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Communications

Discovery of FAK Inhibitors Using Structure Based Drug Design

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Focal adhesion kinase (FAK) is a 125-kDa non-receptor protein tyrosine kinase that associates with both integrin receptors and some growth factor receptor tyrosine kinases to control cell motility, invasion and survival. FAK1 has been demonstrated to modulate cancer cell proliferation, survival, migration and angiogenesis.[1-3] FAK1 is overexpressed in invasive or metastatic breast and colon cancer[4] whereas knocking down FAK1 elevated p53 and p21 levels and reduced cell proliferation. In addition, translocation of nuclear FAK1 facilitates cell survival through enhancing p53 degradation under conditions of cellular stress.[5,6] Small-molecule inhibitors that suppress FAK1 catalytic activity have been developed and reached clinical trials.[7] TAE226 is a small molecule inhibitor of FAK1 (IC_{50} = 5.5 nM) and displayed to inhibit insulin receptor (InsR) and insulin-like growth factor-I receptor (IGF-IR).[8] PF-562,271 are potent inhibitor of FAK1 catalytic activity with IC_{50} of 1.5 nM. PF-562,271 have shown to inhibit phosphorylation of FAK1 at Tyr397 and tumor growth inhibition in *in vivo* model.[9]

ADME/Tox (Absorption, Distribution, Metabolism, Discretion and Toxicity) profiling has become increasingly important for efficient drug discovery and development. Computational methods that can predict cell permeability of novel compounds have been proven to greatly assist lead optimization processes.[10] Fujiwara *et al.* developed an approach involving a combination of molecular orbital (MO) calculation and neural network to predict Caco-2 cell permeability from the molecular 3D structures of compounds with training set 87 compounds.[11] They determined two correlated descriptors (the dipole moment and the polarizability of a molecule) for cell permeability. The permanent dipole moment of a molecule is directly related to solute-solvent interactions.[12] Previously, quantum mechanical (QM) calculation methods are proven to be effective for predicting binding activity between an enzyme and a ligand.[13]

In this work, we synthesized small molecule inhibitors of the FAK1 kinase domain and reported their abilities to block phosphorylation of FAK1 at Tyr397 (pFAK1) in HT29 cancer cell line. The data presented here provide an application of QM methods to inhibit the cellular kinase activity (pFAK1) of FAK1-inhibitors.

The IC_{50} value for TAE226 of FAK1 kinase was determined to be 5.3 nM. TAE226 had strong hydrogen bond interaction with the backbone of Asp564 as well as formed hydrogen bonds with the hinge backbone.[14] We modified a functional group on bis-anilino pyrimidine moiety to increase the hydrophobic interaction with side-chain of gatekeeper residue, Met499. Two inhibitors that were synthesized are also presented in Figure 1. The detailed synthetic procedures and FAK1 kinase assay protocol are described in the

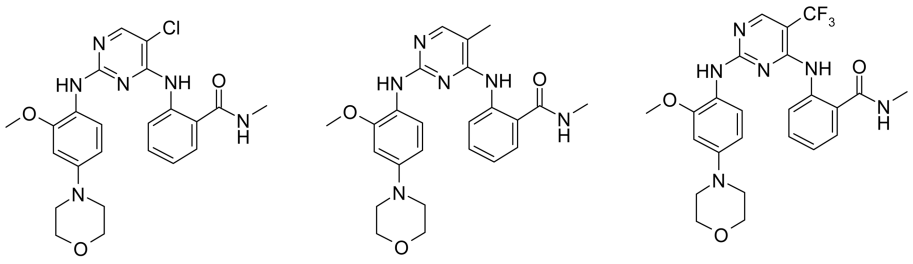

Figure 1. Chemical Structure of TAE226, Inhibitor 1 and 2.

Table 1. IC_{50} values of FAK1 and pFAK1 kinase activity, calculated dipole moment with HF/6-31+G* basis set and Caco-2 permeability predictions

	FAK1 (nM)	pFAK1[a] (μM)	Dipole Moment (HF/6-31+G*)	Caco-2[b] (cm/s × 10^6)
TAE226	5.3	0.07	4.37	3.98
1	7.8	0.16	2.57	11.20
2	4.8	0.01	5.93	16.84

[a]Blocking activity of phosphorylation of FAK1 at Tyr397 (pFAK1) in HT29 cancer cell line. [b]Caco-2 permeability was predicted by PreADMET S/W.[18]

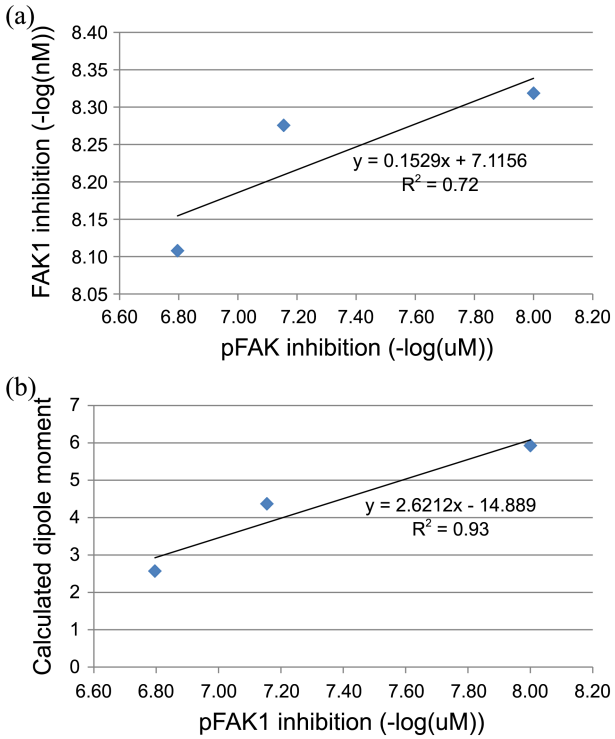

Figure 2. Correlation graphs of pFAK1 inhibition versus other properties: (a) FAK1 kinase inhibition; (b) calculated dipole moment with 6-31+G* basis sets.

Supplemental Materials. The X-ray crystal structure of the TAE226 ligand was obtained from the Protein Data Bank[15] (PDB ID: 2JKK) and was used as an initial structure. The inhibitors were built using Discovery Studio 2.1 (Accelrys),[16] and calculations were carried out using GAUSSIAN03.[17] The geometric optimization and elevation of vibration frequencies were evaluated at the HF level by using the 6-31+G* basis sets. The dipole moments were calculated to investigate the structure activity correlations and the quantum mechanical dipole moments are listed in Table 1.

The FAK1 kinase activities did not show correlation to pFAK1 in HT29 cancer cell line. Compound **1** showed good inhibition of FAK1 kinase (7.8 nM), however the inhibition of pFAK1 (0.16 μM) in HT29 cancer cell line is lower than the others. Methyl substituent on bis-anilino pyrimidine (compound **1**) yielded lower pFAK1 activity than a chloride group on (TAE226). The IC_{50} values for pFAK1 inhibition of CF_3 substituted inhibitor 2 was 0.01 μM, respectively, indicating that pFAK1 inhibition is related with not FAK1 inhibition but also with cell permeability. The correlation between the pFAK1 inhibition and the dipole moment was computed and the results with the 6-31+G* basis set showed relatively high statistical correlation ($R^2 = 0.93$) compare to FAK1 kinase inhibition ($R^2 = 0.72$). Caco-2 permeability of inhibitors was directly predicted by PreADMET S/W.[18] To attain a better comparison, the correlation between the pFAK1 activities and the theoretical dipole moments are plotted in Figure 2.

We have synthesized two bis-anilino pyrimidine compounds as potent FAK1 kinase inhibitors. Optimization of the inhibitor 2 with CF_3 yielding has displayed its dominance in FAK1 and pFAK1 inhibition in comparison to that of the methyl-substituted inhibitor 1. The IC_{50} values of inhibitor 2 were 4.8 nM for FAK1 kinase inhibition and 0.01 μM for pFAK1 kinase inhibition in HT29 cancer cell line.

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