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Imidazo[1,2-*a*]pyridines: A Potent and Selective Class of Cyclin-Dependent Kinase Inhibitors Identified Through Structure-Based Hybridisation

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Abstract—High-throughput screening identified the imidazo[1,2-*a*]pyridine and bisanilinopyrimidine series as inhibitors of the cyclin-dependent kinase CDK4. Comparison of their experimentally-determined binding modes and emerging structure–activity trends led to the development of potent and selective imidazo[1,2-*a*]pyridine inhibitors for CDK4 and in particular CDK2.

Cancer has long been recognized as a disease of aberrant cellular proliferation. It is now known that proteins that regulate proliferation are frequently mutated, deleted or over-expressed in cancer cell lines, and that this leads to the deregulated growth of the tumour cell. Such proteins therefore represent potential targets for therapeutic intervention.

The cyclin-dependent kinases (CDKs) are a family of serine/threonine kinases that are important in controlling entry into and transition through each phase of the cell cycle.[1] Their cellular activity is tightly regulated through a number of mechanisms:[2] phosphorylation by kinases and dephosphorylation by phosphatases; the binding of specific activating proteins called cyclins; and the binding of inhibitory peptides such as members of the p16INK4A and p21CIP/p27KIP families. These inhibitory peptides must be either sequestered or destroyed, and the CDK appropriately phosphorylated and bound to its cyclin, before the enzyme is fully active. The synthesis and degradation of the activating cyclins is tightly controlled; this, together with their specificity for a particular CDK partner, causes first one, and then another member of the CDK family to become active and drive progression through the cell cycle.

The cyclin D1-CDK4/6-pRb signalling axis is a key mediator of growth control in normal cells and a frequent target for mutation in tumours, implicating CDK4 as an important biochemical target for small molecule inhibition of cell cycling. However, since cyclin D-dependent kinases are dispensable in cells lacking functional Rb, some tumours may be refractory to specific inhibition of CDK4. Recently, CDK2 has become an attractive target with the hypothesis that pharmacological inhibition of CDK2 might selectively kill tumour cells in which inactivation or loss of pRB serves to deregulate E2F activity.[3] The development of CDK inhibitors with different selectivity profiles therefore potentially offers the opportunity to treat a wide range of tumour types.

A number of groups have identified CDK inhibitors.[4–18] In this paper we describe how comparison of the binding mode of a weak inhibitor identified through high-throughput screening with that of a more potent series described in earlier work[17,18] led to the identification of the imidazo[1,2-*a*]pyridines as a potent and selective class of CDK inhibitors.

A high-throughput screen of the AstraZeneca compound collection using an in vitro Scintillation Proximity Assay (SPA) to detect the inhibition of CDK4-cyclin D catalysed [γ-33-P]-phosphorylation of a GST-Rb substrate[15] identified the imidazo[1,2-*a*]pyridine **1** as

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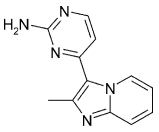
an inhibitor of CDK4 (IC_{50} 8 μM). This prompted us to initiate chemistry to explore the activity of this class of compounds against CDK2 and CDK4.



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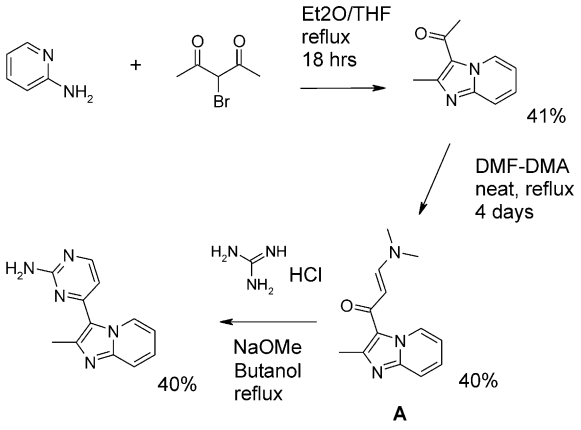
Our initial synthetic goals were to improve potency and demonstrate SAR by varying the substituents at the amino group. The synthetic route to compound **1**[19] is shown in Scheme 1: reaction of 2-amino pyridine with 3-bromo 2,4-pentanedione gave 3-acetyl imidazo[1,2-*a*]-pyridine. Condensation of the methyl ketone with DMF dimethyl acetal gave aminopropenone **A**, which could be cyclised with guanidine to give the desired 3-(pyrimid-4-yl)imidazo[1,2-*a*]pyridine product.



Scheme 1. Synthesis of imidazo[1,2-*a*]pyridine **1**.

Acetylation of the amino group was tolerated, but with little increase in potency over the parent compound **1** (Table 1).

The most significant compound in this series was **2**: although still relatively weak, it was sufficiently potent and soluble to attempt determination of the imidazo[1,2-*a*]pyridine binding mode by X-ray crystallography of the complex with CDK2.[20] In these studies, inactive CDK2 monomer was used as a structural surrogate for activated CDK–cyclin complexes. While the detailed use of a structural surrogate for molecular design will be limited, studies demonstrate that a series' bioactive conformation is typically conserved between the activated CDK2–cyclinA complex and inactive CDK2 monomer,[21,22] and between CDK2 and other kinases with low sequence homology though counter examples do exist.[22]

The crystal structure of CDK2 complexed with **2** revealed the imidazo[1,2-*a*]pyridine binding mode (Fig. 1). The hydrogen-bonding interactions with the protein are between the pyrimidine N1 and Leu83 backbone NH, the 2-amido NH and Leu83 backbone carbonyl O, and the imidazo[1,2-*a*]pyridine N1 and the Lys33 amino group. A water molecule bridges between the 2-amido carbonyl O and the Asp86 side chain (Fig. 1a). The first two hydrogen bonds are also found in the bisanilinopyrimidine binding mode (Fig. 1b) and are consistent with the accepted kinase purine-mimetic pharmacophore. The third had not been observed previously in our studies, but an equivalent interaction is found between an imidazole ring and the analogous lysine in the complexes of pyridinyl-imidazoles with p38.[23]

An earlier study had identified the bisanilinopyrimidines as potent and selective CDK inhibitors.[17,18] A compar-

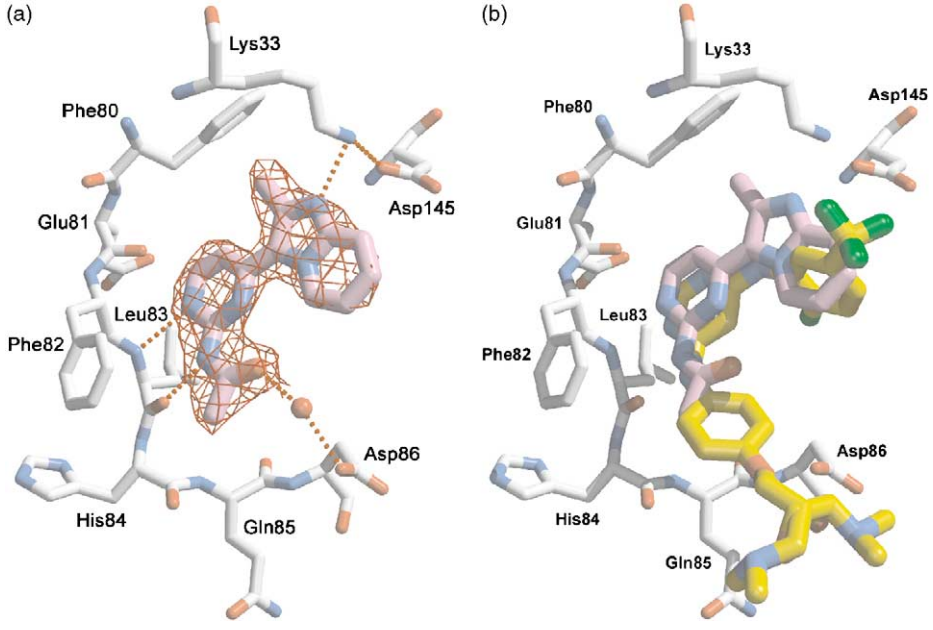
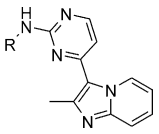


Figure 1. Crystal structure of CDK2 complexed with **2**.[24] (a) Final 2Fo-Fc electron density for the inhibitor **2** (1σ level). (**b**) Superposition (by matching Cα-carbon atoms of residues 80–87) of **2** and a bisanilinopyridine.[17] The bisanilinopyrimidine is modelled as a racemic mixture but this portion of the molecule is poorly ordered in the crystal structure.[17] The figures were prepared using Bobscript and Raster3D.[32,33]

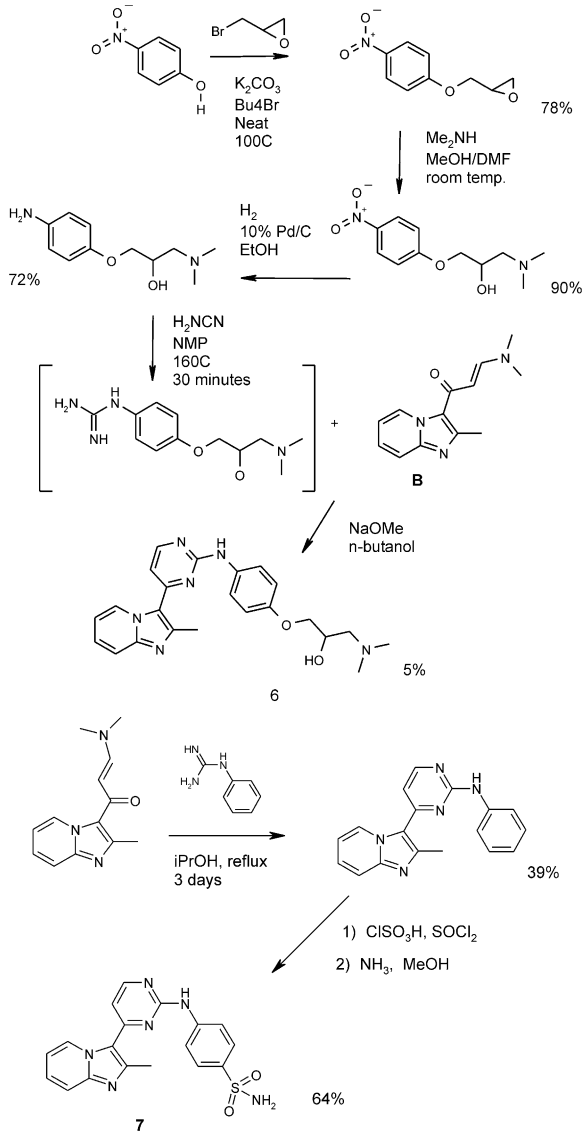
Table 1. Structures and enzyme activity for amino-substituted imidazo[1,2-*a*]pyridines



Compd	CDK4 IC_{50}, μM[a]	CDK2 IC_{50}, μM[a]	R
1	8	4	H
2	8.9	2.9	$COCH_3$
3	40	95	$COCF_3$
4	>10	>10	CONHPh

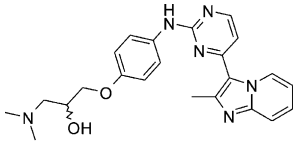
[a]Average of at least two measurements; enzyme protocol.[15]

ison of the bisanilinopyrimidine and imidazo[1,2-*a*]pyridine binding modes (Fig. 1b) suggested a hybridisation strategy: the addition of a 2-aniline group to the pyrimidine ring to replace the 2-amido group. Emerging



Scheme 2. Synthesis of 2-anilino substituted imidazo[1,2-*a*]pyridines **6** and **7**.

SAR in the bisanilinopyrimidine series suggested that CDK2 potency would be improved by a para-sulphonamoyl substituted aniline; whereas CDK4 selectivity would require a para-(3-dimethylamino-2-hydroxypropoxy) substituted aniline. The synthesis of compounds **6** and **7** was prioritised.



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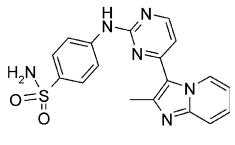

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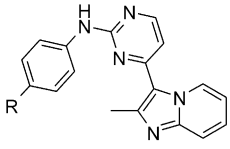
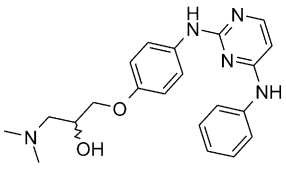
Synthesis of compound **6**[34] began with alkylation of 4-nitrophenol with epibromohydrin. Opening of the epoxide with dimethylamine followed by hydrogenation of the nitro group gave the required aniline. Reaction of this aniline with cyanamide gave the corresponding guanidine, which was reacted in situ with the aminopropenone of the imidazo[1,2-*a*]pyridine **B**. Compound **7**[35] was prepared by reaction of the same imidazo[1,2-*a*]pyridine **B** with phenylguanidine followed by chlorosulphonation of the aniline and reaction with ammonia in methanol (Scheme 2, Table 2).

Addition of the 2-anilino group (**5**) led to only a small increase in potency against CDK4 but a 100-fold increase against CDK2. Varying the anilino para-substituent led to enhanced activity against CDK4 (**6**), or particularly CDK2 (**7**). This trend was analogous to that found in the 2,4-bisanilinopyrimidine series (**8** and **9**), suggesting the binding modes of the anilino groups were similar in the two series. X-ray structure determination of the complexes of **6** and a 2-des methyl analogue[36] of **7** with CDK2 confirmed this interpretation (Fig. 2).

Table 2. Structures and enzyme activity for 2-anilino imidazo[1,2-*a*]-pyridines (IP) and their 2,4-bisanilinopyrimidine (BAP) analogues



5,6,7



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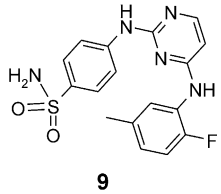

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Compd	Series	R	CDK4 IC_{50}, μM[a]	CDK2 IC_{50}, μM[a]
1	IP		8	4
5	IP	H	3.6	0.036
6	IP	$OCH_2CH(OH)CH_2N(Me)_2$	0.15	0.032
7	IP	SO_2NH_2	2.5	<0.003
8	BAP	$OCH_2CH(OH)CH_2N(Me)_2$	2	32
9	BAP	SO_2NH_2	2.7	0.006

[a]Average of at least two measurements; enzyme protocol.[15]

The hydrogen bonding interactions of the pyrimidine core to the peptide backbone remain the same in these structures as those seen in Figure 1a. In the CDK2 complex with **6**, the direct interaction between the imidazo[1,2-*a*]pyridine ring and the Lys33 amino group is replaced by a water-mediated interaction with the Asp145 side chain, possibly due to the carboxylation of the lysine side chain (Fig. 2a).

In these CDK2 structures, the hydrophobic anilino rings lie against a hydrophobic surface formed by the Phe82, Leu83, Leu134 and Ile10 side chains, and against

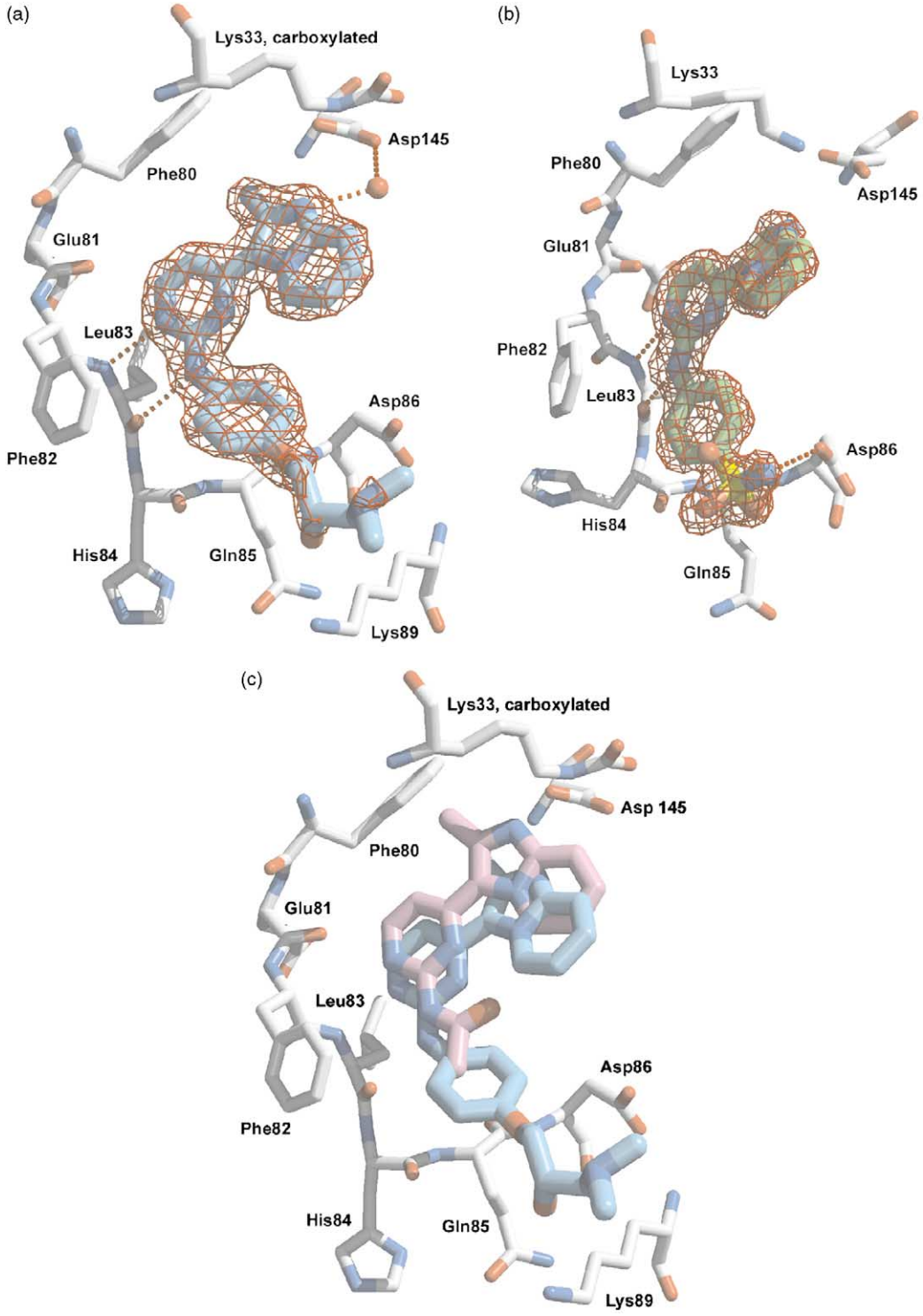

Figure 2. Crystal structures[37] of CDK2 complexed with **6** and a 2-des methyl analogue of **7**. (**a**) Final 2Fo-Fc electron density for the inhibitor **6** (1σ level). In this structure, the Lys33 side chain has become carboxylated. **6** is a racemate (one enantiomer shown) but the molecule is poorly ordered beyond the hydroxyl. (**b**) Initial 2Fo-Fc electron density for the inhibitor 2-des methyl **7** (1.3σ level). (**c**) Superposition (matching Cα-carbon atoms of residues 80–87) of **2** and **6**. The figures were prepared using Bobscript and Raster3D.[32,33]

the peptide backbone of residues 84–86. The desolvation of these hydrophobic surfaces possibly accounts for the increased potency found for compound 5. The smaller increase in CDK4 activity may be due to differences in the hydrophobic residues packing the aniline ring: Phe82 in CDK2 is replaced by His95 in CDK4, and Leu83 by Val96.

The *para*-sulphonamide group in Figure 2b forms hydrogen bonds with the Asp86 backbone NH and with its carboxylic side chain. Davies et al. have described how the aniline packing and sulphonamide binding are optimally arranged in their NU6102 series.[16] Similar interaction geometries are seen in our structure and may account for the increased potency seen for **7** over **5**. Asp86 is conserved between CDK2 and CDK4 suggesting that the improved CDK2 selectivity seen for **7** results from differences in the residues packing the aniline ring.

A number of basic residues lining the CDK2 binding site are replaced by acidic or neutral residues in CDK4. In particular Lys89, which is known to coordinate an acidic substituent in purvalanol B,[40] is replaced by Thr102 in CDK4. The lack of an equivalent lysine side chain at this position, and other differences at the surface of the binding site (CDK2/Lys9- > CDK4/Glu11 and His84- > Asp97) may account for the preference for a basic *para*-substituent by CDK4 (**5** cf **6**).

In conclusion, knowledge of the imidazo[1,2-*a*]pyridine binding mode and its comparison with the bisanilinopyridimidine SAR led to the development of the imidazo[1,2-*a*]pyridine series of CDK inhibitors. Examples from this series have been identified as potent and selective inhibitors of CDK4, while other compounds show particularly potent and selective inhibition of CDK2.

Acknowledgements

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19. Compd **1**: NMR (DMSO-d_6) δ 2.62 (d, 1H), 6.74 (s, 2H), 6.85 (d, 1H), 7.01 (td, 1H), 7.4 (m, 1H), 7.60 (d, 1H), 8.30 (d, 1H), 9.85 (d, 1H); MS (ES^+) 226 $[MH]^+$.
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24. Protein and crystals were obtained according to established procedures.[25,26] Diffraction data were collected on beamline 9.6 at SRS, Daresbury, at 100 K. Data processing, data reduction and structure solution by molecular replacement were carried out using programs from the CCP4 suite.[27] Compound **2** was modelled into electron density using QUANTA.[28] The protein complex model was refined using CNX[29] and Refmac5,[30] and the final structure[31] has been deposited in the Protein Data Bank with deposition code 1ioq together with structure factors and detailed experimental conditions.
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28. Quanta2000, Accelrys.
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31. Crystallographic statistics for 2 are: space group $P2_12_12_1$, unit cell 54.6, 72.7, 72.9 Å, resolution 2.31 Å, 12637 unique reflections from 60244 observations give 93.8% completeness with $R_{merge} = 13.2\%$ and mean $I/\sigma(I)$ of 8.0. The final model containing 2099 protein, 32 water and 20 inhibitor atoms has an R-factor of 23.3% (R_{free} using 5% of data is 27.9%). Mean temperature factor for protein is 36.3 and for ligand is 32.4 $Å^2$.
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34. Compd **6**: NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.40–2.63 (m, 2H), 3.82–4.02 (m, 3H), 6.90 (d, 2H), 7.06 (dd, 1H), 7.30 (d, 1H), 7.50 (dd, 1H), 7.59 (s, 2H), 7.74 (d, 1H), 8.38 (d, 1H), 8.58 (s, 1H), 9.42 (s, 1H); MS (ES+) 405 $[MH]^+$.
35. Compd **7**: NMR (DMSO-d_6) δ 2.64 (s, 3H), 7.05 (dd, 1H), 7.15–7.20 (m, 3H), 7.44 (dd, 1H), 7.64 (d, 1H), 7.74 (d, 2H), 7.92 (d, 2H), 8.68 (d, 1H), 9.75 (d, 1H); MS (ES+) 381 $[MH]^+$.
36. Unexpectedly, soaking with **7** cracked the cdk2 crystals whereas this did not happen with the 2-des methyl analogue. No adverse contacts between the methyl group and the protein could be identified by modelling. The synthetic route and characterisation of the 2-des methyl imidazo[1,2-*a*]pyridine series is the subject of a paper in preparation by the authors; details will be made available on request.
37. Protein and crystals were obtained according to established procedures.[25,26] Diffraction data were collected on beamline 9.6 at SRS, Daresbury, at 100 K. Data processing, data reduction and structure solution by molecular replacement were carried out using programs from the CCP4 suite.[27] Compound **6** is a racemic mixture making the conformation ill-defined beyond the hydroxyl. A single isomer was modelled into electron density using QUANTA.[28] The protein complex models was refined using CNX[29] and Refmac5,[30] and the final structures[38,39] have been deposited in the Protein Data Bank with deposition codes 1oir and 1oit together with structure factors and detailed experimental conditions.
38. Crystallographic statistics for **6** are: space group $P2_12_12_1$, unit cell 53.0, 70.7, 72.9 Å, resolution 1.91 Å, 20394 unique reflections from 95201 observations give 84.8% completeness with $R_{merge} = 5.2\%$ and mean $I/\sigma(I)$ of 21.7. The final model containing 2284 protein, 124 water and 31 inhibitor atoms has an *R*-factor of 19.7% (R_{free} using 5% of data is 23.7%). Mean temperature factor for protein is 14.6 and for ligand is 22.1 $Å^2$.
39. Crystallographic statistics for 2-des methyl analogue of **7** are: space group $P2_12_12_1$, unit cell 53.5, 72.2, 72.2 Å, resolution 1.60 Å, 33885 unique reflections from 179548 observations give 80.7% completeness with $R_{merge} = 3.5\%$ and mean $I/\sigma(I)$ of 17.8. The final model containing 2059 protein, 272 water and 26 inhibitor atoms has an *R*-factor of 26.3% (R_{free} using 5% of data is 25.5%). Mean temperature factor for protein is 19.4 and for ligand is 19.6 $Å^2$.
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