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Lead optimization of purine based orally bioavailable Mps1 (TTK) inhibitors

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ABSTRACT

Efforts to optimize biological activity, novelty, selectivity and oral bioavailability of Mps1 inhibitors, from a purine based lead MPI-0479605, are described in this Letter. Mps1 biochemical activity and cytotoxicity in HCT-116 cell line were improved. On-target activity confirmation via mechanism based G2/M escape assay was demonstrated. Physico-chemical and ADME properties were optimized to improve oral bioavailability in mouse.

Mitotic kinases such as CDKs, Auroras, and PLKs, which are overexpressed in proliferative cancer cells, play a critical role during cell division[1] and are considered attractive targets for inhibition of cancer cell proliferation. There are several mitotic kinase inhibitors that are currently being investigated in the clinic. In this context, we were interested in developing orally bioavailable inhibitors of the mitotic kinase, Mps1 (also known as TTK). Mps1 kinase is a dual specificity serine/threonine and tyrosine protein kinase[2] and is essential for the proper attachment of chromosomes to the mitotic spindle. Inhibiting Mps1 has been shown to cause chromosomal missegregations followed by cell death.[3] Since we started working on this program, several Mps1 kinase inhibitors were reported in the literature such as Reversine,[4] NMS-P715,[5] MPS1-IN-1,[6] and AZ 3146[7] (Fig. 1).

Recently, we disclosed biological data for one of our earlier Mps1 inhibitors, MPI-0479605 (**1**) (Fig. 2).[3] It has potent activity against the Mps1 enzyme (IC_{50} = 0.004 μM) and good cytotoxicity (HCT-116; IC_{50} = 0.1 μM). Commensurate activity in the mechanism-based G2/M escape assay (EC_{50} = 0.3 μM)[8] suggests that cytotoxicity of this compound is due to inhibition of the Mps1 kinase. Based on the in-house as well as invitrogen kinase profiling it had acceptable selectivity against other kinases.[3]

Significantly, MPI-0479605 dosed intra-peritoneally (IP), demonstrated dose dependent and statistically significant tumor growth inhibition (75% TGI @ 150 mg/kg, Q4DX6; 49% TGI @ 30 mg/kg, QDX15) in HCT-116 xenograft studies in mice.[3] As far as ADME data is concerned, **1** has solubility of 16 μM (pH 7.4)[9] and low metabolic stability in mouse liver microsomes (20% remaining at 40 min)[10] and some permeability in the PAMPA assay ($P_{app} = 400 \times 10^{-6}$ cm/s).[11] This was reflected in low (~20%) oral bioavailability in mouse with solubility limited oral pharmacokinetics.[12]

Docking[13] of compound **1** in the ATP-binding site of Mps1 crystal structure 3H9F reveals a three-point hinge binding motif with hydrogen bond acceptor C=O of hinge *i+1*-th residue Glu603 (where *i*-th residue is the gatekeeper Met602), donor NH of *i+3*-th residue Gly605 and acceptor C=O of Gly605 interaction with two nitrogens of the purine core and with C-6 aniline NH, respectively (Fig. 3). These crucial interactions anchor the molecule in the active site, while orienting the C-6 cyclohexyl ring toward the ribose binding pocket, and the C-2 aniline group towards a largely solvent exposed region of the active site.

Since compound **1** shared structural similarity with Reversine (Fig. 1), a known multi-kinase (including Mps1) inhibitor,[4] we needed to structurally modify compound **1** to improve novelty.

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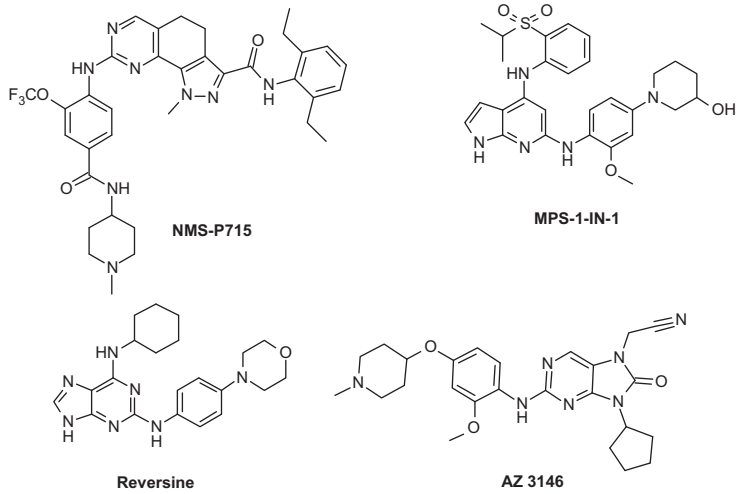
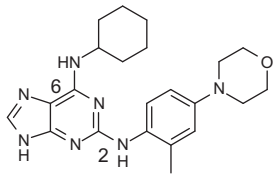


Figure 1. Structures some of the disclosed Mps1 inhibitors.



(1)

Figure 2. Structure of MPI-0479605 (**1**).

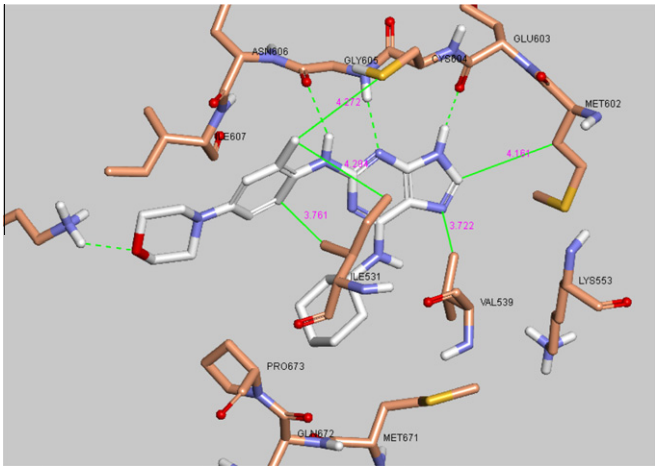


Figure 3. Compound **1** docked in Mps1 crystal structure 3H9F. Protein residues and ligand are displayed as sticks colored by atom type with protein carbons colored gold and ligand carbons colored white.

With **1** as the lead compound, we initiated efforts toward identifying novel, potent, and selective Mps1 kinase inhibitors with improved oral pharmacokinetics.

During this phase of lead optimization efforts, we opted to conserve the three hinge-binding interactions presented by the purine core, and instead focused on specific changes to the C-6 and C-2 positions. Since the primary goal of this optimization campaign was improved oral bioavailability, the effects of substitution on aqueous solubility, metabolic stability and permeability were also assessed.

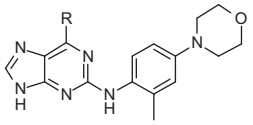
Optimization efforts were begun at the C-6 position and were aimed at identifying a more hydro/amphiphilic replacement for the cyclohexylamino group of compound **1**. Keeping the C-2 group constant as 2-methyl-4-morpholino aniline, systematic changes to the C-6 position were made. The effects of these changes on potency and solubility are summarized in Table 1. Compounds not meeting a 30 nM potency threshold against the Mps1 kinase did not advance into cell-based assays. Introduction of solubilizing groups at the C-6 position, as in compounds **2**, **4** and **7** for example, resulted in significantly diminished activity against Mps1 kinase. Changes to the linking element were similarly deleterious to potency, where N-disubstitution (compounds **5**), and linking through carbon and oxygen, (compounds **3** and **8**, respectively) were not tolerated. Replacement of the cyclohexyl group with short-chain aliphatic, cycloalkyl and aromatic groups led to compounds with significant loss of activity against Mps1 kinase (data not shown). With C-6 proving intolerant to significant change, we settled on cyclohexyl and refocused on modifications to the C-2 aniline moiety as an avenue to improved physico-chemical properties and oral pharmacokinetics.

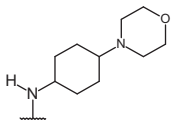
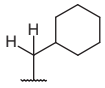
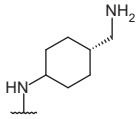
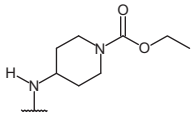
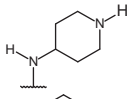
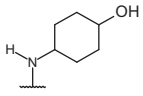
The presence of an *ortho*-substituted aniline at the purine C-2 position is an essential selectivity element of this class of Mps1 inhibitors. This '*ortho* selectivity' effect is illustrated in Table 2 where **1** and des-methyl derivative Reversine **10** were assayed against the related mitotic kinases Aurora A and B, and PLK1. In each instance the selectivity of compound **1** for Mps1 was at least 1000-fold.

The role of the *ortho* substituent in influencing selectivity can be rationalized by molecular modeling studies (Fig. 4). The aniline *ortho* group makes van der Waals contacts with the side chain of hinge residue Cys604, along with the backbone of hinge residue Asn606 and with the δ-methyl group of non-hinge residue Ile531 (see Fig. 3). In Aurora A, respective positions are occupied by residues Tyr212, Pro214 and Leu139.

The steric demands imparted by the Tyr212 hydroxyphenyl group are significantly greater than that presented by the Cys605 thiomethyl group present in Mps1. Tyr212 4-OH group and especially the ring 3-CH position make unfavorable contacts with the aniline *ortho* group. The presence of a proline residue in position *i+4* significantly changes the backbone conformation of hinge residues *i+4* and *i+5* in Aurora A. In addition, the second δ-methyl

Table 1
SAR of the purine C-6 substituent[a]



#	R	Mps1 IC_{50} (μM)	HCT 116 Cytotoxic IC_{50} (μM)	G2/M Chkpt Esc. EC_{50}[8] (μM)	Sol. pH 7.2[9] (μM)
2		0.36			38
3		0.17			19
4		0.035	3.2	2.7	47
5		0.13			23
6		0.028	0.79	1.3	10
7		0.23			69
8		0.014	0.13	0.31	19
9		0.001	0.69	1.5	23

[a] Mean of triplicates values with standard deviation of ±10%.

group of the Leu139 side chain in Aurora A overlaps with the position of *ortho* substituent of compound **1** bound to Mps1 that contains Ile531 residue in the same position (see Fig. 4). As a result the Aurora A ATP binding site does not present a sufficiently large cavity in the hinge loop region to accommodate any heavy atom (non-hydrogen) at the *ortho* position of the C-2 aniline.

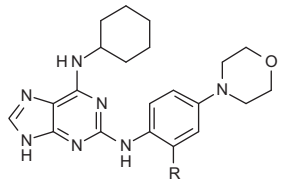
In general, compounds with an *ortho* substituent are expected to demonstrate selectivity for Mps1 versus kinases possessing a large side chain residue (Tyr, Phe) in the *i+3* hinge loop position. For instance Aurora B has active site residues almost identical to those of Aurora A with bulky Phe residue in position *i*+3, which contributes to the observed selectivity for compound **1**. Moderate selectivity is anticipated against kinases containing medium-size side chains (Leu, Ile, Met) at position *i+3*. On the other hand, low sequence identity between Mps1 and PLK1 (25%) explains lack of activity at PLK1 for both Mps1 inhibitors **1** and **10** (see Table 2).

Various *ortho* groups on the aniline moiety were investigated and the results are summarized in Table 3. Relatively small groups such as those present in compounds **1** (methyl), **15** (methoxy) and **16** (ethoxy), were well-tolerated by the Mps1 enzyme and exhibited the desired cellular phenotypes (less than 0.1 μM activity in HCT-116 toxicity and G2/M escape assay). Inhibitors bearing larger substituents, such as, methylsulfone **13** or methyl sulfide **14**, had significantly lower potency. Surprisingly, the trifluoromethoxy and trifluoromethyl derivatives **12** and **17**, demonstrated unacceptably poor cellular activities despite very good biochemical potency (IC_{50} = 0.001 μM and 0.003 μM, respectively). Based on these studies, the *ortho*-methoxy group present in **15** was deemed optimal, and was therefore held constant during subsequent investigations. Not surprisingly, **15** had a similar selectivity profile against related mitotic kinases such as Aurora A & B and PLK1 as observed for compound **1**.

With the optimal *ortho* selectivity element identified, our attention was focused on optimizing the remaining C-2 aniline substituents. We knew from previous studies that only para substituents (to the aniline nitrogen) preserved potency against Mps1. All other regio-isomers explored, di-*ortho* or meta anilino for example, exhibited significantly diminished Mps1 inhibition (by 50- to 100-fold, data not shown). As a result, the search for enhanced physico-chemical properties and oral bioavailability were necessarily focused on modifications to the para-position of the C-2 aniline. To this end, numerous para-position modifications were assessed. It was determined that replacement of the morpholine group, with a substituted piperazine or piperdine, resulted in a series of promising inhibitors, Tables 4 and 5.

Table 4 highlights some of the numerous para-position piperazine analogs that were prepared during this SAR investigation.

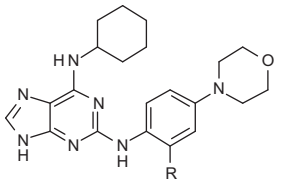
Table 2
Effect of *ortho* substitution on selectivity[a]



	R	Mps IC_{50} (μM)	Aur A IC_{50} (μM)	Aur B IC_{50} (μM)	PLK1 IC_{50} (μM)
10	H	0.004	0.11	0.075	>5
1	Me	0.004	>10	>5	>5

[a] Mean of triplicates values with standard deviation of ±10%.

Table 3
C-2 Aniline *ortho* substitution SAR[a]



	R	Mps1 IC_{50} (μM)	HCT 116 IC_{50} (μM)	G2/M Chkpt Esc. EC_{50} (μM)[8]
1	-Me	0.004	0.1	0.3
12	$-OCF_3$	0.001	0.54	0.38
13	$-SO_2Me$	1		
14	-SMe	0.51		
15	$-OCH_3$	0.004	0.033	0.023
16	$-OCH_2CH_3$	0.001	0.07	0.041
17	$-CF_3$	0.003	0.52	1

[a] Mean of triplicates values with standard deviation of ±10%.

Modifications to the piperazine group were generally well tolerated by the enzyme and had little impact on the Mps1 biochemical and cellular activities suggesting minimal interactions between the protein and this distal portion of the molecule. This assumption is supported by Mps1 docking studies which orient the C-2 aniline, and specifically the para substituents, toward the solvent exposed region of the ATP-binding site. Therefore, we viewed this region as a potential fertile ground for optimization of physico-chemical properties.

Although improvements in solubility were modest, many of the compounds in this series, for example **19**, **20**, **21**, **23**, **25** and **27** demonstrated substantially improved metabolic stability and PAMPA permeability, and these compounds were advanced into mouse PK studies. Compound **22** had good solubility owing to its basic substituent; however, not surprisingly this compound had poor permeability in the PAMPA assay.

Most of the compounds in the piperazine subseries (Table 4) had one or two violations (molecular weight and/or total polar surface area, TPSA) of the Lipinski 'rule of five'.[14] To increase the odds of good oral absorption, we opted to reduce TPSA by replacing the piperazine with a piperidine moiety.

Selected compounds from the piperidine subseries are shown in the Table 5. As mentioned previously, and based on our binding model, substituents in this region of the inhibitor extend into the solvent exposed area of the protein and exert little effect on inhibitory activity. Certain substitutions on the piperidine ring, as exemplified by compounds **30**–**32**, **34** and **36**, resulted in significantly enhanced solubility, PAMPA permeability and metabolic stability. These compounds were advanced into mouse PK studies. Here again, basic amine derivatives, for example **29** and **35**, had good aqueous solubility but at the expense of permeability. Azetidine **37** had lower than desired activity in the mechanism-based assay (G2/M escape assay).

In vitro and mouse xenograft[3] data suggest that a 24 h constant exposure to an Mps1 inhibitor is required for robust responses. With

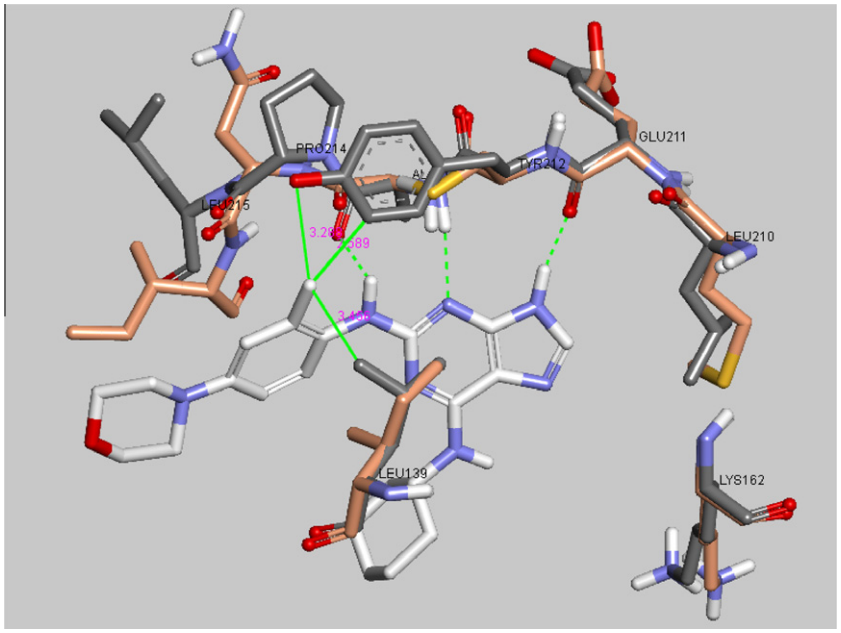
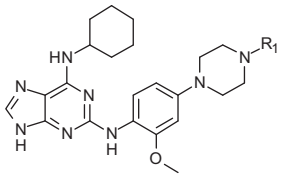
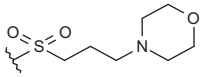
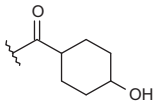
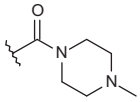
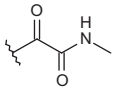
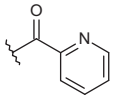
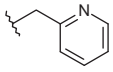
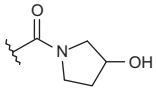
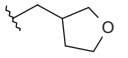


Figure 4. Overlay of ATP binding sites of Mps1 crystal structure 3H9F and Aurora A crystal structure 2NP8 with Compound **1** docked to Mps1. Protein residues and ligand are displayed as sticks colored by atom type with carbons of Mps1 colored gold, carbons of Aurora A colored grey and ligand carbons colored white. Residue labels are shown for Aurora A structure only.

Table 4
C-2 Piperazine substitution SAR[a]



#	R	Mps1 IC_{50} (μM)	HCT 116 Cytotoxic IC_{50} (μM)	G2/M Chkpt Esc. EC_{50}[8] (μM)	Sol. pH 7.2[9] (μM)	PA MPA[b]	MLM stability % rem[c]
18	H	0.001	0.088	0.16	19	NA	NA
19		0.002	0.011	0.001	10	848	77
20		0.002	0.022	0.015	10	725	28
21		0.003	0.015	0.014	19	81	68
22		0.003	0.017	0.018	75	Low	46
23		0.009	0.022	0.029	19	978	50
24		0.003	0.023	0.019	10	NA	17
25		0.009	0.051	0.089	38	976	38
26		0.005	0.027	0.1	19	Low	47
27		0.009	0.066	0.17	5	1874	59

[a] Mean of triplicates values with standard deviation of ±10%.
[b] PAMPA permeability P_{app} 10^{-6} cm/s.[7]
[c] % Remaining in mouse liver microsomal stability assay.

prolonged exposure appearing critical for efficacy, we adopted an abbreviated, mouse PK screening paradigm in which compound plasma levels were evaluated at 4 and 8 h following oral administration at 10 mg/kg.[15] This abbreviated protocol obviated the need for individual IV and oral PK analyses on all compounds meeting the potency and ADME criteria (such as compounds shortlisted for PK studies from Tables 4 and 5). This approach improved the throughput of the compounds going through the PK studies. Table 6 shows the list of compounds along with plasma concentration data at the 4 and 8 h time points.

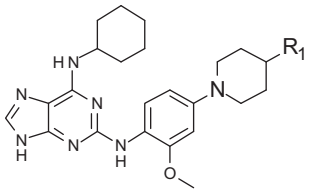
Compounds demonstrating a plasma concentration of less than 10 ng/ml exposure at 8 h were abandoned, since they were not likely to have sufficient exposure to see a biological response. Compound **1** in this study had oral plasma exposure of 105 ng/ml and 72.3 ng/ml at 4 h and 8 h time point respectively. Six Mps1 inhibitors **20**, **28**, **31**, **32**, **33** and **36** (Table 6) demonstrated acceptable plasma levels at the 4 and 8 h time points post oral administration, and these compounds were chosen for more extensive PK studies.[16]

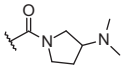
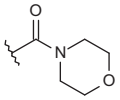
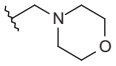
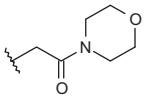
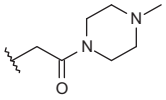
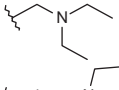
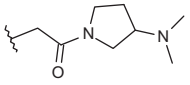
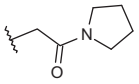
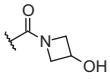
Data from the two point assay format translated well into data obtained from full PK studies (Table 6). Compounds with the highest exposure at the 8 h time point also showed improved bioavailability over Compound **1**. Importantly, compounds **28**, **31** and **36** exhibited oral bioavailability values of 28%, >83%, and 38%, respectively, all of which were improvements over **1**. Compound **31**, which had the highest exposure at the 8 h time point, exhibited slightly higher clearance than desired, but had the highest oral bioavailability (>83%) and a good IV half life in the mouse. As a consequence of these studies, further support for the correlation between PAMPA permeability and oral bioavailability was established. For example, compound **29** had virtually no measurable permeability by PAMPA and it was undetectable in plasma at the 4 and 8 h time points.

A multi-faceted lead optimization strategy resulted in several novel purine-based Mps1 inhibitors with improved physico-chemical properties, for example aqueous solubility, permeability, and oral bioavailability, while maintaining potent and selective activity against the Mps1 target. Details of the efficacy demonstrated by these compounds in tumor xenografts will be communicated in future publications.

The Mps1 inhibitors described in this Letter were prepared according to the synthetic procedures previously described[17] and were adequately characterized.[18]

Table 5
C-2 Piperidine substitution SAR[a]



#	R1	Mps1 IC_{50} (μM)	HCT 116 Cytotoxic IC_{50} (μM)	G2/M Esc. EC_{50}[8] (μM)	Sol. pH 7.2[9] (μM)	PAMPA[b]	MLM stability %[c]
28		0.011	0.022	0.031	10	700	53
29		0.004	0.058	0.077	38	0.0	60
30		0.004	0.05	0.13	19	744	70
31		0.005	0.049	0.12	38	2087	68
32		0.003	0.036	0.07	19	409	61
33		0.005	0.051	0.065	38	530	43
34		0.004	0.074	0.055	19	480	47
35		0.007	0.059	0.097	75	Low	48
36		0.007	0.015	0.026	19	1688	59
37		0.004	0.05	0.26	19	NA	65

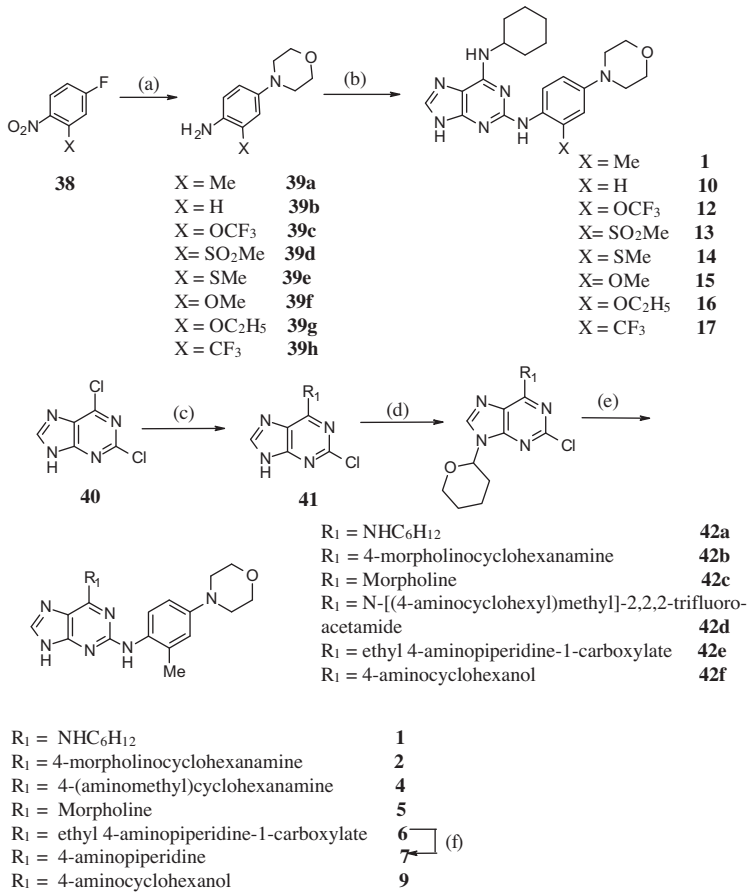
[a] Mean of triplicates values with standard deviation of ±10%.
[b] PAMPA permeability P_{app} 10^{-6} cm/s.[7]
[c] % Remaining in mouse liver microsomal stability assay.

Table 6
Mouse IV and Oral PK parameters

#	4 h Conc. (ng/ml)	8 h Conc. (ng/ml)	IV Cl[a]	IV $T_{1/2}$ (h)	PO AUC[b]	PO C_{max} (μM)	%F
20	155.8	17.5	1032	1.5	1730	1.0	17.9
25	66.3	2.9	NA	NA	NA	NA	NA
28	104.7	44.2	1810	2.2	1714	2.6	31
29	BQL	BQL					
30	16.7	1.68	NA	NA	NA	NA	NA
31	435.2	186.8	1048	2.9	10686	2.6	>83
32	97.04	20.9	1398	0.9	1651	1.4	23.1
33	179.7	39.9	1255	2.8	547	0.3	6.9
36	137.6	69.2	2051	2.0	1774	1.8	36

[a] ml/h/kg.
[b] h.ng/ml.

Briefly, the inhibitors illustrated in Tables 1–3 were prepared according to the synthesis as described in Scheme 1 from appropriate starting materials.[17] Nucleophilic substitution of fluoro group in 4-fluoro-1-nitrobenzene **38** (with suitable *ortho* substituent such as methyl, methoxy etc, next to the nitro group) with various amines such as morpholine followed by palladium catalyzed reduction of nitro group gave desired aniline derivatives **39a–h**. 2,6-dichloropurine **40** was treated under thermal conditions with various amines such as cyclohexyl amine, to get 6-substituted purine derivatives **41**, which upon THP-protection provided key



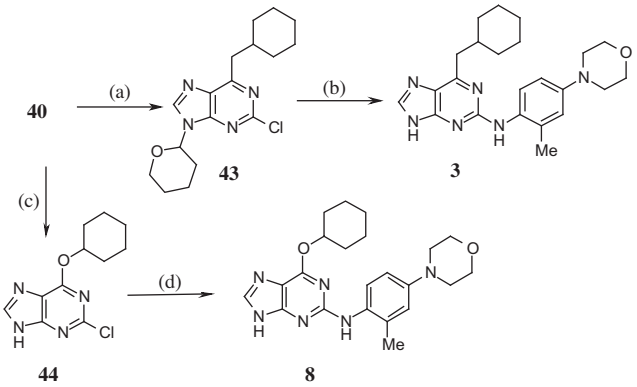
Scheme 1. Reagents and conditions: (a) (i) Morpholine neat, 80 °C, quant.; (ii) 10% Pd/C, MeOH, H_2, balloon, quant.; (b) (i) **42a**, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 80–100 °C, 60–90%; (ii) 4M HCl in 1,4-dioxane, MeOH, RT, 60–80%; (c) Amine, Et_3N, 80 °C, EtOH, 60–80%; (d) dihydropyran (DHP), *p*-TSA (cat), reflux, THF, 50–75%; (e) (i) Aniline **39a**, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 80–100 °C, 60–90%; (ii) 4M HCl in 1,4-dioxane, MeOH, rt, 60–80%; (f) 4 NaOH, MeOH, THF, 65–75%.

intermediates **42a**–**f**. Buchwald coupling of various intermediates **42a**–**f** with aniline derivative **39a** and subsequent THP deprotection under acidic conditions gave the desired products **1**, **2**, **4**–**7** and **9**. On the other hand, compounds **12**–**17** in Table 3 were prepared via Buchwald coupling of aniline derivatives **39b**–**h** with purine intermediate **42a** (Scheme 1) followed by deprotection.

Compound **3** from Table 2 was prepared according to Scheme 2, where Suzuki coupling[19] with cyclohexylmethylboronic acid with THP protected 2,6-dichloro purine gave intermediate **43**. Buchwald coupling of **43** with aniline **39a** furnished desired compound **3**. Compound **8** was obtained via treatment of 2,6-dichloro purine with cyclohexanol in sodium, followed by acid catalyzed C-6 chlorine displacement with aniline **39a** under microwave conditions. Generally, the microwave based acid catalyzed method of introducing aniline group at C-6 position on the purine was less efficient than Buchwald coupling methods.

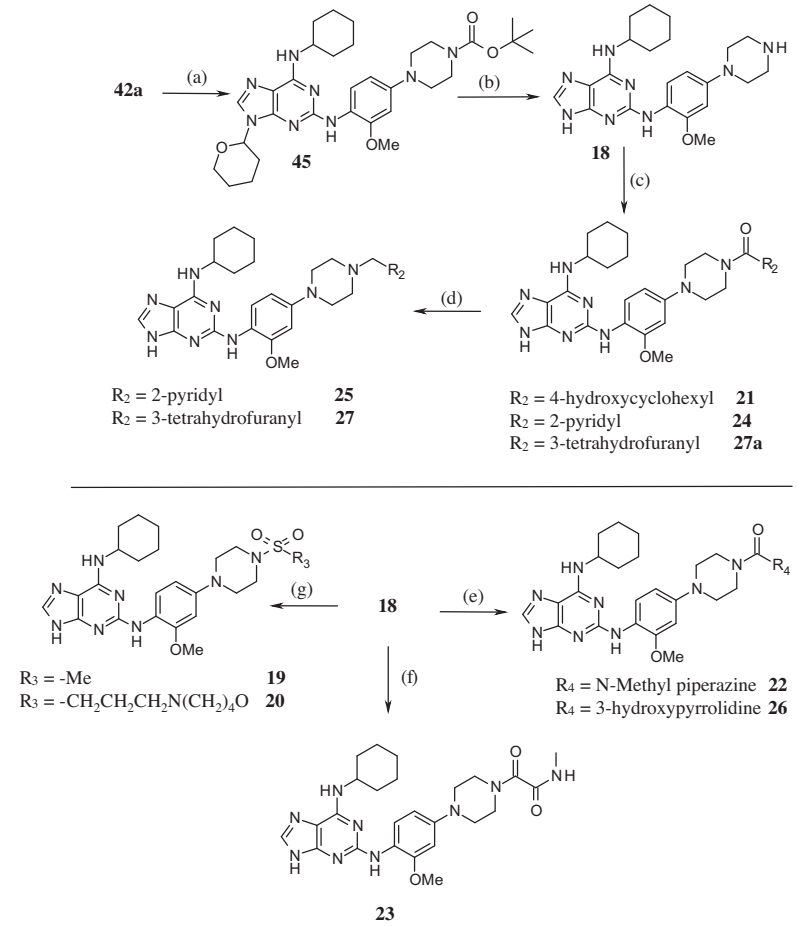
The piperazine analogs shown in the Table 4 were prepared according to Scheme 3. Buchwald coupling of **42a** with *tert*-butyl 4-(4-amino-3-methoxy-phenyl)piperazine-1-carboxylate (prepared employing similar procedure as described for aniline **39f** using *tert*-butyl piperazine-1-carboxylate) followed by deprotection gave key intermediate **18**, which was used for preparing various derivatives shown in the Table 4.

N-linked piperidine analogs shown in the Table 5 were prepared according to Scheme 4. Compound **28** was obtained via Buchwald coupling of 2-methoxy-4-(4-morpholino-1-piperidyl)aniline with purine derivative **42a**. Key intermediate **46** was

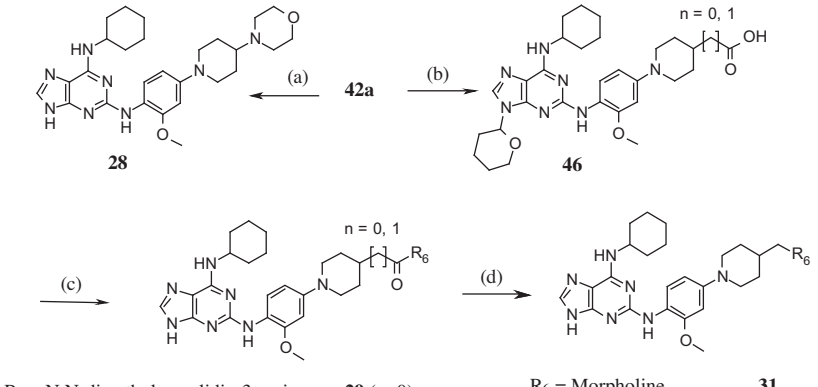


Scheme 2. Reagents and conditions: (a) (i) Dihydropyran (DHP), *p*-TSA (cat), reflux, THF, 75%; (ii) cyclohexylmethylboronic acid, $Pd(PPh_3)_4$, dioxane, 80–110 °C, 55%; (b) (i) Aniline **39a**, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 80–100 °C, 60–70%; (ii) 4M HCl in 1,4-dioxane, MeOH, 80%; (c) cyclohexanol, Na, 90 °C, 53%; (d) **39a**, *p*-TSA (cat), microwave, 135–150 °C, 30 min, 12–30%.

obtained similarly using aniline derivative with appropriate piperidine carboxylic acid ester in the coupling reaction. Finally, amide coupling of acid **46** with various amines such as morpholine followed by hydrolysis of the THP protecting group gave the desired amides **29**, **30**, **32**, **33**, **35**, **36** and **37**. Some of these amides were reduced with $LiAlH_4$ to give aminoalkyl piperadine derivatives such as **31** and **34**.



Scheme 3. Reagents and conditions: (a) *tert*-butyl 4-(4-amino-3-methoxy-phenyl)-piperazine-1-carboxylate, BINAP, $Pd(OAc)_2$, Cs_2CO_3, toluene, 90 °C, 74%; (b) 4M HCl in 1,4-dioxane, MeOH, 80%; (c) Acid, EDCI, HOBt, DIEA, DMF; (d) $LiAlH_4$, THF, rt; (e) Amine, *p*-nitro phenylchloroformate, Et_3N, NMP or Isocyanate, DIEA, DMF, 50–70%; (f) 2-methylamino-2-oxo-acetic acid, EDCI, HOBt, DMF, 65%; (g) sulfonyl chloride, DIEA, DMF, 50–68%.



Scheme 4. Reagents and conditions: (a) (i) 2-Methoxy-4-(4-morpholino-1-piperidyl)aniline, $Pd(OAc)_2$, BINAP, Cs_2CO_3, 80–100 °C, toluene, 60–80%; (ii) 4M HCl 1,4-dioxane, MeOH, 75%; (b) (i) methyl 2-(4-piperidyl)acetate or methyl piperidine-4-carboxylate, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 80–100 °C, 60–75%; (ii) 4M NaOH, MeOH, 50 °C, THF, 70–80%; (c) (i) Amine, DIEA, HATU, DMF, 60–75%; (ii) 4M HCl 1,4-dioxane, MeOH, 75%; (d) $LiAlH_4$, THF, 30–70%.

Acknowledgments

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References and notes

1. (a) Musacchio, A.; Salmon, E. D. *Nat. Rev. Mol. Cell Bio.* **2007**, *8*, 379; (b) Liu, S. T.; Chan, G. K.; Hittle, J. C.; Fujii, G.; Lees, E.; Yen, T. J. *Mol. Biol. Cell* **2003**, *14*, 1638; (c) Malumbres, M. *Physiol. Rev.* **2011**, *91*(3), 973.
2. (a) De Cárcer, G.; Pérez de Castro, I.; Malumbres, M. *Curr. Med. Chem.* **2007**, *14*(9), 969; (b) Malumbres, M.; Barbacid, M. *Curr. Opin. Genet. Dev.* **2007**, *17*, 60; (c) Kang, J.; Yu, H. *J. Biol. Chem.* **2009**, *284*(23), 15359.
3. Tardiff, K. D.; Roger, A.; Cassiano, J.; Roth, B. L.; Cimbora, D. M.; McKinnon, R.; Peterson, A.; Douce, T.; Robinson, R.; Dorweiler, I.; Davis, T.; Hess, M. A.; Ostanin, K.; Papac, D. I.; Baichwal, V.; McAlexander, I.; Willardsen, J. A.; Saunders, M.; Hoarau, C.; Kumar, D. V.; Wettstein, D. A.; Carlson, R. O.; Williams, B. L. *Mol. Cancer Ther.* **2011**, *10*, 2267.
4. Santaguida, S.; Tighe, A.; D'Alise, A. M.; Taylor, S. S.; Muscchio, A. *J. Cell Biol.* **2010**, *190*, 73.
5. Caldarelli, M.; Angiolini, M.; Disingrini, T.; Donati, D.; Guanci, M.; Nuvoloni, S.; Posteri, H.; Quartieri, F.; Silvagni, M.; Colombo, R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4507.
6. Kwiatkowski, N.; Jelluma, N.; Fillippakopouls, P.; Soundararajan, M.; Manak, M. S.; Kwon, M.; Choi, H. G.; Sim, T.; Deveraux, Q. L.; Rottmann, S.; Pellman, D.; Shah, J. V.; Kops, G. J. P. L.; Knapp, S.; Gray, N. S. *Nat. Chem. Biol.* **2010**, *6*, 359.
7. (a) Hewit, L.; Tighe, A.; Santaguida, S.; White, A. M.; Jones, C. D.; Musacchio, A.; Green, S.; Taylor, S. S. *J. Cell Biol.* **2010**, *190*, 25; (b) Lan, W.; Cleveland, D. W. *J. Cell Biol.* **2010**, *190*, 21; (c) Maciejowski, J.; George, K. A.; Terret, M. E.; Zhang, C.; Shokat, K. M.; Jallepalli, P. V. *J. Cell Biol.* **2010**, *190*, 89.
8. *G2/M Checkpoing Escape Assay:* To measure phosphohistone H3 (pHH3), nocodazole-arrested cells were treated for 4 h with MPI-0479605. Cells were fixed with 4% paraformaldehyde for 15 min at 37 °C, permeabilized in 0.2% Triton-X 100 for 5 min, incubated with primary antibodies (rabbit polyclonal anti-phosphohistone H3 (diluted 1:200; Upstate) and mouse monoclonal anti-β-tubulin (diluted 1:2000; Sigma)) for 45 min at 37 °C, followed by a 45 min incubation with Alexa Fluor-conjugated secondary antibodies (Invitrogen). Cells were subsequently stained with Hoechst 33342 dye. The percentage of pHH3-positive cells was quantified by image analysis (BD Pathway High Content Imaging system).
9. Kinetic solubility measurements were obtained using pION instrument at pH 7.4.
10. % Of compound remaining after 40 min of incubation in mouse liver micosomes at 1 μM and the quantitation was done by mass spectrometry.
11. Permeability measurements were obtained using PAMPA assay.
12. Lead identification efforts leading up to MPI-0479605 including its mouse oral PK data will be disclosed in the future publications.
13. *Molecular docking studies were performed using Schrodinger program Glide:* Glide 5.0 User Manual, Schrodinger Press, Schrodinger, L.L.C.: New York, NY 2008. Crystal structure of Mps1 complex with a small molecule inhibitor (PDB ID 3H9F, Ref.[6]) solved with resolution 2.60 Å was used as a target for docking. Docking model of Mps1 complex with compound **1** was further refined using Schrodinger Induced Fit Docking protocol: Sherman, W.; Day, T.; Jacobson, M.P.; Friesner, R.A.; Farid, R.; *J. Med. Chem.*, **2006**, *49*,534–553. The images were made with Accelrys Discovery Studio Visualizer, release 3.2, Accelrys Software Inc., San Diego, USA, 2011.
14. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3.
15. Compounds were dosed in DMA/PEG 400 solution formulation orally in CD-1 mice at 10 mg/kg and the plasma concentrations were determined at 4 and 8 h time points.
16. IV PK: CD-1 mice were dosed in DMA/PEG 400 at 2.5 mg/kg and PO PK: CD-1 mice were dosed in DMA/PEG 400 at 10 mg/kg.
17. Kumar, D. V.; McAlexander, I; Bursavich, M.; Hoarau, C.; Slattum, P.; Gerrish, D.; Lockman, J.; Judd, W.; Saunders, M.; Parker, D.;. Zigar, D.; Kim, I.; Willardsen, J.; Yager, K.; Shenderovich, M.; Williams, B.; Tardiff, K. WO 2010/111406.
18. *All the compounds reported in the paper were purified by reverse phase HPLC and characterized by NMR and HRMS. Characterization data for compound* ***31****:* ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.50 (s, 1H), 8.19 (s, 1H), 7.74 (s, 1H), 6.71 (s, 1H), 6.57 (s, 1H), 3.98 (d, J = 12.8 Hz, 2H), 3.96 (s, 1H), 3.84 (s, 3H), 3.74 (t, J = 11.2 Hz, 4H), 3.48 (d, J = 12 Hz, 2H), 3.10 (m, 4H), 2.72 (s, 1H), 2.0–1.3 (m, 17H). Mass Spec.: Calculated: 521.3347 (M+H) and found 521.3348 (M+H).
19. Molandar, G. A.; Yun, C. S. *Tetrahedron* **2002**, *58*, 1465.