Dihydroxypyrimidine-4-carboxamides as Novel Potent and Selective HIV Integrase Inhibitors

Paola Pace,*[,†] M. Emilia Di Francesco,[†] Cristina Gardelli,[†] Steven Harper,[†] Ester Muraglia,[†] Emanuela Nizi,[†] Federica Orvieto,[†] Alessia Petrocchi,[†] Marco Poma,[†,§] Michael Rowley,[†] Rita Scarpelli,[†] Ralph Laufer,[†] Odalys Gonzalez Paz,[†] Edith Monteagudo,[†] Fabio Bonelli,[†] Daria Hazuda,[‡] Kara A. Stillmock,[‡] and Vincenzo Summa[†]

Istituto Di Ricerche Di Biologia Molecolare, P. Angeletti S.p.A. (Merck Research Laboratories, Rome), Via Pontina Km 30,600, 00040 Pomezia, Italy, and Department of Antiviral Research, Merck Research Laboratories, West Point, Pennsylvania 19486

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Human immunodeficiency virus type-1 (HIV-1) integrase, one of the three constitutive viral enzymes required for replication, is a rational target for chemotherapeutic intervention in the treatment of AIDS that has also recently been confirmed in the clinical setting. We report here on the design and synthesis of *N*-benzyl-5,6-dihydroxypyrimidine-4-carboxamides as a class of agents which exhibits potent inhibition of the HIV-integrase-catalyzed strand transfer process. In the current study, structural modifications on these molecules were made in order to examine effects on HIV-integrase inhibitory potencies. One of the most interesting compounds for this series is 2-[1-(dimethylamino)-1-methylethyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide **38**, with a CIC_{95} of 78 nM in the cell-based assay in the presence of serum proteins. The compound has favorable pharmacokinetic properties in preclinical species (rats, dogs, and monkeys) and shows no liabilities in several counterscreening assays, highlighting its potential as a clinically useful antiviral agent.

Introduction

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of acquired immunodeficiency syndrome (AIDS), which is one of the world's most serious health problems. Since 1981, when it was first reported in a small number of patients, AIDS has now become a major worldwide epidemic with more than 38 million people infected worldwide. FDA-approved therapies target primarily two viral enzymes, HIV reverse transcriptase[1] and HIV protease,[2] to block the viral life cycle. Multidrug cocktails consisting of a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) in combination with two nucleoside reverse transcriptase inhibitors (HAART, highly active antiretroviral therapy) is now the standard for treatment. In 2003 Enfuvirtide was also approved as the first member of a new family of antiretroviral drugs that inhibits HIV-1 replication, preventing viral entry into target cells.[3] Although current therapies are effective in reducing viral load and morbidity and mortality, the long-lived nature of the infection, drug toxicity, and the emergence of multidrug resistant phenotypes underscore the need for innovative antiretroviral strategies targeting alternative steps essential for the viral replication cycle.

HIV-1 integrase catalyzes the insertion of the viral DNA into the genome of the host cell,[4] which is an essential step in the viral replication cycle, and thus HIV-1 integrase is an attractive target for novel chemotherapy. Integration is a multistep process consisting of three biochemical steps: (i) assembly of a complex with specific DNA sequences at the end of HIV-1 long terminal repeat (LTR) regions, (ii) endonucleolytic processing of the viral DNA to remove the terminal dinucleotide from each 3′ end, and after nuclear entry, (iii) strand transfer, in which the viral DNA 3′ ends are covalently linked to the cellular DNA. HIV-1 integrase is a 32-kDa enzyme, which is composed of three structurally and functionally distinct domains, all required for each step of the integration process. Extensive mutagenesis studies mapped the catalytic site to the core domain (residues 50−212), which contains the catalytic residues Asp-64, Asp-116, and Glu-152.[5] These residues are highly conserved in the integrase superfamily and polynucleotide transferases. As with other polynucleotidyl transferases, two metals ions (Mg^{2+}) are present in the active site and are required for both 3′ processing and strand transfer and for the assembly of integrase onto specific viral donor DNA to form a complex competent to carry out either function.[6]

4-Aryl-2,4-diketobutanoic acids (DKAs), the first class of truly HIV-1 strand transfer inhibitors, followed by structurally related 1,3-diaryl-1,3-propanediones, provided the first proof of concept for HIV-1 integrase inhibitors as antiviral agents[7] in the cell-based assay. Because of their structure, DKAs could coordinate one or two metal ions in the HIV integrase active site, and mutations that engender resistance to these prototype inhibitors map to the integrase active site proximal to the residues that coordinate the divalent metals (Asp-64, Asp-116, and Glu-152). Subsequently, our group reported a number of diketoacids as potent inhibitors of NS5b HCV RNA-dependent RNA polymerase.[8] In this enzyme, the residues responsible for the nucleotidyl transfer reaction are found in the palm domain, which contains an Asp-$(Xaa)_4$-Asp motif and the signature Gly-Asp-Asp motif, common to the reverse transcriptase and other viral polymerases. The first aspartate of each motif provides the carboxylate side chains required for coordinating the two metal ions involved in catalysis.[9] A hypothesis to explain inhibition by this class of compounds is that the diketoacid fragment inhibits the RNA-dependent RNA polymerase activity through an interaction with the catalytic metal-ions found in the enzyme active site, as for pyrophosphate analogues such as Foscarnet and phosphonoacetic acid. A similar metal-chelating moiety was also found in another class of NS5b RdRP inhibitors, namely the meconic acid derivatives.[10] Both classes of compounds were further developed, and a more complete understanding of the necessary pharmacophore for the inhibition led

* To whom correspondence should be addressed. Phone: +39 0691093313. Fax: +39 06 91093654. E-mail: paola_pace@merck.com.
† Merck Research Laboratories, Rome.
‡ Merck Research Laboratories, West Point.
§ Present address: Industria Farmaceutica Serono, Italy.

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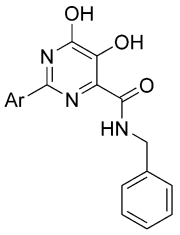


1 Ar = 2-thienyl HEPCPol IC_{50} > 50 μM
HIV Int IC_{50} = 0.08 μM
2 Ar = Ph HEPCPol IC_{50} > 50 μM
HIV Int IC_{50} = 0.07 μM

Figure 1. HIV integrase inhibitors from the HCV polymerase program.

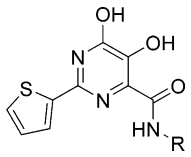
to the discovery of dihydroxypyrimidine-4-carboxylic acid as a suitable replacement of the chelating motif.[11] Given the topological resemblance between HCV polymerase and HIV integrase, both enzymes using a carboxylate-metal ion catalytic mechanism, the dihydroxypyrimidines derivatives and the prototypical scaffolds from the HCV program were screened as potential HIV integrase inhibitors. While the dihydroxypyrimidine carboxylic acid derivatives were almost inactive in the HIV integrase-mediated strand transfer assay, dihydroxypyrimidine-4-carboxamides **1** (Figure 1) were discovered as potent reversible inhibitors of HIV integrase. Extensive structure−activity relationships led to identification of compounds that inhibit HIV integrase in vitro at nanomolar concentrations, block HIV replication in cell culture, and show excellent physicochemical and pharmacokinetic properties.

Results and Discussion

As part of our initial investigations, a collection of dihydroxypyrimidines that had been synthesized as potential HCV NS5B polymerase inhibitors was screened in the HIV-1 integrase assays. All compounds having free carboxylic acids or carboxylic acid isosteres were observed to be weakly active, while the benzyl amide derivative **1** (Figure 1) showed an IC_{50} of 80 nM in the strand transfer assay and in a single cycle infectivity assay[15] had an IC_{50} of 5.0 μM in the presence of 10% fetal bovine serum (FBS) and an IC_{50} of 59 μM in the presence of 50% normal human serum (NHS). Moreover, the benzylamide moiety marked a significant departure from the SAR that had been observed in the NS5b polymerase project, where the free carboxylic acid moiety is crucial for activity.[11] Substitution of the thiophene with a phenyl (**2**, Figure 1) did not affect the potency. In pharmacokinetic studies **1** showed moderate oral bioavailability (15%) and low clearance (5 mL/min/kg, Table 4). Counterscreening studies showed that **1** did not inhibit human DNA polymerase α, β, and γ at 10 μM and was inactive against HIV-RT and HCV polymerase at 50 μM. These findings indicated **1** as a valid lead for the HIV-1 integrase project.

To evaluate the dihydroxypyrimidine-benzylic amide scaffold for development as an HIV-1 integrase inhibitor, a focused library of more than 200 different amides was initially synthesized and screened. The methodology and the scope of this chemistry will be discussed in detail elsewhere.[16] Table 1 collects results from key compounds evaluated at the beginning of our investigation, by assessing carboxamide modifications as an approach to improving the potency of our inhibitors. From this set of compounds, SAR trends started to emerge. It was immediately evident that an aromatic ring separated by at least one sp^3 atom was essential for the activity since the anilide **3** (IC_{50} = 1 μM), the primary amide **4** (IC_{50} = 50 μM) and the cycloalkyl derivative **5** (IC_{50} = 50 μM) lost significant activity.

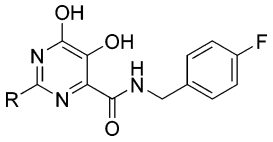
Table 1. Effect of Different Carboxamides on the Inhibition of HIV-1 Integrase Catalytic Strand Transfer Activity



compd	R	inhibition of strand transfer IC_{50} (μM)[a]
1	CH_2Ph	0.08
3	Ph	1.00
4	H	50.0
5	CH_2[c]hexyl	50.0
6	(*S*)$CH(CH_3)Ph$	0.50
7	(*R*)$CH(CH_3)Ph$	5.00
8	CH_2 CH_2Ph	0.02
9	$CH_2C_6H_4$(4-F)	0.01

[a] Assays were performed with recombinant HIV-1 integrase (0.1 μM) preassembled on immobilized oligonucleotides. Inhibitors were added after assembly and washings, and IC_{50} is the concentration of inhibitor that reduces HIV integrase activity by 50%. Results are the mean of at least three independent experiments. SD was always ± 8% of the value. For details, see ref 12.

Table 2. Effect of 2-Substituent on the Inhibition of HIV-1 Integrase Catalytic Strand Transfer Activity



compd	R	inhibition of strand transfer IC_{50} (μM)[a]
9	2-thienyl	0.01
10	H	0.06
11	Me	0.06
12	^{i}Pr	0.10
13	$PhCH_2$	0.05
14	*p*-tolyl	0.02
15	2-thiazolyl	0.02
16	2-pyridyl	0.05

[a] For footnote details, see Table 1.

α-Branching on the benzylic amide was also detrimental (**6**, IC_{50} = 0.5 μM and **7**, IC_{50} = 5 μM). The phenethyl amide **8** (IC_{50} = 0.02 μM) showed slightly improved activity as compared with the benzyl analogue. The 4-fluoro-substituted benzyl amide **9** (IC_{50} = 0.01 μM) was optimal for enzyme inhibition. However, despite the increased in vitro potency, compound **9** showed weak inhibition of the spread of HIV-1 infection in cell culture. The rat pharmacokinetic profile of **9** (Table 4) showed improved oral bioavailability (F = 29%) and similar plasma clearance (11 mL/min/kg) compared to that of **1**.

After optimization of the benzylamide moiety, a significant site for exploring SAR in **9** was the thiophene ring in the 2-position of the pyrimidine core. SAR studies showed that the thiophene ring of **9** could be removed (**10**) or replaced by simple small alkyl groups (**11**, **12**) or benzyl, aryl or heterocycles (**13**−**16**), without loosing significant potency (Table 2). We interpreted the small effect that changing the 2-substituent had on in vitro potency as evidence that this part of the molecule does not interact strongly with the enzyme, and therefore this appeared to be the site that would be most likely to tolerate more dramatic changes influencing the physicochemical properties of the inhibitors aimed to develop compounds with activity

in the cell based assays. Compound **10** can be considered the minimal template of this class of inhibitors capable to retain good inhibitory potency in the enzymatic assay.

Despite the good activity in the enzymatic assay (Quick In), all these compounds were weakly active in the spread assay. The poor activity of our dihydroxypyrimidines in the cell based assay was thought to be due to a suboptimal physical chemical properties that could affect cell permeability and/or to binding to intracellular proteins and also plasma protein present in the cell medium (for example, compounds **9** and **12** are <1% unbound in human plasma). The nonspecific binding of a drug to plasma protein is an important determinant of its biological efficacy since it modulates the availability of the drug to its intended target. This parameter is particularly relevant in the HIV field where it is known that compounds with very high protein plasma protein binding show a marginal activity in the cell based assay in the presence of human serum. In order to assess the role of serum protein on drug availability at a quantitative level, the inhibitor concentration required to inhibit 95% of the spread of HIV-1 infection in MT4 cell culture was measured in the presence of 10% fetal bovine serum (FBS) and 50% normal human serum (NHS) (spread assays). Both assays were important for compound selection, since cell-based activity in the low serum conditions (10% FBS) reflects the ability to cross cell membranes while the high serum conditions (50% NHS) provided efficacy in a physiologically more relevant environment.

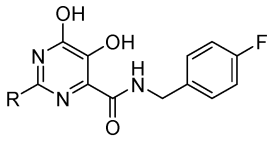
To see whether polar or ionizable groups could affect the physicochemical properties and the protein binding of our inhibitors, therefore improving cell based activity, compounds **17**−**23** were synthesized and tested (Table 3). Introduction of a methoxy group to the 2-benzyl derivative **13** in the benzylic position resulted in compound **17** with activity in 10% FBS in the micromolar range (CIC_{95} = 2.5 μM), while two methoxy groups on the aromatic ring had a negative impact (**18**, CIC_{95} > 10.0 μM). The installation of a basic group resulted in more desirable physical properties, improving cell permeability as demonstrated by compound **19** showing a very low shift between the in vitro and the cell-based assay. This compound inhibited the spread of the virus in low serum conditions with CIC_{95} values of 0.3 μM. The single enantiomers of **19** showed similar activity in all assays suggesting that side of the molecule is not strongly recognized from the integrase enzyme (data not shown). The less basic aniline derivative **20** resulted in a significant loss in potency in the spread assay with respect to **19**. Introduction of the dimethylamine on the *p*-tolyl position as in compound **21** also had a negative impact. Amines different from dimethylamine on the 2-benzyl derivative were acceptable but did not offer any advantage (**22** and **23**).

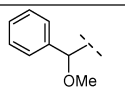
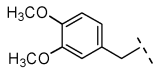
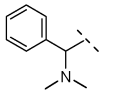
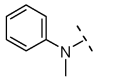
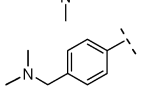
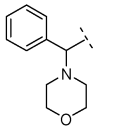
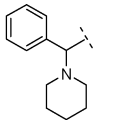
Compound **19** was further characterized with in vivo and in vitro screenings. In pharmacokinetic studies in rats (Table 4), **19** showed good oral bioavailability (F = 59%) and low clearance (Cl_p = 14 mL/min/kg). The profile was even better in dog with improved oral bioavailability (F = 93%) and very low clearance (Cl_p = 0.5 mL/min/kg) and long terminal plasma half-life ($T_{1/2}$ = 6.0 h). Counterscreening studies showed that the compound did not inhibit cytochrome P450s 2C19, 2C9, 2D6, and 3A4.

Despite the promising cell based activity in the presence of 10% FBS, compounds in Table 3 were weakly active when tested using 50% NHS. Our hypothesis that the 2-position substituents were not involved in tight, specific interactions with the enzyme but were rather mobile in nature became particularly important as we assessed whether decreasing the molecular weight of our inhibitors, via removal of the bulky aryl group, would have a favorable impact on cell based potency in the presence of 50% normal human serum. It is indeed known that for lipophilic acids, plasma protein binding correlates to the water/*n*-octanol distribution coefficient (log *P*). Compounds with low lipophilicity or a basic center usually have lower protein binding. We therefore decided to try to improve the activity of **19** by removing the phenyl group to reduce lipophilicity, but maintaining the presence of the beneficial amino group.

A first approach was to construct analogues in which the basic group was embedded in an aliphatic cycle. As shown in Table 5, the 2-piperidyl derivative **24** exhibited higher potency in the in vitro enzyme assay compared to the 3- (**25**) and 4-piperidyl

Table 3. 2-Benzyl, 2-Aniline, and 2-Tolyl Derivatives



compd	R	inhibition of strand transfer IC_{50} (μM)[a]	10% FBS antiviral activity CIC_{95} (μM)[b]
17		0.07	2.50
18		0.15	> 10.0
19		0.20	0.31
20		0.15	2.50
21		0.15	> 10.0
22		0.05	0.63
23		0.10	0.31

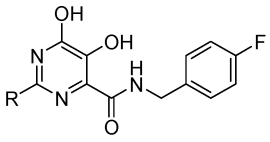
[a] For footnote details, see Table 1. [b] Spread assay: 95% cell culture inhibitory concentrations for inhibition of the spread of HIV-1 infection in cell culture, using HIV-1IIIb and MT-4 T-lymphoid cells, in a medium containing 10% heat-inactivated fetal bovine serum. Results are the mean of at least three independent experiments. SD was always <±10% of the value. For details, see ref 13.

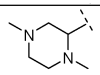
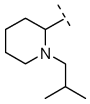
Table 4. Rat and Dog Pharmacokinetic Parameters, Human Plasma Protein Binding, and Log *D*

compd	species	F (%)[c]	$T_{1/2}$ (h)[d]	Cl_p (mL/min/kg)[e]	% plasma protein binding	log *D*
1	rat[a]	15	3	5	nd[f]	nd
9	rat[a]	29	1.3	11	nd	nd
19	rat[a]	59	1.73	14	99.0	2.13
	dog	93	6.78	0.5		
27	rat[a]	27	0.43	75	94.4	0.79
	dog	90	6.0	2		

[a] 2.4 mg/kg (n = 3); vehicle iv DMSO, po 10%DMSO/90%PEG_{200}. [b] 1.2 mg/kg iv, 1.6 mg/kg po (n = 3); vehicle iv DMSO, po 10% DMSO/45% H_2O/45% PEG_{200}. [c] Oral bioavailability. [d] Terminal phase plasma half-life following iv administration. [e] Plasma clearance. [f] Not determined.

Table 5. 2-Position Cyclic Amine Analogues



compd	R	IC_{50} (μM)[a]	CIC_{95}(μM)[b] 10%FBS	50%NHS	compd	R	IC_{50} (μM)[a]	CIC_{95}(μM)[b] 10%FBS	50%NHS
24		0.10	1.46	5.83	**30**		0.23	0.12	0.62
25		0.80	>10	>10	**31**		0.03	0.03	0.23
26		1.23	>10	>10	**32**		0.12	0.15	0.62
27		0.20	0.14	0.40	**33**		0.01	0.06	0.25
28		0.10	0.25	0.50	**34**		0.05	0.12	0.12
29		0.20	1.00	>1.0					

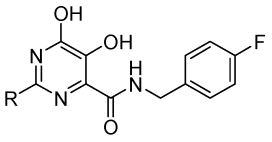
[a] For footnote details, see Table 1. [b] For footnote details, see Table 3.

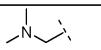
(**26**) counterparts. However, **24** displayed disappointing cell based activities, perhaps due to the presence of multiple H-bond donors. Therefore the NH was methylated (**27**), giving a very pleasing 10-fold improvement in activity in cell based assay under both serum conditions (CIC_{95} = 0.14 and 0.4 μM 10% FBS and 50% NHS, respectively). Alkylation with higher alkyl groups offered no advantages (**28**, **29**). Compound **27**, with a 94.4% human plasma protein binding, apparently shows a better compromise between plasma protein binding and cell penetration, thus retaining sub-micromolar cell based activity under high serum conditions (the resolved enantiomers of **27** both showed equal activity to the racemate; data not shown). When the piperidine ring was replaced by a piperazine (**30**) and a pyrrolidine (**32**), the compounds showed cell based activity in 50% normal human serum similar to that of inhibitor **27**, and replacement with a morpholine (**31**) gave 2-fold improvement in the same assay. Further characterization and evolution of 2-morpholine derivatives will be discussed in a separate publication.[17] A significant observation in this cyclic amine series was the increase in enzyme affinity and in cell based potency through the introduction of an additional methyl in the benzylic position, as shown by comparison of compounds **30** and **33**. On the basis of this finding, the bicyclic achiral derivative **34**, with a quaternary benzylic carbon atom, was prepared and resulted in one of the most potent compounds in this series in the cell based anti-HIV assays, where an IC_{95} of 0.12 μM was measured both in 10% FBS and in 50% NHS. Polar heterocycles such as pyridine could also be envisaged as alternatives to the cyclic amines, but the 2-pyridine derivative **16** (Table 2), while retaining enzymatic affinity, was completely ineffective in the cell based assays. In addition, other less basic and more electron-rich heterocycles (data not shown) were suboptimal at C-2. Taken together, these data show that small, aliphatic tertiary amines and a tetrasubstituted sp^3-carbon at C-2 of the dihydroxypyrimidine core are generally preferred.

Compound **27** was evaluated in pharmacokinetic iv/po studies in rats and dogs. In rats, it was a moderately orally bioavailable compound, with high clearance and rather short half-life. The pharmacokinetic profile was much better in dog showing an outstanding oral bioavailability and very low clearance (Table 4).

A second study was done on smaller acyclic amines in the 2 position (Table 6). A dimethylaminomethyl substituent was found to exhibit acceptable inhibitory potency (**35**), while further separation of the basic amine by means of an ethyl linker as in compound **36** reduced the activity. Mirroring the behavior observed in the cyclic amine derivatives, a quaternary sp^3-carbon spacer afforded a significant increase in cell based potency over a secondary and tertiary analogues (compare **38** vs **35** and **37**). Increasing the size of the *N*-alkyl substituent (**39**) reduced the activity, and, as in the cyclized series, a free NH (**40**) was also detrimental to cell based activity. Attempted substitutions of the *gem*-dimethyl all had a negative impact, since residues like trifluoromethylmethyl (**41**), cyclopropane (**42**), cyclobutane (**43**), cyclopentane (**44**), cyclohexane (**45**), and tetrahydropyran (**46**) led to significant loss in potency in the cell based assay carried out in the presence of 50% normal human serum. The binding of compound **38** to rat, dog, and human plasma was determined by ultrafiltration method. The unbound fraction of the drug in plasma was approximately 17% for rats, 10% for dogs, and 11% for humans. In the cell-based antiviral assay, the addition of 50% normal human serum resulted in less than 1.5 fold reduction in potency for **38**, demonstrating that it is effective in suppressing the spread of HIV-1 infection in cells in the presence of biologically relevant proteins. Overall, 2-[1-(dimethylamino)-1-methylethyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide **38** proved a to be very active compound with

Table 6. 2-Position Acyclic Amine Analogues



compd	R	inhibition of strand transfer IC_{50} (μM)[a]	antiviral activity CIC_{95} (μM)[b] 10% FBS	50% NHS
35		0.20	1.00	>1.00
36		2.00	10.0	>10.0
37		0.01	0.12	0.50
38		0.05	0.06	0.078
39		0.06	0.12	0.50
40		0.04	0.25	1.00
41		0.008	0.06	1.0
42		0.01	0.37	1.00
43		0.052	0.02	0.25
44		0.088	0.06	1.00
45		0.08	0.12	1.00
46		0.026	0.04	0.19

[a] For footnote details, see Table 1. [b] For footnote details, see Table 3.

Table 7. Rat, Dog, and Rhesus Monkey Pharmacokinetic Parameters for Compound **38**

species	dose[a] po	dose[a] iv	F^b (%)	$T_{1/2}$ (h)[c]	Cl_p[d] (mL/min/kg)	nAUC[e] (μM·h·kg/mg)
rat[f]	3	3	28	2.1	16	2.4
dog[g]	2	1	100	4.8	1.9	21.5
Rhesus[h]	1	1	61	1.9	15	1.8

[a] mg/kg body weight; $n = 3$. [b] Oral bioavailability. [c] Terminal phase plasma half-life following iv administration. [d] Plasma clearance. [e] Area under the curve following po administration. [f] Vehicle iv DMSO, po PEG_{200}. [g] Vehicle iv 0.9% NaCl, po 1% methylcellulose. [h] Vehicle iv DMSO, po 0.5% methylcellulose.

virtual no shift in potency from the enzymatic assay (IC_{50} = 50 nM) to the spread cell based assay in the two serum conditions [CIC_{95} = 60 nM (10% FBS) and 78 nM (50% NHS)]. This agent proved to be one of the most potent compounds we found in the cell based anti HIV assay.

The pharmacokinetic profile of **38** was determined in Sprague−Dawley rats, beagle dogs and rhesus monkeys. The results of these experiments are summarized in Table 7. In all three species, the compound had prolonged plasma half-lives, moderate to low clearance and good to excellent oral bioavailability (from 28% in rats to 100% in dogs). AUC and C_{max} were increased linearly (r^2 = 0.997 and 0.998 respectively) in the rat dose proportionality study dosing the compound orally at doses from 1 to 60 mpk (4 and 15mpk were the medium doses) and a similar trend was observed in rhesus monkeys increasing the oral dose from 1 to 10 mpk, confirming high levels of oral absorption. To assess the intrinsic metabolic stability, the compound **38** was evaluated in rat, monkey, dog, and human liver microsomes in the presence of NADPH (no turnover, data not shown) and UDPGA (see also Supporting Information). Higher in vitro metabolism was observed in monkey and rat microsomes, which correlated with the moderate in vivo clearance, while the compound showed very low turnover in dog liver microsomes, as mirrored by the low in vivo clearance. Low human turnover in vitro, in combination with other data not reported here, predicts human pharmacokinetics to be similar to dog, where the compound had 24 h trough blood levels, with a 2 mpk po dose, at or slightly below the CIC_{95} (C_{24h} = 67 nM). The major metabolite in urine (accounting for >90% of the dose on the basis of ^{19}F analysis) was characterized using ^1H, ^{13}C and $^1H-^{13}C$ correlation spectroscopy. These data support the formation of the glucuronide conjugate of the hydroxyl group in position 5 of the pyrimidine ring. The full assignment of all ^1H and ^{13}C signals ascribed to this β-glucuronide was achieved using the crude sample of urine after solid-phase extraction. The same profile was found using the ^3H material.

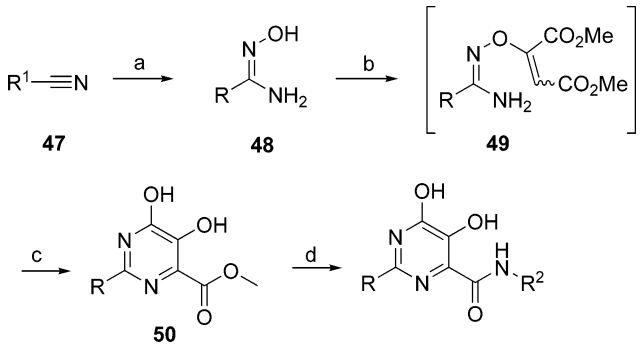
Further profiling of compound **38** revealed that it had no CYP450 liabilities (1A2, 2C19, 2C9, 2D6, and 3A4 IC50 > 20 μM), did not inhibit human DNA polymerases α, β, and γ (IC_{50} > 100 μM) and did not bind the human I_{Kr} potassium channel (IC_{50} > 130 μM) (18). The compound did not react with glutathione after incubation at 37 °C for 24 h and the [^3H]-**38** after administrations in rat (25 mpk, po) did not show measurable covalent binding to liver, kidney, and plasma.

Conclusions

The dihydroxypyrimidine-4-carboxamides were discovered as a new, relevant class for the development of new AIDS therapeutics. In this report we have summarized our efforts toward the optimization of the initial lead *N*-benzyl-5,6-dihydroxy-2-(2-thienyl)pyrimidine-4-carboxamide **1**. On the hypothesis that its physical properties were the basis for its lack of efficacy in the cell based assays, initial SAR allowed the identification of the position where changes affecting the physical properties could be made without compromising the intrinsic in vitro efficacy. Introduction and optimization of amino substituents in the 2-position of the dihydroxypyrimidine core resulted in analogues effective in suppressing the spread of HIV-1 infection in cells. While early analogues suffered high protein binding, reduction of lipophilicity addressed this problem, leading to the discovery of diverse series effective for blockade of the spread of the virus in the presence of serum proteins. SAR optimization provided compound **38** having the best overall potencies in the enzymatic and cellular assays. This compound showed excellent PK properties in rat, dog, and rhesus monkeys, was clean in an extensive panel of counterscreening assays, and therefore may have potential for clinical development as anti-HIV drug with a novel mechanism of action.

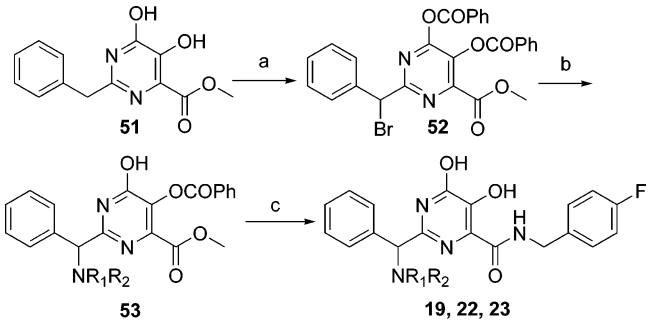
Biology. Compounds were routinely assessed for activity against the purified HIV-1 integrase enzyme.[6] Integrase-mediated strand transfer activity was determined as described in ref 12. Compounds were also tested in HIV-1 replication assays, performed in MT-4 human T-lymphoid cells as described in ref 13. Cells were infected en masse at low multiplicity (0.01).

Scheme 1. General Scheme for the Synthesis of Pyrimidine-4-carboxamide[a]



[a] Reagents and conditions: (a) $H_2NOH{\cdot}HCl$, KOH, MeOH; (b) DMAD, $CHCl_3$, 60 °C; (c) xylene or methanol, reflux; (d) R_2NH_2, methanol, NMP or DMF, 80−90 °C.

Scheme 2. Synthesis of Compounds **19**, **22**, **23**[a]



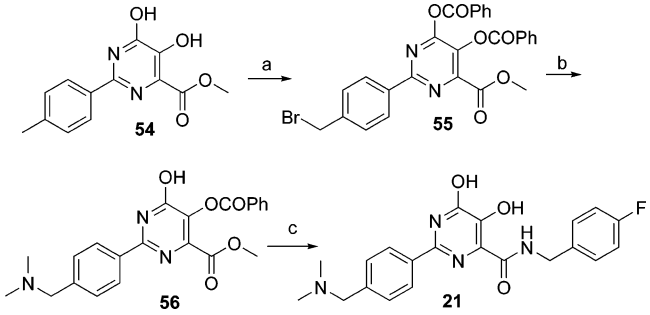
[a] Reagents and conditions: (a) i: PhCOCl, pyridine, THF; ii: *N*-bromosuccinimide, $(PhCO)_2O_2$, CCl_4, 90 °C; (b) R_1R_2NH, THF, rt; (c) 4-F−C_6H_4−CH_2NH_2, DMF, 90 °C.

HIV-1 replication was assessed by p24 core antigen ELISA. Control cultures in the absence of inhibitor were fully infected at 4 days.

Chemistry. For the synthesis of 2-substituted methyl 5,6-dihydroxypyrimidine-4-carboxylates, the method of Culbertson was used[14] (Scheme 1). Therefore, amidoximes **48** were prepared from nitriles **47**, either commercially available or prepared as described in the Experimental Section, and reacted with dimethyl acetylenedicarboxylate (DMAD). The corresponding adducts **49** were not isolated, but directly cyclized in refluxing methanol or xylene to yield the desired pyrimidine methyl esters **50**. Subsequent reaction with the appropriate amine in methanol, *N*-methylpyrrolidone, or *N*,*N*-dimethylformamide gave the required amides. Compounds **1**−**16**, **17**, **18**, **20**, and **24**−**26** (in this case, an additional deprotection step was required) were obtained directly by this route. Schemes 2 and 3 illustrate the chemistry used for the investigation of the 2-substituent SAR in compounds **19** and **21**−**23**. Bis-benzoylation of **51**, followed by bromination under standard conditions, yielded the benzylic bromide derivative **52**. Displacement of the bromine with an amine went smoothly, with concomitant deprotection of the 6-hydroxyl group, affording **53**. Subsequent coupling with the appropriate benzylic amine gave compounds **19**, **22**, and **23**. Compound **21** was accessed by analogous chemistry, starting from methyl 5,6-dihydroxy-2-(4-methylphenyl)pyrimidine-4-carboxylate **54** (Scheme 3) and simultaneous loss of the remaining COPh through amide formation.

For analogues containing a cyclic amine in the 2-position of the dihydroxypyrimidine, synthesis of the dihydroxypyrimidine methyl ester, with the nitrogen protected as Cbz or Boc, was accomplished as usual. In some cases, an additional protection of the 5-hydroxy group as its benzoate ester was required to

Scheme 3. Synthesis of Compound **21**[a]



[a] Reagents and conditions: (a) i: PhCOCl, pyridine, THF; ii: *N*-bromosuccinimide, $(PhCO)_2O_2$, CCl_4, 90 °C; (b) Me_2NH, THF, rt; (c) 4-F-C_6H_4-CH_2NH_2, DMF, 90 °C.

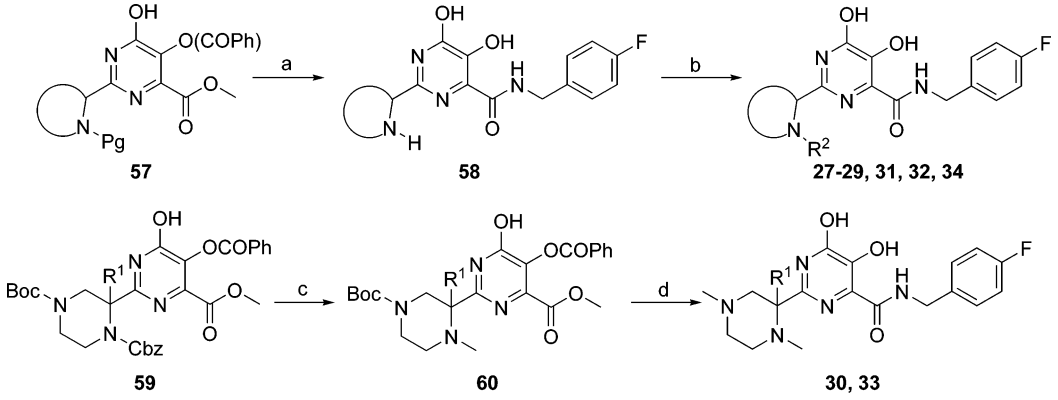
improve the solubility of the compounds and the workup from organic solvents. Removal of the protecting groups and reaction with *p*-fluorobenzylamine afforded intermediates **58**. The basic nitrogen was alkylated via reductive amination, leading to compounds **27**−**29**, **31**, **32**, **34**. Piperazine derivatives **30**, **33** were accessed via sequential deprotection and alkylation steps of the two piperazine nitrogens of **59**, followed by formation of the p-fluorobenzylic amides as usual (Scheme 4).

The synthesis of compounds with an acyclic amine in the 2-position is summarized in Scheme 5. Heating of the pyrimidines **61** with p-fluorobenzylamine produced compounds **62**. Deprotection using standard conditions followed by reductive amination with formaldehyde yielded compounds **35**, **37**, **38**, **41**−**46**. In the preparation of the monomethylated derivative **40**, we were not able to stop the reductive amination of **63** after the introduction of the first methyl. Therefore, a three-step protocol based on reductive amination with 1 equiv of benzaldehyde, followed by a second reductive amination with formaldehyde and final removal of the benzyl group via catalytic hydrogenation, yielded compound **40**. Second reductive amination with acetaldehyde afforded compound **39**. Compound **36** was obtained from **65** via synthesis of the amide, removal of the Boc, and reductive amination.

Experimental Section

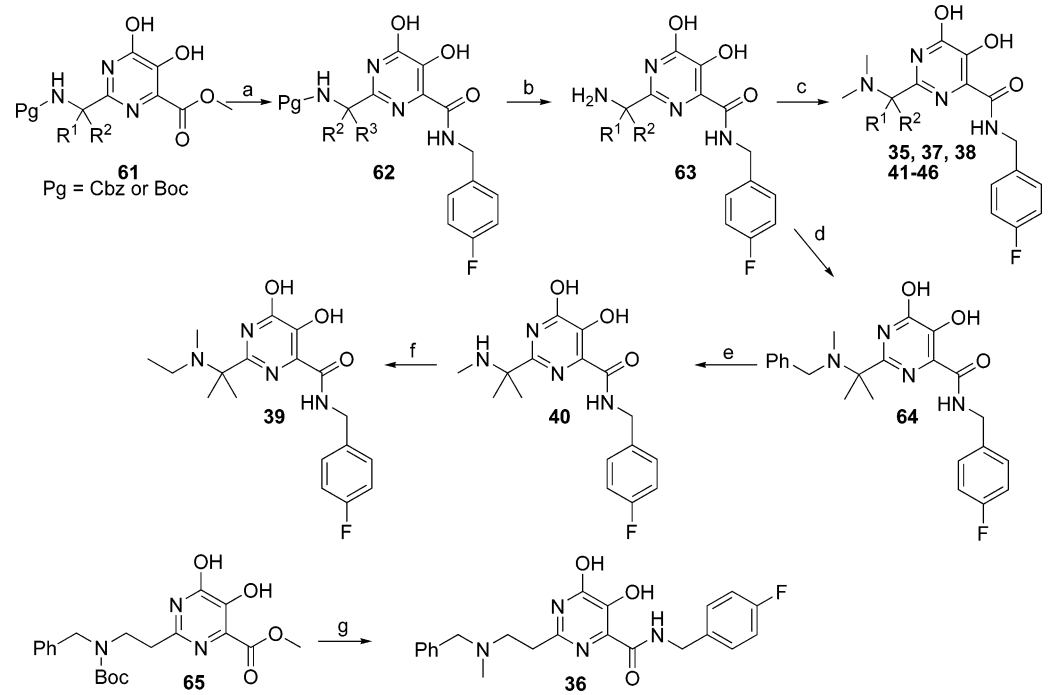
Solvents and reagents were obtained from commercial suppliers and were used without further purification. Flash chromatography purifications were performed on Merck silica gel (200−400 mesh) as the stationary phase or were conducted using prepacked cartridges on a Biotage system, eluting with petroleum ether/ethyl acetate mixtures. HPLC-MS analyses were performed on a Waters Alliance 2795 apparatus, equipped with a diode array and a ZQ mass spectrometer, using an X-Terra C_{18} column (5 μm, 4.6 × 50 mm). The solvent system was acetonitrile−0.1% HCOOH/water−0.1% HCOOH, gradient 10−90% over 6 min with a flow rate of 1 mL/min. Purity of final compounds was more than 99% by area. Preparative reversed-phase high-performance liquid chromatography (RP-HPLC) was performed on a Shimadzu 10AV-VP operating with a flow rate of 15 mL/min and incorporating a diode array detector SPD10AV-VP. The stationary phases used were Waters Symmetry C_{18} 5 μm 19 × 100 mm, C_{18} 7 μm 19 × 150 mm, and C_{18} 7 μm 19 × 300 mm. The mobile phase comprised a linear gradient of binary mixtures of acetonitrile (containing 0.1% TFA) and H_2O (containing 0.1% TFA). Nuclear magnetic resonance spectra ^{1}H NMR recorded at 400 or 300 MHz, ^{13}C NMR recorded at 100 or 75 MHz) were obtained on Bruker AMX spectrometers and are referenced in ppm relative to TMS. Unless indicated, spectra were acquired at 300 K. Low-resolution mass spectra (*m*/*z*) were recorded on a Perkin-Elmer API 100 (electrospray ionization) mass spectrometer. High-resolution mass measurements were carried out by electrospray ionization performed on a TSQ Quantum Ultra AM

Scheme 4. Synthesis of Compounds **27**−**34**[a]



[a] Reagents and conditions: (a) i: H_2, Pd/C, MeOH or CF_3COOH/CH_2Cl_2; ii: 4-F-$C_6H_4CH_2NH_2$,MeOH, NMP or DMF, 80−90 °C; (b) RCHO, $NaCNBH_3$, CH_3COONa (or CH_3COOH, Et_3N), MeOH; (c) i: H_2, Pd/C, MeOH; ii: HCHO, $NaCNBH_3$, CH_3COONa, MeOH; (d) i: CF_3COOH/CH_2Cl_2 (9:1); ii: HCHO, $NaCNBH_3$, CH_3COONa, CH_3OH; iii: 4-F-$C_6H_4CH_2NH_2$, MeOH or NMP, 80−90 °C.

Scheme 5. Synthesis of Compounds **35**−**46**[a]



[a] Reagents and conditions: (a) 4-F-$C_6H_4CH_2NH_2$, CH_3OH, 80 °C; (b) H_2, Pd/C, CH_3OH or CF_3COOH/CH_2Cl_2; (c) HCHO, $NaCNBH_3$, CH_3COOH, Et_3N, CH_3OH; (d) i: PhCHO, $NaCNBH_3$, CH_3COOH, Et_3N, CH_3OH; ii: HCHO, $NaCNBH_3$, CH_3COOH, Et_3N, CH_3OH; (e) H_2, Pd/C, CH_3OH; (f) CH_3CHO, $NaCNBH_3$, CH_3COOH, Et_3N, CH_3OH; (g) i: 4-F-$C_6H_4CH_2NH_2$, CH_3OH, 80 °C; ii: CF_3COOH/CH_2Cl_2; iii: HCHO, $NaCNBH_3$, CH_3COOH, Et_3N, CH_3OH.

operating in enhanced mass resolution mode. The accurate mass measurements were carried out on a chromatographic time scale by means of flow injection of 5 μL of each working solution (20 μg/mL concentration) in acetonitrile on a Waters C_{18} Sunfire (20 × 2.1 mm, 5 μm) column using an isocratic elution (solvent A: solvent B, 50:50) of solvent A: water + 0.1% formic acid and solvent B: acetonitrile + 0.1% formic acid at a flow rate of 1000 μL/min.

The following compounds were prepared according to literature procedures: 1,3-thiazole-2-carbonitrile,[19] 1-[(benzyloxy)carbonyl]-4-(*tert*-butoxycarbonyl)piperazine-2-carboxylic acid,[20] 7-benzyl 1-*tert*-butyl 7-azabicyclo[2.1.1]heptane-1,7-dicarboxylate,[21] 2-amino-2-methylpropanenitrile,[22] *tert*-butyl-3-cyanopiperidine-1-carboxylate,[23] *tert*-butyl-4-cyanopiperidine-1-carboxylate,[24] *tert*-butyl-2-cyanopyrrolidine-1-carboxylate,[25] *tert*-butyl-(1-cyanoethyl)carbamate.[26]

General Procedure for the Synthesis of 2-Substituted Methyl-5,6-dihydroxypyrimidine-4-carboxamide. 2-Substituted methyl 5,6-dihydroxypyrimidine-4-carboxylates **50** were prepared from the appropriate amidoximes following the method described by Culbertson.[14] In a subsequent step, the corresponding 4-carboxamides were obtained by heating the 2-substituted methyl 5,6-dihydroxypyrimidine-4-carboxylate with the appropriate amines in *N*,*N*-dimethylformamide, in *N*-methylpyrrolidone, or in methanol. Amidoximes **48** were prepared from the corresponding nitriles by use of known procedures. Unless differently specified, 1 equiv of potassium hydroxide dissolved in methanol was added to a solution of hydroxylamine hydrochloride (1 equiv) in methanol. The precipitated potassium chloride was removed by filtration, and to the above solution was added the appropriate aryl nitrile (1 equiv). The reaction mixture was stirred at 60 °C for the appropriate time (3−6 h, TLC monitoring). After cooling, the solvent was removed under vacuum, and the residue was triturated with diethyl ether. The precipitate was collected and eventually recrystallized from an appropriate solvent, furnishing the desired amidoxime in 60−70% yield. Usually amidoximes were used without further purification in the reaction with dimethyl acetylenedicarboxylate. Michael adducts **49** were not isolated.

Synthesis of *N*-(4-fluorobenzyl)-5,6-dihydroxy-2-thien-2-ylpyrimidine-4-carboxamide **9** is a representative example.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-thien-2-ylpyrimidine-4-carboxamide (9)**. Step 1: *N*-Hydroxythiophene-2-carboximidamide (1.0 g, 7.0 mmol) was suspended in chloroform (20 mL) and refluxed overnight in the presence of 1.0 equiv of dimethyl acetylenedicarboxylate (0.87 mL, 7.0 mmol). After being cooled to room temperature, volatiles were evaporated and the residue was refluxed in xylene (25 mL) for 3 h. The mixture was cooled to room temperature to allow the formation of a precipitate. Methyl 5,6-dihydroxy-2-thien-2-ylpyrimidine-4-carboxylate was collected by filtration and washed with diethyl ether (0.265 g, 15% yield). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.0 (bs, 1 H), 8.08 (d, J = 3.2 Hz, 1 H), 7.85 (d, J = 4.4 Hz, 1 H), 7.25 (dd, J = 4.9 Hz, 3.9 Hz, 1 H), 3.93 (s, 3 H). Step 2: The solid from above (0.265 g, 1.05 mmol) was dissolved in DMF (3 mL) and 2.0 equiv of 4-fluorobenzylamine (0.24 mL, 2.1 mmol) was added to the stirred solution. The reaction mixture was left overnight at 90 °C. After the mixture was cooled to room temperature, 1 N HCl (2 mL) was added and a solid precipitated from the mixture. This solid was collected by filtration, washed with ethyl ether, and dried under vacuum to afford title compound (0.127 g, 35% yield). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.06 (s, 1 H), 12.38 (s, 1 H), 8.70 (bs, 1 H), 8.03 (d, J = 3.2 Hz, 1 H), 7.81 (d, J = 4.8 Hz, 1 H), 7.39 (dd, J = 5.7, 8.4 Hz, 2 H), 7.20−7.18 (m, 3 H), 4.51 (d, J = 6.3 Hz, 2 H); MS *m*/*z* 346 (M + H)$^+$. HRMS Calcd for $C_{16}H_{13}FN_3O_3S$ (M + H)$^+$: 346.06562. Found: 346.06515.

Unless otherwise specified, the following compounds were prepared according to the above procedure and isolated with similar yields.

***N*-Benzyl-5,6-dihydroxy-2-thien-2-ylpyrimidine-4-carboxamide (1).** As for the preparation of **9**, replacing in step 2 4-fluorobenzylamine with benzylamine. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 13.06 (bs, 1 H), 12.53 (bs, 1 H), 9.19 (t, J = 6.2 Hz, 1 H), 8.06 (d, J = 3.5 Hz, 1 H), 7.82 (d, J = 4.2 Hz, 1 H), 7.44−7.24 (m, 5 H), 7.19 (dd, J = 4.2, 3.5 Hz, 1 H), 4.56 (d, J = 6.2 Hz, 2 H); MS (ES$^+$) *m*/*z* 328 (M + H)$^+$. HRMS Calcd for $C_{16}H_{14}N_3O_3S$ (M + H)$^+$: 328.07504. Found: 328.07608.

***N*-Benzyl-5,6-dihydroxy-2-phenylpyrimidine-4-carboxamide (2).** As for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *N*-hydroxybenzenecarboximidamide and in step 2 4-fluorobenzylamine with benzylamine. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.97 (bs, 1 H), 12.58 (s, 1 H), 9.52 (bs, 1 H), 8.23 (d, J = 8.8 Hz, 2 H), 7.53−7.42 (m, 3 H), 7.38−7.20 (m, 5 H), 4.55 (d, J = 7.5 Hz, 2 H); MS *m*/*z* 322 (M + H)$^+$. HRMS Calcd for $C_{18}H_{16}N_3O_3S$ (M + H)$^+$: 322.11862. Found: 322.11790.

5,6-Dihydroxy-*N*-phenyl-2-(2-thienyl)pyrimidine-4-carboxamide (3). As for the preparation of **9**, replacing in step 2 4-fluorobenzylamine with aniline. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.16 (bs, 1 H), 11.70 (bs, 1 H), 10.13 (s, 1 H), 8.08 (d, J = 3.2 Hz, 1 H), 7.83 (d, J = 4.93 Hz, 1 H), 7.73 (d, J = 7.89 Hz, 2 H), 7.41 (t, J = 7.62 Hz, 2 H), 7.22−7.20 (m, 3 H); MS *m*/*z* 314 (M + H)$^+$. HRMS Calcd for $C_{15}H_{12}N_3O_3S$ (M + H)$^+$: 314.05939. Found: 314.05885.

5,6-Dihydroxy-2-(2-thienyl)pyrimidine-4-carboxamide (4). To a suspension of 0.200 g (0.79 mmol) of methyl 5,6-dihydroxy-2-(2-thienyl)pyrimidine-4-carboxylate (prepared according to the procedure described for compound **9**, step 1) in MeOH (2 mL) was added ammonia (2 N solution in MeOH, 20 equiv) and the resulting reaction mixture was stirred in a sealed vessel at 40 °C for 72 h. The volatiles were removed under reduced pressure, and the solid residue was triturated with Et_2O to give the title compound as a solid product (0.085 g, 45% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.46 (bs, 1H), 7.84 (bs, 1H), 7.81 (d, J = 3.6 Hz, 1H), 7.57 (d, J = 4.9 Hz, 1H), 7.08 (t, J = 3.9 Hz, 1H); MS *m*/*z* 238 (M + H)$^+$. HRMS Calcd for $C_9H_8N_3O_3S$ (M + H)$^+$: 238.02809. Found: 238.02734.

***N*-(Cyclohexylmethyl)-5,6-dihydroxy-2-(2-thienyl)pyrimidine-4-carboxamide (5).** As for the preparation of **9**, replacing in step 2 4-fluorobenzylamine with cyclohexylamine. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.03 (bs, 1 H), 12.62 (bs, 1 H), 8.54 (bs, 1 H), 8.02 (d, J = 3.69 Hz, 1 H), 7.81 (d, J = 4.43 Hz, 1 H), 7.18 (dd, J = 4.92, 3.69 Hz, 1 H), 3.19 (t, J = 6.64 Hz, 2 H), 1.70−1.60 (m, 6 H), 1.21−1.12 (m, 3 H), 0.99−0.91 (m, 2 H); MS *m*/*z* 334 (M + H)$^+$. HRMS Calcd for $C_{16}H_{20}N_3O_3S$ (M + H)$^+$: 334.12199. Found: 334.12042.

5,6-Dihydroxy-*N*-[(1*S*)1-phenylethyl]-2-(2-thienyl)pyrimidine-4-carboxamide (6). As for the preparation of **9**, replacing in step 2 4-fluorobenzylamine with (1*S*)-1-phenylethanamine. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.07 (bs, 1 H), 12.25 (s, 1 H), 8.60 (m, 1 H), 8.03 (d, J = 3.7 Hz, 1 H), 7.80 (d, J = 4.92 Hz, 1 H), 7.44 (d, J = 7.63 Hz, 2 H), 7.37 (t, J = 7.38 Hz, 2 H), 7.28 (t, J = 7.14 Hz, 1 H), 7.18 (t, J = 4.30 Hz, 1 H), 5.17 (q, J = 7.63 Hz, 1 H), 1.57 (d, J = 7.14 Hz, 3 H); MS *m*/*z* 342 (M + H)$^+$. HRMS Calcd for $C_{17}H_{16}N_3O_3S$ (M + H)$^+$: 342.09069. Found: 342.08968.

5,6-Dihydroxy-*N*-[(1*R*)1-phenylethyl]-2-(2-thienyl)pyrimidine-4-carboxamide (7). As for the preparation of **9**, replacing in step 2 4-fluorobenzylamine with (1*R*)-1-phenylethanamine. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.07 (bs, 1 H), 12.25 (s, 1 H), 8.60 (m, 1 H), 8.03 (d, J = 3.7 Hz, 1 H), 7.80 (d, J = 4.92 Hz, 1 H), 7.44 (d, J = 7.63 Hz, 2 H), 7.37 (t, J = 7.38 Hz, 2 H), 7.28 (t, J = 7.14 Hz, 1 H), 7.18 (t, J = 4.30 Hz, 1 H), 5.17 (q, J = 7.63 Hz, 1 H), 1.57 (d, J = 7.14 Hz, 3 H); MS *m*/*z* 342 (M + H)$^+$. HRMS Calcd for $C_{17}H_{16}N_3O_3S$ (M + H)$^+$: 342.09069. Found: 342.09023.

5,6-Dihydroxy-*N*-(2-phenylethyl)-2-(2-thienyl)pyrimidine-4-carboxamide (8). As for the preparation of **9**, replacing in step 2 4-fluorobenzylamine with 2-phenylethanamine. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.08 (Bs, 1 H), 12.58 (bs, 1 H), 8.61 (bs, 1 H), 8.07 (dd, J = 3.6, 0.8 Hz, 1 H, 7.87 (d, J = 4.9 Hz, 1 H), 7.40−7.21 (m, 6 H), 2.48 (bq, J = 7.1 Hz, 2 H), 2.94 (t, J = 7.4 Hz, 2 H);); MS *m*/*z* 342 (M + H)$^+$. HRMS Calcd for $C_{17}H_{16}N_3O_3S$ (M + H)$^+$: 342.09069. Found: 342.08935.

***N*-(4-Fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (10).** Step 1: 2-Ethoxycarbonyl-4,5-dihydroxy-6-(methoxycarbonyl)pyrimidine (0.5 g, 2.06 mmol, prepared according to the procedure described in step 1, compound **9**, by using ethyl amino(hydroxyimino)ethanoate[27] in place of *N*-hydroxythiophene-2-carboximidamide) was suspended in dioxane/THF 2:1 (10 mL) and treated with 1 equiv of 1 N NaOH. After 20 min the mixture was acidified with 1 N HCl and concentrated, and 4,5-dihydroxy-6-(methoxycarbonyl)pyrimidine-2-carboxylic acid was collected by filtration (0.42 g, 95% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.1 (bs, 1 H), 11.1 (bs, 1 H), 3.82 (s, 3 H); MS *m*/*z* 213 (M + H)$^+$. Step 2: The solid from above (0.42 g, 1.98 mmol) was taken up in 1 N HCl (15 mL) and stirred for 6 h at 90 °C. After being cooled to room temperature, the mixture was filtered and the solid was washed with cold 1 N HCl. Evaporation of the filtrate afforded methyl 5,6-dihydroxypyrimidine-4-carboxylate as a solid (0.33 g, 100% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.75 (s, 1 H), 3.82 (s, 3 H). Step 3: Solid from above was dissolved in DMF (4 mL), and 4-fluorobenzylamine (0.45 mL, 3.96 mmol) was added. After 2 h at 90 °C the mixture was evaporated. Compound **10** was isolated in 56% yield (0.28 g) by preparative HPLC (C_{18}, 5 μm, gradient of CH_3CN/H_2O both containing 0.1% trifluoroacetic acid). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.72 (bs, 1 H), 12.54 (bs, 1 H), 9.48 (bs, 1 H), 7.77 (s, 1 H), 7.36 (t, J = 8.0 Hz, 2 H), 7.14 (t, J = 8.8 Hz, 2 H), 4.43 (d, J = 6.3 Hz, 2 H); MS *m*/*z* 264 (M + H)$^+$. HRMS Calcd for $C_{12}H_{11}FN_3O_3$ (M + H)$^+$: 264.07790. Found: 264.07816.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-methylpyrimidine-4-carboxamide (11)**. As for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *N*-hydroxyethanimidamide.[28] ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.58 (bs, 1 H), 12.28 (bs, 1 H), 9.33 (bs, 1 H), 7.36 (dd, J = 8.6 and 5.8 Hz, 2 H), 7.15 (t, J = 8.9 Hz, 2 H), 4.43 (d, J = 6.36 Hz, 2 H), 2.24 (s, 3 H); MS *m*/*z* 278 (M + H)$^+$. HRMS Calcd for $C_{13}H_{13}FN_3O_3$ (M + H)$^+$: 278.09355. Found: 278.09460.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-(1-methylethyl)pyrimidine-4-carboxamide (12)**. As for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *N*-hydroxy-2-methylpropanimidamide. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.53 (bs, 1 H), 12.28 (bs, 1 H), 9.23 (t, J = 6.4 Hz, 1 H), 7.36 (dd, J = 8.1, 5.0 Hz, 2 H), 7.17 (t, J = 8.9 Hz, 2 H), 4.48 (d, J = 6.4 Hz, 2 H), 2.84−2.70 (m, 1 H), 1.19 (d, J = 7.3 Hz, 6 H); MS *m*/*z* 306

$(M + H)^+$. HRMS Calcd for $C_{15}H_{17}FN_3O_3$ $(M + H)^+$: 306.12485. Found: 306.12512.

2-Benzyl-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (13). As for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *N*-hydroxy-2-phenylethanimidamide. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.81 (s, 1 H), 12.32 (s, 1 H), 9.37 (t, $J = 6.2$ Hz, 1 H), 7.42−7.32 (m, 6 H), 7.25 (t, $J = 8.2$ Hz, 1 H), 7.14 (t, $J = 8.8$ Hz, 2 H), 4.46 (d, $J = 6.2$ Hz, 2 H), 3.84 (s, 2 H); MS *m*/*z* 354 $(M + H)^+$. HRMS Calcd for $C_{19}H_{17}FN_3O_3$ $(M + H)^+$: 354.12485. Found: 354.12573.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-(4-methylphenyl)pyrimidine-4-carboxamide (14)**. As for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *N*-hydroxy-4-methylbenzenecarboximidamide. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.90 (bs, 1 H), 12.30 (bs, 1 H), 9.78 (t, $J = 5.3$ Hz, 1 H), 8.24 (d, $J = 8.8$ Hz, 2 H), 7.49 (dd, $J = 5.6$ Hz, 8.7 Hz, 2 H), 7.41 (d, $J = 8.8$ Hz, 2 H), 7.27 (t, $J = 8.8$ Hz, 2 H), 4.64 (d, $J = 5.3$ Hz, 2 H), 2.48 (s, 3 H); MS *m*/*z* 354 $(M + H)^+$. HRMS Calcd for $C_{19}H_{17}FN_3O_3$: 354.12485. Found: 354.12500.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-(1,3-thiazol-2-yl)pyrimidine-4-carboxamide (15)**. As for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *N*-hydroxy-1,3-thiazole-2-carboximidamide. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.24 (bs, 1 H), 12.99 (bs, 1 H), 9.58 (t, $J = 6.7$ Hz, 1 H), 8.28−8.20 (m, 2 H), 7.59 (dd, $J = 8.0$, 5.5 Hz, 2 H), 7.37 (t, $J = 8.0$ Hz, 2 H), 4.72 (d, $J = 6.7$ Hz, 2 H); MS *m*/*z* 347 $(M + H)^+$. HRMS Calcd for $C_{15}H_{12}FN_4O_3S$ $(M + H)^+$: 347.06087. Found: 347.06015.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-pyridin-2-ylpyrimidine-4-carboxamide (16).** As for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *N*-hydroxypyridine-2-carboximidamide. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.98 (bs, 1 H), 12.50 (bs, 1 H), 9.71 (t, $J = 7.4$ Hz, 1 H), 8.72−8.65 (m, 2 H), 8.02 (t, $J = 6.2$ Hz, 1 H), 7.60−7.52 (m, 1 H), 7.38 (dd, $J = 8.3$, 6.5 Hz, 2 H), 7.18 (t, $J = 8.8$ Hz, 2 H), 4.52 (d, $J = 7.4$ Hz, 2 H); MS *m*/*z* 341 $(M + H)^+$. HRMS Calcd for $C_{17}H_{14}FN_4O_3$ $(M + H)^+$: 341.10445. Found: 341.10366.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-[methoxy(phenyl)methyl]-pyrimidine-4-carboxamide (17).** As for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *N*-hydroxy-2-methoxy-2-phenylethanimidamide. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.75 (bs, 1 H), 12.40 (bs, 1 H), 9.27 (bs, 1 H), 7.52 (d, $J = 7.1$ Hz, 2 H), 7.39−7.28 (m, 5 H), 7.18−7.12 (m, 2 H), 5.14 (s, 1 H), 4.52−4.41(m, 2 H), 3.34 (s, 3 H); MS *m*/*z* 384 $(M + H)^+$. HRMS Calcd for $C_{20}H_{19}FN_3O_4$ $(M + H)^+$: 384.135410. Found: 384.13498.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-[methyl(phenyl)amino]-pyrimidine-4-carboxamide (18).** As for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *N*″-hydroxy-*N*-methyl-*N*-phenylguanidine. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.6 (bs, 1 H), 9.09 (bs, 1 H), 7.41−7.35 (m, 4 H), 7.26−7.22 (m, 3 H), 7.17 (t, $J = 8.8$ Hz, 2 H), 4.47 (d, $J = 6.4$ Hz, 2 H), 3.36 (s, 3 H); MS *m*/*z* 369 $(M + H)^+$. HRMS Calcd for $C_{19}H_{18}FN_4O_3$ $(M + H)^+$: 369.13629. Found: 369.13692.

2-(3,4-Dimethoxybenzyl)-*N*-(4-fluorobenzyl)-5,6-dihydroxy-2-methylpyrimidine-4-carboxamide (20). As for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with 2-(3,4-dimethoxyphenyl)-*N*-hydroxyethanimidamide. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.85 (bs, 1 H), 12.12 (bs, 1 H), 9.39 (t, $J = 5.8$ Hz, 1 H), 7.40−7.33 (m, 2 H), 7.17 (t, $J = 8.7$ Hz, 2 H), 7.04 (s, 1 H), 6.88 (t, $J = 8.8$ Hz, 2 H), 4.46 (d, $J = 5.8$ Hz, 2 H), 3.74 (s, 2 H), 3.70 (s, 6 H); MS *m*/*z* 414 $(M + H)^+$; HRMS Calcd for $C_{21}H_{21}FN_3O_5$ $(M + H)^+$: 414.14598. Found: 414.14526.

Synthesis of Compounds 19, 22, and **23.** Synthesis of 2-[(dimethylamino)phenylmethyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide **19** is a representative example. Compounds **22** and **23** were isolated as trifluoroacetate salts by preparative RP HPLC in similar yields to **19**.

2-[(Dimethylamino)phenylmethyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (19). Step 1: Dimethyl acetylenedicarboxylate (0.82 mL, 6.7 mmol) was added to a stirred suspension of *N*′-hydroxy-2-phenylethanimidamide (1.0 g, 6.7 mmol) in chloroform (20 mL), and the reaction mixture was refluxed for 1 h. After complete conversion (TLC monitoring), volatiles were evaporated, p-xylenes (20 mL) were added to the residue, and the solution was refluxed for 6 h. Methyl-2-benzyl-5,6-dihydroxypyrimidine-4-carboxylate **51** precipitated from the solution after cooling to room temperature and was collected by filtration and dried (0.35 g, 20% yield). ^{1}H NMR (DMSO-d_6) δ 12.9 (bs, 1 H), 10.2 (bs, 1 H), 7.40−7.20 (m, 5 H), 3.82 (s, 2 H), 3.80 (s, 3 H); MS *m*/*z* 261 $(M + H)^+$. Step 2: To a stirred solution of methyl 2-benzyl-5,6-dihydroxypyrimidine-4-carboxylate (**51**, 0.35 g, 1.35 mmol) in anhydrous pyridine (3 mL) was added benzoyl chloride (0.78 mL, 6.75 mmol) dropwise with external cooling, and the reaction was stirred overnight at room temperature. The mixture was poured into 1 N HCl and extracted with ethyl acetate. The organic phase was washed with a saturated solution of $NaHCO_3$ and with brine, dried (Na_2SO_4), filtered, and concentrated under vacuum. The residue was purified by flash column chromatography (SiO_2, 80/20 *v*/*v* petroleum ether/ethyl acetate as eluent), yielding methyl 5,6-bis(benzoyloxy)-2-benzylpyrimidine-4-carboxylate as a colorless oil (0.47 g, 75% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (t, $J = 9.0$ Hz, 4 H), 7.62−7.57 (m, 2 H), 7.48−7-40 (m, 6 H), 7.31 (t, $J = 8.9$ Hz, 2 H), 7.28 (d, $J = 8.9$ Hz, 1 H), 4.41 (s, 2 H), 3.91 (s, 3 H). Step 3: A solution of methyl 5,6-bis-(benzoyloxy)2-benzylpyrimidine-4-carboxylate (0.47 g, 1.0 mmol, 1.0 equiv) in carbon tetrachloride (25 mL) was heated up to 90 °C under nitrogen. *N*-Bromosuccinimide (0.18 g, 1.0 mmol) and benzoyl peroxide (0.026 g, 0.1 mmol) were added as dry powder, and the mixture was refluxed for 3 h. Succinimide was removed by filtration, and the filtrate was concentrated and purified by flash column chromatography (SiO_2, 85/15 *v*/*v* petroleum ether/ethyl acetate as eluent), yielding methyl 5,6-bis(benzoyloxy)-2-[bromo-(phenyl)methyl]pyrimidine-4-carboxylate **52** as a white solid (0.47 g, 87% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11 (d, $J = 8.6$ Hz, 2 H), 8.05 (d, $J = 8.6$ Hz, 2 H), 7.79 (d, $J = 8.9$ Hz, 2 H), 7.56−7.49 (m, 2 H), 7.50−7.30 (m, 7 H), 6.30 (s, 1 H), 3.90 (s, 3 H). Step 4: Compound from above (0.47 g, 0.87 mmol) was added to a 2.0 M solution of dimethylamine in THF (2 mL). After the mixture was stirred for 10 min at room temperature, volatiles were evaporated under a stream of nitrogen and the residue was taken up in *N*,*N*-dimethylformamide (2 mL) and treated with 4 equiv of *p*-fluorobenzylamine (0.40 mL, 3.48 mmol). The reaction mixture was stirred at 90 °C for 1 h. After the mixture was cooled to room temperature, compound **19** was isolated in 35% yield (0.15 g) as the trifluoroacetate salt by RP-HPLC (C_{18}, acetonitrile/water containing 0.1% trifluoroacetic acid as eluent). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 13.42 (bs, 1 H), 12.34 (s, 1 H), 10.06 (bs, 1 H), 9.64 (t, $J = 5.9$ Hz, 1 H), 7.52 (s, 5 H), 7.43 (dd, $J = 8.4$ Hz, 5.6 Hz, 2 H), 7.23 (t, $J = 8.8$ Hz, 2 H), 5.28 (s, 1 H), 4.67 (dd, $J = 15.4$ Hz, 6.6 Hz, 1 H), 4.59 (dd, $J = 15.5$ Hz, 6.0 Hz, 1 H), 3.02 (s, 3 H), 2.06 (s, 3 H); ^{13}C NMR (DMSO-d_6, 600 MHz) δ 168.25, 161.32 (d, $J = 242.9$ Hz), 148.41, 144.29, 134.30, 130.89, 130.61, 129.40, 129.24, 129.00 (d, $J = 8.2$ Hz), 125.95, 115.56 (d, $J = 21.4$ Hz), 68.90, 43.30, 41.20, 40.80; MS *m*/*z* 397 $(M + H)^+$. HRMS Calcd for $C_{21}H_{22}FN_4O_3$ $(M + H)^+$: 397.16705. Found: 397.16830.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-[morpholin-4-yl(phenyl)-methyl]pyrimidine-4-carboxamide (22).** As for the preparation of **19**, replacing in step 4 dimethylamine with morpholine. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.30 (bs, 1 H), 9.90 (bs, 1 H), 9.34 (bs, 1 H), 7.63−7.58 (m, 2 H), 7.52−7.32 (m, 5 H), 7.18 (t, $J = 8.8$ Hz, 1 H), 5.39 (bs, 1 H), 4.60−4.39 (m, 2 H), 3.9−3.3 (m, 8 H, partially obscured by water); MS *m*/*z* 439 $(M + H)^+$; HRMS Calcd for $C_{23}H_{24}FN_4O_4$ $(M + H)^+$: 439.17816. Found: 439.17854.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-[phenyl(piperidin-1-yl)-methyl]pyrimidine-4-carboxamide (23).** As for the preparation of **19**, replacing in step 4 dimethylamine with piperidine. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.38 (bs, 1 H), 12.31 (s, 1 H), 9.90−9.70 (bs, 2 H), 7.58−7.40 (m, 7 H), 7.28 (t, $J = 8.9$ Hz, 2 H), 5.28 (bs, 1 H), 4.76−4.63 (m, 1 H), 4.58−4.48 (m, 1 H), 3.80−3.60 (m, 2 H), 3.20−2.80 (m, 2 H), 1.90−1.50 (m, 6 H); MS *m*/*z* 437

$(M + H)^+$; HRMS Calcd for $C_{24}H_{26}FN_4O_3$ $(M + H)^+$: 437.19835. Found: 437.19714.

2-{4-[(Dimethylamino)methyl]phenyl}-*N*-(4-fluorobenzyl)-5,6-dihydroxypyimidine-4-carboxamide (21). Step 1: Dimethyl acetylenedicarboxylate (0.82 mL, 6.7 mmol) was added to a stirred solution of *N*′-hydroxy-4-methylbenzenecarboximidamide (1.0 g, 6.7 mmol) in chloroform (20 mL), and reaction mixture was refluxed for 1 h. After complete conversion (TLC monitoring), volatiles were evaporated, p-xylenes (20 mL) were added to the residue, and the solution was refluxed for 6 h. Methyl 5,6-dihydroxy-2-(4-methylphenyl)pyrimidine-4-carboxylate (**54**) precipitated from the solution after cooling to room temperature and was collected by filtration and dried (0.50 g, 29% yield). MS *m*/*z* 261 $(M + H)^+$. Step 2: A mixture of methyl 5,6-dihydroxy-2-(4-methylphenyl)pyrimidine-4-carboxylate from above (**54**, 0.50 g, 1.9 mmol), benzoyl chloride (0.88 mL, 7.7 mmol), and dry pyridine (1.2 g, 15.2 mmol, 8 equiv) was stirred in dry dichloromethane (3 mL) at room temperature overnight. The reaction mixture was diluted with ethyl acetate, and the organic layer was washed twice with 1 N HCl and once with brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuum. The solid residue was triturated with diethyl ether to give methyl 5,6-bis(benzoyloxy)-2-(4-methylphenyl)pyrimidine-4-carboxylate (0.81 g, 91% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.38 (d, $J = 8.2$ Hz, 2 H), 8.14 (d, $J = 7.44$ z, 2 H), 8.10 (d, $J = 7.44$ Hz, 2 H), 7.62 (m, 2 H), 7.45 (m, 4 H), 7.30 (d, $J = 8.06$ Hz, 2 H), 3.93 (s, 3 H), 2.44 (s, 3 H). Step 3: A suspension of methyl ester from above (0.81 g, 1.7 mmol), an equimolar amount of *N*-bromosuccinimide (0.30 g, 1.7 mmol), and 5% of dibenzoyl hydroperoxide (0.022 g, 0.08 mmol) in carbon tetrachloride (50 mL) was heated at 95 °C. The reaction mixture was refluxed for 2 h and then cooled to room temperature. Succinimide was filtered off, and volatiles were removed in vacuum, yielding 0.80 g of methyl 5,6-bis(benzoyloxy)-2-[4-(bromomethyl)phenyl]pyrimidine-4-carboxylate (**55**, 85% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.47 (d, $J = 8.25$ Hz, 2 H), 8.14 (d, $J = 7.56$ Hz, 2 H), 8.10 (d, $J = 7.50$ Hz, 2 H), 7.63 (m, 2 H), 7.53 (d, $J = 8.23$, 2 H), 7.46 (m, 4 H), 4.56 (s, 2 H), 3.94 (s, 3 H). Step 4: A mixture of the benzyl bromide from above (**55**, 0.80 g, 1.5 mmol) and dimethylamine (3 mL as 2 M solution in THF) was allowed to stir at room temperature overnight. Volatiles were then removed in vacuum, the residue was taken up in DMF (4 mL), and after addition of 4-fluorobenzylamine (0.34 mL, 3.0 mmol) the reaction mixture was stirred at 90 °C overnight. After being cooled to room temperature, the mixture was acidified with 1 N HCl and compound **21** was purified by preparative RP-HPLC (C_{18}, acetonitrile/water containing 0.1% trifluoroacetic acid as eluent) and isolated in 44% yield (0.34 g) as the trifluoroacetate salt. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.00 (bs, 1 H), 12.59 (s, 1 H), 9.69 (bs, 1 H), 9.66 (t, $J = 5.8$ Hz, 1 H), 8.37 (d, $J = 9.7$ Hz, 2 H), 7.62 (d, $J = 9.7$ Hz, 2 H), 7.41 (dd, $J = 8.3$, 5.4 Hz, 2 H), 7.19 (t, $J = 8.4$ Hz, 2 H), 4.54 (d, $J = 5.8$ Hz, 2 H), 4.34 (s, 2 H), 2.77 (s, 6 H); MS *m*/*z* 397 $(M + H)^+$. HRMS Calcd for $C_{21}H_{22}FN_4O_3$ $(M + H)^+$: 397.16705. Found: 397.16830.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-piperidin-2-ylpyrimidine-4-carboxamide (24).** Step 1: Identical procedure used for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with benzyl 2-[amino(hydroxyimino)methyl]-piperidine-1-carboxylate, afforded benzyl 2-(4-{[(4-fluorobenzyl)-amino]carbonyl}-5,6-dihydroxypyrimidin-2-yl)piperidine-1-carboxylate (18% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.67 (bs, 1 H), 12.29 (bs, 1 H), 8.68 (bs, 1 H), 7.35 (dd, $J = 8.4$, 5.6 Hz, 2 H), 7.27 (bs, 5 H), 7.17 (t, $J = 8.8$ Hz, 2 H), 5.12 (d, $J = 12.2$ Hz, 1 H), 5.00−4.93 (m, 2 H), 4.49 (d, $J = 5.8$ Hz, 2 H), 3.93−3.87 (m, 1 H), 3.30−3.27 (m, 1 H), 2.29−2.18 (m, 1 H), 1.88−1.32 (m, 5 H); MS *m*/*z* 481 $(M + H)^+$. Step 2: The compound from the previous step (0.05 g, 0.1 mmol) was taken up in methanol (5 mL) and hydrogenated in the presence of 10% Pd/C at room temperature from 1 h. Catalyst was filtered off, and the filtrate was concentrated and purified by preparative RP-HPLC (C_{18}, acetonitrile/water containing 0.1% trifluoroacetic acid as eluent), giving title compound in 60% yield (0.029 g) as its trifluoroacetate salt. ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 13.1 (bs, 1 H), 12.2 (s, 1 H), 9.38 (t, $J = 6.4$ Hz, 1 H), 9.20−9.10 (m, 1 H), 8.80−8.65 (m, 1 H), 7.41 (dd, $J = 5.6$ Hz, 8.4 Hz, 2 H), 7.17 (t, $J = 8.8$ Hz, 2 H), 4.65−4.43 (m, 2 H), 4.15−4.05 (m, 1 H), 3.49−3.38 (m, 1 H), 3.12−3.00 (m, 1 H), 2.30−2.20 (m, 1 H), 1.88−1.50 (m, 5 H); MS *m*/*z* 347 $(M + H)^+$. HRMS Calcd for $C_{17}H_{20}FN_4O_3$ $(M + H)^+$: 347.15140. Found: 347.15009.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-piperidin-3-ylpyrimidine-4-carboxamide (25).** Step 1: Identical procedure used for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *tert*-butyl 3-[amino(hydroxyimino)methyl]-piperidine-1-carboxylate, afforded *tert*-butyl 3-(4-{[(4-fluorobenzyl)-amino]carbonyl}-5,6-dihydroxypyrimidin-2-yl)piperidine-1-carboxylate (23% yield). MS *m*/*z* 447 $(M + H)^+$. Step 2: The crude compound from the previous step (0.050 g, 0.11 mmol) was stirred in 2 mL of CH_2Cl_2:TFA (8:2) for 1 h at room temperature. Evaporation of volatiles and purification by preparative RP-HPLC (C_{18}, acetonitrile/water containing 0.1% trifluoroacetic acid as eluent) yielded the title compound as its trifluoroacetate salt (0.034 g, 67% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.83 (bs, 1 H), 12.38 (s, 1 H), 9.32 (t, $J = 5.6$ Hz, 1 H), 8.69 (bs, 1 H), 8.50 (bs, 1 H), 7.38 (dd, $J = 8.8$, 5.7 Hz, 2 H), 7.29 (t, $J = 8.8$ Hz, 2 H), 4.58−4.43 (m, 2 H), 3.29−3.17 (m, 3 H), 2.98−2.89 (m, 1 H), 2.88−2.76 (m, 1 H), 2.10−2.00 (m, 1 H), 1.90−1.79 (m, 1 H), 1.72−1.58 (m, 2 H); MS *m*/*z* 347 $(M + H)^+$. HRMS Calcd for $C_{17}H_{20}FN_4O_3$ $(M + H)^+$: 347.15140. Found: 347.15161.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-piperidin-4-ylpyrimidine-4-carboxamide (26).** Step 1: Identical procedure used for the preparation of **9**, replacing in step 1 *N*-hydroxythiophene-2-carboximidamide with *tert*-butyl 4-[amino(hydroxyimino)methyl]-piperidine-1-carboxylate, afforded *tert*-butyl-4-(4-{[(4-fluorobenzyl)-amino]carbonyl}-5,6-dihydroxypyrimidin-2-yl)piperidine-1-carboxylate (27% yield). MS *m*/*z* 447 $(M + H)^+$. Step 2: The crude compound from the previous step (0.050 g, 0.11 mmol) was stirred in 2 mL of CH_2Cl_2:TFA (8:2) for 1 h at room temperature. Evaporation of volatiles and purification by preparative RP-HPLC (C_{18}, acetonitrile/water containing 0.1% trifluoroacetic acid as eluent) yielded the title compound as its trifluoroacetate salt (0.029 g, 58% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.68 (bs, 1 H), 12.36 (s, 1 H), 9.19 (d, $J = 5.8$ Hz, 1 H), 8.58 (bs, 1 H), 8.29 (bs, 1 H), 7.38 (dd, $J = 8.8$, 5.7 Hz, 2 H), 7.18 (t, $J = 8.8$ Hz, 2 H), 4.49 (d, $J = 5.8$ Hz, 2 H), 3.37−3.28 (m, 2 H), 3.01−2.88 (m, 2 H), 2.82−2.75 (m, 1 H), 2.10−2.02 (m, 2 H), 1.99−1.86 (m, 2 H); MS *m*/*z* 347 $(M + H)^+$. HRMS Calcd for $C_{17}H_{20}FN_4O_3$ $(M + H)^+$: 347.15140. Found: 347.15256.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-(1-methylpiperidin-2-yl)-pyrimidine-4-carboxamide (27).** To a solution of compound **24** (0.070 g, 0.15 mmol) in methanol (2 mL) were added formaldehyde (37 wt % in H_2O, 0.065 mL), $NaBH_3CN$ (0.050 g, 0.78 mmol), and NaOAc (0.070 g, 0.87 mmol). The mixture was stirred at room temperature under nitrogen for 12 h and then concentrated and purified by preparative RP-HPLC purification (C_{18}, water/acetonitrile containing 0.1% trifluoroacetic acid as eluent) to isolate title compound in 55% yield (0.039 g) as its trifluoroacetate salt. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.1 (bs, 1 H), 12.2 (s, 1 H), 9.45 (bs, 1 H), 9.34 (t, $J = 6.4$ Hz, 1 H), 7.37 (dd, $J = 5.6$ Hz, 8.4 Hz, 2 H), 7.18 (t, $J = 8.8$ Hz, 2 H), 4.57 (d, $J = 6.4$ Hz, 2 H), 4.05 (bs, 1 H), 3.61 (bd, $J = 12.4$ Hz, 1 H), 3.52−3.50 (m, 1 H), 2.78 (bs, 3 H), 2.16 (d, $J = 13.6$ Hz, 1 H), 1.92−1.80 (m, 2 H), 1.65−1.46 (m, 3 H); MS *m*/*z* 361 $(M + H)^+$. HRMS Calcd for $C_{18}H_{22}FN_4O_3$ $(M + H)^+$: 361.16705. Found: 361.16583.

2-(1-Ethylpiperidin-2-yl)-*N*-(4-fluorobenzyl)-5,6-dihydroxy-pyrimidine-4-carboxamide (28). As for the preparation of **27**, replacing formaldehyde with 1.5 equiv of acetaldehyde (43% yield). ^{1}H NMR (DMSO-d_6, 400 MHz, 330 K) δ 13.11 (bs, 1 H), 12.19 (s, 1 H), 9.29 (bs, 1 H), 8.97 (bs, 1 H), 7.38 (dd, $J = 8.4$, 5.6 Hz, 2 H), 7.19 (t, $J = 8.8$ Hz, 2 H), 4.72−4.63 (m, 1 H), 4.58−4.50 (m, 1 H), 4.11−4.06 (m, 1 H), 3.79−3.71 (m, 1 H), 3.42−3.30 (m, 2 H), 3.09−3.00 (m, 1 H), 2.22−2.14 (m, 1 H), 1.98−1.50 (m, 5 H), 1.16 (t, $J = 6.6$ Hz, 3 H); MS *m*/*z* 375 $(M + H)^+$. HRMS Calcd for $C_{19}H_{24}FN_4O_3$ $(M + H)^+$: 375.18270. Found: 375.18088.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-(1-isobutylpiperidin-2-yl)pyrimidine-4-carboxamide (29).** As for the preparation of **27**, replacing formaldehyde with 1.5 equiv of 2-methylpropanal (22% yield). ^{1}H NMR (DMSO-d_6, 400 MHz, 330 K) δ 12.21 (bs, 1 H), 9.19 (bs, 1 H), 8.47 (bs, 1 H), 7.38 (dd, J = 8.5, 5.7 Hz, 2 H), 7.26 (t, J = 8.9 Hz, 2 H), 4.78−4.68 (m, 1 H), 4.48−4.39 (m, 1 H), 4.08−3.97 (m,1 H), 3.37−3.28 (m, 1 H), 2.92−2.83 (m, 2 H), 2.20−1.40 (m, 8 H), 0.86−0.75 (m, 6 H); MS m/z 403 $(M + H)^+$. HRMS Calcd for $C_{21}H_{28}FN_4O_3$ $(M + H)^+$: 403.21400. Found: 403.21561.

2-(1,4-Dimethylpiperazin-2-yl)-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (30). Step 1: A solution of 1-benzyl 4-*tert*-butyl 2-[amino(hydroxyimino)methyl]piperazine-1,4-dicarboxylate (2 g, 5.3 mmol) in chloroform (30 mL) was treated with dimethyl acetylenedicarboxylate (0.78 mL, 6.36 mmol) and refluxed for 3 h. The reaction mixture was cooled to room temperature and concentrated. The residue was taken up in xylene (30 mL) and refluxed for 24 h. The solvent was removed in vacuum, and the residue was triturated with a 1:1 mixture of Et_2O:*n*-hexane. The solid was filtered off and washed further with Et_2O:*n*-hexane. The crude product was taken up in pyridine (8 mL), and to the stirred solution was added benzoic anhydride (1.8 g, 7.9 mmol). The reaction mixture was stirred at room temperature until the starting material was consumed as determined by MS analysis. The reaction mixture was concentrated, and the oily residue was diluted with EtOAc and washed with 1 N HCl, $NaHCO_3$ (sat. soln), and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated, yielding 0.69 g of crude 1-benzyl-4-*tert*-butyl-2-[5-(benzoyloxy)-4-hydroxy-6-(methoxycarbonyl)pyrimidin-2-yl]piperazine-1,4-dicarboxylate (LC-MS analysis, about 22% yield). MS m/z 593 $(M + H)^+$. Step 2: 1-Benzyl-4-*tert*-butyl-2-[5-(benzoyloxy)-4-hydroxy-6-(methoxycarbonyl)pyrimidin-2-yl]piperazine-1,4-dicarboxylate from the previous step was treated with TFA/CH_2Cl_2 1:1 (10 mL). After being stirred 1 h at room temperature, the solution was evaporated to the crude methyl 5-(benzoyloxy)-2-{1-[(benzyloxy)carbonyl]piperazin-2-yl}-6-hydroxypyrimidine-4-carboxylate, which was further reacted without further purification. MS m/z 493 $(M + H)^+$. Step 3: The crude product from step 2 was dissolved in methanol (5 mL) and treated with $NaCNBH_3$ (0.10 g, 1.62 mmol), NaOAc (0.150 g, 1.86 mmol), and formaldehyde (37 wt % in H_2O, 0.2 mL). After being stirred 1 h at room temperature, the mixture was evaporated and the crude residue containing (LC-MS analysis) methyl 5-(benzoyloxy)-2-{1-[(benzyloxy)carbonyl]-4-methyl-piperazin-2-yl}-6-hydroxypyrimidine-4-carboxylate was further reacted in the next step. MS m/z 507 $(M + H)^+$. Step 4: Crude material from the previous step was dissolved in methanol (10 mL) and hydrogenated at atmospheric pressure in the presence of 10% Pd/C overnight. Crude methyl 5,6-dihydroxy-2-(4-methyl-piperazin-2-yl)pyrimidine-4-carboxylate was obtained by filtration and evaporation of the filtrate. MS m/z 269 $(M + H)^+$. Step 5: Crude product from step 4 was dissolved in methanol (10 mL) and treated with $NaCNBH_3$ (0.10 g, 1.62 mmol), NaOAc (0.15 g, 1.86 mmol), and formaldehyde (37 wt % in H_2O, 0.2 mL), and the resulting mixture was stirred at room temperature for 2.5 h. After evaporation, the crude residue containing (LC-MS analysis) methyl 2-(1,4-dimethylpiperazin-2-yl)-5,6-dihydroxypyrimidine-4-carboxylate was taken on without purification. MS m/z 283 $(M + H)^+$. Step 6: Crude product from the previous step was dissolved in *N*-methylpyrrolidone (6 mL/mmol) and treated with 4-fluorobenzylamine (0.40 mL, 3.48 mmol). The mixture was stirred at 90 °C overnight and then cooled to room temperature, and an aliquot of the crude reaction mixture was purified by preparative RP-HPLC (C_{18}, acetonitrile/water containing 0.1% trifluoroacetic acid as eluent) to obtain 0.015 g of 2-(1,4-dimethylpiperazin-2-yl)-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (**30**) as its trifluoroacetate salt. ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 12.5 (bs, 1 H), 7.38 (dd, J = 5.8, 8.8 Hz, 2 H), 7.17 (t, J = 8.8 Hz, 2 H), 4.58−4.40 (m, 2 H), 3.66 (bs, 1 H), 3.55−3.35 (m, 3 H), 3.20 (d, J = 13.3 Hz, 1 H), 3.03 (t, J = 11.7 Hz, 1 H), 2.79 (s, 3 H), 2.85−2.70 (m, 1 H), 2.33 (bs, 3 H); MS m/z 376 $(M + H)^+$. HRMS Calcd for $C_{18}H_{23}FN_5O_3$ $(M + H)^+$: 376.17794. Found: 376.17925.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-(4-methylmorpholin-3-yl)pyrimidine-4-carboxamide (31).** Step 1: A solution of *tert*-butyl-3-[amino(hydroxyimino)methyl]morpholine-4-carboxylate (2.0 g, 7.3 mmol) in chloroform (25 mL) was treated with dimethyl acetylenedicarboxylate (1.08 mL, 8.76 mmol) and refluxed for 3 h. The reaction mixture was cooled to room temperature and concentrated. The residue was taken up in xylene (25 mL) and refluxed for 24 h. The solvent was removed in vacuum, and the crude residue was treated with 20 mL of a mixture of trifluoroacetic acid:dichloromethane:water (65:35:10) at room temperature for 15 min. The reaction mixture was concentrated, and the residue was taken up in Et_2O and evaporated. A solid residue corresponding to the TFA salt of methyl 5,6-dihydroxy-2-morpholin-3-ylpyrimidine-4-carboxylate was obtained after trituration with Et_2O (1.35 g, 50% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.24 (bs, 1 H), 10.54 (bs, 1 H), 9.54 (bs, 2 H), 4.34 (d, J = 6.9 Hz, 1 H), 4.24 (dd, J = 12.2, 3.2 Hz,1 H), 3.93 (d, J = 11.2 Hz, 1 H), 3.84 (s, 3 H), 3.75 (t, J = 10.3 Hz, 1 H), 3.58 (t, J = 10.5 Hz, 1 H), 3.32 (d, J = 12.8 Hz, 1 H), 3.20 (td, J = 11.0, 3.7 Hz, 1 H); MS m/z 256 $(M + H)^+$. Step 2: The compound from step 1 was dissolved in dry methanol (20 mL) and treated with 4-fluorobenzyl amine (0.83 mL, 7.30 mmol) at 90 °C for 1 h. After being cooled to room temperature, the reaction mixture was concentrated and the residue triturated with Et_2O. *N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-morpholin-3-ylpyrimidine-4-carboxamide was isolated in 90% yield (1.5 g) by preparative RP-HPLC (C_{18}, acetonitrile/water containing 0.1% trifluoroacetic acid as eluent) as its trifluoroacetate salt. ^{1}H NMR (DMSO-d_6+TFA, 400 MHz) δ 9.63 (bs, 1 H), 9.4 (t, J = 6.07 Hz, 1 H), 9.2 (bs, 1 H), 7.39 (dd, J = 8.31, 5.76 Hz, 2 H), 7.18 (t, J = 8.84 Hz, 2 H), 4.59 (dd, J = 15.53, 6.80 Hz, 2 H), 4.53 (dd, J = 15.36, 6.24 Hz, 1 H), 4.37 (bs, 1 H), 4.24 (dd, J = 12.41, 3.24 Hz, 1 H), 3.99 (d, J = 12.04 Hz, 1 H), 3.74 − 3.62 (m, 2 H), 3.41 (d, J = 13.09 Hz, 1 H), 3.40 (bs, 1 H); MS m/z 349 $(M + H)^+$. Step 3: To a methanolic solution of compound from above (0.200 g, 0.43 mmol) were added formaldehyde (37 wt % in H_2O, 0.2 mL), $NaBH_3CN$ (0.140 g, 2.24 mmol), and NaOAc (0.20 g, 2.49 mmol). The mixture was stirred at room temperature under nitrogen for 12 h and then concentrated and purified by preparative RP-HPLC purification (C_{18}, water acetonitrile containing 0.1% trifluoroacetic acid as eluent), yielding *N*-(4-fluorobenzyl)-5,6-dihydroxy-2-(4-methylmorpholin-3-yl)pyrimidine-4-carboxamide (**31**) as a its trifluoroacetate salt (0.14 g, 67% yield). ^{1}H NMR (DMSO-d_6+TFA, 400 MHz) δ 9.2 (bt, 1 H), 7.40 (dd, J 0 8.38, 5.75 Hz, 2 H), 7.16 (t, J = 8.84 Hz, 2 H), 4.57 (d, J = 6.34 Hz, 2 H), 4.27 (dd, J = 10.03, 3.55 Hz, 1 H), 4.22 (dd, J = 12.82, 3.22 Hz, 1 H), 4.10 (d, J = 13.73 Hz, 1 H), 3.77 (t, J = 11.84 Hz, 1 H), 3.65−3.60 (m, 2 H), 3.41 (td, J = 12.54, 3.67 Hz, 1 H), 2.87 (s, 3 H); MS m/z 363 $(M + H)^+$. HRMS Calcd for $C_{17}H_{20}FN_4O_4$ $(M + H)^+$: 363.14631. Found: 363.14565.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-(1-methylpyrrolidin-2-yl)pyrimidine-4-carboxamide (32).** Step 1: A solution of *tert*-butyl-2-[amino(hydroxyimino)methyl]pyrrolidine-1-carboxylate (2.0 g, 8.7 mmol) in chloroform (25 mL) was treated with dimethyl acetylenedicarboxylate (1.28 mL, 10.44 mmol) and refluxed for 3 h. The reaction mixture was cooled to room temperature and concentrated. The residue was taken up in xylene (30 mL) and refluxed for 24 h. The solvent was removed in vacuum, and the crude residue was taken up in pyridine (10 mL). To the solution was added benzoic anhydride (2.9 g, 13 mmol, 1.5 equiv), and the reaction mixture was stirred at room temperature until the starting material was consumed as determined by MS analysis. The reaction mixture was concentrated, and the oily residue was diluted with EtOAc and washed with 1 N HCl, $NaHCO_3$ (sat. soln), and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated, and the residue was purified by flash column chromatography (SiO_2, petroleum ether/ethyl acetate 60/40 *v*/v as eluent), to obtain the methyl 5-(benzoyloxy)-2-[1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-6-hydroxypyrimidine-4-carboxylate as a yellow solid (0.73 g, 19% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.08 (bs, 1 H), 8.18 (d, J = 7.6 Hz, 2 H), 7.64 (t, J = 7.6 Hz, 1 H), 7.50 (t, J = 7.6 Hz, 2 H), 4.80−4.60 (m, 1 H), 3.82 (s, 3 H), 3.60−3.50 (m, 1 H), 3.40−

3.20 (m, 1 H), 2.50−2.10 (m, 2 H), 2.00−1.70 (m, 2 H), 1.50 (s, 9 H); MS *m*/*z* 444 (M + H)$^+$. Step 2: Methyl 5-(benzoyloxy)-2-[1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-6-hydroxypyrimidine-4-carboxylate from above (0.73 g, 1.64 mmol) was treated with 20 mL of CH_2Cl_2:TFA (7:3) at 0 °C. The solution was allowed to warm to room temperature and stirred for 1 h. Volatiles were removed under reduced pressure, the residue was triturated with Et_2O, and methyl 5-(benzoyloxy)-6-hydroxy-2-pyrrolidin-2-yl-pyrimidine-4-carboxylate was collected by filtration (0.54 g, 96% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.14 (d, J = 7.5 Hz, 2 H), 7.67 (t, J = 7.6 Hz, 1 H), 7.50 (dd, J = 7.6, 7.6 Hz, 2 H), 4.99 (dd, J = 14.9, 7.3 Hz, 1 H), 3.78 (s, 3 H), 3.60−3.40 (m, 2 H), 2.60−2.45 (m, 1 H), 2.40−2.30 (m, 1 H), 2.20−2.10 (m, 2 H); MS *m*/*z* 344 (M + H)$^+$. Step 3: A solution of compound from step 2 (0.10 g, 0.29 mmol) in MeOH (3 mL) was treated with 4-fluorobenzylamine (0.10 mL, 0.87 mmol), and the solution was refluxed for 5 h. After being cooled to room temperature, the reaction mixture was concentrated under reduced pressure, and the product *N*-(4-fluorobenzyl)-5,6-dihydroxy-2-pyrrolidin-2-ylpyrimidine-4-carboxamide that precipitated was collected by filtration (0.046 g, 48% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.55 (bs, 1 H), 7.35 (dd, J = 13.7, 7.8 Hz, 2 H), 7.16 (dd, J = 17.5, 8.8 Hz, 2 H), 4.60−4.40 (m, 2 H), 4.06 (dd, J = 14.0, 6.9 Hz, 1 H), 3.15−3.10 (m, 1 H), 3.00−2.90 (m, 1 H), 2.20−2.10 (m, 1 H), 1.90−1.70 (m, 3 H); MS *m*/*z* 333 (M + H)$^+$. Step 4: To a stirred solution of the above compound (0.046 g, 0.14 mmol) in MeOH (2 mL) were added Et_3N (0.02 mL, 0.14 mmol), NaOAc (0.018 g, 0.22 mmol), AcOH glacial (0.015 mL), formaldehyde (37 wt % in H_2O, 0.03 mL), and $NaBH(AcO)_3$ (0.046 g, 0.22 mmol). The reaction mixture was stirred at room temperature overnight and then poured into aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was collected, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by preparative RP-HPLC (C_{18}, water/acetonitrile with 0.1% trifluoroacetic acid as eluent) to give the *N*-(4-fluorobenzyl)-5,6-dihydroxy-2-(1-methylpyrrolidin-2-yl)pyrimidine-4-carboxamide (**32**) as its trifluoroacetate salt (0.037 g, 57% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.20 (bs, 1 H), 12.50 (bs, 1 H), 10.0−9.70 (m, 1 H), 9.63 (bs, 1 H), 7.35 (dd, J = 13.8, 8.2 Hz, 2 H), 7.18 (dd, J = 17.5, 8.8 Hz, 2 H), 4.54 (m, 2 H), 4.40 (dd, J = 15.7, 7.7 Hz, 1 H), 3.82−3.70 (m, 1 H), 3.40−3.20 (m, 1 H), 2.94 (s, 3 H), 2.60−2.50 (m, 1 H), 2.20−1.80 (m, 3 H); MS *m*/*z* 347 (M + H)$^+$. HRMS Calcd for $C_{17}H_{20}FN_4O_3$ (M + H)$^+$: 347.15140. Found: 347.15021.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-(1,2,4-trimethylpiperazin-2-yl)pyrimidine-4-carboxamide (33).** Step 1: A solution of 1-benzyl 4-*tert*-butyl-2-[amino(hydroximino)methyl]-2-methylpiperazine-1,4-dicarboxylate (2.0 g, 5.10 mmol) in chloroform (30 mL) was treated with dimethyl acetylenedicarboxylate (0.75 mL, 6.12 mmol) and refluxed for 3 h. The reaction mixture was cooled to room temperature and concentrated. The residue was taken up in xylene (30 mL) and refluxed for 24 h. The solvent was removed in vacuum, and the crude residue was dissolved in pyridine (8 mL) and treated with benzoic anhydride (1.73 g, 7.65 mmol). The reaction mixture was stirred at room temperature overnight and then concentrated under reduced pressure and partitioned between EtOAc and 1 N HCl. The organic layer was dried (Na_2SO_4), filtered, and evaporated, and the 1-benzyl 4-*tert*-butyl 2-[5-(benzoyloxy)-4-hydroxy-6-(methoxycarbonyl)pyrimidin-2-yl]-2-methylpiperazine-1,4-dicarboxylate was purified by flash chromatography on silica gel eluting with petroleum ether/ethyl acetate 70:30 *v*/*v* (0.37 g, 12% yield). ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 12.96 (bs, 1 H), 8.11−8.04 (m, 2 H), 7.79−7.73 (m, 1 H), 7.66−7.58 (m, 2 H), 7.37−7.22 (m, 5 H), 5.03 (s, 2 H), 4.00−3.91 (m, 1 H), 3.80−3.52 (m, 7 H), 3.47−3.40 (m, 1 H), 1.65 (s, 3 H), 1.35 (s, 9 H); MS *m*/*z* 607 (M + H)$^+$. Step 2: ompound from above (0.37 g, 0.61 mmol) was dissolved in ethyl acetate (15 mL) and hydrogenated at 1 atm on 10% w/w Pd/C overnight. Catalyst was filtered off and solvent evaporated to methyl 5-(benzoyloxy)-2-[4-(*tert*-butoxycarbonyl)-2-methylpiperazin-2-yl]-6-hydroxypyrimidine-4-carboxylate (0.29 g, 100% yield). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz, 340 K) δ 8.11−8.04 (m, 2 H), 7.81−7.74 (m, 1 H), 7.66−7.58 (m, 2 H), 4.22 (d, J = 14.4 Hz, 1 H), 3.80 (s, 3 H), 3.75−3.67 (m, 2 H), 3.63−3.44 (m, 2 H), 3.32−3.24 (m, 1 H), 1.68 (s, 3 H), 1.38 (s, 9 H); MS *m*/*z* 473 (M + H)$^+$. Step 3: Crude material from above (0.29 g, 0.61 mmol) was dissolved in methanol (10 mL), and to the stirred mixture were added $NaCNBH_3$ (0.054 g, 0.85 mmol), NaOAc (0.158 g, 1.95 mmol), and 37% water solution of formaldehyde (0.1 mL). The reaction mixture was stirred for 0.5 h at room temperature, volatiles were evaporated, and the residue containing methyl 2-[4-(*tert*-butoxycarbonyl)-1,2-dimethylpiperazin-2-yl]-5,6-dihydroxypirimidine-4-carboxylate (LC-MS analysis) was triturated with Et_2O. MS *m*/*z* 383 (M + H)$^+$. Step 4: Crude material obtained in the previous step (assuming quantitative yield) was dissolved in methanol (15 mL) and treated with an excess of 4-fluorobenzylamine (0.21 mL, 1.8 mmol, about 3 equiv). The mixture was refluxed overnight, cooled to room temperature, and concentrated, allowing the precipitation of crude *tert*-butyl 3(4-{-[(4-fluorobenzyl)amino]carbonyl}-5,6-dihydropyrimidin-2-yl)-3,4-dimethylpiperazine-1-carboxylate as a solid, which was filtered off and washed with Et_2O (0.150 g). MS *m*/*z* 476 (M + H)$^+$. Step 5: Crude material from the previous step was stirred in 10 mL of CH_2Cl_2/TFA (1:1) for 1 h. Evaporation of the solvent afforded 2-(1,2-dimethylpiperazin-2-yl)-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide as a crude compound (0.11 g). MS *m*/*z* 376 (M + H)$^+$. Step 6: Crude material obtained in step 5 was dissolved in methanol (5 mL), and triethylamine (0.19 mL, 1.3 mmol), $NaCNBH_3$ (0.054 g, 0.85 mmol), NaOAc (0.158 g, 1.95 mmol), and 37% aqueous HCHO (0.15 mL) were added to the solution under stirring. The reaction mixture was stirred at room temperature overnight and then evaporated under reduced pressure to an oily residue. An aliquot of this residue was purified by preparative RP-HPLC (C_{18}, acetonitrile/water containing 0.1% trifluoroacetic acid as eluent), yielding 0.014 g of *N*-(4-fluorobenzyl)-5,6-dihydroxy-2-(1,2,4-trimethylpiperazin-2-yl)pyrimidine-4-carboxamide (**33**) as its trifluoroacetate salt. ^{1}H NMR (CD_3CN + TFA, 600 MHz, 280 K) δ 7.50−7.38 (m, 2 H), 7.13−7.07 (m, 2 H), 4.66−4.51 (m, 2 H), 4.00−3.72 (m, 4.3 H), 3.60−3.56 (t, J = 12.7 Hz. 0.7 H), 3.49−3.44 (t, J = 15.5 Hz, 1 H), 3.04 (s, 2 H), 2.91 (s, 1 H), 2.73 (s, 1 H), 2.69 (bs, 2 H), 2.05 (s, 1 H), 1.95 (2 H, partially obscured by solvent); MS *m*/*z* 390 (M + H)$^+$. HRMS Calcd for $C_{19}H_{25}FN_5O_3$ (M + H)$^+$: 390.19359. Found: 390.19353.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-(7-methyl-7-azabicyclo-[2.1.1]hept-1-yl)pyrimidine-4-carboxamide (34).** Step 1: A solution of benzyl 1-[amino(hydroxyimino)methyl]-7-azabicyclo[2.2.1]-heptane-7-carboxylate (3 g, 10.4 mmol) in chloroform (45 mL) was treated with dimethyl acetylenedicarboxylate (1.52 mL, 12.4 mmol) and refluxed for 5 h. The reaction mixture was cooled to room temperature and concentrated. The residue was taken up in xylene (75 mL) and refluxed overnight. The solvent was removed in vacuum, and the residue was dissolved in pyridine (10 mL) and treated with 2 equiv of benzoic anhydride (4.7 g, 20.7 mmol). The reaction mixture was stirred at room temperature for 3 h and then concentrated under reduced pressure, and the residue was taken up in ethyl acetate and washed with 1 N HCl and saturated $NaHCO_3$ aqueous solution. The organic phase was dried over Na_2SO_4, filtered, and concentrated. Benzyl 1-[5-(benzoyloxy)-4-hydroxy-6-(methoxycarbonyl)pyrimidin-2-yl]-7-azabicyclo[2.2.1]heptane-7-carboxylate was purified by flash column chromatography (SiO_2, 60/40 petroleum ether/ethyl acetate as eluent) and obtained in 20% yield (1.04 g, 2.1 mmol). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.38 (s, 1 H), 8.09 (d, J = 7.5 Hz, 2 H), 7.80 (t, J = 7.5 Hz, 1 H), 7.64 (t, J = 7.5 Hz, 2 H), 7.40−7.22 (m, 5 H), 5.00 (s, 2 H), 4.40 (t, J = 4.3 Hz, 1 H), 3.76 (s, 3 H), 2.32−2.11 (m, 2 H), 1.95−1.79 (m, 4 H), 1.66−1.51 (m, 2 H); MS *m*/*z* 504 (M + H)$^+$. Step 2: The compound from the previous step (0.200 g, 0.40 mmol) was taken up in methanol (5 mL) and hydrogenated under H_2 atmosphere in the presence of Pd/C 10% (10% w/w) at room temperature for 2 h. Catalyst was filtered off, and filtrate was evaporated under reduced pressure. Residue was dissolved in methanol (5 mL) and treated with about 3.5 equiv of *p*-fluorobenzylamine (0.16 mL, 1.40 mmol). The reaction mixture was refluxed overnight and then cooled to room temperature, allowing the precipitation of a solid, which was

recovered by filtration and washed with Et_2O. The solid was taken up in methanol (5 mL) and treated with $NaCNBH_3$ (0.035 g, 0.56 mmol), NaOAc (0.052 g, 0.64 mmol), and 37% aqueous solution of formaldehyde (0.04 mL). The reaction mixture was stirred at room temperature overnight. The cooled reaction mixture was purified by preparative RP-HPLC (C_{18}, acetonitrile/water containing 0.1% trifluoroacetic acid as eluent), and the product *N*-(4-fluorobenzyl)-5,6-dihydroxy-2-(7-methyl-7-azabicyclo[2.2.1]hept-1-yl)-pyrimidine-4-carboxamide (**34**) was isolated as its trifluoroacetate salt in 25% yield (0.048 g). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.9 (bs, 1 H), 12.2 (s, 1 H), 10.95 (bs, 1 H), 9.66 (bs, 1 H), 7.47−7.40 (m, 2 H), 7.21−7.12 (m, 2 H), 4.50 (d, J = 6.0 Hz, 2 H), 4.17 (bs, 1 H), 2.67 (s, 3 H), 2.45−2.1 (m, 6 H), 1.95−1.80 (m, 2 H); MS *m*/*z* 373 (M + H)$^+$. HRMS Calcd for $C_{19}H_{22}FN_4O_3$ (M + H)$^+$: 373.16705. Found: 373.16714.

Synthesis of Compounds 35, 37, 38, 41−46. Synthesis of 2-[1-(dimethylamino)-1-methylethyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (**38**) is a representative example. Compounds **35**, **37**, **41−46** were isolated as trifluoroacetate salts by preparative RP HPLC in yields similar to **38**.

2-[1-(Dimethylamino)-1-methylethyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (38). Step 1: A solution of benzyl [2-amino-2-(hydroxyimino)-1,1-dimethylethyl]carbamate (10 g, 40 mmol) in chloroform (250 mL) was treated with dimethyl acetylenedicarboxylate (5.90 mL, 48 mmol) and stirred at 60 °C for 3 h. The mixture was cooled to room temperature and concentrated. The residue was taken up in xylene (0.300 mL) and refluxed overnight. After the mixture was cooled to room temperature, methyl 2-(1-{[(benzyloxy)carbonyl]amino}-1-methylethyl)-5,6-dihydroxypyrimidine-4-carboxylate precipitated as a brown solid and was collected by filtration and washed with diethyl ether (5.92 g, 41% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.58 (bs, 1 H), 10.21 (bs, 1 H), 7.45−7.25 (m, 5 H), 4.98 (s, 2 H), 3.81 (s, 3 H), 1.48 (s, 6 H); MS *m*/*z* 362 (M + H)$^+$. Step 2: A solution of the above compound (2.0 g, 5.54 mmol) in methanol (75 mL) was treated with 2 equiv of 4-fluorobenzylamine (1.26 mL, 11.08 mmol) and refluxed overnight. After evaporation of the solvent, the residue was poured in EtOAc and washed with 1 N HCl and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated under vacuum. The solid residue was triturated with diethyl ether to give benzyl 1-(4-{[(4-fluorobenzyl)amino]carbonyl}-5,6-dihydroxypyrimidin-2-yl)-1-methylethylcarbamate (2.16 g, 86% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.4 (bs, 1 H), 12.3 (bs, 1 H), 9.20 (bs, 1 H), 7.5−7.25 (m, 7 H), 7.16 (t, J = 8.8 Hz, 2 H), 4.97 (s, 2 H), 4.48 (d, J = 6.4 Hz, 2 H), 1.51 (s, 6 H); MS *m*/*z* 455 (M + H)$^+$. Step 3: To a solution of compound from above (2.16 g, 4.76 mmol) in methanol (100 mL) was added 10% Pd/C (10% by weight). The flask was evacuated, filled with hydrogen, and stirred under hydrogen atmosphere at room temperature for 1 h. The catalyst was filtered off and washed with cold methanol, the filtrate was evaporated to dryness, and the residue was triturated with Et_2O to obtain 2-(1-amino-1-methylethyl)-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide as a pale yellow solid in quantitative yield (1.52 g). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.36 (t, J = 8.2 Hz, 2 H), 7.16 (t, J = 8.8 Hz, 2 H), 4.46 (d, J = 6.2 Hz, 2 H), 1.43 (s, 6 H); MS *m*/*z* 321 (M + H)$^+$. Step 4: To a stirred solution of compound from step 3 (1.52 g, 4.76 mmol) in methanol (250 mL) was added an excess (about 11 equiv) of acetic acid and subsequently $NaBH_3CN$ (2.39 g, 38.08 mmol) and formaldehyde (37 wt % in H_2O, 0.9 mL). The mixture was stirred at room temperature for 5 days, concentrated by rotary evaporation, and purified by preparative RP-HPLC (C_{18}, water/acetonitrile with 0.1% trifluoroacetic acid as eluent). Collection and lyophilization of appropriate fractions afforded compound **38** as its trifluoroacetate salt in about 90% yield (1.98 g). The white powder was dissolved in 1 N HCl and lyophilized again to be converted into the corresponding hydrochloride salt. Mp: 218 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.4 (s, 1 H), 10.2 (bs, 2 H), 7.41 (dd, J = 8.3 Hz, 6.9 Hz, 2 H), 7.16 (t, J = 8.3 Hz, 2 H), 4.50 (d, J = 5.9 Hz, 2 H), 2.73 (s, 6 H), 1.60 (s, 6 H); MS *m*/*z* 349 (M + H)$^+$. HRMS Calcd for $C_{17}H_{22}FN_4O_3$ (M + H)$^+$: 349.16705. Found: 349.16675.

2-[(Dimethylamino)methyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (35). As for the preparation of **38**, replacing in step 1 benzyl [2-amino-2-(hydroxyimino)-1,1-dimethylethyl]carbamate with benzyl [2-amino-2-(hydroxyimino)ethyl]-carbamate. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.15 (bs, 1 H), 12.33 (bs, 1 H), 9.46 (t, J = 7.9 Hz, 1 H), 7.34 (dd, J = 8.8, 5.6 Hz, 2 H), 7.19 (t, J = 8.9 Hz, 2 H), 4.56 (d, J = 7.9 Hz, 2 H), 4.27 (s, 2 H), 2.93 (s, 6 H); MS *m*/*z* 321 (M + H)$^+$. HRMS Calcd for $C_{15}H_{18}FN_4O_3$ (M + H)$^+$: 321.13575. Found: 321.13562.

2-[1-(Dimethylamino)ethyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (37). As for the preparation of **38**, replacing in step 1 benzyl [2-amino-2-(hydroxyimino)-1,1-dimethylethyl]carbamate with *tert*-butyl [2-amino-2-(hydroxyimino)1-methylethyl]carbamate, and in step 3 removal of the Boc protecting group was obtained by stirring in a CH_2Cl_2:TFA 8:2 solution at room temperature for 1 h. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.05 (bs, 1 H), 12.27 (bs, 1 H), 9.51 (t, J = 6.21 Hz, 1 H), 7.37 (dd, J = 8.41, 5.56 Hz, 2 H), 7.19 (t, J = 8.79 Hz, 2 H), 4.59−4.48 (m, 2 H), 4.22 (bs, 1 H), 2.73 (s, 6 H), 1.46 (d, J = 6.73 Hz, 3 H); MS *m*/*z* 335(M + H)$^+$. HRMS Calcd for $C_{16}H_{20}FN_4O_3$ (M + H)$^+$: 335.15140. Found: 335.15030.

2-[1-(Dimethylamino)-2,2,2-trifluoro-1-methylethyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (41). As for the preparation of **38**, replacing in step 1 benzyl [2-amino-2-(hydroxyimino)-1,1-dimethylethyl]carbamate with *tert*-butyl{1-[amino(hydroxyimino)methyl]-2,2,2-trifluoro-1-methylethyl}-carbamate, and in step 3 removal of the Boc protecting group was obtained by stirring in a CH_2Cl_2:TFA 8:2 solution at room temperature for 1 h. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 12.46 (bs, 1 H), 12.26 (bs, 1 H), 8.73 (bs, 1 H), 7.39 (dd, J = 8.4, 6.0 Hz, 2 H), 7.17 (t, J = 9.0 Hz, 2 H), 4.52−4.48 (m, 2 H), 2.28 (s, 6 H), 1.57 (s, 3 H); MS *m*/*z* 403 (M + H)$^+$.

2-[1-(Dimethylamino)cyclopropyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (42). As for the preparation of **38**, replacing in step 1 benzyl [2-amino-2-(hydroxyimino)-1,1-dimethylethyl]carbamate with benzyl {1-[amino(hydroxyimino)-methyl]cyclopropyl}carbamate. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.48 (bs, 1 H), 9.40 (bs, 1 H), 7.41 (dd, J = 8.3 Hz, 6.9 Hz, 2 H), 7.16 (t, J = 8.3 Hz, 2 H), 4.53 (d, J = 6.2 Hz, 2 H), 2.75 (bs, 6 H), 1.40 (bs, 4 H); MS *m*/*z* 347 (M + H)$^+$. HRMS Calcd for $C_{17}H_{20}FN_4O_3$ (M + H)$^+$: 347.15140. Found: 347.15079.

2-[1-(Dimethylamino)cyclobutyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (43). As for the preparation of **38**, replacing in step 1 benzyl [2-amino-2-(hydroxyimino)-1,1-dimethylethyl]carbamate with benzyl {1-[amino(hydroxyimino)-methyl]cyclobutyl}carbamate. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.90 (bs, 1 H), 12.65 (bs, 1 H), 10.35 (bs, 1 H), 9.43 (bs, 1 H), 7.37 (dd, J = 8.4, 5.5 Hz, 2 H), 7.16 (t, J = 8.85 Hz, 2 H), 4.49 (d, J = 5.97 Hz, 2 H), 2.86 (bs, 2H), 2.63 (s, 6 H), 2.61−2.49 (m, 2 H, partially obscured by solvent), 1.70−1.63 (m, 2 H); MS *m*/*z* 361 (M + H)$^+$. HRMS Calcd for $C_{18}H_{22}FN_4O_3$ (M + H)$^+$: 361.16705. Found: 361.16815.

2-[1-(Dimethylamino)cyclopentyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (44). As for the preparation of **38**, replacing in step 1 benzyl [2-amino-2-(hydroxyimino)-1,1-dimethylethyl]carbamate with benzyl {1-[amino(hydroxyimino)-methyl]cyclopentyl}carbamate. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.80−12.40 (bs, 2 H), 10.89 (bs, 1 H), 9.85 (bs, 1 H), 7.48 (dd, J = 8.4, 6.0 Hz, 2 H), 7.12 (t, J = 8.8 Hz, 2 H), 4.49 (d, J = 8.0 Hz, 2 H), 2.80−2.64 (s, 6 H + m, 2 H), 2.22−2.05 (m, 2 H), 1.88−1.70 (m, 2 H), 1.68−1.50 (m, 2 H);); MS *m*/*z* 375 (M + H)$^+$. HRMS Calcd for $C_{19}H_{24}FN_4O_3$ (M + H)$^+$: 375.18270. Found: 375.18109.

2-[1-(Dimethylamino)cyclohexyl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (45). As for the preparation of **38**, replacing in step 1 benzyl [2-amino-2-(hydroxyimino)-1,1-dimethylethyl]carbamate with benzyl {1-[amino(hydroxyimino)-methyl]cyclohexyl}carbamate.^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.62 (bs, 1 H), 9.82 (bs, 1 H), 9.41 (bs, 1 H), 7.39 (dd, J = 8.3 Hz, 6.9 Hz, 2 H), 7.15 (t, J = 8.3 Hz, 2 H), 4.51 (d, J = 6.0 Hz, 2 H), 3.09 (bd, 2 H), 2.68 (s, 6 H), 1.80−1.47 (m, 5 H), 1.20−

1.02 (m, 3 H); MS *m*/*z* 389 (M + H)$^+$. HRMS Calcd for $C_{20}H_{26}FN_4O_3$ (M + H)$^+$: 389.19835. Found: 389.19928.

2-[4-(Dimethylamino)tetrahydro-2*H*-pyran-4-yl]-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (46). As the procedure used for the preparation of **38**, replacing in step 1 benzyl [2-amino-2-(hydroxyimino)-1,1-dimethylethyl]carbamate with *tert*-butyl{4-[amino(hydroxyimino)methyl]tetrahydro-2*H*-pyran-4-yl}-carbamate, and in step 3 removal of the Boc protecting group was obtained by stirring in a CH_2Cl_2:TFA 8:2 solution at room temperature for 1 h. ^{1}H NMR (DMSO-d_6+TFA, 300 MHz) δ 9.85 (bs, 1 H), 9.51 (t, J = 6.26 Hz, 1 H), 7.37 (dd, J = 8.54, 5.71 Hz, 2 H), 7.17 (t, J = 8.85 Hz, 2 H), 4.51 (d, J = 6.26 Hz, 2 H), 3.93−3.90 (m, 2 H), 3.19−3.11 (m, 4 H), 2.71 (s, 6 H), 1.89−1.82 (m, 2 H); MS *m*/*z* 391 (M + H)$^+$. HRMS Calcd for $C_{19}H_{24}FN_4O_4$ (M + H)$^+$: 391.17761. Found: 391.17679.

2-{2-[Benzyl(methyl)amino]ethyl}-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (36). Step 1: A solution of *tert*-butyl [3-amino-3-(hydroxyimino)propyl]benzylcarbamate (1 g, 3.4 mmol) in chloroform (30 mL) was treated with dimethyl acetylenedicarboxylate (0.5 mL, 4.09 mmol) and stirred at 60 °C for 3 h. The reaction mixture was cooled to room temperature and concentrated. The residue was taken up in xylene (50 mL) and refluxed overnight. The solvent was removed in vacuum, and a brown solid was formed upon trituration with CH_2Cl_2:Et_2O 50:50. The solid was collected by filtration, taken up in methanol (30 mL), treated with an excess of *p*-fluorobenzylamine (1.2 mL, 10.2 mmol), and refluxed overnight. After evaporation of the solvent, the residue was poured in EtOAc and washed with 1 N HCl and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude residue was stirred in a 80:20 mixture of CH_2Cl_2:TFA (20 mL) for 0.5 h. After evaporation of volatiles, the residue was purified by preparative RP HPLC, yielding 2-[2-(benzylamino)ethyl]-*N*-(4-fluorobenzyl)5,6-dihydroxypyrimidine-4-carboxamide as its trifluoroacetate salt (0.087 g, 5% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.73 (bs, 1 H), 12.38 (bs, 1 H), 9.34 (bs, 1 H), 8.71 (bs, 1 H), 7.47 (s, 5 H), 7.37 (dd, J = 8.5, 5.4 Hz, 2 H), 7.28 (t, J = 8.9 Hz, 2 H), 4.51 (d, J = 6.3 Hz, 2 H), 4.29 (bs, 2 H), 3.48 (bs, 2 H), 2.93−2.88 (m, 2 H); MS *m*/*z* 397 (M + H)$^+$. Step 2: The compound from the previous step (0.087 g, 0.17 mmol) was taken up in methanol (2 mL) and treated with $NaCNBH_3$ (0.013 g, 0.20 mmol), NaOAc (0.022 g, 0.27 mmol), and 37% aqueous solution of formaldehyde (0.02 mL). The mixture was stirred at room temperature for 1 h, concentrated, and purified by preparative RP HPLC (C_{18}, acetonitrile/water containing 0.1% trifluoroacetic acid as eluent) to isolate compound **36** as its trifluoroacetate salt (0.022 g, 25% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.71 (bs, 1 H), 12.32 (bs, 1 H), 9.40 (bs, 1 H), 9.30 (bs, 1 H), 7.48 (s, 5 H), 7.36 (dd, J = 8.3 Hz, 6.9 Hz, 2 H), 7.16 (t, J = 8.3 Hz, 2 H), 4.59−4.49 (m, 3 H), 4.23 (bs, 1 H), 3.78 (bs, 2 H), 3.03 (bs, 2 H), 2.68 (bs, 3 H); MS *m*/*z* 411 (M + H)$^+$. HRMS Calcd for $C_{22}H_{24}FN_4O_3$ (M + H)$^+$: 411.18270. Found: 411.18300.

***N*-(4-Fluorobenzyl)-5,6-dihydroxy-2-[1-methyl-1-(methylamino)ethyl]pyrimidine-4-carboxamide (40).** Step 1: To a stirred solution of 0.250 g (0.78 mmol) of 2-(1-amino-1-methylethyl)-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (prepared as described in the synthesis of compound **38**, steps 1−3) in methanol (5 mL), benzaldehyde (0.08 mL g, 0.78 mmol), triethylamine (0.11 mL, 0.78 mmol), acetic acid (0.78 mmol), and $NaBH_3CN$ (0.147 g, 2.34 mmol) were added. The reaction mixture was stirred 4 h at room temperature, until disappearance of starting material (LC-MS monitoring). Formaldehyde (0.06 mL) (37 wt % in H_2O) was added to the reaction, and resulting mixture was stirred at room temperature overnight. After evaporation of volatiles, 2-{1-[benzyl(methyl)amino]-1-methylethyl}-*N*-(4-fluorobenzyl)-5,6-dihydroxypyri midine-4-carboxamide was isolated in 50% yield (0.21 g) as its TFA salt by preparative RP HPLC (C_{18}, gradient acetonitrile/water 0.1% TFA as eluent, from 80/20 to 20/80 in 18 min). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.80 (bs, 1 H), 12.18 (bs, 1 H), 9.75 (bs, 1 H), 9.28 (bs, 1 H), 7.45−7.35 (m, 5 H), 7.25−7.19 (m, 4 H), 4.62 (d, J = 6.5 Hz, 2 H), 4.40−4.32 (m, 1 H), 4.08−3.95 (m, 1 H), 2.81 (s, 3 H), 1.83 (s, 3 H), 1.58 (s, 3 H); MS *m*/*z* 425 (M + H)$^+$. Step 2: A solution of compound from the previous step (0.21 g, 0.39 mmol) in methanol (5 mL) was treated with 10% Pd/C, and the mixture was stirred for 2 h under H_2 atmosphere. Catalyst was filtered off, and filtrate was evaporated under vacuum. Compound **40** was isolated as its TFA salt by preparative RP HPLC (0.15 g, 85% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.83 (bs, 1 H), 12.31 (bs, 1 H, 9.46 (t, J = 6.27 Hz, 1 H), 9.02 (bs, 2 H), 7.37 (dd, J = 8.48, 5.59 Hz, 2 H), 7.18 (t, J = 8.82 Hz, 2 H), 4.54 (d, J = 6.28 Hz, 2 H), 2.48 (s, 3 H), 1.58 (s, 6 H);); MS *m*/*z* 335 (M + H)$^+$. HRMS Calcd for $C_{16}H_{20}FN_4O_3$ (M + H)$^+$: 335.15140. Found: 335.15222.

2-{1-[Ethyl(methyl)amino]-1-methylethyl}-*N*-(4-fluorobenzyl)-5,6-dihydroxypyrimidine-4-carboxamide (39). A solution of **40** (20 mg, 0.04 mmol) in methanol (1 mL) was treated with acetaldehyde (0.03 mL, 0.05 mmol), Et_3N (0.011 mL, 0.08 mmol), AcOH (0.05 mL, 0.08 mmol), and $NaBH_3CN$ (0.007 g, 0.12 mmol). The resulting mixture was stirred 5 h at room temperature. After evaporation of volatiles, compound **65** was isolated as its TFA salt by preparative RP-HPLC (0.010 g, 54% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.78 (bs, 1 H), 12.30 (bs, 1 H), 10.49 (bs, 1 H), 10.18 (bs, 1 H),), 7.44 (dd, J = 8.48 Hz, 5.58 Hz, 2 H), 7.16 (t, J = 8.80 Hz, 2 H), 4.62−4.51 (m, 1 H), 4.42−4.32 (m, 1 H), 3.21−3.11 (m, 1 H), 2.98−2.96 (m, 1 H), 2.78 (s, 3 H), 1.66 (s, 3 H), 1.58 (s, 3 H), 1.27 (t, J = 5.6 Hz, 3 H); MS *m*/*z* 362 (M + H)$^+$. HRMS Calcd for $C_{18}H_{24}FN_4O_3$ (M + H)$^+$: 363.18270. Found: 363.18353.

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Supporting Information Available: Synthesis and analytical data for the preparation of nitriles and amidoximes intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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