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Design and synthesis of 7*H*-pyrrolo[2,3-*d*]pyrimidines as focal adhesion kinase inhibitors. Part 2

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Abstract—A series of 2-amino-9-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidines were designed and synthesized as focal adhesion kinase (FAK) inhibitors using molecular modeling in conjunction with a co-crystal structure. Chemistry was developed to introduce functionality onto the 9-aryl ring, which resulted in the identification of potent FAK inhibitors. In particular, compound **32** possessed single-digit nanomolar IC_{50} and represents one of the most potent FAK inhibitors discovered to date.

Focal adhesion kinase (FAK) is a protein-tyrosine kinase that is found at the sites of cellular contact and is phosphorylated in response to cell attachment.[1–3] FAK plays an important role in cellular movement and survival pathways. FAK is a potential target for the treatment of both primary cancers and the prevention of tumor metastasis.[4–7] In the previous communication, we reported the synthesis of a series of 7*H*-pyrrolo[2,3-*d*]pyrimidines as inhibitors of FAK.[8] Among these, compound **1** exhibited submicromolar inhibitory activity against FAK (Fig. 1). A co-crystal structure of compound **1** with FAK was also successfully obtained.[9] Molecular modeling based on the co-crystal structure suggested that the inhibitory activity of this series of compounds could potentially be improved by introducing additional interactions between the K454, D564, R550 or E502 amino acid residues of the kinase and the inhibitor.

Our initial attempt focused on forming a salt bridge or hydrogen bond to K454 by introducing a carboxylate to the 8-position of pyrrolopyrimidine (**7**, Scheme 1).

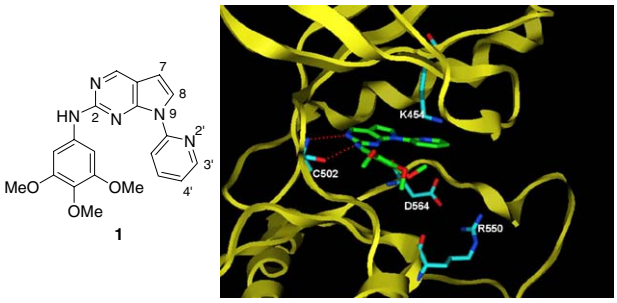

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Figure 1. Structure of pyrrolopyrimidine **1** and its complex with FAK.

Compound **7** was synthesized in six steps starting from commercially available 3-bromo-2-oxo-propionic acid **2**.[10] After a two-step conversion of **2** to methyl enol ether ester **3**,[11] the corresponding vinyl stannane **4** was prepared by reaction with tributylstannyl copper. Palladium-catalyzed coupling of **4** with bromopyrimidine **5** gave the corresponding vinyl ether in good yield.[8,12] Treatment of the resulting vinyl ether with hydrochloric acid furnished the pyrrolopyrimidinyl ester **6**, which upon saponification, led to the desired carboxylic acid **7**. Contrary to our expectation, the appendage of the carboxy group at position 8 resulted in a complete loss of inhibitory activity (Table 1).

Keywords: 7*H*-Pyrrolo[2,3-*d*]pyrimidines; Focal adhesion kinase (FAK); Molecular modeling; SAR; Kinase inhibitor.
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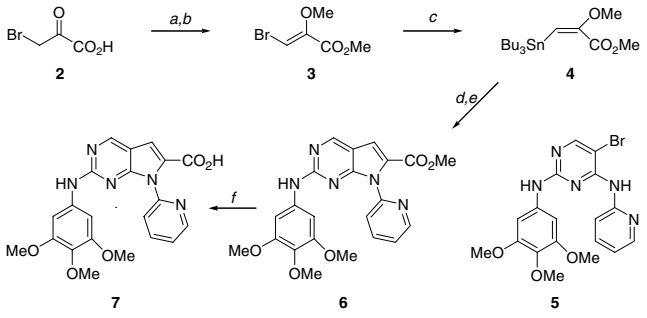
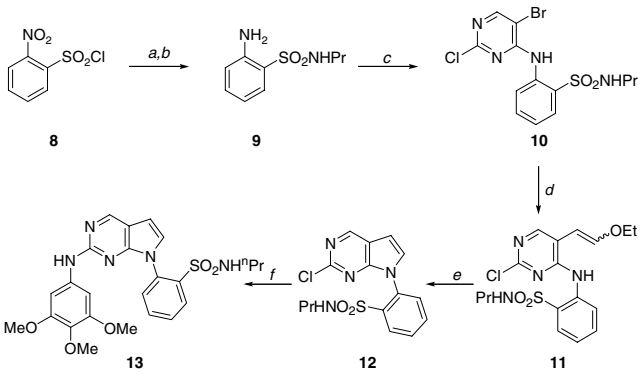


Scheme 1. Synthesis of **6** and **7**. Reagents and conditions: (a) $(MeO)_3CH$ (6.0 equiv), MeOH, H_2SO_4 (cat), reflux, 4 h; (b) *p*-TSOH (cat), 210 °C, 3 h, 68% for two steps; (c) $CuSnBu_3$, THF, −78 °C to 25 °C, 3 h, 47%; (d) **5** (1.0 equiv), $Pd(PPh_3)_4$ (cat), toluene, reflux, 4 h, 81%; (e) HCl (3.0 N), *i*-PrOH, reflux, 4 h, 80%; (f) NaOH (2.0 N), MeOH, 25 °C, 5 h, 86%.

Table 1. Enzymatic IC_{50} against FAK for inhibitors **1**–**32**[8]

Cpd	IC_{50} (μM)	Cpd	IC_{50} (μM)	Cpd	IC_{50} (μM)
1	0.212	**18b**	1.53	**23l**	0.983
6	>10	**18c**	5.12	**23m**	0.434
7	>10	**18d**	7.32	**23n**	0.882
13	3.50	**18e**	0.831	**24a**	1.81
16a	2.81	**18f**	1.51	**24b**	0.421
16b	>10	**18g**	3.23	**24c**	0.117
16c	4.05	**18h**	6.50	**24d**	5.80
16d	>10	**18i**	2.84	**24e**	0.727
16e	0.727	**19**	0.115	**24f**	1.33
16f	>10	**20**	0.232	**24g**	>10
16g	0.504	**21**	0.119	**24h**	0.635
16h	0.314	**22a**	>10	**24i**	0.318
16i	2.82	**22b**	0.342	**25a**	0.331
17a	0.221	**22c**	>10	**25b**	0.345
17b	0.235	**22d**	>10	**25c**	0.063
17c	0.126	**23a**	>10	**25d**	0.236
17d	0.063	**23b**	0.262	**25e**	0.339
17e	0.093	**23c**	0.365	**28a**	7.55
17f	1.507	**23d**	0.322	**28b**	>10
17g	0.038	**23e**	5.06	**28c**	0.182
17h	0.037	**23f**	4.11	**28d**	0.634
17i	0.035	**23g**	0.029	**28e**	0.397
17j	2.71	**23h**	0.038	**28f**	0.278
17k	>10	**23i**	0.073	**28g**	0.242
17l	>10	**23j**	0.298	**28h**	0.544
18a	0.507	**23k**	0.054	**32**	0.004

We next tried to introduce a contact to either K454 or E502 by incorporating a sulfonamide appendage on the 9-phenyl moiety (**13**, Scheme 2). Compound **13** was synthesized in six steps from commercially available sulfonyl chloride **8**. Selective displacement of 5-bromo-2,4-dichloropyrimidine with aniline **9** gave the corresponding anilinyl pyrimidine **10** in good yield. Stille coupling of the resulting 5-bromopyrimidine led to vinyl ether **11**, which was cyclized to pyrrolopyrimidine **12** in excellent yield using hydrochloric acid. Displacement of the chlorine in **12** with 3,4,5-trimethoxyaniline gave the desired sulfonamide analog **13** in moderate yield. Again, to our disappointment, sulfonamide **13** displayed significantly reduced FAK inhibitory activity relative to **1** (Table 1).



Scheme 2. Synthesis of **13**. Reagents and conditions: (a) *n*-$PrNH_2$ (1.5 equiv), Et_3N (2 equiv), 23 °C, 5 h; (b) 10% Pd/C (cat), H_2, EtOH, 25 °C, 16 h; (c) 5-bromo-2,4-dichloropyrimidine (1.0 equiv), 80% AcOH, 50 °C, 16 h, 52% for three steps; (d) $EtOCHCHSnBu_3$ (1.2 equiv), $Pd(PPh_3)_4$, (cat) toluene, 110 °C, 1 h, 83%; (e) HCl (3.0 N), *i*-PrOH, reflux, 4 h, 90%; (f) 3,4,5-trimethoxyaniline (1.5 equiv), *t*-BuOK, 1,4-dioxane, 70 °C, 8 h, 45%.

In an effort to understand why our designed inhibitors **7** and **13** were significantly less active than our lead compound **1**, we used molecular mechanics to study the conformational preferences of our inhibitors. These studies suggested that the diminished FAK inhibitory activity might result from the compounds being predisposed to a conformation less favorable to binding. Specifically, the mechanics calculations suggest that the 9-pyridyl ring in **1** and the 9-aryl ring in **13** have significantly different orientations in the ground state (Fig. 2). The aryl ring in **13** is nearly perpendicular to the pyrrolopyrimidine ring in contrast to the nearly planar orientation of the pyridine ring in **1**. Similar results were obtained for compound **7**. Since these conformational changes are likely caused by the steric hindrance created between the pyrrole moiety and the 9-aryl moiety, we decided to pursue substituents that would minimize these interactions.

A library of analogs with various substitutions on the 9-phenyl moiety (2′, 3′ and 4′) was synthesized using copper-mediated coupling of phenyl bromides with pyrrolopyrimidine intermediate **15** (Scheme 3). Consistent with what was observed for **13**, most of the analogs with ortho substitutions (**16a**–**16h**) exhibited weak inhibitory activity. Among these analogs, only the ester (**16h**) and nitrile (**16g**) analogs showed 0.314 μM and 0.504 μM IC_{50}, respectively. In contrast, most of the 3′-substituted analogs (**17a**–**17l**) demonstrated comparable or improved inhibitory activity compared to **1** (0.212 μM). In particular, the carboxy analogs, **17g**–**17i**, represent significantly improved potency, suggesting that these pyrrolopyrimidines might have gained additional interactions with the enzyme via these substitutions. The 4′-substituted analogs (**18a**–**18i**) did not exhibit any improved activity. With this information in hand, we prepared an additional library of 3′-substituted analogs with various functional groups that have the potential to form interactions with the enzyme. The nitrile analogs (**17c** and **17d**) were thus converted into the corresponding tetrazoles (**19**–**21**) in good yields by treatment with

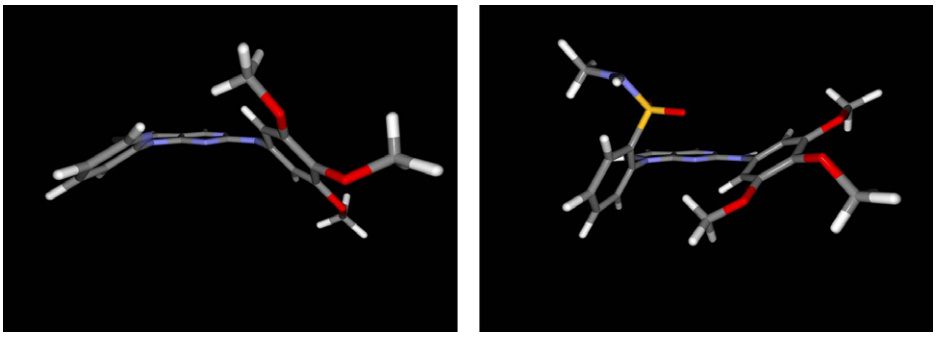
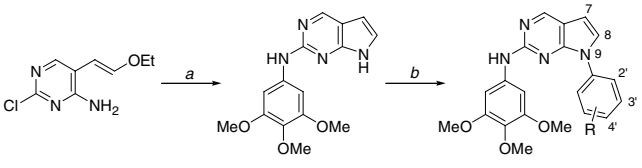


Figure 2. Ground state conformations of **1** and **13**.[13]



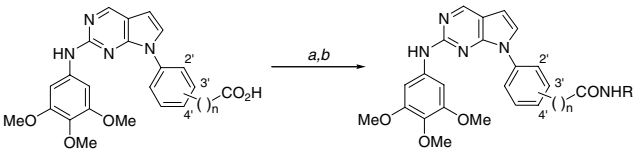
16a: R=2'-Me
16b: R=2'-Et
16c: R=2'-NO_2
16d: R=2'-NH_2
16e: R=2'-CH_2OH
16f: R=2'-NHAc
16g: R=2'-CN
16h: R=2'-CO_2Me
16i: R=2'-CO_2H
17a: R=3'-Me
17b: R=3'-CF_3
17c: R=3'-CN
17d: R=3'-CH_2CN
17e: R=3'-$(CH_2)_2CN$
17f: R=3'-CO_2Me
17g: R=3'-CO_2H
17h: R=3'-CH_2CO_2H
17i: R=3'-$(CH_2)_2CO_2H$
17j: R=3'-SO_2NHMe
17k: R=3'-NH_2
17l: R=3'-CH_2NH_2
18a: R=4'-Me
18b: R=4'-Et
18c: R=4'-*n*Bu
18d: R=4'-*t*Bu
18e: R=4'-SMe
18f: R=4'-CF_3
18g: R=4'-NO_2
18h: R=4'-CO_2Me
18i: R=4'-CO_2H

Scheme 3. Synthesis of 2,9-disubstituted pyrrolopyrimidines **16**–**18**. Reagents and conditions: (a) 3,4,5-trimethoxyaniline (1.5 equiv), HCl (1.0 equiv), *n*-BuOH, 110 °C, 4 h, 90%; (b) ArBr (1.5 equiv), 1,4-dioxane, CuI (0.1 equiv), K_3PO_4 (2.0 equiv), *trans*-1,2-diaminocyclohexane (0.1 equiv), 100 °C, 5 h, 40–85%.

tributyltin azide (Scheme 4). Compared to the corresponding nitriles, tetrazole **20** lost activity slightly, while **19** and **21** remained approximately equipotent.

A variety of amide analogs (**22**–**25**) were prepared from the carboxy analogs **16i**, **17g**–**17i** by reaction with various simple and functionalized amines (Scheme 5). Among the ortho analogs (**22a**–**22d**), only the relatively small methyl amide (**22b**) exhibited moderate activity. While most of the analogs with substitutions at the 3′ position (**23**–**25**) have reduced potency, a number of them (**23g**–**23i** and **23k**) retained their activity. Since all of these more potent analogs contain basic pendant amines of various chain lengths, it is likely that they are directed towards solvent. While these modifications did not improve the potency, they could serve as useful handles to modulate the physicochemical properties of these pyrrolopyrimidines at a later stage.

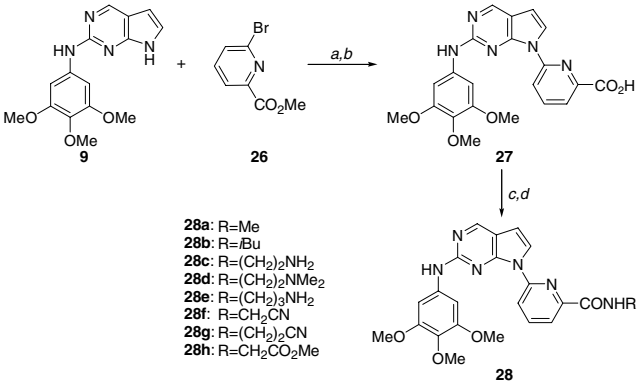
Scheme 4. Synthesis of **19**–**21**. Reagents and conditions: (a) *n*-Bu_3SnN_3, xylene, reflux, 20 h, 70–82%.



22a: R=H
22b: R=Me
22c: R=$(CH_2)_2NH_2$
22d: R=$(CH_2)_3NMe_2$
23a: R=H
23b: R=Me
23c: R=Et
23d: R=*c*Pr
23e: R=*i*Bu
23f: R=*c*Pent
23g: R=$(CH_2)_2NH_2$
23h: R=$(CH_2)_2NMe_2$
23i: R=$(CH_2)_2N(CH_2)_4$
23j: R=$(CH_2)_2NHAc$
23k: R=$(CH_2)_3NMe_2$
23l: R=$(CH_2)_4NMe_2$
23m: R=$(CH_2)_2OH$
23n: R=$(CH_2)_3OH$
24a: R=Me
24b: R=Et
24c: R=*c*Pr
24d: R=*c*Pent
24e: R=$(CH_2)_2NH_2$
24f: R=$(CH_2)_3NH_2$
24g: R=$(CH_2)_3NMe_2$
24h: R=CH_2CO_2Me
24i: R=$(CH_2)_2CO_2Me_2$
25a: R=Me
25b: R=Et
25c: R=*i*Bu
25d: R=$(CH_2)_2NMe_2$
25e: R=CH_2CO_2Me

Scheme 5. Synthesis of **22**–**25**. Reagents and conditions: (a) $(COCl)_2$, DMF (cat), CH_2Cl_2, 25 °C, 3 h; (b) RNH_2 (1.5 equiv), *i*-Pr_2NEt (20.0 equiv), 25 °C, 4 h, 45–85% for two steps.

Since a 2-pyridyl moiety at the 9-position is more favorable over the corresponding phenyl substitution at the same position,[8] we next prepared a series of analogs which contain both the 2-pyridinyl ring and the 3′-substitutions (**28a**–**28h**, Scheme 6). Coupling of pyrrolopyrimidine intermediate **9** with 2-bromopyridine **26**, followed by hydrolysis of the resulting methyl ester, furnished the corresponding carboxy analog **27** in good overall yield. The acid chloride was then



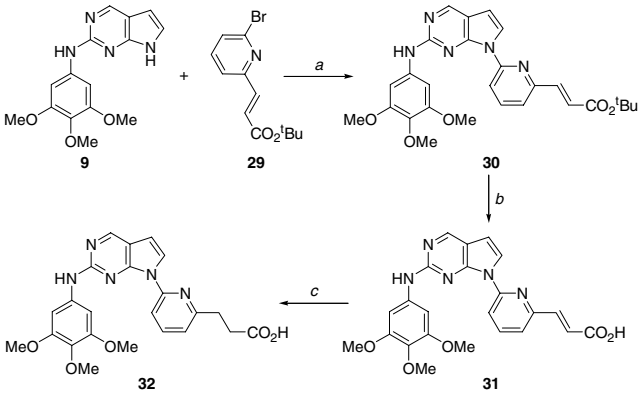
28a: R=Me
28b: R=*i*Bu
28c: R=$(CH_2)_2NH_2$
28d: R=$(CH_2)_2NMe_2$
28e: R=$(CH_2)_3NH_2$
28f: R=CH_2CN
28g: R=$(CH_2)_2CN$
28h: R=CH_2CO_2Me

Scheme 6. Synthesis of **28**. Reagents and conditions: (a) **26** (1.2 equiv), 1,4-dioxane, CuI (0.1 equiv), K_3PO_4 (2.0 equiv), *trans*-1,2-diaminocyclohexane (0.1 equiv), 100 °C, 4 h, 60%; (b) NaOH (3.0 N), MeOH, 25 °C, 5 h, 85%; (c) $(COCl)_2$, DMF (cat), CH_2Cl_2, 25 °C, 3 h; (d) RNH_2 (1.5 equiv), 25 °C, 2 h, 75–90%.

formed from **27** using oxalyl chloride, and its reaction with various amines produced the amide analogs **28a**–**28h**. Contrary to our expectations of simple additivity, all these analogs had reduced FAK inhibitory activity. One potential explanation is that an unfavorable conformation is induced through a lone-pair interaction between the pyridyl nitrogen and adjacent carbonyl oxygen.

In order to test this hypothesis, an analog containing both a 2-pyridine ring and an extended carboxy group (**32**) was synthesized from **7** (Scheme 7). To our delight, compound **32** exhibits single-digit nanomolar IC_{50} against FAK and represents one of the most potent FAK enzyme inhibitors reported to date. Molecular modeling studies suggest that the carboxy group could make a salt bridge with K454 (Fig. 3).

In summary, we have demonstrated how a structure-based design approach can be used to design pyrrolo[2,3-*d*]pyrimidines which possess potent FAK inhibitory activity. Various acidic or basic moieties could be incorporated onto the 9-aryl ring to modulate the interactions with the enzyme and potentially their physicochemical properties. Our studies suggest that the effect of the 2-pyridine nitrogen is sensitive to its environment, and that the positioning and orientation of a carboxy group are critical for optimal interactions with the kinase. Future studies will focus on optimizing the selectivity against other kinases and the cellular activities of the pyrrolo[2,3-*d*]pyrimidines.



Scheme 7. Synthesis of **32**. Reagents and conditions: (a) **29** (1.2 equiv), 1,4-dioxane, CuI (0.1 equiv), K_3PO_4 (2.0 equiv), *trans*-1,2-diaminocyclohexane (0.1 equiv), 100 °C, 4 h, 80%; (b) TFA, CH_2Cl_2, 25 °C, 3 h, 80%; (c) 10% Pd/C (cat), H_2, EtOH, 25 °C, 8 h, 76%.

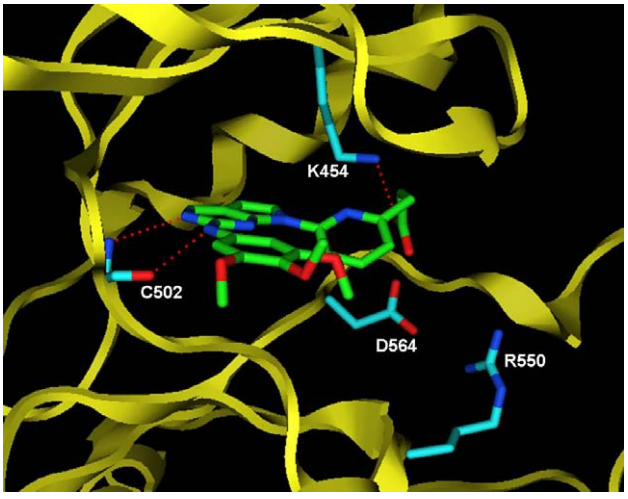


Figure 3. Docking of pyrrolopyrimidine **32** into the active site of FAK.[14]

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13. Ground state energies and conformations were calculated using OPLS2003 forcefield with quantum mechanical ESP charges. Quantum mechanical calculations were based on DFT with 6-31G** basis set under continuum solvation model ($\varepsilon = 80.4$). The conformational energy penalty was obtained from internal energy difference between the bound and free forms of each ligand molecule including solvation. Quantum mechanical charges were important for proper assessments of energies since charge distributions had significant influence on desolvation energies. Desolvation and torsional energy penalty were the two most important factors that control the activity difference among this class of compounds.
14. Computational docking: protein coordinates for docking were obtained from the crystal structure of FAK in complex with pyrrolopyrimidine **1**. The binding site for docking was defined as the collection of amino acids of FAK within 10 Å radius centered on the bound ligand **1**. Waters and **1** were deleted from the pdb file and hydrogen atoms were added to the amino acid residues. Compound 3D coordinates were generated using the MOE program. Docking was performed using the default parameter settings of GOLD v2.2 (Cambridge Crystallographic Data Centre). All atom types and charges were assigned in GOLD. 1,00,000 independent genetic algorithm (GA) runs were performed for the ligand. The radius of the search was set to 10 Å.