

Structure-based design of a new class of highly selective aminoimidazo[1,2-*a*]pyridine-based inhibitors of cyclin dependent kinases

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Abstract—Structure-based design approach was successfully used to guide the evolution of imidazopyridine scaffold yielding new structural class of highly selective inhibitors of cyclin dependent kinases that were able to form a new interaction with an identified residue of the protein, Lys89. Compounds from this series have shown no detectable effect when tested against a representative set of other serine/threonine kinases such as GSK3 β , CAMKII, PKA, PKC- $\alpha,\beta,\epsilon,\gamma$. Compound **2i** inhibits proliferation in HCT 116 cells in tissue culture. Synthesis, co-crystal structure of CDK2 in complex with compound **2i**, and preliminary SAR study are disclosed. © 2005 Elsevier Ltd. All rights reserved.

Cyclin-dependent kinases (CDK) and their cyclin partners are key players in regulating the entry into, passage through, and exit from the cell cycle.¹ Because of their critical role in the regulation of cell cycle and the observed expression/activity pattern in most human cancers, considerable effort has been focused on the development of small molecule inhibitors.² However, the number of structural classes that act as CDK inhibitors is limited, and most of them derive from relatively nonspecific protein kinase inhibitor scaffolds that also inhibit CDKs, such as staurosporins,³ flavonoids,⁴ indigoids,⁵ paulones,⁶ and purines.⁷ Considerable efforts are still devoted to the search for new structural classes of cyclin-dependent kinase inhibitors⁸ with potentially new selectivity profiles and new physiochemical properties.

We recently described the discovery of a new structural class of protein serine/threonine kinase inhibitors comprising of an aminoimidazo[1,2-*a*]pyridine nucleus.⁹

Compounds represented by structure **1** were demonstrated to inhibit cyclin dependent kinases by competing with ATP for binding to a catalytic subunit of the protein. In view of these results, we sought to utilize the structural information from the crystal structure of compound **1** bound to monomeric human CDK2 to identify new potential binding sites with greater impact on selectivity (Fig. 1).

Our initial hypothesis was: could the internal hydrogen bonds in the aminoimidazo[1,2-*a*]pyridine **1**, be replaced by a newly identified external interaction with Lys89? This might also hold the amino hydrogen in a *syn* position with the nitrogen at the 1-position, favoring the key interactions with Leu83. Herein we report our efforts to test this hypothesis. The synthesis of our target molecules is outlined in Scheme 1. In path A, reaction of 2-amino-5-iodo-pyridine **3** with *p*-tolylsulfonyl chloride in pyridine and subsequent treatment with iodoacetamide in the presence of Hünig's base in DMF provided the corresponding carbamide¹⁰ **4** in good yields. Conversion of **4** to the desired 2-(*N*-trifluoroacetyl-amino)imidazopyridine **5** was accomplished by treatment with trifluoroacetic anhydride in refluxing methylene

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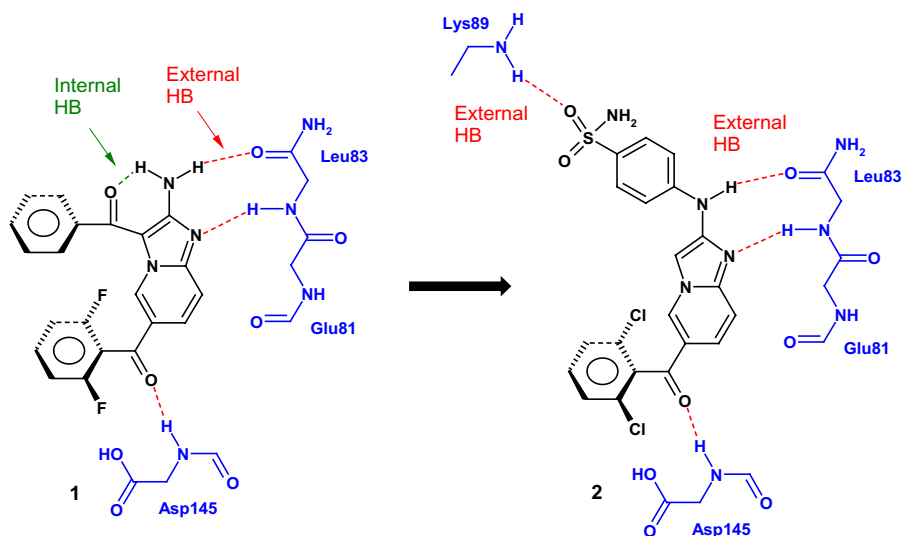
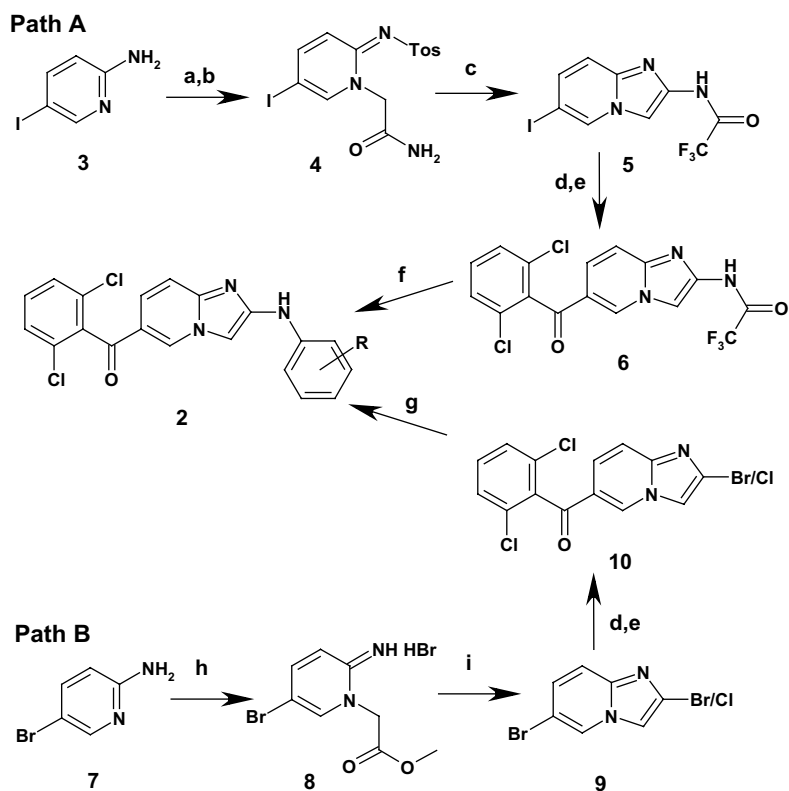


Figure 1. Two-dimensional representation of the binding mode of imidazopyridines **1** and **2** with hCDK2.

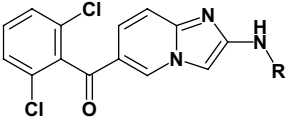
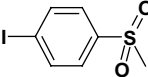
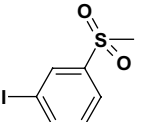
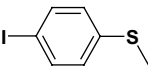
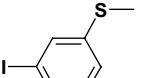
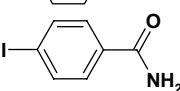
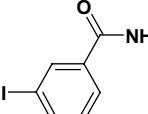
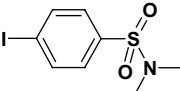
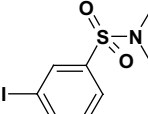
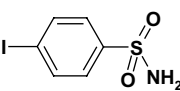
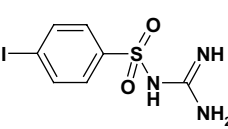
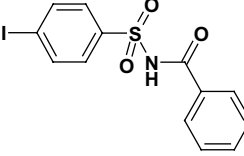
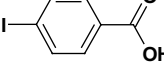
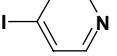
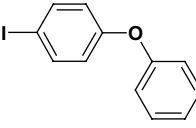


Scheme 1. Synthesis of phenylaminoimidazo[1,2-*a*]pyridines: (a) TsCl, py, 89%; (b) ICH₂CONH₂, DIPEA, DMF, 86%; (c) TFAA, CH₂Cl₂, 63%; (d) *i*-PrMgCl, THF, 2,6-Cl₂-C₆H₃CHO, 81%; (e) MnO₂, CH₂Cl₂, 79%; (f) ArNH₂, >250 °C; (g) BrCH₂CO₂CH₃, 97%; (h) POCl₃, 105 °C, 98%; (i) ArNH₂, CoCl₂, Ph₂O, 150–200 °C, 20–90%.

chloride. Metalation of compound **5** with *i*-propyl magnesium chloride and subsequent reaction with 2,6-dichlorobenzaldehyde followed by oxidation with manganese dioxide furnished the diaryl ketone **6**. The trifluoroacetamide group was then substituted by reaction with a selection of anilines at high temperature to give the corresponding phenylaminoimidazo[1,2-*a*]pyridines **2**. Alternatively, compounds **2** were also prepared

using a cobalt-mediated amination as illustrated in path **B**. Reaction of the commercially available 2-amino-5-bromopyridine **7** with methyl bromoacetate and subsequent treatment with phosphorus oxychloride gave a 1:3 mixture of 2-bromo and 2-chloro imidazo[1,2-*a*]pyridines **9** in good yield. A halogen–metal exchange study on building block **9** showed that use of *i*-propyl magnesium chloride is most effective for chemoselective

Table 1. Enzyme inhibition data for compounds **2a–n**

				
Compds	R	E/CDK2 Inhibition IC ₅₀ (μM) ^a	D1/CDK4 Inhibition IC ₅₀ (μM) ^a	B/CDK1 Inhibition IC ₅₀ (μM) ^a
2a		1.21	1.22	0.52
2b		2.86	3.14	1.7
2c		13.85	7.40	3.04
2d		17.73	11.90	4.8
2e		1.63	1.61	0.58
2f		2.72	3.95	1.92
2g		1.54	2.16	0.97
2h		12.09	>20	9.26
2i		0.56	1.34	0.39
2j		0.25	1.29	0.16
2k		0.67	3.84	0.25
2l		0.74	4.99	0.45
2m		>20	>20	>20
2n		>20	>20	>20
	Flavopiridol	0.96	0.18	0.20

^a Values are means of three experiments, see Ref. 9 for assays protocol.

functionalization at position-6. Reaction of compound **5** with *i*-propyl magnesium chloride and subsequent reaction with 2,6-dichlorobenzaldehyde followed by oxidation with manganese dioxide furnished the diaryl ketone **10** in 65% yield from **9**. The Br/Cl group was then substituted by reaction with a selection of anilines in the presence of cobalt chloride in diphenyl ether at 150–200 °C.¹¹

Our assumption was tested through both SAR studies and X-ray crystallographic analysis. Initial compounds that were designed to satisfy the new interaction with Lys89 were the methyl sulfones **2a** and **2b**. As listed in Table 1, these two compounds showed moderate activity against cyclin dependent kinases CDK2, CDK4, and CDK1, **2a** (IC_{50} = 1.21, 1.22, and 0.52 μ M) and **2b** (IC_{50} = 2.86, 3.14, and 1.7 μ M).

Interestingly when the oxygens were removed (sulfide analogues **2c** and **2d**) the activity dropped significantly.

Phenyl sulfonamide and carboxylic amides were also found to be potent against cyclin dependent kinases. Our initial data suggests the substitution at the *para*-position to be more favorable for CDKs inhibition. In addition, incorporation of proton prone to forming stronger hydrogen bond such as in **2i**, **2j**, **2k**, and **2l** increases potency.

The crystal structure of compound **2i** bound to monomeric human CDK2 was solved to reveal the basis for the binding behavior.¹² Inhibitor **2i** was found to occupy the ATP binding site of CDK2 as shown in Figure 2. In particular, the N-1 of the imidazopyridines acts as a hydrogen bond acceptor with the backbone NH of Leu83 (heavy atom distance of 2.95 Å), and the amino group donates a hydrogen bond to the backbone car-

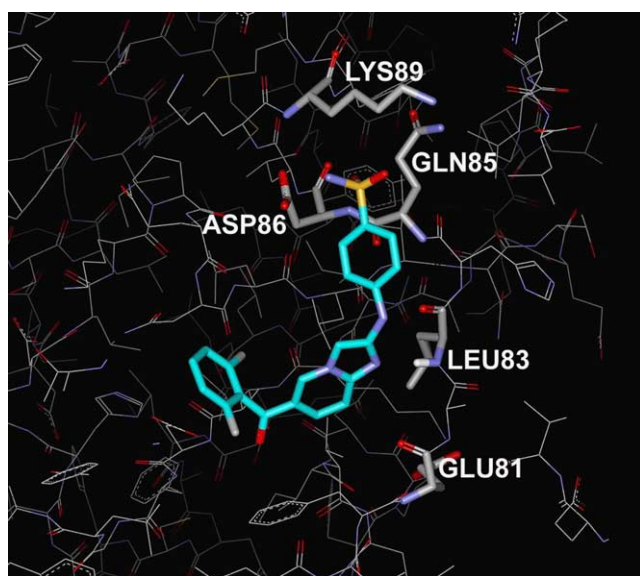


Figure 2. Crystal structure of imidazopyridine **2i** bound to CDK2. The compound occupies the space of the ATP-pocket. The inhibitor carbon atoms are in light blue, the nitrogen atoms are in blue, the chlorine atoms are in grey, the oxygen atoms are in red and the sulfur atoms are in yellow. The protein carbon atoms are in grey.

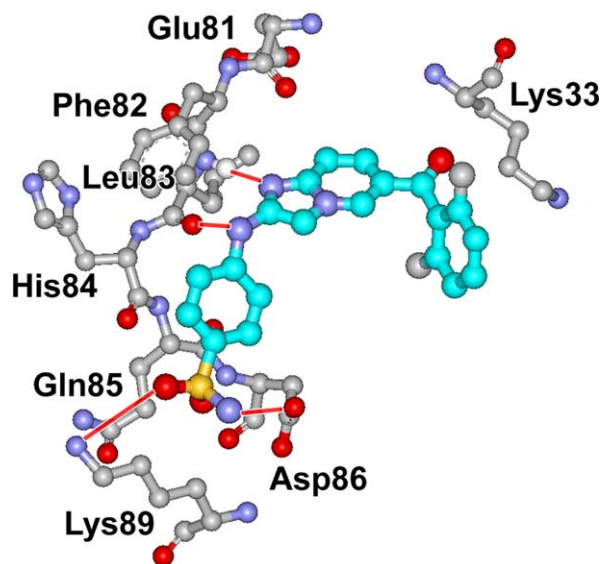


Figure 3. Binding of imidazopyridine **2i** to CDK2 highlighting the surrounding residues and hydrogen bond interactions (red lines).

bonyl oxygen of Leu83 (heavy atom distance of 2.61 Å). In addition, there is an indication of hydrogen bond interactions between the sulfonamide group and both the Lys89 (O–N distance of 4.13 Å) and Asp86 (N–O distance of 3.44 Å) as shown in Figure 3. The Lys89 bridges the inhibitor sulfonamide and the surface Arg297 guanidinium group (N–N distance of 4.54 Å). The side chain of Asp145 is 3.32 Å from one of the aromatic chlorine, which creates speculation of the potential protonation of the Asp145 side chain.

X-ray crystallographic analysis, preliminary SAR study, conformational analysis and docking studies furnished a number of guidelines for analogue design. A complete loss of affinity was found when the 2-position was either 4-aminopyridine (compound **2m**) or phenyl ether (**2n**).

This is not unexpected as the pyridine of the compound **2m** is too short to reach the Lys89 and adequately form a hydrogen bond, while compound **2n** has the bulkier hydrophobic phenyl group, which does not have enough room to be accommodated in this area.

The original lead compound **1**⁹ was used to validate our CDK2 docking model using the CDocker methodology.¹³ Using this CDK2 docking model, new proposed molecules were docked to find their optimal binding geometry. Then their binding affinity was predicted using the DoMCoSAR (Docking mode that correlates with SAR) methodology.¹¹ DoMCoSAR uses a statistical model based on the energetics of the ligand/protein residue interactions. The comparison of the binding geometries of the CDocker methodology, however, has shown good ability to predict a mode that can be confirmed by crystallography. The results of the prediction versus the original lead compound **1**¹⁰ and the crystalized compound **2i** are shown in Figure 4. The prediction agrees with the crystallographic results with 1.06 RMS, with the main deviation being a slight distortion of the sulfonamide.

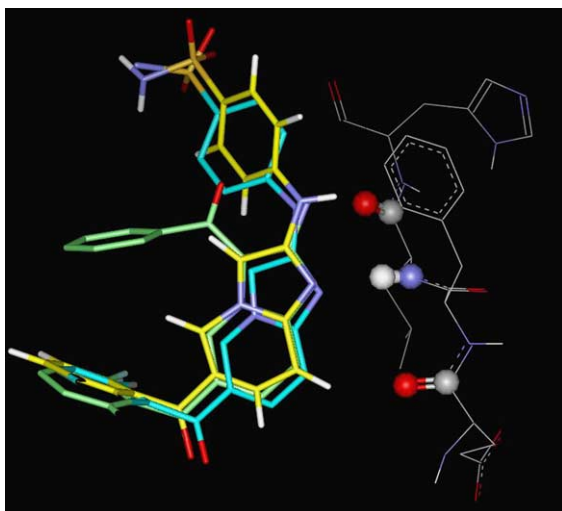


Figure 4. Comparison of the prospective predicted binding geometry of the difluoroanalog of **2i** (carbons in yellow) and the observed crystal structures of the original lead compound **1** (carbons in green) and the **2i** compound (carbons in light blue) bound to CDK2.

Of particular interest is the observed specificity of this class of compounds for CDKs over other kinases. Compound **2i** has shown no detectable effect when tested against a representative set of other serine/threonine kinases such as GSK3 β , CAMKII, PKA, PKC- α , β , ϵ , γ . This compound inhibits proliferation in HCT 116 cells in tissue culture as determined by an MTT assay ($IC_{50} = 5.5 \mu M$).

In conclusion, we have identified a highly selective structural class of cyclin dependent kinase inhibitors by modifying aminoimidazo[1,2-*a*]pyridines scaffold. Structural-based design approach was successfully used to investigate and design new compounds that were able to form a new interaction with an identified residue of the protein, Lys89. This new interaction eliminated the need for internal hydrogen bonds. These compounds were found to compete with ATP for binding to a catalytic subunit of the protein. Initial structure features required for activity were defined. Further studies, including in vivo activities and additional structure-activity relationships are underway.

Acknowledgements

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References and notes

- (a) Sherr, C. J. *Science* **1996**, *274*, 1672; (b) Grana, X.; Reddy, E. P. *Oncogene* **1995**, *11*, 211; (c) Hunter, T. *Cell* **1997**, *88*, 333; (d) Malumbres, M.; Barbacid, M. *Nat. Rev. Cancer* **2001**, *1*, 222; (e) Stein, G.; Baserga, R.; Giordano, A.; Denhardt, D. T. *The Molecular Basis of Cell Cycle Control*; John Wiley & Sons: New York, 1998; p 389; (f)

- Pines, J. *Trends Biochem. Sci.* **1993**, *18*, 195; (g) Gillet, C. E.; Barnes, D. M. *J. Clin. Pathol.* **1998**, *51*, 310.
- (a) Gray, N.; Detivaud, L.; Doerig, C.; Meijer, L. *Curr. Med. Chem.* **1999**, *6*, 859; (b) Knockaert, M.; Greengard, P.; Meijer, L. *Trends Pharmacol. Sci.* **2002**, *23*, 417; (c) Lees, J. A.; Weinberg, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4221; (d) Kong, N.; Fotouhi, N.; Wovkulich, P. M.; Roberts, J. *Drugs Future* **2003**, *9*, 881; (e) Shapiro, G. I. *Mol. Biol. Intel. Unit* **2002**, *15*, 208.
- (a) Gadbois, D.; Hamaguchi, J. R.; Swank, R. A.; Bradbury, E. M. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 80–85; (b) Meijer, L.; Thunnissen, A. M.; White, A. W.; Garnier, M.; Nikolic, M.; Tsai, L. H.; Walter, J.; Cleverley, K. E.; Salinas, P. C.; Wu, Y. Z.; Biernat, J.; Mandelkow, E. M.; Kim, S.-H.; Pettit, G. R. *Chem. Biol.* **2000**, *7*, 51.
- (a) Sedlacek, H. H.; Czech, J.; Naik, R.; Kaur, G.; Worland, P.; Losiewicz, M.; Parker, B.; Carlson, B.; Smith, A.; Senderowicz, A.; Sausville, E. *Int. J. Oncol.* **1996**, *9*, 1143; (b) Shapiro, G. I. *Clin. Cancer Res.* **2004**, *10*, 4270; (c) Newcomb, E. W. *Anti-Cancer Drugs* **2004**, *15*, 411.
- Hoessel, R.; Leclerc, S.; Endicott, J.; Nobel, M.; Lawrie, A.; Tunnah, P.; Leost, M. E. D.; Marie, D.; Marko, D.; Niederberger, E.; Tang, W.; Eisenbrand, G.; Meijer, L. *Nat. Cell Biol.* **1999**, *1*, 60.
- Zaharevitz, D.; Gussio, R.; Leost, M.; Senderowicz, A.; Lahusen, T.; Kunick, C.; Meijer, L.; Sausville, E. A. *Cancer Res.* **1999**, *59*, 2566.
- Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J.; Donella-Deana, A.; Pinna, L.; Letham, D.; Kato, J.; Detivaud, L.; Leclerc, S. *Eur. J. Biochem.* **1994**, *224*, 771.
- (a) Misra, R. N.; Xiao, H.-y.; Williams, D. K.; Kim, K. S.; Lu, S.; Keller, K. A.; Mulheron, J. G.; Batorsky, R.; Tokarski, J. S.; Sack, J. S.; Kimball, S. D.; Lee, F. Y.; Webster, K. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2973; (b) Anderson, M.; Beattie, J. F.; Breault, G. A.; Breed, J.; Byth, K. F.; Culshaw, J. D.; Ellston, R. P. A.; Green, S.; Minshull, C. A.; Norman, R. A.; Paupit, R. A.; Stanway, J.; Thomas, A. P.; Jewsbury, P. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3021.
- (a) Hamdouchi, C.; Keyser, H.; Collins, E.; Jaramillo, C.; de Diego, E.; Spencer, C.; Dempsey, J.; Anderson, B.; Leggett, T.; Stamm, N.; Schultz, R.; Watkins, S.; Cocke, K.; Lemke, S.; Burke, T.; Beckmann, R.; Dixon, J.; Gurganus, T.; Rankl, N.; Houck, K. A.; Zhang, F.; Vieth, M.; Espinosa, J.; Timm, D.; Campbell, R.; Patel, B.; Brooks, H. B. *Mol. Cancer Ther.* **2004**, *3*, 1; (b) Jaramillo, C.; de Diego, E.; Hamdouchi, C.; Collins, E.; Keyser, H.; Sanchez-Martinez, C.; delPrado, M.; Norman, B.; Brooks, H.; Watkins, S.; Spencer, C.; Dempsey, J.; Anderson, B.; Leggett, T.; Schultz, R.; Watkins, S.; Lemke, S.; Burke, T.; Beckmann, R.; Dixon, J.; Gurganus, T.; Rankl, N.; Houck, K. A.; Zhang, F.; Vieth, M.; Espinosa, J.; Timm, D.; Campbell, R.; Patel, B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6095.
- (a) Hamdouchi, C.; Sanchez, C.; Ezquerra, J. *Synthesis* **1998**, 867; (b) Hamdouchi, C.; de Blas, J.; del Prado, M.; Gruber, J.; Heinz, B. A.; Vance, L. *J. Med. Chem.* **1999**, *42*, 50.
- Final compounds were characterized by 400/500 MHz 1H NMR, HRMS, and LC/MS (97%).
- The crystal structure of compound **2i** bound to monomeric human CDK2 has been deposited in the Protein Data Bank with deposition code 1YKR together with structure factors and detailed experimental condition.
- Wu, G.; Robertson, D. H.; Brooks, C. L., III; Vieth, M. *J. Comput. Chem.* **2003**, *24*, 1549.