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# Structure-guided design of pyrazolo[1,5-*a*]pyrimidines as inhibitors of human cyclin-dependent kinase 2

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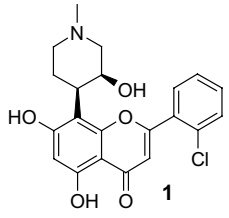
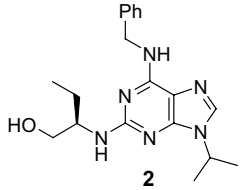
**Abstract**—The protein structure guided design of a series of pyrazolo[1,5-*a*]pyrimidines with high potency for human cyclin-dependent kinase 2 (CDK2) is described. Some examples were shown to inhibit the growth of human colon tumour cells, were equipotent for CDK1 and were selective against GSK-3β and other kinases.


Cyclin-dependent kinases (CDKs) play a central role in mammalian cell division.[1] Recent knock-out mouse experiments, however, suggest significant functional redundancy within this family of kinases.[2,3] Many questions have therefore been raised regarding the optimal selectivity profile for CDK inhibitors aimed at yielding safe and effective anti-cancer agents. Pragmatically, absolute selectivity has yet to be achieved and most efforts have focused on using CDK2 as a template, given its ready applicability to protein structure guided drug design.[4]

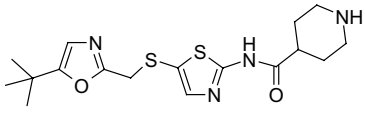
Inhibitors with varying CDK selectivity profiles are now being evaluated in clinical trials. Flavopiridol (**1**)[5] (phase I/II) displays modest selectivity for CDKs over other kinases and inhibits many members within the CDK family, including those without cell-cycle roles. Roscovitine/CYC-202 (**2**)[6] (phase I/II) and BMS-387032 (**3**)[7] (phase I) represent *bona fide* CDK inhibitors currently in clinical development. Both compounds **2** and **3** inhibit CDK2 more effectively than CDK1, roscovitine (**2**) has limited potency on cells and BMS-387032 (**3**) inhibits the related but therapeutically antagonistic glycogen synthase kinase-3β (GSK-3β) at concentrations only modestly in excess of those required to inhibit CDK2.[8] BMS-387032 (**3**) is also subject to PGP-mediated drug resistance.[9] Given these issues, there is still scope for further research towards finding the ideal CDK inhibitor for cancer therapy. We describe herein the discovery of a series of compounds with high potency for CDK2, which in some cases were shown to inhibit the growth of human colon tumour cells and were equipotent against CDK1, a kinase that partially overlaps in function with CDK2.[10] Many examples were also shown to be selective against GSK-3β and other kinases.



1



2



3

*Keywords*: Pyrazolo[1,5-*a*]pyrimidine; CDK2; Cyclin-dependent kinase.
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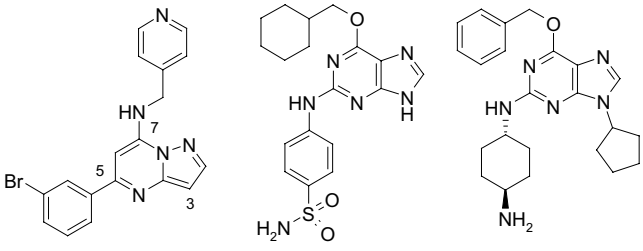
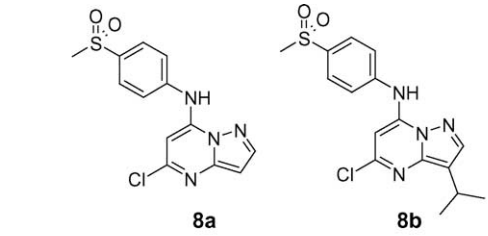
The pyrazolo[1,5-*a*]pyrimidine **4** arose as a hit from a high-throughput screen of a commercially available kinase-directed library. It was found to have an $IC_{50}$ of 1.8 μM against CDK2, but inhibited GSK-3β with similar potency ($IC_{50}$ 0.96 μM).[11,12] Compound **4** was also shown to inhibit the growth of human colon tumour cells (HCT116) in a sulforhodamine B (SRB) growth inhibition assay[13] ($GI_{50}$ 7.9 μM).[14] Compound **4** is structurally similar to roscovitine (**2**), and other purine-based competitor compounds such as NU-6102 (**5**)[15] and H717 (**6**).[16] It was decided to explore the SAR around the 3-, 5- and 7-positions of the pyrazolo[1,5-*a*]pyrimidine template, with a view to optimizing potency and selectivity for CDK2 and tumour cell growth inhibition.




The 7-Cl of 5,7-dichloropyrazolo[1,5-*a*]pyrimidines of type **7**[17] underwent facile displacement with a range of anilines to afford compounds of type **8** (Scheme 1). The 5-Cl could be displaced with aliphatic amines by heating with microwaves in a Smith Synthesizer™ at 180 °C in 1,4-dioxane and acetonitrile in the presence of triethylamine, resulting in compounds of type **9** (X = NH). Alternatively, ethers of type **9** (X = O) could be synthesized by treating a 1,4-dioxane–acetonitrile solution of the chloride **8** with an alcohol in the presence of sodium hydride and triethylamine, and then heating with microwaves to 180 °C as described previously.

The sulfonylphenylamino analogue **8a** was initially synthesized, and this inhibited CDK2 with an $IC_{50}$ of 2.0 μM and had a similar potency ($IC_{50}$ 3.1 μM) against GSK-3β. An X-ray crystallographic structure[18] was obtained, following co-crystallization of the compound with inactive monomeric human CDK2 prepared in-house[19] (Fig. 1). The key 'donor–acceptor–donor' binding motif between the 7-NH and Leu83 backbone carbonyl, a pyrazole N and Leu83 backbone NH, and the aromatic C2-H and the Glu81 backbone carbonyl was observed. This type of interaction motif is a well-recognized feature of a number of ATP competitive kinase ligands, and the importance of C–H···O interactions is now documented.[20] In addition, a hydrogen bond between a sulfone oxygen and Asp86 [analogous to the sulfonamide O atom of NU-6102 (**5**)] was present, and the compound showed good steric complimentarity with the active site cleft. Overlaying this structure with that of roscovitine (**2**) bound to CDK2 indicated that elaboration at the 3-position of the pyrazolopyrimidine ring would allow interaction with the Phe80 residue. Compound **8b** incorporated an isopropyl group at the 3-position and, encouragingly, this increased the CDK2 potency approximately 10-fold to 0.23 μM, and reduced GSK-3β potency to 11 μM. This resulted in a molecule, which was now almost 50-fold selective over GSK-3β, more potent than roscovitine (**2**) (CDK2 $IC_{50}$ 0.65 μM, GSK-3β $IC_{50}$ 34 μM)[8] and more selective over GSK-3β than NU-6102 (**5**) (CDK2 $IC_{50}$ 0.045 μM, GSK-3β $IC_{50}$ 0.040 μM).[8]



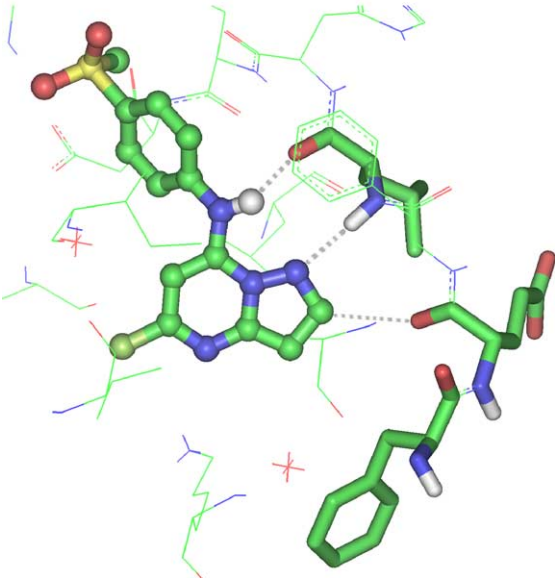
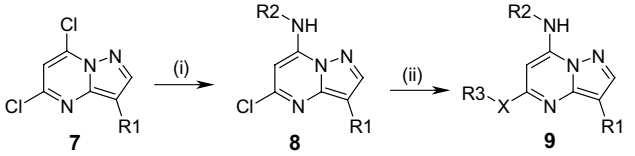



**Figure 1.** Compound **8a** bound to inactive monomeric human CDK2, with key kinase donor–acceptor–donor motif interactions indicated, and potential for elaboration to improve contact with Phe80 side chain.




**Scheme 1.** Reagents and conditions: (i) $R^2NH_2$, EtOH, 70–90%; (ii) (for X = NH) $R^3NH_2$, $Et_3N$, MeCN, 1,4-dioxane, μWave @ 180 °C, 3 h, 18–82%; (for X = O) $R^3OH$, $Et_3N$, NaH, $Et_3N$, MeCN, 1,4-dioxane, μWave @ 180 °C, 3 h, 7–20%.

Replacement of the methyl sulfone with a dimethylsulfonamide and incorporation of the *trans*-4-aminocyclohexylamino moiety, as found in H717 (**6**), into the 5-position of the pyrazolopyrimidine ring afforded compound **9a**. This compound had improved potency

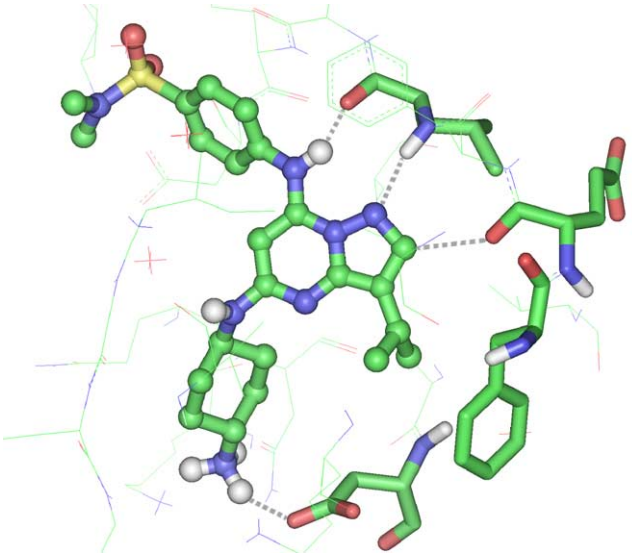
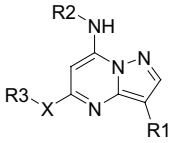


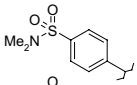
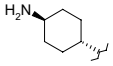
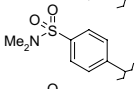
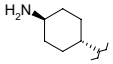
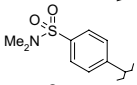
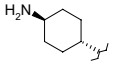
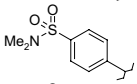
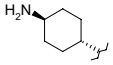
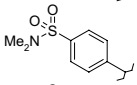
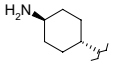
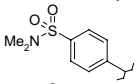
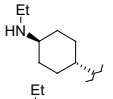
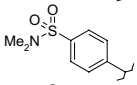
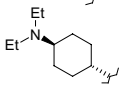
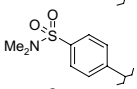
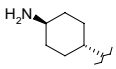
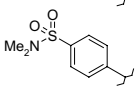
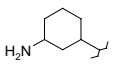
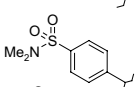
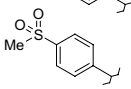
**Figure 2.** Compound **9a** bound to inactive monomeric human CDK2, showing the kinase motif interaction and improved steric complementarity with the Phe80 side chain. The salt bridge from the ligand primary amine to Asp145 is also highlighted.

for CDK2 ($IC_{50}$ 0.059 μM), possibly as a result of an additional interaction between the charged amino group attached to the cyclohexyl ring, which interacts via a salt bridge to Asp145, and a hydrogen bond with Asn132, as observed in an X-ray structure (Fig. 2).[18] In addition, the crystal structure of compound **9a** in complex with CDK2 clearly shows the improved interaction between Phe80 and the isopropyl group, in an analogous fashion to the roscovitine complex. These changes also benefited the selectivity against GSK-3β ($IC_{50}$ 40 μM) and the cellular activity. Compound **9a** had a $GI_{50}$ of 0.58 μM, a considerable improvement in comparison to that of roscovitine (**2**) ($GI_{50}$ 15 μM).[8] In terms of broader specificity versus kinases, **9a** inhibited CDK5 with equal potency to CDK2, but was 3-fold selective versus CDK7, >20-fold selective versus CDK6 and >50-fold selective versus Chk1, PKA, IKKα, MEK1, CK2, JNK1α, PKCα, MAPK1 and PDGFR. High concentrations of **9a** (32 μM), however, strongly inhibited CK1, Rsk1, Chk2 and Flt3.

Table 1 contains the compounds synthesized, along with details of potency for CDK2, selectivity against GSK-3β

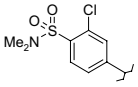
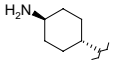
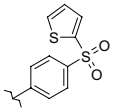
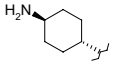
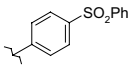
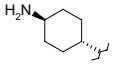
**Table 1.** Enzyme activity (CDK2 and GSK-3β) and cell-based inhibition in HCT116 (colon) cell line for pyrazolo[1,5-*a*]pyrimidines **9a**–**n**[a]




| No. | X | $R^1$ | $R^2$ | $R^3$ | CDK2 $IC_{50}$ μM[a,b] | GSK-3β $IC_{50}$ μM[c] | HCT 116 $GI_{50}$ μM |
|---|---|---|---|---|---|---|---|
| **9a** | NH | $^i$Pr |  |  | 0.059 | 40 | 0.58 |
| **9b** | NH |  |  |  | 0.004 | 22 | 0.54 |
| **9c** | NH | CN |  |  | 0.007 | 0.46 | 11 |
| **9d** | NH | Cl |  |  | 0.008 | 5.1 | 2.7 |
| **9e** | NH | Br |  |  | 0.010 | 3.4 | 0.91 |
| **9f** | NH | $^i$Pr |  |  | 0.061 | >50 | 1.2 |
| **9g** | NH | $^i$Pr |  |  | 0.092 | >50 | 16 |
| **9h** | O | $^i$Pr |  |  | 0.017 | 28 | 0.71 |
| **9i** | NH | $^i$Pr |  |  | 2.1 | >50 | 3.4 |
| **9j** | O | $^i$Pr |  | $Me_2CHCH_2$ | 1.2 | 50 | 7.6 |
| **9k** | O | $^i$Pr |  | $Me_2CHCH_2$ | 1.2 | 12 | 7.8 |

(*continued on next page*)

**Table 1** (*continued*)

| No. | X | $R^1$ | $R^2$ | $R^3$ | CDK2 $IC_{50}$ μM[a,b] | GSK-3β $IC_{50}$ μM[c] | HCT 116 $GI_{50}$ μM |
|---|---|---|---|---|---|---|---|
| **9l** | NH | Br |  |  | 0.002 | 8.7 | 0.34 |
| **9m** | NH | Cl |  |  | 0.011 | 4.0 | 0.12 |
| **9n** | NH | Br |  |  | 0.016 | 5.3 | 0.08 |

[a] All $IC_{50}$ and $GI_{50}$ values are the mean of at least two determinations, and are rounded to two significant figures where appropriate.
[b] [ATP] 100 μM ($K_m$ 50 μM).
[c] [ATP] 10 μM ($K_m$ 10 μM).

and inhibition of tumour cell growth. All compounds were also screened against Chk1 and PKA, and shown to be >50-fold selective for CDK2.

Replacement of the 3-isopropyl substituent with cyclopentyl (**9b**), nitrile (**9c**), chloro (**9d**) or bromo (**9e**) all enhanced the enzyme potency significantly, but did not enhance cellular activity. We gathered additional information on the selectivity of compound **9e**, which was >12-fold less potent versus CDK5 and >30-fold less potent versus CDK1, CDK6, CDK7, Abl1, IKKβ, MEK1, FRFR-3, p70S6Kinase, PKCγ, c-Raf, CK1δ, MAPK1, MAPKAP2, PAK2 and NEK2. Compound **9e**, however, retained the ability of compound **9a** to inhibit Chk2 (90% inhibition @ 300 nM).

A number of modifications were carried out on the primary amine group of compound **9a**, which was considered to be a potential metabolic liability. These generally retained the enzyme potency, but reduced the cellular activity dramatically. For example, mono-ethylation of the primary amine[21] to give compound **9f** resulted in the same potency against CDK2 (0.061 μM), but the $GI_{50}$ dropped to 1.2 μM. Diethylation to give **9g** resulted in a slight drop in CDK2 potency (0.092 μM), but the $GI_{50}$ fell to 16 μM.

The linker at the 5-position was also varied to an O atom and, in comparison with **9a**, compound **9h** displayed a marginally increased potency against CDK2 (0.017 μM), but made no significant difference to the cellular potency ($GI_{50}$ 0.71 μM).

Minor changes, such as moving the primary amine of compound **9a** to the 3-position of the cyclohexyl ring (**9i**), or total replacement with a 2-methylpropanyloxy side chain (**9j**), were detrimental to CDK2 and cellular potency. The dimethyl sulfonamide group of compound **9j** could be replaced with a methyl sulfone (**9k**) with no significant effect on CDK2 potency or cellular activity.

Incorporation of a chlorine atom into the 2-position of the benzenesulfonamide ring of **9e** gave the most potent CDK2 inhibitor **9l** (CDK2 $IC_{50}$ 2 nM, $GI_{50}$ 0.34 μM). Compounds **9m** ($GI_{50}$ 0.12 μM) and **9n** ($GI_{50}$ 0.08 μM), which incorporated an arylsulfonylphenylamino substituent at the 7-position and a 3-Cl or Br atom, respectively, had the greatest cellular activity.

Selected compounds were also assayed for their ability to inhibit human CDK1. Compounds **9a** and **9n** inhibited CDK1 and CDK2 with approximately equal potency, whilst compound **9e** was around 60 times less potent versus CDK1. Compounds **9a**, **9e** and **9n** were capable of downregulating phosphorylation on threonine 821 of the retinoblastoma protein in HCT116 cells (a site phosphorylated by CDK2), when added at a low multiple (4×) of the $GI_{50}$. Moreover, at $1 \times GI_{50}$, compound **9n** caused an accumulation of cells in the G2/M phase of the cell cycle, a result consistent with the expected mode of action for a dual CDK1/CDK2 inhibitor. The effects of our compounds on cells will be fully described in a forthcoming paper.

Compounds **9a** and **9f** were administered intravenously and orally to male SD rats at a dose of 1 mg/kg. These compounds displayed poor bioavailability and were not progressed.

In conclusion, knowledge of the binding mode of a series of pyrazolo[1,5-*a*]pyrimidines to CDK2 was gained by X-ray structures, which assisted the design of highly potent inhibitors for this kinase. Some examples were shown to inhibit the growth of human colon tumour cells, were equipotent against CDK1 and were selective against GSK-3β and other kinases.

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