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# Chemically diverse Group I p21-activated kinase (PAK) inhibitors impart acute cardiovascular toxicity with a narrow therapeutic window

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ABSTRACT

p21-activated kinase 1 (PAK1) has an important role in transducing signals in several oncogenic pathways. The concept of inhibiting this kinase has garnered significant interest over the past decade, particularly for targeting cancers associated with PAK1 amplification. Animal studies with the selective Group I PAK (pan-PAK1, 2, 3) inhibitor G-5555 from the pyrido[2,3-d]pyrimidin-7-one class uncovered acute toxicity with a narrow therapeutic window. To attempt mitigating the toxicity we introduced significant structural changes, culminating in the discovery of the potent pyridone side chain analog G-9791. Mouse tolerability studies with this compound, other members of this series, and compounds from two structurally distinct classes revealed persistent toxicity and a correlation of minimum toxic concentrations and PAK1/2 mediated cellular potencies. Broad screening of selected PAK inhibitors revealed PAK1, 2, and 3 as the only overlapping targets. Our data suggest acute cardiovascular toxicity resulting from the inhibition of PAK2, which may be enhanced by PAK1 inhibition, and cautions against continued pursuit of pan-Group I PAK inhibitors in drug discovery.

## Introduction

The p21-activated kinases (PAKs) are serine/threonine kinases in the STE20 kinase family that have generated significant interest as therapeutic targets in cancer.[1-3] The six PAK family members are divided into two subgroups, group I (PAK1-3) and group II (PAK4-6), based on sequence homology and distinct autoinhibitory regions in group I and II PAKs. PAKs are key signal transducers in several oncogenic and survival pathways, including Ras, Raf, NFκB, Akt, Bad, and p53.[1] As downstream effectors of the Rho family GTPases Cdc-42, Rac1 and Rho A, they govern the reorganization of the actin cytoskeleton induced by growth factors and thus, have fundamental roles in regulating cytoskeletal dynamics, cell shape, adhesion and migration.[4] Moreover, PAK1 is implicated in cellular processes that directly contribute to tumorigenesis, including growth factor pathways, cell proliferation, and pro-survival signaling.[5] Genomic amplification and over-expression of PAK1 are prevalent in luminal breast cancer[2] and other malignancies, and data from us and others demonstrated a functional dependence of PAK1-amplified cell lines on PAK1 expression and activity for cell survival and transformation.[6,7] Not only is PAK function increased in many human cancers, but occurrence of PAK amplification also correlates with advanced tumor grade and, inversely, patient survival.[1,8] As a result, PAKs, and PAK1 in particular, have been the subject of significant drug discovery efforts.[3, 9,10]

We recently described the discovery of a potent and selective inhibitor of the group I PAKs (PAK1, 2, and 3), G-5555 (**1**).[11] Compound **1** possesses a favorable in vitro pharmacology and in vivo DMPK profile, rendering it a useful tool for target validation studies. In an array of 23 breast cancer cell lines, **1** had significantly greater growth inhibitory activity in cell lines that were PAK-amplified compared to non-amplified lines.[12]

In an H292 non-small cell lunger cancer (NSCLC) xenograft study in mice, **1** inhibited phosphorylation of the PAK1/2 downstream substrate mitogen-activated protein kinase 1 (MEK1) S298 and, when administered at an oral dose of 25 mg/kg b.i.d., imparted 60% tumor growth inhibition in this model[13] and a PAK1 amplified breast cancer xenograft model, MDA-MB-175.[12] Unfortunately, doses greater than 25 mg/kg b.i.d. and 30 mg/kg q.d. were not tolerated, and doses of 40 and 50 mg/kg q.d. were associated with death of the majority of study animals within 2-4 hours after dosing. Death in these mice was typically preceded by hunched posture, hypoactivity, lowered body temperature, and blood that was dark and difficult to draw, indicative of decreased blood flow and cardiac output. Concomitant pharmacokinetic studies revealed a $C_{max}$ of approximately 50 μM, corresponding to a $C_{max,u}$ ($C_{max}$ unbound) of ~150 nM as minimum toxic concentration.

Although screening data from a kinase and receptor pharmacology panel revealed no immediate explanation or hypothesis,[11] our initial speculation for the cause of the toxicity was off-target activity. We decided to address this possibility through chemical modification, and specifically, variation of the head and tail groups in **1**. Compound **1** and analogs in this series possess a lipophilic head group, and it was deemed advantageous to replace this moiety with a more polar group to improve drug properties and better balance solubility, clearance, and permeability requirements. To strike this balance in compound **1** we had to resort to the introduction of an unorthodox 5-amino-1,3-dioxanyl tail, a product of considerable fine-tuning. A more polar head group was expected to broaden the range of options for the tail group and thus, address the goal of introducing structural diversity.

## Chemistry

The central pyrido[2,3-d]pyrimidin-7-one core of compounds **1** to **26** was accessed through cyclocondensation of pyrimidine amino aldehyde building blocks **I-4** or **I-25** with arylacetic acid esters **I-6**, **I-9** or **I-11**. Pyrimidine amino aldehydes **I-4a** and **I-4b** were synthesized by reacting 4-chloro-2-(methylthio)pyrimidine-5-carboxylic acid ester **I-1** with ammonia or ethylamine to form **I-2a** and **I-2b**, ester reduction to a methylalcohol (**I-3a**, **I-3b**) in the penultimate and conversion to an aldehyde functionality in the final step (Scheme 1).

A chemoselective Reformatsky-Negishi reaction[14] provided the foundation to conveniently access the aryl acetic esters **I-9** and **I-11**. Ethyl 2-bromoacetate **I-5** was converted into the corresponding 2-bromozincacetate, which was further reacted with 4-bromo-2-chloro-1-iodobenzene using palladium-catalyzed coupling to form **I-6a**. C-linked analogs were accessed by converting **I-6a** into boronic ester **I-7** and subsequent Suzuki reaction to form **I-9a** and **I-9b**. Ullmann coupling with 3-methylpyridin-2-one (**I-10a**) or 3-methylpyridin-2-one (**I-10b**) yielded aryl acetic esters **I-11a** and **I-11b** (Scheme 2).

Compound **2** was synthesized starting with cyclocondensation between **I-9a** and **I-4a** followed by *N*-alkylation to provide **I-13**. Oxidation of the thiomethyl group, subsequent SnAr reaction with methylamine, and deprotection yielded compound **2** (Scheme 3).

*N*-linked head group analogs **4** and **7-10** were accessed using a sequence that was similar to the previously described one; however for the cyclo-condensation reaction with pyrimidine amino aldehyde **I-4a**, the derivatizable bromo head group aryl acetic acid ester **I-6a** was used in the first step to generate **I-16**. *N*-alkylation to append the tail moiety (**I-17**) was followed by Buchwald-Hartwig or Ullmann coupling to install several *N*-linked head groups. The final steps d, e, and f paralleled steps c, d, and e in the sequence described in Scheme 3 (Scheme 4a). As illustrated in

the synthetic sequence for compounds **5** and **6**, Ullmann coupling also succeeded with the left-hand-side methylamino group already installed (Scheme 4b).

*N*-ethyl substituted core analogs **11** and **12** were synthesized by cyclo-condensation reaction between pyrimidine aldehyde **I-4b** containing a pre-formed ***N***-ethyl group and aryl acetic ester **I-6a**. The subsequent transformations b through d paralleled the steps c through e described in Scheme 3. For compound **12**, oxetan-3-amine was used in the final $S_NAr$ step (Scheme 5).

Compounds **13**-**15**, **18**, **19**, **23**, **25**, and **26** were made analogously to compound **8** using appropriate tail group precursors, while compounds **16**, **17**, and **22** required a different method because of difficulties introducing the amine chain via nucleophilic substitution. The sequence to access the latter compounds started with an $S_NAr$ reaction of pyrimidine aldehyde **I-28** with fully elaborated tail group amino building blocks to form **I-29a** and **I-29b**. Subsequent steps were similar to the transformations described in the previous schemes (Scheme 6).

## Results and Discussion

We began our head group SAR explorations using the pyrido[2,3-d]pyrimidin-7-one core substituted with a simple aminopropyl tail group, as earlier studies had found this group to impart robust potency and solubility (Table 1).[11] In addition to PAK1 potency[15] and associated ligand lipophilic efficiency (LLE), we monitored selectivity versus PAK4 as representative of a Group II PAK, and Lymphocyte-specific protein-tyrosine kinase (LCK), a persistent off-target of compounds in this series. The primary goals of this exploration were to increase LLE and decrease human liver microsome (HLM) turnover of reference compound **2**, an analog substituted with the default 6-methyl-2-pyridyl head group. Our initial design idea was replacement of this head group with *N*-linked heterocyclic systems containing a flanking

carbonyl group. For such systems we predicted a similar dihedral angle between the middle aryl and terminal ring compared to analogs with terminal pyridine rings and, most importantly, an opportunity for the carbonyl oxygen to engage in a cation-dipole interaction with the catalytic lysine side chain.

First, we established that presence of a head group is necessary for both potency and selectivity (R = H; compound **3**). Introduction of a simple *N*-linked pyrrolidone group (compound **4**), while reducing potency versus **2**, maintained LLE and, likely as result of greater polarity, increased metabolic stability. As we knew that an appropriately placed methyl substituent can boost activity by filling a lipophilic pocket in the head group binding area,[11] we prepared the *N*-methyl substituted imidazolinone **5** and imidazolone **6**, and observed further increased potency/LLE. We were particularly pleased with the data of the unsaturated imidazolone system, with an LLE of 6.7, significant selectivity vs. both PAK4 and LCK, and moderate-to-low HLM clearance. As this SAR data suggested advantages of unsaturated versus saturated ring systems we turned to exploring unsaturated 6-membered ring systems, such as pyridones. Consistent with the SAR for the 5-membered ring systems, introduction of a methyl group at the 3-position of pyridone analog **7** increased potency and selectivity (compound **8**). We were further heartened by the high HLM stability of this compound. Encouraged by the fact that a significant amount of polarity was tolerated in this area of the molecule, we introduced an additional nitrogen atom at two different ring positions, resulting in the pyrazinone analog **9** and pyrimidinone analog **10**. Both compounds showed comparable PAK1 potency to their pyridone counterpart **8**, combined with exquisite selectivity and low HLM- predicted clearance.

An X-ray co-crystal structure of compound **9** bound to PAK1 (PDB: 5IME; resolution = 2.2 Å) and superposition of this structure with the co-crystal structure of compound **1** bound to PAK1[11]

is shown in Figure 2. The most striking difference between these structures is the orientation of the tail groups; while the 5-amino-1,3-dioxanyl motif of compound **1** forms a hydrogen bond interaction with the backbone carbonyl of Asp393 (not shown), the aminopropyl group of **9** forms a hydrogen bond with the DFG-aspartate side chain (Asp407). The pyrazinone head group of **9** is tightly packed against the α-C-helix in the pocket surrounded by Lys299, Glu315, Ile316 and Val342, and the general shape of the protein residues lining the deep pocket is qualitatively similar in both structures. The main difference is the carbonyl group in the pyrazinone group with the oxygen atom projecting a 3.0 Å cation dipole interaction toward the catalytic lysine 299 side chain, which had been our original design objective. Furthermore, the crystal structure of compound **9** shows an out-of-plane (OOP) angle of 65° for the biaryl system (pyrazinone-phenyl), which is very close to the calculated ground state and similar to the observed[10] and calculated OOP angle for compound **8** (see quantum-mechanical calculations in the supplemental material). Having identified less lipophilic head groups than pyridyl, we turned our attention to replacing the strongly basic amine tail (cpKa ~ 11) with less basic or neutral tail groups to balance polarity and permeability (Table 2). Among the head groups identified above, the pyridone group was deemed best suited to reach a desirable logD range and hence was kept constant. A particular focus in this effort was optimization of cellular potency, as determined from the inhibition of phosphorylation of the PAK1/2 downstream substrate MEK1 S298.

Introduction of a simple ethyl tail group (compound **11**) imparted single digit nanomolar PAK1 potency and high permeability in Madin-Darby canine kidney (MDCK) cells, however less desirable cellular potency (pMEK $IC_{50}$ = 340 nM) and high HLM-predicted clearance. Earlier SAR studies had found the methyl group as hinge binder substituent, $R^1$, to impart optimal properties[10] but oxetanyl was found to be an alternative option with the potential to

increase cellular potency and selectivity. We tried oxetanyl as replacement for methyl in **11** (compound **12**), and indeed selectivity vs. LCK and cell potency were both increased, however HLM-predicted clearance remained high. Furthermore, both compounds showed poor aqueous solubility. To address these shortcomings, we turned to tail groups that possessed a low level of polarity. The hydroxyethyl analog **13** exhibited insufficient potency and strikingly lower MDCK permeability compared to its ethyl tail counterpart **11**. Both liabilities were addressed with the corresponding methoxyethyl analog **14**, but cell potency was still poor. We successfully replaced the methyl hinge binder group $R^1$ with oxetanyl, leading again to a gain in selectivity versus LCK and cell potency (compound **15**). Both compounds showed improved aqueous solubility versus their ethyl tail counterparts (80 and 38 μM, respectively, at pH 7.4).

Our earlier work had demonstrated that amine containing tail groups can have a profound effect on potency in this series, even if basicity is substantially attenuated,[11] and we decided to re-explore this direction. Introduction of two fluorine atoms to the tail group of **8**, as realized in compound **16**, led to a drop of pKa from ~11 to 7.3, resulting in a large gain in permeability from 0.1 to 8.4 $10^{-6}$ cm/s (MDCK A to B).[16] This compound was not sufficiently potent but we were encouraged by the compatibility of the pyridone head group with a low pKa amine tail group with respect to cell permeability. Further variation of the tail group led to compounds with double digit nanomolar cellular potency. While the *N*-methyl ethoxyazetidine analog **18** was hampered by low MDCK permeability and high efflux, presumably because of high basicity, the less basic tail analogs **17** and **19** (**G-9791**) possessed good permeability. A clear standout was the 3-fluoroazetidine tail analog **19**, a weakly basic compound with high biochemical and cellular potency, and good solubility.

Based on its favorable LLE and strong cell potency, we moved forward with evaluating **19** in a mouse pharmacokinetic study using a standard oral dose of 25 mg/kg. Disappointingly, the animal with the highest exposure in this study group (n=3) (plasma concentration at the 15 min time point was 2.75 μM), died at ~ 2 hours post dosing. The other two study mice survived (plasma concentrations were 2.24 and 1.71 μM, respectively), however, their blood was darkened and difficult to draw, similar to what was observed in the efficacy study with PAK inhibitor **1**. A total drug concentration of 2.75 μM corresponds to an unbound concentration of 88 nM, a value close to the $IC_{50}$ in the phospho-MEK cellular assay (33 nM). This close relationship between minimum adverse free $C_{max}$ and cellular $IC_{50}$ paralleled the observations for compound **1**. Considering the two major structural differences between **19** and **1**, the possibility of on-target toxicity became a growing concern, and hence we selected a structurally diverse set of PAK inhibitors for single-dose tolerability studies in mice (Tables 3). All compounds were ATP competitive inhibitors and, as a result of high homology in the ATP binding site, showed similar inhibition of PAK1 and PAK2. Tolerability studies were conducted at multiple doses, using either oral (p.o.) or intraperitoneal (i.p.) dosing as appropriate, and minimum unbound drug concentrations associated with adverse effects ($C_{max,adverse,u}$) were compared to pMEK $IC_{50}$ values.

As shown in Table 3, none of the compounds investigated, except an inactive control compound (**26**) with close structural similarity to one of the active compounds tested (**21**) were tolerated, and in many instances dosing resulted in lethality. Since all compounds shown in Table 3 stem from the same class (pyrido[2,3-d]pyrimidin-7-ones), we conducted similar tolerability studies with molecules from two structurally distinct series, **27**, a PAK inhibitor from the aminopyridine hinge binder class previously pursued by us,[13] and PF-3758309 (**28**), a pan-

PAK inhibitor that had entered Phase I clinical studies, but was later discontinued (Table 4).[17] As it was challenging to achieve sufficient drug exposures with these compounds in mice by oral dosing, both compounds were administered intraperitoneally (i.p.). Both compounds, at 25 and 50 mg/kg i.p., for **27** and **28**, respectively, displayed intolerability similar to the compounds shown in Table 3. Analyzing the entire data set in more detail, we noticed a striking correlation between $C_{max,adverse,u}$ and pMEK $IC_{50}$ values (Figure 3). Compounds with higher cell potency caused adverse effects at lower concentrations while compounds with reduced cell potency were better tolerated. This relationship was chemical class independent, and the value for $C_{max,\ adverse,u}$ was consistently in a range of 1 to 4-fold over the pMEK $IC_{50}$ value. This clearly supported our on-target toxicity hypothesis.

To rule out coincidental overlap of off-target activities we performed broader off-target profiling of three structurally distinct compounds, **1**, **23**, a compound with a polar head group and devoid of a basic amine tail, and **28**, a pan-PAK inhibitor from an entirely different series. This included testing in a large kinase panel as well as a secondary pharmacology screening panel. As illustrated in the Venn diagrams in Figure 4, the only overlapping kinase activities were PAK1, 2, and 3, and there was no overlap in activities within the secondary pharmacology panel. In addition, **1** was tested in a specific panel of pharmacology assays related to ion channel targets with known cardiac functions (hERG, hNav1.1, hNav1.5, hCav3.2, $Na^+/Ca^{2+}$ ATPase, $Na^+/K^+$ ATPase), and no significant activities were observed (see supporting information for details). Thus, no apparent shared biological targets outside of Group I PAK isoforms could be identified for this group of compounds.

In-life observations in the described mouse tolerability experiments suggested implication of the cardiovascular system and, to further investigate the nature of the toxicity, we examined the

effects of tool compound **1** on a Langendorff heart preparation isolated from rats. The Langendorff heart assay is a widely used *ex vivo* model that allows the examination of cardiac hemodynamic and electrocardiographic parameters without the confounding reflex components that are present in an intact animal (such as neuronal and hormonal effects),[18] and we expected to gain clarity on the mode of toxicity from such a study. Hearts from Sprague Dawley rats were paced via the right atrium at 10-15% above the intrinsic heart rate and exposed to increasing concentrations of compound **1** (0, 0.5, 1.5, 5 and 15 μM, n=5) for 15 minutes at each concentration. Exposure to 1.5, 5 and 15 μM of **1** resulted in a significant increase of 64-83% in coronary perfusion pressure (CPP). Notable decreases in left ventricular developed pressure (LVDP), dP/dt maximum, dP/dt minimum, dP/dtmax/P, and dP/dtmax/P at 40 mmHg as well as increases in left ventricular systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP) were observed. In addition, drug concentrations of 5 and 15 μM led to significant increases of 37 and 34%, respectively, in PR interval, and loss of 1:1 atrioventricular conduction occurred in one heart at these concentrations. Ventricular fibrillation (VF) occurred in another heart after perfusion with 5 μM of **1**. There were no substantive effects at concentrations of ≤ 15 μM of **1** on HR or QRS duration. In summary, compound **1** caused a decline of the left ventricle function, arrhythmia, and heart failure in isolated rat hearts, consistent with the acute adverse effects observed in mice. In addition, the lowest concentration associated with effects (0.5 – 1.5 μM) lays within only 3-10-fold of $C_{max,adverse,u}$ (150 nM). While we did not perform systematic rat tolerability studies, one of the PAK1 inhibitors shown in Table 3, FRAX1036 (**20**),[19] was investigated in a rat single-dose experiment and found to cause lethality at a $C_{max}$ of 4 μM, corresponding to a $C_{max,adverse,u}$ of 0.076 μM. This is again in close proximity to the pMEK S298 $IC_{50}$ of this compound, 0.22 μM. Furthermore, rats dosed with **20** displayed similar clinical

signs as the mice (slow blood flow slow and darkened blood, hypoactivity, and hunched posture). This suggests that the acute cardiovascular toxicity imparted by PAK1/2 inhibitors is not limited to mice. The inter-species homology of PAK1 and 2 is high (species conservation vs. human PAK1: mouse 99.26%, rat 99.08%; PAK2: mouse 97.14%, rat 96.56%),[3] and pharmacological translatability was therefore expected.

PAK tissue expression is also consistent with the on-target toxicity hypothesis. PAK1 and PAK2 are broadly expressed, with PAK1 levels being high in brain, muscle, and heart,[20] and PAK2 levels high in endothelial cells.[21] Conversely, PAK3 is almost exclusively found in the brain,[21] and based on this and the fact that our compounds are unlikely to be brain exposed due to considerable efflux (e.g., MDCK-MDR1 A-B flux is 0.3 $10^{-6}$ cm/s and the efflux ratio 68.9 for compound **1**) we ruled out its association with the toxicity findings. PAK1 knockout mice are viable, but recent studies with PAK1-deficient mice revealed vulnerability to cardiac hypertrophy and fast progression to heart failure under sustained pressure overload, in addition to susceptibility to ischemia and reperfusion injury.[20] This is consistent with the recently discovered role of PAK1 in cardiac physiology, which includes the regulation of cardiac ion channel and actomyosin function.[20] PAK2 has an important role in angiogenesis during development[1] and PAK2 knockout mice die early in embryogenesis due to multiple developmental abnormalities, most prominently those involving defective vascularization.[21, 22] In adult mice, PAK2 plays important roles in maintaining the integrity of blood vessels, and ubiquitous PAK2 deletion in adult conditional knockout mice also resulted in lethality.[23] Given these known functions of PAK1 and 2 in heart and vasculature, toxicity as a consequence of inhibition of both targets was plausible.

## Conclusions

The question of whether toxicity imparted by small molecules is caused by on-target or off-target pharmacological effects is critical for stop/go decisions of drug discovery projects. On-target toxicity is related to an exaggerated pharmacological effect on the primary target and typically not possible to eliminate. Off-target toxicities may be related to the chemical, structural, or physicochemical properties and therefore are considered solvable with chemical modifications.[24] Common key characteristics of on-target toxicities are outlined in Table 5. For on-target toxicity, the target is usually expressed in the tissue associated with toxicity, and the toxicity is oftentimes consistent with the phenotype observed in knockout models. As discussed above, both PAK1 and PAK2 are broadly expressed and modulate cardiac and vasculature function and/or development. In addition, if toxicity is on-target, the therapeutic window is typically expected to be narrow,[25] as it was the case with the PAK1/2 inhibitors, with ratios between $C_{max,adverse,u}$ and pMEK $IC_{50}$ values within 1-4. Furthermore, the ratios remained constant across 11 compounds with PAK1 potency spanning > 2 log units. In the example of a structurally closely related inactive/active pair (**26** and **21**), only the active analog **21** was toxic in vivo. Another indication of toxicity being on-target is its presence with two or more chemical series. Here we have shown that compounds from three chemotypes with no apparent common off-targets were all acutely toxic in mice. The weight of evidence thus suggests that the toxicity caused by pan-Group I PAK inhibitors is on-target.

One hypothesis is that combined inhibition of PAK1 and PAK2 cause the observed toxicity. It is unlikely that inhibition of PAK1 alone suffices in driving the observed toxicity, as PAK1 knockout mice, in contrast to PAK2 knockout mice, are viable. However, we cannot rule out the possibility that developmental compensation could mask the effects of PAK1 deletion on

cardiovascular function.[26] Another hypothesis is that PAK2 inhibition alone is driving the toxicity. PAK2 is not only critical in embryonic vasculature development,[21, 22] but is also important for the integrity and function of blood vessels in adult mice, and inducible ubiquitous PAK2 deletion was lethal in adult mice.[23]

Both hypotheses lead to the possibility that selective PAK1 inhibition may *not* result in cardiovascular toxicity and might therefore offer a viable therapeutic strategy. In fact, allosteric PAK1 isoform selective inhibitors with biochemical selectivity versus PAK2 of > 50-fold were recently reported,[27] although no appreciable inhibition of phosphorylation of the downstream substrate MEK1 S298 was observed. The authors concluded that for cell lines that are dependent on and express both PAK1 and PAK2, a dual PAK1/2 inhibitor would likely be necessary for driving cellular efficacy. Data from us and others indicate that PAK1 amplified tumors are highly dependent on PAK1 signaling[6,28] suggesting that this may be one possibility where a PAK1 isoform specific inhibitor could be therapeutically viable, but additional work is needed to validate this proposition.

In summary, this study describes the structural diversification of our previous lead compound **1**, a Group I PAK inhibitor from the pyrido[2,3-d]pyrimidin-7-one class, in an attempt to dial out acute toxicity that was initially believed to be off-target. Considerable chemical variation of both head and tail groups yielded compound **19**, a potent Group I PAK inhibitor with improved cellular potency and solubility, but with a similar toxicity profile. Mouse tolerability studies with nine PAK1/2 inhibitors from the pyrido[2,3-d]pyrimidin-7-one series and two compounds from structurally distinct chemical classes revealed consistent acute toxicity, and we found that unbound $C_{max}$ associated with adverse effects in mice correlated with cellular inhibition of phospho-MEK1 S298. A mechanistic study in isolated rat hearts with compound **1** uncovered a

dose-dependent decline of function in the left ventricle, arrhythmia and heart failure, in alignment with the acute lethality in mouse tolerability studies. PAK1 and 2 are expressed in heart and vasculature tissues; PAK1 is known to be involved in cardiac function and PAK2 has a role in maintaining vascular integrity. As PAK1 knock-out mice are viable and PAK2 knock-out mice lethal it is likely that PAK2 inhibition is the major contributor to the toxicity. The only potential path forward for Group I PAK inhibitors might be with a selective PAK1 inhibitor in the context of PAK1 amplified tumors. However, given that PAK1 has been implicated in cardiac function,[20] a compound with such a profile would need to be carefully evaluated to ensure it has an acceptable safety profile. Although kinases have been implicated in cardiovascular functions,[29] we are not aware of kinase inhibitors that elicited cardiovascular on-target toxicity of similar acuteness and severity as observed with pan-Group I PAK inhibitors. This suggests fundamentally important functions of PAK2, either alone or in combination with PAK1, in cardiovascular physiology.

## EXPERIMENTAL SECTION

All chemicals were purchased from commercial suppliers and used as received. Flash chromatography was carried out with prepacked silica cartridges from either ISCO or SiliCycle on an ISCO Companion chromatography system using gradient elution. NMR spectra were recorded on a Bruker AV III 400 NMR spectrometer at 300K or 350K and referenced to tetramethylsilane. Preparative HPLC was performed on a Polaris C18 5 μm column (50 mm × 21 mm), eluting with mixtures of water/acetonitrile. All final compounds were purified to >95% chemical and optical purity, as assayed by LC/MS. This analysis was performed on an Agilent 1100 HPLC coupled with Agilent MSD mass spectrometer using ESI as the ionization source. The LC separation was performed using an Agilent ZORBAX SB-C18, 1.8 mm, 30 × 2.1 mm column with a flow rate of 0.4 mL/min. The gradient consisted of 3−97% acetonitrile in water, each containing 0.05% TFA, over 7 min; 97% acetonitrile was then maintained for 1.5 min, followed by re-equilibration at 3% acetonitrile for 1.5 min. The LC column temperature was maintained at 40 °C. UV absorbance was measured at 220 and 254 nm along with a full scan mass spectrum. For select compounds, a longer gradient LC was used (method B). In these cases, the gradient consisted of 2−98% acetonitrile in water, each containing 0.05% TFA, over 25.5 min; 98% acetonitrile was then maintained for 2.5 min, followed by re-equilibration at 2% acetonitrile for 1.5 min. UV and MS detection was performed as before. The synthesis of compound **1** was described in an earlier publication,[11] and compound **28** was prepared according to a procedure described in the literature.[17] Compound **20** was obtained from Afraxis, Inc.

**Ethyl 4-amino-2-(methylthio)pyrimidine-5-carboxylate (I-2a).** To a 2 L three-necked flask was added ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate (**I-1**, 100 g, 0.43 mol), THF

(500 mL), and triethylamine (186 mL, 1.29 mol). The ammonium hydroxide (28% in water, 400 mL) was added by portions to keep the internal temperature below 30 °C. After 2 h, water (1000 mL) was added and THF was distillated out under vacuum. The resulted solid was filtrated and dried under vacuum at 50 °C to afford the title compound (90 g, 99% yield). LCMS (ESI): *m/z* = 213.9 $[M+1]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.57 (s, 1H), 8.03 (br, 1H), 7.65 (br, 1H), 4.27 (q, *J* = 7.5 Hz, 2H), 2.46 (s, 3H), 1.29 (t, *J* = 7.2 Hz, 3H).

**Ethyl 4-(ethylamino)-2-(methylthio)pyrimidine-5-carboxylate (I-2b).** To a 3 L round bottom flask equipped with a mechanical stirrer under $N_2$ atmosphere was charged 4-chloro-2-methylthio-5-pyrimidinecarboxalate (**I-1**, 200 g, 0.86 mol), trimethylamine (3.0 equiv, 352 mL), then $NH_2$.HCl (140.2 g, 2.0 equiv) in DCM (5 mL/g, 1000 mL) was added into the solution at 0-5 $^o$C. After the addition, the mixture was warmed to 20-25 $^o$C and the mixture was stirred at this temperature for the completion. The reaction was quenched with water, washed with aq. $NH_4Cl$ and then the DCM layer was concentrated under reduced pressure to give a light yellow liquid (191 g, 92% yield) which was used for the next step without further purification, $^1$H NMR (400 MHz, DMSO-$d_6$): δ 8.51 (s, 1H), 8.29 (t, 1H, *J* = 5.4 Hz), 4.27 (q, 2H, *J* = 7.2 Hz), 3.49-3.54 (m, 2H), 2.47 (s, 3H), 1.30 (t, 3H *J* = 7 Hz), 1.16 (q, 3H, *J* = 7.2 Hz).

**(4-Amino-2-(methylthio)pyrimidin-5-yl)methanol (I-3a).** To a 3 L three-necked flask was added lithium aluminum hydride (**I-2a**, 25.7 g, 0.68 mol) and THF (1 L) and the suspension was cooled to −10−0 °C. A solution of ethyl 4-amino-2-(methylthio)pyrimidine- 5-carboxylate (120 g, 0.56 mol) in THF (1 L) was slowly added through an addition funnel to the stirring mixture. The reaction mixture was then allowed to warm to 20−25 °C. After 2 h, the mixture was cooled to 0 °C, and slowly quenched by water (26 mL). Then 10% sodium hydroxide (26 mL) was added and agitated for 2 h, which was followed by the addition of 78 mL (water). After filtration,

the solvent was removed to dryness to afford 80 g crude product. This was used in the next step without further purification. LCMS (ESI): *m/z* = 171.8 $[M+1]^+$.

**(4-(Ethylamino)-2-(methylthio)pyrimidin-5-yl)methanol (I-3b).** To a 3 L round bottom flask equipped with a mechanical stirrer under $N_2$ atmosphere was charged lithium alumimum hydride (1.5 equiv, 28.4 g) and THF (960 mL) and the mixture was cooled down at below 10 °C. A solution of ethyl 4-(ethylamino)-2-(methylthio)pyrimidine-5-carboxylate (**I-2b**) in THF (8 mL/g, 960 mL) was added into the solution while maintaining the inner temperature at 0-10 °C. The reaction mixture was then warmed to 20 °C and stirred at this temperature for 1 h. The reaction was determined complete by HPLC. The reaction mixture was cooled to 0-10 °C and quenched with 20 mL water and 10% aq sodium hydroxide (20 mL). The mixture was filtered and the wet cake was washed with THF (300 mL × 2). The filtrate was concentrated to remove the solvent to afford the title compound as white solid (85.2 g, 86% yield). $^1$H NMR (400 MHz, DMSO-*d6*): δ 7.82 (s, 1H), 6.82 (t, 1H, *J* = 5.4 Hz), 5.12 (t, 1H, *J* = 5.4 Hz), 4.30 (d, 2H, *J* = 5.2 Hz), 3.39 (q, 2H, *J* = 6Hz), 2.41 (s, 3H), 1.14 (t, 3H, *J* = 7.2 Hz).

**4-Amino-2-(methylthio)pyrimidine-5-carbaldehyde (I-4a).** To a 3 L three-necked flask was added (4-amino-2-(methylthio)pyrimidin-5-yl)methanol (**I-3a,** 100 g, 0.58 mol), activated manganese dioxide (152 g, 1.75 mol), and THF (2000 mL). The mixture was heated to 40−45 °C for 16 h. The reaction mixture was cooled to 20−25 °C and filtered through celite pad and rinsed with THF (400 mL × 3). The combined filtrate was concentrated to dryness. Reslurrying in 200 mL EtOAc/*n*-heptane (1:5) at 20−25 °C yielded pure product (51 g, 51.6% yield). LCMS (ESI): *m/z* =169.8 $[M+1]^+$; $^1$H NMR (400 MHz, $CDCl_3$): δ 9.80 (s, 1H), 8.44 (s, 1H), 8.22 (br, 1H), 5.77 (br, 1H), 2.57 (s, 3H).

**4-(Ethylamino)-2-(methylthio)pyrimidine-5-carbaldehyde (I-4b).** To a 3 L round bottom flask

equipped with a mechanical stirrer under $N_2$ atmosphere was charged (4-(ethylamino)-2-(methylthio)pyrimidin-5-yl)methanol (**I-3b**, 150 g, 1.0 equiv), activated manganese dioxide (5.0 equiv, 327 g) and THF (15 mL/g, 2250 mL). The mixture was heated to 40 $^{o}$C for 16 h for completion. The mixture was then cooled to 20 $^{o}$C and filtered through a pad of celite. The cake was rinsed with 500 mL (2x) of THF. The combined filtrate was concentrated to remove solvent to give a light yellow solid. The solid was re-slurried in EtOAc to obtain a white solid (123 g, 82% yield). $^{1}$H NMR (400 MHz, $CDCl_3$): δ 9.71 (s, 1H), 8.30 (s, 1H), 3.49-3.53 (m, 2H), 2.57 (s, 3H), 1.30 (t, 3H, $J$ = 7.4 Hz).

**Ethyl 2-(4-bromo-2-chlorophenyl)acetate (I-6a)**. To a 3 L three-necked flask under nitrogen was added zinc dust (117 g, 1.79 mol), THF (0.3 L). Chlorotrimethylsilane (10.4 mL) was added dropwise to control the internal temperature below 27 °C. The mixture was allowed to stir at 25−27 °C for 30 min and then heated to 30 °C. A solution of ethyl bromoacetate (150 g, 0.90 mol) in THF (1.2 L) was slowly added dropwise to the reaction mixture (the internal temperature should be below 50 °C). After the addition was complete, the reaction mixture was allowed to cool back to 25−30 °C. The mixture was filtered through celite pad under nitrogen to afford a yellow (occasionally also greenish or orange) solution of bromo-(2-ethoxy-2-oxoethyl)zinc. The concentration was titrated to be 0.50 M.

To a 3 L three-necked flask under nitrogen were added 4-bromo-2-chloro-1-iodo-benzene (80 g, 0.25 mol), bis(dibenzylideneacetone)palladium (7.2 g, 0.0125 mol), 4,5-bis(diphenyl-phosphino)-9,9-dimethylxanthene (Xantphos, 7.2 g, 0.0125 mol), and THF (800 mL). The mixture was degassed and backfilled with nitrogen three times. Bromo-(2-ethoxy-2-oxoethyl)zinc (960 mL, 0.48 mol) was added to the reaction mixture, followed by heating to 65 °C. The reaction was complete after 1 h. The reaction mixture was cooled to 30 °C and

quenched with aq. 1N HCl (400 mL) and 25% brine (400 mL). The organic layer was separated, filtered through a celite pad, and concentrated under vacuum. The crude material was purified by silica column chromatography (0−30% EtOAc/*n*-heptane) to afford the product as light yellow oil (60 g, 86% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, $J$ = 2.0 Hz, 1H), 7.29 (dd, $J_1$ = 8.0 Hz, $J_2$ = 2.0 Hz, 1H), 7.08 (d, $J$ = 8 Hz, 1H), 4.10 (q, $J$ = 6.8 Hz, 2H), 3.64 (s, 2H), 1.18 (t, $J$ = 6.8 Hz, 3H).

**Ethyl 2-(2-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetate (I-7).** To a 500 mL three-necked flask was added ethyl 2-(4-bromo-2-chlorophenyl)acetate (**I-6a**, 50 g, 0.18 mol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (62.5 g, 0.24 mol), potassium acetate (33 g, 0.34 mol), 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride DCM complex (10.4 g, 0.012 mol) and 1,4-dioxane (250 mL). The mixture was degassed and backfilled with nitrogen three times. It was then heated at refluxing for 20 h. This mixture was filtered. 25% brine (200 mL) was added, and the resulted mixture was extracted with EtOAc (300 mL × 3). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to give crude product. The crude material was then purified by silica column chromatography (5%−25% EtOAc/n-heptane) to afford the title compound (54 g, 90% yield). LCMS (ESI): *m/z* =324.6 $[M+1]^+$.

**Ethyl 2-(2-methyl-4-(6-methylpyridin-2-yl)phenyl)acetate (I-9a).** To a 2000mL jacket reactor equipped with a mechanical stirrer under an atmosphere of $N_2$ was charged ethyl 2-(2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetate (**I-7**, 60 g, 1.0 equiv) and 2-chloro-6-methylpyridine (**I-8a**, 25 g, 1.0 equiv) followed by 1200 mL of toluene, 120 mL of EtOH and 240 mL of water. To the mixture was charged Pd(dppf)$Cl_2$ (12 g, 20 wt%) in one portion. The reactor was filled with $N_2$ and subsequently heated until complete of consumption of starting

material was observed (deemed to complete when s.m./product <10%). The reaction mixture was cooled to 20 °C and undissolved substances filtered out. The filtrate was concentrated to remove the solvent, and the residue purified by silica gel chromatograpgy to give the title compound in 97.7% purity (54 g, 51% yield). $^1$H NMR (400 MHz, DMSO-*d6*): δ 7.89 (s, 1H), 7.81-7.84 (m, 1H), 7.73 (t, 2H, *J* = 4.6 Hz), 7.29 (d, 1H, *J* = 8 Hz), 7.20 (dd, 1H, $J^1$ = 1.4 Hz, $J^2$ = 7 Hz), 4.10 (q, 2H, *J* = 7.2 Hz), 3.73 (s, 2H), 2.53 (s, 3H), 2.31 (s, 3H), 1.19 (t, 3H, *J* = 7.2 Hz). LCMS (ESI): *m/z* = 270.2 $[M+1]^+$.

**Ethyl 2-(2-chloro-4-(6-methylpyrazin-2-yl)phenyl)acetate (I-9b).** To a 2 L three-necked flask was added ethyl 2-(2-chloro-4-(4,4,5,5-tetramethyl-1,3,2- dioxaborolan-2-yl)phenyl)acetate (**I-7**, 54 g, 0.17 mol), potassium acetate (33 g, 0.34 mol), 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride DCM complex (10.4 g, 0.012 mol), 2-chloro-6 methylpyrazine (**I-8b**, 21.3 g, 0.17 mol) and 1,4-dioxane/water (2:1, 750 mL). The mixture was degassed and backfilled with nitrogen (3×). Then it was heated to 100 °C for 15 h. After cooling to 20−25 °C the mixture was diluted with water (200 mL) and extracted with EtOAc (300 mL × 3). The combined organic layers were washed with 25% brine (100 mL × 2), and dried over anhydrous sodium sulfate. The crude material was purified by silica gel column chromatography (5%−25% EtOAc/*n*-heptane), followed by slurrying in *n*-heptane. The title compound was obtained as white solid (18 g, 37.5% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.81 (s, 1H), 8.42 (s, 1H), 8.10(d, *J* = 1.6 Hz, 1H), 7.85 (d, *J* = 8 Hz, 1H), 7.43 (d, *J* = 8 Hz, 1H), 4.20 (q, *J* = 7.2 Hz, 2H), 3.84 (s, 2H), 2.64 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H).

**Methyl 2-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)acetate (I-11a).** A mixture of methyl 2-(4-bromo-2-chlorophenyl)acetate (**I-6b**, prepared analogously to **I-6a**, 100 mg, 0.38 mmol), 3-methylpyridin-2(1H)-one (**I-10a**, 62 mg, 0.57 mmol), $N^1$,$N^2$-dimethylethane-1,2-

diamine (18 mg, 0.20 mmol), copper(I) iodide (20 mg, 0.10 mmol) and potassium phosphate (550 mg, 1.71 mmol) in 1,4-dioxane (1.5 mL) was stirred in a sealed tube at 110 °C under a nitrogen atmosphere for 48 h. After cooling to rt and filtration, the filtrate was concentrated and purified by silica gel chromatography using DCM/methanol (100:1) as eluting solvents to afford the title compound as yellow solid (60 mg, 54% yield). LCMS (ESI): *m/z* = 292.1 [M+1].

**Methyl 2-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)phenyl)acetate (I-11b).** A mixture of methyl 2-(4-bromo-2-chlorophenyl)acetate (**I-6b**, 792 mg, 3.0 mmol), 3-methylpyrazin-2(1H)-one (**I-10b**, 660 mg, 6.0 mmol), $N^1,N^2$-dimethylethane-1,2-diamine (105.6 mg, 1.2 mmol), copper(I) iodide (114.6 mg, 0.60 mmol) and potassium phosphate (2.89 g, 9.0 mmol) in 1,4-dioxane (10 mL) was stirred at 110 °C under nitrogen atmosphere for 2 d. After cooling to rt and filtration, the filtrate was concentrated and purified by silica gel chromatography using DCM/methanol (100:1) as eluting solvents to afford the title compound as yellow solid (560 mg, 64 % yield). LCMS (ESI): *m/z* = 293.1 [M+1].

**6-(2-Chloro-4-(6-methylpyridin-2-yl)phenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (I-12).** To a 3L round bottom reactor was charged 4-amino-2-(methylthio)pyrimidine-5-carbaldehyde (**I-4a**, 115.8 g, 684.5 mmol), ethyl 2-(2-chloro-4-(6-methylpyridin-2-yl)-phenyl)acetate (**I-9a**, 238 g, 1.2 equiv) and $K_2CO_3$ (283.8 g, 3 equiv) followed DMF (600 mL). The mixture was stirred at 110 $^0$C for 21 h under a nitrogen atmosphere and progression monitored by HPLC. After completion, DMF was partially removed *in vacuo*. HCl (12 M, 170 mL) and $H_2O$ (1 L) were added to the mixture, and the reaction mixture was filtered. The cake was reslurried in sat. aq. $NaHCO_3$ (1 L) and water (2 L), respectively and filtered. The cake was dried at 50°C in vacuum to give the title compound as brown solid (260 g, 96.0% yield). $^1$H NMR (400 MHz, DMSO-*d6*): δ 8.90 (s, 1H), 8.24 (d, 1H, *J* = 1.6 Hz), 8.10 (t, 2H, *J* = 4.8 Hz),

7.99 (s, 1H), 7.821-7.89 (m, 3H), 7.53 (d, 1H, $J$ = 8 Hz), 7.29 (d, 1H, $J$ = 7.6 Hz), 2.51 (s, 3H), 2.38 (s, 3H).

***tert*-Butyl 3-(6-(2-chloro-4-(6-methylpyridin-2-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (I-13).** A mixture 6-(2-chloro-4-(6-methylpyridin-2-yl)phenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (**I-12**, 193 mg, 0.49 mmol), cesium carbonate (478 mg, 1.47 mmol) and 3-(*tert*-butoxycarbonylamino)propyl 4-methylbenzene-sulfonate (200 mg, 0.61 mmol) in 1,4-dioxane (5 mL) was stirred at 85 °C overnight. After filtration and concentration, the residue was purified by silica gel chromatography using DCM/MeOH (40:1 to 20:1) as eluting solvents to afford the title compound as a brown solid (300 mg, 90% yield). LCMS (ESI): $m/z$ = 496.0 $[M-55]^+$.

***tert*-Butyl 3-(6-(2-chloro-4-(6-methylpyridin-2-yl)phenyl)-2-(methylsulfinyl)-7-oxopyrido-[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (I-14).** To a solution of *tert*-butyl 3-(6-(2-chloro-4-(6-methylpyridin-2-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl-carbamate (300 mg, 0.54 mmol) in dry DCM (10 mL) was added in portions *m*-CPBA (164 mg, 0.80 mmol, 85% wt). The reaction mixture was stirred at rt for 30 min. Water (50 mL) was added and the solution was extracted with DCM (2×50 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford the title compound as a white solid (280 mg, crude), which was used in the next step without further purification. LCMS (ESI): $m/z$ = 512.0 $[M-55]^+$.

***tert*-Butyl 3-(6-(2-chloro-4-(6-methylpyridin-2-yl)phenyl)-2-(methylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (I-15).** A mixture of 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-ethyl-2-(methylsulfinyl)pyrido[2,3-d]pyrimidin-7(8H)-one (280 mg, 0.49 mmol) and methyl amine (30% wt in EtOH, 5 mL) in THF (20 mL) was stirred at 50°C

overnight. After concentration under reduced pressure, the residue was purified by preparative HPLC to afford the title compound as a yellow oil (150 mg, crude), which was used in the next step without further purification. LCMS (ESI): *m/z* = 480.1 $[M-55]^+$.

**6-(4-Bromo-2-chlorophenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (I-16).** To a 2 L round bottom flask equipped with a mechanical stirrer under $N_2$ atmosphere was charged 4-amino-2-methylsulfanyl-pyrimidine-5-carbaldehyde (80.00 g, 1.0 equiv), potassium carbonate (196 g, 3.0 equiv), DMF (5 mL/g, 400 mL), and methyl 2-(4-bromo-2-chloro-phenyl)acetate (1.0 equiv, 131 g). The mixture was heated to 120 °C. The reaction was complete in 8 h based on HPLC analysis and then cooled to ambient temperature. Water (2400 mL, 30 vol) was added, and the mixture was stirred for 30 min. The mixture was stirred for an additional 1 h, filtered and the wet cake was rinsed with water (150 mL × 2). The solids were recrystallized in THF/heptane and dried at 50 °C under vacuum to afford the product as a light orange solid (151 g, 83% yield). $^1$H NMR (400 MHz, DMSO-*d6*): δ 12.70 (s, 1H), 8.90 (s, 1H), 7.98 (s, 1H), 7.85 (s, 1H), 7.65 (d, 1H, *J* = 8 Hz), 7.38 (d, 1H, *J* =8 Hz).

***tert*-Butyl (3-(6-(4-bromo-2-chlorophenyl)-2-(methylthio)7-oxopyrido[2,3-*d*]pyrimidin-8(7*H*)-yl)propyl)carbamate (I-17).** To a 1L 3-neck round-bottom flask was added 6-(4-bromo-2-chlorophenyl)-2(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (**I-16**; 26.0 g, 67.9 mmol), cesium carbonate powder (66.4 g, 3.00 equiv), *tert*-butyl *N*-(3-bromopropyl)carbamate (24.3 g, 1.50 equiv) and DMF (390 mL). The mixture was stirred at rt for 17 h and quenched with $H_2O$ (390 mL). The mixture was then extracted with DCM (300 mL) and phases were separated. The aqueous layer was extracted with DCM (100 mL) and the combined organic layers were washed with $H_2O$ (100 mL × 3) and brine (100 mL). The organic layer was filtered through a 2-inch silica gel pad and washed with DCM (100 mL × 2), 2.5% MeOH in DCM (100 mL × 2) and 5%

MeOH in DCM (100 mL). The filtrate was washed with $H_2O$ (200 mL × 2) followed by $H_2O$ (300 mL × 2) and dried over anhydrous $Na_2SO_4$. The solution was concentrated to dryness to afford the title compound as an orange oil (51.0 g, 74% yield). LCMS (ESI): *m/z* = 441.0 [M-Boc+H]$^+$; $^1$H NMR (300 MHz, $CDCl_3$): δ 8.67 (s, 1H), 7.67 (d, *J* = 1.9 Hz, 1H), 7.65 (s, 1H), 7.49 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 1H), 5.26 (s, 1H), 4.56 (t, *J* = 6.4 Hz, 2H), 3.13 (q, *J* = 6.3 Hz, 2H), 2.65 (s, 3H), 2.03–1.92 (m, 3H), 1.43 (s, 9H).

***tert*-Butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (I-19a).** A mixture of *tert*-butyl 3-(6-(4-bromo-2-chlorophenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (**I-17**, 590 mg, 1.10 mmol), pyrrolidin-2-one (187.2 mg, 2.2 mmol), tris(dibenzylideneacetone)di-palladium(0) (100.7 mg, 0.11 mmol), dimethylbisdiphenylphosphinoxanthene (127.1 mg, 0.22 mmol) and cesium carbonate (1.08 g, 3.3 mmol) in toluene (10 mL) was stirred in a sealed at 100 °C overnight. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography using DCM/MeOH (20:1) as eluting solvents to afford the title compound (600 mg, 99% yield) as a yellow solid. LCMS (ESI): *m/z* = 488 [M-55]$^+$.

***tert*-Butyl 3-(6-(2-chloro-4-(2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (I-19b).** A mixture of *tert*-butyl 3-(6-(4-bromo-2-chlorophenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (**I-17**, 212 mg, 0.40 mmol), pyridin-2(1H)-one (**I-18b**, 76 mg, 0.80 mmol), cuprous iodide (14.8 mg, 0.08 mmol), $N^1$,$N^2$-dimethylethane-1,2-diamine (14.3 mg, 0.16 mmol) and potassium phosphate (254 mg, 1.2 mmol) in dioxane (2 mL) was stirred in a sealed tube at 110 °C overnight. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography using DCM: MeOH (20:1) as eluting solvents to afford the title compound as a

yellow solid (157 mg, 71% yield). LCMS (ESI): *m/z* = 570.1 $[M+17]^+$.

***tert*-Butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylsulfinyl)-7-oxopyrido-[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (I-20a).** A mixture of *tert*-butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl-carbamate (**I-19a**, 200 mg, 0.37 mmol) and *m*-CPBA (112 mg, 0.56 mmol, 85% wt) in DCM (10 mL) was stirred at r.t. overnight. Saturated sodium bicarbonate and DCM (40 mL) were added. The separated organic layer was washed with brine and dried over sodium sulfate. After concentration under reduced pressure, *tert*-butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (250 mg, crude) was obtained as a white solid, which was used in the next step without further purification. LCMS (ESI): *m/z* = 505.1 $[M-55]^+$.

***tert*-Butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylsulfinyl)-7-oxo-pyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (I-20b).** A mixture of *tert*-butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)-propylcarbamate (**I-19b**, 80 mg, 0.14 mmol) and *m*-CPBA (42 mg, 0.21 mmol, 85% wt) in DCM (5 mL) was stirred at r.t. overnight. Saturated sodium bicarbonate and DCM (20 mL) was added. The separated organic layer was washed with brine and dried over sodium sulfate. After concentration under reduced pressure, the title compound (100 mg, crude) was obtained as a white solid, which was used in the next step without further purification. LCMS (ESI): *m/z* = 515.1 $[M-55]^+$.

***tert*-Butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylsulfinyl)-7-oxopyrido-[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (I-21a).** A mixture of *tert*-butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-

yl)propylcarbamate (**I-20a**, 250 mg, crude) and methyl amine (30% wt in EtOH, 5 mL) in MeOH at was stirred overnight at r.t. after concentration under reduced pressure, the title compound (150 mg, crude) was obtained as yellow oil, which was used in the next step without further purification. LCMS (ESI): *m/z* = 472.0 $[M-55]^+$.

***tert*-Butyl 3-(6-(2-chloro-4-(2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)-7-oxopyrido-[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (I-21b).** A mixture of *tert*-butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (**I-20b**, 100 mg, crude) and methyl amine (30% wt in EtOH, 2 mL) in MeOH at was stirred overnight at r.t. After concentration under reduced pressure, it was afforded the title compound (110 mg, crude) as yellow oil, which was used in the next step without further purification. LCMS (ESI): *m/z* = 482.0 $[M-55]^+$.

***tert*-butyl (3-(6-(4-bromo-2-chlorophenyl)-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl)carbamate (I-22).** To a 1L 3-neck round-bottom flask was added *tert*-butyl *N*-[3-[6-(4-bromo-2-chlorophenyl)-2-methylsulfanyl-7-oxo-pyrido[2,3-*d*]pyrimidin-8-yl]propyl]-carbamate (**I-17**, 23.4 g, 74 wt%, 32.1 mmol) and $CH_3CN$ (350 mL). The mixture was cooled to 0−5 °C and *m*-CPBA (9.62 g, 70 wt%, 1.20 equiv) was added. The mixture was stirred for 30 min and quenched with a mixture of satd. $Na_2SO_3$ (75 mL) and $H_2O$ (75 mL). The organic layer was separated and washed with a mixture of satd. $Na_2SO_3$ (50 mL) and $H_2O$ (50 mL), then dried over anhydrous $MgSO_4$ (10.0 g) and filtered. The filtrate was concentrated to ca. 275 mL and used directly in the next step.

**6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylamino)pyrido[2,3-*d*]pyrimidin7(8*H*)-one (I-23).** To the above $CH_3CN$ solution of crude **I-22** was added $MeNH_2$ (12.1 mL, 33 wt% in EtOH, 3.0 equiv) at 0−10 °C and the mixture was stirred at 10−20 °C for 30 min. The mixture

was then concentrated to a red oil, diluted with DCM (50 mL) and passed through a 2-inch silica gel pad eluting with DCM (100 mL × 2) followed by 2.5% MeOH in DCM (100 mL x5). The combined organic fractions were concentrated to dryness and triturated in heptane/EtOAc (50:1). The solids were filtered and dried under vacuum at 50 °C to afford the title compound as a yellow solid (16.0 g, 94% yield). LCMS (ESI): *m/z* = 424.0 $[M\text{-Boc+H}]^+$; $^1$H NMR (300 MHz, $CDCl_3$) δ 8.47 (br s, 1H), 7.65 (d, *J* = 1.9 Hz, 1H), 7.53 (s, 1H), 7.46 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 1H), 5.56 (br s, 1H), 4.51 (br s, 2H), 3.18−3.08 (m, 4H), 1.98 (br s, 3H), 1.44 (s, 9H).

***tert*-Butyl (3-(6-(2-chloro-4-(3-methyl-2-oxoimidazolidin-1-yl)phenyl)-2-(methylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7*H*)-yl)propyl)carbamate (I-24a).** A suspension of *tert*-butyl *N*-[3-[6-(4-bromo-2-chloro-phenyl)-2-(methylamino)-7-oxo-pyrido[2,3-d]pyrimidin-8-yl]propyl]-carbamate (**I-23**, 108 mg, 207 µmol), 1-methylimidazolidin-2-one (43.1 mg, 430 µmol, 2.1 equiv), tris(dibenzylideneacetone)-dipalladium(0) (17.4 mg, 18.8 µmol, 0.09 equiv), 4,5-bis(di-phenylphosphino)-9,9-dimethylxanthene (18.1 mg, 31.3 µmol, 0.15 equiv) and cesium carbonate (204 mg, 627 µmol, 3.0 equiv) in degassed toluene (4.0 mL) was heated to 100 °C for 6 h and allowed to cool. The mixture was diluted with EtOAc, washed with water and brine, dried over sodium sulfate, filtered and concentrated *in vacuo*, and used directly in the next step.

**6-(4-Bromo-2-chlorophenyl)-8-ethyl-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (I-25).** To a 2 L round-bottom flask was added 4-(ethylamino)-2-(methylthio)pyrimidine-5-carbaldehyde (62.0 g, 0.314 mmol), potassium carbonate (3.0 equiv, 130 g), DMF (400 mL), and ethyl 2-(4-bromo-2-chloro-phenyl) acetate (**I-6b**, 1.0 equiv, 87 g). The mixture was heated to 120 °C. The reaction was completed in 2 h based on HPLC analysis and was cooled to ambient temperature. Water (2400 mL, 30 vol) was added and the mixture was stirred for 30 min, solid

was collected by filtration, and the brown solid was reslurried in EtOH (81.5 g, 92% yield). $^1$H NMR (400 MHz, DMSO-*d6*) δ 8.944 (s, 1H), 8.031 (s, 1H), 7.866 (s, 1H), 7.654 (d, 1H, *J* = 1.2 Hz), 7.407 (d, 1H, *J* = 6.8 Hz), 2.637 (s, 2H), 1.263 (t, 3H, *J* = 7 Hz).

**6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-ethyl-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (I-26).** A mixture of 6-(4-bromo-2-chlorophenyl)-8-ethyl-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (**I-25**, 400 mg, 0.98 mmol), 3-methylpyridin-2(1H)-one (214 mg,1.96), cuprous iodide (37 mg,0.2 mmol), $N^1$,$N^2$-dimethylethane-1,2-diamine (35 mg, 0.39 mmol) and potassium phosphate (623 mg, 2.94 mmol) in dioxane (4 mL) was stirred in a sealed tube at 110 °C overnight. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography using DCM:EtOAc (2:1) as eluting solvents to afford the title compound as a yellow solid (300 mg, 35% yield). LCMS (ESI): *m/z* = 439.1 $[M+1]^+$.

**6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-ethyl-2-(methylsulfinyl)pyrido-[2,3-d]pyrimidin-7(8H)-one (I-27).** To a solution of 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-ethyl-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (**I-26**, 100 mg, 0.23 mmol) in dry DCM (5 mL) was added in portions *m*-CPBA (70 mg, 0.34 mmol, 85% wt). The reaction mixture was stirred at rt for 30 min. Water (50 mL) was added and the solution was extracted with DCM (2×50 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford the title compound (150 mg, crude) as a white solid, which was used in the next step without further purification. LCMS (ESI): *m/z* =455.1 $[M+1]^+$.

**4-(3-(Bis(4-methoxybenzyl)amino)-2,2-difluoropropylamino)-2-(methylthio) pyrimidine-5-carbaldehyde (I-29a).** A mixture of 2,2-difluoro-$N^1$,$N^1$-bis(4-methoxybenzyl)propane-1,3-

diamine (160 mg, 0.45 mmol), 4-chloro-2-(methylthio)pyrimidine-5-carbaldehyde (**I-28**, 96 mg, 0.50 mmol), triethylamine (160 mg, 1.38 mmol), $H_2O$ (160 mg, 9.2 mmol), THF (6 mL) and *i*-PrOH (2 mL) was stirred at 0°C for 8 h. Then water and EtOAc (100 mL) were added. The organic phase was separated and dried over $Na_2SO_4$. After concentration under reduced pressure, the residue was purified by silica gel chromatography using petroleum ether/EtOAc (1:0 to 3:1) as eluting solvent to afford the title compound as a yellow oil (130 mg, 56% yield). LCMS (ESI): *m/z* = 502 $[M+1]^+$.

***tert*-Butyl 3,3-difluoro-4-((5-formyl-2-(methylthio)pyrimidin-4-ylamino)methyl)piperidine-1-carboxylate (I-29b).** To a solution of 4-chloro-2-(methylthio)pyrimidine-5-carbaldehyde (**I-28**, 100 mg, 0.53 mmol) in dry THF (5 mL) and propan-2-ol (5 mL) was added triethylamine (161 mg, 1.59 mmol) and *tert*-butyl 4-(aminomethyl)-3,3-difluoropiperidine-1-carboxylate (139 mg, 0.56 mmol) at rt under a nitrogen atmosphere. The reaction solution was stirred at rt overnight. Water (50 mL) was added, and the solution was extracted with DCM (3×50 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated to afford the title compound as yellow solid (200 mg, 94% yield). LCMS (ESI): *m/z* = 403.3 $[M+1]^+$.

**8-(3-(Bis(4-methoxybenzyl)amino)-2,2-difluoropropyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (I-30a).** A mixture of 4-(3-(bis(4-methoxybenzyl)amino)-2,2-difluoropropylamino)-2-(methylthio) pyrimidine-5-carbaldehyde (**I-29a**, 130 mg, 0.26 mmol), methyl 2-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)acetate (95 mg, 0.31 mmol), $K_2CO_3$ (107 mg, 0.78 mmol) and DMF (4 mL) was stirred at 95°C for 16 h. After filtration, water and EtOAc (150 mL) was added. The organic phase was separated, dried over $Na_2SO_4$ and concentrated under reduced pressure. The

residue was purified by silica gel chromatography using DCM: EtOAc (1:0 to 2:1) as eluting solvent to afford the title compound as a yellow solid (110 mg, 57% yield). LCMS (ESI): *m/z* = 744 $[M+1]^+$.

***tert*-Butyl 4-((6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxo-pyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (I-30b).** A mixture of *tert*-butyl 3,3-difluoro-4-((5-formyl-2-(methylthio)pyrimidin-4-ylamino)methyl)-piperidine-1-carboxylate (**I-29b**, 200 mg, 0.54 mmol), potassium carbonate (206 mg, 1.49 mmol) and methyl 2-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)acetate (174 mg, 0.60 mmol) in DMF (10 mL) was stirred at 90°C overnight. The reaction was diluted with EtOAc (50 mL) and water. The separated organic layer was dried over anhydrous sodium sulfate and evaporated in *vacuo*. The residue was purified by silica gel chromatography using EtOAc: petroleum ether (1:1) as eluting solvents to afford the title compound as a yellow solid (200 mg, 62% yield). LCMS (ESI): *m/z* = 644.3 $[M+1]^+$.

***tert*-Butyl 4-((6-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxo-pyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (I-30c).** A mixture of *tert*-butyl 3,3-difluoro-4-(((5-formyl-2-(methylthio)pyrimidin-4-yl)amino)methyl)-piperidine-1-carboxylate (**I-29b**, 300 mg, 0.81 mmol), methyl 2-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)phenyl)acetate (261 mg, 0.9 mmol) and potassium carbonate (309 mg, 2.23 mmol) in DMF (15 mL) was stirred at 90 °C overnight. The reaction mixture was diluted with EtOAc (50 mL) and water. The separated organic layer was dried over anhydrous sodium sulfate and evaporated in *vacuo*. The residue was purified by silica gel chromatography using EtOAc/petroleum ether (1:1) as eluting solvents to afford the title compound as a yellow solid (400 mg, 83% yield).

***tert*-butyl (*S**)-4-((6-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (I-30d).** **I-30c** was resolved by chiral preparative HPLC to afford the two enantiomers, *tert*-butyl (*S**)-4-((6-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]-pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate and *tert*-butyl (*R**)-4-((6-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]-pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (100 mg each) with unknown absolute configuration. LCMS (ESI): *m/z* = 589.1 $[M-55]^+$. The following steps describe the synthetic steps leading to the active enantiomer **22**, arbitraily assigned (*S**)-configuration.

***tert*-Butyl (4*S**)-4-((6-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)phenyl)-2-(methyl-sulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (I-31).** A mixture of (*S**)-4-((6-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)-phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (**I-30d**, 100 mg, 0.155 mmol) and *m*-CPBA (89.5 mg, 0.23 mmol, 85% wt) was stirred at rt for 30 min. Water (20 mL) was added, and the solution was extracted with DCM (2×20 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford the title compound (90 mg, crude) as a white solid, which was used in the next step without further purification. LCMS (ESI): *m/z* = 606.0 $[M-55]^+$.

**8-(3-Amino-2,2-difluoropropyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (I-32a).** To a solution of 8-(3-(bis(4-methoxybenzyl)amino)-2,2-difluoropropyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (**I-30a**, 110 mg, 0.15 mmol) in $MeNH_2$ (30%wt in MeOH, 6 mL) was stirred at 80°C for 8 h. After cooling to r.t, the mixture

was concentrated under reduced pressure to afford the title compound (100 mg, crude) as a yellow oil, which was used in the next step without further purification. LCMS (ESI): *m/z* = 727 $[M+1]^+$.

***tert*-Butyl 4-((6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (I-32b).** A solution of *tert*-butyl-4-((6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (**I-30b**, 200 mg, 0.31 mmol) in methanamine (3 mL, 2M in THF) was stirred at 80 °C overnight in a sealed tube. The reaction mixture was concentrated in *vacuo* to give the title compound (200 mg, crude) as yellow solid, which was used in the next step without further purification. LCMS (ESI): *m/z* = 627.1 $[M+1]^+$.

***tert*-Butyl (*S**)-4-((6-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)phenyl)-2-(oxetan-3-ylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (I-32c).** A mixture of *tert*-butyl (4*S*)-4-((6-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)-phenyl)-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoro-piperidine-1-carboxylate (**I-31**, 90 mg, 0.16 mmol), oxetan-3-amine (35 mg, 0.48 mmol) and ethyldiisopropylamine (62 mg, 0.48 mmol) in THF (5 mL) was stirred at 70 °C overnight. After concentration under reduced pressure, the title compound (100 mg, crude) was obtained as a yellow oil, which was used in the next step without further purification.

**8-(3-Aminopropyl)-6-(2-chloro-4-(6-methylpyridin-2-yl)phenyl)-2-(methylamino)pyrido-[2,3-d]pyrimidin-7(8H)-one (2).** A mixture of 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-ethyl-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (150 mg, crude) and TFA

(3 mL) in DCM (30 mL) was stirred at rt for 1 h. After concentration under reduced pressure, the residue was purified by preparative HPLC to afford the title compound as a white solid (46 mg, 20% yield). LCMS (ESI): *m/z* = 435.0 $[M+1]^+$ ; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.47 (s, 1H), 8.11 (d, *J* = 1.6 Hz, 1H), 7.90 (dd, *J* = 1.6 Hz, 8Hz, 1H), 7.65 (t, *J* = 8 Hz, 1H), 7.56 (s, 1H), 7.52 (d, *J* = 8 Hz, 1H), 7.45 (d, *J* = 8 Hz, 1H), 7.13 (d, *J* = 8 Hz, 1H), 5.61-5.60 (m, 1H), 4.56 (m, 2H), 3.11 (d, *J* = 4.8 Hz, 3H), 2.74 (s, 2H), 2.63 (s, 3H), 1.98 (s, 2H).

**8-(3-Aminopropyl)-6-(2-chlorophenyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (3).**

Step 1: *tert*-Butyl (3-(6-bromo-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl)-carbamate. Solid 6-bromo-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (1.34 g, 4.91 mmol), *tert*-butyl N-(3-bromopropyl)carbamate (1.19 g, 5.01 mmol), and cesium carbonate (1.66 g, 5.10 mmol) were suspended together in DMF (5.0 mL) and the mixture stirred at ambient temperature for 21 h. The mixture was diluted with water and extracted with 2-methyltetrahydrofuran (2x). The combined organic phases were washed with water and brine, dried over sodium sulfate, filtered and concentrated *in vacuo* to afford a cream-colored solid (2.095 g, 99% yield). $^1$H NMR (400 MHz, DMSO-*d6*) δ 8.87 (s, 1H), 8.53 (s, 1H), 6.82 (t, *J* = 5.9 Hz, 1H), 4.39 – 4.30 (m, 2H), 3.00 (q, *J* = 6.6 Hz, 2H), 2.61 (s, 3H), 1.77 (p, *J* = 7.0 Hz, 2H), 1.37 (s, 9H). LCMS (ESI) *m/z* = 429/431 $[M+1]^+$.

Step 2: *tert*-Butyl (3-(6-bromo-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl)carbamate *and* tert-butyl (3-(6-bromo-2-(methylsulfonyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl)carbamate. To a solution of *tert*-butyl (3-(6-bromo-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl)carbamate (2.09 g, 4.88 mmol) in THF (20 mL) at ambient temperature was added solid *m*-CPBA (1.32 g, 5.88 mmol) in several portions over ~5

min. LCMS analysis after 30 min indicated complete conversion to a 1.9:1 mixture (254 nm) of sulfoxide product ($MH^+$ 445/447) with sulfone product (MH+461/463) and a minor amount of starting material ($MH^+$ 429/431). After 60 min, the mixture was diluted with 2-methyltetrahydrofuran, washed with saturated aqueous sodium bicarbonate and brine, dried over sodium sulfate, filtered and concentrated *in vacuo* to afford a pale yellow foam (2.46 g, 109% crude yield) that was used in the next step without purification.

Step 3: *tert*-Butyl (3-(6-bromo-2-(methylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl)-carbamate. The crude 1.9:1 mixture of *tert*-butyl (3-(6-bromo-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl)carbamate AND *tert*-butyl (3-(6-bromo-2-(methylsulfonyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl)carbamate (1.94 g, 3.84 mmol) from step 2 was treated with methylamine (2.0 mol/L) in THF (9.0 mL, 18 mmol) at ambient temperature to give a clear golden solution. After 1 h, the mixture had become thick and a cream-colored solid formed. The mixture was treated with saturated aqueous sodium bicarbonate and water and extracted with EtOAc (200 mL). The separated organic phase was washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo* to afford a white solid (1.47 g, 93% yield). $^1$H NMR (400 MHz, DMSO-*d6*) δ 8.68 – 8.55 (m, 1H), 8.28 (s, 1H), 7.96 – 7.68 (m, 1H), 6.82 – 6.73 (m, 1H), 4.35 – 4.19 (m, 2H), 3.03 – 2.92 (m, 2H), 2.89 (d, $J$ = 4.7 Hz, 3H), 1.82 – 1.69 (m, 2H), 1.36 (s, 9H). LCMS (ESI) $m/z$ = 412/414 $[M+1]^+$.

Step 4: 8-(3-Aminopropyl)-6-bromo-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one. *tert*-Butyl (3-(6-bromo-2-(methylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propyl)carbamate (340 mg, 0.823 mmol) was treated with a solution of TFA (2.0 mL, 26 mmol) in dichloromethane (4 mL) at ambient temperature for 30 min. The mixture was concentrated *in vacuo* to yield a brown oil and further treated with saturated aqueous sodium bicarbonate followed by extraction

with DCM (2x). The combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo* to afford a cream colored solid (154.6 mg, 60% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.50 – 8.31 (m, 1H), 7.90 (s, 1H), 5.63 (s, 1H), 4.61 – 4.38 (m, 2H), 3.08 (d, *J* = 5.0 Hz, 3H), 2.73 (t, *J* = 6.9 Hz, 2H), 2.00 – 1.87 (m, 2H), 1.81 – 1.64 (m, 3H). LCMS (ESI) *m/z* = 312/314 $[M+1]^+$.

Step 5: 8-(3-aminopropyl)-6-(2-chlorophenyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 8-(3-aminopropyl)-6-bromo-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (38.6 mg, 0.124 mmol), (2-chlorophenyl)boronic acid (25.2 mg, 0.161 mmol), and bis(di-tert-butyl(4-dimethylaminophenyl)phosphine)dichloropalladium(II) (8.79 mg, 0.124 mmol) in acetonitrile (1.5 mL) was treated with aqueous sodium carbonate (1.0 mL, 1.0 M), followed by heating under microwave irradiation (CEM, 300 watts) at 100 ºC for 15 min. The mixture was diluted with EtOAc, and the upper organic phase decanted from the aqueous phase, dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude residue was submitted to purification by reverse phase HPLC (C18, gradient of acetonitrile in water with 0.1% ammonium hydroxide) to afford the title compound (11.6 mg, 27% yield). $^1$H NMR (400 MHz, DMSO-*d6*) δ 8.73 – 8.60 (m, 1H), 7.87 – 7.68 (m, 1H), 7.77 (s, 1H), 7.55 – 7.49 (m, 1H), 7.45 – 7.35 (m, 3H), 4.42 – 4.26 (m, 2H), 2.92 (d, *J* = 4.7 Hz, 3H), 2.65 – 2.55 (m, 2H), 1.89 – 1.74 (m, 2H). LCMS (ESI): *m/z* = 344.1 $[M+1]^+$.

**8-(3-Aminopropyl)-6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (4).**

A mixture of *tert*-butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (**I-21a**, 150 mg, crude) and TFA (2 mL) in DCM (20 mL) was stirred for 2 h. After concentration under reduced pressure, the residue was

purified by preparative HPLC to afford 8-(3-aminopropyl)-6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one as a white solid (60.4 mg, 50% yield). $^1$H NMR (500 MHz, MeOD-$d_4$) $\delta$ 8.59 (s, 1H), 7.95 (d, $J$ = 2.5 Hz, 1H), 7.74 (s, 1H), 7.61 (dd, $J$ = 2.5, 9 Hz,1H), 7.40 (d, $J$ = 9 Hz, 1H), 4.62-4.44 (m, 2H), 3.97 (t, $J$ = 7.5 Hz, 2H), 3.11-3.00 (m, 3H), 2.76-2.67 (m, 2H), 2.65(t, $J$ = 8 Hz, 2H), 2.25 (q, $J$ = 7.5 Hz, 2H), 2.08-1.89 (m, 2H). LCMS (ESI): $m/z$ = 427.0 $[M+1]^+$.

**8-(3-Aminopropyl)-6-[2-chloro-4-(3-methyl-2-oxo-imidazol-1-yl)phenyl]-2-(methylamino)-pyrido[2,3-d]pyrimidin-7-one (5).**

The residue **I-24a** described above was treated with a solution of hydrogen chloride in dioxane (4M, 4 mL) at ambient temperature, causing the rapid formation of white solid. The mixture was stirred vigorously for 60 min and concentrated *in vacuo*. The residue was treated with saturated aqueous sodium bicarbonate and extracted with EtOAc and 2-methyltetrahydrofuran to which methanol was added. The combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo* to an orange solid (63 mg) which was submitted to purification by reverse phase HPLC (C18, gradient of acetonitrile in water with 0.1% ammonium hydroxide) to afford the title compound as a white solid (14.4 mg, 16% yield for 2 steps). $^1$H NMR (400 MHz, DMSO-*d*6) $\delta$ 8.71 – 8.59 (m, 1H), 7.84 (d, $J$ = 2.2 Hz, 1H), 7.82 – 7.62 (m, 1H), 7.73 (s, 1H), 7.48 (dd, $J$ = 8.6, 2.3 Hz, 1H), 7.32 (d, $J$ = 8.5 Hz, 1H), 4.41 – 4.23 (m, 2H), 3.83 (dd, $J$ = 9.2, 6.6 Hz, 2H), 3.52 – 3.43 (m, 2H), 2.91 (d, $J$ = 4.7 Hz, 3H), 2.79 (s, 3H), 2.60 – 2.49 (m, 2H), 1.82 – 1.73 (m, 2H). LCMS (ESI) $m/z$ = 442.2 $[M+1]^+$.

**8-(3-Aminopropyl)-6-(2-chloro-4-(3-methyl-2-oxo-2,3-dihydro-1*H*-imidazol-1-yl)phenyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8*H*)-one (6).** The title compound was obtained analogously to compound **5** using appropriate starting materials. $^1$H NMR (400 MHz, DMSO-*d6*)

δ 8.74 – 8.60 (m, 1H), 8.02 (d, *J* = 2.2 Hz, 1H), 7.87 – 7.80 (m, 1H), 7.79 (s, 1H), 7.76 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.45 (d, *J* = 8.3 Hz, 1H), 7.17 (d, *J* = 3.1 Hz, 1H), 6.79 (d, *J* = 3.2 Hz, 1H), 4.43 – 4.26 (m, 2H), 3.21 (s, 3H), 2.92 (d, *J* = 4.7 Hz, 3H), 2.60 – 2.51 (m, 2H), 1.83 – 1.70 (m, 2H). LCMS (ESI) *m/z* = 440.2 $[M+1]^+$.

**8-(3-Aminopropyl)-6-(2-chloro-4-(2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)pyrido-[2,3-d]pyrimidin-7(8H)-one (7).**

A mixture of *tert*-butyl 3-(6-(2-chloro-4-(2-oxopyrrolidin-1-yl)phenyl)-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)propylcarbamate (110 mg, crude) and TFA (1 mL) in DCM (10 mL) was stirred for 2 h. After concentration under reduced pressure, the residue was purified by preparative HPLC to afford the title compound as a white solid (25.7 mg, 29% yield). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.67 (s, 1H), 7.88 (d, *J* = 4.0 Hz, 1H), 7.83 (s, 1H), 7.75 (dd, *J* = 4.5, 12.5 Hz,1H), 7.72 (d, *J* = 1.5 Hz, 1H), 7.67 (s, 1H), 7.66 (s, 1H), 7.45 (dd, *J* = 4.5, 8.5 Hz, 1H), 6.51 (d, *J* = 9 Hz, 1H), 6.35 (t, *J* = 6 Hz, 1H),4.71-4.31 (m, 2H), 3.00 (s, 3H), 2.64-2.58 (m, 2H), 1.80-1.78 (m, 2H). LCMS (ESI): *m/z* = 436.9 $[M+1]^+$.

**8-(3-Aminopropyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methyl-amino)pyrido[2,3-d]pyrimidin-7(8H)-one (8).** The title compound was prepared in a similar fashion as compound **7** using appropriate starting materials (Scheme 4a). LCMS (ESI): *m/z* = 450.9 $[M+1]^+$. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.67 (s, 1H), 8.40 (s, 1H), 7.95-7.94 (m, 1H), 7.87 (s,1H), 7.66 (d, *J* = 2 Hz, 1H), 7.58 (d, *J* = 5.5 Hz, 1H), 7.53 (d, *J* = 8 Hz, 1H), 7.46-7.42 (m, 2H), 6.28 (t, *J* = 7 Hz, 1H), 4.42-4.39 (m, 2H), 2.93 (d, *J* = 4.5 Hz, 3H), 2.77-2.74 (m, 2H), 2.06 (s, 3H), 2.08-1.89 (m, 2H).

**8-(3-Aminopropyl)-6-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)phenyl)-2-(methyl-amino)pyrido[2,3-d]pyrimidin-7(8H)-one (9).** The title compound was prepared in a similar

fashion as compound **7** using appropriate starting materials (Scheme 4a). LCMS (ESI): *m/z* = 452.2 $[M+1]^+$. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.60-7.28 (m, 5H), 7.20-7.10 (m, 1H), 5.54 (s,1H), 4.57-4.54 (m, 2H), 3.12 (d, *J* = 5.1 Hz, 3H), 2.00-1.94 (m, 2H), 2.76-2.67 (m, 2H), 2.65(t, *J* = 8 Hz, 2H), 2.25 (q, *J* = 7.5 Hz, 2H), 2.08-1.89 (m, 2H).

**8-(3-Aminopropyl)-6-(2-chloro-4-(5-methyl-6-oxopyrimidin-1(6H)-yl)phenyl)-2-(methyl-amino)pyrido[2,3-d]pyrimidin-7(8H)-one (10).** The title compound was prepared in a similar fashion as compound **7** using appropriate starting materials (Scheme 4a). LCMS (ESI): *m/z* = 452.0 $[M+1]^+$. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.55 (s, 1H), 8.09 (s, 1H), 7.86 (d, *J* = 6.3 Hz, 1H), 7.61 (s, 2H), 7.59-7.51 (m, 1H), 7.35 (dd, *J* = 2.1 ,5.4 Hz,1H), 5.68-5.64 (m, 1H), 4.58-4.46 (m, 2H), 3.11 (d, *J* = 5.1 Hz, 3H), 2.77-2.71 (m, 2H), 2.43-2.40 (m, 2H), 2.15 (s, 3H), 2.09-1.01 (m, 2H).

**6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-ethyl-2-(methylamino)pyrido-[2,3-d]pyrimidin-7(8H)-one (11).**

A mixture of 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-ethyl-2-(methylsulfinyl)-pyrido[2,3-d]pyrimidin-7(8H)-one (**I-27**, 150 mg, 0.33 mmol), methyl amine (30% wt in EtOH, 2 mL) and in THF (10 mL) was stirred at 50 °C overnight. After concentration under reduced pressure, the residue was purified by preparative HPLC to afford the title compound as a white solid (99 mg, 71% yield). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.58 (s, 1H) 7.53 (d, *J* = 2.0 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.35 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.28 (d, *J* = 7.0 Hz, 1H), 7.23 (d, *J* = 7.0 Hz, 1H), 6.21-6.18 (t, *J* = 7.0 Hz, 1H), 5.53-5.52 (m, 1H), 4.52-4.51 (m, 2H), 3.12-3.11 (d, *J* = 5 Hz, 3H), 2.20 (s, 3H), 1.37-1.35 (m, 3H). LCMS (ESI): *m/z* = 422.1 $[M+1]^+$.

**6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-ethyl-2-(methylamino)pyrido-[2,3-d]pyrimidin-7(8H)-one (12).** A mixture of 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-

yl)phenyl)-8-ethyl-2-(methylsulfinyl)pyrido[2,3-d]pyrimidin-7(8H)-one (**I-27**, 150 mg, 0.33 mmol), oxetan-3-amine (36 mg, 0.5 mmol), ethyldiisopropylamine (128 mg, 0.99 mmol) and THF (10 mL) was stirred at 50°C overnight. After concentration under reduced pressure, the residue was purified by preparative HPLC to afford the title compound as a white solid (38 mg, 25% yield). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.59 (s, 1H) 7.54 (d, *J* = 2.0 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.38 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.28 (d, *J* = 7.0 Hz, 1H), 7.24 (d, *J* = 7.0 Hz, 1H), 6.21-6.18 (t, *J* = 7.0 Hz, 1H), 5.20-5.18 (m, 1H), 5.05-5.03 (t, *J* = 7.0 Hz, 2H), 4.69-4.67 (m, 2H), 4.47-4.43 (m, 2H), 2.20 (s, 3H), 1.35-1.32 (t, *J* = 7.0 Hz, 3H). LCMS (ESI): *m/z* = 464.2 $[M+1]^+$.

**6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-hydroxyethyl)-2-(methyl-amino)pyrido[2,3-d]pyrimidin-7(8H)-one (13).**

Step 1: 6-(4-Bromo-2-chlorophenyl)-8-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-2-(methylthio)-pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(4-bromo-2-chlorophenyl)-2-(methylthio)-pyrido[2,3-d]pyrimidin-7(8H)-one (**I-16**, 300, 0.785 mmol), (2-bromoethoxy)(*tert*-butyl)-dimethylsilane (374 mg, 1.57 mmol) and cesium carbonate (765 mg, 2.36) in DMF (15 mL) was stirred at 80 °C for 2 h. After filtration, water and EtOAc (40 mL) was added. The organic layer was separated, washed with brine, dried over sodium sulfate and concentrated under reduced pressure to afford 6-(4-bromo-2-chlorophenyl)-8-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (300 mg, crude) as brown solid, which was used in the next step without further purification. LCMS (ESI): *m/z* = 540.1 $[M+1]^+$.

Step 2: 8-(2-((*tert*-Butyldimethylsilyl)oxy)ethyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(4-bromo-2-chloro-phenyl)-8-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-

one (300 mg, 0.68 mmol) 3-methylpyridin-2(1H)-one (150 mg,1.36 mol), cuprous iodide (26 mg, 0.136 mmol), $N^1,N^2$-dimethylethane-1,2-diamine (55 mg, 0.63 mmol) and potassium phosphate (460 mg, 2.18 mmol) in dioxane (10 mL) was stirred in a sealed tube at 100 °C overnight. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography using DCM/EtOAc (2:1) as eluting solvents to afford 8-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)pyrido-[2,3-d]pyrimidin-7(8H)-one as a yellow solid (250 mg, 80% yield). LCMS (ESI): *m/z* = 485.0 $[M+17]^+$.

Step 3: 8-(2-((*tert*-Butyldimethylsilyl)oxy)ethyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylsulfinyl)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 8-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (250 mg, 0.44 mol) and *m*-CPBA (135.6 mg, 0.66 mmol, 85% wt). The reaction mixture was stirred at rt for 30min. Water (50 mL) was added and the solution was extracted with DCM (2×50 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylsulfinyl)pyrido[2,3-d]-pyrimidin-7(8H)-one (200 mg, crude) as a white solid, which was used in the next step without further purification. LCMS (ESI): *m/z* = 585.1 $[M+1]^+$.

Step 4: 8-(2-((*tert*-Butyldimethylsilyl)oxy)ethyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 8-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylsulfinyl)pyrido[2,3-d]pyrimidin-7(8H)-one (200 mg, crude) and methyl amine (30% wt in EtOH, 5 mL) and in THF (15 mL) was stirred at 50°C overnight. After concentration under

reduced pressure, the title compound was obtained as yellow oil, which was used in the next step without further purification. LCMS (ESI): *m/z* = 552.1 $[M+1]^+$.

Step 5: 6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-hydroxyethyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 8-(2-((*tert*-butyldimethylsilyl)oxy)-ethyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)pyrido[2,3-d]-pyrimidin-7(8H)-one (220 mg, 0.50 mmol) and HCl (6 M in dioxane, 1.2 mL, 5 mmol) in dioxane (5 mL) was stirred at 70 °C for 2 h. After concentration under reduced pressure, the residue was purified by preparative HPLC to afford 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-hydroxyethyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one as a white solid (28 mg, 12% yield). LCMS (ESI): *m/z* = 438.1 $[M+1]^+$. $^1$H NMR (400 MHz, MeOD-$d_4$) δ 8.62 (s, 1H), 7.82 (s, 1H), 7.62 (d, *J* = 2 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.53-7.51 (m, 2H), 7.43 (d, *J* = 2 Hz, 8.4 Hz, 1H), 6.44 (t, *J* = 6.8 Hz, 1H), 4.67 (s, 2H), 3.91 (s, 2H), 3.07 (s, 3H), 2.19 (s, 3H).

**6-(2-Chloro-4-(5-methyl-6-oxopyrimidin-1(6H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (14).**

Step 1: 6-(4-Bromo-2-chlorophenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(4-bromo-2-chlorophenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (**I-16**, 300 mg, 0.78) 1-bromo-2-methoxyethane (162 mg, 1.18 mmol) and cesium carbonate (760 mg, 2.34 mmol) in DMF (5 mL) was stirred at 80 °C overnight. After filtration, EtOAc (20 mL) and water was added. The organic layer was washed with brine, dried over sodium sulfate and concentrated under reduced pressure to afford the title compound (300 mg, crude) as brown solid, which was used in the next step without further purification. LCMS (ESI): *m/z* = 440.1 $[M+1]^+$.

Step 2: 6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(4-bromo-2-chlorophenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (290 mg, 0.66 mmol), and 3-methylpyridin-2(1H)-one (145 mg,1.32 mol), cuprous iodide (25 mg, 0.132 mmol), $N^1,N^2$-dimethylethane-1,2-diamine (50 mg, 0.57 mmol) and potassium phosphate (419.7 mg, 1.98 mmol) in dioxane (10 mL) was stirred in a sealed tube at 100 °C overnight. After filtration and concentration under reduced pressure, the residue was purified by silica gel chromatography using DCM: EtOAc (4:1 to 2:1) as eluting solvents to afford the title compound (150 mg, 49%) as a yellow solid. LCMS (ESI): *m/z* = 485.0 $[M+17]^+$.

Step 3: 6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylsulfinyl)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(2-chloro-4-(5-methyl-6-oxo-pyrimidin-1(6H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (150 mg, 0.32 mmol) and *m*-CPBA (98.7 mg, 0.48 mmol, 85% wt) was stirred at rt for 30 min. Water (50 mL) was added, and the solution was extracted with DCM (2×50 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford the title compound (120 mg, crude) as a white solid, which was used in the next step without further purification. LCMS (ESI): *m/z* = 486.0 $[M+1]^+$.

Step4: 6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylsulfinyl)pyrido[2,3-d]pyrimidin-7(8H)-one (120 mg, crude) and methyl amine (30% wt in EtOH, 5 mL) and in THF (10 mL) was stirred at 50°C overnight. After concentration under reduced pressure, the residue was purified by preparative HPLC to afford the title compound as a white solid (77.6 mg, 54% yield). LCMS

(ESI): *m/z* = 452.1[M+1]$^+$. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.59 (s, 1H), 7.53 (d, *J* = 1.6 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.36 (dd, *J* = 2 Hz, 8 Hz, 1H), 7.30-7.22 (m, 2H), 6.20 (t, *J* = 6.8 Hz, 1H), 5.54 (s, 1H), 4.70 (s, 2H), 3.78 (s, 2H), 3.42 (s, 3H), 3.11 (d, *J* = 4.8 Hz, 3H), 2.20 (s, 3H).

**6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(oxetan-3-ylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (15).**

Step 1: 6-(4-Bromo-2-chlorophenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one. A solution of 6-(4-bromo-2-chlorophenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (1.30 g, 3.40 mmol), 1-bromo-2-methoxyethane (0.94 g, 6.79 mmol) and cesium carbonate (4.43 g, 13.59 mmol) in DMF (10 mL) was stirred at 85°C overnight. The reaction was diluted with EtOAc (20 mL) and water. The separated organic layer was dried over anhydrous sodium sulfate and concentrated in *vacuo*. The residue was purified by silica chromatography using EtOAc: petroleum ether (1:5) as eluting solvents to give the title compound as a yellow solid (1.20 g, 80% yield). LCMS (ESI): *m/z* =440.1 [M+1]$^+$.

Step 2: 6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(4-bromo-2-chlorophenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (320 mg, 0.73 mmol), 3-methylpyridin-2-ol (119 mg, 1.09 mmol), copper(I) iodide (28 mg, 0.15 mmol), $N^1$,$N^2$-dimethylethane-1,2-diamine (19 mg, 0.22 mmol) and cesium carbonate (710 mg, 2.18 mmol) in 1,4-dioxane (10 mL) was stirred at 110 °C overnight. The reaction was diluted with EtOAc (50 mL) and water. The separated organic layer was dried over anhydrous sodium sulfate and concentrated in *vacuo*. The residue was purified by silica chromatography using EtOAc/petroleum ether (1:6) as eluting solvents to give 6-(2-chloro-4-(3-methyl-2-oxopyridin-

1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one as a yellow solid (200 mg, 59 % yield). LCMS (ESI): *m/z* = 469.1 $[M+1]^+$.

Step 3: 6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylsulfinyl)pyrido[2,3-d]pyrimidin-7(8H)-one. To a mixture of 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (200 mg, 0.43 mmol) in dry DCM (5 mL), *m*-CPBA (130 mg, 0.64 mmol, 85% of wt) was added in portions. The reaction mixture was stirred at rt for 30 min. Water (50 mL) was added, and the aqueous layer was extracted with DCM (2×50 mL). The combined organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford the title compound as a white solid (200 mg, 97% yield). LCMS (ESI): *m/z* = 485.1 $[M+1]^+$.

Step 4: 6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(oxetan-3-ylamino)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylsulfinyl)pyrido[2,3-d]pyrimidin-7(8H)-one (200 mg, 0.41 mmol), oxetan-3-amine (60 mg, 0.82 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (213 mg, 1.65 mmol) in THF (10 mL) was stirred at 50 °C overnight. The reaction was diluted with EtOAc (40 mL) and water. The separated organic layer was dried over anhydrous sodium sulfate and concentrated in *vacuo*. The residue was purified by preparative HPLC to afford the title compound as a white solid (120 mg, 59% yield). $^1$H NMR (400 MHz, MeOD-$d_4$) δ 8.67 (s, 1H), 8.45 (s, 1H), 7.83 (s, 1H), 7.62 (d, *J* = 1.2 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 7.53-7.51 (m, 2H), 7.44 (dd, , *J* = 3.0, 8.4 Hz, 1H), 6.43 (t, , *J* = 6.8 Hz, 1H), 5.21-5.12 (m, 1H), 5.01 (t, *J* = 6.4 Hz, 2H), 4.84-4.80 (m, 2H), 4.66 (t, *J* = 6.0 Hz, 2H), 3.75(t, *J* = 6.0 Hz, 2H), 3.40 (s, 3H), 2.19 (s, 3H). LCMS (ESI): *m/z* = 494.1 $[M+1]^+$.

**8-(3-Amino-2,2-difluoropropyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-**

**(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (16).**

Step 1: 2,2-Difluoro-$N^1$,$N^1$-bis(4-methoxybenzyl)propane-1,3-diamine. To a solution of 3-(bis(4-methoxybenzyl)amino)-2,2-difluoropropanamide (500 mg, 1.37 mmol) in THF (5 mL) was added $BH_3$ (3 mL, 1M in THF) portion-wise. The mixture was stirred at rt for 1 h. Then the mixture was quenched with MeOH and stirred at 60 °C for 1 h. After cooling to r.t, the mixture was concentrated under reduced pressure to afford 2,2-difluoro-$N^1$,$N^1$-bis(4-methoxybenzyl)propane-1,3-diamine (160 mg, crude) as colorless oil, which was used in the next step without further purification. LCMS (ESI): *m/z* = 350 $[M+1]^+$.

Step 2. 8-(3-Amino-2,2-difluoropropyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (**16**). A solution of 8-(3-amino-2,2-difluoro-propyl)-6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (**I-32a**, 100 mg, 0.14 mmol) and TFA (3 mL) was stirred at 95°C for 8 h in a sealed tube. After cooling to r.t, the mixture was concentrated under reduced pressure. The residue was purified by preparative HPLC to afford the title compound as a white solid (25 mg, 40% yield). LCMS (ESI): *m/z* = 486.0 $[M+1]^+$. $^1$H NMR (400 MHz, MeOD-$d_4$) δ 8.63 (s, 1H), 7.87 (s, 1H), 7.63 (d, *J* = 2 Hz, 1H), 7.57 (d, *J* = 8 Hz, 1H), 7.56-7.51 (m, 2H), 7.44 (dd, *J* = 2 Hz, *J* = 8 Hz, 1H), 6.44 (t, *J* = 6.8 Hz, 1H), 5.15-4.93 (m, 2H), 3.11-2.91 (m, 5H), 2.19 (s, 3H).

**6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-((3,3-difluoropiperidin-4-yl)methyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (17).**

A mixture of *tert*-butyl 4-((6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methyl-amino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (**I-32b**, 180 mg, 0.29 mmol) in DCM (4 mL) and TFA (2 mL) was stirred at rt for 2 h. The solution concentrated in *vacuo* and the residue was purified by preparative HPLC to afford the title

compound as a wheat solid (100 mg, 66% yield). $^1$H NMR (400 MHz, MeOD-$d_4$) δ 8.49 (s, 1H), 7.69 (s, 1H), 7.50 (s, 1H), 7.44-7.38 (m, 3H), 7.31 (dd, *J* = 2.0, 8.0 Hz, 1H), 6.31 (t, *J* = 7.2 Hz, 1H), 4.72-4.56 (m, 2H), 3.4-3.00 (m, 1H), 2.95 (s, 3H), 2.90-2.86 (m, 1H), 2.80 (dd, *J* = 13.6, 29.6 Hz, 2H), 2.42-2.39 (m, 1H), 2.06 (s, 3H). 1.62-1.50 (m, 2H). LCMS (ESI): *m/z* = 527.1 $[M+1]^+$.

**3-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-7-(methylamino)-1-(2-(1-methyl-azetidin-3-yloxy)ethyl)-1,6-naphthyridin-2(1H)-one (18).**

Step 1: *tert*-Butyl 3-(2-hydroxyethoxy)azetidine-1-carboxylate. Tetrabutylammonium bromide (148 mg, 0.46 mmol) was added to a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (4 g, 23 mmol) in triethylamine (2.467 g, 24 mmol). The reaction mixture was stirred for 5 h, followed by addition of 1,3-dioxolan-2-one (2.228 g, 25 mmol) and heating at 100 °C for 2.5 d. The mixture was then concentrated in vacuo and purified by flash column chromatography, eluting with (MeOH/DCM = 0% to 3% in 40 min), to give the title compound as an orange colored oil (2.63 g, 53% yield). LCMS (ESI): *m/z* = 162.1 $[M-55]^+$.

Step 2: *tert*-Butyl 3-(2-(tosyloxy)ethoxy)azetidine-1-carboxylate. A mixture of *tert*-butyl 3-(2-hydroxyethoxy)azetidine-1-carboxylate (2.630 g, 12.1 mmol), 4-methylbenzene-1-sulfonyl chloride (2.768 g, 14.5 mmol), triethylamine (3.5 mL, 24.2 mmol), *N,N*-dimethyl-4-aminopyridine (370 mg, 3.025 mmol) in DCM (36 mL) and THF (9 mL) was stirred at 40 °C overnight. The reaction mixture was then concentrated, diluted with DCM (50 mL) and washed with an aqueous HCl solution (0.5 M, 40 mL). The aqueous layer was then extracted with DCM (2 × 30 mL) and the combined organic layers washed with a sodium bicarbonate solution (50 mL), brine (50 mL), dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash column chromatography, eluting with (EtOAc / mixture of PE/DCM (1/1) = 3%

to 6%), to give the title compound as a yellow oil (2.36 g, 52% yield). LCMS (ESI): *m/z* = 316.0 $[M-55]^+$.

Step 3: *tert*-Butyl 3-(2-(6-(4-bromo-2-chlorophenyl)-2-(methylthio)-7-oxopyrido[2,3-d]-pyrimidin-8(7H)-yl)ethoxy)azetidine-1-carboxylate. To a solution of 6-(4-bromo-2-chloro-phenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (**I-16**, 191 mg, 1.6 mmol) in DMF (15 mL) was added sodium hydride (60% wt, 160 mg, 4 mmol) at rt. The mixture was stirred at 50 ºC for 30 min. After cooling to rt, *tert*-butyl 3-(2-(tosyloxy)ethoxy)azetidine-1-carboxylate (1485 mg, 4 mmol) was added, and the mixture was stirred at 85 ºC for 2 h. The reaction mixture was poured into ice water. The aqueous layer was extracted with EtOAc (3×100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel chromatography using petroleum ether: EtOAc (6:1 to 4:1) as eluting solvents to afford the title compound as a pale yellow oil (572 mg, 61% yield). LCMS (ESI): *m/z* = 539.0 $[M-55]^+$.

Step 4: *tert*-Butyl 3-(2-(6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)ethoxy)azetidine-1-carboxylate. A mixture of *tert*-butyl 3-(2-(6-(4-bromo-2-chlorophenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)ethoxy)-azetidine-1-carboxylate (572 mg, 0.98 mmol), 3-methylpyridin-2-ol (180 mg, 1.97 mmol), dimetylethylenediamine (34.6 mg, 0.39 mmol), cuprous iodide (37.4 mg, 0.196 mmol) and potassium phosphate (623 mg, 2.94 mmol) in dioxane (10 mL) was stirred under a nitrogen atmosphere at 110 ºC overnight. After filtration, water and EtOAc (50 mL) were added. The organic layer was washed with brine and dried over sodium sulfate. After concentration, the residue was purified by silica gel chromatography using petroleum ether: EtOAc (1:1) as eluting solvents to afford the title compound as an orange solid (356 mg, 59% yield). LCMS (ESI): *m/z*

= 556.1 $[M-55]^+$.

Step 5: *tert*-Butyl 3-(2-(6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)ethoxy)azetidine-1-carboxylate. A mixture of *tert*-butyl 3-(2-(6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)ethoxy)azetidine-1-carboxylate (356 mg, 0.58 mmol) and *m*-CPBA (70% wt, 172 mg, 0.7 mmol) in DCM (5 mL) was stirred at rt overnight. Water and EtOAc (20 mL) were added. The organic layer was washed with saturated sodium bicarbonate, brine and dried over sodium sulfate. After concentration, the title compound (410 mg, crude) was obtained as a yellow solid, which was used in the next step without further purification. LCMS (ESI): *m/z* = 571.0 $[M-55]^+$.

Step 6: *tert*-Butyl 3-(2-(6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)ethoxy)azetidine-1-carboxylate. A mixture of *tert*-butyl 3-(2-(6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylsulfinyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)ethoxy)azetidine-1-carboxylate (410 mg, crude) and methyl amine (30% wt in methanol, 162 mg, 1.74 mmol) in THF (5 mL) was stirred at 40 ºC for 2 h. After concentration, the title compound (450 mg, crude) was obtained as a yellow solid, which was used in the next step without further purification. (ESI): *m/z* = 538.1 $[M-55]^+$.

Step 7. 1-(2-(Azetidin-3-yloxy)ethyl)-3-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-7-(methylamino)-1,6-naphthyridin-2(1H)-one. A mixture of *tert*-butyl 3-(2-(6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)ethoxy)azetidine-1-carboxylate (450 mg, crude) and TFA (2 mL) in DCM (20 mL) was stirred at rt for 1 h. Neutralization with $NH_4OH$ and concentration afforded the title compound (500 mg, crude) as a yellow solid, which was used in the next step without further purification. LCMS

(ESI): *m/z* = 492.1 $[M+1]^+$.

Step 8: 3-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-7-(methylamino)-1-(2-(1-methylazetidin-3-yloxy)ethyl)-1,6-naphthyridin-2(1H)-one. A mixture of 1-(2-(azetidin-3-yloxy)ethyl)-3-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-7-(methylamino)-1,6-naphthyridin-2(1H)-one (356 mg, crude) and formaldehyde (37% wt in water, 470.4 mg 5.8 mmol) in MeOH (4 mL) was stirred at 50 °C for 2 h. Then $NaBH_4$ (44 mg, 1.16 mmol) was added, and the reaction mixture was stirred overnight. After filtration and concentration, the residue was purified by preparative HPLC to afford the title compound as a white solid (70.2 mg, 24% yield). LCMS (ESI): *m/z* = 506.9 $[M+1]^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.65 (s, 1H), 7.98-7.81 (m, 2H), 7.66 (d, *J* = 2 Hz, 1H), 7.58 (dd, *J* = 1.2Hz, 6.8 Hz, 1H), 7.52 (d, *J* = 8 Hz, 1H), 7.46-7.39 (m, 2H), 4.53-4.37 (m, 2H), 4.16-4.05 (m, 1H), 3.66-3.52 (m, 2H), 3.51-3.44 (m, 2H), 2.93 (d, *J* = 4.8 Hz, 3H), 2.74-2.66 (m, 2H), 2.20 (s, 3H), 2.06 (s, 3H).

**6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-(3-fluoro-1-methylazetidin-3-yl)ethyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (19).**

Step 1: *tert*-Butyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate. Methyl acetate (455 mg, 6.14 mmol) was added drop-wise to the solution of lithium bis(trimethylsilyl)amide in THF (6.43 mL, 1 mol/L, 6.43 mmol) at -78 °C. After stirring at -78 °C for 10 min, *tert*-butyl 3-oxoazetidine-1-carboxylate (1000 mg, 5.85 mmol) was added and the reaction was stirred for 15 min. Then the reaction was stirred at 0 °C for 2 h. The mixture was poured into ice water. The aqueous layer was extracted with EtOAc (3×50 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to afford *tert*-butyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate as a yellow oil (932 mg, crude) as colorless oil, which was used in the next step without purification. LCMS (ESI): *m/z* = 190.0 [M-55].

Step 2: *tert*-Butyl 3-fluoro-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate. Diethylamino-sulfur trifluoride (735 mg, 4.56 mmol) was added drop-wise to a solution of *tert*-butyl 3-hydroxy-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate (932 mg, crude, 3.8 mmol) in DCM (240 mL) at -78 °C. After stirring for 3 h, the mixture was poured into ice water. The aqueous layer was extracted with EtOAc (3×50 mL). The combined organic layers were dried over sodium sulfate and concentrated to afford the title compound as a yellow oil (768 mg, crude) as colorless oil, which was used in the next step without purification. LCMS (ESI): *m/z* = 192.1 [M-55].

Step 3: *tert*-Butyl 3-fluoro-3-(2-hydroxyethyl)azetidine-1-carboxylate. Lithium aluminum hydride (354 mg, 9.33 mmol) was added to a solution of crude *tert*-butyl 3-fluoro-3-(2-methoxy-2-oxoethyl)azetidine-1-carboxylate (768 mg, crude, 3.11 mmol) in THF (50 mL) portion-wise at 0 °C, and the reaction was stirred for 30 min. The mixture was poured into ice water (200 mL), and the aqueous layer extracted with EtOAc (3×50 mL). The combined organic layers were dried over $Na_2SO_4$ and concentrated to afford the title compound as a yellow solid (546 mg, crude) as colorless oil, which was used in the next step without purification. LCMS (ESI): *m/z* = 192.1 [M-55].

Step 4: *tert*-Butyl 3-fluoro-3-(2-(tosyloxy)ethyl)azetidine-1-carboxylate. A mixture of *tert*-butyl 3-fluoro-3-(2-hydroxyethyl)azetidine-1-carboxylate (546 mg, crude, 2.49 mmol), tosyl chloride (522 mg, 2.74 mmol), *N,N*-dimethylpyridin-4-amine (31 mg, 0.25 mmol) and triethylamine (755 mg, 7.48 mmol) in DCM (450 mL) was stirred at rt for 18 h. The reaction mixture was concentrated and the residue was purified by silica gel chromatography using petroleum ether: EtOAc (3:1) as eluting solvents to afford the title compound as a white solid (266 mg, 29 % yield). LCMS (ESI): *m/z* = 318.1 [M-55].

Step 5: *tert*-Butyl 3-(2-(6-(4-bromo-2-chlorophenyl)-2-(methylthio)-7-oxopyrido[2,3-d]-pyrimidin-8(7H)-yl)ethyl)-3-fluoroazetidine-1-carboxylate. A mixture of 6-(4-bromo-2-chlorophenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (**I-16**, 300 mg, 0.785 mmol), 3-fluoro-3-(2-(tosyloxy)ethyl)azetidine-1-carboxylate (352 mg, 0.942 mmol) and cesium carbonate (768 mg, 2.36 mmol) in DMF (10 mL) was stirred at 80 °C for 2 h. After cooling to rt, water was added. The aqueous layer was extracted with (3×50 mL). The combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by silica gel chromatography using petroleum ether: EtOAc (3:1) as eluting solvents to afford the title compound as a yellow solid (303 mg, 66 % yield). LCMS (ESI): *m/z* = 529.0, 527.0 $[M+1]^+$.

Step 6: *tert*-Butyl 3-(2-(6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)ethyl)-3-fluoroazetidine-1-carboxylate. A mixture of *tert*-butyl 3-(2-(6-(4-bromo-2-chlorophenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)-ethyl)-3-fluoroazetidine-1-carboxylate (303 mg, 0.52 mmol), 3-methylpyridin-2-ol (**I-18c**, 68 mg, 0.624 mmol), $N^1,N^2$-dimethylethane-1,2-diamine (18 mg, 0.208 mmol), copper(I) iodide (20 mg, 0.104 mmol) and potassium phosphate (220 mg, 1.04 mmol) in 1,4-dioxane (10 mL) was stirred in the sealed tube at 110 °C under nitrogen for 48 h. After cooling to rt, the mixture was concentrated and the residue purified by silica gel chromatography using DCM/methanol (15:1) as eluting solvents to afford the title compound as a yellow solid (287 mg, 90 % yield). LCMS (ESI): *m/z* = 629.2 [M+18].

Step 7: 6-(2-Chloro-4-(2-oxopyridin-1(2H)-yl)phenyl)-8-(2-(3-fluoroazetidin-3-yl)ethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one 2,2,2-trifluoroacetate. A mixture of *tert*-butyl 3-(2-(6-(2-chloro-4-(3-ethyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)ethoxy)azetidine-1-carboxylate (287 mg, 0.469 mmol) in TFA (1 mL) and

DCM (10 mL) was stirred at rt for 2 h. The reaction mixture was concentrated to afford the title compound (257 mg, crude) as a yellow solid, which was used in the next step without purification. LCMS (ESI): *m/z* = 512.2 $[M+1]^+$.

Step 8: 6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-(3-fluoro-1-methyl-azetidin-3-yl)ethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(2-chloro-4-(2-oxopyridin-1(2H)-yl)phenyl)-8-(2-(3-fluoroazetidin-3-yl)ethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one 2,2,2-trifluoroacetate (257 mg, crude, 0.469 mmol) and formaldehyde (516 mg, 40% wt in $H_2O$, 4.69 mmol) in methanol (50 mL) was stirred at rt for 1 h. Then sodium triacetoxyborohydride (995 mg, 4.69 mmol) was added. The mixture was stirred at rt for 18 h, and water was then added. The aqueous layer was extracted with EtOAc (3×50 mL). The combined organic layers were dried over sodium sulfate and concentrated to afford the title compound as a yellow solid (217 mg, crude), which was used in the next step without further purification. LCMS (ESI): *m/z* = 526.2 $[M+1]^+$.

Step 9: 6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-(3-fluoro-1-methyl-azetidin-3-yl)ethyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (**19**).

A mixture of 6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-(3-fluoro-1-methyl-azetidin-3-yl)ethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (180 mg, crude, 0.343 mmol) in methanamine (40 % wt in ethanol, 5 mL) was stirred in a sealed tube at 80 °C for 18 h. After cooling to rt, the mixture was concentrated and the residue was purified by preparative HPLC to afford the title compound as a white solid (76 mg, 44 % yield). LCMS (ESI): *m/z* = 509.2 $[M+1]^+$. $^1$H NMR (400 MHz, MeOD-$d_4$) δ 8.61 (brs, 1H), 7.81 (s, 1H), 7.62 (d, *J* = 2.0 Hz, 1H), 7.57-7.51 (m, 3H), 7.43 (dd, *J* = 2.0, 8.0 Hz, 1H), 6.43 (t, *J* = 6.8 Hz, 1H), 4.63 (brs, 2H), 3.63-3.57 (m, 2H), 3.42-3.33 (m, 2H), 3.08 (s, 3H), 2.43 (s, 3H), 2.43-2.33 (m, 2H), 2.19 (s, 3H).

**(*R*)-6-(2-Chloro-4-(3-ethyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)-8-(morpholin-2-ylmethyl)pyrido[2,3-d]pyrimidin-7(8H)-one (21).**

Step 1: (*R*)-*tert*-butyl 2-(tosyloxymethyl)morpholine-4-carboxylate. A mixture of (*R*)-*tert*-butyl 2-(hydroxymethyl)morpholine-4-carboxylate (6.1 g, 28.1 mmol), 4-methylbenzene-1-sulfonyl chloride (8.05 g, 42.1 mmol), triethylamine (5.7 g, 56.2 mmol) and *N,N*-dimethyl-4-amino-pyridine (340 mg, 2.8 mmol) in DCM (150 mL) was stirred at rt overnight. The reaction mixture was then concentrated in *vacuo*. The residue was purified by silica gel chromatography using petroleum ether:/EtOAc (5:1) as eluent to afford the title compound as a colorless oil (6.0 g, 57% yield). LCMS (ESI): *m/z* = 316.0 $[M-55]^+$.

Step 2: (*S*)-*tert*-Butyl 2-((6-(4-bromo-2-chlorophenyl)-2-(methylthio)-7-oxopyrido[2,3-d]-pyrimidin-8(7H)-yl)methyl)morpholine-4-carboxylate. A mixture of 6-(4-bromo-2-chloro-phenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin7(8H)-one (**I-16**, 765 mg, 2.0 mmol), (*R*)-*tert*-butyl 2-(tosyloxymethyl)morpholine-4-carboxylate (1.49 g, 4.0 mmol) $Cs_2CO_3$ (986 mg, 3.0 mmol) in DMF (10 mL) was stirred at 60 °C overnight. Saturated $NH_4Cl$ solution (80 mL) was added to the reaction mixture. The aqueous phase was extracted with EtOAc (3×80 mL). The combined organic layers were washed with brine, dried and concentrated under reduced pressure. The residue was purified by silica gel chromatography using petroleum ether/EtOAc (1:1) as eluting solvents to afford the title compound (753 mg, 64% yield). LCMS (ESI): *m/z* = 582.2 $[M+1]^+$.

(*S*)-*tert*-Butyl 2-((6-(4-bromo-2-chlorophenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)morpholine-4-carboxylate was converted into the final product using similar procedures as used for the synthesis of compound **7** described above.

LCMS (ESI): *m/z* = 507.2 $[M+1]^+$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.58 (s, 1H), 7.53

(d, *J* = 2 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.37 (dd, *J* = 1.6 Hz, 8 Hz, 1H), 7.26-7.22 (m, 2H), 5.52 (s, 1H), 4.61-4.60 (m, 2H), 4.01-3.90 (m,2H), 3.57 (t, *J* = 10.8 Hz, 1H), 3.12 (d, *J* = 4.4 Hz, 3H), 2.93-2.87 (m, 2H), 2.82-2.78 (m, 2H), 2.61 (q, *J* = 7.6 Hz, 2H), 1.30-1.21 (m, 3H).

**(*S**)-6-(2-Chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-8-(2-hydroxyethyl)-2-(methyl-amino)pyrido[2,3-d]pyrimidin-7(8H)-one (22) – Absolute configuration unknown and arbitrarily assigned.**

A mixture of *tert*-butyl (*S*)-4-((6-(2-chloro-4-(3-methyl-2-oxopyrazin-1(2H)-yl)phenyl)-2-(oxetan-3-ylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)-3,3-difluoropiperidine-1-carboxylate (**I-32c**, 100 mg, crude) and TFA (2 mL) in DCM (20 mL) was stirred for 2 h. After concentration, the residue was purified by preparative HPLC to afford the title compound as a white solid (38 mg, 43% yield). LCMS (ESI): *m/z* = 570.2 $[M+1]^+$. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.54(s, 1H), 7.62(s, 1H), 7.57 (d, *J* = 2.0 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.44 (dd, *J* = 2.0, 8.4 Hz,2H), 7.29(d, *J* = 4.4 Hz 1H), 7.08 (d, *J* = 4.4 Hz 1H), 6.17-5.94 (m, 1H), 5.28-4.94 (m, 3H), 4.88-4.43 (m, 5H), 3.27-2.96 (m, 2H), 2.56-2.40(m, 4H).

**6-(2-Chloro-4-(5-methyl-6-oxopyrimidin-1(6H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methyl-amino)pyrido[2,3-d]pyrimidin-7(8H)-one (23).**

Step 1: 6-(2-Chloro-4-(5-methyl-6-oxopyrimidin-1(6H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(4-bromo-2-chlorophenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (see preparation of compound **14**, step 1) (200 mg, 0.45 mmol), and 5-methylpyrimidin-4(3H)-one (99 mg,0.9 mol), cuprous iodide (17 mg,0.09 mmol), (1*R*,2*R*)-$N^1$,$N^2$-dimethylcyclohexane-1,2-diamine (35 mg, 0.39 mmol) and potassium phosphate (28.6 mg,0.135 mmol) in dioxane (2 mL) was stirred in a sealed tube at 100 °C overnight. After filtration and concentration under reduced pressure, the residue

was purified by silica gel chromatography using DCM: EtOAc (2:1) as eluting solvents to afford the title compound as a yellow solid (120 mg, 56% yield). LCMS (ESI): *m/z* = 470.0 $[M+1]^+$.

Step 2: 6-(2-Chloro-4-(5-methyl-6-oxopyrimidin-1(6H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylsulfinyl)pyrido[2,3-d]pyrimidin-7(8H)-one. A mixture of 6-(2-chloro-4-(5-methyl-6-oxopyrimidin-1(6H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (120 mg, 0.26 mmol) and *m*-CPBA (78 mg, 0.38 mmol, 85% wt). The reaction mixture was stirred at rt for 30 min. Water (50 mL) was added and the solution was extracted with DCM (2×50 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford the title compound (100 mg, crude) as a white solid, which was used in the next step without further purification. LCMS (ESI): *m/z* = 486.0 $[M+1]^+$.

Step 3: 6-(2-Chloro-4-(5-methyl-6-oxopyrimidin-1(6H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (**23**). A mixture of 6-(2-chloro-4-(5-methyl-6-oxopyrimidin-1(6H)-yl)phenyl)-8-(2-methoxyethyl)-2-(methylsulfinyl)pyrido[2,3-d]pyrimidin-7(8H)-one (100 mg, crude) and methyl amine (30% wt in EtOH, 3 mL) and in THF (10 mL) was stirred at 50 °C overnight. After concentration under reduced pressure, the residue was purified by preparative HPLC to afford the title compound as a white solid (19 mg, 16% yield). LCMS (ESI): *m/z* = 453.2 $[M+1]^+$. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.07 (s, 1H), 7.84 (s, 1H), 7.60 (s, 1H), 7.56-7.53 (m, 2H), 7.34 (dd, *J* = 2 Hz, 4 Hz, 1H), 5.50 (s, 1H), 4.70 (s, 2H), 3.78 (s,2H), 3.42 (s, 3H), 3.12 (d, *J* = 4.8 Hz, 3H), 2.15 (s, 3H).

**8-(((1r,4r)-4-Aminocyclohexyl)methyl)-6-(2-chloro-4-(6-methylpyrazin-2-yl)phenyl)pyrido-[2,3-d]pyrimidin-7(8H)-one (24).**

Step 1: *tert*-Butyl (1r,4r)-4-((6-(2-chloro-4-(6-methylpyrazin-2-yl)phenyl)-7-oxopyrido[2,3-

d]pyrimidin-8(7H)-yl)methyl)cyclohexylcarbamate. A mixture of *tert*-butyl (1r,4r)-4-((6-(2-chloro-4-(6-methylpyrazin-2-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)-methyl)cyclohexylcarbamate (prepared similarly to **I-13**) (500 mg, 0.82 mmol) and Raney-Nickel (50 mg in water) was stirred in methanol (20 mL) and $NH_3$ (30% wt in methanol, 5 mL) at rt under a hydrogen atmosphere for 12 h. After filtration and concentration, the title compound (220 mg, crude) was obtained as yellow oil.

Step 2: 8-(((1r,4r)-4-Aminocyclohexyl)methyl)-6-(2-chloro-4-(6-methylpyrazin-2-yl)phenyl)-pyrido[2,3-d]pyrimidin-7(8H)-one (**24**). A mixture of *tert*-butyl (1r,4r)-4-((6-(2-chloro-4-(6-methylpyrazin-2-yl)phenyl)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)cyclohexylcarbamate (220 mg, 0.39 mmol) and TFA (2 mL) in DCM (20 mL) was stirred for 3 h. After concentration, the residue was purified by preparative HPLC to afford the title compound as a yellow solid (104.3 mg, 58% yield). LCMS (ESI): *m/z* = 461.1 $[M+1]^+$. $^1$H NMR (500 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.91 (s, 1H), 8.85 (s, 1H), 8.45 (s, 1H), 8.20 (d, *J* = 1.5 Hz, 1H), 7.98 (dd, *J* = 1.5 Hz, *J* = 8 Hz, 1H), 7.78 (s, 1H), 7.52 (d, *J* = 8 Hz, 1H), 4.44 (d, *J* = 7 Hz, 2H), 2.69-2.60 (m, 4H), 2.04-1.93 (m, 1H), 1.89-1.83 (m, 2H), 1.75-1.69 (m, 2H), 1.31-1.21 (m, 2H), 1.09-1.00 (m, 2H).

**(S)-6-(2-Cyclopropyl-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)-8-(morpholin-2-ylmethyl)pyrido[2,3-d]pyrimidin-7(8H)-one (25).**

Step 1. (R)-*tert*-Butyl 2-((6-(4-bromo-2-chlorophenyl)-2-(methylthio)-7-oxopyrido[2,3-d]-pyrimidin-8(7H)-yl)methyl)morpholine-4-carboxylate. A mixture of 6-(4-bromo-2-chloro-phenyl)-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (**I-16**, 500 mg, 1.31 mmol), (*S*)-*tert*-butyl 2-(tosyloxymethyl)morpholine-4-carboxylate (730 mg, 1.97 mmol) and cesium carbonate (850 mg, 2.62 mmol) in DMF (20 mL) was stirred at 50 °C overnight. After cooling to rt, the mixture was poured into water (200 mL). The aqueous layer was extracted with EtOAc

(3×50 mL), and the combined organic layers were dried over $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography using petroleum ether/EtOAc (3:1) as eluting solvents to afford the title compound as yellow oil (535 mg, 69 % yield). LCMS (ESI): *m/z* = 591.1 $[M+1]^+$.

Step 2: (R)-*tert*-Butyl 2-((6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)morpholine-4-carboxylate. A mixture of (R)-*tert*-butyl 2-((6-(4-bromo-2-chlorophenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)morpholine-4-carboxylate (430 mg, 0.73 mmol), 3-methylpyridin-2-ol (**I-18c**, 119 mg, 1.1 mmol), $N^1,N^2$-dimethylethane-1,2-diamine (18 mg), copper(I) iodide (20 mg) and potassium phosphate (470 mg, 1.46 mmol) in 1,4-dioxane (15 mL) was stirred in a sealed tube at 110 °C under nitrogen for 48 h. After cooling to rt, the mixture was concentrated and the residue purified by silica gel chromatography using DCM/methanol (100:3) as eluting solvents. This afforded the title compound as yellow oil (300 mg, 67 % yield). LCMS (ESI): *m/z* = 610.2 [M+1].

Step 3: (R)-*tert*-Butyl 2-((6-(2-cyclopropyl-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)morpholine-4-carboxylate. A mixture of (*R*)-*tert*-butyl 2-((6-(2-chloro-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methyl-thio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)morpholine-4-carboxylate (300 mg, 0.49 mmol), cyclopropylboronic acid (94 mg, 1.1 mmol), $Pd(OAc)_2$ (20 mg) and 2-dicyclohexylphosphino-2',6'-di-*i*-propoxy-1,1'-biphenyl (20 mg) in 1,4-dioxane (15 mL) was stirred in the sealed tube at 100 °C under nitrogen for 48 h. After cooling to rt, the mixture was concentrated and purified by silica gel chromatography using DCM/methanol (100:3) as eluting solvents to afford the title compound as yellow oil (290 mg, 96 % yield). LCMS (ESI): *m/z* =

616.2 $[M+1]^+$.

Step 4: (R)-*tert*-Butyl 2-((6-(2-cyclopropyl-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)morpholine-4-carboxylate. A solution of (*R*)-*tert*-butyl 2-((6-(2-cyclopropyl-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylthio)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)morpholine-4-carboxylate (290 mg, 0.47 mmol) in methanamine (40 % in ethanol, 5 mL) was stirred in a sealed tube at 80 °C for 18 h. After cooling to rt, the mixture was concentrated and the residue was purified by silica gel chromatography using DCM/methanol (100:3) as eluting solvents to afford the title compound as yellow oil (160 mg, 57 % yield). LCMS (ESI): *m/z* = 599.2 $[M+1]^+$.

Step 5: (*S*)-6-(2-Cyclopropyl-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)-8-(morpholin-2-ylmethyl)pyrido[2,3-d]pyrimidin-7(8H)-one (**25**). A solution of (*R*)-*tert*-butyl 2-((6-(2-cyclopropyl-4-(3-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)-7-oxo-pyrido[2,3-d]pyrimidin-8(7H)-yl)methyl)morpholine-4-carboxylate (160 mg, 0.27 mmol) in TFA (5 mL) and DCM (10 mL) was stirred at rt for 2 h. The reaction mixture was concentrated and purified by preparative HPLC to afford the title compound as white solid (81 mg, 60% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.75 (s, 1H), 7.84 -7.72 (m, 2H), 7.51 (dd, *J* = 1.2, 6.8 Hz, 1H), 7.40 (dd, *J* = 1.2, 6.8 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.21 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.96 (d, *J* = 2.0 Hz, 1H), 6.24 (t, *J* = 6.8 Hz, 1H), 4.61 – 4.40 (m, 1H), 4.35-4.43 (m, 1H), 3.88-3.73 (m, 2H), 3.19 (s, *3*), 2.93-2.65 (m, 5H), 2.21(s, 3H), 1.98-1.78 (m, 1H), 1.40-1.37 (m, 2H), 1.35-1.25 (m, 2H). LCMS (ESI): *m/z* = 499.3 $[M+1]^+$.

**(*R*)-6-(2-Chloro-4-(4-methyl-2-oxopyridin-1(2H)-yl)phenyl)-2-(methylamino)-8-(morpholin-2-ylmethyl)pyrido[2,3-d]pyrimidin-7(8H)-one (26).** The synthetic procedure was similar as for compound **21** using appropriate starting materials LCMS (ESI): *m/z* =493.1 $[M+1]^+$. $^1$H NMR

(400 MHz, MeOD-$d_4$) δ 8.63 (s, 1H), 7.85 (s, 1H), 7.62 (d, $J$ = 2 Hz, 1H), 7.57-7.55 (m, 2H), 7.33 (dd, $J$ = 2 Hz, 8.4 Hz, 1H), 6.49 (s, 1H), 6.41 (dd, $J$ = 2 Hz, 6.8 Hz, 1H), 4.82-4.64 (m, 1H), 4.62-4.43 (m,1H), 4.35-4.17 (m, 1H), 4.16-4.07 (m, 1H), 3.86-3.74 (m, 1H), 3.42-3.35 (m, 1H), 3.30-3.10 (m, 3H), 3.08 (s, 3H), 2.33 (s, 3H).

**_N_2-(((1r,4r)-4-Aminocyclohexyl)methyl)-N4-(4-(trifluoromethyl)pyridin-2-yl)pyrimidine-2,4-diamine (27).**

Step 1: 2-Chloro-*N*-(4-(trifluoromethyl)pyridin-2-yl)pyrimidin-4-amine. To a solution of NaH (60%, 59 mg, 2.5 mmol, 1.2 equiv) in dry DMF (10 mL) at 0 $^{o}$C was added 4-(trifluoro-methyl)pyridin-2-amine (200 mg, 1.23 mmol, 1.0 equiv). The mixture was stirred at 0 $^{o}$C for 30 min and 2,4-dichloropyrimidine (191 mg, 1.29 mmol, 1.05 equiv) then added. The mixture was stirred at rt for 16 h and then poured into 25 mL of water. The aqueous layer was extracted with EtOAc (50 mL × 3). The organic layers were combined, dried over $Na_2SO_4$ and concentrated. The residue was purified by silica gel chromatography using DCM: methanol (20:1) as eluting solvents to afford 2-chloro-*N*-(4-(trifluoromethyl)pyridin-2-yl)pyrimidin-4-amine as a yellow solid (100 mg, 30 % yield). LCMS (ESI): $m/z$ = 275.0 $[M+1]^+$.

Step 2: *tert*-Butyl (1r,4r)-4-((4-(4-(trifluoromethyl)pyridin-2-ylamino)pyrimidin-2-ylamino)-methyl)cyclohexylcarbamate. A mixture of 2-chloro-*N*-(4-(trifluoromethyl)pyridin-2-yl)-pyrimidin-4-amine (80 mg, 0.29 mmol), *tert*-butyl (1r,4r)-4-(aminomethyl)cyclohexylcarbamate (99 mg, 0.43 mmol) and diisopropylethylamine (0.5 mL) in *i*-propanol (2 mL) was stirred in a sealed tube under a nitrogen atmosphere. The mixture was stirred at 130 °C for 16 h and then concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford the title compound as yellow oil (100 mg, 74% yield). MS (ESI): $m/z$ = 467.2 $[M+1]^+$.

Step 3: *N*2-(((1r,4r)-4-Aminocyclohexyl)methyl)-*N*4-(4-(trifluoromethyl)pyridin-2-yl)-

pyrimidine-2,4-diamine (**27**). To a solution of *tert*-butyl (1r,4r)-4-((4-(4-(trifluoromethyl)pyridin-2-ylamino)pyrimidin-2-ylamino)methyl)cyclohexylcarbamate (100 mg, 0.21 mmol) in 1,4-dioxane (5.0 mL) at 0 °C was added hydrogen chloride (5.0 mL, 4.0 M in dioxane). The mixture was stirred at rt for 3 h and concentrated under reduced pressure. To the residue was added saturated $NaHCO_3$ solution and $CH_2Cl_2$ (20 mL). The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by preparative HPLC to afford the title compound as a white solid (30.0 mg, 38% yield). MS (ESI): *m/z* = 367.2 $[M+1]^+$. $^1$H NMR (500 MHz, MeOD-$d_4$) δ 8.69 (brs, 1H), 8.48 (d, *J* = 5.0 Hz, 1H), 7.93 (d, *J* = 5.5 Hz, 1H), 7.23 (d, *J* = 4.5, 1H), 6.41 (brs, 1H), 3.50-3.20 (m, 2H), 2.90-2.80 (m, 1H), 2.10-1.95 (m, 4H), 1.70-1.55 (m, 1H), 1.40-1.05 (m, 4H).

Biochemical and cellular assays were conducted as described previously.[11] Pharmacokinetic and toxicokinetic studies with compounds **1, 12, 19, 20-28** were carried out in female CD-1® or BALB/c mice obtained from Charles River Laboratories. Mice were administered test compounds via the intra-peritoneal (IP) route or oral gavage (PO) (n = 3 per group) using the vehicles and doses outlined in Table S3 (Supporting Information) and dose volumes of 5 or 10 mL/kg. Following administration of the compounds, unless otherwise noted, serial blood sampling was applied: 15 µL of blood was collected at each time point via tail nick and placed into a prefilled tube containing 60 µL of EDTA water. The sampling time points were 0.25, 0.5, 1, 3, 6, 8, and 24 hours and 0.5, 1, 3, and 6 hours post-dose for PK and toxicokinetic studies, respectively. The diluted blood samples were stored at –70 to –80°C until analysis. Compound concentrations in the blood samples were determined using LC/MS/MS. Pharmacokinetic

parameters were determined using standard non-compartmental methods using WinNonlin, Version 6.4 (Certara USA, Inc., Princeton, NJ).

## ASSOCIATED CONTENT

Supporting Information. Kinase profiling and secondary pharmacology data for selected compounds. Pharmacokinetic/ tolerability data for compounds from Tables 3 and 4. Experimental details on Langendorff heart experiments with compound **1**. Crystallographic parameters associated with the X-ray structure of compound **9**. Rotational scans for model systems related to compounds **1** and **9**.

New PDB ID Code: 5IME. Authors will release the Atomic Coordinates and experimental data upon article publication.

## ABBREVIATIONS

CL, clearance; DCM, dichloromethane; DFG, peptide sequence consisting of aspartate−phenylalanine−glycine; DIPEA, diisopropylethylamine; equiv, equivalent; hERG, human ether-a-go-go-related gene; HLM, human liver microsomes; KO, knock-out; LCK, Lymphocyte-specific protein tyrosine kinase; LDA, lithium diisopropylamide; LLE, ligand lipophilic efficiency; *m*-CPBA, *meta*-chloroperbenzoic acid; MDCK, Madin-Darby canine kidney; n.d., not determined; PAK, p21-activated kinase; PK, pharmacokinetics; QM, quantum mechanics, quantum-mechanical; SAR, structure-activity relationship; $S_NAr$, nucleophilic aromatic substitution; THF, tetrahydrofuran; TsOH, toluenesulfonic acid; tPSA, topological polar surface area; Xantphos, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene.

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**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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27. Karpov, A. S.; Amiri, P.; Bellamacina, C.; Bellance, M. H.; Breitenstein, W.; Daniel, D.; Denay, R.; Fabbro, D.; Fernandez, C.; Galuba, I.; Guerro-Lagasse, S.; Gutmann, S.; Hinh, L.; Jahnke, W.; Klopp, J.; Lai, A.; Lindvall, M. K.; Ma, S.; Möbitz, H.; Pecchi, S.; Rummel, G.; Shoemaker, K.; Trappe, J.; Voliva, C.; Cowan-Jacob, S. W.; Marzinzik, A. L. Optimization of a

dibenzodiazepine hit to a potent and selective allosteric PAK1 Inhibitor. *ACS Med. Chem. Lett.* **2015,** *6*, 776-781.

28. Prudnikova, T. Y.; Villamar-Cruz, O.; Rawat, S. J.; Cai, K. Q.; Chernoff, J. Effects of p21-activated kinase 1 inhibition on 11q13-amplified ovarian cancer cells. *Oncogene* **2016,** *35*, 2178-2185.

29. Force, T.; Kolaja, K. L. Cardiotoxicity of kinase inhibitors: the prediction and translation of preclinical models to clinical outcomes. *Nat. Rev. Drug Discov.* **2011,** *10*, 111-126.

30. Implicit assumption is that blood/plasma (B/P) ratio for all compounds in this table is approximately 1. For spot-checking, the blood/plasma ratio was experimentally determined for three representative compounds (**1**, **12**, and **20**) and indeed found to be close to **1**. Specifically, the B/P ratios were 0.76, 0.78, and 1.1 for **1**, **12**, and **20**, respectively.

## FIGURES

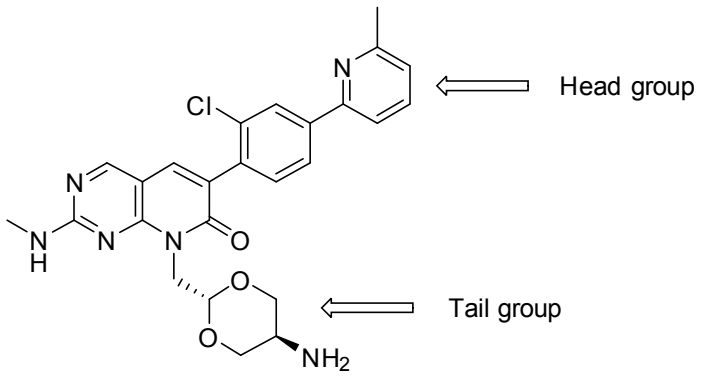



**Figure 1.** Previously reported Group I PAK inhibitor G-5555 (**1**).

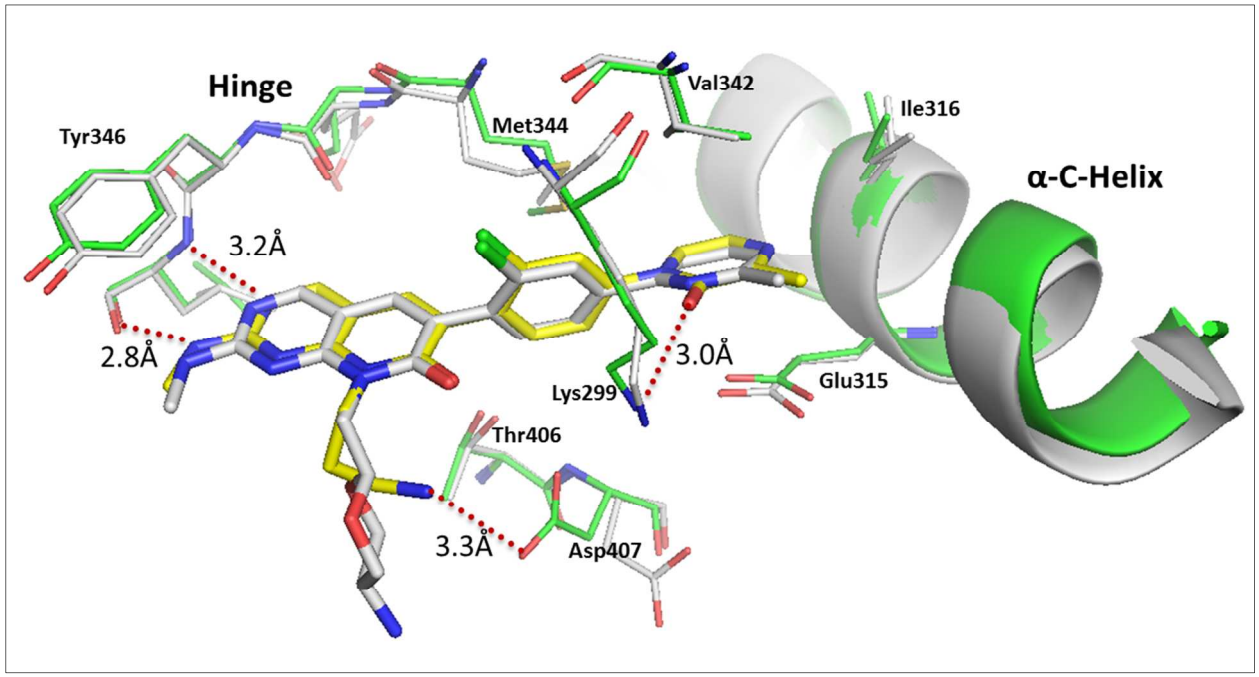



**Figure 2.** Co-crystal structure of compound **9** (yellow) in complex with PAK1 (green) (PDB: 5IME) superimposed with previously reported ligand-PAK1 structure of compound **1** with PAK1[11] (ligand and protein in gray; PDB: 5DEY). Hydrogen bonding interactions for the PAK1 / **9** co-crystal structure are shown as dotted red lines.

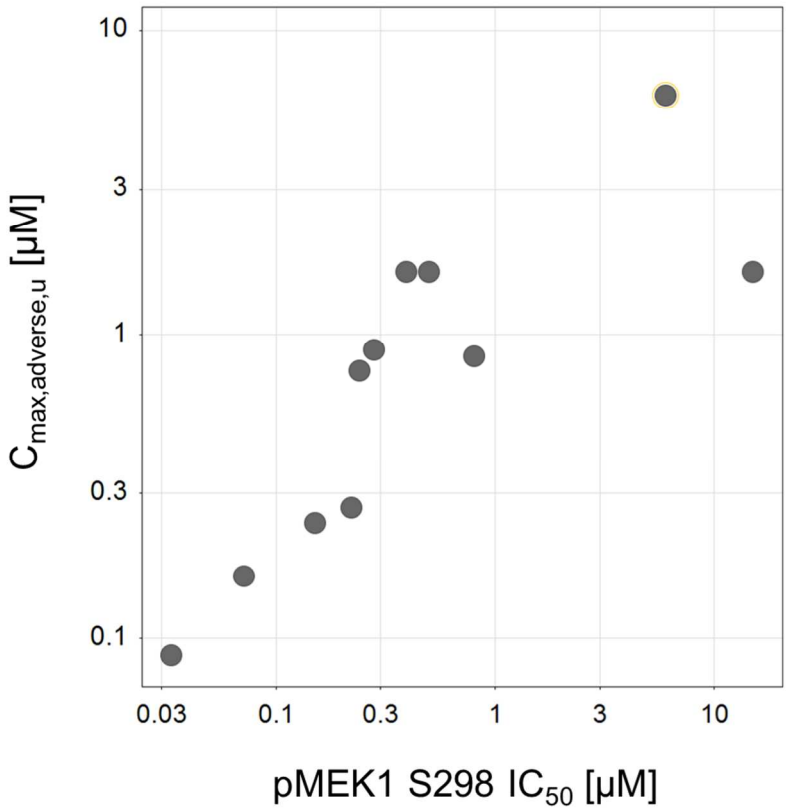



**Figure 3.** Plot showing the relationship between the lowest individual unbound $C_{max}$ associated with acute adverse effects determined in multi-group mouse pharmacokinetics/tolerability studies and the phospho-MEK1 S298 $IC_{50}$ of 11 test compounds. The plot shows data from all compounds from Table 3 and 4 except inactive control compound **26**.

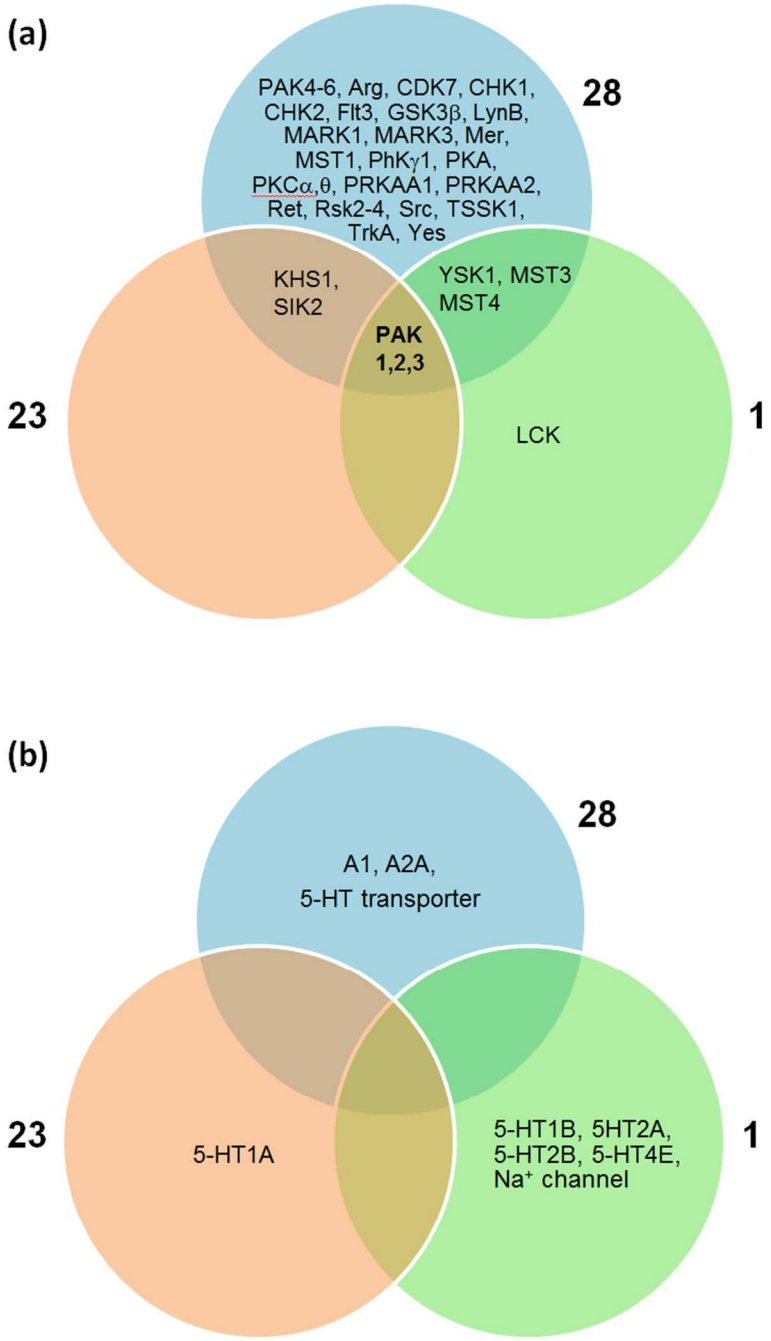
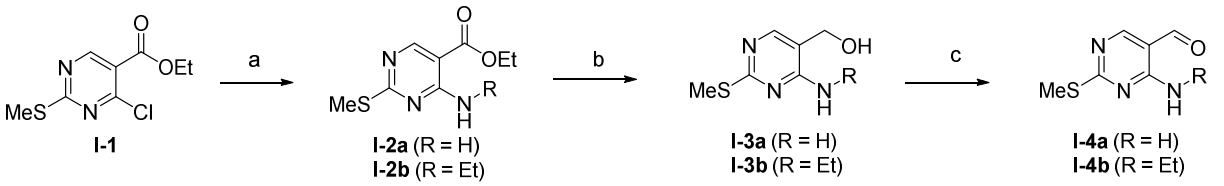
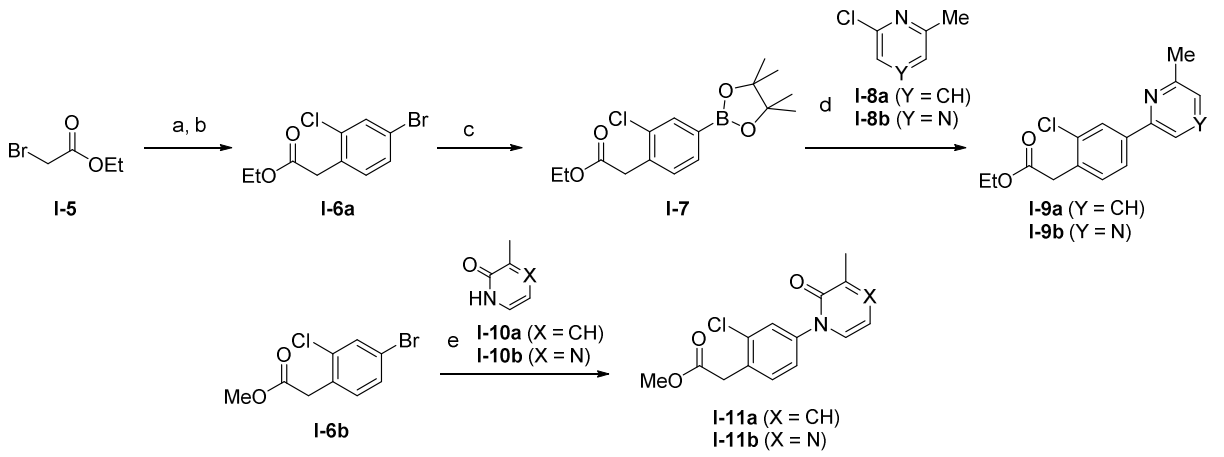



**Figure 4.** (a) Venn diagram showing the kinases inhibited at > 75% at a test concentration of 0.1 μM (compound **1**) and 1 μM (compounds **23** and **28**). The kinase screening panel contained 237 kinases for compound **1**, 96 kinases for compound **23** and 145 kinases for compound **28**. The panel used for **1** included all the 96 kinases tested for **23** and all 145 kinases tested for **28**. All kinases that were inhibited by compound **1** at > 75%, were included in the panels of the other two compounds. (b) Venn diagram showing the targets from a secondary pharmacology screening panel modulated at > 75% at a test concentration of 10 μM (52 targets tested overall). Details are provided in the supporting information.

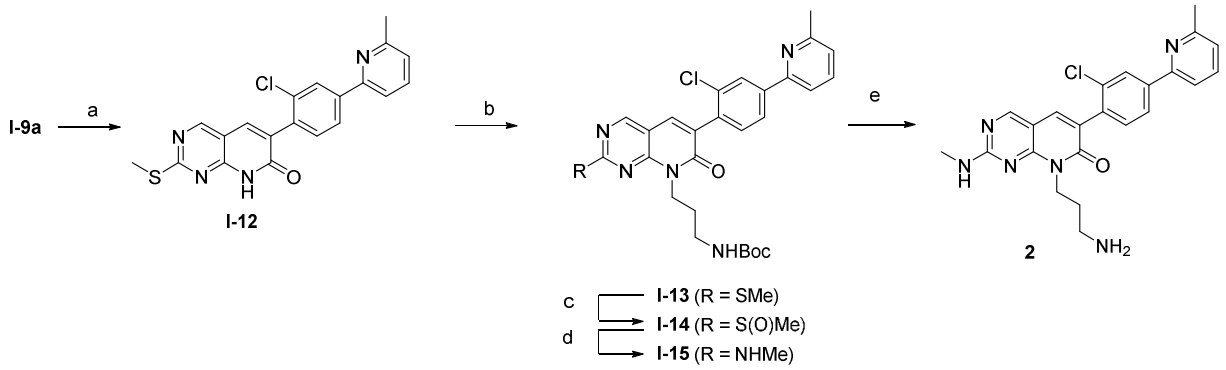
## SCHEMES



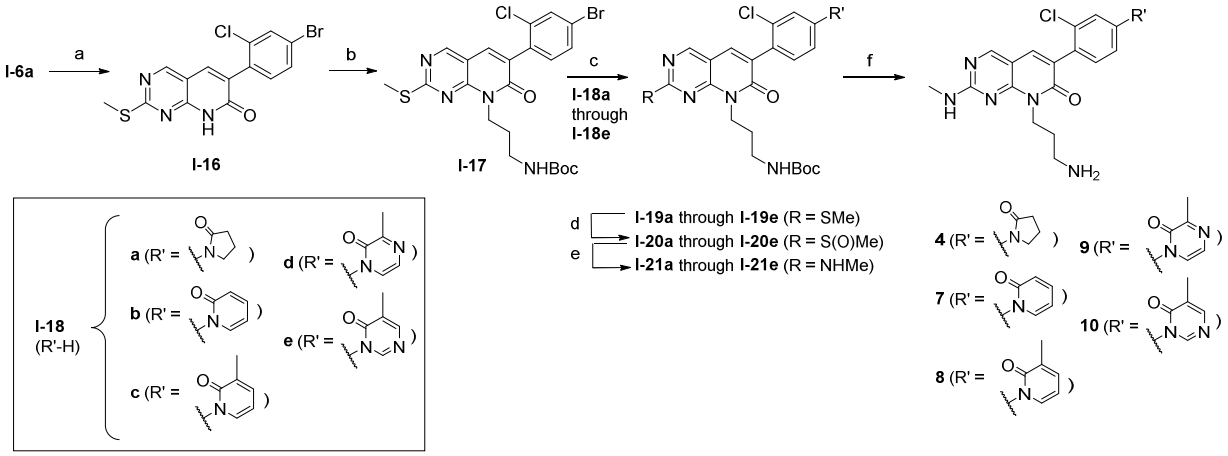

**Scheme 1.** (a) $NH_3$ or $NH_2Et$, THF, 2 h, 83-99% (b) $LiAlH_4$, THF, -10 °C to rt, 2 h, 83-86% (c) $MnO_2$, THF, 40 °C, 16 h, 52-82%.



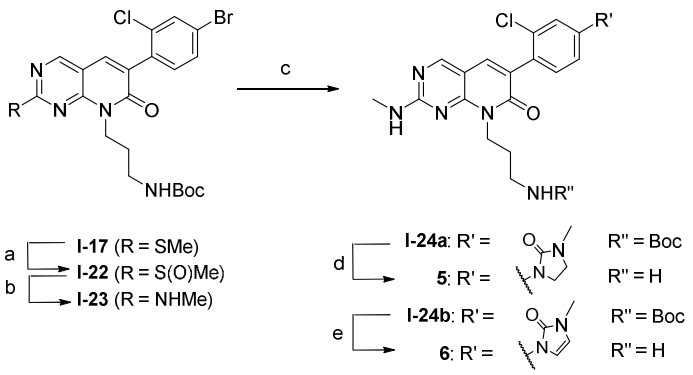

**Scheme 2.** (a) Zn dust, cat TMSCl, THF. (b) cat $Pd(dba)_2$, Xantphos, THF, 4-bromo-2-chloro-1-iodobenzene, 86% from 2 steps. (c) Bispinacolato diboron, $Pd(dppf)Cl_2$, KOAc, dioxane, 90%. (d) $Pd(dppf)Cl_2$, KOAc, 2-chloro-6-methylpyridine (**I-8a**) or 6-chloro-2-methylpyrazine (**I-8b**), dioxane, $H_2O$, 38-51%. (e) 3-Methylpyridin-2(1H)-one (**I-10a**) or 3-methylpyrazin-2(1H)-one (**I-10b**), CuI, *N,N*-dimethylethane-1,2-diamine, $K_3PO_4$, dioxane, 110 °C, 12 h, 54-64%.



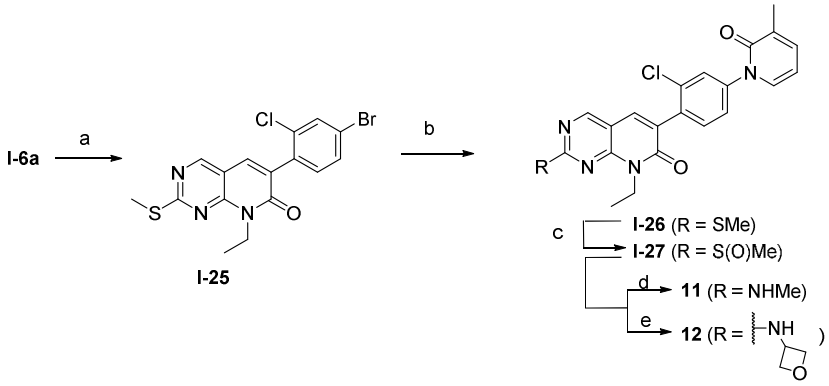

**Scheme 3.** (a) **I-4a**, $K_2CO_3$, DMF, 96%. (b) 3-((*tert*-butoxycarbonyl)amino)propyl 4-methylbenzenesulfonate, $Cs_2CO_3$, dioxane, 85 °C, 12 h, 90%. (c) *m*-CPBA, 30 min, rt. (d) $MeNH_2$, THF, 50 °C, 15 h. (e) TFA, $CH_2Cl_2$, rt, 1 h, 20% over three steps.



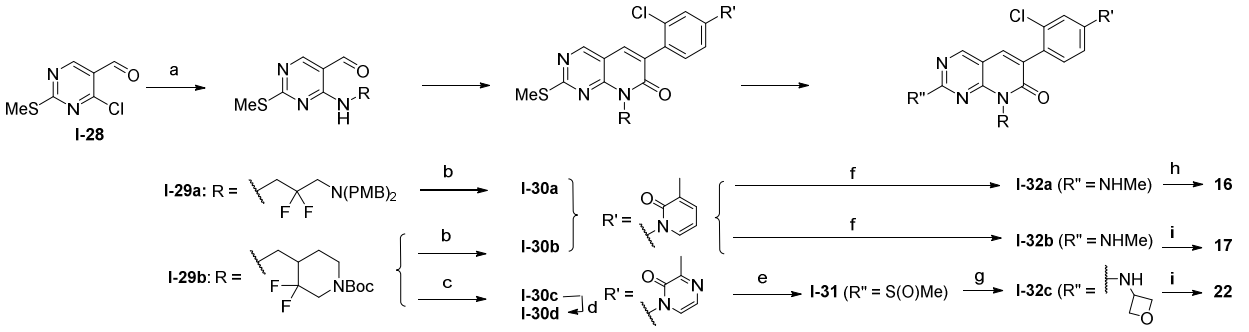

**Scheme 4a.** (a) **I-4a**, $K_2CO_3$, DMF, 83%. (b) *tert*-butyl *N*-(3-bromopropyl)carbamate or 3-(*tert*-butoxycarbonylamino)propyl 4-methylbenzenesulfonate, $Cs_2CO_3$, DMF; 85 °C, 12-17 h, >90%. (c) using **I-18a** (pyrrolidinone) as starting material: cat $Pd_2(dba)_3$, Xantphos, $Cs_2CO_3$, toluene, 100 °C, 6-12 h, 99%; using **I-18b** through **I-18e** (R'-H) as starting materials: CuI, *N,N*-dimethylethane-1,2-diamine, $K_3PO_4$, dioxane, 110 °C, 12 h, 32-71%. (d) *m*-CPBA, 30 min, rt. (e) $MeNH_2$, THF, 50 °C, 15 h. (f) TFA or HCl, dioxane, 1-2 h, 16% over three steps (for **4**); 29-65% over three steps (for **7**-**10**).




**Scheme 4b.** (a) *m*-CPBA, 30 min, rt. (b) $MeNH_2$, $CH_3CN$, EtOH, 10-20 °C, 30 min, 94% over two steps. (c) 3-Methyl-1*H*-imidazolidin-2-one or 3-methyl-1*H*-imidazol-2-one, cat $Pd_2(dba)_3$, Xantphos, $Cs_2CO_3$, toluene, 100 °C, 6 h. (d) HCl, dioxane, 1 h, 16% over two steps. (e) TFA, $CH_2Cl_2$, rt, 17% over two steps.



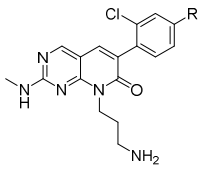

**Scheme 5.** (a) **I-4b**, $K_2CO_3$, DMF, 92%. (b) 3-Methylpyridin-2(1H)-one, CuI, *N,N*-dimethylethane-1,2-diamine, $K_3PO_4$, dioxane, 110 °C, 12 h, 35%. (c) *m*-CPBA, 30 min, rt. (d) $MeNH_2$, THF, 50 °C, 15 h, 71% over two steps. (e) Oxetan-3-amine, DIPEA, THF, 50 °C, 15 h, 25% over two steps.




**Scheme 6.** (a) For **I-29a**: 2,2-difluoro-$N^1$,$N^1$-bis(4-methoxybenzyl)propane-1,3-diamine, $NEt_3$, THF, *i*PrOH, 8 h, 56%; for **I-29b**: *tert*-butyl 4-(aminomethyl)-3,3-difluoropiperidine-1-carboxylate, $NEt_3$, THF, *i*PrOH, 12 h, 94%. (b) **I-11a**, $K_2CO_3$, DMF, 12 h, 57% (**I-30a**), 62% (**I-30b**). (c) **I-11b**, $K_2CO_3$, DMF, 12 h, 83%. (d) **I-30c** was separated into enantiomers by chiral HPLC, and the single enantiomers with unknown absolute stereochemistry, each obtained in 25% from the racemate, were used in the following steps. Only the yields for the intermediates leading to the active enantiomer **22** are shown. (e) *m*-CPBA, 30 min, rt. (f) $MeNH_2$, THF, 80 °C, 8-15 h. (g) Oxetan-3-amine, DIPEA, THF, 70 $^0$C, 15 h. (h) TFA, 95 °C, 8 h, 40% over two steps (**16**). (i) TFA, $CH_2Cl_2$, 2 h, 66% over two steps (**17**), 43% over three steps (**22**).

## TABLES

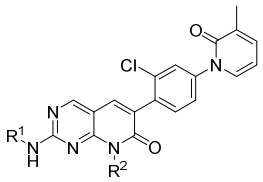
**Table 1.** Head group SAR studies.

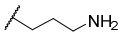
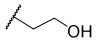
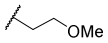
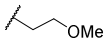
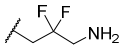
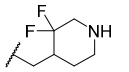
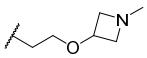
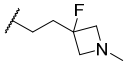



| | R | PAK1[a] $K_i$ (nM) | Selectivity (x-fold) vs PAK4 | Selectivity (x-fold) vs LCK | LLE[b] | HLM CLhep [mL/min/kg][c] |
|---|---|---|---|---|---|---|
| **2** |  | 3.3 | 449 | 53 | 5.0 | 10.1 |
| **3** | H | 382 | >7.6 | 0.4 | 4.2 | 2.2 |
| **4** |  | 89 | >33 | 25 | 5.3 | 4.8 |
| **5** |  | 56 | >52 | 10 | 5.5 | 10.6 |
| **6** |  | 13 | >232 | 76 | 6.7 | 5.2 |
| **7** |  | 36 | >82 | 86 | 5.6 | 4.8 |
| **8** |  | 3.5 | 517 | 221 | 6.2 | 3.0 |
| **9** |  | 4.6 | >642 | 429 | 7.0 | 1.8 |
| **10** |  | 6.7 | >443 | 282 | 7.2 | 4.7 |

[a]PAK1 assay results represent the geometric mean of a minimum of two determinations performed in duplicate. [b]LLE = –log $K_i$ – ClogP. [c]Human liver microsome-derived predicted hepatic clearance.

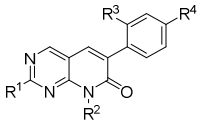
**Table 2.** Tail group SAR studies.

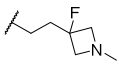
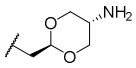
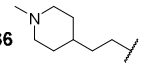
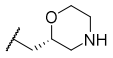
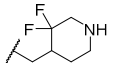
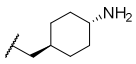
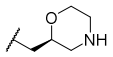
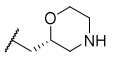
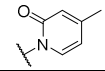


| | $R^1$ | $R^2$ | PAK1[a] $K_i$ (nM) | Selectivity vs LCK (x-fold) | LLE[b] | pMEK[c] $IC_{50}$ (nM) | cpKa[d] | logD[e] (7.4) | Solubility at pH 7.4 (μM)[f] | MDCK perm. A to B / efflux[g] | HLM CLhep [mL/min/kg][h] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **8** | Me |  | 3.5 | 221 | 6.2 | 602 | 11 | 0.05 | 82 | 0.1 (4.3) | 10.1 |
| **11** | Me | Et | 8 | 53 | 4.7 | 340 | <5 | 2.1 | 2.5 | 28 (0.4) | 13.4 |
| **12** |  | Et | 6.3 | 123 | 5.2 | 150 | <5 | 2.5 | 2.6 | 37 (0.4) | 13.0 |
| **13** | Me |  | 17 | 63 | 5.6 | 930 | <5 | 2.2 | 15 | 5.5 (2.8) | 14.6 |
| **14** | Me |  | 13 | 38 | 5.1 | 450 | <5 | 2.2 | 80 | 27 (0.5) | 14.4 |
| **15** |  |  | 5.9 | 158 | 5.7 | 290 | <5 | 1.9 | 38 | 7.6 (2) | 11.6 |
| **16** | Me |  | 9 | 56 | 5.5 | 460 | 7.3 | 1.8 | 51 | 8.4 (1.8) | 12.1 |
| **17**[i] | Me |  | 2.7 | 20 | 5.3 | 78 | 6.9 | 0.9 | 115 | 9.7 (1.3) | 15.4 |
| **18** | Me |  | 4.4 | 86 | 5.9 | 88 | 8.7 | 0.63 | 71 | 0.2 (26) | 3.4 |
| **19 G-9791** | Me |  | 0.95 | 57 | 5.9 | 33 | 7.8 | 1.8 | 117 | 6.9 (2) | 12.9 |

[a]PAK1 assay results represent the geometric mean of a minimum of two determinations performed in duplicate. [b]LLE = –log $K_i$ – ClogP. [c]Cellular assay to determine the inhibition of the phosphorylation of residue S298 of MEK1 in EBC1 cells. Data results represent the geometric mean of a minimum of two determinations performed in duplicate. [d]Calculated pKa using MoKa software (version 1.1.0, Molecular Discovery). [e]Measured logD at pH7.4. [f]Kinetic solubility. [g]Compounds were incubated with Madin−Darby canine kidney cells (MDCK) at 10 μM test concentration over 60 min; shown is the value of the rate of apical−basolateral (A−B) flux with units $\times 10^{-6}$ cm/s (apparent permeability, $P_{app}$). Efflux is defined as the ratio of the basolateral-apical (B-A) flux divided by the apical-basolateral (A-B) flux. [h]Human liver microsome-derived predicted hepatic clearance. [i]Compound is a racemic mixture.

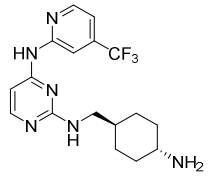
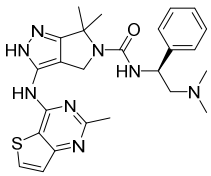
**Table 3.** PAK inhibitors tested in single dose mouse PK/tolerability studies.



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | PAK1[a] $K_i$ (nM) | PAK2[a] $K_i$ (nM) | pMEK[b] $IC_{50}$ (μM) | Mouse $f_u$[c] | $C_{max,adverse}$[d] (μM) | $C_{max,adverse,\ u}$[e] (μM) |
|---|---|---|---|---|---|---|---|---|---|---|
| **19** **G-9791** |  |  | Cl |  | 0.95 | 2.0 | 0.033 | 0.032 | 2.8 | 0.088 |
| **1** **G-5555** |  |  | Cl |  | 4.4 | 11 | 0.071 | 0.003 | 55 | 0.16 |
| **12** |  | Et | Cl |  | 7.5 | 21 | 0.15 | 0.006 | 41 | 0.24 |
| **20** **FRAX1036** |  | Et | Cl |  | 23 | 48 | 0.22 | 0.017 | 16 | 0.27 |
| **21** |  |  | Cl |  | 11 | 9.5 | 0.24 | 0.027 | 28 | 0.76 |
| **22**[f] |  |  | Cl |  | 5.2 | 4.6 | 0.28 | 0.076 | 12 | 0.89 |
| **23** |  |  | Cl |  | 22 | 39 | 0.80 | 0.059 | 14 | 0.85 |
| **24** | H |  | Cl |  | 372 | 940 | 6.0 | 0.104 | 59 | 6.1 |
| **25** |  |  |  |  | 950 | 3,670 | 15 | 0.025 | 63 | 1.6 |
| **26** |  |  | Cl |  | 943 | 2,300 | >30 | 0.143 | n/a[g] | n/a |

[a]PAK1 and PAK2 assay results represent the geometric mean of a minimum of two determinations performed in duplicate. [b]Cellular assay to determine the inhibition of the phosphorylation of residue S298 of MEK1 in EBC1 cells. Data results represent the geometric mean of a minimum of two determinations performed in duplicate. [c]Fraction unbound determined in a mouse plasma protein binding experiment. [d]Lowest individual $C_{max}$ associated with acute adverse effects (moribund animals or lethality) determined in a multi-group mouse pharmacokinetics/ tolerability study. Compounds were either dosed orally (**1**, **19**, **20**, **23**) or intraperitoneally (**12**, **21**, **22**, **24**, **25**, **26**), and blood sampling was performed serially. Further details are given in the supporting information. [e]Unbound $C_{max,adverse}$ calculated as total $C_{max,adverse}$ × mouse $f_u$.[30] [f]Compound is the single active enantiomer of unknown absolute configuration. PAK1 $K_i$ of the opposite enantiomer is 12 nM. [g]Compound was tolerated up to the maximum $C_{max}$ achieved (40 μM, corresponding to $C_{max,u}$ of 5.7 μM).

**Table 4**. PAK inhibitors from distinct chemical series tested in single dose mouse PK/tolerability studies.

| | | PAK1[a] $K_i$ (nM) | PAK2[a] $K_i$ (nM) | pMEK[b] $IC_{50}$ (μM) | Mouse $f_u$[c] | $C_{max,\ adverse}$[d] (μM) | $C_{max,adverse,\ u}$[e] (μM) |
|---|---|---|---|---|---|---|---|
| **27** |  | 26 | 225 | 0.393 | 0.063 | 26[f] | 1.6 |
| **28** **PF-3758309** |  | 38 | 139 | 0.498 | 0.163 | ~10[g] | 1.6 |

[a]PAK1 and PAK2 assay results represent the geometric mean of a minimum of two determinations performed in duplicate. [b]Cellular assay to determine the inhibition of the phosphorylation of residue S298 of MEK1 in EBC1 cells. Data results represent the geometric mean of a minimum of two determinations performed in duplicate. [c]Fraction unbound determined in a mouse plasma protein binding experiment. [d]Lowest individual $C_{max}$ associated with acute adverse effects determined in a multi-group mouse pharmacokinetics/ tolerability study. Both compounds were dosed intraperitoneally and blood sampling was performed serially for **27** and in a parallel fashion for **28**. Further details are given in the supporting information. [e]Unbound $C_{max,adverse}$ calculated as total $C_{max,adverse}$ × mouse $f_u$.[30] [f]$C_{max,adverse}$ from animals dosed with 50 mg/kg. All animals in this group were moribund shortly after dosing and were euthanized during the course of the study. Blood sampling had to be performed retroorbitally. [g]As blood sampling was performed in a parallel fashion for **28**, monitoring the progression of individual animals was not possible. All animals in the high-dose group (50 mg/kg, dosed i.p.) were found to be lethargic and challenging to bleed due to low blood flow. Three out of the 24 study animals died during the course of the study. The aggregated $C_{max}$ from this group was approximately 10 μM.

**Table 5.** Key characteristics of on-target toxicity and specific observations made in the investigation of potential on-target toxicity caused by PAK1/2 inhibitors.

| On-target toxicity characteristic | Observation made |
|---|---|
| Toxicity consistent with knock-out model | PAK1 KO - compromised cardiac function upon challenge, abnormal basal $[Ca^{2+}]_i$ transient, prone to $Ca^{2+}$ overload and arrhythmia<br><br>PAK2 KO – embryonically lethal (vasculature development)<br><br>PAK2 inducible KO – lethal in adult mice (vascular integrity) |
| Target expressed in tissue with toxicity | PAK1 and 2 are widely expressed, including the cardiovascular system |
| Toxicity observed with different chemotypes | Three chemotypes, pyridonopyrimidines, aminopyridine, and PF-3758309 were all acutely toxic/lethal |
| Toxicity absent with an inactive analog | Inactive/active pair: only the active analog **21** was toxic; the counterpart **26** devoid of activity in the cellular assay (pMEK) was not |
| Toxic concentration tracks with potency | Minimum concentration to obtain adverse effects parallels pMEK potency for 11 compounds (1-4 × pMEK $IC_{50}$) |

## Table of Contents Graphic