Molecular Dynamics, Density Functional, ADMET Predictions, Virtual Screening, and Molecular Interaction Field Studies for Identification and Evaluation of Novel Potential CDK2 Inhibitors In Cancer Therapy†

Vinicius Barreto da Silva,‡ Daniel Fábio Kawano,‡ Adriane da Silveira Gomes,‡ Ivone Carvalho,‡ Carlton Anthony Taft,§ and Carlos Henrique Tomich de Paula da Silva*,‡

Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café, s/n, Monte Alegre 14040-903, Ribeirão Preto-SP, Brazil, and Centro Brasileiro de Pesquisas Físicas, Rua Dr. Xavier Sigaud, 150, Urca 22290-180, Rio de Janeiro-RJ, Brazil

Received: February 3, 2008; Revised Manuscript Received: May 23, 2008

In this work, we have used molecular dynamics, density functional theory, virtual screening, ADMET predictions, and molecular interaction field studies to design and propose eight novel potential inhibitors of CDK2. The eight molecules proposed showed interesting structural characteristics that are required for inhibiting the CDK2 activity and show potential as drug candidates for the treatment of cancer. The parameters related to the Rule of Five were calculated, and only one of the molecules violated more than one parameter. One of the proposals and one of the drug-like compounds selected by virtual screening indicated to be promising candidates for CDK2-based cancer therapy.

1. Introduction

In recent years, much attention has turned toward the proteins that control the cell cycle progression as rational targets for anticancer drug discovery, with large participation of computer-aided drug design techniques.[1−4] Virtual screening methods are capable of exploring and exploiting molecular similarity. They can be used to analyze and predict biologically active compounds and correlate structural features and chemical properties of molecules with specific activities. Since it is increasingly recognized that simply synthesizing and screening of more and more compounds does not necessarily provide a sufficiently large number of high-quality leads and, ultimately, clinical candidates, much effort is spent in developing and implementing computational concepts that help to identify and refine leads.[1] The retinoblastoma (Rb) has been identified as one of the most important pathways in controlling normal cell proliferation, and although few human tumors contain a mutation of the Rb gene itself, the majority of human malignancies have derangement in Rb function due to "hyperactivation" of cyclin-dependent kinases. The Rb negatively regulates the cellular G1/S transition of the proliferative cell cycle and is required for proper differentiation of certain cell types, including skeletal muscle, adipocytes, and keratinocytes. It becomes hyperphosphorylated in the late G1 phase and remains hyperphosphorylated in S, G2, and M phases. Cyclin/cyclin-dependent kinases complexes complement each other to achieve complete Rb hyperphosphorylation in late G1, inactivating its growth-suppressive function and allowing cell cycle progression.[5,6]

The cyclin-dependent kinases (CDKs) are a class of serine−threonine kinases that are responsible for the progression of cells through the various phases and transitions of the cell cycle. As the name implies, the activity of these kinases as well as their subcellular localization and substrate specificity depend upon the presence of a proteic regulatory subunit called cyclin. To date, different cyclins (A, B1, B2, D, and E) and CDKs (CDK1−CDK11) have been identified in human cells, but the number of complexes they form is limited. CDK2, for example, complexes only with cyclin A or cyclin E.[7,8]

As well as other kinases, CDKs have a tertiary structure composed of a small amino-terminal lobe and a larger carboxy-terminal lobe. However, two modifications make them inactive in the absence of cyclin: (1) a large T-loop blocks the binding of the protein's substrate (ATP) at the entrance of the active-site cleft, and (2) several important amino acid side chains in the active site are incorrectly positioned. Cyclin linkage and phosphorylation of a conserved residue T160 stabilizes the complex and makes it active, inducing conformational changes to avoid the steric hindrance caused by the T-loop, moving the amino acid side chains to the correct position, allowing the docking of ATP in the active-site cleft.[4,9,10]

Since cancer is essentially a disease of uncontrolled cell growth and CDKs play a central role in cell growth regulation, such as DNA replication and chromosome separation, it is not surprising that CDK's activity is deregulated in tumors. It was demonstrated that the physiologic levels of the coactivator and the inhibitor of CDK2, cyclin E and p27, respectively, are altered in breast, colon, non-small-cell lung, gastric, prostate, bladder, non-Hodgkin's lymphoma, ovarian, and other cancers.[11−15]

According to these findings, small molecules that provide the inhibition of the complex CDK2−cyclin A are expected to be useful as antitumor agents.[16] Actually, several CDK inhibitors are known (Figure 1), of which roscovitine (1),[17] olomoucine (2),[18,19] isopentenyladenine (3),[18] flavopiridol (4),[20,21] staurosporine (5),[22] and indirubin (6)[23] are important examples. Flavopiridol, roscovitine, and UCN-01, a hydroxyl analogue of staurosporine, are reported to be under clinical evaluation.[24] Since all compounds have the same mode of action, competing with ATP for the kinase binding site, structure-based drug design approaches have been used to help in the recent development

† Part of the special section for the "Symposium on Energetics and Dynamics of Molecules, Solids and Surfaces".

* To whom correspondence should be addressed.
‡ Universidade de São Paulo.
§ Centro Brasileiro de Pesquisas Físicas.

10.1021/jp8011969 CCC: $40.75
Published on Web 08/13/2008

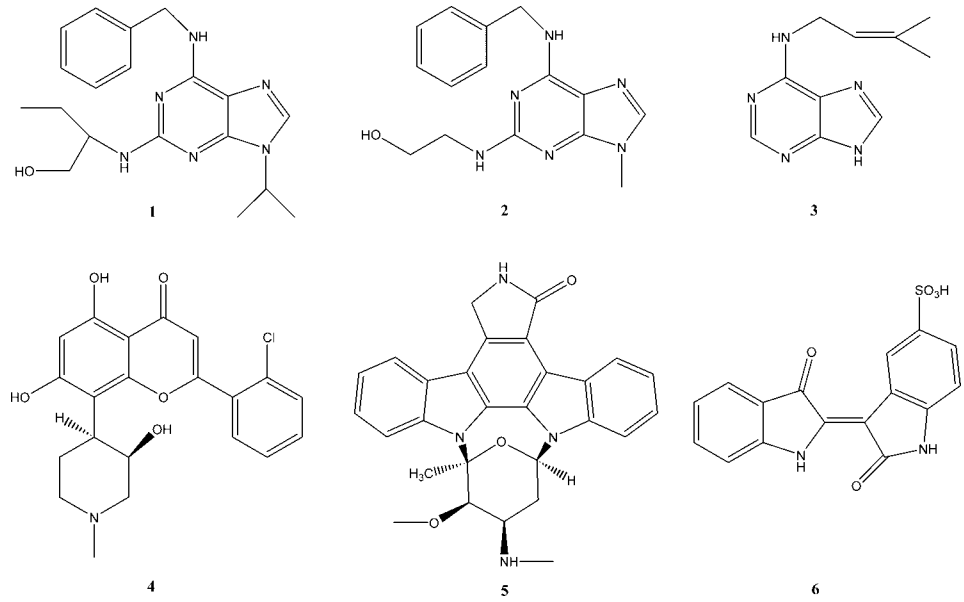

Figure 1. CDK2 inhibitors: roscovitine (1), olomoucine (2), isopentenyladenine (3), flavopiridol (4), staurosporine (5), and indirubin (6).

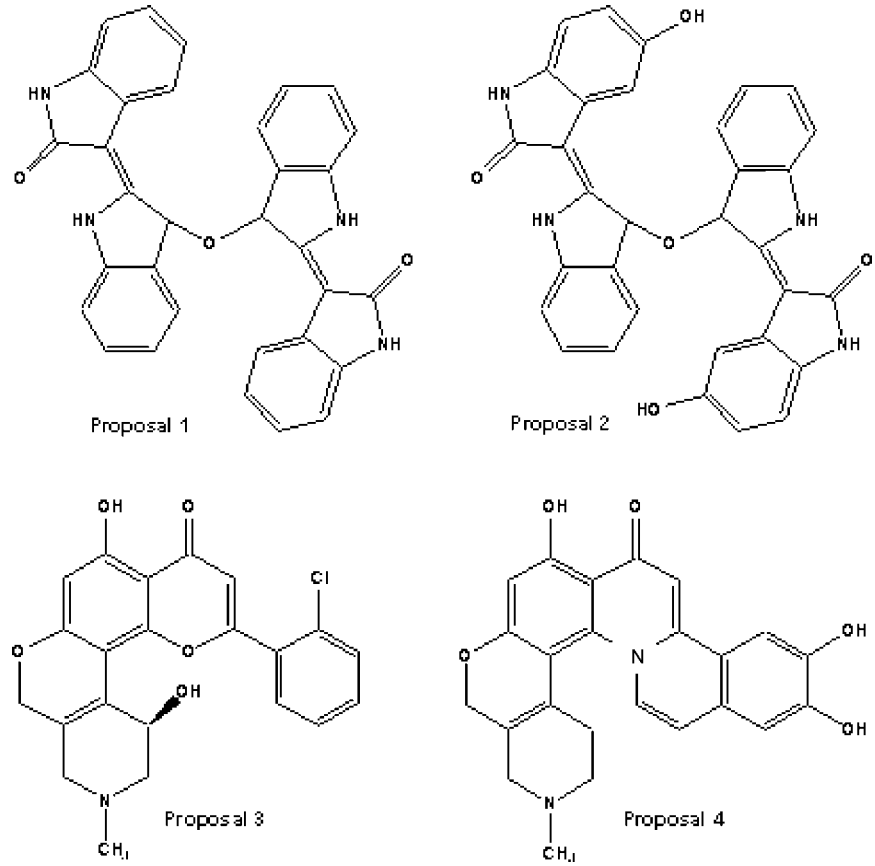

Figure 2. Linear structures of four proposals with potential CDK2 inhibitory activity.

of CDK2 inhibitors, with the contribution of CDK2 crystallized structures available in PDB.[24]

In this work, we propose eight novel potential CDK2 inhibitors, four using an in silico synthesis approach and four selected from large databases via virtual screening. These novel compounds were investigated using computational strategies such as molecular docking, molecular interaction fields, drug-like properties, identification of toxicophoric chemical groups, and molecular dynamics. The molecules presented here can be prepared and tested, representing novel drug candidates in cancer therapy.

2. Methodology

CDK2 structures in complexes with indirubin-5-sulfate and staurosporine selected from Protein Data Bank (PDB code 1E9H) were analyzed using DS ViewerPro 5.0[25] and Insight II softwares.[26] Flexible docking simulations were performed with GOLD 3.1.1[27] for four of the most potent CDK2 inhibitors and derivative compounds proposed by us. The CDK2 inhibitors submitted to docking simulations were indirubin-5-sulfonate, (*R*)-roscovitine, flavopiridol, and staurosporine. The proposed compounds, designed by molecular modifications to these CDK2

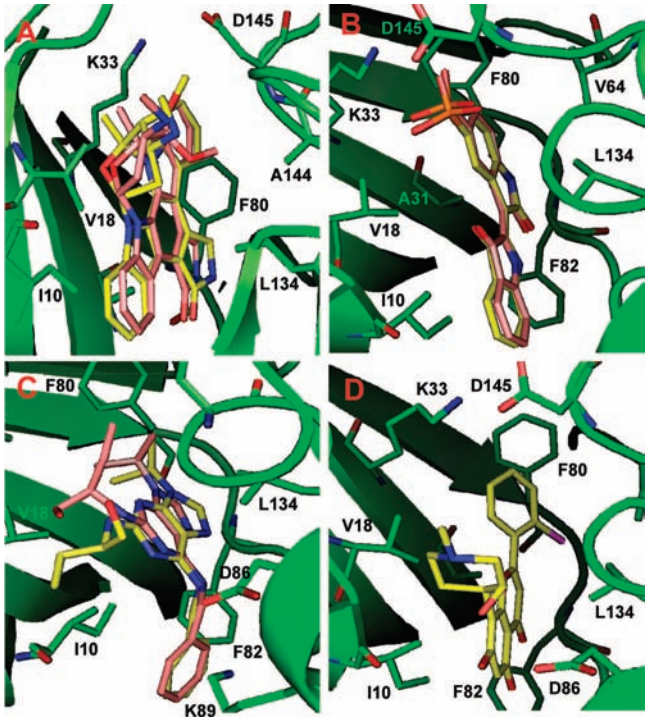

Figure 3. Top-ranked solutions of reported CDK2 inhibitors obtained in flexible docking simulations superimposed to their respective crystallographic orientations in the CDK2 active site. The carbon atoms of crystal structures are colored in pink, and the carbon atoms of the suggested orientations are colored in yellow; (A) staurosporine, (B) indirubin-5-sulfonate, (C) (*R*)-roscovitine, and (D) flavopiridol.

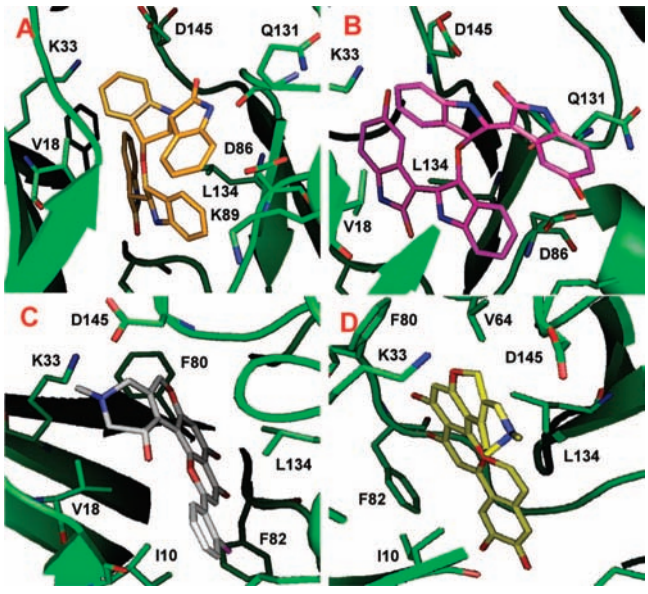

Figure 4. Orientations of highest score obtained in the docking simulations for (A) proposal 1, (B) proposal 2, (C) proposal 3, and (D) proposal 4.

inhibitors, were optimized at the B3LYP/6-31G* level of calculation using Gaussian 03 software.[28] GOLD software was used, as well, to perform virtual screening simulations with the Ilibdiverse and Kinaset virtual collections of compounds from the ChemBridge database.[29] The docking simulations were performed inside of a sphere of 12 Å radius centered at carbon gamma of the L134 side chain of the CDK2 structure downloaded from PDB (PDB code 1E9H). Hydrogen atoms were added to the protein structure using the Sybyl 7.3 package[30,31] after the removal of the ligand and crystallographic waters. The

TABLE 1: Description of Chemical Class, IC50, and ChemScore Values Obtained in Docking Simulations for Four Reported Potent Inhibitors of CDK2

inhibitor	chemical class	IC50(μM)	ChemScore	rmsd
staurosporine	indolocarbazole	0.007	41.5	0.91
Indirubin-5-sulfonate	oxindole	0.04	35.0	0.38
flavopiridol	flavone	0.4	34.2	
(*R*)-roscovitine	purine	0.7	22.2	1.85

GOLD software performs flexible docking using a genetic algorithm, and it was originally optimized from a set of 305 complex structures with coordinates deposited in PDB. We used populations of 100 conformers, 100 000 operations, 95 mutations, and 95 crossovers. For virtual screening, the orientation of highest score was selected by GOLD for each of the best 30 compounds thus ranked from the databases. These molecules selected in the virtual screening simulations were assessed individually for rescoring and reranking. For docking simulations with the inhibitors, our proposals, and the structures filtered by virtual screening, the five orientations of highest score were selected. These selections were made by the ChemScore function. On the basis of this function, the software classifies the orientations of the molecules by a decreasing ordering of affinity (the fitness) with the binding site of CDK2. The ChemScore function was originally parametrized against the experimental binding affinities for a test set of 82 protein−ligand complexes. For the calculation of physical−chemical properties, we used DSviewerPro 5.0. The molecular interaction fields were obtained using the software Almond[30,31] from the Sybyl 7.3 package. Two prototypical probes have been used used, DRY (representing hydrophobic interactions) and carbonyl oxygen (representing hydrogen bonding acceptor groups). Toxicity predictions were performed with DEREK expert system software,[32] which identifies potential toxicity by analyzing chemical toxicophoric groups present in a molecule using a high-throughput screening strategy in a knowledge-based system looking for specific end points, including carcinogenicity, chromosome damage, genotoxicity, mutagenicity, neurotoxicity, hepatotoxicity, teratogenicty, irritancy, reproductive toxicity, respiratory sensitization, skin sensitization, and thyroid toxicity.

Molecular dynamics simulations (MD) were performed using the discover module of the Insight II package. Previously, the energy of the CDK2−compound 4 complex was minimized using 1000 steps of a combined steepest-descent/conjugate gradient algorithm and the Discover/CVFF force field of Insight II. An implicit solvent condition with a dielectric constant of 80 (water) was employed. No constraints were made during any optimization procedure. We subsequently made a 1500 ps MD simulation of our novel AChE potential inhibitor with an equilibration phase of 80 ps at 298 K. The NBO partial atomic charges of the ligand, calculated at the B3LYP/6-31G* level were used, and the atomic charges for the receptor atoms were obtained using the all-atom force field CVFF. The coordinates of the system were saved every 1.5 ps during the simulations. From the molecular trajectory generated by the molecular dynamics simulation, we analyzed the root-mean-square deviations of two main AChE−compound 4 hydrogen bonds, as well as the total energy of its complex with CDK2 as a function of time.

3. Results and Discussion

Docking Simulations. Flexible docking simulations were performed in order to evaluate the viability of four novel

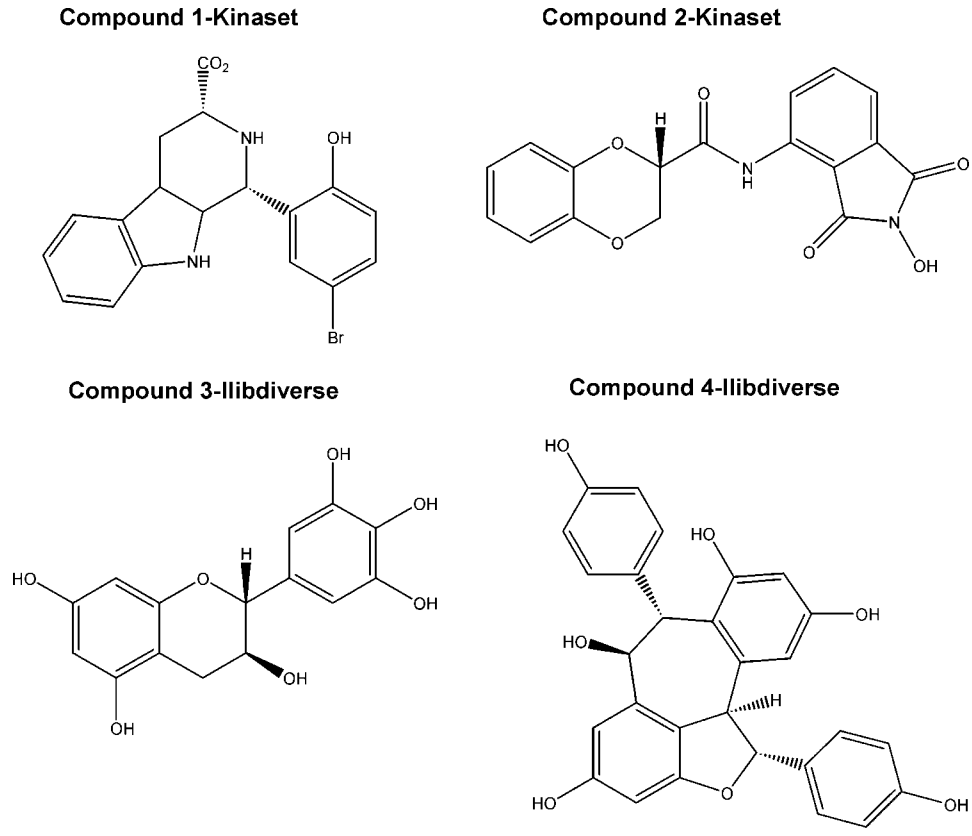

Figure 5. Structures of four molecules selected by virtual screening.

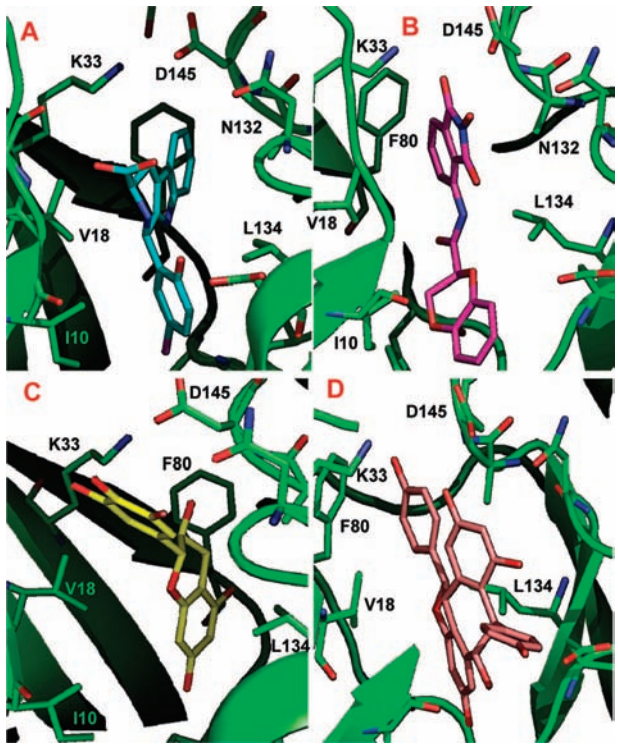

Figure 6. Top-ranked orientations of four potential inhibitors of CDK2 selected by virtual screening simulations.

compounds on inhibition of CDK2 activity as well as to suggest a binding mode for these new structures proposed in this work. These novel potential CDK2 inhibitors were designed by classical strategies of molecular modifications. The compounds proposed are derivatives of two or three different chemical classes of CDK2 inhibitors, such as flavopiridol and indirubin-5-sulfonate. The linear structures of the four proposals are showed in Figures 1 and 2.

TABLE 2: Parameters Related to the Rule of Five for Reported CDK2 Inhibitors, Proposed Derivatives, and Compounds Selected via Virtual Screening Simulations

compounds	molecular weight	no. H bond acceptors	no. H bond donors	log *P*
flavopiridol	401.851	7	3	2.95
indirubin-5-sulfonate	342.336	5	3	0.76
(*R*)-roscovitine	354.459	4	3	2.62
staurosporine	466.544	4	2	4.15
proposal 1	510.556	3	4	3.17
proposal 2	542.556	5	6	2.63
proposal 3	411.846	7	2	2.87
proposal 4	418.431	7	3	2.26
compound 1	386.228	5	3	4.17
compound 2	340.297	6	2	1.58
compound 3	306.277	7	6	1.65
compound 4	470.484	7	6	4.36

For docking simulations, we used the structure of CDK2 in complex with cyclin A and indirubin-5-sulfonate (PDB code 1E9H). The CDK2 structure in this complex is represented by its active form, which is in complex with a cyclin subunit. It is phosphorylated at the T160 residue, which becomes essential to asses our proposals.

Previously, docking simulations were performed for four experimentally validated inhibitors of CDK2 in order to asses docking accuracy and its ability to predict binding affinities. The biological activity (IC50) values reported for these compounds and the respective ChemScores obtained with the simulations as well as the rmsd values are presented in Table 1. The ChemScore function was able to rank these compounds in good agreement with their experimental binding affinity values.

Figure 3 shows the orientations of highest scores suggested by docking using the ChemScore function superimposed on the

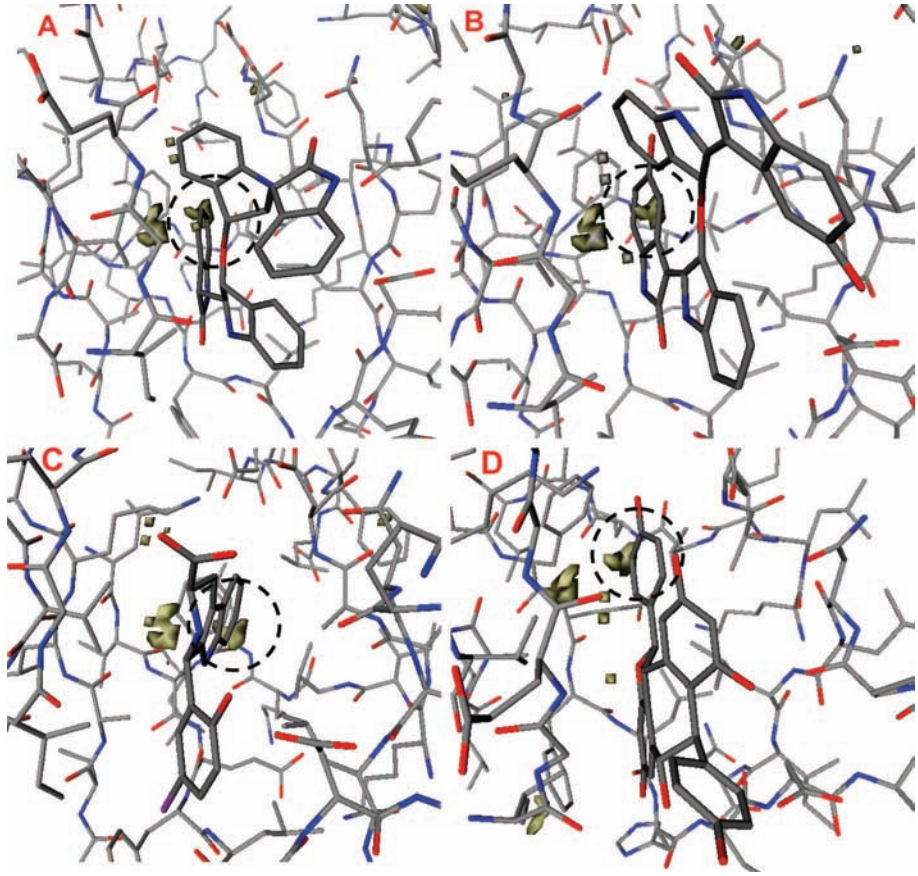

Figure 7. Molecular interaction fields generated with DRY probe in phase with the top-ranked orientations of proposal 1 (A), proposal 2 (B), compound 1 (C), and compound 4 (D).

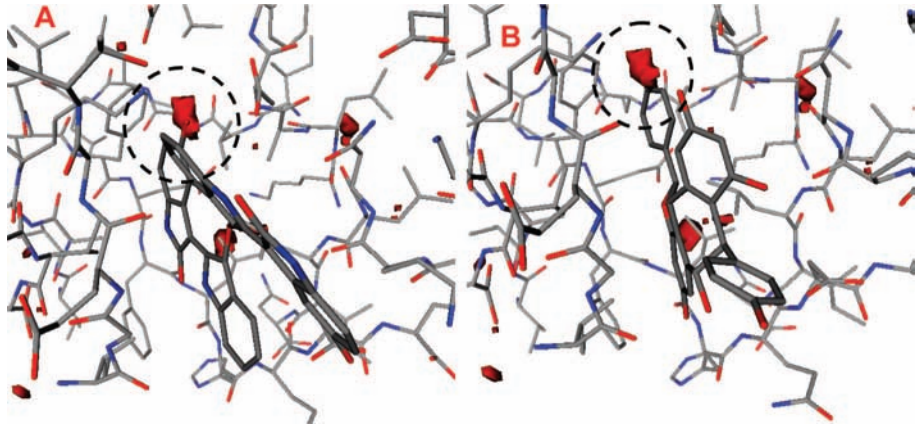

Figure 8. Molecular interaction fields generated with carbonyl oxygen probe in phase with the top-ranked orientations of proposal 2 (A) and compound 4 (B).

crystallographic orientations of the ligands. The docked pose of flavopiridol is shown alone because there does not exist any crystal structure available for this ligand in complex with CDK2. In this analysis, the ChemScore function proved to be a good and accurate method to orient the ligands in the active site of CDK2 protein.

We have proposed molecular modifications in flavopiridol and indirubin-5-sulfonate molecules in order to design four novel compounds with potential CDK2 inhibitory activity. Figure 4 shows the top-ranked orientations obtained in the docking simulations for the designed structures.

Regarding our proposals, all of them showed docked poses close to the crystallographic orientations reported for the crystallographic CDK2 inhibitors, maintaining the key interactions observed in the various classes of inhibitors of this kinase. In this way, all of these proposals as well as the compounds selected by virtual screening demonstrated a complementarity to the CDK2 hinge region very close to that observed for classical CDK2 inhibitors such as flavopiridol and indirubin-5-sulfonate. Proposals 3 and 4 as well as compound 3 (selected using virtual screening) established the best interactions with the residues 80−84. In particular, E81 and L83 comprise the most common binding site of the CDK2 inhibitors, known as a "molecular fork".[33]

In addition to retaining the main interactions in the adenine binding site of CDK2, these proposals are also complementary to residues outside of this region that are known to stabilize the ATP triphosphate side chain, mainly K33 and D145. These

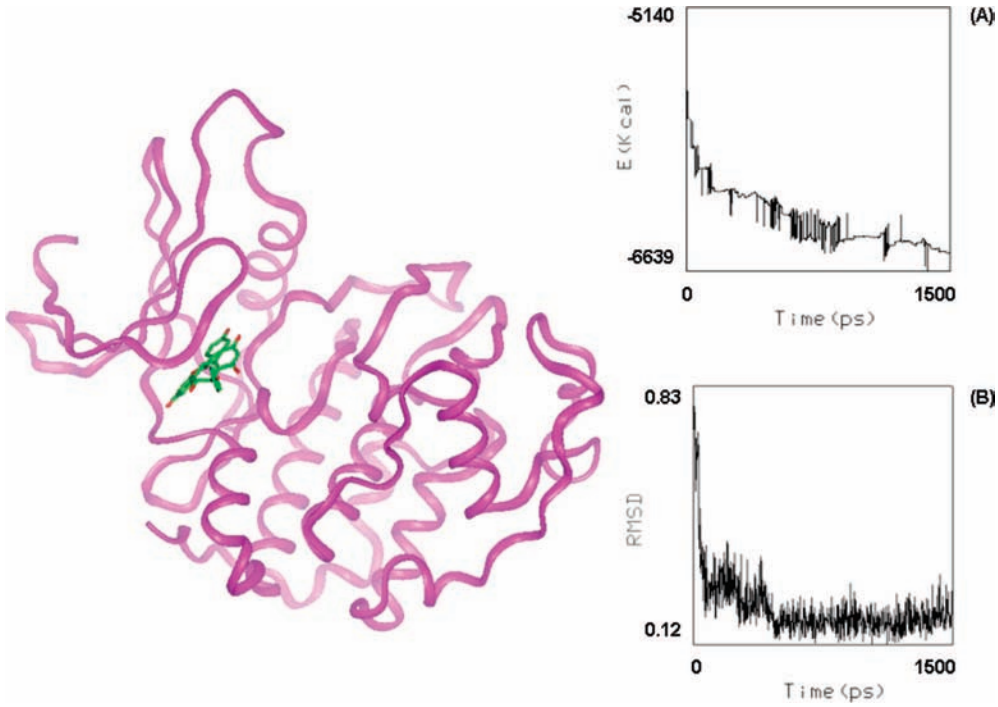

Figure 9. (A) Plot of the total energy versus time (simulation time) for the CDK2−compound 4 complex. (B) Plot of the root-mean-square deviation (in angstroms) versus time for the atoms of compound 4 regarding its initial atomic coordinates.

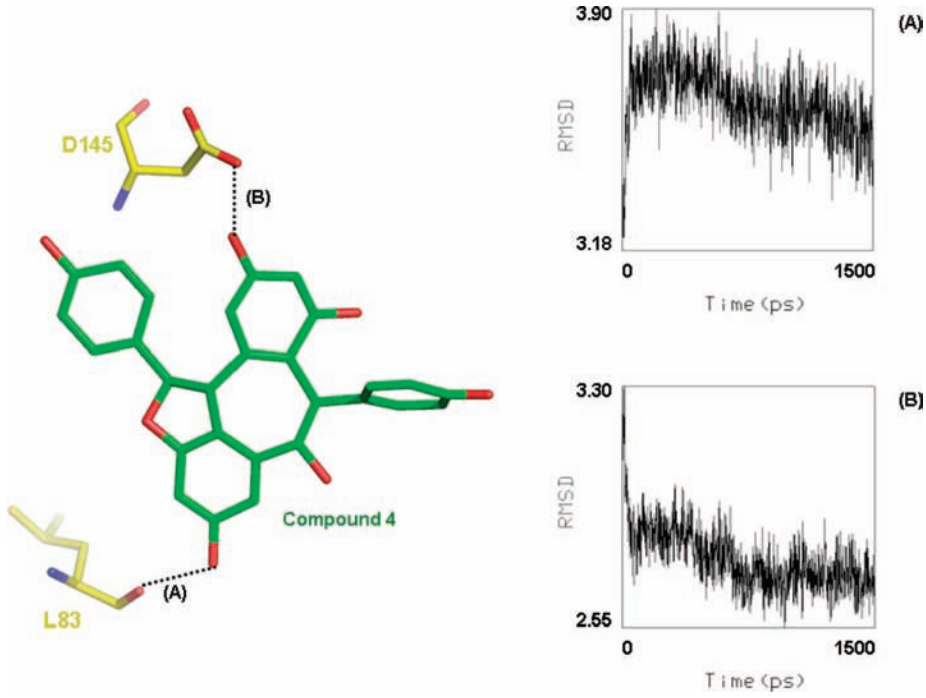

Figure 10. (A) Plot of the root-mean-square deviation versus time for the hydrogen bonding interaction of compound 4 with L83. (B) Plot of the root-mean-square deviation (in angstroms) versus time for the hydrogen bonding interaction of compound 4 with D145.

two residues belong to a triad of catalytic site residues (K33, E51, and D145) that are conserved among eukaryotic CDKs.[34] They are essential for the correct orientation of ATP phosphate in order to phosphorylate T160, an event necessary for stabilization and activation of the CDK2 complex. Mutation of these residues results in inactive kinases even though the cyclin A binding is still conserved.[35] Furthermore, K33 and D145 make significant interactions with the side chains of CDK2 small inhibitors and are considered to be residues targeted for increasing the potency of cyclin-dependent kinase inhibitors.[36]

Proposal 1 is a derivative of indirubin-5-sulfonate, which was designed as a twin derivative as the main molecular modification, where the oxygen atoms of the original carbonyl groups, present in the oxindole rings, form an ether bridge. The sulfonate group present in indirubin-5-sulfonate is not present in the other two oxindoles rings of our proposed molecule in order for the molecule to have more hydrophobic properties. The best orientation for proposal 1 obtained in the docking simulations showed a ChemScore value of 36.4. This value is higher than that obtained for indirubin-5-sulfonate.

Proposal 2 is very similar to proposal 1, and it is a derivative of indirubin-5-sulfonate as well. In this molecule, a hydroxyl group was added in the oxindoles rings, replacing the sulfonate group in the indirubin molecule. The ChemScore value obtained for proposal 2 (22.2) is lower than that obtained for indirubin. Nevertheless, a new interaction was identified in the binding mode suggested by docking for proposal 2. In this molecule (Figure 3B), the added hydroxyl group is placed closer to the K33 and D145 residues (Figure 4B) than the sulfonate group present in indirubin-5-sulfonate (Figure 1B).

Proposal 3 is a derivative from flavopiridol and showed a ChemScore value of 32.4, a value slightly lower than that obtained for flavopiridol. This molecule was built from a simple molecular modification of the flavopiridol structure, where the aromatic ring is condensed to the pyridine ring in order to introduce rigidity.

Proposal 4 is also a derivative of flavopiridol and is very similar to proposal 3. In this designed molecule, the rings remained condensed, and the chlorine group has been removed. Two new hydroxyl groups were added to an aromatic ring in order to explore interactions with K33 and D145 residues of CDK2. The ChemScore value obtained for proposal 4 is 37.5. This proposal has the highest score of all of our proposals.

Virtual Screening. Virtual screening simulations were performed using the approach of high throughput flexible docking with GOLD software in order to select compounds that could interact with CDK2 and that have therapeutic potential against cancer. For these simulations, two collections of compounds were used, Ilibdiverse and Kinaset. The Ilibdiverse collection provides about 1200 compounds with drug-like features, especially oral bioavailability and blood−brain barrier penetration.[29] Kinaset is a fragment collection of the ChemBridge database,[29] containing approximately 12 000 compounds originally validated in silico. The compounds of the Kinaset collection show pharmacophoric groups that are required to give possibilities for specific interactions with members of the kinase protein family.

Four compounds were selected in the virtual screening simulations. Figure 5 shows the structures of these selected molecules. Compounds 1 and 2 are provided from the Kinaset database, while compounds 3 and 4 were selected from the Ilibdiverse database.

The compounds selected by virtual screening share structural similarity with the inhibitors described in the literature, such as flavopiridol, staurosporine, and indirubin-5-sulfonatre, where there is a rigid system of condensed rings containing polar substituent groups. The orientations of these compounds, suggested by rescoring in flexible docking simulations, appear to maintain the key interactions with CDK2 observed for the reported inhibitors as well as our proposals previously described in this work. Figure 6 presents the top-ranked orientations obtained by rescoring simulations with GOLD software. The ChemScore values obtained for compounds 1, 2, 3, and 4 were 29.9, 25.73, 30.30, and 31.81, respectively.

Physical−Chemical Properties. We have proposed four novel potential ligands for CDK2 and also have identified, by virtual screening simulations, four molecules that could show activity against this enzyme. In an attempt to design new potential CDK2 inhibitors, the drug-like characteristics should be investigated in order to asses the efficiency of new molecules that could become drugs. For our four proposals and the four compounds selected in virtual screening, the parameters of the Lipinski's Rule of Five (RO5) were calculated,[37] which are common to the oral bioavailable drugs, such as molecular weight lower than 500, log *P* lower than 5, the number of hydrogen bond acceptors equal to or less than 10, and the number of hydrogen bond donors equal to or less than 5. The RO5 values calculated for our molecules are shown in Table 2. The molecules presented here showed good drug-like properties, except for proposal 2, which has violated more than one parameter of the rule.

Molecular Interaction Field Studies. Molecular interaction fields (MIFs) were generated for the binding site of CDK2 structure in order to support our findings. MIFs were obtained using two relevant probes, DRY (representing hydrophobic interactions) and carbonyl oxygen (representing hydrogen bonding acceptor groups).

The results obtained for the DRY probe are shown in Figure 7. The molecules that presented the best agreement with the MIFS were proposal 1, proposal 2, compound 1, and compound 4. The orientations of these structures showed aromatic rings in a particular region of the CDK2 active site that is relevant for hydrophobic interactions. MIFs obtained with the DRY probe were generated mainly by the influence of F81 residue, which is located in a favorable region of interaction with inhibitors, just between the "molecular fork" (E81, F82, and L83)[33] an the triad of catalytic residues (K33, E51, and D145)[34] described above.

The results obtained for the carbonyl oxygen probe (to investigate potential binding sites of hydrogen bonding acceptor groups) are shown in Figure 8. The molecules that presented the best agreement with the MIFs calculated with this probe were proposal 2 and compound 4. The orientations of these two molecules indicate a hydroxyl group in the most relevant region defined by this chemical probe. In this region, the hydroxyl groups of the potential ligands can play a role as hydrogen bonding acceptor groups. MIFs represented in Figure 8 were generated by the region composed of the triad of catalytic residues[34] that accommodate hydroxyl groups of compounds 2 and 4. Considering these two chemical probes analyzed in the molecular interaction fields studies, proposal 2 and compound 4 seem to be the most promising CDK2 inhibitor candidates.

Toxicity Predictions. Considering flavopiridol, the toxicity prediction performed with the DEREK system reveals a plausible skin sensitization effect due to the presence of a resorcinol substructure. Resorcinols are hydroxyphenols which have the potential to react with other skin proteins like other simple phenols. The activity of such compounds has been demonstrated in several skin sensitization assays. The phenolic radical mechanism for simple phenols is also appropriate for resorcinols.[38]

A plausible chromosome damage alert was generated for the indirubin that is explained by the presence of a substituted vinyl ketone in its chemical structure. On the basis of published toxicity data for methylvinyl ketone and ethylvinyl ketone, vinyl ketones show a general trend toward mutagenicity.[39] Considering that indirubin presents a beta substitution of the vinyl double bond, it would be conceivable to observe that this fact could lead to a loss of activity in mutagenicity as a consequence of the steric and/or electronic effects of the substituents. Additionally, the presence of a substituent in the α position, as can be observed in the indirubin structure, may be expected to result in a similar loss of effect.[40]

Regarding the roscovitine chemical structure, the major toxicophoric alert was generated due to the presence of a pyrimidine. Some pyrimidine derivatives, including uracil and thymidine, have been shown to have carcinogenic potential and induce bladder tumors in rats. However, depending on the

groups attached to the pyrimidine ring, these effects can be minimized, like the anticancer agent and pyrimidine derivative 5-fluoruracil, which has been shown to lack significant carcinogenic activity in rats.[41] In this way, it is possible to identify a potential carcinogenic activity in roscovitinine related to its chemical structure with the DEREK system, which should be verified carefully in experimental tests. The DEREK system simulation for staurosporin revealed no plausible end-point prediction for its chemical structure and only a doubted nephropathy effect in mammals.

Considering the chemical structure of the eight potential inhibitors investigated in this work, the most prominent toxicophoric alerts were generated for compounds 3 and 4. In relation to compound 3, a catechol derivative ring in its structure was identified as a potential risk to cause carcinogenic effects. Some catechol derivatives, including caffeic acid and 4-methylcatechol, can produce, after chronic repeated oral exposure, carcinogenic effects in rodents, although some other cathecol derivatives, such as dopamine, are not carcinogenic. Given that cathecol functionality is generally not genotoxic in conventional assays, the relevance to human health is not clearly stablished.[42] The DEREK system analysis revealed a potential risk of chromosome damage due to the presence of alkylphenol groups in compound 4. Activity in the chromosome aberration test is generally observed for alkylphenols with a log *P* value of 3 or less,[42] different from compound 4 that presents a log *P* value of 4.36. There are a number of mechanisms by which alkylphenols may induce chromosome damage. Some findings suggest that activity may be related to oxidation and the formation of reactive metabolites, including quinols or quinine methides.[43] Phenolic compounds may also have the potential to be uncouplers of oxidative phosphorylation[44] and have been shown to generate reactive oxygen species via the interaction of phenoxyl radicals with cellular thiols.[45]

Molecular Dynamics Simulations. The results obtained also were supported by molecular dynamics simulations for the CDK2−compound 4 (Figure 9). Theoretically (considering docking simulations, molecular interaction fields, and molecular dynamics), compound 4 is the best potential inhibitor candidate among the compounds proposed by us and the compounds selected by virtual screening. Thermodynamic properties such as temperature and energy were monitored during the MD simulations, and all converged to stable values. Our results suggest that compound 4 is theoretically stable inside of the CDK2 active site, with small and stable rmsd (root-mean-square deviation) values along the time as well as the energy of the complex decreasing during the simulation. According to the study performed by Alzate-Morales et al.,[46] the energy interaction of some inhibitors comprising the active site of CDK2 is strongly correlated with biological activity. Considering this fact, the hydrogen bonding interactions of compound 4 with L83 and D145 were evaluated during the trajectory (Figure 10). The stability of this compound in the active site is mainly due to stable and strong hydrogen bond interactions between compound 4 and L83 as well as D145 residues (∼2.8 Å between aromatic hydroxyl oxygens of compound 4 and the carbonyl oxygen of L83 as well as the carboxy oxygen of D145), indicating this compound as a potential CDK2 inhibitor.

Conclusions

In this work, we have used molecular dynamics, density functional theory, ADMET predictions, molecular interaction field studies, flexible docking, as well as virtual screening to design and propose eight novel potential inhibitors of CDK2 with drug-like properties. The eight molecules proposed showed interesting structural characteristics that could be required for inhibiting CDK2 activity. After toxicophoric analysis of the most promising candidate, compound 4 does not show an alarming toxicity result and even no more risk than any of the four known inhibitors of CDK2 evaluated in this study. Consequently, compound 4 could be a promising drug candidate for the treatment of cancer.

Acknowledgment. We acknowledge financial support from CNPq, CAPES, FAPESP, and FAPERJ.

References and Notes

(1) (a) Taft, C. A., Silva, C. H.T.P., Eds.; *Current Methods in Medicinal Chemistry and Biological Physics*; Research Signpost: Kerala, India, 2007; Vol. 1. (b) International Invited MiniReview: Taft, C. A.; Silva, V. B; Silva, C. H. T. P. Topics in Computer-Aided Drug Design. *J. Pharm. Sci.* **2008**, *97*, 1089.

(2) Invited International Review: Taft, C. A.; Silva, C. H. T. P. Cancer and Aids: New Trends in Drug Design and Chemotherapy. *Curr. Comput.-Aided Drug Des.* **2006**, *2*, 307, and references therein.

(3) Silva, V. B.; Taft, C. A.; Silva, C. H. T. P. *J. Phys. Chem. A* **2008**, *112*, 2007.

(4) Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. *J. Med. Chem.* **2000**, *43*, 1.

(5) Hatakeyama, M.; Weinberg, R. A. *Prog. Cell Cycle Res.* **1995**, *1*, 9.

(6) Tetsu, O.; McCormick, F. *Cancer Cell* **2003**, *3*, 233.

(7) Pevarello, P.; Brasca, M. G.; Amici, R.; Orsini, P.; Traquandi, G.; Corti, L.; Piutti, C.; Sansonna, P.; Villa, M.; Pierce, B. S.; Pulici, M.; Giordano, P.; Martina, K.; Fritzen, E. L.; Nugent, R. A.; Casale, E.; Cameron, A.; Ciomei, M.; Roletto, F.; Isacchi, A.; Fogliatto, G.; Pesenti, E.; Pastori, W.; Marsiglio, A.; Leach, K. L.; Clare, P. M.; Fiorentini, F.; Varasi, M.; Vulpetti, A.; Warpehoski, M. A. *J. Med. Chem.* **2004**, *47*, 3367.

(8) Moshinsky, D. J.; Bellamacina, C. R.; Boisvert, D. C.; Huang, P.; Hui, T.; Jancarik, J.; Kim, S.; Rice, A. G. *Biochem. Biophys. Res. Commun.* **2003**, *310*, 1026.

(9) Brown, N. R.; Noble, M. E. M.; Endicott, J. A.; Johnson, L. N. *Nat. Cell Biol.* **1999**, *1*, 438.

(10) Brown, N. R.; Noble, M. E. M.; Lawrie, A. M.; Morris, M. C.; Tunnah, P.; Divita, G.; Johnson, L. N.; Endicott, J. A. *J. Biol. Chem.* **1999**, *274*, 8746.

(11) Catzavelos, C.; Bhattacharya, N.; Ung, Y. C.; Wilson, J. A.; Roncari, L.; Sandhu, C.; Shaw, P.; Yeger, H.; Morava-Protzner, I.; Kapusta, L.; Franssen, E.; Pritchard, K. I.; Slingerland, J. M. *Nat. Med.* **1997**, *3*, 227.

(12) Quintanilla-Martinez, L.; Thieblemont, C.; Fend, F.; Kumar, S.; Pinyol, M.; Campo, E.; Jaffe, E. S.; Raffeld, M. *Am. J. Pathol.* **1998**, *153*, 175.

(13) Cordon-Cardo, C.; Koff, A.; Drobnjak, M.; Capodieci, P.; Osman, I.; Millard, S. S.; Gaudin, M. F.; Zhang, Z.; Massague, J.; Scher, H. I. *J. Natl. Cancer Inst.* **1998**, *90*, 1284.

(14) Cote, R. J.; Shi, Y.; Groshen, S.; Feng, A.; Carlos, C.; Skinner, D.; Lieskovosky, G. *J. Natl. Cancer Inst.* **1998**, *90*, 916.

(15) Flrenes, V. A.; Maelandsmo, G. M.; Kerbel, R. S.; Slingerland, J. M.; Nesland, J. M.; Holm, R. *Am. J. Pathol.* **1998**, *153*, 305.

(16) Pevarello, P.; Brasca, M. G.; Orsini, P.; Traquandi, G.; Longo, A.; Nesi, M.; Orzi, F.; Piutti, C.; Sansonna, P.; Varasi, M.; Cameron, A.; Vulpetti, A.; Roletto, F.; Alzani, R.; Ciomei, M.; Albanese, C.; Pastori, W.; Marsiglio, A.; Pesenti, E.; Fiorentini, F.; Bischoff, J. R.; Mercurio, C. *J. Med. Chem.* **2005**, *48*, 2944.

(17) Azevedo, W. F.; Leclerc, S.; Meijer, L.; Havlicek, L.; Strnad, M.; Kim, S. H. Inhibition of Cyclin-Dependent Kinase by Purine Analogues. *Eur. J. Biochem.* **1997**, *243*, 518.

(18) Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J. J.; Donella-Deana, A.; Pinna, L.; Letham, D. S.; Kato, J. Y.; Detivaud, L.; Leclerc, S.; Meijer, L. *Eur. J. Biochem.* **1994**, *224*, 771.

(19) Schulze-Gahmen, U.; Brandsen, J.; Jones, H. D.; Morgan, D. O.; Meijer, L.; Vesely, J.; Kim, S. H. *Proteins* **1995**, *22*, 378.

(20) Sedlacek, H. H.; Czech, J.; Naik, R.; Kaur, G.; Worland, P.; Losiewicz, M.; Parker, B.; Carlson, B.; Smith, A. *Int. J. Oncol.* **1996**, *9*, 1143.

(21) Carlson, B. A.; Dubay, M. M.; Sausville, E. A.; Brizuela, L.; Worland, P. J. *Cancer Res.* **1996**, *56*, 2973.

(22) Lawrie, A. M.; Noble, M. E.; Tunnah, P.; Brown, N. R.; Johnson, L. N.; Endicott, J. A. *Nat. Struct. Biol.* **1997**, *4*, 796.

(23) Hoessel, R.; Leclerc, S.; Endicott, J. A.; Nobel, M. E. M.; Lawrie, A.; Tunnah, P.; Leost, M.; Damiens, E.; Marie, D.; Marko, D.; Niederberger, E.; Tang, W.; Eisenbrand, G.; Meijer, L. *Nat. Cell Biol.* **1999**, *1*, 60.

(24) Fischer, P. M.; Gianella-Borradori, A. *Expert Opin. Invest. Drugs* **2003**, *12*, 955–70.
(25) *Discovery Studio ViewerPro*; Accelrys Inc: San Diego, CA, 2002.
(26) *Insight II*; Accelrys: San Diego, CA, 2005.
(27) Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Mulrray, C. W.; Taylor, R. D. *Proteins: Struct., Funct., Genet.* **2003**, *52*, 609.
(28) Frisch, M. J. Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision A.1; Gaussian, Inc.: Pittsburgh, PA, 2003.
(29) *ChemBridge*; Chembridge Corporation: San Diego, CA, 2007.
(30) *Sybyl User Guide*, version 7.3; Tripos Inc: CA, 2006.
(31) Pastor, M.; Cruciani, G.; Mclay, I.; Pickett, S.; Clementi, S. *J. Med. Chem.* **2000**, *43*, 3233.
(32) *DEREK and METEOR 8.0*; LHASA Limited: Leeds, U.K., 2004.
(33) Canduri, F.; de Azevedo, W. F. *Curr. Comput.-Aided Drug Des.* **2005**, *1*, 53–64.
(34) Taylor, S. S.; Radzio-Andzelm, E. *Structure* **1994**, *2* (5), 345–355.
(35) Jeffrey, P. D.; Russo, A. A.; Polyak, K.; Gibbs, E.; Hurwitz, J.; Massague, J.; Pavletich, N. P. *Nature* **1995**, *376*, 313–320.
(36) Sridhar, J.; Akula, N.; Pattabiraman, N. *AAPS J.* **2006**, *8* (1), 204–221.
(37) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
(38) Barratt, M. D.; Basketter, D. A. *Contact Dermatitis* **1992**, *27*, 98.
(39) Deininger, C.; Eder, E.; Neudecker, T.; Hoffman, C. *J. Appl. Toxicol.* **1990**, *10*, 167.
(40) Eder, E.; Deininger, C. *Mutat. Res.* **2000**, *470*, 29.
(41) Toyoda, K.; Shibutani, M.; Sato, H.; Uneyama, C.; Takahashi, M.; Hayashi, Y.; Hirose, M. *Food Chem. Toxicol.* **2000**, *38*, 187.
(42) Kusakabe, H.; Yamakage, K.; Wakuri, S.; Sasaki, K.; Nakagawa, Y.; Watanabe, M.; Hayashi, M.; Sofuni, T.; Ono, H.; Tanaka, N. *Mutat. Res.* **2002**, *517*, 187.
(43) Thompson, D. C.; Perera, K.; London, R. *Chem. Res. Toxicol.* **1995**, *8*, 55.
(44) Terada, H. *Environ. Health Perspect.* **1990**, *87*, 213.
(45) Stoyanovsky, D. A.; Goldman, R.; Claycamp, H. G.; Kagan, V. E. *Arch. Biochem. Biophys.* **1995**, *317*, 315.
(46) Alzate-Morales, J. H.; Contreras, R.; Soriano, A.; Tunon, I.; Silla, E. *Biophys. J.* **2007**, *92*, 430.

JP8011969