Introduction of a Methyl Group Curbs Metabolism of Pyrido[3,4-d]pyrimidine Monopolar Spindle 1 (MPS1) Inhibitors and Enables the Discovery of the Phase 1 Clinical Candidate N^2-(2-Ethoxy-4-(4-methyl-4H-1,2,4-triazol-3-yl)phenyl)-6-methyl-N^8-neopentylpyrido[3,4-d]pyrimidine-2,8-diamine (BOS172722)


1 Cancer Research UK Cancer Therapeutics Unit at The Institute of Cancer Research, London SM2 5NG, United Kingdom
2 Division of Structural Biology, The Institute of Cancer Research, London SW3 6JB, United Kingdom
3 The Breakthrough Breast Cancer Research Centre, Division of Breast Cancer Research, The Institute of Cancer Research, London SW3 6JB, United Kingdom

ABSTRACT: Monopolar spindle 1 (MPS1) occupies a central role in mitosis and is one of the main components of the spindle assembly checkpoint. The MPS1 kinase is an attractive cancer target, and herein, we report the discovery of the clinical candidate BOS172722. The starting point for our work was a series of pyrido[3,4-d]pyrimidine inhibitors that demonstrated excellent potency and kinase selectivity but suffered from rapid turnover in human liver microsomes (HLM). Optimizing HLM stability proved challenging since it was not possible to identify a consistent site of metabolism and lowering lipophilicity proved unsuccessful. Key to overcoming this problem was the finding that introduction of a methyl group at the 6-position of the pyrido[3,4-d]pyrimidine core significantly improved HLM stability. Met ID studies suggested that the methyl group suppressed metabolism at the distant aniline portion of the molecule, likely by blocking the preferred pharmacophore through which P450 recognized the compound. This work ultimately led to the discovery of BOS172722 as a Phase 1 clinical candidate.
proliferative cancer but, on its own, does frequently not lead to durable responses, particularly in the metastatic setting. Triple negative breast cancer thus remains a high medical need, and new effective therapeutic regimens are needed.

The work presented here builds upon a series of pyrido[3,4-d]pyrimidines that we recently disclosed. Advanced compounds in this series showed excellent potency in biochemical and cellular assays, exemplified by 5 (Figure 2); which was effective in inhibiting MPS1 in vivo. However, this series in general, and 5 in particular, suffered from key liabilities that prevented further development, specifically high turnover in human microsomes as well as excessive lipophilicity.

Herein, we describe our optimization of the pyrido[3,4-d]pyrimidine series culminating in the discovery of a Phase 1 clinical candidate compound.

**CHEMISTRY**

Des methyl pyrido[3,4-d]pyrimidine compounds were made using the route shown in Scheme 1. Two complementary approaches could be used to gain access to the des methyl compounds. First, the amine was introduced into 7 by displacement of the chloride, followed by m-CPBA oxidation to give sulfone 9. Displacement of the sulfone with the appropriate formamide under NaH/THF conditions gave rise to the desired des methyl pyrido[3,4-d]pyrimidine compounds (Scheme 1). Alternatively, the steps could be reversed, carrying out the m-CPBA oxidation as the first step to afford sulfone 18. Displacement with the appropriate formamide could then be carried out as previously, before introducing the amine at the final step, through reaction of the chloro-intermediate 20 with neopentylamine in NMP at elevated temperatures.

Compounds in the 6-methyl pyrido[3,4-d]pyrimidine series were prepared from the key intermediate 8-chloro-2-(methylthio)pyrido[3,4-d]pyrimidine 22, the synthesis of which we have previously reported. Treatment of this intermediate with an amine at elevated temperature in NMP gave rise to sulides 23−25. Oxidation of these compounds with m-CPBA afforded sulfones 26−28, which were ideally set up to undergo selective displacement. The aniline moiety was introduced using the corresponding formamide with either NaH in THF or cesium carbonate in DMSO, affording the desired 6-methyl pyrido[3,4-d]pyrimidines 34−42 (Scheme 2).

Again the order of steps could be reversed with the oxidation with m-CPBA being carried out first to give sulfone 43, which could then undergo the same coupling with the appropriate formamide as previously described using NaH in THF. Displacement of the chloro intermediate 44 was then carried out with amines at elevated temperatures in NMP (Scheme 3).

All amines used were commercially available, and the formamides were synthesized from the corresponding anilines by refluxing in formic acid. The anilines were prepared by standard transformations (see Supporting Information for procedures).

**RESULTS AND DISCUSSION**

We routinely tested our compounds in a caliper-based MPS1 kinase assay at 1 mM ATP. As described in our preceding publication, this relatively high ATP concentration was
necessary due to the high potency of advanced compounds ($K_i < 1 \text{nM}$) that was beyond the dynamic range of the assay at lower ATP concentrations. Furthermore, we progressed compounds of sufficient potency to an MSD-based cellular assay that measured autophosphorylation of ectopically expressed MPS1 in HCT116 cells.\textsuperscript{16} In addition, we routinely determined selectivity against CDK2, a cell cycle kinase with a high homology to MPS1 in terms of the ATP binding domain. Since the CDK2 assay was run at much lower ATP concentrations, we converted the IC$_{50}$ values from the MPS1 assay (at 1 mM ATP) and those from the CDK2 assay (at 10 $\mu$M ATP) into $K_i$ values and used these calculated $K_i$s to assess the selectivity ratio.

Our key goal for the optimization of the pyrido[3,4-$d$]-pyrimidines series was to significantly improve the low human liver microsomal stability observed for compound 5 and other compounds in this series. Extensive attempts to identify major metabolites showed multiple oxidations and dealkylation products but failed to identify a consistent site of metabolism. Furthermore, we had not observed a correlation between stability in human microsomes and lipophilicity indicating that lowering LogP was not a promising approach. We thus suspected that rapid turnover was driven by recognition of a specific pharmacophore within our series and decided to systematically derivatize the molecule to discover modifications that would block this recognition and increase the metabolic stability.

We started by altering the five-membered ring heterocycle of 5 maintaining a neopentyl substituent in the 8-position of the pyrido[3,4-$d$]pyrimidine core. These compounds are summarized in Table 1. Methylated and dimethylated pyrazole containing compounds (21, 14, and 15) showed only relatively modest activity in the high ATP assay, as well as in the cellular assay. The imidazole substituted compound (16) demonstrated excellent levels of biochemical activity, as well as showing promising levels of selectivity over CDK2 and cellular activity. Finally, triazole containing 17, while exhibiting nanomolar potency for CDK2, stood out in terms of its single digit nanomolar potency in the cellular assay and represents one of the most potent MPS1 inhibitors known to us. In fact, the potency of this compound was beyond the lower range of our biochemical assay even at 1 mM ATP. The reason why replacing the pyrazole moiety in 21 with imidazole (15) and particularly triazole (16) significantly boosted biochemical activity remained unclear, and crystal structures (\textit{vide infra}) did not provide any additional insights.

We tested the human liver microsome (HLM) stability of compounds 16 and 17, but both still underwent extensive metabolism showing C$_{1\text{m}}$ values of 79 and 92 $\mu$L/min/mg protein, respectively. Nevertheless, the comparably low molecular weight (418) and lipophilicity (3.6) made 17 an excellent starting point for further investigation, and we decided to improve CDK2 selectivity and HLM stability.

In order to develop hypotheses on how to reduce CDK2 activity, we superimposed X-ray structures of our compounds\textsuperscript{17} with published CDK2 structures (Figure 3).\textsuperscript{29,30} Fourteen residues are different within the ATP binding pockets of MPS1 and CDK2, including the gatekeeper residue, which is Met602 in MPS1 and a bulkier phenylalanine (Phe80) in CDK2 (Figure 3). We hypothesized that introducing a methyl group at the 6-position of the pyrido[3,4-$d$]pyrimidine core would be less tolerated in CDK2 than in MPS1, due to a clash with the CDK2 Phe80 gatekeeper residue.

We thus set out to prepare a series of methyl substituted compounds. The significant investigations required to access pyrido[3,4-$d$]pyrimidines with substitution in this position were disclosed by us recently.\textsuperscript{28}  

\begin{scheme}
\begin{center}
\includegraphics[width=\textwidth]{Scheme1.pdf}
\end{center}
\end{scheme}

\textsuperscript{a}Reagents and conditions: (i) amine, NMP, 100–130 °C; (ii) m-CPBA, CH$_2$Cl$_2$, 0 °C–r.t.; (iii) ArNHCHO 10–13 or 19, NaH, THF, 0 °C–r.t.
Table 2 shows the biochemical and cellular results for two initial proof-of-concept compounds made as matched pairs to the corresponding unsubstituted compounds (16 and 17). Both compounds show potent biochemical inhibition of MPS1 and lower but still acceptable levels of inhibition in cells. As hypothesized, both 6-methylated compounds (34 and 35) demonstrated a significant improvement in selectivity for MPS1 over CDK2 ($K_i$ ratio is between 500 and 7600). Even more importantly and somewhat unexpectedly, we observed a large improvement in HLM metabolism for the 6-methylated compounds. Compounds 34 and 35 represented by far the most stable compounds we had observed and thus a breakthrough in terms of optimizing the up to this point persisting HLM liability. We thus decided to focus on the methylated amino-pyrido[3,4-d]pyrimidine core.

To understand why these compounds showed greatly improved stability, we identified the metabolites for the matched pair 17 and 35. Interestingly, these experiments showed that the introduction of the methyl group completely changed the nature of the main metabolites in HLM (Figure 4). Incubation of compound 17 with HLM primarily led to metabolites in which the triazole and aniline moieties were oxidized (Figure 4). In sharp contrast, treatment of 35 with HLM led to oxidation on the neopentyl chain followed by loss of the entire chain (Figure 4). Importantly, the metabolic hotspots are not only different for these compounds but, in both cases, also distant from the position of the newly introduced methyl group of 35. This observation thus suggests that the reduction of HLM metabolism is not due to blocking of a metabolically labile position (a commonly applied strategy, particularly using fluorine atoms) but instead to blocking of the...
pharmacophore through which 17 is recognized and bound. This hypothesis is consistent with the nature of P450 enzymes where substrate recognition and catalytic sites are spatially separated.

Compound 35 thus represented a significant step forward, and we explored whether HLM stability, CDK2 selectivity, cellular potency, and solubility could be further optimized. Table 3 summarizes modifications of the methoxy group and the triazole ring substituents. Based on existing SAR, we hypothesized that introducing an ethoxy group in place of the methoxy group of 35 improves selectivity further. The corresponding ethoxy compound (36) showed similar levels of biochemical potency (IC₅₀ 11 vs 13 nM), albeit with a slight drop in cellular potency (P-MPS1 IC₅₀ 63 vs 30 nM). As hypothesized, this transformation resulted in an improved selectivity window over CDK2 (Kᵢ ratio 500 (35) vs 46 (36)) and significantly improved stability in HLM.

Introduction of a methyl group onto the triazole ring (37) resulted in very similar levels of potency to 35 both in the biochemical and cellular assays (Table 3), albeit with an increase in lipophilicity (ALogP = 4.49 vs 3.88). The bicyclic triazole derivatives 38 and 39 were also potent biochemical inhibitors but showed significantly weaker inhibition in cells.

Interestingly, this matched pair (38 and 39) also demonstrated a similar increase in selectivity between methoxy and ethoxy derivatives. Finally, adding a basic dimethylamino group to improve solubility (40) resulted in loss of cellular potency (P-MPS1 IC₅₀ 230 nM) possibly due to a decrease in cellular permeability of the more polar dimethylamine tail group, though the solubility (77.6 μM [HPLC method, 1% DMSO, 10 mM PBS, pH 7.4]) of this compound was greatly improved in comparison to 35.

From this investigation, 36 emerged as an attractive compound, and we tested if the overall properties could be further optimized by modification of the neopentyl amine. Table 4 shows a representative set of amine substitutions at the 8-position of the pyrido[3,4-d]pyrimidine core. Compound 45 bears the same branched primary amine used in our previously reported MPS1 inhibitor 5. Compound 45 demonstrated good activity against MPS1 in the biochemical and cellular assays (Table 4) but exhibits poor solubility (2.2 μM), a possible consequence of the increased ALogP (4.61).

The introduction of secondary amines including pyrrolidine (46) and substituted azetidines (47, 48, and 42) exhibited varied activity against MPS1. Pyrrolidine containing 46 displayed a significant decrease in the biochemical and cellular

### Table 1. Biochemical and Cellular Data for Aniline Modifications on Neopentyl-Substituted Core

<table>
<thead>
<tr>
<th>Compd</th>
<th>R¹</th>
<th>MPS1 IC₅₀</th>
<th>CDK2 IC₅₀</th>
<th>P-MPS1 IC₅₀</th>
<th>ALogP</th>
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<td></td>
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<td>24</td>
<td>2,800</td>
<td>1,400</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>79</td>
<td>0.78</td>
<td>3,000</td>
<td>1,500</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>8.5</td>
<td>0.084</td>
<td>86</td>
<td>43</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>≤ 6.8</td>
<td>≤ 0.07</td>
<td>16</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*Results are in nM unless otherwise stated and are mean for n ≥ 3, or mean values of two independent determinations or samples run n = 1. For SD (n ≥ 3) and individual determinations (n = 2), see Table S1. “P-MPS1” indicates an electrochemiluminescence mesoscale discovery (MSD)-based cellular assay that measured autophosphorylation of ectopically expressed MPS1 in HCT116 cells. Kᵢs were calculated from the IC₅₀ using the Cheng–Prusoff equation.*
Compound 47 exhibited excellent activity (P-MPS1 IC\text{50} 37 nM) and good levels of selectivity over CDK2 coupled with an increased solubility of 31.5 μM, possibly due to the decreased ALogP of 3.85. However, movement of the gem dimethyl group one carbon round the ring into the α-position (48) resulted in loss of all MPS1 activity (P-MPS1 IC\text{50} 3.8 μM). Cyano-substituted azetidine compound (42) also showed acceptable levels of potency (P-MPS1 IC\text{50} 110 nM) and selectivity over CDK2 (CDK2 IC\text{50} 3.8 μM).

Figure 3. Top: Superimposed crystal structure of MPS1 (green) bound to a pyrido[3,4-d]pyrimidine core (carbon atoms colored green), extracted from PDB code 5EH0, onto the structure of CDK2 (blue), extracted from PDB code 1H08 (ligand hidden for clarity), showing the different gatekeeper residues present in MPS1 and CDK2. Bottom: 6-position methyl group on pyrido[3,4-d]pyrimidine core.

Figure 4. Results of a Met ID study showing oxidation products for the match pair of compounds 17 and 35 after treatment with HLM.

Table 2. Biochemical and Cellular Data for Two Matched Pairs of Compounds Containing H or Me at the 6-Position of the Pyrido[3,4-d]pyrimidine Core

<table>
<thead>
<tr>
<th>Compd</th>
<th>Structure</th>
<th>Biochemical activity</th>
<th>P-MPS1 IC\text{50}</th>
<th>AlogP</th>
<th>Cl\text{max} (μL/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MPS1 IC\text{50}</td>
<td>CDK2 IC\text{50}</td>
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<td>mouse human</td>
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<tr>
<td>16</td>
<td></td>
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<tr>
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<td>13 0.13 130 65</td>
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<td>3.88</td>
<td>41.1 23.8</td>
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</table>

"Results are in nM unless otherwise stated and are mean for n ≥ 3, or mean values of two independent determinations or samples run n = 1. For SD (for n ≥ 3) and individual determinations (n = 2), see Table S2. "P-MPS1" indicates an electrochemiluminescence mesoscale discovery (MSD)-based cellular assay that measured autophosphorylation of ectopically expressed MPS1 in HCT116 cells. K_s were calculated from the IC_{50} using the Cheng–Prusoff equation."
2.70 μM), combined with a much lower AlogP of 3.25. However, this reduced AlogP did not translate into increased solubility, with only showing a solubility of 6.8 μM. Introduction of a polar spirocyclic amine (41) resulted in a dramatic drop in lipophilicity (AlogP = 3.08), which, as expected, translated into an increase in solubility (56.2 μM).

To understand the observed MPS1 SAR (Table 4), we solved the crystal structure of compound 36 bound to MPS1 (Figure 5A). As expected, the binding mode of 36 was nearly identical to that of the previously described pyrido[3,4-d]pyrimidine inhibitors.17 The pyrido[3,4-d]pyrimidine scaffold of 36 binds to the hinge region, the 6-methyl group important for CDK2 selectivity and for the reduction in HLM metabolism, is located close to the side chain of the gatekeeper residue Met602, and the ethoxy moiety also important for selectivity binds in the selectivity pocket above the hinge. Somewhat surprisingly, the triazole moiety was not engaged in a hydrogen bond, and thus the X-ray structure did not explain why replacing the pyrazole of 21 with triazole (17, Table 1) led to a significant increase in activity (vide supra). Together with previously reported structures of CCT251455 (4) and (5), the X-ray structure of 36 allowed us to rationalize the SAR summarized in Table 4. As previously described for compound 5, the neopentyl chain binds to a hydrophobic pocket that is created by a reordering of the MPS1 activation loop into an inactive conformation (Figure 5B).16,17 The shape of the neopentyl chain represents an excellent match to this pocket resulting in several hydrophobic contacts explaining why this moiety is critical for activity. The two azetidine derivatives 47 and 48 exemplify the importance of the correct shape of the amine substituent for potent inhibition. The 3,3-dimethylazetidine substituent of derivative 47 can be regarded as a constrained mimetic of the neopentyl chain that can likely engage in similar hydrophobic contacts, and 47 maintains potent inhibition. The 2,2-dimethylazetidine moiety of 48, however, differs significantly in its overall shape from the neopentyl chain leading to less favorable interactions and a 40-fold higher IC₅₀.

To investigate which of the potent and selective compounds in Table 4 can be progressed further, we tested the stability in liver microsomes. Gratifyingly, all compounds tested showed satisfactory stability (Clₘ₀ < 45 μL/min/mg protein in mouse and Clₘ₀ < 26 μL/min/mg protein in human) (Table 4). This represented a vast improvement over the human intrinsic
Table 4. Biochemical and Cellular Data for Amine Modifications Based on 36<sup>a</sup>

<table>
<thead>
<tr>
<th>Compd</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Biochemical activity</th>
<th>P-MPS1 IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>AlogP</th>
<th>Cl&lt;sub&gt;int&lt;/sub&gt; (µL/min/mg protein)</th>
<th>Solubility µM</th>
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<td>MPS1 (1 mM ATP) CDK2</td>
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<td>K&lt;sub&gt;4&lt;/sub&gt;</td>
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<td></td>
<td>11 0.11 1,020 510</td>
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<tr>
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<td></td>
<td>8.9 0.088 1,600 810</td>
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<sup>a</sup>Results are in nM unless otherwise stated and are mean for n ≥ 3, or mean values of two independent determinations or samples run n = 1. For SD (for n ≥ 3) and individual determinations (n = 2), see Table S4. "P-MPS1" indicates an electrochemiluminescence mesoscale discovery (MSD)-based cellular assay that measured autophosphorylation of ectopically expressed MPS1 in HCT116 cells.

Figure 5. (A) Crystal structure of 36 bound to MPS1 (pdb code 6H3K). Compound 36 is shown with carbon atoms in yellow. Selected amino acids are shown in sea green and are labeled. Key hydrogen bonds are indicated as black dotted lines. (B) Close up of the neopentyl binding pocket. The neopentyl substituent is enveloped by residues Met671 and Pro673 from the activation loop. Protein surface is displayed as a transparent blue surface. The compound surface is shown in transparent yellow.
clearance values seen for compound 5 (Clint 151.2 μL/min/mg protein), the starting point of our investigation.

With a number of active, selective, and soluble compounds in hand, we decided to investigate the mouse and rat pharmacokinetics (PK) of a selection of compounds (36, 41, 45, and 47) at 5 mg/kg administered both intravenous (iv) and orally (po) (Table 5). The resulting data showed moderate clearance for all compounds in both mouse (8.4−22.6 mL/min/kg) and rat (6.00−10.1 mL/min/kg). All compounds with the exception of 41 showed high oral bioavailability in both species (63−92%) with moderate volumes of distribution (Table 5). The plasma protein binding for all four of these compounds was high (>98%), the lowest unsurprisingly shown by that presenting the lowest AlogP (41). This corresponded to a higher blood clearance and shorter half-life (0.66 h) for 41 compared to 36 (2.68 h).

We thus decided to progress both 36 and 41 to a single dose pharmacokinetic/pharmacodynamic (PK/PD) experiment in DLD1 xenografts to investigate if the in vitro and PK profiles translated into sustained inhibition of MPS1 in vivo (Figure 6). We recently disclosed a xenograft model to assess modulation of MPS1 activity in vivo. Briefly, this model (Dox-DLD1) measures the level of MPS1 autophosphorylation in DLD1 cancer cells and, importantly, overcomes the issue of low MPS1 levels through doxycycline inducible expression of the kinase. We tested both compounds at 25 mg/kg and in addition compound 36 at 50 mg/kg. The data are summarized in Figure 6. At 25 mg/kg, both compounds led to a pronounced reduction of MPS1 autophosphorylation after 6

Table 5. Mouse and Rat Blood Pharmacokinetics of 36, 41, 45, and 47 at 5 mg/kg iv and po, unless Otherwise Stated

<table>
<thead>
<tr>
<th>Compd</th>
<th>Structure</th>
<th>species</th>
<th>t1/2 (h)</th>
<th>Cl (mL/min/kg)</th>
<th>Cmax (nmol/L)</th>
<th>AUClast (h·nmol/L)</th>
<th>PPB (%)</th>
<th>Vss (L/kg)</th>
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</table>

Notes:
- 2.5 mg/kg (iv). b1 mg/kg (iv). cCompounds were administered iv and po (Mouse, 0.1 mL/10 g in 10% DMSO, 5% Tween 20 in saline; Rat, 0.05 mL/10 g in 10% DMSO, 5% Tween 20 in saline). dAUClast 6 h for mouse, 24 h for rat, unless otherwise stated. eAUClast 6 h.

Figure 6. Bar charts show the ratio of phosphorylated-MPS1/total-MPS1 levels (gray) in the Dox-DLD1 model after treatment with 36 (left) and 41 (right) at specified doses and time points. The plasma levels at the respective time points are given in the table below the bar chart.
h. Consistent with its longer half-life, only 36 showed significant inhibition at 24 h. As expected, the 50 mg/kg dose of 36 resulted in an increased suppression of MPS1 autophosphorylation at 6 and 24 h. The comparison of the plasma levels determined in the PK/PD study (Figure 6) after blood to plasma correction with the compound levels determined in the PK study described above and performed at lower doses (Table 5) was consistent with linear PK.

The robust modulation of the PD biomarkers observed for 36 prompted us to focus on this compound since a long duration of action is desirable for cell cycle targets. At 1 mg/kg iv and 5 mg/kg po, 36 showed complete bioavailability (100%), low clearance (1.2 mL/min/kg), a moderate volume of distribution (1.1 L/kg), and a 12 h half-life (Table 6) in a dog PK study.

Table 6. Dog Blood Pharmacokinetics not Dogblood 36 at 1 mg/kg iv and 5 mg/kg po

<table>
<thead>
<tr>
<th>Compd</th>
<th>Structure</th>
<th>species</th>
<th>f1/2 (h)</th>
<th>C1 (mL/min/kg)</th>
<th>Cmax (μmol/L)</th>
<th>AUClast (h*μmol/L)</th>
<th>Vd (L/kg)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td><img src="image" alt="Structure" /></td>
<td>dog</td>
<td>12</td>
<td>1.2</td>
<td>7770</td>
<td>147035</td>
<td>1.1</td>
<td>100</td>
</tr>
</tbody>
</table>

The compound was administered as the dichloride salt in saline containing 10% DMSO. AUClast 48 h.

We were intrigued by the high bioavailability of 36, particularly given its poor solubility. Interestingly, pKa values for 36 were determined as 6.22 and 2.63. This suggests that, while the compound primarily exists as the free base at physiological pH, it is protonated in the acidic environment of the stomach, likely accelerating the dissolution and enabling high bioavailability despite modest solubility at physiological pH. This is an attractive feature of the pyrido[3,4-d]pyrimidine scaffold since it avoids the well-recognized risk associated with drugs carrying a positive charge at physiological pH. This is an interesting example where metabolism is not primarily driven by hydrophobicity but by recognition of a specific pharmacophore distant from the site of metabolism. These results underscore the importance of systematic chemical modification to solve high metabolic turnover.

Further optimization led to a set of compounds with promising in vitro profile, and we progressed a number of selected compounds to PK and subsequently PK/PD experiments. Compound 36 emerged as our candidate showing excellent PK in mouse, rat, and dog. Data showing robust efficacy of 36 in combination with paclitaxel in in vivo models will be published shortly.

Interestingly, 36 showed very good bioavailability in all three species despite very modest solubility at physiological pH. We attribute this observation to the weakly basic character of 36 (pKa = 6.22) and other compounds in this series, which is likely accelerating their dissolution in the acidic environment of the stomach.

The synthesis of 36 has been scaled to the kilogram range, and the compound is currently in Phase 1 clinical trials.

### EXPERIMENTAL SECTION

**General Chemistry Information.** Starting materials, reagents, and solvents for reactions were reagent grade and used as purchased. Chromatography solvents were HPLC grade and were used without further purification. Thin layer chromatography (TLC) analysis was performed using silica gel 60 F-254 thin layer plates. Flash column chromatography was carried out using columns prepacked with 40–63 μm silica. Microwave-assisted reactions were carried out using a Biotage Initiator microwave system. LCMS and HRMS analyses were performed on a HPLC system with diode array detector operating at 254 nm, fitted with a reverse-phase 50 × 4.6 mm column at a temperature of 22 °C, connected to a time of flight (ToF) mass spectrometer (ESI). The following solvent system, at a flow rate of 2 μL/min, was used: solvent A, methanol; solvent B, 0.1% formic acid in water. Gradient elution was as follows: 1.9 (A:B) to 9.1 (A:B) over 2.5 min, 9.1 (A:B) for 1 min then reversion back to 1.9 (A:B) over 0.3 min, 1.9 (A:B) for 0.2 min. 1H NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer using an internal deuterium lock. NMR data is given as follows: chemical shift (δ) in ppm, multiplicity, coupling constants (J) given in Hz, and integration. The purity of final compounds was determined by HPLC as described above and is ≥95% unless specified otherwise.

### CONCLUSIONS

We describe herein the discovery of our MPS1 inhibitor 36 (BOS172722). The starting point for the work described here was a series of previously reported pyrido[3,4-d]pyrimidine inhibitors. These earlier compounds already showed promising in vitro potency and selectivity but suffered from a number of liabilities, particularly high lipophilicity and rapid metabolism in HLM. Optimizing HLM metabolism proved challenging since commonly used approaches, such as identification of metabolites and lowering lipophilicity, did not help. Key to overcoming this problem was the serendipitous finding that introduction of a methyl group at the 6-position of the pyrido[3,4-d]pyrimidine core significantly improved HLM stability. Met ID studies suggested that the methyl group suppressed metabolism at the distant aniline portion of the molecule, likely by blocking the preferred pharmacophore through which P450 recognized the compound. Compound 17 is thus an interesting example where metabolism is not primarily driven by hydrophobicity or the presence of a particular metabolic hotspot but by recognition of a specific pharmacophore distant from the site of metabolism. These results underscore the importance of systematic chemical modification to solve high metabolic turnover.

Further optimization led to a set of compounds with promising in vitro profile, and we progressed a number of selected compounds to PK and subsequently PK/PD experiments. Compound 36 emerged as our candidate showing excellent PK in mouse, rat, and dog. Data showing robust efficacy of 36 in combination with paclitaxel in in vivo models will be published shortly.

### EXPERIMENTAL SECTION

**General Chemistry Information.** Starting materials, reagents, and solvents for reactions were reagent grade and used as purchased. Chromatography solvents were HPLC grade and were used without further purification. Thin layer chromatography (TLC) analysis was performed using silica gel 60 F-254 thin layer plates. Flash column chromatography was carried out using columns prepacked with 40–63 μm silica. Microwave-assisted reactions were carried out using a Biotage Initiator microwave system. LCMS and HRMS analyses were performed on a HPLC system with diode array detector operating at 254 nm, fitted with a reverse-phase 50 × 4.6 mm column at a temperature of 22 °C, connected to a time of flight (ToF) mass spectrometer (ESI). The following solvent system, at a flow rate of 2 μL/min, was used: solvent A, methanol; solvent B, 0.1% formic acid in water. Gradient elution was as follows: 1.9 (A:B) to 9.1 (A:B) over 2.5 min, 9.1 (A:B) for 1 min then reversion back to 1.9 (A:B) over 0.3 min, 1.9 (A:B) for 0.2 min. 1H NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer using an internal deuterium lock. NMR data is given as follows: chemical shift (δ) in ppm, multiplicity, coupling constants (J) given in Hz, and integration. The purity of final compounds was determined by HPLC as described above and is ≥95% unless specified otherwise.

### CONCLUSIONS

We describe herein the discovery of our MPS1 inhibitor 36 (BOS172722). The starting point for the work described here was a series of previously reported pyrido[3,4-d]pyrimidine inhibitors. These earlier compounds already showed promising in vitro potency and selectivity but suffered from a number of liabilities, particularly high lipophilicity and rapid metabolism in HLM. Optimizing HLM metabolism proved challenging since commonly used approaches, such as identification of metabolites and lowering lipophilicity, did not help. Key to overcoming this problem was the serendipitous finding that introduction of a methyl group at the 6-position of the pyrido[3,4-d]pyrimidine core significantly improved HLM stability. Met ID studies suggested that the methyl group suppressed metabolism at the distant aniline portion of the molecule, likely by blocking the preferred pharmacophore through which P450 recognized the compound. Compound 17 is thus an interesting example where metabolism is not primarily driven by hydrophobicity or the presence of a particular metabolic hotspot but by recognition of a specific pharmacophore distant from the site of metabolism. These results underscore the importance of systematic chemical modification to solve high metabolic turnover.

Further optimization led to a set of compounds with promising in vitro profile, and we progressed a number of selected compounds to PK and subsequently PK/PD experiments. Compound 36 emerged as our candidate showing excellent PK in mouse, rat, and dog. Data showing robust efficacy of 36 in combination with paclitaxel in in vivo models will be published shortly.

Interestingly, 36 showed very good bioavailability in all three species despite very modest solubility at physiological pH. We attribute this observation to the weakly basic character of 36 (pKa = 6.22) and other compounds in this series, which is likely accelerating their dissolution in the acidic environment of the stomach.

The synthesis of 36 has been scaled to the kilogram range, and the compound is currently in Phase 1 clinical trials.
Reaction mixture was diluted with EtOAc and water, dried (MgSO₄), for Amine Displacement on mL) was added appropriate amine (2 equiv) and triethylamine (5 equiv). J = 6.0 Hz, 1H), 4.07 (s, 3H), 3.87 (s, 3H), 3.44 (s, 2H), 1.10 (s, 9H).

**Preparation of Compounds in Scheme 1. General Procedure for Nalt Mediated Displacement on 9 (Compounds 14-17).**

To a cooled (0 °C) solution of appropriate formamide (1 equiv) in THF (3 mL) was added sodium hydride (2.7 mg, 0.11 mmol, 60% dispersion in ME0H, 1 M NH, in ME0H) and if necessary, followed by SCX-2 cartridge (ME0H, 1 M NH, in ME0H) to afford the title compounds.

**General Procedure for m-CPBA Oxidation on 23-25.** To a cooled (0 °C) solution of appropriate sulfone (1 equiv) in CH₂Cl₂ (10 mL) was added CPBA (3 equiv). The reaction mixture was stirred for 18 h, while slowly warming to r.t. In some instances the further m-CPBA was needed to achieve full conversion. The reaction mixture was quenched with water and CH₂Cl₂, and the combined organic layers were washed with aq sat. NaHCO₃, dried (MgSO₄), and concentrated in vacuo. The residue was purified by flash column chromatography (0–10% MeOH in CH₂Cl₂). Yield = 66%. HRMS (ESI) m/z calc for C₁₆H₂₁N₄O₅ (M + H) 317.1431, found 318.1019, found 318.1006; ¹H NMR (500 MHz, CD₃OD) δ 9.46 (s, 1H), 7.08 (s, 1H), 4.54 (br s, 4H), 3.44 (s, 3H), 2.56 (s, 3H), 1.80 (s, 3H).

**Preparation of Compounds in Scheme 2. General Procedure for Amine Displacement on 22.** To a solution of 8-chloro-6-methyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-2-amine (25.6 mg, 0.083 mmol) in DMSO (20 mL) was added appropriate amine (2 equiv) and triethylamine (5 equiv). The reaction mixture was heated to 100 °C for 36 h. The reaction mixture was diluted with EtOAc and water, dried (MgSO₄), and concentrated in vacuo. The residue was purified by flash column chromatography (0–50% EtOAc in cyclohexane) to afford the title compounds.

**General Procedure**

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followed by SCX-2 cartridge (MeOH, 1 M NH3 in MeOH) to afford the title compounds.

N°-(2-Methoxy-4-(4-methyl-4H-1,2,4-triazol-3-yl)phenyl)-6-methyl-N°-neopentylpyrido[3,4-d]pyrimidine-2,8-diamine 35.

Using 6-methyl-2-(methylsulfonyl)-N°-neopentylpyrido[3,4-d]pyrimidine-8-amine 26 and N°-(2-ethoxy-4-(4-methyl-4H-1,2,4-triazol-3-yl)phenyl)formamide 13. Yield = 48%. HRMS (ESI) m/z calculated for C29H24N8O2 (M + H) 501.1714, found 501.1715; 1H NMR (500 MHz, CD3OD) δ 8.91 (s, 1H), 8.72 (d, J = 8.5 Hz, 1H), 8.65 (s, 1H), 7.42 (d, J = 2.0 Hz, 1H), 7.34 (d, J = 8.5, 2.0 Hz, 1H), 6.71 (d, J = 1.0 Hz, 1H), 4.07 (s, 3H), 3.48 (s, 2H), 2.53 (s, 3H), 2.45 (s, 3H), 2.39 (s, 2H), 1.10 (s, 9H).

N°-(2-Ethoxy-4-(4-methyl-4H-1,2,4-triazol-3-yl)phenyl)-6-methyl-N°-neopentylpyrido[3,4-d]pyrimidine-2,8-diamine 36. Using 6-methyl-2-(methylsulfonyl)-N°-neopentylpyrido[3,4-d]pyrimidine-8-amine 26 and N°-(2-ethoxy-4-(4-methyl-4H-1,2,4-triazol-3-yl)phenyl)formamide 29. Yield = 38%. HRMS (ESI) m/z calculated for C28H25N9O2 (M + H) 518.3227, found 518.3217; 1H NMR (500 MHz, CD3OD) δ 9.06 (s, 1H), 8.57 (d, J = 8.5 Hz, 1H), 8.56 (s, 1H), 7.39 (d, J = 2.0 Hz, 1H), 7.36 (d, J = 8.5, 2.0 Hz, 1H), 6.78 (s, 1H), 4.31−4.27 (m, 6H), 3.87 (s, 3H), 3.69 (app t, J = 5.5 Hz, 4H), 2.44 (s, 3H), 1.89 (app t, J = 5.5 Hz, 4H), 1.54 (t, J = 7.0 Hz, 3H).

Preparation of Compounds in Scheme 3. 8-Chloro-6-methyl-(methylsulfonyl)pyrido[3,4-d]pyrimidine 43. A suspension of 8-chloro-6-methyl-2-(methylthio)pyrido[3,4-d]pyrimidine 22 (1.13 g, 5.01 mmol) in CH2Cl2 (50 mL) was treated with m-CPBA (77% w/w, 2.60 g, 11.57 mmol) at 0 °C. The reaction mixture was stirred for 18 h, while slowly warming to r.t. The reaction mixture was quenched with H2O and extracted with CH2Cl2. The combined organic layers were washed with sat. NaHCO3, dried (MgSO4), and concentrated in vacuo. The residue was purified by flash column chromatography (30–70% EtOAc in cyclohexane) to afford the title compound (972 mg, 75%). HRMS (ESI) m/z calculated for C26H31N8O2S (M + H) 487.2511, found 487.2509; 1H NMR (500 MHz, CD3OD) δ 9.82 (s, 1H), 8.46 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 8.5, 2.0 Hz, 1H), 7.39 (d, J = 2.0 Hz, 1H), 6.91 (s, 1H), 4.75 (d, J = 9.0 Hz, 2H), 4.39 (g, J = 9.0 Hz, 2H), 4.28 (g, J = 7.0 Hz, 2H), 3.88 (s, 3H), 2.47 (s, 3H), 1.78 (s, 3H), 1.53 (t, J = 7.0 Hz, 3H).

General Procedure for Amine Displacement on 44 (Compounds 45–48). To a solution of the appropriate chloro compound (1 equiv) in NMP (3 mL) was added the appropriate amine or salt thereof (2–5 equiv) and triethylamine (5 equiv). The reaction mixture was heated to 100 °C in a closed cap vial for 18 h. The reaction mixture was diluted with EtOAc and water. The organic layer was washed with brine, dried (MgSO4), and concentrated in vacuo. The residue was purified by flash column chromatography (eluting with the appropriate solvent system) and, if necessary, followed by SCX-2 cartridge (MeOH, 1 M NH3 in MeOH).

Using 6-methyl-2-(methylsulfonyl)-N°-neopentylpyrido[3,4-d]pyrimidine-8-amine 26 and N°-(4-(2-(dimethylamino)ethyl)-4-methyl-4H-1,2,4-triazol-3-yl)-2-ethoxyphenyl)formamide 33. Purified by reductive phase flash column chromatography (0–100% MeOH in CH2Cl2, followed by 0–30% 2 M methanolic ammonia in CH2Cl2). Yield = 18%. HRMS (ESI) m/z calculated for C28H25N9O2 (M + H) 518.3236, found 518.3237; 1H NMR (500 MHz, CD3OD) δ 9.05 (s, 1H), 8.75 (d, J = 8.5 Hz, 1H), 7.34 (d, J = 1.8 Hz, 1H), 7.28 (d, J = 8.5, 1.8 Hz, 1H), 6.71 (s, 1H), 4.30 (g, J = 7.0 Hz, 2H), 3.73 (s, 3H), 3.47 (s, 2H), 3.09 (app t, J = 7.5 Hz, 2H), 2.91 (app t, J = 7.5 Hz, 2H), 2.44 (s, 3H), 2.42 (s, 6H), 1.56 (t, J = 7.0 Hz, 3H), 1.09 (s, 9H).
1H NMR (500 MHz, (CD3)2SO) δ 9.15 (s, 1H), 8.57 (s, 1H), 8.43 (d, J = 8.3 Hz, 1H), 7.41 (d, J = 1.9 Hz, 1H), 7.31 (dd, J = 8.2, 1.9 Hz, 1H), 6.72 (d, J = 1.0 Hz, 1H), 6.43 (d, J = 9.5 Hz, 1H), 4.23 (q, J = 6.9 Hz, 2H), 4.13 (d, J = 9.4, 6.6 Hz, 1H), 3.78 (s, 3H), 2.38 (s, 3H), 1.41 (t, J = 6.9 Hz, 3H), 1.16 (d, J = 6.6 Hz, 3H), 1.00 (s, 9H).

N-2-(Ethoxy-4-(4-methyl-1H-1,2,4-triazol-3-yl)phenyl)-6-methyl-8-(pyrrolidin-1-yl)pyrido[3,4-d]pyrimidin-2-amine 46. Using 8-chloro-N-(2-ethoxy-4-(4-methyl-1H-1,2,4-triazol-3-yl)-6-methylpyrido[3,4-d]pyrazin-2-amine 44 and pyridolidine at 130 °C. Yield = 70%. HRMS (ESI) m/z calculated for C24H30N8O (M + 2H)/2 223.1266, found 223.1261; 1H NMR (500 MHz, CD3OD) δ 9.0, 7.5 Hz, 2H), 1.71 (s, 6H), 1.54 (t, J = 7.0 Hz, 3H), 1.38 (s, 6H), 1.16 (s, 9H).

Biochemical Assays. MPS1 and CDK2 counterscreen assay were performed as previously described.32

PK/PD Experiments. These experiments were conducted as previously described.33

ASSOCIATED CONTENT

# Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.8b00690.

Molecular formula strings (CSV)

Experimental procedures and analytical data for all formamides (10−13 and 29−33) and corresponding intermediates, CYP, hERG activity, and P-gp efflux data as well as kinase selectivity profiling of 36. Tables with standard deviations for all compounds as well as antiproliferative (GI50) data for several compounds (PDF)

Accession Codes

The PDB ID code for 36 bound to MPS1 is 6H3K.

AUTHOR INFORMATION

Corresponding Author
*Tel: +44 (0)20 8772 4353. E-mail: swen.hoelder@icr.ac.uk.

ORCID

Julian Blagg: 0000-0002-7409-0323

Swen Hoelder: 0000-0001-8636-1488

Present Address


Notes

The authors declare the following competing financial interest(s): The authors are current or former employees of The Institute of Cancer Research, which has a commercial interest in the development of kinase inhibitors.
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ABBREVIATIONS USED

CDK2, cyclin-dependent kinase 2; Cl, clearance; hERG, human Ether-à-go-go-Related Gene; HLM, human liver microsomes; L.E., ligand efficiency; MLM, mouse liver microsomes; MPS, monopolar spindle kinase; MSD, MesoScale Discovery; MT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PARP, poly ADP ribose polymerase; PLK1, polo-like kinase; PTEN, phosphatase and tensin homologue; SAC, spindle assembly checkpoint; RLM, rat liver microsomes; V_{ss}, volume of distribution

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