A single amino acid substitution makes ERK2 susceptible to pyridinyl imidazole inhibitors of p38 MAP kinase

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Abstract

Mitogen-activated protein (MAP) kinases are serine/threonine kinases that mediate intracellular signal transduction pathways. Pyridinyl imidazole compounds block pro-inflammatory cytokine production and are specific p38 kinase inhibitors. ERK2 is related to p38 in sequence and structure, but is not inhibited by pyridinyl imidazole inhibitors. Crystal structures of two pyridinyl imidazoles complexed with p38 revealed these compounds bind in the ATP site. Mutagenesis data suggested a single residue difference at threonine 106 between p38 and other MAP kinases is sufficient to confer selectivity of pyridinyl imidazoles. We have changed the equivalent residue in human ERK2, Q105, into threonine and alanine, and substituted four additional ATP binding site residues. The single residue change Q105A in ERK2 enhances the binding of SB202190 at least 25,000-fold compared to wild-type ERK2. We report enzymatic analyses of wild-type ERK2 and the mutant proteins, and the crystal structure of a pyridinyl imidazole, SB203580, bound to an ERK2 pentamutant, I103L, Q105T, D106H, E109G, T110A. These ATP binding site substitutions induce low nanomolar sensitivity to pyridinyl imidazoles. Furthermore, we identified 5-iodotubercidin as a potent ERK2 inhibitor, which may help reveal the role of ERK2 in cell proliferation.

Keywords: asthma; ATP binding site; cancer; ERK2; iodotubercidin; MAP kinase; p38; pyridinyl imidazole

Mammalian mitogen-activated protein (MAP) kinases are serine/threonine kinases that mediate intracellular signal transduction pathways (Cobb & Goldsmith, 1995; Davis, 1995). Members of the MAP kinase family share sequence similarity and conserved structural domains, and include the ERK, JNK, and p38 kinases. JNKs and p38 kinases are activated in response to the pro-inflammatory cytokines TNF- α and interleukin-1, and by cellular stress such as heat shock, hyperosmolarity, ultraviolet radiation, lipopolysaccharides, and inhibitors of protein synthesis (Derijard et al., 1994; Han

et al., 1994; Raingeaud et al., 1995; Shapiro & Dinarello, 1995). In contrast, ERKs are activated by mitogens and growth factors (Boke-meyer et al., 1996).

ERK2 is a widely distributed protein kinase that achieves maximum activity when both Thr183 and Tyr185 are phosphorylated by the upstream MAP kinase kinase, MEK1 (Anderson et al., 1990; Crews et al., 1992). Upon activation, ERK2 phosphorylates many regulatory proteins, including the protein kinases Rsk90 (Bjorbaek et al., 1995) and MAPKAP2 (Rouse et al., 1994), and transcription factors such as ATF2 (Raingeaud et al., 1996), Elk-1 (Raingeaud et al., 1996), c-Fos (Chen et al., 1993), and c-Myc (Oliver et al., 1995). ERK2 is also a downstream target of the Ras/Raf-dependent pathways (Moodie et al., 1993), and may help relay the signals from these potentially oncogenic proteins. ERK2 has been shown to play a role in the negative growth control of breast cancer cells (Frey & Mulder, 1997), and hyperexpression of ERK2 in human breast cancer has been reported (Sivaraman et al., 1997). Activated ERK2 has also been implicated in the proliferation of endothelin-stimulated airway smooth muscle cells, suggesting a role for this kinase in asthma (Whelchel et al., 1997).

The crystal structures of unphosphorylated p38 (Wilson et al., 1996; Wang et al., 1997) (Brookhaven PDB entry, 1WFC) and ERK2 (Zhang et al., 1994) (Brookhaven PDB entry, 1ERK) have been solved. Recently, a phosphorylated ERK2 crystal structure

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Abbreviations: ATF2, activating transcription factor 2; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; E-64, 1-(1-*trans*-epoxysuccinyl-L-leucylamino)-4-guanidinobutane; EGFR, epidermal growth factor receptor; ERK, extracellular-signal regulated kinase; ERK2(5X), ERK2 pentamutant I103L, Q105T, D106H, E109G, T110A; IL, interleukin; Ito, 5-iodotubercidin; JNK, Jun N-terminal kinase; LDH, lactate dehydrogenase; LPS, lipopolysaccharides; MAP, mitogen-activated protein; MAPKAP2, MAPK-activated protein kinase-2; β -ME, β -mercaptoethanol; MKK, MAP kinase kinase; PCR, polymerase chain reaction; PEGMME2000, polyethylene glycol 2000 monomethyl ether; PEP, phosphoenol pyruvate; PMSF, phenylmethylsulfonyl fluoride; PK, pyruvate kinase; RSK90, 90 kDa ribosomal s6 kinase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TNF- α , tumor necrosis factor- α .

has also been solved (Canagarajah et al., 1997). The fold and topology of ERK2 are similar to p38 (Wilson et al., 1996), and the two proteins are 48% identical in amino acid sequence.

p38 was identified as a kinase that was phosphorylated on tyrosine following stimulation of monocytes by LPS (Lee et al., 1994). p38 kinase was cloned (Han et al., 1994) and shown to be the target for pyridinyl imidazole compounds that block the production of IL-1 β and TNF- α by monocytes stimulated with LPS (Lee et al., 1994). SB203580, a 2,4,5-triarylimidazole (Fig. 1A), is a potent p38 kinase inhibitor that is selective relative to other kinases, including other closely related MAP kinases such as the p38 γ isoform (Cuenda et al., 1995, 1997; Wilson et al., 1997). The structure of SB203580 in complex with p38 has been reported (Tong et al., 1997). The crystal structure of a different pyridinyl imidazole compound, VK-19,911, 4-(4-fluorophenyl)-1-(4-piperidinyl)-5-(4-pyridyl)-imidazole (Fig. 1A) in complex with p38 (Fig. 2B) has also been described (Wilson et al., 1997). These structures identified the residues important for binding pyridinyl imidazoles (Fig. 2B). Many of these residues are conserved in ERK2 (Fig. 1B), but there are enough differences that binding of pyridinyl imidazole compounds does not occur. Based on the structures and sequence differences, we have generated a series of ERK2 kinase mutants with unchanged catalytic properties that are sensitive to pyridinyl imidazole compounds. We report the crystal structure of an ERK2 pentamutant, I103L, Q105T, D106H, E109G, T110A, bound

to SB203580 (Fig. 2C), along with enzymatic analyses of the wild-type and mutant proteins. We find that a single amino acid substitution, Q105T or Q105A, is sufficient to make ERK2 susceptible to pyridinyl imidazole inhibitors of p38 MAP kinase. We also show that 5-iodotubercidin is an excellent inhibitor of ERK2 and the ATP-binding site mutants. The data presented here should help improve our understanding of MAPK signal transduction pathways and aid in the design of specific MAP kinase inhibitors.

Results and discussion

Members of the MAP kinase family share conserved structural domains and a high degree of sequence similarity, particularly in the ATP binding site (Fig. 1B). Pyridinyl imidazole compounds are potent and specific p38 kinase inhibitors and two crystal structures of different pyridinyl imidazoles bound to the ATP site of p38 have been solved (Tong et al., 1997; Wilson et al., 1997). Comparison of the ERK2/ATP (Fig. 2A) and p38/VK-19,911 (Fig. 2B) crystal structures identified T106 of p38 as an important residue for the specificity of this class of compounds (Wilson et al., 1997). These observations suggested that the ATP-binding site of ERK2 could be modified to mimic p38 kinase. To test this hypothesis, we used site-directed mutagenesis to change the equivalent residue in ERK2, glutamine 105, into threonine (Q105T) and alanine (Q105A). Four other nonconserved residues in the ATP site of p38 were also changed to make a pentamutant, ERK2(5X), I103L, Q105T, D106H, E109G, T110A (Fig. 1B).

Fig. 1. A: Chemical structures of ERK2 and p38 inhibitors (1) 5-iodotubercidin, (2) VK-19,911, (3) SB203580, and (4) SB202190. **B:** Sequence alignment of ERK2 and p38 between residues 100–114 (human ERK2 numbering). ERK2 mutations are shown in bold.

Inhibition of ERK2, ERK2 mutants, and p38 kinase by pyridinyl imidazoles

Quantification of the yields of purified phosphorylated ERK2 and ERK2 mutants indicates that the latter are phosphorylated in vitro as efficiently as wild-type enzyme by MEK1(DD). The kinase activity of the ERK2 mutants are comparable to wild-type enzyme (Fig. 4). However, ERK2(Q105T) shows a 640- to 2,500-fold increased binding affinity for the three pyridinyl imidazoles tested (Table 1), using a lower limit of 20 μ M for wild-type ERK2 inhibition. ERK2(Q105A) is even more sensitive to this compound class, exhibiting 1,800- to 25,000-fold increased binding (Table 1). Mutation of residues, I103L, D106H, E109G, T110A, in addition to Q105T, produced an enzyme most sensitive to the pyridinyl imidazoles, ranging from 6.9 nM for VK-19,911 to 0.4 nM for SB202190. The K_i values correspond to a 2,900- to 50,000-fold increase in potency of binding of these compounds. These results indicate that the larger glutamine side chain at residue 105 accounts for the resistance of ERK2 to pyridinyl imidazoles.

Interactions of SB203580 and ERK2(5X)

The crystal structure reveals the interactions that lead to potent binding of the pyridinyl imidazole compound, SB203580, with residues in the ATP site of ERK2(5X) (Fig. 2C). The para-fluorophenyl ring of SB203580 is shielded from solvent and is within favorable van der Waals distance (<4.5 Å) of the carbon atoms of eight ERK2 side chains; V37, A50, K52, I82, I84, L101, and T105 (Fig. 2C). Comparing this structure with that of wild-type ERK2/ATP (Fig. 2A) shows that the larger glutamine side chain at position 105 in the wild-type protein would prohibit binding of SB203580 by blocking access to the pocket filled by the

Table 1. K_M for ATP-binding, inhibition constants for Itu, and pyridinyl imidazole inhibition of ERK2, ERK2 mutants, and p38 kinase

Enzyme	K_M for ATP (μ M)	Inhibition constants, K_i (nM)			
		Itu	VK-19,911	SB203580	SB202190
ERK2(wild-type)	76 $\pm$ 14	525 $\pm$ 46	^a Nil	Nil	Nil
ERK2(Q105A)	51 $\pm$ 6	385 $\pm$ 111	10.8 $\pm$ 2.4	1.2 $\pm$ 0.3	0.81 $\pm$ 0.19
ERK2(Q105T)	33 $\pm$ 4	686 $\pm$ 59	31.0 $\pm$ 4.3	13.0 $\pm$ 3.6	6.8 $\pm$ 0.6
ERK2(5X)	26 $\pm$ 2	638 $\pm$ 192	6.9 $\pm$ 1.7	0.76 $\pm$ 0.14	0.4 $\pm$ 0.04
p38	260 $\pm$ 30	Nil	60 $\pm$ 20	100 $\pm$ 30	30 $\pm$ 8

^aNil indicates no inhibition at 20 μ M.

para-fluorophenyl ring. Additional contacts are made between the pyridine ring and V39, A52, I84, L106, M108, and L156, while the four-substituted phenyl ring of SB203580 contacts only L156 and C166. The interactions of the methane-sulfonyl group are more extensive, and this group is nearby to D167, N154, S153, and K151. The imidazole ring contacts V39, K54, L156, and C166, and appears to assist in binding by positioning the three substituents. Despite the high binding affinity, only one hydrogen bond is made between SB203580 and ERK2(5X) (Fig. 2C). A comparison of the interactions of SB203580 with ERK2(5X) (Fig. 2C) to those between VK-19,911 and p38 (Fig. 2B) shows that the two compounds bind to the different proteins in a similar fashion.

Active site titration of ERK2(5X) with the pyridinyl imidazole SB202190

Active site titrations are valuable for assessing enzyme integrity. SB202190 is a potent inhibitor of ERK2(5X) and is an ideal titrant for this enzyme. Figure 3 shows that 50 nM phosphorylated ERK2(5X) is completely inhibited by 45 nM SB202190. Addition of equimolar unphosphorylated ERK2(5X) causes the intercept to shift to 106 nM, indicating that SB202190 binds to only one high affinity site per enzyme, and binds to both unphosphorylated and phosphorylated enzyme with similar affinities. In agreement, mixing 25 nM of each enzyme form causes the initial velocity to decrease by 50%, but the intercept remains at 50 nM inhibitor.

Substrate recognition

The ATF2 transcription factor is a good substrate for p38 kinase and is rapidly phosphorylated (Fig. 4, lane 6). In contrast, at similar concentrations the wild-type and mutant ERK2 proteins phosphorylate ATF2 weakly (Fig. 4, lanes 2–5). The structures of ERK2 and p38 kinase show that the substituted residues are unlikely to contact the protein substrate, so no change in protein substrate specificity is expected. The K_M for ATP binding to ERK2 is similar for the wild-type and mutant proteins (Table 1) with two exceptions. Substitution of the Q105 side chain with alanine caused a marginal decrease in K_M for ATP binding (Table 1), and the Q105T mutation caused a 2.5-fold decrease in K_M for ATP. The Thr105 side chain in the ERK2 mutants may interact favorably with ATP, as it does with inhibitor.

Inhibition of ERK2 by 5-iodotubercidin

Itu is a potent inhibitor of adenosine kinase (K_i = 30 nM) (Newby et al., 1983; Cottam et al., 1993) and Ser/Thr-specific kinases such

as casein kinases 1 and 2, and the insulin receptor kinase fragment with IC₅₀s ranging from 0.4 to 28 μ M (Massillon et al., 1994). We have found that Itu is a potent, competitive inhibitor of ERK2, exhibiting a K_i of 525 nM. Thus, Itu may help probe the role of ERK2 hyperexpression in cell proliferation. This represents the most potent ERK2 inhibitor published to date, and is the first reported observation that Itu inhibits a MAP kinase. In contrast, Itu does not inhibit p38 kinase up to 20 μ M (Table 1). The similar K_i values observed for Itu inhibition of ERK2 and the ERK2 mutants suggest the mutated residues play little or no part in Itu binding.

Conclusion

In summary, the essential role of Q105 in conferring specificity against pyridinyl imidazole binding to ERK2 is clearly demonstrated. The single amino acid substitution Q105T renders ERK2 susceptible to pyridinyl imidazole inhibitors, and introduction of a small side chain in Q105A causes a further 3- to 10-fold improvement in pyridinyl imidazole binding. The latter represents a 25,000-fold increase in SB202190 binding compared to wild-type ERK2. Substitution of four other residues in the ATP site slightly improves ERK2 inhibition by the pyridinyl imidazoles (up to 50,000-fold).

Materials and methods

Cloning, mutagenesis, expression of kinases

Plasmid pG-MEK1glu containing cDNA for a constitutively active mutant of mouse MEK1 (S218D, S222D) (Huang & Erikson, 1994) was obtained from Dr. R.L. Erikson (Harvard University), and pET-BS-(His)₆-MEK1 was constructed for bacterial expression of N-terminally (His)₆-tagged MEK1 (DD). A human ERK2 cDNA was cloned into pT7Blue (Novagen, Madison, Wisconsin) by reverse transcription and subsequent PCR of total RNA prepared from peripheral lymphocytes, and a (His)₆ affinity tag and a thrombin cleavage site were introduced at the N-terminus of the translation product for bacterial expression via pET-ERK2. To facilitate construction of ERK2 mutants, a silent mutation was introduced into the ERK2 cDNA that provided a new *Hind*III restriction site near the region of mutations. This ERK2 variant (ERK2-HIII) and several ERK2 mutants were generated by PCR using PT7Blue-ERK2 as template, a forward primer containing an internal *Sac*II site and reverse primers containing a *Hind*III site, and one or several mutated nucleotides. Each of these *Sac*II-*Hind*III PCR fragments was ligated with a *Hind*III-*Msc*I PCR fragment into *Sac*II-*Msc*I double-

digested pT7Blue-ERK2 to yield subclones for the ERK2-HIII variant, and the ERK2(Q105T), ERK2(Q105A), and ERK2(5X) mutants. These were used to isolate *SacII-XhoI* ERK2 cDNA frag-

ments that were ligated into the appropriate restriction sites of pET-ERK2 for bacterial expression of (His)₆-tagged recombinant proteins.

Fig. 2. See figure caption on facing page.

Fig. 3. Active site titration of SB202190 binding to ERK2(5X). (A) 50 nM phosphorylated ERK2(5X) (closed circles), (B) 50 nM unphosphorylated ERK2(5X) (open circles), and (C) 25 nM unphosphorylated ERK2(5X) (closed squares). Enzyme was incubated with sub-stoichiometric SB202190 for 15 min at 30 °C, and assayed for residual kinase activity.

Purification of MEK1(DD), ERK2, and ERK2 mutants

E. coli cells were resuspended in lysis buffer (50 mM HEPES, pH 7.8, 10% glycerol (v/v), 250 mM NaCl, 5 mM β -ME, 5 mM imidazole, 0.1 mM PMSF, 2 μ g/mL pepstatin, 1 μ g/mL E-64, and leupeptin). Cells were disrupted using a French press and centrifuged at $35,000 \times g$ for 60 min. The supernatant was incubated overnight with 1 mL Talon resin (Clontech, Palo Alto, California)/5 mg protein. The resin was washed with 20 column volumes (cv) of lysis buffer, and 20 cv of wash buffer (lysis buffer at pH 7.5, with 10 mM imidazole). Protein was eluted in 3 cv with wash buffer adjusted to pH 8.0 and 100 mM imidazole. SDS-PAGE gels were used to identify MEK1(DD) fractions, which were concentrated by ultrafiltration. MEK1(DD) was loaded onto a Superdex-75 column (60 $\times$ 1.6 cm, Pharmacia, Uppsala, Sweden) equilibrated with 20 mM HEPES, pH 7.5, 10% glycerol, 100 mM NaCl, and 2 mM DTT at 1 mL/min. ERK2 kinases were affinity purified as described for MEK1(DD), then diluted to 25 mM NaCl with 20 mM HEPES, pH 8.0, 10% glycerol, and 2 mM DTT (buffer A), and loaded onto a MonoQ (HR 5/5) anion-exchange column equil-

Fig. 4. Substrate specificity of ERK2, ERK2 mutants, and p38 kinase measured as 32 P incorporation into ATF2. Wild-type ERK2 and the ERK2 mutants were phosphorylated by the constitutively active mutant of MEK1 in vitro and purified as described in Materials and methods. In vitro phosphorylation of p38 MAP kinase by the constitutively active mutant of MKK6 was performed as described (Wilson et al., 1997). Reaction conditions for ATF2 phosphorylation are presented in Materials and methods.

ibrated in buffer A. After washing with 5% buffer B (buffer A + 1 M NaCl), ERK2 proteins were eluted in a 5–20% buffer B gradient developed over 60 min at 0.5 mL/min.

In vitro phosphorylation of ERK2 proteins

ERK2 was diluted to 0.5 mg/mL in 50 mM HEPES, pH 8.0, 10% glycerol, 100 mM NaCl, 2 mM DTT, 10 mM $MgCl_2$. Activation was initiated by addition of 2.5 mM ATP and a 1/25 molar ratio of MEK1(DD) for 1 h at 25 °C. Activated ERK2 proteins were diluted to 25 mM NaCl and purified by anion-exchange as described.

Kinase assays

A coupled spectrophotometric assay was used in which ADP generated by ERK2 or p38 kinase was converted to ATP by PK with

Fig. 2 (on facing page). **A:** Stereo drawing of the ERK2 active site. The refined model of the ERK2/ATP complex in the region of the ATP binding site is displayed. Some side chains in contact with ATP are identified by their one-letter code and sequence position. Carbon atoms are colored tan, nitrogen blue, oxygen red, and sulfur yellow. ATP is displayed in licorice sized bonds. Hydrogen bonds between ATP and enzyme are highlighted by dashed lines. Water molecules in the vicinity of the ligand, and residues 30–34 of the phosphate anchor, were omitted for clarity. **B:** Stereo drawing of the p38 active site. The refined model of the VK-19,911 binding site in p38 is displayed. Some side chains in contact with the inhibitor are identified by their one-letter code and by sequence position. Carbon atoms are colored tan, nitrogen blue, oxygen red, sulfur yellow, and fluorine green. The inhibitor is displayed in licorice sized bonds. Hydrogen bonds between the inhibitor and enzyme are shown as dashed lines. The distance between the amide-nitrogen of M109 and the four-position nitrogen of the pyridine ring is 2.98 Å. Water molecules in the vicinity of the ligand were omitted for clarity. **C:** Stereo drawing of the ERK2(5X)/SB203580 complex. The refined model of ERK2 in the region of the SB203580 binding site is displayed. Some side chains in contact with the inhibitor are identified by their one-letter code and by sequence position. Carbon atoms are colored tan, nitrogen blue, oxygen red, sulfur yellow, and fluorine green. The inhibitor is displayed in licorice sized bonds. The residues mutated in ERK2(5X), I103L, Q105T, D106H, E109G, T110A are colored orange and displayed in licorice sized bonds. The view is approximately the same as in Figures 2A and 2B. The distance between the amide-nitrogen of M108 and the four-position nitrogen of the pyridine ring is 2.94 Å. Larger side chains at position 105, like Gln in ERK2 and Met in JNK and p38 γ , block pyridinyl imidazole binding by occupying the space filled by the para-fluorophenyl ring.

the concomitant production of pyruvate from PEP. LDH reduces pyruvate to lactate with the oxidation of NADH. NADH depletion was monitored at 340 nm using a microplate reader for 20 min at 30 °C. Reactions were in 100 mM HEPES, pH 7.6, 10 mM MgCl₂, and started by addition of 100 μ M ATP. PK (100 μ g/mL), LDH (50 μ g/mL), PEP (2 mM), and NADH (140 μ M) were added in large excess. Addition of 200 μ M KRELVEPLTPSGEAPNQALLR substrate, corresponding to an EGF receptor peptide (Gonzalez et al., 1991), allowed measurement of kinase activity. In K_i determinations, E + I was preincubated for 15 min at 30 °C prior to assay by addition of ATP. Inhibition constants were determined by fitting kinetic data to the Morrison tight-binding equation (Morrison & Walsh, 1988) using KineTic (BioKin, Madison, Wisconsin). ³²P incorporation into ATF2 (0.1 mg/mL) by 7.5 nM phosphorylated kinase was assayed for 10 min at 30 °C in 50 mM HEPES, pH 7, 10 mM MgCl₂, and 2 mM DTT. Reactions were stopped by addition of SDS-PAGE buffer and run on 4–20% Precast gels (Novex, San Diego, California), and visualized by autoradiography.

Crystallization and structure determination of the ERK2(5X)/SB203580 complex

Crystals of unphosphorylated ERK2(5X) were grown by vapor diffusion when protein (14 mg/mL in 20 mM Tris, pH 7.0, 5 mM DTT, 200 mM NaCl) was mixed with reservoir (100 mM HEPES, pH 7.2, 28–30% (v/v) PEGMME2000, 200 mM (NH₄)₂SO₄, 20 mM β -ME) at a equal volume ratio of protein solution to reservoir and allowed to stand at room temperature. Prior to X-ray data collection at –169 °C, a single crystal was equilibrated for 48 h in 100 mM HEPES pH 7.0, 200 mM (NH₄)₂SO₄, 28% PEGMME2000, 5% glycerol, 2% DMSO, and 1 mM SB203580.

X-ray data were collected on an Raxis IIC image plate and processed and scaled using DENZO and SCALEPACK (Otwinowski & Minor, 1996). The crystals had space group symmetry P2₁, with unit cell dimensions $a = 48.6$ Å, $b = 69.7$ Å, $c = 60.3$ Å, and $b = 109.25$. R -merge for the data was 3.2%, with $I/\sigma(I) = 8.9$ at 1.95 Å resolution. The X-ray data comprised 26,737 unique reflections with $|F| > \sigma(F)$ derived from 69,783 intensity measurements. The data were 96.7% complete overall, and 83.2% complete in the 2.01–1.95 Å resolution shell. X-ray coordinates of unphosphorylated ERK2 were used to construct a model for the refinement of the inhibited ERK2(5X) complex. All thermal factors were set to 20.0 Å². The R -factor after the rigid body and positional refinement was 30% for 10–2.4 Å data. The resolution of the maps and model was gradually increased to 2.0 Å resolution by cycles of model building, positional refinement, and thermal factor refinement, interspersed with torsional dynamics runs. XPLOR was used for model refinement (Brünger, 1992). Our current ERK2(5X) model in complex with SB203580 contains 334 protein residues, 283 water molecules, one sulfate molecule, and one inhibitor molecule, and has an R -factor of 21.3% (R -free = 28.6%) versus all data with $|F| > \sigma(F)$ between 6–2.0 Å resolution (23,621 reflections). PROCHECK and XPLOR were used to analyze the model stereochemistry. Ninety percent of the ERK2 residues were located in the most favored region of the phi-psi plot, and 11% in the additional allowed regions. Deviations from ideal bond lengths and angles were 0.009 Å and 1.5°, respectively, and other indications of stereochemistry were average or better than average for a structure determined at 2.0 Å resolution. No electron density was

observed for ERK2(5X) amino acids 1–13, 31–33, and 328–335, so these residues were not included in the model. The coordinates of the ERK2(5X)/SB203580 complex have been deposited in the Protein Data Bank, accession number (1PME).

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