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Discovery of Pyrazolo[1,5-*a*]pyrimidine TTK Inhibitors: CFI-402257 is a Potent, Selective, Bioavailable Anticancer Agent

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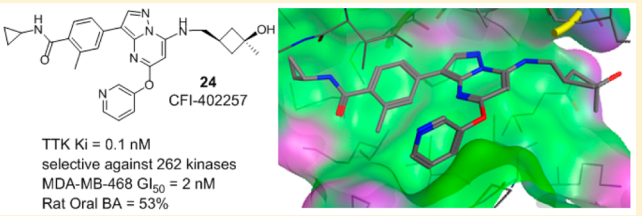
Supporting Information

ABSTRACT: This work describes a scaffold hopping exercise that begins with known imidazo[1,2-*a*]pyrazines, briefly explores pyrazolo[1,5-*a*][1,3,5]triazines, and ultimately yields pyrazolo[1,5-*a*]pyrimidines as a novel class of potent TTK inhibitors. An X-ray structure of a representative compound is consistent with $1^1/_2$ type inhibition and provides structural insight to aid subsequent optimization of in vitro activity and physicochemical and pharmacokinetic properties. Incorporation of polar moieties in the hydrophobic and solvent accessible regions modulates physicochemical properties while maintaining potency. Compounds with enhanced oral exposure were identified for xenograft studies. The work culminates in the identification of a potent (TTK K_i = 0.1 nM), highly selective, orally bioavailable anticancer agent (CFI-402257) for IND enabling studies.

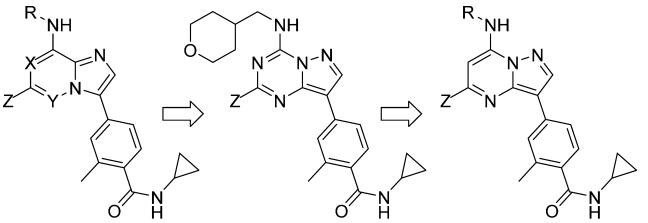


KEYWORDS: *TTK inhibitors, CFI-402257, pyrazolo[1,5-a]pyrimidines, $1^1/_2$ type inhibitors*

Threonine tyrosine kinase (TTK) has been a drug discovery focus[1,2] in our laboratory since it was identified by an internal RNAi screen.[3] The growth of 7 of 12 breast cancer cell lines was inhibited, and subsequent soft agar experiments showed that TTK siRNA inhibits colony size and number. These effects are associated with a disabled mitotic checkpoint, resulting in chromosome segregation errors, aneuploidy, and cell death.[3] In vivo, interfering RNA meditates growth suppression of breast cancer xenografts.[3] These results are supported by the fact that TTK is an essential chromosomal regulator[4,5] and is overexpressed in aneuploid tumors.[6−10] High TTK levels correlate with a high tumor grade[11] and poor patient outcomes.[6]

The potential of this anticancer target is underscored by the large number of inhibitors disclosed.[1,2,12−18] We pursued indazole based inhibitors which culminated in the identification of a preclinical candidate.[2] The role of TTK is supported by proof-of-concept studies wherein inhibitors display cell growth inhibition in vitro and tumor growth inhibition in vivo.[1,2,13−19] Moreover, several laboratories have selected TTK inhibitors for clinical trials.[18,19] Our continued interest in TTK inhibitors led to a scaffold hopping exercise (Chart 1). The imidazo[1,2-*a*]pyrazines, originally disclosed in patent applications,[20,21] were the starting point for the pyrazolo[1,5-*a*][1,3,5]triazines.

Chart 1. TTK Inhibitor Scaffold Hopping



These inhibitors possess desirable attributes including biochemical potency, cell activity, and oral bioavailability (*vide infra*). However, rodent pharmacokinetic (PK) data indicates that certain members suffer from dissolution limiting exposure. To address this problem a further variation on the theme was

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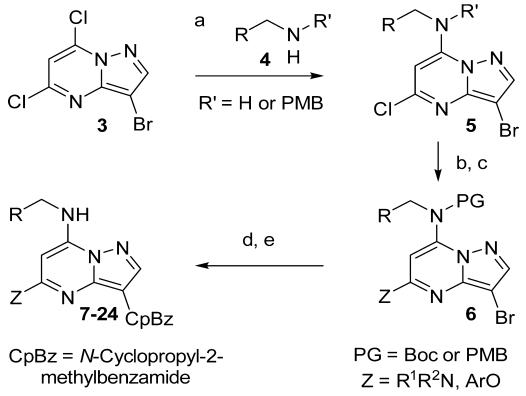
examined. Inspired by the imidazo[1,2-*b*]pyridazines[20−22] we reasoned that a carbon for nitrogen switch at position 3 of the pyrazolo[1,5-*a*][1,3,5]-triazines[23] would result in potent TTK inhibitors (Chart 1), i.e., the pyrazolo[1,5-*a*]pyrimidines described herein. A secondary benefit could accrue in that the basicity and solubility (at low pH) of the latter could be increased relative to the former.

The synthesis of pyrazolo[1,5-*a*]pyrimidines (Scheme 1) begins with 3-bromo-5,7-dichloro-pyrazolo[1,5-*a*]pyrimidine **3**

Scheme 1. Synthesis of Pyrazolopyrimidines[a]



[a]Reagents and conditions: (a) DCM, DIPEA, 0 °C, 84−100%; (b) Boc2O, TEA, DCM, 40−100%; (c) R1R2NH, DIPEA or ArOH, K2CO3, 46−100%; (d) N-cyclopropyl-2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide, Pd(dppf)Cl2·CH2Cl2, K3PO4, H2O, THF; (e) TFA, DCE or TFA, DCM, 10−68% over 2 steps.

and proceeds by sequential displacement of halides. Intermediate **5** is obtained by nucleophilic aromatic substitution by amine **4** at the 7-position followed by protection of the nitrogen, as required. Subsequent displacement at the 5-position is affected by amines or aryloxy anions to give secondary anilines or ethers, respectively. Intermediate **6** is subjected to Suzuki−Miyaura coupling and deprotection to yield the desired products **7**−**24**.

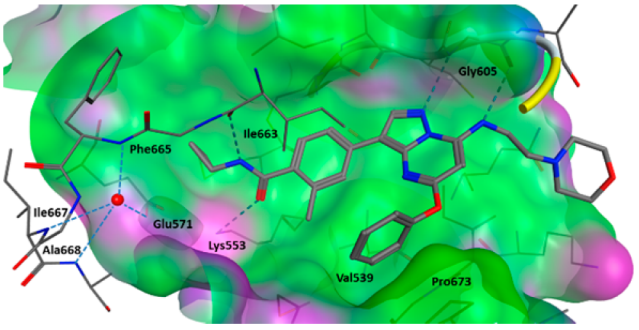


Figure 1. TTK/compound **9** cocrystallized X-ray structure (PDB: 4ZEG) with intermolecular H-bonds indicated.

Pyrazolopyrimidines **7** and **8** were compared to their respective pyrazolotriazine congeners **1** and **2** (Table 1). The former pair was comparable to the latter in potency (IC_{50}) with much improved cell activity (GI_{50}) and higher oral exposure in mice. Nonetheless, it appears that the bioavailability of compound **7** is dissolution limited as we were unable to achieve plasma levels high enough to reach a maximum tolerated dose (MTD). Accordingly, a lead optimization program designed to modify the physicochemical properties was initiated. Related work[24] had demonstrated the strict requirement for the *N*-cyclopropyl-2-methylbenzamide at position 3 of the imidazo-[1,2-*b*]pyridazine inhibitors, ruling out further modifications here. Therefore, we modulated physicochemical properties by incorporating polar, basic, solubilizing elements into the hydrophobic and solvent exposed areas of the compounds.

To this end we applied the strategy of appending a moderately basic morpholino function at the solvent accessible region. Compounds **9** and **10** were potent TTK inhibitors, but only the latter matched the inhibition of cancer cell growth achieved by **7**. Compound **10** was orally bioavailable in mice (Table 1) and displayed a dose-dependent increase in plasma concentrations; an MTD of 6.5 mg/kg QD was established.

In an effort to better understand the TTK binding interactions of our pyrazolo[1,5-*a*]pyrimidine inhibitors, a cocrystallized X-ray structure was obtained with compound **9** (Figure 1). A unique feature of this structure is a bound water not previously observed.[1,2] The molecule is positioned to interact with NHs of the activation loop and the side chain of Glu571, but its role is uncertain in that the angles for the formation of hydrogen bonds are not ideal. The phenyloxy ring makes extensive hydrophobic interactions with Pro673 and the side chain of Val539.

The cyclopropylbenzamide carbonyl and NH coordinate with the side chain of Lys553 and Ile663 carbonyl, respectively. This indicates a highly structured activation loop and is consistent with the strict requirement for the cyclopropane amido group in this region. Two hydrogen bonds from the anilino and pyrazolo nitrogens of **9** to the hinge (Gly605) are observed. The morpholino group is in a solvent exposed region suggesting that polar substituents will be tolerated here.

Further inspection of the X-ray structure indicated that the "hydrophobic region", occupied by Z, was bounded by polar residues. This pocket corresponds to the ribose binding region of the enzyme, suggesting that polar moieties would be tolerated. Entries **11**−**16** in Table 1 explore the effect of introducing hydroxy alkyl amino hydrophobes. Compound **11** was significantly less potent than **8**, but the SAR predicted that we could increase potency by incorporating additional hydrophobic bulk. This expectation was realized (compounds **12**−**14**) and optimized by judicious selection of stereochemistry (cf. **13** and **14**). Further variations on this theme yielded inhibitors **15** and **16** with equivalent enzymatic potency. However, the former suffered from reduced cell activity and oral exposure.

A third approach to modulating physicochemical properties while maintaining in vitro potency and bioavailability was pursued. Hydroxyl functions (e.g., cyclohexanol) are added to the solvent accessible region of the molecule. Simultaneously, a weak base (pyridyl) is incorporated into the aromatic hydrophobe. Both stereoisomers, **17** and **18**, are potent TTK inhibitors but only the cis isomer **17** demonstrated desirable cell activity and oral exposure. Unfortunately **17** was rapidly converted to the trans isomer in vivo.

Isomerization was not seen in the cyclobutane analogues **19** and **20** but at the cost of significantly lower oral exposure. We postulated that oral bioavailability would be increased by morphing the hydroxyl cyclobutanes to tertiary alcohols as is found in compounds **21**−**24**. In fact, these compounds are potent inhibitors of TTK; the cis congeners **23** and **24** proved to be the most orally bioavailable in mice. The latter also had the best activity against our sentinel panel of cancer cell lines.

Head to head, the solubilities of the hydroxyl containing compounds, at physiologic pH, were not higher than their tetrahydropyranyl counterparts. However, with the incorpo-

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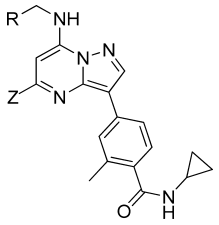
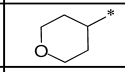
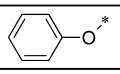
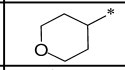
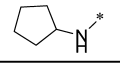
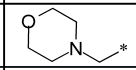
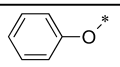
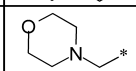
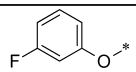
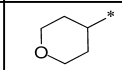
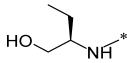
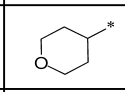
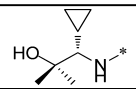
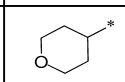
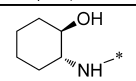
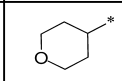
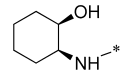
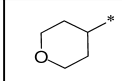
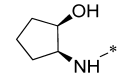
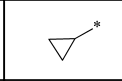
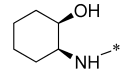
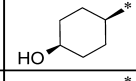
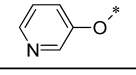
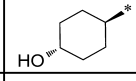
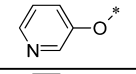
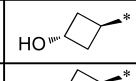
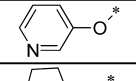
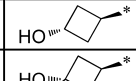
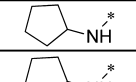
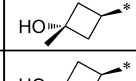
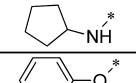
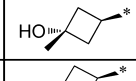
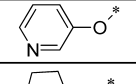
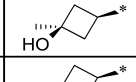
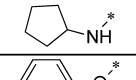
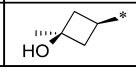
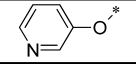
Table 1. In Vitro and in Vivo Data for Pyrazolo[1,5-*a*][1,3,5]triazine and Pyrazolo[1,5-*a*]pyrimidine TTK Inhibitors



Entry	R	Z	TTK IC_{50} (nM)	Cancer Cell GI_{50} (μM)			ACD Calc. Physicochemical Properties[25]		Mouse PK (@10 mg/kg PO)	
				MDA-MB-468	HCT116	OVCAR-3	Sol. (μg/mL) pH 7.4/2.0	pKa	Cmax (μg/mL)	AUC (μg.h/mL)
1	Not applicable		1.4	0.16	0.014	0.19	1.1/1.5	1.6	0.89	1.5
7			2.5	0.013	0.003	0.022	0.5/12	3.4	0.56	3.1
2	Not applicable		5.8	0.32	0.13	0.48	3.6/28	2.7	0.43	0.53
8			3.2	0.036	0.015	0.09	3.1/88	3.2	2.3	4.0
9			3.0	0.018	0.011	0.024	1.6/12,000	6.7	1.0	2.7
10			2.2	0.02	0.001	0.26	1.5/11,000	6.7	1.2	3.6
11			21	0.48	0.21	1.1	27/1,100	3.5	ND	ND
12			7.0	0.06	0.02	0.08	10/470	3.5	5.5	20
13			13	0.18	0.13	0.34	13/410	3.3	ND	ND
14			2.0	0.003	0.001	0.009	13/410	3.3	2.2	2.4
15			2.7	0.062	0.059	0.079	23/480	3.2	1.3	1.5
16			2.2	0.02	0.009	0.015	5.3/170	3.4	1.7	3.1
17			1.8	0.019	0.017	0.043	2.1/360	3.7	0.75	3.4
18			2.8	0.16	0.05	0.27	2.1/360	3.7	0.19	0.70
19			1.1	0.002	0.002	0.011	5.4/930	3.7	0.12	0.13
20			2.4	0.035	0.014	0.068	5.4/930	3.7	0.18	0.13
21			5.3	0.13	0.049	0.21	3.6/110	3.2	ND	ND
22			2.4	0.071	0.04	0.10	3.3/560	3.7	1.8	3.9
23			2.6	0.057	0.014	0.087	3.6/110	3.2	2.9	7.3
24			1.7	0.002	0.002	0.004	3.3/560	3.7	2.5	7.5

ration of weak bases, increases in solubility were manifest at the acidic pH of the gastric milieu (Table 1) and allowed for the preparation of salts.

We chose four potent compounds with good oral exposure for further evaluation (Table 2). They were tested against a panel of human kinases at 1 μM (Table S3). Compound **10** was the least

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Table 2. In Vitro Data, Rat and Dog PK, and Mouse Xenograft Results for Selected TTK Inhibitors

study\entry		10	12	16	24
kinases selectivity[a]		6/262	1/262	4/262	0/262
CYP 3A4 IC_{50} (μM)		0.78	3.0	2.0	0.51
rat PK[b]	Cl (mL/min/kg)	34	66	22	14
	PO AUC_{INF} (h·μg/mL)	1.9	0.49	3.7	3.4
	BA (%)	77	38	94	53
dog PK[b]	Cl (mL/min/kg)	29	13	7.9	4.9
	PO AUC_{INF} (h·μg/mL)	2.2	6.9	14	25
	BA (%)	77	98	95	114
HCT116/HT29 xenografts % TGI @ MTD (mg/kg)[c]		72/41 @ 6.5	70/38 @ 15	72/53 @ 15	68/60 @ 6.5

[a]Number with >50% inhibition @ 1 μM, [ATP] = K_m. [b]IV dose: 1 mg/kg. PO dose: 5 mg/kg. [c]Results at day 21.

selective, yet only inhibited six of the 262 kinases tested (>50% @ 1 μM), whereas compound **24** inhibited none. The high selectivity is attributed, in part, to the $1^1/_2$ type nature of the binding; the *N*-cyclopropyl-2-methylbenzamide group inserts deeply into the ATP binding site of TTK. As illustrated by our X-ray structure, the cyclopropyl-2-methylbenzamide moiety and its binding pocket are highly complementary.

The short listed compounds were tested in a pair of xenograft models at their estimated maximum tolerated dose (MTD). Similar results were obtained for all four compounds in the HCT116 xenograft, i.e., between 68 and 72% tumor growth inhibition (TGI). A wider range of TGI was observed in the HT29 model, but all four compounds had modest responses overall. In contrast the PK parameters determined for dog spanned an order of magnitude. The very high oral exposure seen in mouse with compound **12** did not carry over to rat, the low AUC apparently driven by high clearance. On balance, two compounds (**16**, **24**) distinguished themselves from their comparators (**10**, **12**) with high oral exposure and low clearance in rat and dog.

Given its in vitro potency (TTK IC_{50} = 1.7 nM), exquisite selectivity, and PK parameters, pyrazolo[1,5-*a*]pyrimidine **24** was deemed to have the best balance of properties for subsequent preclinical evaluation. One potential issue, CYP inhibition, was examined in more detail. Compound **24**, had no activity against CYPs 1A2 and 2D6, inhibited 2C9 and 2C19 with IC_{50}s of 13 and 8 μM, respectively. The level of CYP 3A4 activity observed was substrate dependent with IC_{50}s of 0.51 and 14 μM for BFC and DBF, respectively. In comparison, the TTK K_i for compound **24** equals 0.1 nM; however, further studies may be required to understand its potential for drug−drug interactions.

Compound **24** was tested against a panel of cancer cell lines from different pathologies and shown to be a potent inhibitor of cell growth (Table S1). The molecule is an intracellular inhibitor of exogenous TTK autophosphorylation with no effect on Aurora kinase A or B or Histone H3 phosphorylation.[26] This selective inhibitor has the expected mechanism of action in cancer cells and a phenotype consistent with the inhibition of TTK;[26] experimental details will be disclosed in due course.

The performance of compound **24** (designated CFI-402257) in the HCT116 colorectal cancer xenograft model is illustrated in Figure S1. A dose-dependent increase in TGI versus vehicle was observed at day 21: TGI = 39% @ 5.5 mg/kg (p = 0.08) as compared to 68% at 6.5 mg/kg (p = 0.01). There was minimal weight loss (<10%), and no signs of overt toxicity. This level of single agent activity upon daily oral dosing is comparable to published TTK inhibitors.[17,18] On the strength of the cumulative data, CFI-402257 was selected for IND enabling studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00485.

Experimental methods, extended GI_{50} panel (Table S1), X-ray data (Table S2), kinase screen (Table S3), and HCT116 xenograft (Figure S1) (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

ATP, adenosine-5′-triphosphate; AUC, area under the curve; BA, bioavailability; BFC, 7-benzyloxy-4-trifluoromethylcoumarin; Boc, *tert*-butoxycarbonyl; Cl, clearance; CYP, cytochrome P450; DBF, dibenzylfluorescein; DCE, dichloroethylene; DCM, dichloromethane; DIPEA, N,N-diisopropylethylamine; dppf, 1,1′-bis(diphenylphosphino)ferrocene; GI_{50}, half-maximal cell growth inhibitory concentration; IV, intravenous; MTD, maximum tolerated dose; Mps1, monopolar spindle 1; PDB, Protein Data Bank; PG, protecting group; PMB, *p*-methoxybenzyl; PO, per os (by mouth); QD, quaque die (once daily); RNAi, RNA interference; SAR, structure−activity relationship; siRNA, small interfering RNA; TGI, tumor growth inhibition; THF, tetrahydrofuran; TEA, triethylamine; TFA, trifluoroacetic acid; TTK, threonine tyrosine kinase

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