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Discovery of 2-(6-{[(6-Fluoroquinolin-2-yl)methyl]amino}bicyclo[3.1.0]hex-3-yl)-*N*-hydroxypyrimidine-5-carboxamide (CHR-3996), a Class I Selective Orally Active Histone Deacetylase Inhibitor

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A novel series of HDAC inhibitors demonstrating class I subtype selectivity and good oral bioavailability is described. The compounds are potent enzyme inhibitors (IC_{50} values less than 100 nM), and improved activity in cell proliferation assays was achieved by modulation of polar surface area (PSA) through the introduction of novel linking groups. Employing oral pharmacokinetic studies in mice, comparing drug levels in spleen to plasma, we selected compounds that were tested for efficacy in human tumor xenograft studies based on their potential to distribute into tumor. One compound, **21r** (CHR-3996), showed good oral activity in these models, including dose-related activity in a LoVo xenograft. In addition **21r** showed good activity in combination with other anticancer agents in in vitro studies. On the basis of these results, **21r** was nominated for clinical development.

Introduction

Histone deacetylases (HDACs[a]) are a family of epigenetic regulator enzymes, the inhibition of which leads to a reversal of tumor suppressor silencing, cell cycle arrest, differentiation, and/or apoptosis.[1] Because of these effects, which manifest themselves most strikingly in transformed cells, HDAC inhibition has emerged as a strategy to reverse aberrant epigenetic changes associated with cancer.[2,3] A number of HDAC inhibitors have shown antitumor activity in animal models of human cancer and are now in clinical trials (Figure 1).[4] The first such agent to reach pivotal clinical trials was the hydroxamate, SAHA[5−7] (vorinostat); this drug has been approved for use in the treatment of cutaneous T-cell lymphoma. The cyclic depsipeptide romidepsin has been registered for the treatment of the same disease. A wealth of preclinical data suggest that HDAC inhibitors have potential against a wide range of tumor types either alone or in combination with other therapies.[8]

The HDACs comprise three separate classes of enzyme.[9] Both class I and class II enzymes are metalloenzymes containing a catalytic zinc atom. There are thought to be at least 11 isoforms of the metalloenzyme HDAC proteins: class I HDACs (HDACs 1, 2, 3, and 8) are ubiquitously expressed and generally detected in the nucleus, while class II HDACs (HDACs 4, 5, 6, 7, 9, and 10) can shuttle between the cytoplasm and nucleus. HDAC11 has a related catalytic domain but has only weak homology with the others. The class III enzymes are not metalloenzymes and, while relevant to certain human diseases, are not yet strongly implicated in cancer.

The major objectives of our discovery efforts toward therapeutically useful HDAC inhibitors were to achieve good intratumoral distribution of drug via oral administration and class I isoform selectivity. The latter goal was driven by the hypothesis that these isoforms are those most predominantly involved in cellular proliferation.[10] It has been suggested from extensive analysis of published protein and mRNA expression of HDAC isoforms that class I HDACs are often overexpressed in tumors compared to corresponding normal tissue, and this overexpression generally correlates with poor prognosis. In contrast, expression of class II HDACs may be associated with better prognosis, suggesting that their inhibition may not be useful in cancer therapy.[11]

Chemistry

We designed a series of *N*-hydroxypyrimidine-5-carboxamides as potential HDAC inhibitors based on an analysis of all previously described families that assessed their ability to

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[a] Abbreviations: HDAC, histone deacetylase; PSA, polar surface area; SAHA, suberoylanilide hydroxamic acid; HDACi, histone deacetylase inhibitor; TBME, *tert*-butyl methyl ether; TFA, trifluoroacetic acid; DMAP, 4-dimethylaminopyridine; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, hydroxybenzotriazole; mCPBA, *m*-chloroperoxybenzoic acid; ZBG, zinc binding group; AUC, area under curve; Cl, clearance; *F*, oral bioavailability; HLM, human liver microsomes; MLM, mouse liver microsomes; BOC, *tert*-butoxycarbonyl; Boc_2O, di-*tert*-butyl dicarbonate; EtOAc, ethyl acetate; THF, tetrahydrofuran; DMF, dimethylformamide; DMSO, dimethylsulfoxide; EGF, epidermal growth factor; PBS, phosphate buffered saline; FACS, fluorescence-activated cell sorting; ND, not determined; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PARP, poly ADP-ribose polymerase.

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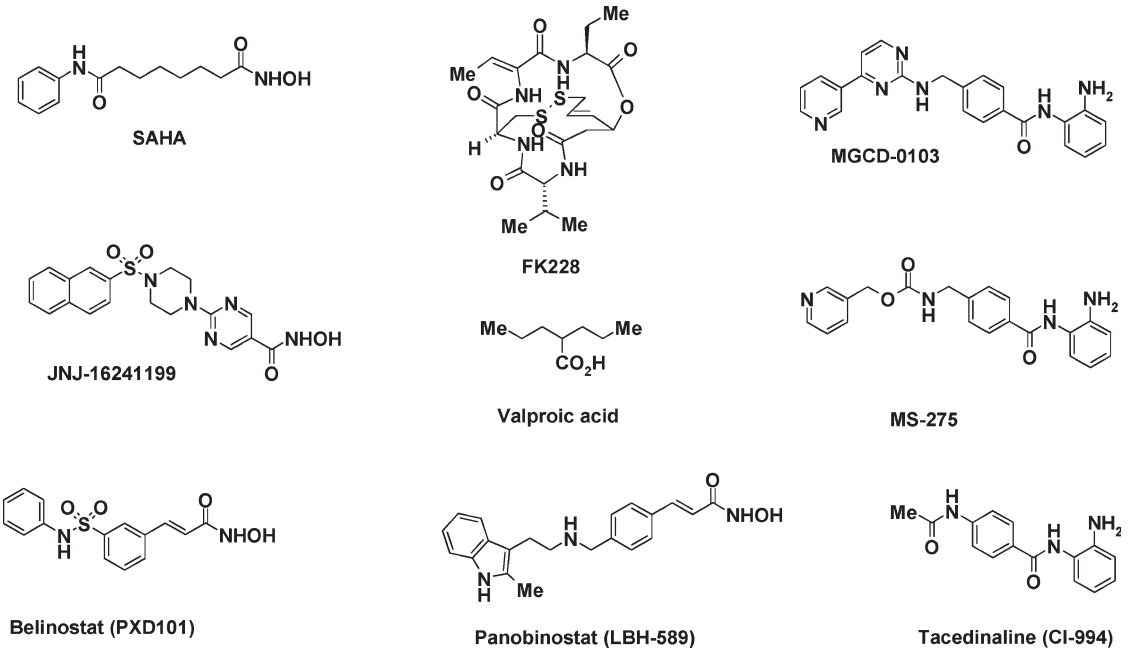


Figure 1. HDAC inhibitors under clinical investigation.

meet our goal of robust physicochemical properties to facilitate improved cell permeability and oral dosing as well as to provide novelty. We decided to utilize the pyrimidine hydroxamate moiety as the zinc-binding group (ZBG) in our approach, as this seemed likely to be the most metabolically stable functionality that would be compatible with good HDAC inhibitory activity. The Johnson & Johnson group has previously reported on HDAC inhibitors from this chemical class.[12−14] In parallel to our work,[15] this group has pursued a strategy aimed at improving the pharmacodynamic properties of their initial lead series through introduction of an ionizable group to improve solubility.[16]

Work on the project was initiated with docking studies using an available structure of the class I isoform HDAC8[17] based on coordination of the pyrimidine hydroxamate unit to the zinc ion at the base of the catalytic site. We believed that this would permit the identification of novel linker groups that would appropriately position the inhibitor headgroup in the hydrophobic region at the entrance of the active site. These studies indicated that the hexahydropyrrolo[3,4-*c*]pyrrole and azabicyclo[3,1,0]hexane linkers (Figure 2) were of a requisite length to provide good interaction between aryl- or heteroaryl substituents and this hydrophobic surface. We have previously described the optimization of the hexahydropyrrolo-[3,4-*c*]pyrrole linked compounds.[18] In this article we detail the development of the azabicyclo[3,1,0]hexane series toward a compound that has progressed to clinical study.

Our approach to the synthesis of the pyrimidine hydroxamate target molecules was dependent on the availability of several key intermediates (Scheme 1). Intermediate **2** was prepared from commercially available ethyl 4-chloro-2-(methylsulfanyl)pyrimidine-5-carboxylate in a two step procedure involving zinc mediated removal of the 4-chloro substituent followed by subsequent oxidation of the 2-methanesulfanyl group. The route to our linker unit required the synthesis of the Boc-protected 6-amino-3-aza-bicyclo[3.1.0]hexane **8** following a multistep procedure starting from *N*-benzylmaleimide via the known intermediate **6**.[19] Selenium dioxide mediated oxidation of 6-fluoro-2-methylquinoline gave the aldehyde intermediate

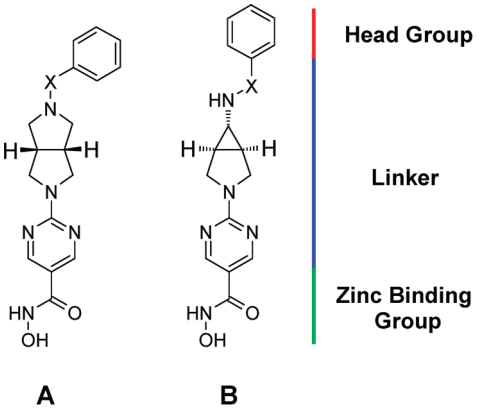
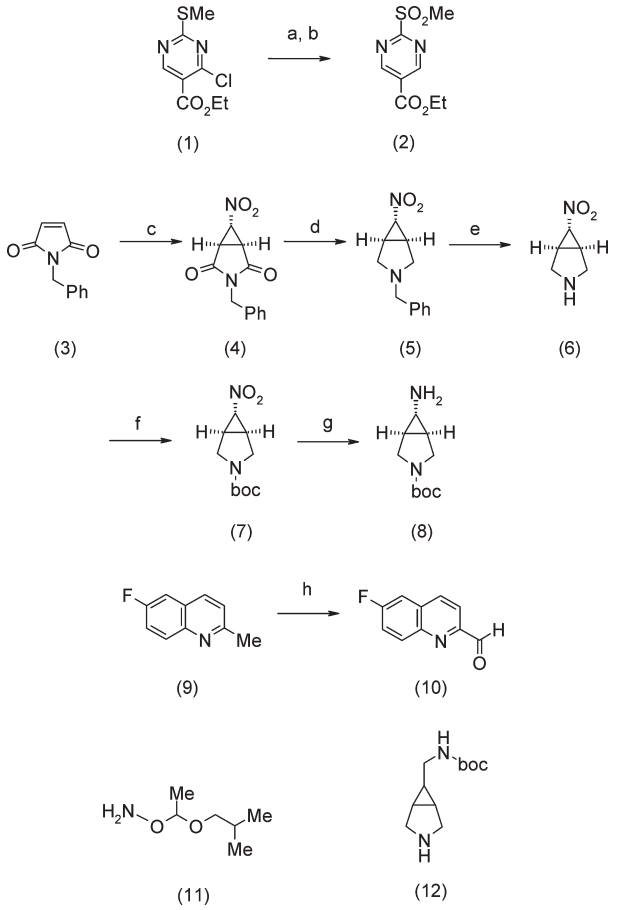


Figure 2. Structural components of pyrimidine hydroxamate HDAC inhibitors containing hexahydropyrrolo[3,4-*c*]pyrrole (A) and azabicyclo[3,1,0]hexane (B) linkers.

10 in excellent yield. The differentially Boc-protected methylaminoazabicyclohexane derivative **12** was available from a previously described method,[20] as was *O*-(1-isobutoxyethyl)-hydroxylamine **11**.[21] This was employed to introduce the hydroxamate functionality in the final steps of our analogue preparation.

Sulfonation of the amino group of compound **8** with aryl- or heteroarylsulfonyl chlorides followed by removal of the Boc group gave suitable intermediates **13a**,**b** for condensation with the pyrimidine intermediate **2** through displacement of the 2-methanesulfonyl substituent (Scheme 2). Base hydrolysis of the intermediate ester gave carboxylic acids **14a**,**b** which were coupled with the protected hydroxylamine **11** to give intermediates that could be purified via flash chromatography on silica. Subsequent deprotection of the hydroxamate was facile under acidic conditions, giving target molecules **15a** and **15b**. The corresponding amide analogues **18a**−**c** were prepared by similar methodology beginning with amidation of **8** with readily available acid chlorides. In the case of the alkylamine analogues **21a**−**r** the six-step sequence from **8** proved to be a robust and

Scheme 1. Preparation of Intermediates[a]



[a] Reagents and conditions: (a) Zn powder, H_2O, *t*-BuOH, THF, 85 °C, 57%. (b) *m*CPBA, THF, 0 °C, 77%. (c) $BrCH_2NO_2$, K_2CO_3, CH_3CN, room temp, 42%. (d) $NaBH_4$, $BF_3 \cdot$ THF, 45−50 °C, 42%. (e) $CH_3CHClOCOCl$, $ClCH_2CH_2Cl$, 55−65 °C, 25%. (f) Boc_2O, DMAP, CH_2Cl_2, room temp, 92%. (g) 10% Pd−C, H_2, EtOH, room temp, 95%. (h) SeO_2, dioxane, 100 °C, 87%.

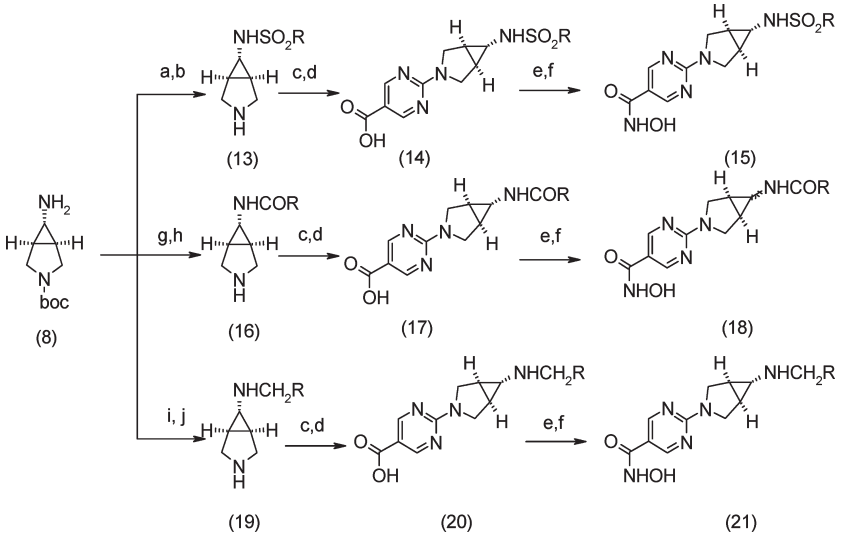
efficient process in which the intermediates **19** and **20** could generally be reached without the need for a purification step. The conformation of the azabicyclo[3,1,0]hexane linker unit was determined by X-ray diffraction studies on compound **21r**. The constrained three-membered ring was observed in the *exo*-conformation, consistent with previous reports.[19,22]

Methylation of the intermediate sulfonamide, formed by the reaction of amine **8** and 2-naphthylenesulfonyl chloride provided **22**, facilitating the synthesis of final hydroxamate **23** (Scheme 3). The condensation of **8** with 1-chloroisoquinoline furnished the desired aminoisoquinoline intermediate **24**, allowing access to final compound **25**. The extended methylamino analogue **27** was obtained by the reaction of Boc-protected azabicyclo[3,1,0]hexane **12** with the 2-methanesulfonyl intermediate **2** followed by subsequent ester hydrolysis to give intermediate **26**. The hydroxamate functionality of **27** was introduced as for previous analogues.

Results and Discussion

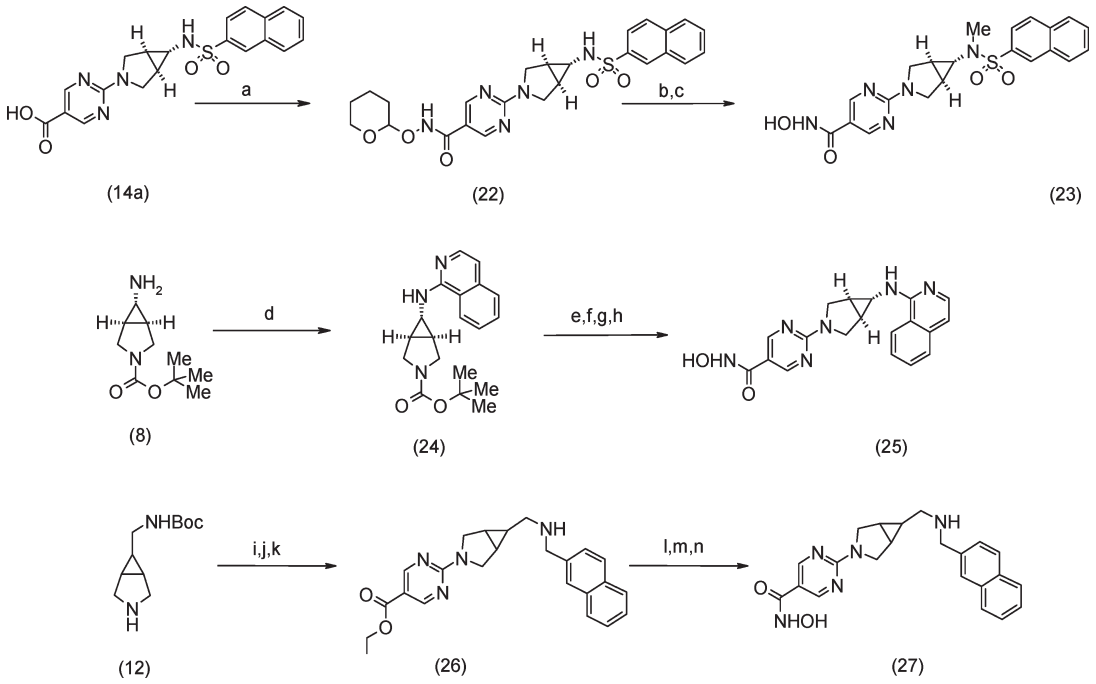
The intrinsic HDAC potency of test compounds was established through IC_{50} determination (Table 1) in an assay utilizing nuclear extract from HeLa cells. Similar levels of inhibition were observed for the sulfonamide **15a**, amide **18a**, and alkylamino **21a** compounds, confirming a hypothesis from our modeling studies that all these linkers from the azabicyclohexane spacer would place the 2-naphthyl headgroup in a similar position at the surface of the protein (Figure 3). However, we did observe that the cell-to-enzyme potency ratio (GI_{50}(HCT116)/IC_{50}(HDAC)) was reduced for the naphthylmethylene analogue **21a** when growth inhibition of the HCT116 human colon carcinoma cell line was examined. The cell-to-enzyme ratios for **15a**, **18a**, and **21a** were 73, 32, and 13, respectively. We attributed this observation to the lower calculated PSA of **21a** in comparison to **15a** and **18a**. This hypothesis appears to be supported by an analysis of an expanded panel of 84 pyrimidine hydroxamates made in this project. Although the absolute correlation between PSA and cell-to-enzyme ratio is relatively weak, the trends for

Scheme 2. Preparation of Pyrimidine Hydroxamate Analogues[a]



[a] Reagents and conditions: (a) RSO_2Cl, triethylamine, CH_2Cl_2, 0 °C to room temp. (b) TFA, CH_2Cl_2, room temp. (c) Ethyl 2-(methylsulfonyl)pyrimidine-5-carboxylate **2**, K_2CO_3, CH_3CN, room temp. (d) NaOH, MeOH, THF, room temp. (e) *O*-(1-Isobutoxyethyl)hydroxylamine, EDC hydrochoride, HOBt, triethylamine, room temp. (f) TFA, CH_2Cl_2, room temp or 4 M HCl in dioxane, CH_2Cl_2, room temp. (g) ROCl, pyridine, 0 °C. (h) 4 M HCl in dioxane, CH_2Cl_2, room temp. (i) RCHO, $NaBH_4$, MeOH, NaOH, room temp, 58%. (j) 4 M HCl in dioxane, CH_2Cl_2, room temp.

Scheme 3. Preparation of Further Pyrimidine Hydroxamate Analogues[a]



[a] Reagents and conditions: (a) 2-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine, EDC hydrochloride, HOBt, DMF, room temp, 90%. (b) NaH, $(MeO)_2SO_2$, THF, room temp. (c) TFA, CH_2Cl_2, MeOH, room temp, 3% over two steps. (d) 1-Chloroisoquinoline, 100 °C. (e) Ethyl 2-(methylsulfonyl)pyrimidine-5-carboxylate **2**, K_2CO_3, CH_3CN, DMF, room temp. (f) NaOH, THF, H_2O, room temp, 30% over three steps. (g) *O*-(1-Isobutoxyethyl)hydroxylamine, EDC hydrochloride, HOBt, triethylamine, room temp, 36%. (h) 4 M HCl in dioxane, room temp, 58%. (i) Ethyl 2-(methylsulfonyl)pyrimidine-5-carboxylate **2**, K_2CO_3, CH_3CN, DMF, room temp, 90%. (j) 4 M HCl in dioxane, CH_2Cl_2, room temp, 97%. (k) 2-Naphthaldehyde, $NaBH_4$, MeOH, room temp. (l) NaOH, THF, H_2O, room temp. (m) *O*-(1-Isobutoxyethyl)hydroxylamine, EDC hydrochloride, HOBt, triethylamine, room temp, 46% over two steps. (n) 4 M HCl in dioxane, CH_2Cl_2, room temp, 50%.

low cell-to-enzyme ratios, arbitrarily set at < 20, were made evident by binning PSA data (Figure 4). For 53 compounds that had cell-to-enzyme ratio of less than 20 (Figure 4a), 58% had a PSA value of less than 100 Å^2. In comparison, for the 31 compounds with a ratio greater than 20, 81% of compounds had a PSA of > 100 Å^2 (Figure 4b), suggesting that targeting a lower PSA value was important for good cell activity. Since these lower PSA values are achieved by the introduction of the alkylamino linkage, we decided to focus our optimization on these derivatives. We also believed that the more lipophilic nature of these analogues resulting from reduced PSA in conjunction with the introduction of the ionizable aminoalkyl linker would perhaps lead to improved distribution of drug to the tumor.[23] Expansion of the SAR of compound **21a** indicated that many aryl and heteroaryl groups are tolerated as the aminoalkyl substituent and that good cell-to-enzyme ratios are maintained.

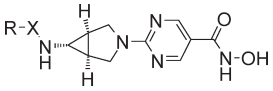
An additional factor limiting cell penetration of **15a** may have been the ionization of the sulfonamide linker. The calculated pK_a for this group is 3.4, suggesting that it will be negatively charged at physiological pH. We therefore decided to investigate whether methylation of the sulfonamide nitrogen would mitigate this effect. The HDAC IC_{50} value of 23 nM for **23** (Table 2) indicated that while the introduction of the methyl substituent was not detrimental to potency, activity in the cell growth inhibition assay was still moderate at 1 μM. This result enhanced our belief that high PSA limits cell penetration of these compounds.

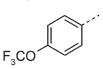
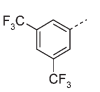
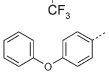
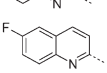
We next decided to examine the effect of linker length on potency through the synthesis of compounds **25** and **27**. The truncated aminoisoquinoline compound **25** maintained good potency against the target, in relation to the aminoalkyl analogues **21a**−**r**. The introduction of an additional methylene unit in compound **27** led to an HDAC IC_{50} value of 32 nM which compared favorably to the potency of **21a** which inhibits the enzyme with a potency of 14 nM. Thus, it would appear that HDAC activity is relatively insensitive to linker length in this series. It has been reported that, at least for HDAC8, the protein surface at the opening to the active site is malleable allowing it to accommodate binding of a variety of ligands.[24] Thus, the narrow range of inhibition within our series is unsurprising.

Pharmacokinetic Data. With good enzyme and cell potency demonstrated, we decided to investigate the exposure of this series of compounds by the oral route of administration to ascertain which compounds to progress to efficacy studies. With many compounds of similar potency available, we pursued a triaging strategy based on comparison of the AUCs of these analogues following oral cassette dosing at 3 mg/kg in mice, the best compounds being shown in Table 3. Interestingly **21r** had significantly higher exposure in the spleen in comparison to the 2-naphthyl analogue **21a**. This presumably reflects the ability of the fluoroisoquinoline group in **21r** to prevent the rapid oxidative metabolism of the naphthyl headgroup observed for **21a** in microsomal studies (data not shown). From this work **21f** and **21r** were identified as lead compounds for progression.

Further in vitro profiling of **21f** and **21r** indicated that both compounds demonstrated moderate to good permeability in a Caco-2 monolayer assay, particularly in comparison to the prototypical sulfonamide inhibitor **15a** (Table 4). Good metabolic stability in mouse and human liver microsomes was also

Table 1. Inhibition of HDAC Activity IC_{50}, Growth Inhibition GI_{50} Values in Human Colorectal Cancer Cell Line HCT116, and PSA Values for Compounds **15a**,**b**, **18a**−**c**, and **21a**−**r**



Compound	R	X	HDAC IC_{50} (nM)[a]	HCT116 GI_{50} (nM)[b]	Cell/ enzyme ratio[c]	PSA (Å^2)
15a		-SO_2-	6.5±0.9	440	73	133
15b		-SO_2-	27	>10000	>370	161
18a		-CO-	13	410	32	107
18b		-CO-	40	3600	90	136
18c		-CO-	57	5100	89	107
21a		-CH_2-	12.4±4.2	160	13	90
21b		-CH_2-	10	120	12	90
21c		-CH_2-	34	280	8	90
21d		-CH_2-	44	160	4	90
21e		-CH_2-	27	380	14	90
21f		-CH_2-	13±3.2	210	16	90
21g		-CH_2-	14	250	18	90
21h		-CH_2-	34	700	21	90
21i		-CH_2-	16	200	13	90
21j		-CH_2-	30	270	9	114
21k		-CH_2-	15	120	8	100
21l		-CH_2-	20	190	9.5	99
21m		-CH_2-	22	410	18	90
21n		-CH_2-	16	200	12.5	90
21o		-CH_2-	16	120	7.5	99
21p		-CH_2-	14	56	4	90
21q		-CH_2-	9	ND[d]	-	103
21r		-CH_2-	8±1.8	72	9	103

[a] Values are the mean of two independent determinations or mean ± SD of three or more experiments. The K_i for **21r** was 4.8 nM. SAHA was used as a positive control: IC_{50} = 87 ± 13 nM. [b] n = 6. SAHA gave a GI_{50} (mean of three experiments) in these assays of 920 ± 120 nM. [c] Cell/enzyme ratio = (HCT116 GI_{50})/(HDAC IC_{50}). [d] Not determined.

observed, particularly for **21r**, with a significant amount of parent drug remaining after 30 min of incubation in both preparations. The glucoronide was the major metabolite identified in mouse liver microsomes with smaller amounts of the amide and carboxylic acid being observed (Figure 5). No detectable metabolites were identified from human microsomes. Compounds **21f** and **21r** also displayed favorable drug−drug interaction profiles, with no significant inhibitory activity being observed against CYP2C19, CYP2D6, and CYP3A4 at concentrations below 10 μM. The equilibrium solubility[25] of **21a** and **21r** was measured in phosphate buffer

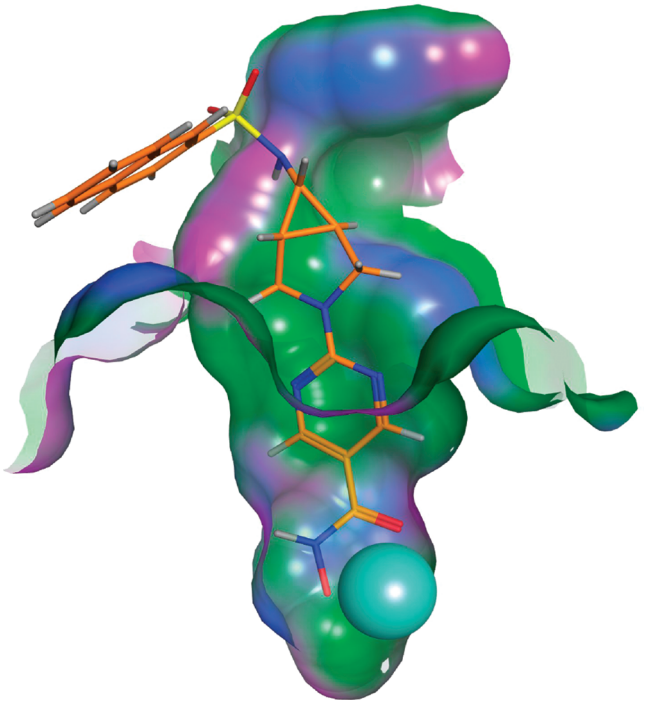


Figure 3. Proposed binding mode of **15a** with HDAC8 (cross section). The Zn^{2+} ion is shown as a sphere and the protein as a surface.

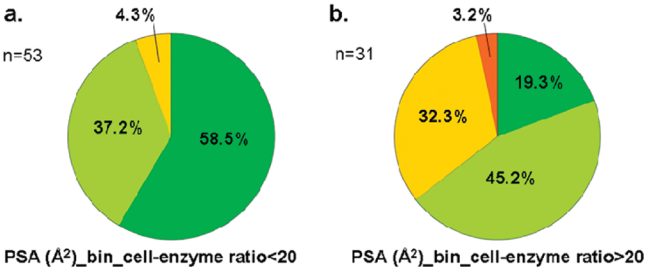


Figure 4. Pie charts showing the percentage distribution of pyrimidine hydroxamate compounds in PSA bins. Compounds are divided between those with cell-to-enzyme ratio of < 20 (a) and those with cell-to-enzyme ratio of > 20 (b). Compounds with PSA of 70−100 Å^2 are represented in bright green, 100−130 Å^2 in light green, 130−160 Å^2 in amber, and 160−190 Å^2 in red. Pie charts were generated with Vortex (Dotmatics Ltd., U.K.).

Table 2. Inhibition of HDAC Activity (IC_{50}), Growth Inhibition (GI_{50}) in Human Colorectal Tumour Cell Line HCT116, and PSA Values for Compounds **23**, **25**, and **27**

compd	HDAC IC_{50} (nM)[a]	HCT116 GI_{50} (nM)[b]	cell/enzyme ratio[c]	PSA (Å^2)
23	23	1000	44	124
25	18	130	7	103
27	32	370	12	90

[a] Values are the mean of two independent determinations. SAHA was used as a positive control: IC_{50} = 87 ± 13 nM. [b] n = 6. SAHA gave a GI_{50} (mean of three experiments) in these assays of 920 ± 120 nM. [c] Cell/enzyme ratio = (HCT116 GI_{50})/(HDAC IC_{50}).

at pH 5.5 and 7.4. The solubility of **21a** was pH dependent as expected, but the solubility of **21r** was surprisingly poor, especially at the lower pH. Despite this, we were encouraged to profile the in vivo PK of **21f** and **21r** in other species to further assess their suitability as potential drug candidates. We were pleased to find that the oral exposure demonstrated in the mouse oral PK studies translated into good bioavailability for both compounds in the dog, with F values of 56% and 40% for **21f** and **21r**, respectively. In the rat, **21r** also demonstrated good bioavailability (F = 27%); **21f** was not tested (Table 5).

Pharmacology. We were keen to determine the HDAC isoform selectivity of **21f** and **21r**, and so their IC_{50} values against a panel of HDAC isoforms were determined (Table 6). We were pleased to observe that **21f** and **21r** showed excellent potency against the class I isoforms, HDACs 1, 2, and 3, and good selectivity over HDACs 5 and 6, members of the class II family. These two compounds were ~700-fold selective for HDAC1 compared to HDAC6, whereas SAHA showed equipotent activity. To further investigate this subtype selectivity at the cellular level, we used the HCT116 cell line to evaluate the acetylation status of histone H3 in comparison to that of the HDAC6-specific substrate α-tubulin. We found that **21f**, **21r**, and SAHA have almost equivalent effects on histone acetylation at equitoxic doses whereas SAHA has a greater effect on tubulin acetylation (Figure 6). The inability of **21f** and **21r** to induce significant acetylation of α-tubulin confirmed that they are not effective inhibitors of HDAC6. The cleavage of PARP was also observed in HCT116 cells (Figure 7), this being indicative of apoptosis.

Given the important role of HDACs in tumor cell proliferation, we measured the antiproliferative effects of **21f** and **21r**, again in comparison to SAHA, against an expanded panel of transformed human cell lines originating from both solid and hematological cancers (Table 7). As shown, **21r** exhibits growth inhibition in the range 31−750 nM and was generally 2−3 times more potent than **21f** across the cell panel. In comparison to SAHA, **21r** was 5−20 times more potent.

With attention turning increasingly toward combination trials of HDAC inhibitors[26] we decided to investigate the synergy between our lead compounds with other anticancer agents. HDAC inhibitors have been reported to act in a synergistic manner with other antitumor agents in a number of in vitro studies.[27] Therefore, conventional Chou and Talalay median effect analysis[28] was conducted following the generation of appropriate cell proliferation data using **21f** and **21r** in combination with erlotinib[29] (EGF receptor kinase inhibitor), decitabine[30] (DNA methyltransferase inhibitor), and tosedostat[31] (aminopeptidase inhibitor). As indicated by the combination index (CI),[32] good additivity and synergy were seen across a panel of human cancer cell lines (Table 8). The most striking synergy was observed with a combination of **21r** and tosedostat against cells originating from pancreatic carcinoma and malignant melanoma.

Flow cytometric analysis was employed to determine the relative abilities of **21f**, **21r**, and SAHA to induce cell cycle arrest (Table 9), following the treatment of HCT116 cells with 300 nM inhibitor for 15 h. An increase in cells in the sub-G1 phase was observed for all three compounds, but only **21r**

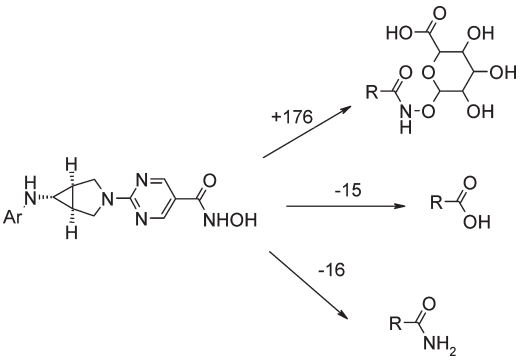


Figure 5. Metabolism of **21f** and **21r** in the mouse.

Table 3. AUC Values from Mouse po Cassette Studies[a]

	AUC_{0-t} (ng·h/mL)		
compd	plasma	spleen	spleen/plasma ratio
21a	50	145	2.9
21f	58	436	7.5
21k	83	251	3.0
21o	52	219	4.2
21q	42	294	7.0
21r	93	775	8.3

[a] Cassettes comprised four compounds dosed at 3 mg/kg po to CD1 mice.

Table 5. Pharmacokinetic Parameters of Compounds **21f** and **21r** in Rat[a] and Dog[b]

	iv (1 mg/kg)		po (10 mg/kg)		
	AUC_{0-t} (ng·h/mL)	Cl ((mL/min)/kg)	AUC_{0-t} (ng·h/mL)	$t_{1/2}$ (h)	F (%)
dog **21f**	265	63	1475	ND[c]	56
dog **21r**	241	70	950	0.9	40
rat **21r**	124	134	102	5.2	27

[a] The vehicle for rat iv and po dosing was 1% DMSO-HPβC (11.25% w/v). [b] The vehicle for dog iv dosing was 5% DMSO-HPβC (11.25% w/v) and 5% DMSO-saline for the po arm. [c] Not determined.

Table 6. Inhibition of HDAC Isoforms (IC_{50} in nM)[a]

HDAC	**21f**	**21r**	SAHA
1	6	3	30
2	14	4	24
3	10	7	150
5	500	200	3000
6	3300	2100	30

[a] Assays were performed by BPS Biosciences (San Diego, CA) using human recombinant enzyme.

Table 4. In Vitro ADME Properties of HDAC Inhibitors

	Caco-2 P_{app} (cm/s)		in vitro Cl (% remaining 30 min)		CYP IC_{50} (μM)			solubility (μg/mL)	
compd	A2B	B2A	MLM[a]	HLM[b]	2C19	2D6	3A4	pH 5.5	pH 7.4
15a	< 0.5	8.9	66	43	10−50	10−50	10−50	74	34
21a	3	20	100	40	> 50	> 50	> 50	208	12
21f	24	27	43	38	50	10−50	> 50	ND[c]	ND[c]
21r	9	17	31	21	> 50	> 50	10−50	3	1

[a] MLM = mouse liver microsome. [b] HLM = human liver microsome. [c] Not determined.

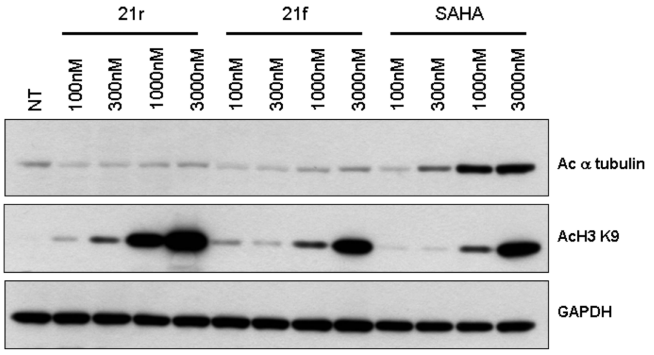


Figure 6. Substrate selectivity of **21f** and **21r** in HCT116 cells. Human HCT116 colon carcinoma cells were incubated with the indicated concentrations of **21f**, **21r**, and SAHA for 30 h. Total cell lysates were prepared and analyzed by SDS−PAGE and Western blotting using antibodies to acetylated H3K9 (Abcam 1:5000 dilution) and acetylated α-tubulin (Abcam, 1:500 dilution). GAPDH was used as a loading control. Similar results were obtained using a fixed cell ELISA (see Supporting Information).

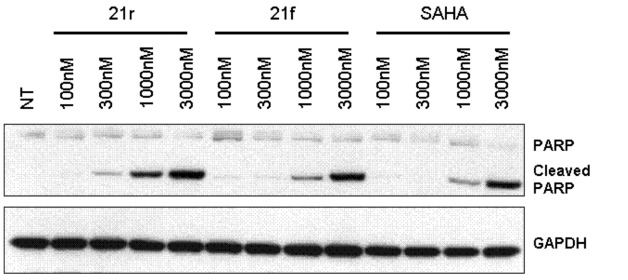


Figure 7. Induction of PARP cleavage by **21f** and **21r** in comparison to SAHA. Human HCT-116 colon carcinoma cells were incubated with the indicated concentrations of **21f**, **21r**, and SAHA for 30 h. Total cell lysates were prepared and analyzed by SDS−PAGE and Western blotting using antibodies to PARP and cleaved PARP (both Cell Signaling, 1:1000 dilution). GAPDH was used as a loading control.

Table 7. Antiproliferative Activity of **21f** and **21r** in a Panel of Human Tumour and Leukemic Cell Lines (GI_{50} in nM)[a]

cell line	origin	**21f**	**21r**	SAHA
HCT116	colon carcinoma	210	72	740
LoVo	colon carcinoma	310	140	1700
A2780	ovarian adenocarcinoma	410	210	3150
Du145	prostate adenocarcinoma	92	48	1050
PC3	prostate adenocarcinoma	260	160	2000
HepG2	hepatocellular adenocarcinoma	470	230	4600
MG63	osteocarcinoma	1600	750	$>10\ \mu$M
MiaPaca-2	pancreatic carcinoma	620	280	3500
A549	lung adenocarcinoma	500	250	5700
COR-L23	large cell lung carcinoma	210	105	1100
COR-L23 "R"	doxorubicin resistant variant	1100	560	3300
Molt-4	acute lymphoblastic leukemia	71	31	670
Hut78	T-cell lymphoma	60	31	295
U937	histiocytic lymphoma	120	52	450
MRC5- SV 2	transformed lung fibroblast	ND[b]	110	2100

[a] $n = 6$ replicates measured by WST-1 assay. [b] Not determined.

showed accumulation at G2/M compared to nontreated cells at this concentration and time-point.

The promising pharmacological and PK profiles of **21f** and **21r** prompted an in vivo efficacy study of these compounds against some of the human cancer cell lines employed in our in vitro cell growth inhibition studies. Initially we wanted to ensure that pharmacodynamic effects observed in tumor growth models were consistent with HDAC inhibition. Female CrTac:NCr-*Fox1*(*nu*) mice with established HCT116 tumor xenografts were dosed orally on a daily schedule for 16 days with 50 mg/kg **21f**. Although the efficacy of **21f** in this study was disappointing (Figure 8a), the compound was well tolerated and an increase in histone acetylation was observed in the tumors of **21f**-treated animals relative to vehicle animals for at least 4 h (Figure 8b). As expected from in vitro studies, α-tubulin acetylation was unchanged in the tumors from **21f**-treated mice compared with controls. Concentrations of **21f** in the spleen were 4−14 times higher than those in plasma, consistent with our earlier cassette PK study (Table 3). The measured tumor concentration of **21f** was sustained for at least 4 h at levels more than 4 times greater than its IC_{50}, consistent with the observed duration of increased histone acetylation.

In a second HCT-116 mouse xenograft study (Figure 9a) we compared the efficacy of **21f** (75 mg/kg po q.d.) with **21r** (50 mg/kg po q.d.). We found that **21r**, even at a lower dose than **21f**, resulted in almost complete suppression of tumor growth. Examination of tumor concentrations revealed that **21r** (133 ng/mL) achieved levels almost 5 times higher in comparison to the higher dose of **21f** (28 ng/mL). The ratio of tumor to plasma concentrations for **21r** (Figure 9b) also reflected our earlier studies of distribution to spleen (Table 3). Given that **21r** in general showed greater in vitro potency and enhanced distribution to tumor compared to **21f**, this result was perhaps unsurprising. We therefore decided to examine the efficacy of **21r** in a second human tumor xenograft model. A study was performed to evaluate the effect of a lower dose level of **21r** on subcutaneous growth of a LoVo human colorectal xenograft in female BALB/c nude mice (Figure 10). The compound was administered orally at 50 and 25 mg/kg daily, with **21r** again substantially reducing tumor volume and demonstrating an apparent dose dependency. As a result of these observations **21r** (CHR-3996)[15] was selected as our clinical candidate.

Conclusion

Through an optimization process we have identified **21r** as a 7 nM inhibitor of HDAC with a class I isotype selectivity that shows good potency for the inhibition of tumor cell growth. We have demonstrated that the compound can be delivered via oral administration with good distribution to tissue as measured by drug level in the mouse spleen and xenograft tissue and that the compound shows complete inhibition of growth in two human tumor xenograft models. **21r** has been selected for a phase 1 clinical trial and represents a highly promising new agent with the potential to be applied to a broad range of human cancers.

Experimental Section

Biological Methods. Measurement of HDAC Activity. The HeLa cell nuclear extract (catalog no. CC-01−20-50) was obtained from Cilbiotech S.A., Mons, Belgium. Histone deacetylase (HDAC) activity was determined using the Fluor De Lys system with the substrate (catalog no. KI-104) and the developer concentrate (catalog no. KI-105) purchased from Biomol International, Palatine House, Matford Court, Exeter, U.K. All assays were run in polystyrene, flat-bottomed Immulon 2HB 96-well plates purchased from Thermo Life Sciences, Milford, MA, U.S. The fluorimetric assay of HDAC activity was carried out as described in the Biomol technical manual. The Fluor De Lys substrate, developer concentrate, and HeLa cell nuclear extract were all diluted with the assay buffer (25 mM Tris-HCl,

Table 8. In Vitro Synergy Studies between HDAC Inhibitors and Anticancer Agents

combination	schedule	cell line	type of cancer	CI[b] **21f** (mean)	CI **21r** (mean)
HDACi plus erlotinib	HDACi (24 h), washout then erlotinib (6 days)	H358	NSCLC	0.32	0.28
		H520	NSCLC	0.54	0.85
		DU-145[a]	prostate carcinoma	0.63	0.61
HDACi plus decitabine	decitabine (48 h) then decitabine plus HDACi (72 h)	MDA-MB-231	breast carcinoma	0.94	0.773
		MDA-MB-435	breast ductal carcinoma	0.74	1.097
HDACi plus tosedostat	HDACi (24 h), washout then CHR-2797 (72 h)	BxPC-3	pancreatic carcinoma	ND[c]	0.262
		RPMI-7951	malignant melanoma	ND[c]	0.288
		H23	NSCLC	ND[c]	0.780

[a] 5 day erlotinib for DU-145. [b] CI values indicate the degree of synergy according to the following system. CI: >1.1, antagonism; 0.90−1.10, nearly additive; 0.70−0.90, slight to moderate synergism; 0.30−0.70, synergism; 0.10−0.30, strong synergism; <0.10, very strong synergism. [c] Not determined.

Table 9. Cell Cycle Effects of **21f** and **21r**[a]

	HCT116 cells after 15 h of drug treatment (%)			
compd	sub-G1	G1	S	G2/M
vehicle treated	5	36	21	38
21f	23	33	11	33
21r	21	21	10	48
SAHA	19	35	14	32

[a] Human HCT116 colon carcinoma cells were incubated with 300 nM inhibitor. DNA content of nuclei was evaluated using propidium iodide staining followed by flow cytometry analysis, and the number of cells in sub-G1, G1, S, and G2/M phases was calculated as a percentage of control. Results are expressed as percent of total cells, and a representative experiment out of three is shown.

137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, pH 8.0). Compounds (30 μM in 0.3% DMSO) were preincubated for 5 min with the diluted enzyme (10 μg of HeLa cell nuclear extract protein) at room temperature in a 15 μL volume. The reaction was initiated by the addition of the substrate (15 μL, 0.125 mM). After 15 min of incubation at room temperature the developer concentrate (25 μL), which contained 2 μM trichostatin A to stop the reaction, was added and the plate shaken. After the mixture was left to stand for 10 min, the fluorescence signal was read on a Victor II plate reader with excitation at 355 nm and emission at 455 nm. On each assay plate, column 1 contained DMSO alone for the totals and column 12 had buffer added rather than enzyme to generate the blank values. For the IC_{50} determinations, each compound was assayed at eight concentrations in duplicate and the data were analyzed in Graph Pad Prism. Preliminary kinetic assays with the Fluor de Lys substrate produced a K_m of 60 ± 10 μM (Biomol K_m = 54 μM), so all assays were run at a substrate concentration of 62.5 μM. Under these conditions, enzyme activity was linear up to 15 min of incubation and 40 μg of protein. Measurement of inhibitor activity for HDAC isoforms was performed by BPS Biosciences (San Diego, CA) using human recombinant enzymes in a fluorogenic assay kit. Protocols are available at http://www.bpsbioscience.com.

Cellular Proliferation Assays. Cells were seeded in 96-well BD-Falcon plates (Becton Dickinson) at a density of (1−5) × 10^3 cells per well in the appropriate serum-containing culture medium and cultured at 37 °C in a humidified 5% (v/v) CO_2 incubator for 24 h. Samples (50 μL) of different HDAC inhibitor concentrations (0.6−10000 nM), diluted in the relevant culture medium, were transferred to the wells containing the cells for a further 72 h. During the final 4 h of the incubation, cells were pulsed with 10 μL of the tetrazolium salt Cell Proliferation Reagent, WST-1 (Roche). In the presence of viable cells WST-1 is metabolized to formazan product which is measured spectrophotometrically at 450 nm against a background media control (reference wavelength of 650 nm). Antiproliferative effects of HDAC inhibitors on the cells were expressed as percentage inhibition of the vehicle response and were the plotted as the mean ± SD, n = 6 replicates. Results are summarized as GI_{50} values, i.e., the concentration of inhibitor that inhibits 50% of the vehicle response.

Western Blotting and Flow Cytometry. HCT116 cells were seeded at 2 × 10^4 cells/mL and 24 h later treated with the stated doses of compound or vehicle for 6, 15, or 30 h. Samples were harvested on ice and washed twice with ice cold PBS before (a) lysing the pellet in RIPA buffer to prepare SDS−PAGE samples or (b) fixing pellet overnight at 1 × 10^6 cells/mL in 70% ethanol/30% PBS (ice cold) before preparing samples for FACS. Gel samples were loaded at 10 μg/lane on 4−12% acrylamide gels, transferred to nitrocellulose, and stained with primary antibodies to acetylated α-tubulin, acetylated H3 lysine 9 (K9), GAPDH, and PARP. Signal was visualized using ECL and film. FACS samples were prepared by washing twice with PBS before resuspending cells gently in propidium iodide/RNase solution for 30 min before reading the output using a FACScanto. Cells were gated to give populations of sub-G1, G1, S, and G2/M phases. Numbers shown represent the percent of each population from the total number of single cells counted (10 000 per sample).

Thymidine Incorporation Proliferation Assays for Synergy Studies. Cells were seeded in 96-well BD-Falcon plates (Becton Dickinson, Oxford, U.K.) at a density of (1−5) × 10^3 cells per well in the appropriate serum containing culture medium and cultured at 37 °C in a humidified 5% CO_2 incubator for 24 h. Compounds to be tested were serially diluted in the relevant culture medium and then added to the wells for a further 72 h before pulsing the cells for 4 h with 0.4 μCi per well of ^{3}H-methylthymidine (specific activity 1 mCi/mL Amersham Biosciences, U.K.). For synergy experiments, minor dosing modifications were carried out to take account of the necessary scheduling differences required when studying more than one drug combination. Cells were then harvested onto glass fiber filter mats (GF/C Perkin-Elmer LAS, U.K.) using a Tomtec harvester and then counted on a 1450 MicroBeta scintillation counter to determine the amount of radioactive thymidine incorporated into cellular DNA. Data were expressed as a mean percentage ± SD, relative to vehicle response, which measures the amount of ^{3}H-thymidine incorporation into cells in the presence of medium containing 0.1% DMSO and no test compound. Results are calculated as the GI_{50} or concentration of compound that inhibited the vehicle response by 50%.

In Vitro ADME Studies. Microsomal stability studies were performed using published methods.[33] Inhibition studies for CYP2D6 and 3A4 were performed using previously described methods.[34] IC_{50} values for CYP2C19 were determined by Cyprotex (U.K.).

Human Tumour Xenograft Studies. Female CrTac:NCr-*Fox1*-(*nu*) athymic mice 6−8 weeks of age bred in-house at ICR were inoculated bilaterally sc with 2 million human HCT116 colon carcinoma cells. Daily treatment with **21f** (50 mg/kg po) commenced on day 6, when tumors were established (approximately 5 mm mean diameter). Controls received an equal volume of

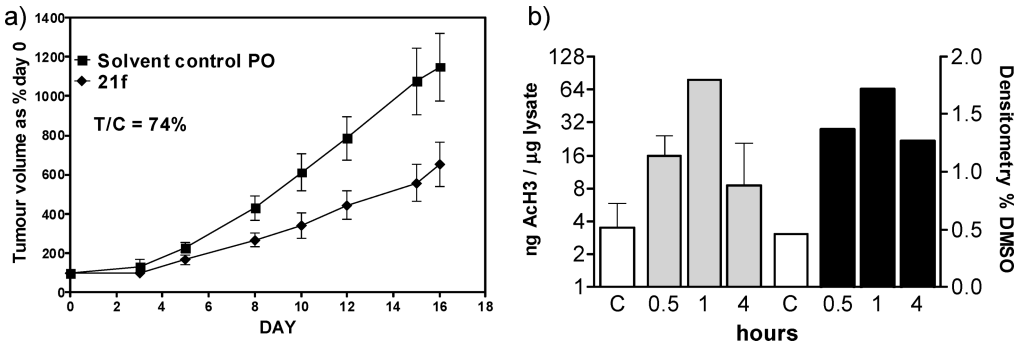


Figure 8. (a) Efficacy of **21f** 50 mg/kg po daily and (b) histone acetylation in HCT116 tumor tissue from mice treated with **21f**. (a) Relative tumor volumes in HCT116 human tumor mouse xenograft study. Compound **21f** (50 mg/kg po in 10% DMSO-saline) was administered for 16 days to female CrTac:NCr-*Fox1*(*nu*) mice bearing established HCT116 colon carcinoma xenografts. (b) Histone acetylation in HCT116 tumor tissue treated with **21f** as in (a). Tumour tissue was obtained, and lysates were prepared 0.5, 1.0, and 4 h following the last dose of **21f**. The samples were analyzed using a quantitative sandwich ELISA with electrochemiluminescent end point (see Supporting Information). Histone acetylation was also measured by Western blot using an antibody to acetylated histone H3 (Abcam) and GAPDH as loading control (see Supporting Information). Gray bars are **21f**-treated samples. Black bars show densitometry results from the Western blot normalized to GAPDH, and white bars represent the samples from vehicle control animals. C = samples from vehicle treated mice.

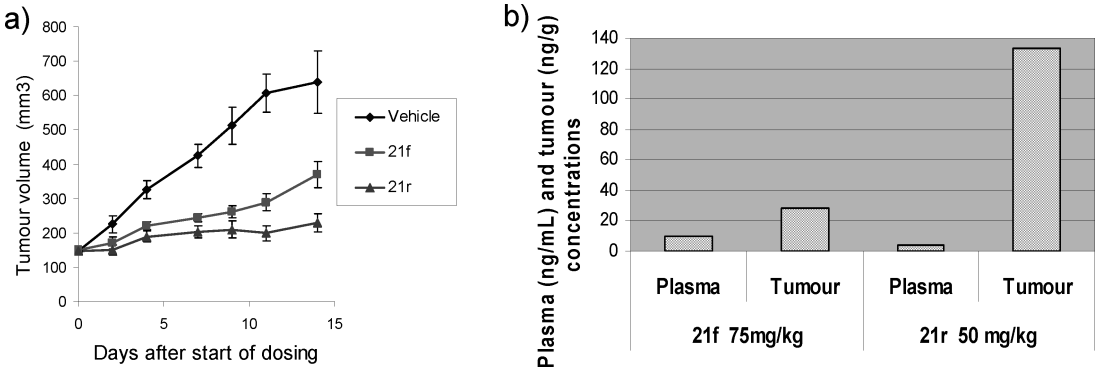


Figure 9. (a) Relative tumor volumes in HCT116 tumor xenografts after treatment with **21f** and **21r**. Compound **21f** (75 mg/kg po) and **21r** (50 mg/kg po) were administered for 14 days to female nude BALB/c mice in 5% DMSO-saline bearing the HCT116 colon carcinoma xenograft (EpiStem Ltd.). (b) Plasma and tumor concentrations of **21f** and **21r**.

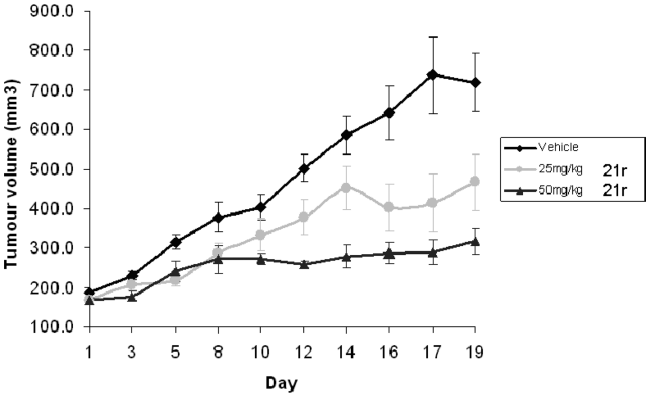


Figure 10. Growth curves of LoVo human colon xenografts after treatment with **21r**. Relative tumor volumes in LoVo tumor xenograft study. Compound **21r** (25 and 50 mg/kg po) was administered for 19 days to female nude BALB/c mice in 5% DMSO-saline bearing the LoVo colon carcinoma xenograft (EpiStem Ltd.).

vehicle alone, 10% DMSO in saline). Tumor measurements and body weights were recorded three times weekly. The study was terminated on day 16, and plasma, spleen, and tumor samples were obtained and stored frozen for later PK and biomarker assays. Additional efficacy studies in HCT116 and LoVo human tumor xenograft bearing mice using **21r** were carried out at Epistem Ltd. (U.K.).

Chemistry General Methods. All nonaqueous reactions were carried out under a nitrogen atmosphere unless otherwise noted. All solvents employed were commercially available HPLC grade. ^{1}H NMR spectra were determined with a Bruker AV spectrometer at 300 MHz. Chemical shifts are reported in parts per million (δ) relative to residual chloroform (7.26 ppm) or dimethylsulfoxide (2.54 ppm) as internal reference with coupling constants (J) reported in hertz (Hz). The peak shapes are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Analytical HPLC/MS was performed on an Agilent HP1100 LC system using reverse phase Luna C18 columns (3 μm, 50 mm $\times$ 4.6 mm), gradient 5−95% B (A = water/0.1% formic acid, B = acetonitrile/0.1% formic acid) over 2.25 min, flow rate of 2.25 mL/min. UV spectra were recorded at 220 and 254 nm using a G1315B DAD detector. Mass spectra were obtained over the range m/z 150−800 on a LC/MSD SL G1956B detector. Data were integrated and reported using ChemStation and ChemStation Data Browser software. The purity of all the above-mentioned compounds was found to be above 95%. Reverse phase HPLC purifications were performed on Gilson preparative systems using reverse phase Axia prep Luna C18 columns (10 μm, 100 mm $\times$ 21.2 mm), gradient 0−100% B (A = water/0.05% TFA, B = acetonitrile/0.05% TFA) over 10 min, flow rate of 25 mL/min, monitored by UV detection at 220 or 254 nm. Thin-layer chromatography (TLC) analysis was performed with Kieselgel 60 F_{254} (Merck) plates and visualized using UV light. The terms "concentrated" and "evaporated" refer to removal of solvents using a rotary

evaporator at water aspirator pressure with a bath temperature equal to or less than 40 °C. Unless otherwise noted, reagents were obtained from commercial sources and were used without further purification.

Modeling and Calculated Properties. Compounds were docked with Glide, version 55211 (Schrodinger Inc., Portland, OR, U.S.) using the SP settings. The protein structure used was PDB code 1T67, which was prepared in MOE, version 2008.1001 (Chemical Computing Group, Montreal, Canada). Initially the software perceived the ligand as having a double bond between the N and O atoms of the hydroxamic acid. This was manually adjusted to a single bond, and the oxygen was assigned a formal negative charge. The Protonate 3D tool was then used to assign the remaining ionization states, add hydrogen atoms, and adjust tautomers and conformers of His, Asn, and Gln residues. This structure was then transferred to Maestro (Schrodinger Inc., Portland, OR, U.S.) where all waters were removed except Wat392 (the 11th one as read from the PDB file). This water appears to play an important role in the ligand binding by bridging between its amide oxygen and His180. The positions of the hydrogen atoms were then refined using the OPLS2005 force field in Maestro's protein preparation wizard before generating a Glide grid for docking purposes. This included a constraint to ensure that the ligands bind to the zinc ion of the active site.

PSA values (Å^2) were calculated using Pipeline Pilot, version 7.01.100 (Accelrys, Cambridge, U.K.). pK_a values were calculated using Structure Designer (ACD Labs, Toronto, Canada).

Method A. Reductive Amination Reactions of Compound (8). Compound **8** (1.2 equiv) was stirred in MeOH at room temperature under N_2, and aldehyde (1 equiv) was added. The resultant solution was stirred for 3 h. After this time, $NaBH_4$ (1.7 equiv) was added and the solution stirred for 10 min. Then 1 M NaOH (3−4 equiv) was added, forming an opaque white solution that was stirred for 20 min. Water was then added and the solution extracted with Et_2O. The combined organic extracts were dried ($MgSO_4$) and solvent was removed in vacuo to give the product which was either used without purification or purified by flash column chromatography.

Method B. Boc Deprotection of N-Substituted *tert*-Butyl 6-Amino-3-azabicyclo[3,1,0]hexane-3-carboxylates. A solution of the Boc-protected 6-aminoazabicyclo[3,1,0]hexane (1 equiv) was stirred in 4 M HCl in dioxane (8 equiv) under N_2 at room temperature for 0.5 h. The solvent was removed under reduced pressure to give the product amine hydrochloride salt which was used without purification.

Method C. Reaction of N-Substituted 6-Amino-3-azabicyclo-[3,1,0]hexanes with Ethyl 2-(Methylthio)pyrimidine-5-carboxylate and Hydrolysis of the Resulting Ethyl 2-{6-Amino]-3-azabicyclo-[3.1.0]hex-3-yl}pyrimidine-5-carboxylates. K_2CO_3 (3 equiv) was added to a stirred suspension of the N-substituted 6-amino-3-azabicyclo[3.1.0]hexane trifluoroacetate (1 equiv) in CH_3CN at room temperature under N_2. A solution of compound **2** (1 equiv) in CH_3CN was added dropwise over 5 min, leading to the formation of a precipitate which was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc, giving a suspension that was washed with water. The precipitate in the organic phase was isolated by filtration, washed with TBME, and air-dried to give the product. The product may be used without purification or purified by flash column chromatography. Aqueous 1 M NaOH (10 equiv) was added to a solution of the resulting product (1 equiv) in THF and MeOH at room temperature. The reaction mixture was stirred at room temperature for 18 h. The organic solvents were removed in vacuo, and the resultant aqueous solution was acidified to pH ∼5 with 1 M aq HCl. The precipitate was isolated by filtration, washed with H_2O, and dried by azeotroping with toluene, giving the product which was used without purification or purified by trituration.

Method D. Hydroxamate Formation Using *O*-(1-Isobutoxyethyl)-hydroxylamine (7) Followed by Deprotection. The pyrimidine-5-carboxylate (1 equiv) was stirred with EDC hydrochloride (1.2 equiv) and HOBt (1.2 equiv) in DMF at room temperature under a nitrogen atmosphere. *O*-(1-Isobutoxyethyl)hydroxylamine **7** (5 equiv) was then added followed by triethylamine (5 equiv) and the mixture left to stir for 16 h. The mixture was then diluted with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, and solvent was removed in vacuo. The residue was purified by column chromatography using an eluent of 0−10% MeOH in CH_2Cl_2 to give the product. The protected hydroxamate product (1 equiv) was stirred in CH_2Cl_2 at room temperature and 4 M HCl in dioxane (2 equiv) added. The mixture was stirred for 10 min and then the solvent removed in vacuo. The residue was purified by preparative HPLC to give the product as a TFA salt.

Ethyl 2-(Methylsulfonyl)pyrimidine-5-carboxylate 2: Step 1. To a suspension of ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate **1** (5.0 g, 21.5 mmol) in THF (25 mL) at 85 °C was added slowly dropwise powdered zinc (7.0 g, 107.7 mmol) in water (25 mL). Following complete dissolution of the zinc powder, *tert*-BuOH (1 mL) was added and heating was continued at 90 °C with vigorous stirring for 18 h. The mixture was cooled to room temperature and filtered through a Celite pad which was washed with further EtOAc (20 mL). The combined filtrates were concentrated, redissolved in EtOAc (30 mL), and washed with water. The EtOAc layer was dried (Na_2SO_4), filtered, and concentrated to dryness and the residue purified by flash column chromatography [eluting with 0−100% EtOAc in hexane] to give ethyl 2-(methylthio)pyrimidine-5-carboxylate as a colorless oil (2.45 g, 57%). m/z 199 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.0 (2H, s), 4.35 (2H, q, J = 7.2 Hz), 2.60 (3H, s), 1.30 (3H, t, J = 7.2 Hz).

Step 2. To a stirred solution of ethyl 2-(methylthio)pyrimidine-5-carboxylate (2.9 g, 14.64 mmol) in dry THF (76 mL) was added 77% mCPBA (11.0 g, 64.4 mmol) at 0 °C under N_2. The reaction mixture was allowed to warm to room temperature, stirred for 2 h before evaporating to dryness. The residue was purified by flash column chromatography [eluting with, 100% hexane followed by 1:5:3 CH_2Cl_2/heptane/Et_2O, followed by 1:1:1 CH_2Cl_2/heptane/Et_2O] to give compound **2** as a white solid (10.0 g, 66%). m/z 231 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.50 (2H, s), 4.40 (2H, q, J = 7.2 Hz), 3.50 (3H, s), 1.40 (3H, t, J = 7.2 Hz).

***tert*-Butyl 6-Aminoazabicyclo[3.1.0]hexane-3-carboxylate 8. Step 1. Preparation of 3-Benzyl-6-nitro-3-azabicyclo[3.1.0]hexane-2,4-dione 4.** To a solution of N-benzylmaleimide (5.0 g, 26.7 mmol) in CH_3CN (334 mL) was added bromonitromethane (1.87 mL, 26.7 mmol). K_2CO_3 (3.69 g, 26.7 mmol) was added and the reaction mixture was vigorously stirred at room temperature. After 4 h an additional portion of bromonitromethane (0.2 mL, 2.8 mmol) was added. Further bromonitromethane portions (0.2 mL, 2.8 mmol) were added at further 4 h intervals (x4). After 48 h the reaction mixture was evaporated to dryness and the residue was purified by column chromatography [eluting with, 100% CH_2Cl_2], giving **4** as a white solid (3.0 g, 42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.35−7.24 (5H, m), 4.52 (2H, s), 4.41 (1H, s,), 3.33 (2H, s).

Step 2. Preparation of 3-Benzyl-6-nitro-3-azabicyclo[3.1.0]hexane 5. To a solution of 3-benzyl-6-nitro-3-azabicyclo[3.1.0]hexane-2,4-dione **4** (3.0 g, 12.2 mmol) in dry THF (30 mL) was added $NaBH_4$ (1.15 g, 30.48 mmol). The mixture was stirred for 15 min at room temperature under N_2. $BF_3 \cdot$THF complex (3.15 mL, 13.4 mmol) was added dropwise and the mixture heated at 40 °C for 4 h. Further $NaBH_4$ (0.15 g, 3.96 mmol) was added followed by $BF_3 \cdot$THF complex (0.32 mL, 1.4 mmol). Heating was continued at 45 °C for 30 min. A mixture of THF/H_2O (1:1 v/v, 60 mL) was added dropwise with stirring. The resulting mixture was heated at 50 °C for 1 h before standing at room temperature for 16 h. The THF was removed under reduced pressure and the resulting aqueous mixture was extracted with EtOAc (3 × 50 mL), dried ($MgSO_4$) and evaporated to dryness to give **5** as a yellow oil (2.47 g, 93%). m/z 219 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.63 (2H, br m), 7.44−7.42 (3H, m), 5.38 (1H, br s), 4.33 (2H, br s), 3.57 (4H, br s), 2.91 (24H, s).

Step 3. Preparation of 6-Nitro-3-azabicyclo[3.1.0]hexane Hydrochloride 6. To a solution of 3-benzyl-6-nitro-3-azabicyclo[3.1.0]-hexane **5** (2.47 g, 11.3 mmol) in 1,2-dichloroethane (5 mL) was added 1-chloroethylchloroformate (1.83 mL, 16.9 mmol) at 0 °C. The reaction mixture was heated to 55 °C for 4 h. Further 1-chloroethylchloroformate (0.5 mL) was added, and heating continued for 2 h. The reaction mixture was then evaporated to dryness. MeOH (15 mL) was added, and the reaction mixture was heated at 65 °C for 3 h. Concentrated HCl (1 mL) was added and the mixture stirred at room temperature for 2 h. A precipitate formed which was isolated by filtration and washed with Et_2O to give **6** as a gray powder (464 mg, 25%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.50 (2H, br s), 4.75 (1H, s), 3.60−3.31 (4H, m), 2.90 (2H, s).

Step 4. Preparation of *tert*-Butyl 6-Nitro-3-azabicyclo[3.1.0]-hexane-3-carboxylate 7. 6-Nitro-3-azabicyclo[3.1.0]hexane hydrochloride **6** (464 mg, 2.82 mmol) was suspended in dry CH_2Cl_2 (10 mL) and cooled to 0 °C. Di-*tert*-butyl dicarbonate (677 mg, 3.10 mmol) was added followed by DMAP (1 crystal) and triethylamine (0.43 mL, 3.10 mmol). The reaction mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure to give a white solid. Purification by flash column chromatography [eluting with 25% EtOAc in heptane) gave **7** as a white solid (592 mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.10 (1H, t), 3.77 (2H, br m), 3.51 (2H, br d), 2.67 (2H, s), 1.46 (9H, s).

Step 5. *tert*-Butyl 6-Aminoazabicyclo[3.1.0]hexane-3-carboxylate 8. *tert*-Butyl 6-nitro-3-azabicyclo[3.1.0]hexane-3-carboxylate **7** (592 mg, 2.59 mmol) was reduced in the presence of 10% Pd/C (20 mg) in EtOH (5 mL) under H_2 (balloon pressure) for 18 h at room temperature. The catalyst was removed by filtration through Celite washing with EtOH and the filtrate concentrated to give **8** as a pale yellow oil (487 mg, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.54 (1H, d, J = 10.6 Hz), 3.51 (1H, d, J = 10.7 Hz), 3.38−3.32 (2H, br m), 2.12 (1H, t, J = 0.8 Hz), 2.01−1.93 (2H,br m), 1.49 (2H, t, J = 10.5 Hz), 1.44 (9H, s).

6-Fluoroquinoline-2-carbaldehyde 10. 6-Fluoro-2-methylquinoline (1.0 g, 6.2 mmol) was stirred in dioxane (50 mL) at room temperature under a nitrogen atmosphere. SeO_2 (0.86 g, 7.4 mmol) was added and the mixture heated to 100 °C for 1 h, during which time the mixture changed from an opaque brown color to a clear dark red solution. The mixture was cooled to room temperature, filtered and the filtrate concentrated in vacuo. The residue was purified by column chromatography [eluting with 0−3% MeOH in CH_2Cl_2] to give the product as an off-white solid (944 mg, 87%). m/z 239 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.11 (1H, s), 8.60 (1H, d, J = 11.0 Hz), 8.32 (1H, dd, J = 7.6, 11.0 Hz), 8.04 (1H, d, J = 11.0 Hz), 7.96 (1H, dd, J = 4.4, 11.0 Hz), 7.85 (1H, td, J = 4.4, 11.0 Hz).

***N*-Hydroxy-2-{6-[(2-naphthylsulfonyl)amino]-3-azabicyclo[3.1.0]-hex-3-yl}pyrimidine-5-carboxamide Trifluoroacetate 15a: Step 1. 6-[(2-Naphthylsulfonyl)amino]-3-azabicyclo[3.1.0]hexane Trifluoroacetate 13a.** 2-Naphthalenesulfonyl chloride (20.07 g, 97.39 mmol) was added to a solution of *tert*-butyl 6-aminoazabicyclo-[3.1.0]hexane-3-carboxylate **5** (1.69 g, 8.54 mmol) and triethylamine (24.7 mL, 177.07 mmol) in anhydrous CH_2Cl_2 (270 mL) at 0 °C under N_2, giving a light brown solution which was allowed to warm to room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and saturated $NaHCO_3$ (200 mL). The organic phase was separated and washed with H_2O (2 × 200 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo to give a pale yellow oil. Trituration with 5% TBME in heptane (50 mL) gave a solid which was isolated by filtration and washed with heptane to afford **13a** as a white solid (27.99 g, 82%). m/z 389 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.47 (1H, m), 8.02−7.99 (2H, m), 7.93 (1H, d, J = 7.9 Hz), 7.85 (1H, m), 7.68−7.62 (2H, m), 5.12 (1H, br s), 3.56−3.41 (2H, m), 3.34−3.29 (2H, m), 1.96 (1H, m), 1.82 (2H, m), 1.35 (9H, s). A solution of *tert*-butyl 6-[(2-naphthylsulfonyl)amino]-3-azabicyclo[3.1.0]hexane-3-carboxylate (27.50 g, 68.43 mmol) in 20% TFA in CH_2Cl_2 (300 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and then CH_2Cl_2 (100 mL) was added and re-evaporated in vacuo three times to remove excess TFA, giving **13a** as the trifluoroacetate salt (crude yield 34.0 g). This product was used in the next step without purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.20 (1H, br s), 8.48 (1H, s), 8.33 (1H, br s), 8.23−8.18 (3H, m), 8.08 (1H, d, J=8.7 Hz), 7.83 (1H, d, J=7.9 Hz), 7.75−7.70 (2H, m), 3.22 (4H, m), 2.20 (1H, m), 1.86 (2H, s).

Step 2. Preparation of 2-{6-[(2-Naphthylsulfonyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxylic Acid 14a. Following method C, K_2CO_3 (28.33 g, 205.29 mmol) was added to a stirred suspension of 6-[(2-naphthylsulfonyl)amino]-3-azabicyclo[3.1.0]-hexane trifluoroacetate **13a** (27.50 g, 68.43 mmol) in CH_3CN (300 mL) at room temperature under N_2. A solution of compound **2** (15.74 g, 68.43 mmol) in CH_3CN (50 mL) was added dropwise over 5 min, leading to the formation of a precipitate which was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc (250 mL), giving a suspension which was washed with water (2 × 250 mL). The precipitate in the organic phase was isolated by filtration, washed with TBME, and air-dried to give ethyl 2-{6-[(2-naphthylsulfonyl)amino]-3-azabicyclo-[3.1.0]hex-3-yl}pyrimidine-5-carboxylate as a white solid (25.40 g, 85%). m/z 439 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.70 (2H, s), 8.50 (1H, m), 8.22−8.15 (3H, m), 8.04 (1H, d, J = 8.3 Hz), 7.84 (1H, m), 7.67−7.71 (2H, m), 4.23 (2H, q, J = 7.3 Hz)), 3.70−3.67 (2H, m), 3.53−3.50 (2H, m), 1.90 (1H, m), 1.82 (2H, s), 1.26 (3H, t, J = 7.3 Hz). Aqueous 1 M NaOH (500 mL) was added to a solution of ethyl 2-{6-[(2-naphthylsulfonyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxylate (25.40 g, 58.00 mmol) in THF (500 mL) and MeOH (100 mL) at room temperature, and the reaction mixture was stirred for 18 h. The organic solvents were removed in vacuo, and the resultant aqueous solution was acidified to pH 5 with 1 M aqueous HCl. A heavy white precipitate was formed which was isolated by filtration, washed with H_2O, and dried by azeotroping with toluene to give 2-{6-[(2-naphthylsulfonyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxylic acid **14a** as a white solid (22.19 g, 93%). m/z 411 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.83 (2H, s), 8.66 (1H, s), 8.36−8.31 (3H, m), 8.21 (1H, d, J = 8.7 Hz), 8.01 (1H, m), 7.88−7.84 (2H, m), 3.89−3.82 (2H, m), 3.67−3.64 (2H, m), 2.05 (1H, s), 1.97 (2H, s).

Step 3. Preparation of *N*-Hydroxy-2-[6-(naphthalene-2-sulfonylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxamide 15a. Following method D, EDC hydrochloride (7.27 g, 37.9 mmol) was added to a suspension of 2-{6-[(2-naphthylsulfonyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxylic acid **14a** (22.19 g, 54.12 mmol) in anhydrous CH_2Cl_2 (100 mL) and anhydrous THF (500 mL) at room temperature under N_2. Triethylamine (22.6 mL, 162.14 mmol) was added followed by HOBt (8.78 g, 64.97 mmol) and *O*-(1-isobutoxyethyl)hydroxylamine **7** (8.9 mL, 64.89 mmol). After being stirred at room temperature for 6 days, the reaction mixture was re-treated with EDC hydrochloride (10.25 g, 53.47 mmol), HOBt (7.22 g, 53.43 mmol), and triethylamine (19.4 mL, 139.0 mmol). After being stirred for another 3 days at room temperature, the reaction mixture was evaporated to dryness and suspended in EtOAc (100 mL) and water (100 mL). A white solid was collected by filtration, washed with EtOAc (50 mL), water (50 mL), MeOH (50 mL), and dried in vacuo to give a white solid. This was stirred in EtOH (1500 mL) at 60 °C for 1 h, giving a white suspension which was cooled to room temperature, filtered, and air-dried to give *N*-(1-isobutoxyethoxy)-2-[6-(naphthalene-2-sulfonylamino)-3-azabicyclo-[3.1.0]hex-3-yl]pyrimidine-5-carboxamide **15a** as a white solid (24.0 g, 87%). m/z 511 $[M + H]^+$. A solution of 4 M HCl in dioxane (350 mL, 1.4 mol) was added in portions over 5 min to *N*-(1-isobutoxyethoxy)-2-[6-(naphthalene-2-sulfonylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxamide (10.0 g, 19.65 mmol) at room temperature with vigorous stirring under N_2. After 2 h CH_2Cl_2 (20 mL) was added followed by another portion of 4 M HCl in dioxane (20 mL) and stirring was continued for a further 2.5 h. The reaction mixture was evaporated in vacuo to ∼200 mL volume, and CH_2Cl_2 (200 mL) was added in portions effecting precipitation.

The resultant white precipitate was isolated by filtration and washed with CH_2Cl_2 (5 mL), giving **15a** as a white powder (6.25 g, 75%). *m/z* 426 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 11.02 (1H, br s), 8.97 (1H, br s), 8.58 (2H, s), 8.50 (1H, s), 8.24−8.10 (3H, m), 8.05 (1H, d, J = 8.8 Hz), 7.83 (1H, dd, J = 1.8, 8.8 Hz), 7.80−7.65 (2H, m), 3.65 (2H, d, J = 12.0 Hz), 3.45 (2H, d, J = 12.0 Hz), 3.20 (1H, s), 1.80 (1H, s). Anal. Calcd for $C_{21}H_{26}ClN_5O_6S$: C, 49.27; H, 5.12; Cl, 6.92; N, 13.68. Found: C, 49.70; H, 5.09; Cl, 7.10; N, 13.55.

***N*-Hydroxy-2-{6-[(naphthalene-2-carbonyl)amino]-3-azabicyclo-[3.1.0]hex-3-yl}-pyrimidine-5-carboxamide 18a.** To a stirred solution of *tert*-butyl 6-aminoazabicyclo[3.1.0]hexane-3-carboxylate **8** (300 mg, 1.51 mmol) in pyridine (1 mL) at 0 °C was added 2-naphthoyl chloride (316 mg, 1.66 mmol). The mixture was stirred for 1 h, and water (8 mL) was then added. The resulting precipitate was collected by filtration and dried under vacuum to afford *tert*-butyl 6-(2-naphthoylamino)-3-azabicyclo[3.1.0]hexane-3-carboxylate (406 mg, 76%). ^1H NMR (300 MHz, C*D*Cl$_3$) δ: 8.26 (1H, s), 7.88−7.95 (3H, m), 7.81 (1H, d, J = 1.8, 8.8 *Hz*), 7.60−7.54 (2H, m), 6.37 (1H, br s), 3.82 (2H, d, J = 11.4 Hz), 3.50−3.40 (2H, m), 2.71 (1H, d), 1.85 (2H, s), 1.47 (9H, s). Following method B, *tert*-butyl 6-(2-naphthoylamino)-3-azabicyclo[3.1.0]hexane-3-carboxylate (406 mg, 1.15 mmol) was stirred in 4 M HCl in dioxane (10 mL) to give *N*-(3-azabicyclo[3.1.0]hex-6-yl)naphthalene-2-carboxamide hydrochloride **16a** as a pink solid which was carried forward without purification. *m/z* 353 $[M + H]^+$. Following method C, compound **16a** (1.15 mmol), compound **2** (350 mg, 1.15 mmol), and K_2CO_3 (3.9 g, 28.75 mmol) gave ethyl 2-[6-(2-naphthoylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylate (343 mg, 74% over two steps). *m/z* 403 $[M + H]^+$. Ethyl 2-[6-(2-naphthoylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylate (343 mg, 0.85 mmol) was hydrolyzed with 6 M NaOH (5 mL, 30 mmol) to give 2-[6-(2-naphthoylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylic acid **17a** (167 mg, 52%) which was carried forward without purification. *m/z* 375 $[M + H]^+$. Following method D, 2-[6-(2-naphthoylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylic acid **17a** (167 mg, 0.45 mmol), HOBt (83 mg, 0.54 mmol), EDC hydrochloride (103 mg, 0.54 mmol), triethylamine (314 μL, 2.25 mmol), and *O*-(1-isobutoxyethyl)hydroxylamine (309 μL, 2.25 mmol) gave *N*-(1-isobutoxyethoxy)-2-[6-(2-naphthoylamino)-3-azabicyclo-[3.1.0]hex-3-yl]pyrimidine-5-carboxamide (58 mg, 26%). *m/z* 490 $[M + H]^+$. *N*-(1-Isobutoxyethoxy)-2-[6-(2-naphthoylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxamide (58 mg, 0.12 mmol) was stirred in 4 M HCl in dioxane (60 mL, 0.29 mmol) at room temperature for 5 min. The solvent was removed in vacuo and the residue dried under reduced pressure to give *N*-hydroxy-2-{6-[(naphthalene-2-carbonyl)amino]-3-aza-bicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxamide **18a** (27 mg, 59%). *m/z* 390 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 11.05 (1H, br s), 9.01 (1H, br s), 8.74 (1H, d, J = 3.9 Hz), 8.69 (2H, s), 8.43 (1H, br s), 8.05−7.55 (4H, m), 7.55−7.66 (2H, m), 3.95 (2H, d, J = 11.6 Hz), 3.70−3.60 (2H, m), 2.70−2.64 (1H, m), 2.04 (2H, br s).

***N*-Hydroxy-2-{6-[(2-naphthylmethyl)amino]-3-azabicyclo[3.1.0]-hex-3-yl}pyrimidine-5-carboxamide 21a.** Following method A, *tert*-butyl 6-aminoazabicyclo[3.1.0]hexane-3-carboxylate **8** (200 mg, 1.01 mmol), 2-naphthaldehyde (148 mg, 0.96 mmol), and sodium borohydride (61 mg, 1.62 mmol) gave *tert*-butyl 6-(naphth-2-ylmethyl)-3-azabicyclo[3.1.0]hexane-3-carboxylate as a colorless oil which was used in the subsequent step without purification. *m/z* 339 $[M + H]^+$. Following method B, *tert*-butyl 6-(naphth-2-ylmethyl)-3-azabicyclo-[3.1.0]hexane-3-carboxylate (0.96 mmol) was stirred in 4 M HCl (2 mL) in dioxane at room temperature for 0.5 h to give *N*-naphth-2-ylmethyl-3-azabicyclo[3.1.0]hexan-6-amine hydrochloride **19a** which was used in the next step without purification, *m/z* 239 $[M + H]^+$. Following method C, *N*-naphth-2-ylmethyl-3-azabicyclo[3.1.0]-hexan-6-amine hydrochloride (0.96 mmol), ethyl 2-(methylsulfonyl)pyrimidine-5-carboxylate **2** (221 mg, 0.96 mmol), and potassium carbonate (1.32 g, 9.6 mmol) gave ethyl 2-[6-(naphth-2-ylmethyl)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylate as a yellow oil which was used in the next step without purification. *m/z* 389 $[M + H]^+$. Ethyl 2-[6-(naphth-2-ylmethyl)-3-azabicyclo-[3.1.0]hex-3-yl]pyrimidine-5-carboxylate (0.96 mmol) was hydrolyzed with 1 M NaOH (10 mL, 10 mmol) in THF (10 mL) to give 2-[6-(naphth-2-ylmethyl)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylic acid **20a** (70 mg, 19% over 4 steps) as a colorless solid which was used in the subsequent step without purification. *m/z* 361 $[M + H]^+$. Following method D, 2-{6-[(2-naphth-2-ylmethyl)-amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxylic acid (70 mg, 0.194 mmol) was stirred with EDC hydrochloride (56 mg, 0.29 mmol) and HOBt (39 mg, 0.29 mmol), triethylamine (135 μL, 0.97 mmol), and *O*-(1-isobutoxyethyl)hydroxylamine (270 μL, 1.94 mmol). In this case after quenching the reaction with water (10 mL) the layers were separated and the aqueous layer was re-extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with 0.5 M HCl and the organic layer was dried ($MgSO_4$) and evaporated in vacuo to give *N*-hydroxy-2-{6-[(2-naphthylmethyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxamide **21a** (15 mg, 20%). *m/z* 376 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 11.10 (1H, br s), 9.61 (1H, br s), 9.01 (1H, br s), 8.66 (2H, s), 8.07 (1H, s), 7.97 (3H, m), 7.67 (1H, dd, J = 8.4, 1.8 Hz), 7.57 (2H, m), 4.41 (2H, s), 3.80 (2H, d, J = 11.7 Hz), 3.56 (2H, d, J = 11.7 Hz), 2.57 (1H, m), 2.22 (2H, br s).

2-{6-[(4-Chlorobenzyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}-*N*-hydroxypyrimidine-5-carboxamide Bistrifluoroacetate 21f. Following method A, *tert*-butyl 6-aminoazabicyclo[3.1.0]hexane-3-carboxylate **8** (200 mg, 1.01 mmol), 4-chlorobenzaldehyde (135 mg, 0.96 mmol), and sodium borohydride (36 mg, 0.96 mmol) gave *tert*-butyl 6-(4-chlorobenzylamino)-3-azabicyclo[3.1.0]hexane-3-carboxylate which was used in the subsequent step without purification. *m/z* 323/325 $[M + H]^+$. Following method B, *tert*-butyl 6-(4-chlorobenzylamino)-3-azabicyclo[3.1.0]hexane-3-carboxylate (0.96 mmol) was stirred in 4 M HCl in dioxane (2 mL) at room temperature for 0.5 h to give *N*-(4-chlorobenzyl)-3-azabicyclo-[3.1.0]hexan-6-amine hydrochloride **19f** which was used in the next step without purification. *m/z* 223/225 $[M + H]^+$. Following method C, *N*-6-(4-chlorobenzyl)-3-azabicyclo[3.1.0]hexan-6-amine hydrochloride **19f** (0.96 mmol), ethyl 2-(methylsulfonyl)pyrimidine-5-carboxylate **2** (221 mg, 0.96 mmol), and potassium carbonate (1.32 g, 9.6 mmol) gave ethyl 2-{6-[(4-chlorobenzyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxylate as a yellow oil which was used in the next step without purification. *m/z* 373/375 $[M + H]^+$. Ethyl 2-{6-[(4-chlorobenzyl)amino]-3-azabicyclo[3.1.0]-hex-3-yl}pyrimidine-5-carboxylate (0.96 mmol) was hydrolyzed with 1 M NaOH (10 mL, 10 mmol) in THF (10 mL) to give 2-{6-[(4-chlorobenzyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxylic acid **20f** (166 mg, 50% over four steps) as a colorless solid which was used in the subsequent step without purification. *m/z* 345/347 $[M + H]^+$. Following method D, 2-{6-[(4-chlorobenzyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxylic acid **20f** (122 mg, 0.14 mmol), EDC hydrochloride (121 mg, 0.63 mmol), HOBt (81 mg, 0.63 mmol), *O*-(1-isobutoxyethyl)hydroxylamine (373 μL, 2.62 mmol), and triethylamine (365 μL, 2.62 mmol) gave 2-{6-[(4-chlorobenzyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}-*N*-(1-isobutoxyethoxy)pyrimidine-5-carboxamide (128 mg, 58%) after purification by flash column chromatography [eluting with 0−10% MeOH/CH_2Cl_2]. *m/z* 460/462 $[M + H]^+$. 2-{6-[(4-chlorobenzyl)amino]-3-azabicyclo[3.1.0]-hex-3-yl}-*N*-(1-isobutoxyethoxy)pyrimidine-5-carboxamide (128 mg, 0.28 mmol) was stirred with TFA (0.5 mL) in 1:1 v/v CH_2Cl_2/MeOH (4 mL) overnight. The solvent was removed in vacuo and the residue purified by preparative HPLC to give 2-{6-[(4-chlorobenzyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}-*N*-hydroxypyrimidine-5-carboxamide bistrifluoroacetate **21f** (41 mg, 25%) as a colorless solid. *m/z* 360/362 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 11.07 (1H, br s), 9.26 (1H, br s), 9.04 (1H, br s), 8.67 (2H, s), 7.54 (4H, m), 4.27 (2H, s), 3.71 (2H, d, J = 11.7 Hz), 3.57 (2H, d, J = 11.7 Hz) 2.57 (1H, br s), 2.13 (2H, br s).

2-(6-{[(6-Fluoroquinolin-2-yl)methyl]amino}bicyclo[3.1.0]hex-3-yl)-*N*-hydroxypyrimidine-5-carboxamide Trifluoroacetate 21r.

Following method A, 6-fluoroquinoline-2-carbaldehyde **10** (265 mg, 1.51 mmol) was stirred in MeOH (10 mL) with *tert*-butyl 6-amino-3-azabicyclo[3,1,0]hexane-3-carboxylate **8** (300 mg, 1.51 mmol) at room temperature for 3 h. $NaBH_4$ (91 mg, 2.42 mmol) was then added in one portion and stirring continued for 10 min. The reaction was then carefully quenched with 1 M NaOH (5 mL), and stirring continued for 20 min. The mixture was then diluted with H_2O (50 mL) and extracted with Et_2O (2 × 100 mL). The combined organic layers were dried over $MgSO_4$ and solvent was removed in vacuo to give *tert*-butyl 6-{[(6-fluoroquinolin-2-yl)methyl]amino}-3-azabicyclo[3.1.0]hexane-3-carboxylate as a colorless oil which was used in the next step without purification. m/z 358 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 8.30 (1H, d, J = 8.7 Hz), 8.02 (1H, m), 7.79−7.61 (3H, m), 4.72 (1H, d, J = 6.5 Hz), 3.98 (2H, s), 1.78 (1H, s), 1.50 (2H, s), 1.31 (9H, s). Following method B, *tert*-butyl 6-{[(6-fluoroquinolin-2-yl)methyl]-amino}-3-azabicyclo[3.1.0]hexane-3-carboxylate (1.51 mmol) was stirred in 4 M HCl in dioxane (3 mL) at room temperature for 30 min. Solvent was then removed in vacuo and the residue dried to give *N*-[(6-fluoroquinolin-2-yl)methyl]-3-azabicyclo[3.1.0]hexan-6-amine hydrochloride **19r** which was used in the next step without purification. m/z 258 $[M^+ + H]$. Following method C, *N*-[(6-fluoroquinolin-2-yl)methyl]-3-azabicyclo[3.1.0]hexan-6-amine hydrochloride **19r** (1.51 mmol) was stirred in a 1:1 v/v DMF/CH_3CN (20 mL) with K_2CO_3 (2.1 g, 15.1 mmol) at room temperature under a nitrogen atmosphere for 10 min. Ethyl 2-(methylsulfonyl)pyrimidine-5-carboxylate **2** (350 mg, 1.51 mmol) was then added and the mixture stirred for a further 10 min. The reaction was the quenched with H_2O (50 mL) and extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over $MgSO_4$ and solvent was removed in vacuo to give the ethyl 2-(6-{[(6-fluoroquinolin-2-yl)methyl]amino}-3-azabicyclo[3.1.0]hex-3-yl)pyrimidine-5-carboxylate as an orange oil which was used in the next step without purification. m/z 408 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 8.71 (2H, s), 8.32 (1H, d, J = 9.8 Hz), 8.07−7.95 (2H, m), 7.81−7.71 (2H, m), 4.72 (1H, d, J = 7.6 Hz), 4.23 (2H, q, J = 8.7 Hz), 3.53 (2H, d, J = 12 Hz), 3.33 (2H, s), 1.88 (1H, br s), 1.71 (2H, br s), 1.24 (3H, t, J = 8.7 Hz). Ethyl 2-(6-{[(6-fluoroquinolin-2-yl)methyl]-amino}-3-azabicyclo[3.1.0]hex-3-yl)pyrimidine-5-carboxylate was stirred in 1 M NaOH/THF (7 mL/7 mL) at room temperature for 16 h. The mixture was the acidified to pH 3 with 1 M HCl which caused a precipitate to form. This was filtered off and dried to give 2-(6-{[(6-fluoroquinolin-2-yl)methyl]amino}-3-azabicyclo[3.1.0]-hex-3-yl)pyrimidine-5-carboxylic acid **20r** (193 mg, 34% over three steps) as a light yellow solid and used without purification. m/z 380 $[M + H]^+$. Following method D, 2-(6-{[(6-fluoroquinolin-2-yl)methyl]amino}-3-azabicyclo[3.1.0]hex-3-yl)pyrimidine-5-carboxylic acid **20r** (193 mg, 0.51 mmol) was stirred with EDC hydrochloride (117 mg, 0.61 mmol) and HOBt (117 mg, 0.61 mmol) in DMF (10 mL) at room temperature under a nitrogen atmosphere. *O*-(1-Isobutoxyethyl)hydroxylamine (352 μL, 2.55 mmol) was then added followed by triethylamine (355 μL, 2.55 mmol). The mixture was stirred for 16 h, then diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried over $MgSO_4$, and solvent was removed in vacuo. The residue was purified by flash column chromatography [eluting with 0−10% MeOH in CH_2Cl_2] to give 2-(6-{[(6-fluoroquinolin-2-yl)methyl]-amino}-3-azabicyclo[3.1.0]hex-3-yl)-*N*-(1-isobutoxyethoxy)pyrimidine-;5-carboxamide (130 mg, 52%) as a light yellow oil. m/z 495 $[M + H]$. ^1H NMR (300 MHz, DMSO-d_6) δ: 8.69 (2H, s), 8.30 (1H, d, J = 9.8 Hz), 8.02 (1H, dd, J = 6.5, 9.8 Hz), 7.74 (1H, dd, J = 3.3, 9.8 Hz), 7.67−7.60 (2H, m), 4.90 (1H, q, J = 5.4, 9.7 Hz), 4.01 (2H, br s), 3.63 (2H, s), 3.49 (2H, d, J = 10.1 Hz), 1.87 (1H, br s), 1.70 (2H, br s), 1.30 (3H, d, J = 5.4 Hz), 0.85 (3H, s), 0.83 (3H, s). 2-(6-{[(6-Fluoroquinolin-2-yl)methyl]amino}-3-azabicyclo[3.1.0]hex-3-yl)-*N*-(1-isobutoxyethoxy)pyrimidine-5-carboxamide (130 mg, 0.26 mmol) was stirred in CH_2Cl_2 (2 mL) at room temperature, and 4 M HCl in dioxane (130 μL, 0.52 mmol) added. The mixture was stirred for 10 min and then the solvent removed in vacuo. The residue was purified by preparative HPLC to give 2-(6-{[(6-fluoroquinolin-2-yl)methyl]amino}bicyclo[3.1.0]hex-3-yl)-*N*-hydroxypyrimidine-5-carboxamide trifluoroaceate **21r** as a light orange solid (25 mg, 19%). m/z 395 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 11.09 (1H, br s), 9.61 (1H, br s), 9.01 (1H, br s), 8.67 (2H, s), 8.48 (1H, d, J = 8.4 Hz), 7.88 (1H, dd, J = 9.3 Hz, 2.7 Hz), 7.75 (1H, td, J = 8.7 Hz, 3.0 Hz), 7.66 (1H, d, J = 8.4 Hz), 4.69 (2H, bs), 3.88 (2H, d, J = 11.7 Hz), 3.60 (2H, d, J = 11.7 Hz), 2.30 (2H, s,), 2.75 (1H, s).

N-Hydroxy-2-{6-[methyl(naphthalene-2-sulfonyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxamide 23: Step 1. Preparation of *N*-(Tetrahydro-2*H*-pyran-2-yloxy)-2-{6-[(naphthalene-2-sulfonyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxamide 22. 2-{6-[(2-Naphthylsulfonyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxylic acid **14a** (1.0 g, 2.4 mmol) was stirred with EDC hydrochloride (556 mg, 2.9 mmol) and HOBt (392 mg, 2.9 mmol) in DMF at room temperature under a nitrogen atmosphere. 2-(Tetrahydro-2*H*-pyran-2-yl)hydroxylamine (1.4 g, 12 mmol) was then added followed by triethylamine (1.66 mL, 12 mmol), and the mixture was left to stir for 16 h. The mixture was then diluted with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), and solvent was removed in vacuo. The residue was purified by flash column chromatography [eluting with 0−10% MeOH in CH_2Cl_2] to give **22** (1.11 g, 90%). m/z 511 $[M + H]^+$.

Step 2. Preparation of *N*-(Tetrahydro-2*H*-pyran-2-yloxy)-2-{6-[methyl(naphthalene-2-sulfonyl)amino]-3-azabicyclo[3.1.0]-hex-3-yl}pyrimidine-5-carboxamide. NaH (147 mg, 3.75 mmol) was washed with heptanes and suspended in THF (10 mL). *N*-(Tetrahydro-2*H*-pyran-2-yloxy)-2-[6-(naphthalene-2-sulfonylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxamide **22** (1.11 g, 2.2 mmol) was then added, followed by addition of dimethyl sulfate (0.22 mL, 2.3 mmol). The mixture was stirred for 48 h and then poured into CH_2Cl_2 (150 mL), and 2.4 M ammonium chloride (50 mL) was added. The aqueous layer was further extracted with CH_2Cl_2 (150 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to give an orange oil. This was carried forward without purification.

Step 3. Preparation of *N*-Hydroxy-2-{6-[methyl(naphthalene-2-sulfonyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxamide 23. To *N*-(tetrahydro-2H-pyran-2-yloxy)-2-{6-[methyl-(naphthalene-2-sulfonyl)amino]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxamide was added TFA/CH_2Cl_2/MeOH (5 mL, 1:1:1 v/v/v mixture). The solution was stirred at room temperature for 2 h. The mixture was then concentrated under reduced pressure and purified by reverse phase HPLC to yield **23** (34 mg, 3% yield over two steps). m/z 440 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 11.05 (1H, br s), 9.01 (1H, br s), 8.61 (2H, s), 8.52−8.49 (1H, m), 8.28−8.22 (1H, m), 8.18 (1H, d, J = 8.7 Hz), 8.06 (1H, m), 7.82 (1H, dd, J = 1.8, 8.6 Hz), 7.66−7.84 (2H, m), 3.78 (2H, d, J = 11.8 Hz), 3.58 (2H, m), 2.77 (3H, s), 2.25 (2H, m), 1.55 (1H, m).

N-Hydroxy-2-[6-(quinolin-2-ylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxamide 25: Step 1. Preparation of *tert*-Butyl 6-(quinolin-2-ylamino)-3-azabicyclo[3.1.0]hexane-3-carboxylate 24. *tert*-Butyl 6-aminoazabicyclo[3.1.0]hexane-3-carboxylate **8** (200 mg, 1.01 mmol) was combined with 2-chloroquinoline (328 mg, 2.02 mmol), and the two solids melted at 100 °C for 16 h. The mixture was then cooled and the residue purified by flash column chromatography [eluting with, 0−3% MeOH/CH_2Cl_2] to give compound **24** as a brown oil (320 mg, 97%). m/z 326 $[M + H]^+$.

Step 2. Preparation of (3-Azabicyclo[3.1.0]hex-6-yl)quinolin-2-yl-amine. Following method B, *tert*-butyl 6-(quinolin-2-ylamino)-3-azabicyclo[3.1.0]hexane-3-carboxylate (320 mg, 0.98 mmol) was stirred in 4 M HCl in dioxane (2 mL) at room temperature under N_2 for 15 min. The solvent was then removed in vacuo and the residue dried under high-vacuum to give crude (3-azabicyclo-[3.1.0]hex-6-yl)quinolin-2-yl-amine (220 mg) which was used in the next step without purification. m/z 226 $[M + H]^+$.

Step 3. Preparation of Ethyl 2-[6-(Quinolin-2-ylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylate. (3-Azabicyclo[3.1.0] hex-6-yl)quinolin-2-ylamine (220 mg, 0.98 mmol) was stirred in

CH_3CN (10 mL) and DMF (10 mL) at room temperature under N_2. Potassium carbonate (1.35 g, 9.8 mmol) was then added and the mixture stirred for 15 min. Ethyl 2-(methylsulfonyl)pyrimidine-5-carboxylate **2** (227 mg, 0.98 mmol) was then added to the reaction mixture, and stirring continued for 30 min. The mixture was then diluted with H_2O (100 mL) and extracted twice with EtOAc (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and solvent was removed in vacuo to give ethyl 2-[6-(quinolin-2-ylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylate as a light brown solid which was used in the next step without purification. m/z 376 $[M + H]^+$.

Step 4. Preparation of 2-[6-(Quinolin-2-ylamino)-3-azabicyclo-[3.1.0]hex-3-yl]pyrimidine-5-carboxylic Acid. Ethyl 2-[6-(quinolin-2-ylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylate (0.98 mmol) was stirred in THF (10 mL) and H_2O (10 mL) at room temperature for 64 h. The mixture was then acidified to pH 3 using 1 M HCl and the solvent removed in vacuo to give a brown solid. The solid was collected and washed with a little H_2O to give the title compound as a brown solid (103 mg, 30% over three steps). m/z 348 $[M + H]^+$.

Step 5. Preparation of *N*-(1-Isobutoxyethoxy)-2-[6-(quinolin-2-ylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxyamide. 2-[6-(Quinolin-2-ylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylic acid (103 mg, 0.29 mmol) was stirred in DMF (10 mL) at room temperature under a nitrogen atmosphere. EDC hydrochloride (67 mg, 0.35 mmol) and HOBt (47 mg, 0.35 mmol) were added, and the mixture was stirred for 10 min. *O*-(1-Isobutoxyethyl)hydroxylamine (200 μL, 1.45 mmol) and triethylamine (202 μL, 1.45 mmol) were then added, and the mixture was stirred for 16 h. The mixture was then diluted with H_2O (100 mL) and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$), and solvent was removed in vacuo. The residue was purified by column chromatography [eluting with 0−10% MeOH/CH_2Cl_2] to give the title compound as a white solid (48 mg, 36%). m/z 463 $[M + H]^+$.

Step 6 Preparation of *N*-Hydroxy-2-[6-(quinolin-2-ylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxamide 25. *N*-(1-Isobutoxyethoxy)-2-[6-(quinolin-2-ylamino)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxyamide (48 mg, 0.1 mmol) was stirred in CH_2Cl_2 (2 mL) at room temperature under a nitrogen atmosphere. Then 4 M HCl in dioxane (20 μL, 0.2 mmol) was added, immediately causing a solid to precipitate. The mixture was stirred for 10 min and then solvent removed in vacuo. CH_2Cl_2 (10 mL) was added to the residue and the precipitate filtered and dried to give compound **25** as a white solid (21 mg, 58%). m/z 363 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 12.96 (1H, br s), 11.12 (1H, br s), 10.24 (1H, br s), 8.33 (1H, d, J = 8.8 Hz), 8.28 (2H, s), 8.09 (1H, d, J = 8.0 Hz), 7.94 (1H, d, J = 8.0 Hz), 7.81 (1H, t, J = 7.6 Hz), 7.53 (1H, t, J = 7.6 Hz), 7.15 (1H, d, J = 8.8 Hz), 4.27 (2H, d, J = 11.7 Hz), 3.63 (2H, d, J = 11.7 Hz), 2.99 (1H, m), 2.19 (2H, br s).

***N*-Hydroxy-2-{6-[(naphthalen-2-ylmethylamino)methyl]-3-azabicyclo[3.1.0]hex-3-ylpyrimidine-5-carboxamide 27: Step 1. Preparation of Ethyl 2-(6-{[(*tert*-Butoxycarbonyl)amino]methyl}-3-azabicyclo-[3.1.0]hex-3-yl)pyrimidine-5-carboxylate.** Compound **12** (2.27 g, 10.7 mmol) was stirred in CH_3CN/DMF (60 mL, 1:1) with potassium carbonate (4.44 g, 32.1 mmol) at room temperature under a nitrogen atmosphere for 10 min. Ethyl 2-(methylsulfonyl)pyrimidine-5-carboxylate **2** (2.48 g, 10.7 mmol) was then added and the mixture stirred for 30 min. The mixture was then diluted with H_2O (100 mL) and extracted with EtOAc (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was purified by column chromatography [eluting with 0−5% MeOH in CH_2Cl_2] to give the title compound as a white solid (3.5 g, 90%). m/z 363 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 8.76 (2H, s), 6.92 (1H, m), 4.26 (2H, q, J = 7.2 Hz), 3.82 (2H, d, J = 11.7 Hz), 3.54 (2H, d, J = 11.7 Hz), 2.93 (2H, m), 1.61 (2H, m), 1.38 (9H, s), 1.28 (3H, t, J = 7.2 Hz), 0.70 (1H, m).

Step 2. Preparation of Ethyl 2-[6-(Aminomethyl)-3-azabicyclo-[3.1.0]hex-3-yl]pyrimidine-5-carboxylate. Ethyl 2-(6-{[(*tert*-butoxycarbonyl)amino]methyl}-3-azabicyclo[3.1.0]hex-3-yl)pyrimidine-5-carboxylate (1.7 g, 4.7 mmol) was stirred in CH_2Cl_2 (20 mL) at room temperature under a nitrogen atmosphere. Then 4 M HCl in dioxane (2.35 mL, 9.4 mmol) was added, immediately causing a solid to precipitate. The mixture was left to stir for 30 min, and then the solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ (100 mL). The organic layer was then dried (Na_2SO_4) and the solvent removed in vacuo to give the title compound as an orange solid (1.2 g, 97%). m/z 263 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 8.76 (2H, s), 4.26 (2H, q, J = 7.2 Hz), 3.82 (2H, d, J = 11.7 Hz), 3.55 (2H, d, J = 11.7 Hz), 3.33 (4H, m), 1.58 (2H, m), 1.29 (3H, t, J = 7.2 Hz), 0.64 (1H, m).

Step 3. Preparation of 2-[6-(Aminomethyl)-3-azabicyclo[3.1.0]-hex-3-yl]pyrimidine-5-carboxylic Acid 26. Ethyl 2-[6-(aminomethyl)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylate (200 mg, 0.76 mmol) was stirred with 2-naphthaldehyde (119 mg, 0.76 mmol) in MeOH (10 mL) at room temperature under a nitrogen atmosphere for 16 h. Sodium borohydride (46 mg, 1.22 mmol) was then added and the mixture stirred for 10 min. Saturated aqueous NH_4Cl (20 mL) was added and the mixture stirred for 20 min. The mixture was then diluted with H_2O (50 mL) and extracted with Et_2O (2 × 100 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was removed in vacuo to give the title compound as a yellow oil which was used in the next step without purification. m/z 403 $[M + H]^+$.

Step 4. Preparation of 2-(6-{[(Naphthalen-2-ylmethyl)amino]-methyl}-3-azabicyclo[3.1.0]hex-3-yl)pyrimidine-5-carboxylic Acid. 2-[6-(Aminomethyl)-3-azabicyclo[3.1.0]hex-3-yl]pyrimidine-5-carboxylic acid **26** (0.76 mmol) was stirred in THF (6 mL) and 1 M NaOH (6 mL) at room temperature for 16 h. The mixture was then acidified to pH 3 with 2 M HCl, causing a solid to precipitate. This was collected and dried to give the title compound as a white solid (72 mg, 25% over two steps) which was used in the next step without purification. m/z 375 $[M + H]^+$.

Step 5. Preparation of *N*-(1-Isobutoxyethoxy)-2-(6-{[(naphthalen-2-ylmethyl)amino]methyl}-3-azabicyclo[3.1.0]hex-3-yl)pyrimidine-5-carboxamide. 2-(6-{[(Naphthalen-2-ylmethyl)amino]methyl}-3-azabicyclo[3.1.0]hex-3-yl)pyrimidine-5-carboxylic acid (72 mg, 0.19 mmol) was stirred with EDC hydrochloride (44 mg, 0.23 mmol) and HOBt (31 mg, 0.23 mmol) in DMF (10 mL) at room temperature under a nitrogen atmosphere for 10 min. *O*-(1-Isobutoxyethyl)hydroxylamine (131 μL, 0.95 mmol) was then added followed by triethylamine (132 μL, 0.95 mmol) and the mixture allowed to stir for 64 h. The mixture was then diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was purified by column chromatography [eluting with 0−10% MeOH/CH_2Cl_2] to give the title compound as a colorless oil (43 mg, 46%). m/z 490 $[M + H]^+$.

Step 6. Preparation of *N*-Hydroxy-2-{6-[(naphthalen-2-ylmethyl-amino)methyl]-3-azabicyclo[3.1.0]hex-3-yl}pyrimidine-5-carboxamide 27. *N*-(1-Isobutoxyethoxy)-2-(6-{[(naphthalene-2-ylmethyl) amino]-methyl}-3-azabicyclo[3.1.0]hex-3-yl)pyrimidine-5-carboxamide (43 mg, 0.09 mmol) was stirred in CH_2Cl_2 (2 mL) at room temperature under a nitrogen atmosphere, and 4 M HCl in dioxane (45 μL, 0.18 mmol) was added. This immediately caused a solid to precipitate. The mixture was allowed to stir for 10 min and then the solvent was removed in vacuo to give the title compound **27** as a white solid (16 mg, 50%). m/z 390 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ: 11.07 (1H, br s), 9.11 (2H, br s), 8.66 (2H, s), 8.03 (2H, m), 7.95 (2H, m), 7.68 (1H, m), 7.58 (2H, m), 4.34 (2H, m), 3.88 (2H, d, J = 11.7 Hz), 3.55 (2H, m), 2.99 (2H, m), 2.50 (1H, m), 1.91 (2H, m).

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Supporting Information Available: Methods for fixed cell and electrochemiluminescent ELISA; results showing effects of **21f** and **21r** on proliferation of a P-glycoprotein isogenic cancer cell line pair; in vitro effects on histone and α-tubulin acetylation; in vivo PK of **21f** and effects on histone H3 and α-tubulin acetylation in samples from a HCT116 human tumor mouse xenograft efficacy study; synthesis and analytical data for **15b**, **18b**−**c**, **21b**−**e**, and **21g**−**21q**; single crystal X-ray structure of **21r**. This material is available free of charge via the Internet at http://pubs.acs.org.

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