylethynyl)pyrimidin-4-ylamine (16a). To a solution of **15a** (611 mg, 1.70 mmol) in DME (30 mL) and 2 M Na_2CO_3 (5 mL) at rt were added 4-dimethylaminophenylboronic acid [586-77-6] (1.16 g, 5.80 mmol) and $Pd(PPh_3)_4$ (308 mg, 0.267 mmol), and the mixture heated to 80 °C for 28 h. The mixture was partitioned between H_2O and CH_2Cl_2, the organic phase dried (Na_2SO_4), concentrated, and the residue purified by chromatography (elution with 2% then 5% MeOH–CH_2Cl_2) to afford 275 mg (41%) of **16a**: R_f 0.13 (5% MeOH–CH_2Cl_2). Material was converted to the HCl salt for biological evaluation. light yellow solid. ^1H NMR (300 MHz, DMSO-d_6) δ 8.25 (s, 1H, =N–C*H*=N), 7.98 (d, 1H, J = 2.1, 2′-pyridyl-*H*), 7.36 (dd, 1H, J = 9.1, 2.4, 4′-pyridyl-*H*), 7.25 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.85 (AA′BB′, 2H, J = 8.9, Ar*H*), 6.83 (d, 1H, J = 8.9, 5′-pyridyl-*H*), 3.66 (app t, 4H, J = 4.4, OCH_2CH_2N), 3.50 (app t, 4H, J = 4.4, OCH_2CH_2N),

2.96 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (100 MHz, C_6D_5N) δ 163.2 (s), 158.6 (s), 157.8 (d), 152.2 (d), 150.8 (s), 147.7 (s), 140.4 (d), 131.2 (d), 122.0 (s), 121.1 (s), 112.9 (d), 107.9 (s), 106.4 (d), 92.8 (s), 90.4 (s), 66.5 (t), 45.2 (t), 40.2 (q). MS (DCI/NH_3) *m/z* 401 $(M+H)^+$. Anal. Calcd for $C_{23}H_{24}N_6O\cdot3.0HCl$: C, 54.35; H, 5.62; N, 16.28. Found: C, 54.18; H, 5.34; N, 16.48.

5.2.44. 6-(6-Morpholin-4-ylpyridin-3-ylethynyl)-5-thiophen-2-ylpyrimidin-4-ylamine (16b). Starting with **15a**, the title compound was prepared according to the procedure described for **16a** substituting thiophene-2-boronic acid [6165-68-0] for 4-dimethylaminophenylboronic acid to give 61 mg (20%) of **16b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (s, 1H, =N–C*H*=N), 7.98 (d, 1H, *J* = 2.0, 2′-pyridyl-*H*), 7.78 (dd, 1H, *J* = 3.0, 3.0, 4′-thienyl-*H*), 7.39 (dd, 1H, *J* = 8.8, 2.4, 4′-pyridyl-*H*), 7.23 (m, 2H, 3′- and 5′-thienyl-*H*), 6.83 (d, 1H, *J* = 8.8, 5′-pyridyl-*H*), 6.82 (br s, 2H, NH_2), 3.66 (app t, 4H, *J* = 4.4, OCH_2CH$_2$N), 3.52 (app t, 4H, *J* = 4.4, OCH$_2$CH_2N). MS (DCI/NH_3) *m/z* 364 $(M+H)^+$. Anal. Calcd for $C_{19}H_{17}N_5OS\cdot0.6H_2O\cdot0.05PPh_3$: C, 61.70; H, 4.93; N, 18.08. Found: C, 61.44; H, 4.55; N, 17.95.

5.2.45. 6-(6-Morpholin-4-ylpyridin-3-ylethynyl)-5-thiophen-3-ylpyrimidin-4-ylamine (16c). Starting with **15a**, the title compound was prepared according to the procedure described for **16a** substituting thiophene-3-boronic acid [6165-69-1] for 4-dimethylaminophenylboronic acid to give 58 mg (30%) of **16c**: white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.30 (s, 1H, =N–C*H*=N), 8.01 (dd, 1H, *J* = 2.2, 2′-pyridyl-*H*), 7.73 (m, 2H, thienyl-*H*), 7.41 (dd, 1H, *J* = 8.9, 2.3, 4′-pyridyl-*H*), 7.25 (m, 1H, thienyl-*H*), 6.84 (d, 1H, *J* = 8.8, 5′-pyridyl-*H*), 6.65 (br s, 2H, NH_2), 3.67 (app t, 4H, *J* = 4.4, OCH_2CH$_2$N), 3.52 (app t, 4H, *J* = 4.0, OCH$_2$CH_2N). MS (DCI/NH_3) *m/z* 364 $(M+H)^+$. Anal. Calcd for $C_{19}H_{17}N_5OS\cdot0.6H_2O\cdot0.1PPh_3$: C, 62.38; H, 4.96; N, 17.49. Found: C, 62.23; H, 4.92; N, 17.66.

5.2.46. 5-Benzo[*b*]thiophen-2-yl-6-(6-morpholin-4-ylpyridin-3-ylethynyl)pyrimidin-4-ylamine (16d). Starting with **15a**, the title compound was prepared according to the procedure described for **16a** substituting benzo[*b*]thiophene-2-boronic acid [98437-23-1] for 4-dimethylaminophenylboronic acid to give 254 mg (35%) of **16d**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.36 (s, 1H, =N–C*H*=N), 8.05 (m, 1H, Ar*H*), 7.93 (m, 1H, Ar*H*), 7.88 (d, 1H, *J* = 2.4, 2′-pyridyl-*H*), 7.58 (s, 1H, 3′-thienyl-*H*), 7.42 (m, 2H, Ar*H*), 7.28 (dd, 1H, *J* = 9.2, 2.4, 4′-pyridyl-*H*), 7.01 (br s, 2H, NH_2), 6.76 (d, 1H, *J* = 9.2, 5′-pyridyl-*H*), 3.63 (app t, 4H, *J* = 4.4, OCH_2CH$_2$N), 3.47 (app t, 4H, *J* = 4.1, OCH$_2$CH_2N). MS (DCI/NH_3) *m/z* 414 $(M+H)^+$. Anal. Calcd for $C_{23}H_{19}N_5OS\cdot0.2H_2O\cdot0.2MeOH$: C, 65.80; H, 4.81; N, 16.54. Found: C, 66.11; H, 4.59; N, 16.27.

5.2.47. 5-Benzofuran-2-yl-6-(6-morpholin-4-ylpyridin-3-ylethynyl)pyrimidin-4-ylamine (16e). Starting with **15a**, the title compound was prepared according to the procedure described for **16a** substituting 2-benzofuranboronic acid [100124-06-9] for 4-dimethylaminophenylboronic acid to give 75 mg (18%) of **16e**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.38 (s, 1H, =N–C*H*=N), 8.10 (d, 1H, *J* = 2.2, 2′-pyridyl-*H*), 7.75 (dd, 1H, *J* = 7.3, 0.7, 4′ or 7′-Ar*H*), 7.66 (dd, 1H, *J* = 7.7, 0.7, 4′ or 7′-Ar*H*), 7.48 (dd, 1H, *J* = 9.2, 2.6, 4′-pyridyl-*H*), 7.35 (d, 1H, *J* = 0.8, 3′-furanyl-*H*), 7.34 (m, 2H, Ar*H*), 7.34 (br s, 2H, NH_2), 6.84 (d, 1H, *J* = 8.8, 5′-pyridyl-*H*), 3.66 (app t, 4H, *J* = 4.4, OCH_2CH$_2$N), 3.52 (app t, 4H, *J* = 4.4, OCH$_2$CH_2N). MS (DCI/NH_3) *m/z* 398 $(M+H)^+$. Anal. Calcd for $C_{23}H_{19}N_5O_2\cdot0.4H_2O$: C, 68.27; H, 4.93; N, 17.31. Found: C, 68.20; H, 4.74; N, 17.23.

5.2.48. 6-(4-Dimethylaminophenylethynyl)-5-(3-nitrophenyl)pyrimidin-4-ylamine (16f). To a solution of **15b** (515 mg, 1.62 mmol) and 3-nitrophenyl boronic acid ([13331-27-6]; 1.37 g, 8.21 mmol) in DME (5 mL) and 2 M Na_2CO_3 (5 mL) was added $Pd(PPh_3)_4$ (100 mg, 0.0087 mmol) and the mixture heated to reflux for 12 h. The reaction mixture was cooled, partitioned between H_2O and CH_2Cl_2. The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (elution with $CHCl_3$ then 10% MeOH–$CHCl_3$). The appropriate fractions were collected and concentrated. The resulting solid was triturated with Et_2O, sonicated (to eliminate trapped triphenylphosphine), filtered, and dried to give 67 mg (11%) of **16f**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.37 (s, 1H, =N–C*H*=N), 8.33 (ddd, 1H, *J* = 8.3, 2.5, 1.2, 4′-Ar*H*), 8.26 (dd, 1H, *J* = 1.8, 1.8, 2′-Ar*H*), 7.92 (ddd, 1H, *J* = 7.8, 1.2, 1.2, 6′-Ar*H*), 7.82 (dd, 1H, *J* = 8.0, 8.0, 5′-Ar*H*), 6.96 (AA′BB′, 2H, *J* = 8.9, Ar*H*), 6.77 (br s, 2H, NH_2), 6.61 (AA′BB′, 2H, *J* = 8.9, Ar*H*), 2.92 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.9 (s), 157.6 (d), 150.7 (s), 148.1 (s), 146.9 (s), 137.2 (d), 135.9 (s), 132.7 (d), 130.2 (d), 125.0 (d), 122.9 (d), 116.7 (s), 111.7 (d), 106.3 (s), 96.6 (s), 85.9 (s), 39.5 (q). MS (DCI/NH_3) *m/z* 360 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}N_5O_2\cdot0.4H_2O\cdot0.2$ Et_2O: C, 65.50; H, 5.23; N, 18.36. Found: C, 65.41; H, 4.93; N, 18.58.

5.2.49. 5-(3-Aminophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-ylamine (16g). Starting with **15b**, the title compound was prepared according to the procedure described for **16f** substituting 3-aminophenylboronic acid [206658-89-1] for 3-nitrophenyl boronic acid. After aqueous workup, the residue was purified by chromatography (elution with 5% MeOH–CH_2Cl_2). The appropriate fractions were collected and concentrated. The resulting solid was triturated with Et_2O, sonicated (to eliminate trapped triphenylphosphine), filtered, and dried to afford 708 mg (68%) of **16g**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.27 (s, 1H, =N–C*H*=N), 7.15 (dd, 1H, *J* = 7.8, 7.8, 5′-Ar*H*), 7.05 (AA′BB′, 2H, *J* = 9.2, Ar*H*), 6.64 (AA′BB′, 2H, *J* = 9.2, Ar*H*), 6.62 (m, 2H, 2′-and 4′-Ar*H*), 6.51 (ddd, 1H, *J* = 7.5, 1.7, 1.0, 6′-Ar*H*), 6.34 (br s, 2H, NH_2), 5.20 (br s, 2H, NH_2), 2.93 (s, 6H, N(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.9 (s), 156.6 (d), 150.5 (s), 148.9 (s), 146.2 (s), 134.3 (s), 132.8 (d), 129.1 (d), 119.7 (s), 116.8 (d), 114.9 (d), 113.6 (d), 111.6 (d), 107.1 (s), 95.5 (s), 86.5 (s), 39.3 (q). MS (DCI/NH_3) *m/z* 330 $(M+H)^+$. Anal. Calcd for $C_{20}H_{19}N_5\cdot0.5H_2O\cdot0.15$

Et_2O: C, 70.79; H, 6.20; N, 20.02. Found: C, 70.71; H, 5.82; N, 19.87.

5.2.50. [5-(4-Chlorophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-yl]methylamine (17a). Starting with **4d**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method B) with excess methylamine (40% in H_2O). Residue was purified by chromatography (elution with 2.5% MeOH–CH_2Cl_2) to give 232 mg (47%) of **17a**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.41 (s, 1H, =N–C*H*=N), 7.59 (AA′BB′, 2H, J = 8.8, Ar*H*), 7.41 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.97 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.64 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.47 (q, 1H, J = 4.1, N*H*CH_3), 2.93 (s, 6H, N(CH_3)$_2$), 2.78 (d, 3H, J = 4.7, NHCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7 (s), 157.1 (d), 150.6 (s), 145.3 (s), 132.8 (s), 132.6 (s), 132.5 (d), 132.1 (d), 128.7 (d), 118.9 (s), 111.7 (d), 106.7 (s), 95.8 (s), 86.0 (s), 39.5 (q), 27.8 (q). MS (DCI/NH_3) m/z 363 (M+H)$^+$. Anal. Calcd for $C_{21}H_{19}ClN_4$: C, 69.51; H, 5.28; N, 15.44. Found: C, 69.20; H, 5.04; N, 15.20.

5.2.51. [5-(4-Chlorophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-yl]dimethylamine (17b). Starting with **4d**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method B) with excess dimethylmethylamine (40% in H_2O). Residue was purified by chromatography (elution with 2.5% MeOH–CH_2Cl_2) to provide 277 mg (54%) of **17b**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (s, 1H, =N–C*H*=N), 7.56 (AA′BB′, 2H, J = 8.1, Ar*H*), 7.45 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.02 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.65 (AA′BB′, 2H, J = 9.2, Ar*H*), 2.94 (s, 6H, ArN(CH_3)$_2$), 2.75 (s, 6H, C(4)-N(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.5 (s), 156.0 (d), 150.7 (s), 149.2 (s), 136.0 (s), 133.3 (d), 131.6 (d), 128.2 (d), 118.8 (s), 111.5 (d), 108.0 (s), 97.9 (s), 86.2 (s), 40.5 (q), 39.9 (q), one quaternary carbon overlapped. MS (DCI/NH_3) m/z 377 (M+H)$^+$. Anal. Calcd for $C_{22}H_{21}ClN_4 \cdot 0.25H_2O$: C, 69.28; H, 5.68; N, 14.69. Found: C, 69.05; H, 5.68; N, 14.64.

5.2.52. [5-(4-Chlorophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-yl]cyclohexylamine (17c). Starting with **4d**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method B) using cyclohexylamine ([108-91-8]; 2.0 equiv). Residue was purified by chromatography (elution with 5% MeOH–CH_2Cl_2) to provide 134 mg (23%) of **17c**: yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H, =N–C*H*=N), 7.50 (AA′BB′, 2H, J = 8.6, Ar*H*), 7.38 (AA′BB′, 2H, J = 8.6, Ar*H*), 7.09 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.55 (AA′BB′, 2H, J = 8.9, Ar*H*), 4.59 (d, 1H, J = 8.0, N*H*CH(CH_2)$_2$), 4.00 (m, 1H, NHC*H*(CH_2)$_2$), 2.95 (s, 6H, N(CH_3)$_2$), 1.97 (m, 2H, cyclohexyl-*H*), 1.70-1.59 (m, 3H, cyclohexyl-*H*), 1.46-1.36 (m, 2H, cyclohexyl-*H*), 1.18-1.05 (m, 3H, cyclohexyl-*H*). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.0 (s), 157.4 (d), 150.7 (s), 146.4 (s), 134.4 (s), 133.3 (d), 132.3 (s), 131.5 (d), 129.4 (d), 118.5 (s), 111.5 (d), 108.0 (s), 97.5 (s), 85.7 (s), 49.4 (d), 40.0 (q), 32.9 (br t), 25.5 (br t), 24.8 (br t). MS (DCI/NH_3) m/z 431 (M+H)$^+$. Anal. Calcd for $C_{26}H_{27}ClN_4 \cdot 0.5H_2O$: C, 70.98; H, 6.41; N, 12.73. Found: C, 70.78; H, 6.17; N, 12.63.

5.2.53. [5-(4-Chlorophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-yl]-[2-(1*H*-imidazol-4-yl)ethyl]amine (17d). Starting with **4d**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method B) using histamine ([56-92-8]; 2.1 equiv). Residue was purified by chromatography (elution with 5% MeOH–CH_2Cl_2) to provide 180 mg (30%) of **17d**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.89 (br s, 1H, N*H*), 8.40 (s, 1H, =N–C*H*=N), 7.58 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.49 (d, 1H, J = 1.0, imidazoyl-*H*), 7.38 (AA′BB′, 2H, J = 8.5, Ar*H*), 6.98 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.78 (br s, 1H, imidazoyl-*H*), 6.65 (AA′BB′, 2H, J = 8.8, Ar*H*), 3.52 (m, 2H, NHC$H_2$$CH_2$), 2.93 (s, 6H, N($CH_3$)$_2$), 2.71 (m, 2H, NHC$H_2CH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.2 (s), 157.1 (d), 150.6 (s), 145.6 (s), 134.9 (s), 134.5 (d), 132.9 (s), 132.6 (d), 132.5 (s), 132.0 (d), 128.7 (d), 118.9 (s), 116.1 (d), 111.7 (d), 106.7 (s), 96.0 (s), 86.0 (s), 40.6 (t), 39.5 (q), 26.1 (t). MS (DCI/NH_3) m/z 443 (M+H)$^+$. Anal. Calcd for $C_{25}H_{23}ClN_6 \cdot 0.1$EtOH: C, 67.02; H, 5.62; N, 18.03. Found: C, 67.12; H, 5.54; N, 17.78.

5.2.54. [5-(4-Chlorophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-yl]-[2-(1*H*-indol-3-yl)ethyl]amine (17e). Starting with **4d**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method B) using tryptamine ([61-54-1]; 2.1 equiv). Residue was purified by chromatography (elution with 2.5% MeOH–CH_2Cl_2) to provide 252 mg (38%) of **17e**: yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.78 (br s, 1H, N*H*), 8.44 (s, 1H, =N–C*H*=N), 7.55 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.54 (m, 1H, Ar*H*), 7.35 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.33 (m, 1H, Ar*H*), 7.13 (d, 1H, J = 2.0, C=C*H*-NH), 7.06 (ddd, 1H, J = 7.1, 7.1, 1.4, Ar*H*), 6.98 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.97 (m, 1H, Ar*H*), 6.65 (AA′BB′, 2H, J = 9.2, Ar*H*), 6.50 (br t, 1H, J = 5.8, N*H*), 3.59 (m, 2H, NHC$H_2$$CH_2$), 2.93 (s, 6H, N($CH_3$)$_2$), 2.91 (m, 2H, NHC$H_2CH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.2 (s), 157.1 (d), 150.6 (s), 145.6 (s), 136.2 (s), 132.8 (s), 132.6 (d), 132.5 (s), 132.0 (d), 128.7 (d), 127.2 (s), 122.6 (d), 120.8 (d), 118.8 (s), 118.3 (d), 118.1 (d), 111.71 (s), 111.67 (d), 111.2 (d), 106.7 (s), 95.9 (s), 86.0 (s), 41.3 (t), 39.5 (q), 24.6 (t). MS (DCI/NH_3) m/z 492 (M+H)$^+$. Anal. Calcd for $C_{30}H_{26}ClN_5 \cdot 0.4CH_2Cl_2$: C, 69.42; H, 5.14; N, 13.31. Found: C, 69.58; H, 5.04; N, 13.49.

5.2.55. {4-[5-(4-Chlorophenyl)-6-morpholin-4-ylpyrimidin-4-ylethynyl]phenyl}dimethylamine (17f). Starting with **4d**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method B) with excess morpholine (11 equiv). Residue was purified by chromatography (elution with 30% EtOAc–hexane) to afford 79 mg (60%) of **17f**: light yellow solid; R_f 0.16 (50% EtOAc–hexane). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.55 (s, 1H, =N–C*H*=N), 7.60 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.53 (AA′BB′, 2H, J = 8.5, Ar*H*), 7.07 (AA′BB′, 2H, J = 8.8, Ar*H*), 6.67 (AA′BB′, 2H, J = 8.8, Ar*H*), 3.47

(app t, 4H, $J = 4.4$, OCH_2CH_2N), 3.20 (app t, 4H, $J = 4.7$, OCH_2CH_2N), 2.94 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4 (s), 156.3 (d), 150.8 (s), 149.0 (s), 135.2 (s), 132.8 (d), 132.6 (s), 131.4 (d), 128.5 (d), 120.1 (s), 111.7 (d), 106.3 (s), 97.1 (s), 86.3 (s), 65.5 (t), 47.3 (t), 39.5 (q). MS (DCI/NH_3) m/z 419 $(M+H)^+$. Anal. Calcd for $C_{24}H_{23}ClN_4O \cdot 0.24EtOAc$: C, 68.12; H, 5.71; N, 12.73. Found: C, 68.31; H, 5.74; N, 12.41.

5.2.56. {4-[5-(4-Chlorophenyl)-6-piperazin-1-ylpyrimidin-4-ylethynyl]phenyl}dimethylamine (17g). Starting with **4d**, the title compound was prepared according to the general procedure described for the aminolysis reaction (method B) with excess piperazine (5 equiv). Residue was purified by chromatography (elution with 5% MeOH–CH_2Cl_2) to provide 174 mg (38%) of **17g**: yellow solid. ^1H NMR (300 MHz, DMSO-d_6) δ 8.50 (s, 1H, =N–C*H*=N), 7.59 (AA′BB′, 2H, $J = 8.5$, Ar*H*), 7.51 (AA′BB′, 2H, $J = 8.5$, Ar*H*), 7.06 (AA′BB′, 2H, $J = 8.8$, Ar*H*), 6.67 (AA′BB′, 2H, $J = 9.2$, Ar*H*), 3.14 (app t, 4H, $J = 5.1$, $HNCH_2CH_2N$), 2.94 (s, 6H, $N(CH_3)_2$), 2.55 (app t, 4H, $J = 5.1$, $HNCH_2CH_2N$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.5 (s), 156.2 (d), 150.7 (s), 148.8 (s), 135.5 (s), 132.7 (d), 132.4 (s), 131.4 (d), 128.4 (d), 120.0 (s), 111.7 (d), 106.4 (s), 96.8 (s), 86.4 (s), 47.9 (t), 44.8 (t), 39.5 (q). MS (DCI/NH_3) m/z 418 $(M+H)^+$. Anal. Calcd for $C_{24}H_{24}ClN_5 \cdot 0.3H_2O$: C, 68.09; H, 5.86; N, 16.54. Found: C, 68.47; H, 5.79; N, 16.18.

5.2.57. 4-[5-(4-Chlorophenyl)-6-(4-dimethylaminophenylethynyl)pyrimidin-4-yl]piperazine-1-sulfonic acid dimethylamide (17h). To a solution of **17g** (134 mg, 0.321 mmol) in Et_3N (10 mL) at 0 °C was added *N*,*N*-dimethylsulfamoyl chloride (38 μL, 0.35 mmol), the reaction mixture warmed to rt and stirred overnight. Solvent was removed and the residue purified by chromatography (elution with 3% MeOH–CH_2Cl_2) to give 130 mg (77%) of **17h**: yellow solid. ^1H NMR (300 MHz, DMSO-d_6) δ 8.56 (s, 1H, =N–C*H*=N), 7.61 (AA′BB′, 2H, $J = 8.5$, Ar*H*), 7.54 (AA′BB′, 2H, $J = 8.5$, Ar*H*), 7.07 (AA′BB′, 2H, $J = 8.8$, Ar*H*), 6.67 (AA′BB′, 2H, $J = 9.2$, Ar*H*), 3.28 (app t, 4H, $J = 5.4$, $Me_2NSO_2NCH_2CH_2N$), 3.02 (app t, 4H, $J = 5.1$, $Me_2NSO_2NCH_2CH_2N$), 2.95 (s, 6H, $ArN(CH_3)_2$), 2.73 (s, 6H, $(CH_3)_2NSO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4 (s), 156.3 (d), 150.8 (s), 149.1 (s), 135.0 (s), 132.8 (d), 132.7 (s), 131.4 (d), 128.6 (d), 120.4 (s), 111.7 (d), 106.3 (s), 97.3 (s), 86.3 (s), 46.4 (t), 45.2 (t), 39.5 (q), 37.7 (q). MS (DCI/NH_3) m/z 525 $(M+H)^+$. Anal. Calcd for $C_{26}H_{29}ClN_6O_2S$: C, 59.47; H, 5.57; N, 16.01. Found: C, 59.31; H, 5.68; N, 15.84.

5.2.58. 6-(4-Dimethylaminophenyl)-5-(4-dimethylaminophenylethynyl)pyrimidin-4-ylamine (20). Starting with 4-methoxy-6-(4-dimethylaminophenyl)-5-(4-dimethylaminophenylethynyl)-pyrimidine (**S60**), the title compound was prepared according to the general procedure described for the aminolysis reaction (method A) to afford **20**: ^1H NMR (300 MHz, DMSO-d_6) δ 8.32 (s, 1H, =N–C*H*=N), 8.13 (AA′BB′, 2H, $J = 8.6$, Ar*H*), 7.43 (AA′BB′, 2H, $J = 8.6$, Ar*H*), 7.00 (br s, 2H, NH_2), 6.80 (AA′BB′, 2H, $J = 9.0$, Ar*H*), 6.73 (AA′BB′, 2H, $J = 8.6$, Ar*H*), 3.00 (s, 6H, $N(CH_3)_2$), 2.95 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.8 (s), 155.3 (d), 151.2 (s), 150.1 (s), 132.1 (d), 129.9 (d), 124.8 (s), 111.7 (d), 110.7 (d), 110.5 (s), 108.9 (s), 100.7 (s), 81.7 (s), 81.3 (s), 39.7 (q), one methyl overlapped.

Acknowledgments

The authors would like to thank members of the Abbott Structural Chemistry group (Dr. Tom Pagano, Dave Whittern, Jan Waters, and Carmina Presto) for helpful NMR and mass spec characterization.

Supplementary data

Experimental details and characterization for all intermediates can be found in the supplementary section of this report. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.12.029.

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