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Design and synthesis of 7*H*-pyrrolo[2,3-*d*]pyrimidines as focal adhesion kinase inhibitors. Part 1

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Abstract—A series of 2-amino-9-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidines were designed and synthesized to target focal adhesion kinase (FAK). A number of these pyrrolopyrimides exhibited low micromolar inhibitory activities against focal adhesion kinase, and their preliminary SAR was established via systematic chemical modifications. The 2-amino-9-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidines represent a new class of kinase inhibitors.
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Focal adhesions are found at the cell membrane where the cytoskeleton interacts with the proteins of the extracellular matrix. The clustering of integrins at these sites attracts a large complex of proteins which regulate processes such as anchorage-dependent proliferation and cell migration. Signal transduction mediated by interactions between cells and the extracellular matrix (ECM) at focal adhesions is an important determinant of cell fate. Focal adhesion kinase (FAK) was first discovered in 1992 and was implicated in integrin signaling.[1–3] It is a 125 kDa protein tyrosine kinase recruited at an early stage to focal adhesions and is phosphorylated in response to cell attachment and mediates focal adhesion formation.[4,5] FAK is known to promote cellular movement and survival. In a variety of human epithelial and mesenchymal tumors, such as melanoma, lymphoma, and multiple myeloma, FAK is highly active. Moreover, increased FAK expression correlates with increased invasiveness and increased ability of cancer to metastasize. Inhibition of FAK signaling in vitro induces cell growth arrest, reduces motility, and in certain contexts causes cell death in cancer cell lines.[6–12] In the mouse xenograft studies using human melanoma cells, antisense oligonucleotides against FAK inhibited the growth of the primary tumor and virtually eliminated metastases with few adverse effects to normal tissues.[13,14] These observations suggest that FAK represents a promising therapeutic opportunity for both the treatment of primary disease and the prevention of metastatic disease.

While much of research has been performed to elucidate the biological roles of FAK, the only documented FAK inhibitors are described by AstraZeneca and a representative structure is shown in Figure 1 (**1a**).[15]

Despite screening historical kinase-directed medicinal chemical libraries of inhibitors, we were unable to discover interesting starting points for lead optimization. To circumvent this problem, we employed a rational

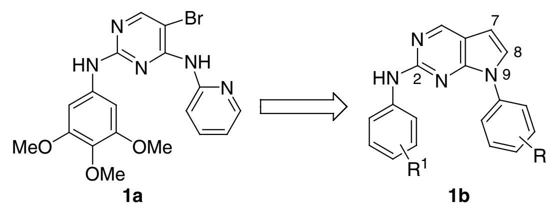

Figure 1.

Keywords: Focal adhesion kinase; Inhibition; 7*H*-Pyrrolo[2,3-*d*]pyrimidines; SAR.

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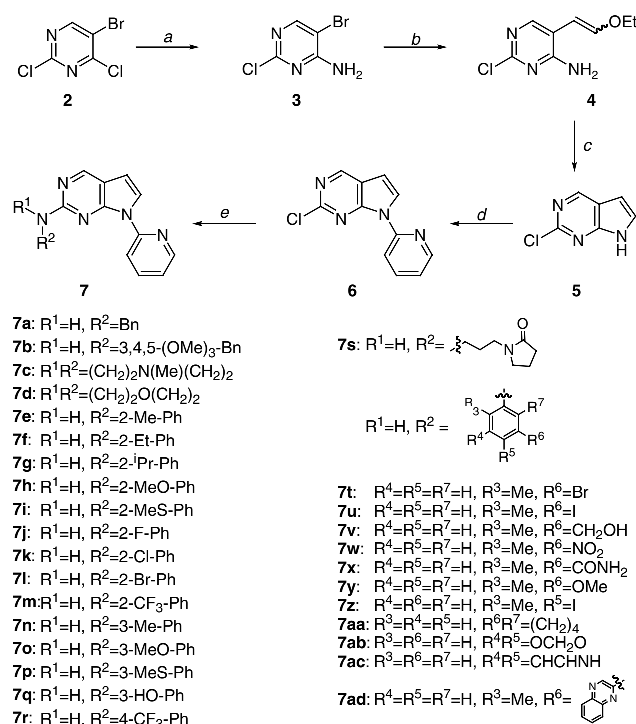
design approach where we introduced a five-membered ring to bridge the pyrimidine 4 and 5 positions (Fig. 1) to yield the pyrrolopyrimidine **1b**. Although pyrrolopyrimidines have been reported as kinase inhibitors,[16] 2-amino-9-arylpyrrolopyrimidines have not been documented previously. The route used to prepare pyrrolopyrimidine analogs is shown in Scheme 1. Starting from commercially available 5-bromo-2,4-dichloropyrimidine (**2**), the chlorine at the 5 position was regioselectively displaced by an amino group in excellent yield. Palladium-catalyzed cross coupling of the resulting pyrimidine intermediate with vinyl stannane gave the corresponding vinyl ether **4**, which was cyclized to furnish pyrrolopyrimidine intermediate **5** upon treatment with hydrochloric acid.[17] The 2-pyridyl moiety was first introduced at the 9-position via copper-mediated coupling,[18] followed by the displacement of the remaining chlorine with various amines to provide the targeted pyrrolopyrimidines (**7a–7ad**). While many of these analogs did not show significant inhibitory activities against FAK in a biochemical time-resolved fluorescence assay, several compounds exhibited submicromolar IC_{50}s (Table 1, **7e**, **7h**, **7n**, **7o**, **7x**, **7y**, **7aa**, and **7ad**). This was very encouraging as they could serve as starting points for optimization. The preliminary data suggested that a phenyl moiety as R^2 is preferred for inhibitory activity (Scheme 1 and Table 1).

We next examined the effect of different substitutions at the 9-position. The synthesis of these analogs is shown

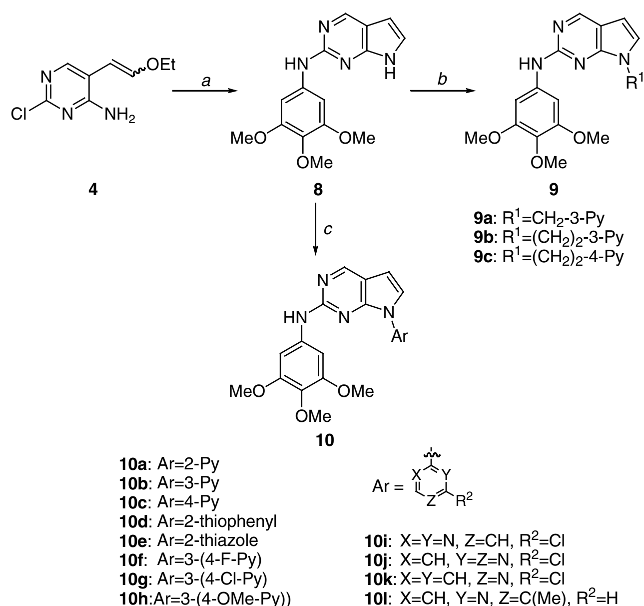


Scheme 1. Synthesis of pyrrolopyrimidines **7a–7ad**. Reagents and conditions: (a) NH_3 (l), THF, 25 °C, 15 h, 94%; (b) $EtOCHCHSnBu_3$ (1.2 equiv), $Pd(PPh_3)_4$ (0.05 equiv), toluene, 110 °C, 16 h, 58%; (c) HCl (3.0 N), ^{i}PrOH, reflux, 2 h, 96%; (d) 2-bromopyridine (1.5 equiv), 1,4-dioxane, CuI (0.1 equiv), K_3PO_4 (20 equiv), *trans*-1,2-diaminocyclohexane (0.1 equiv), 90 °C, 4 h, 89%; (e) R_1R_2NH (2.0 equiv), KO^{t}Bu, THF, reflux, 7 h, 30–65%.

Table 1. Inhibitory activities of pyrrolo[2,3-*d*]pyrimidines[21]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1a	0.1	**7aa**	0.3
7a	>10	**7ab**	>10
7b	>10	**7ac**	>10
7c	>10	**7ad**	0.6
7d	>10	**9a**	7.9
7e	0.5	**9b**	>10
7f	0.9	**9c**	7.5
7g	>10	**10a**	0.2
7h	0.7	**10b**	0.1
7i	1.0	**10c**	9.0
7j	1.2	**10d**	>10
7k	0.7	**10e**	0.5
7l	1.5	**10f**	0.8
7m	7.0	**10g**	0.5
7n	0.7	**10h**	0.3
7o	0.6	**10i**	>10
7p	2.0	**10j**	0.8
7q	>10	**10k**	0.8
7r	>10	**10l**	>10
7s	>10	**15**	>10
7t	>10	**16**	0.2
7u	3.0	**18**	>10
7v	>10	**19**	0.1
7w	>10	**23**	>10
7x	0.4	**24**	0.2
7y	0.2	**25**	0.2
7z	8.0	**26**	>10

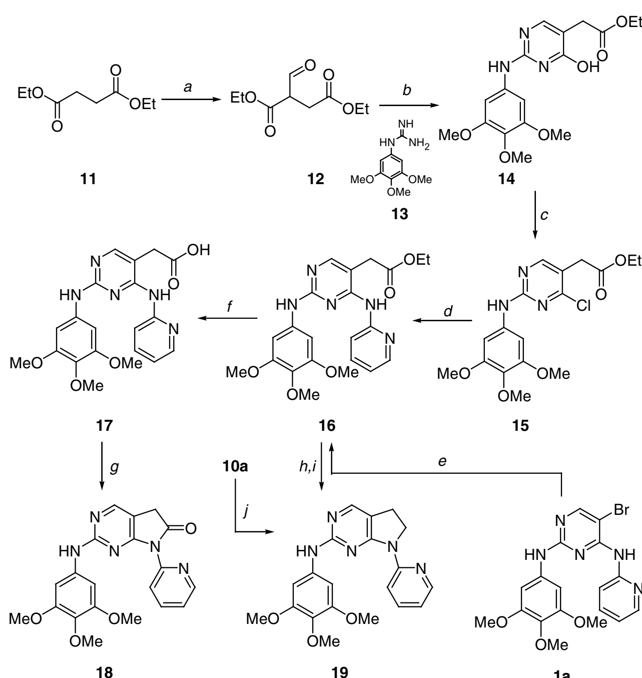
in Scheme 2. Vinyl ether **4** was converted into aniline-substituted pyrrolopyrimidine **8** in one pot in the presence of hydrochloric acid. Pyridine analogs (**9a–9c**) with 1, 2 or 3 methylene linkers were prepared from **8** via a Mitsunobu reaction. All three analogs showed weak or no activity. These data suggest that the distance of the pyridine moiety from the pyrrolopyrimidine is important for favorable interactions with the enzyme. To further probe this region, different aryl moieties could be introduced via copper-mediated coupling to give **10a–10l** in moderate to good yields.[18] Among these compounds, the simple 2- and 3-pyridyl analogs (**10a** and **10b**) possessed IC_{50} of 0.2 and 0.1 μM against FAK, respectively. As the 4-pyridyl analog **10c**, exhibited significantly diminished activities (9.0 μM), it suggested that the position of pyridine nitrogen plays an important role via either hydrogen bonding or electrostatic interactions. Several electron-donating or -withdrawing substitutions were introduced to the 3-pyridyl ring (**10f–10h**) to probe the electronic preference of the 9-position. The conclusion was that while all substitutions reduced activity, an electron-donating group (**10h**) is favored over an electron-withdrawing group (**10f**). Heterocycle substitutions lead to variable results, thiophene (**10d**) lost activity, while thiazole (**10e**) retained activity. The activity of thiazole (**10e**) provided further evidence for the importance of a basic nitrogen in this region of the inhibitor. Two regioisomeric pyrimidine analogs were also prepared. While **10i** completely lost the activity, its isomer **10j** still showed submicromolar activities, which again suggests the importance of the position of the nitrogen atom in this region.



Scheme 2. Synthesis of pyrrolopyrimidines **9a**–**9c** and **10a**–**10l**. Reagents and conditions: (a) 3,4,5-trimethoxyaniline (1.1 equiv), ^{n}BuOH, HCl (1.0 equiv), 110 °C, 4 h, 76%; (b) R^1OH (1.3 equiv), PPh_3 (1.5 equiv), DEAD, THF, 25 °C, 12 h, 30%; (c) 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, 4 h, 40–90%.

With the preliminary SAR information on the substitutions in hand, we decided to explore the importance of pyrrole moiety on the interaction with the enzyme. The first two analogs designed to address this question are **18** and **19** and their synthesis is shown in Scheme 3. Condensation of succinic acid diethyl ester with ethyl formate led to aldehyde intermediate **12**, which was reacted with trimethoxyguanidine **13** to furnish the trisubstituted pyrimidine **14** in moderate yield. The hydroxy group was then converted into chlorine in excellent yield using phosphorus oxychloride. Palladium-catalyzed coupling of the resulting pyrimidine chloride with 2-aminopyridine led to 2,4-diaminopyrimidine **16** in good yield.[19] Compound **16** could also be prepared in one step from **1a** through reaction with stannane acetate under the Negishi–Reformatsky coupling conditions.[20] Sponification of the ethyl ester followed by the lactam formation using thionyl chloride gave the target molecule **18**. Compound **19** was prepared from **16** in two steps: (1) reduction of the ester by LAH to the corresponding alcohol and (2) Mitsunobu cyclization. Compound **19** could also be synthesized in excellent yield from **10a** by palladium-catalyzed hydrogenation. Enzymatic assay against FAK demonstrated that **18** was inactive, while **19** still retained similar biological activity. These data suggest that the electronics on the pyrrolidine nitrogen or the conformation of the pyrrolidine, and possibly sterics in this region, is crucial for the activity. The electron-withdrawing 8-carbonyl is detrimental to the interaction with the enzyme.

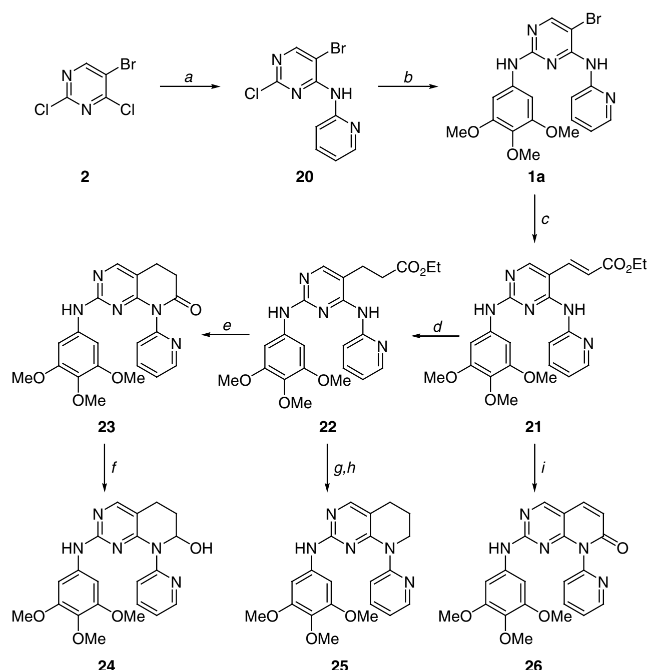
Several additional analogs (**23**–**26**) were synthesized to further explore the SAR in this region (Scheme 4). Selective displacement of 5-bromo-2,4-dichloro-pyrimidine (**2**) with 2-aminopyridine gave **20** in moderate yield.



Scheme 3. Synthesis of **18** and **19**. Reagents and conditions: (a) NaOEt, Et_2O, HCO_2Et, 25 °C, 14 h, 54%; (b) **13** (0.7 equiv), NaOEt, EtOH, relux, 3 h, 55%; (c) $POCl_3$, 40 °C, 2 h, 94%; (d) 2-aminopyridine (1.3 equiv), $Pd_2(dba)_3$ (0.1 equiv), BINAP (0.2 equiv), Cs_2CO_3 (1.4 equiv), toluene, 80 °C, 2 h, 70%; (e) $^nBu_3SnCH_2CO_2Et$ (1.5 equiv), $Pd_2(dba)_3$ (0.1 equiv), DMF, 80 °C, 16 h, 30%; (f) NaOH (1.0 N), EtOH, 25 °C, 1 h, 60%; (g) $SOCl_2$ (3.0 equiv), CH_2Cl_2, 25 °C, 2 h, 50%; (h) LAH (4.0 equiv), THF, 25 °C, 2 h, 63%; (i) DEAD (1.5 equiv), PPh_3 (1.5 equiv), THF, 25 °C, 58%; (j) H_2, Pd/C (10%), EtOAc, AcOH (5–10 equiv), 25 °C, 12 h, 91%.

Additional displacement of the remaining chlorine in **20** with trimethoxyaniline led to diaminopyrimidine **1a** in good yield.[15] Heck coupling of **1a** with ethyl acrylate gave the corresponding olefin intermediate **21**. Hydrogenation of the double bond followed by the treatment of the resulting ester led to the lactam target **23**. Partial reduction of the lactam with DIBAL-H furnished the corresponding pyrimidinol **24**. The ester moiety in **22** can be completely reduced using LAH. Cyclization of the resulting alcohol under the Mitsunobu conditions gave **25** in moderate yield. Target **26** was obtained by directly treating ester **21** with sodium ethoxide. Consistent with the loss of FAK activity for 8-keto compound **18**, neither **23** nor **26** exhibited significant FAK inhibitory activity. As had been observed for the saturated pyrrolidinyl analog **19**, the corresponding fused piperidinyl analog **24** also exhibited an IC_{50} of 0.2 μM.

In summary, we have discovered that 2-amino-9-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidines can be elaborated into moderately potent FAK inhibitors based on rational design. Efficient and flexible chemistries have been developed to synthesize pyrrolopyrimidine analogs with various substitutions at the 2 and 9 positions. These compounds are different from the previously reported pyrrolopyrimidines-based kinase inhibitors, which possess characteristic 3,8, or 3,9 disubstitutions. Thus, 2-amino-9-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidines represent a new class of kinase inhibitors. Preliminary SAR studies sug-



Scheme 4. Synthesis of compounds **23**–**26**. Reagents and conditions: (a) 2-aminopyridine (1.2 equiv), DIEPA, MeOH, 100 °C, 16 h, 40%; (b) 3,4,5-trimethoxyaniline (2.0 equiv), ^{n}BuOH, 16 h, reflux, 85%; (c) CH_2CHCO_2Et (10.0 equiv), $Pd(OAc)_2$ (0.01 equiv), CH_3CN, reflux, 16 h, 75%; (d) H_2, Pd/C (10%), EtOH, 25 °C, 8 h, 85%; (e) NaOEt (4.0 equiv), EtOH, 80 °C, 16 h, 30%; (f) DIBAL-H (3.0 equiv), THF, 25 °C, 2 h, 55%; (g) LAH (4.0 equiv), THF, 25 °C, 2 h, 60%; (h) DEAD (1.5 equiv), PPh_3 (1.5 equiv), THF, 25 °C, 40%; (i) NaOEt (4.0 equiv), EtOH, 80 °C, 16 h, 60%.

gested that a combination of an electron-rich arylamino group at the 2-position and pyridinyl group at the 9-position resulted in moderately potent FAK inhibitors (**7aa**, **10a**, **10b**, **10h**, **19**, **24** and **25**). It appears that the correct positioning of the substituents at the 9-N and the electronics on the N are important for the inhibitory activities against FAK, and the oxo-analogs (**18** and **26**) exhibited significantly reduced activities. Interestingly, the aromaticity of the pyrrole moiety does not have big impact on the activity (**10a**, **10b** vs **19**, **24**, and **25**).

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21. A TR-FRET-based FAK kinase assay was used to measure the potency of the FAK inhibitors. Briefly, 15 μL of assay mixture containing 133 nM of the FAK substrate peptide (Biotin-Ahx-SETDDYAEIID) and 2.4 μg/mL of FAK in assay buffer (20 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 2 mM $MnCl_2$, 50 μM Na_3VO_4, 0.01% BSA, and 0.05% Tween 20) was added into a 384-well plate, followed by the addition of 0.5 μL of compounds in DMSO. After incubation at room temperature for 20 min, the kinase reaction was initiated by the addition of 5 μL of 40 μM ATP. The kinase reaction was performed at 37 °C for 2 h and then stopped by the addition of the stop solution mixture containing 0.15 nM Eu-PT66 (Perkin-Elmer), 1.5 μg/mL SA-APC in detection buffer (10 mM Tris–HCl, pH 7.4, 6.25 mM EDTA, 0.01% BSA, and 0.05% Tween 20). The plate was incubated at room temperature for 1 h and the TR-FRET signal was detected with an Acquest plate reader.