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Synthesis and optimization of substituted furo[2,3-*d*]-pyrimidin-4-amines and 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines as ACK1 inhibitors

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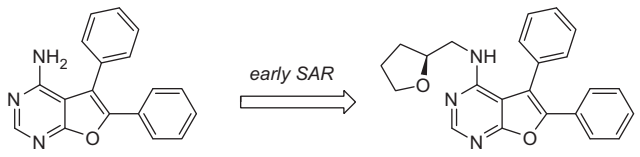
ABSTRACT

Two classes of ACK1 inhibitors, 4,5,6-trisubstituted furo[2,3-*d*]pyrimidin4-amines and 4,5,6-trisubstituted 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines, were discovered and evaluated as ACK1 inhibitors. Further structural refinement led to the identification of potent and selective dithiolane inhibitor **37**.

ACK1 (Activated Cdc42-associated tyrosine Kinase 1) is a non-receptor tyrosine kinase that has attracted substantial interest as a target for drug discovery research in recent years.[1] Amplification of the ACK1 gene in primary tumors has been reported to correlate with poor prognosis.[2] The expression of activated ACK1 in LNCaP cells dramatically promotes prostate tumorigenesis.[3a,b] The Ack1 inhibitor inhibits androgen receptor transcription activity.[3c] Furthermore, ACK1 is a mediator of EGF signals to Rho family GTP-binding proteins through phosphorylation and activation of guanine nucleotide exchange factors (GEFs) such as Dbl.[4] These findings suggest that ACK1 is a potential target for developing anti-cancer therapeutics. The crystal structures of the human ACK1 kinase domains in both the unphosphorylated and phosphorylated states have been solved.[5] In this Letter, the synthesis and SAR of two classes of ACK1 inhibitors are discussed.

A high-throughput small molecule ACK1 biochemical inhibition screen was performed in-house and led to the identification of 1 μM inhibitor furanopyrimidine **1** (Fig. 1). Further binding studies found compound **1** to be both ATP-competitive and reversible. Early structure-activity relationship (SAR) work was performed upon this promising initial hit and revealed that appropriate *N*-substitution could significantly enhance ACK1 inhibition levels. Indeed, this effort led to the identification of potent (*S*)-*N*-(tetrahydrofuran-2-yl)methyl) furanopyrimidine **2** (ACK1 K_i = 0.01 μM, Fig. 1). Subsequent SAR studies addressed the modification of other regions of the lead structure as well as further refinement of the *N*-substituent and are described below.

After the discovery of potent furanopyrimidine **2**[6], we initially sought to assess the viability of related core structures in order to expand upon our available chemical space. We evaluated these analogs in both ACK1 biochemical and cellular autophosphorylation assays.[7] To this end, a number of new bicyclic structures were



1
ACK1 K_i = 1 μM

2
ACK1 K_i = 0.01 μM

Figure 1. Initial HTS hit **1** and subsequent derivative **2** resulting from amino group substitution.

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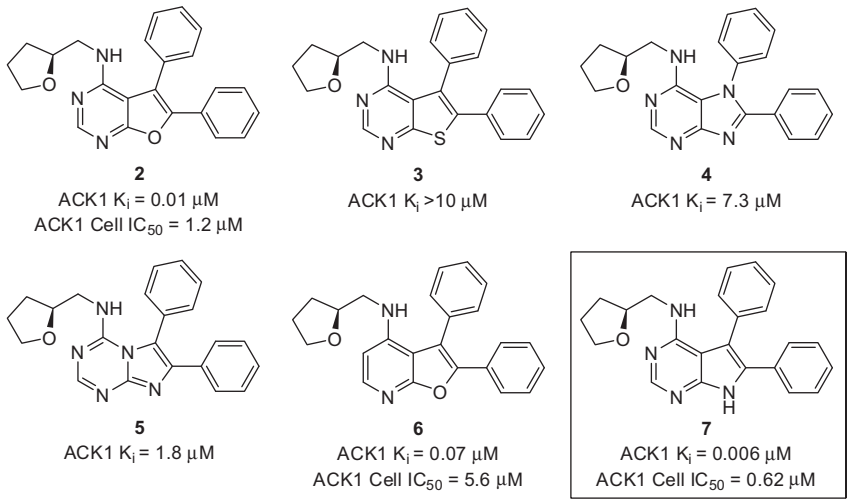
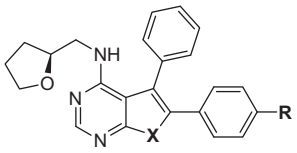


Figure 2. Various furanopyrimidine core changes that were tested. The biochemical and cellular potencies of pyrrolopyrimidine **7** were found to be similar to those of furanopyrimidine **2.**

Table 1
ACK1 inhibition for (*S*)-5,6-diphenyl-*N*-((tetrahydrofuran-2-yl)methyl)furo[2,3-*d*]pyrimidin-4-amines and (*S*)-5,6-diphenyl-*N*-((tetrahydrofuran-2-yl)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines with selected 6-aryl substituents **R**



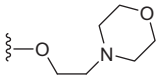
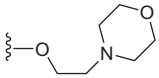
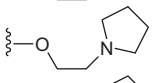
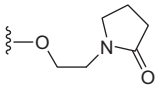
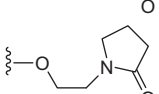
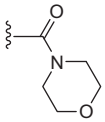
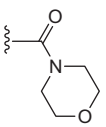
Compound	X	**R**	ACKi K_i (μM)[a]	ACKi Cell IC_{50} (μM)[b]
2	O	H	0.01	1.2
7	NH	H	0.006	0.62
8	O	OMe	0.005	0.55
9	NH	OMe	0.006	0.35
10	O	SO_2Me	0.008	0.25
11	NH	SO_2Me	0.007	0.10
12	NH	SO_2NMe_2	0.002	0.09
13	O	SO_2NHMe	0.004	0.30
14	NH	SO_2NHMe	0.002	0.04
15	O	$O(CH_2)_2NMe_2$	0.005	0.03
16	NH	$O(CH_2)_2NMe_2$	0.006	0.02
17	O		0.004	0.16
18	NH		0.006	0.08
19	NH		0.006	0.06
20	O		0.004	0.23
21	NH		0.004	0.02
22	O	$C(O)NMe_2$	0.01	0.18
23	NH	$C(O)NMe_2$	0.002	0.02
24	O	C(O)NHMe	0.013	2.05
25	NH	C(O)NHMe	0.003	0.04
26	O		0.006	0.19

Table 1 (*continued*)

Compound	X	**R**	ACKi K_i (μM)[a]	ACKi Cell IC_{50} (μM)[b]
27	NH		0.006	0.02

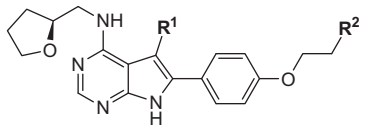
[a] Values are means of at least two experiments.
[b] Average of at least two IC_{50} values.

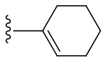
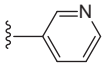
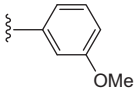
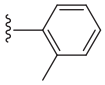
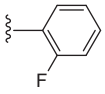
synthesized and evaluated (Fig. 2). Changes that exhibited little or no ACK1 inhibition include thienopyrimidine **3**, imidazopyrimidine **4**, and imidazotriazine **5**. Furanopyridine **6** proved to be a potent ACK1 inhibitor in vitro (ACK1 K_i = 0.07 μM, Fig. 2) but showed a significant erosion in cellular activity relative to furanopyrimidine **2**. Gratifyingly, it was discovered that pyrrolopyrimidine **7** was roughly equipotent to furanopyridine **2** in both biochemical and cellular assays, and thus represented a second core scaffold for further SAR exploration.

A large number of substituents were introduced at the *para*-position of the 6-phenyl ring for both the furanopyrimidine **2** and pyrrolopyrimidine **7** lead structures (Table 1). While methoxy and methanesulfonyl groups were tolerated in both series (**8**–**11**), significant improvements in both biochemical and cellular ACK1 inhibition were observed for more polar sulfonamides attached to the pyrrolopyrimidine core structure (**12** and **14**, ACK1 K_i = 0.002 μM and ACK1 Cell IC_{50} <0.10 μM in both cases). Building upon this finding, a series of polar 2-aminoethoxy groups was examined in the hope of improving cellular activity (**15**–**21**). It was found that a 2-dimethylaminoexthoxy moiety induced very high levels of ACK1 inhibition in enzymatic and cellular assays for both core structures (**15** and **16**, ACK1 Cell IC_{50} <0.03 μM in both cases). Moreover, the attachment of a 2-pyrrolidinonylethoxy group to the pyrrolopyrimidine scaffold was highly favorable to cellular ACK1 inhibition (**21**, ACK1 Cell IC_{50} = 0.02 μM). In addition, a number of amides were examined (**22**–**27**), and several were shown to be quite potent in the pyrrolopyrimidine series (**23**, **25** and **27**, ACK1 Cell IC_{50} = 0.02–0.04 μM).

Table 2 summarizes the results from a broad survey of C-5 modifications of the pyrrolopyrimidine scaffold. ACK1 inhibition was diminished substantially when the C-5 phenyl moiety was replaced with a cyclopropyl group (**30**), while substituents with a

Table 2
ACK1 inhibition for (*S*)-6-ethylphenyoxy-*N*-((tetrahydrofuran-2-yl)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine with selected 5-position substituents $\mathbf{R}^1$ and 6-ethoxyphenyl substituents $\mathbf{R}^2$



Compound	$\mathbf{R}^1$	$\mathbf{R}^2$	ACKi K_i (μM)[a]	ACKi Cell IC_{50} (μM)[b]
16	Ph	NMe_2	0.006	0.02
19	Ph		0.006	0.06
28		NMe_2	0.01	0.50
29		NMe_2	0.02	0.24
30		NMe_2	1.98	*n.d.*
31			0.04	0.39
32			0.01	0.16
33			0.02	0.16
34			0.004	0.04

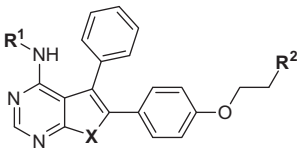
[a] Values are means of at least two experiments.
[b] Average of at least two IC_{50} values.

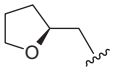
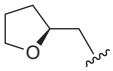
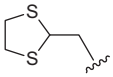
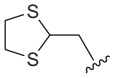
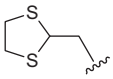
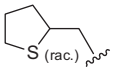
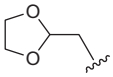
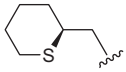
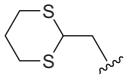
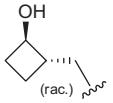
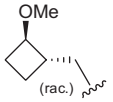
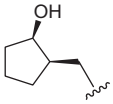
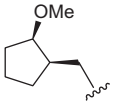
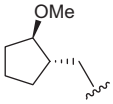
similar ring size to phenyl were tolerated (**28**–**29**). This result may be due to an increase in unfavorable steric interactions between the C-5 and C-6 groups in the case of $\mathbf{R}^1$ = cyclopropyl since early co-crystallographic studies suggested that the C-5 and C-6 aryl substituents are orthogonally disposed, where the C-6 aryl ring adopts a coplanar orientation relative to the pyrrolopyrimidine core.[8] Various electron-rich and electron-poor six-membered aryl and pyridyl groups in this position were also investigated (**31**–**34**), and one compound of this series, *ortho*-fluorophenyl analog **34**, proved to be a potent ACK1 inhibitor.

A wide range of aliphatic substituents $\mathbf{R}^1$ was tolerated on the pyrimidine ring C-4 amino group (Table 3). In general, four and five-membered rings with a methylene linker were well tolerated, while the larger six-membered ring derivatives exhibited reduced ACK1 inhibition. While a (*S*)-tetrahydrofuranylmethyl group was reasonable starting point for both core structures (**15**–**16**), further SAR identified the 1,3-dithiolanylmethyl group as the most potent amino substituent discovered to date (**35**–**37**). Indeed, dithiolane **35** in particular is highly potent in the biochemical assay (K_i = 0.3 nM) as well as the cellular assay (IC_{50} = 5 nM). The exquisite potency of **35** is consistent with molecular modeling results; while the C3-methylene of the (*S*)-tetrahydrofuranylmethyl group was postulated to sterically clash with the L259 residue of ACK1, a dithiolane moiety was predicted to avoid this unfavorable interaction.

Since pyrrolopyrimidine dithiolanes **35** and **37** both displayed particularly excellent levels of ACK1 inhibition, these analogs were viewed as potential candidates for further investigation in tumor xenograft experiments. While in vitro metabolic studies indicated that pyrrolidine **37** was predicted to be significantly more stable

Table 3
ACK1 inhibition for 6-ethylphenyloxy-5-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines and 6-ethylphenoxy-5-phenyl furo[2,3-*d*]pyrimidin-4-amines with selected *N*-substituents $\mathbf{R}^1$ and 6-ethoxyphenyl substituents $\mathbf{R}^2$



Compound	X	$\mathbf{R}^1$	$\mathbf{R}^2$	ACKi K_i (μM)[a]	ACKi Cell IC_{50} (μM)[b]
15	O		NMe_2	0.005	0.03
16	NH		NMe_2	0.006	0.02
35	NH		NMe_2	0.0003	0.005
36	O		NMe_2	0.0002	0.01
37	NH			0.0003	0.01
38	O		NMe_2	0.008	3.00
39	O		NMe_2	0.013	2.00
40	O		NMe_2	0.069	7.16
41	O		NMe_2	0.004	0.55
42	NH		NMe_2	0.03	0.41
43	NH		NMe_2	0.02	0.42
44	NH		NMe_2	0.04	0.46
45	NH		NMe_2	0.05	0.78
46	NH		NMe_2	0.05	1.30

[a] Values are means of at least two experiments.
[b] Average of at least two IC_{50} values.

in rat hepatocytes relative to dimethylamine **35**, unfortunately both **35** and **37** displayed high iv clearances in Sprague–Dawley rats (Table 4). Subsequent in vitro rat liver S9 metabolite identification studies of **35** indicated that extensive oxidation of both the dithiolane ring and the dimethylamino group occurred. In terms of kinase selectivity, compound **37** was reasonably selective (K_i = 0.3 nM) for ACK1 relative to the following related kinases: LCK

Table 4
Sprague–Dawley rat pharmacokinetic parameters and predicted rat hepatocyte clearances of pyrrolopyrimidine **35** and **37** (1 mg/kg iv dose)[a]

Compound	Cl (L/h∗kg)	V_{ss} (L/kg)	MRT (h)	Predicted Cl in rat hepatocytes (μL/min∗10^{-6} cells)
35	7.5	26.6	3.6	2.5
37	6.8	15.6	2.3	0.16

[a] Three animals were used per study.

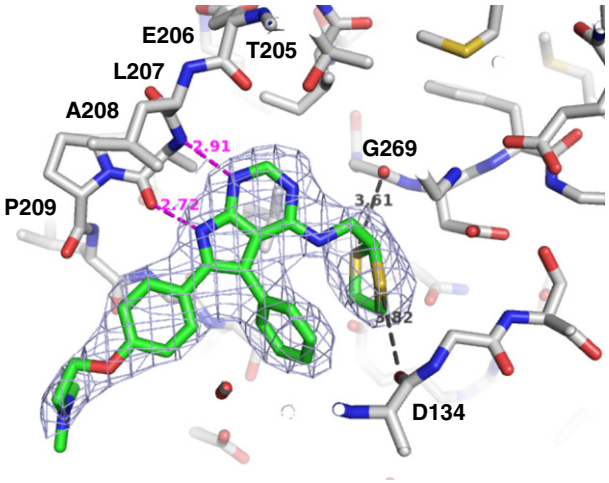
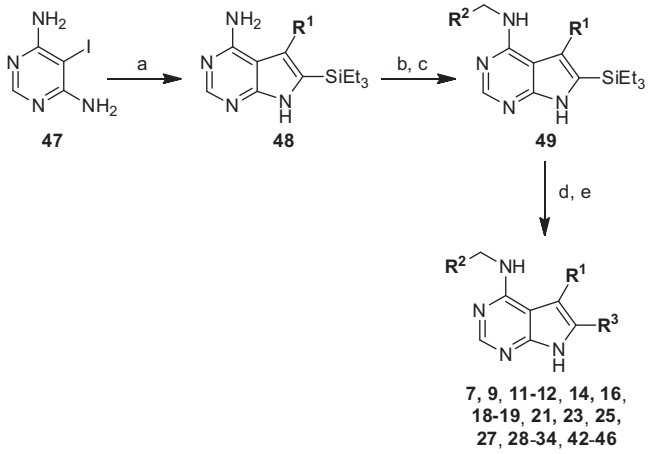


Figure 3. X-ray co-crystal structure of compound **35** bound to human ACK1 kinase domain at 2.5 Å resolution. RCSB file name (4EWH).

(K_i = 138 nM), JAK3 (K_i = 6.5 nM), KDR (K_i = 380 nM), and TIE2 (K_i = 200 nM).

The X-ray co-crystal structure of dithiolane **35** bound to the human ACK1 catalytic kinase domain was obtained at 2.5 Å resolution (Fig. 3). Compound **35** is anchored into the ATP-binding site of the ACK1 protein via two key protein-ligand hinge interactions: the pyrrolopyrimidine NH acts as a hydrogen bond donor to the carbonyl oxygen of A208, while the pyrimidine N-1 accepts a hydrogen bond from the backbone amide NH of L207 (shown in purple dashed lines). The rest of the compound makes numerous van der Waals interactions with the protein, including two close van der Waals contacts between the sulfur atoms of the dithiolane moiety and the carbonyl oxygen atoms of D134 and G269 (shown in grey dashed lines). These dithiolane interactions are postulated



Scheme 1. Reagents and conditions: (a) 1-$\mathbf{R^1}$-2-(triethylsilyl)acetylene (2.5 equiv), Pd(dppf)Cl_2 (0.1 equiv), Na_2CO_3 (2 equiv), LiCl, DMF, 95 °C; (b) $\mathbf{R^2}CO_2H$, EDC, HOBT, DMAP (cat), DMF; (c) $LiAlH_4$, THF, 0–50 °C, 2 h; (d) NIS, DMF, rt to 45 °C; (e) $\mathbf{R^3}B(OH)_2$ (1.5 equiv), Pd(dppf)Cl_2 (0.1 equiv), 2 M aq Na_2CO_3 (2.5 equiv), LiCl (3 equiv), 1:1 toluene/EtOH, 80 °C.

to account for the high levels of biochemical and cellular inhibition observed for **35**.[9] The structure also shows that the 2-dimethylaminoexthoxy group is exposed to solvent.

The formation of indole-type derivatives by the intramolecular palladium-catalyzed Heck reaction has reported.[10] Figure 4 shows our general route used to access the 4-aminopyrrolopyrimidine core structure from 4,6-diamino-5-iodopyrimidine (**47**)[11] via intramolecular Heck chemistry. Indeed, the coupling of an appropriately-substituted 2-triethylsilylalkyne and organopalladium complex **47a** (generated by an oxidative addition of compound **47** with a palladium (0) species) gives the π-alkyne-σ-organo-palladium (0) complex **47b**, which transforms into carbopalladium adduct **47c**. Intermediate **47c** subsequently forms a nitrogen-containing palladacycle **47d** via iodide displacement from the palladium by one of the pendant nitrogen nucleophiles. The palladacycle **47d** then converts to the 6-triethylsilyl-4-aminopyrrolopyrimidine product **47e** by reductive elimination.[12] This method is highly regioselective to form 5-subsituted-6-(triethylsilyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine of general structure **47e** due to coordination effects in the carbo-palladation step.[10,13] Our optimized protocol is as follows: 4,6-diamino-5-iodopyrimidine is treated with a triethylsilylalkyne (2.5 equiv), sodium carbonate (2.0 equiv), LiCl (1 equiv) and 10 mol % of Pd(dppf)Cl_2 in DMF and then heated at 95 °C overnight. The yields of the reaction ranged from good to excellent.

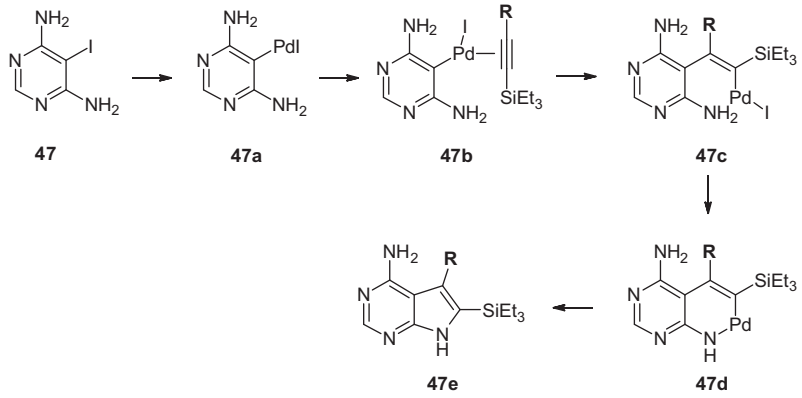
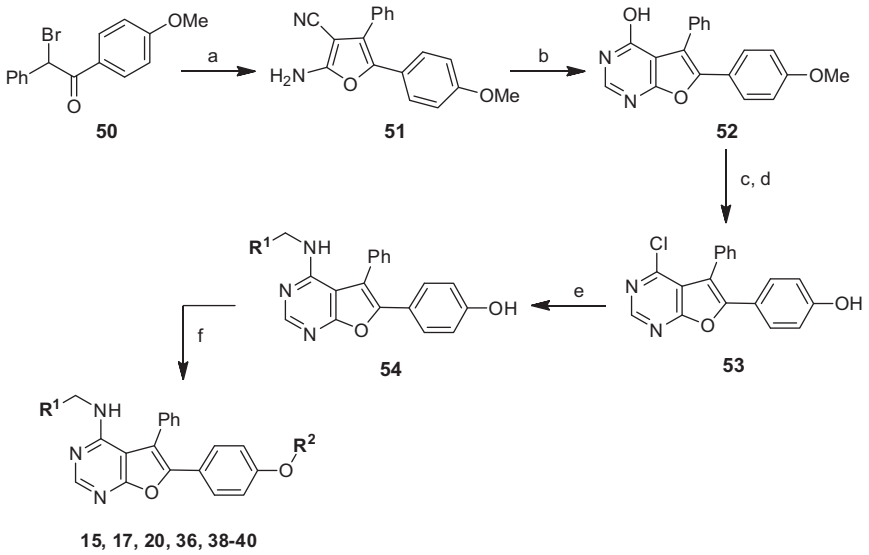
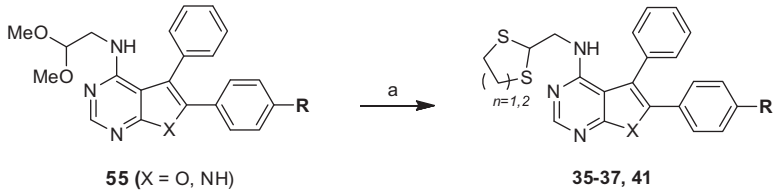


Figure 4. Proposed intermediates in the intramolecular palladium-catalyzed formation of 4-aminopyrrolopyrimidines.



Scheme 2. Reagents and conditions: (a) malononitrile, Et_3N; (b) HCO_2H, reflux; (c) $POCl_3$, 105 °C; (d) BBr_3, DCM; (e) $\mathbf{R^1}CH_2NH_2$, *n*-BuOH; (f) Cs_2CO_3, $\mathbf{R^2}$Br, DMF.



Scheme 3. Reagents and conditions: (a) 1,2-ethanedithiol or 1,3-propanedithiol, p-TsOH, toluene, reflux.

Our general route to ACK1 pyrrolopyrimidine inhibitors is shown in Scheme 1. After palladium-catalyzed pyrrolopyrimidine formation as described above, *N*-monoalkylation of the 4-amino group of amine **48** was achieved by stepwise acylation and reduction to give the *N*-alkylated product **49**. Subsequent transformation of the C-6 triethylsilyl group of **49** into the corresponding iodide followed by a Suzuki coupling with an appropriate arylboronic acid provided the pyrrolopyrimidine analogs listed in Tables 1–3.

Many of the furanopyrimidine analogs with alkoxy substituents attached to the 4-position of the 6-phenyl ring that are listed in Table 1 and Table 3 were prepared by the sequence shown in Scheme 2. Specifically, condensation of acetophenone **50**[14] with malononitrile afforded furan **51** which was further transformed into furanopyrimidine **52** by exposure to refluxing formic acid. Subsequent chlorination, demethylation and *N*-alkylation with an appropriate amine provided amine **54**, which was then *O*-alkylated with an appropriate alkyl bromide in the presence of cesium carbonate to generate the target molecules. Note that 1,3-dithiolane derivatives **35**–**37** and **41** were generated from 1,1-dimethylacetal intermediate **55** by treatment with either 1,2-ethanedithiol or 1,3-propanedithiol and *p*-toluenesulfonic acid in refluxing toluene (Scheme 3). [6a,c]

In conclusion, two novel series of furo[2,3-*d*]pyrimidin4-amines and 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines which exhibit potent in vitro inhibitor activity against ACK1 have been identified and evaluated. 1,3-Dithiolane-substituted pyrrolopyrimidine **37** displays excellent ACK1 cellular inhibition, good kinase selectivity, and a suitable in vitro metabolic profile. Unfortunately, the pharmacokinetic profile of **37** was poor and prevented this inhibitor from being further evaluated in tumor xenograft studies.

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Biochem. **2007**, *367*, 179–189 (b) ACK1 enzymatic assay K_i determination: The assay utilizes a protein expressed in baculovirus infected Hi-5 cells (a fusion of an N-terminal $(His)_6$ Tag with amino acids 117 to 489 of ACK1) purified by affinity chromatography on a Ni-NTA column. The substrate for the reaction is ACK1 itself (autophosphorylation) and poly-Glutamic acid-Tyrosine (PGT (4:1), Sigma catalog #PO275). The PGT is coated to Nunc 96 well plates at 80 mg/mL overnight at 4 °C. The morning after coating, the plates are washed twice, and 80 mL reaction buffer (10 mM Hepes, pH 7.6; 20 mM $MgCl_2$; 75 mM NaCl, 0.125% TWEEN20 (polyoxyethylene sorbitan monolaurate); 1 mM DTT) with 5 μM ATP are added to each well. Test compounds are added in 10 μL DMSO, and the reaction is started by addition of 10 μL kinase in assay buffer. The reaction proceeds 2 h at room temperature. Next, the plates are washed four times, and the level of tyrosine phosphorylation in a given well is quantified by standard ELISA assay utilizing a phosphotyrosine antibody (PY20, Pierce). (c) ACK1 cell based assay IC_{50} determination: The assay is based on the dependence of certain transformed cell lines (e.g. C8 cells, a Ras and E1A transformed fibroblast line) on ACK1 for survival under low serum conditions, whereas other cell lines (e.g. HeLa) do not. This dependency was confirmed utilizing ACK1 specific siRNAs. For this assay, test (C8) and control (HeLa) cell lines are seeded in 96 well tissue culture plates (BD Falcon) at a density of 2 to 4×10^4 in DMEM/F12 (C8) or DMEM (HeLa) with 0.125% FCS in the presence of ACK1 inhibitors (final DMSO concentration is 0.5%, all tissue culture media are from Cellgro). After 20 to 24 h incubation at 37 °C and 5% CO_2, cell viability is determined using the Cytotox One kit (Promega) according to the manufacturer's instructions.

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