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COMMUNICATIONS

Published on Web 04/12/2006

Searching for Cyclin-Dependent Kinase Inhibitors Using a New Variant of the Cope Elimination

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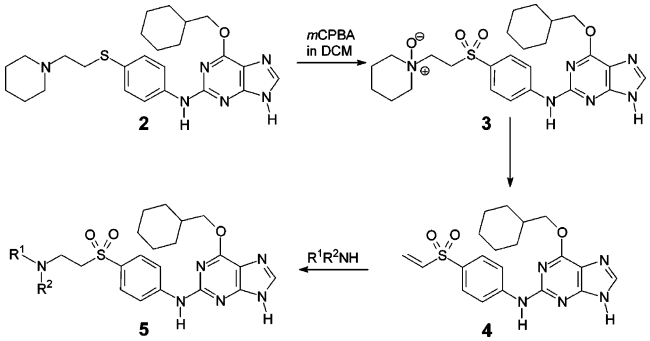
The defective functioning of cyclin-dependent kinases (CDKs) compromises normal cell cycle progression[1,2] and is associated with the molecular pathology of cancer.[3,4] During late G_1, the CDK2/cyclin E complex sustains hyperphosphorylation of the retinoblastoma tumor suppressor protein (pRb), which controls levels of the transcription factor E2F. Once cells enter S phase, CDK2/cyclin A phosphorylates and consequently deactivates E2F. Deregulated and elevated levels of E2F transcriptional activity lead to cell death via apoptosis. This suggests that inhibition of CDK2 may have a therapeutic benefit by eliciting tumor cell apoptosis.[5] Numerous CDK inhibitors have been reported,[6,7] the most common being those selective for CDK2 and CDK4. In this paper, we describe a synthetic strategy that has been developed for use in a multiple-parallel format and is applicable for generating a variety of compound libraries. This has enabled the discovery of new, potent inhibitors of CDK2.

With the finding that O^6-cyclohexylmethylguanine **1a** (Figure 1) is a moderate inhibitor of CDK1 and CDK2 [K_i = 12 and 5 μM, respectively),[8] a rational design approach was implemented guided by the crystal structure of **1a** bound to monomeric CDK2.[9] These studies suggested that introduction of arylamino groups at C-2 of the purine, especially those substituted at the 4-position, would confer enhanced potency. We found that sulfonyl [e.g., **1b**; IC_{50} (CDK2) = 63 nM] and sulfonamido substituents [e.g., **1c**; IC_{50} (CDK2) = 5 nM] enhanced activity. Crystallographic studies of **1c** bound to fully activated CDK2/cyclin A revealed that the enhanced potency relative to **1a** is primarily a result of two additional hydrogen bonds to Asp86 and stacking interactions that these bonds promote between the arylamino ring of **1c** and the CDK2 backbone.[9] The sulfonamido oxygen of **1c** accepts a hydrogen bond from the backbone NH of Asp86, while a sulfonamido NH atom donates a hydrogen bond to the side chain carboxylate of Asp86. Examination of the X-ray structure suggested that N-substituents on the sulfonamido group would protrude toward the surface of the C-terminal domain of CDK2. Further exploration in this region, which differs among CDKs, was expected to lead to inhibitors with enhanced selectivity for CDK2, as well as improved pharmaceutical properties.

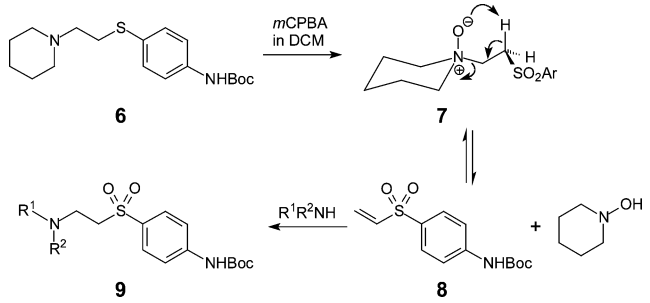
Whereas elaboration of the sulfonamide of **1c** was relatively straightforward,[10] an innovative approach was required to enable the generation of libraries of sulfone-based compounds. To achieve this goal, we conceived a procedure whereby oxidation of the β-piperidinoethylsulfide **2** with >3 molar equiv of 3-chloroperbenzoic acid would give *N*-oxide sulfone **3**. Cope-type elimination[11] of **3** would afford vinylsulfone **4** that could be captured by a variety of amines leading to products **5** (Scheme 1). The planned chemistry was initially established using [4-(2-piperidin-1-ylethylsulfanyl)-phenyl] carbamic acid *tert*-butyl ester **6** as a model substrate. Triple oxidation of **6** resulted in the corresponding tri-oxygenated species (**7**), which spontaneously decomposed to *N*-hydroxypiperidine and vinyl sulfone **8**, the structure of which was confirmed by X-ray analysis.[12] When vinyl sulfone **8** was mixed with *N*-hydroxypiperidine, **7** was partially regenerated, showing that these compounds are in equilibrium (ca. 1:1 ratio of **7** and **8** according to LC-MS). However, addition of an amine to the mixture gave the corresponding adducts **9a**−**9e** in excellent yield (77−91%) and purity (Scheme 2 and Supporting Information). These results confirmed the

Scheme 1. Synthesis of β-Aminoethylsulfone-Based Inhibitors of CDK2 **5a**−**5w**[a]



[a] e.g. **5b**, R^1R^2= $CH_2CH_2NMeCH_2CH_2$; **5e**, $R^1 = R^2$ = Et; **5h**, R^1 = 3-hydroxypropyl, R^2 = H; **5l**, R^1 = 2-acetamidoethyl, R^2 = H; **5o**, R^1R^2= $CH_2CH_2CH(CH_2CH_2OH)CH_2CH_2$; **5s**, R^1 = 2-dimethylaminoethyl, R^2 = Me; **5t**, $R^1R^2 = (CH_2)_6$; **5w**, $R^1 = R^2$ = 2-hydroxyethyl.

Scheme 2. Model Reactions with 4-(2-Piperidin-1-ylethylsulfanyl)-phenyl] Carbamic Acid *tert*-Butyl Ester **6** Leading to **9**[a]



[a] e.g. **9a**, R^1 = 2-hydroxyethyl, R^2 = H; **9b**, $R^1R^2 = CH_2CH_2OCH_2CH_2$; **9c**, R^1 = cyclopentyl, R^2 = H.

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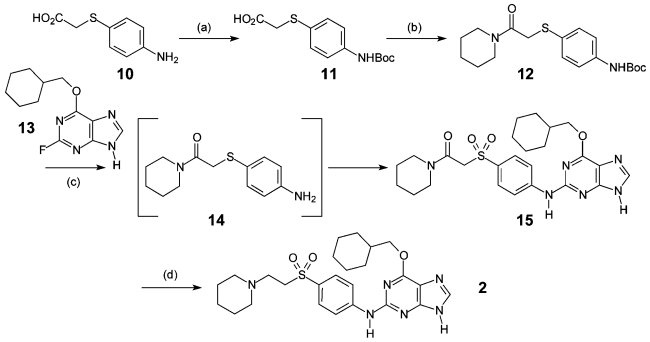
10.1021/ja060595j CCC: $33.50

Scheme 3. Synthesis of β-Piperidinoethylsulfide **2**[a]



[a] Reagents and conditions: (a) (i) Boc_2O, NEt_3, dioxane/water, rt, (ii) 3 M HCl; (b) (i) $SOCl_2$, DMF, THF, (ii) piperidine, THF, rt; (c) **13**, TFA, TFE, reflux; (d) $LiAlH_4$, THF, rt.

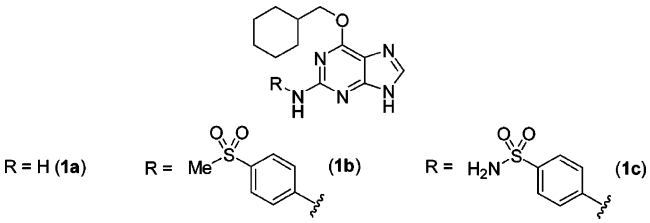


Figure 1. Lead CDK2 inhibitors identified by a structure-based design strategy.

existence of the equilibrium described, while providing a basis for the application of similar chemistry to the purine scaffold.

We were gratified to find that the chemistry planned for **2** proceeded exactly as intended to provide sulfones **5a**−**5w** (Scheme 1 and Supporting Information). To access the β-piperidinoethylsulfide **2** (Scheme 3), commercially available 4-aminophenylthioacetic acid (**10**) was N-protected with *t*-butoxycarbonyl (Boc) to give **11**, which was converted into amide **12** by reaction of an intermediate acid chloride with piperidine. On heating **12** with fluoropurine **13** under the trifluoroacetic acid/2,2,2-trifluoroethanol protocol previously described,[13] in situ removal of the Boc group released aniline **14**, which reacted with **13** to furnish the amide **15**. Reduction of **15** afforded the corresponding tertiary amine **2**. Application of the methodology developed with **6** to the purine **2** (Scheme 1) gave compounds **5a**−**5c** in acceptable yields (>65%) and sufficiently pure after HPLC for biological evaluation. The methodology was then implemented in a multiple-parallel format, allowing the synthesis of compounds **5d**−**5w** in high purity and satisfactory yields. The "one-pot" procedure developed is applicable to numerous scenarios in the context of drug development.

Inhibitors **5a**−**5w** displayed a range of activities against CDK2, the structure−activity relationship (SAR) appearing to favor the incorporation of smaller, less sterically hindered groups (e.g., **5h**; IC_{50} = 45 nM). Increasingly bulky groups (e.g., **5t**; IC_{50} = 1.78 μM) largely abolished activity. The structure of the CDK2/cyclin A/**5h** complex provided a starting point from which to rationalize the observed SAR (Figure 2). There are three hydrogen bonds between the guanine N9, N3, and the NH of the C2-substituted anilino group and the backbone carbonyl of Glu81 and the amide NH and carbonyl group of Leu83, respectively.[8−10] The position of the anilino group is similar to that of **1c** bound to CDK2/cyclin A[9] and enables the 3-hydroxypropyl group of the aminoethylsulfonyl substituent to form multiple polar contacts, notably with Asp86 and Lys89. Mimicking the interactions of the **1c** sulfonamide group, the backbone amide NH and side chain carboxylate of Asp86 can hydrogen bond to a sulfone oxygen and the NH of the ethylamino group, respectively. The other sulfone oxygen is then

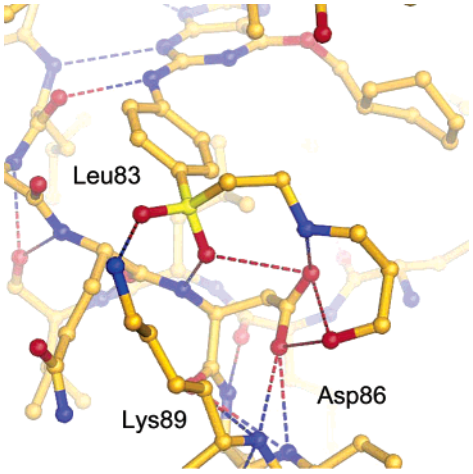


Figure 2. Structure of **5h** bound to CDK2/cyclin A. Dotted lines represent polar contacts <3.4 Å.

positioned to interact with the ϵ-NH_2 group of Lys89 (the first observation of such an interaction in this series) and the terminal OH group with the side chain of Asp86. All these interactions result in the extended anilino substituent adopting an ordered conformation on the CDK2 surface. However, the lower potency for **5h** compared to that of **1c** suggests that this network of polar contacts is insufficient to compensate for the loss of more favorable interactions between CDK2 and the purine and anilino rings of **1c**. An overlay of the two inhibitors bound to CDK2/cyclin A showed that their relative binding orientations do differ.

Application of the methodology described to the development of inhibitors of other CDKs is in progress.

Acknowledgment. We thank Cancer Research UK, BBSRC (A.H.), and EU FP 6 (A.E.) for financial support.

Supporting Information Available: Details of preparative procedures/spectroscopic data for compounds **5a**−**5w** and **9a**−**9d**, CDK2 inhibitory data for **5a**−**5w**; crystallization of a T160pCDK2/cyclin A/**5h** complex, X-ray crystallography data collection and processing; complete refs 8−10. The coordinates of the CDK2/cyclin A/**5h** complex have been deposited in the Protein Data Bank under ID code 2G9X. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA060595J