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Identification of allosteric ERK2 inhibitors through in silico biased screening and competitive binding assay

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ABSTRACT

Extracellular signal-regulated kinase 2 (ERK2) is a drug target for type 2 diabetes mellitus. A peptide-type ERK2 inhibitor (PEP) was discovered in the previous study through the knowledge-based method and showed physiological effects on the *db*/*db* mice model of type 2 diabetes. Here, the crystal structure showed that PEP bound to the allosteric site without the interruption of the ATP competitive inhibitor binding to ERK2. An in silico biased-screening using the focused library rendered three compounds with inhibitory activity of IC_{50} <100 μM. Among them, two compounds revealed the concentration-dependent competition with PEP and could be lead compounds for antidiabetic medicine.

Extracellular signal-regulated kinase 2 (ERK2) controls vital physiological processes via phosphorylation of various proteins including downstream kinases and transcription factors.[1] ERK2 is a drug target for miscellaneous diseases such as cancers and inflammation disorders.[2] ATP competitive inhibitors have been discovered for these diseases to date. FR180204 showed anti-inflammatory effects in cell-functional assays.[3] SCH772984 induced the in vivo tumor regression in xenograft models.[4] The National Cancer Institute drug dictionary declared that GDC-0994 and BVD-523 are currently in clinical trials for cancers in the USA.[5]

A text-mining exploration showed that the disruption of ERK2 phosphorylation of hepatic signal transducer and activator of transcription 3 (STAT3) was efficacious against type 2 diabetes mellitus.[6] At that time, we planned to develop allosteric inhibitors instead of ATP competitive ERK2 inhibitors, based on the unique substrate recognition mechanism. ERK2 anchors substrates such as STAT3 via a consensus sequence of $Lys(Arg)_{2-3}$-X_{1-6}-Φ_a-X-Φ_b (Φ: hydrophobic amino acid, X: any amino acid) at the allosteric site[7] which is distinct from the phosphorylation site recognizing the Ser(Thr)-Pro sequence of the substrate (Fig. 1a). The peptide inhibitor (PEP) with the amino acid sequence of KKYILAL had been designed to interfere with this trap on the basis of STAT3 sequence, and exerted effects in vitro and in vivo.[6] PEP had a significant effect on the *db*/*db* mice, a standard animal model for type 2 diabetes.[6] Most peptide inhibitors are available for biological experiments but have disadvantageous pharmacokinetic properties. Therefore, we planned to exploit the non-peptide type small molecule inhibitor based on the structural information.

The crystal structural analysis[8] at 2.98 Å resolution depicts the PEP binding region adjacent to the ATP binding site of ERK2 in which an ATP analog inhibitor, 5′-iodotubercidin (5-IOD), binds (Fig. 1a). Hydrophobic residues of the peptide inhibitor are located in the shallow pocket configured by the hydrophobic residues Leu106, His116, Phe120, Leu148, and Cys152 (Fig. 1b). The positive-charged lysine residues of the peptide inhibitor were located in the negative charge pool involving Glu72, Asp153, Asp308, and Asp311 (Fig. 1b). The peptides from MAP kinase phosphatase 3 (MKP3),[9] MAP kinase kinase 2 (MAP2K2),[10] ribosomal protein S6 kinase (S6K),[11] and MAP kinase signal-integrating kinase 1 (MNK1),[11] as well as PEP, bind to ERK2 at the hydrophobic pocket and negative charge pool, although the binding conformations of these peptides vary. The MKP3 and MAP2K2 peptides and PEP configured a β-strand, while the S6K and MNK1 peptides had a helical conformation when binding to ERK2. Namely, the peptide-binding allosteric site is biologically significant for ERK2. Therefore, in silico screening in regard to the allosteric site is likely to be a noteworthy approach to discovering new chemical entities as ERK2 inhibitors despite of the fact that the shallow allosteric site is rather disadvantageous for in silico screening.

Abbreviations: ERK2, extra-cellular signal-regulated kinase 2; STAT3, hepatic signal transducer and activator of transcription 3; 5-IOD, 5′-iodotubercidin; PEP, designed peptide inhibitor; pNA, p-nitroaniline.

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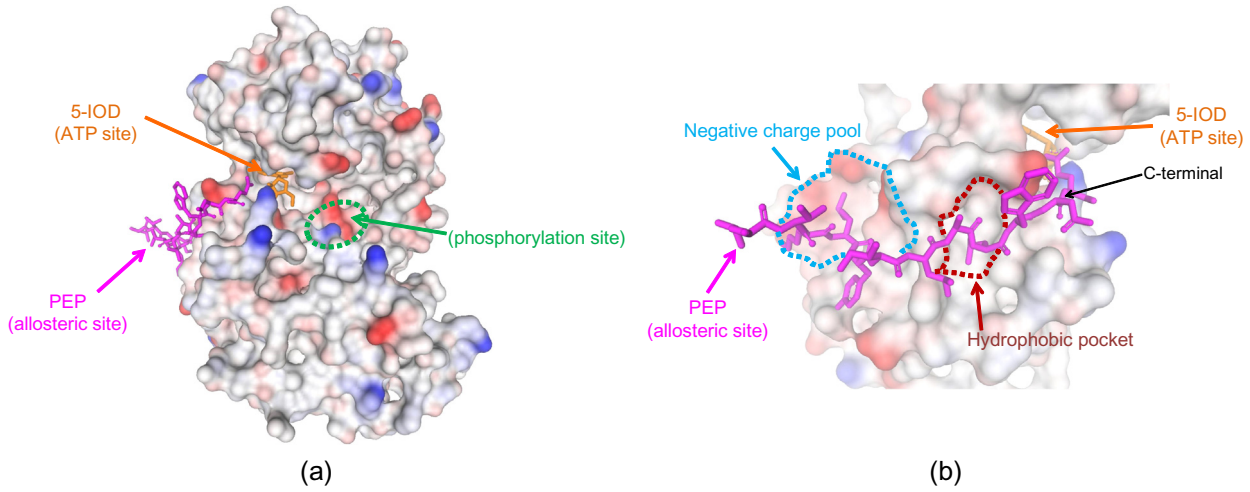

Figure 1. Crystal structure of the ternary complex of ERK2, 5′-iodotubercidin (5-IOD), and peptide-type allosteric inhibitor (PEP). (a) Overall structure; (b) a closed-up view around the peptide inhibitor binding region.

Table 1
Inhibitory activities and ligand efficacies of ERK2 inhibitors

	PEP[a]	5-IOD[b]	In silico Hit-1	In silico Hit-2	In silico Hit-3
IC_{50} (μM)	9.8	1.2	11	82	47
LE[c]	0.11	0.41	0.21	0.19	0.17

[a] Designed peptide inhibitor;
[b] 5′-Iodotubercidin;
[c] Ligand efficacy of the inhibitors.[20]

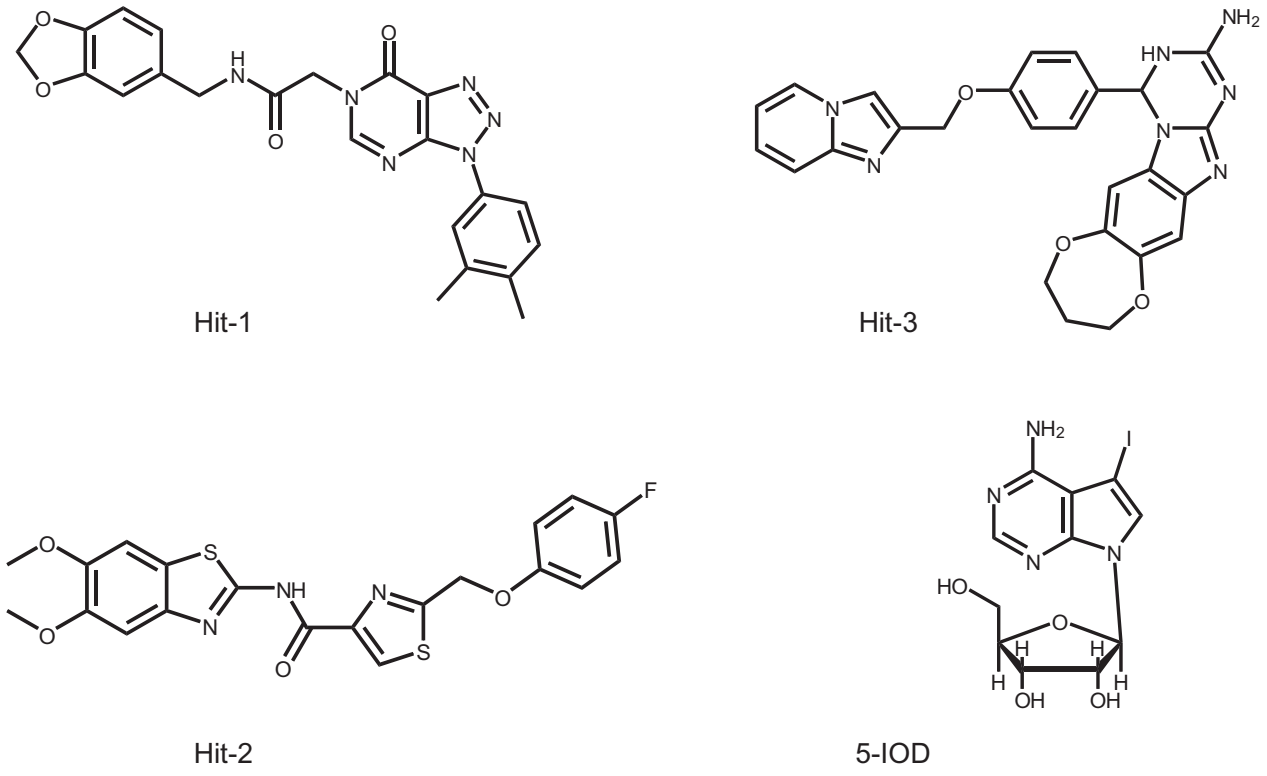

Figure 2. Chemical structures of in silico hit compounds and 5-IOD.

A lot of the ATP site binders are probably contained in the commercial-based chemical libraries. Actually, a non-biased preliminary screening conferred the several ATP binders although they also docked into the allosteric site. Before in silico screening, to preferentially obtain allosteric binders we prepared a focused library derived from the ZINC drug-like library (release 8, June