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Imidazo[1,2-*b*]pyridazines: a potent and selective class of cyclin-dependent kinase inhibitors

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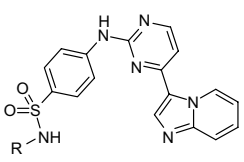
Abstract—Modification of imidazo[1,2-*a*]pyridine CDK inhibitors lead to identification of less lipophilic imidazo[1,2-*b*]pyridazine series of CDK inhibitors. Although several equivalent compounds from these two series have similar structure and show similar CDK activity, the SAR of the two series differs significantly. Protein inhibitor structure determination has confirmed differences in binding mode and given some understanding of these differences in SAR. Potent and selective imidazo[1,2-*b*]pyridazine inhibitors of CDK2 have been identified, which show >1 μM plasma levels following a 2 mg/kg oral dose to mice.

In the preceding paper[1] we describe the in vitro SAR and optimisation of imidazo[1,2-*a*]pyridines (**1**) as cyclin-dependent kinases (CDK) inhibitors. Such inhibitors are expected to be useful as anti-tumour agents due to the key role that the CDK enzymes play in controlling the cell cycle.[2] Furthermore, it has been shown that inhibition of CDK2 activity can lead to the selective killing of tumour cells with deregulated E2F-1 activity.[3] A key question in the optimisation of CDK inhibitors is whether such agents will be most useful following an acute single dose or following regular daily dosing as a chronic therapy. This would be an important determinant in deciding whether CDK inhibitors should be optimised as agents for intravenous or for oral delivery. Our assessment was that regular dosing would be optimally required and that CDK inhibitors with oral activity should be sought. The physico-chemical properties of the imidazo[1,2-*a*]pyridine CDK inhibitors reported in the preceding paper[1] were not ideally suited for optimisation as orally active agents, and we sought to modify these compounds to reduce the lipophilicity of the core structure.



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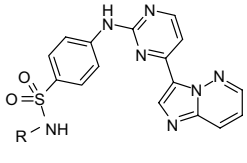

2

We reasoned that introduction of additional heteroatoms into the pyridine ring of the imidazo[1,2-*a*]pyridine system should be undertaken and this work would lead to the identification of imidazo[1,2-*b*]pyridazine CDK inhibitors (**2**).

Initial examples of the new imidazo[1,2-*b*]pyridazines showed similar potency to that shown by corresponding imidazo[1,2-*a*]pyridines[1] (Table 1).

The preparation of compounds of structure **2** and **3** proceeded via the key methyl ketone intermediate **C** (Scheme 1). 3-Amino-6-chloropyridazine was condensed with DMF·DMA to give the amidine intermediate **A**, which was reacted with chloroacetone through initial quaternisation on the pyridazine 2-N followed by deprotonation and attack on the amidine leading to cyclisation with loss of dimethamine to give imidazo[1,2-*b*]pyridazine **B**.[5] The chloro group was removed by

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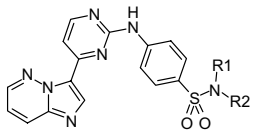
Table 1. Structures and enzyme activity for imidazo[1,2-*b*]pyridazines



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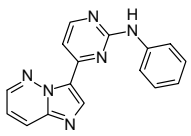
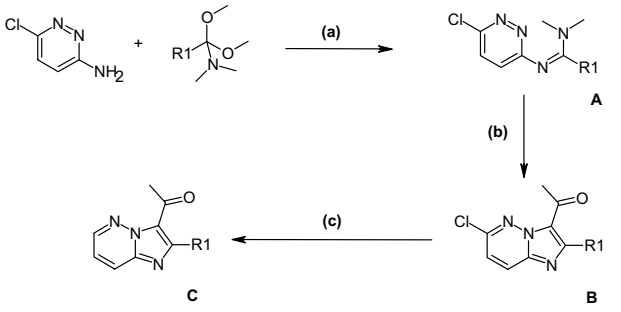


3

Compd	R1	R2	CDK2 IC_{50}, μM[a]	MCF-7 prolif. IC_{50}, μM[b]
2a	H	H	<0.003	
2b	Me	H	<0.003	
2c	$(CH_2)_2OMe$	H	<0.003	0.39
2d	$(CH_2)_2NMe_2$	H	0.008	
2e	$(CH_2)_3NMe_2$	H	<0.003	0.43
2f	$(CH_2)_2OMe$	Me	0.6	>5
3			1.0	

[a] Average of at least two measurements; enzyme protocol Ref. 4.
[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.

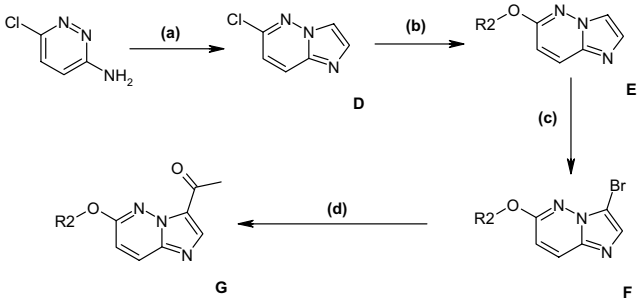


Scheme 1. Synthesis of imidazo[1,2-*b*]pyridazine intermediate **C**. Reagents and conditions: (a) toluene, reflux, 2.5 h, 80–95%; (b) R1 = H: chloroacetone, KI, DMF, 80 °C, 5 h, 58%; R1 = Me: chloroacetone, KBr, EtOH, reflux, 5 h, 79%; (c) H_2 1 atm, Pd/C, Et_3N, 20 °C, 2 h, 90–92%.

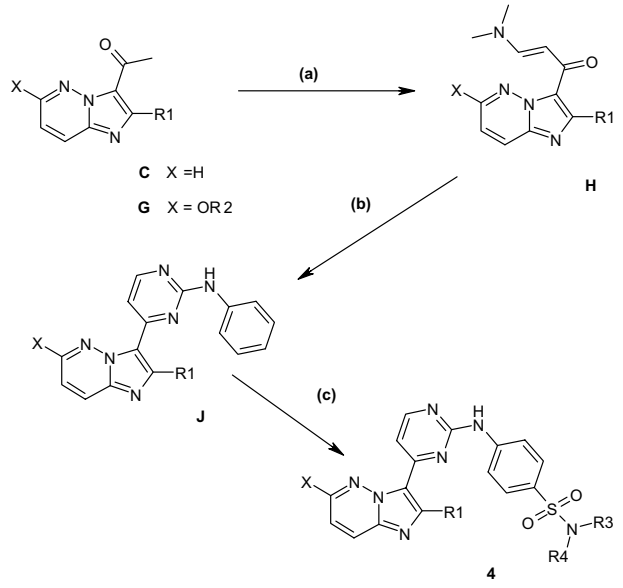
hydrogenolysis to give key methyl ketone **C**. Introduction of a methyl substituent in the 2-position of **C** was achieved by an analogous procedure but starting with DMA·DMA (Scheme 1).

Following the SAR shown by the imidazo[1,2-*a*]pyridine series,[1] we wanted to take advantage of the reactivity of the chloropyridazine to introduce substituents to the 5-position of the imidazo[1,2-*b*]pyridazine ring system. This was achieved (Scheme 2) by first forming the 5-chloroimidazopyridazine **D**.

The eventual 5-substituent was introduced by displacement of the chloro group of **D** with alkoxide nucleophiles. Introduction of the acetyl substituent was then carried out by bromination, followed by transmetallation with isopropyl magnesium bromide[6] and reaction with the appropriate acetamide to give the methyl ketone **G**. The final conversion of the methyl ketones to final compounds **4** was performed (Scheme 3) via an analogous route to that developed for imidazo[1,2-*a*]pyridine CDK inhibitors.[1]



Scheme 2. Synthesis of imidazo[1,2-*b*]pyridazine intermediate **G**. Reagents and conditions: (a) chloroacetaldehyde, *n*-BuOH, 120 °C, reflux, 18 h, 78%; (b) R1 = Me: NaOMe, MeOH, 20 °C, 18 h, 91%; R1 = CH_2CF_3: CF_3CH_2OH, NaH, DMF, 20 °C, 18 h, 71%; (c) NBS, $CHCl_3$, 20 °C, 2 h, 76–93%; (d) (i) *i*-PrMgBr, THF, −40 °C, 2 h; (ii) add MeCON(OMe)Me, −40 to 20 °C, 4 h, 43–62%.

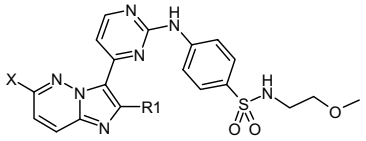


Scheme 3. Synthesis of imidazo[1,2-*b*]pyridazines **3**. Reagents and conditions: (a) DMF·DMA, 100 °C, 20–44 h, 86–88%; (b) phenylguanidine hydrocarbonate, DMA, 100–160 °C, 5–7 h, 40–80%; (c) (i) $ClSO_3H$, $SOCl_2$, 0 °C to reflux, 1 h; (ii) R3(R4)NH, MeOH, 0–20 °C, 24 h, 58–88%.

The biological results (Table 1) appeared to support our initial expectation that the binding mode and SAR of the imidazo[1,2-*b*]pyridazine series would mirror that of the earlier imidazo[1,2-*a*]pyridine series. However, it was obvious that the imidazo[1,2-*b*]pyridazine showed a significantly greater dependence on the presence of the 4-sulfonamide substituent (SO_2NH) on the aniline (compare compds **2f** and **2c**, and compds **3** and **2a**) than shown by the imidazo[1,2-*a*]pyridines.[1]

Also based on the SAR shown by the imidazo[1,2-*a*]pyridine series,[1] it was our expectation that introduction of 5-substituents to the imidazo[1,2-*b*]pyridazine ring system would be at least tolerated, and potentially beneficial for CDK2 activity. However, the results for compounds such as **4a** (Table 2) show a major loss in

Table 2. Structures and enzyme activity for substituted imidazo[1,2-*b*]-pyridazines



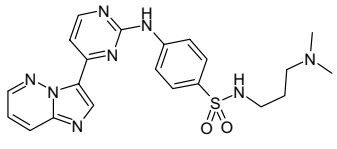
4

Compd	R1	X	CDK2 IC_{50}, μM[a]	CDK1 IC_{50}, μM[a]	MCF-7 prolif. IC_{50}, μM[b]
2c	H	H	<0.003	0.04	0.39
4a	H	MeO	0.6		
4b	Me	H	0.003	0.3	0.23

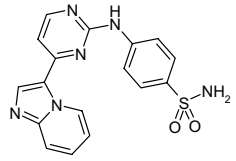
[a] Average of at least two measurements; enzyme protocol Ref. 4.
[b] IC_{50} for inhibition of BrdU incorporation to MCF-7 cells following 3 day exposure to test compound; average of at least two measurements.

potency for CDK2 inhibition. Also contrary to the SAR shown by analogous imidazo[1,2-*a*]pyridine inhibitors, was the equivalent if not improved CDK2 activity shown when a methyl group was introduced to the 2-position of the imidazo[1,2-*b*]pyridazine ring system (compd **4b**) compared to the 2-H derivative (compd **2c**) (Table 2).

From the data generated (Tables 1 and 2) it is clear that significant differences in SAR exist between the imidazo[1,2-*b*]pyridazine series reported here and the imidazo[1,2-*a*]pyridine series reported in the preceding paper.[1] The inverted tolerability shown by the two series to substitution at the 2- and 5-positions of the imidazopyrid(az)ine rings systems indicated some likely change in conformation and binding mode of the 6–5 ring system in the two series. To help explain and explore these differences we undertook structural studies, based on previous work with CDK2 X-ray crystallography,[7–9] to investigate binding mode of the complex with the representative imidazo[1,2-*b*]pyridazine **2e**. This was compared to the structure generated for the parent imidazo[1,2-*a*]pyridine **5**.[10]



2e



5

The crystal structures of CDK2 complexed with **2e** (Fig. 1) and with **5** (Fig. 2) show that the key hydrogen-bonding interactions between the pyrimidine N1 and Leu83 backbone NH and between the anilino NH and Leu83 backbone carbonyl O are maintained in both complexes. However, as suggested by the SAR discussed above, the orientation of the imidazo[1,2-*b*]pyridazine ring of **2e** is indeed in an inverted orientation relative to

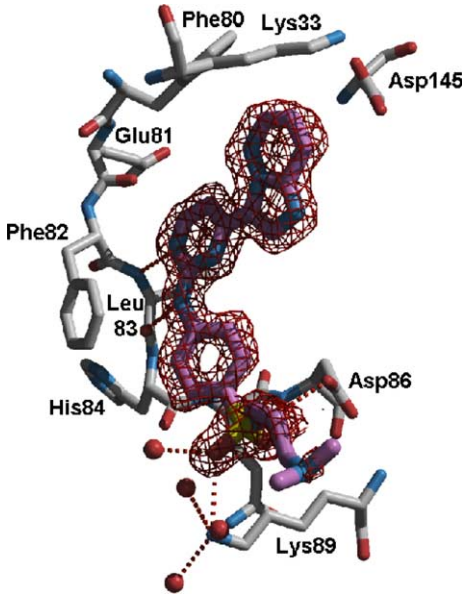


Figure 1. Crystal structures of CDK2 complexed with **2e**:[11] Final $2F_o - F_c$ electron density for the inhibitor **2e** (1.3σ level). The figures were prepared using Bobscript and Raster3D.[18,19]

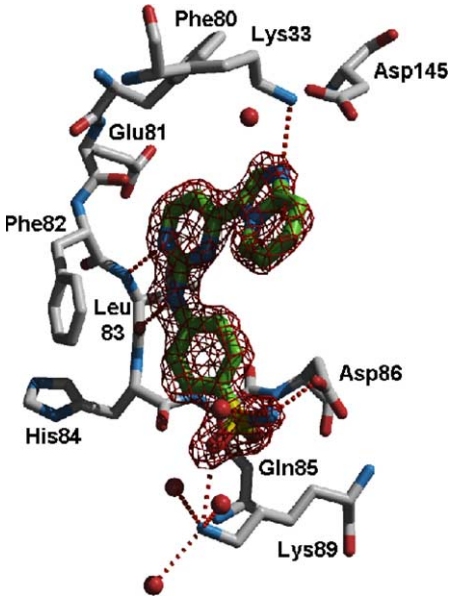


Figure 2. Crystal structures of CDK2 complexed with **5**:[10] Final $2F_o - F_c$ electron density for the inhibitor **5** (1.3σ level). The figures were prepared using Bobscript and Raster3D.[18,19]

the imidazo[1,2-*a*]pyridine ring of **5**. The lack of tolerance of 5-substituents on the imidazo[1,2-*b*]pyridazine shown by compd **4a** is clearly understood as this substituent is directed towards Phe80 at the closed end of the binding pocket, while the 2-substituent tolerated in the imidazo[1,2-*b*]pyridazine (compd **4b**) is directed towards the open end of the binding pocket.

The binding mode of imidazo[1,2-*b*]pyridazine **2e** shows loss of the H-bonding interaction between the N-1 of the imidazole ring and the Lys33 amino group. SAR from

Table 3. PK summary of **2c** and **4c** following 2 mg/kg po to mice[a]

Compd	AUC 0–6 h, μM h	C_{max}, μM	T_{max}, h	Approx. $T_{1/2}$, h
2c	9.4	3.2	0.5	3.0
4b	20	9.9	0.5	3.3

the imidazo[1,2-*a*]pyridine series[1] indicated this H-bond interaction to be important for CDK2 activity, however the edge-to-face interaction of the pyridazine ring of **2e** with the phenyl group of Phe80 that is gained must provide compensatory binding free energy. The change in binding conformation of the imidazo[1,2-*b*]pyridazine **2e** compared to the imidazo[1,2-*a*]pyridine **5** can be understood in terms of the electrostatic repulsion that would exist between the N4 of the imidazo[1,2-*b*]pyridazine and the N3 of the pyrimidine ring of **2e** if it were to adopt the binding mode of imidazo[1,2-*a*]pyridine such as **5**. While the absence of a *peri*-hydrogen at the 5-position of the 5,6-ring system in imidazo[1,2-*b*]pyridazines, such as **2e**, also makes the adopted conformation (Fig. 1) more favourable.

For both the imidazo[1,2-*b*]pyridazine **2e** and imidazo[1,2-*a*]pyridine **5**, the sulfonamide group forms hydrogen bonds with the Asp86 backbone NH and its carboxylic side chain and also with the side chain NH of Lys89 (Figs. 1 and 2). Though some subtle differences in binding and orientation are apparent, the cause of the difference in dependence on sulfonamide group for binding potency shown by the two series is not clear.

The properties and activities of compounds **2c** and **4b** have been characterised in more detail. Both compounds show selectivity for CDK2 over CDK1, though this appears to be greater for **4b** (Table 2). Examining activity against a broader range of kinases, **2c** gave an IC_{50} of >10 μM against the following kinases: Csk, EGFR, FAK, FGFR-1, IGF-1R, JAK3, Src, vAbl, Zap70, p38a, JNK1 and PKA. Significantly, compounds from the imidazo[1,2-*b*]pyridazine series show improved blood levels following oral dosing over those shown by the imidazo[1,2-*a*]pyridine series. Both compounds **2c** and **4b** show significant blood levels in mice following oral dosing (Table 3).

In conclusion, imidazo[1,2-*b*]pyridazines CDK2 inhibitors have been discovered and optimised. Compounds **2c** and **4b** have been characterised as potent and selective inhibitors of CDK enzymes, which show significant plasma levels following oral dosing. These compounds provide useful leads for the discovery and development of orally active CDK inhibitors.

References and notes

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17. Crystallographic statistics for compd **2e** are: space group $P2_12_12_1$, unit cell 54.2, 72.9, 73.3 Å, resolution 1.6 Å, 33,057 reflections from 72,225 observations give 85% completeness with $R_{merge} = 5.4\%$ and mean $I/\sigma(I)$ of 13.2. The final model containing 2153 protein, 212 water and 32 inhibitor atoms has an *R*-factor of 22.0% (R_{free} using 5% of the data 25.5%). Mean temperature factors for the protein and the ligand are 23.7 and 20.4 Å^2, respectively.
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