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Identification of cylin-dependent kinase 1 inhibitors of a new chemical type by structure-based design and database searching

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Summary

We have selected cyclin-dependent kinase 1 (CDK1), an enzyme participating in the regulation of the cell cycle, as a target in our efforts to discover new antitumor agents. By exploiting available structural information, we designed an ATP-site directed ligand scaffold that allowed us to identify 4-(3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-benzenesulfonamide as a new potent inhibitor of CDK1 in a subsequent database search. The synthesis and testing of some analogues confirmed the interest of this class of compounds as novel CDK1 inhibitors.

Abbreviations: ATP – adenosine triphosphate; CDK – cyclin-dependent kinase; EGF-R – epidermal growth factor receptor; PKA – cyclic AMP-dependent protein kinase; PKC – protein kinase C; CA – chemical abstracts.

Introduction

The cell division cycle is controlled by a complex molecular machinery involving a variety of regulatory proteins that constitute a source of potential targets in anticancer drug research [1, 2]. In particular, due to their crucial role in regulating the progression of the cell through the different phases of the cycle, cyclin-dependent kinases (CDKs) have recently attracted a lot of attention [3]. The enzymatic activity of one of these kinases, CDK1, is essential for the G2/M phase transition of the cycle [4]. Inhibitors of CDK1 can induce the death of deregulated cancer cells by blocking them at this late stage of the cycle just before mitosis [5]. One of our strategies, to discover new antitumor agents, is based on this concept. Thus, we have initiated efforts to identify small organic molecules that inhibit CDK1 by binding to its ATP (cofactor) pocket. These include the optimization of established leads [6–8] like the purine derivative olomoucine (**1**) [9] but also the search for new chemical classes of inhibitors. We report here the results of work addressing the latter aspect by a combination of structure-based design and database searching.

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Structural basis

Small molecules of different chemical types have been reported to inhibit CDK1 and the closely related enzyme CDK2 [10]. The structural basis of the inhibition capacity of some of them has been elucidated by protein X-ray crystallography. This is the case for **1** [11] and the *des*-chloro analogue (**2**) [12] of flavopiridol (**3**), a flavonoid compound undergoing clinical evaluation [13]. The structures of their complex with CDK2 have been determined revealing that these inhibitors interact with the ATP binding site of the enzyme in the way shown in Figure 1. As can be seen, both inhibitors form a pair of bidentate hydrogen bonds with the backbone of the amino acid stretch that connects the N- and C-terminal domains of the kinase, the so called 'hinge' region. Both **1** and **2** accept a hydrogen bond from the backbone amide of Leu 83: **2** using the oxygen atom of the chromenone carbonyl function as the acceptor and **1** the purine N7 atom. However, they donate hydrogen bonds to different residues of the hinge region. The

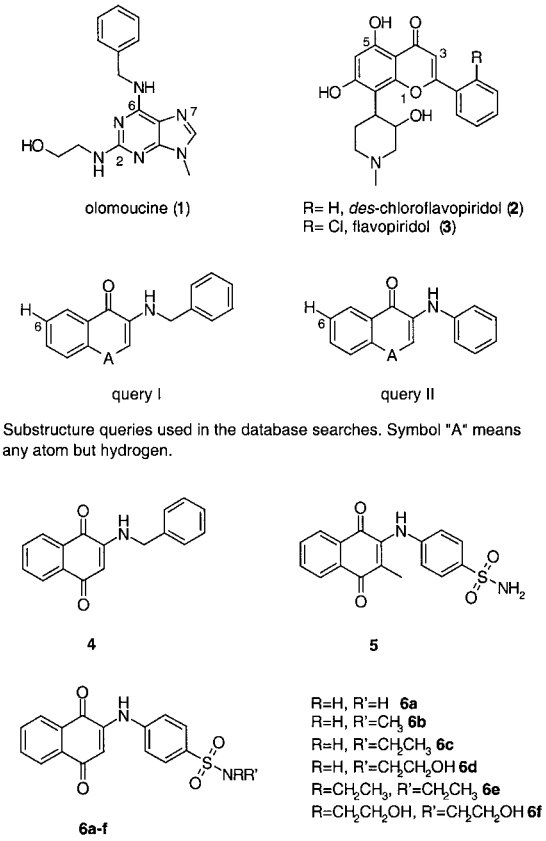


Chart 1.

C6 benzylamine group of **1** engages the carbonyl of Leu 83 while the 5-hydroxy substituent of **2** interacts with the carbonyl of Glu 81. The bidentate hydrogen bonds position the conjugated bicyclic systems of the inhibitors in the same region of the pocket, precisely in the hydrophobic environment provided by the side chains of residues Ala 31, Val 64, Phe 80, Leu 134 and Ala 144. These normally serve to bind the adenine moiety of ATP. In addition, the benzyl group of **1** and the 2-phenyl substituent of **2** extend in the same direction making hydrophobic contacts with Ile 10.

Scaffold design

The overlay of **1** and **2** representing their relative binding modes in the ATP pocket of CDK2 suggested the design of hybrid molecules. In particular, we noticed in Figure 1 the proximity of the C3 position of the chromenone moiety of **2** and the C6 position of the purine ring of **1**. This led to the idea of introducing a secondary amine functionality at the C3 position of **2** in order to mimic the benzylamine group of **1** in terms of hydrogen bonding with the carbonyl group of Leu 83 and hydrophobic interactions with Ile 10. Further elaboration of this idea, supported by docking studies [14], confirmed the attractiveness of the molecular scaffold shown in Figure 2 in our search for new CDK1 inhibitors. As can be appreciated in this figure, the designed scaffold forms a pair of bidentate hydrogen bonds with the backbone of Leu 83, as **1** does, while the bicyclic system occupies the hydrophobic region of the ATP pocket. Positions 1 and 8 of the scaffold are potential attachment points for chemical moieties interacting with the same regions of the cavity as the C2 chain of **1** and the piperidyl moiety of **2**, respectively. Moreover, lipophilic R substituents on the amine are ideally positioned to contact Ile 10.

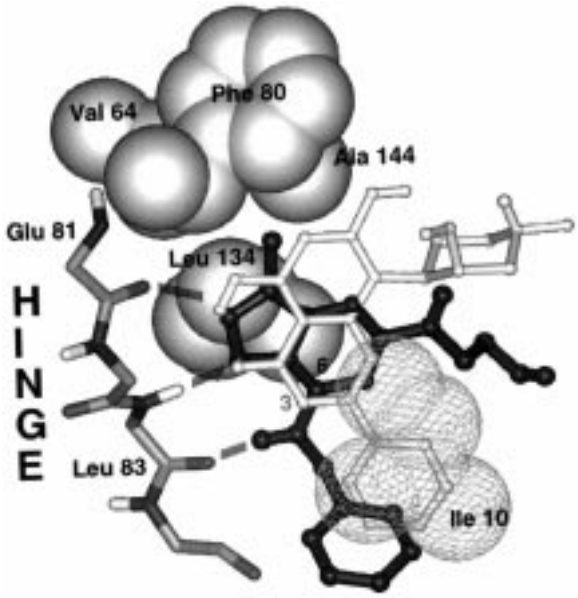


Figure 1. Relative binding modes of **1** (dark) and **2** (light) in the ATP pocket of CDK2. Hydrogen bonds formed with the hinge region are indicated as dashed lines. The side chain of Ile 10 which is located above the planes of the inhibitors bicyclic systems is represented in mesh style.

Identification of new inhibitors by database searching

We checked if compounds corresponding to the designed scaffold were available in the chemical archives of Novartis by performing substructure searches using the two queries drawn in Chart 1. In the first one, a benzyl group was taken as the amine susbtituent to search for exact mimics of **1**. In the second one, a phenyl substituent was used because we had previously found that this modification leads to more potent CDK1 inhibitors in the olomoucine series [6–8]. We excluded substituents at position 6 of the bicyclic system because in our binding model this position was too close to residue Phe 80 to tolerate any additional bulk. Heteroaromatic rings as alternatives to the phenyl part of the bicycle were also excluded due to the purely hydrophobic environment of this part of the scaffold in the model.

These searches returned several compounds, all possessing a naphthoquinone moiety as the conjugated bicyclic system. Only two of them, compounds **4** and **5**, were available for testing. The unavailable derivatives were analogues of **5** having a chloro instead of a methyl substituent in the quinone moiety or a different substituent on the anilino ring.

Table 1. IC_{50} values (μM units) in CDK1 and T24 cell proliferation inhibition assays

	1	**3**	**4**	**5**	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**
CDK1	7	0.4	34	0.6	0.5	0.8	0.3	1.0	5.3	3.8
T24	70	0.1	–	0.1	0.1	0.5	0.9	0.9	2.3	0.6

To have an idea of which other existing chemical classes of compounds, not represented in the Novartis compound collection, would match the designed scaffold, the Chemical Abstracts (CA) registry was also searched using queries I and II. Besides naphthoquinones that represented the majority of hits, molecules having the following core bicyclic systems were retrieved: 4H-1-Benzopyran-4-one, 4H-1-Benzothiopyran-4-one, 1,4-Naphthoquinone imine and 4 (1H)-Quinolone.

When tested compounds **4** and **5** turned out to inhibit CDK1 with IC_{50} values of 34 μM and 0.6 μM respectively. The potency of these inhibitors is comparable to that of **1** (7 μM) [9] or **3** (0.4 μM) [10], proving the utility of the designed scaffold in the identification of new CDK1 inhibitors.

The fact that **5** displays the same level of potency as **3** is remarkable. The two inhibitors are assumed to form the same number of hydrogen bonds with the backbone of the protein in the hinge region. In addition to a common bicyclic conjugated system occupying the hydrophobic region of the ATP pocket, they both possess an aryl moiety that can make hydrophobic contacts with Ile 10. However, **5** lacks the piperidyl moiety of **3** which is seen to make favorable interactions with the enzyme in the X-ray structure of CDK2 in complex with the *des*-chloro analogue **2**. As illustrated in Figure 3, docking **5** into the ATP pocket suggested a contribution of the sulfonamide group to potency through hydrogen bonding interactions with Lys 89 and Asp 86, two residues located at the entrance of the pocket.

Furthermore, **5** appeared to selectively inhibit CDK1 as testified by IC_{50} values greater than 10 μM in inhibition tests involving other kinases. These include CDK4 and kinases that do not belong to the CDK family (PKCα, PKA and EGF-R). The much weaker potency of **5** against CDK4 may be ascribed, at least partially, to the lack of proper hydrogen bond acceptor group in this enzyme to interact with the sulfonamide substituent. The amino acid correspond-

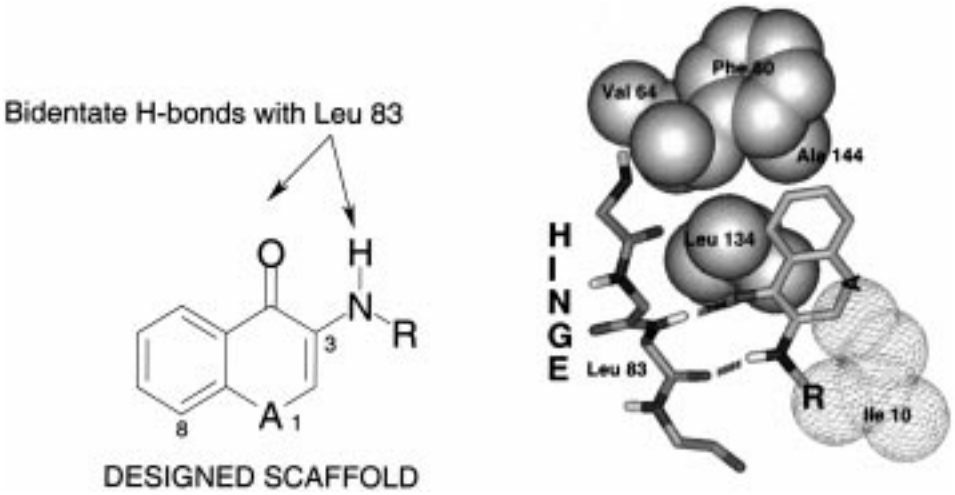


Figure 2. Designed ligand scaffold. Position 'A' can be carbon, nitrogen, oxygen or sulfur.

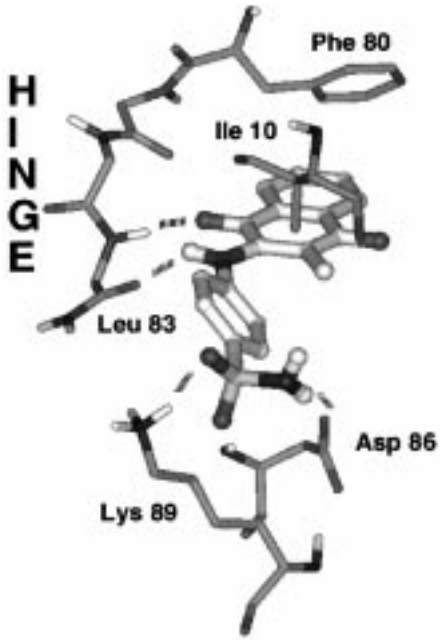


Figure 3. Compound **5** docked in the ATP binding pocket. Hydrogen bonds are indicated as dashed lines.

ing to Lys 89 in CDK4 is a shorter and less polar threonine.

5 was also tested for its ability to inhibit the proliferation of human bladder carcinoma T24 cells *in vitro* (Table 1). In this assay, **5** was also equipotent to the well established CDK inhibitor flavopiridol.

Analogue synthesis and binding mode validation by X-ray crystallography

Encouraged by the significant biological activity of **5**, we envisaged the synthesis of some analogues. The sulfonamide part of the molecule was the most accessible, in terms of synthesis, to introduce substituents. Modification of this group would provide information on its role in the CDK1 inhibitory activity of **5**. Morever, it gave a handle to modulate the physico-chemical properties of the compound in the perspective of tests in cell-based assays. Thus, derivatives **6a-f** (Chart 1) were prepared. The sulfonamide groups were selected based on visual inspection of the model of **5** docked in the ATP pocket of CDK2. With the groups introduced in **6b**, **6c** and **6e**, we wanted to ascertain the existence of the postulated hydrogen bond with Asp 86 while the groups with alcohol functionalities introduced in **6d** and **6f** were attempts to gain additional hydrogen bond interactions with the enzyme. In analogues **6a-f**, the methyl group present in position 3 of the naphthoquinone moiety of **5** was omitted because in our model we did not see any favorable interaction between this group and the ATP binding site. Some of the compounds proved to have activity in the submicromolar range both in inhibiting CDK1 and the proliferation of bladder carcinoma T24 cells (Table 1), thereby confirming the interest of the naphthoquinone scaffold in our search for new antitumor agents.

CDK2 cocrystallisation experiments were attempted with several of the synthesized compounds. With one of them, **6d**, crystals of suitable quality for

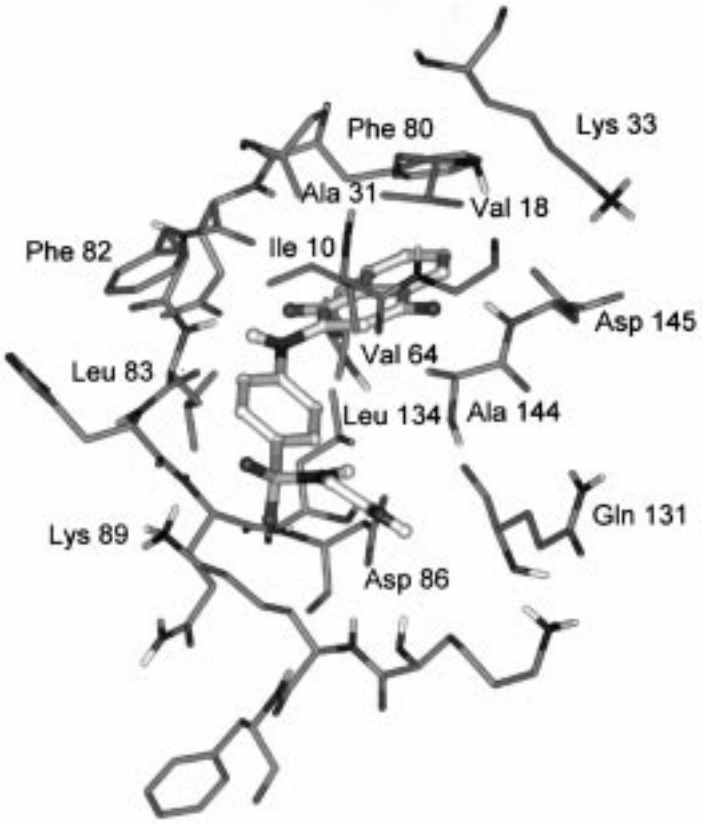


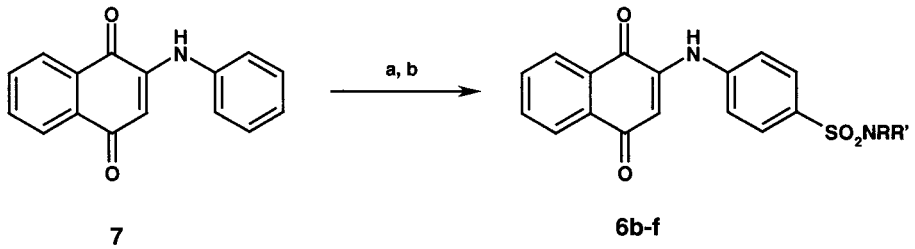
Figure 4. X-ray crystal structure of CDK2 in complex with compound **6d**. Only the amino acids within a distance of 6 Å of **6d** are represented.

X-ray diffraction were obtained. These allowed us to solve a structure of CDK2 ligated by **6d** at 3.0 Å resolution. As shown in Figure 4, the crystal structure fully confirmed the binding mode that was assumed for the naphthoquinone inhibitors based on the design hypothesis. As predicted, the acceptor-donor system formed by the 1-keto and 2-amino groups of the naphthoquinone makes bidentate hydrogen bonds with the backbone of Leu 83. The expected hydrophobic interactions between the bicycle and Ala 31, Val 64, Phe 80, Leu 134 and Ala 144 are also observed. As for the *p*-sulfonamide phenyl moiety, the major hydrophobic contact with the side chain of Ile 10 is present while the sulfonamide goup is engaged in multiple hydrogen bond interactions with Lys 89 and Asp 86 as anticipated by modeling. Precisely, the SO_2 oxygen atoms accept a hydrogen bond from the side chain amino group of Lys 89 and for one of them, also from the backbone NH of Asp 86. The sulfonamide NH donates a hydrogen bond to the side chain of Asp 86. However, the alcohol chain appended to it does not interact with the protein and extends towards the solvent.

The structure-CDK1 activity relationships reported in Table 1 are consistent with the crystal structure. Compounds **6e** and **6f** have a tertiary sulfonamide group. Hence, contrary to the primary and secondary sulfonamide analogues **5** and **6a-d**, they cannot form the hydrogen bond with the side chain of Asp 86. As a result, they are less potent than the latter in inhibiting the enzyme. Introducing a single substituent on the sulfonamide group (compounds **6b-d** compared to **6a)** has no significant effect on potency in agreement with the observation that the alcohol chain of **6d** does not interact with the protein in the crystal structure.

Conclusion

In summary, exploiting X-ray crystal structure information on an homologous enzyme and mining the compound collection of our company, we were able to identify CDK1 inhibitors of a novel chemical type that



7 6b-f

Scheme 1. (a) HSO_3CI, 60 °C, 2h, 85%; (b) amines (NHRR), THF, 1h, rt, 50–75%.

show antiproliferative activity *in vitro*. The existence of related classes of compounds equally matching the designed scaffold in the CA registry suggests that other CDK1 inhibitors could be found based on the same approach. This work illustrates the power of structure-based design in lead discovery.

Experimental section and methods used

Chemistry

Compounds **4**, **5** and **6a** (R=R′=H, Chart 1) are not novel. Their synthesis and characterization have previously been described in a different context than CDK1 inhibition in references [15] and [16]. Sulfonamides **6b-f** were prepared in two steps starting from commercially available 2-phenylamino-1,4-naphthoquinone (**7**, Aldrich) as outlined in Scheme 1. First, **7** was treated with chlorosulfonic acid to give the sulfochloride, which then was reacted with various amines to furnish the desired sulfonamides in good yields.

Biology

The experimental details of enzyme purification and inhibition assays used in this study are described in reference [17] for CDK1, in reference [18] for PKCα and EGF-R and finally in reference [19] for PKA. The details of the CDK4/cyclin D1 enzyme inhibition assay can be found in reference [20].

The compounds were tested for antiproliferative activity on the human bladder carcinoma T24 cells as described in reference [7].

Molecular modeling

The modeling work was performed in MacroModel v.4.0 [21] (‘in house’ version enhanced for graphics by A. Dietrich-Unpublished results). The ligands were manually docked in the ATP pocket of CDK2 (PDB structure used: 1HCK). Then, energy minimization of the complexes was performed using the AMBER* force field [22] in conjunction with the GB/SA water solvation model [23]. Only residues of the ATP pocket within 6 Å of the initial position of the ligand were included in the calculations. The 2-dimensional queries shown in Chart 1 and the subsequent substructure searches were made using the ISIS DRAW-ISIS BASE software, version 2.1.3d (MDL). The full Novartis corporate database of compounds was searched. The search in the CA database was performed with SciFinder. 250 hits were obtained in the latter search. No chemical programmes have yet been initiated around the retrieved structures besides the naphthoquinones. Because the designed scaffold (Figure 2) is a flat aromatic structure, it was not necessary to perform 3-D database searches.

Table 2. Analytical data for compounds **6a–f**

Compound	ESI-MS (pos. mode) m/z $[M+H]^+$	M.p. (°C)[a]
6a	329	303
6b	343	246–249
6c	357	220–223
6d	373	203–206
6e	385	198–200
6f	417	198–205

[a]Decomposition.

X-ray crystallography

Human CDK2 was expressed in Sf9 insect cells using recombinant baculovirus encoding CDK2. The protein was purified and crystallized according to the protocols described in reference [24]. The crystals obtained belonged to the space group $P2_12_12_1$with unit-cell dimensions $a = 73.3$ Å $\pm$ 0.8, $b = 72.1$ Å

± 0.7, c = 53.6 Å ± 0.3, $\alpha = \beta = \gamma = 90°$C. The inhibitor was soaked in these apo-CDK2 crystals and diffraction data was collected. The structure of the inhibitor-CDK2 complex was solved by molecular replacement. The data collection and structure determination statistics are the following:

X-ray source	FR591 rotating anode X-ray generator
Temperature	105 K
Resolution	3.0 Å
Rsym	12.9%
Completeness	76.0%
Unique reflections	4523
Reflections/atoms	2.0
Rfactor	24.0%
Rfree	35.8%
Δbond	0.009%
Δangle	1.54°
Waters	none
Residues	1-36, 45-150, 163-296

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