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European Journal of Medicinal Chemistry 38 (2003) 245–252
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Synthesis and structure-activity relationships of 5-heteroatom-substituted pyridopyrimidines as adenosine kinase inhibitors

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Received 18 November 2002; received in revised form 8 January 2003; accepted 9 January 2003

Abstract

Under stressful conditions, many cells release adenosine to minimize tissue damage. Inhibition of intracellular adenosine kinase (AK) increases the local extracellular concentration of adenosine and its effect on traumatized tissue. The synthesis and SAR of a new series of pyridopyrimidines for the inhibition of AK are described. It was found that a range of analogs with position five substituted by an amine or ether functionality increased aqueous solubility while retaining the in vitro potency of initial leads. A narrower range of analogs was active in vivo in a rat inflammatory hyperalgesia model.

Keywords: Adenosine; Pyridopyrimidines; Hyperalgesia

1. Introduction

Under stressful conditions (e.g., pain, inflammation, ischemia or mechanical trauma) many cells release adenosine in order to minimize tissue damage [1]. Adenosine stimulates specific receptors (A_1, A_{2A}, A_{2B}, and A_3) on the outer surface of cell membranes, resulting in decreased excitatory amino acid release, neutrophil degranulation, and superoxide production [2]. As the therapeutic utility of direct acting adenosine receptor agonists has been limited by unacceptable side effects, our approach was to enhance the naturally beneficial effects of adenosine at sites of trauma or hyperexcitability by inhibiting its metabolism [3–5]. The primary enzyme responsible for the inactivation of adenosine is adenosine kinase (AK), which phosphorylates adenosine to AMP [6]. Therefore, we sought to discover potent and selective AK inhibitors for the treatment of pain and inflammation.

A novel set of heteroatom-substituted pyridopyrimidine analogs of previously described potent and selective AK inhibitors **1a** (ABT-702) and **1b** (Fig. 1) [7–10] was designed with the goal of maintaining in vitro potency, enhancing the basicity of the pyridopyrimidine ring system, and increasing solubility and absorption. It has already been found that medium to large-sized lipophilic substituents in position five of the pyridopyrimidine ring system likely provide an additional interaction with a binding pocket in AK [10], and that replacement with an even slightly polar substituent provided compounds with reduced in vitro activity [9]. However, C-5 analogs with a heteroatom directly attached to the pyridopyrimidine were projected to increase the basicity in the ring system and form more soluble salts. As the receptor site has been found to tolerate either aromatic or aliphatic substituents at C-5, both aromatic and aliphatic groups were attached to the heteroatom with the aim of balancing solubility with potency. In the present paper we describe the synthesis,

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0223-5234/03/$ - see front matter
doi:10.1016/S0223-5234(03)00019-9

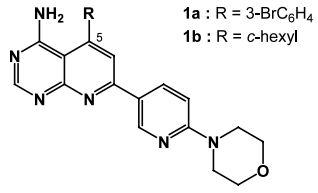


Fig. 1. Lead compounds **1a** and **1b**.

solubility, and in vitro activity of these novel, potent AK inhibitors.

2. Chemistry

The original synthesis of the lead compounds had required the condensation of a substituted 1,1-dicyanoethylene **2** and acetylpyridine **3** with ammonium acetate (Fig. 2). However, our first attempts to prepare 5-heteroatom analogs in a similar manner failed to produce the desired product and afforded unsubstituted **4** instead. One plausible mechanism is consistent with actual observations: After **2** reacts with **3** to give intermediate **I**, ammonia can condense with **I** to provide dihydropyridine **II**, which then readily air oxidizes to pyridine **5** if **R** is not a heteroatom. On the other hand, if **R** is a readily eliminated group the second pathway is favoured and results in **4**.

In order to compensate for this elimination, we used as a starting material a compound where two thiomethyl groups were attached to the dicyanoethylene, with the aim that after one was eliminated the second would remain and could be substituted at a later time. But when 2,2-dicyano-1,1-dithiomethylethylene (**6**) was used under identical reaction conditions, no products were formed.

Tominaga reported in 1987 [11] that **6** reacted with acetone in the presence of KOH in DMSO to give **7** after an acid quench (Fig. 3). We hypothesized that a similar reaction of **6** with **3** and ammonium acetate might produce **5a** instead. What was obtained, in poor yield, was an intermediate which possessed a mass equivalent to the sum of the masses of **6** and **3**, with no sign of ammonia incorporation. The absence of evidence for

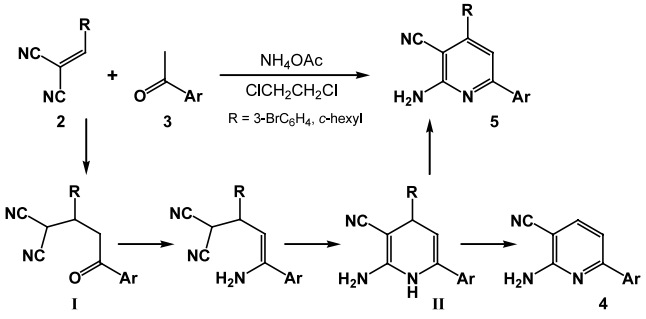


Fig. 2. Mechanisms for the formation of pyridines **4** and **5**.

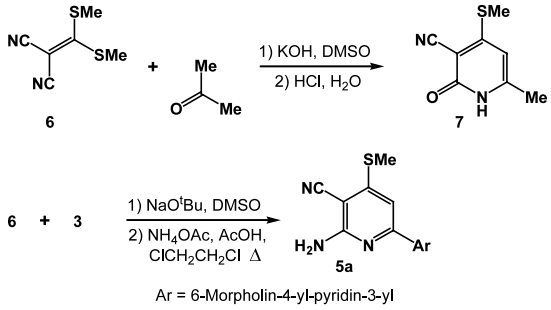


Fig. 3. Preparation of aryl methylsulphides **7** and **5a**.

methylenic protons alpha to a carbonyl suggested that a cyclization had already taken place. When this intermediate was refluxed with ammonium acetate in dichloroethane, **5a** was obtained. Later, the overall yield was improved by changing the base in the first step to sodium *tert*-butoxide and by adding acetic acid in the second.

2-Aminopyridine **5a** was heated with catalytic ammonium sulphate in triethyl orthoformate to afford the imino ether, which was immediately converted to the amidine by addition of 2 M ammoniacal ethanol (Fig. 4). The crude product was subsequently heated in dichlorobenzene to provide the desired pyridopyrimidine **8** in an overall yield of 23% from **3**. The oxidation of **8** to **9** with Oxone® ($2KHSO_5/KHSO_4/K_2SO_4$) required a mixture of three solvents to keep all of the reactive species in solution, but succeeded in 74% yield. Compounds **8** and **9** were both used to prepare the targeted series of compounds.

Attempted substitution of the methyl sulphide in **8** with piperidine in either refluxing butanol or diglyme failed. Once the substrate was changed to the more electrophilic **9**, substitution of the sulphone with most primary aliphatic amines occurred smoothly in hot DMSO to give **10** (Fig. 5).

Secondary aliphatic amines reacted similarly, but in poor yield. Therefore, an alternative synthetic route was explored, wherein most secondary amines were first reacted with **6** to give intermediate **11** (Fig. 5). **11** was then stirred with acetylpyridine **3** in DMSO and treated with lithium *tert*-butoxide for 2 h, after which the reaction was quenched with acetic acid and heated with

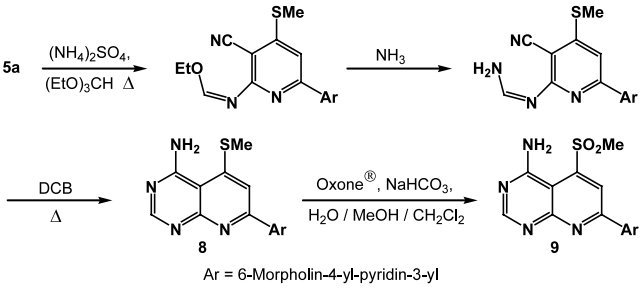


Fig. 4. The synthesis of sulphone **9**.

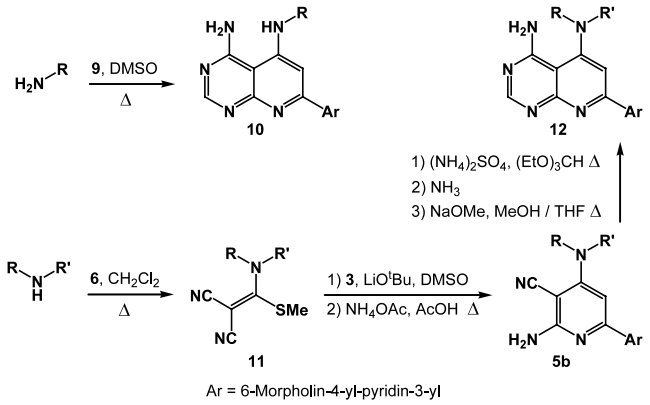


Fig. 5. The syntheses of 5-alkylamino-pyridopyrimidines.

ammonium acetate to afford **5b**. Heating **5b** with ammonium sulphate in triethyl orthoformate afforded the imino ether, and this could be converted to the amidine by addition of 2 M ammoniacal ethanol as with **5a**. These amidines were then heated with sodium methoxide in THF/MeOH to obtain the desired **12**.

The less reactive anilines reacted with **9** in poor yield and only after deprotonation with KH (Fig. 6). The reaction was sometimes found to be accelerated upon addition of diglyme or moderate heating.

Sodium alkoxides reacted cleanly with sulphide **8** to give ethers **14** (Fig. 6). Propyl ether **14a** was prepared by adding the sulphide to a solution of sodium metal in propanol and then refluxing for 2.5 h. Cyclohexyl ether **14b** was obtained by dissolving sodium hydride and cyclohexanol into THF, adding a DMSO solution of **8**, and then refluxing overnight. As expected, phenol reacted with sulphone **9** in the presence of KH and in good yield, providing **15**.

3. Results and discussion

Compounds **10a**–**g**, **12a**–**i**, **13a**–**e**, **14a**–**c**, and **15** were converted to a hydrochloride salt and tested for inhibition of AK in both enzyme and intact (IMR) cell assays

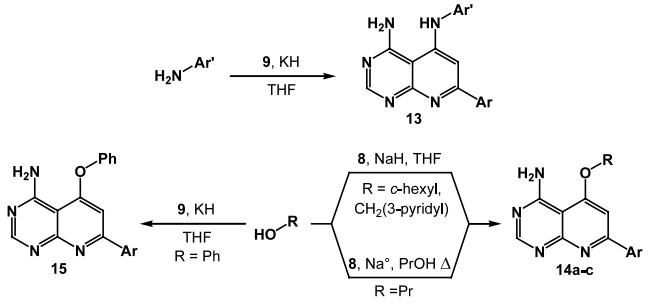


Fig. 6. The synthesis of anilines **13** and ethers **14** and **15**.

by methods previously described (Tables 1 and 2) [7]. The whole cell assay was conducted to ascertain whether the compounds were able to penetrate cell membranes and inhibit AK intracellularly.

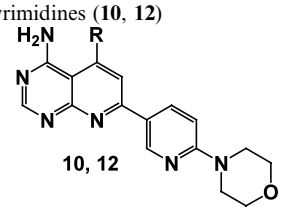
The biological data for series **10** and **12** reveal a general trend among those compounds found potent in both assays: They possess the same type of moderately large hydrophobic substituents found in the lead compounds. For example, an increase in the size of the substituent ring in **12a**–**d** from five to eight enhances potency at the cytosolic AK from 81 to 4 nM, and in the whole cell assay from 560 to 32 nM; however, compound **12e**, which possesses a larger substituent, has a substantially lesser potency. This preference for moderately-sized groups carries over to monosubstituted derivatives **10a** and **10b**, where the larger **10b** is more potent than its smaller analog. An apparent exception in this trend is **10c**, anomalously less potent than **10a**, with a similar trend from **10f** to **10g**, **10h** to **10i**, and **12d** to **12f**. One explanation consistent with these data is that the extra degrees of freedom in the larger systems lower affinity for the enzyme.

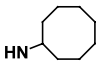
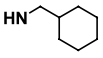
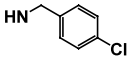
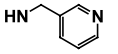
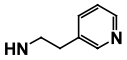
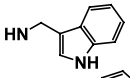
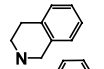
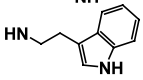
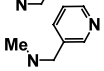
Arylamines **13** were all found to be either comparable or inferior to compounds **10** and **12**, and at least appear to favour unsubstituted rings and disubstitution of the nitrogen (Table 2). 2-trifluoromethylaniline **13b** is less potent than aniline **13a** in both assays, with an IC_{50} of 270 nM vs. 83 nM in broken cells and an IC_{50} of 650 nM vs. 340 nM in whole cells. Disubstituted anilines **13d-e** showed approximately two-fold greater affinities for AK than the parent **13a**, and were also considerably more soluble than **13b**. The indoline **13e** was even soluble to the extent of 100 mg mL^{-1}.

Few ethers were prepared, but it was found that both aliphatic and aromatic ethers could be potent AK antagonists with moderate solubility (Table 2). Cyclohexyl **14b** is ten-fold more potent at AK than the smaller propyl **14a**, as would be expected from the trend among the amines. But although the potency of ethers **14b** and **15** were significantly greater than their nitrogen analogs, **14c** binds much more poorly than does amine **10f** (140 nM vs 9 nM in broken cells and >2000 nM vs 220 nM in the whole cell assay).

A number of these new AK inhibitors were assayed in a carrageenan-induced thermal hyperalgesia model of inflammatory pain (Table 3): In this model a dilute saline solution of λ-carrageenan was injected into the plantar surface of a rat's hindpaw. After 2 h radiant heat from a projection bulb was focused on the same paw, and the time elapsed until its quick withdrawal was measured [8]. Withdrawal latencies were increased by four compounds when administered intraperitoneally at 30 μmol kg^{-1} (**10d**, **10f**, **12c**, **13e**). Neither of the ethers tested demonstrated efficacy, but at least one compound from each amine series did. While this may in part be due to the increased solubility of the amines, solubility

Table 1
In vitro activity of substituted 5-alkylamnio-pyridopyrimidines (**10**, **12**)



	R	AK(IC_{50}) (nM)[7]	IMR(IC_{50}) (nM)[7]	Solubility (mg/mL)[a]		R	AK(IC_{50}) (nM)[7]	IMR(IC_{50}) (nM)[7]	Solubility (mg/mL)[a]
10a[c]		46	1000	8	**12a**[c]		81	560	100
10b[c]		33	120	20	**12b**[b]		16	160	> 100
10c[c]		120	410	> 50	**12c**[c]		10	76	> 100
10d[d]		11	160	---	**12d**[b]		4	32	> 100
10e[b]		3	43	> 200	**12e**[b]		180	370	---
10f[e]		9	220	> 200	**12f**[b]		47	450	< 5
10g[d]		52	520	---	**12g**[b]		28	130	< 1
10h[f]		10	370	---	**12h**[b]		9	45	> 100
10i[f]		23	930	---	**12i**[c]		8	400	100

[a] In water at room temp. [b] 2 HCl salt. [c] 3 HCl salt. [d] 4 HCl salt. [e] 5 HCl salt. [f] free base.

and in vitro potency in themselves could not guarantee in vivo activity (e.g., **10e**, **12h**).

In summary, we were successful in the preparation of compounds which possessed significant in vitro and in vivo activity (e.g., **10f** carrageenan-induced inflammatory hyperalgesia $ED_{50} = 10$ μmol kg^{-1} i.p.), and displayed high solubility (e.g., **10f** > 200 mg mL^{-1}).

4. Experimental protocols

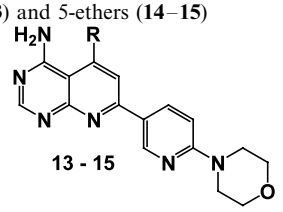
^{1}H-NMR spectra were measured on 300 MHz spectrometers, using tetramethylsilane as internal reference; chemical shifts are expressed in ppm. Elemental combustion analyses, carried out on each of the final compounds as either the free base or solvated salt, were obtained for C, H, and N from Robertson Microlit Laboratories, Inc., and were within ±0.4% of the theoretical values. Column chromatography was carried out on EM Science silica gel 66 (230–400 mesh).

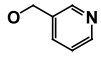
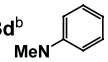
*4.1. 6-Amino-4-methylsulfanyl-6′-morpholin-4-yl-[2,3′]bipyridinyl-5-carbonitrile (**5a**)*

2-(Bis-methylsulfanyl-methylene)-malononitrile (**6**) (851 mg, 5.00 mmol), 1-(6-morpholin-4-yl-pyridin-3-yl)-ethanone (**3**) (1031 mg, 5.00 mmol), and sodium *tert*-butoxide (961 mg, 10.0 mmol) were stirred together in DMSO (5 mL) for 1 h. The mixture was diluted with water (40 mL) and acidified by dropwise addition of acetic acid. The solids were collected by filtration, dissolved into methanol–dichloromethane, concentrated to an oil and triturated from dichloromethane with ether. The resulting tan precipitate was collected by filtration, washed with additional dichloromethane–ether, and dried under vacuum (738 mg).

This intermediate (731 mg) and ammonium acetate (1050 mg, 13.6 mmol) were then suspended into a mixture of acetic acid (5 mL) and dichloroethane (15 mL), and heated at 95 °C for 4 h. Additional ammonium acetate (150 mg, 1.9 mmol) was added, and the mixture was heated at 100 °C for another hour, cooled

Table 2
In vitro activity of 5-arylamino-pyridopyrimidines (**13**) and 5-ethers (**14**–**15**)



	R	AK(IC_{50}) (nM)[7]	IMR(IC_{50}) (nM)[7]	Solubility (mg/mL)[a]		R	AK(IC_{50}) (nM)[7]	IMR(IC_{50}) (nM)[7]	Solubility (mg/mL)[a]
13a[c]		83	340	---	**14a**[b]		40	---	15
13b[b]		270	650	< 2	**14b**[b]		4	95	10
13c[d]		210	380	---	**14c**[d]		140	>2000	---
13d[b]		20	230	15	**15**[c]		11	96	---
13e[b]		39	160	100					

[a] In water at room temperature. [b] 2 HCl salt. [c] 3 HCl salt. [d] 4 HCl salt.

to room temperature and partitioned between dichloromethane and sufficient saturated aqueous sodium bicarbonate to keep the aqueous phase basic to litmus. The aqueous phase and insoluble solids were extracted with dichloromethane (4 ×) and the remaining solids were discarded. The combined organic phases were dried (Na_2SO_4) and concentrated to give the title compound as a yellow solid (538 mg, 33% yield). ^{1}H-NMR (DMSO-d_6): δ 8.90 (d, $J = 3$ Hz, 1H), 8.23 (dd, $J = 9$ Hz/3 Hz, 1H), 6.98 (s, 1H), 6.93 (d, $J = 9$ Hz, 1H), 6.83 (s, 2H), 3.71 (m, 4H), 3.57 (m, 4H), 2.65 (s, 3H).

Table 3
In vivo activity of selected pyridopyrimidines

Compound	Rat carrageenan hyperalgesia ED_{50} (μmol kg^{-1}), i.p. [a]
10d	30 *
10e	NS@30 [b]
10f	10 **
12c	30 *
12h	NS@30
13d	> 30 **
13e	30 **
14b	NS@30 [c]
15	NS@30

[a] Values were obtained with six animals at each dose. ED_{50}s were calculated as the dose giving half maximal effect.
[b] Not significant at 30 μmol kg^{-1}.
[c] Compound administered subcutaneously.
* $P < 0.01$.
** $P < 0.05$.

*4.2. 5-Methylsulfanyl-7-(6-morpholin-4-yl-pyridin-3-yl)-pyrido[2,3-d]pyrimidin-4-ylamine (**8**)*

6-Amino-4-methylsulfanyl-6′-morpholin-4-yl-[2,3′]bipyridinyl-5-carbonitrile (**5a**) (535 mg, 1.63 mmol) and ammonium sulphate (40 mg, 0.3 mmol) were suspended in triethyl orthoformate (10 mL) and heated at 140 °C for 1 h. The mixture was cooled to room temperature, treated with 2 M ammonia in ethanol (5 mL, 10 mmol), and stirred for 5 h. Hexanes (5 mL) was added and the resulting yellow powder was collected by filtration and rinsed with ether (462 mg).

This intermediate was then suspended into *o*-dichlorobenzene (6 mL), heated at 160 °C for 3.5 h, and cooled to room temperature. 50% Ether in dichloromethane (6 mL) was added and the resulting brown solid was collected by filtration. The filtrate was concentrated and diluted with ether (5 mL) to provide a second crop of product. A third crop was obtained by trituration with hexanes. The combined crude solids were combined (407 mg, 70%) and used without further purification. ^{1}H-NMR (DMSO-d_6): δ 9.06 (d, $J = 2$ Hz, 1H), 8.43 (s, 1H), 8.42 (dd, $J = 9$ Hz/2 Hz, 1H), 7.67 (s, 1H), 7.64 (bs, 2H), 6.99 (d, $J = 9$ Hz, 1H), 3.73 (m, 4H), 3.62 (m, 4H), 2.83 (s, 3H).

*4.3. 5-Methanesulfonyl-7-(6-morpholin-4-yl-pyridin-3-yl)-pyrido[2,3-d]pyrimidin-4-ylamine (**9**)*

5-Methylsulfanyl-7-(6-morpholin-4-yl-pyridin-3-yl)-pyrido[2,3-d]pyrimidin-4-ylamine (**8**) (404 mg, 1.14

mmol) was dissolved into a mixture of dichloromethane (25 mL) and methanol (20 mL). Saturated aqueous sodium bicarbonate (5 mL) and a solution of Oxone® (1400 mg, 2.28 mmol) in water (10 mL) were added sequentially, and the resulting heterogeneous mixture was vigorously stirred for 25 min before being partitioned between chloroform and water. The mixture was worked up as usual to yield a crude yellow–brown powder (328 mg, 74%) which was used without further purification. ^{1}H-NMR (DMSO-d_6): δ 9.05 (d, $J = 2$ Hz, 1H), 8.60 (s, 1H), 8.42 (dd, $J = 9$ Hz/2 Hz, 1H), 8.37 (s, 1H), 8.33 (bs, 2H), 7.04 (d, $J = 9$ Hz, 1H), 3.72 (m, 4H), 3.66 (m, 4H), 3.64 (s, 3H).

4.4. Representative procedure for the preparation of monosubstituted amines

4.4.1. N^5-Cyclohexyl-7-(6-morpholin-4-yl-pyridin-3-yl)-pyrido[2,3-d]pyrimidine-4,5-diamine (**10a**)

5-Methanesulfonyl-7-(6-morpholin-4-yl-pyridin-3-yl)-pyrido[2,3-d]pyrimidin-4-ylamine (**9**) (270 mg, 0.70 mmol) and cyclohexylamine (400 μL, 3.50 mmol) were dissolved into DMSO (7 mL) and heated at 100 °C for 40 min, then at 130 °C for another hour. The reaction mixture was cooled to room temperature, partitioned between dichloromethane and water, and worked up as usual. The organic phases were concentrated and chromatographed on silica with a gradient of 2–8% methanol–dichloromethane to give a yellow powder (131 mg, 46%). ^{1}H-NMR ($CDCl_3$): δ 8.69 (d, $J = 2$ Hz, 1H), 8.10 (m, 1H), 7.55 (bs, 1H), 6.67 (d, $J = 7$ Hz, 1H), 6.62 (s, 1H), 3.83 (m, 4H), 3.60 (m, 4H), 3.49 (m, 1H), 2.04 (m, 2H), 1.79 (m, 2H), 1.66 (m, 1H), 1.25–1.50 (m, 5H).

4.5. Representative procedure for the preparation of disubstituted amines

4.5.1. 6′Amino-6″-morpholin-4-yl-3,4,5,6-tetrahydro-2H-[1,4′;2′,3″]terpyridine-5′-carbonitrile (**5b**, RNR′ = piperidinyl)

2-(Bis-methylsulfanyl-methylene)-malononitrile (**6**) (3400 mg, 20 mmol) and piperidine (2.0 mL, 20 mmol) were refluxed in dichloromethane (80 mL) for 90 min. The reaction mixture was brought to room temperature and concentrated under vacuum to provide **11** (4030 mg), which was used without further purification.

This intermediate, 1-(6-morpholin-4-yl-pyridin-3-yl)-ethanone (**3a**) (3090 mg, 15.0 mmol), and lithium *tert*-butoxide (2400 mg, 30.0 mmol) were dissolved into DMSO (30 mL) and stirred at room temperature for 2.5 h. Ammonium acetate (11.56 g, 150 mmol) and acetic acid (80 mL) were added and the resulting mixture was refluxed for 3 h, cooled to room temperature, partitioned between 1 M aqueous sodium hydroxide (1 L) and dichloromethane, and worked up as usual. The organic phases were concentrated and chromatographed on silica with 50% ethyl acetate–hexanes to give the title compound (4130 mg, 73%). ^{1}H-NMR (DMSO-d_6): δ 8.82 (d, $J = 2$ Hz, 1H), 8.18 (dd, $J = 9$ Hz/2 Hz, 1H), 6.89 (d, $J = 9$ Hz, 1H), 6.63 (s, 1H), 6.46 (bs, 2H), 3.71 (m, 4H), 3.54 (m, 4H), 3.39 (m, 4H), 1.62 (m, 6H).

4.5.2. 7-(6-Morpholin-4-yl-pyridin-3-yl)-5-piperidin-1-yl-pyrido[2,3-d]pyrimidin-4-ylamine (**12b**)

6′-Amino-6″-morpholin-4-yl-3,4,5,6-tetrahydro-2H-[1,4′;2′,3″]terpyridine-5′-carbonitrile (**5b**, RNR′ = piperidinyl) (3740 mg, 10.26 mmol) and ammonium sulphate (300 mg, 2.3 mmol) were refluxed in triethyl orthoformate (100 mL) for 3 h. The reaction mixture was cooled to room temperature, treated with 2.0 M ammonia in ethanol, and stirred for 3 h. Hexanes was added and the resulting powder was collected by filtration and dried under vacuum (3640 mg).

This intermediate was then suspended in THF (25 mL), treated with 1 M sodium methoxide in methanol (50 mL), refluxed for 2.5 h, and cooled to room temperature. The reaction mixture was neutralized with 1 M aqueous HCl and extracted with dichloromethane. The combined extractions were dried ($MgSO_4$), concentrated, and chromatographed with a gradient of 2.5–5% (5% aqueous ammonium hydroxide in ethanol)/dichloromethane to afford the title compound (3360 mg, 83%). ^{1}H-NMR (DMSO-d_6): δ 9.02 (d, $J = 3$ Hz, 1H), 8.40 (dd, $J = 9$ Hz/3 Hz, 1H), 8.38 (s, 1H), 8.08 (s, 1H), 7.96 (s, 1H), 7.54 (s, 1H), 6.97 (d, $J = 9$ Hz, 1H), 3.72 (m, 4H), 3.60 (m, 4H), 3.32 (m, 2H), 2.81 (m, 2H), 1.65–1.88 (m, 6H).

4.6. Representative procedure for the preparation of anilines

4.6.1. 7-(6-morpholin-4-yl-pyridin-3-yl)-N^5-phenyl-pyrido[2,3-d]pyrimidine-4,5-diamine (**13a**)

5-Methanesulfonyl-7-(6-morpholin-4-yl-pyridin-3-yl)-pyrido[2,3-d]pyrimidin-4-ylamine (**9**) (270 mg, 0.7 mmol) and aniline (320 μL, 3.5 mmol) were suspended in THF (7 mL) and treated with potassium hydride in mineral oil (ca. 100 mg, 2.5 mmol). After 40 min the reaction mixture was partitioned between pH 6 aqueous potassium phosphate buffer (10 mL) and dichloromethane, and worked up as usual. The organic phases were concentrated and chromatographed on silica with a gradient of 2–6% methanol–dichloromethane to give an impure yellow powder (119 mg, 42%) which could be recrystallized from ethyl acetate–chloroform. ^{1}H-NMR ($CD_3OD/CDCl_3$): δ 8.35 (d, $J = 2$ Hz, 1H), 8.16 (s, 1H), 7.69 (m, 1H), 7.37 (dd, $J = 8$ Hz/8 Hz, 2H), 7.11 (t, $J = 8$ Hz, 1H), 7.00 (d, $J = 8$ Hz, 2H), 6.67 (d, $J = 9$ Hz, 1H), 6.35 (bs, 1H), 3.82 (m, 4H), 3.58 (m, 4H).

Table 4
1H NMR data for compounds **10**, **12**–**15**

10a ($CDC1_3$) δ 1.25–1.50 (m, 5H), 1.66 (m, 1H), 1.79 (m, 2H), 2.04 (m, 2H), 3.49 (m, 1H), 3.60 (m, 4H), 3.83 (m, 4H), 6.62 (s, 1H), 6.67 (d, $J = 7$ Hz, 1H), 7.55 (s, 1H), 8.10 (m, 1H), 8.69 (d, $J = 2$ Hz, 1H).

10b ($CD_3OD/CDC1_3$) δ 1.50–1.88 (m, 12H), 1.92–2.05 (m, 2H), 3.64 (m, 4H), 3.73 (m, 1H), 3.85 (m, 4H), 6.65 (s, 1H), 6.77 (d, $J = 9$ Hz, 1H), 8.09–8.24 (m, 2H), 8.72 (d, $J = 2$ Hz, 1H).

10c ($CD_3OD/CDC1_3$) δ 1.00–1.39 (m, 5H), 1.65–1.92 (m, 6H), 3.15 (d, $J = 7$ Hz, 2H), 3.61 (m, 4H), 3.84 (m, 4H), 6.70 (s, 1H), 6.75 (d, $J = 9$ Hz, 1H), 8.03–8.26 (m, 2H), 8.75 (d, $J = 2$ Hz, 1H).

10d ($CD_3OD/CDC1_3$) δ 3.61 (m, 4H), 3.83 (m, 4H), 4.57 (s, 2H), 6.61 (s, 1H), 6.72 (d, $J = 9$ Hz, 1H), 7.31–7.35 (m, 4H), 8.05 (dd, $J = 9$ Hz/3 Hz, 1H), 8.12 (s, 1H), 8.59 (d, $J = 3$ Hz, 1H).

10e ($CD_3OD/CDC1_3$) δ 3.64 (m, 4H), 3.84 (mm, 4H), 4.81 (s, 2H), 6.73 (s, 1H), 6.74 (d, $J = 9$ Hz, 1H), 7.00 (dd, $J = 5$ Hz/4 Hz, 1H), 7.09 (dd, $J = 4$ Hz/1 Hz, 1H), 7.26 (dd, $J = 5$ Hz/1 Hz, 1H), 8.04 (m, 1H), 8.20 (s, 1H), 8.63 (d, $J = 2$ Hz, 1H).

10f ($CD_3OD/CDC1_3$) δ 3.62 (m, 4H), 3.84 (m, 4H), 4.68 (s, 2H), 6.56 (s, 1H), 6.73 (d, $J = 9$ Hz, 1H), 7.37 (dd, $J = 8$ Hz/5 Hz, 1H), 7.84 (ddd, $J = 8$ Hz/2 Hz/2 Hz, 1H), 7.94 (dd, $J = 9$ Hz/3 Hz, 1H), 8.14 (s, 1H), 8.50 (dd, $J = 5$ Hz/2 Hz, 1H), 8.58 (d, $J = 3$ Hz, 1H), 8.64 (d, $J = 2$ Hz, 1H).

10g ($CD_3OD/CDC1_3$) δ 3.11 (t, 2H), 3.60–3.68 (m, 6H), 3.85 (t, $J = 7$ Hz, 4H), 6.58 (s, 1H), 6.75 (d, $J = 9$ Hz, 1H), 7.32 (m, 1H), 7.69 (m, 1H), 8.04 (dd, $J = 9$ Hz/2 Hz, 1H), 8.10 (s, 1H), 8.45 (m, 1H), 8.51 (d, $J = 2$ Hz, 1H), 8.66 (d, $J = 2$ Hz, 1H).

10h (Acetone/DMSO-d_6) δ 3.48 (m, 4H), 3.63 (m, 4H), 4.68 (s, 2H), 6.75 (d, $J = 6$ Hz, 1H), 6.91 (t, $J = 6$ Hz, 1H), 7.01 (m, 2H), 7.30 (d, $J = 6$ Hz, 1H), 7.35 (s, 1H), 7.60 (d, $J = 6$ Hz, 1H), 7.80 (s, 1H), 8.24 (dd, $J = 9$ Hz/3 Hz, 1H), 8.88 (d, $J = 3$ Hz, 1H).

10i (Acetone-d_6) δ 3.18 (t, $J = 6$ Hz, 2H), 3.55 (m, 4H), 3.72 (m, 6H), 6.79 (d, $J = 9$ Hz, 1H), 6.85 (s, 1H), 7.00 (m, 1H), 7.08 (m, 1H), 7.28 (s, 1H), 7.35 (d, $J = 9$ Hz, 1H), 7.54 (d, $J = 9$ Hz, 1H), 7.95 (bs, 1H), 8.13 (d, $J = 9$ Hz, 1H), 8.84 (s, 1H).

12b (DMSO-d_6) δ 1.65–1.88 (m, 6H), 2.81 (m, 2H), 3.32 (m, 2H), 3.60 (m, 4H), 3.72 (m, 4H), 6.97 (d, $J = 9$ Hz, 1H), 7.54 (s, 1H), 7.96 (s, 1H, NH_2), 8.08 (s, 1H,NH_2), 8.38 (s, 1H), 8.40 (dd, $J = 9$ Hz/3 Hz, 1H), 9.02 (d, $J = 3$ Hz, 1H).

12c (DMSO-d_6) δ 1.59–1.72 (m, 4H), 1.72–1.84 (m, 4H), 3.3–3.52 (m, 4H), 3.59 (m, 4H), 3.72 (m, 4H), 6.97 (d, $J = 9$ Hz, 1H), 7.51 (s, 1H), 7.95 (s, 2H, NH_2), 8.32 (s, 1H), 8.37 (dd, $J = 9$ Hz/2 Hz, 1H), 8.99 (d, $J = 2$ Hz, 1H).

12d (DMSO-d_6) δ 1.54–1.66 (m, 6H), 1.66–1.79 (m, 4H), 3.35–3.58 (m, 4H), 3.58 (m, 4H), 3.72 (m, 4H), 6.97 (D, $J = 9$ Hz, 1H), 7.54 (s, 1H), 7.91 (s, 2H, NH_2), 8.30 (s, 1H), 8.38 (dd, $J = 9$ Hz/3 Hz, 1H), 8.99 (d, $J = 3$ Hz, 1H).

12e (DMSO-d_6) δ 0.90 (t, $J = 7$ Hz, 3H), 1.24–1.49 (m, 7H), 1.80–1.90 (m, 2H), 2.73–2.86 (m, 2H), 3.29–3.4 (m, 2H), 3.59 (m, 4H), 3.72 (m, 4H), 6.98 (d, $J = 9$ Hz, 1H), 7.55 (s, 1H, NH_2), 8.38 (s, 1H) 8.40 (dd, $J = 9$ Hz/2 Hz, 1H), 9.02 (d, $J = 2$Hz, 1H).

12f (DMSO-d_6) δ 0.83 (t, $J = 7$ Hz, 6H), 1.15–1.30 (m, 4H), 1.30–1.58 (m, 4H), 2.98–3.17 (m, 2H), 3.28–3.46 (m, 2H), 3.60 (m, 4H), 3.73 (m, 4H), 6.99 (d, $J = 9$ Hz, 1H), 7.66 (s, 1H), 8.03 (s, 1H, NH_2), 8.32 (s, 1H, NH_2), 8.36 (s, 1H), 8.40 (dd, $J = 9$ Hz/2 Hz, 1H), 9.02 (d, $J = 2$ Hz, 1H).

12g (DMSO-d_6) δ 1.04–1.96 (m, 10H), 2.82 (s, 3H), 3.12 (m, 1H), 3.59 (m, 4H), 3.72) (m, 4H), 6.98 (d, $J = 9$ Hz, 1H), 7.61 (s, 1H), 7.95 (s, 1H, NH_2), 8.26 (s, 1H, NH_2), 8.36 (s, 1H), 8.40 (dd, $J = 9$ Hz/2 Hz, 1H), 9.02 (d, $J = 2$ Hz, 1H).

12h (DMSO-d_6) δ 2.92 (m, 1H), 3.21 (m, 1H), 3.57–3.78 (m, 10H), 4.26 (d, $J = 15$ Hz, 1H), 4.42 (d, $J = 15$ Hz, 1H), 6.99 (d, $J = 9$ Hz, 1H), 7.22 (s, 4H), 7.64 (s, 1H), 7.96 (s, 2H, NH_2), 8.40 (dd, $J = 9$ Hz/2 Hz, 1H), 8.41 (s, 1H), 9.02 (d, $J = 2$ Hz, 1H).

12i (DMSO-d_6) δ 2.81 (s, 3H), 3.60 (m, 4H), 3.72 (m, 4H), 4.35 (d, $J = 14$ Hz, 1H), 4.58 (d, $J = 14$ Hz, 1H), 6.97 (d, $J = 9$ Hz, 1H), 7.31 (dd, $J = 8$ Hz/5 Hz, 1H), 7.53 (s, 1H), 7.71 (ddd, $J = 8$ Hz/2 Hz/2 Hz, 1H), 8.11 (s, 1H, NH_2), 8.26 (s, 1H, NH_2), 8.34 (dd, $J = 9$ Hz/3 Hz, 1H) 8.38 (s, 1H), 8.14 (dd, $J = 5$ Hz/2 Hz, 1H), 8.55 (d, $J = 2$ Hz, 1H), 8.95 (d, $J = 2$ Hz, 1H).

13a ($CD_3OD/CDCl_3$) δ 3.58 (m, 4H), 3.82 (m, 4H) 6.35 (s, 1H), 6.67 (d, $J = 9$ Hz, 1H), 7.00 (d, $J = 8$ Hz, 2H), 7.11 (t, $J = 8$ Hz, 1H), 7.37 (dd, $J = 8$ Hz/8 Hz, 2H), 7.69 (m, 1H), 8.16 (s, 1H), 8.35 (d, $J = 2$ Hz, 1H).

13b ($CD_3OD/CDCl_3$) δ 3.58 (m, 4H), 3.82 (m, 4H), 6.10 (s, 1H), 6.67 (d, $J = 9$ Hz, 1H), 7.02 (d, $J = 8$ Hz, 1H), 7.15 (dd, $J = 8$ Hz/8 Hz, 1H), 7.49 (dd, $J = 8$ Hz/8 Hz, 1H), 7.62–7.71 (m, 2H), 8.20 (s, 1H), 8.32 (d, $J = 2$Hz, 1H)

13c ($CD_3OD/CDCl_3$) δ 3.60 (m, 4H), 3.82 (m, 4H), 6.13 (s, 1H), 6.69 (d, $J = 9$ Hz, 1H), 7.40–7.45 (m, 2H), 7.65 (m, 1H), 8.20 (s, 1H), 8.22–8.31 (m, 2H), 8.34 (d, $J = 2$ Hz, 1H).

13d ($CDCl_3$) δ 3.30 (s, 3H), 3.64 (m, 4H), 3.82 (m, 4H), 6.02 (bs, 1H, NH_2), 6.72 (d, $J = 9$ Hz, 1H), 6.97 (d, $J = 8$ Hz, 2H), 7.07 (t, $J = 8$ Hz, 1H), 7.32 (dd, $J = 8$ Hz/8 Hz, 2H), 7.45 (s, 1H), 7.81 (bs, 1H, NH_2), 8.51 (dd, $J = 9$ Hz/3 Hz, 1H), 8.69 (s, 1H), 8.75 (d, $J = 2$ Hz, 1H).

13e (DMSO-d_6) δ 3.04–3.3 (m, 2H), 3.59 (m, 4H), 3.71 (m, 4H), 3.96–4.21 (m, 2H), 6.45 (d, $J = 8$ Hz, 1H), 6.88 (dd, $J = 8$ Hz/8 Hz, 1H), 6.97 (d, $J = 9$ Hz, 1H), 7.00 (dd, $J = 8$ Hz/8 Hz, 1H), 7.29 (d, $J = 8$ Hz, 1H), 7.44 (bs, 1H, NH_2), 7.79 (s, 1H), 7.91 (bs, 1H, NH_2), 8.37 (dd, $J = 9$ Hz/3 Hz, 1H), 8.43 (s, 1H), 8.99 (d, $J = 3$ Hz, 1H).

14a (DMSO-d_6) δ 1.05 (t, $J = 7$ Hz, 3H), 1.92 (qt, $J = 7$ Hz/7 Hz, 2H), 3.59 (m, 4H), 3.72 (m, 4H), 4.40 (t, $J = 7$ Hz, 2H), 6.98 (d, $J = 9$ Hz, 1H), 7.36 (s, 1H, NH_2), 7.50 (s, 1H), 8.06 (s, 1H, NH_2), 8.40 (s, 1H), 8.42 (dd, $J = 9$ Hz/3 Hz, 1H), 9.06 (d, $J = 3$ Hz, 1H).

14b (DMSO-d_6) δ 1.27–1.77 (m, 8H), 2.00–2.11 (m, 2H), 3.59 (m, 4H), 3.72 (m, 4H), 5.08 (m, 1H), 6.98 (d, $J = 9$ Hz, 1H), 7.35 (s, 1H, NH_2), 7.53 (s, 1H), 8.07 (s, 1H, NH_2), 8.40 (s, 1H), 8.42 (dd, $J = 9$ Hz/3 Hz, 1H), 9.06 (d, $J = 3$ Hz, 1H).

14c ($CD_3OD/CDCl_3$) δ 3.67 (m, 4H), 3.86 (m, 4H), 5.47 (s, 2H), 6.80 (d, $J = 9$ Hz, 1H), 7.32 (s, 1H), 7.50 (dd, $J = 8$ Hz/5 Hz, 1H), 7.94 (m, 1H), 8.50 (dd, $J = 9$ Hz/2 Hz, 1H), 8.58 (s, 1H), 8.70 (m, 1H), 8.79 (d, $J = 2$ Hz, 1H), 8.87 (d, $J = 2$ Hz, 1H).

15 ($CD_3OD/CDCl_3$) δ 3.61 (m, 4H), 3.82 (m, 4H), 6.74 (d, $J = 9$ Hz, 1H), 6.86 (s, 1H), 7.28 (d, $J = 8$ Hz, 2H), 7.44 (t, $J = 8$ Hz, 1H), 7.58 (dd, $J = 8$ Hz/8 Hz, 2H), 8.34 (dd, $J = 9$ Hz/3 Hz, 1H), 8.55 (d, $J = 3$ Hz, 1H), 8.59 (s, 1H).

4.7. Representative procedure for the preparation of alkyl ethers

*4.7.1. Cyclohexyloxy-7-(6-morpholin-4-yl-pyridin-3-yl)-pyrido[2,3-d]pyrimidin-4-ylamine (**14b**)*

A mixture of 5-methylsulfanyl-7-(6-morpholin-4-yl-pyridin-3-yl)-pyrido[2,3-d]pyrimidin-4-ylamine (**8**) (177 mg, 0.50 mmol), cyclohexanol (260 μL, 2.5 mmol), and sodium hydride in mineral oil (ca. 70 mg, 3 mmol) was suspended in a mixture of THF (5 ml) and DMSO (3 mL). After the mixture was refluxed overnight, it was cooled to room temperature, diluted with water, filtered, worked up as usual, and concentrated to provide the title compound (144 mg, 70%). ^{1}H-NMR (DMSO-d_6): δ

9.06 (d, $J = 3$ Hz, 1H), 8.42 (dd, $J = 9$ Hz/3 Hz, 1H), 8.40 (s, 1H), 8.07 (bs, 1H), 7.53 (s, 1H), 7.35 (bs, 1H), 6.98 (d, $J = 9$ Hz, 1H), 5.08 (m, 1H), 3.72 (m, 4H), 3.59 (m, 4H), 2.00–2.11 (m, 2H), 1.27–1.77 (m, 8H).

*4.7.2. 7-(6-morpholin-4-yl-pyridin-3-yl)-5-phenoxy-pyrido[2,3-d]pyrimidin-4-ylamine (**15**)*

A suspension of 5-methanesulphonyl-7-(6-morpholin-4-yl-pyridin-3-yl)-pyrido[2,3-d]pyrimidin-4-ylamine (**9**) (386 mg, 1.0 mmol) and phenol (471 mg, 5.0 mmol) in THF (10 mL) was briefly sonicated to affect small particle size. Potassium hydride in mineral oil (ca. 200 mg, 5 mmol) was added to the rapidly stirred suspension. Bubbling quickly ensued. After 15 min the reaction mixture was partitioned between pH 6 aqueous potassium phosphate buffer (40 mL) and dichloromethane (40 mL), and worked up as usual. The organic phases were concentrated and chromatographed on silica with a gradient of 0–6% methanol–dichloromethane to give the title compound as an impure yellow powder (263 mg, 65%) which could be recrystallized from ethyl acetate–chloroform. ^{1}H-NMR ($CD_3OD/CDCl_3$): δ 8.59 (s, 1H), 8.55 (d, $J = 3$ Hz, 1H), 8.34 (dd, $J = 9$ Hz/3 Hz, 1H), 7.58 (dd, $J = 8$ Hz/8 Hz, 2H), 7.44 (t, $J = 8$ Hz, 1H), 7.28 (d, $J = 8$ Hz, 2H), 6.86 (s, 1H), 6.74 (d, $J = 9$ Hz, 1H), 3.82 (m, 4H), 3.61 (m, 4H) (Table 4).

References

[1] M. Williams, M.F. Jarvis, Biochem. Pharmacol. 59 (2000) 1173.
[2] G.W. Bong, S. Rosengren, G.J. Firestein, Clin. Invest. 98 (1996) 2779.
[3] E.A. Kowaluk, M.F. Jarvis, Exp. Opin. Invest. Drug 9 (2000) 551.
[4] P. Marangos, Med. Hypotheses 32 (1990) 45.
[5] K. Mullane, M. Young, Drug Dev. Res. 23 (1993) 336.
[6] J.R.S. Arch, E.A. Newsholme, Essays Biochem. 14 (1978) 82.
[7] M.F. Jarvis, H. Yu, K. Kohlhaas, K. Alexander, C.-H. Lee, M. Jiang, S.S. Bhagwat, M. Williams, E.A. Kowaluk, J. Pharmacol. Exp. Ther. 295 (2000) 1156.
[8] E.A. Kowaluk, J. Mikusa, C.T. Wismer, C.Z. Zhu, E. Schweitzer, J.J. Lynch, C.-H. Lee, M. Jiang, S.S. Bhagwat, A. Gomtsyan, J. McKie, B.F. Cox, J. Polakowski, G. Reinhart, M. Williams, M.F. Jarvis, J. Pharmacol. Exp. Ther. 295 (2000) 1165.
[9] M. Cowart, C.-H. Lee, G.A. Gfesser, E.K. Bayburt, S.S. Bhagwat, A.O. Stewart, H. Yu, K.L. Kohlhaas, S. McGaraughty, C.T. Wismer, J. Mikusa, C. Zhu, K.M. Alexander, M.F. Jarvis, E.A. Kowaluk, Bioorg. Med. Chem. 11 (2001) 83.
[10] C.-H. Lee, M. Jiang, M. Cowart, G. Gfesser, R. Perner, K.H. Kim, Y.G. Gu, M. Williams, M.F. Jarvis, E.A. Kowaluk, A.O. Stewart, S.S. Bhagwat, J. Med. Chem. 44 (2001) 2133.
[11] Y. Tominaga, M. Kawabe, A. Hosomi, J. Heterocyclic Chem. 24 (1987) 1325.