

# Binding Mode of the 4-Anilinoquinazoline Class of Protein Kinase Inhibitor: X-ray Crystallographic Studies of 4-Anilinoquinazolines Bound to Cyclin-Dependent Kinase 2 and p38 Kinase

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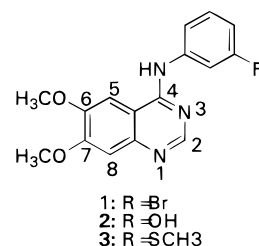
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4-Anilinoquinazolines represent an important class of protein kinase inhibitor. Modes of binding for two members of this inhibitor class were determined by X-ray crystallographic analysis of one inhibitor (4-[3-hydroxyanilino]-6,7-dimethoxyquinazoline) in complex with cyclin-dependent kinase 2 (CDK2) and the other (4-[3-methylsulfanylanilino]-6,7-dimethoxyquinazoline) in complex with p38 kinase. In both inhibitor/kinase structures, the 4-anilinoquinazoline was bound in the ATP site with the quinazoline ring system oriented along the peptide strand that links the two domains of the protein and with the anilino substituent projecting into a hydrophobic pocket within the protein interior. In each case, the nitrogen at position-1 of the quinazoline accepted a hydrogen bond from a backbone NH (CDK2, Leu-83; p38, Met-109) of the domain connector strand, and aromatic hydrogen atoms at C2 and C8 interacted with backbone carbonyl oxygen atoms of the peptide strand. The anilino group of the CDK2-bound compound was essentially coplanar with the quinazoline ring system and occupied a pocket between Lys-33 and Phe-80. For the p38-bound inhibitor, the anilino group was angled out of plane and was positioned between Lys-53 and Thr-106 in a manner similar to that observed for the aryl substituent of the pyridinylimidazole class of inhibitor.

## Introduction

Selective, reversible phosphorylation of protein hydroxyl groups is one of the primary mechanisms for cellular signal transduction and regulation of a variety of cellular events.<sup>1–3</sup> Consequently, selective inhibitors of particular protein kinases may have therapeutic value in a wide range of disease states, such as cancer, diabetes, and arthritis.<sup>4–10</sup> To date, the large majority of known protein kinase inhibitors are competitors of ATP.<sup>6,11,12</sup> The ATP binding site of protein kinases is relatively conserved;<sup>13,14</sup> therefore inhibitor selectivity has been an important concern. Among the many reported classes of protein kinase inhibitors, the 4-anilinoquinazolines have played a particularly important role in demonstrating the potential for selectivity and potency.<sup>15–17</sup> For example, Fry and co-workers identified 4-anilinoquinazoline **1** as a highly potent and selective inhibitor of the epidermal growth factor receptor (EGFR) kinase.<sup>18</sup> The compound has an EGFR kinase IC<sub>50</sub> of 29 pM and shows little effect on the activity of platelet-derived growth factor receptor, fibroblast growth factor receptor, colony-stimulating factor receptor, and c-Src kinases at a concentration of 50  $\mu$ M.<sup>18</sup> Of relevance to this report, compound **1** inhibits cyclin-dependent kinase 2 (CDK2) and p38 kinase with IC<sub>50</sub> values of 250 and 6.3  $\mu$ M, respectively.



The available evidence indicates that the 4-anilinoquinazolines bind to protein kinases in the ATP binding site, and molecular models of inhibitor/kinase complexes have been created.<sup>19–21</sup> The two reported efforts to predict the binding mode for this general class of inhibitor came to different conclusions as to the orientation of the inhibitor within the ATP binding site of homology models of EGFR tyrosine kinase.<sup>19–21</sup>

In this paper, we report crystal structures of two serine/threonine protein kinases – CDK2<sup>22</sup> and p38<sup>23</sup> – each in complex with a 4-anilinoquinazoline (compounds **2** and **3**, respectively). The interactions between kinase and inhibitor were similar in both complexes and closely corresponded to those proposed by Palmer and co-workers for a series of 4-anilinoquinazolines binding to EGFR kinase on the basis of molecular modeling and structure–activity relationships.<sup>19</sup> Although the two compounds used in these crystallographic studies were relatively weak inhibitors of the CDK2 and p38 kinases, the observed mode of binding may be of relevance to other kinases for this class of inhibitor and should be useful for the design of novel inhibitors.

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**Table 1.** Crystallographic Data for the **2**/CDK2 and **3**/p38 Complexes

|                      | CDK2                                       | p38   |
|----------------------|--|---|
| space group          | $P2_12_12_1$                               | $P2_12_12_1$                                |
| cell                 | $a = 72.75$ , $b = 74.07$ ,<br>$c = 54.00$ | $a = 45.17$ , $b = 84.86$ ,<br>$c = 127.01$ |
| molecules/asu        | 1  | 1   |
| resolution           | 10–2.2 Å                                   | 10–2.6 Å                                    |
| no. of observations  | 160 386                                    | 88 853                                      |
| no. of unique reflns | 14 980                                     | 14 136                                      |
| completeness         | 97%  | 90%   |
| $R_{\text{merge}}$   | 9.3%                                       | 6.5%  |
| $R_{\text{factor}}$  | 18.5%                                      | 20.6%                                       |
| $R_{\text{free}}$    | 22.7%                                      | 25.1%                                       |
| average $B$          | 32.8°                                      | 19.6°                                       |
| rms bonds            | 0.009 Å                                    | 0.010 Å                                     |
| rms angles           | 1.3°                                       | 1.3°  |

## Results and Discussion

The protein structures described herein are in keeping with those reported previously by other investigators for CDK2<sup>24–28</sup> and p38.<sup>29–32</sup> Thus the discussion will focus primarily on the inhibitors and their interactions with the two enzymes but will also include differences in protein conformation where significant.

**Structure of Compound **2** Bound to CDK2.** The X-ray structure of the CDK2/compound **2** complex was solved at 2.2 Å resolution with an  $R$  factor of 18.5% at the current level of refinement (see Table 1 for data collection and refinement details). This monomeric form of the kinase is catalytically inactive, but the ATP binding site is structurally similar to that of the activated cyclin A complex.<sup>33,34</sup> However, the region of the ATP site involving Lys-33, Glu-51, and Asp-145 does undergo structural alterations upon complexation with cyclin A. Thus, analysis of inhibitor interactions with that part of the cleft must be viewed with some caution.

The 4-anilinoquinazoline **2**, which inhibited CDK2 with an  $IC_{50}$  of 1  $\mu$ M, was found to bind in the ATP site with the quinazoline ring interacting with the  $\beta$ -strand of CDK2 (residues 81–83) that links the two domains of the protein, as illustrated in Figure 1. The 3'-hydroxyanilino group at the 4-position of the quinazoline ring projected into the interior of the protein toward Phe-80, and its phenyl ring was approximately coplanar with the quinazoline ring system as defined by torsion angles  $N3-C4-C1'-C2' = 5^\circ$  and  $C4-C1'-C2'-C3' = -180^\circ$ . This essentially planar conformation was similar to the lowest-energy conformation of 4-anilinoquinazoline as calculated by quantum mechanical optimization using the 6-31G\* basis set and was consistent with an NMR analysis of 4-anilino-6,7-dimethoxyquinazoline.<sup>35</sup> The 6,7-dimethoxy substituents were near the opening to the active site with methyl groups close to the plane of the quinazoline ring system, in concert with conformations of *o*-dimethoxy groups observed in small molecule crystal structures.<sup>36</sup>

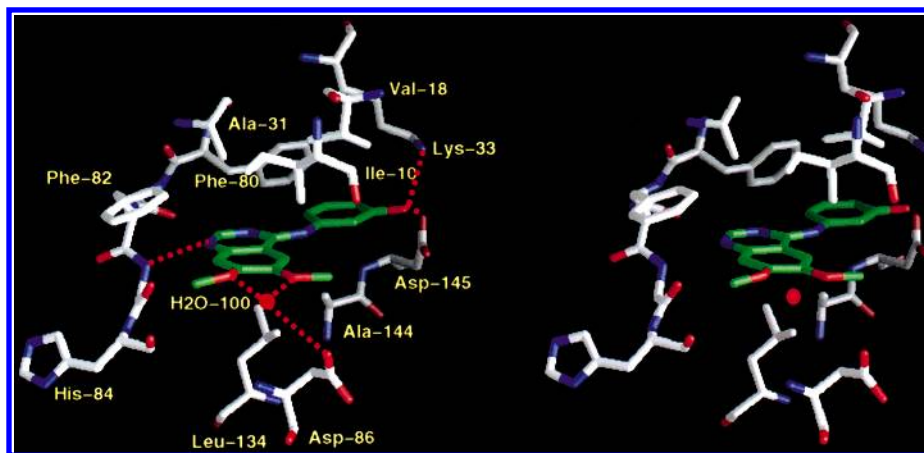
A number of interactions between compound **2** and CDK2 appeared to control binding. A hydrogen bond between quinazoline N1 and the backbone NH of Leu-83 linked inhibitor to enzyme. Two aromatic  $CH\cdots O=C$  interactions<sup>37,38</sup> also appeared to play a role in binding; the carbons at positions 2 and -8 of the quinazoline ring were 3.1 and 2.9 Å from the carbonyl oxygens of Glu-81 and Leu-83, respectively. A similar interaction is observed in the CDK2 complex with the purine

derivative olomoucine in which C8 of olomoucine is found in close proximity to the carbonyl oxygen of Glu-81.<sup>39</sup> In both cases, polarization of the aromatic CH by adjacent ring nitrogens may enhance the interaction. The 3'-hydroxy group of the anilino substituent donated a hydrogen bond to the side chain of Asp-145 and accepted one from Lys-33, although those interactions may be altered in the complex with activated enzyme because the corresponding residues are found in a significantly different orientation in the latter form of the protein. The phenyl moiety of compound **2** interacted with the phenyl group of Phe-80 in an approximately edge-to-face orientation<sup>40</sup> and was also in close contact with, and sandwiched between, the side chains of Val-18 and Ala-144. The methoxy groups of the inhibitor were at the solvent interface with a water molecule bridged between them. The methyl groups were 3–5 Å from the side chains of Ile-10, Phe-82, and Leu-134 and the backbone of His-84. The side chains of Ile-10, Ala-31, Phe-82, and Leu-134 surrounded the quinazoline ring system of the inhibitor as shown in Figure 1.

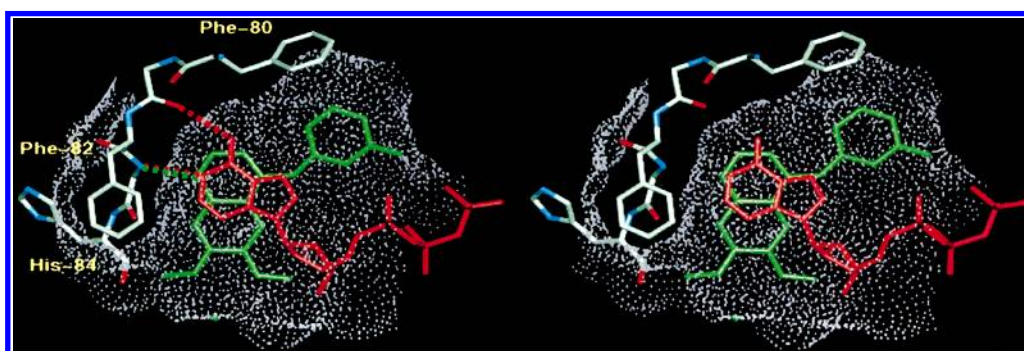
The binding of compound **2** in monomeric CDK2 is compared to that of ATP in the fully activated CDK2/cyclin A heterodimer<sup>33</sup> in Figure 2. The adenine ring system of ATP was approximately coplanar with the quinazoline ring system of compound **2**, but overlap of the two compounds in the binding site of CDK2 was limited primarily to the pyrimidine ring found in each. The N1 atoms of both ATP and quinazoline **2** accept a hydrogen bond from the backbone NH of Leu-83. A hydrogen of ATP's 6-amino group donates a hydrogen bond to the backbone carbonyl oxygen of Glu-81 and is similar in position to the hydrogen at C2 of the inhibitor. The C2 hydrogen of ATP is in close proximity to the carbonyl oxygen of Leu-83, analogous to C8 of the inhibitor. The hydroxyphenyl group and the dimethoxybenzo moiety of the inhibitor had no counterpart in ATP, and conversely, no part of the inhibitor corresponded to the ribosyltriphosphate portion of ATP. Thus, the inhibitor takes advantage of significant portions of the binding site that ATP does not and vice versa.

Relatively small protein conformational differences observed in the two liganded (ATP and compound **2**) complexes of CDK2 appeared to reflect the structural differences of the two ligands. For example, the side chain orientation of His-84 was somewhat different in the two complexes and may be related to the different position of the peptide unit that links residues 83 and 84. The carbonyl oxygen of that amide group interacts with the aromatic hydrogen at C8 of the inhibitor and the aromatic hydrogen at C2 of ATP. The position of the peptide unit appeared to adjust for an optimal interaction in each case. Similarly, the two ligands differentially affected the side chain conformation of Lys-33. In the CDK2/compound **2** complex, the amino group of Lys-33 was found almost 4 Å away from its position in the corresponding ATP complex in which it is hydrogen-bonded to the  $\alpha$ -phosphate moiety of ATP. A potential steric clash with the anilino group of inhibitor **2** presumably forced Lys-33 to adopt an alternative conformation.

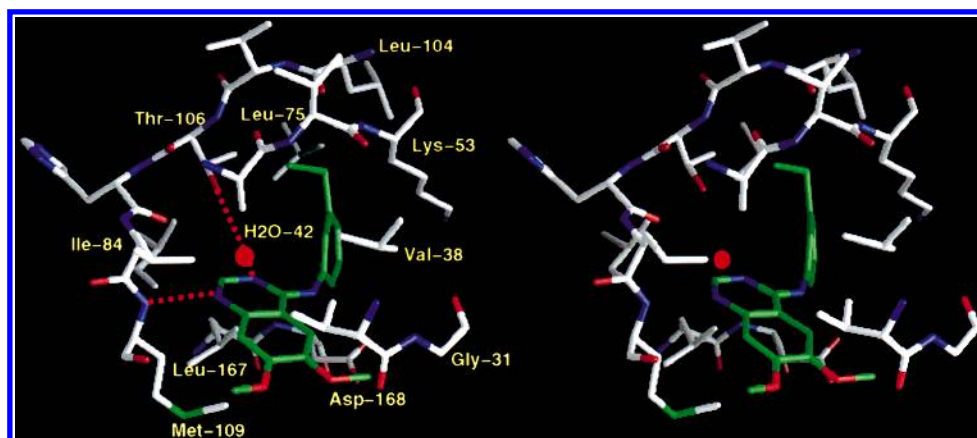
**Structure of Compound **3** Bound to p38.** The structure of p38 in complex with quinazoline **3** was solved at 2.8 Å resolution and an  $R$  factor of 20.6% at



**Figure 1.** Stereoview of compound **2** in complex with CDK2. Oxygen atoms are shown in red, nitrogens in blue, protein carbons in white, and inhibitor carbons in green. Hydrogen atoms are not shown. Hydrogen bonds are indicated by red dashed lines. Water molecules are represented as red spheres.



**Figure 2.** Stereoview of a superposition of compound **2**/CDK2 and ATP/CDK2/cyclin A complexes. Compound **2** and its hydrogen bond to protein backbone are shown in green. ATP and its associated hydrogen bonds are shown in red. The color scheme for protein atoms is as described in Figure 1. The molecular surface of the binding site is represented by white dots.



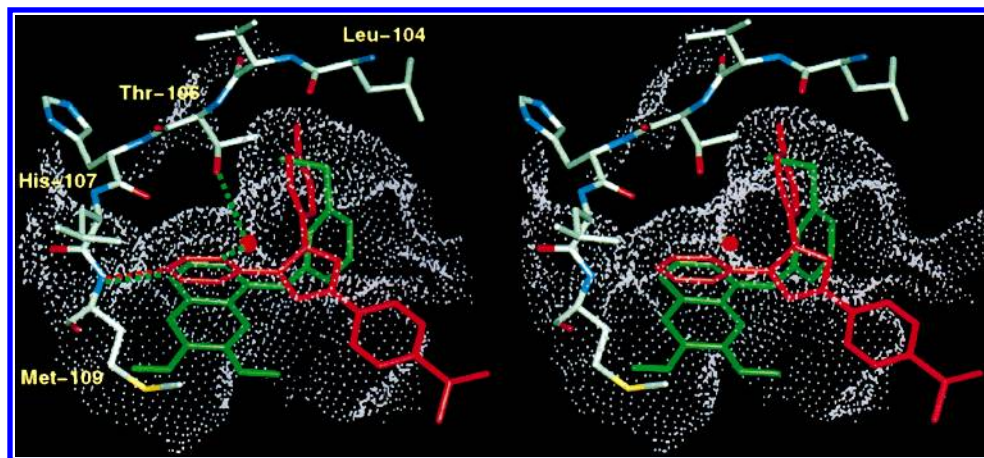
**Figure 3.** Stereoview of compound **4** bound to p38. Graphics details are as described in Figure 1.

the current level of refinement (see Table 1). The p38 protein was in the nonphosphorylated, catalytically inactive form corresponding to that in previously reported crystal structures.<sup>29–32,41</sup> In keeping with previous studies of p38, the structure reported here showed a relatively open ATP binding site. The structural relevance of this inactive form of p38 to the phosphorylated active form is not known, although members of the pyridinylimidazole class of inhibitor, which compete with ATP, have similar affinity for the active and inactive forms of the enzyme.<sup>29,42</sup> However, until structures of activated p38 are determined, conclusions drawn from structures of inhibitor complexes with the inactive form of p38 should be tempered with an

appropriate level of skepticism regarding relevance to experimental inhibition of p38 activity.

The quinazoline ring system of compound **3**, which inhibited p38 with an  $IC_{50}$  of 5  $\mu$ M, was found to interact with p38 in a manner similar to the interaction observed for compound **2** in complex with CDK2. As shown in Figure 3, the nitrogen at position-1 of the inhibitor accepted a hydrogen bond from the backbone NH of Met-109 of p38, with an N–N distance of 2.8 Å. The carbons at positions-2 and -8 were 3.3 and 3.7 Å from the backbone carbonyl oxygen atoms of His-107 and Met-109, respectively. In addition, the nitrogen at position-3 was hydrogen bonded to a buried water molecule, which in turn was linked to the side chain of Thr-106. Such





**Figure 4.** Stereoview of a superposition of p38 complexes with compounds **3** and **4**. Graphics details are as described in Figure 2, with compound **3** in green and compound **4** in red.

an interaction with N3 was not observed in the CDK2/compound **2** complex in which the corresponding protein residue is Phe-80.

As in the CDK2/compound **2** complex, the dimethoxy groups of inhibitor **3** were in a coplanar conformation. Unlike the fully planar conformation of quinazoline **2**, the anilino group of quinazoline **3** was tilted out of the plane of the quinazoline ring system (torsion angles  $N3-C4-C1'-C2' = 21^\circ$  and  $C4-C1'-C2'-C3' = 57^\circ$ ). Similar nonplanar conformations were predicted by Palmer and co-workers in their modeling studies of anilinoquinazolines bound to the EGFR kinase.<sup>19</sup> The methylthio substituent was out of plane with a  $C2'-C3'-S-CH_3$  torsion angle of  $-39^\circ$ .

The quinazoline core was sandwiched in a hydrophobic environment formed by the side chains of Val-30, Val-38, Ala-51, Met-109, and Leu-167 which are all within 4 Å of the inhibitor. The methoxy substituents were again at the solvent interface but with some protein contacts. The 6-methoxy group was within 4 Å of Val-30 and Gly-31, and the methyl group of the 7-methoxy substituent was in close contact with the carbonyl oxygen of Met-109.

The 3'-methylthiophenyl group of compound **3** was oriented into a large pocket at the back of the binding site. This part of the p38 binding site is formed by Val-38, Val-52, Lys-53, Glu-71, Leu-75, Ile-84, Leu-104, Thr-106, and Asp-168 and appears to play an important role in binding the pyridinylimidazole class of inhibitor.<sup>29,32,41</sup> For example, the 4'-fluorophenyl group of compound **4** binds in this pocket as reported by Tong and co-workers.<sup>32</sup> A comparison of compounds **3** and **4**

group of compound **4** and the 3'-methylthiophenyl group of compound **3** were slightly angled with respect to one another and occupied different regions of the large pocket at the back of the site. The methylthio group of inhibitor **3** was positioned in the same general vicinity as the C3', C4', and C5' part of the fluorophenyl ring of compound **4**. The dimethoxybenzo piece of quinazoline **3** had no counterpart in compound **4**, and the phenyl-methylsulfoxide moiety of inhibitor **4** had no counterpart in compound **3**.

## Conclusions

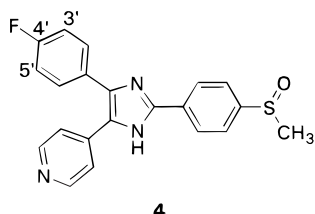
The structures reported here represent the first experimental determination of the kinase binding mode for the 4-anilinoquinazoline class of protein kinase inhibitor. The quinazoline ring system was found to bind to two different serine/threonine kinases – CDK2 and p38 – in essentially the same way, with the fused ring oriented along and interacting with the peptide strand that connects the two domains of the protein. In both structures the inhibitor's anilino substituent projected into a pocket deep inside the ATP binding site, which ATP does not occupy. The observed binding mode was similar to that predicted by Palmer and co-workers from molecular modeling studies of 4-anilinoquinazolines bound to EGFR kinase.<sup>19</sup> Thus, these experimental structures provide confirmation of the theoretical studies and may serve as a basis for the design of novel inhibitors.

## Experimental Section

**Chemistry.** Compounds **2**<sup>43</sup> and **3** were prepared from 4-chloro-6,7-dimethoxyquinazoline and the corresponding aniline according to literature procedures.<sup>44</sup>

**X-ray Crystallography.** Human CDK2 was expressed in *T. ni* cells using a baculovirus vector. The protein was expressed and purified as previously described.<sup>45</sup> Crystals of the apo-protein were obtained by the hanging drop vapor diffusion method. Protein (~10 mg/mL in 20 mM HEPES, pH 7.5, 1 mM EDTA) was mixed with an equal volume of well solution (10% PEG3340) and equilibrated at 20 °C. Crystals grew within 1 day. Prior to data collection, crystals were soaked for 24 h with a 5 mM inhibitor solution and then transferred to a cryogenic solution containing 25% glycerol in 10% PEG3340.

Human p38 was expressed and purified as follows. All procedures were performed at 4 °C. Ninety grams of *E. coli* cells expressing 6 × histidine-tagged p38 were lysed in 5



in their respective complexes with p38 is shown in Figure 4. The pyridine ring of compound **4** was in a position similar to the pyrimidine ring of quinazoline **3**, both accepting a hydrogen bond from the backbone NH of Met-109. The phenyl rings of the 4'-fluorophenyl

volumes of 20 mM HEPES, 325 mM NaCl, and 25 mM imidazole, pH 7.5, using two passes through a cell homogenizer. The clarified supernatant was passed over a 40-mL nickel chelating Sepharose column (Pharmacia-Amersham; 2.5 × 8 cm), washed with lysis buffer, and step-eluted with lysis buffer containing 100 mM imidazole. The eluate was diluted 5-fold with 25 mM HEPES and 1 mM DTT, pH 7.5 (buffer A), and loaded onto a 50-mL Q Sepharose HP column (Pharmacia-Amersham; 2.5 × 10 cm). The column was washed with buffer A until baseline absorbance was observed and then was washed with buffer A containing 200 mM NaCl. p38 was step-eluted using buffer A containing 300 mM NaCl. After diluting the p38 eluate 5-fold with buffer A, the protein was loaded onto a 50-mL Blue Sepharose column (Pharmacia-Amersham; 2.5 × 10 cm). The column was washed with buffer A until baseline absorbance, then eluted with a 0–1 M NaCl gradient over 10 column volumes. The main p38 fraction as judged by SDS–PAGE was concentrated in a Centrprep 10 concentrator (Amicon) and then loaded onto an 1800-mL Superdex 200 gel filtration column (Pharmacia-Amersham; 5 × 92 cm) equilibrated in 25 mM HEPES, 200 mM NaCl, and 1 mM DTT, pH 7.5. The protein eluted as a single peak and was judged to be >95% pure by densitometry scanned SDS gels, Edman sequencing, and electrospray ionization mass spectrometry. The purified p38 was aliquoted and stored at –80 °C.

The p38 protein (~10 mg/mL in 50 mM HEPES, pH 7.5, 300 mM NaCl, 1 mM DTT) was mixed with a 3-fold molar excess of inhibitor dissolved in DMSO. Crystals were grown by vapor diffusion by mixing the protein/inhibitor complex with an equal volume of well solution (30% PEG600, 250 mM NH<sub>4</sub>SO<sub>4</sub>, 100 mM HEPES, pH 7.5) and equilibrating at 20 °C. Crystals grew within 1 week. Prior to data collection, crystals were briefly soaked in a solution consisting of 25% glycerol, 30% PEG600, 250 mM NH<sub>4</sub>SO<sub>4</sub>, and 100 mM HEPES, pH 7.5.

Data were collected at –170 °C on an RAXIS II area detector using a Rigaku rotating anode X-ray generator and processed with DENZO and SCALEPAK.<sup>46</sup> The structures were solved by molecular replacement using Brookhaven Database coordinates files 1HCL and 1WFC as starting models for CDK2 and p38, respectively. Both structures were built using QUANTA<sup>47</sup> and refined with XPLOR 3.1<sup>48</sup> and CNS 0.5.<sup>49,50</sup> Data collection and refinement statistics are presented in Table 1. Coordinates have been deposited in the Protein Data Bank (CDK2, 1DI8; p38, 1DI9).

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## References

- (1) *Protein kinases*; Woodgett, J. R., Ed.; Oxford University Press: New York, 1994.
- (2) *The protein kinase factsbook: Protein-serine kinases*; Hardie, G., Hanks, S., Eds.; Academic Press Inc.: San Diego, CA, 1995.
- (3) *The protein kinase factsbook: Protein-tyrosine kinases*; Hardie, G., Hanks, S., Eds.; Academic Press Inc.: San Diego, CA, 1995.
- (4) Fry, D. W.; Kraker, A. J.; Connors, R. C.; Elliott, W. L.; Nelson, J. M.; Showalter, H. D.; Leopold, W. R. Strategies for the discovery of novel tyrosine kinase inhibitors with anticancer activity. *Anticancer Drug Des.* **1994**, *9*, 331–351.
- (5) Gray, N. S.; Wodicka, L.; Thunnissen, A.-M. W. H.; Norman, T. C.; Kwon, S.; Espinoza, F. H.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S.-H.; Lockhart, D. J.; Schultz, P. G. Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science* **1998**, *281*, 533–538.
- (6) Groundwater, P. M.; Solomons, K. R. H.; Drewe, J. A.; Munawar, M. A. Protein kinase inhibitors. *Prog. Med. Chem.* **1996**, *33*, 233–329.
- (7) Lawrence, D. S.; Niu, J. Protein kinase inhibitors: The tyrosine-specific protein kinases. *Pharmacol. Ther.* **1998**, *77*, 81–114.
- (8) Lee, J. C.; Adams, J. L. Inhibitors of serine/threonine kinases. *Curr. Opin. Biotechnol.* **1995**, *6*, 657–661.
- (9) Patrick, D. R.; Heimbrook, D. C. Protein kinase inhibitors for the treatment of cancer. *Drug Discov. Today* **1996**, *1*, 325–330.
- (10) Adams, J. L.; Lee, D. Recent progress towards the identification of selective inhibitors of serine/threonine kinases. *Curr. Opin. Drug Discov. Dev.* **1999**, *2*, 96–109.
- (11) Traxler, P. M. Protein tyrosine kinase inhibitors in cancer treatment. *Expert Opin. Ther. Pat.* **1997**, *7*, 571–588.
- (12) Myers, M. R.; He, W.; Hulme, C. Inhibitors of tyrosine kinases involved in inflammation and autoimmune disease. *Curr. Pharm. Des.* **1997**, *3*, 473–502.
- (13) Wei, L.; Hubbard, S. R.; Smith, R. F.; Ellis, L. Protein kinase superfamily -- comparisons of sequence data with three-dimensional structures. *Curr. Opin. Struct. Biol.* **1994**, *4*, 450–455.
- (14) Hanks, S. K. Eucaryotic protein kinases. *Curr. Opin. Struct. Biol.* **1991**, *1*, 369–383.
- (15) Wakeling, A. E.; Barker, A. J.; Davies, D. H.; Brown, D. S.; Green, L. R.; Cartledge, S. A.; Woodburn, J. R. Specific inhibition of epidermal growth factor receptor tyrosine kinase by 4-anilinoquinazolines. *Breast Cancer Res. Treat.* **1996**, *38*, 67–73.
- (16) Denny, W. A.; Rewcastle, G. W.; Bridges, A. J.; Fry, D. W.; Kraker, A. J. Structure–activity relationships for 4-anilinoquinazolines as potent inhibitors at the ATP binding site of the epidermal growth factor receptor in vitro. *Clin. Exp. Pharm. Physiol.* **1996**, *23*, 424–427.
- (17) 4-Anilinoquinazoline derivatives. *Expert Opin. Ther. Pat.* **1998**, *8*, 475–478.
- (18) Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambrosio, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. A specific inhibitor of the epidermal growth factor receptor. *Science* **1994**, *265*, 1093–1095.
- (19) Palmer, B. D.; Trumpp-Kallmeyer, S.; Fry, D. W.; Nelson, J. M.; Showalter, H. D. H.; Denny, W. A. Tyrosine kinase inhibitors. 11. Soluble analogues of pyrrolo- and pyrazoloquinazolines as epidermal growth factor receptor inhibitors: Synthesis, biological evaluation, and modeling of the mode of binding. *J. Med. Chem.* **1997**, *40*, 1519–1529.
- (20) Traxler, P.; Green, J.; Mett, H.; Sequin, U.; Furet, P. Use of a pharmacophore model for the design of EGFR tyrosine kinase inhibitors: Isoflavones and 3-phenyl-4(1H)-quinolones. *J. Med. Chem.* **1999**, *42*, 1018–1026.
- (21) Traxler, P. M.; Furet, P.; Mett, H.; Buchdunger, E.; Meyer, T.; Lydon, N. 4-(Phenylamino)pyrrolopyrimidines: potent and selective, ATP site directed inhibitors of the EGF-receptor protein tyrosine kinase. *J. Med. Chem.* **1996**, *39*, 2285–2292.
- (22) Morgan, D. O. Cyclin-dependent kinases: Engines, clocks, and microprocessors. *Annu. Rev. Cell Dev. Biol.* **1997**, *13*, 261–291.
- (23) New, L.; Han, J. The p38 MAP kinase pathway and its biological function. *Trends Cardiovasc. Med.* **1998**, *8*, 220–228.
- (24) Schulze-Gahmen, U.; De Bondt, H. L.; Kim, S.-H. High-resolution crystal structures of human cyclin-dependent kinase 2 with and without ATP: Bound waters and natural ligand as guides for inhibitor design. *J. Med. Chem.* **1996**, *39*, 4540–4546.
- (25) 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.
- (26) De Azevedo, W. F.; Leclerc, S.; Meijer, L.; Havlicek, L.; Strnad, M.; Kim, S.-H. Inhibition of cyclin-dependent kinases by purine analogues. Crystal structure of human cdk2 complexed with roscovitine. *Eur. J. Biochem.* **1997**, *243*, 518–526.
- (27) De Azevedo, W. F.; Mueller-Dieckmann, H.-J.; Schulze-Gahmen, U.; Worland, P. J.; Sausville, E.; Kim, S.-H. Structural basis for specificity and potency of a flavanoid inhibitor of human CDK2, a cell cycle kinase. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*.
- (28) Lawrie, A. M.; Noble, M. E. M.; 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.
- (29) Wilson, K. P.; McCaffrey, P. G.; Hsiao, K.; Pazhanisamy, S.; Galullo, V.; Bemis, G. W.; Fitzgibbon, M. J.; Caron, P. R.; Murcko, M. A.; Su, M. S. S. The structural basis for the specificity of pyridinylimidazole inhibitors of p38 MAP kinase. *Chem. Biol.* **1997**, *4*, 423–431.
- (30) Wilson, K. P.; Fitzgibbon, M. J.; Caron, P. R.; Griffith, J. P.; Chen, W.; McCaffrey, P. G.; Chambers, S. P.; Su, M. S.-S. Crystal structure of p38 mitogen-activated protein kinase. *J. Biol. Chem.* **1996**, *271*, 27696–27700.
- (31) Wang, Z.; Harkins, P. C.; Ulevitch, R. J.; Han, J.; Cobb, M. H.; Goldsmith, E. J. The structure of mitogen-activated protein kinase p38 at 2.1-Å resolution. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2327–2332.
- (32) Tong, L.; Pav, S.; White, D. M.; Rogers, S.; Crane, K. M.; Cywin, C. L.; Brown, M. L.; Pargellis, C. A. A highly specific inhibitor of human p38 MAP kinase binds in the ATP pocket. *Nat. Struct. Biol.* **1997**, *4*, 311–316.

- (33) Russo, A. A.; Jeffrey, P. D.; Pavletich, N. Structural basis of cyclin-dependent kinase activation by phosphorylation. *Nat. Struct. Biol.* **1996**, *3*, 696–700.
- (34) Jeffrey, P. D.; Russo, A. A.; Polyak, K.; Gibbs, E.; Hurwitz, J.; Massague, J.; Pavletich, N. Mechanism of CDK activation revealed by the structure of a cyclin A-CDK2 complex. *Nature* **1995**, *376*, 313–320.
- (35) Myers, M. R.; Setzer, N. N.; Spada, A. P.; Persons, P. E.; Ly, C. Q.; Maguire, M. P.; Zulli, A. L.; Cheney, D. L.; Zilberstein, A.; Johnson, S. E.; Franks, C. F.; Mitchell, K. J. The synthesis and SAR of new 4-(N-alkyl-N-phenyl)amino-6,7-dimethoxyquinazolines and 4-(N-alkyl-N-phenyl)aminopyrazolo[3,4-*d*]pyrimidines, inhibitors of CSF-1R tyrosine kinase activity. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 421–424.
- (36) Anderson, G. M.; Kollman, P. A.; Domelsmith, L. N.; Houk, K. N. Methoxy group nonplanarity in o-dimethoxybenzenes. Simple predictive models for conformations and rotational barriers in alkoxyaromatics. *J. Am. Chem. Soc.* **1979**, *101*, 2344–2352.
- (37) Thomas, K. A.; Smith, G. M.; Thomas, T. B.; Feldman, R. J. Electronic distributions within protein phenylalanine aromatic rings are reflected by three-dimensional oxygen atom environments. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 4843–4847.
- (38) Lamb, M. L.; Jorgensen, W. L. Investigations of neurotrophic inhibitors of FK506 binding protein via Monte Carlo simulations. *J. Med. Chem.* **1998**, *41*, 3928–3939.
- (39) Schulze-Gahmen, U.; Brandsen, J.; Jones, H. D.; Morgan, D. O.; Meijer, L.; Vesely, J.; Kim, S.-H. Crystal structures of cyclin-dependent protein kinase 2 in complex with ATP and two inhibitors, olomoucine and isopentenyladenine. *Proteins* **1995**, *22*, 378–391.
- (40) Burley, S. K.; Petsko, G. A. Weakly polar interactions in proteins. *Adv. Protein Chem.* **1988**, *39*, 125–189.
- (41) Wang, Z.; Canagarajah, B. J.; Boehm, J. C.; Kassisa, S.; Cobb, M. H.; Young, P. R.; Abdel-Meguid, S.; Adams, J. L.; Goldsmith, E. J. Structural basis of inhibitor selectivity in MAP kinases. *Structure* **1998**, *6*, 1117–1128.
- (42) Frantz, B.; Klatt, T.; Pang, M.; Parsons, J.; Rolando, A.; Williams, H.; Tocci, M. J.; O'Keefe, S. J.; O'Neill, E. A. The activation state of p38 mitogen-activated protein kinase determines the efficiency of ATP competition for pyridinylimidazole inhibitor binding. *Biochemistry* **1998**, *37*, 13846–13853.
- (43) Narla, R. K.; Liu, X.-P.; Myers, D. E.; Uckun, F. 4-(3'-Bromo-4'-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline: a novel quinazoline derivative with potent cytotoxic activity against human glioblastoma cells. *Clin. Cancer Res.* **1994**, *4*, 1405–1414.
- (44) Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Showalter, H. D.; Fry, D. W.; Kraker, A. J.; Denny, W. A. Tyrosine kinase inhibitors: 8. An unusually steep structure–activity relationship for analogues of 4-(3-bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a potent inhibitor of the epidermal growth factor receptor. *J. Med. Chem.* **1996**, *39*, 267–276.
- (45) Bourne, Y.; Watson, M. H.; Hickey, M. J.; Holmes, W.; Rocque, W.; Reed, S. I. Crystal structure and mutational analysis of the human CDK2 kinase complex with cell cycle-regulatory protein CksHs1. *Cell* **1996**, *84*, 863–874.
- (46) Otwinowski, Z. In *Oscillation data reduction program*; Sawyer, L., Isaacs, N., Burley, S., Eds.; CCP4 Study Weekend; SERC Daresbury Laboratory: Daresbury, U.K., 1993; pp 56–62.
- (47) *QUANTA User Guide*; Molecular Simulations: San Diego, CA, 1996.
- (48) Brunger, A. T. *XPLOR Version 3.1 Manual*; Yale University Press: New Haven, CT, 1992.
- (49) Brunger, A. T. The free *R* value: a novel statistical quantity for assessing the accuracy of crystal structures. *Nature* **1992**, *355*, 472–474.
- (50) Pannu, N. S.; Read, R. J. Improved structure refinement through maximum likelihood. *Acta Crystallogr.* **1996**, *A52*, 659–668.

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