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Fragment-based discovery of focal adhesion kinase inhibitors

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ABSTRACT

Chemically diverse fragment hits of focal adhesion kinase (FAK) were discovered by surface plasmon resonance (SPR) screening of our in-house fragment library. Site specific binding of the primary hits was confirmed in a competition setup using a high-affinity ATP-site inhibitor of FAK. Protein crystallography revealed the binding mode of 41 out of 48 selected fragment hits within the ATP-site. Structural comparison of the fragment binding modes with a DFG-out inhibitor of FAK initiated first synthetic follow-up optimization leading to improved binding affinity.

The nonreceptor tyrosine kinase FAK (EC 2.7.10.2) is composed by an N-terminal FERM domain (Four-point-one, ezrin, radixin, moesin), which regulates the enzymatic activity of its C-terminal kinase domain (KD).[1,2] Direct interaction of the FERM domain with the kinase C-lobe occludes the ATP-binding site and maintains an auto-inhibited conformation also by protecting the activation loop from phosphorylation by Src kinase. The auto-inhibitory state can be released upon interaction with FERM binding partners such as integrins and growth factors, underlining the important role of FAK in integrating diverse cellular signalling pathways. Disruption of the FERM–KD interaction leads to auto-phosphorylation of Tyr397 in the linker region and exposure of the activation loop. This is followed by binding of Src and phosphorylation of Tyr576 and Tyr577 within the FAK activation loop resulting in full catalytic activation of the enzyme.[3,4] Apart from playing a key role in regulation of normal cellular activities such as adhesion, migration and survival, FAK is also involved in cancer cell invasion, metastasis and survival. Therefore, FAK has been reported as attractive target for oncology and small molecule inhibitors are already in clinical phase-1 testing.[5–7]

Fragment-based lead discovery (FBLD) is considered as valuable technology within the pharmaceutical industry and has been reported to deliver lead series for a variety of drug targets.[8–10] FBLD uses a diverse library ($\sim 10^3$ molecules) of small molecules with MW typically below 250 Da for screening in contrast to high-throughput screening of large libraries ($\sim 10^6$ molecules). Fragments allow the sampling of a greater portion of chemical diversity and exploration of more binding motifs within the target. Even weak binding interactions of fragments to the protein target can be detected by applying high compound concentrations in biophysical methods such as NMR, surface plasmon resonance (SPR), or X-ray crystallography.[8–10]

In our FBLD campaign, we screened a library of 1920 fragments against the immobilized kinase domain of FAK using SPR (Fig. 1a).[11] Fragments were screened at a fixed concentration of 2 mM to remove 'sticky' and other bad behaving compounds.[12] At such high fragment concentrations, 80% of the fragments showed binding to the FAK surface. Therefore, the remaining fragments were tested again at 2 mM but in the presence of FAK-inhibitor **I** (Fig. 1b), which has been published by Roberts et al. at Pfizer (PF-562,271) as high-affinity ATP-competitive binder (IC_{50} = 1.5 nM).[13] The number of 'false-positive' hits (e.g., unspecific binders) could be dramatically decreased by using the FAK-inhibitor **I** for SPR competition experiments and fragments binding specifically within the ATP-pocket were identified. As a result, 180 fragments with a corresponding reduction in their binding levels in the presence of the competitor were selected as primary hits. The primary fragment hits were evaluated in further detail by SPR in titration series of 11 concentrations (1.95 μM to 2.0 mM). Steady state dissociation constants ($K_{D,ss}$) were determined by plotting the binding levels at the end of the association phase against the concentration (see Supplementary Fig. 3S for details) and then fitting the data to a single-site binding isotherm.

The affinity determination by SPR confirmed 105 hits with $K_{D,ss}$ values in the range of 13 μM to 3.5 mM, which were considered as

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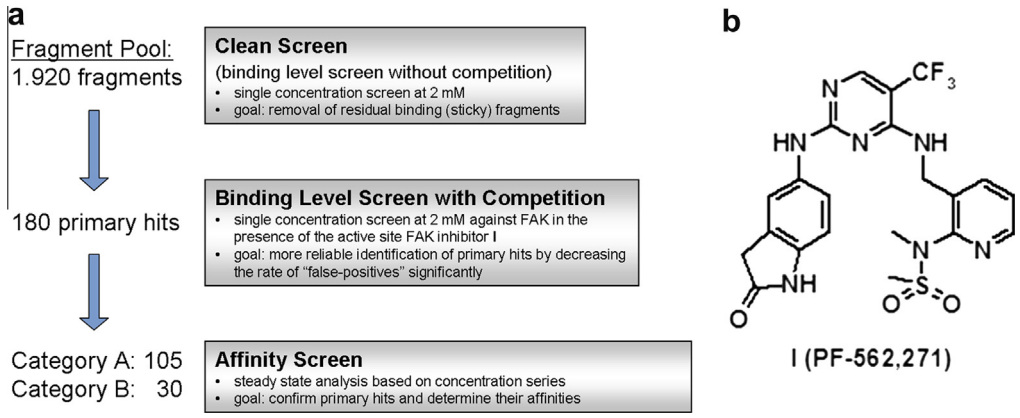

Figure 1. (a) FAK fragment screening strategy and results. (b) Structure of the FAK-inhibitor **I** (PF-562,271) used for SPR competition titration.

'category A' hits with highest confidence in binding.[14] In addition, 30 'category B' hits were discovered with the same characteristics as the 'category A' hits, but with limited compound solubility in DMSO or aqueous solution. We selected 48 'category A' and 2 'category B' hits by chemical diversity based on the Murcko framework representation and ligand efficiency (LE, range between 0.21 and

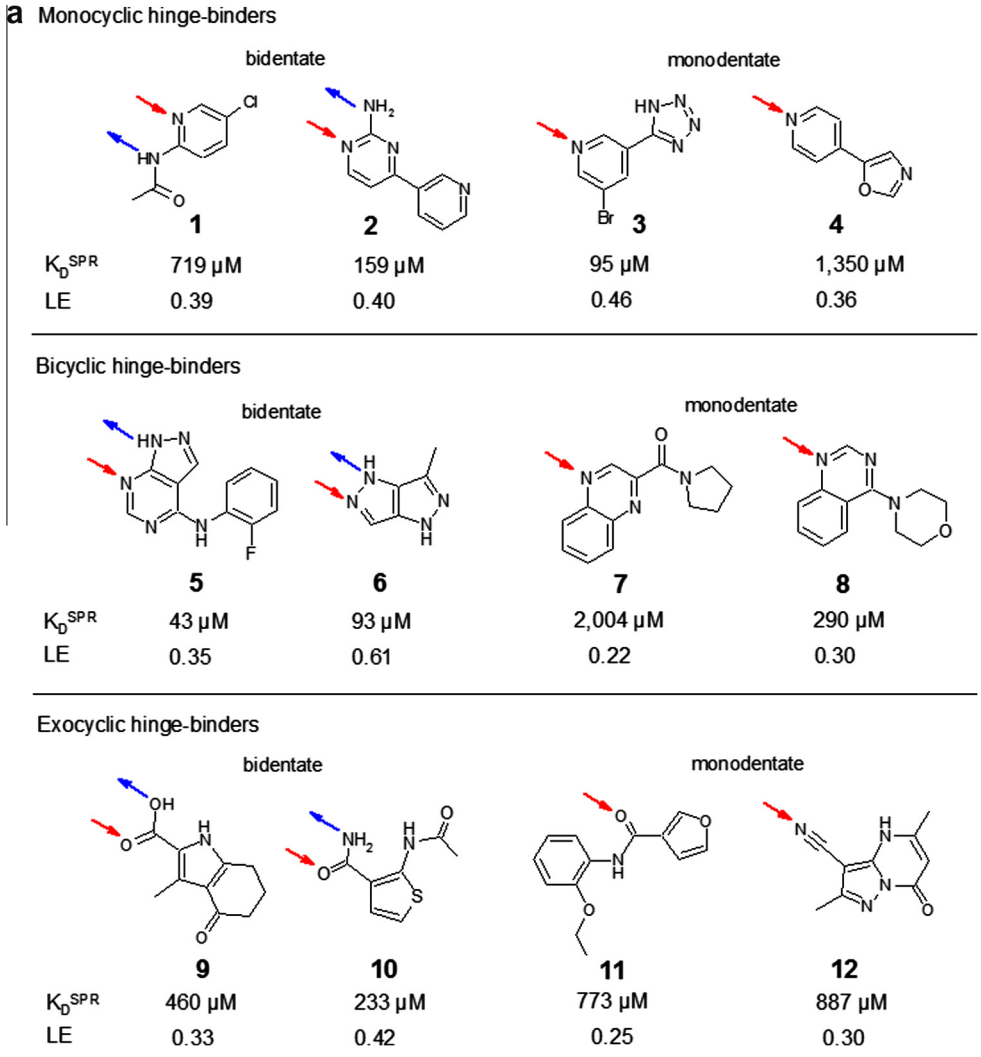

Figure 2. (a) Classification of representative FAK fragment-screening hits by the number of H-bond interactions (mono- and bidentate) to the hinge region (blue: donor contact, red: acceptor contact). (b) The X-ray structure of FAK in complex with fragment **6** at 2.7 Å resolution reveals alternative fragment binding orientations in the two monomers of the asymmetric unit (red: monomer A, green: monomer B). In both orientations, bi-dentate H-bond contacts (distances in Å) are formed to the hinge region (Glu500 and Cys502). The 2Fo–Fc electron density maps of the two binding modes are contoured at 1σ are shown in blue. (c) X-ray structure of FAK in complex with fragment **3** at 2.9 Å resolution (PDB ID: 4K8A) indicates one classical and two non-classical H-bonds (distances in Å) between the pyridine ring and the hinge region (Glu500 & Cys502). The 2Fo–Fc electron density map contoured at 1σ is shown in blue.