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Imidazo[1,2-*a*]pyridines. Part 2: SAR and optimisation of a potent and selective class of cyclin-dependent kinase inhibitors

Kate F. Byth, Janet D. Culshaw, Stephen Green, Sandra E. Oakes and Andrew P. Thomas*

AstraZeneca, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

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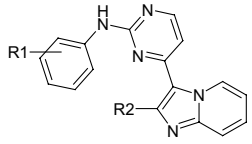
Abstract—Exploration of SAR and optimisation of the imidazo[1,2-*a*]pyridine CDK inhibitors has lead to the discovery of novel, potent and selective inhibitors of the cyclin-dependent kinase CDK2. Understanding of SAR has identified positions of substitution, which allow modification of physical properties and offer the potential for in vivo optimisation.

The normal progression through the cell cycle leading to cell division is a remarkably ordered process regulated by the sequential activation and deactivation of several members of the cyclin-dependent kinase (CDK) family.[1] For example, CDK2 plays an important role in several stages of the cell cycle. The CDK2/cyclin E complex contributes to pRb phosphorylation to enable the G1/S-phase transition and activate the transcription factor E2F.[2] CDK2 then associates with cyclin A promoting uninterrupted passage through the S-phase and appropriately timed deactivation of E2F.[3] Recent studies using a dominant negative CDK2 have shown that CDK2 also plays an important role in the entry and possibly progression in the G2/M-phase.[4]

It is a feature of most cancer cells that they exhibit a deregulation of CDK function and cell cycle control. Virtually all cancers exhibit at least one alteration in CDK function, through upregulation of cyclin effectors such as cyclins D and E, the loss of negative regulators such as p16 and p27 or genetic mutations to CDK substrates.[3,5] Evidence from in vitro studies suggests that inhibition of CDK2 selectively kills tumour cells with deregulated E2F-1 activity.[6] CDK inhibitors are therefore particularly attractive as potential therapeutic agents[7] and may offer new opportunities for selective and tolerable therapy for human cancer.

As a result of these findings, there is much interest in the development of small molecule CDK inhibitors and a number of groups have identified CDK inhibitors from a variety of structure classes.[6–17] In an earlier communication we described the discovery of prototype 2-anilino-4-(3-imidazo[1,2-*a*]pyridyl)pyrimidine CDK inhibitors.[17] In this paper we describe the optimisation and structure–activity relationships (SAR) of these imidazo[1,2-*a*]pyridines, as a potent and selective class of CDK inhibitors.

The initial lead structures of this series[17] are represented by structure **1**, where R2 = Me.



1

The important contribution of the aniline and pyrimidine groups to the binding and activity of these compounds has been shown in our earlier work.[17] Our initial goals in this work were to develop chemistry that would allow exploration of the role of the aniline substituent (R1 on compound **1**) and the substitution on imidazopyridine ring; and to further improve potency. The initial synthetic route to compounds **1** is shown in Scheme 1: the 3-acetyl imidazo[1,2-*a*]pyridines **A** were prepared by reaction of 2-amino pyridine with 3-chloro-2,4-pentanedione for the case R2 is methyl or by acetylation of the parent imidazo[1,2-*a*]pyridine for the case where R2 is hydrogen.[18] The latter reaction gave varying degrees of conversion and although it was possible to force this

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* Corresponding author. Tel.: +44-(0)1625-515170; fax: +44-(0)1625-513910; e-mail: andrew.p.thomas@astrazeneca.com

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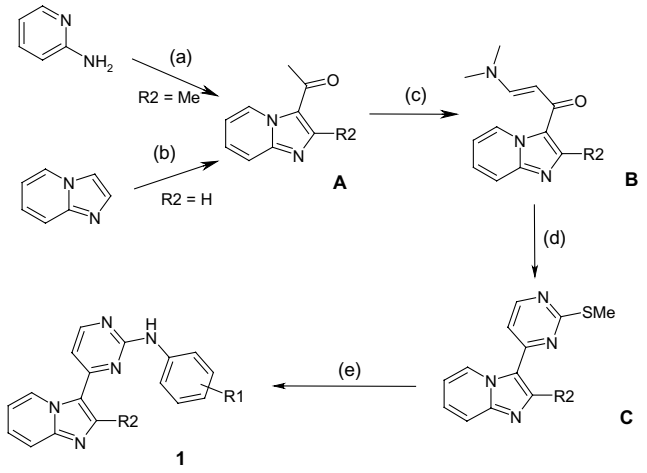


Scheme 1. Synthesis of imidazo[1,2-*a*]pyridines **1**. Reagents and conditions: (a) 3-chloro-2,4-pentanedione, Et_2O/THF, reflux, 12 h, 44%; (b) acetic anhydride, $AlCl_3$, CH_2Cl_2, 5 °C, reflux, 2 h, 40–80%; (c) DMF, DMA, reflux, 3 days, 50–70%; (d) (i) thiourea, NaOMe, *n*-BuOH, 85 °C, 2–20 h, (ii) cool to 30 °C, MeI, 3 h, 80–93%; (e) R1-substituted aniline, $NaN(SiMe_3)_2$, NMP, 150 °C, 3 h, 3–12%.

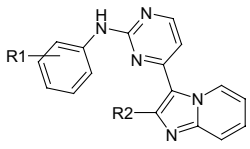
reaction close to completion, it was practically easier to carry forward crude product to the following reaction. Condensation of methyl ketone **A** with *N*,*N*-dimethylformamide dimethyl acetal gave aminopropenone **B**, which could be cyclised with thiourea and methylated in the same pot to give the key intermediate **C**.[19] The thiomethyl group could be displaced, albeit in low yield, by reaction with aniline anions at high temperature to give the compounds **1**.

Compounds were assayed using a scintillation proximity assay (SPA) to detect the inhibition of CDK2-cyclin E catalysed [γ-33-P]-phosphorylation of a GST–Rb substrate.[20]

Initial results demonstrated the sensitivity of substituents R1 and R2 in structure **1** (Table 1). These results show that removal of the methyl group from the 2-position of the imidazo[1,2-*a*]pyridine leads to a significant increase in potency (compare compounds **1a**,**c**,**e** with **1f**,**g**,**h**). Summarising a broad range of aniline substituents that were investigated, substitution at the 2-position of the aniline group was found to be detrimental to CDK2 activity (compare **1a** with **1b**) while potency was relatively insensitive to substitution of relatively small and nonpolar substituents at the 3- and 4-positions (compare **1a** with **1c** and **1d**). However, the 4-sulfamoyl group was found to significantly improve activity, compounds **1e** and **1h**. Similar SAR has been previously reported.[21] The importance of the imidazo[1,2-*a*]pyridine group to binding was also investigated. Introduction of alternative heterocycles that did not have an equivalent to the N-1 hydrogen bond acceptor of the imidazo[1,2-*a*]pyridine were very much less active (data not shown), while even the apparently modest isomeric change to a benzimidazole group (compound **2**) led to a significant decrease in activity (compare **2** with **1g**). The straightforward, though low yielding, synthesis of compound **2** is shown in Scheme 2.

Given the interesting levels of activity shown by sulfonamide **1h** and the particularly poor reaction in the conversion of intermediate **C** to **D** in Scheme 1, improvements in synthetic route were required to facilitate preparation of required compounds. Alternative routes from the aminopropenone intermediate **B** to final targets were developed (Schemes 3 and 4).

Table 1. Structures and enzyme activity for imidazo[1,2-*a*]pyridines



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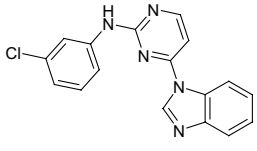
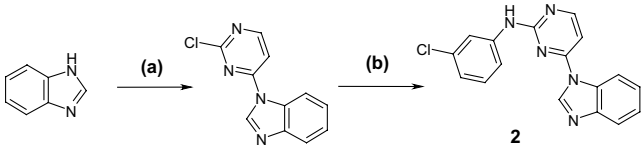


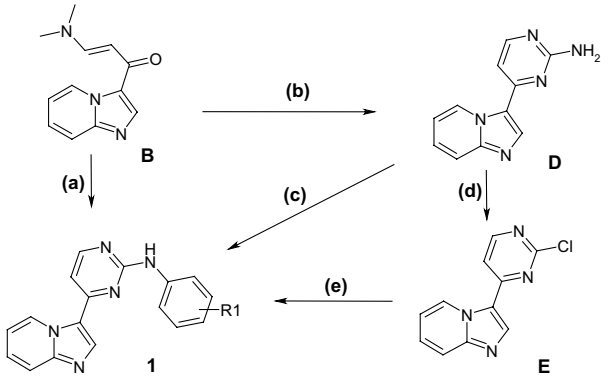
2

Compd	R1	R2	CDK2 IC_{50} (μM)[a]
1a	H	Me	0.21
1b	2-Cl	Me	8.8
1c	3-Cl	Me	0.15
1d	4-Cl	Me	0.62
1e	4-SO_2NH_2	Me	0.038
1f	H	H	<0.003
1g	3-Cl	H	0.005
1h	4-SO_2NH_2	H	<0.003
2			0.68

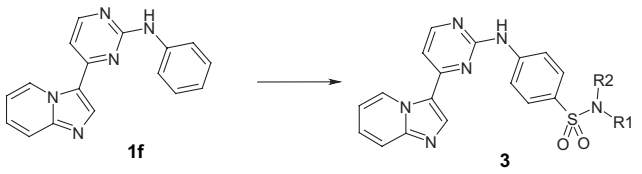
[a] Average of at least two measurements; enzyme protocol.[20]



Scheme 2. Synthesis of benzimidazole **2**. Reagents and conditions: (a) NaH, 2,4-dichloropyrimidine, DMF, 0 °C 3 h, 8%; (b) 3-chloroaniline, *n*-BuOH, reflux, 5 h, 13%.



Scheme 3. Improved synthesis of imidazo[1,2-*a*]pyridines **1** (R2 = H). Reagents and conditions: (a) Phenylguanidine, H_2CO_3, DMA, 130 °C, 24 h, 49–74%; (b) guanidine, HCl, NaOMe, *n*-BuOH/MeOH, reflux, 48 h, 67%; (c) substituted bromo (or iodo)benzene, $Pd_2(dba)_3$, BINAP, NaO-*t*-Bu, Toluene, 100 °C, 24 h, 30–42%; (d) (i) $NaNO_2$, AcOH, 60 °C, 3 h, 83%; (ii) $POCl_3$, PCl_5, reflux, 24 h, 69–80%; (e) R1-substituted aniline, $Pd_2(dba)_3$, BINAP, NaO-*t*-Bu, Toluene, 100 °C, 24 h, 40–53%.

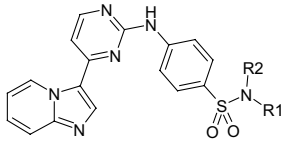


Scheme 4. Synthesis of sulfonamides **3**. Reagents and conditions: (i) $ClSO_3H$, $SOCl_2$, reflux, 1 h; (ii) evaporate; (iii) add R1(R2)NH, MeOH, 0–20 °C, 1 h, 43–87%.

Reaction of the aminopropenone **B** with simple phenyl guanidines (R1 = H or 3-Cl) proceeded smoothly.[19] Alternatively the aminopyrimidine **D** could be similarly be prepared from guanidine. Intermediate **D** could be converted in to target 1 (where R1 = *N*-alkyl-sulfamoyl) by two routes. Direct coupling with 4-bromo (or iodo) phenyl-sulfonamides under Buchwald conditions[22] proceeded in moderate yield. Alternatively **D** could be converted to the 2-chloropyrimidine **E** by hydrolysis[23] and chlorination. Chloropyrimidine **E** was surprisingly inert to conventional acid or base catalysed displacement reactions, however, target compounds **1** could be prepared by using Buchwald conditions.[22] Finally, simple variation of the sulfonamide substituent could be achieved from the parent aniline **1f** by chlorosulfonation and in situ reaction with the required amine[24] (Scheme 4).

Representative results from sulfonamides of structure **3** (Table 2) show that CDK2 enzyme activity and effects on cellular proliferation are relatively insensitive to sulfonamide substitution. This is consistent with the binding mode shown by compound **1h** in the crystal structure of its complex with CDK2,[17] where the sulfonamide substituent is directed out towards solvent. However, this does allow such substituents to provide the means to modify physical properties by incorporation of basic residues such as in **3d** and **3e**. The reduced serum protein binding of these two compounds contributes to the increased cellular potency shown by these compounds.

Table 2. Structures and biological activity for sulfonamides **3**



3

Compd	R1	R2	CDK2 IC_{50} (μM)[a]	MCF-7 prolif. IC_{50} (μM)[b]
1h	H	H	<0.003	0.26
3a	Me	H	<0.003	0.26
3b	$(CH_2)_2OMe$	H	0.012	0.39
3c	$(CH_2)_3OMe$	H	0.004	0.6
3d	$(CH_2)_2NMe_2$	H	0.005	0.07
3e	$(CH_2)_3NMe_2$	H	<0.003	0.07
3f	$(CH_2)_3NMe_2$	Me	0.005	0.44

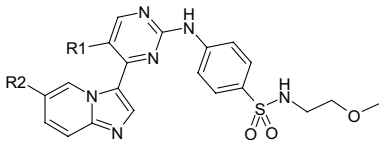
[a] Average of at least two measurements; enzyme protocol.[20]
[b] IC_{50} for inhibition of BrdU incorporation to MCF-7 cells following 3 day exposure to test compound; average of at least two measurements.

The relatively similar activity of compounds **3e** and **3f** demonstrates that the NH of the sulfonamide is not essential for CDK2 activity. This result confirms the SAR shown by compounds **1f** and **1h** (Table 1) where the sulfonamide group does not appear to add greatly to potency in the more optimised unsubstituted (R2 = H) imidazo[1,2-*a*]pyridines, this is despite the sulfonamide group showing three hydrogen bonding interactions with the CDK2 protein.[17] From this we conclude that the sulfonamide group acts primarily to increase kinase selectivity rather than increase potency in more optimised compounds. Increased selectivity being due to the fact that only those kinases, which are able to accommodate the hydrogen bonding needs of the sulfonamide group can bind these inhibitors. This proposition is confirmed by selectivity measurements in kinase panels and in cellular assays. For example, the sulfonamides such as **3c** and **3d** show greater differential effects on the viability of proliferating cells relative to nonproliferating cells than imidazopyridines that do not carry a sulfonamide substituent (data not shown). Proliferating cells being sensitive to CDK inhibition while nonproliferating cells should not be.

From understanding of the binding interactions with the CDK2 enzyme[17], it was of interest to explore the effect of substitution at the 5-position of the pyrimidine and 5-position of the imidazo[1,2-*a*]pyridine; R1 and R2, respectively, in structure **4**. Compounds **4a** and **4b** were prepared from reaction of **D** with the appropriate *N*-halosuccinimide and conversion to final product by steps (d) and (e) in Scheme 3. Compound **4c** was prepared from **4b** by displacement of the bromo group with sodium phenylthiolate. 2-Amino-5-bromopyridine was condensed with 2-bromoacetaldehyde diethylacetal to give 5-bromoimidazo[1,2-*a*]pyridine, which was similarly taken through to Compound **4d**. Compounds **4e**–**g** were prepared from **4d** either by palladium catalysed cyanide displacement[25] or by sodium thiolate displacement.

The activity of these compounds (Table 3) shows that relatively small substituents in the 5-position of the

Table 3. Structures and enzyme activity for imidazo[1,2-*a*]pyridines **4**



4

Compd	R1	R2	CDK2 IC_{50} (μM)[a]
3b	H	H	0.005
4a	Cl	H	<0.003
4b	Br	H	<0.003
4c	SPh	H	>10
4d	H	Br	<0.003
4e	H	CN	<0.003
4f	H	SEt	<0.003
4g	H	SPh	0.016

[a] Average of at least two measurements; enzyme protocol.[20]

Table 4. Biological characterisation of compounds **3c** and **3d**

Compd	CDK2 IC_{50} (μM)	CDK4 IC_{50} (μM)	CDK1 IC_{50} (μM)	MCF-7 prolif. IC_{50} (μM)[a]	S249-T252 Phos. IC_{50} (μM)[b]
3c	0.004	3.1	0.006	0.6	0.4
3d	0.005	0.26	0.015	0.07	0.05

[a] IC_{50} for inhibition of BrdU incorporation to MCF-7 cells following 3 day exposure to test compound; average of at least two measurements.
[b] IC_{50} for inhibition of phosphorylation of S249-T252 site on Rb protein in MCF-7 cells following 2 h exposure to test compound.

pyrimidine (compounds **4a** and **4b**) are tolerated or beneficial for activity while larger groups such as in **4c** are very much less active. This is consistent with this substituent approaching Phe80 at the closed end of the binding pocket.[17] Substitution at the 5-position of the imidazo[1,2-*a*]pyridine, compounds **4d**–**g**, is less sensitive to the introduction of larger groups, which is consistent with this group being directed towards solvent at the open end of the binding pocket.[17]

Finally, the biological profiles of compounds **3c** and **3d** were more fully characterised (Table 4). These results (Table 4) show that compounds **3c** and **3d** inhibit CDK1 with similar potency to CDK2 though show selectivity with respect to CDK4. Both compounds block the cell cycle at G1, S and G2/M-phases (data not shown), and at concentrations that inhibit proliferation show inhibition of CDK-dependent phosphorylation of the Rb protein within 2 h of drug exposure. These observations are consistent with these compounds acting as direct CDK inhibitors in cells.

In conclusion, imidazo[1,2-*a*]pyridines have been optimised and characterised as potent inhibitors of CDK enzymes and provide useful leads for the discovery of orally active CDK inhibitors.

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