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Structure-based *de novo* design and synthesis of aminothiazole-based p38 MAP kinase inhibitors

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ABSTRACT

p38 mitogen-activated protein kinase (MAPK) is a promising target for the development of therapeutics for various immunological diseases. We designed and synthesized aminothiazole-based p38 MAPK inhibitors of with IC_{50} values ranging from 0.1 to 2 μM by means of the structure-based *de novo* design of phenyl-(2-phenylamino-thiazol-5-yl)-methanone scaffold. Because these newly identified inhibitors were also screened for having desirable physicochemical properties as a drug candidate, they deserve consideration for further investigation as anti-inflammatory drugs. Structural features relevant to the stabilization of the newly identified inhibitors in the ATP-binding site of p38 MAPK are discussed in detail.

p38 mitogen-activated protein kinase (MAPK) plays a key role in the regulation of inflammatory processes, and is responsible for the pathogenesis of various inflammatory diseases including rheumatoid arthritis (RA), multiple sclerosis, and neuropathic pain.[1] Actually, a high-level expression of cyclooxygenase-2 and interleukin-6 (IL-6) in the synovial cell of RA stems from the activation of p38 MAPK by macrophage migration inhibitory factor, which is a key regulatory cytokine in innate and adaptive immune responses.[2] It was also demonstrated that the inhibition of p38 MAPK had the effect of decreasing the production of tumor necrosis factor-α and IL-1, which culminated in the suppression of various inflammatory diseases.[3,4] Further persuasive evidence was provided from clinical trials to support that the inhibition of p38 MAPK activity would be an effective therapeutic strategy for the treatment of RA.[2] p38 MAPK has thus served as a promising target for the development of therapeutics for various immunological diseases.[5]

Accordingly, a great deal of efforts has been devoted to the discovery of p38 MAPK inhibitors. Since the discovery of a pyridinylimidazole-based inhibitor,[6] potent and structurally diverse p38 MAPK inhibitors have been reported including diaryl pyrazole,[7] fused pyrazole,[8] dibenzeno[*a*,*d*]cycloheptenone,[9] *N*-aryl pyridinone,[10,11] pyridopyridazin-6-one,[12] methylphenylpyridazin-3-one,[13] dibenzosuberone,[14] and pyrimidinylisoxazole[15] moieties as a key structural element. A rational fragment-based design approach was also applied using structural information on p38 MAPK.[16]

A few years ago, we identified novel classes of p38 MAPK inhibitors based on the virtual screening with docking simulations between p38 MAPK and putative inhibitors.[17] The most potent inhibitor found in this study was (4-amino-2-phenylamino-thiazol-5-yl)-phenylmethanone (**1**). We undertake a structure-based *de novo* design of aminothiazole **1** as the molecular core using the modified scoring function. Even with 3D structure of the target protein, the results of *de novo* design have not always been successful due to the imperfections in the scoring function to compute the binding free energy between the target protein and a putative ligand.[18] This inaccuracy can be attributed in a large part to the underestimation of ligand solvation effects in protein–ligand binding, which leads to the overestimation of the binding affinity of a ligand with polar moieties.[19] Therefore, to enhance the efficiency of *de novo* design, we modified the scoring function by the addition of a proper solvation free energy term in the scoring function. This augmentation of the scoring function makes it possible to calculate the desolvation cost for a ligand to be bound to the receptor protein. On the basis of the results for docking simulations with the improved scoring function, we will also address the binding forces that are responsible for stabilization of the newly identified inhibitors in the ATP-binding site of p38 MAPK.

3-D atomic coordinates in the X-ray crystal structure of p38α MAPK in complex with a potent inhibitor (PDB code: 3FMK) were

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used as the receptor model in the *de novo* design for our structure-activity relationship (SAR) study. The structure-guided design of new p38 MAPK inhibitors was performed in three steps. First, the structural modifications were made to the initial p38 MAPK inhibitor (**1**) to obtain the better scaffold from which a number of new potent inhibitors could be derivatized. This led to the identification of phenyl-(2-phenylamino-thiazol-5-yl)-methanone (**2**) as a promising scaffold for designing new p38 MAPK inhibitors.

In the second step, various derivatives of **2** were generated based on the calculated binding mode of **2** in the ATP-binding site of p38 MAPK. This started with the structural analysis of the ATP-binding pocket using the POCKET module of the LigBuilder program.[20] The structure of p38 MAPK in complex with **2** obtained from docking simulations with the AutoDock program[21] served as the input to find the key interaction residues in the ATP-binding site. To generate various derivatives of **2** as the candidates for a potent inhibitor, the proper chemical moieties at the substitution positions were selected with the genetic algorithm. To score and rank the generated derivatives, we used the empirical scoring function suggested by Wang et al. that included van der Waals, hydrogen bond, electrostatic, and entropic terms.[22] To reduce the computational burden, only the generated derivatives that could satisfy the bioavailability rules as a drug candidate[23] were selected for further analysis. Based on the filtration criteria, we obtained 2259 derivatives that were estimated to have higher inhibitory activity against p38 MAPK than **2**.

Although the effects of ligand solvation have been shown to be critically important in protein–ligand association,[19] the current scoring function of the LigBuilder program lacks a solvation term. In the third step of *de novo* design, therefore, the derivatives of **2** generated with LigBuilder were further screened with a new binding free energy function constructed by combining an appropriate solvation free energy term to the original scoring function of the AutoDock program. This modified scoring function can be expressed as follows:[24]

$$\Delta G_b^{aq} = W_{vdW}\sum_{i=1}\sum_{j=1}\left(\frac{A_{ij}}{r_{ij}^{12}}-\frac{B_{ij}}{r_{ij}^{6}}\right) + W_{hbond}\sum_{i=1}\sum_{j=1}E(t)\left(\frac{C_{ij}}{r_{ij}^{12}}-\frac{D_{ij}}{r_{ij}^{10}}\right) + W_{elec}\sum_{i=1}\sum_{j=1}\frac{q_i q_j}{\varepsilon(r_{ij})r_{ij}} + W_{tor}N_{tor} + \sum_{i=1}S_i\left(O_i^{\max}-\sum_{j\neq i}V_j e^{-\frac{r_{ij}^2}{2\sigma^2}}\right) \quad (1)$$

In the calculation of the molecular solvation free energies of the derivatives of **2**, we used the atomic parameters developed by Park because they proved to be useful for estimating the solvation free energies of various organic molecules in SAMPL4 blind prediction challenge.[25] This modification of the scoring function seems to have an effect of raising the probability of finding the actual p38 MAPK inhibitors because the overestimation of the binding affinity of a polar group can be avoided effectively. Indeed, the superiority of this modified scoring function to the previous one was well-appreciated in recent studies for virtual screening of kinase inhibitors.[26]

Although **1** is a submicromolar p38 MAPK inhibitor, it seems to be a poor molecular core to serve as a starting point of drug discovery due to the presence of an amino moiety on the central thiazole ring. This functional group is actually responsible for high desolvation cost by facilitating the formation of multiple hydrogen bonds with water molecules,[25] which would have the effect of limiting the inhibitory activity of a candidate inhibitor derived from **1**. Therefore, we decided to make some structural modifications of **1** to obtain the better inhibitor scaffold than **1** with similar biochemical potency. This could be made possible by the structure-based *de novo* design with two constraints. First, we selected only the structures similar to **1** with Tanimoto coefficient higher than 8.0 to ensure tight binding in the ATP binding site of p38 MAPK. The output structures were then screened further for having the lower ΔG_b^{aq} values than **1** to select the new scaffolds with the increased binding affinity.

Due to the two severe constraints imposed in the *de novo* design, we were able to find only one structure (**2**) that was anticipated to be a promising scaffold from which new potent p38 MAPK inhibitors could be derivatized. As can be seen in Figure 1, it differs from **1** only in the lack of $-NH_2$ moiety on the central thiazole ring. The calculated binding free energy and solvation free energy (ΔG^{sol}) of **2** are shown in Table 1 in comparison with those of **1**. Keeping it in mind that the binding free energy of a protein–ligand complex in aqueous solution (ΔG_b^{aq}) can be approximated as the difference between that in the gas phase (ΔG_b^{gas}) and the ΔG^{sol} value of ligand, we computed the two energy components separately to estimate their relative contributions to ΔG_b^{aq}. Judging from the lower ΔG_b^{gas} value of **1** than **2**, the former seems to bind more tightly in the ATP-binding site of p38 MAPK than the latter. However, the biochemical potency of **1** seems to be limited by the relatively high desolvation cost ($-\Delta G^{sol}$) for binding to p38 MAPK. As a consequence, **1** and **2** are predicted to be almost equally potent p38 MAPK inhibitors. This result exemplifies the necessity of the solvation free energy term in the scoring function to estimate the relative inhibitory activities. The significant contribution of ΔG^{sol} to ΔG_b^{aq} also indicates that in order to enhance the potency of a p38 MAPK inhibitor with structural modifications, the resulting increase in the strength of enzyme-inhibitor interactions should be sufficient to surmount the increased stabilization in aqueous solution. In the *de novo* design to find new potent p38 MAPK inhibitors, we selected **2** as the molecular core instead of **1** because the former had the more chance for derivatization than the latter due to the structural simplicity.

Using the binding free energy function in Eq. 1, the derivatives of **2** generated with LigBuilder were rescored according to the binding affinity in the ATP-binding site of p38 MAPK. Top-ranked derivatives (100 compounds) were then selected as virtual hits, which were inspected for synthetic availabilities. Finally, twenty derivatives of **2** were synthesized and tested for inhibitory activity against p38 MAPK.[27] The general synthetic route for the preparation of substituted aminothiazole derivatives is shown in Scheme 1.

To address the possibility of tight binding of **2** in the ATP-binding site, we carried out docking simulations between p38 MAPK and **2** using the modified scoring function shown in Eq. 1.

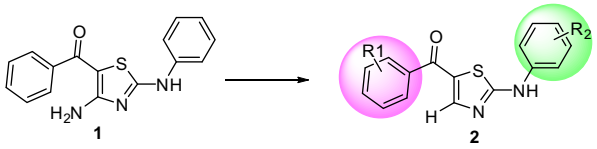

Figure 1. Structural modifications of **1** to find the better inhibitor scaffold for *de novo* design.

Table 1
Calculated ΔG_b^{gas}, ΔG^{sol}, and ΔG_b^{aq} values of **2** in comparison with those of **1**. Each energy value is given in kcal/mol

Scaffold	ΔG_b^{gas}	ΔG^{sol}	ΔG_b^{aq}
1	−24.8	−13.7	−11.1
2	−21.4	−10.1	−11.3