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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%).

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]

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; *m*CPBA, *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-2*H*-pyran; TTK, Tyrosine Threonine Kinase.

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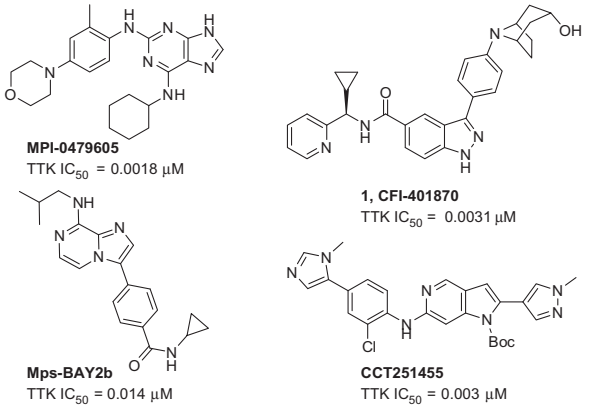


Chart 1. Selected examples of published TTK inhibitors.

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] (Fig. 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.

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]

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

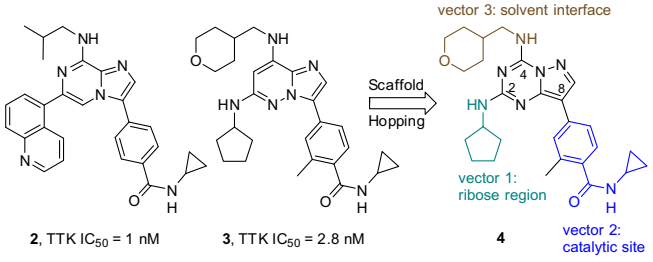


Chart 2. Scaffold hopping exercise yields pyrazolo[1,5-*a*]triazines.

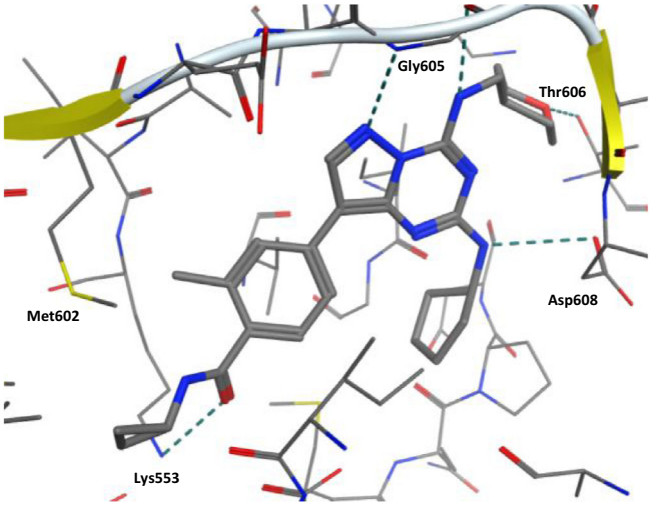
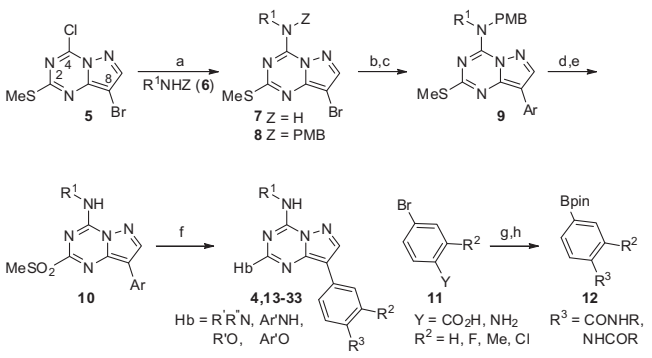


Figure 1. Glide XP-predicted docking pose of compound **4** in a crystal structure of TTK (PDB code: 4O6L).



Scheme 1. Synthesis of pyrazolo[1,5-*a*][1,3,5]triazines. Reagents and conditions: (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) *m*CPBA, 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 = NH_2); (h) Pd(dppf)Cl_2·CH_2Cl_2, B_2Pin_2, KOAc, DMF, Δ.

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).

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-2*H*-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