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Structure-based design of potent CDK1 inhibitors derived from olomoucine

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Summary

Cyclin-dependent kinase 1 (CDK1), an enzyme participating in the regulation of the cell cycle, constitutes a possible target in the search for new antitumor agents. Starting from the purine derivative olomoucine and following a structure-based approach, potent inhibitors of this enzyme were rapidly identified. The molecular modeling aspects of this work are described.

Introduction

Cyclin-dependent kinases (CDKs) play an important role in the regulation of the division cycle of mammalian cells [1, 2]. Inhibitors of these enzymes block the progression of the cell cycle and are therefore considered to be of potential interest as novel antiproliferative agents in anticancer drug research [3–5]. In particular, we have selected CDK1, whose activity is essential for the G2/M phase transition of the cycle, as a target for a medicinal chemistry effort. Our goal is to identify potent and selective low molecular weight inhibitors of CDK1 that act by preventing the binding of the cofactor ATP to the enzyme. As part of this effort, we engaged in a chemistry program to enhance the potency of olomoucine (see Figure 1 for chemical structures of compounds discussed through the article), a micromolar, highly selective inhibitor of CDK1, identified by Vesely et al. in a screen of purine derivatives [6]. The chemistry and biology aspects of this work have recently been published [7]. As a complement, we would like to report here the molecular modeling work underlying the early part of the program during which the inhibitory potency of olomoucine was improved by two orders of magnitude by synthesizing only a few designed analogues.

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Basis for a structure-based optimization of olomoucine

The three-dimensional structure of CDK1 has not yet been determined. However, several X-ray crystal structures of the closely related CDK2 enzyme have been solved [8–13]. These include the structure of the apoenzyme and those of CDK2 in complex with ATP and various inhibitors including olomoucine. The latter [12] shows that olomoucine binds in the large cleft at the interface of the N- and C-terminal domains of the protein that forms the binding site for ATP. This was not unexpected for an inhibitor derived from adenine. However, as illustrated by a model in Figure 2, although the purine ring of olomoucine occupies roughly the same location as that of ATP in the cleft, their relative orientations with respect to the protein differ. In both ligands the adenine moiety forms two hydrogen bonds in a bidentate manner with the backbone of the amino acid stretch connecting the N- and C-terminal domains of the kinase. This stretch, known as the hinge region, comprises residues 81 to 86 in CDK2. By analogy to the mode of base pairing of adenine nucleotides, the binding mode of ATP can be qualified as being of the 'Watson-Crick' type. The adenine N1 atom accepts a hydrogen bond from the amide NH of Leu 83 while the N6 amino group donates one to the backbone carbonyl of Glu 81. In contrast, olomoucine adopts a 'Hoogsteen' type binding mode in

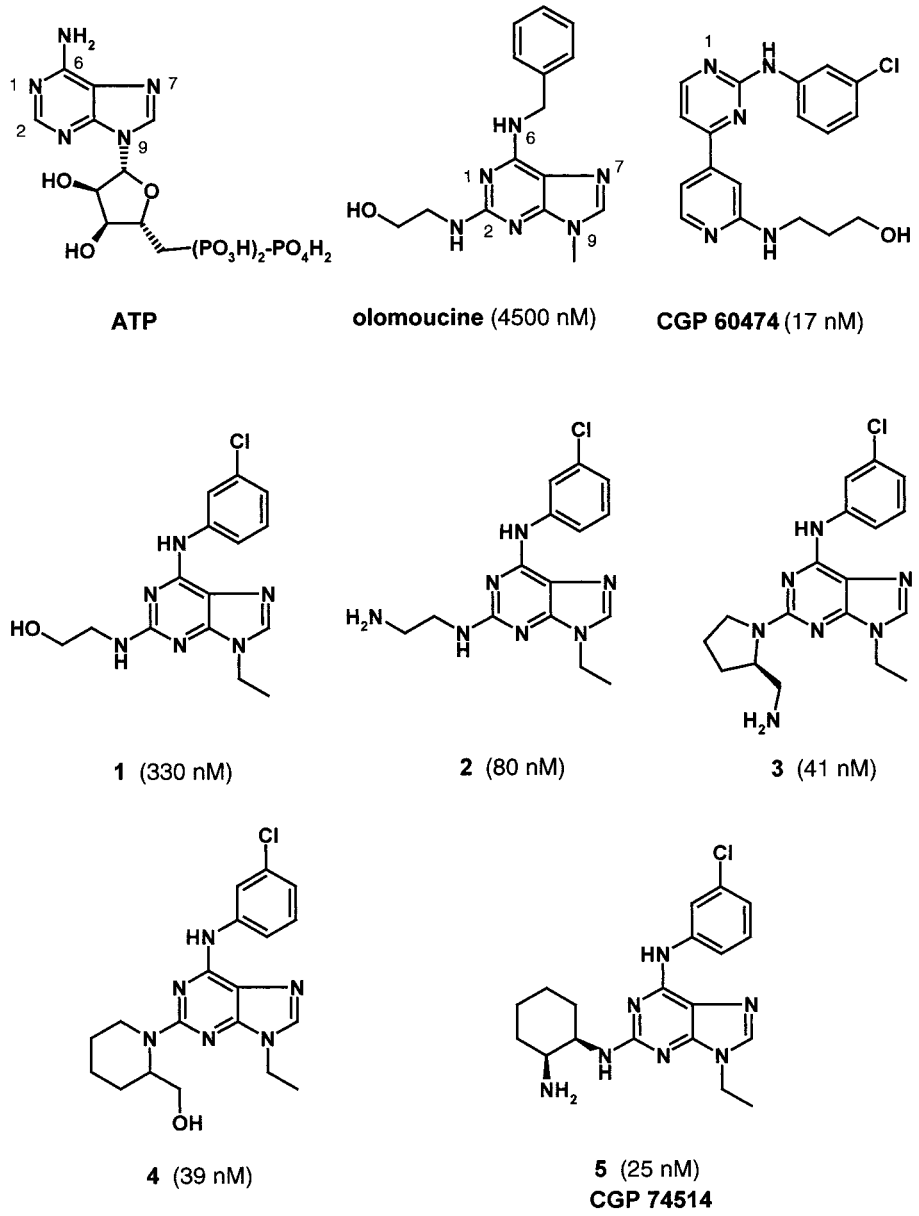

Figure 1. Chemical structures and CDK1 inhibitory activities of discussed molecules. The synthesis and biological testing of the olomoucine derivatives are described in Reference 7.

which the N7, and not the N1 atom, accepts the hydrogen bond from the NH group of Leu 83 while N6 forms a hydrogen bond with the backbone carbonyl of the same residue. Modeling suggests that this situation can be ascribed to the presence of a bulky benzyl substituent at position N6 of olomoucine. Docking of the inhibitor in the cleft following the same hydrogen bonding pattern as that used by ATP leads to a severe steric clash between the benzyl substituent and some residues including Phe 80. In addition, it is known that introducing a substituent at the N6 position of adenine disfavors the conformation in which the amino proton is *syn* with respect to the N1 atom as required in a binding mode of the 'Watson-Crick' type [14].

Based on this information and sequence alignment, a three-dimensional model of the ATP binding pocket of CDK1 containing bound olomoucine was constructed as described in the next section. The model

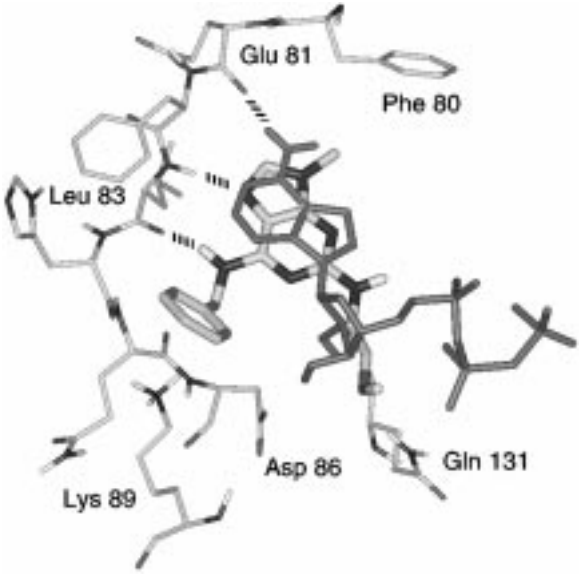

Figure 2. Relative binding modes of olomoucine and ATP (dark) in the active site of CDK2. The hydrogen bonds involving the adenine moieties of the ligands and the hinge region of the kinase are represented as dashed lines.

was subsequently used to design modifications at the synthetically accessible N9, N6 and C2 positions of olomoucine with the objective of improving its CDK1 inhibitory activity.

Construction of the model

As can be seen in Figure 3, where the sequences of human CDK1 and CDK2 are aligned according to the scheme of Hanks et al. [15], the ATP binding sites of these two enzymes are very similar. They present only two differences, at positions 84 and 85, in their amino acid composition. These differences can be considered as minor since the side chains of the amino acids in positions 84 and 85 project outside the ATP binding pocket and are not involved in ligand binding in any of the published X-ray crystal structures of CDK2. This, in addition to the high degree of conservation observed in the tertiary structures of protein kinases [16, 17], supports the use of CDK2 as an appropriate template to model the ATP binding site of CDK1.

Although the X-ray crystal structure of human CDK2 in complex with olomoucine has been reported, the coordinates of the complex have not been made available. However, those of the apoenzyme and the ATP complex have been deposited at the Brookhaven Protein Databank (entry codes 1HCL and 1HCK respectively). Judging from these structures and the analysis reported in Reference 12, it can be concluded that ligand binding does not induce major structural changes of the enzyme. The only significant changes observed are adjustments of the side chain conformations of some residues including Ile 10, Lys 33, Lys 89, Gln 131 and Asn 132. Thus, a model of the ATP binding site of CDK1 in complex with olomoucine was constructed as summarized below.

Starting from the coordinates of the CDK2-ATP complex (1HCK), only the residues of the protein having an atom within a distance of 6 Å of any atom of ATP were kept (residues in the boxes in Figure 3). ATP was replaced by olomoucine docked in the orientation and conformation shown in Figure 2. The conformations of the side chains of residues Ile 10, Lys 89, Gln 131 and Asn 132, which are different in the ATP and olomoucine complexes, were adjusted to those observed in the olomoucine complex according to the indications given in Reference 12. Then, residues His 84 and Gln 85, the only residues of the ATP binding pocket that are different in CDK1, were mutated to Ser and Met respectively to conform to the sequence of CDK1. The final complex was energy minimized keeping the amino acids of the protein rigid.

Methods

The modeling work was performed in MacroModel v.4.0 [18]. The complexes formed between ligands and the ATP pocket of CDK1 were energy minimized. The ligand was allowed to move freely upon minimization while the atoms of the protein residues were constrained by application of a parabolic force constant of 50 kJ/Å. Energies were calculated using the AMBER* force field [19] in conjunction with the GB/SA water solvation model [20].

For each designed ligand, a full conformational search of the isolated molecule was performed, applying the water solvation model, to make sure that the modeled CDK1 bound conformation corresponds to a low-energy conformational minimum (calculated energy within 3 kcal/mol of that of the global minimum). In compounds **3** and **4**, the conformations presenting pseudo-axial and axial positions of the methylamino and methylhydroxy moieties, respectively, had the lowest energy due to destabilization of the pseudo-equatorial and equatorial conformations by steric hindrance with the adjacent pyrimidine ring.

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CDK1:    1 MEDYTKIEKIGEGTYGVVYKGRHKTTGQVVAMKKIRLE  38
CDK2:    1 MENFQKVEKIGEGTYGVVYKARNKLTGEVVALKKIRLD  38

CDK1:   39 SEEEGVPSTAIREISLLKELRHPNIVSLQDVLMQDSRL  76
CDK2:   39 TETEGVPSTAIREISLLKELNHPNIVKLLDVIHTENKL  76

CDK1:   77 YLIFEFLSMDLKKYLDSIPPGQYMDSSLVKSYLYQILQ 114
CDK2:   77 YLVFEFLHQDLKKFMDASALTG-IPLPLIKSYLFQLLQ 113

CDK1:  115 GIVFCHSRRVLHRDLKPQNLLIDDKGTIKLADFGLARA 152
CDK2:  114 GLAFCHSHRVLHRDLKPQNLLINTEGAIKLADFGLARA 151

CDK1:  153 FGIPIRVYTHEVVTLWYRSPEVLLGSARYSTPVDIWSI 190
CDK2:  152 FGVPVRTYTHEVVTLWYRAPEILLGCKYYSTAVDIWSL 189

CDK1:  191 GTIFAELATKKPLFHGDSEIDQLFRIFRALGTPNNEVW 228
CDK2:  190 GCIFAEMVTRRALFPGDSEIDQLFRIFRTLGTPDEEVW 227

CDK1:  229 PEVESLQDYKNTFPKWKPGSLASHVKNLDENGLDLLSK 266
CDK2:  228 PGVTSMPDYKPSFPKWARQDFSKVVPPLDEDGRSLLSQ 265

CDK1:  267 MLIYDPAKRISGKMALNHPYF                   287
CDK2:  266 MLHYDPNKRISAKAALAHPFF                   286
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Figure 3. Alignment of the sequences of human CDK1 (SWISS-PROT code P06493) and human CDK2 (SWISS-PROT code P24941). The amino acid residues inside boxes form the ATP binding sites of these kinases (defined from the X-ray structure of the ATP-CDK2 complex 1HCK as residues having an atom within a distance of 6 Å of any atom of ATP).

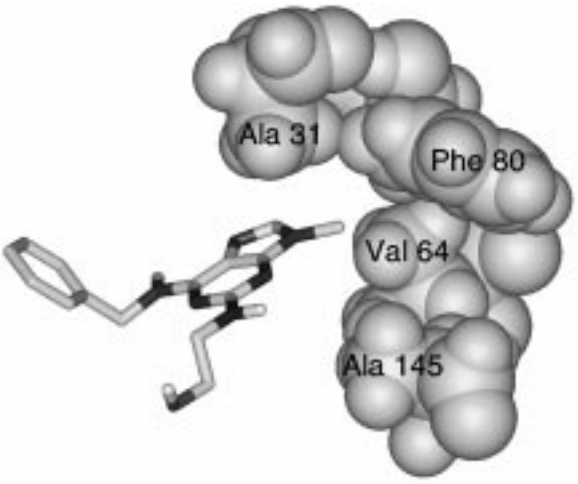

Figure 4. Environment of the methyl group in position N9 in the model of the CDK1-olomoucine complex.

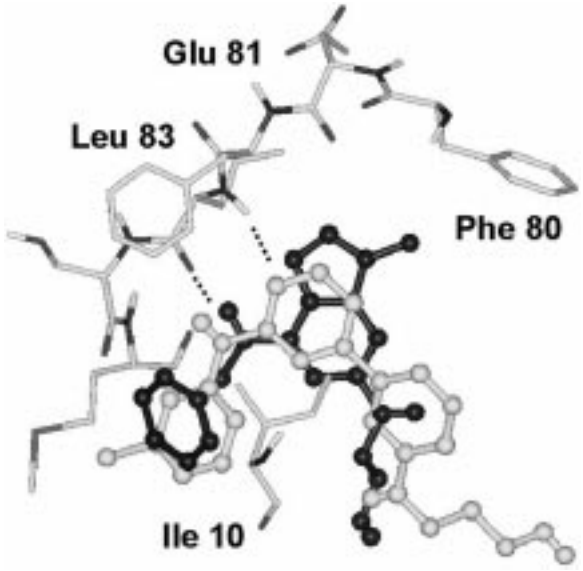

Figure 5. Model of the relative binding modes of CGP 60474 (light) and olomoucine (dark) in the ATP binding site of CDK1.

N9 modification

Inspection of the model of the CDK1-olomoucine complex suggested that the N9 position offered few opportunities to increase the number of favorable binding interactions of the inhibitor with the enzyme. The N9 methyl group of olomoucine lies close to the side chains of residues Phe 80, Ala 31, Val 64 and Ala 145 (Figure 4). Modeling experiments supported the possibility to increase the size of the substituent by one atom in either one of the two directions perpendicular to the plane of the purine ring. However, an increase of more than one atom in any direction caused severe steric clashes with the above residues. For this reason, no substantial chemistry effort was undertaken to derivatize the N9 position. The optimization of the other positions of the inhibitor was carried out with ethyl at N9. In agreement with the model, replacing the N9 methyl substituent of olomoucine by ethyl is tolerated. This modification leads to a slight gain in CDK1 inhibitory activity (twofold increase).

N6 modification

By screening compounds synthesized in other protein kinase inhibitor projects of the company, some phenylamino-pyrimidine derivatives were identified to possess high CDK1 inhibitory activity. With an IC_{50} value of 17 nM, CGP 60474 is representative of these compounds [21, 22]. It was therefore of interest to model the binding mode of this type of inhibitor in the ATP binding site of CDK1. Interactive docking studies guided by structure–activity relationships, in particular the fact that methylation of the anilino nitrogen abolishes activity, led to the model shown in Figure 5. As can be seen, a binding mode similar to that of olomoucine, including the formation of bidentate hydrogen bonds with the backbone of residue Leu 83, was assumed. Precisely, the pyrimidine N1 atom of CGP 60474 accepts a hydrogen bond from the amide NH of Leu 83 while its anilino nitrogen hydrogen bonds to the carbonyl group of the residue. These hydrogen bonds position the chlorophenyl moiety of the inhibitor in the same region of the binding site as that occupied by the benzyl group of olomoucine. An alternative ATP-like binding mode in which the anilino nitrogen donates a hydrogen bond to the backbone oxygen of Glu 81 was discarded because it required an orientation of the molecule placing the chlorophenyl moiety in severe steric conflict with residue Phe 80.

The equivalent positions of the anilino nitrogen of CGP 60474 and the N6 atom of olomoucine in the model gave support to the idea of synthesizing a hybrid molecule in which the chlorophenyl ring of CGP 60474 replaces the benzyl substituent at position N6 of olomoucine. Structural information indicates that the benzylic phenyl ring of olomoucine is involved in an amino-aromatic interaction with the side chain of Lys 89. The ring also makes multiple hydrophobic contacts with Ile 10. Replacing the benzyl group of olomoucine by phenyl in the model suggests a loss of this amino-aromatic interaction. However, the phenyl group directly attached to N6 is still able to make extensive hydrophobic contacts with Ile 10 and some additional ones with Phe 82. Moreover, this modification provides a conformationally more rigid compound with the associated entropic advantage by conjugation of the anilino moiety with the purine ring. This can explain why compound **1**, when synthesized, turned out to be our first olomoucine analogue with submicromolar potency (330 nM).

C2 modification

As illustrated in Figure 2, the CDK2 crystal structures on which our model is based indicate that the C2 position of the purine ring of olomoucine faces the part of the catalytic cleft that normally interacts with the ribose and triphosphate moieties of ATP. The hydroxyethylamino chain attached to this position of the inhibitor forms a hydrogen bond with the amide group of the side chain of Gln 131. In the CDK2-ATP complex, it is the main chain amide carbonyl of Gln 131 that makes a hydrogen bond with the ligand, precisely with one of the hydroxy groups of the ribose moiety. By changing the conformation of the hydroxyethylamino chain of olomoucine in our model, we noticed that its terminal hydroxy group could also be placed within hydrogen bonding distance from the backbone carbonyl of Gln 132 (Gln 132 corresponds to CDK2 residue Gln 131 in the numbering of CDK1). This required to decrease the value of the N-C-C-O dihedral angle from 180° (extended) to 60° (gauche +). In this alternative conformation of the chain, in addition, the hydroxy group was within hydrogen bonding distance from the side chain carboxylate group of Asp 86, a residue proximal to Gln 132. This observation led to the idea of replacing the hydroxy by an amino group capable of donating two hydrogen bonds, one to the carbonyl of Gln 132, the other to the carboxylate of Asp 86, as shown in Figure 6. It was expected that the additional hydrogen bond formed with Asp 86 would be beneficial. In full agreement with this concept, the designed amino modification produced a fourfold increase of the potency of compound **1**, the amino analogue **2** having an IC_{50} value of 80 nM.

Aiming at further improvement of inhibitory activity, we envisaged restricting the conformational freedom of the C2 chain in **2**. To this end two types of cyclizations were designed, both favoring the adoption by the chain of the conformation required to achieve the two hydrogen bonds with Gln 132 and Asp 86. The first one, represented by compound **3**, consisted of a five-membered ring including the first two atoms of the chain. In addition to favoring a pseudo-axial position of the methylamino moiety necessary to place the amino group within hydrogen bonding distance of Gln 132 and Asp 86, the ring made hydrophobic contacts with Val 18 and Gly 11 in our model (Figure 7). When synthesized, using a proline intermediate having the required stereochemistry, **3** turned out to be twice more active than parent compound **2**, an encouraging result. Saturated five-membered rings are

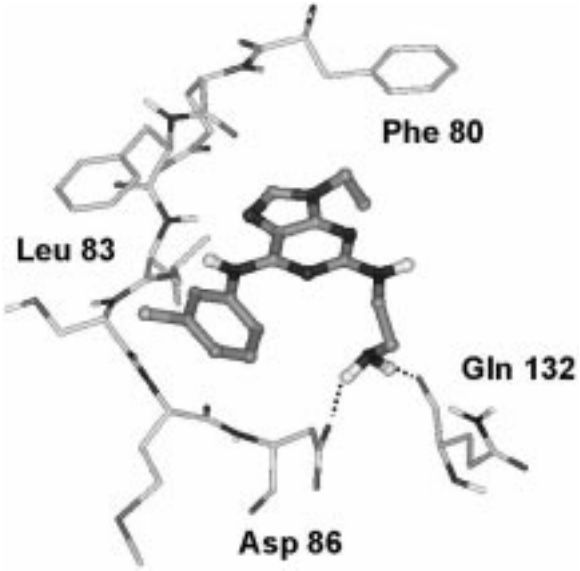

Figure 6. Model of designed compound **2** bound to the ATP pocket of CDK1. The amino group of **2** forms hydrogen bonds with Asp 86 and Gln 132, indicated as dashed lines.

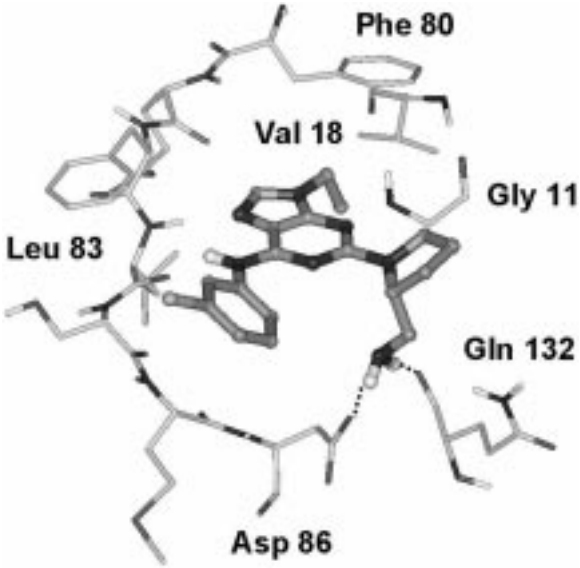

Figure 7. Model of designed compound **3** bound to the ATP pocket of CDK1. The hydrogen bonds formed with Asp 86 and Gln 132 appear as dashed lines.

notorious for their plasticity. We felt that a more rigid six-membered ring analogue of **3**, by losing less entropy upon binding, would probably be more active. For reasons of synthesis, this idea was realized with a terminal hydroxy group on the C2 moiety instead of the more favorable amino one. Impressively, the resulting compound **4**, tested as a mixture of enantiomers, was one order of magnitude more potent than the non-cyclized parent hydroxy compound **1**, thus validating the design.

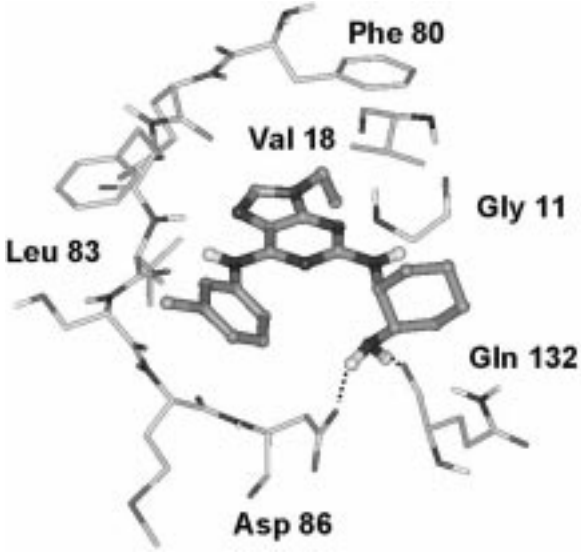

Figure 8. Model of designed compound **5** bound to the ATP pocket of CDK1. The hydrogen bonds formed with Asp 86 and Gln 132 appear as dashed lines.

The second type of cyclization involved the construction of a six-membered ring between the two carbon positions of the chain. As shown in Figure 8, attachment of a (1S, 2S) cis-diaminocyclohexane moiety, in a proper conformation, to the C2 position of the purine ring allows the formation of the targeted hydrogen bonds with Glu 132 and Asp 86. As in the first type of cyclization, our model suggested that the cyclohexyl ring could make favorable hydrophobic contacts with residues Val 18 and Gly 11. As expected, compound **5** (CGP 74514), synthesized on the basis of this design, was more potent than **2**. With an IC_{50} value reaching 25 nM, a fourfold increase in inhibitory activity was obtained, making **5** one of the most potent CDK1 inhibitors reported to date [23–27]. Furthermore, **5** maintained the selectivity profile displayed by olomoucine [6]. As indicated in Table 3 of Reference 7, **5** exhibits selectivity ratios of more than 100 against kinases belonging to different families (PKCα, PKA and EGF-R) while it inhibits CDK2 (IC_{50} = 9 nM) as potently as CDK1.

Conclusions

The notion that a large number of compounds needs to be synthesized to optimize the in vitro potency of

a lead structure is still widely accepted in contemporary medicinal chemistry. The work reported here shows that this is not necessarily true. Appropriate use of structural information and molecular modeling allowed us to increase the CDK1 inhibitory potency of olomoucine by several orders of magnitude through the introduction of a few designed modifications. This illustrates the effectiveness of the structure-based approach in lead optimization problems.

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