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Cell Penetrant Inhibitors of the KDM4 and KDM5 Families of Histone Lysine Demethylases. 2. Pyrido[3,4-*d*]pyrimidin-4(3*H*)-one Derivatives

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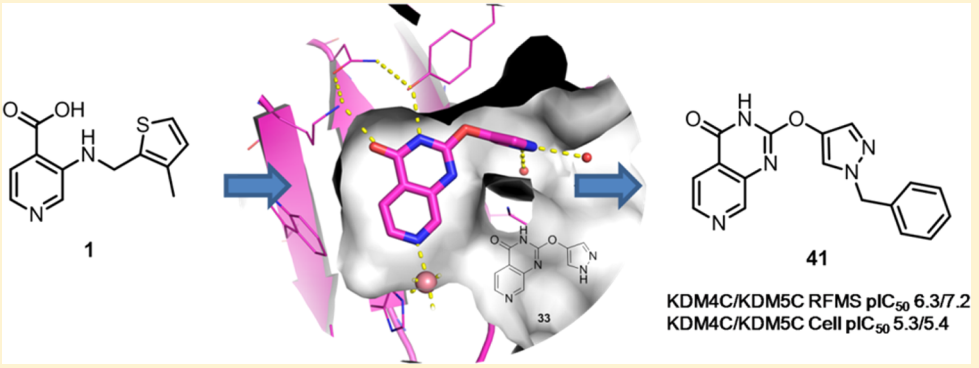
Supporting Information



ABSTRACT: Following the discovery of cell penetrant pyridine-4-carboxylate inhibitors of the KDM4 (JMJD2) and KDM5 (JARID1) families of histone lysine demethylases (e.g., **1**), further optimization led to the identification of non-carboxylate inhibitors derived from pyrido[3,4-*d*]pyrimidin-4(3*H*)-one. A number of exemplars such as compound **41** possess interesting activity profiles in KDM4C and KDM5C biochemical and target-specific, cellular mechanistic assays.

INTRODUCTION

There is currently rapidly expanding interest in how epigenetic control of gene expression is implicated in various disease states.[1−3] As a consequence, "probe molecules" with proven cellular activity against a specific epigenetic target or family or targets, for example, the "writers", "erasers", or "readers" of post-translational modifications of nuclear histone proteins, can play a key role in understanding how modulation of such targets could affect disease progression. In the preceding article[4] we reported on our initial efforts to identify inhibitors of the KDM4 (JMJD2) family of histone lysine demethylases that led to the discovery and optimization of a series of cell-active 3-aminopyridine-4-carboxylate based inhibitors of both the KDM4 and the KDM5 (JARID1) families, for example, compounds **1** and **2** (Figure 1).

While these compounds may prove useful for further in vitro phenotypic exploration of the roles of KDM4 and KDM5 enzymes, we were keen to determine whether we could make further improvements through the design of less acidic and thus potentially more cell penetrant compounds, targeting cellular activity $pIC_{50} \geq 6$ ($IC_{50} \leq 1\ \mu M$). In a similar, recently reported approach, optimization of the broad spectrum 2-oxoglutarate-dependent dioxygenase inhibitor 8-hydroxyquinoline-5-carboxylic acid, which included removal of the carboxylate, led to increased cellular activity and a reduced differential between cellular and biochemical activity against members of the KDM4 family.[5]

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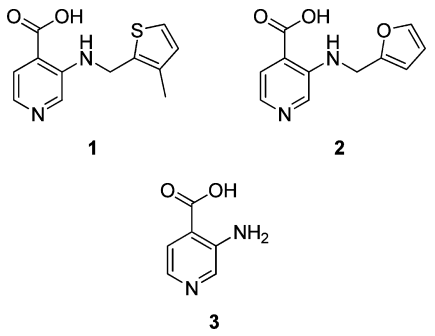


Figure 1. Pyridine-4-carboxylic acid inhibitors of KDM4 and KDM5 families of histone lysine demethylases (KDMs).

RESULTS AND DISCUSSION

As described in the preceding article,[4] we found that the fragment core of KDM4C cell active molecules **1** and **2**, 3-amino-4-pyridinecarboxylic acid **3**, was an inhibitor of the KDM4 family with micromolar activity in our biochemical RapidFire mass spectrometry (RFMS)-based assays.[6] Selectivity vs the related Jumonji enzyme KDM6B (JMJD3) and the prolyl hydroxylase EGLN3 (also known as PHD3), a more distantly related 2-oxoglutarate (2-OG) dependent dioxygenase enzyme, was encouraging with activity against these targets assessed using RFMS and homogeneous time-resolved fluorescence (HTRF) assay formats, respectively[4] (Table 1).

Table 1. Inhibition Profile of 3-Amino-4-pyridinecarboxylic Acid 3 in KDM4 and KDM6B RFMS Assays and an EGLN3 HTRF Assay[4,7]

	pIC_{50}
KDM4A	6.3
KDM4C	6.2
KDM4D	5.8
KDM4E	5.9
KDM6B	4.2[a]
EGLN3	<4.3

[a]4.2, n = 12; <4.0, n = 12.

With the aim of improving cell penetration, we targeted a range of less acidic, bicyclic fragments based on **3** that conserved the key sp^2 nitrogen atom that interacts with the catalytic metal within the active site and that also contained functionality that could potentially form similar interactions with K210 and Y136 as shown for **3** bound to KDM4D (Figure 2). Compounds **4**, **6**, and **7** were synthesized according to Scheme 1. Thus, pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **4** was prepared utilizing a literature method involving treatment of 3-amino-4-pyridinecarboxylic acid with formamidine acetate[8] in a moderate yield of 39%. Pyrido[3,4-*d*]pyridazin-1(2*H*)-one **6** was prepared via a three-step literature-based synthesis[9] wherein 4-pyridinecarboxylic acid was derivatized as the *N*-phenylamide **10** and then deprotonated with *n*-BuLi and treated with *N*,*N*-dimethylformamide to give the crude isoindolin-1-one intermediate **11**.

Reaction with aqueous hydrazine then gave the target bicycle **6**. 1*H*-Pyrazolo[3,4-*c*]pyridin-3(2*H*)-one **7** was prepared in two steps from 3-amino-4-pyridinecarboxylic acid via conversion of the amine to the hydrazine **12** by treatment with sodium nitrite and sulfur dioxide followed by ring closure under acidic conditions in 25% overall yield.[10] Additionally, 2,6-naphthyridin-1(2*H*)-one **5** was sourced commercially. The data from screening compounds **4**–**7** in the KDM4C RFMS biochemical assay are shown in Table 2. Compounds **5**–**7** did not show detectable activity in this assay, indicating the preference for an sp^2 nitrogen atom at the 1-position. The pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **4** showed encouraging activity in the KDM4C assay with pIC_{50} = 5.7 and was tested in the RFMS assays for other KDM4 family members giving pIC_{50} of 5.9, 5.0, and 5.1 for KDM4A, -D, and -E, respectively. Additionally, **4** gave no detectable activity in our KDM6B and EGLN3 assays (pIC_{50} <4.0 and <4.3, respectively).

X-ray crystallography of **4** bound into KDM4D confirmed that the compound, as designed, adopts a very similar binding mode to the pyridine carboxylate **3** (Figure 3a). It was also apparent from this structure that **4** possessed a high level of shape complementarity in the region of the 5- and 6-positions that offered little scope for further substitution (Figure 3b). Indeed, the introduction of methyl substituents into either of

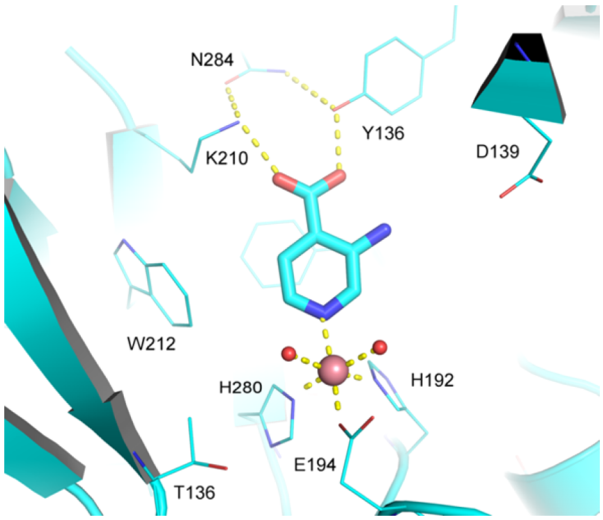
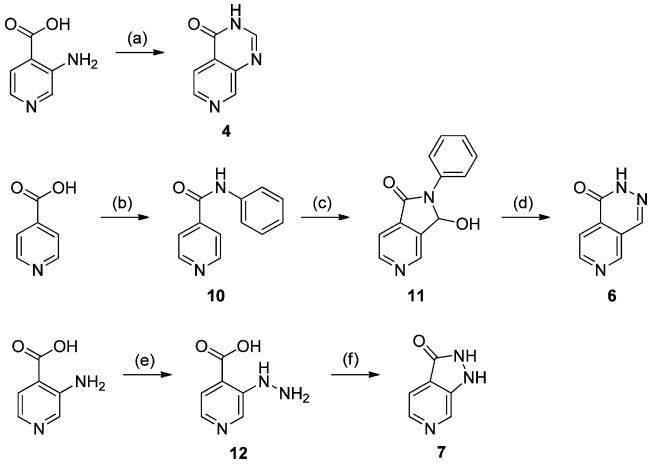


Figure 2. X-ray crystal structure of **3** bound to KDM4D active site (PDB code 5FP9).

Scheme 1. Synthesis of Bicyclic Pyridine Derivatives 4, 6, and 7[a]



[a]Conditions: (a) $HC(NH)NH_2$.HOAc, *N*,*N*-dimethylacetamide, 150 °C, 16 h; (b) 1,1′-CDI, $PhNH_2$, 2Me-THF, 50 °C, 20 h; (c) *n*-BuLi, DMF, THF, −70 to 0 °C; (d) 35% N_2H_4 in water, reflux, 2 h; (e) conc HCl, water, $NaNO_2$, SO_3, rt; (f) 2 M HCl_{aq}, reflux, 5 h.

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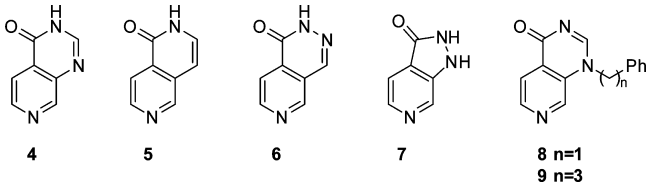
Table 2. Activity of Bicyclic Analogues 4−9 vs KDM4C[7]



compd	KDM4C RFMS pIC_{50}
4	5.7
5	<4.0[a]
6	<4.0
7	<4.0
8	4.6
9	4.0, <4.0

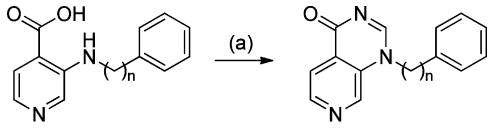
[a]See ref 11.

these positions resulted in IC_{50} values greater than 100 μM (data not shown). The cLogP and $ChromLogD_{pH7.4}$ of **4** are −0.7 and −0.6, respectively, and these values are suboptimal for passive permeability into cells. Thus, the 1-, 2-, and 8-positions appeared the most viable for exploration of the SAR and modulation of physicochemical properties.

We hypothesized that substitution of the nitrogen atom at the 1-position would give a tautomeric derivative of **4** that could still potentially form suitable interactions with the key lysine and tyrosine residues, even though this substitution would result in the loss of the hydrogen bond donor (HBD) at the 3-position; indeed 3-amino-4-pyridinecarboxylic acid **3** will predominantly exist as the ionized form under physiological conditions and thus will not have hydrogen bond donor capability in the equivalent position. The 1-benzyl and 1-(3-phenylpropyl) derivatives of **4**, compounds **8** and **9**, were synthesized via cyclization of the corresponding 3-amino-4-pyridinecarboxylic acids **13** and **14** with formamide in the presence of phosphorus oxychloride, as shown in Scheme 2. However, we were somewhat surprised to find that these analogues **8** and **9** showed considerably reduced potency at KDM4C when compared to core **4** (Table 2) and the parent acids **13** and **14** (pIC_{50} = 7.0, 6.7, respectively).

These data indicated the importance of the HBD at the 3-position. Therefore, keeping this in place, we next synthesized a small set of compounds with substituents at the 8-position, including some that could potentially combine with the nitrogen atom in the 7-postion of the core to form bidentate

Scheme 2. Synthesis of 1-Benzyl and 1-Phenylpropyl Analogues 8 and 9[a]



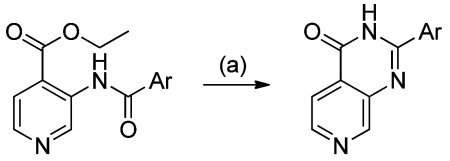
13 n=1
14 n=3

8 n=1
9 n=3

[a]Conditions: (a) $HCONH_2$, $POCl_3$, 0 °C to rt, 16.5 h.

interactions with the active site iron atom. However, this proved unsuccessful with all compounds prepared giving $pIC_{50} \leq 4.6$ (Supporting Information Table 1). We then turned our attention to exploration of the 2-postion of compound **4**, and the effects of a directly attached aryl were investigated in the first instance with the synthesis of key exemplars shown in Scheme 3. The ethyl 3-amido-4-pyridine carboxylates were reacted with methanolic ammonia[12] followed by aqueous sodium hydroxide to furnish compounds **15**−**17** in yields of 7−60%.

Scheme 3. Synthesis of Aryl and Hetaryl Analogues 15−17[a]



15-17

[a]Conditions: (a) NH_3 (7 M in MeOH), rt, 4 h, or 0.88 NH_3, MeOH, rt, 21.5 h, then NaOH (10 M_{aq}), EtOH.

The introduction of a phenyl ring into the 2-position, **15** resulted in 10-fold lower potency at KDM4C (pIC_{50} = 4.7) when compared to **4** (Table 3). However, some of the activity could be regained by moving to the 3-pyridyl analogue **16** with pIC_{50} = 5.1 at KDM4C.

A variety of substituents was investigated to determine if any further increases in potency could be achieved. Meta substitution of a phenyl or 3-pyridyl ring relative to the position of attachment to the core was most beneficial, with compound **17** giving the best albeit a modest 4-fold increase in potency to pIC_{50} = 5.7 (IC_{50} = 2 μM) (Table 3). To assess

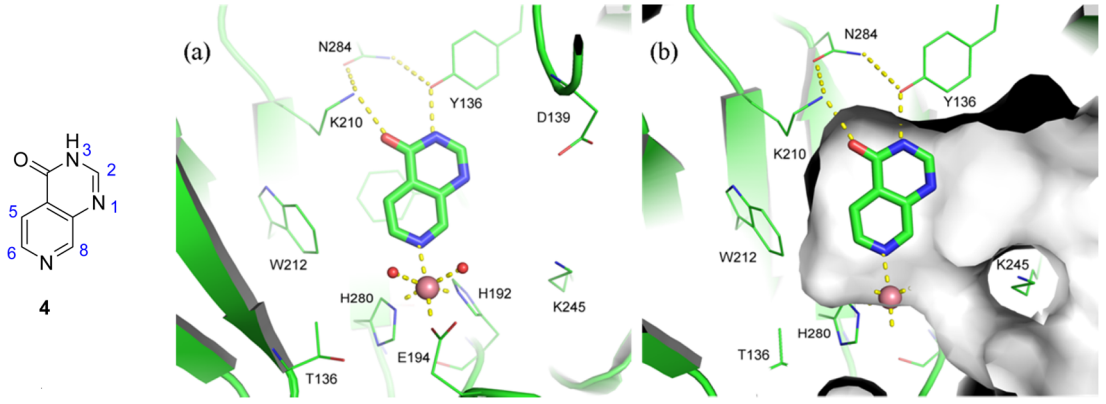


Figure 3. X-ray structure of **4** bound to KDM4D active site (PDB code 5FPA).

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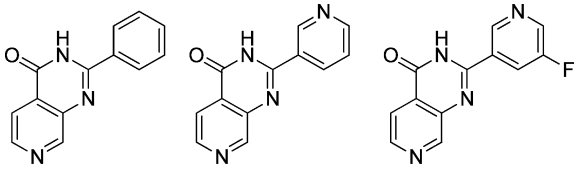
Table 3. Activity of 2-Aryl Analogues 15−17 vs KDM4C[7]



15 16 17

compd	KDM4C RFMS pIC_{50}
4	5.7
15	4.7
16	5.1
17	5.7

cellular activity, compound **17** was tested in the previously described KDM4C mechanistic, high content imaging assay.[4] In this assay U2OS cells were subjected to a Bacmam-mediated transfection with full-length HALO-tagged KDM4C in the presence of test compound at 37 °C for 24 h, followed by determination of the level of global $H3K9Me_3$ demethylation compared to positive and negative controls. Unfortunately the level of biochemical activity observed for **17** (pIC_{50} = 5.7) did not translate into measurable cellular activity in this assay (pIC_{50} < 4.0).

As part of an investigation into a range of small aliphatic substituents at the 2-postion of **4**, a series of compounds was prepared wherein an ethyl group was linked to the core through CH_2, NH, S, and O linker groups. The methylene linked analogue **18** was prepared in a manner similar to that utilized for the directly attached aryl analogues shown in Scheme 3, whereas the heteroatom linked compounds **19**−**21** were prepared as outlined in Scheme 4. Thus, 3-amino-4-pyridinecarboxylic acid was cyclized with thiourea to give the thione **22**.[13] Methylation with iodomethane and finally treatment with ethylamine or sodium ethoxide under microwave heating then furnished **19** and **21**, respectively. Thioethyl analogue **20** was prepared by treatment of **22** with iodoethane under basic conditions.

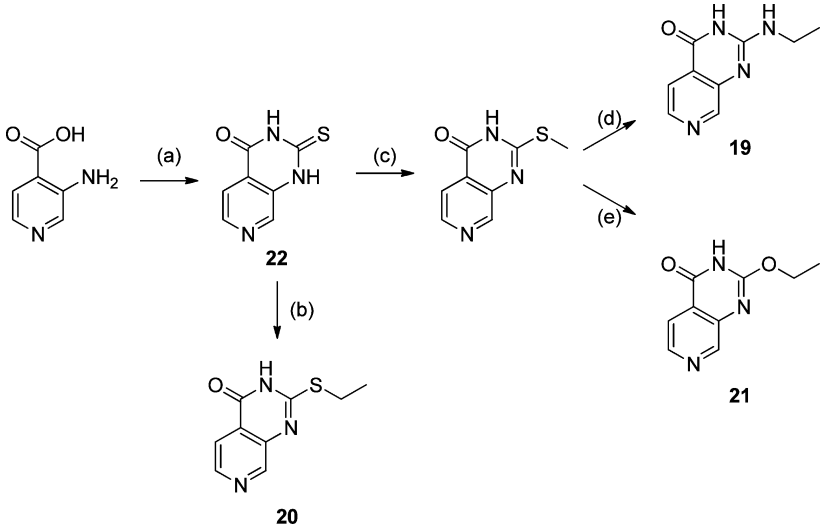
The S- and O-linked analogues **20** and **21** gave the highest level of activity in the KDM4C RFMS assay (pIC_{50} = 6.1 and 6.0, respectively), followed by the NH analogue **19** and then the CH_2 analogue **18**, which was 12- to 16-fold less active (Table 4). The increase in potency from CH_2 through to O and S corresponds to a decrease in the pK_a value of the 3-NH, and we hypothesize that the increased acidity contributes to a stronger H-bonding interaction between this NH and the key tyrosine residue described previously. Furthermore, the O-linked analogue **21** was also starting to show detectable activity in the KDM4C cell based assay on some test occasions, albeit at a low level.

Ethoxy analogue **21** was used as a starting point to further optimize biochemical and cellular potency in this series, and a set of O-linked six-membered ring containing analogues was synthesized as outlined in Scheme 5. The thioethyl analogue **20** was oxidized with either *m*CPBA or peracetic acid to give either the sulfonyl intermediate **20a** or mixtures of the sulfonyl and sulfinyl intermediates **20b**. The proportions of each of the oxidized sulfur species present in the crude product mixture **20b** were variable between reaction runs, and the final step, treatment with an appropriate nucleophile, was carried out directly on isolated crude material due to the relative instability of these species (see Experimental Section for details).

Optimization of this route was required with some of the less reactive nucleophiles. Thus, **20** was protected with the SEM group to give **23**, and this was then reacted with *m*CPBA. The crude oxidized intermediate **23a** was then reacted with the required alcohol in the presence of cesium carbonate, and a final Lewis acid-mediated deprotection step furnished the required product. The data obtained from testing these analogues in the KDM4C RFMS and cell imaging assays are shown in Table 5.

The phenyl analogue **24** gave similar potency to the ethyl compound **21** with pIC_{50} = 6.2 in the KDM4C RFMS biochemical assay and encouragingly showed improved activity in the cellular assay with pIC_{50} = 4.9. The benzyl analogue **25** was less potent in both assay formats. The cyclohexyl and cyclohexylmethyl analogues **26** and **27** also showed decreased

Scheme 4. Synthesis of S-, N-, and O-Linked Analogues 19−21[a]



[a]Conditions: (a) $H_2NC(S)NH_2$, 160 °C, 16 h, 77%; (b) EtI, 1 M $NaOH_{aq}$, MeOH, rt, 1 h, 88%; (c) MeI, 1 M $NaOH_{aq}$, MeOH, rt, 16 h, 64%; (d) $EtNH_2$ (2 M in THF), microwave, 120 °C, 4 h, then 150 °C, 4 h, 63%; (e) NaOEt (21% w/w in EtOH), microwave, 140 °C, 4 h, 14%.

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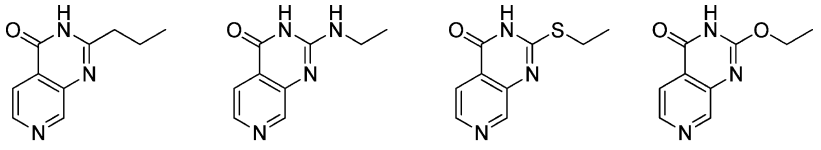
Table 4. KDM4C Biochemical and Cell Activity of 18−21[7]

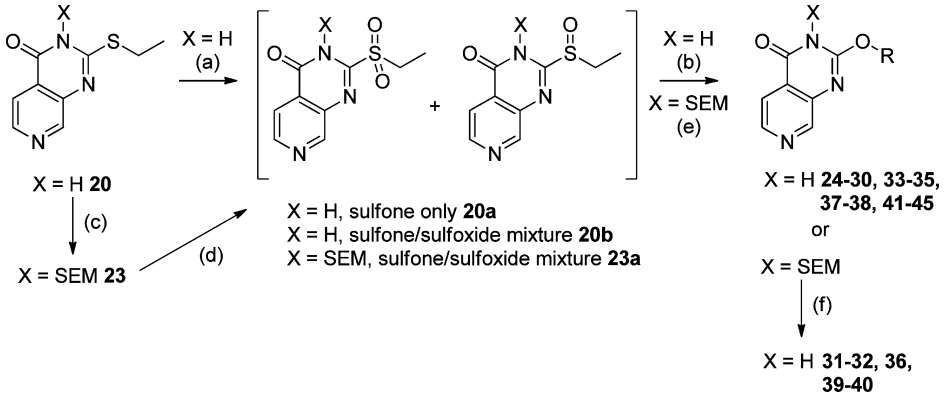


18 19 20 21

compd	KDM4C RFMS pIC_{50}	KDM4C cell pIC_{50}	pK_a
4	5.7	<4.0	8.3
18	4.9	nd[a]	8.8
19	5.5	nd[a]	8.6
20	6.1	<4.0	6.8
21	6.0	4.2[b]	7.5

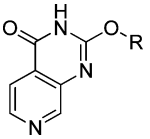
[a]nd = not determined. [b]4.2, n = 3; <4.0, n = 3.

Scheme 5. Synthesis of Aryloxy, Hetaryloxy, and Alkoxy Analogues 24−43[a]



[a]Conditions: (a) (i) *m*CPBA, NMP, rt, 3 h or (ii) mCPBA, THF, rt, overnight or (iii) 39% $MeCO_3H$ in AcOH, THF, 3 Å molecular sieves, rt, overnight; (b) ROH, NaH, DMF, 110 °C, overnight; (c) SEM-Cl, K_2CO_3, DMF, 50 °C, overnight; (d) *m*CPBA, THF, rt, 1 h; (e) ROH, $CsCO_3$, 1,4-dioxane, 60 °C, 3 h; (f) $MgBr_2.Et_2O$, $MeNO_2$, 95 °C, 1 h.

Table 5. KDM4C Biochemical and Cell Activity with Lipophilicity Parameters of Analogues 24-32[7]



compd	R	KDM4C RFMS pIC_{50}	KDM4C cell pIC_{50}	cLogP	$ChromLogD_{pH7.4}$
24	Ph	6.2	4.9	1.4	1.2
25	CH_2Ph	5.7	<4.0	1.6	2.8
26	*c*-hexyl	5.5	4.6	2.3	3.7
27	CH_2-*c*-hexyl	5.5[a]	<4.0	2.9	4.5
28	3-pyridyl	6.3	4.9	−0.1	−0.2
29	4-ClPh	6.3	5.0	2.1	2.0
30	(3-OH)Ph	6.7	5.0	0.8	0.3
31	(4-Cl-3-OH)Ph	6.4	5.4	1.4	1.1
32	(6-^{i}Pr)-3-pyridyl	6.0	5.4	1.4	1.5

[a]5.5, n = 2; <4, n = 1.

potency in the KDM4C RFMS assay compared to the phenyl compound **24**. Introduction of a nitrogen atom into the phenyl ring was well tolerated with the 3-pyridyl compound **28** possessing a similar profile to **24** in terms of both biochemical and cellular potency. Substitution of the phenyl ring also gave analogues of interest with the 4-chlorophenyl analogue **29** being equipotent with the phenyl compound **24**. Introduction of the 3-hydroxy substituent **30** gave a 3-fold increase in potency to pIC_{50} = 6.7. Combining these two substituents, however, did not result in additive SAR with compound **31** possessing intermediate KDM4C RFMS activity, pIC_{50} = 6.4, although this combination of substituents did result in a modest improvement in cellular activity with **31** giving pIC_{50} = 5.4 in the KDM4C cell imaging assay. A similar trend was noted for compound **32** whereby addition of a 6-isopropyl substituent to **28** gave a compound with cell activity pIC_{50} = 5.4.

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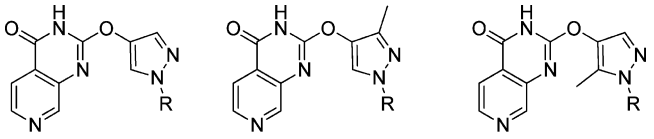
Table 6. KDM4C Biochemical and Cell Activity with Lipophilicity Parameters of Analogues 33−45[7]



33-43 44 45

compd	R	KDM4C RFMS pIC_{50}	KDM4C cell pIC_{50}	cLogP	$ChromLogD_{pH7.4}$
33	H	6.4	5.1	−0.5	−0.6
34	Me	6.4	5.1	−0.5	−0.3
35	^{i}Pr	6.3	5.3	0.4	0.7
36	*c*-pentyl	6.2[a]	5.7	1.0	1.7
37	*c*-Hex	6.4	5.4	1.6	2.2
38	*c*-Hep	6.3	5.6	2.1	2.9
39	CH_2(*c*-hexyl)	6.2	5.3	2.2	2.9
40	Ph	6.4	5.4	1.5	1.9
41	CH_2Ph	6.3	5.3	1.5	1.8
42	CH_2(3-OMe)Ph	6.2[b]	5.2	1.4	2.0
43	CH_2-3-Pyr	6.2	4.9	0.0	0.3
44	Me	5.6	<4.0	−0.2	0.0
45	Me	5.7	nd[c]	−0.2	−0.2

[a]6.2, n = 8; <4.0, n = 1. [b]6.2, n = 9; <4.0 n = 1;. [c]nd = not determined.

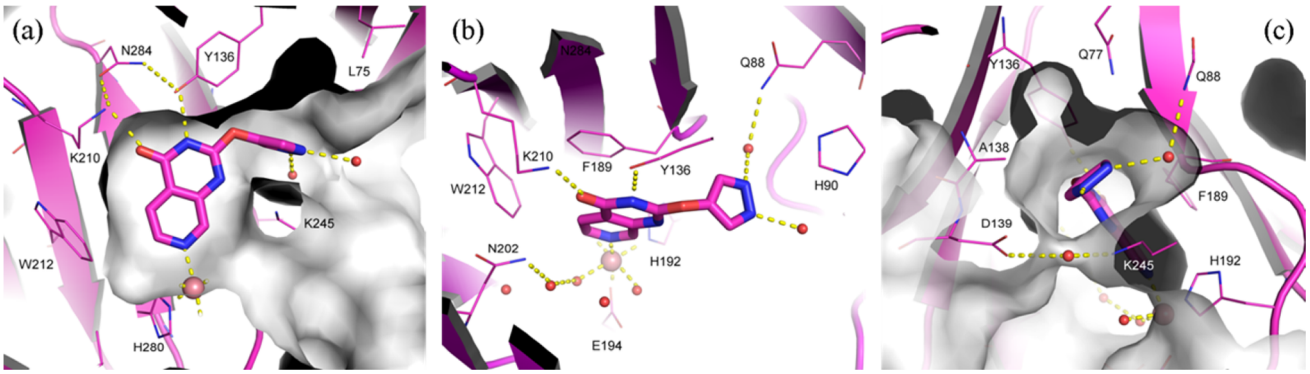


Figure 4. X-ray structure of **33** bound to KDM4D active site (PDB code 5FPB).

In a parallel investigation, a series of pyrazolyloxy derivatives of **4** was also prepared (Scheme 5), and the data from testing these compounds are shown in Table 6. Somewhat surprisingly the parent pyrazole **33** showed a relatively high level of cellular activity in the KDM4C cell imaging assay given its highly polar nature (pIC_{50} = 5.1, cLogP = −0.5, ChromLogD = −0.6). A crystal structure of **33** bound to KDM4D showed excellent shape complementarity with the protein (Figure 4a). One pyrazole nitrogen forms a water-bridged interaction to Q88 with limited potential for substitution. In contrast the other nitrogen provides a vector for growth out toward solvent and the opportunity to pick up additional interactions (Figure 4b). As expected, N-substitution of the pyrazole ring was well tolerated when this substitution gave the 4-pyrazolyl isomers; for example, Me **34**, *i*-Pr **35**, *c*-pentyl **36**, phenyl **40**, and benzyl **41** substitution gave compounds with biochemical potency in the range pIC_{50} = 6.2−6.4. The introduction of methyl groups into the 3- and 5- positions of the pyrazole ring (**44**, **45**) was not beneficial to activity due to the restricted size of the pocket in these regions as illustrated in Figure 4c. We observed that the effects of increasing lipophilicity on cellular potency were small; thus, by comparison of **36**, **37**, **38**, and **39**, there was a 16-fold increase in ChromLogD but KDM4C cell imaging activity remained broadly stable at pIC_{50} = 5.3−5.7 (IC_{50} = 2−5 μM). This suggests these compounds may be actively transported into cells and that increasing potency through modifications immediately adjacent to the 2-OG binding pocket may be challenging. These data also indicate a significant component of activity for this series resides in the fragment-like core that mimics the key interactions of 2-OG within the KDM4 enzymes.

Indeed, from examination of X-ray crystallography data of a number of examples, including the 3-pyridine analogue **28**, phenol analogue **30**, and the pyrazole **33**, we found heteroatoms strategically placed to form direct or water-mediated hydrogen bonding interactions with the protein were successful in making these contacts. However, at best, only modest potency gains were observed when compared with the core **4** and the phenoxy analogue **24**. Therefore, substitution into this region may produce sufficient entropic and enthalpic penalties to attenuate the positive effects of forming a range of positive interactions (H-bonding, van der Waals), giving rise to relatively flat SAR. Nevertheless, the cell activity demonstrated by a range of these compounds and their novelty as non-carboxylate KDM4 inhibitors led us to further profile key exemplars as shown in Table 7. Also included is the inactive,

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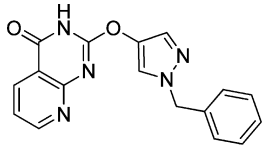
Table 7. Further Profiling of Cell Active Pyridopyrimidinone Inhibitors 31, 32, 36, 39, and 41 with "Negative Control" Analogue 46[7]



46

target	assay type	value	31	32	36	39	41	46
KDM4A	RFMS	pIC_{50}	6.4	5.8	6.1	6.2	6.2	<4.0
KDM4C	RFMS	pIC_{50}	6.4	6.0	6.2[a]	6.2	6.3	<4.0[b]
KDM4D	RFMS	pIC_{50}	6.5	6.2	6.3	6.4	6.4	<4.0
KDM4E	RFMS	pIC_{50}	6.9	6.4	6.4	6.3	6.3	<4.0
KDM6B	RFMS	pIC_{50}	<4.0	nd[e]	<4.0	<4.0	<4.0	<4.0
KDM5C	RFMS	pIC_{50}	6.7	6.9	6.9	7.1	7.2	4.5[c]
EGLN3	HTRF	pIC_{50}	<4.0	nd[e]	<4.0	<4.0	<4.0	<4.0
KDM4C	cell imaging	pIC_{50}	5.4	5.4	5.7	5.3	5.3	<4.0
KDM5C	cell imaging	pIC_{50}	4.5[d]	4.3	5.3	5.6	5.4	<4.0

[a]6.2, n = 8; <4.0, n = 1. [b]<4.0, n = 5; 4.4, n = 1. [c]4.5, n = 3; <4.0, n = 7. [d]4.5, n = 2; <4.0 n = 4. [e]nd = not determined.

"negative control" analogue **46** of compound **41** wherein the nitrogen atom has been translocated from the 7- to the 8-position of the core and is therefore no longer able to interact with the active site iron.

While these compounds are equipotent across the KDM4 family and have maintained high levels of selectivity vs KDM6B and the more distantly related prolyl hydroxylase EGLN3, we also found that like the 3-amino-4-pyridinecarboxylic acids described previously,[4] they are potent inhibitors of the H3K4 demethylase KDM5C (JARID1C). These molecules also show activity in the KDM5C cell imaging assay[4] but in general with a larger differential between biochemical and cellular potency values for this target than for KDM4C. Additionally, the six-membered aryl analogues **31** and **32** appear ~10-fold selective for KDM4C over KDM5C in the cell assay, as they showed a larger drop in potency from biochemical to cell assay for KDM5C than the pyrazolyloxy analogues **36**, **39**, and **41** which appear equipotent for both targets in this format. The reasons for this observation are not clear, as both assays utilize the same cell type, but as previously suggested in the accompanying article,[4] differences in affinity for the natural cofactor 2-OG and/or differences in expression levels of the enzymes in the cells could potentially play a role.

Compounds **31**, **32**, **36**, **39**, and **41** have been tested against our in-house panel of nonrelated drug and liability targets (Supporting Information Tables 2–6) and show a range of broadly selective profiles.

CONCLUSION

We have designed and synthesized a range of non-carboxylate inhibitors of the KDM4 (JMJD2) and KDM5 (JARID1) families of histone lysine demethylases based on pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **4**.[14] A number of exemplars possess interesting activity profiles in target-specific, cellular mechanistic assays against overexpressed KDM4C (compounds **31**, **32**, **36**, **39**, and **41**, IC_{50} = 2–5 μM) and against overexpressed KDM5C (compounds **36**, **39**, and **41**, IC_{50} = 2.5–5 μM). As described above, a limited set of biochemical assays was used to assess selectivity during this program of work with encouragingly low levels of activity observed vs KDM6B and EGLN3. To enable assessment of selectivity against the broader class of 2-OG dependent dioxygenases, including other KDM enzymes, a chemical proteomics approach has been developed in our laboratories, and the activity/selectivity profile determined for the 1-benzylpyrazolyloxy derivative **41** using this approach will be published in due course.[15]

Compound **41** and its analogues, for example, those shown in Table 7, may have utility in probing the effects of inhibition of KDM4 and KDM5 enzymes in a cellular phenotypic context when used in combination with an inactive control molecule such as **46**. Additionally, these compounds may also serve as a staging point in the design and synthesis of further nonacidic inhibitors of these two families or indeed other members of the broader 2-OG utilizing class of enzymes.

EXPERIMENTAL SECTION

Chemistry. All commercial chemicals and solvents are reagent grade and were used without further purification unless otherwise specified. Reactions were monitored by thin-layer chromatography on 0.2 mm silica gel plates (POLYGRAM SIL G/UV254, Macherey-Nagel) and were visualized with UV light. Compounds were typically purified by automated flash silica chromatography (Biotage SP4), manual chromatography on prepacked cartridges (SPE), or mass directed autopreparative chromatography (MDAP). Where specifically indicated the following MDAP methods were used. For the formic method, the HPLC analysis was conducted on a Sunfire C18 column (150 mm × 30 mm i.d., 5 μm packing diameter) at ambient temperature, eluting with 0.1% formic acid in water and 0.1% formic acid in acetonitrile using an elution gradient. The UV detection was an averaged signal from wavelength of 210–350 nm. The mass spectra were recorded on a Waters ZQ mass spectrometer using alternate-scan positive and negative electrospray. Ionization data were rounded to the nearest integer. For the high pH method, the HPLC analysis was conducted on a XBridge C18 column (100 mm × 30 mm i.d., 5 μm packing diameter) at ambient temperature, eluting with 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution, and acetonitrile using an elution gradient. The UV detection was an averaged signal from wavelength of 210–350 nm. The mass spectra were recorded on a Waters ZQ mass spectrometer using alternate-scan positive and negative electrospray. Ionization data were rounded to the nearest integer. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker DPX-400 spectrometer at 400 and 126 MHz, respectively, or a Bruker AV-600 spectrometer at 600 and 150 MHz, respectively. Chemical shifts are reported in parts per million (ppm, δ units). Splitting patterns are designated as s, singlet; d,

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doublet; t, triplet; q, quartet; m, multiplet; br, broad; etc. LCMS spectra were recorded on an Acquity UPLC BEH C18 column (50 mm × 2.1 mm i.d., 1.7 μm packing diameter) at 40 °C. The UV detection was a summed signal from wavelength of 210−350 nm. The mass spectra were recorded on a Waters ZQ mass spectrometer using alternate-scan positive and negative electrospray. Ionization data were rounded to the nearest integer. As specifically indicated, the compounds were eluted by one of the following LCMS methods. For the formic method, elution was with 0.1% v/v solution of formic acid in water (solvent A) and 0.1% v/v solution of formic acid in acetonitrile (solvent B) using the following elution gradient 0−1.5 min 3−100% B, 1.5−1.9 min 100% B, 1.9−2.1 min 3% B at a flow rate of 1 mL/min. For the high pH method, elution was with 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution (solvent A) and acetonitrile (solvent B) using the following elution gradient 0−1.5 min 1−97% B, 1.5−1.9 min 97% B, 1.9−2.1 min 100% B at a flow rate of 1 mL/min. For the TFA method, elution was with 0.1% v/v solution of trifluoroacetic acid in water (solvent A) and 0.1% v/v solution of trifluoroacetic acid in acetonitrile (solvent B) using the following elution gradient: 0−1.5 min 3−100% B, 1.5−1.9 min 100% B, 1.9−2.0 min 100−3% B at a flow rate of 1 mL/min. High resolution mass spectra (HRMS) were acquired as profile data using a Thermo Scientific LTQ Orbitrap mass spectrometer, equipped with an ESI interface, over a mass range of 120−1000 Da at a mass resolution of 100 000. A commercial calibration solution (Pierce LTQ ESI positive ion calibration solution) was used to externally calibrate the instrument prior to analysis. Ionization was achieved with a spray voltage of 4 kV, a capillary voltage of 45 V, tube lens voltage of 110 V, and sheath and auxiliary gas flows of 40 and 20 (arbitrary units), respectively. The capillary temperature was maintained at 300 °C. The elemental composition was calculated using Xcalibur (version 2.0.7) for the $[M + H]^+$ and the mass error quoted as ppm; an error of <5 ppm indicates that the measured mass is consistent with the proposed formula. Melting point analysis was carried out using a Stuart SMP40 melting point apparatus, and melting points are uncorrected. The purity of all compounds screened in the biological assays was found to be ≥95% by LCMS analysis unless otherwise specified.

Pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 4. Formamidine acetate (1.507 g, 14.48 mmol) was added in a single portion to a stirred suspension of 3-aminoisonicotinic acid (1 g, 7.24 mmol) in *N*,*N*-dimethylacetamide (8 mL) at rt under N_2. The resultant suspension was heated to 150 °C for 16 h. The resulting solution was then cooled to rt which resulted in a suspension forming. Water was added, followed by sat. $NaHCO_3$ (aq), and the resulting suspension was filtered. The collected solid was washed with sat. $NaHCO_3$ (aq), H_2O, MeOH, and Et_2O to give **4** as a pale brown solid (418 mg, 39%). LCMS (formic method) retention time 0.33 min, $[M + H]^+ = 148$. ^1H NMR (600 MHz, DMSO-d_6) δ ppm: 7.96 (d, J = 5.1 Hz, 1H), 8.23 (s, 1H), 8.67 (d, J = 5.1 Hz, 1H), 9.06 (s, 1H), 12.60 (br s, 1H).

2,6-Naphthyridin-1(2*H*)-one, 5. Sourced from DL chiral chemicals. LCMS (high pH method) retention time 0.44 min, $[M + H]^+ = 147$. ^1H NMR (600 MHz, DMSO-d_6) δ ppm: 6.67 (d, J = 7.3 Hz, 1H), 7.33 (d, J = 7.3 Hz, 1H), 7.97 (d, J = 5.5 Hz, 1H), 8.62 (d, J = 5.5 Hz, 1H), 9.06 (s, 1H).

Pyrido[3,4-*d*]pyridazin-1(2*H*)-one, 6. *Step 1.* Isonicotinic acid (5 g, 40.6 mmol) and 1,1′-carbonyldiimidazole (7.90 g, 48.7 mmol) were stirred in 2-methyl-THF (50 mL), and the mixture was heated to 50 °C for 30 min. Aniline (4.82 mL, 52.8 mmol) was added, and the mixture was stirred at 50 °C for 20 h. The mixture was cooled to rt, and a solid precipitated. The organic mixture was washed with water and brine and dried using $MgSO_4$. The aqueous washings were extracted with DCM, combined with the washed organic layer, and evaporated to give a brown solid. The solid was triturated with EtOAc and the resulting solid washed with EtOAc and dried via filtration to give *N*-phenylisonicotinamide **10** as a white crystalline solid (5.96 g, 70%). LCMS (formic method) retention time 0.55 min, $[M + H]^+ = 199$. ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.19−7.25 (m, 3H), 7.41 (t, J = 8.0 Hz, 2H), 7.66 (d, J = 8.1 Hz, 2H), 7.72 (dd, J = 4.6, 1.0 Hz, 2H), 7.93 (br s, 1H), 8.81 (dd, J = 4.8, 1.0 Hz, 1H).

Step 2. *N*-Phenylisonicotinamide **10** (1.14 g, 5.75 mmol) was dissolved in THF (36 mL) under N_2, and the mixture cooled to −70 °C. *n*-Butyllithium (7.91 mL, 12.65 mmol) was added cautiously keeping the reaction temperature below −60 °C. The reaction was stirred at −30 °C for 30 min and warmed to 0 °C for 5 min. The reaction was cooled to −70 °C and DMF (0.891 mL, 11.50 mmol) added cautiously. The reaction was allowed to warm to rt overnight, acidified to pH 2 with 2 M HCl(aq) and the organic layer separated. The aqueous layer was extracted with DCM, and the organic phases were dried and concentrated. The residue was purified by silica gel column chromatography, eluting with a gradient of 75−100% EtOAc/cyclohexane followed by 0−10% DCM/MeOH to give crude 3-hydroxy-2-phenylisoindolin-1-one **11** as a brown solid (84 mg, 21%). LCMS (TFA method) retention time 0.52 min, $[M + H]^+ = 227$.

Step 3. The crude 3-hydroxy-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-1-one **11** (84 mg) was stirred in 35% hydrazine in water (2.4 g, 26.2 mmol) and the mixture heated to reflux under N_2 for 2 h. The mixture was azeotroped to dryness with toluene and the residue purified by MDAP (high pH method) to give **6** as a pale brown solid (7.3 mg). LCMS (high pH method) retention time 0.39 min, 94% pure by area, $[M + H]^+ = 195$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 8.06 (d, J = 5.3 Hz, 1H), 8.53 (s, 1H), 8.98 (d, J = 5.3 Hz, 1H), 9.33 (d, J = 0.8 Hz, 1H), 13.00 (br s, 1H).

1*H*-Indazol-3(2*H*)-one, 7. *Step 1.* Concentrated HCl (22 mL, 264 mmol) was added to a stirred suspension of 3-aminoisonicotinic acid (2.8 g, 20.27 mmol) in water (20 mL) and the resulting suspension cooled in an ice bath. Sodium nitrite (1.6 g, 23.19 mmol) was added cautiously and the resulting solution stirred for 1 h. The solution was added dropwise to a solution of water while sparging with SO_2(g), and the resulting suspension was stirred for 1 h and allowed to stand overnight. The suspension was stirred for 5 min and filtered; the resulting filter cake was washed with water, water/MeOH (1:1) and dried in vacuo to give 3-hydrazinylisonicotinic acid dihydrochloride **12** as a yellow solid (3.02g, 56%). ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 8.02 (br s, 2H), 8.74 (s, 1H), 8.93 (br s, 1H).

Step 2. 3-Hydrazinylisonicotinic acid dihydrochloride **12** (1 g, 4.42 mmol) was added to a stirred solution of 2 M HCl (aq) (50 mL, 100 mmol) and the mixture heated to reflux for 5 h. The solution was evaporated in vacuo to a yellow solid. This solid was suspended in water (30 mL) and 50% NaOH (aq) added until the resulting suspension was adjusted to pH 7. The precipitate was recrystallized from the solution to give **7** as an orange solid (269 mg, 45%). LCMS (high pH method) retention time 0.19 min, $[M + H]^+ = 135$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 7.61 (dd, J = 5.5, 1.3 Hz, 1H), 8.10 (d, J = 5.4 Hz, 1H), 8.81 (d, J = 1.2 Hz, 1H), 10.87 (br s, 1H), 12.14 (br s, 1H).

1-Benzylpyrido[3,4-*d*]pyrimidin-4(1*H*)-one, 8. *Step 1.* 3-Fluoroisonicotinic acid (250 mg, 1.772 mmol) was suspended in benzylamine (0.5 mL, 4.57 mmol) and irradiated in a microwave at 150 °C for 2 h. The crude mixture was dissolved in MeOH and applied to an aminopropyl column. The column was washed with MeOH and then 10% 2 M HCl (aq) in MeOH. The appropriate fractions were combined and evaporated to give a yellow oil, 3-(benzylamino)isonicotinic acid (323 mg, 80% yield). LCMS (TFA method) retention time 0.56 min, $[M + H]^+ = 229$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 4.63 (s, 2H), 7.26−7.32 (m, 1H), 7.34−7.45 (m, 7 H), 7.47−7.52 (m, 2H), 7.96 (s, 2H), 8.23 (s, 1H), 8.34 (br s, 2H).

Step 2. 3-(Benzylamino)isonicotinic acid (76 mg, 0.333 mmol) was suspended in formamide (0.5 mL, 12.54 mmol) under N_2 and the mixture cooled to ∼0 °C in an ice bath. Phosphorus oxychloride (0.124 mL, 1.332 mmol) was added dropwise. The mixture was stirred for 30 min at 0 °C followed by a further 16 h at rt. The reaction mixture was concentrated, and the residue purified by MDAP (high pH method) to give **8** (12.5 mg, 15% yield) as a cream solid. LCMS (TFA method) retention time 0.59 min, $[M + H]^+ = 238$. ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 5.38 (s, 2H), 7.28−7.23 (m, 3H), 7.43−7.37 (m, 2H), 8.13 (d, J = 5.2 Hz, 1H), 8.41 (s, 1H), 8.71 (d, J = 5.2 Hz, 1H), 8.79 (s, 1H).

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1-(3-Phenylpropyl)pyrido[3,4-*d*]pyrimidin-4(1*H*)-one, 9. *Step 1.* A suspension of 3-fluoroisonicotinic acid (308 mg, 2.183 mmol) in 3-phenylpropan-1-amine (0.5 mL, 2.183 mmol) was heated by microwave irradiation in a sealed vial to 120 °C for 8 h. The reaction mixture was diluted with DCM and purified using silica gel column chromatography, eluting with a gradient of 0−10% MeOH/DCM to give 3-((3-phenylpropyl)amino)isonicotinic acid (510 mg, 91% yield) as a yellow oil. LCMS (TFA method) retention time 0.1 min, $[M + H]^+$ = 257.

Step 2. 3-((3-Phenylpropyl)amino)isonicotinic acid (153 mg, 0.597 mmol) was dissolved in formamide (2 mL, 50.2 mmol) and cooled to 0−5 °C under N_2. Phosphorus oxychloride (0.14 mL, 1.502 mmol) was added dropwise, and the mixture was stirred at 0−5 °C for 30 min, followed by a further 1 h at rt. A further portion of phosphorus oxychloride (0.17 mL, 1.824 mmol) was added and the mixture stirred at rt for 16 h. The reaction mixture was concentrated and purified by MDAP (high pH method) to give **9** (26.7 mg, 17% yield). LCMS (formic method) retention time 0.71 min, $[M + H]^+$ = 266. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.29 (dt, J = 7.3 Hz, 5.3 Hz, 2H), 2.79 (t, J = 7.3 Hz, 2H), 4.16 (t, J = 5.3 Hz, 2H), 7.38−7.16 (m, 5 H), 8.10 (d, J = 5.1 Hz, 1H), 8.15 (s, 1H), 8.72 (d, J = 5.1 Hz, 1H), 8.75 (s, 1H).

2-Phenylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 15. *Step 1.* Benzoyl chloride (0.227 mL, 1.956 mmol) was added dropwise to a stirred biphasic solution of ethyl 3-aminoisonicotinate (250 mg, 1.504 mmol) in DCM (5 mL) and sat. K_2CO_3 (aq) (5 mL) at rt. After stirring rapidly at rt for 1 h, H_2O and DCM were added. The separated aqueous phase was extracted with DCM, the combined organic phase was passed through a hydrophobic frit and evaporated under reduced pressure to give an orange oil. This oil was purified by silica gel column chromatography using a gradient of 0−30% EtOAc/cyclohexane to give ethyl 3-benzamidoisonicotinate (196 mg, 48%). LCMS (formic method) retention time 1.01 min, $[M + H]^+$ = 271. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.48 (t, J = 7.1 Hz, 3H), 4.50 (q, J = 7.1 Hz, 2H), 7.28 (s, 2H), 7.50 (t, J = 8.0 Hz, 1H), 7.53−7.67 (m, 4H), 7.88 (d, J = 5.1 Hz, 1H), 8.07 (dd, J = 7.8, 1.5 Hz, 2H), 8.12 (dd, J = 8.3, 1.5 Hz, 1H), 8.50 (d, J = 5.1 Hz, 1H), 10.27 (s, 1H), 11.67 (br s, 1H)

Step 2. 7 M NH_3 in methanol (10 mL, 70.0 mmol) was added in a single portion to a flask containing ethyl 3-benzamidoisonicotinate (196 mg, 0.725 mmol) at rt. The resultant solution was stirred at rt for 4 h, and then the solvent was evaporated under reduced pressure to give a white solid. The solid was dissolved in EtOH, and then NaOH (aq) (0.73 mL, 10 M in H_2O, 4.35 mmol) was added dropwise. The resultant solution was stirred at rt for 1 h and then allowed to stand at rt for 12 h. 2 M HCl (aq) (4 mL) was added, and the resultant solution was evaporated under reduced pressure to give a yellow solid. The solid was dissolved in EtOH and passed through an aminopropyl column (20 g) that had been prewashed with EtOH. The column was eluted with EtOH. The appropriate fractions were combined and evaporated under reduced pressure to give a white solid (100 mg). The solid was suspended in DMSO and filtered. The collected solid was washed with DMSO, MeOH, and finally Et_2O. The solid was then air-dried under vacuo to give **15** as a white solid (11 mg, 7%). LCMS (formic method) retention time 0.66 min, $[M + H]^+$ = 224. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.53−7.66 (m, 3H), 7.99 (dd, J = 5.3, 0.8 Hz, 1H), 8.20 (dd, J = 8.4, 1.5 Hz, 2H), 8.67 (d, J = 5.3 Hz, 1H), 9.13 (s, 1H), 12.85 (br s, 1H).

2-(Pyridin-3-yl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 16. *Step 1.* Nicotinoyl chloride hydrochloride (268 mg, 1.504 mmol) was added to a stirred suspension of ethyl-3-amino-4-pyridine carboxylate (250 mg, 1.505 mmol) in DCM (10 mL) at 0 °C under N_2. The solution was then stirred for 5 min after which the flask was removed from the cooling bath and stirring was continued at rt for 3 h. Saturated $NaHCO_3$ (aq) and DCM were added, and the separated aqueous phase was extracted with DCM. The combined organic phases were passed through a hydrophobic frit and evaporated under reduced pressure to give a brown oil (508 mg). The oil was purified by silica gel column chromatography, eluting with a gradient of 0−100% EtOAc/cyclohexane to give ethyl 3-(nicotinamido)isonicotinate as a light yellow solid (222 mg, 54%). LCMS (high pH method) retention time 0.79 min, $[M + H]^+$ = 272.

Step 2. Ammonium hydroxide (0.032 mL, 0.818 mmol) was added to a solution of ethyl 3-(nicotinamido)isonicotinate (222 mg, 0.818 mmol) in MeOH and stirred at rt for 1.5 h. An additional amount of ammonium hydroxide (1 mL) was added to the reaction mixture and stirred at rt for 20 h. The solvent was removed under reduced pressure after which the crude solid was dissolved in EtOH and stirred at 0 °C for 5 min. Then NaOH(aq) (0.50 mL, 10 M in H_2O, 4.09 mmol) was added dropwise and stirred at 0 °C for 5 min after which the flask was removed from the cooling bath and stirred at rt for 3 h. 2 M HCl (aq) was added dropwise, and the resultant solution was loaded onto an aminopropyl column. The column was eluted with MeOH, and the appropriate fractions were combined and evaporated under reduced pressure to give a white solid (201 mg). The crude solid was purified by MDAP (high pH method) to give a white solid (104 mg). The white solid was then dissolved in MeOH and loaded onto a SCX cartridge that had been prewashed with MeOH. The column was washed with MeOH and then 2 M NH_3 in MeOH. The appropriate fraction were combined and evaporated under reduced pressure to give **16** as a white solid (25 mg, 14%). LCMS (high pH method) retention time 0.43 min, $[M + H]^+$ = 225. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.59 (dd, J = 7.8, 4.8 Hz, 1H), 7.97 (d, J = 5.0 Hz, 1H), 8.51 (d, J = 7.8 Hz, 1H), 8.64 (d, J = 5.0 Hz, 1H), 8.76 (d, J = 3.8 Hz, 1H), 9.11 (s, 1H), 9.32 (s, 1H).

2-(5-Fluoropyridin-3-yl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 17. *Step 1.* Ethyl 3-aminoisonicotinate (300 mg, 1.805 mmol), 5-fluoronicotinic acid (289 mg, 1.986 mmol), and *N*-ethyl-*N*-isopropylpropan-2-amine (0.946 mL, 5.42 mmol) were dissolved in DCM (6 mL). 1-Propanephosphonic acid cyclic anhydride (1.505 mL, 2.53 mmol) was added, and the mixture was stirred at rt for 5 h. Further 5-fluoronicotinic acid (130 mg), *N*-ethyl-*N*-isopropylpropan-2-amine (0.315 mL), and 1-propanephosphonic acid cyclic anhydride (0.5 mL) were added, and the mixture was stirred for 16 h. The reaction was diluted with sat. $NaHCO_3$ (aq) and extracted with DCM. The organic phase was dried using a hydrophobic frit and concentrated in vacuo. The crude was purified using silica gel column chromatography, eluting with a gradient of 20−60% EtOAc/cyclohexane to give ethyl 3-(5-fluoronicotinamido)isonicotinate (343 mg, 66%) as a yellow solid. LCMS (formic method) retention time 0.64 min, $[M + H]^+$ = 290.

Step 2. Ethyl 3-(5-fluoronicotinamido)isonicotinate (343 mg, 0.711 mmol) was dissolved in 7 M NH_3 in MeOH (6 mL, 42.0 mmol) and allowed to stir at rt for 16 h. 10 M NaOH (0.711 mL, 7.11 mmol) was added and the mixture stirred for 24 h. The basic mixture was neutralized with 2 M HCl (aq) and filtered, washed with water followed by MeOH to give the **17** as a white solid (64 mg, 37%). LCMS (formic method) retention time 0.56 min, $[M + H]^+$ = 243. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.01 (dd, J = 5.3, 0.8 Hz, 1H), 8.42 (ddd, J = 9.8, 2.5, 1.8 Hz, 1H), 8.71 (d, J = 5.1 Hz, 1H), 8.82 (d, J = 2.8 Hz, 1H), 9.16 (s, 1H), 9.21 (t, J = 1.5 Hz, 1H), 13.06−13.10 (m, 1H).

2-Propylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 18. *Step 1.* To ethyl 3-aminoisonicotinate (200 mg, 1.204 mmol) in EtOAc (15 mL) was added butyric acid (0.221 mL, 2.407 mmol), *N*-ethyl-*N*-isopropylpropan-2-amine (0.841 mL, 4.81 mmol), and 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphinane 2,4,6-trioxide (2.87 mL, 4.81 mmol). The mixture was heated to 60 °C under N_2 for 72 h. The reaction mixture was partitioned between $NaHCO_3$ (aq) and EtOAc. The aqueous phase was washed with EtOAc, and the combined organic fractions were washed with water and evaporated to afford a dark brown oil. The crude oil was purified by silica gel column chromatography, eluting with a gradient of 0−80% EtOAc/cyclohexane to give ethyl 3-butyramidoisonicotinate (109 mg, 38%) as a yellow oil. LCMS (formic method) retention time 0.85 min, $[M + H]^+$ = 237.

Step 2. Ethyl 3-butyramidoisonicotinate (109 mg, 0.461 mmol) was taken up in 7 M NH_3 in MeOH (15 mL, 105 mmol) and stirred at 50 °C for 16 h. After this time the solvent was evaporated in vacuo to afford a green/gray crystalline solid. The crude product was recrystallized from MeOH and dried in vacuo to afford **18** (47 mg, 54%) as a green crystalline solid. LCMS (formic method) retention

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time 0.51 min, $[M + H]^+$ = 190. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 0.95 (t, J = 7.5 Hz, 3H), 1.72−1.82 (m, 2H), 2.62 (t, J = 7.6 Hz, 2H), 7.91 (d, J = 5.1 Hz, 1H), 8.61 (d, J = 5.1 Hz, 1H), 8.99 (s, 1H), 12.49 (br s, 1H).

2-Thioxo-2,3-dihydropyrido[3,4-*d*]pyrimidin-4(1*H*)-one, 22. 3-Aminoisonicotinic acid (5 g, 36.2 mmol) and thiourea (3.31 g, 43.4 mmol) were stirred at 160 °C for 16 h. The reaction was cooled to 100 °C and was treated with water and then cooled to rt. The resulting precipitate was removed by filtration and dried to give the **22** as a light brown solid (5.711 g, 88%). LCMS (formic method) retention time 0.38 min, $[M + H]^+$ = 180. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.78 (dd, J = 5.1, 0.7 Hz, 1H), 8.48 (d, J = 5.1 Hz, 1H), 8.72 (s, 1H), 12.71 (br s, 1H), 12.90 (br s, 1H).

2-(Ethylamino)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 19. *Step 1.* To a suspension of 2-thioxo-2,3-dihydropyrido[3,4-*d*]pyrimidin-4(1*H*)-one **22** (5.7 g, 31.8 mmol) in a mixture of 1 M NaOH (50 mL, 50.0 mmol) and MeOH (50 mL) was added methyl iodide (2.98 mL, 47.7 mmol) dropwise, and the reaction was stirred at rt under N_2 for 16 h. The reaction was adjusted to pH 7 by the addition of 1 M HCl (aq) and the MeOH removed under reduced pressure. The resulting precipitate was removed by filtration, washed with water, and then dried in a vacuum oven to give 2-(methylthio)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one as a white solid (3.91 g, 95%). LCMS (formic method) retention time 0.51 min, $[M + H]^+$ = 194. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.61 (s, 3H), 7.86 (d, J = 5.1 Hz, 1H), 8.57 (d, J = 5.1 Hz, 1H), 8.92 (s, 1H), 12.93 (br s, 1H).

Step 2. 2-(Methylthio)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (100 mg, 0.518 mmol) was placed in a microwave vial, and ethylamine (2 M solution in THF) (2 mL, 4.00 mmol) was added. The reaction was irradiated at 120 °C for 4 h followed by 150 °C for 4 h. The reaction was diluted with MeOH and eluted through an SCX column, eluting with MeOH and 2 M NH_3 in MeOH. The methanolic ammonia fraction was evaporated to give an orange solid. This solid was purified using silica gel column chromatography, eluting with a gradient of 0−5% DCM/MeOH to give **19** as a white solid (82 mg, 83%) . LCMS (formic method) retention time 0.37 min, $[M + H]^+$ = 191. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.16 (t, J = 7.1 Hz, 3H), 3.34−3.42 (m, 2H), 6.44 (br s, 1H), 7.68 (d, J = 5.1 Hz, 1H), 8.25 (d, J = 5.1 Hz, 1H), 8.64 (s, 1H), 11.17 (br s, 1H).

2-Ethoxypyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 21. 2-(Methylthio)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (100 mg, 0.518 mmol) (see compound **19**, step 1, for preparation) and NaOEt (1950 μL, 21% w/w in EtOH, 5.18 mmol) were placed in a microwaveable vial and irradiated at 140 °C for 4 h. The reaction was concentrated to an orange solid that was suspended in MeOH; the resulting precipitate was removed by filtration and the filtrate concentrated to give a crude solid. This solid was purified using MDAP (formic method). Appropriate fractions were combined and eluted through an NH_2 SPE column with MeOH. The MeOH was concentrated and dried to give **21** as a white solid (14 mg, 14%). LCMS (formic method) retention time 0.49 min, $[M + H]^+$ = 192. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.36 (t, J = 7.1 Hz, 3H), 4.47 (q, J = 7.1 Hz, 2H), 7.83 (d, J = 5.1 Hz, 1H), 8.47 (d, J = 5.1 Hz, 1H), 8.82 (s, 1H), 12.50−12.66 (m, 1H).

2-(Ethylthio)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 20. 2-Thioxo-2,3-dihydropyrido[3,4-*d*]pyrimidin-4(1*H*)-one **22** (1.355 g, 7.56 mmol) was taken up in MeOH (20 mL) and 1 M NaOH (20 mL, 20.00 mmol) and stirred at rt for 5 min. Ethyl iodide (0.733 mL, 9.07 mmol) was added dropwise, and the reaction was stirred at rt for 1 h. The reaction was concentrated to remove the MeOH, and the resulting yellow solution was adjusted to pH 6 with 2 M HCl (aq). A yellow precipitate formed which was removed by filtration and dried to give the **20** as a yellow solid (1.479 g, 94%). LCMS (formic method) retention time 0.62 min, $[M + H]^+$ = 208. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.36 (t, J = 7.5 Hz, 3H), 3.24 (q, J = 7.5 Hz, 2H), 7.87 (d, J = 5 Hz, 1H), 8.56 (d, J = 5 Hz, 1H), 8.91 (s, 1H), 12.80−12.92 (m, 1H).

Representative Procedure for the Oxidation of 20 to 2-(Ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 20a. A round-bottom flask was charged with 2-(ethylthio)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20** (2 g, 80% w/w, 7.72 mmol) in THF (100 mL), and *m*CPBA (77% in water) (4.33 g, 19.3 mmol) was added, and the slurry was stirred at rt for 3 h. The slurry was diluted with an equal volume of IPA (100 mL) and was concentrated under reduced pressure. The slurry was rediluted with IPA (100 mL) and was concentrated in vacuo again. The precipitated solid was isolated by filtration, washed with IPA (20 mL) followed by Et_2O (20 mL), and dried under suction to give **20a** as a white free-flowing powder (2.1 g, ∼80% w/w) . LCMS (formate method) retention time 0.43 min, $[M + H]^+$ = 240.

Representative Procedure for the Oxidation of 20 to a Mixture of 2-(Ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(Ethylsulfinyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 20b. 39% Peracetic acid in AcOH (2.054 mL, 12.06 mmol) was added to a stirred suspension of 2-(ethylthio)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20** (1g, 4.83 mmol) in THF (50 mL) over 3 Å molecular sieves (1 g). The resulting suspension was stirred for 24 h. A further portion of 39% peracetic acid in AcOH (2.054 mL, 12.06 mmol) was added and the mixture stirred for 16 h. The mixture was filtered and the resulting solid washed with toluene, methyl *tert*-butyl ether and dried under vacuo to give crude **20b** as an almost white solid (1.84 g, ∼40% w/w) which was used directly in the subsequent steps without further purification. The crude mixture contained a 2:1 ratio of 2-(ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(ethylsulfinyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one. LCMS (TFA method) retention time 0.40 min, $[M + H]^+$ = 224 (sulfoxide), and retention time 0.42 min, $[M + H]^+$ = 240 (sulfone).

Further Representative Procedure for the Oxidation of 20 to a Mixture of 2-(Ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(Ethylsulfinyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 20b. *m*CPBA (2 g, 11.59 mmol) was added to a stirred suspension of 2-(ethylthio)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20** (1g, 4.82 mmol) in THF (50 mL), and the mixture was stirred for 16 h. The resulting fine suspension was filtered, and the filtrate was stirred with solid sodium metabisulfite (2 g, 10.52 mmol) for 5 min, filtered, and evaporated to a brown slurry. This slurry was triturated with IPA, filtered, and washed with further IPA to give the crude **20b** as a pale yellow solid (674 mg, ∼85% w/w) which was used in subsequent reactions without further purification. The crude mixture contained a 4:1 ratio of 2-(ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(ethylsulfinyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one. LCMS (TFA method) retention time 0.41 min, $[M + H]^+$ = 224 (sulfoxide), and retention time 0.43 min, $[M + H]^+$ = 240 (sulfone).

2-Phenoxypyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 24. A reaction tube was charged with phenol (71 mg, 0.754 mmol), DMF (5 mL), and NaH (51 mg, 60% w/w in oil, 1.275 mmol). The reaction was stirred at rt for 3 h. After this time a mixture of 2-(ethylsulfonyl)-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(ethylsulfinyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one **20b** (150 mg, 0.477 mmol) was added. The reaction was stirred at 110 °C for 2 days. Further phenol (30 mg, 0.319 mmol) and NaH (51 mg, 60% w/w in oil, 1.275 mmol) were added, and the reaction was stirred at 110 °C for 16 h. The reaction was concentrated and slurried in hot DMSO before the precipitated solid was removed by filtration through a plug of cotton wool. The filtrate was purified by MDAP (formic method) to give **24** as a tan solid (13 mg, 11%). LCMS (formic method) retention time 0.66 min, $[M + H]^+$ = 240. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.16−7.24 (m, 3H), 7.41 (t, J = 8.0 Hz, 2H), 7.74 (d, J = 5.1 Hz, 1H), 8.29 (br s, 2H), 8.59 (s, 1H).

2-(Benzyloxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 25. Phenylmethanol (0.048 mL, 0.460 mmol) and NaH (20.0 mg, 60% w/w in oil, 0.500 mmol) were added to DMF (2.5 mL) at 0 °C and stirred for 5 min. A mixture of 2-(ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(ethylsulfinyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20b** (100 mg, 0.307 mmol) was added to DMF (2.5 mL), and this suspension was added to the reaction mixture. The reaction was heated to 110 °C for 1 h and then cooled to rt and stirred for 2.5 days. Further phenylmethanol (0.048 mL, 0.460 mmol) and NaH (16.7 mg, 60% w/w in oil) were added to DMF (1 mL), and this was added to the reaction mixture. The reaction was heated to 110 °C and stirred for 2 h. The reaction was quenched with water and extracted with EtOAc.

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The organic phase was washed with 10% LiCl (aq), brine and dried using a hydrophobic frit. The solvent was removed in vacuo and the resulting solid triturated with MeOH to give **25** (21 mg, 27%) as a white solid. LCMS (formic method) retention time 0.76 min, $[M + H]^+$ = 254. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 5.51 (s, 2H), 7.35−7.45 (m, 3H), 7.53 (d, J = 6.8 Hz, 2H), 7.86 (d, J = 5.1 Hz, 1H), 8.51 (d, J = 5.1 Hz, 1H), 8.88 (s, 1H).

2-(Cyclohexyloxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 26. Cyclohexanol (62.8 mg, 0.627 mmol) was taken up in NMP (2 mL) and treated with NaH (25.08 mg, 60% w/w in oil, 0.627 mmol). The reaction was then treated with a mixture of 2-(ethylsulfonyl)pyrido-[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(ethylsulfinyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one **20b** (50 mg, 0.166 mmol) and allowed to stand at rt for 16 h. The reaction was diluted with water and was extracted with EtOAc, and the organic layer was washed with brine, dried using a hydrophobic frit, and concentrated to an oil. This oil was purified using silica gel column chromatography, eluting with a gradient of 0−50% EtOAc/cyclohexane to give **26** (3 mg, 7%) as a white solid. LCMS (formic method) retention time 0.82 min, $[M + H]^+$ = 246. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.23−1.35 (m, 1H), 1.36−1.47 (m, 2H), 1.50−1.60 (m, 3H), 1.70−1.80 (m, 2H), 1.99 (m, J = 3.5 Hz, 2H), 5.13−5.20 (m, 1H), 7.83 (d, J = 5.1 Hz, 1H), 8.47 (d, J = 5.1 Hz, 1H), 8.82 (s, 1H), 12.50 (br s, 1H).

2-(Cyclohexylmethoxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 27. Cyclohexylmethanol (0.085 mL, 0.692 mmol) in anhydrous DMF (2 mL) was added to NaH (50.2 mg, 60% w/w in oil, 1.255 mmol) under N_2. After 10 min of stirring a mixture of 2-(ethylsulfonyl)-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(ethylsulfinyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one **20b** (150 mg, 0.570 mmol) in DMF (2 mL) was added and the reaction mixture heated at 110 °C under N_2 for 7 h. Further NaH (25 mg, 60% w/w in oil) was added and the reaction mixture heated at 110 °C under N_2 for 72 h. The reaction mixture was quenched with water. Sat. $NaHCO_3$(aq), 10% LiCl (aq), and brine were added. The product was extracted with EtOAc and passed through a hydrophobic frit and concentrated. The resulting crude was purified by silica gel column chromatography, eluting with a gradient of 0−75% EtOAc/cyclohexane to give **27** (8 mg, 5% yield) as a white powder. LCMS (formic method) retention time 0.94 min, $[M + H]^+$ = 260. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.00−1.12 (m, 2H), 1.14−1.32 (m, 3H), 1.62−1.69 (m, 1H), 1.69−1.84 (m, 5 H), 4.24 (d, J = 6.1 Hz, 2H), 7.83 (d, J = 4.8 Hz, 1H), 8.48 (d, J = 5.1 Hz, 1H), 8.83 (s, 1H).

2-(Pyridin-3-yloxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 28. Pyridin-3-ol (44 mg, 0.463 mmol) in DMF (1 mL) was added dropwise over 0.5 min to a stirred suspension of NaH (34 mg, 60% w/w in oil, 0.850 mmol) in DMF (2 mL) at rt under N_2. After 30 min, a mixture of 2-(ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(ethylsulfinyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20b** (100 mg, 0.380 mmol) in DMF (1 mL) was added. The reaction was heated to 110 °C for 18 h under N_2 then cooled to rt. Water, sat. $NaHCO_3$ (aq), 10% LiCl (aq), brine (aq), and EtOAc were added to the reaction mixture, and the phases were separated. The aqueous portion was extracted with EtOAc and then adjusted to pH 7 and further extracted with EtOAc. All of the organic phases were combined, passed through a hydrophobic frit, and the solvent was evaporated in vacuo to give a cream solid. The solid was purified by silica gel column chromatography, eluting with a gradient of 0−10% MeOH/DCM to give **28** (30 mg, 29%) as a white solid. LCMS (formic method) retention time 0.46 min, $[M + H]^+$ = 241. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.57 (dd, J = 8.3, 4.8 Hz, 1H), 7.86−7.92 (m, 2H), 8.53−8.58 (m, 2H), 8.65 (d, J = 2.8 Hz, 1H), 8.70 (s, 1H), 13.26 (br s, 1H).

2-(4-Chlorophenoxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 29. 2-(Ethylthio)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20** (200 mg, 0.965 mmol) was added to THF (10 mL), and *m*CPBA (77% in water) (416 mg, 2.413 mmol) was added with continuous stirring. The reaction was stirred at rt under N_2 for 4 h. Further *m*CPBA (77% in water) (0.965 mmol) was added, and the reaction was stirred for 1.5 h. The resultant slurry was filtered using a hydrophobic frit and washed with THF. The filtrate was diluted with an equal volume of IPA and concentrated to a slurry in vacuo. The slurry was diluted with IPA and concentrated in vacuo to a minimum volume. The precipitated solid was isolated by filtration, washed with IPA followed by Et_2O, and dried under vacuo to give the crude sulfone/sulfoxide intermediate **20b** as a white powder. The 4-chlorophenol (0.095 mL, 0.965 mmol) was taken up in DMF (5 mL), and NaH (77 mg, 60% w/w in oil, 1.930 mmol) was added with continuous stirring at rt. The reaction was left stirring for 5 min, and then the intermediate white powder **20b** was added. The reaction was heated to 110 °C and stirred for 16 h. The reaction was allowed to cool to rt, and MeOH was added. The solvent was removed in vacuo and the crude product was purified by silica gel column chromatography, eluting with a gradient of 0−100% EtOAc/cyclohexane followed by 0−5% MeOH/DCM to give **29** (17 mg, 6%) as a white solid. LCMS (formic method) retention time 0.80 min, $[M + H]^+$ = 274/276. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.41 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 7.89 (d, J = 5.1 Hz, 1H), 8.53 (d, J = 5.1 Hz, 1H), 8.70 (s, 1H), 13.15 (br s, 1H).

2-(3-Hydroxyphenoxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 30. To NaH (80 mg, 60% w/w in oil, 1.998 mmol) at rt under N_2 was added resorcinol (55 mg, 0.499 mmol) in DMF (4 mL). After 20 min, 2-(ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20a** (520 mg, 0.999 mmol) was added, and the reaction was heated to 110 °C for 16 h. The reaction was quenched with water and the solvent was evaporated in vacuo affording a red/black solid. This was taken up in MeOH and filtered. The filtrate was purified by silica gel column chromatography, eluting with a gradient of 0−20% MeOH/DCM to give crude **30**. The crude was purified by MDAP (formic method) to give **30** (8 mg, 6%) as a pink solid. LCMS (formic method) retention time 0.55 min, $[M + H]^+$ = 256. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 6.69−6.76 (m, 3H), 7.25 (t, J = 8.0 Hz, 1H), 7.87 (d, J = 5.1 Hz, 1H), 8.50 (d, J = 5.1 Hz, 1H), 8.70 (s, 1H), 9.71−9.84 (m, 1H).

2-((1*H*-Pyrazol-4-yl)oxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 33. *Step 1.* Tosic acid monohydrate (0.294 g, 1.546 mmol) and 3,4-dihydro-2*H*-pyran (2.82 mL, 30.9 mmol) were added to a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (3 g, 15.46 mmol), and the resulting suspension was heated to 75 °C with a drying tube attached for 3 h. The resulting solution was cooled, washed with sat. $NaHCO_3$ (aq) and brine, then dried ($MgSO_4$) and evaporated in vacuo to a pale brown oil. The residue was purified using silica gel column chromatography, eluting with a gradient of 0−33% EtOAc/cyclohexane to give crude 1-(tetrahydro-2*H*-pyran-2-yl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (2.85 g) as a colorless oil. LCMS (high pH method) retention time1.03 min, $[M + H]^+$ = 279.

Step 2. A premixed solution of 2 M NaOH (5.98 mL, 11.96 mmol) and 30% H_2O_2 (aq) (1.222 mL, 11.96 mmol) was added to a solution of crude 1-(tetrahydro-2*H*-pyran-2-yl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (2.85g) from step 1, in THF (50 mL). The resulting suspension was stirred for 2 h. The reaction mixture was adjusted to pH 9 with 2 M HCl (aq) and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated to a colorless oil. This oil was purified by silica gel column chromatography, eluting with a gradient of 25−75% EtOAc/cyclohexane to give 1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-ol (1.12g, 90%) as a white solid. LCMS (high pH method) retention time 0.51 min, $[M + H]^+$ = 169.

Step 3. NaH (182 mg, 60% w/w in oil, 4.55 mmol) was added cautiously to a solution of 1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-ol (249 mg, 1.480 mmol) in DMF (5 mL) under N_2, and the resulting suspension was stirred for 5 min. A mixture of 2-(ethylsulfonyl)-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(ethylsulfinyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one **20b** (300 mg, 1.137 mmol) was added, and the resulting suspension was heated to 110 °C for 1.5 h, cooled to rt, acidified with 1 M HCl (5 mL), and neutralized to pH 7 with sat. aqueous $NaHCO_3$. The resulting suspension was evaporated in vacuo to dryness and purified by silica gel column chromatography, eluting with a gradient of 0−10% DCM/MeOH to give 2-((1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (93 mg, 23%) as an off white solid. LCMS (high pH method) retention time 0.53 min, $[M + H]^+$ = 314.

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Step 4. 2 M aq HCl (0.064 mL, 0.128 mmol) was added to a stirred suspension of 2-((1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-pyrazol-4-yl)oxy)-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (40 mg, 0.128 mmol) in MeOH (2 mL). The suspension was stirred for 16 h and the resulting solution concentrated in vacuo. The residue was dry-loaded on to silica column and then eluted with a gradient of 0−20% DCM/MeOH to a give **33** (16 mg, 49%) as a white solid. LCMS (TFA method) retention time 0.36 min, $[M + H]^+$ = 230. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.66 (br s, 1H), 7.88 (d, J = 5.1 Hz, 1H), 8.03 (br s, 1H), 8.54 (d, J = 5.1 Hz, 1H), 8.83 (s, 1H), 13.06−13.09 (m, 1H).

2-((1-Methyl-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 34. *Step 1.* 1-Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1.01g, 4.89 mmol) was dissolved in THF (10 mL). 2 M NaOH (aq) (4.89 mL, 9.79 mmol) and 30% H_2O_2 (aq) (1 mL, 9.79 mmol) were added, and the reaction was stirred for 2 h at rt. The reaction was adjusted to pH 2 with 2 M HCl (aq) and then partitioned between water and DCM. The aqueous layer was extracted with DCM and the combined organics were washed with sat. $Na_2S_2O_5$ (aq) and concentrated in vacuo to give a crude residue. This residue was purified by silica gel column chromatography, eluting with a gradient of 0−70% EtOAc/cyclohexane to give 1-methyl-1*H*-pyrazol-4-ol (48 mg, 0.489 mmol) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.31 (br s, 3*H*) 6.94 (s, 1H), 7.13 (s, 1H), 8.28 (br s, 1H).

Step 2. 1-Methyl-1*H*-pyrazol-4-ol (48 mg, 0.489 mmol) and NaH (33.4 mg, 60% w/w in oil, 0.835 mmol) were added to DMF (4 mL) and stirred at rt for 5 min. 2-(Ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20a** (100 mg, 0.284 mmol) was taken up in DMF (1 mL) and added to the reaction mixture which was heated to 110 °C under N_2 for 4 h. 1-Methyl-1*H*-pyrazol-4-ol (20 mg, 0.201 mmol) and NaH (16.7 mg, 60% w/w in oil, 0.416 mmol) were added, and the reaction was stirred at 110 °C under N_2 for 16 h. The reaction was quenched with water and extracted into EtOAc. The aqueous layer was adjusted to pH 4 with 2 M HCl (aq) and extracted with EtOAc. The combined organics were washed with 10% LiCl (aq) and brine and dried using a hydrophobic frit. The solvent was removed in vacuo to give a solid which was purified by silica gel column chromatography, eluting with a gradient of 0−10% MeOH/DCM to yield crude product. This crude product was further purified by MDAP (formic method) to give a white solid. This solid was taken up in a minimum amount of MeOH and DMSO and eluted through an NH_2 SPE column with 2 M NH_3 in MeOH. All fractions were collected and the solvent was removed in vacuo to give **34** as a white solid. (11 mg, 16%). LCMS (formic method) retention time 0.45 min, $[M + H]^+$ = 244. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.86 (s, 3H), 7.58 (s, 1H), 7.88 (d, J = 5.3 Hz, 1H), 8.03 (s, 1H), 8.55 (d, J = 5.1 Hz, 1H), 8.84 (s, 1H), 13.05 (br s, 1H).

2-((1-Isopropyl-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 35. *Step 1.* 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1.5 g, 7.73 mmol) and Cs_2CO_3 (3.78 g, 11.6 mmol) were suspended in MeCN (15 mL) and stirred at rt for 10 min. 2-Bromopropane (1.097 mL, 11.6 mmol) was added and the reaction was stirred at 60 °C for 16 h. Further 2-bromopropane (0.6 mL, 6.4 mmol) was added and the reaction stirred at 60 °C for 16 h. The reaction was cooled and triturated with Et_2O to give 1-isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1.31g, 62%) as a brown oil. LCMS (formic method) retention time 0.98 min, $[M + H]^+$ = 237.

Step 2. A mixture of 1-isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1 g, 4.24 mmol), 30% H_2O_2 (aq) (0.865 mL, 8.47 mmol), and 2 M NaOH (aq) (4.24 mL, 8.47 mmol) in THF (5 mL) was stirred at rt for 5 h. The reaction was adjusted to pH 2 with 2 M HCl (aq) and extracted with DCM and EtOAc. The combined organics were washed with 10% $Na_2S_2O_5$ (aq) and dried using a hydrophobic frit. The solvent was removed in vacuo to give 1-isopropyl-1*H*-pyrazol-4-ol (850 mg, 159%) as an orange oil. LCMS (formic method) retention time 0.43 min, $[M + H]^+$ = 127.

Step 3. 1-Isopropyl-1*H*-pyrazol-4-ol (116 mg, 0.919 mmol) and NaH (33.4 mg, 60% w/w in oil, 0.835 mmol) were added to DMF (5 mL) at rt and stirred for 5 min. A mixture of 2-(ethylsulfonyl)pyrido-[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(ethylsulfinyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one **20b** (200 mg, 0.342 mmol) was added and the reaction was stirred for 16 h under N_2. Further NaH (16.7 mg, 60% w/w in oil, 0.417 mmol) was added, and the reaction was stirred for a further 4 h. The reaction was cooled to rt, and MeOH was added. The solvent was removed in vacuo and the crude solid was purified by silica gel column chromatography, eluting with a gradient of 0−100% EtOAc/cyclohexane to give **35** (22 mg, 24%) as a white solid. LCMS (formic method) retention time 0.60 min, $[M + H]^+$ = 272. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.45 (d, J = 6.6 Hz, 6H), 4.50 (dt, J = 13.3, 6.6 Hz, 1H), 7.61 (s, 1H), 7.88 (d, J = 5.1 Hz, 1H), 8.08 (s, 1H), 8.54 (d, J = 5.3 Hz, 1H), 8.85 (s, 1H), 13.06 (br s, 1H).

2-((1-Cycloheptyl-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one, 38. *Step 1.* To 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1 g, 5.15 mmol) and cycloheptanol (0.626 mL, 5.15 mmol) in THF (10 mL) were added triphenylphosphine (2.70 g, 10.31 mmol) and (*E*)-di-*tert*-butyldiazene-1,2-dicarboxylate (1.187 g, 5.15 mmol). The reaction mixture was stirred under N_2 at rt for 48 h. The reaction mixture was concentrated under reduced pressure to give a yellow gum which was purified by silica gel column chromatography, eluting with a gradient of 0−100% EtOAc/cyclohexane to give a mixture of 1-cycloheptyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole and (1-cycloheptyl-1*H*-pyrazol-4-yl)boronate (ratio 5:1) (717 mg, 28%) as a white solid. LCMS (formic method) retention time 0.74 min, $[M + H]^+$ = 209, retention time 1.26 min, $[M + H]^+$ = 291.

Step 2. To a solution of the mixture of 1-cycloheptyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole/(1-cycloheptyl-1*H*-pyrazol-4-yl)boronate from step 1 (717 mg, 1.007 mmol) in THF (10 mL) was added 2 M NaOH (aq) (1.511 mL, 3.02 mmol) and 30% H_2O_2 (aq) (0.206 mL, 2.015 mmol). The solution was stirred at rt for 2 h. The reaction was treated with sat. Na_2SO_3 (aq) and was then acidified to pH 2 with 2 M HCl (aq). The reaction was extracted with EtOAc. The organic phase was dried over $MgSO_4$ and concentrated to give a crude residue. This residue was purified by silica gel column chromatography, eluting with a gradient of 0−100% EtOAc/cyclohexane to give 1-cycloheptyl-1*H*-pyrazol-4-ol (422 mg) as a colorless gum that was used in the next step without further purification. LCMS (formic method) retention time 0.77 min, $[M + H]^+$ = 181.

Step 3. To a suspension of 1-cycloheptyl-1*H*-pyrazol-4-ol (69 mg, 0.306 mmol) in DMF (10 mL) was added NaH (61.2 mg, 60% w/w in oil, 1.531 mmol). The slurry was stirred for 15 min at rt under N_2. Then 2-(ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20a** (95 mg, 0.337 mmol) was added, and the reaction mixture was stirred for 2 days under N_2 at 110 °C. The reaction was quenched by adding MeOH, and the resultant solution was concentrated in vacuo to give a black solid. This solid was purified by reverse phase column chromatography, eluting with 0−50% MeCN/H_2O to give crude desired product. This crude product was further purified using a MDAP (high pH method) to give **38** (14 mg, 14%) as a light brown solid. LCMS (formic method) retention time 0.87 min, $[M + H]^+$ = 326. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.47−1.57 (m, 2H), 1.57−1.67 (m, 3H), 1.70−1.80 (m, 2H), 1.89−1.99 (m, 2H), 2.00−2.08 (m, 2H), 4.34 (tt, J = 9.5, 4.7 Hz, 1H), 7.58 (s, 1H), 7.87 (d, J = 5.1 Hz, 1H), 8.06 (s, 1H), 8.52 (d, J = 5.1 Hz, 1H), 8.83 (s, 1H).

2-((1-Cyclohexyl-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one, 37. *Step 1.* 1-Cyclohexyl-1*H*-pyrazol-4-ol was prepared using a similar method to that described for 1-cycloheptyl-1*H*-pyrazol-4-ol described in the preparation of compound **38**. LCMS (formic method) retention time 0.69 min, $[M + H]^+$ = 167.

Step 2. 2-(Ethylthio)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20** (387 mg, 1.868 mmol) was taken up in THF (10 mL). *m*CPBA (77% in water) (1172 mg, 5.23 mmol) was added, and the mixture was stirred at rt for 4 h by which time LCMS showed conversion of **20** to the sulfone intermediate **20a**. The solvent was evaporated in vacuo, and the residue was triturated with IPA and filtered. The collected solid was washed with IPA and Et_2O and air-dried affording a pale yellow powder. To this powder was added 1-cyclohexyl-1*H*-pyrazol-4-ol (207 mg, 0.747 mmol) and NaH (59.8 mg, 60% w/w in oil, 1.494 mmol) in

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DMF (4 mL). The mixture was heated to 110 °C under N_2 for 16 h. The reaction was poured into 10% LiCl (aq) and extracted with EtOAc. The organic fractions were dried using a hydrophobic frit and concentrated to a brown oil. This oil was purified by MDAP (formic method) to give **37** (10 mg, 4%) as a off-white solid. LCMS (formic method) retention time 0.79 min, $[M + H]^+$ = 312. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.22 (qt, J = 12.9, 3.3 Hz, 1H), 1.41 (qt, J = 12.9, 3.3 Hz, 2H), 1.63−1.71 (m, 2H), 1.75 (dd, J = 12.5, 3.3 Hz, 1H), 1.83 (dt, J = 12.9, 3.3 Hz, 2H), 2.04 (dd, J = 12.6, 2.6 Hz, 2H), 4.12 (tt, J = 11.6, 3.8 Hz, 1H), 7.59 (s, 1H), 7.86 (dd, J = 5.1, 0.7 Hz, 1H), 8.06 (s, 1H), 8.51 (d, J = 5.1 Hz, 1H), 8.83 (s, 1H).

2-((1-Benzyl-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 41. *Step 1.* 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (500 mg, 2.58 mmol) was taken up in MeOH (5 mL) and 1 M NaOH (aq) (5.15 mL, 5.15 mmol). The reaction mixture was stirred for 5 min at rt. Then (bromomethyl)benzene (0.368 mL, 3.09 mmol) was added and the reaction stirred at rt for 16 h. Further 1 M NaOH (aq) (2.58 mL, 2.58 mmol) and (bromomethyl)benzene (0.307 mL, 2.58 mmol) were added, and the reaction was stirred at rt for 2.5 days. The reaction was adjusted to pH 5 with 2 M HCl (aq) and then extracted with EtOAc. The solvent was removed in vacuo and the crude residue was purified by silica gel column chromatography, eluting with a gradient of 0−25% EtOAc/cyclohexane to give 1-benzyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (170 mg, 23%) as a white solid. LCMS (formic method) retention time 1.09 min, $[M + H]^+$ = 285.

Step 2. 1-Benzyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (170 mg, 0.598 mmol) was dissolved in THF (5 mL) and cooled to 0 °C. 2 M NaOH (aq) (0.598 mL, 1.197 mmol) and 30% H_2O_2 (aq) (0.122 mL, 1.197 mmol) were added, and the reaction was stirred at rt for 40 min. The reaction was adjusted to pH 2 with 2 N HCl (aq) and then partitioned with water and DCM. The organic phase was dried using a hydrophobic frit and concentrated to give a crude residue. This residue was purified using silica gel column chromatography, eluting with a gradient of 0−50% EtOAc/cyclohexane to give 1-benzyl-1*H*-pyrazol-4-ol as a white solid (80 mg, 77%). LCMS (formic method) retention time 0.63 min, $[M + H]^+$ = 175.

Step 3. 1-Benzyl-1*H*-pyrazol-4-ol (80 mg, 0.459 mmol) and NaH (33.4 mg, 60% w/w in oil, 0.835 mmol) were added to DMF (4 mL) and stirred at rt for 5 min. 2-(Ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20a** (100 mg, 0.305 mmol) dissolved in DMF (1 mL) was added to the reaction mixture which was heated to 110 °C and stirred for 5 h. Further NaH (17 mg, 60% w/w in oil, 0.417 mmol) was added and the reaction stirred for a further 18 h. The reaction was quenched with water and extracted into EtOAc. The organic phase was washed with 10% LiCl (aq) and then washed with brine and concentrated to give a yellow solid. This solid was triturated in MeOH to give **41** (33 mg, 34%) as a white solid. LCMS (formic method) retention time 0.74 min, $[M + H]^+$ = 320. HRMS: $C_{17}H_{14}N_5O_2$ requires $[M + H]^+$ 320.1142, found 320.1142 (error −0.1 ppm). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 5.34 (s, 2H), 7.27−7.34 (m, 3H), 7.35−7.41 (m, 2H), 7.65 (s, 1H), 7.88 (d, J = 5.1 Hz, 1H), 8.19 (s, 1H), 8.54 (d, J = 4.8 Hz, 1H), 8.82 (s, 1H), 13.1 (br s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 162.7, 154.5, 149.4, 144.9, 143.3, 137.8, 135.6, 131.4, 129.0, 128.2, 128.1, 125.3, 122.3, 118.9, 56.0. Mp 214−216 °C.

2-((1-(3-Methoxybenzyl)-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one, 42. *Step 1.* To a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1 g, 5.15 mmol) in MeCN (10 mL) were added Cs_2CO_3 (2.52 g, 7.73 mmol) and 1-(bromomethyl)-3-methoxybenzene (0.794 mL, 5.41 mmol). The reaction mixture was stirred for 24 h at 80 °C. The resultant slurry was concentrated and partitioned between water and EtOAc. The organic phase was concentrated to give crude residue which was purified using silica gel column chromatography, eluting with a gradient of 0−100% EtOAc/cyclohexane to give a three-component mixture of (1-(3-methoxybenzyl)-1*H*-pyrazol-4-yl)boronic acid with 1-(3-methoxybenzyl)-1*H*-pyrazole and 1-(3-methoxybenzyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (9:1:4) (1.04g, 27%) as a colorless solid. LCMS (formic method) retention time 1.09 min, $[M^+ H]^+$ = 314, retention time 0.63 min, $[M + H]^+$ = 232, retention time 0.83 min, $[M + H]^+$ = 188.

Step 2. To the mixture of (1-(3-methoxybenzyl)-1*H*-pyrazol-4-yl)boronic acid, 1-(3-methoxybenzyl)-1*H*-pyrazole, and 1-(3-methoxybenzyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (9:1:4) from step 1 (1.044 g, 3.32 mmol) in THF (10 mL) were added 2 M NaOH (aq) (4.98 mL, 9.97 mmol) and 30% H_2O_2 (aq) (0.679 mL, 6.65 mmol). The reaction mixture was stirred for 2 h at rt and was quenched by adding sat. Na_2SO_3 (aq) and adjusted to pH 4 with 2 M HCl (aq). The resultant solution was concentrated and partitioned between water and EtOAc. The organics were dried over Na_2SO_4 and concentrated to give crude residue. This residue was purified by silica gel column chromatography, eluting with a gradient of 0−100% EtOAc/cyclohexane to give 1-(3-methoxybenzyl)-1*H*-pyrazol-4-ol (390 mg, 58%) as a white solid. LCMS (formic method) retention time 0.66 min, $[M + H]^+$ = 205.

Step 3. To a solution of 1-(3-methoxybenzyl)-1*H*-pyrazol-4-ol (93 mg, 0.455 mmol) in DMF (15 mL) was cautiously added NaH (48 mg, 60% w/w in oil, 1.200 mmol). The mixture was stirred at rt for 10 min. Then 2-(ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20a** (143 mg, 84% w/w, 0.501 mmol) was added and the reaction stirred at 110 °C for 16 h. Further 2-(ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20a** (74 mg, 84% w/w, 0.455 mmol) was added and the mixture heated at 110 °C for a further 16 h and then quenched with MeOH and concentrated. The resulting residue was partitioned with EtOAc and water. The organic layer was dried using a hydrophobic frit and concentrated to give crude product. This was purified by MDAP (formic method) to give **42** (38 mg, 24%) as a white solid. LCMS (formic method) retention time 0.77 min, $[M + H]^+$ = 350. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.75 (s, 3H), 5.31 (s, 2H), 6.83−6.86 (m, 2H), 6.87−6.91 (m, 1H), 7.29 (t, J = 8.0 Hz, 1H), 7.65 (d, J = 0.9 Hz, 1H), 7.88 (dd, J = 5.1, 0.9 Hz, 1H), 8.19 (s, 1H), 8.54 (d, J = 5.1 Hz, 1H), 8.82 (s, 1H), 13.09 (br s, 1H).

2-((1-(Pyridin-3-ylmethyl)-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 43. *Step 1.* 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1 g, 5.15 mmol) was dissolved in MeCN (20 mL) and was treated with Cs_2CO_3 (5.04 g, 15.46 mmol) followed by 3-(bromomethyl)pyridine hydrobromide (2.61 g, 10.31 mmol). The mixture was stirred at rt for 16 h. The insoluble materials were removed by filtration and the filtrate was concentrated in vacuo to give an orange oil. The oil was purified by silica gel column chromatography using a gradient of 0−100% EtOAc/cyclohexane to give 3-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-1-yl)methyl)pyridine (316 mg, 22% yield) as a colorless oil. LCMS (formic method) retention time 0.65 min, $[M + H]^+$ = 286.

Step 2. 3-((4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-1-yl)methyl)pyridine (316 mg, 0.831 mmol) was dissolved in THF (10 mL) and was treated with 2 M NaOH (aq) (1.7 mL, 3.40 mmol) and 30% H_2O_2 (aq) (0.170 mL, 1.662 mmol) and the mixture stirred at rt for 1 h. The reaction was neutralized with 2 M HCl (aq), and the sample was loaded onto an SCX column and eluted with MeOH then 2 M NH_3 in MeOH. The appropriate fractions were combined and evaporated to give 1-(pyridin-3-ylmethyl)-1*H*-pyrazol-4-ol (178 mg, 122% yield) as a colorless oil which solidified. LCMS (high pH method) retention time 0.42 min, $[M + H]^+$ = 176.

Step 3. A round-bottom flask was charged with 2-(ethylthio)pyrido-[3,4-*d*]pyrimidin-4(3*H*)-one **20** (210 mg, 1.013 mmol), THF (10 mL), and *m*CPBA (77% in water) (568 mg, 2.53 mmol). The slurry was stirred at rt for 4 h. IPA (10 mL) was added, and the mixture was concentrated to approximately 7 mL. Further IPA (10 mL) was added, and the solution was again concentrated in vacuo to approximately 7 mL. The resultant slurry was filtered; the residue was washed with Et_2O and dried under vacuo to give sulfone/sulfoxide intermediate **20b**. A round-bottom flask was charged with 1-(pyridin-3-ylmethyl)-1*H*-pyrazol-4-ol (178 mg, 1.013 mmol), DMF (10 mL), and NaH (81 mg, 60% w/w in oil, 2.027 mmol). The mixture was stirred at rt for 20 min prior to the addition of the above prepared intermediate **20b**. The reaction was heated at 110 °C under N_2 for 16 h. The reaction was cooled to rt and was quenched with water. The volatiles were removed in vacuo to leave a brown residue. The residue was triturated with hot

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EtOH, and the resulting slurry was filtered. The filtrate was concentrated in vacuo to give a red residue which was purified by MDAP (high pH method) to give **43** (59 mg, 18%) as a yellow solid. LCMS (high pH method) retention time 0.46 min, purity 91% by area, $[M + H]^+$ = 321. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 5.40 (s, 2H), 7.41 (dd, J = 7.7, 4.5 Hz, 1H), 7.67 (s, 1H), 7.70 (dt, J = 8.0, 1.5 Hz, 1H), 7.88 (d, J = 5.1 Hz, 1H), 8.26 (s, 1H), 8.54 (d, J = 5.1 Hz, 2H), 8.56 (d, J = 1.7 Hz, 1H), 8.82 (s, 1H), 13.12 (br s, 1H).

2-((1,3-Dimethyl-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one, 44. *Step 1.* A premixed solution of 30% H_2O_2 (aq) (0.270 mL, 2.64 mmol) and 2 M NaOH (aq) (1.322 mL, 2.64 mmol) was added to a solution of 1,3-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (534 mg, 2.404 mmol) in THF (10 mL). The resulting suspension was stirred for 2 h and evaporated to dryness. The residue was purified by silica gel column chromatography, eluting with a gradient of 50−100% EtOAc/cyclohexane to give 1,3-dimethyl-1*H*-pyrazol-4-ol (205 mg, 72%) as a white solid. LCMS (TFA method) retention time 0.23 min, $[M + H]^+$ = 112.

Step 2. NaH (115 mg, 60% w/w in oil, 2.88 mmol) was added to a solution of 1,3-dimethyl-1*H*-pyrazol-4-ol (105 mg, 0.936 mmol) in DMF (5 mL) under N_2, and the resulting suspension was stirred for 5 min. A mixture of 2-(ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one and 2-(ethylsulfinyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20b** (150 mg, 0.568 mmol) was added, and the resulting suspension was heated to 110 °C for 1.5 h. The mixture was cooled to rt, acidified to pH 4 with 2 M HCl (aq), and diluted with EtOAc. The aqueous layer was separated, and the organic layer was washed with water and brine and then dried ($MgSO_4$) and evaporated to give a dark red solid. This solid was purified using silica gel column chromatography, eluting with a gradient of 0−6% DCM/MeOH to give an off white solid which was triturated with *tert*-butylmethyl ether and dried to give **44** (9 mg, 5%) as a white solid. LCMS (TFA method) retention time 0.45 min, $[M + H]^+$ = 258. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.07 (s, 3H), 3.78 (s, 3H), 7.88 (d, J = 4.6 Hz, 1H), 7.91 (s, 1H), 8.53 (d, J = 5.1 Hz, 1H), 8.81 (s, 1H), 13.06 (br s, 1H).

2-((1,5-Dimethyl-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one, 45. *Step 1.* To a solution of 1,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (500 mg, 2.251 mmol) in THF (10 mL) were added 2 M NaOH (aq) (3.38 mL, 6.75 mmol) and 30% H_2O_2 (aq) (0.460 mL, 4.50 mmol). The solution was stirred for 2 h at rt. The excess of H_2O_2 was quenched by adding sat. Na_2SO_3 (aq), and the mixture was adjusted to pH 4 with 2 M HCl (aq). The mixture was extracted with EtOAc, dried over $MgSO_4$, and concentrated to a crude residue which was purified using silica gel column chromatography, eluting with a gradient of 0−100% EtOAc/cyclohexane to give 1,5-dimethyl-1*H*-pyrazol-4-ol (120 mg, 35%) as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ ppm: 2.18 (s, 3 H), 3.69 (s, 3 H), 7.01 (s, 1 H).

Step 2. To 1,5-dimethyl-1*H*-pyrazol-4-ol (88 mg, 0.785 mmol) in DMF (10 mL) was added NaH (23.54 mg, 60% w/w in oil, 0.981 mmol). The suspension was stirred for 15 min under N_2 at rt. 2-(Ethylsulfonyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20a** (258 mg, 80% w/w, 0.863 mmol) was added, and the reaction mixture was stirred overnight at 110 °C. Further NaH (23.54 mg, 60% w/w in oil, 0.981 mmol) was added, and the reaction mixture was stirred for 20 h at 100 °C under N_2. 1,5-Dimethyl-1*H*-pyrazol-4-ol (44 mg, 0392 mmol) was added, and the reaction mixture was stirred overnight under N_2 at 110 °C. MeOH and water were added to the reaction which was then concentrated to give a crude solid. This solid was purified by MDAP (formic method) to give **45** (6.3 mg, 3%) as a white solid. LCMS (formic method) retention time 0.49 min, $[M + H]^+$ = 258. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.16 (s, 3H), 3.76 (s, 3H), 7.47 (s, 1H), 7.84 (d, J = 5.2 Hz, 1H), 8.46 (d, J = 5.2 Hz, 1H), 8.73 (s, 1H).

2-((1-Benzyl-1*H*-pyrazol-4-yl)oxy)pyrido[2,3-*d*]pyrimidin-4(3*H*)-one, 46. *Step 1.* 2-Aminonicotinic acid (5g, 36.2 mmol) and urea (12.70 g, 145 mmol) were stirred at 180 °C for 15 min, then at 200 °C for 15 min and then 210 °C for 1 h. The reaction was cooled to rt. To the resulting solid was added 2 M NaOH (approximately 50 mL), and the mixture was stirred at 50 °C until all the solid had dissolved. The solution was cooled again and was saturated with CO_2 until a cream colored precipitate had formed. The solid was isolated by filtration and dried in vacuo to give pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4.8 g, 82%) as a white solid. LCMS (formic method) retention time 0.36 min, $[M + H]^+$ = 164. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 5.43 (br s, 1H), 7.01 (dd, J = 7.6, 4.7 Hz, 1H), 8.13 (dd, J = 7.6, 2.0 Hz, 1H), 8.48 (dd, J = 4.7, 1.96 Hz, 1H), 10.96 (br s, 1H).

Step 2. Pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (1g, 6.13 mmol) was taken up in phosphorus oxychloride (20 mL, 215 mmol) and stirred at 110 °C under N_2 for 16 h. The reaction was azeotroped with toluene. The residue was treated with 2 N NaOH (20 mL, 40.0 mmol) and stirred at rt for 2 h. The mixture was adjusted to pH 5 with 2 M HCl (aq) and extracted with EtOAc. The combined organic fractions were dried using a hydrophobic frit and concentrated in vacuo to give 2-chloropyrido[2,3-*d*]pyrimidin-4(3*H*)-one as a pale yellow solid (723 mg, 65%). LCMS (formic method) retention time 0.39 min, $[M + H]^+$ = 182. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.58 (dd, J = 7.8, 4.7 Hz, 1H), 8.49 (dd, J = 7.8, 2.0 Hz, 1H), 8.94 (dd, J = 4.7, 2.0 Hz, 1H), 13.59 (br s, 1H).

Step 3. 1-Benzyl-1*H*-pyrazol-4-ol (767 mg, 2.203 mmol) (see compound **41**, steps 1 and 2, for preparation) was taken up in DMF (4 mL). NaH (44.1 mg, 60% w/w in oil, 1.101 mmol) was added, and after 5 min 2-chloropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (200 mg, 1.101 mmol) was added. The mixture was heated to 110 °C under N_2 for 2 days. The reaction was filtered, and the solvent was evaporated in vacuo. The residue was purified by MDAP (formic method) to give **46** (87 mg, 25%). LCMS (formic method) retention time 0.71 min, $[M + H]^+$ = 320. HRMS: $C_{17}H_{14}N_5O_2$ requires $[M + H]^+$ 320.1142, found 320.1142 (error −0.1 ppm). ^{1}H NMR (400 MHz, MeOH-d_4) δ ppm: 5.35 (s, 2H), 7.30−7.34 (m, 3H), 7.35−7.40 (m, 2H), 7.45 (dd, J = 7.8, 4.7 Hz, 1H), 7.70 (s, 1H), 8.20 (s, 1H), 8.57 (dd, J = 7.8, 2.0 Hz, 1H), 8.78 (dd, J = 4.7, 2.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 164.1, 158.5, 156.0, 155.9, 137.8, 136.3, 135.5, 131.6, 129.0, 128.2, 128.1, 122.3, 121.2, 115.1, 56.0. Mp 265−270 °C (dec).

2-(Ethylthio)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 23. A mixture of 2-(ethylthio)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **20** (5 g, 24.13 mmol), DMF (50 mL), K_2CO_3 (6.67 g, 48.3 mmol), and SEM chloride (4.22 g, 25.3 mmol) was stirred at 50 °C for 3 h. The reaction temperature was increased to 80 °C, and heating continued for a further 4 h. The reaction was cooled to rt prior to the addition of further SEM chloride (2 g, 12.00 mmol). The mixture was stirred at 50 °C under N_2 for 16 h. The reaction was cooled to rt, and further SEM chloride (0.8 g, 4.80 mmol) was added dropwise. The reaction was stirred at 50 °C for 4 h. The reaction was cooled to rt, the insoluble material was removed by filtration through Celite (EtOAc eluent), and the filtrate was concentrated in vacuo to give an orange oil. This oil was purified using silica gel column chromatography, eluting with a gradient of 0−20% EtOAc/cyclohexane to give **23** (6.36 g, 78% yield) as a yellow oil which solidified on standing. LCMS (formic method) retention time 1.37 min, $[M + H]^+$ = 338. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.01−0.04 (m, 10H), 1.01 (t, J = 8.5 Hz, 2H), 1.44−1.50 (m, 4 H), 3.35 (q, J = 7.5 Hz, 2H), 3.73 (t, J = 8.0 Hz, 2H), 5.63 (s, 2H), 7.98 (d, J = 5.1 Hz, 1H), 8.61 (d, J = 5.1 Hz, 1H), 9.00 (s, 1H).

2-(4-Chloro-3-hydroxyphenoxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 31. *Step 1.* 5-Bromo-2-chlorophenol (2g, 9.64 mmol) was dissolved in DMF (20 mL). (2-(Chloromethoxy)ethyl)trimethylsilane (1.922 mL, 10.60 mmol) and K_2CO_3 (2.66 g, 19.28 mmol) were added, and the mixture was stirred at rt for 20 h. 10% aq LiCl (40 mL) was added, and the aqueous portion was extracted with EtOAc (40 mL). The organic layer was passed though a hydrophobic frit and was evaporated in vacuo affording a colorless oil. This was purified by silica gel column chromatography, eluting with 0−30% EtOAc/cyclohexane to give (2-((5-bromo-2-chlorophenoxy)methoxy)ethyl)trimethylsilane (3.23g) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.00 (s, 9H), 0.96 (m, 2H), 3.79 (m, 2H), 5.26 (s, 2H), 7.06 (dd, J = 8.0, 2 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 2.0 Hz, 1H).

Step 2. (2-((5-Bromo-2-chlorophenoxy)methoxy)ethyl)-trimethylsilane (1 g, 2.96 mmol) was dissolved in 2-methyl-THF

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(15 mL). Bis(pinocolato)diboron (0.759 g, 2.99 mmol), $Pd(dppf)Cl_2$ (0.108 g, 0.148 mmol), and potassium acetate (0.436 g, 4.44 mmol) were added, and the mixture was heated to 80 °C under nitrogen for 16 h. The reaction was filtered through Celite and the filtrate was concentrated in vacuo affording a colorless oil which was purified by silica gel column chromatography, eluting with 0−15% EtOAc/cyclohexane. Relevant fractions were combined and evaporated in vacuo to give (2-((2-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)methoxy)ethyl)trimethylsilane as a colorless oil (409 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.00 (s, 9H), 0.97 (m, 2H), 1.33 (s, 12H), 3.83 (m, 2H), 5.33 (s, 2H), 7.37 (m, 2H), 7.55 (s, 1H).

Step 3. (2-((2-Chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)methoxy)ethyl)trimethylsilane (409 mg, 0.776 mmol) was dissolved in THF. 30% w/w H_2O_2 (159 μL, 1.552 mmol) and 2 M NaOH (776 μL, 1.552 mmol) were added, and the mixture was stirred at rt for 4 h and then left standing at rt for 2 days. Water (20 mL) was added, and the mixture was extracted with DCM (30 mL). The organic layer was passed through a hydrophobic frit and was evaporated in vacuo affording a colorless oil which was purified by silica gel column chromatography, eluting with a gradient of 0−20% EtOAc/cyclohexane to give 4-chloro-3-((2-(trimethylsilyl)ethoxy)-methoxy)phenol as a colorless oil (135 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.00 (s, 9H), 0.96 (m, 2H), 3.79 (m, 2H), 5.26 (s, 2H), 4.98 (s, 1H), 6.42 (dd, J = 8, 2.0 Hz, 1H), 6.74 (d, J = 2.0 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H).

Step 4. 2-(Ethylthio)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido-[3,4-*d*]pyrimidin-4(3*H*)-one **23** (332 mg, 0.982 mmol) was taken up in 2-methyl-THF (10 mL). *m*CPBA (77% in water) (339 mg, 1.965 mmol) was added, and the mixture was stirred at rt for 2 h. The reaction mixture was washed with sat. $NaHCO_3$ (aq), and the organic layer was passed through a hydrophobic frit before being evaporated in vacuo to give a yellow oil. To the yellow oil was added 4-chloro-3-((2-(trimethylsilyl)ethoxy)methoxy)phenol (135 mg, 0.491 mmol) in 1,4-dioxane (10 mL) and Cs_2CO_3 (320 mg, 0.982 mmol). The mixture was heated to 60 °C under N_2 for 16 h. The reaction was filtered, and the solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography, eluting with a gradient of 0−30% EtOAc/cyclohexane to give 2-(4-chloro-3-((2-(trimethylsilyl)ethoxy)-methoxy)phenoxy)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (217 mg, 80%). LCMS (formic method) retention time 1.64 min, $[M + H]^+$ = 550.

Step 5. 2-(4-Chloro-3-((2-(trimethylsilyl)ethoxy)methoxy)-phenoxy)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (217 mg, 0.394 mmol) was taken up in nitromethane (10 mL). Magnesium bromide diethyl etherate (306 mg, 1.183 mmol) was added, and the mixture was stirred at 95 °C under nitrogen for 16 h. The solvent was evaporated in vacuo and the residue was purified by silica gel column chromatography with a gradient of 0−100% EtOAc/cyclohexane to give **31** as a pale brown solid (45 mg, 39%). LCMS (formic method) retention time 0.67 min, $[M + H]^+$ = 290. HRMS: $C_{13}H_9ClN_3O_3$ requires $[M + H]^+$ 290.0327, found 290.0329 (error 0.6 ppm). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 6.82 (dd, J = 8.7, 2.7 Hz, 1H), 6.94 (d, J = 2.8 Hz, 1H), 7.43 (d, J = 8.6 Hz, 1H), 7.89 (d, J = 5.1 Hz, 1H), 8.53 (d, J = 5.1 Hz, 1H), 8.72 (s, 1H), 10.57 (s, 1H), 13.09 (br s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 162.4, 155.0, 154.2, 150.8, 149.4, 145.0, 143.4, 130.7, 125.3, 118.8, 117.7, 114.0, 110.8. Mp 268 °C (dec).

2-((6-Isopropylpyridin-3-yl)oxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 32. *Step 1.* A round-bottom flask was charged with 6-bromopyridin-3-ol (2 g, 11.49 mmol), polymer-bound triphenylphosphine (3 mmol/g) (9.20 g, 27.6 mmol), DCM (70 mL), and benzyl alcohol (1.45 mL, 13.95 mmol). To the mixture was added diisopropyl azodicarboxylate (2.68 mL, 3.79 mmol) dropwise. The slurry was stirred at rt under a blanket of N_2 for 2 days. The insoluble materials were removed by filtration through a hydrophobic frit, and the filtrate was concentrated in vacuo to give a brown oil. The oil was purified by silica gel column chromatography, eluting with a gradient of 0−40% EtOAc/cyclohexane to give 5-(benzyloxy)-2-bromopyridine (1.98 g, 65% yield) as a colorless solid. LCMS (high pH method) retention time 1.16 min, $[M + H]^+$ = 264/266.

Step 2. A round-bottom flask was charged with 5-(benzyloxy)-2-bromopyridine (1 g, 3.79 mmol), 4,4,5,5-tetramethyl-2-(prop-1-en-2-yl)-1,3,2-dioxaborolane (950 mg, 5.65 mmol), 1,4-dioxane (20 mL), tetrakis(triphenylphosphine)palladium(0) (0.088 g, 0.076 mmol), and 2 M Na_2CO_3 (5.68 mL, 11.36 mmol). The mixture was degassed and was warmed to 90 °C under a blanket of N_2 overnight. The mixture was cooled to rt, diluted with EtOAc and water, and the layers were mixed and separated. The organics were washed with brine, passed through a hydrophobic frit, and concentrated in vacuo to give a yellow oil. The oil was purified by silica gel column chromatography, eluting with a gradient of 0−25% EtOAc/cyclohexane to give 5-(benzyloxy)-2-(prop-1-en-2-yl)pyridine (768 mg, 63% yield) as a colorless oil. This material contained unreacted starting material 5-(benzyloxy)-2-bromopyridine (~20% by LCMS) and was used in the next step without further purification. LCMS (high pH method) retention time 1.18 min, $[M + H]^+$ = 264/266, starting material; retention time 1.24 min $[M + H]^+$ = 226, product.

Step 3. A hydrogenation flask was charged with 5-(benzyloxy)-2-(prop-1-en-2-yl)pyridine (664 mg, 2.063 mmol) from step 2, EtOH (10 mL), and palladium hydroxide on carbon (66 mg, 0.470 mmol). A hydrogenation head was fitted, and the reaction was stirred at rt under an atmosphere of H_2 overnight. The mixture was passed through Celite (EtOAc eluent), and the filtrate was concentrated in vacuo to give an off-white solid. The sample was purified by silica gel column chromatography, eluting with a gradient of 0−40% EtOAc/cyclohexane to give 6-isopropylpyridin-3-ol (228 mg, 81% yield) as a white solid. LCMS (formic method) retention time 0.34 min, $[M + H]^+$ = 138.

Step 4. 2-(Ethylthio)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido-[3,4-*d*]pyrimidin-4(3*H*)-one **23** (237 mg, 0.702 mmol), THF (10 mL), and *m*CPBA (77% weight in water) (393 mg, 1.755 mmol) were stirred at rt for 1 h. The mixture was concentrated in vacuo to half volume, then diluted with DCM. The organics were washed with sat. $NaHCO_3$ (aq), dried using a hydrophobic frit, and concentrated to give a yellow oil. This oil was dissolved in 1,4-dioxane (10 mL). 6-Isopropylpyridin-3-ol (96 mg, 0.702 mmol) and Cs_2CO_3 (686 mg, 2.107 mmol) were added, and the reaction was stirred at 60 °C under N_2 for 3 h. The reaction was diluted with EtOAc and the organics were washed with sat. $NaHCO_3$ (aq) and brine, dried using a hydrophobic frit, and concentrated in vacuo to give a yellow solid. This solid was purified using silica gel column chromatography, eluting with a gradient of 0−20% EtOAc/cyclohexane to give 2-((6-isopropylpyridin-3-yl)oxy)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (122 mg, 0.180 mmol, 25.7% yield) as a colorless oil. LCMS (formic method) retention time 1.31 min, $[M + H]^+$ = 413.

Step 5. To 2-((6-Isopropylpyridin-3-yl)oxy)-3-((2-(trimethylsilyl)-ethoxy)methyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (122 mg, 0.180 mmol) in nitromethane (10 mL) was added magnesium bromide diethyl etherate (140 mg, 0.541 mmol), and the reaction was stirred at 95 °C for 1 h. The mixture was cooled to rt and concentrated to give a tan residue. The residue was slurried in MeOH and the solid was isolated by filtration, washed with MeOH, and dried to give **32** (25 mg, 49%) as a buff solid. LCMS (formic method) retention time 0.63 min, $[M + H]^+$ = 283. HRMS: $C_{15}H_{15}N_4O_2$ requires $[M + H]^+$ 283.1190, found 283.1191 (error 0.4 ppm). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.28 (d, J = 6.9 Hz, 6 H), 3.10 (dt, J = 13.9, 6.9 Hz, 1H), 7.43 (d, J = 8.6 Hz, 1H), 7.78 (dd, J = 8.6, 2.9 Hz, 1H), 7.90 (d, J = 5.9 Hz, 1H), 8.53 (d, J = 7.8 Hz, 2H), 8.71 (s, 2H), 13.23 (br s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 164.5, 162.6, 155.3, 149.2, 146.7, 145.0, 143.2, 142.7, 130.6, 125.3, 122.0, 118.9, 35.5, 23.0. Mp 248 °C (dec).

2-((1-Cyclopentyl-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one, 36. *Step 1.* 1-Cyclopentyl-1*H*-pyrazol-4-ol was prepared using a similar method to that described for 1-cycloheptyl-1*H*-pyrazol-4-ol described in the preparation of compound **38**. LCMS (formic method) retention time 0.60 min, $[M + H]^+$ = 153.

Step 2. To a solution of mCPBA (77% in water) (664 mg, 2.96 mmol) in 2-methyl-THF, (5 mL) was added 2-(ethylthio)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **23**

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(400 mg, 1.185 mmol). The resultant suspension was stirred under N_2 for 1 h. The reaction was diluted with 2-methyl-THF (20 mL) and washed with sat. $NaHCO_3$ (aq); the organic layer was passed through a hydrophobic frit and the solvent was evaporated to give a pale yellow solid. This solid was redissolved in 1,4-dioxane (5 mL), and then Cs_2CO_3 (1158 mg, 3.56 mmol) and 1-cyclopentyl-1*H*-pyrazol-4-ol (180 mg, 1.185 mmol) were added. The resultant suspension was stirred at 60 °C under N_2 for 16 h. The reaction was concentrated and purified by MDAP (formic method) to give 2-((1-cyclopentyl-1*H*-pyrazol-4-yl)oxy)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (87 mg, 0.203 mmol) as a clear oil. LCMS (formic method) retention time 1.33 min, $[M + H]^+$ = 428.

Step 3. To a solution of 2-((1-cyclopentyl-1*H*-pyrazol-4-yl)oxy)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (87 mg, 0.203 mmol) in nitromethane (5 mL) at 0 °C was added magnesium bromide diethyl etherate (112 mg, 0.610 mmol) portionwise. The reaction was stirred at 95 °C for 2 h. The solvent was removed and the crude purified by MDAP (formic method) to give **36** (21 mg, 34%) as a cream solid. LCMS (formic method) retention time 0.74 min, $[M + H]^+$ = 298. HRMS: $C_{15}H_{16}N_5O_2$ requires $[M + H]^+$ 298.1299, found 298.1298 (error −0.3 ppm). ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 1.62−1.68 (m, 2H), 1.76−1.83 (m, 2H), 1.90−2.00 (m, 2H), 2.04−2.14 (m, 2H), 4.69 (quin, J = 7.0 Hz, 1H), 7.60 (s, 1H), 7.87 (dd, J = 5.1, 0.7 Hz, 1H), 8.07 (s, 1H), 8.51 (d, J = 5.1 Hz, 1H), 8.82 (s, 1H), 13.04−13.11 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 163.7, 155.7, 149.4, 144.4, 143.9, 135.5, 130.7, 125.2, 120.6, 118.9, 63.1, 32.9, 24.2. Mp 224−228 °C (decomp).

2-((1-(Cyclohexylmethyl)-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one, 39. *Step 1.* 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (2 g, 10.31 mmol) was taken up in DMF (30 mL) and cooled to 0 °C. NaH (0.824 g, 60% w/w in oil, 20.61 mmol) was added portionwise, and the reaction was stirred at rt under N_2 for 30 min until effervescence ceased. (Bromomethyl)-cyclohexane (1.5 mL, 10.75 mmol) was added slowly, and the reaction was stirred under the same conditions for 2.3 h. Further (bromomethyl)cyclohexane (0.5 mL) was added and the reaction stirred under the same conditions for 1 h. Water was added to the reaction followed by extraction with EtOAc. The organics were washed with 10% LiCl (aq) followed by brine and dried by passing through a hydrophobic frit. The solvent was removed in vacuo to give a pale yellow oil which was purified using silica gel column chromatography, eluting with a gradient of 0−100% EtOAc/cyclohexane to give 1-(cyclohexylmethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole as a colorless oil (1.41g). LCMS (formic method) retention time 1.27 min, $[M + H]^+$ = 291.

Step 2. 1-(Cyclohexylmethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1.41 g, 4.86 mmol) was taken up in THF (10 mL), and 2 M NaOH (4.86 mL, 9.72 mmol) was added. H_2O_2 (30% w/w, 0.993 mL, 9.72 mmol) was slowly added, and the reaction was stirred at rt under $N_2$for 1 h. The reaction was adjusted to pH 2 using 2 M HCl (aq) and was partitioned between water and DCM. The aqueous layer was extracted into DCM (×3), and the combined organics were passed through a hydrophobic frit. The solvent was removed in vacuo to give a pale yellow oil which was purified using silica gel column chromatography, eluting with a gradient of 0−40% EtOAc/cyclohexane to give 1-(cyclohexylmethyl)-1*H*-pyrazol-4-ol as a colorless oil (893 mg). LCMS (formic method) retention time 0.81 min, $[M + H]^+$ = 181.

Step 3. 2-(Ethylthio)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido-[3,4-*d*]pyrimidin-4(3*H*)-one **23** (500 mg, 1.481 mmol) was taken up in THF (10 mL). *m*CPBA (77% in water) (830 mg, 3.70 mmol) was added, and the reaction was stirred for 1 h under N_2. The reaction was concentrated to half volume and diluted with DCM. The organics were washed with sat. $NaHCO_3$ (aq), dried using a hydrophobic frit, and concentrated to give a yellow residue. The yellow residue was taken up in 1,4-dioxane (20 mL), and 1-(cyclohexylmethyl)-1*H*-pyrazol-4-ol (401 mg, 2.222 mmol) was added to the resulting solution, followed by Cs_2CO_3 (1448 mg, 4.44 mmol). The reaction was stirred at 60 °C under N_2 for 16 h. The reaction was cooled to rt and was diluted with EtOAc. The organics were washed with sat. $NaHCO_3$ (aq), and the aqueous layer was extracted with further EtOAc. The combined organic layers were washed with brine, dried using a hydrophobic frit, and concentrated. The residue was purified using silica gel column chromatography, eluting with a gradient of 0−30% EtOAc/cyclohexane to give 2-((1-(cyclohexylmethyl)-1*H*-pyrazol-4-yl)oxy)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (138 mg, 0.303 mmol). LCMS (formic method) retention time 1.43 min, $[M + H]^+$ = 456.

Step 4. 2-((1-(Cyclohexylmethyl)-1*H*-pyrazol-4-yl)oxy)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (138 mg, 0.303 mmol) was taken up in nitromethane (10 mL) and the resulting solution cooled to 0 °C. Magnesium bromide diethyl etherate (235 mg, 0.909 mmol) was added and the reaction stirred at rt for 1 h under N_2 and then at 95 °C for 1 h. The reaction was cooled to rt and the solvent removed in vacuo to give a yellow solid. The solid was triturated with MeOH to give **39** (53 mg, 54%) as a yellow solid. LCMS (formic method) retention time 0.90 min, $[M + H]^+$ = 326. HRMS: $C_{17}H_{20}N_5O_2$ requires $[M + H]^+$ 326.1612, found 326.1609 (error −0.2 ppm). ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 0.90−1.03 (m, 2H), 1.12−1.27 (m, 3H), 1.54 (d, J = 11.3 Hz, 2H), 1.63 (d, J = 8.6 Hz, 1H), 1.69 (d, J = 12.2 Hz, 2H), 1.83 (dtt, J = 14.8, 7.4, 7.4, 3.6, 3.6 Hz, 1H), 3.95 (d, J = 7.3 Hz, 2H), 7.60 (s, 1H), 7.88 (d, J = 5.1 Hz, 1H), 8.04 (s, 1H), 8.54 (d, J = 5.1 Hz, 1H), 8.82 (s, 1H), 13.07 (br s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 162.4, 154.3, 149.4, 145.0, 143.2, 135.1, 130.7, 125.3, 122.4, 118.9, 58.4, 38.7, 30.3, 26.4, 25.6. Mp 242−244 °C.

2-((1-Phenyl-1*H*-pyrazol-4-yl)oxy)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, 40. *Step 1.* A round-bottom flask was charged with aniline (0.840 mL, 9.2 mmol), water (4 mL), and HCl (37%) (2 mL, 65.8 mmol). The stirred solution was cooled to 0 °C in an ice bath. To the cold solution was added sodium nitrite (0.635 g, 9.20 mmol) in water (5 mL), dropwise over a period of 10 min, maintaining the temperature at 0 °C. A solution of 4-chloro-3-oxobutanoic acid (1.44 g, 10.55 mmol) in water (5 mL) was added dropwise, also at 0 °C. Finally, a solution of sodium acetate (1.51 g, 18.41 mmol) in water (10 mL) was added dropwise over a period of 30 min. The mixture was warmed to rt and stirred overnight. The mixture was partitioned with EtOAc, the layers were mixed and separated, and the aqueous portion was re-extracted with EtOAc. The organics were combined and passed through a hydrophobic frit and were concentrated in vacuo to give crude (*E*)-1-chloro-3-(2-phenylhydrazono)propan-2-one (1.6 g) as a red solid. LCMS (formic method) retention time 0.97 min, $[M + H]^+$ = 197/199.

Step 2. A round-bottom flask was charged with ground NaOH (0.82 g, 20.50 mmol) and MeOH (10 mL). The slurry was stirred at rt for 30 min prior to the portionwise addition of (*E*)-1-chloro-3-(2-phenylhydrazono)propan-2-one (1.6 g, 8.14 mmol) from step 1. The vessel was sealed and stirred at rt over the weekend. The solvent was removed in vacuo and the residue slurried with water. The insoluble materials were removed by filtration, and the filtrate was neutralized with concentrated HCl. The aqueous portion was extracted with EtOAc (×2), and the organics were combined, washed with brine, and passed through a hydrophobic frit. Concentration of the filtrate in vacuo gave crude 1-phenyl-1*H*-pyrazol-4-ol as a red solid (866 mg). LCMS (formic method) retention time 0.69 min, $[M + H]^+$ = 161.

Step 3. A mixture of 2-(ethylthio)-3-((2-(trimethylsilyl)ethoxy)-methyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **23** (200 mg, 0.593 mmol), THF (15 mL), and *m*CPBA (77% weight in water) (332 mg, 1.481 mmol) was stirred at rt for 1 h. The mixture was concentrated to half the volume and was diluted with DCM, washed with sat. $NaHCO_3$ (aq), dried using a hydrophobic frit, and concentrated to give an orange oil. The oil was redissolved in 1,4-dioxane (15 mL) and was treated with 1-phenyl-1*H*-pyrazol-4-ol (95 mg, 0.593 mmol) and Cs_2CO_3 (579 mg, 1.778 mmol). The reaction was stirred at 60 °C under N_2 for 16 h. The reaction was cooled to rt and diluted with EtOAc and water; the organic phase was washed with brine, dried using a hydrophobic frit, and concentrated in vacuo to give an orange oil. This oil was purified using silica gel column chromatography, eluting with a gradient of 0−20% EtOAc/cyclohexane to give 2-((1-

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phenyl-1*H*-pyrazol-4-yl)oxy)-3-((2-(trimethylsilyl)ethoxy)methyl)-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (89 mg, 34.5% yield) as a colorless oil. LCMS (formic method) retention time 1.36 min, $[M + H]^+ = 436$.

Step 4. To 2-((1-phenyl-1*H*-pyrazol-4-yl)oxy)-3-((2-(trimethylsilyl)-ethoxy)methyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (89 mg, 0.153 mmol) and nitromethane (10 mL) was added magnesium bromide diethyl etherate (119 mg, 0.460 mmol), and the slurry was heated to 95 °C under N_2 for 1 h. The reaction was cooled to rt and concentrated to give an orange solid. The solid was triturated with MeOH to give **40** (22 mg, 47%) as a buff solid. LCMS (formic method) retention time 0.79 min, $[M + H]^+ = 306$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.35 (t, J = 7.5 Hz, 1H), 7.54 (t, J = 8.1 Hz, 2H), 7.89 (dd, J = 7.8, 1.2 Hz, 2H), 7.91 (dd, J = 5.1, 0.7 Hz, 1H), 7.99 (s, 1H), 8.57 (d, J = 5.1 Hz, 1H), 8.83 (s, 1H), 8.90 (s, 1H), 13.23 (br s, 1H).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01538.

LCMS spectra and selectivity profiling data for **31**, **32**, **36**, **39**, and **41**; KDM4C RFMS assay data for compounds **47**–**49**; X-ray crystallography methods and data for compounds **3**, **4**, and **33** bound to KDM4D (PDF)

Molecular formula strings (CSV)

Accession Codes

X-ray crystal structures have been deposited in the Protein Data Bank as follows: compound **3** bound to KDM4D, PDB code 5FP9; compound **4** bound to KDM4D, PDB code 5FPA; compound **33** bound to KDM4D, PDB code 5FPB.

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Notes

The authors declare the following competing financial interest(s): All authors except C.J.S. were GlaxoSmithKline and Cellzome full-time employees at the time this work was carried out.

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■ ABBREVIATIONS USED

KDM, lysine demethylase; JMJD, Jumonji domain-containing protein; RFMS, RapidFire mass spectrometry; HTRF, homogeneous time-resolved fluorescence; JARID, Jumonji AT-rich interactive domain; 2-OG, 2-oxoglutarate; EGLN, egg-laying deficiency protein ninelike protein; PHD, prolyl hydroxylase domain protein; SCX, strong cation exchange; SPE, solid phase extraction; SEM, ((trimethylsilyl)ethoxy)methyl

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(7) pIC_{50} data reported are the mean of at least two values with SEM $\leq$ 0.3 in all cases with the following exceptions: KDM4A RFMS n = 1 for **4**, **32**; KDM4C RFMS n = 1 for **6**, **9**; EGLN3 HTRF n = 1 for **31**, **36**, **39**, **46**. The pIC_{50} data reported from the earlier KDM4C RFMS assay format (ref 11) for compounds **6** and **9** are the mean of at least two values with SEM $\leq$ 0.3. In a similar early KDM4A RFMS format compound **4** gave pIC_{50} = 5.7, n = 4, SEM < 0.1.

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were <4.0, 4.8, and 4.3, respectively and these data are similar to those reported in Table 2 for the optimized format where pIC_{50} values were <4.0, 4.6, and 4.3, respectively.

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