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Crystal structure of human ERK2 complexed with a pyrazolo[3,4-*c*]pyridazine derivative

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Abstract—A series of pyrazolopyridazine compounds were briefly investigated as ERK2 inhibitors. The crystal structure of ERK2 complexed with an allyl derivative was determined. The compound induces structural change including movement of the glycine-rich loop and peptide flip between Met108-Glu109. As a result, the newly formed subsite can recognize small hydrophobic substituents but not hydrophilic ones.

Extracellular signal-regulated kinase 2 (ERK2, E.C. 2.7.1.37) is a member of the Ras/Raf/MEK1(or MEK2)/ERK1(or ERK2) mitogen-activated protein kinase (MAPK) signaling pathways. These pathways contribute to the coordination and regulation of cell growth and differentiation in response to extracellular stimulation.[1] They function as an integral step in the formation, progression, and survival of tumors, in addition to being intracellular mediators in signaling aberrations in many inflammatory processes.[2] Based on experimental evidence, the MAPK signaling pathways represent an attractive target for pharmacological intervention in proliferative and inflammatory diseases.[3]

Recently, we discovered that a 1*H*-pyrazolo[3,4-*c*]pyridazine derivative **1** acted as a potent ERK2 inhibitor that dose-dependently depressed AP-1-dependent transcription induced by TGFβ1, as an inflammatory mediator.[4] Compound **1** showed ATP-competitive inhibition in a Lineweaver–Burk analysis and was the first compound with high selectivity toward many other kinases. Among known ERK2 inhibitors such as olomoucine and 5-indotubercidin, which have low selectivity. The compound is useful as a tool to elucidate the roles of ERK in a variety of cellular events. X-ray analysis[4] confirmed that it bound to the ATP binding site of the enzyme with a novel binding mode. The phenyl moiety and the pyrazolopyridazine moiety bind to the bottom of the active site, which is structurally rigid. On the other hand, the pyrazolopyridazine moiety binds to the entrance region of the active site, which consists of structurally flexible loops.

Kinases, including ERK2, have a common architecture, and contain an ATP binding site consisting of flexible loops known as a glycine-rich loop and an activation loop. The glycine-rich loop has various conformations according to the binding of different inhibitors. The flexibility allows recognition of a variety of structural compounds. The glycine-rich loop in the **1**/ERK2 complex is different from those in the previously reported structures.[5]

For a brief investigation on the structural flexibility of the glycine-rich loop, compounds **2**–**4**, with various substituents at the 1-position of the pyrazolopyridazine ring, were synthesized.[6] The respective inhibitory activities[7] are shown in Table 1.

Compounds **2** and **3** have inhibitory activity higher than that of **1**, although activity reduction would be expected due to the steric hindrance between these compounds and the protein (Fig. 1). The substituents at the 1H position would obstruct the Cδ atom of Ile31 and the carbonyl group of Met108 if the structure in the

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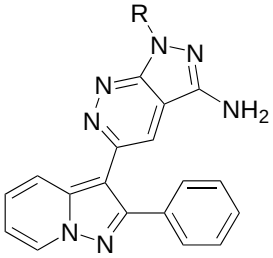
Table 1. Classical complement inhibition for compounds **1**–**4**



Compound	R	IC_{50} (μM)
1	H	1.9
2	Me	1.4
3	$CH_2CH{=}CH_2$	0.56
4	CH_2CH_2OH	5.1

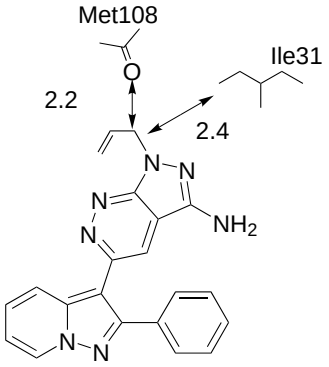


Figure 1. Steric hindrance when there is no structural change. Distances are shown in Å. The model of compound **3** was calculated based upon the coordinates of compound **1** in the **1**/ERK2 complex and was superposed onto the ERK2 protein in the **1**/ERK2 complex.

1/ERK2 complex were rigid. That is, conformational change of the protein may occur. Alternatively, a structural difference may be brought about by the interaction mode.

The crystal structure of the **3**/ERK2 complex[8] reveals that **3** binds to the protein in the same manner as **1**, except around the allyl moiety (Fig. 2). All interactions of the **1**/ERK2 complex are conserved in the **3**/ERK2 complex. The amino group forms a hydrogen bond with the carbonyl of Asp106 and the amide carbonyl of Gln105. The N2 atom of the pyrazolopyridazine ring interacts with the NH of Met108. This interaction is important for the recognition of various inhibitors by their respective kinases.[9] The N2 atom of the pyrazolopyridine ring interacts with the Lys54. The phenyl group binds in the hydrophobic subsite consisting of Val39, Ile103 and the methylene chains of Lys54 and Gln105. These portions of the protein are rigid in the **1**/ERK2 and **3**/ERK2 complexes. On the other hand, the allyl group fits well into the newly formed hydrophobic subsite consisting of Leu107, Leu156, and the peptide backbone of Glu109 and Thr110 (Fig. 3). The methylene part of the allyl group has a hydrophobic interaction with Leu107. The vinyl group also has a hydrophobic interaction with Leu156 and has van der Waals contacts with the peptide backbone of Glu109 and Thr110.

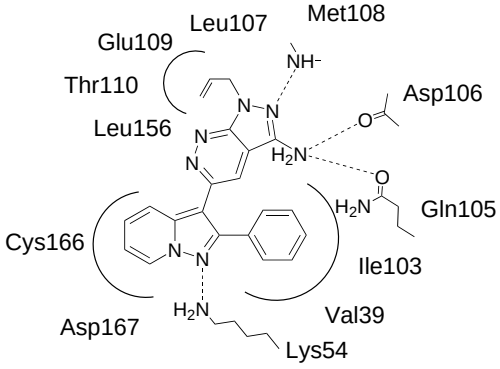


Figure 2. Interaction pattern of the X-ray structure.

As a result, the allyl group of the inhibitor moves the Ile31 residue and the carbonyl group of Met108 away from this position in the **1**/ERK2 complex (Fig. 4). The Cα atom of Ile31 in the glycine-rich loop is pushed out from the position it occupies in **1**/ERK2 by 2.0 Å. The whole of the glycine-rich loop is moved out with the Ile31, but this movement has no influence on the molecular recognition pattern. The carbonyl group of Met108 is directed to the opposite side of the allyl group, but the Cα atoms of both ends of the peptide bond are fixed in the two complexes. That is, the peptide flip between Met108 and Glu109 occurs as observed in p38 mitogen-activated protein kinase complexed with a quinazolinone inhibitor.[10] Furthermore, the peptide flip in the p38 kinase occurs between Met109 and Gly110, and the large residue makes the peptide flip energetically unfavorable, based on the mutational investigations at Gly110. In the case of the **3**/ERK2 complex, the peptide flip occurs even though the peptide consists of large residues. Overall, two disadvantageous factors in the allyl group binding are settled by this motion. This structural

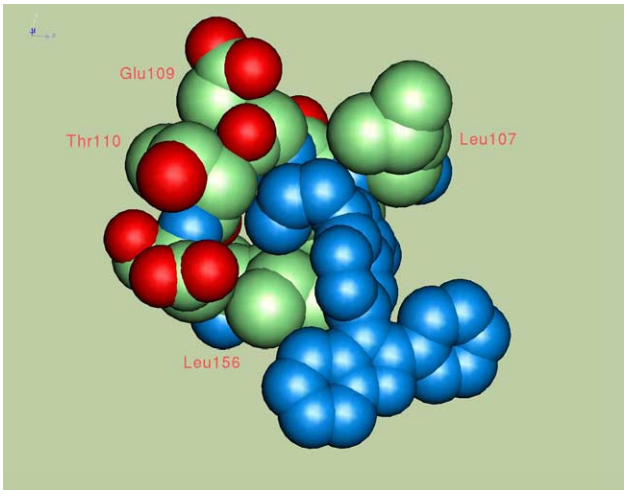


Figure 3. Structure around the allyl group in the **3**/ERK2 complex (van der Waals sphere models).