Discovery of 4-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)benzamides as Novel, highly potent and selective, orally bioavailable inhibitors of Tyrosine Threonine Kinase, TTK

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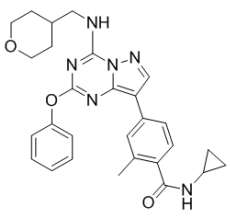
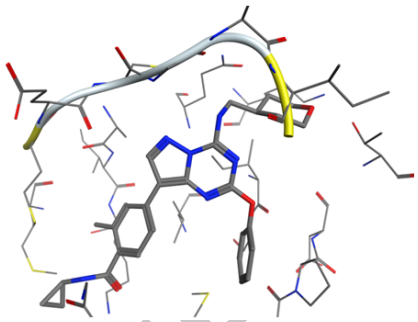


25, CFI-401980
TTK K_i = 0.8 nM
HCT116 GI_{50} = 13 nM
rat PK: oral BA = 97 %



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Discovery of 4-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)benzamides as Novel, highly potent and selective, orally bioavailable inhibitors of Tyrosine Threonine Kinase, TTK.

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ABSTRACT

TTK/Mps1 is a key kinase controlling progression of cell division via participation in the mitotic spindle assembly checkpoint and is overexpressed in a number of human cancers. Herein we report the discovery of 4-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)benzamides as a potent, novel class of TTK inhibitors. The series was identified by means of bioisosteric replacement of the related imidazopyrazine and imidazopyridazine scaffolds. Optimization led to the identification of compounds with excellent potency (K_i = 0.8 nM) and exceptional kinase selectivity. The SAR indicates a strong dependence of activity on the presence of the N-cyclopropyl-2-methylbenzamide moiety delineating the geometry for 1½ type kinase inhibitor. Molecular modeling indicates the extensive and optimal contacts, mediated through H-bonds and hydrophobic interactions, are responsible for the selectivity and potency of the inhibitors. The compounds demonstrate a strong anti-proliferative activity in a panel of human cancer cell lines (HCT116 GI_{50} < 15 nM) and good rodent pharmacokinetics (oral %F 97 %)

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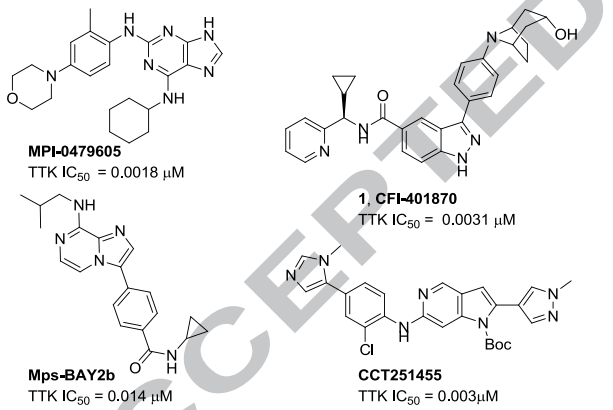
Tyrosine Threonine Kinase (TTK, also known as Monopolar spindle protein 1, Mps1) is a conserved dual-specificity kinase[1] essential for proper distribution of chromosomes to daughter cells during mitosis via the spindle assembly checkpoint (SAC).[2,3] Elevated levels of TTK contribute to abnormal number of chromosomes, a trait referred to as aneuploidy. This characteristics is frequently present in solid tumors[4] and has been identified as predictor of poor prognosis in a number of cancers.[5-8] Furthermore, overexpression of TTK, common in a variety of tumors,[9-13] is correlated with high histological grade, poor patient prognosis and may promote initiation, survival of genomically unstable and aneuploid cancer cells.[9] Inhibition of the kinase activity causes severe chromosome segregation defects that lead to cancer cell death. Recognizing the role of TTK in tumorigenesis, we and other groups have been actively pursuing TTK inhibitors for the treatment of cancer. A number of potent TTK inhibitors have been reported in the literature (Chart 1) and recently the first two examples have entered clinical trials.[14-18]

We have previously disclosed our discovery path to CFI-401870, a small molecule, indazole-based TTK inhibitor that was selected as a preclinical development candidate (Chart 1).[15,19] Having completed our work on the indazole series, we turned our attention to the development of a backup series. We considered a diverse set of scaffolds but were particularity drawn to imidazopyrazines **2** and imidazopyridazine **3** originally disclosed in patent applications by Bayer AG and Oncotherapy Science, Inc. (Charts 2).[20,21] We recognized that these bicyclic ring systems represented relatively unexplored templates for the presentation of TTK binding elements.[16,22]

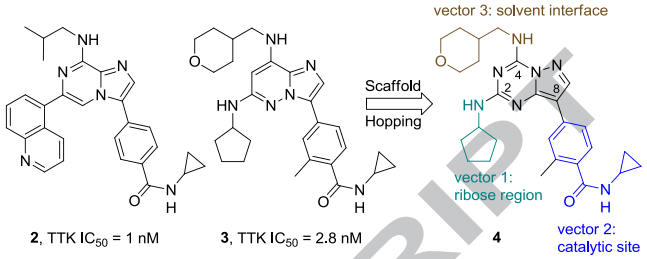
Chart 1. Selected examples of published TTK inhibitors



We selected the pyrazolo[1,5-a][1,3,5]triazin-4-amine scaffold **4** (Chart 2) following analysis of molecular docking results and calculated physicochemical properties of a large set of bioisosteric rings. Based on molecular docking of **4** into the active site of TTK[23] **(Figure 1),** multiple hydrogen bond interactions are predicted between the ligand and the enzyme: two bonds to the hinge residue Gly605 and two to the solvent accessible Asp608 and Thr606. The ligand is also expected to participate in a number of hydrophobic interactions with the amino acid residue side chains: Val539/Ile663, Pro673 and Leu654/Ile531 engage in contacts with the phenyl, cyclopentyl and triazine rings, respectively.

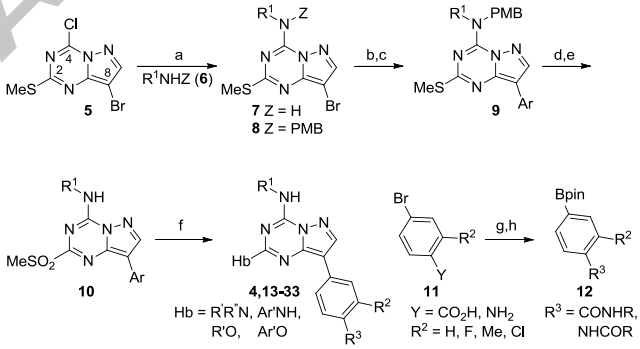
Most intriguing, is the role of the N-cyclopropyl-2-methyl-benzamide moiety. This group inserts deep into the back pocket of the TTK active site; the carbonyl makes an H-bond to the catalytic Lys553 and the cyclopropyl group engages in hydrophobic interactions with Met602 and the Lys553 side chain. Hence, based on the fact that N-cyclopropyl-benzamide formulates type 1½ binding in a restricted back pocket,[24] selective TTK inhibitors were anticipated.

Chart 2. Scaffold hopping exercise yields pyrazolo[1,5-a]triazines



The 4-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)benzamide scaffold, although not previously described, was deemed to be accessible from a synthetic standpoint. We demonstrated that the desired molecules could be synthesized in a highly convergent sequence starting from 8-bromo-4-chloro-2-(methylthio)pyrazolo[1,5-a]-[1,3,5]triazine **5** (Scheme 1). This highly functionalized core was subjected to three key steps: two S_NAr transformations and one cross-coupling reaction. More specifically, a S_NAr displacement of 4-Cl in **5** with amine **6** was followed by a Suzuki-Miyaura cross coupling at C8 position of bicycle **8**.[25]

Scheme 1. Synthesis of pyrazolo[1,5-a][1,3,5]triazines



a. DCM, DIPEA, 0 °C; b. if Z = H then PMBCl, K_2CO_3, DMF; c. ArBpin (**12**), Pd(dppf)Cl_2•CH_2Cl_2, K_3PO_4, H_2O, THF, Δ, μw; d. TFA, DCM; e. mCPBA, DCM; f. R'R"NH, THF, 35 °C (Hb = R'R"N) or Ar'NH_2, NMP, Δ (Hb = Ar'NH) or Ar'OH, DBU, DME, Δ (Hb = Ar'O) or R'OH, NaH, DMF/THF 0 °C to rt (Hb = R'O); g. TBTU, DIPEA, RNH_2 (for Y = CO_2H) or RCO_2H (for Y = NH2); h. Pd(dppf)Cl_2•CH_2Cl_2, B_2Pin_2, KOAc, DMF, Δ

Deprotection of the PMB group and an oxidation of 2-methylthio group in **9** to the corresponding sulfone **10** typically preceded the ultimate S_NAr event leading to fully functionalized inhibitors **(4,13-33)**. Introduction of PMB group was required to enable the Suzuki-Miyaura cross coupling of intermediates **8** and **12**. Most expeditiously the protecting group was incorporated in the amine **6**. The required boronate esters **12** were straightforwardly synthesized from aniline or benzoic acid **11** in an amide coupling followed by a Miyaura borylation step.

We synthesized **4** as a proof of concept inhibitor and were gratified to find that it inhibited TTK at IC_{50} of 5.8 nM with moderate antiproliferative effects in cell culture (Table 1). Based on the binding model, the subsequent lead optimization process focused on three binding vectors: 1. C2-hydrophobic

substitution extending into kinase ribose biding subpocket, 2. C8 phenylcarboxamide, reaching the kinase catalytic site, and 3. C4-group extending from the hinge region to the protein-solvent interface (Chart 2).

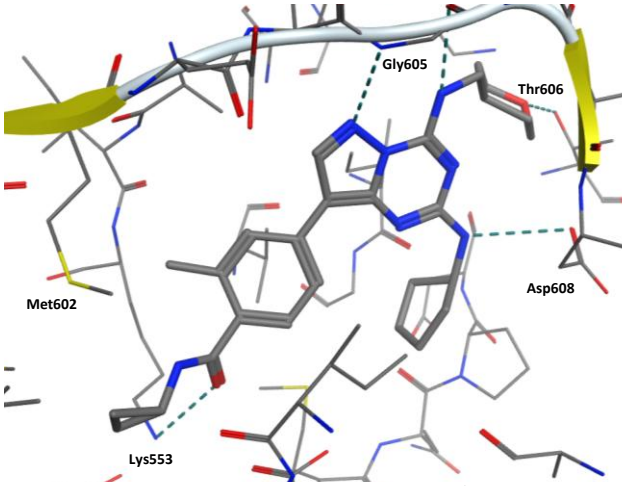


Figure 1. Glide XP-predicted docking pose of compound 4 in a crystal structure of TTK (PDB code: 4O6L)

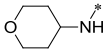
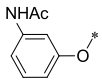
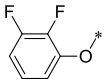
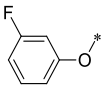
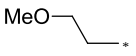
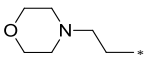
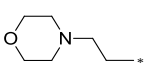
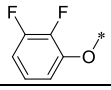
For the C2-substituent, nitrogen could be replaced with oxygen without a loss of activity as demonstrated by **13.** Expansion to six-membered rings was also tolerated, as in examples **14**-**15,** but without a further improvement over **4**. A conservative increase in polarity was acceptable as for example, in tetrahydro-2H-pyran-4-amine **14**. Further substitution of the linking C2-nitrogen was detrimental (e.g. morpholine **17** and N-methyl **16**).

The role of the terminal carboxamide (R^3) is illustrated by comparing compounds **4** and **18**-**21** (Table 1). Of the two possible amide connectivities, carboxamides **18** and **20** are significantly less active than their corresponding benzamide isomers **19** (eightfold) and **4** (350-fold). A loss of an order of magnitude in activity is observed by replacing the cyclopropyl ring in **21** with an isopropyl group, **19**. All together, these findings are consistent with our binding model i.e. two favorable hydrogen bonds to the amide and the existence of a narrow subpocket accommodating the small c-propyl ring.

An increase in activity was observed with aromatic rings at C2, as demonstrated by compounds **23**-**25**. This trio also illustrates the role of the ortho-substituents (R^2) of the C8-benzene ring. A threefold increase in potency for chloro **24** and methyl **25** over the proto congeners **21** and **23** is seen. Notwithstanding similar IC_{50} values for **24** and **25**, the ortho methyl substituted **25** demonstrated greater antiproliferative effects in the panel of human cancer cell lines (Table 1).

The benefit of introducing an aromatic ring at C2 is limited only to ethers as illustrated by a matched pair of C2-aniline **26** and phenoxy **25**. In addition, an attempt to reduce the hydrophobic character of the C2 arylether resulted in a decrease in inhibition; for example, 3-acetamidophenyloxy (**27**) was about an order of magnitude less active than the corresponding phenoxy **25**. Cell activity was more profoundly affected. In contrast, introduction of fluorine substituents on the phenyl ethers afforded compounds with subnanomolar activity (**28**-**30**). However, their measured IC_{50}s must be viewed as apparent values because they approach the nominal concentration of enzyme in the assay.

Table 1. In vitro inhibitory activity of pyrazolo[1,5-a][1,3,5]triazin-8-ylbenzamides **10**.

cpd	R^1	R^2	R^3	Hb	TTK IC_{50} (μM)	Cancer Cell GI_{50} (μM)		
						MDA-MB 231	HCT116	OVCAR-3
4	4-THP-CH_2–*	Me	(C=O)NHc-Pr		0.0058	0.17	0.13	0.48
13	4-THP-CH_2–*	Me	(C=O)NHc-Pr		0.0023	0.23	0.16	0.59
14	4-THP-CH_2–*	Me	(C=O)NHc-Pr		0.009	0.54	0.28	0.78
15	4-THP-CH_2–*	Me	(C=O)NHc-Pr		0.0026	0.27	0.17	0.47
16	4-THP-CH_2–*	F	(C=O)NHc-Pr		0.015	ND	ND	ND
17	4-THP-CH_2–*	Cl	(C=O)NHc-Pr		0.032	0.45	0.44	0.07
18	4-THP-CH_2–*	H	NH(C=O)*i*-Pr		1.7	ND	ND	ND
19	4-THP-CH_2–*	H	(C=O)NH*i*-Pr		0.23	ND	ND	ND
20	4-THP-CH_2–*	Me	NH(C=O)c-Pr		2.0	ND	ND	ND
21	4-THP-CH_2–*	H	(C=O)NHc-Pr		0.010	0.69	0.39	1.7
22	4-THP-CH_2–*	F	(C=O)NHc-Pr		0.007	1.4	0.85	1.7
23	4-THP-CH_2–*	H	(C=O)NHc-Pr	PhO	0.0049	0.35	0.20	0.91
24	4-THP-CH_2–*	Cl	(C=O)NHc-Pr	PhO	0.0017	0.23	0.16	0.40
25	4-THP-CH_2–*	Me	(C=O)NHc-Pr	PhO	0.0014	0.032	0.013	0.19
26	4-THP-CH_2–*	Me	(C=O)NHc-Pr	PhNH	0.011	1.2	0.69	1.7
27	4-THP-CH_2–*	Me	(C=O)NHc-Pr		0.0084	1.6	1.3	1.2
28	4-THP-CH_2–*	Me	(C=O)NHc-Pr		0.0004	0.012	0.009	0.037
29	4-THP-CH_2–*	Me	(C=O)NHc-Pr		0.0007	0.016	0.009	0.058
30	4-THP-CH_2–*	Me	(C=O)NHc-Pr		0.00094	0.047	0.021	0.19
31		H	(C=O)NHc-Pr	PhO	0.014	ND	ND	ND
32		H	(C=O)NHc-Pr	PhO	0.024	0.96	0.88	0.41
33		Me	(C=O)NHc-Pr		0.0022	0.040	0.014	0.17

We noted that several properties were sensitive to subtle changes to the position of fluorine on the ring. The difluoro phenyl **28** and meta monofluorinated compound **29** were equipotent against TTK and cancer cells. However of the two inhibitors, **29** was significantly more active against CYP 3A4

(Table 2). By contrast the ortho monofluorinated compound **30** was not a potent inhibitor of CYP3A4. This advantage also held in comparison to the des fluoro analog **25**.

Table 2. ADME and pharmacokinetic properties of pyrazolo[1,5-a][1,3,5]triazin-8-ylbenzamides.

entry	CYP 3A4 IC_{50} (μM)	microsomal $T_{1/2}$ (min) m/r/h	mouse PK (10 mg/kg)	
			Cmax (μg/mL)	AUC (μg•h/mL)
4	19	14/11/>60	0.43	0.53
25	0.8	46/>60/>60	0.89	1.53
28	>20	28/40/>60	1.60	4.0
29	0.47	30/39/>60	ND	ND
30	9.3	17/43/>60	0.61	1.9

For groups at C4 (R^1), the vector projects towards the protein-solvent interface, this region was not extensively explored. Nonetheless the cyclic N-((tetrahydro-2H-pyran-4-yl)methyl) was preferred over its acyclic variant (e.g. **31** vs **23,** Table 1). For basic solubilizing groups (e.g. morpholinoethyl) over an order of magnitude loss in TTK inhibition was observed (compound **32**). Activity could be recouped in this case by using difluorophenoxy ring as the C-2 hydrophobe, as in entry **33**.

A subset of TTK inhibitors (**4**, **25**, **29**, **30** and **33**) were tested against additional human cancer cell lines from colon, ovary and breast (Table 3). The results show that these molecules were particularly effective against HT-29 and PA-1 and less active against MDA-MB-468. Nonetheless these data indicate a general applicability of this class of inhibitors against a broader spectrum of cancers.

Table 3. Cancer cell growth inhibition (GI_{50}) for selected compounds

GI_{50} (μM)	**4**	**25**	**29**	**30**	**33**
colon HT-29	0.084	0.015	0.007	0.020	0.021
ovarian PA-1	0.053	0.030	0.006	0.017	0.017
breast MDA-MB-468	0.32	0.16	0.23	0.12	0.15

The pharmacokinetic properties of selected compounds were assessed in rodents. In mouse, compound **25** as well as other phenyl ethers (**25**, **28,** **30**, Table 2) displayed high oral exposure. In the rat, high bioavailability (97 %), low clearance (19 mL/(min·kg)) and moderate half-life (1.5 h) were observed for compound **25**. Compound **25** displayed relatively high plasma protein binding (98.4 % in mouse, 97.2 % in human). It should be noted that the linear dose-AUC dependence for **25** only extended to 70 mg/kg effectively thwarting our efforts to establish the maximum tolerated dose, a prerequisite to the in vivo efficacy studies.[26]

Figure 2. Heat map showing % inhibition of 55 human kinases at a screening concentration of 1 μM for compound 25 (bottom row) in comparison to 2 (top). For each kinase [ATP] = Km. Compounds were individually sorted.

A kinetic analysis of TTK inhibitor **25** confirmed the potency (K_i = 0.77 ± 0.5 nM) and ATP competitive nature of the binding.[25] Compound **25** was also tested against a panel of protein kinases and, as illustrated by the heat map in Figure 2, did not inhibit any of the fifty five targets at concentration of 1 μM.[25] In the dose response assays, the compound exhibited IC_{50} greater than 50 μM against PLK4, AURKA, AURKB/INCENP. Based on a cell cycle analysis, we concluded that compound **25** caused massive aneuploidy and cell death, at concentrations between 100 and 200 nM (Figure 3). The compound exhibited low to moderate levels of inhibition of Cyp450 isoforms (Table 2) with over three orders of magnitude separation over TTK activity.

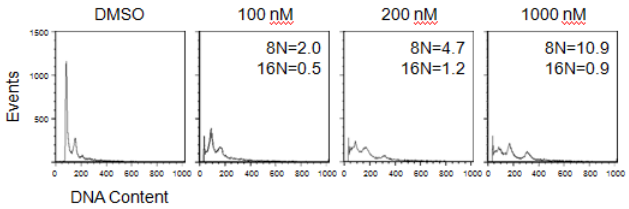


Figure 3. Cell cycle analysis of HCT116 colon cancer cells treated with compound 25 at given concentrations. Percentage of 8 N and 16 N cells, at 48 h, normalized to DMSO control.

In summary, a scaffold hopping exercise led to the discovery of a new class of TTK inhibitor, namely 4-(4-aminopyrazolo[1,5-a][1,3,5]triazin-8-yl)benzamides. The lead optimization of this novel scaffold resulted in preparation of very potent and strikingly selective molecules with good oral exposure in rodents. The strength of these results set stage to our work on related pyrazolo[1,5-a]pyrimidines, culminating in the identification of a development candidate, CFI-402257.[27]

Supplementary Material

Synthetic, analytical methods and data are provided. Inhibition data of 55-member kinase panel for compounds **25**. Steady State kinetics results for compound **25**. This material is available free of charge via the Internet at http://....

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Abbreviations

Ar, aryl; ATP, Adenosine-5'-triphosphate; AUC, area under the curve; BA, bioavailability; CYP, Cytochrome P450; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCM, dichloromethane; DIPEA, diisopropylethylamine; Δ heat; DME, 1,2-dimethoxyethane; DMF, N,N-dimethylformamide; DMSO, dimethyl sulfoxide; dppf, 1,1'-bis(diphenylphosphino) ferrocene; h, hour or human (in MS); GI_{50}, half maximal cell growth inhibitory concentration; Hb, hydrophobe; IC_{50}, half maximal inhibitory concentration; m, mouse; mCPBA, meta-chloroperoxybenzoic acid; μw, microwave irradiation; Mps1, Monopolar spindle protein 1; ND, not determined; PDB,

protein data bank; PK, pharmacokinetics; pin, pinacol; PMB, para–methoxybenzyl; r, rat; rt, room temperature; S_NAr, Nucleophilic Aromatic Substitution; TBTU, *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; THF, tetrahydrofuran; THP, tetrahydro-2H-pyran; TTK, Threonine Tyrosine Kinase.

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