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Structural insights into IKKβ inhibition by natural products staurosporine and quercetin

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ABSTRACT

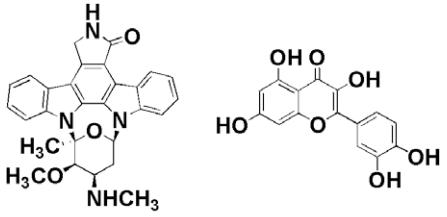
This Letter describes the results of two combined approaches: homology modeling and molecular docking studies, in order to propose the molecular basis of IKKβ inhibition by staurosporine and quercetin as ATP-competitive inhibitors. The results provides a rationale and structural frameworks for designing potent ATP binding-site inhibitors of IKKβ, which is an attractive drug target for inflammatory diseases and has been found to be responsible for some of the already observed pharmacological effects for marketed drugs.

The nuclear transcription factor NF-κB has a central role in the autoimmune, inflammatory and destructive mechanisms that drive the progression of diseases such as rheumatoid arthritis and cancer.[1] Normally, NF-κB is held in an inactive state in the cytoplasm by IκB inhibitory proteins. In response to specific external stimuli, including tumor necrosis factor-α (TNF) and interleukin-1, the IκB component of the complex is phosphorylated and degraded, resulting in the translocation of NF-κB into the nucleus and induction of gene transcription.[1] The signal-induced phosphorylation of IκB involves two IκB kinases, IKKα and IKKβ.[2]

Various studies indicate that IKKβ plays the dominant role in the proinflammatory signal-induced phosphorylation of the IκB protein. On the other hand, IKKα is dispensable for these functions, but is essential for developing the epidermis and its derivatives.[3] IKKβ has been found to be responsible for some of the observed anti-inflammatory properties of marketed drugs such as aspirin and salicylates.[4]

A large number of natural compounds have been suggested to interfere with the cascade leading to NF-κB activation and gene transcription.[5] Staurosporine **1** (Fig. 1), a microbial alkaloid that was isolated from *Streptomyces staurosporeus*,[6] and quercetin **2** (Fig. 1), a flavonoid that occurs in many fruits and vegetables,[7] have shown anti-inflammatory responses as observed in various animal models, including experimental arthritis and colitis.[8] These pharmacological effects are consistent with the strong inhibition of IKKβ by staurosporine (IC_{50} = 1.6 μM, *Ki* = 172 nM) and quercetin (IC_{50} = 4 μM), reported by Peet and Li in 1999.[9]

These studies revealed that staurosporine **1** (Fig. 1) is an ATP-competitive inhibitor of IKKβ whereas quercetin **2** (Fig. 1) showed a mixed inhibition mechanism towards ATP. Also, they have shown



(1) Staurosporine (2) Quercetin

Figure 1. Chemical structures of the natural products staurosporine and quercetin.

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that the binding site of quercetin overlaps with both ATP and IκBα binding sites.[9]

However, the structural basis of IKKβ inhibition by staurosporine and quercetin has not been established yet, and the proposal of their putative binding modes would be of great value to the comprehension of their mechanism of action and would provide information for the further design of bioactive molecules. A crystal structure of IKKβ is not available yet. However, it is possible to obtain experimental structural data from related kinases.

In this Letter, we describe the results of two combined approaches, homology modeling and molecular docking studies, of the ATP and IKKβ inhibitors, staurosporine and quercetin. Homology modeling studies of IKKβ have been described in the literature for the design of new inhibitors. However, in these studies the metal ions have not been included in the ATP binding-site[10a,b] and/or the ATP phosphate groups were considered fully protonated,[10a] in spite to be well known that these groups are fully ionized at biophase.[10c] Two Mg^{2+} ions are present in the phosphate binding site of related kinases and this region is conserved throughout the family. A few studies have reported that the Mg^{2+} ions are coordinated with ATP phosphate groups, suggesting the importance of inclusion of these ions in theoretical calculations.[11]

For the construction of the 3D homology model of IKKβ the catalytic domain sequence was collected from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) (code AAC51860.1).[12] The sequence similarity search of IKKβ catalytic domain was performed using the BLAST tool, available in Swiss-Model.[13] The serine/threonine protein Chk2 (30% identical in the kinase domain to IKKβ) was chosen as the template because it has received the highest BLAST scores among the template sequences in the database, and also for its good resolution, 2.25 Å. The crystal structure of Chk2 in complex with ADP was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/) (PDB code 2CN5).[14] The 3D model was constructed with the First Approach Mode of the Swiss-Model server[15] which includes model generation with ProModII and energy minimization with GROMOS96.[16] The coordinates of the metals and coordination waters were added manually in the pdb file. The structural analysis was carried out with the Ramanchandran Plot on Swiss-PDB Viewer software[17] version 4.0.1 and Procheck version 3.5.4 on the Structural Analysis and Verification Server (http://nihserver.mbi.ucla.edu/SAVES/).

The ChemScore fitness function in GOLD docking software,[18] version 3.1.0 was used to score the docked compounds because of its ability to predict with good accuracy the binding modes of known ligands, being particularly sensitive to metal parameters. [18] The active site was defined as all atoms within 10 Å from Asn150, which was used as the reference residue. This is conserved throughout the kinase family and is found to be coordinated with Mg^{2+} in the phosphate binding site.[11a] The metals' coordination geometries were assigned as octahedral and the water orientation was set to *Spin*. Under GOLD software, consecutive dockings using the same input files will not give identical solutions or fitness scores, and the reason for this is related to the way in which GOLD determines solutions. In order to solve this problem, five consecutive dockings using the same input files were carried out and the best ranking energy value from each run was collected. The binding energy was obtained as a mean of these values. The protein-ligand complex with the most favorable free energy of binding (ΔG_{bind}) among the top scored complexes in the five runs was used for further visual inspection.

The structures of ATP, staurosporine and quercetin were extracted from the crystal structures of the kinases GCN2 (PDB code 1ZYD),[19] CDK2 (PDB code 1AQ1),[20] HCK (PDB code 2HCK),[21] respectively. The atomic charges were assigned with the semi-empirical method AM1[22] using MOPAC in SYBYL 8.0.[23] ATP formal charges were assigned as −4.

The structural analysis of the homology model with Procheck showed 85.3% of the residues in the most favorable geometric regions and the Ramachandran Plot showed only two non-glycine residues outside the most favored regions in comparison to the plot of Chk2 (see Supplementary data for further details).[24] Comparison of our homology model for IKKβ and the template Chk2 showed a RMS deviation of 0.11 Å for Cα atoms. Analysis of both structures revealed their structural similarity.

The interaction of ATP with IKKβ catalytic domain obtained in the docking study is illustrated in Figure 2.

The molecular docking simulation of ATP suggested slightly distorted octahedral coordination geometry of $Mg^{+2}(1)$ and six oxygen atoms (Mg···O = 1.6–2.6 Å), one from each of the β- and γ-phosphates, one from each of protein kinase-conserved Asn150 (catalytic loop) and Asp166 and two from well ordered water molecules. The distance from the $Mg^{+2}(2)$ ion to β-phosphate group was greater than 3.5 Å, which is too long for direct coordination. It is possible that the metal ion binds in a fully hydrated form and that water molecules mediate the metal coordination. The coordination environment is similar to other known kinases (Fig. 2).[11]

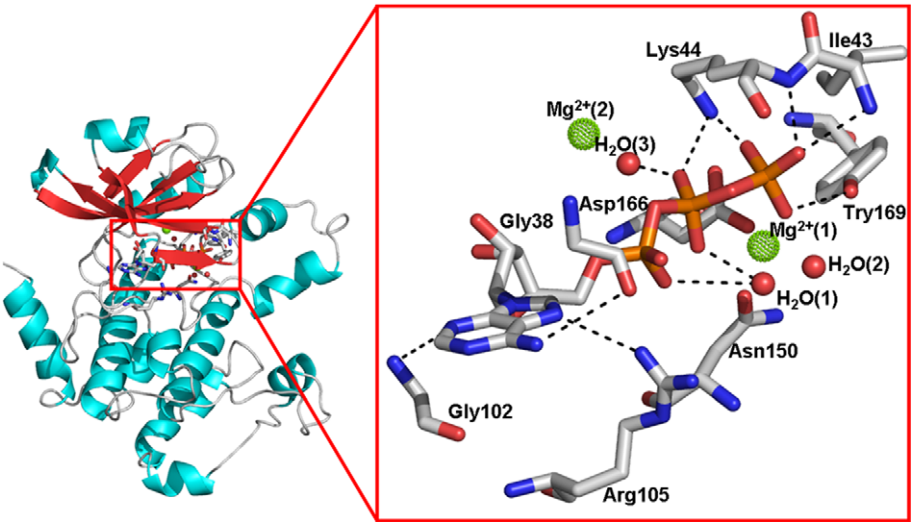

Figure 2. The putative binding mode of ATP with IKKβ. The hydrogen atoms were removed for visualization facility. The figure was generated by Pymol.[25]

The role of the conserved Lys44 for IKKβ activity has been described. Mercurio et al.,[2] have previously shown that IKKβ with a mutation in Lys44 failed to activate NF-kB. In our docking results Lys44 participated on ATP recognition through H-bonding with the oxygen of β- and γ-phosphates. Other residues interacting with the ATP phosphate groups are Ile43 and Tyr169, and there are also coordinating waters. Additionally, H-bonding interactions of the adenosine ring of ATP with residues Gly38, Gly102 and Arg105 are also present. The calculated ΔG_{bind} was −25.41 kJ/mol (Fig. 2).[24]

The docking analysis was used herein to predict binding affinities and gain structural insight into staurosporine and quercetin binding modes, as ATP-site inhibitors. The binding energies are correlated with the experimental activities of these compounds, which suggest that ChemScore function was able to correctly estimate the relative binding affinities (Table 1).

Staurosporine contains very few rotatable bonds. However, in the docking simulation of this compound in the ATP binding site, two different poses have been observed in the molecular ensemble (Fig. 3A and B). In the first one, the lactam and methylamine groups interact within the ATP binding site (Fig. 3A). The lactam ring hydrogen bonds to residues Glu149 and Asn150; and methylamine with Cys59. Additionally, the oxygen atom of the tetrahydropyran ring is within H-bonding distance to Cys46 (S···O = 1.64 Å).

Asn150 is almost completely conserved throughout the kinase family and is found to be coordinated with Mg^{2+} in the phosphate binding site.[11a] The molecular interaction with this residue was explored as a strategy to improve the activity of IKKβ inhibitors and can explain the inhibitory activity of staurosporine.[26]

Molecular interaction with the residue Glu149 is frequent with known inhibitors in molecular docking simulations,[10a] supporting its importance for IKKβ inhibition. Also, the distance between the lactam oxygen and $Mg^{2+}(1)$ (Mg···O = 2.58 Å) suggests an ion-dipole interaction (Fig. 3A).

The second binding mode observed for staurosporine and IKKβ in the ATP binding site shows a shift of the lactam ring towards Gly102, in an H-bonding interaction that has also been observed for the adenosine ring of ATP.

The superposition of staurosporine and ATP in the first binding mode (Fig. 3C) almost complete site occupancy, blocking ATP binding. Nevertheless, the superposition of staurosporine with ATP in the second binding mode (Fig. 3D) shows that the lactam ring is in the same position as the adenosine ring of ATP, which is in accordance to what has been reported for other kinases by Lawrie et al.[20]

The analysis of the binding mode of quercetin (Fig. 4A) showed H-bonding interactions with Glu39, Gly102, and with a coordination water $H_20(1)$. Gly102 participates on ATP recognition by IKKβ and the structures of quercetin and ATP superimposed in the ATP binding site show a similar binding mode. An additional ion-dipole interaction between one of the oxygens of the catechol ring of quercetin and $Mg^{+2}(1)$ (Mg···O = 2.50 Å) has been observed (Fig. 4A).

These observations suggest that quercetin binding may overlap with the ATP binding site, explaining the inhibitory activity of this compound (Fig. 4B).

The combination of homology modeling and molecular docking helped us gain further insight into binding of ATP with IKKβ since the inclusion of metal ions showed a different binding mode from what has been reported previously.[10]

Also, we have shown that the inhibitory activity of staurosporine and quercetin towards IKKβ may be explained based principally on H-bonding interactions and ATP binding-site occupancy by these compounds. Finally, these studies provided information for the further design of bioactive molecules based on interactions

Table 1
Docking energies and experimental activities of staurosporine and quercetin

Compounds	ΔG_{bind}[a] (kJ/mol)	IC_{50}[b] (μM)
Staurosporine	−46.07	1.6
Quercetin	−30.32	4.0

[a] Values are the mean of five consecutive dockings using ChemScore function available in GOLD software version 3.1.0. Please see Supplementary data for further details.[24]

[b] IC_{50} values were obtained from Ref. 9.

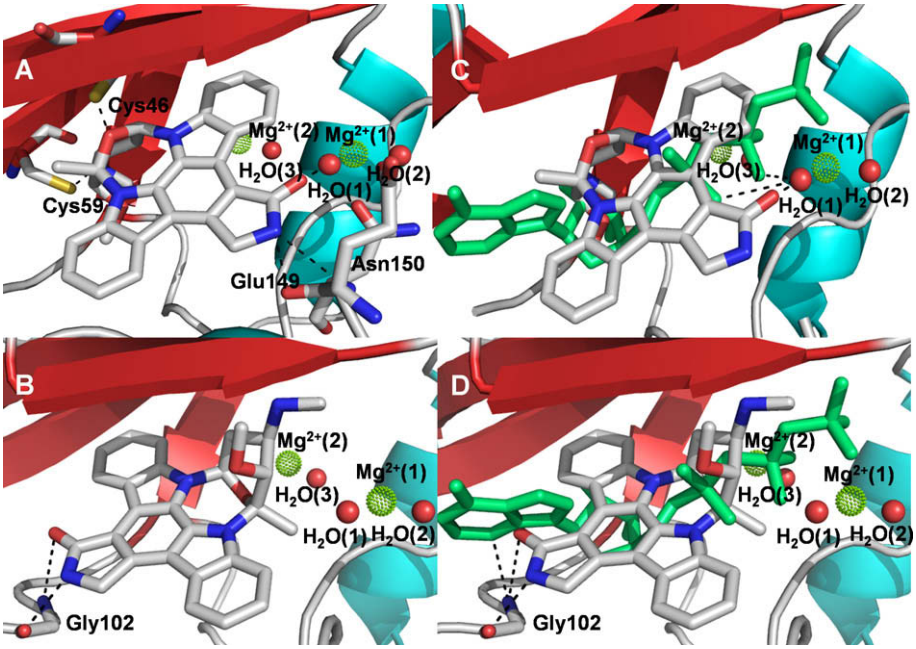

Figure 3. (A, B) The top scored binding modes of staurosporine (color by element) in IKKβ site. (C, D) Superposition of ATP (color green) and staurosporine (color by element) in both binding modes in IKKβ site. The hydrogen atoms were removed for visualization facility. The figure was generated by Pymol.[25]

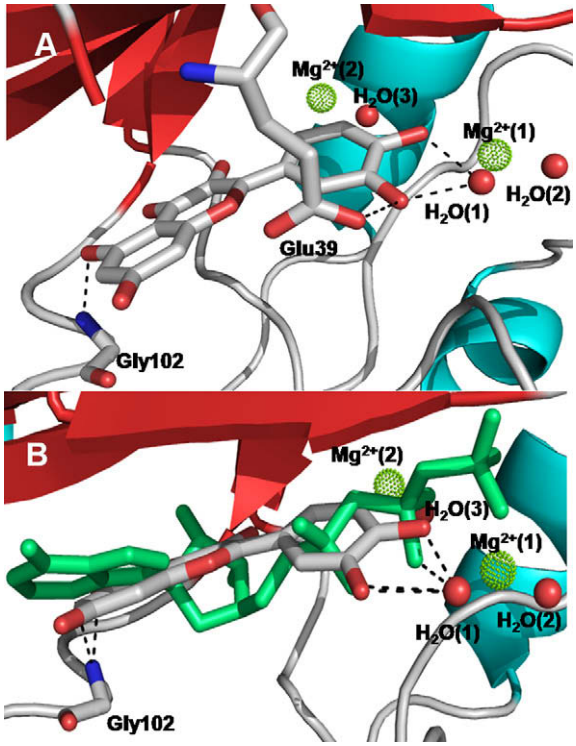

Figure 4. (A) The binding model of quercetin (color by element) in IKKβ site. (B) Superposition of ATP (color green) and quercetin (color by element). The hydrogen atoms were removed for visualization facility. The figure was generated by Pymol.[25]

performed by these natural products in the ATP binding-site of IKKβ.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.076.

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