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Imidazole pyrimidine amides as potent, orally bioavailable cyclin-dependent kinase inhibitors

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ABSTRACT

The development of a novel series of imidazole pyrimidine amides as cyclin-dependent kinase (CDK) inhibitors is described. The series was found to have much improved CDK2 inhibition and potent in vitro anti-proliferative effects against cancer cell lines. Control of overall lipophilicity was important to achieve good in vitro potency along with acceptable physiochemical properties and margins against inhibition of both CYP isoforms and the hERG potassium ion channel. A compound with an attractive overall balance of properties was profiled in vivo and possessed suitable physiochemical and pharmacokinetic profiles for oral dosing.

The cyclin-dependent kinase (CDK) family is a group of serine–threonine protein kinases with roles in the coordination of the eukaryotic cell cycle and transcriptional regulation. The proper regulation of CDKs involved in the cell cycle is crucial for the ordered execution of each phase of the cycle. CDKs are initially activated by formation of a complex with a cyclin partner protein, followed by phosphorylation to yield the fully active complex. The transient expression of the cyclin partner proteins at specific stages of the cell cycle allows the normal process of cell division to proceed under strict control.

A characteristic of cancer is uncontrolled cell growth and proliferation and most cancer cells show deregulation of CDKs. Extensive profiling of tumour tissue has repeatedly identified components of the CDK machinery that are altered in cancer. This commonly occurs through amplification of cyclin effectors such as cyclins D and E, the inactivation of endogenous inhibitors such as p16 and p27, or genetic mutations to CDK substrates. Because of their critical role in the regulation of the cell cycle and the observed expression/activity pattern in most human cancers, considerable effort has been focused on the development of small molecule CDK inhibitors as potential therapeutic agents.[1]

As a result, a number of CDK inhibitors, such as flavopiridol **1**, roscovitine (CYC202) **2**, BMS-387032 (SNS-032) **3** and R547 **4** have progressed into clinical trials for the treatment of cancer (Fig. 1). Recently, flavopiridol was demonstrated to have activity in patients with refractory chronic lymphocytic leukaemia (CLL).[2]

Our own efforts in this area began with the imidazo[1,2-*a*]pyridine (**5**, A = CH) and imidazo[1,2-*b*]pyridazine (**5**, A = N) CDK inhibitors which were described in earlier letters (Fig. 2).[3–5]

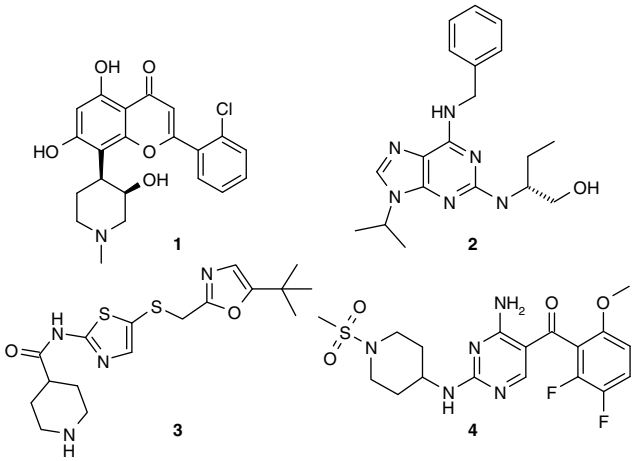

Figure 1. CDK inhibitors tested in clinical trials.

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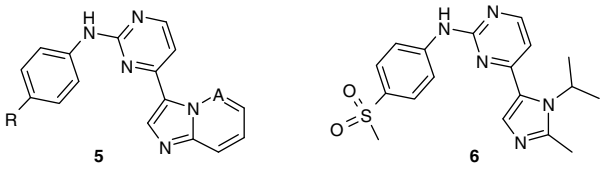


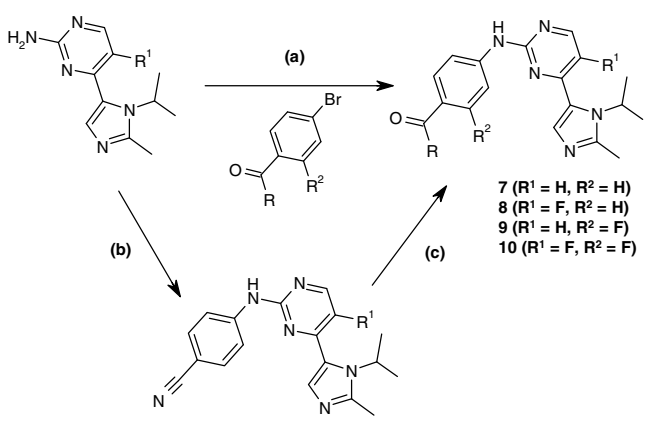
Figure 2. Previous AstraZeneca CDK inhibitor series.

Improvements in physical properties and cellular potency were achieved and led to the discovery of the imidazole sulphone AZD5438 **6**, which was investigated further as an orally bioavailable anti-cancer agent.[6]

The replacement of the sulphone in **6** with a piperazine ring resulted in a new series of potent CDK inhibitors with further improvements in physical properties, which were suitable for oral dosing.[7] We decided to investigate if there were other groups that would provide suitable replacements for sulphone. Amide linkers gave an attractive lipophilicity range and would also be able to form similar protein–ligand interactions that were key for potency and selectivity in the sulphonamide and sulphone series. Using the routes shown in Scheme 1, an initial set of alkyl amides (**7a**–**e**) were synthesised in the imidazole-5*H*-pyrimidine series (Table 1).

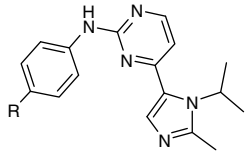
Excellent levels of enzyme potency against CDK2 were observed for the simple primary, secondary and tertiary amides **7a**–**c**, which translated into good anti-proliferative effects in LoVo cancer cells. Encouragingly, the levels of potency observed were similar to, or exceeded, the clinical candidate AZD5438 **6**. Larger alkyl groups generally gave lower levels of CDK2 potency **7d**. The targeting of enzyme solvent exposed regions with hydrophilic groups is commonly used in kinase inhibitors to improve physical properties without impacting potency. Incorporation of a hydrophilic amide substituent **7e** retained good enzyme activity but with reduced anti-proliferative cellular potency compared to **7a–d**, due to lower cellular permeability (data not shown). Generally, low levels of activity against the hERG potassium ion channel were observed for the less lipophilic examples and overall offered a significant improvement in hERG binding over AZD5438 **6**. As a number of these initial examples also possessed excellent levels of solubility with no significant CYP inhibition,[9] we were encouraged to explore the series further.

The routes used to synthesise imidazole pyrimidine amides are shown in Scheme 1. Palladium catalysed coupling of the previously reported 4-imidazoyl-2-aminopyrimidines (R^1 = F or H)[7,10] with suitably substituted 4-bromobenzamides under Buchwald–Hartwig conditions gave the desired products in good to excellent yield. The required 4-bromobenzamides were readily obtained by reacting the corresponding acid and amine together using standard amide coupling conditions. This route was generally applicable, except for the primary amides (**7a** and **8a**), which gave exclusively bis-arylation of the aminopyrimidine in the palladium catalysed coupling step. To avoid these problems a two-step process was used, first coupling 4-bromobenzonitrile, then hydrolysing the nitrile to the primary amide under basic conditions to give **7a** and **8a**.



Scheme 1. Synthesis of imidazole amides **7**–**10**. Reagents and conditions: (a) $Pd(OAc)_2$, Xantphos, Cs_2CO_3, 1,4-dioxane, 75–83%; (b) 4-bromobenzonitrile, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, 1,4-dioxane, 81%; (c) KOH, EtOH, water, 53%.

Table 1
CDK inhibition profile for compounds **7a**–**e**.

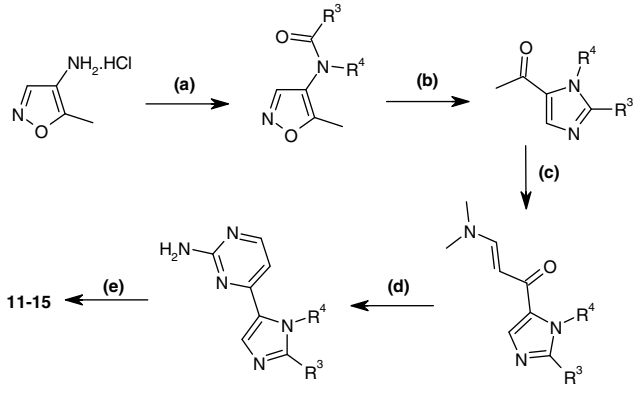


Compound	R	CDK2 IC_{50} (μM)	LoVo IC_{50} (μM)[a]	hERG IC_{50} (μM)[b]	Solubility[c] (μM)
6	$-SO_2Me$	<0.006	0.80	3.2	588[d]
7a	$-CONH_2$	0.003	0.70	17	140
7b	–CONHMe	0.003	0.47	>30	540
7c	$-CONMe_2$	0.008	0.48	18	>2300
7d	$-CONH^cPr$	0.017	0.60	7	70
7e	$-CONHC_2H_4OH$	0.002	5.7	>32	1600

[a] IC_{50} for inhibition of BrdU incorporation to LoVo cells following 48 h exposure to test compound.
[b] hERG patch-clamp assay.[8]
[c] Aqueous equilibrium solubility measured over 24 h.
[d] Mesylate salt.

As the methylamide **7b** had an attractive balance of potency and physical properties, we decided to maintain this amide substituent whilst exploring substitution around the imidazole ring.

Variation of the imidazole substitution to obtain compounds **11**–**15** was synthetically more challenging and used the routes shown in Scheme 2. The substituent R^4 was introduced by reductive amination of 5-methylisoxazole-4-amine, followed by amide bond formation to incorporate the substituent R^3. Hydrogenolysis of the isoxazole followed by base-assisted cyclisation gave suitably substituted imidazole methyl ketones. Treatment with DMF–DMA gave the aminopropanones which on cyclisation with guanidine carbonate gave the corresponding 2-aminopyrimidine imidazoles. These were coupled with 4-bromobenzamides using similar



Scheme 2. Synthesis of substituted imidazole aminopyrimidines **11**–**15**. Reagents and conditions: (a) i—R^4 = ^{c}Pentyl: cyclopentanone, $NaCNBH_3$, MeOH, 69%; R^4 = ^{i}Pr: acetone, NaOAc, $NaCNBH_3$, AcOH, MeOH, 59%; ii—R^3 = ^{c}Pr: cyclopropylcarbonyl chloride, NEt_3, DCM, 40%; R^3 = Me: Ac_2O, NaOAc, AcOH, 91%; (b) i—H_2, 4 atm, 10% Pd/C, EtOH, 44–84%; ii—NaOH, 1,4-dioxane, 54–68%; (c) DMF–DMA, DMF, 75–96%; (d) guanidine carbonate, BuOH, 81–83%; (e) $Pd(OAc)_2$, Xantphos, Cs_2CO_3, 1,4-dioxane, 33–71%.

conditions to those shown in Scheme 1 to give the substituted imidazole amides (**11–15**).

The importance of an α-branched substituent in the R^4 position is demonstrated by the reduction in potency moving from isopropyl (**7b**, Table 1) to ethyl substitution (**11,** Table 2). Larger groups such as **12** did not show any improvements in potency whereas the α-branched cyclopentyl **13** returned potency back to low nanomolar levels. These observations can be attributed to α-branched substituents providing an appropriately sized lipophilic group to give optimal hydrophobic contacts with the glycine-rich loop around the ATP-ribose binding domain.

Structural studies indicated that larger groups than methyl might be tolerated at the imidazole R^3 position. This potential alternative route to the solvent exposed region was attractive as it would give additional options to modify the physical properties of the series. In addition, it offered the opportunity to block a potential site of metabolism and modulate pharmacokinetic (PK) properties. However, we were surprised to find that substitution at the R^3 position, even with comparatively small groups (**14** and **15**), led to a relatively large reduction in CDK2 potency. These data indicated that the combination of (R^4 = *^{i}Pr and* R^3 = Me) gave the optimal balance of CDK inhibition and physical properties in this series, so we retained this R^3/R^4 combination to explore variation at other positions.

The potency benefits of substituting the central pyrimidine core with fluorine has been reported previously.[7] This is believed to be due to a stronger interaction with the hinge region of the protein through acidification of the aniline N–H by the electronegative fluorine atom. Consequently, the 5-fluoropyrimidine benzamides (R^1 = F) (Table 3) were attractive targets and they were synthesised using the routes shown in Scheme 1.

Comparing each 5-H pyrimidine (**7a–c**, Table 1) with the equivalent 5-F pyrimidine **8a–c** shows a small but significant increase in enzyme potency against CDK2. These potency increases, along with improved cellular permeability through increased lipophilicity, gave larger improvements in anti-proliferative activity with compounds starting to approach a cellular potency of 100 nM. However, the increased lipophilicity of 5-F pyrimidines compared to 5-H pyrimidines also results in a decrease in aqueous solubility and an increase in hERG activity.

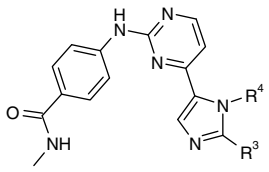
An increase in potency was also observed by substitution with fluorine ortho to the secondary amide **9b**, a smaller potency increase was seen with the tertiary amide **9c**. This indicated that an internal fluorine–hydrogen bond between the secondary amide and adjacent fluorine atom could be stabilising the biologically active conformation. Incorporating both of these beneficial fluorine substitutions into the same molecule gave compounds **10a–c**. Both the primary amide **10a** and the methylamide **10b** possessed excellent levels of enzyme and anti-proliferative activity. Again, the tertiary amide **10c** showed a smaller benefit and had a similar profile to the unsubstituted compound **8c**. Again, the disadvantage of increasing lipophilicity is observed in the lower levels of solubility of **10a–c** but with smaller effects on hERG activity.

Interestingly, methyl substitution adjacent to the amide **16** led to a large increase in solubility, despite the increase in lipophilicity compared to **9c**. We hypothesised that this is due to a steric clash between the methyl group and the dimethylamide adversely affecting packing in the solid state. This possible change in conformation could also be responsible for the much lower activity observed against the hERG ion channel (cf. **9c** with **16**). Similar beneficial effects on potency and hERG activity were observed with other *ortho*-substituted tertiary amides (data not shown) but substitution was generally detrimental for potency so they were not pursued further.

A number of compounds had suitable characteristics for further progression, but the methylamide **9b** appeared to have the best overall balance of enzyme and cellular potency, along with suitable physical properties. Additional selectivity screening against a range of other kinases showed that within the CDK kinase family the methylamide **9b** showed greater than 50 fold selectivity for CDK2 over CDK1 and CDK4 (Table 4). Good levels of selectivity against non-CDK family kinases were also observed when **9b** was tested against a broader panel of kinases, with IC50s of >10 μM against the kinases: Csk, EGFR, FGFR-1, IGF-1R, JAK2, Zap70 and PKA. No issues with plasma protein binding (PPB) were observed, with high levels of free drug across a number of species.

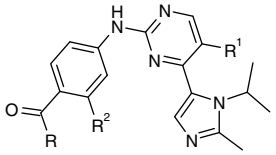
With a promising in vitro profile the methyl amide **9b** progressed into in vivo studies. Good pharmacokinetic (PK) parameters were observed with moderate to low clearance in nude mouse and rat (Table 5). Rat bioavailability was moderate but im-

Table 2
CDK inhibition profile for compounds **11**–**15**.



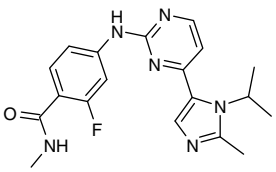
Compound	R^3	R^4	CDK2 IC_{50} (μM)	LoVo IC_{50} (μM)	Solubility (μM)
11	Me	Et	0.056	3.1	35
12	Me	^{c}PrCH$_2$	0.042	2.1	27
13	Me	^{c}Pentyl	0.002	0.19	24
14	^{c}Pr	^{i}Pr	0.081	0.66	10
15	CH_2OMe	^{i}Pr	0.062	2.9	24

Table 3
CDK inhibition profile for compounds **8**–**10** and **16**.



Compound	R^1	R^2	R	CDK2 IC_{50} (μM)	LoVo IC_{50} (μM)	hERG IC_{50} (μM)	Solubility (μM)
8a	F	H	NH_2	<0.001	0.27	20	36
8b	F	H	NHMe	0.003	0.19	12	14
8c	F	H	NMe_2	0.002	0.13	11	11
9a	H	F	NH_2	<0.001	nd	10	33
9b	H	F	NHMe	<0.001	0.14	16	71
9c	H	F	NMe_2	0.006	0.36	3	130
10a	F	F	NH_2	<0.001	0.10	20	6
10b	F	F	NHMe	<0.001	0.07	19	4
10c	F	F	NMe_2	0.002	0.17	6	16
16	F	Me	NMe_2	0.009	2.3	>32	>2600

Table 4
Additional data for the methylamide **9b**.



Parameters	
CDK2 (IC_{50}, μM)	<0.001
CDK1 (IC_{50}, μM)	0.06
CDK4 (IC_{50}, μM)	0.05
Log*D*	3.0
PPB (% free) mouse/rat/dog/human	12/8/30/8

Table 5
Pharmacokinetic parameters of the methylamide **9b**.

PK parameters	Mouse iv[a]	Rat iv[b]	Rat oral	Rat oral
Dose (μmol/kg)	2.0	1.0	5	20
T1/2 (h)	16.7	5.8	—	—
Cl (mL/min/kg)	18.5	11.9	—	—
Vss (L/kg)	9.5	1.7	—	—
AUC (μM h)	1.8	1.4	1.5	13.7
Bioavailability (%)	—	—	21	47

[a] Female nude.
[b] Male Han-Wistar.

proved at higher doses indicating that the compound was a suitable probe compound for additional in vivo efficacy studies.

In conclusion, we have discovered a new series of imidazole pyrimidine amides that show excellent inhibition of CDK2 and anti-proliferative activity along with good physical properties and acceptable pharmacokinetics that are suitable for dosing orally.

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