Pyrrolo[2,3-*a*]carbazoles as Potential Cyclin Dependent Kinase 1 (CDK1) Inhibitors. Synthesis, Biological Evaluation, and Binding Mode through Docking Simulations

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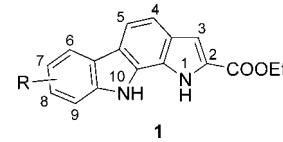
Pyrrolo[2,3-*a*]carbazole derivatives were synthesized, and their effects on CDK1/cyclinB activity were evaluated. The most potent and efficacious inhibitor was found to be ethyl 9-chloro-1*H*-pyrrolo[2,3-α]carbazole-2-carboxylate (**1e**), exhibiting an IC_{50} in the low micromolar range and leading to 90% at higher concentrations. Using a computational model for CDK1−**1e**, binding we have observed that **1e** exhibited two likely binding modes in the ATP-binding cleft that involve interactions with Lys130, Thr14, and Asp146 of the enzyme.

Introduction

Cyclin dependent kinases (CDKs), a family of serine/threonine kinases, play a pivotal role in the regulation of cell cycle progression. In addition, CDKs are also implicated in apoptosis, neuronal cell physiology, differentiation, and transcription.[1] Enzymatic activity of CDKs is tightly regulated by several means including binding to cyclins, phosporylation by CDK activating kinases (CAKs), dephosphorylation by phosphatases, and association with negative regulatory proteins (CDK inhibitors, CKIs).[2] Among the 11 standard CDKs that have been identified in the human genome,[3] CDKs 1−4 and 6 have been proposed to control the transition from one stage of the cell cycle to the next after forming complexes with the appropriate cyclin subunit. Deregulation of CDKs and their modulators occurs in many human tumors[4] and in a number of proliferative and neurodegenerative[5] disorders. Thus, CDKs represent attractive targets for therapeutic intervention. Currently, much effort has focused on the discovery of new chemical inhibitors of CDKs that could act as anticancer agents. Up to date, several scaffolds have been recognized as efficient inhibitors of these enzymes,[6,7] with several of them being in the late phases of clinical trials.[8,9] Purines, pyrimidines, flavonoids, indolocarbazole analogues, and other heterocyclic compounds (butyrolactone, fascaplysin, paullones) comprise typical representatives with well established CDK inhibitory activity. Particularly, most of the already known CDK inhibitors (natural products or/and synthetic molecules) are flat, small heterocycles that act by competing with ATP for the kinase ATP-binding site. Although some inhibit CDK activity in nanomolar concentrations, optimizing potency and selectivity against individual CDK family members remains a challenge. Initial structure-based drug discovery efforts focused on CDK2 inhibition, as it was the first member of CDK family for which the crystal structure was resolved.[10] Several small molecules have been cocrystallized with CDK2,[11–15] and information derived from these complexes has elucidated key interactions needed for effective inhibitor binding; these data have greatly contributed to the design and optimization of new lead compounds. Moreover, sequence alignment and homology modeling have revealed a high degree of similarity among CDK2 and CDK1.

On the basis of the above-mentioned data and considering that CDK1 is sufficient to drive the mammalian cell cycle,[16] we aimed at synthesizing new compounds with potential CDK1 inhibitory activity. We considered pyrrolo[2,3-*a*]carbazole core **1** as a promising scaffold, as it bears structural features that could potentially contribute to the development of interactions with the ATP-binding site of CDKs. Herein, we are reporting a slightly modified synthesis method for pyrrolo[2,3-*a*]carbazole analogues[17] along with preliminary results concerning their ability to inhibit CDK1 in vitro. Moreover, using a docking simulation approach, we have tried to address the structural basis of CDK1−inhibitor interactions.



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Results and Discussion

Chemistry. The general methodology for the synthesis of pyrrolo[2,3-*a*]carbazole analogues is outlined in Scheme 1. The key step for the construction of the functionalized tetracyclic pyrrolocarbazole core involves Fischer indolization of tetrahydroindole ketone **3** with various arylhydrazine derivatives.

Previously reported[17] synthetic approach to pyrrolo[2,3-*a*]carbazole core was based on the employment of the tetrahydroindole ketone **2**[18] as building block in Fischer indolization with various arylhydrazines. However, the fragility of ketone **2**'s corresponding arylhydrazones prompted us to explore the utilization of a protective group for the pyrrole nitrogen atom. The inserted group was expected to contribute to the stabilization of the pyrrole ring and hence to the increased stability of arylhydrazones under the subsequent Fischer indolization conditions. For this approach, the application of the *p*-methoxybenzyl

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[a] Abbreviations: PMB, *p*-methoxybenzyl group; PPSE, polyphosphoric acid trimethylsilyl ester; TFA, trifluoroacetic acid; UCN01, 7-hydroxystaurosporin.

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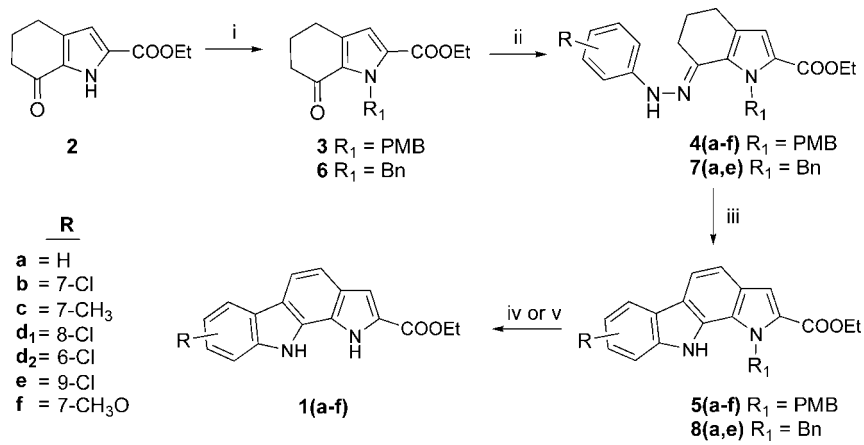
Scheme 1[a]



[a] Conditions. (i) *t*-BuOK, 18-crown-6, PMB-Br or Bn-Br, Et_2O, room temp, 2.5 h; (ii) $ArNHNH_2 \cdot HCl$, AcONa, EtOH/TFA, reflux, 2–4 h; (iii) PPSE, CH_3NO_2, reflux, 4–44 h. (iv) R_1 = PMB: anisole, H_2SO_4, TFA, 0 °C, 0.5 h and then room temp, 2–3.5 h. (v) R_1 = Bn: $AlCl_3$, C_6H_6, 0 °C, 15 min and then 50 °C, 0.5 h.

(PMB[a]) moiety as a protecting group for the pyrrole nitrogen atom was initially investigated, considering that the latter has been used successfully in the chemistry of nitrogen heterocycles.[19–21] Formation of the PMB[a] ketone **3** was achieved according to the method of Guida and Mathre[22] with minor modifications. Specifically, treatment of **2** with freshly prepared *p*-methoxybenzyl bromide (PMB-Br)[23] in the presence of potassium *tert*-butoxide (*t*-BuOK) and 18-crown-6 in diethyl ether under phase transfer conditions afforded the *N*-PMB ketone **3** in good yield (75%). Condensation of **3** with various substituted arylhydrazines in the presence of anhydrous sodium acetate and trifluoroacetic acid in ethanol afforded the intermediate arylhydrazones **4a**−**f**. Subsequently, following an one-pot procedure, the crude mixture of **4a**−**f** was employed in Fischer cyclization conditions using freshly prepared polyphosphoric acid trimethylsilyl ester (PPSE) as an aprotic catalyst and nitromethane as a solvent.[17,24] Only the *N*(1)-PMB pyrrolo[2,3-*a*]carbazole derivatives **5a**−**f** were obtained in all cases, in contrast to our previously reported method[17] where the Fischer indolization of the ketone **2**'s corresponding arylhydrazones gave the desired fully aromatized products accompanied by the corresponding 4,5-dihydro ones. Finally, cleavage of the protective group by treatment with TFA in the presence of anisole and concentrated sulfuric acid[19] afforded the final products **1a**−**f** in very good yields (83–96%).

The benzyl (Bn) group was alternatively investigated as a possible protective group for ketone **2**, but it proved to be inferior to the PMB group because the debenzylation procedure[25] of the *N*(1)-benzylpyrrolo[2,3-*a*]carbazole derivatives **8a** and **8e** led to a complex mixture of the unprotected final products and polyalkylated byproduct. Isolation of the desired derivatives **1a** and **1e** proved to be insufficient with the conventional methods, and it was accomplished only using HPLC chromatography. Thus, the PMB group facilitated the preparation of the functionalized pyrrolocarbazole core in an efficient and convenient manner without affecting functional groups of target molecules or rendering their isolation unattainable. Moreover, it contributed to the complete aromatization of the Fischer indolization products and to the avoidance of the subsequent aromatization process involved in the previously reported method.[17]

Biological Activity. Compounds **1a**−**f** were tested for their inhibitory activity in vitro against the CDK1/cyclin B complex using the MESACUP cdc2 kinase assay kit (see Supporting Information). Compounds **1a**−**c**, **1d$_1$**, **1d$_2$**, and **1f** were inactive against CDK1/cyclin B (Figure 1A). Interestingly, **1e** showed a concentration-dependent, statistically significant inhibitory activity against CDK1/cyclin B (Figure 1). As estimated from Figure 1B, the concentration of **1e** that caused CDK1 inhibition by 50% was 15 μM, while the reference compound olomoucine exhibits an inhibitory activity against the same complex with IC_{50} = 7 μM.[26]

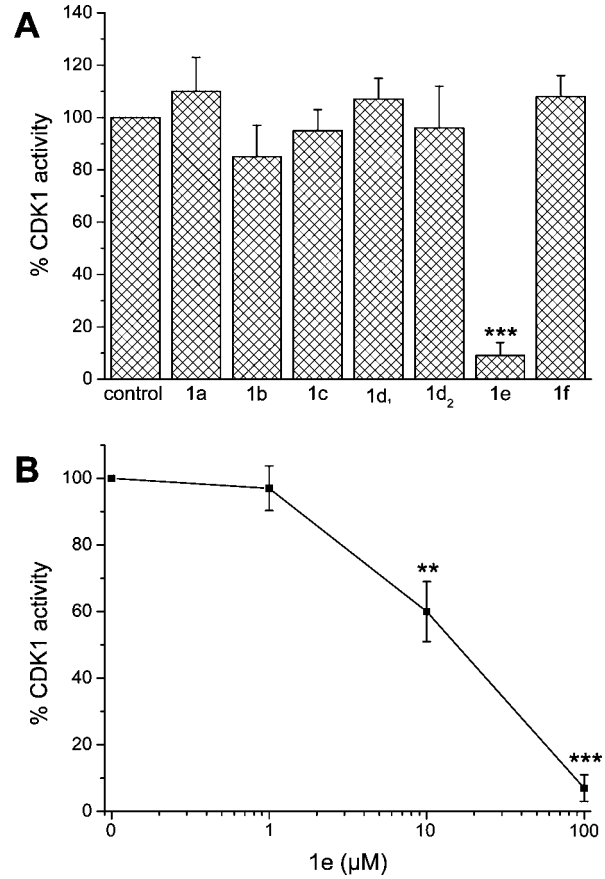


Figure 1. (A) Effect of the tested compounds at 100 μM on the activity of CDK1. (B) Concentration-dependent inhibition of CDK1 by **1e**. Results are expressed as the mean ± SEM of the % CDK1 activity. Asterisks in all cases denote a statistically significant difference from the untreated samples (control): (*) $P < 0.05$; (***) $P < 0.001$.

Moreover, we tested the **1e** against the CDK1/cyclin B1 complex, using an additonal in vitro kinase reaction approach

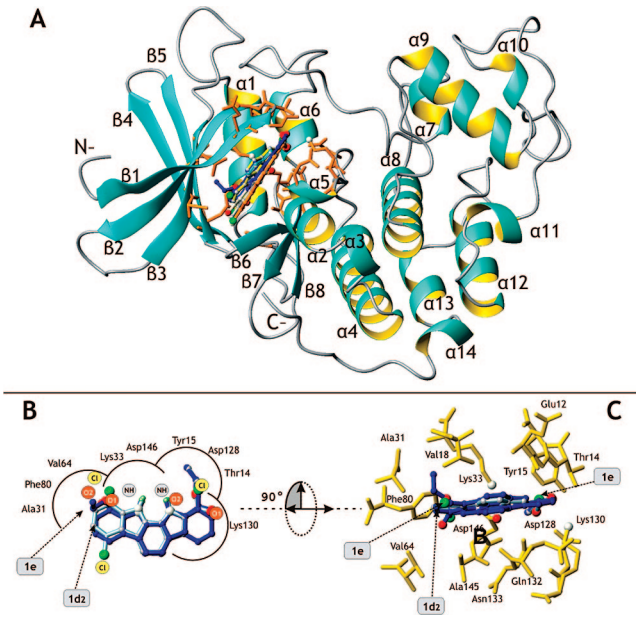


Figure 2. (A) Ribbon representation of CDK1 homology model generated with MOLMOL.[32] Docked **1** conformers of lowest energy are illustrated to be in the ATP-binding cleft. Side chains of CDK1 residues involved in CDK1−**1** interaction (within 4.0 Å) are also shown (residues Glu12, Gly13, Thr14, Tyr15, Val18, Ala31, Lys33, Val64, Phe80, Asp128, Lys130, Gln132, Asn133, Asp146, (B, C) Views differing by 90° of the lowest energy conformation for $\mathbf{1d_2}$ in light blue and for **1e** in blue, anchored to the CDK1 ATP-binding cleft. Residues that surround the ligands and atoms of the pyrrolo[2,3-α]carbazole derivatives involved in the CDK1−ligand interactions (B) are denoted. Residues that are found within 4 Å from the ligands are shown as yellow sticks (C).

(see Supporting Information). This compound inhibited CDK1 activity by 50% when used at 30 μM. **1e** also proved to be inactive against CDK1/cyclin A and CKD1/cyclin E complexes. To determine whether **1e** also affects the activity of receptor tyrosine kinases, we tested it on EGFR, IGFR, FGFR, KDR, Flt-4, and Tie2 (data not shown). In all cases **1e** proved to be inactive up to 50 μM using a previously described methodology.[27]

Molecular Modeling. Interaction of pyrrolo[2,3-*a*]carbazoles derivatives and CDK1 was studied through molecular modeling and docking simulations using a CDK2-based homology mode of CDK1.[28] CDK1 (297 residues) exhibits 63.6% sequence identity with CDK2 (298 residues; see Supporting Information). The structure of CDK2 in complex with ATP (PDB code 1B38)[29] was chosen with the criterion that it is the best representative model to address the ATP binding site. The ribbon representation of the energy minimized CDK1 model is shown in Figure 2A.

On the basis of the homology model of CDK1, efforts were focused on the generation of the protein–substrate complexes through a docking simulation approach. All seven compounds (see Supporting Information for experimental details) exhibit a remarkable preference for binding to the ATP-binding cleft. The compounds used are classified according to the substitution at the D ring of the pyrrolo[2,3-*a*]carbazole core and the nature of the substituent. All derivatives exhibit docking energies from −11.0 to −12.8 kcal/mol, with **1a** exhibiting the lowest docking energy. Additionally, derivative **1e**, which proved to be the most potent inhibitor of CDK1 as described above, exhibited the highest docking energy. However, docking simulations of **1e** suggest two likely placements into the CDK1 ligand binding site. With respect to the conformer with the highest docked energy (**1e-I**), the compound's second placement (**1e-II**) (Figure 2B,C) resulted in the overlap of three out of four rings in a way that (i) rings B are well fitted and (ii) ring A of **1e-I** is well fitted to ring C of **1e-II** and vice versa (ring C of **1e-I** is perfectly fit ted to ring A of **1e-II**). The two orientations differ in terms of docked energy by less than 0.8 kcal/mol. Among the seven compounds studied here, only **1e** exhibits remarkably high inhibition properties, up to 90% inhibition of CDK1 (Figures 1 and 2B,C).

The most important CDK1−substrate simulated interactions involve residues Ile10, Glu12, Gly13, Thr14, Tyr15, Val18, Ala31, Lys33, Val64, Phe80, Glu81, Phe82, Leu83, Asp86, Asp128, Lys130, Gln132, Asn133, Ala145, Asp146, and Leu149 (Figure 2B,C). In all cases, Val18, Lys33, Lys130, Asn133, Ala145, and Asp146 are predicted to exhibit major contacts with the pyrrolo[2,3-*a*]carbazole core inside the ATP-binding cleft. These residues define the following substrate binding subsites: (i) a hydrophilic region consisting of residues Glu12-Tyr15, (ii) the polar area comprising Asp128, Lys130, Gln132, and Asn133, (iii) the Asp146 docking site favorable for H-bond formation, and (iv) the Phe80 to Leu83 fragment.

According to the analysis of the CDK2-ATP crystal structure,[30] the corresponding CDK1 residues that might be important for ATP-binding are Asp86 and Gln132 (Gln131 in CDK2) and Lys33 and Asp146 (Asp145 in CDK2). From those, Lys33, Lys130, and Asp146 are predicted to possess a key role in positioning and binding of **1e** through hydrogen bonds (Figure 2B,C). It is noteworthy that N1 and N10 of the pyrrolo[2,3-*a*]carbazole scaffold are predicted to be in a favorable position to form hydrogen bonds with side chain carboxylic oxygen atoms of Asp146. In CDK1−**1e-I** simulated complex Asp146 Oδ2 is in H-bond distance with N10 and N1 atoms (2.6 and 2.9 Å). In contrast, in CDK1−**1e-II** complex Asp146 both carboxylic oxygens are predicted to be close to both pyrrole nitrogens of the ligand; Oδ1 atom is at 3.2 and 4.2 Å to N1 and N10, respectively, and Oδ2 at 2.5 and 2.6 Å to N10 and N1, respectively (Figure 2B,C). An examination of all available CDK2−inhibitor crystal structures[7] has revealed that such an interaction between both carboxylic oxygens of Asp145 (Asp146 in CDK1) and inhibitor is not present. These data underline the role of Asp145 in CDK1−inhibitor complexes, suggesting a tight interaction of **1e** active compound presented herein with ATP-binding cleft in CDK1. Asp146 (Asp145 in CDK2), which has been reported to be coordinated to Mg^{2+} and to make contact with the ATP triphosphate group through the metal ion in the CDK2−ATP complex, is far from staurosporine in CDK2−staurosporine complex (~6.0 Å to the closest staurosporine atom). The same residue in CDK2−UCN01 complex is H-bonded through its backbone nitrogen to a water molecule, which is found to be close to the 7-hydroxyl group of UCN01.

Focusing on the most potent derivative **1e**, it is noted that in simulated **1e-I** placement the ligand exhibits some H-bond interactions with (i) side chain amine protons of Lys33, (ii) the Thr14 amide HN and the O1 (C=O group), (iii) side chain amine protons of Lys130 and the O2 oxygen of all compounds, while it accommodates the carboxyl ester $-CH_3$ toward the aromatic ring of Tyr15 and between the backbone of Asp128 and the side chains of Asp146. The C=O moiety is close to Lys130 side chain $-NH_3^+$ group with distance and geometry favorable for H-bonding. In such a conformation the chlorine atom of **1e-I** orients toward the Phe80 ring (in 3.4 Å distance). In CDK1−**1e-II** simulated complex, the ligand orients its carboxyl ester chain perpendicular to the aromatic moiety close

to Val18, Lys33, and Phe80, while the chlorine atom is found close (~3.6 Å) to the positively charged $-NH_3^+$ group of Lys130.

Conclusions

While aiming to generate agents with inhibitory CDK1 activity, we designed and synthesized, following a modified route, a novel series of compounds bearing the pyrrolo[2,3-*a*]carbazole core. Our initial design was confirmed by biological results where one of the studied derivatives (**1e**) exhibited inhibitory potency against CDK1/cycline B, causing 50% inhibition when used at 30 μM. At concentrations up to 100 μM the rest of the compounds proved to be inactive, while **1e** at this high concentration caused 90% inhibition. In subsequent experiments, **1e** proved to be inactive against a panel of kinases (CDK1/cyclin A, CDK1/cyclin E, EGFR, IGFR, FGFR, KDR, Flt-4, and Tie2) when used at 50 μM, demonstrating selectivity toward the CDK1/cycline B complex.

By use of a homology model of CDK1, CDK1−pyrrolo[2,3-*a*]carbazole complexes were modeled. The structural analysis of the simulated complexes attempts to illustrate the physicochemical and structure determinants for **1e**'s high inhibitory potential toward CDK1. Additionally, this study provides a different interaction platform between CDK1 and a potential inhibitor bearing the pyrrolo[2,3-*a*]carbazole scaffold. Since no X-ray structure of CDK1 is available, all data for structure-based inhibitor design are based on CDK2−substrate complexes and on simulated complex of CDK1 homology models with heterocycle ligands.[7,29,30] According to these studies, substrate binding into ATP binding pocket of CDK2 implicates Lys33, Glu81, Leu83, Asp86, and Gln131 and according to CDK1−paullone complexes, Glu8, Lys20, Leu83, Asp86, Lys89, and Gln300.[31] The pyrrolo[2,3-α]carbazole derivative **1e** docking studies suggest that this compound does not preferentially fit into the Glu81-Leu83 cleft but exploits remarkably the potential of Lys130, Thr14, and Asp146 side chains in positioning and binding. Structural modifications of the basic pyrrolo[2,3-α]carbazole core are under investigation aiming at the determination of structure–activity relationships and optimization of the inhibitory potency or/and selectivity, and they will be reported in due course.

Experimental Section

The target compounds **1a**−**f** have been synthesized previously[17] using a different synthetic approach.

General Procedure for the Preparation of Arylhydrazones 4a−**f and 7a,e.** To a solution of ketone (**3** or **6**) (0.61 mmol) in absolute ethanol (4.3 mL) was added trifluoroacetic acid (2.38 mmol, 0.18 mL), and the mixture was stirred at room temperature under an argon atmosphere. A suspension of the appropriate arylhydrazine (1 mmol) and sodium acetate anhydrous (1.22 mmol, 100 mg) in absolute ethanol (4.3 mL) was added dropwise over 15 min. The mixture was stirred at 80 °C for 2–4 h under argon atmosphere. After the completion of the reaction (TLC monitoring), solvents were removed under reduced pressure, dichloromethane (5 mL) was added, and evaporation was repeated. The crude mixture was dried in vacuo.

General Procedure for the Preparation of N-Protected Pyrrolo[2,3-*a*]carbazole Derivatives 5a−**f, and 8a,e. Fischer Indolization.** To a solution of freshly prepared PPSE in dry nitromethane (2 mL), a solution of the crude mixture of arylhydrazone in dry nitromethane (6 mL) was added at one portion at 100 °C, and the resulting mixture was heated to reflux for 4–44 h under argon atmosphere. The reaction mixture was quenched with ice–water and extracted with ethyl acetate three times. The combined organic extracts were washed with water and brine and dried over anhydrous sodium sulfate. The solvent was evaporated. The crude residue was purified by column chromatography using toluene/ethyl acetate gradient as eluent (from 100/0 to 90/10) to afford the title compounds **5a**−**f** and **8a**,**e**.

Ethyl 9-Chloro-1-(4-methoxybenzyl)-1*H*,10*H*-pyrrolo[2,3-*a*]-carbazole-2-carboxylate (5e). Reaction time: 15 h. 21% yield. IR (film): 3391, 1702, 1244, 813, 739 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.19 (br s, 1H), 7.84 (d, 1H, $J = 7.8$ Hz), 7.72 (d, 1H, $J = 8.5$ Hz), 7.48–7.45 (m, 2H), 7.24–7.18 (m, 3H), 7.07 (t, 1H, $J = 7.8$ Hz), 6.85 (d, 2H, $J = 8.6$ Hz), 6.09 (s, 2H), 4.33 (q, 2H, $J = 7.1$ Hz), 3.69 (s, 3H), 1.35 (t, 3H, $J = 7.1$ Hz). ^{13}C NMR ($CDCl_3$): δ 162.0, 159.4, 136.0, 130.2, 127.4, 127.3, 127.1, 125.7, 125.4, 125.1, 123.8, 120.9, 120.6, 118.1, 116.5, 115.3, 115.2, 114.5, 112.6, 60.7, 55.3, 49.3, 14.4. LC−MS (ESI+) *m*/*z* 433.10 [M + H^+]. Anal. ($C_{25}H_{21}ClN_2O_3$) C, H, N.

General Procedure for the Preparation of the Pyrrolo[2,3-*a*]carbazole Derivatives 1a−**f.** To a stirred mixture of the protected **5a**−**f** (0.05 mmol) in trifluoroactic acid (0.6 mL) at 0 °C under argon atmosphere, anisole (0.136 mmol, 148 μL) and concentrated sulfuric acid 7.5 μl) were added. The mixture was stirred 0.5 h at 0 °C and then for 2–3.5 h at room temperature. The solution was then added dropwise to an ice-cooled solution of saturated aqueous sodium bicarbonate (3 mL), and the mixture was extracted with ethyl acetate three times. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude product was purified by column chromatography using toluene/ethyl acetate gradient as eluent (from 100/0 to 80/20) to give the unprotected products **1a**−**f**. The analytical and spectroscopic data of the title compounds were identical to those previously reported.[17]

Ethyl 9-Chloro-1*H*,10*H*-pyrrolo[2,3-*a*]carbazole-2-carboxylate (1e). White solid, 95% yield. Mp 285–286 °C (EtOAc). IR(KBr): 3427, 3288, 1682, 1206, 748 cm^{-1}. ^1H NMR (DMSO-d_6): δ 11.67 (s, 1H, NH), 11.26 (s, 1H, NH), 8.10 (d, 1H, $J = 7.8$ Hz), 7.85 (d, 1H, $J = 8.5$ Hz), 7.52–7.43 (m, 2H), 7.34 (s, 1H), 7.22 (t, 1H, $J = 7.8$ Hz), 4.40 (q, 2H, $J = 6.8$ Hz), 1.39 (t, 3H, $J = 7.1$ Hz). ^{13}C NMR (DMSO-d_6): δ 161.0, 134.8, 125.8, 125.7, 125.4, 123.8, 123.3, 120.1, 118.5, 118.3, 115.3, 114.2, 114.0, 109.3, 109.2, 60.5, 14.2. LC−MS (ESI+) *m*/*z* 312.88 [M + H^+]. Anal. ($C_{17}H_{13}ClN_2O_2$) C, H, N.

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Supporting Information Available: Synthetic procedures, spectroscopic data for new compounds, biological assays details, and computational methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Knockaert, M.; Greengard, P.; Meijer, L. Pharmacological inhibitors of cyclin-dependent kinases. *Trends Pharmacol. Sci.* **2002**, *43*, 417–427.
(2) Pavletich, N. P. Mechanisms of Cyclin-dependent kinase regulation: structures of Cdks, their cyclin activators, and Cip and INK4 inhibitors. *J. Mol. Biol.* **1999**, *287*, 821–828.
(3) Malumbres, M.; Barbacid, M. Mammalian cyclin-dependent kinases. *Trends Biochem. Sci.* **2005**, *30*, 630–641.
(4) Malumbres, M.; Barbacid, M. To cycle or not to cycle: a critical decision in cancer. *Nat. Rev. Cancer* **2001**, *1*, 222–231.
(5) Lee, K.-Y.; Clark, A. W.; Rosales, J. L.; Chapman, K.; Fung, T.; Johnston, R. N. Elevated neuronal Cdc2-like kinase activity in the Alzheimer disease brain. *Neurosci. Res.* **1999**, *34*, 21–29.

(6) Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. Cyclin-dependent kinase inhibitors: useful targets in cell cycle regulation. *J. Med. Chem.* **2000**, *43*, 1–18.
(7) Huwe, A.; Mazitschek, R.; Giannis, A. Small molecules as inhibitors of cyclin-dependent kinases. *Angew. Chem., Int. Ed.* **2003**, *42*, 2122–2138.
(8) Fischer, P. M.; Gianella-Borradori, A. CDK inhibitors in clinical development for the treatment of cancer. *Expert Opin. Invest. Drugs* **2003**, *12*, 955–970.
(9) Sausville, E. A. Cyclin-dependent kinase modulators studied at the NCI: pre-clinical and clinical studies. *Curr. Med. Chem.: Anti-Cancer Agents* **2003**, *3*, 47–56.
(10) De Bondt, H. L.; Rosenblatt, J.; Jancarik, J.; Jones, H. D.; Morgan, D. O.; Kim, S. H. Crystal structure of cyclin-dependent kinase 2. *Nature* **1993**, *363*, 595–602.
(11) Lawrie, A. M.; Noble, M. E.; Tunnah, P.; Brown, N. R.; Johnson, L. N.; Endicott, J. A. Protein kinase inhibition by staurosporine revealed in details of the molecular interaction with CDK2. *Nat. Struct. Biol.* **1997**, *4*, 796–801.
(12) De Azevedo, W. F., Jr.; Mueller-Dieckmann, H. J.; Schulze-Gahmen, U.; Worland, P. J.; Sausville, E.; Kim, S. H. Structural basis for specificity and potency of a flavonoid inhibitor of human CDK2, a cell cycle kinase. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2735–2740.
(13) Meijer, L.; Thunnissen, A. M. W. H.; 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. Inhibition of cyclin-dependent kinases, GSK-3β and CK1 by hymenialdisine, a marine sponge constituent. *Chem. Biol.* **2000**, *7*, 51–63.
(14) D'Alessio, R.; Bargiotti, A.; Metz, S.; Brasca, M. G.; Cameron, A.; Ermoli, A.; Marsiglio, A.; Polucci, P.; Roletto, F.; Tibolla, M.; Vazquez, M.l L.; Vulpetti, A.; Pevarello, P. Benzodipyrazoles: a new class of potent CDK2 inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1315–1319.
(15) 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. 3-Aminopyrazole inhibitors of CDK2/cyclin A as antitumor agents. 1. Lead finding. *J. Med. Chem.* **2004**, *47*, 3367–3380.
(16) Santamaria, D.; Barriere, C.; Cerqueira, A.; Hunt, S.; Tardy, C.; Newton, K.; Caceres, J. F.; Dubus, P.; Malumbres, M.; Barbacid, M. Cdk1 is sufficient to drive the mammalian cell cycle. *Nature* **2007**, *448*, 811–815.
(17) Fousteris, M. A.; Koutsourea, A. I.; Arsenou, E. S.; Leondiadis, L.; Nikolaropoulos, S. S.; Stamos, I. K. Pyrrolocarbazole analogs of aromatic skeleton of indolocarbazole natural products. *J. Heterocycl. Chem.* **2004**, *41*, 349–353.
(18) Tani, M.; Ariyasu, T.; Ohtsuka, M.; Koga, T.; Ogawa, Y.; Yokoyama, Y.; Murakami, Y. New strategy for indole synthesis from ethyl pyrrole-2-carboxylate (synthetic studies on indoles and related compounds. XXXIX). *Chem. Pharm. Bull.* **1996**, *44*, 55–61.
(19) Forbes, I. T.; Johnson, C. N.; Thompson, M. Syntheses of functionalized pyrido[2,3-*b*]indoles. *J. Chem. Soc., Perkin Trans. 1* **1992**, 275–281.
(20) Subramanyam, C. 4-Methoxybenzyl, a versatile protecting group for the regiospecific lithiation and functionalization of pyrazoles. *Synth. Commun.* **1995**, *25*, 761–774.
(21) Eskildsen, J.; Kristensen, J.; Vedso, P.; Begtrup, M. Convergent synthesis of 3-arylated 1-hydroxypyrazoles via 3-metalated pyrazole-1-oxides. *J. Org. Chem.* **2001**, *66*, 8654–8656.
(22) Guida, W. C.; Mathre, D. J. Phase-transfer alkylation of heterocycles in the presence of 18-crown-6 and potassium *tert*-butoxide. *J. Org. Chem.* **1980**, *45*, 3172–3176.
(23) Raul, F.; Schneider, Y.; Brouillard, R.; Fougerousse, A.; Chabert, P.; Gonzalez, E. Method for the Synthesis of (*Z*)-3,5,4′-Trimethoxystilbene, a Trimethyl Derivative of Resveratrol, the Resulting Compound, and Uses of Said Compound, in Particular as a Medicine, and Particularly as an Anticancer Drug. PCT Int. Appl. WO 03/031381A2, CAN 138:321056, 2003.
(24) Bergman, J.; Pelcman, B. Synthesis of indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazoles by double Fischer indolizations. *J. Org. Chem.* **1989**, *54*, 824–828.
(25) Watanabe, T.; Kobayashi, A.; Nishiura, M.; Takahashi, H.; Usui, T.; Kamiyama, I.; Mochizuki, N.; Noritake, K.; Yokoyama, Y.; Murakami, Y. Synthetic studies on indoles and related compounds. XXVI. The debenzylation of protected indole nitrogen with aluminum chloride. (2). *Chem. Pharm. Bull.* **1991**, *39*, 1152–1156.
(26) Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J. J.; Donella-Deana, A.; Pinna, L.; Letham, D. S.; Kato, J.; Detivaud, L.; Leclerc, S.; Meijer, L. Inhibition of cyclin-dependent kinases by purine analogs. *Eur. J. Biochem.* **1994**, *224*, 771–786.
(27) Gourzoulidou, E.; Carpintero, M.; Baumhof, P.; Giannis, A.; Waldmann, H. Inhibition of angiogenesis-relevant receptor tyrosine kinases by sulindac analogues. *ChemBioChem* **2005**, *6*, 527–531.
(28) Cavalli, A.; Dezi, C.; Folkers, G.; Scapozza, L.; Recanatini, M. Three-dimensional model of the cyclin-dependent kinase 1 (CDK1): ab initio active site parameters for molecular dynamics studies of CDKs. *Proteins* **2001**, *45*, 478–485.
(29) Brown, N. R.; Noble, M. E.; Lawrie, A. M.; Morris, M. C.; Tunnah, P.; Divita, G.; Johnson, L. N.; Endicott, J. A. Effects of phosphorylation of threonine 160 on cyclin-dependent kinase 2 structure and activity. *J. Biol. Chem.* **1999**, *274*, 8746–8756.
(30) Davies, T. G.; Pratt, D. J.; Endicott, J. A.; Johnson, L N.; Noble, M. E. Structure-based design of cyclin-dependent kinase inhibitors. *Pharmacol. Ther.* **2002**, *93*, 125–133.
(31) Kunick, C.; Zeng, Z.; Gussio, R.; Zaharevitz, D.; Leost, M.; Totzke, F.; Schachtele, C.; Kubbutat, M. H.; Meijer, L.; Lemcke, T. Structure-aided optimization of kinase inhibitors derived from alsterpaullone. *ChemBioChem* **2005**, *6*, 541–549.
(32) Koradi, R.; Billeter, M.; Wüthrich, K. MOLMOL: a program for display and analysis of macromolecular structures. *J. Mol. Graphics* **1996**, *14*, 51–55.

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