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Article

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Design, synthesis, and biological evaluation of first-in-class dual acting histone deacetylases (HDACs) and phosphodiesterase 5 (PDE5) inhibitors for the treatment of Alzheimer's disease

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ABSTRACT

Simultaneous inhibition of phosphodiesterase 5 (PDE5) and histone deacetylases (HDAC) has recently been validated as a potentially novel therapeutic approach for Alzheimer's Disease (AD). To further extend this concept, we designed and synthesized the first chemical series of dual acting PDE5 and HDAC inhibitors, and we validated this systems therapeutics approach. Following the implementation of structure- and knowledge-based approaches, initial hits were designed and were shown to validate our

hypothesis of dual *in vitro* inhibition. Then, an optimization strategy was pursued to obtain a proper tool compound for *in vivo* testing in AD models. Initial hits were translated into molecules with adequate cellular functional responses (histone acetylation and cAMP/cGMP response element-binding (CREB) phosphorylation in the nanomolar range), an acceptable therapeutic window (>1 log unit) and the ability to cross the blood-brain barrier, leading to the identification of **7** as a candidate for *in vivo* proof-of-concept testing (described in ref 23).

INTRODUCTION

Multitarget drugs have emerged as an innovative therapeutic approach for Alzheimer's disease (AD) due to the complex etiology of this neurodegenerative disease and its multifactorial progression.[1] As observed for other conditions (e.g., complex diseases), it is becoming clear that AD therapies should focus on the simultaneous modulation of multiple targets implicated in the disease.[1] Among these targets, phosphodiesterases (PDEs)[2] and epigenetic targets, primarily histone deacetylases (HDACs)[3–6], have recently attracted much potential therapeutic interest to restore memory function.

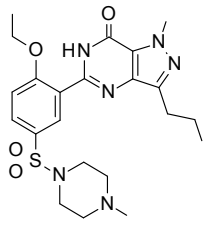
PDEs hydrolyze the second messengers cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) and are extensively distributed in the brain.[7] In fact PDE5, a cGMP-specific phosphodiesterase, is up-regulated in the brains of AD patients compared with age-matched healthy control subjects.[8] Consequently, cGMP levels, but not cAMP levels, are significantly decreased in the cerebrospinal fluid (CSF) of AD patients when compared with non-demented controls.[8] Inhibition of phosphodiesterase-5 (PDE5), a cGMP-specific phosphodiesterase, elevates cGMP

levels, which may ultimately promote gene transcription by directly and/or indirectly activating CREB.[2] Moreover, by favoring the inactive form of GSK3β (phosphorylated at GSK3β-Ser9), PDE5 inhibition decreases levels of phosphorylated Tau (pTau).[9,10] Specific PDE5 inhibitors (sildenafil **1**, vardenafil **2** and tadalafil **3**, Chart 1) approved for the treatment of erectile dysfunction and pulmonary arterial hypertension[7] have been shown to improve memory performance and/or enhance synaptic plasticity and cognitive function in different animal models of AD.[9–11]

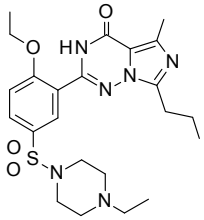
Histone deacetylases (HDACs) comprise a family of 18 genes in humans and are divided into four groups: class I (HDACs 1, 2, 3, 8), class IIa (HDACs 4, 5, 7, 9), class IIb (HDACs 6, 10) and class IV (HDAC11). HDACs are epigenetic modulators that deacetylate lysine residues in histone and non-histone substrates. Although already a proven strategy for the treatment of cancers,[12] inhibition of HDACs has attracted much interest for the treatment of neurodegenerative disorders[12] in recent years, with class I HDACs and HDAC6 being implicated in AD memory-related dysfunction. Class I HDACs, particularly HDAC2, predominantly localize in the nucleus and reduce the transcription of CREB-regulated genes that are important for learning and memory,[13,14] and HDAC1 activity may be neuroprotective.[15] Notably, HDAC2 and HDAC6 are over-expressed in the cortex and hippocampus of AD patients, although the cause and effect of this up-regulation remain unknown.[14,16] Chronic treatment with suberoylanilide hydroxamic acid **4** (SAHA; vorinostat, Chart 1), a clinically approved pan-HDAC inhibitor for the treatment of cutaneous T cell lymphoma (CTCL), enhanced memory in animal models.[13] HDAC6, the major cytoplasmatic deacetylase in mammalian cells, targets α-tubulin, among other proteins. Increasing α-tubulin acetylation via HDAC6 inhibition may facilitate the amelioration of tau[17,18] and amyloid pathologies[19,20] by promoting tau clearance and decreasing Aβ levels, respectively.

In this context, we have recently demonstrated the beneficial synergistic effects of concomitant HDAC and PDE5 inhibition in the Tg2576 murine model of AD using **3** and the pan-HDAC inhibitor **4**, thereby establishing the basis for a potential new symptomatic and disease-modifying strategy to treat AD.[21] Based on these results and considering that i) toxicity is associated with strong inhibition of HDAC class I isoforms,[22] ii) simultaneous inhibition of HDAC and PDE5 exerts a synergistic effect on histone acetylation[21] and thus strong inhibition of HDAC class I is not required, iii) histone acetylation in conjunction with CREB activation, achieved through PDE5 inhibition, may facilitate the transcription of specific memory-related genes,[23] iv) inhibition of HDAC6 does not affect cell survival[22] and may facilitate the degradation of misfolded proteins (such as Aβ and pTau),[19,20] v) HDAC inhibitors show poor permeability[24,25] and brain availability[26] and vi) a single agent does not lead to the additive toxicity that is often observed with combination therapy,[27] our next step was to obtain brain-penetrating dual inhibitors with moderate HDAC class I activity as well as potent HDAC6 and PDE5 inhibition. Thus, we set out with the goal of designing novel dual PDE5 and HDAC inhibitors with the appropriate profiles for potency, selectivity and pharmacokinetic properties to consider for *in vivo* testing in AD mouse models. Compound **7** (CM-414**,** Chart 1) fulfilled these requirements in terms of primary activities (IC_{50} values of 60 nM, 310 nM, 490 nM, 322 nM and 91 nM against PDE5, HDAC1, HDAC2, HDAC3 and HDAC6, respectively), crossing the blood-brain barrier (BBB), inducing AcH3K9 acetylation and CREB phosphorylation in the hippocampus and rescuing long-term potentiation (LTP) in APP/PS1 mice. With additional consideration of its adequate ADME and pharmacokinetic profiles, **7** was selected for *in vivo* proof of concept (PoC) testing.[23] In this article, we present a detailed account of the

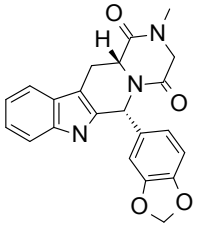
discovery of this novel first-in-class chemical series with dual acting PDE5 and HDAC inhibitory activities, from initial hits (e.g., **13c**) to lead identification (**7**).



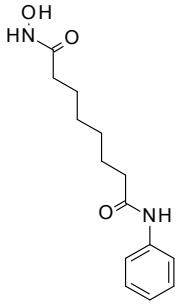
1
Sildenafil
PDE5A IC_{50} = 8.5 nM



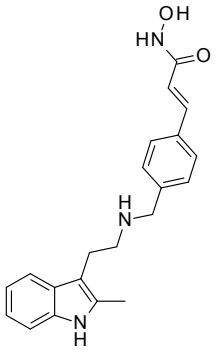
2
Vardenafil
PDE5A IC_{50} = 0.89 nM



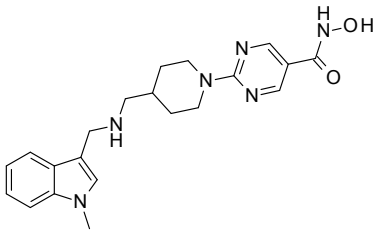
3
Tadalafil
PDE5A IC_{50} = 9.4 nM



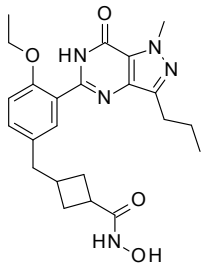
4
SAHA
HDAC1 IC_{50} = 30nM
HDAC2 IC_{50} = 170nM
HDAC3 IC_{50} = 100nM
HDAC6 IC_{50} = 38nM



5
Panobinostat
HDAC1 IC_{50} = 2.5nM
HDAC2 IC_{50} = 13.2nM
HDAC3 IC_{50} = 2.1nM
HDAC6 IC_{50} = 10.5nM



6
Quisinostat
HDAC1 IC_{50} = 0.11nM
HDAC2 IC_{50} = 0.33nM
HDAC3 IC_{50} = 4.86nM
HDAC6 IC_{50} = 76.8nM



7
CM-414
PDE5A IC_{50} = 60nM
HDAC1 IC_{50} = 310nM
HDAC2 IC_{50} = 490nM
HDAC3 IC_{50} = 322nM
HDAC6 IC_{50} = 91nM

Chart 1. Known PDE5 inhibitors shown to improve memory (**1**, **2**, **3**), HDAC inhibitors (**4**, **5**, **6**) and the structure of a novel therapeutic tool **7**. PDE5 IC_{50} inhibition values taken from Ref. [28] for **1**, **2**, **3**. HDAC inhibition IC_{50} values for **4** extracted from Ref. [29], values for **5** extracted from Ref. [30] and values for **6** extracted from Ref. [31].

RESULTS

Rational Design

One interesting approach to generate multivalent ligands is to combine key structural features facilitating binding to HDAC and PDE into one molecule to obtain a new chemical entity; however, this is not a straightforward procedure, and we must identify the appropriate common features, substitution sites and growing vectors to maintain primary activities without interference. Indeed, in this particular case, given the structures of both protein families (see below), the design process goes one step further than simply incorporating the pharmacophoric features of HDAC inhibitors (HDACi) and PDE inhibitors into one molecule as the sum of two parts to obtain a single agent with dual activity. This strategy has been particularly useful to derive bifunctional HDACi anticancer agents due to the presence of large hydrophobic patches at the HDAC surface rim.[32] We envisioned using the sildenafil central core to append HDAC pharmacophores.

However, before commencing any design and synthetic efforts around the sildenafil scaffold, we confirmed that the synergestic effects achieved by **3** and **4** [21] on the induction of histone 3 acetylation at lysine 9 (AcH3K9) are mechanism-of-action (MoA)-dependent; then, a combination of **4** and **1** was tested and quantified using

AlphaLisa technology in the SH-SY5Y neuroblastoma cell line. After treating cells with 50 nM of **4**, AcH3K9 marks increased by 1.5-fold compared to non-treated cells (Figure 1), and this induction was significantly stronger (*P* value < 0.01) when combined with concentrations of **1** higher than 400 nM (1.95-fold change over vehicle-treated cells at 400 nM of compound **1**, Figure 1).

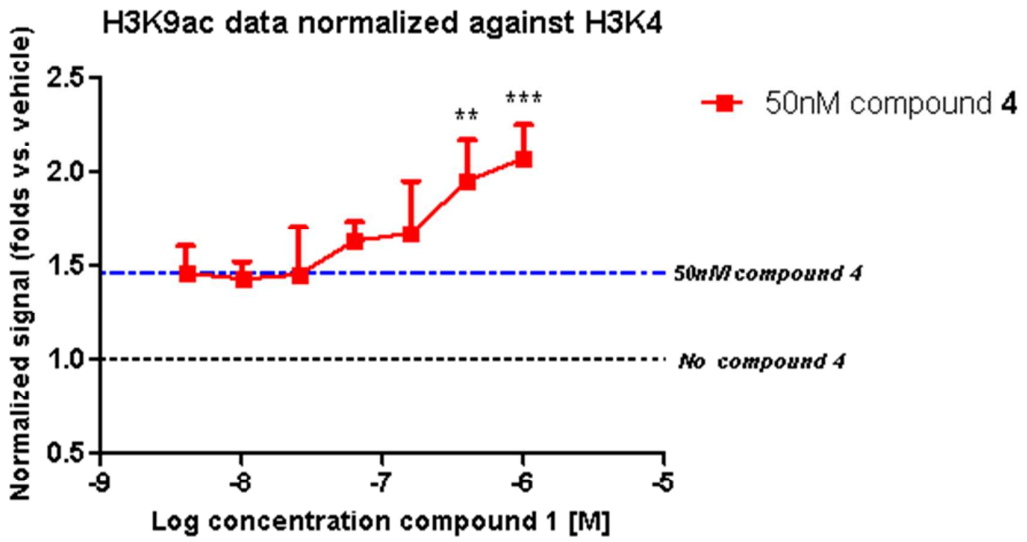


Figure 1. Detection of AcH3K9 assayed using SH-SY5Y cells and AlphaLisa technology. SH-SY5Y cells were treated with **4** and **1** for 2 hours (** p≤0.01, ***p≤0.001).

Once the synergistic effect between **4** and **1** was confirmed, our design strategy commenced. Typically, the classical pharmacophore for HDACi consists of a hydrophobic recognition capping group (also known as a surface recognition motif) that is able to interact with the rim of the catalytic tunnel, a zinc-binding group (ZBG) that is able to complex the Zn^{2+} ion at the bottom of the catalytic cavity and a hydrophobic linker connecting the two parts along the 11-Å hydrophobic channel.[12,33] There are

various ZBGs for HDACi, including hydroxamic acids, aminobenzamides, carboxylates (short-chain fatty acids), electrophilic ketons, thiols, mercaptoacetamides and 3-hydroxypyridin-2-thiones.[34] Initially, and for this proof-of-concept series, the hydroxamic moiety was chosen as a ZBG because it is one of most well-established functionalities for chelating the zinc ion at the catalytic site of HDACs. Examination of the crystal structure of **1** bound to PDE5 (PDB entry 1TBF[35], Figure 2a) suggested that linking the hydroxamic moiety to the methylpiperazine would project this ZBG substituent into the solvent region and be well tolerated from a potency perspective. From the viewpoint of HDAC inhibitory activity, the sildenafil core would serve as a cap group. As a straightforward strategy for HDACi linker design, different linker moieties contained in reported potent HDAC inhibitors were analyzed in the context of the structure of HDAC2 complexed with **4** (PDB entry 4LXZ[36], Figure 2b), considering the 11-Å cavity length.

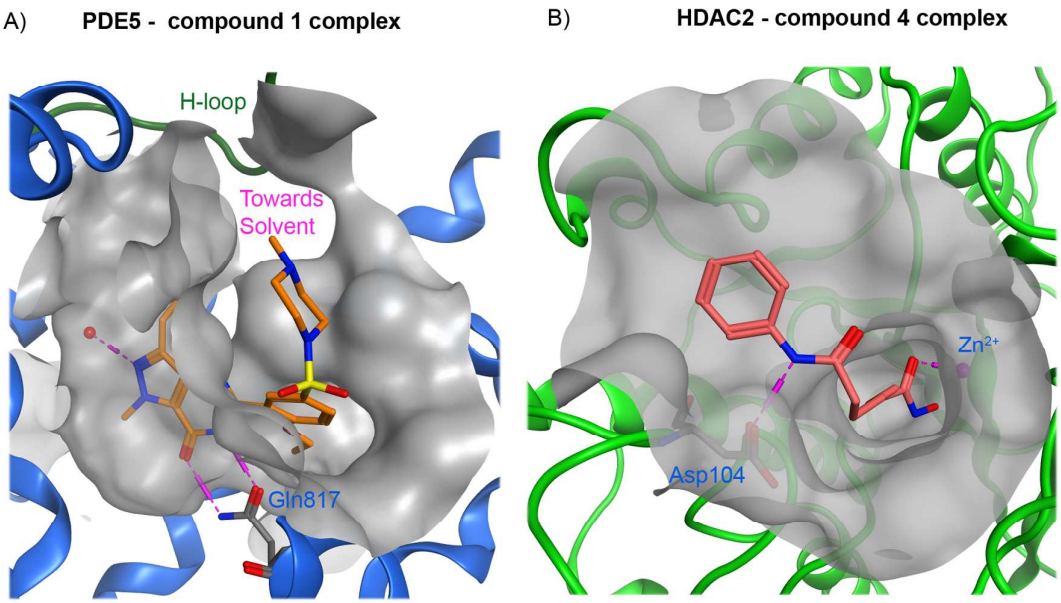
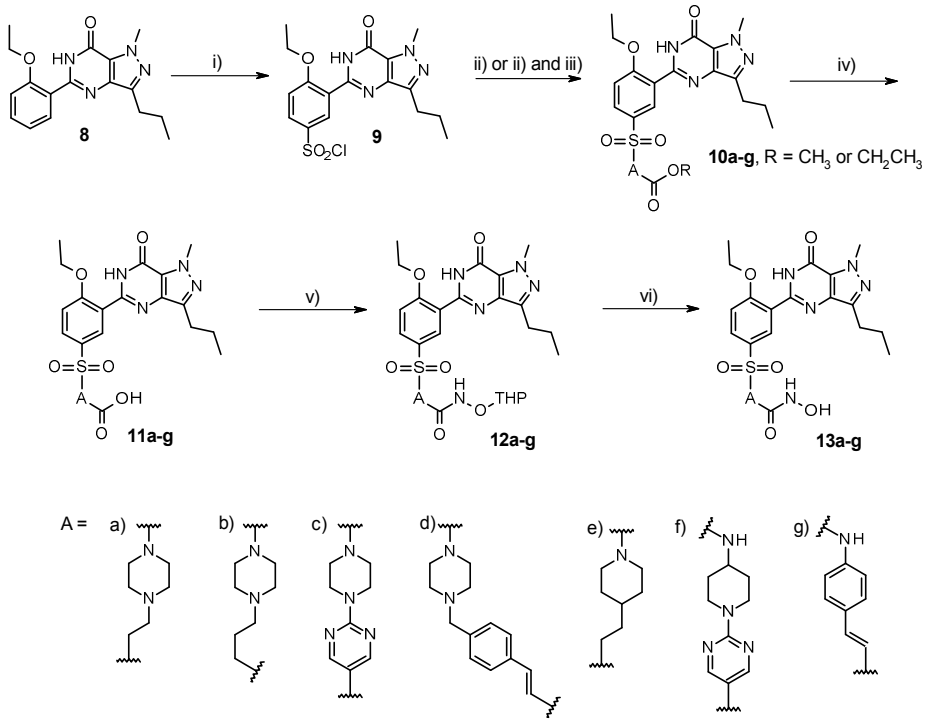


Figure 2. (A) Crystal structure of **1** in the PDE5 cavity (PDB entry 1TBF[35]). The pyrazolopyrimidinone group of **1** makes bidentate H-bonds with the conserved Q817.

The piperidinylsulfonamide group is solvent-oriented towards the H-loop region (residues 660-683). (B) Complex of **4** and HDAC2 (PDB entry 4LXZ[36]). The NH of the amide group of **4** makes an H-bond contact with the well-conserved residue Asp104 of HDAC2 (Asp99 for HDAC1 and Asp567 for HDAC6).

This combination of structure-based and knowledge-based approaches, together with consideration to synthetic accessibility, enabled the rapid design of potential dual PDE5/HDAC inhibitors. We envisioned attaching the following to the piperidinylsulfonamide group of **1**: flexible alkyl linkers of varying lengths as in compound **4**, a cinnamic hydroxamic acid analog as in **5**[30] and pyrimidylhydroxamic acids as in **6**[31], resulting in novel N-4-substituted-piperazine derivatives **13a-13d** as potential dual PDE5/HDAC inhibitors. These compounds were synthesized from commercially available 5-(2-ethoxyphenyl)-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**8**) (Scheme 1). Selective sulfonylation at the 5'-position of the phenyl ring afforded **9**, which was converted into esters **10a-10d** via reactions with appropriated amines. Then, the corresponding carboxylic acids were obtained through hydrolysis and transformed into the THP-protected hydroxamic acids **12a-12d** by reacting with *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine ($THPONH_2$) using EDC/HOBt as the coupling system. Final deprotection under acidic conditions afforded us the desired compounds **13a-13d**.

Scheme 1.

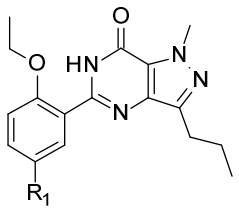


Conditions: i) $ClSO_3H$, rt, 2 h; ii) corresponding amine, Et_3N (optional), EtOH, MW, 100 ºC, 1-2 h; iii) ethyl 3-bromopropanoate or ethyl 4-bromobutanoate, K_2CO_3, CH_3CN, MW, 100 ºC, 2 h; iv) $LiOH{\cdot}H_2O$, THF/MeOH/H_2O (10:1:5), rt, overnight; v) EDC·HCl, HOBt, $THPONH_2$, NMM, DMF, rt, overnight; vi) HCl/1,4-dioxane (2.0 M), 1,4-dioxane or CH_2Cl_2 (optional) rt, 3 h.

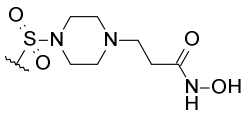
Compounds **13a-13d** were evaluated for their inhibition against PDE5, HDAC1, HDAC2 and HDAC6 activity (Table 1). Purified full-length recombinant human HDAC proteins were used to monitor HDAC activity (HDAC1, 2 and 6 were routinely included in our screening funnel, and HDAC3 activity was evaluated for selected compounds). As shown in Table 1, all of these compounds are potent PDE5 inhibitors, with IC_{50} values in the low nanomolar range (2-3 nM), comparable to **1** (IC_{50} is 8.5 nM[28] or 4 nM in our assay set-up, see Methods). Moreover, these compounds exhibit HDAC inhibitory activity with different profiles for potency and isoform selectivity, validating our initial hypothesis to design dual PDE5/HDAC inhibitors. Alkyl (**13a, 13b**) linkers resulted in low micromolar or mid-nanomolar inhibitors (**13b** against HDAC6). The

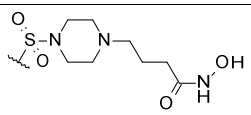
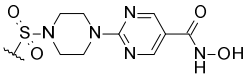
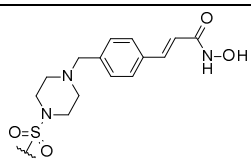
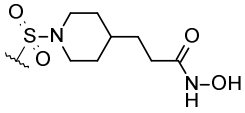
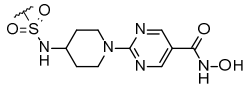
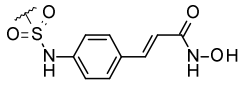
comparison of **13a** with **13b** suggests that increasing the length of the linker may have a positive effect on the HDAC activities of the three isoforms. However, due to the size of these derivatives, this strategy was not further contemplated. The cinnamic derivative **13d** showed similar potency against HDAC1 and HDAC2 compared to alkyl derivatives but a remarkable potency against HDAC6 (IC_{50} value of 89 nM), with >1 log units of selectivity over the class I HDACs (HDAC1 and HDAC2). Conversely, the pyrimidylhydroxamic **13c** showed excellent potency against HDAC1 (IC_{50} of 8 nM), comparable to that of the standard compound**4** (IC_{50} of 11 nM, Chart 1), and less potency against HDAC2 (117 nM) and HDAC6 (268 nM). The suitable potency of this pyrimidylhydroxamic moiety against class I HDAC isoforms has also been previously reported for **6** and related analogues.[31,37]

Table 1. Initial set of potential PDE5/HDAC inhibitors bearing a sulfonamide moiety.



1'

Cpd	R1	PDE5 A IC_{50} nM	HDAC1 IC_{50} nM	HDAC2 IC_{50} nM	HDAC3-NCOR2 IC_{50} nM*	HDAC6 IC_{50} nM
13a		3	10500	>20000		2360

13b		2	1100	4640		360
13c		3	8	117	36	268
13d		2	1340	6970		89
13e		0.5	406	1940		87
13f		0.6	57	341	54	59
13g		1	356	1310		84

* HDAC3_NCOR2 values obtained at BPS.[38]

Hit Explosion. Exploring the 5'-position of the phenyl ring of compound 1 (R1 at 1') and SAR analysis.

Encouraged by these early *in vitro* results for our dual acting compounds that were initial hits, our strategy focused on identifying molecules with previously defined primary activities (moderate HDAC class I as well as potent HDAC6 and PDE5 inhibitors) that were CNS-penetrating. The physicochemical properties of compounds

13a-13d are far outside the traditional range for CNS drugs, with high topological surface areas (TPSA > 90 Å^2) and high molecular weights (MW > 450).[39,40] Thus we sought to explore different alternatives for the piperidinylsulfonamide group of compound **1**, not only to optimize potency and examine different *in vitro* HDAC inhibitory profiles but also to obtain derivatives that demonstrated a reduced polar surface area to de-risk poor blood-brain barrier (BBB) penetration.

From the viewpoint of PDE5 activity, based on the crystal structure of compound **1** bound to PDE5 (Figure S1) and the previous analysis of structure-activity relationships (SAR)[41], the piperidinylsulfonamide group of compound **1** is not essential for potent PDE5 inhibition. Moreover, several published complexes of ligands **1**[35,42,43] and **2**[44] with PDE5 have exhibited significantly different orientations of the methylpiperazine portion of both ligands, stressing the potential of PDE5 to accommodate different substituents at this region. Thus, our SAR strategy to effectively balance dual PDE5/HDAC potency, differential selectivity profiles *versus* HDACs and ADME properties focused on i) exploring different attachment points (connecting bonds) and enabling different geometries at the 5'-position of the phenyl ring of molecule **1** (sulfonamide-, amine-, ether- and carbon-linked substituents) as well as ii) varying the substituents acting as HDAC linkers to occupy the catalytic channel between the ZBG and the surface recognition motif of HDACi (that corresponds to the sildenafil core, acting as a driving force for PDE binding). Moreover, in this case many analogues can be easily synthesized due to the selectivity of electrophilic attack on the 5'-position of the phenyl ring of intermediate **8**.

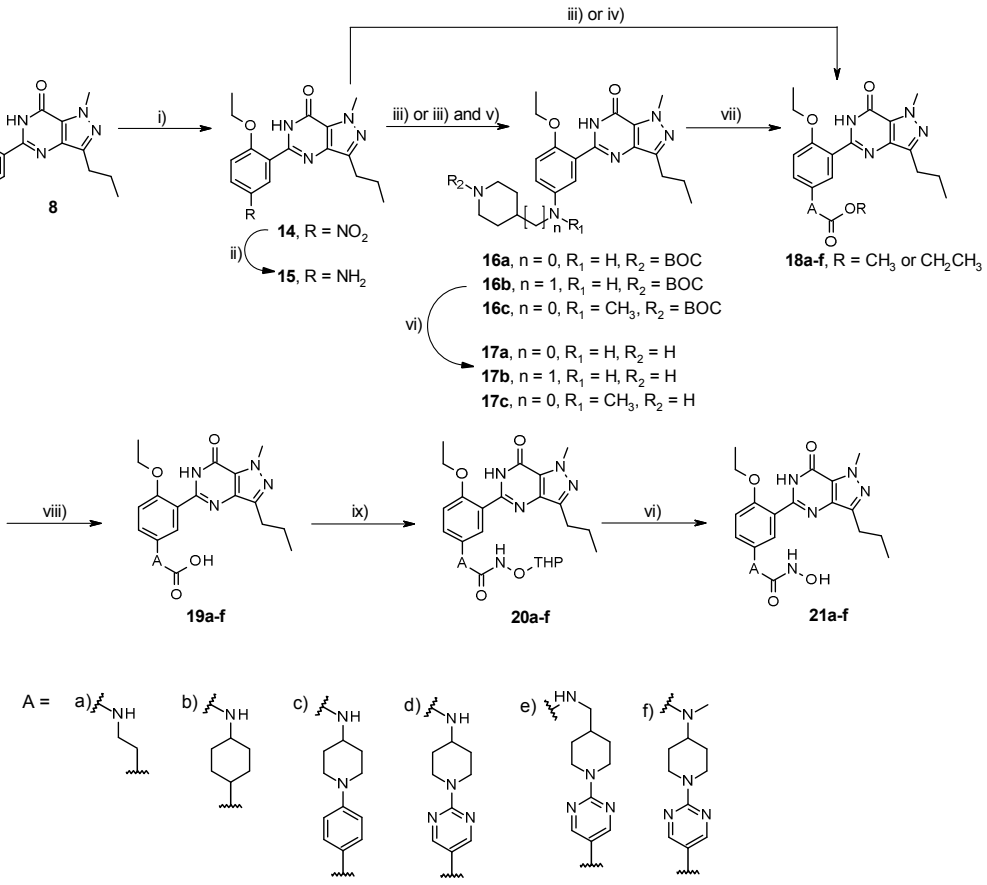
As a first initial exploration, the sulfonamide linker was retained with variations in the piperidinyl ring designed to: i) increase its hydrophobicity by removing the positively charged nitrogen (**13e**) and ii) increase the flexibility between the sildenafil core (capping group) and the hydrophobic linker binding in the HDAC catalytic channel by introducing a secondary sulfonamide as an attachment point (**13f**), together with increasing the planarity of the HDACi linker by removing the piperidinyl group (**13g**). The synthesis of these compounds was performed as previously described for hydroxamic acids **13a-13d** by coupling benzenesulfonyl chloride (**9**) with appropriated amines (Scheme 1), and the *in vitro* evaluation is listed in Table 1. These three derivatives (**13e-13g**) resulted in potent HDAC6 inhibitors with IC_{50} values < 100 nM. Concerning class I HDACs, derivatives **13e** and **13g** exhibited enhanced potency (with IC_{50} values of approximately 400 nM against HDAC1) compared to their less-hydrophobic parent compounds **13a** and **13d** (with IC_{50} >= 1300 nM against HDAC1), suggesting that a positively charged amine at this position of the class I HDAC channel is not well tolerated. Conversely, the secondary sulfonamide bearing a pyrimidylhydroxamic group **13f** was less potent against HDAC1 compared to the privileged substructure conferring potent class I HDAC activity in **13c** (57 nM *versus* 8 nM; 0.8 log units). As expected, all of these modifications exerted minor influences on PDE5 activity.

We next examined a variety of heteroatom (nitrogen and oxygen) bonded substituents at the 5'-position of the phenyl ring of compound **1** (Table 2): secondary amines (**21a-21e**), linear tertiary amines (**21f**), cyclic tertiary amines (**30a-30e**) and ethers (**30f-30g, 37**). Together with the heteroatom connection, a variety of alkyl- (**21a, 30a, 30b**), cycloalkyl- (**21b**, **30f**), phenyl- (**30g**), piperidylphenyl- (**21c**), piperidylpyrimidine-

(**21d**, **21e**, **21f**) and nitrogen-bonded spiro substituents (**30c**, **30d**, **30e**) (Table 2) were selected to cover a large variety of hydrophobic, electronic, and steric properties that might result in different HDAC profiles, as previously obtained for compounds in Table 1.

Amines **21a-21f** were synthesized as illustrated in Scheme 2. Intermediate **8** was converted into amine **15** via nitration and reduction of the nitro group. Then, esters **18a-18f** were obtained through reductive amination (and subsequent BOC-deprotection and coupling with ethyl 2-chloropyrimidine-5-carboxylate in the case of esters **18d**, **18e** and **18f**). These intermediates were transformed into desired hydroxamic acids via ester hydrolysis, reaction with $THPONH_2$ and acidic cleavage of the protecting group.

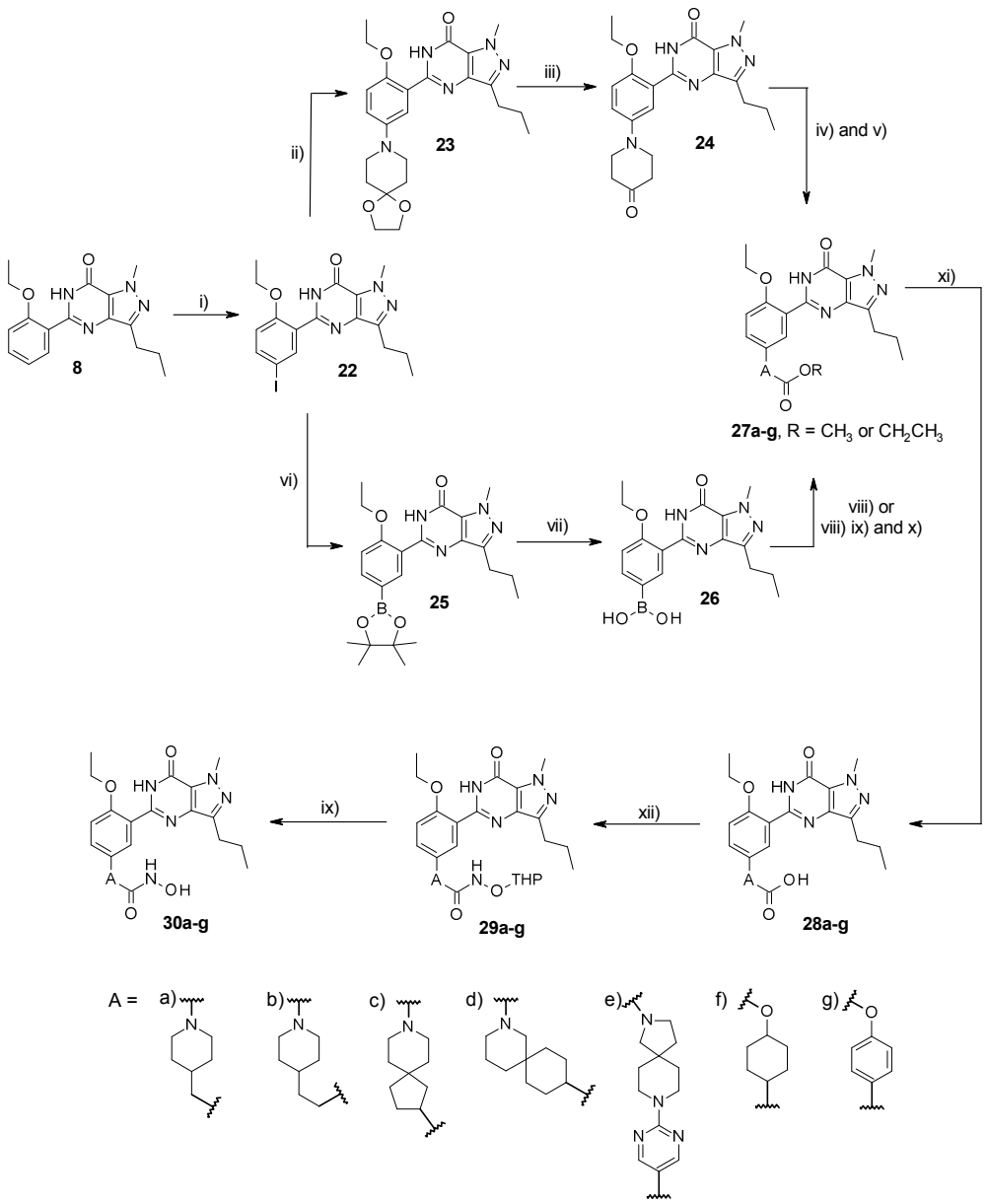
Scheme 2.



Conditions: i) H_2SO_4, KNO_3, 0 ºC, 20 minutes; ii) Pd/C, H_2 (1 atm), MeOH, rt, overnight; iii) corresponding carbonyl compound, AcOH, $NaBH(OAc)_3$, CH_2Cl_2, rt, overnight; iv) 3,3-dimethoxypropanoate, TFA, Et_3SiH, CH_2Cl_2, rt, overnight; v) paraformaldehyde, AcOH, $NaBH(OAc)_3$, CH_2Cl_2, 60 ºC, overnight; vi) HCl/EtOAc (1.0 or 4.0 M), rt, 1-4 h; vii) K_2CO_3, ethyl 2-chloropyrimidine-5-carboxylate, CH_3CN, 40 ºC, overnight; viii) $LiOH \cdot H_2O$, THF/MeOH/H_2O (3:3:2 or 3:1:1), 25-40 ºC, overnight; ix) EDC·HCl, HOBt, $THPONH_2$, NMM, DMF, rt, overnight.

The synthesis of tertiary amines **30a-30e** and ethers **30f** and **30g** was performed as shown in Scheme 3 from iodure **22**. This compound was transformed into boronic acid **26**, and then esters **27b-27g** were obtained through reactions with different amines or alcohols. Conversely, ester **27a** was synthesized from iodure **22** after Buchwald–Hartwig amination with 1,4-dioxa-8-azaspiro[4.5]decane, acidic deprotection, Horner–Wadsworth–Emmons reaction with methyl 2-diethoxyphosphorylacetate and reduction of the double bond under H_2. Finally, hydroxamic acids **30a-30g** were prepared from esters **27a-27g** employing the strategy previously described.

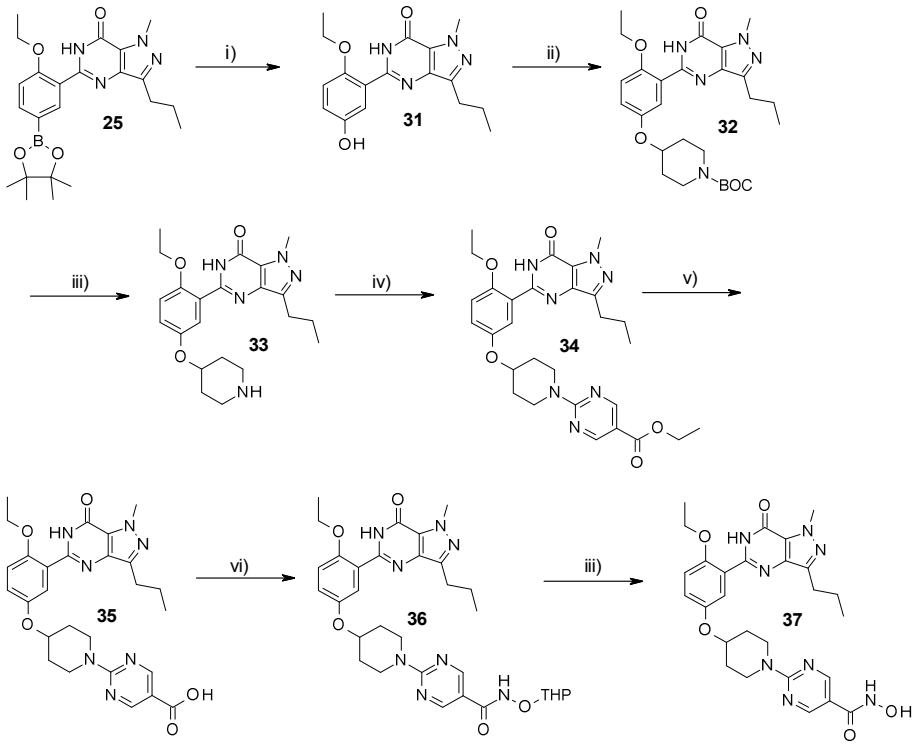
Scheme 3.



Conditions i) NIS, TFA, 0 ºC, then rt, overnight; ii) *t*-BuOK, $Pd_2(dba)_3$, 1,4-dioxa-8-azaspiro[4.5]decane, xantphos, toluene, 120 ºC, MW, 1 h; iii) HCl/THF (6.0 M), 70 ºC, overnight; iv) methyl 2-diethoxyphosphorylacetate, NaH, THF, 0 ºC, 1 h, then **24**, rt, overnight; v) Pd/C, MeOH, H_2 (1 atm), rt, 3 h; vi) 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi-1,3,2-dioxaborolane, $PdCl_2(dppf)$, KOAc, 1,4-dioxane, 80-100 ºC, 48 h; vii) $NaIO_4$, NH_4OAc, acetone, 25 ºC, 16 h; viii) corresponding alcohol or amine, $Cu(OAc)_2$, Et_3N, DMAP (optional), 4 Å MS, CH_2Cl_2, O_2 (1 atm), rt, 2-12 h; ix) HCl/EtOAc (1.0, 2.0 or 4.0 M), rt, 1-2 h; x) K_2CO_3, ethyl 2-chloropyrimidine-5-carboxylate, CH_3CN, 60 ºC, overnight; xi) $LiOH{\cdot}H_2O$, MeOH/THF/H_2O (3:1:3, 1:3:1, 3:3:2), rt, overnight; xii) EDC·HCl, HOBt, $THPONH_2$, NMM, DMF, rt, overnight.

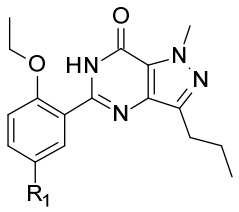
The synthetic route for ether **37** with a pyrimidyl group is outlined in Scheme 4. Starting from boronic ester **25**, alcohol **31** was obtained by oxidation. Then, ether **32** was prepared by the Mitsunobu reaction. Acidic removal of the BOC protecting group led us to amine **33**, which was coupled with ethyl 2-chloropyrimidine-5-carboxylate. The resulting ester, **34**, was converted into the carboxylic acid **35**, which was finally transformed in the desired hydroxamic acid **37** via the THP-protected intermediate **36**.

Scheme 4.

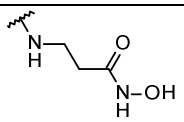
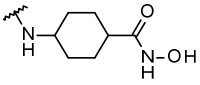
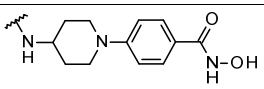
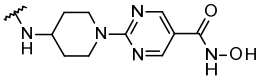
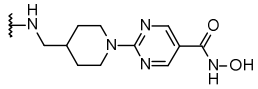
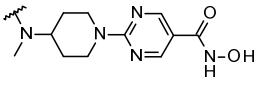
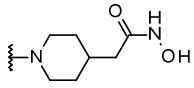


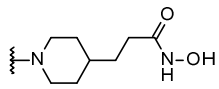
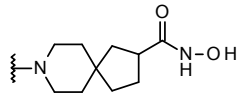
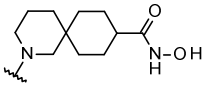
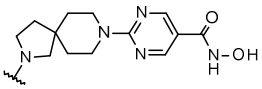
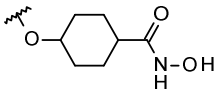
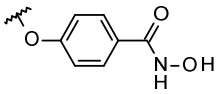
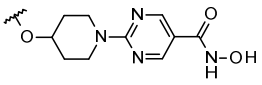
Conditions: i) NaOH (aq, 4.0 M), H_2O_2, H_2O, rt, overnight; ii) *tert*-butyl 4-hydroxypiperidine-1-carboxylate, PPh_3, DEAD, toluene, 110 ºC, 1 h; iii) HCl/1,4-dioxane (4.0 M), rt, 1-2 h; iv) ethyl 2-chloropyrimidine-5-carboxylate, K_2CO_3, CH_3CN, rt, 3 h; v) $LiOH \cdot H_2O$, THF/MeOH/H_2O (10:1:3), rt, overnight; vi) EDC·HCl, HOBt, $THPONH_2$, NMM, DMF, rt, overnight.

Table 2. SAR of heteroatom-bonded substituents as dual PDE5/HDAC inhibitors.



1'

Cpd	R1	PDE5 A IC_{50} nM	HDAC1 IC_{50} nM	HDAC2 IC_{50} nM	HDAC3-NCOR2 IC_{50} nM	HDAC6 IC_{50} nM
21a		44	7080	>20000		10400
21b		22	5980	>20000		12700
21c		20	7810	17500		1600
21d		5	68	490	31	441
21e		17	118	712		709
21f		10	25	166	43	584
30a		65	5860	>20000		1090

30b		24	603	2030		1060
30c		12	12900	>20000		4340
30d		40	9270	>20000		10100
30e		74	443	1850		1900
30f		11	2440	9830		2330
30g		23	566	2250		196
37		10	66	432		373

Regarding PDE5 activity, all compounds in Table 2 retained potent activities in the low nanomolar range (IC_{50} < 75 nM); however, the replacement of the sulfonamide group tended to result in a slight decrease in the potency for PDE5 compared to **1**, particularly for compounds **21a**, **30a**, **30d** and **30e**, which exhibited > 1 log unit of decreased potency.

As anticipated, diverse responses in HDAC activity were observed for the compounds in Table 2. This differential HDAC inhibitory profile is largely attributable to the nature of the linker groups bearing the hydroxamic acid, predictably lying deep in the hydrophobic catalytic channel of the HDACs, rather than due to the influence of the

heteroatom attached to the sildenafil core, closer to the rim surface according to the proposed binding mode. Thus, differences of low significant in terms of inhibitory activity against the three isoforms (HDAC1, HDAC2 and HDAC6) are observed when comparing the secondary amines **21b** (5980 nM, >20000 nM, 12700 nM) and **21e** (118 nM, 712 nM, 709 nM) with their corresponding ether-linked matched pairs **30f** (2440 nM, 9830 nM, 2330 nM) and **37** (66 nM, 432 nM, 373 nM), although a preference for the more liphophilic ethers can be acknowledged.

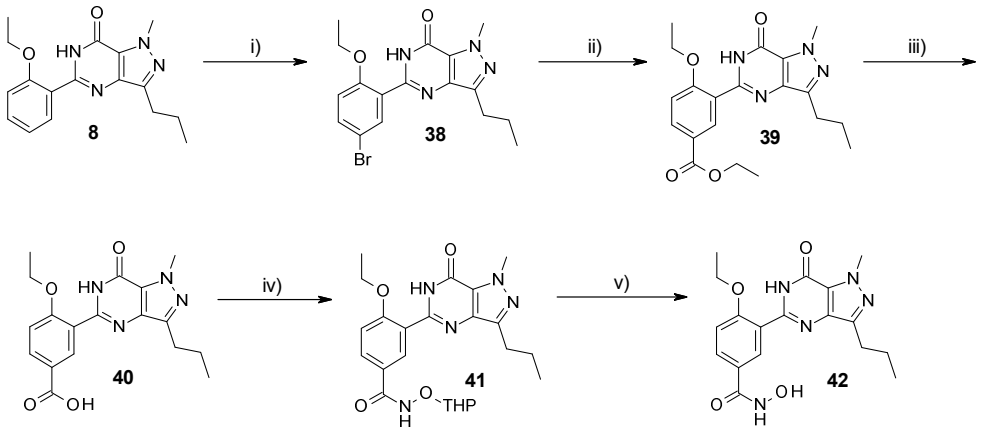
Concerning the linkers entering deep into the HDAC catalytic channel, the class I HDAC potency trend towards pyrimidylhydroxamic acids was replicated in the case of nitrogen- and oxygen-bonded variants (**21d-21f, 30e, 37**). These analogues were the most potent compounds in Table 2 against HDAC1 and HDAC2 isoforms, with IC_{50} values close to or below 100 nM for HDAC1 and in the mid-nanomolar range for HDAC2 and HDAC6, with the exception of the spiro-linked pyrimidylhydroxamic **30e**, which exhibited reduced potency against the three isoforms, likely due to conformational constraints to achieve optimal chelation geometry. The impact of this pyrimidyl group for HDAC activity is clearly recognized when replacing it (**21d**) with a phenyl group (**21c**); derivative **21c** demonstrated decreased potency against HDAC1, HDAC2 and HDAC6 by more than 2, 1.5 and 0.5 log units, respectively. The good potency of the pyrimidyl group could not be attributed to a plausible explicit hydrogen-bond contact between any nitrogen of the pyrimidine ring and HDAC residues in the catalytic pocket. Additionally, the catalytic channel of the three isoforms is highly conserved such that this class selectivity can be attributed to a particular residue. The decreased pKa of the hydroxamic group (from 8.73 in **21c** to 7.83 in **21d**, as calculated with Pipeline Pilot[45]) might play a role in the good class I potency of the pyrimidyl group, although it does not definitively explain the selectivity profile.[46]

Conversely, small linear alkyl (**21a, 30a, 30b**) and cycloalkyl (**21b**, **30f**) derivatives were weak micromolar HDAC inhibitors or even inactive against HDAC2 (Table 2), although increasing the flexible chain length tended to improve HDAC1 and HDAC2 potency compared to shorter linkers (e.g., compare **30b** with **30a**). Conformationally constrained spiros (**30c**, **30d**) exhibited no improvement in HDAC potency. Among the derivatives in Table 2, the optimal linker for HDAC6 was the small phenyl group of **30g**, which had an IC_{50} value of 196 nM. Replacement of the planar phenyl ring in **30g** by cyclohexyl in derivative **30f** was detrimental for HDAC inhibitory activity, particularly for HDAC6 (IC_{50} of **30f** of 2330 nM). This fully agrees with previous findings regarding the preference of HDAC6 isoforms for aromatic groups over alkyl groups[47] and inspired us to guide the rational design of HDAC6 selective inhibitors (manuscript in preparation). The hydrophobic nature of the tunnel channel, flanked by Phe150 in HDAC1 (Phe155 and Phe620 for HDAC2 and HDAC6, respectively) and Phe205 in HDAC1 (Phe210 and Phe680 for HDAC2 and HDAC6, respectively) as well as the different conformation of the hydroxamic acid group for optimal bidentate coordination with Zn metal might explain the preference for phenyl over cyclohexyl linkers. In summary, despite their reduced polar surface area, compounds in Table 2 showed no clear HDAC improvement *in vitro* compared to compounds in Table 1.

The next stage of our SAR exploration focused on carbon-linked substituents at the 5'-position of the phenyl ring (Table 3) covering both linear alkyl chains (**48a, 48b**) and methylene-homologated rings (**7, 48c-48m, 52a-52b**) as well as more rigid derivatives with carbon-bonded rings at the 5' position (**48n-48o, 52c-52f**). Additionally, the hydroxamic moiety was directly attached to the phenyl ring of sildenafil (**42**), resulting in an inactive derivative against HDAC2 and HDAC6 and low affinity for HDAC1

(IC_{50} of 6420 nM, Table 3). According to our modelling studies, the ethoxyphenyl ring and sildenafil core causes steric clashes that prevent optimal positioning of the ZBG within the HDAC cavity. This compound, **42**, was prepared from bromide **38** via reaction with $Pd(dppf)_2Cl_2$ and Et_3N in EtOH under a CO atmosphere, ester hydrolysis, treatment with $THPONH_2$ and acidic removal of the THP-protecting group (Scheme 5).

Scheme 5.



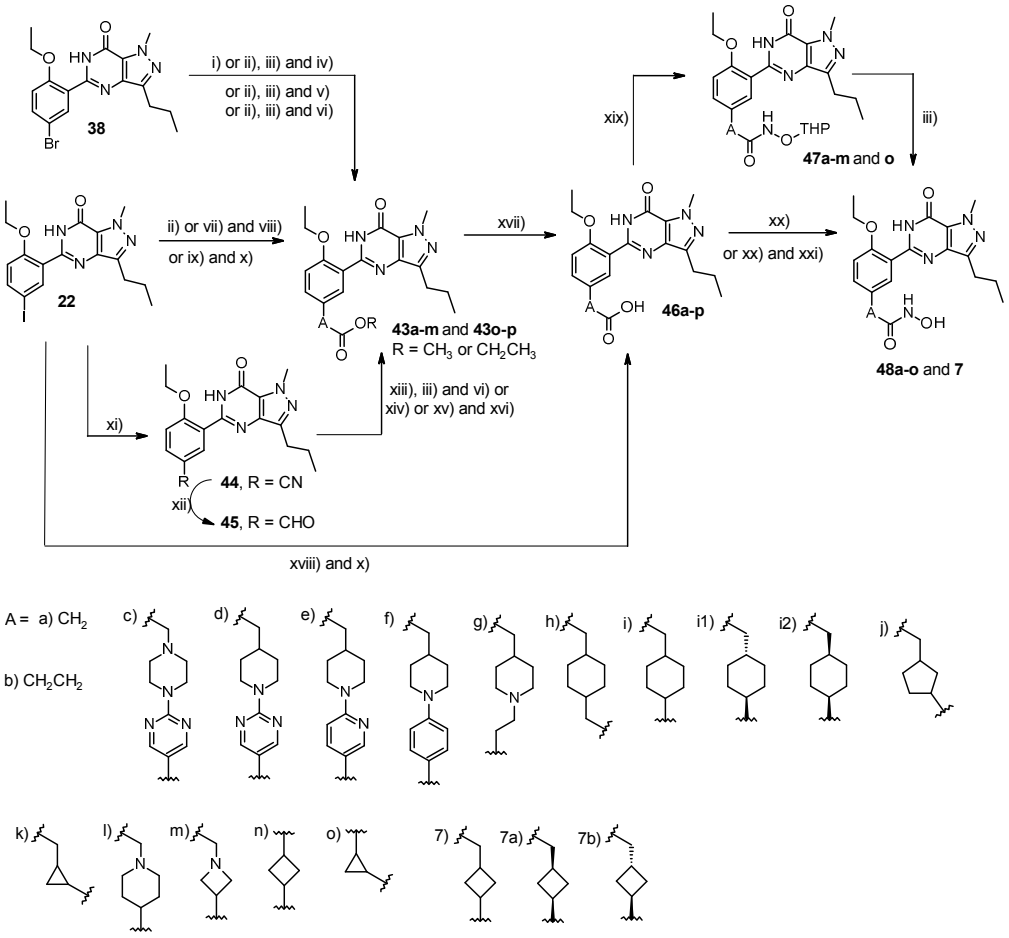
Conditions: i) Br_2, AcOH, rt, overnight; ii) $Pd(dppf)Cl_2$, Et_3N, EtOH, CO (atm), 80 ºC, overnight; iii) $LiOH{\cdot}H_2O$, THF/MeOH/H_2O (3:1:1), 40 ºC, overnight; iv) EDC·HCl, HOBt, $THPONH_2$, NMM, DMF, rt, overnight; v) HCl/EtOAc (4.0 M), rt, 1 h.

Hydroxamic acids **48a-48o** and **7** were synthesized as illustrated in Scheme 6. In this case, bromine **38** and iodine **22** were employed as starting materials to prepare esters **43a-43m** and **43o-43p**. Esters **43a** and **43d-43g** were obtained from bromide **38** through a Negishi reaction or Suzuki coupling. Intermediates **43b**, **43h-43k** and **43p** were prepared from iodide **22** by different methods. The key intermediate **22** was also transformed into aldehyde **45**, and then ester **43c** could be synthesized. Esters **43l**, **43m** and **43o** were also prepared from aldehyde **45**, in this case via reductive amination, aldolic condensation and cyclopropanation, respectively. Then, carboxylic acids **46a-**

46m and **46o-46p** were isolated as previously described after reaction with LiOH. Conversely, carboxylic acid **46n** was directly obtained from iodide **22** after reaction with ethyl 2-formylcyclopropanecarboxylate and reduction with Et_3SiH. Finally, the desired hydroxamic acids **48a-48o** and **7** were achieved through a THP-protected intermediate or by direct reaction with NH_2OH hydrochloride.

Hydroxamic acids **48i1** and **48i2** (trans and cis isomers) were obtained directly after preparative HPLC purification of the crude reaction mixture. Conversely, hydroxamic acids **7a** and **7b** (cis and trans isomers too) could be isolated after supercritical fluid chromatography (SFC), although the stereochemistry of these two pairs of isomers could not be confirmed and was randomly assigned.

Scheme 6.

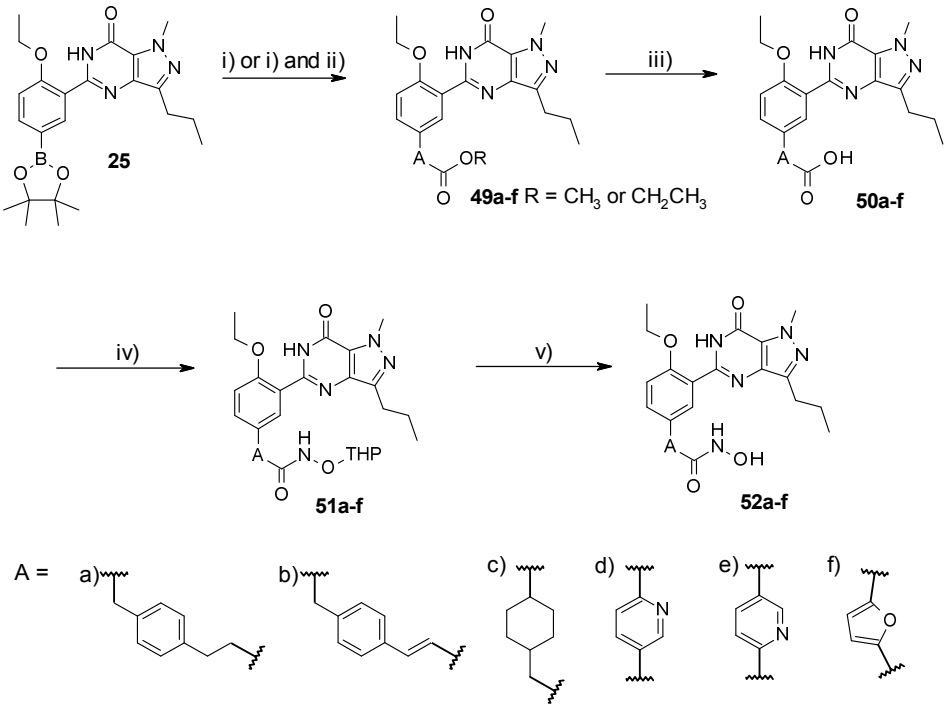


Conditions: i) bromo-(2-ethoxy-2-oxo-ethyl)zinc, $Pd_2(dba)_3$, xantphos, THF, 80 ºC, overnight; ii) corresponding borane reagent, xantphos, Na_2CO_3, $Pd_2(dba)_3$, 1,4-dioxane/H_2O (10:1, 6:1 or 5:1), reflux, overnight; iii) HCl/EtOAc (0.2, 1.0, 2.0 or 4.0 M), 0-25 ºC, 1-3 h; iv) corresponding chloride, K_2CO_3, CH_3CN, 40-100 ºC, overnight; v) CAN, ethyl prop-2-enoate, DIEA, 80 ºC, overnight; vi) (4-methoxycarbonylphenyl)boronic acid, $Cu(OAc)_2$, Et_3N, CH_2Cl_2, O_2 (1 atm), rt, overnight; vii) ethyl acrylate, POT, Et_3N, DMF, 100 ºC, overnight; vii) Pd/C, H_2 (1 atm), MeOH, rt, overnight; ix) n-BuLi, THF, -70 ºC, 10 minutes, then -40 ºC, 1 h, then ethyl 2-formylcyclopropanecarboxylate, rt, 15 h; x) TFA, Et_3SiH, CH_2Cl_2, 0 ºC, then rt, 10 h; xi) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 ºC, overnight; xii) DIBAL-H, CH_2Cl_2, 0 ºC, then rt, overnight; xiii) *tert*-butyl piperazine-1-carboxylate, $Ti[OCH(CH_3)_2]_4$, toluene, rt, 90 minutes, then $NaBH(OAc)_3$, rt, overnight; xiv) corresponding amine, AcOH, $NaBH(OAc)_3$, CH_2Cl_2, rt, overnight; xv) ethyl 2-diethoxyphosphorylacetate, NaH, THF, 0 ºC, 1 h, then **45**, rt, overnight; xvi) trimethyloxosulfonium iodide, NaH, DMSO, 40 ºC, 12 h; xvii) $LiOH \cdot H_2O$, MeOH/THF/H_2O (1:3:1 or 3:3:2), rt or 40 ºC, overnight; xviii) n-BuLi, THF, -70 ºC, then -40 ºC, 1 h, then *tert*-butyl 3-

oxocyclobutanecarboxylate, rt, 15 h; xix) EDC·HCl, HOBt, $THPONH_2$, NMM, DMF, rt, overnight; xx) BOP, DIEA, NH_2OH·HCl, DMF, 80 ºC, overnight; xxi) SFC separation.

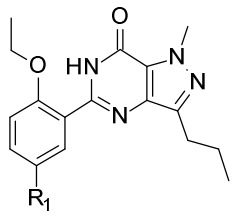
A similar synthetic route was used to prepare compounds **52a-52f**. As shown in Scheme 7, ester functionality was conferred via the reaction of boronic ester **25** with the appropriated bromide, chloride or triflate and subsequent hydrogenation in the case of ester **49f**. Then, a three-step protocol (hydrolysis, reaction with $THPONH_2$ and acidic deprotection) led us to the desired hydroxamic acids **52a-52f**.

Scheme 7.

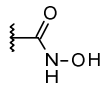
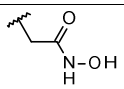
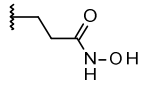
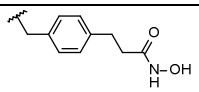
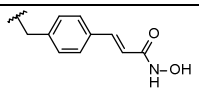
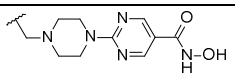
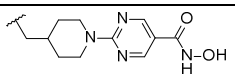
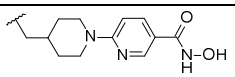
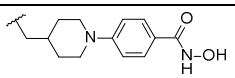
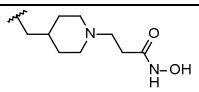


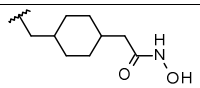
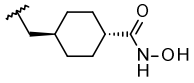
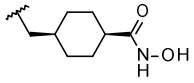
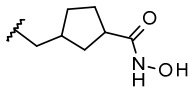
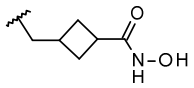
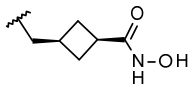
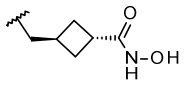
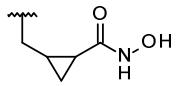
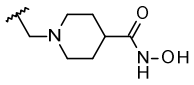
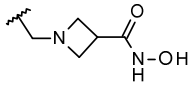
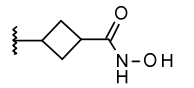
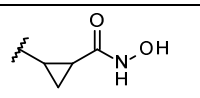
Conditions: i) corresponding bromide, chloride or triflate, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane/H_2O (5:2) or 1,4-dioxane, 85 ºC, MW, 1 h, or conventional heating, 80 ºC, overnight; ii) Pd/C, H_2 (1 atm), MeOH, rt, 1 h; iii) LiOH·H_2O, THF/MeOH/H_2O (3:3:2 or 3:1:1), rt, overnight; iv) EDC·HCl, HOBt, $THPONH_2$, NMM, DMF, rt, overnight; v) HCl/EtOAc (2.0 or 4.0 M) or HCl/1,4-dioxane (4.0 M), rt, 1 h.

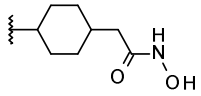
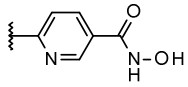
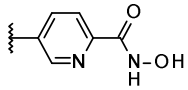
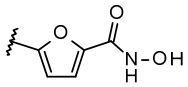
Table 3. SAR of carbon-linked dual PDE5/HDAC inhibitors.



1'

Cpd	R1	PDE5 A IC_{50} nM	HDAC1 IC_{50} nM	HDAC2 IC_{50} nM	HDAC3-NCOR2 IC_{50} nM	HDAC6 IC_{50} nM
42		5	6420	>20000		>20000
48a		19	>20000	>20000		>20000
48b		46	4810	>20000		2120
52a		13	2420	>20000	>20000	2350
52b		122	1530	>20000		1790
48c		7	14	89		379
48d		7	63	335	51	1250
48e		57	1880	6430		395
48f		121	>20000	>20000		>20000
48g		57	>20000	>20000		>20000

48h		38	1530	>20000		344
48i1		13	672	>20000		515
48i2		22	346	>20000		57
48j		165	3510	13900		416
7		60	310	490	322	91
7a		34	225	729	279	143
7b		45	326	1220	239	126
48k		39	2610	14100		1920
48l		207	7620	>20000		9640
48m		303	>20000	>20000		>10000
48n		17	554	1860		130
48o		20	8750	>20000		5370

52c		70	6910	>20000		5130
52d		4	354	1870		79
52e		4	2360	>20000		861
52f		5	>20000	>20000		5570

As with previous attachment points and connections at the 5' position, reported in Table 2, short linear alkyl chains (**48a**, **48b**) and homologated arylalkyl chains (**52a**, **52b**) resulted in weak micromolar (HDAC1 and HDAC6) and even inactive HDAC2 compounds. Strikingly, the methylene-linked cinnamic derivative **52b** was far less potent against HDAC6 (IC_{50} of 1790 nM) than the corresponding sulfonamide-linked derivatives **13d** and **13g** as shown in Table 1 (with HDAC6 IC_{50} values of 89 and 84 nM, respectively), likely as a result of the different positioning of the sildenafil capping group on the surface area due to the different geometries of the connecting bonds.

Once again, the influence of the pyrimidylhydroxamic moiety on class I activity was clearly recognized for compounds **48c** and **48d** (IC_{50} values of 14 and 63 nM for HDAC1 and 89 and 335 nM for HDAC2, respectively). Regarding the nitrogen-linked pair (**21d** *versus* **21c**), replacement of the pyrimidine ring (**48d**) by pyridine (**48e**) and phenyl (**48f**) progressively decreased HDAC activity against the three isoforms, in agreement with a trend towards increased basicity of the pKa of the hydroxamic acid

group (7.85 **48d** > 8.29 **48e** > 8.73 **48f**). The role of the heteroatom connecting the ethoxyphenyl ring of sildenafil to the linker moiety exerted a minor influence; derivatives **21d** (-NH-), **37** (-O-) and **48d** (-CH_2-) exhibited similar potencies (< 0.3 log units difference) against HDAC1 (< 100 nM) and HDAC2 (300-500 nM). For HDAC6, the replacement of the heteroatoms with carbon (**48d**) caused a drop in inhibitory activity (1250 nM), which confirmed this compound as one of the most selective class I inhibitors over HDAC6 over the course of this study (absolute pIC50 difference of 1.4 log units between HDAC1 and HDAC6).

The *cis*-cyclohexylmethyl derivative **48i2** (stereochemistry not confirmed and randomly assigned as *cis*- in comparison with *trans*-**48i1**) was found to be one of the most potent HDAC6 inhibitors in our exploration (HDAC6 IC_{50} of 57 nM), with a greatly reduced molecular weight and polar surface area (MW = 467.56 Da and TPSA = 118 $Å^2$) compared to other compounds with similar HDAC6 potency in Table 1, such as **13d** (MW = 635.74 Da and TPSA = 167 $Å^2$) or **13g** (MW = 552.60 and TPSA = 172 $Å^2$). Thus, given its good HDAC potency and improved physicochemical properties, we decided to systematically reduce the ring size of the cycloalkyl (**48j**, **7**, **48k**). It was not possible to observe a shared ring size SAR between the three HDAC isoforms, but the cyclobutylmethyl **7** achieved the best compromise in terms of HDAC activity as a mid-nanomolar pan-HDAC inhibitor with potent inhibition of HDAC6, although exhibiting reduced potency against PDE5 compared to compound **1** (IC_{50} of 60 nM). Based on its potency, the corresponding *cis*- and *trans*- forms of **7** were separated by SFC. As shown in Table 3, no significant differences in HDAC potency against HDAC1, HDAC2 and HDAC6 (<0.4 log units difference) were observed between the racemic **7** and the *cis*-**7a**

and *trans*-**7b** forms. Thus, given its simpler accessibility, the parent derivative **7** was selected for further studies.

Replacement of the two best 4- (**7**) and 6-membered (**48i2**) cycloalkyl rings with azetidine (**48m**) and piperidine (**48l**) caused a dramatic lost of potency against all HDACs (micromolar range or inactive compounds) as well as PDE5 (mid-nanomolar range) (Table 3). These data suggest that positively charged groups entering deep into the channel of the HDAC catalytic center are disfavored. Additionally, direct connection of the cycloalkyl rings to the 5'-position of the phenyl ring tended to reduce HDAC potency compared to the pair-matched set of methylene-homologated derivatives: **48n** *versus* **7**, **48o** *versus* **48k** and **52c** *versus* **48h**.

Finally, we carried out a small investigation of heteroaryl rings directly bonded at the 5' position: 2-pyridine (**52d**), 3-pyridine (**52e**) and 2-furan (**52f**). As shown in Table 3, these derivatives recovered PDE5 inhibitory activity comparable to that of **1** (in the 1-10 nM range), although achieving variable results in terms of HDAC activity: while the 2-pyridine **52d** exhibited good (<100 nM at HDAC6) and modest (HDAC1, HDAC2) potency, the furane **52f** was a weak micromolar (HDAC6) or inactive (HDAC1, HDAC2) inhibitor.

Concerning HDAC isoform selectivity, the most remarkable trend among these compounds was observed for the pyrimidylhydroxamic derivatives (**13c, 21d, 21e, 21f, 30e, 37, 48c, 48d**), which exhibited > 0.6 log units of selectivity for HDAC1 over HDAC6, with some compounds (**13c**, **21f**, **48c**, **48d**) possessing more than 1 log unit of preference for the HDAC1 isoform. Interestingly, similar HDAC1 and HDAC6

activities (IC_{50} values of 57 nM and 59 nM, respectively) were found for the secondary sulfonamide **13f** bearing this pyrimidylhydroxamic moiety. Conversely, a certain trend for HDAC6 preference over HDAC1 was observed for the carbon-linked aliphatic rings in Table 3, with derivatives **48h**, **48i2**, **48j**, and **48n** demonstrating an absolute pIC50 difference of more than 0.6 log units between HDAC6 and HDAC1. Our efforts to develop dual PDE5-HDAC6 selective inhibitors and examine analogues, focusing on this type of linker substituent, will be reported in due course (manuscript in preparation).

Cytotoxicity and Cellular Functional Response: effects on histone acetylation and CREB phosphorylation

Compounds were selected to be assayed in a cellular context based on a well-balanced compromise between favorable PDE inhibition (IC_{50}< 100 nM), HDAC potency against at least one isoform (preferably with $IC_{50} \leq 500$ nM) and structural diversity (e.g., **30a**, **52e**).

Unlike HDAC6 inhibition,[22] inhibition of HDAC class I isoforms is associated with toxicity,[22,48] and this was a major concern when investigating this novel therapeutic approach for neurodegenerative disorders. Thus, we routinely screened the cytotoxicity of selected compounds in the healthy hepatic cell line THLE-2 (Table 4) after 72 hours of incubation, and for those compounds demonstrating LC_{50} values higher than 5000 nM in THLE-2 cells, their cytotoxicity was also evaluated in primary neuronal cultures of glia cells (Table 4). This threshold was established on the basis of the LC_{50} values exhibited by the standard compound **4** (3590 nM, Table 4), which was our initial

reference compound to study the synergistic effects induced by inhibiting both PDE5 and HDACs.[21] With the exception of the 3-pyrido derivative **52e** (LC_{50} of 161 nM) and the pyrimidylhydroxamic acid **48c** (LC_{50} of 422 nM), all compounds exhibited middling (1000 – 5000 nM) or low (> 5000 nM) THLE-2 cytotoxicity. In general, a certain correlation exists between THLE-2 cytotoxicity and potent HDAC1 inhibition, as observed for the potent HDAC1 pyrimidylhydroxamic derivatives (**13f** < **21d** < **37** < **48d** < **13c** < **48c** in order of compounds exhibiting less to more THLE-2 cytotoxicity). Obviously, differences not only in primary biochemical activities (e.g. HDAC1 inhibition) but also in permeability may play a major role in the cytotoxicity observed as well as in the corresponding functional responses. Thus, the passive membrane permeability (P_e) of these molecules was measured *in-vitro* in a parallel artificial membrane permeation assay (PAMPA) (Table 4). PAMPA was performed using a brain polar lipid (BPL) membrane which is particularly suited for predicting brain permeability; therefore, providing an additional value to our priorization process: identification of compounds with higher probability to cross the BBB. In general, our compounds demonstrated low (Pe < 10 nm/s) or moderate (10 < Pe < 30 nm/s) permeability comparable to that of **1** (Pe = 27.5 nm/s), a well-characterized CNS-penetrating drug. [49] These ranges to classify poor (Pe < 10 nm/s), moderate (10 < Pe < 30 nm/s) and good (30 nm/s) cellular permeation were established on the basis of the permeability values determined for known commercial drugs with either high or low brain penetration[50] and corrected based on internally tested permeability values. Compound **4** exhibited low permeation (Pe is 2.3 nm/s), in agreement with its established permeability classification (class IV) according to the Biopharmaceutical Classification System[24,25] and supportive of its poor brain availability[26] despite its demonstrated effect in improving cognitive function and rescuing memory function.[5] As

anticipated, compounds with the highest TPSA values (**13c**, **13f,** and **13g** with TPSA values of 193, 201 and 172 $Å^2$, respectively) demonstrated the poorest cellular permeation (Pe < 10 nm/s). Other derivatives with TPSA attributes similar to that of **1** (117 $Å^2$) and increased lipophilicity (calculated LogD at pH 7.4[45]> 3.5 but less than 5), such as **30g**, **48h** and **48i1**, exhibited excellent permeability (Pe > 30 nm/s), in agreement with findings by GSK for molecules containing an ionizable group.[51] In general, the increased lipophilicity of our dual inhibitors compared to that of compound **4** (LogD of 2), enabled moderate to good permeability. For example, the lead compound **7** (Pe = 15.7 nm/s; TPSA of 118 $Å^2$ and LogD of 3.4) exhibited improved PAMPA results over **4**, although the results were slightly worse than those for sildenafil (Pe is 27.5 nm/s), which lacks the ionizable hydroxamic acid group.

Considering both the moderate permeability and the weak potency of compound **52e** against class I HDACs (HDAC1 IC_{50} of 2360 nM and inactive against HDAC2), there is no clear explanation for the idiosyncratically high cytotoxicity observed for this compound, particularly when it is compared with its closest analogue **52d** (IC_{50} of 354 nM and 1870 nM against HDAC1 and HDAC2, respectively), which has similar permeation and an improved HDAC profile; **52e** is 1.3 log units less cytotoxic than **52d** in THLE-2 cells. A good correlation was observed between THLE-2 cytotoxicity and cytotoxicity in neurons and glia cells from WT mice, with absolute pLC_{50} difference values between both cell lines < 0.40 for all tested compounds. Thus, THLE-2 cytotoxicity can be used as a good marker of neuronal cytotoxicity, reducing the need to screen all compounds against a primary culture. The cytotoxicity of compound **7** was also evaluated after 24 and 48 hours of incubation, and no effect was detected (LC_{50} > 100 μM). Taken alone, these data suggest that there is potential to obtain HDAC

inhibitors with moderate to low cytotoxicity, thus demonstrating an acceptable therapeutic window (see below).

To test the functional responses of these molecules, the cellular activity of selected compounds was assessed; we then measured their ability to induce histone and α-tubulin acetylation in SH-SY5Y neuroblastoma cells and evaluated the functional consequence of cellular class I HDAC and HDAC6 inhibition, respectively (Table 4). Compounds were incubated at three different concentrations (100, 500, 1000 nM) for two hours, and western blotting assays were carried out to quantify the levels of acetylated histone 3 at Lys 9 (AcH3K9), which has been implicated in cognition enhancement[13,21], and acetylated α-tubulin at Lys 40 (AcTub) (Table 4). In each case, the data were normalized to total histone 3 (H3, for AcH3K9) or actin (for AcTub) and expressed as the mean fold change *versus* control vehicle-treated cultures, with values greater than 1 indicating the induction of acetylation. In general, compounds in Table 4 induced histone and tubulin acetylation in a concentration-dependent manner, with minor variations for those compounds demonstrating a weak effect on cellular acetylation (values ~1-fold change). However, there were also exceptional cases in which this dose-response behaviour was not observed (e.g., the drop in α-tubulin acetylation of compound **30g** from a 4.6- to 2.9-fold change at 500 nM and 1000 nM, respectively). We attribute these observations to the selected incubation time (after several trials, all functional responses were measured after two hours of incubation), as we have observed a strong impact of this parameter on the induction of acetylation marks (*data not shown*), which may ultimately reflect the influence of the association and dissociation kinetic rates (k_{on}/k_{off}) of the HDAC inhibitors on their corresponding targets.[36]

One of the desirable characteristics for our final tool compound was to possess an acceptable therapeutic window, i.e., a high toxicity/function ratio. Given that some of our compounds exhibited low cytotoxicity in the 5–10 μM range, optimal compounds were required to elicit significant functional responses on cellular acetylation at a dose of 500 nM to enable a minimal therapeutic window of 1 log unit for this cell line. Relative to the standard compound **4** (12.5- and 11.9-fold induction of AcH3K9 and AcTub at 500 nM), compound **7** was optimal among all compounds presented in Table 4, with a well-balanced profile against both marks (7.4- and 12.7-fold change at this concentration). When comparing alpha technology and western blotting assays, compound **7** achieved a 2.4-fold increase in H3K9 acetylation at 400 nM over non-treated cells with alpha technology; in fact, the significant induction of AcH3K9 was obtained from 64 nM.[23] Other compounds, such as the potent class I compounds with a pyrimidylhydroxamic acid moiety, demonstrated even greater potency when increasing the histone acetylation of neurons (**48c** and **48d**, with 21.1 and 8.1-fold values) but had only a minor influence on the tubulin marker (2.8 and 2.0-fold change, respectively) and demonstrated a reduced therapeutic window (above all **48c**) than inhibitor **7**. Conversely, the potent HDAC6 inhibitor **52d** exhibited an interesting cellular profile in terms of tubulin acetylation (increased 16.6-fold) but exerted a minor effect on histone acetylation (likely due to its decreased class I HDAC activity compared to **7**). At this step in the project, a cellular functional response for both marks, as achieved with **4**, which has demonstrated *in vivo* efficacy[21], was required to progress to *in vivo* efficacy studies.

As seen in Table 4, a weak correlation was observed between *in vitro* HDAC6 potency and the induction of α-tubulin acetylation. Compounds demonstrating IC_{50} values against HDAC6 close to or below 100 nM (**52d**, **7**, **48n**) produced >6-fold induction of

tubulin acetylation at 500 nM (Table 4), with the exception of the derivatives **13f** and **13g**, which, despite being potent HDAC6 inhibitors, exerted weak cellular effects attributable to the fact that these two compounds are those possessing the poorest permeability (Pe < 5 nm/s) in Table 4. Derivative **48i2**, with an HDAC6 IC_{50} of 57 nM, was not tested because its cytotoxicity (LC_{50} of 2650 nM) was not acceptable and its corresponding pseudoenantiomer **48i1** (with similar class I HDAC profile) showed no potent induction of histone acetylation. Conversely, this trend between *in vitro* potency and cellular AcH3K9 was not detected for either HDAC1 or HDAC2. Not all potent pyrimidilhydroxamic acid-bearing compounds (with IC_{50} HDAC1 and HDAC2 <100 and <500 nM, respectively), such as **13c**, **13f** and **21d**, were able to achieve a histone acetylation change similar to that of **48c**, **48d** and **37**. For some compounds, permeability plays a role (**13f**, **13g**), but this is clearly not the only factor involved, as highlighted for the pairwise comparison between the secondary amine-linked **21d** and the carbon-linked **48d** pyrimidyl hydroxamic compounds, with similar class I HDAC biochemical profiles (Tables 2 and 3) and reduced permeability of **48d** *versus* **21d** (4.1 *versus* 13.4 nm/s). Additionally, compared to lead compound **7,** the reduced cellular response of the more permeable and more HDAC1-potent oxygen-linked pyrimidylhydroxamic derivative **37** (7.4- *versus* 4.5-fold change in histone acetylation at 500 nM) is striking, although other permeability-related factors, such as active transport (P-gp efflux), which we identified for **7**, might also play a role. Conversely, this differential functional response may also mirror the impact of the different kinetic binding rates of our compounds, a characteristic that we have recently started to explore (as reported for **7**, residence time[23]). Moreover, the non-specific contribution of each HDAC to H3K9 deacetylation complicates the analysis of the functional responses determined for these compounds.

To further validate the PDE5 inhibitory activity of these compounds and to determine whether this activity translates to a functional cell-based response, the effects of the compounds at 500 nM on pCREB-Ser133 in SH-SY5Y neuroblastoma cells were also examined after 30 min and 2 hours of incubation (Table 4). As a reference, the equipotent low nanomolar PDE5 inhibitors **1** and **3** enhanced pCREB 1.9 times (30 min) and 1.4 times (2 hours) over vehicle controls. As expected, compound **4** had no effect. Compared to the notable alterations in epigenetic marks, the effects observed on pCREB had a narrower window for improvement and were also highly affected by the chosen incubation time. Thus, we targeted a minimal fold change of 1.4 (as observed with **3**) at any incubation time. This response was achieved by most tested compounds in Table 4 (with the exception of **13c**, **48i1**, and **52d**). However, two compounds that demonstrated strong stimulation of CREB phosphorylation (**21d**, **52e**) did not progress based on their poor induction of acetylation hallmarks and their cytotoxicity.

To further characterize the translational potential of the compounds reported in Table 4, which had good potency in cell-based assays and an acceptable therapeutic window (**7** and **37**), we examined their effect on wild type (WT) neurons exposed to different concentrations of compounds for 2 hours. As previously reported, our lead compound **7** led to a 190% increase in AcH3K9 at 10 nM[23], whereas compound **37** reached its maximum effect at 100 nM (170% increase). Thus, there is consistency between the acetylation responses observed for this pair of compounds in SH-SY5Y neuroblastoma and WT neurons.

Table 4. Functional cellular profile of the initial set of PDE5/HDAC inhibitors.

Cpd	**THLE-2 LC_{50} nM**	**Primary Neurons LC_{50} nM**	**AcH3K9 levels** (fold-change over basal (1)) **100 nM 500 nM 1000 nM**	**AcTub levels** (fold-change over basal (1)) **100 nM 500 nM 1000 nM**	**pCREB levels** (fold-change over basal (1)) **at 500 nM 30 min 2 hours**	**PAMPA P_e (nm/s)**
1	>100000	N.D.	N.D.	N.D.	1.9 0.6	27.5
3	60300	110000	N.D.	N.D.	1.3 1.4	26.8
4	3590	4910	4.0 12.5 13.9	5.0 11.9 27.6	N.D.	2.3
13c	1460	2150	1.2 1.0 2.4	1.1 0.9 2.3	0.7 0.7	7.2
13f	11900	4950	0.9	1.1	0.5	1.0

			1.1 1.7	1.1 1.3	1.9	
13g	27400	30300	0.8 1.2 1.0	0.4 0.6 0.8	N.D.	2.3
21d	2570	N.D.	1.2 1.8 5.1	1.4 2.8 2.7	0.9 2.1	13.4
30a	88700	>100000	1.6 1.3 1.8	2.6 2.4 3.6	1.5 0.7	4.9
30g	13400	15100	1.3 2.7 2.5	2.8 4.6 2.9	1.4 1.5	36.8
37	2210	N.D.	1.8 4.6 13.0	1.0 1.8 2.1	0.4 1.7	30.5
48c	422	N.D.	14.4 21.1 45.6	1.2 2.8 6.7	1.6 1.4	8.9
48d	1830	N.D.	3.7 8.1 18.9	0.4 2.0 3.1	2.1 1.4	4.1
48h	17000	38300	0.3	1.0	1.1	31.2

			0.8 1.4	1.7 1.1	1.3	
48-i1	9280	9020	1.4 3.0 3.8	1.9 1.5 1.7	0.5 0.5	35.1
48-i2	2650	N.D.	N.D.	N.D.	N.D.	N.D.
7	7200	17700	1.2 7.4 19.9	1.5 12.7 17.8	1.5 1.2	15.7
48n	13500	15300	1.1 1.3 3.7	4.3 6.4 10.2	1.3 1.0	13.7
52d	3690	N.D.	1.7 2.2 3.3	16.9 16.6 21.4	0.5 0.2	9.2
52e	161	N.D.	0.8 1.3 0.9	0.8 3.0 11.7	1.4 2.1	15.2

N.D.= Not determined.

ADME Profiling of compounds 37 and therapeutic tool 7

Based on its good cellular response for the induction of epigenetic hallmarks with an acceptable therapeutic window (approximately 1 log unit), we determined the *in vivo* CNS penetration of compounds **7** and **37** in mice after intraperitoneal administration at a dose of 40 mg/Kg by determining the logBB, where BB is the ratio of the brain to plasma concentration. Both compounds exhibited poor central access with logBB values at each corresponding time to reach maximum plasma concentrations (Tmax) of -1.87 (**7**, at Tmax = 10 min)[23] and -1.43 (**37**, at Tmax = 15 min). The average total brain concentrations of compounds **7** and **37** were 248[23] and 71 nmol/Kg, respectively. Functional responses in the CNS were explored at different time points. In the case of compound **37**, the maximum functional response in the hippocampus (40% increase in AcH3K9 and 110% increase in pCREB-Ser133 phosphorylation relative to the controls) was observed 1 h after administration, while for the lead compound, there was a 98% increase in AcH3K9 and a 148% increase in pCREB-Ser133 phosphorylation relative to the controls 30 min after administration.[23] On the other hand, taking into account that other phosphodiesterase isoforms such as PDE9 and PDE6 also hydrolyse cGMP, the effects of **7** and **37** on these two targets were assessed. In fact, **7** does not inhibit PDE9 (IC_{50} > 10μM);[23] but, its activity *vs* PDE6 is quite potent (IC_{50} is 2.6 nM). Compound **37** is even better inhibitor of PDE6 than **7** (> 1 log unit), its IC_{50} is 0.13 nM, and shows moderate acitivity against PDE9 (its IC_{50} is 4.8 μM). Additionally, considering that PDE3A is a phosphodiesterase isoform that hydrolyses cAMP and cGMP and is involved in cardiac contractility[52] (its inhibition may lead to unwanted cardiac side-effects), thus important from cardiovascular safety perpective, we also tested these two selected molecules **7** and **37** *vs* PDE3A. Compound **7** shows a moderate inhibition against PDE3A, IC_{50} is 1.8 μM, to be improved; however, **37** inhibits PDE3A at mid-nanomolar range, IC_{50} is 750 nM. Given the low concentration reached by **37** in the

brain, its cytotoxicity (2210 nM in THLE-2) and worse off-target selectivity profiling than **7**, this compound did not progress further. The low brain permeation of compound **7** may be attributable to its still relatively high TPSA (118 $Å^2$), as its molecular weight (439.51 Da) and lipophilicity (predicted LogD at pH=7.4 is 3.37) are in line with commonly accepted ranges for CNS penetration (MW < 450 Da and a $LogD_{7.4}$ ranging between 1 and 3 are commonly recommended).[40] Note that the TPSA of our compounds is biased by the explored sildenafil core (**1'** substructure, unmodified), which is close to surpassing the commonly accepted values for CNS-penetrating drugs[39,53], and the mandatory ZBG; thus, further exploration of the substituents of the pyrazolopyrimidinone core will also be required to optimize BBB penetration (this exploration is currently on-going). Conversely, in addition to the moderate passive diffusion of **7** (Pe, in PAMPA, is 15.7 nm/s), a Caco-2 permeability assay revealed a low Pe value of 0.46 ($\times 10^{-6}$ cm/s) and clear evidence of active transport: the efflux ratio is 41.3.[23] Therefore, together with an improvement in passive permeability, the optimization process will require overcoming the P-gp efflux.

CONCLUSION

Based on structural information as well as the available structure−activity relationship data for HDAC and PDE5 inhibitors, we designed a novel first-in-class chemical series of dual inhibitors (Figure 3).

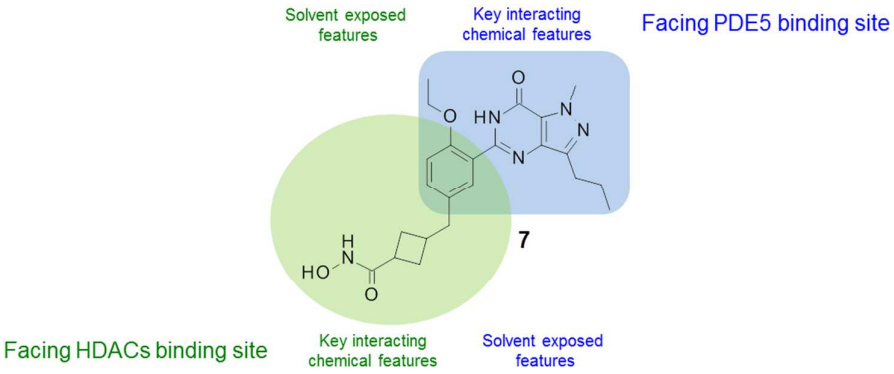


Figure 3. Description of the proposed binding modes, represented by the selected chemical probe **7**, and key interacting features according to each target binding site.

SAR analyses around the growing vector (R1) borne by the initially explored structure **1′** led us to evolve from initial hit compounds (e.g., **13b** and **13c**) and achieve a lead molecule, **7**, through an iterative multifactorial optimization process. We have demonstrated that significant acetylation of histone 3 is achieved through moderate HDAC class I but potent PDE5 inhibition, attributable to the synergistic effects between HDAC class I and PDE5; thus, the toxicity associated with HDAC class I inhibition is minimized.

Despite its non-optimal logBB value (<-1),[40] compound **7** was shown to achieve corresponding functional responses *in vivo* (i.p.; 40 mg/Kg): these included the induction of AcH3K9 (98% increase over non-treated mice) and increased pCREB

(148% increase over non-treated mice) in the hippocampus.[23] Then, considering primary activities (moderate HDAC class I and potent HDAC6 as well as PDE5 inhibition), *in vitro* and *in vivo* functional responses, ADME properties, CNS penetration, pharmacokinetics profiles and therapeutic windows,[23] **7** was identified as a lead compound for *in vivo* PoC.

As previously reported[23], treatment with **7** rescued the memory impairment exhibited by Tg2575 mice, prevented disruptions in synaptic plasticity and induced memory-related genes; in addition, **7** provokes a significant reduction in amyloid and tau pathology as well as a reversion of the reduced dendritic spine density.

In summary, we have described the discovery of a first-in-class chemical series of dual inhibitors and identified **7** as a lead compound. This molecule was utilized as a therapeutic tool compound and validated our systems therapeutics approach, targeting two independent but synergistic enzymatic activities, as a potential new symptomatic- and disease-modifying strategy to treat AD.[23] Chronic treatment of Tg2576 mice with **7** diminished brain Aβ and pTau levels, increased the inactive form of GSK3β, reverted the decrease in dendritic spine density on hippocampal neurons and it reversed their cognitive deficits, at least in part by inducing the expression of genes related to synaptic transmission; in fact, **7** rescued *ex-vivo* the impaired long-term potentiation evident in hippocampal slices from APP/PS1 mice.[23] In addition, **7** can be used as a chemical probe to further elucidate the mechanisms of its targets (HDACs and PDE) in AD and represents an adequate starting point to launch an AD drug discovery program aimed at identifying optimized molecules with the target compound profile described herein.

EXPERIMENTAL SECTION

Chemistry. General Procedure.

Unless otherwise noted, all reagents and solvents were of the highest commercial quality and used without further purification. All experiments dealing with moisture sensitive compounds were conducted under N_2. The reactions were monitored by thin layer chromatography (TLC) on silica gel-coated plates (Merck 60 F254) using reagent grade solvents. Flash column chromatography was performed on silica gel, particle size 60 Å, mesh = 230-400 (Merck) under standard techniques. Automated flash column chromatography was performed using ready-to-connect cartridges from Varian, on irregular silica gel, particle size 15-40 μm (normal phase disposable flash columns) on a Biotage SPX flash purification system. Microwave-assisted reactions were performed in a Biotage Smith Synthesis microwave reactor. Melting points were monitored with Olympus PH2 microscope connected to a Mettler FP80 hot stage and an FP80 central processor. The NMR spectroscopic data were recorded on a Bruker AV400 or VARIAN 400MR spectrometer with standard pulse sequences, operating at 400 MHz. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. The abbreviations used to explain multiplicities are s = singlet, d = doublet, t = triplet, m = multiplet. Coupling constants (J) are in hertz. HPLC-analysis was performed using a Shimadzu LC-20AB or LC-20AD with a Luna-C18(2), 5um, 2.0*50mm column at 40 ºC and UV detection at 215, 220 and 254 nm. Flow from the column was split to a MS spectrometer. The MS detector (Agilent 1200, 6110MS or Agilent 1200, 6120MS Quadropole) was configured with an electrospray source or API/APCI. N_2 was used as the nebulizer gas. The source temperature was maintained at 50 ºC. Data acquisition was accomplished with ChemStation LC/MSD quad software. All tested compounds possessed a purity of at least 95% established by HPLC, unless otherwise noted. Reported yields were not optimized, the emphasis being on purity of product rather than quantity.

3-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclobutanecarbohydroxamic acid (7)

To a solution of compound **46p** (1.1 g, 2.6 mmol) in DMF (60 mL) were added BOP (2.3 g, 5.2 mmol), DIEA (4.5 g, 35 mmol) and $NH_2OH{\cdot}HCl$ (1.8 g, 26 mmol) and the mixture was stirred at 80 ^oC overnight. Then, the solution was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **7** (530 mg, 46%) as a yellow solid; m.p.: 118-119 ^oC. ^1H NMR (MeOD, 400 MHz): δ 7.75-7.74 (m, 1H), 7.35-7.33 (m, 1H), 7.11-7.09 (m, 1H), 4.25-4.21 (m, 5H), 2.93-2.89 (m, 2H), 2.81-2.74 (m, 3H), 2.54-2.52 (m, 1H), 2.36-2.34 (m, 1H), 2.20-2.18 (m, 1H), 2.02-1.99 (m, 2H), 1.87-1.82 (m, 2H), 1.48-1.45 (m, 3H), 1.05-1.01 (m, 3H). ^{13}C NMR (DMSO-d6, 400MHz): δ 14.7 (CH_3), 15.4 (CH_3), 22.6, 28.00, 30.0, 31.4, 33.0, 33.4, 38.7 (NCH_3), 41.8, 64.9 (CH_2O), 113.6, 123.1, 123.2, 125.0, 130.8, 132.4, 138.8, 145.6, 150.5 (CO), 154.5, 155.5, 171.5 (CONHOH). ESI-MS *m/z* 440.2 $[M+H]^+$ calc. for $C_{23}H_{29}N_5O_4$.

3-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclobutanecarbohydroxamic acid (7a)

From **7** (530 mg), pure isomer **7a** (9.8 mg, 1.8%) was obtained by SFC (see protocol in supporting information) as a yellow solid; m.p.: 148-149 ^oC. According to SFC purification method, Rt is 3.28. ESI-MS *m/z* $[M + H]^+$: 440.2 calc. for $C_{23}H_{29}N_5O_4$. Purity is 96.51% according to HPLC analytical method (described in supporting information); where Rt is 2.80. ^1H NMR (MeOD, 400 MHz): δ 7.74 (d, J = 2 Hz, 1H),

7.33-7.31 (m, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 4.23-4.17 (m, 5H), 3.01-2.99 (m, 1H), 2.90-2.87 (m, 2H), 2.81-2.79 (m, 2H), 2.76-2.72 (m, 1H), 2.35-2.33 (m, 2H), 1.98-1.97 (m, 2H), 1.85-1.79 (m, 2H), 1.45 (t, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 7.2 Hz, 3H).

3-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclobutanecarbohydroxamic acid (7b)

From **7** (530 mg), pure isomer **7b** (113 mg, 21%) was obtained by SFC (see protocol in supporting information) as a white solid; m.p.: 178-179 ^{o}C. According to SFC purification method, Rt is 3.03. ESI-MS *m/z* $[M + H]^+$: 440.2 calc. for $C_{23}H_{29}N_5O_4$. Purity is 98.18% according to HPLC analytical method (described in supporting information); where Rt is 2.63. ^{1}H NMR (MeOD, 400 MHz): δ 7.73 (s, 1H), 7.32-7.29 (m, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 4.22-4.17 (m, 5H), 2.90-2.86 (m, 2H), 2.81-2.79 (m, 1H), 2.74-2.72 (m, 2H), 2.52 (m, 1H), 2.18-2.17 (m, 2H), 2.02-1.99 (m, 2H), 1.85-1.79 (m, 2H), 1.45 (t, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 7.2 Hz, 3H).

4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)benzenesulfonyl chloride (9)

Commercially available 5-(2-ethoxyphenyl)-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**8**) (2.5 g, 8.0 mmol) was added into $ClSO_3H$ (10 mL) at ice-water and stirred at room temperature for 2 hours. The reaction mixture was quenched with water, and then filtrated. The filtrate cake was collected and dried under vacuum to give the desired product **9** (2.0 g, 61%). ^{1}H NMR (MeOD, 400 MHz): δ 7.94-7.92 (dd, *J* = 1.6 Hz, 7.6 Hz, 1H), 7.52 (m, 1H), 7.11-7.09 (d, *J* = 8.8 Hz 1H), 4.25 (m, 5H), 2.89 (t, 2H), 1.85 (m, 2H), 1.50 (t, 3H), 0.99 (t, 3H). ESI-MS *m/z* 411 $[M+H]^+$ calc. for $C_{17}H_{19}ClN_4O_4S$

Ethyl 3-[4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]propanoate (10a)

To a solution of **9** (0.41 g, 1 mmol) in EtOH (273 mL) was added piperazine (0.256 g, 2.9 mmol) and the mixture was stirred at 100 ^{o}C under MW for 1 hour. Then, the reaction mixture was concentrated to give the desired intermediate 5-(2-ethoxy-5-piperazin-1-ylsulfonyl-phenyl)-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (0.4 g, 87%). ^{1}H NMR (MeOD, 400 MHz): δ 8.18-8.17 (d, *J* = 2.4 Hz, 1H), 8.00 (dd, *J* = 2.8 Hz, 9.2 Hz, 1H), 7.40-7.38 (d, *J* = 8.8 Hz, 1H) 4.32 (q, 2H), 4.27 (s, 3H), 3.40 (s, 8H), 2.87 (t, 2H), 1.81 (m, 2H), 1.45 (t, 3H), 0.99 (t, 3H). MS *m/z* 461 $[M+H]^{+}$ calc. for $C_{21}H_{28}N_6O_4S$. To a solution of this intermediate (300 mg, 0.651 mmol) in CH_3CN (10 mL) were added K_2CO_3 (271 mg, 1.95 mmol) and ethyl 3-bromopropanoate (177 mg, 0.976 mmol). Then the mixture was stirred at 100 ^{o}C for 2 hours under MW and concentrated to give compound **10a** (260 mg, 71%). ESI-MS *m/z* 561 $[M+H]^{+}$ calc. for $C_{26}H_{36}N_6O_6S$. This intermediate was used in the next step without further characterization.

Ethyl 4-[4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]butanoate (10b)

To intermediate 5-(2-ethoxy-5-piperazin-1-ylsulfonyl-phenyl)-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (described before in **10a** synthesis) (500 mg, 1.09 mmol) dissolved in CH_3CN (10 mL) were added K_2CO_3 (453 mg, 3.28 mmol) and ethyl 4-bromobutanoate (320 mg, 1.64 mmol) and the mixture was stirred at 100 ^{o}C for 2 hours under MW. Then, the reaction mixture was concentrated to give compound **10b**

(300 mg, 48%). ESI-MS *m/z* 575 $[M+H]^+$ calc. for $C_{27}H_{38}N_6O_6S$. This intermediate was used in the next step without further characterization.

Ethyl 2-[4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]pyrimidine-5-carboxylate (10c)

To a solution of **9** (0.41 g, 1 mmol) in EtOH (273 mL) were added ethyl 2-piperazin-1-ylpyrimidine-5-carboxylate (**Int. 1**, synthesis described in supporting information) (0.472 g, 2 mmol) and Et_3N (303 mg, 3 mmol). The mixture was stirred at 100 ^oC under MW for 2 hours. Then, the reaction mixture was concentrated under vacuum to give compound **10c** (0.4 g, 65%). ESI-MS *m/z* 611 $[M+H]^+$ calc. for $C_{28}H_{34}N_8O_6S$. This intermediate was used in the next step without further characterization.

Ethyl (*E*)-3-[4-[[4-[4-ethoxy-3-(3-ethyl-1-methyl-7-oxo-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]methyl]phenyl]prop-2-enoate (10d)

To a solution of **9** (0.41 g, 1 mmol) in EtOH (273 mL) were added ethyl (*E*)-3-[4-(piperazin-1-ylmethyl)phenyl]prop-2-enoate (**Int. 2**, synthesis described in supporting information (0.548 g, 2 mmol) and Et_3N (303 mg, 3 mmol) and the reaction mixture was stirred at 100 ^oC under MW for 2 hours. Then, the reaction mixture was concentrated under vacuum to give the desired compound **10d** (0.35 g, 55%). ESI-MS *m/z* 635 $[M+H]^+$ calc. for $C_{32}H_{38}N_6O_6S$. This intermediate was used in the next step without further characterization.

Methyl 3-[1-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonyl-4-piperidyl]propanoate (10e)

To a solution of **9** (0.41 g, 1 mmol) in EtOH (10 mL) was added methyl 3-(4-piperidyl)propanoate (**Int. 3**, synthesis described in supporting information) (0.185 g, 1 mmol), and the mixture was stirred at 100 ^{o}C under MW for an hour. Then, the reaction mixture was concentrated under vacuum to give compound **10e** (0.4 g, 71%). ^{1}H NMR (MeOD, 400 MHz): δ 8.16-8.15 (d, J = 2.4 Hz, 1H), 7.92-7.86 (m, 1H), 7.39-7.33 (d, J = 8.6 Hz, 1H), 4.32 (q, 2H), 4.24 (s, 3H), 3.78-3.72 (m, 2H), 3.62 (s, 3H), 2.93-2.85 (t, 2H), 2.37-2.27 (m, 4H), 1.87-1.75 (m, 4H), 1.58-1.52 (m, 2H), 1.47 (t, 3H), 1.24-1.21 (m, 3H), 1.04-0.96 (t, 3H). ESI-MS *m/z* 546 $[M+H]^{+}$ calc. for $C_{26}H_{35}N_5O_6S$

Ethyl 2-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylamino]-1-piperidyl]pyrimidine-5-carboxylate (10f)

To a solution of **9** (0.41 g, 1 mmol) in EtOH (273 mL) were added ethyl 2-(4-amino-1-piperidyl)pyrimidine-5-carboxylate (**Int. 4**, synthesis described in supporting information) (0.510 g, 2 mmol) and Et_3N (303 mg, 3 mmol). Then the reaction mixture was stirred at 100 ^{o}C under MW for 2 hours and concentrated under vacuum to give the desired compound **10f** (0.41 g, 65%). ESI-MS *m/z* 625 $[M+H]^{+}$ calc. for $C_{29}H_{36}N_8O_6S$. This intermediate was used in the next step without further characterization.

Ethyl (*E*)-3-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylamino]phenyl]prop-2-enoate (10g)

To a solution of **9** (0.41 g, 1 mmol) in EtOH (10 mL) were added ethyl (*E*)-3-(4-aminophenyl)prop-2-enoate (**Int. 5**, synthesis described in supporting information) (0.191 g, 1 mmol) and Et_3N (303 mg, 3 mmol) and the reaction mixture was stirred at 100 ^{o}C under MW for 1 hour. Then the solution was concentrated under vacuum to give

compound **10g** (0.3 g, 54%). ESI-MS *m/z* 566 $[M+H]^+$ calc. for $C_{28}H_{31}N_5O_6S$. This intermediate was used in the next step without further characterization.

3-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]propanoic acid (11a)

To a solution of compound **10a** (1.0 g, 1.78 mmol) in THF/MeOH/H_2O (10:1:5, 16 mL) was added $LiOH·H_2O$ (374 mg, 8.91 mmol). The resulting mixture was stirred at room temperature overnight. After TLC showed that most of the staring materials were consumed completely, the mixture was diluted with water and adjusted pH to 2-3 with 1 N HCl. The mixture was extracted with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **11a** (600 mg, 63%). ESI-MS *m/z* 533 $[M+H]^+$ calc. for $C_{24}H_{32}N_6O_6S$. This intermediate was used in the next step without further characterization.

4-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]butanoic acid (11b)

To a solution of compound **10b** (200 mg, 0.35 mmol) in THF/MeOH/H_2O (10:1:5, 16 mL) was added $LiOH·H_2O$ (73.2 mg, 1.7 mmol) and the resulting mixture was stirred at room temperature overnight. After TLC showed that most of the staring materials were consumed completely, the mixture was diluted with water and adjusted pH to 2-3 with 1 N HCl. The mixture was extract with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **11b** (130 mg, 68%). ESI-MS *m/z* 547 $[M+H]^+$ calc. for $C_{25}H_{34}N_6O_6S$. This intermediate was used in the next step without further characterization.

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]pyrimidine-5-carboxylic acid (11c)

To a solution of compound **10c** (500 mg, 0.82 mmol) in THF/MeOH/H_2O (10:1:5, 16 mL) was added LiOH·H_2O (168 mg, 4.1 mmol). The resulting mixture was stirred at room temperature overnight. After TLC showed that most of the staring materials were consumed completely, the mixture was diluted with water and adjusted pH to 2-3 with 1 N HCl. The mixture was extracted with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **11c** (300 mg, 63%). ESI-MS *m/z* 583 $[M+H]^+$ calc. for $C_{26}H_{30}N_8O_6S$. This intermediate was used in the next step without further characterization.

(*E*)-3-[4-[[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]methyl]phenyl]prop-2-enoic acid (11d)

To a solution of compound **10d** (300 mg, 0.473 mmol) in THF/MeOH/H_2O (10:1:5, 16 mL) was added LiOH·H_2O (94.6 mg, 2.36 mmol). The resulting mixture was stirred at room temperature overnight. After TLC showed that most of the staring materials were consumed completely, the mixture was diluted with water and adjusted pH to 2-3 with 1 N HCl. The mixture was extracted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **11d** (150 mg, 51%). ESI-MS *m/z* 621 $[M+H]^+$ calc. for $C_{31}H_{36}N_6O_6S$. This intermediate was used in the next step without further characterization.

3-[1-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonyl-4-piperidyl]propanoic acid (11e)

To a solution of compound **10e** (250 mg, 0.44 mmol) in THF/MeOH/H_2O (10:1:5, 16 mL) was added LiOH·H_2O (92 mg, 2.2 mmol). The resulting mixture was stirred at room temperature overnight. After TLC showed that most of the staring materials were consumed completely, the mixture was diluted with water and adjusted pH to 2-3 with 1 N HCl. The mixture was extracted with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **11e** (200 mg, 86%). ESI-MS *m/z* 532 $[M+H]^+$ calc. for $C_{25}H_{33}N_5O_6S$. This intermediate was used in the next step without further characterization.

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylamino]-1-piperidyl]pyrimidine-5-carboxylic acid (11f)

To a solution of compound **10f** (400 mg, 0.64 mmol) in THF/MeOH/H_2O (10:1:5, 16 mL) was added LiOH·H_2O (132 mg, 3.2 mmol). The resulting mixture was stirred at room temperature overnight. After TLC showed that most of the staring materials were consumed completely, the mixture was diluted with water and adjusted pH to 2-3 with 1 N HCl. The mixture was extracted with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **11f** (300 mg, 79%). ESI-MS *m/z* 597 $[M+H]^+$ calc. for $C_{27}H_{32}N_8O_6S$. This intermediate was used in the next step without further characterization.

(*E*)-3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylamino]phenyl]prop-2-enoic acid (11g)

To a solution of compound **10g** (200 mg, 0.354 mmol) in THF/MeOH/H_2O (10:1:5, 16 mL) was added LiOH·H_2O (50.3 mg, 1.2 mmol). The resulting mixture was stirred at room temperature overnight. After TLC showed that most of the staring materials were

consumed completely, the mixture was diluted with water and adjusted pH to 2-3 with 1 N HCl. The mixture was extract with EtOAc, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **11g** (120 mg, 63%). ESI-MS *m/z* 538 $[M+H]^+$ calc. for $C_{26}H_{27}N_5O_6S$. This intermediate was used in the next step without further characterization.

3-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]-*N*-tetrahydropyran-2-yloxy-propanamide (12a)

To a solution of compound **11a** (100 mg, 0.19 mmol) in DMF (10 mL) were added EDC·HCl (48 mg, 0.25 mmol), HOBt (33.5 mg, 0.25 mmol), $THPONH_2$ (48 mg, 0.41 mmol) and NMM (84.8 mg, 0.84 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was diluted with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **12a** (100 mg, 84%). ESI-MS *m/z* 632 $[M+H]^+$ calc. for $C_{29}H_{41}N_7O_7S$. This intermediate was used in the next step without further characterization.

4-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]-*N*-tetrahydropyran-2-yloxy-butanamide (12b)

To a solution of compound **11b** (300 mg, 0.548 mmol) in DMF (10 mL) were added EDC·HCl (126 mg, 0.658 mmol), HOBt (88 mg, 0.658 mmol), $THPONH_2$ (125 mg, 1.07 mmol) and NMM (221 mg, 2.192 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was diluted with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **12b** (200 mg, 57%).

ESI-MS *m/z* 646 $[M+H]^+$ calc. for $C_{30}H_{43}N_7O_7S$. This intermediate was used in the next step without further characterization.

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]-*N*-tetrahydropyran-2-yloxy-pyrimidine-5-carboxamide (12c)

To a solution of compound **11c** (582 mg, 1 mmol) in DMF (10 mL) were added EDC·HCl (230 mg, 1.2 mmol), HOBt (162 mg, 1.2 mmol), $THPONH_2$ (229 mg, 1.9 mmol) and NMM (303 mg, 3 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was diluted with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **12c** (300 mg, 44%). ESI-MS *m/z* 682 $[M+H]^+$ calc. for $C_{31}H_{39}N_9O_7S$. This intermediate was used in the next step without further characterization.

(*E*)-3-[4-[[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]methyl]phenyl]-*N*-tetrahydropyran-2-yloxy-prop-2-enamide (12d)

To a solution of compound **11d** (100 mg, 0.161 mmol) in DMF (10 mL) were added EDC·HCl (69 mg, 0.36 mmol), HOBt (48.6 mg, 0.36 mmol), $THPONH_2$(42 mg, 0.36 mmol) and NMM (40.4 mg, 0.4 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was diluted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give pure compound **12d** (70 mg, 61%). ESI-MS

m/z 720 $[M+H]^+$ calc. for $C_{36}H_{45}N_7O_7S$. This intermediate was used in the next step without further characterization.

3-[1-[4-Ethoxy-3-(3-ethyl-1-methyl-7-oxo-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonyl-4-piperidyl]-*N*-tetrahydropyran-2-yloxy-propanamide (12e)

To a solution of compound **11e** (100 mg, 0.18 mmol) in DMF (10 mL) were added EDC·HCl (43.3 mg, 0.22 mmol), HOBt (30.5 mg, 0.22 mmol), $THPONH_2$ (32 mg, 0.27 mmol) and NMM (57 mg, 0.56 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was diluted with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give pure compound **12e** (100 mg, 90%). ESI-MS *m/z* 617 $[M+H]^+$ calc. for $C_{29}H_{40}N_6O_7S$. This intermediate was used in the next step without further characterization.

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylamino]-1-piperidyl]-*N*-tetrahydropyran-2-yloxy-pyrimidine-5-carboxamide (12f)

To a solution of compound **11f** (597 mg, 1 mmol) in DMF (10 mL) were added EDC·HCl (230 mg, 1.2 mmol), HOBt (162 mg, 1.2 mmol), $THPONH_2$ (229 mg, 1.9 mmol) and NMM (303 mg, 3 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was diluted with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **12f** (300 mg, 44%). ESI-MS *m/z* 696 $[M+H]^+$ calc. for $C_{32}H_{41}N_9O_7S$. This intermediate was used in the next step without further characterization.

(*E*)-3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylamino]phenyl]-*N*-tetrahydropyran-2-yloxy-prop-2-enamide (12g)

To a solution of compound **11g** (120 mg, 0.223 mmol) in DMF (10 mL) were added EDC·HCl (69 mg, 0.36 mmol), HOBt (48.6 mg, 0.36 mmol), $THPONH_2$ (42 mg, 0.36 mmol) and NMM (50 mg, 0.5 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was diluted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **12g** (100 mg, 70%). ESI-MS *m/z* 637 $[M+H]^+$ calc. for $C_{31}H_{36}N_6O_7S$. This intermediate was used in the next step without further characterization.

3-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]propanehydroxamic acid (13a)

A solution of compound **12a** (100 mg, 0.15 mmol) in HCl/1,4-dioxane (2.0 M, 10 mL) was stirred at room temperature for 3 hours. Then, the reaction mixture was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to give desired compound **13a** (62.5 mg, 76%). ^1H NMR (MeOD, 400 MHz): δ 8.18-8.17 (d, J = 2.4 Hz, 1H), 8.00 (dd, J = 2.8 Hz, 9.2 Hz, 1H), 7.40-7.38 (d, J = 8.8 Hz, 1H) 4.32 (q, 2H), 4.27 (s, 3H), 3.91-3.30 (m, 10H), 2.87 (t, 2H), 2.53 (m, 2H), 1.81 (m, 2H), 1.45 (t, 3H), 0.99 (t, 3H). ESI-MS *m/z* 548.3 $[M+H]^+$ calc. for $C_{24}H_{33}N_7O_6S$

4-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]butanehydroxamic acid (13b)

To a solution of compound **12b** (100 mg, 0.155 mmol) in 1,4-dioxane (10 mL) was added HCl/1,4-dioxane (2.0 M, 3 mL) and the solution was stirred at room temperature for 3 hours. Then, the mixture was concentrated to give the crude product which was purified through preparative HPLC (method 1 described in supporting information) to give the desired compound **13b** (80 mg, 92%). ^{1}H NMR (MeOD, 400 MHz): δ 8.18-8.17 (d, *J* = 2.4 Hz, 1H), 8.00 (dd, *J* = 2.8 Hz, 9.2 Hz, 1H), 7.40-7.38 (d, *J* = 8.8 Hz, 1H), 4.32 (q, 2H), 4.27 (s, 3H), 4.10-3.30 (m, 6H), 3.15 (t, 3H), 2.85 (t, 3H), 2.31 (m, 2H), 1.98 (m, 2H), 1.85-1.75 (m, 2H), 1.45 (t, 3H), 0.99 (t, 3H). ESI-MS *m/z* 562.1 $[M+H]^+$ calc. for $C_{25}H_{35}N_7O_6S$

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]pyrimidine-5-carbohydroxamic acid (13c)

To a solution of compound **12c** (200 mg, 0.293 mmol) in 1,4-dioxane (10 mL) was added HCl/1,4-dioxane (2.0 M, 10 mL) and the mixture was stirred at room temperature for 3 hours. Then, the reaction mixture was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to give compound **13c** (62.5 mg, 36%). ^{1}H NMR (DMSO, 400 MHz): δ 12.16 (s, 1H), 11.08 (s, 1H), 9.03 (s, 1H), 8.65 (s, 2H), 7.85 (m, 2H), 7.36-7.34 (d, *J* = 8.8 Hz, 1H), 4.22 (q, 2H), 4.15 (s, 3H), 3.92 (s, 4H), 3.00 (s, 4H), 2.75 (t, 2H), 1.75 (m, 2H), 1.25 (t, 3H), 0.95 (t, 3H). ESI-MS *m/z* 598.1 $[M+H]^+$ calc. for $C_{26}H_{31}N_9O_6S$

(*E*)-3-[4-[[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylpiperazin-1-yl]methyl]phenyl]prop-2-enehydroxamic acid (13d)

A solution of compound **12d** (50 mg, 0.069 mmol) in HCl/1,4-dioxane (2.0 M, 10 mL) was stirred at room temperature for 3 hours. Then, the reaction mixture was concentrated to give the crude product which was purified through preparative HPLC (method 1 described in supporting information) to give compound **13d** (20 mg, 45%). ^{1}H NMR (MeOD, 400 MHz): δ 8.19 (s, 1H), 7.95 (m, 1H), 7.75-7.32 (m, 6H), 6.53-6.49 (d, *J* = 15.6 Hz, 1H), 4.42-4.21 (m, 7H), 3.95-3.32 (m, 7H), 3.19-3.02 (m, 1H), 2.92-2.75 (m, 2H), 1.85-1.72 (m, 2H), 1.48 (t, 3H), 0.93 (t, 3H). ESI-MS *m/z* 636.1 $[M+H]^+$ calc. for $C_{31}H_{37}N_7O_6S$

3-[1-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonyl-4-piperidyl]propanehydroxamic acid (13e)

A solution of compound **12e** (100 mg, 0.162 mmol) in HCl/1,4-dioxane (2.0 M, 10 mL) was stirred at room temperature for 3 hours. Then, the reaction mixture was concentrated to give the crude product which was purified through preparative HPLC (method 1 described in supporting information) to give the desired compound **13e** (40 mg, 46%). ^{1}H NMR (MeOD, 400 MHz): δ 8.18-8.17 (d, *J* = 2.4 Hz, 1H), 8.00 (dd, *J* = 2.8 Hz, 9.2 Hz, 1H), 7.40-7.38 (d, *J* = 8.8 Hz, 1H), 4.32 (q, 2H), 4.27 (s, 3H), 3.75 (m, 2H), 2.87 (t, 2H), 2.31 (m, 2H), 2.15 (m, 2H), 1.81 (m, 4H), 1.53 (m, 2H), 1.45 (t, 3H), 1.31-1.21 (m, 3H), 0.99 (t, 3H). ESI-MS *m/z* 547.1 $[M+H]^+$ calc. for $C_{25}H_{34}N_6O_6S$

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylamino]-1-piperidyl]pyrimidine-5-carbohydroxamic acid (13f)

To a solution of compound **12f** (200 mg, 0.288 mmol) in CH_2Cl_2 (10 mL) was added HCl/1,4-dioxane (2.0 M, 5 mL) and the solution was stirred at room temperature for 3 hours. Then, the reaction mixture was concentrated to give the crude product which was

purified through preparative HPLC (method 1 described in supporting information) to give compound **13f** (50 mg, 30%). ^{1}H NMR (DMSO, 400 MHz): δ 12.21 (s, 1H), 11.06 (s, 1H), 8.64 (s, 2H), 8.02 (s, 1H), 7.94-7.91 (d, *J* = 8.4 Hz, 1H), 7.85-7.83 (d, *J* = 6.8 Hz, 1H), 7.35-7.33 (d, *J* = 8.4 Hz, 1H), 4.45 (m, 2H), 4.22 (q, 2H), 4.15 (s, 3H), 3.35 (m, 1H), 3.15 (m, 2H), 2.75 (t, 2H), 1.75 (m, 4H), 1.45 (m, 5H), 0.93 (t, 3H). ESI-MS *m/z* 612.1 $[M+H]^+$ calc. for $C_{27}H_{33}N_9O_6S$

(*E*)-3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonylamino]phenyl]prop-2-enehydroxamic acid (13g)

A solution of compound **12g** (100 mg, 0.16 mmol) in HCl/1,4-dioxane (2.0 M, 10 mL) was stirred at room temperature for 3 hours. Then, the reaction mixture was concentrated and purified by preparative HPLC (method 1 described in supporting information) to give compound **13g** (47 mg, 53%). ^{1}H NMR (MeOD, 400 MHz): δ 8.25 (s, 1H), 7.91-7.88 (d, *J* = 10.8 Hz, 1H), 7.49-7.39 (m, 3H), 7.29-7.18 (m, 3H), 6.32-6.25 (d, *J* = 15.6 Hz, 1H), 4.22 (q, 2H), 4.15 (s, 3H), 2.85 (t, 2H), 1.85 (m, 2H), 1.45 (t, 3H), 0.96 (t, 3H). ESI-MS *m/z* 553.0 $[M+H]^+$ calc. for $C_{26}H_{28}N_6O_6S$

5-(2-Ethoxy-5-nitro-phenyl)-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (14)

To a solution of compound **8** (1.0 g, 3.21 mmol) in concentrated sulfuric acid (5 mL) was added KNO_3 (324 mg, 3.21 mmol) in portions at 0 ^oC, then the reaction mixture was stirred at 0 ^oC for 20 minutes. Then the mixture was poured into ice water and extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **14** (1.10 g, 96%) as a white solid. ^{1}H NMR ($CDCl_3$ 400 MHz): δ 10.78 (s, 1H), 9.34 (s, 1H), 8.36-

8.33 (m, 1H), 7.17-7.14 (d, J = 9.2 Hz, 1H), 4.45-4.40 (m, 2H), 4.29 (s, 3H), 2.98-2.95 (m, 2H), 1.93-1.86 (m, 2H), 1.69-1.59 (m, 3H), 1.07-1.04 (m, 3H). ESI-MS *m/z* 358 $[M+H]^+$ calc. for $C_{17}H_{19}N_5O_4$

5-(5-Amino-2-ethoxy-phenyl)-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (15)

To a solution of compound **14** (700 mg, 1.961 mmol) in MeOH (20 mL) was added Pd/C (0.5 g) at H_2 atmosphere (1 atm) and the mixture was stirred at room temperature overnight. Then the mixture was filtered and the filtrate was concentrated to give the crude compound **15** (605 mg, 94%) as a white solid which was used for the next step directly. ^{1}H NMR (MeOD 400 MHz): δ 7.47 (s, 1H), 6.98-6.96 (m, 1H), 6.91-6.88 (m, 1H), 4.23 (s, 3H), 4.16-4.11 (m, 2H), 2.89-2.85 (m, 2H), 1.86-1.77 (m, 2H), 1.45-1.41 (m, 3H), 1.02-0.98 (m, 3H). ESI-MS *m/z* 328 $[M+H]^+$ calc. for $C_{17}H_{21}N_5O_2$

***Tert*-butyl 4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]piperidine-1-carboxylate (16a)**

To a solution of **15** (200 mg, 0.61 mmol) in anhydrous CH_2Cl_2 (20 mL) were added *tert*-butyl 4-oxopiperidine-1-carboxylate (145 mg, 0.73 mmol), AcOH (cat) and $NaBH(OAc)_3$ (259 mg, 1.22 mmol), and the mixture was stirred at room temperature overnight. Then, the mixture was extracted with CH_2Cl_2 and the organic layer was washed with aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by preparative TLC to give pure compound **16a** (300 mg, 96%) as a yellow solid. ESI-MS *m/z* 511 $[M+H]^+$ calc. for $C_{27}H_{38}N_6O_4$. This intermediate was used in the next step without further characterization.

Tert-butyl 4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6_H_-pyrazolo[4,3-_d_]pyrimidin-5-yl)anilino]methyl]piperidine-1-carboxylate (16b)

To a solution of compound **15** (400 mg, 1.22 mmol) in anhydrous CH_2Cl_2 (20 mL) were added *tert*-butyl 4-formylpiperidine-1-carboxylate (311 mg, 1.46 mmol), AcOH (cat) and $NaBH(OAc)_3$ (519 mg, 2.44 mmol), and the mixture was stirred at room temperature overnight. Then, the mixture was extracted with CH_2Cl_2 and the organic layer was washed with aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by preparative TLC to give pure compound **16b** (450 mg, 70%) as a yellow solid. ESI-MS *m/z* 525 $[M+H]^+$ calc. for $C_{28}H_{40}N_6O_4$. This intermediate was used in the next step without further characterization.

Tert-butyl 4-[4-ethoxy-_N_-methyl-3-(1-methyl-7-oxo-3-propyl-6_H_-pyrazolo[4,3-_d_]pyrimidin-5-yl)anilino]piperidine-1-carboxylate (16c)

Compound **16a** (500 mg, 0.98 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL) and paraformaldehyde (132 mg, 1.471 mmol), AcOH (cat) and $NaBH(OAc)_3$ (416 mg, 1.960 mmol) were sequentlialy added. Then, the mixture was stirred at 60 ^oC overnight and extracted with CH_2Cl_2. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by preparative TLC to give pure compound **16c** (288 mg, 56%) as a yellow oil. ESI-MS *m/z* 525 $[M+H]^+$ calc. for $C_{28}H_{40}N_6O_4$. This intermediate was used in the next step without further characterization.

5-[2-Ethoxy-5-(4-piperidylamino)phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (17a)

A solution of compound **16a** (300 mg, 0.59 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour and then concentrated to give compound **17a** (240 mg, 99%) as a white solid. ESI-MS *m/z* 411 $[M+H]^+$ calc. for $C_{22}H_{30}N_6O_2$. This intermediate was used in the next step without further characterization.

5-[2-Ethoxy-5-(4-piperidylmethylamino)phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (17b)

A solution of compound **16b** (450 mg, 0.86 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then, the reaction mixture was concentrated to give the crude compound **17b** (350 mg, 96%) as a yellow solid. ESI-MS *m/z* 425 $[M+H]^+$ calc. for $C_{23}H_{32}N_6O_2$. This intermediate was used in the next step without further characterization.

5-[2-Ethoxy-5-[methyl(4-piperidyl)amino]phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (17c)

A solution of compound **16c** (288 mg, 0.55 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour and then concentrated to give compound **17c** (220 mg, 94%) as a white solid. ESI-MS *m/z* 425 $[M+H]^+$ calc. for $C_{23}H_{32}N_6O_2$. This intermediate was used in the next step without further characterization.

Methyl 3-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]propanoate (18a)

To a solution of compound **15** (500 mg, 1.53 mmol) in CH_2Cl_2 (16 mL) under N_2 were added methyl 3,3-dimethoxypropanoate (274 mg, 1.85 mmol), TFA (8 mL) and Et_3SiH (534 mg, 4.6 mmol) and the reaction mixture was stirred at room temperature overnight. Then, the mixture was concentrated, diluted with H_2O and adjusted pH to 7 with aqueous $NaHCO_3$. The solution was then extracted with CH_2Cl_2 and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **18a** (620 mg, 98%). ESI-MS *m/z* 414 $[M+H]^+$ calc. for $C_{21}H_{27}N_5O_4$. This intermediate was used in the next step without further characterization.

Ethyl 4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]cyclohexanecarboxylate (18b)

To a solution of compound **15** (350 mg, 1.07 mmol) in anhydrous CH_2Cl_2 (20 mL) were added ethyl 4-oxocyclohexanecarboxylate (218 mg, 1.28 mmol), AcOH (cat) and $NaBH(OAc)_3$ (454 mg, 2.14 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was extracted with CH_2Cl_2 and the organic layer was washed with aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4,filtered and concentrated to give the crude compound which was purified by preparative TLC to give pure compound **18b** (400 mg, 78%) as a yellow oil. ESI-MS *m/z* 482 $[M+H]^+$ calc. for $C_{26}H_{35}N_5O_4$. This intermediate was used in the next step without further characterization.

Methyl 4-[4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]benzoate (18c)

To a solution methyl 4-(4-oxo-1-piperidyl)benzoate (**Int. 6**, synthesis described in supporting information) (250 mg, 1.07 mmol) in CH_2Cl_2 (20 mL) were added **15** (150

mg, 0.45 mmol) and AcOH (2 drop), and the solution was stirred at room temperature for 2 hours. Then, $NaBH(OAc)_3$ (391 mg, 1.85 mmol) was added to the solution and the mixture was stirred at room temperature overnight. The mixture was quenched with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give pure compound **18c** (80 mg, 32%) as a pale yellow solid. ESI-MS *m/z* 545.2 $[M+H]^+$ calc. for $C_{30}H_{36}N_6O_4$. This intermediate was used in the next step without further characterization.

Ethyl 2-[4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]pyrimidine-5-carboxylate (18d)

To a solution of compound **17a** (240 mg, 0.58 mmol) in CH_3CN (20 mL) were added K_2CO_3 (161 mg, 1.17 mmol) and ethyl 2-chloropyrimidine-5-carboxylate (109 mg, 0.58 mmol), then the mixture was stirred at 40 ^oC overnight. After LC-MS showed the starting material was consumed completely, the mixture was extracted with EtOAc and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **18d** (263 mg, 80%) as a yellow oil. ESI-MS *m/z* 561 $[M+H]^+$ calc. for $C_{29}H_{36}N_8O_4$. This intermediate was used in the next step without further characterization.

Ethyl 2-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]methyl]-1-piperidyl]pyrimidine-5-carboxylate (18e)

To a solution of compound **17b** (350 mg, 0.82 mmol) in CH_3CN (20 mL) were added K_2CO_3 (228 mg, 1.65 mmol) and ethyl 2-chloropyrimidine-5-carboxylate (154 mg, 0.82 mmol) and the mixture was stirred at 40 ^oC overnight. Then, the mixture was extracted

with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **18e** (392 mg, 83%) as a yellow solid. ESI-MS *m/z* 575 $[M+H]^+$ calc. for $C_{30}H_{38}N_8O_4$. This intermediate was used in the next step without further characterization.

Ethyl 2-[4-[4-ethoxy-*N*-methyl-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]pyrimidine-5-carboxylate (18f)

To a solution of compound **17c** (220 mg, 0.52 mmol) in CH_3CN (20 mL) were added K_2CO_3 (143 mg, 1.03 mmol) and ethyl 2-chloropyrimidine-5-carboxylate (97 mg, 0.52 mmol) and the mixture was stirred at 40 ^oC overnight. Then, the mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **18f** (250 mg, 84%) as a yellow solid. ESI-MS *m/z* 575 $[M+H]^+$ calc. for $C_{30}H_{38}N_8O_4$. This intermediate was used in the next step without further characterization.

3-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]propanoic acid (19a)

To a solution of compound **18a** (620 mg, 1.5 mmol) in THF/MeOH/H_2O (3:3:2, 32 mL) was added $LiOH \cdot H_2O$ (645 mg, 15 mmol). The resulting mixture was stirred at room temperature overnight. Then the mixture was diluted with water and adjusted pH to 3-4 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound

19a (580 mg, 96%) as a pale yellow oil. ESI-MS *m/z* 400 $[M+H]^+$ calc. for $C_{20}H_{25}N_5O_4$. This intermediate was used in the next step without further characterization.

4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]cyclohexanecarboxylic acid (19b)

To a solution of compound **18b** (400 mg, 0.832 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (349 mg, 8.32 mmol) and the reaction mixture was stirred at 40 ^oC overnight. Then, the solution was concentrated and the residue was diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. Then, the mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **19b** (380 mg, 99% crude) as a yellow solid. ESI-MS *m/z* 454 $[M+H]^+$ calc. for $C_{24}H_{31}N_5O_4$. This intermediate was used in the next step without further characterization.

4-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]benzoic acid (19c)

To a solution of **18c** (80 mg, 0.15 mmol) in THF/MeOH/H_2O (3:3:2, 8 mL) was added LiOH·H_2O (63 mg, 1.5 mmol) and the resulting mixture was stirred at room temperature overnight. Then, the mixture was diluted with water and adjusted pH to 6~7 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the desired product **19c** (70 mg, 90%). ESI-MS *m/z* 531.2 $[M+H]^+$ calc. for $C_{29}H_{34}N_6O_4$. This intermediate was used in the next step without further characterization.

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]pyrimidine-5-carboxylic acid (19d)

To a solution of compound **18d** (263 mg, 0.47 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (197 mg, 4.70 mmol) and the reaction mixture was stirred at 40 ^oC overnight. Then, the mixture was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. The solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **19d** (230 mg, 92%) as a yellow solid. ESI-MS *m/z* 533 $[M+H]^+$ calc. for $C_{27}H_{32}N_8O_4$. This intermediate was used in the next step without further characterization.

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]methyl]-1-piperidyl]pyrimidine-5-carboxylic acid (19e)

To a solution of compound **18e** (392 mg, 0.68 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (287 mg, 6.83 mmol) and the reaction mixture was stirred at 40 ^oC overnight. Then, the reaction mixture was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. Then, the mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **19e** (350 mg, 94%) as a red solid. ESI-MS *m/z* 547 $[M+H]^+$ calc. for $C_{28}H_{34}N_8O_4$. This intermediate was used in the next step without further characterization

2-[4-[4-Ethoxy-*N*-methyl-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]pyrimidine-5-carboxylic acid (19f)

To a solution of compound **18f** (250 mg, 0.43 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (193 mg, 4.60 mmol) and the reaction mixture was stirred at 40 ^oC overnight until LC-MS showed the starting material was consumed completely. Then, the solution was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **19f** (220 mg, 93%) as a yellow solid. ESI-MS *m/z* 547 $[M+H]^+$ calc. for $C_{28}H_{34}N_8O_4$. This intermediate was used in the next step without further characterization.

3-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-*N*-tetrahydropyran-2-yloxy-propanamide (20a)

To a solution of **19a** (580 mg, 1.45 mmol) in DMF (40 mL) were added EDC·HCl (560 mg, 2.9 mmol), HOBt (392 mg, 2.9 mmol), $THPONH_2$ (340 mg, 2.9 mmol) and NMM (505 mg, 5.0 mmol). The mixture was stirred at room temperature overnight, then quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give pure compound **20a** (630 mg, 87%) as pale yellow oil. ESI-MS *m/z* 499 $[M+H]^+$ calc. for $C_{25}H_{34}N_6O_5$. This intermediate was used in the next step without further characterization.

4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-*N*-tetrahydropyran-2-yloxy-cyclohexanecarboxamide (20b)

To a solution of compound **19b** (380 mg, 0.84 mmol) in DMF (10 mL) were added EDC·HCl (322 mg, 1.68 mmol), HOBt (226 mg, 1.68 mmol), $THPONH_2$ (196 mg, 1.68 mmol) and NMM (254 mg, 2.51 mmol), and the mixture was stirred at room

temperature overnight. Then, the solution was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **20b** (120 mg, 26%) as a yellow oil. ESI-MS *m/z* 553 $[M+H]^+$ calc. for $C_{29}H_{40}N_6O_5$. This intermediate was used in the next step without further characterization.

4-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]-*N*-tetrahydropyran-2-yloxy-benzamide (20c)

To a solution of **19c** (70 mg, 0.13 mmol) in DMF (10 mL) were added EDC·HCl (50 mg, 0.26 mmol), HOBt (35 mg, 0.26 mmol), $THPONH_2$ (31 mg, 0.26 mmol) and NMM (41 mg, 0.4 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give compound **20c** (50 mg, 61%) as a pale yellow solid. ESI-MS *m/z* 630.3 $[M+H]^+$ calc. for $C_{34}H_{43}N_7O_5$. This intermediate was used in the next step without further characterization.

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]-*N*-tetrahydropyran-2-yloxy-pyrimidine-5-carboxamide (20d)

To a solution of **19d** (230 mg, 0.43 mmol) in DMF (10 mL) were added EDC·HCl (166 mg, 0.86 mmol), HOBt (117 mg, 0.86 mmol), $THPONH_2$ (102 mg, 0.86 mmol) and NMM (131 mg, 1.30 mmol) and the mixture was stirred at room temperature overnight. Then, the solution was quenched with water and extracted with EtOAc. The organic

layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **20d** (102 mg, 37%) as a yellow solid. ESI-MS *m/z* 632 $[M+H]^+$ calc. for $C_{32}H_{41}N_9O_5$. This intermediate was used in the next step without further characterization.

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]methyl]-1-piperidyl]-*N*-tetrahydropyran-2-yloxy-pyrimidine-5-carboxamide (20e)

To a solution of compound **19e** (350 mg, 0.64 mmol) in DMF (10 mL) were added EDC·HCl (246 mg, 1.28 mmol), HOBt (173 mg, 1.28 mmol), $THPONH_2$ (150 mg, 1.28 mmol) and NMM (194 mg, 1.92 mmol) and the mixture was stirred at room temperature. Then, the solution was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **20e** (350 mg, 85%) as a yellow oil. ESI-MS *m/z* 646 $[M+H]^+$ calc. for $C_{33}H_{43}N_9O_5$. This intermediate was used in the next step without further characterization.

2-[4-[4-Ethoxy-*N*-methyl-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]-*N*-tetrahydropyran-2-yloxy-pyrimidine-5-carboxamide (20f)

To a solution of compound **19f** (220 mg, 0.40 mmol) in DMF (10 mL) were added EDC·HCl (155 mg, 0.80 mmol), HOBt (109 mg, 0.80 mmol), $THPONH_2$ (95 mg, 0.80 mmol) and NMM (122 mg, 1.20 mmol), and the mixture was stirred at room temperature overnight until LC-MS showed the starting material was consumed

completely. Then, the mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **20f** (220 mg, 85%) as a yellow solid. ESI-MS *m/z* 646 $[M+H]^+$ calc. for $C_{33}H_{43}N_9O_5$. This intermediate was used in the next step without further characterization.

3-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]propanehydroxamic acid (21a)

A solution of compound **20a** (300 mg, 0.6 mmol) in HCl/EtOAc (1.0 M, 40 mL) was stirred at room temperature for 4 hours, then concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **21a** (41.2 mg, 16%) as white solid; ; m.p.: 150-151 ^oC. ^1H NMR (MeOD, 400 MHz): δ 7.75 (d, *J* = 2.8 Hz, 1H), 7.34-7.31 (m, 1H), 7.22 (d, *J* = 8.8 Hz, 1H), 4.28-4.20 (m, 5H), 3.65-3.59 (m, 2H), 2.90-2.86 (m, 2H), 2.52-2.49 (m, 2H), 1.86-1.77 (m, 2H), 1.49-1.44 (m, 3H), 1.00 (t, *J* = 8 Hz, 3H). ESI-MS *m/z* 415.1 $[M+H]^+$ calc. for $C_{20}H_{26}N_6O_4$

4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]cyclohexanecarbohydroxamic acid (21b)

A solution of compound **20b** (120 mg, 0.217 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then the mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **21b** (30 mg, 29%) as a white solid; m.p.: 131.5-132.5 ^oC. ^1H NMR (MeOD, 400 MHz): δ 7.94 (s, 1H), 7.51-7.48 (m, 1H),

7.33-7.31 (d, *J* = 8.8 Hz, 1H), 4.30-4.26 (m, 2H), 4.24 (s, 3H), 3.63-3.58 (m, 1H), 2.90-2.86 (m, 2H), 2.40-2.39 (m, 1H), 2.05-2.00 (m, 4H), 1.86-1.83 (m, 4H), 1.81-1.79 (m, 2H), 1.50-1.47 (m, 3H), 1.02-0.98 (m, 3H). ESI-MS *m/z* 469.2 $[M+H]^+$ calc. for $C_{24}H_{32}N_6O_4$

4-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]benzenecarbohydroxamic acid (21c)

A solution of compound **20c** (50 mg, 0.08 mmol) in HCl/EtOAc (1.0 M, 10 mL) was stirred at room temperature for 1 hour. Then the mixture was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **21c** (14.6 mg, 32%) as a white solid; m.p.: 196-197 ^oC. ^1H NMR (DMSO, 400 MHz): δ 11.86 (s, 1H), 10.92 (s, 1H), 8.90-8.60 (m, 1H), 7.64-7.61 (m, 2H), 7.20-7.10 (m, 1H), 7.10-6.90 (m, 3H), 6.90-6.70 (m, 1H), 4.14 (s, 3H), 4.10-4.00 (m, 2H), 3.90-3.75 (m, 2H), 3.00-2.85 (m, 2H), 2.80-2.70 (m, 2H), 2.52-2.40 (m, 1H), 2.05-1.90 (m, 2H), 1.80-1.70 (m, 2H), 1.50-1.35 (m, 2H), 1.35-1.20 (m, 3H), 0.95-0.85 (m, 3H). ESI-MS *m/z* 546.2 $[M+H]^+$ calc. for $C_{29}H_{35}N_7O_4$

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]pyrimidine-5-carbohydroxamic acid (21d)

A solution of compound **20d** (102 mg, 0.16 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour, then concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **21d** (50 mg, 57%) as a white solid; m.p.: 131-132 ^oC. ^1H NMR (MeOD, 400 MHz): δ 8.67 (s, 2H), 7.94 (s, 1H), 7.51-7.49 (d, *J* = 8.4 Hz, 1H), 7.33-7.31 (d, *J* = 9.2 Hz, 1H), 4.98-4.95 (m, 2H), 4.30-4.26 (m, 2H), 4.23 (s,

3H), 3.86-3.80 (m, 1H), 3.09-3.03 (m, 2H), 2.89-2.86 (m, 2H), 2.13-2.10 (m, 2H), 1.84-1.79 (m, 2H), 1.63-1.61 (m, 2H), 1.50-1.47 (m, 3H), 1.01-0.98 (m, 3H). ESI-MS *m/z* 548.1 $[M+H]^+$ calc. for $C_{27}H_{33}N_9O_4$

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]methyl]-1-piperidyl]pyrimidine-5-carbohydroxamic acid (21e)

A solution of compound **20e** (350 mg, 0.54 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then the solution was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **21e** (190 mg, 62%) as a yellow solid; m.p.: 158-159 ^oC. ^1H NMR (MeOD, 400 MHz): δ 8.65 (s, 2H), 7.95 (s, 1H), 7.50-7.48 (d, *J* = 8.4 Hz, 1H), 7.30-7.27 (d, *J* = 9.2 Hz, 1H), 4.92-4.88 (m, 2H), 4.28-4.25 (m, 2H), 4.23 (s, 3H), 3.32-3.31 (m, 2H), 3.03-2.97 (m, 2H), 2.89-2.85 (m, 2H), 2.13 (s, 1H), 1.96-1.93 (m, 2H), 1.84-1.79 (m, 2H), 1.50-1.48 (m, 3H), 1.34-1.32 (m, 2H), 1.01-0.98 (m, 3H). ESI-MS *m/z* 562.2 $[M+H]^+$ calc. for $C_{28}H_{35}N_9O_4$

2-[4-[4-Ethoxy-*N*-methyl-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)anilino]-1-piperidyl]pyrimidine-5-carbohydroxamic acid (21f)

A solution of compound **20f** (220 mg, 0.34 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then the mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **21f** (94 mg, 49%) as a red solid; m.p.: 101-102 ^oC. ^1H NMR (MeOD, 400 MHz): δ 8.66 (s, 2H), 8.11 (s, 1H), 7.77-7.74 (m, 1H), 7.41-7.39 (d, *J* = 9.2 Hz, 1H), 5.05-5.01 (m, 2H), 4.32-4.27 (m, 2H), 4.24 (s, 3H), 4.08-4.01 (m, 1H), 3.37 (s, 3H), 3.31-2.96 (m, 2H), 2.89-2.85 (m, 2H), 2.16-2.13 (m,

2H), 1.83-1.78 (m, 2H), 1.64-1.61 (m, 2H), 1.50-1.47 (m, 3H), 1.01-0.97 (m, 3H). ESI-MS *m/z* 562.2 $[M+H]^+$ calc. for $C_{28}H_{35}N_9O_4$

5-(2-Ethoxy-5-iodo-phenyl)-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (22)

To a solution of **8** (10 g, 32 mmol) in TFA (50 mL) was added NIS (8.6 g, 38.4 mmol) at 0 ^oC and the solution was stirred at room temperature overnight. Then, the mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **22** (11 g, 79%) as a white solid. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.66-8.40 (m, 1H), 7.73-7.70 (m, 1H), 6.81-6.70 (m, 1H), 4.40-4.10 (m, 5H), 3.00-2.85 (m, 2H), 1.95-1.75 (m, 2H), 1.60-1.50 (m, 3H), 1.10-1.00 (m, 3H). ESI-MS *m/z* 439.1 $[M+H]^+$ calc. for $C_{17}H_{19}IN_4O_2$

5-[5-(1,4-Dioxa-8-azaspiro[4.5]decan-8-yl)-2-ethoxy-phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (23)

To a solution of compound **22** (1.7 g, 3.87 mmol) in toluene (10 mL) were added *t*-BuOK (7.74 mL, 1.0 M, 7.74 mmol), $Pd_2(dba)_3$ (355 mg, 0.387 mmol), 1,4-dioxa-8-azaspiro[4.5]decane (1.1 g, 7.74 mmol) and xantphos (671 mg, 1.16 mmol), and the solution was heated to 120 ^oC for 1 hour with a microwave reactor. Then, the mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **23** (1.4 g, 80%) as a yellow solid. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.09-8.07 (m, 1H), 7.15-7.05 (m, 1H), 7.00-6.90 (m, 1H), 4.27 (s, 3H), 4.26-4.20 (m, 2H), 4.05-3.95 (m, 4H), 2.40-2.25 (m,

4H), 3.00-2.90 (m, 2H), 1.95-1.80 (m, 6H), 1.70-1.65 (m, 1H), 1.60-1.50 (m, 3H), 1.10-1.00 (m, 3H). ESI-MS *m/z* 454.2 $[M+H]^+$ calc. for $C_{24}H_{31}N_5O_4$

5-[2-Ethoxy-5-(4-oxo-1-piperidyl)phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (24)

A solution of compound **23** (1.4 g, 3.1 mmol) in HCl (6.0 M in THF, 10 mL) was stirred at 70 ^oC overnight. Then, the solution was concentrated to give the crude product which was purified by column chromatography to obtain pure compound **24** (1.1 g, 85%) as white solid. ESI-MS *m/z* 410.2 $[M+H]^+$ calc. for $C_{22}H_{27}N_5O_3$. This intermediate was used in the next step without further characterization.

5-[2-Ethoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (25)

A mixture of compound **22** (10 g, 22.82 mmol), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (8.69 g, 34.23 mmol), KOAc (6.72 g, 68.46 mmol) and $Pd(dppf)Cl_2$ (3.34 g, 4.56 mmol, 0.20 eq) in 1,4-dioxane (150 mL) was degassed and purged with N_2 for 3 times. Then, the mixture was stirred at 80-100 °C for 48 hours under N_2 atmosphere. Then, the mixture was extracted with EtOAc and the organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give compound **25** (9 g, 90%) as a purple solid. ESI-MS *m/z* 439.2 $[M+H]^+$ calc. for $C_{23}H_{31}BN_4O_2$. This intermediate was used in the next step without further characterization.

[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]boronic acid (26)

To a solution of compound **25** (6.00 g, 13.7 mmol) in acetone (60 mL) was added $NaIO_4$ (3.51 g, 16.4 mmol) and NH_4OAc (3.69 g, 47.9 mmol) and the mixture was stirred at 25 °C for 16 hours. Then, the mixture was concentrated in vacuum and filtered through a Glass funnel. The filtrate was concentrated to give compound **26** (3.50 g, 9.83 mmol, 71%) gray solid. ESI-MS *m/z* 357.7 $[M+H]^+$ calc. for $C_{17}H_{21}BN_4O_2$. This intermediate was used in the next step without further characterization.

Ethyl 2-[1-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-4-piperidyl]acetate (27a)

To a solution of methyl 2-diethoxyphosphorylacetate (279 mg, 1.34 mmol) in THF (20 mL) was added NaH (54 mg, 60% in mineral oil, 1.34 mmol) at 0 ^oC and the mixture was stirred at 0 ^oC for 1 hour. Then a solution of **24** (500 mg, 1.22 mmol) in THF (5 mL) was added at 0 ^oC and the reaction was stirred at room temperature overnight. The mixture was quenched with aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by the column chromatography to give intermediate ethyl 2-[1-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-4-piperidylidene]acetate (260 mg, 45%) as a white solid. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.07-8.06 (m, 1H), 7.09-7.05 (m, 1H), 7.05-6.95 (m, 1H), 5.75 (s, 1H), 4.40-4.10 (m, 7H), 3.40-3.25 (m, 4H), 3.25-3.15 (m, 2H), 3.00-2.90 (m, 2H), 2.55-2.50 (m, 2H), 1.95-1.70 (m, 2H), 1.60-1.50 (m, 4H), 1.35-1.20 (m, 3H), 1.10-1.00 (m, 3H). ESI-MS *m/z* 480.2 $[M+H]^+$ calc. for $C_{26}H_{33}N_5O_4$. To a solution of this intermediate (140 mg, 0.29 mmol) in MeOH (40 mL) was added Pd/C (0.3 g) and the solution was stirred at room temperature for 3 hours under H_2 atmosphere (1 atm). Then, the solution was filtered and the filtrate was concentrated to give compound **27a**

(100 mg, 71%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.06-8.05 (m, 1H), 7.08-7.06 (m, 1H), 6.97-6.95 (m, 1H), 4.23-4.14 (m, 7H), 3.64-3.61 (m, 2H), 2.96-2.93 (m, 2H), 2.79-2.76 (m, 2H), 1.91-1.86 (m, 5H), 1.64-1.54 (m, 5H), 1.30-1.27 (m, 5H), 1.06-1.03 (m, 3H). ESI-MS *m/z* 482.2 $[M+H]^+$ calc. for $C_{26}H_{35}N_5O_4$

Ethyl 3-[1-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-4-piperidyl]propanoate (27b)

To a solution of **26** (120 mg, 0.34 mmol), $Cu(OAc)_2$ (127 mg, 0.7 mmol), Et_3N (101 mg, 1.0 mmol) and 4Å molecular sieves (400 mg) in anhydrous CH_2Cl_2 (40 mL) was added ethyl 3-(4-piperidyl)propanoate (75 mg, 0.4 mmol) under O_2 condition. Then, the mixture was stirred at room temperature for 2 hours. Then the mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to give pure compound **27b** (15 mg, 9%). ESI-MS *m/z* 496.2 $[M+H]^+$ calc. for $C_{27}H_{37}N_5O_4$. This intermediate was used in the next step without further characterization.

Ethyl 8-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-8-azaspiro[4.5]decane-3-carboxylate (27c)

To a solution of compound **26** (200 mg, 0.56 mmol), $Cu(OAc)_2$ (217 mg, 1.2 mmol), Et_3N (152 mg, 1.5 mmol) and 4Å molecular sieves (800 mg) in anhydrous CH_2Cl_2 (65 mL) was added ethyl 8-azaspiro[4.5]decane-3-carboxylate (144 mg, 0.68 mmol) under O_2 condition. Then, the mixture was stirred at room temperature for 3.5 hours. Then, the mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was

purified by preparative TLC to give pure compound **27c** (135 mg, 46%). ESI-MS *m/z* 522.1 $[M+H]^+$ calc. for $C_{29}H_{39}N_5O_4$. This intermediate was used in the next step without further characterization.

Methyl 2-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-2-azaspiro[5.5]undecane-9-carboxylate (27d)

To a solution of compound **26** (200 mg, 0.56 mmol), $Cu(OAc)_2$ (127 mg, 0.7 mmol), Et_3N (152 mg, 1.5 mmol) and 4Å molecular sieves (200 mg) in anhydrous CH_2Cl_2 (40 mL) was added methyl 2-azaspiro[5.5]undecane-9-carboxylate (**Int. 7**, synthesis described in supporting information) (120 mg, 0.57 mmol) under O_2 condition. Then, the mixture was stirred at room temperature for 2 hours. Then, the mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to give pure compound **27d** (62 mg, 21%). ESI-MS *m/z* 522.2 $[M+H]^+$ calc. for $C_{29}H_{39}N_5O_4$. This intermediate was used in the next step without further characterization.

Ethyl 2-[2-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-2,8-diazaspiro[4.5]decan-8-yl]pyrimidine-5-carboxylate (27e)

To a solution of compound **26** (356 mg, 1.0 mmol), $Cu(OAc)_2$ (217 mg, 1.2 mmol), Et_3N (152 mg, 1.5 mmol) and 4Å molecular sieves (600 mg) in anhydrous CH_2Cl_2 (60 mL) was added *tert*-butyl 2,8-diazaspiro[4.5]decane-8-carboxylate (270 mg, 1.1 mmol) under O_2 condition and then the mixture was stirred at room temperature for 1.5 hours. Then, the mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product

which was purified by preparative HPLC (method 1 described in supporting information) to give pure intermediate *tert*-butyl 2-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6H-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-2,8-diazaspiro[4.5]decane-8-carboxylate (310 mg, 56%). ESI-MS *m/z* 551.3 $[M+H]^+$ calc. for $C_{30}H_{42}N_6O_4$. Then, a solution of this intermediate (310 mg, 0.56 mmol) in HCl/EtOAc (1.0 M, 40 mL) was stirred at room temperature for 2 hours and concentrated. Finally, the residue was dissolved in CH_3CN (60 mL) and K_2CO_3 (194 mg, 1.4 mmol) was added. Then, ethyl 2-chloropyrimidine-5-carboxylate (120 mg, 0.64 mmol) was added and the reaction mixture was stirred at 60 ^oC overnight. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give compound **27e** (130 mg, 39%, 2 steps). ESI-MS *m/z* 601.2 $[M+H]^+$ calc. for $C_{32}H_{40}N_8O_4$. This intermediate was used in the next step without further characterization.

Ethyl 4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]cyclohexanecarboxylate (27f)

To a solution of compound **26** (1.0 g, 2.81 mmol) in anhydrous CH_2Cl_2 (50 mL) were added ethyl 4-hydroxycyclohexanecarboxylate (**Int. 8**, synthesis described in supporting information) (500 mg, 2.91 mmol), $Cu(OAc)_2$ (632 mg, 3.49 mmol), DMAP (71 mg, 0.58 mmol), Et_3N (1.18 g, 11.6 mmol) and 4Å molecular sieves (2.5 g), and the mixture was stirred at room temperature for 3 hours under O_2 atmosphere. Then, the reaction was quenched with water and filtered; the resulting mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by preparative HPLC

(method 1 described in supporting information) to give pure compound **27f** (440 mg, 31%) as a white solid. ESI-MS *m/z* 483 $[M+H]^+$ calc. for $C_{26}H_{34}N_4O_5$. This intermediate was used in the next step without further characterization.

Ethyl 4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]benzoate (27g)

To a solution of compound **26** (250 mg, 0.7 mmol) in CH_2Cl_2 (50 mL) were added ethyl 4-hydroxybenzoate (83 mg, 0.5 mmol), $Cu(OAc)_2$ (127 mg, 0.7 mmol), Et_3N (253 mg, 2.5 mmol) and 4Å molecular sieves (0.5 g). Then, the mixture was stirred at room temperature overnight under O_2 protection. Then, the reaction mixture was filtered and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **27g** (115 mg, 48%) as yellow solid. ESI-MS *m/z* 477.2 $[M+H]^+$ calc. for $C_{26}H_{28}N_4O_5$. This intermediate was used in the next step without further characterization.

2-[1-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-4-piperidyl]acetic acid (28a)

To a solution of compound **27a** (100 mg, 0.21 mmol) in THF/MeOH/H_2O (3:3:2, 8 mL) was added $LiOH·H_2O$ (88 mg, 2.1 mmol) and the resulting mixture was stirred at room temperature overnight. Then, the mixture was diluted with water and adjusted pH to 6~7 with 1 N HCl. The solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford compound **28a** (90 mg, 95%). ESI-MS *m/z* 454.2 $[M+H]^+$ calc. for $C_{24}H_{31}N_5O_4$. This intermediate was used in the next step without further characterization.

3-[1-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-4-piperidyl]propanoic acid (28b)

To a solution of compound **27b** (15 mg, 0.03 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (22 mg, 0.5 mmol) and the reaction mixture was stirred at room temperature overnight. Then, the reaction mixture was concentrated, diluted with H_2O and adjusted pH to 3 with 1 N HCl. Then, the solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **28b** (15 mg, 99% crude). ESI-MS *m/z* 468.3 $[M+H]^+$ calc. for $C_{25}H_{33}N_5O_4$. This intermediate was used in the next step without further characterization.

8-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-8-azaspiro[4.5]decane-3-carboxylic acid (28c)

To a solution of compound **27c** (135 mg, 0.26 mmol) in MeOH/THF/H_2O (1:3:1, 30 mL) was added LiOH·H_2O (130 mg, 3 mmol) and the reaction mixture was stirred at room temperature overnight. Then, the reaction mixture was concentrated, diluted with H_2O and adjusted pH to 3-4 with 1 N HCl. Then, the solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **28c** (120 mg, 93%). ESI-MS *m/z* 494.2 $[M+H]^+$ calc. for $C_{27}H_{35}N_5O_4$. This intermediate was used in the next step without further characterization.

2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-2-azaspiro[5.5]undecane-9-carboxylic acid (28d)

To a solution of compound **27d** (62 mg, 0.12 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (86 mg, 2 mmol) and the reaction mixture was stirred at room temperature overnight. Then, the mixture was concentrated, diluted with H_2O and adjusted pH to 3 with 1 N HCl. Then, the solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **28d** (48 mg, 79%). ESI-MS *m/z* 508.2 $[M+H]^+$ calc. for $C_{28}H_{37}N_5O_4$. This intermediate was used in the next step without further characterization.

2-[2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-2,8-diazaspiro[4.5]decan-8-yl]pyrimidine-5-carboxylic acid (28e)

To a solution of compound **27e** (130 mg, 0.22 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (95 mg, 2.2 mmol) and the reaction mixture was stirred at room temperature overnight. Then, the mixture was concentrated, diluted with H_2O and adjusted pH to 3 with 1 N HCl. Then, the solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound **28e** (95 mg, 77%). ESI-MS *m/z* 573.2 $[M+H]^+$ calc. for $C_{30}H_{36}N_8O_4$. This intermediate was used in the next step without further characterization.

4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]cyclohexanecarboxylic acid (28f)

To a solution of compound **27f** (440 mg, 0.91 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (384 mg, 9.13 mmol) and the reaction mixture was stirred at room temperature overnight until LC-MS showed the starting material was consumed

completely. Then, the reaction mixture was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. Then, the solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **28f** (400 mg, 96%) as a yellow solid. ESI-MS *m/z* 455 $[M+H]^+$ calc. for $C_{24}H_{30}N_4O_5$. This intermediate was used in the next step without further characterization.

4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]benzoic acid (28g)

To a solution of compound **27g** (115 mg, 0.24 mmol) in MeOH/THF/H_2O (3:1:3, 15 mL) was added LiOH·H_2O (102 mg, 2.42 mmol) and the reaction mixture was stirred at room temperature overnight until LC-MS showed the starting material was consumed completely. Then, the reaction mixture was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. Then, the solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **28g** (100 mg, 93%) as a yellow solid. ESI-MS *m/z* 449 $[M+H]^+$ calc. for $C_{24}H_{24}N_4O_5$. This intermediate was used in the next step without further characterization.

2-[1-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-4-piperidyl]-*N*-tetrahydropyran-2-yloxy-acetamide (29a)

To a solution of compound **28a** (90 mg, 0.2 mmol) in DMF (10 mL) were added EDC·HCl (77 mg, 0.4 mmol), HOBt (54 mg, 0.4 mmol), $THPONH_2$ (47 mg, 0.4 mmol) and NMM (62 mg, 0.6 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction mixture was quenched with water and extracted with

EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give compound **29a** (70 mg, 64%) as a pale yellow solid. ESI-MS *m/z* 553.3 $[M+H]^+$ calc. for $C_{29}H_{40}N_6O_5$. This intermediate was used in the next step without further characterization.

3-[1-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-4-piperidyl]-*N*-tetrahydropyran-2-yloxy-propanamide (29b)

To a solution of compound **28b** (15 mg, 0.03 mmol) in DMF (15 mL) were added EDC·HCl (20 mg, 0.1 mmol), HOBt (14 mg, 0.1 mmol), $THPONH_2$ (12 mg, 0.1 mmol) and NMM (16 mg, 0.15 mmol), and the mixture was stirred at room temperature overnight. Then, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **29b** (22 mg, 99% crude). ESI-MS *m/z* 567.2 $[M+H]^+$ calc. for $C_{30}H_{42}N_6O_5$. This intermediate was used in the next step without further characterization.

8-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-*N*-tetrahydropyran-2-yloxy-8-azaspiro[4.5]decane-3-carboxamide (29c)

To a solution of compound **28c** (120 mg, 0.24 mmol) in DMF (20 mL) were added EDC·HCl (93 mg, 0.48 mmol), HOBt (65 mg, 0.48 mmol), $THPONH_2$ (56 mg, 0.48 mmol) and NMM (62 mg, 0.6 mmol), and the mixture was stirred at room temperature overnight. Then, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **29c** (142 mg, 99%). ESI-MS *m/z* 593.2

$[M+H]^+$ calc. for $C_{32}H_{44}N_6O_5$. This intermediate was used in the next step without further characterization.

2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-*N*-tetrahydropyran-2-yloxy-2-azaspiro[5.5]undecane-9-carboxamide (29d)

To a solution of compound **28d** (48 mg, 0.095 mmol) in DMF (15 mL) were added EDC·HCl (39 mg, 0.2 mmol), HOBt (27 mg, 0.2 mmol), $THPONH_2$ (24 mg, 0.2 mmol) and NMM (40 mg, 0.4 mmol), and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **29d** (52 mg, 90%). ESI-MS *m/z* 607.5 $[M+H]^+$ calc. for $C_{33}H_{46}N_6O_5$. This intermediate was used in the next step without further characterization.

2-[2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-2,8-diazaspiro[4.5]decan-8-yl]-*N*-tetrahydropyran-2-yloxy-pyrimidine-5-carboxamide (29e)

To a solution of compound **28e** (95 mg, 0.17 mmol) in DMF (30 mL) were added EDC·HCl (68 mg, 0.35 mmol), HOBt (48 mg, 0.35 mmol), $THPONH_2$ (41 mg, 0.35 mmol) and NMM (61 mg, 0.6 mmol), and the mixture was stirred at room temperature overnight. Then, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **29e** (100 mg, 87%). ESI-MS *m/z* 672.2

$[M+H]^+$ calc. for $C_{35}H_{45}N_9O_5$. This intermediate was used in the next step without further characterization.

4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]-*N*-tetrahydropyran-2-yloxy-cyclohexanecarboxamide (29f)

To a solution of compound **28f** (400 mg, 0.88 mmol) in DMF (10 mL) were added EDC·HCl (338 mg, 1.76 mmol), HOBt (238 mg, 1.76 mmol), $THPONH_2$ (206 mg, 1.76 mmol) and NMM (267 mg, 2.64 mmol), and the mixture was stirred at room temperature overnight. Then, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **29f** (400 mg, 82%) as a white solid. ESI-MS *m/z* 554 $[M+H]^+$ calc. for $C_{29}H_{39}N_5O_6$. This intermediate was used in the next step without further characterization.

4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]-*N*-tetrahydropyran-2-yloxy-benzamide (29g)

To a solution of compound **28g** (100 mg, 0.22 mmol) in DMF (10 mL) were added EDC·HCl (86 mg, 0.45 mmol), HOBt (60 mg, 0.45 mmol), $THPONH_2$ (52 mg, 0.45 mmol) and NMM (68 mg, 0.67 mmol), and the mixture was stirred at room temperature overnight. Then, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **29g** (82 mg, 67%) as a yellow solid. ESI-MS *m/z* 548

$[M+H]^+$ calc. for $C_{29}H_{33}N_5O_6$. This intermediate was used in the next step without further characterization.

2-[1-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-4-piperidyl]ethanehydroxamic acid (30a)

A solution of compound **29a** (70 mg, 0.13 mmol) in HCl/EtOAc (2.0 M, 10 mL) was stirred at room temperature for 1 hour. Then, the solution was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **30a** (22.9 mg, 35%) as a white solid; m.p.: 196-197 ^oC. ^1H NMR (MeOD, 400 MHz): δ 8.19-8.17 (m, 1H), 7.84-7.80 (m, 1H), 7.40-7.36 (m, 1H), 4.35-4.15 (m, 5H), 3.75-3.65 (m, 4H), 2.90-2.80 (m, 2H), 2.35-2.05 (m, 5H), 1.80-1.70 (m, 4H), 1.50-1.40 (m, 3H), 1.05-0.95 (m, 3H). ESI-MS *m/z* 469.2 $[M+H]^+$ calc. for $C_{24}H_{32}N_6O_4$.

3-[1-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-4-piperidyl]propanehydroxamic acid (30b)

A solution of compound **29b** (22 mg, 0.04 mmol) in HCl/EtOAc (1.0 M, 10 mL) was stirred at room temperature for 1 hour and then concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **30b** (5.6 mg, 29%) as a white solid; m.p.: 168-169 ^oC. ^1H NMR (MeOD, 400 MHz): δ 8.15 (s, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.36 (d, J = 9.2 Hz, 1H), 4.31-4.25 (m, 2H), 4.24 (s, 3H), 3.73-3.70 (m, 2H), 3.63-3.57 (m, 2H), 2.90-2.86 (m, 2H), 2.22-2.18 (m, 4H), 1.82-1.79 (m, 2H), 1.72-1.67 (m, 5H), 1.50-1.46 (m, 3H), 1.02-0.95 (m, 3H). ESI-MS *m/z* 483.2 $[M+H]^+$ calc. for $C_{25}H_{34}N_6O_4$

8-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-8-azaspiro[4.5]decane-3-carbohydroxamic acid (30c)

A solution of compound **29c** (142 mg, 0.24 mmol) in HCl/EtOAc (1.0 M, 25 mL) was stirred at room temperature for 1 hour and then concentrated to give crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **30c** (44.8 mg, 37%). ^{1}H NMR (MeOD, 400 MHz): δ 8.18 (d, *J* = 2.4 Hz, 1H), 7.86-7.83 (m, 1H), 7.37 (d, *J* = 8.8 Hz, 1H), 4.31-4.26 (m, 2H), 4.23 (s, 3H), 3.67-3.65 (m, 4H), 2.90-2.86 (m, 2H), 2.80-2.74 (m, 1H), 2.00-1.80 (m, 12H), 1.50-1.46 (m, 3H), 1.02-0.98 (m, 3H). ESI-MS *m/z* 509.2 $[M+H]^+$ calc. for $C_{27}H_{36}N_6O_4$. Purity 98.56%.

2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-2-azaspiro[5.5]undecane-9-carbohydroxamic acid (30d)

A solution of compound **29d** (52 mg, 0.086 mmol) in HCl/EtOAc (1.0 M, 20 mL) was stirred at room temperature for 1 hour. Then, the reaction mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **30d** (7.8 mg, 17%) as a white solid; m.p.: 117-118 ^{o}C. ^{1}H NMR (MeOD, 400 MHz): δ 8.05 (s, 1H), 7.71 (d, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 9.2 Hz, 1H), 4.29-4.24 (m, 5H), 3.58-3.51 (m, 4H), 2.91-2.87 (m, 2H), 2.14-1.83 (m, 5H), 1.81-1.79 (m, 2H), 1.69-1.66 (m, 4H), 1.57-1.56 (m, 2H), 1.49-1.46 (m, 5H), 1.00 (t, *J* = 7.2 Hz, 3H). ESI-MS *m/z* 523.3 $[M+H]^+$ calc. for $C_{28}H_{38}N_6O_4$

2-[2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-2,8-diazaspiro[4.5]decan-8-yl]pyrimidine-5-carbohydroxamic acid (30e)

A solution of compound **29e** (100 mg, 0.15 mmol) in HCl/EtOAc (1.0 M, 20 mL) was stirred at room temperature for 2 hours. Then, the mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **30e** (15.4 mg, 17%) as yellow solid; m.p.: 198-199 ^{o}C. ^{1}H NMR (MeOD, 400 MHz): δ 8.66 (s, 2H), 7.30 (m, 1H), 7.10 (m, 1H), 6.90 (m, 1H), 4.23-4.14 (m, 5H), 3.97 (m, 4H), 3.49 (m, 2H), 3.45-3.35 (m, 2H), 2.89 (m, 2H), 2.05 (m, 2H), 1.82-1.72 (m, 6H), 1.42 (m, 3H), 1.01 (m, 3H). ESI-MS *m/z* 588.3 $[M+H]^+$ calc. for $C_{30}H_{37}N_9O_4$. Purity 94.60%.

4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]cyclohexanecarbohydroxamic acid (30f)

A solution of compound **29f** (200 mg, 0.36 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour and then the mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **30f** (57.3 mg, 34%) as a white solid; m.p.: 181-182 ^{o}C. ^{1}H NMR (MeOD, 400 MHz): δ 7.60-7.58 (m, 1H), 7.14-7.09 (m, 2H), 4.57 (s, 2H), 4.22 (s, 3H), 4.19-4.16 (m, 2H), 2.91-2.87 (m, 2H), 2.24-1.60 (m, 10H), 1.47-1.44 (m, 3H), 1.03-0.99 (m, 3H). ESI-MS *m/z* 470.3 $[M+H]^+$ calc. for $C_{24}H_{31}N_5O_5$

4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]benzenecarbohydroxamic acid (30g)

A solution of compound **29g** (82 mg, 0.15 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then, the mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in

supporting information) to obtain pure compound **30g** (35 mg, 50%) as a white solid; m.p.: 163.5-164.5 ^{o}C. ^{1}H NMR (MeOD, 400 MHz): δ 7.76-7.74 (d, *J* = 8.4 Hz, 2H), 7.69 (s, 1H), 7.23 (s, 2H), 7.04-7.02 (d, *J* = 8.4 Hz, 2H), 4.28-4.24 (m, 2H), 4.21 (s, 3H), 2.85-2.81 (m, 2H), 1.79-1.72 (m, 2H), 1.51-1.47 (m, 3H), 0.97-0.93 (m, 3H). ESI-MS *m/z* 464.2 $[M+H]^+$ calc. for $C_{24}H_{25}N_5O_5$

5-(2-Ethoxy-5-hydroxy-phenyl)-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (31)

To a solution of compound **25** (4.39 g, 10 mmol) in H_2O (50 mL) were added aqueous NaOH (4.0 M, 13 mmol) and H_2O_2 (494 mg, 13 mmol). The reaction mixture was stirred at room temperature overnight. Then, Na_2SO_3 solution was added and the mixture was stirred for 2 hours. Then, the reaction mixture was extracted with EtOAc. The organic phase was dried by Na_2SO_4, filtered and concentrated to give compound **31** (2.0 g, 61%). ESI-MS *m/z* 329 $[M+H]^+$ calc. for $C_{17}H_{20}N_4O_3$. This intermediate was used in the next step without further characterization.

***Tert*-butyl 4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]piperidine-1-carboxylate (32)**

To a solution of compound **31** (328 mg, 1 mmol) in anhydrous toluene (15 mL) were added *tert*-butyl 4-hydroxypiperidine-1-carboxylate (**Int. 9**, synthesis described in supporting information) (230 mg, 1.1 mmol), PPh_3 (316 mg, 1.2 mmol) and DEAD (225 mg, 1.2 mmol) and the reaction mixture was stirred at 110 ^{o}C for one hour. Then, the reaction mixture was concentrated under vacuum and purified by column chromatography to give the desired compound **32** (300 mg, 59%). ESI-MS *m/z* 512

$[M+H]^+$ calc. for $C_{27}H_{37}N_5O_5$. This intermediate was used in the next step without further characterization.

5-[2-Ethoxy-5-(4-piperidyloxy)phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (33)

To a solution of compound **32** (205 mg, 0.4 mmol) in 1,4-dioxane (15 mL) was added HCl/1,4-dioxane (4.0 M, 10 mL). The reaction mixture was stirred at room temperature for 2 hours. Then the reaction mixture was concentrated to give compound **33** (150 mg 91%). ESI-MS *m/z* 412 $[M+H]^+$ calc. for $C_{22}H_{29}N_5O_3$. This intermediate was used in the next step without further characterization.

Ethyl 2-[4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]-1-piperidyl]pyrimidine-5-carboxylate (34)

To a solution of compound **33** (120 mg, 0.3 mmol) in CH_3CN (15 mL) were added K_2CO_3 (138 mg, 1 mmol) and ethyl 2-chloropyrimidine-5-carboxylate (88 mg, 0.45 mmol). The solution was stirred at room temperature for 3 hours. Then, the mixture was concentrated and purified by column chromatography to give compound **34** (150 mg, 90%) as a yellow solid. ESI-MS *m/z* 562 $[M+H]^+$ calc. for $C_{29}H_{35}N_7O_5$. This intermediate was used in the next step without further characterization.

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]-1-piperidyl]pyrimidine-5-carboxylic acid (35)

To a solution of compound **34** (281 mg, 0.5 mmol) in THF/MeOH/H_2O (10:1:3 mL) was added $LiOH{\cdot}H_2O$ (107 mg, 2.5 mmol) and the resulting mixture was stirred at room temperature overnight. Then, the mixture was diluted with water and adjusted pH to 2-3

with 1 N HCl. The solution was extracted with EtOAC and the combined organic phase was concentrated to give compound **35** (150 mg, 56%). ESI-MS *m/z* 534 $[M+H]^+$ calc. for $C_{27}H_{31}N_7O_5$. This intermediate was used in the next step without further characterization.

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]-1-piperidyl]-*N*-tetrahydropyran-2-yloxy-pyrimidine-5-carboxamide (36)

To a solution of compound **35** (200 mg, 0.37 mmol) in DMF (15 mL) were added EDC·HCl (124 mg, 0.61 mmol), HOBt (82.2 mg, 0.61 mmol), $THPONH_2$ (63.2 mg, 0.54 mmol) and NMM (251 mg, 2.5 mmol), and the mixture was stirred at room temperature overnight. Then, the solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative HPLC (method 2 described in supporting information) to give compound **36** (150 mg, 64%). ESI-MS *m/z* 633 $[M+H]^+$ calc. for $C_{32}H_{40}N_8O_6$. This intermediate was used in the next step without further characterization.

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenoxy]-1-piperidyl]pyrimidine-5-carbohydroxamic acid (37)

A solution of compound **36** (100 mg, 0.16 mmol) in HCl/1,4-dioxane (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then, the reaction mixture was concentrated to give the desired crude product which was purified by preparative HPLC (method 3 described in supporting information) to give compound **37** (40 mg, 46%). ^1H NMR (MeOD, 400 MHz): δ 8.58 (s, 2H), 7.62 (s, 1H), 7.14 (m, 2H), 4.65 (m, 1H), 4.21 (m,

7H), 3.84 (m, 2H), 2.88 (m, 2H), 2.05 (m, 2H), 1.82 (m, 4H), 1.47 (t, 3H), 0.99 (t, 3H). ^{13}C NMR (DMSO-d6, 400MHz): δ 14.7 (CH_3), 15.5 (CH_3), 22.6, 28.0, 31.1, 38.7 (NCH_3), 48.2, 65.5 (CH2O), 73.7, 115.3, 119.0, 124.3, 125.1, 138.7, 145.7, 150.0 (CO), 151.3, 154.5, 158.0, 162.1, 182.8 (CONHOH). ESI-MS *m/z* 549.3 $[M+H]^+$ calc. for $C_{27}H_{32}N_8O_5$

5-(5-Bromo-2-ethoxy-phenyl)-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (38)

To a solution of compound **8** (2 g, 6.41 mmol) in AcOH (30 mL) was added Br_2 (1.25 g, 7.69 mmol) slowly and the reaction mixture was stirred at room temperature overnight. Then, Na_2SO_3 (378 mg, 3 mmol) and water were added into the reaction and the mixture was stirred at room temperature for 2 hours. Then, the solution was concentrated under vacuum and extracted with EtOAc. The organic layer was washed by water, dried with anhydrous Na_2SO_4, filtered and concentrated to give compound **38** (2 g, 80%). ESI-MS *m/z* 391 $[M+H]^+$ calc. for $C_{17}H_{19}BrN_4O_2$. This intermediate was used in the next step without further characterization.

Ethyl 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)benzoate (39)

To a solution of compound **38** (350 mg, 0.897 mmol) in EtOH (30 mL) was added Et_3N (227 mg, 2.243 mmol) and $Pd(dppf)Cl_2$ (146 mg, 0.199 mmol) at CO atmosphere, then the mixture was stirred at 80 ^oC overnight under CO protection. Then, the mixture was filtered and concentrated and the residue was extracted with EtOAc. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give the pure compound **39**

(254 mg, 74%) as a white solid. ESI-MS *m/z* 385 $[M+H]^+$ calc. for $C_{20}H_{24}N_4O_4$. This intermediate was used in the next step without further characterization.

4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)benzoic acid (40)

To a solution of compound **39** (254 mg, 0.661 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (278 mg, 6.61 mmol) and the reaction mixture was stirred at 40 ^oC overnight. Then, the mixture was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **40** (220 mg, 94%) as a white solid. ESI-MS *m/z* 357 $[M+H]^+$ calc. for $C_{18}H_{20}N_4O_4$. This intermediate was used in the next step without further characterization.

4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-N-tetrahydropyran-2-yloxy-benzamide (41)

To a solution of compound **40** (220 mg, 0.618 mmol) in DMF (10 mL) was added EDC·HCl (237 mg, 1.236 mmol), HOBt (167 mg, 1.236 mmol), $THPONH_2$ (145 mg, 1.236 mmol) and NMM (187 mg, 1.854 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **41** (140 mg, 50%) as a yellow solid. ESI-MS *m/z* 456 $[M+H]^+$ calc. for $C_{23}H_{29}N_5O_5$. This intermediate was used in the next step without further characterization.

4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)benzenecarbohydroxamic acid (42)

A solution of compound **41** (140 mg, 0.308 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then, the mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **42** (85 mg, 74%) as a white solid; m.p.: 204.5-205.5 ^{o}C. ^{1}H NMR (DMSO, 400 MHz): δ 8.99 (s, 1H), 8.01 (s, 1H), 7.90-7.87 (m, 1H), 7.21-7.19 (d, *J* = 8.8 Hz, 1H), 4.19-4.13 (m, 2H), 4.16 (s, 3H), 2.80-2.76 (m, 2H), 1.79-1.70 (m, 2H), 1.34-1.31 (m, 3H), 0.96-0.92 (m, 3H). ESI-MS *m/z* 372.1 $[M+H]^+$ calc. for $C_{18}H_{21}N_5O_4$

Ethyl 2-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]acetate (43a)

To a solution of compound **38** (500 mg, 1.28 mmol), $Pd_2(dba)_3$ (118 mg, 0.12 mmol) and xantphos (147 mg, 0.25 mmol) in anhydrous THF (30 mL) was added bromo-(2-ethoxy-2-oxo-ethyl)zinc (**Int. 10**, synthesis described in supporting information) (58.6 mmol in 20 mL of THF) under N_2 protection and the mixture was stirred at 80 ^{o}C overnight. Then, the mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **43a** (270 mg, 53%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.10 (s, 1H), 8.35 (s, 1H), 7.41-7.38 (m, 1H), 7.02-7.00 (d, *J* = 8.4 Hz, 1H), 4.32-4.26 (m, 5H), 4.21-4.16 (m, 2H), 2.96-2.92 (m, 2H), 1.89-1.85 (m, 2H), 1.67-1.58 (m, 5H), 1.30-1.27 (m, 3H), 1.06-1.02 (m, 3H). ESI-MS *m/z* 399 $[M+H]^+$ calc. for $C_{21}H_{26}N_4O_4$

Ethyl 3-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]propanoate (43b)

A mixture of compound **22** (100 mg, 0.23 mmol), ethyl acrylate (71 mg, 0.71 mmol), tri-o-tolylphosphine (28 mg, 0.091 mmol) and Et_3N (81 mg, 0.80 mmol) was heated in a heavy-walled Pyrex tube at 100 ^oC overnight under N_2 protection. Then, the mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by preparative TLC to give pure intermediate ethyl (*E*)-3-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]prop-2-enoate (85 mg, 90%) as a yellow solid. ^1H NMR ($CDCl_3$, 400 MHz): δ 10.97 (s, 1H), 8.56 (s, 1H), 7.72-7.68 (d, *J* = 16.0 Hz, 1H), 7.61-7.59 (d, *J* = 8.8 Hz, 1H), 7.04-7.02 (d, *J* = 8.8 Hz, 1H), 6.43-6.39 (d, *J* = 16.4 Hz, 1H), 4.32-4.26 (m, 4H), 4.25 (s, 3H), 3.46 (s, 2H), 1.90-1.84 (m, 2H), 1.60-1.57 (m, 3H), 1.36-1.32 (m, 3H), 1.06-1.02 (m, 3H). ESI-MS *m/z* 411 $[M+H]^+$ calc. for $C_{22}H_{26}N_4O_4$. This compound (85 mg, 0.21 mmol) was then dissolved in MeOH (10 mL) and Pd/C (30 mg) was added at H_2 atmosphere (1 atm). Then the mixture was stirred at room temperature overnight. Then, the mixture was filtered and the filtrate was concentrated to give the desired compound **43b** (81 mg, 93%) as a yellow solid. ESI-MS *m/z* 413 $[M+H]^+$ calc. for $C_{22}H_{28}N_4O_4$. This intermediate was used in the next step without further purification.

Ethyl 2-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]piperazin-1-yl]pyrimidine-5-carboxylate (43c)

To a solution of compound **45** (400 mg, 1.176 mmol) in anhydrous toluene (20 mL) was added *tert*-butyl piperazine-1-carboxylate (325 mg, 1.764 mmol) and $Ti[OCH(CH_3)_2]_4$

(500 mg, 1.764 mmol) and the mixture was stirred at room temperature for 90 minutes under N_2 protection. Then, $NaBH(OAc)_3$ (499 mg, 2.352 mmol) was added and the mixture was stirred at room temperature overnight. Then, the mixture was extracted with EtOAc three times and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by preparative TLC to obtain pure intermediate *tert*-butyl 4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]piperazine-1-carboxylate (450 mg, 75%) as a white solid. ESI-MS *m/z* 511 $[M+H]^+$ calc. for $C_{27}H_{38}N_6O_4$. Then, a solution of this intermediate (450 mg, 0.882 mmol) in HCl/EtOAc (4.0 M, 10 mL) was stirred at room temperature for 1 hour. The mixture was concentrated to give intermediate 5-[2-ethoxy-5-(piperazin-1-ylmethyl)phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (340 mg, 94%) as a white solid. ESI-MS *m/z* 411 $[M+H]^+$ calc. for $C_{22}H_{30}N_6O_2$. Finally, to a solution of this compound (125 mg, 0.307 mmol) in CH_3CN (20 mL) was added K_2CO_3 (85 mg, 0.614 mmol) and ethyl 2-chloropyrimidine-5-carboxylate (57 mg, 0.307 mmol) and the mixture was stirred at 60 ^oC overnight. Then, the mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to obtain pure compound **43c** (150 mg, 87%) as a yellow solid. ESI-MS *m/z* 561 $[M+H]^+$ calc. for $C_{29}H_{36}N_8O_4$. This intermediate was used in the next step without further characterization.

Ethyl 2-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]pyrimidine-5-carboxylate (43d)

To a solution of compound **38** (10.14 g, 26 mmol), $Pd_2(dba)_3$ (733 mg, 0.8 mmol), xantphos (926 mg, 1.6 mmol) and Na_2CO_3 (6.4 g, 60 mmol) in 1,4-dioxane/H_2O (6:1,

70 mL) was added freshly prepared *tert*-butyl 4-(9-borabicyclo[3.3.1]nonan-9-ylmethyl)piperidine-1-carboxylate (**Int. 9**, synthesis described in supporting information**)** (31 mmol in 62 mL of THF) and the mixture was heated at reflux overnight. Then, the mixture was filtered and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by column chromatography to obtain pure intermediate *tert*-butyl 4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]piperidine-1-carboxylate (7.1 g, 53%) as a pale yellow oil. ESI-MS *m/z* 454.1 [M-55] calc. for $C_{28}H_{39}N_5O_4$. Then, a solution of this intermediate (500 mg, 0.982 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then, the mixture was concentrated to give intermedaiate 5-[2-ethoxy-5-(4-piperidylmethyl)phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (400 mg, 99%) as a white solid. ESI-MS *m/z* 410 $[M+H]^+$ calc. for $C_{23}H_{31}N_5O_2$. Finally, to a solution of this compound (400 mg, 0.978 mmol) in CH_3CN (20 mL) was added K_2CO_3 (270 mg, 1.956 mmol) and ethyl 2-chloropyrimidine-5-carboxylate (182 mg, 0.978 mmol) and the mixture was stirred at 40 ^oC overnight. Then, the mixture was extracted with EtOAc and the organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **43d** (450 mg, 82%) as a white solid. ESI-MS *m/z* 560 $[M+H]^+$ calc. for $C_{30}H_{37}N_7O_4$. This intermediate was used in the next step without further characterization.

Methyl 6-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]pyridine-3-carboxylate (43e)

To a solution of intermediate 5-[2-ethoxy-5-(4-piperidylmethyl)phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (synthesis described in **43d**) (200 mg, 0.489 mmol) in CH_3CN (20 mL) was added K_2CO_3 (135 mg, 0.978 mmol) and methyl 6-chloronicotinate (100 mg, 0.587 mmol) and the mixture was stirred at 100 ^oC overnight. Then, the mixture was extracted with EtOAc and the organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to obtain pure compound **43e** (150 mg, 56%) as a white solid. ESI-MS *m/z* 545 $[M+H]^+$ calc. for $C_{30}H_{36}N_6O_4$. This intermediate was used in the next step without further characterization.

Methyl 4-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]benzoate (43f)

To a solution of intermediate 5-[2-ethoxy-5-(4-piperidylmethyl)phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (synthesis described in **43d**) (200 mg, 0.49 mmol) in CH_2Cl_2 (15 mL) was added (4-methoxycarbonylphenyl)boronic acid (180 mg, 1 mmol), $Cu(OAc)_2$ (90 mg, 0.5 mmol) and Et_3N (260 mg, 2.5 mmol) and the mixture was stirred at room temperature overnight under O_2. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to obtain pure compound **43f** (100 mg, 38%) as a yellow solid. ESI-MS *m/z* 544.2 $[M+H]^+$ calc. for $C_{31}H_{37}N_5O_4$. This intermediate was used in the next step without further characterization.

Ethyl 3-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]propanoate (43g)

Freshly prepared *tert*-butyl 4-(9-borabicyclo[3.3.1]nonan-9-ylmethyl)piperidine-1-carboxylate (**Int. 12**, synthesis described in supporting information) (31 mmol in 62 mL of THF) was added to a mixture of **38** (10.14 g, 26 mmol), $Pd_2(dba)_3$ (733 mg, 0.8 mmol), xantphos (926 mg, 1.6 mmol) and Na_2CO_3 (6.4 g, 60 mmol) in 1,4-dioxane/H_2O (6:1, 70 mL). The resulting mixture was stirred at reflux overnight. Then the solution was filtered and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by column chromatography to give intermediate *tert*-butyl 4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]piperidine-1-carboxylate (7.1 g, 45%) as a pale yellow oil. ESI-MS *m/z* 454.1 $[M+H-C(CH_3)_3]^+$ calc. for $C_{28}H_{39}N_5O_4$. This intermediate (500 mg, 0.98 mmol) was dissolved in HCl/EtOAc (4.0 M, 5 mL) and stirred at room temperature for 1 hour. Then, the mixture was concentrated to give corresponding deprotected amine 5-[2-ethoxy-5-(4-piperidylmethyl)phenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (400 mg, 99%) as a white solid. MS *m/z* 410 $[M+H]^+$ calc. for $C_{24}H_{31}N_5O_4$. Finally this intermediate (240 mg, 0.59 mmol) was dissolved in CAN (15 mL) and ethyl prop-2-enoate (180 mg, 1.8 mmol) and DIEA (290 mg, 2.24 mmol) were added. The mixture was stirred at 80 ^oC overnight. Then, the mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give compound **43g** (100 mg, 34%) as a pale yellow solid. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.14-8.13 (m, 1H), 7.18-7.15 (m, 1H), 6.93-6.90 (m, 1H), 4.26-4.10 (m, 6H), 3.64 (s, 3H), 3.40 (s, 1H), 3.20-3.05 (m, 2H), 2.95-2.80 (m, 4H), 2.75-2.60 (m, 2H), 2.60-2.50 (m, 2H), 2.35-2.15 (m, 2H), 1.90-1.50 (m, 10H), 1.05-0.95 (m, 3H). ESI-MS *m/z* 510.2 $[M+H]^+$ calc. for $C_{28}H_{39}N_5O_4$

Ethyl 2-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclohexyl]acetate (43h)

Freshly prepared ethyl 2-[4-(9-borabicyclo[3.3.1]nonan-9-ylmethyl)cyclohexyl]acetate (**Int. 13**, synthesis described in supporting information) (2.74 mmol in 10 mL of THF) was added into a mixture of **22** (1.2 g, 2.74 mmol), $Pd_2(dba)_3$ (275 mg, 0.3 mmol), xantphos (122 mg, 0.21 mmol) and Na_2CO_3 (583 mg, 5.5 mmol) in 1,4-dioxane/H_2O (5:1, 24 mL). The resulting mixture was stirred at reflux overnight. Then, the mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by column chromatography to give pure compound **43h** (300 mg, 23%) as a pale yellow oil. ESI-MS *m/z* 495.3 $[M+H]^+$ calc. for $C_{28}H_{38}N_4O_4$. This intermediate was used in the next step without further characterization.

Ethyl 4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclohexanecarboxylate (43i)

Freshly prepared ethyl 4-(9-borabicyclo[3.3.1]nonan-9-ylmethyl)cyclohexanecarboxylate (**Int. 14**, synthesis described in supporting information) (3 mmol in 10 mL of THF) was added into a mixture of **22** (1 g, 2.3 mmol), $Pd_2(dba)_3$ (80 mg, 0.1 mmol), xantphos (122 mg, 0.2 mmol) and Na_2CO_3 (668 mg, 6.3 mmol) in 1,4-dioxane/H_2O (5:1, 24 mL) and the resulting mixture was stirred at reflux overnight. Then the reaction mixture was filtered and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by column chromatography to give pure compound **43i** (400 mg, 36%) as a pale yellow oil. ESI-

MS *m/z* 481.3 $[M+H]^+$ calc. for $C_{27}H_{36}N_4O_4$. This intermediate was used in the next step without further characterization.

Methyl 3-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclopentanecarboxylate (43j)

Freshly prepared methyl 3-(9-borabicyclo[3.3.1]nonan-9-ylmethyl)cyclopentanecarboxylate (**Int. 15**, synthesis described in supporting information) (5 mmol in 10 mL of THF) was added into a mixture of **22** (2.2 g, 5 mmol), $Pd_2(dba)_3$ (400 mg, 0.4 mmol), xantphos (600 mg, 1.0 mmol) and Na_2CO_3 (2.1 g, 19 mmol) in 1,4-dioxane/H_2O (10:1, 44 mL). The resulting mixture was stirred at reflux overnight. Then, the solution was filtered and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by column chromatography to afford pure compound **43j** (600 mg, 27%) as a pale yellow oil. ESI-MS *m/z* 453.3 $[M+H]^+$ calc. for $C_{25}H_{32}N_4O_4$. This intermediate was used in the next step without further purification.

Ethyl 2-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclopropanecarboxylate (43k)

n-BuLi (1.1 mL, 2.7 mmol, 2.5 M) was added to a stirred suspension of **22** (1.1 g, 2.5 mmol) in THF (40 mL) at -70 ^oC over a period of 10 minutes under N_2. The resulting solution was stirred at -40 ^oC for 1 hour, and then ethyl 2-formylcyclopropanecarboxylate (375 mg, 2.64 mmol, predominantly trans) in THF (10 mL) was added over a period of 5 minutes under N_2. The resulting solution was stirred at room temperature for 15 hours. Then, the reaction was quenched with aqueous NH_4Cl and extracted with EtOAc. The combined organic phase was washed with saturated

brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography to give intermediate alcohol ethyl 2-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-hydroxy-methyl]cyclopropanecarboxylate (210 mg, 19%). ESI-MS *m/z* 455.1 $[M+H]^+$ calc. for $C_{24}H_{30}N_4O_5$. Then this compound (210 mg, 0.46 mmol) was dissolved in TFA (8 mL) and a solution of Et_3SiH (8 mL) in CH_2Cl_2 (8 mL) was added dropwise at 0 ^oC. The reaction mixture was stirred at room temperature for another 10 hours. Then, the solution was quenched by aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic phase was washed with saturated brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **43k** (135 mg, 67%). ESI-MS *m/z* 439.1 $[M+H]^+$ calc. for $C_{24}H_{30}N_4O_4$. This intermediate was used in the next step without further purification.

Ethyl 1-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]piperidine-4-carboxylate (43l)

To a solution of compound **45** (250 mg, 0.736 mmol) in anhydrous CH_2Cl_2 (30 mL) were added ethyl piperidine-4-carboxylate (97 mg, 0.62 mmol), AcOH (cat) and $NaBH(OAc)_3$ (260 mg, 1.22 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was extracted with CH_2Cl_2 and the organic layer was washed with aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by preparative TLC to give pure compound **43l** (150 mg, 42%) as a white solid. ESI-MS *m/z* 482 $[M+H]^+$ calc. for $C_{26}H_{35}N_5O_4$. This intermediate was used in the next step without further characterization.

Methyl 1-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]azetidine-3-carboxylate (43m)

To a solution of compound **45** (1 g, 2.9 mmol) in anhydrous CH_2Cl_2 (50 mL) were added methyl azetidine-3-carboxylate (677 mg, 5.9 mmol), AcOH (cat) and $NaBH(OAc)_3$ (1 g, 4.7 mmol) and the mixture was stirred at room temperature overnight until LC-MS showed the starting material was consumed completely. Then, the mixture was extracted with CH_2Cl_2 and the organic layer was washed with aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by preparative TLC to give pure compound **43m** (0.8 g, 63%) as a white solid. ESI-MS *m/z* 440 $[M+H]^+$ calc. for $C_{23}H_{29}N_5O_4$. This intermediate was used in the next step without further characterization.

Ethyl 2-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]cyclopropanecarboxylate (43o)

To a solution of ethyl 2-diethoxyphosphorylacetate (2.1 g, 9.5 mmol) in THF (60 mL) was added NaH (0.48 g 60% in mineral oil, 12 mmol) at 0 ^oC and the solution was stirred at 0 ^oC for 1 hour. Then a solution of compound **45** (3.2 g, 9.4 mmol) in THF (10 mL) was added at 0 ^oC and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give intermediate ethyl (*E*)-3-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]prop-2-enoate (3.1 g, 80%) as a white solid. ESI-MS *m/z* 411.2 $[M+H]^+$ calc. for $C_{22}H_{26}N_4O_4$. Then, trimethyloxosulphonium iodide (1.85 g, 8.4 mmol) was added to a stirred suspension of NaH (340 mg 60% in mineral

oil, 8.4 mmol) in DMSO (50 mL) at 40 ^{o}C over a period of 15 minutes under N_2. The resulting solution was stirred at 40 ^{o}C for 1 hour, and then intermediate ethyl (*E*)-3-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]prop-2-enoate (2.3 g, 5.6 mmol) in DMSO (10 mL) was added over a period of 10 minutes under N_2. The reaction mixture was stirred at 40 ^{o}C for another 12 hours. Then, the reaction was quenched by ice slowly and extracted with EtOAc. The combined organic phase was washed with saturated brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by column chromatography to give pure compound **43o** (1.82 g, 77%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.26-8.22 (m, 1H), 7.17-7.14 (m, 1H), 6.97-6.94 (m, 1H), 4.32-4.17 (m, 7H), 2.97-2.93 (m, 2H), 2.60-2.56 (m, 1H), 1.92-1.86 (m, 3H), 1.60-1.56 (m, 4H), 1.30-1.26 (m, 4H), 1.07-1.04 (m, 3H), 0.88 (m, 1H). ESI-MS *m/z* 425.1 $[M+H]^+$ calc. for $C_{23}H_{28}N_4O_4$

Ethyl 3-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclobutanecarboxylate (43p)

Freshly prepared ethyl 3-(9-borabicyclo[3.3.1]nonan-9-ylmethyl)cyclobutanecarboxylate (**Int. 17**, synthesis described in supporting information) (21.4 mmol in 40 mL of THF) was added to a mixture of **22** (7.82 g, 17.8 mmol), xantphos (2.55 g, 4.40 mmol), $Pd_2(dba)_3$ (1.63 g,1.78 mmol) and Na_2CO_3 (5.67 g, 53.5 mmol) in 1,4-dioxane/H_2O (10:1, 44 mL). The resulting mixture was stirred at reflux overnight. Then, the reaction mixture was filtered and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by column chromatography to give pure compound **43p** (4.5 g, 56%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.13 (s, 1H), 8.22 (s, 1H), 7.23-7.21 (m, 1H), 6.96-6.94

(m, 1H), 4.28 (s, 3H), 4.15-4.10 (m, 3H), 2.98-2.94 (m, 2H), 2.82-2.75 (m, 2H), 2.55-2.53 (m, 1H), 2.37-2.32 (m, 2H), 1.90-1.87 (m, 2H), 1.60-1.57 (m, 5H), 1.28-1.25 (m, 5H), 1.07-1.04 (m, 3H). ESI-MS *m/z* 453.3 $[M+H]^+$ calc. for $C_{25}H_{32}N_4O_4$

4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)benzonitrile (44)

To a solution of compound **22** (20.0 g, 46 mmol) in DMF (100 mL) were added $Zn(CN)_2$ (10.6 g, 92 mmol) and $Pd(PPh_3)_4$ (5.1 g, 4.6 mmol) and the mixture was stirred at 80 ^oC overnight under N_2 protection. Then, the mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by column chromatography to give pure compound **44** (10 g, 67%) as a white solid. ESI-MS *m/z* 338 $[M+H]^+$ calc. for $C_{18}H_{19}N_5O_2$. This intermediate was used in the next step without further characterization.

4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)benzaldehyde (45)

To a solution of compound **44** (10 g, 29.6 mmol) in anhydrous CH_2Cl_2 (120 mL) was added DIBAL-H (34.8 mL, 1.0 M in toluene, 34.8 mmol) slowly at 0 ^oC, then the mixture was stirred at room temperature overnight under N_2 protection until HPLC showed the starting material was consumed completely. Then, the mixture was poured into 2 N HCl, extracted with CH_2Cl_2 and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by column chromatography to give pure compound **45** (1 g, 10%) as a

white solid. ESI-MS *m/z* 341 $[M+H]^+$ calc. for $C_{18}H_{20}N_4O_3$. This intermediate was used in the next step without further characterization.

2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]acetic acid (46a)

To a solution of compound **43a** (270 mg, 0.68 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (285 mg, 6.78 mmol) and the reaction mixture was stirred at room temperature overnight. Then, the solution was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **46a** (230 mg, 92%) as a white solid. ESI-MS *m/z* 371 $[M+H]^+$ calc. for $C_{19}H_{22}N_4O_4$. This intermediate was used in the next step without further characterization.

3-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]propanoic acid (46b)

To a solution of **43b** (81 mg, 0.20 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (84 mg, 2.0 mmol) and the mixture was stirred at 40 ^oC overnight. Then, the solution was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **46b** (60 mg, 78%) as a yellow solid. ESI-MS *m/z* 385 $[M+H]^+$ calc. for $C_{20}H_{24}N_4O_4$. This intermediate was used in the next step without further purification.

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]piperazin-1-yl]pyrimidine-5-carboxylic acid (46c)

To a solution of compound **43c** (150 mg, 0.268 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (112 mg, 2.68 mmol) and the reaction mixture was stirred at room temperature overnight until LC-MS showed the starting material was consumed completely. Then, the mixture was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. Then, the solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **46c** (130 mg, 91%) as a white solid. ESI-MS *m/z* 533 $[M+H]^+$ calc. for $C_{27}H_{32}N_8O_4$. This intermediate was used in the next step without further characterization.

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]pyrimidine-5-carboxylic acid (46d)

To a solution of compound **43d** (450 mg, 0.805 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (338 mg, 8.05 mmol) and the reaction mixture was stirred at 40 ^oC overnight until LC-MS showed the starting material was consumed completely. Then, the mixture was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. Then, the solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **46d** (400 mg, 94%) as a white solid. ESI-MS *m/z* 532 $[M+H]^+$ calc. for $C_{28}H_{33}N_7O_4$. This intermediate was used in the next step without further characterization.

6-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]pyridine-3-carboxylic acid (46e)

To a solution of compound **43e** (150 mg, 0.276 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (116 mg, 2.76 mmol) and the reaction mixture was stirred at room temperature overnight until LC-MS showed the starting material was consumed completely. Then, the mixture was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. The solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **46e** (130 mg, 89%) as a white solid. ESI-MS *m/z* 531 $[M+H]^+$ calc. for $C_{29}H_{34}N_6O_4$. This intermediate was used in the next step without further characterization.

4-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]benzoic acid (46f)

To a solution of compound **43f** (100 mg, 0.18 mmol) in THF/MeOH/H_2O (1:3:1, 8 mL) was added LiOH·H_2O (76 mg, 1.8 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was diluted with water and adjusted pH to 6-7 with 1 N HCl. The solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the desired product **46f** (80 mg, 84%). ESI-MS *m/z* 530.2 $[M+H]^+$ calc. for $C_{30}H_{35}N_5O_4$. This intermediate was used in the next step without further characterization.

3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]propanoic acid (46g)

To a solution of compound **43g** (100 mg, 0.2 mmol) in THF/MeOH/H_2O (3:3:2, 8 mL) was added LiOH·H_2O (88 mg, 2 mmol) and the resulting mixture was stirred at room temperature overnight. Then the mixture was diluted with water and adjusted pH to 6~7

with 1 N HCl. The mixture was extracted with EtOAc and the organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the desired product **46g** (100 mg, 99%). ESI-MS *m/z* 482.2 $[M+H]^+$ calc. for $C_{26}H_{35}N_5O_4$. This intermediate was used in the next step without further characterization.

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclohexyl]acetic acid (46h)

To a solution of compound **43h** (300 mg, 0.61 mmol) in THF/MeOH/H_2O (3:3:2, 16 mL) was added LiOH·H_2O (260 mg, 6.1 mmol) and the resulting mixture was stirred at room temperature overnight. Then the mixture was diluted with water and adjusted pH to 6~7 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the desired product **46h** (260 mg, 91%). ESI-MS *m/z* 467.3 $[M+H]^+$ calc. for $C_{26}H_{34}N_4O_4$. This intermediate was used in the next step without further characterization.

4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclohexanecarboxylic acid (46i)

To a solution of **43i** (400 mg, 0.84 mmol) in THF/MeOH/H_2O (3:3:2, 16 mL) was added LiOH·H_2O (361 mg, 8.6 mmol) and the resulting mixture was stirred at room temperature overnight. Then, the mixture was diluted with water and adjusted pH to 3~4 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered concentrated and purified by preparative TLC to afford the desired compound **46i** (350 mg, 93%). ESI-MS *m/z* 453.3 $[M+H]^+$ calc. for $C_{25}H_{32}N_4O_4$. This intermediate was used in the next step without further characterization.

3-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclopentanecarboxylic acid (46j)

To a solution of compound **43j** (600 mg, 1.33 mmol) in THF/MeOH/H_2O (3:3:2, 16 mL) was added LiOH·H_2O (560 mg, 13.3 mmol) and the resulting mixture was stirred at room temperature overnight. Then, the mixture was diluted with water and adjusted pH to 3~4 with 1 N HCl. The solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by preparative TLC to afford the desired product **46j** (500 mg, 86%). ESI-MS *m/z* 439.2 $[M+H]^+$ calc. for $C_{24}H_{30}N_4O_4$. This intermediate was used in the next step without further purification.

2-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclopropanecarboxylic acid (46k)

To a solution of compound **43k** (135 mg, 0.31 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (130 mg, 3 mmol) and the reaction mixture was stirred at room temperature overnight. Then, the mixture was concentrated, diluted with H_2O and adjusted pH to 3-4 with 1N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **46k** (125 mg, 98%). ESI-MS *m/z* 411.1 $[M+H]^+$ calc. for $C_{22}H_{26}N_4O_4$. This intermediate was used in the next step without further purification.

1-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]piperidine-4-carboxylic acid (46l)

To a solution of compound **43l** (150 mg, 0.31 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (131 mg, 3.12 mmol) and the reaction mixture was stirred at room temperature overnight. Then, the solution was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **46l** (130 mg, 92%) as a white solid. ESI-MS *m/z* 454 $[M+H]^+$ calc. for $C_{24}H_{31}N_5O_4$. This intermediate was used in the next step without further characterization.

1-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]azetidine-3-carboxylic acid (46m)

To a solution of compound **43m** (800 mg, 1.8 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (763 mg, 18 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction mixture was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. The solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **46m** (800 mg, 99% crude) as a white solid. ESI-MS *m/z* 426 $[M+H]^+$ calc. for $C_{22}H_{27}N_5O_4$. This intermediate was used in the next step without further characterization.

3-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]cyclobutanecarboxylic acid (46n)

n-BuLi (2.6 mL, 6.5 mmol, 2.5 M) was added to a stirred suspension of compound **22** (2.63 g, 6.0 mmol) in THF (60 mL) at -70 ^oC over a period of 5 minutes under N_2. The resulting solution was stirred at -40 ^oC for 1 hour, and then *tert*-butyl 3-

oxocyclobutanecarboxylate (**Int. 16**, synthesis described in supporting information) (1.1 g, 6.5 mmol) in THF (10 mL) was added over a period of 5 minutes under N_2. The resulting solution was stirred at room temperature for 15 hours. The reaction was quenched with aqueous NH_4Cl and then extracted with EtOAc. The combined organic phase was washed with saturated brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by column chromatography to give pure intermediate *tert*-butyl 3-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*h*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-3-hydroxy-cyclobutanecarboxylate (830 mg, 29%). ESI-MS *m/z* 483.2 $[M+H]^+$ calc. for $C_{26}H_{34}N_4O_5$. To a solution of this intermediate (700 mg, 1.45 mmol) in TFA (8 mL) was added a solution of Et_3SiH (8 mL) in CH_2Cl_2 (8 mL) dropwise at 0 ^oC. The reaction mixture was stirred at room temperature for another 10 hours. Then, the reaction was quenched with aqueous $NaHCO_3$ slowly and extracted with CH_2Cl_2. The combined organic phase was washed with saturated brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **46n** (512 mg, 86%). ESI-MS *m/z* 411.1 $[M+H]^+$ calc. for $C_{22}H_{26}N_4O_4$. This intermediate was used in the next step without further characterization.

2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]cyclopropanecarboxylic acid (46o)

To a solution of compound **43o** (1.82 g, 4.3 mmol) in MeOH/THF/H_2O (1:3:1, 60 mL) was added $LiOH·H_2O$ (2.2 g, 52 mmol) and the reaction mixture was stirred at room temperature overnight. Then the mixture was concentrated, diluted with H_2O and adjusted pH to 3-4 with 1 N HCl. The solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **46o** (1.62 g, 95%). ESI-MS *m/z* 397.3 $[M+H]^+$ calc. for

$C_{21}H_{24}N_4O_4$. This intermediate was used in the next step without further characterization.

3-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclobutanecarboxylic acid (46p)

To a solution of compound **43p** (4.5 g, 9.94 mmol) in THF/MeOH/H_2O (3:3:2, 60 mL) was added LiOH·H_2O (4.17 g, 99.4 mmol) and the resulting mixture was stirred at room temperature overnight. Then, the mixture was diluted with water and adjusted pH to 3~4 with 1 N HCl. The solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford compound **46p** (3.8 g, 90%) ESI-MS *m/z* 425.3 $[M+H]^+$ calc. for $C_{23}H_{28}N_4O_4$. This intermediate was used in the next step without further purification.

2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-*N*-tetrahydropyran-2-yloxy-acetamide (47a)

To a solution of compound **46a** (115 mg, 0.31 mmol) in DMF (10 mL) were added EDC·HCl (119 mg, 0.62 mmol), HOBt (84 mg, 0.62 mmol), $THPONH_2$ (73 mg, 0.62 mmol) and NMM (94 mg, 0.93 mmol) and the mixture was stirred at room temperature overnight. Then, the solution was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give the desired **47a** (116 mg, 79%) as a white solid. ESI-MS *m/z* 470 calc. for $C_{24}H_{31}N_5O_5$. This intermediate was used in the next step without further characterization.

3-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-*N*-tetrahydropyran-2-yloxy-propanamide (47b)

To a solution of compound **46b** (60 mg, 0.156 mmol) in DMF (10 mL) were added EDC·HCl (60 mg, 0.31 mmol), HOBt (42 mg, 0.31 mmol), $THPONH_2$ (36 mg, 0.31 mmol) and NMM (48 mg, 0.47 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give the corresponding **47b** (60 mg, 80%) as a white solid. ESI-MS *m/z* 484 $[M+H]^+$ calc. for $C_{25}H_{33}N_5O_5$. This intermediate was used in the next step without further purification.

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]piperazin-1-yl]-*N*-tetrahydropyran-2-yloxy-pyrimidine-5-carboxamide (47c)

To a solution of compound **46c** (130 mg, 0.244 mmol) in DMF (10 mL) was added EDC·HCl (94 mg, 0.488 mmol), HOBt (66 mg, 0.488 mmol), $THPONH_2$ (57 mg, 0.488 mmol) and NMM (74 mg, 0.732 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to obtain pure compound **47c** (120 mg, 78%) as a white solid. ESI-MS *m/z* 632 $[M+H]^+$ calc. for $C_{32}H_{41}N_9O_5$. ^1H NMR ($CDCl_3$, 400 MHz): δ 11.10 (s, 1H), 9.19 (s, 1H), 8.69 (s, 2H), 8.34 (s, 1H), 7.50-7.48 (d, *J* = 6.8 Hz, 1H), 7.03-7.01 (d, *J* = 8.4 Hz, 1H), 5.03 (s, 1H), 4.31-4.29 (m, 2H), 4.26 (s, 3H), 3.98-3.90 (m, 4H), 3.61 (s, 2H), 3.47 (s, 2H), 2.93-2.91 (m, 2H), 2.60-2.52 (m, 4H), 2.00-1.84 (m, 8H), 1.60-1.57 (m, 3H), 1.04-1.00 (m, 3H).

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]-*N*-tetrahydropyran-2-yloxy-pyrimidine-5-carboxamide (47d)

To a solution of compound **46d** (400 mg, 0.753 mmol) in DMF (10 mL) was added EDC·HCl (289 mg, 1.507 mmol), HOBt (203 mg, 1.507 mmol), $THPONH_2$ (176 mg, 1.507 mmol) and NMM (228 mg, 2.259 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to obtain pure compound **47d** (400 mg, 84%) as a white solid. ESI-MS *m/z* 631 $[M+H]^+$ calc. for $C_{33}H_{42}N_8O_5$. This intermediate was used in the next step without further characterization.

6-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]-*N*-tetrahydropyran-2-yloxy-pyridine-3-carboxamide (47e)

To a solution of compound **46e** (130 mg, 0.245 mmol) in DMF (10 mL) was added EDC·HCl (94 mg, 0.490 mmol), HOBt (66 mg, 0.490 mmol), $THPONH_2$ (58 mg, 0.490 mmol) and NMM (74 mg, 0.735 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to obtain pure compound **47e** (100 mg, 65%) as a white solid. ESI-MS *m/z* 630 $[M+H]^+$ calc. for

$C_{34}H_{43}N_7O_5$. This intermediate was used in the next step without further characterization.

4-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]-*N*-tetrahydropyran-2-yloxy-benzamide (47f)

To a solution of compound **46f** (80 mg, 0.15 mmol) in DMF (10 mL) was added EDC·HCl (60 mg, 0.3 mmol), HOBt (40 mg, 0.3 mmol), $THPONH_2$ (30 mg, 0.3 mmol) and NMM (50 mg, 0.45 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to obtain pure compound **47f** (60 mg, 64%) as a pale yellow solid. ESI-MS *m/z* 629.2 $[M+H]^+$ calc. for $C_{35}H_{44}N_6O_5$. This intermediate was used in the next step without further characterization.

3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]-*N*-tetrahydropyran-2-yloxy-propanamide (47g)

To a solution of compound **46g** (100 mg, 0.2 mmol) in DMF (10 mL) were added EDC·HCl (77 mg, 0.4 mmol), HOBt (54 mg, 0.4 mmol), $THPONH_2$ (47 mg, 0.4 mmol) and NMM (62 mg, 0.6 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give the desired **47g** (80 mg, 69%) as a pale yellow solid. ESI-MS *m/z* 581.3 $[M+H]^+$ calc.

for $C_{31}H_{44}N_6O_5$. This intermediate was used in the next step without further characterization.

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclohexyl]-*N*-tetrahydropyran-2-yloxy-acetamide (47h)

To a solution of compound **46h** (260 mg, 0.56 mmol) in DMF (20 mL) were added EDC·HCl (215 mg, 1.12 mmol), HOBt (151 mg, 1.12 mmol), $THPONH_2$ (131 mg, 1.12 mmol) and NMM (170 mg, 1.68 mmol) and the mixture was stirred at room temperature overnight. Then, the mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give the desired **47h** (200 mg, 62%) as a pale yellow solid. ESI-MS *m/z* 566.3 $[M+H]^+$ calc. for $C_{31}H_{43}N_5O_5$. This intermediate was used in the next step without further characterization.

4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-*N*-tetrahydropyran-2-yloxy-cyclohexanecarboxamide (47i)

To a solution of compound **46i** (350 mg, 0.77 mmol) in DMF (20 mL) were added EDC·HCl (292 mg, 1.54 mmol), HOBt (207 mg, 1.54 mmol), $THPONH_2$ (180 mg, 1.54 mmol) and NMM (170 mg, 1.68 mmol) and the mixture was stirred at room temperature overnight. Then, the solution was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give the desired **47i** (200 mg, 47%) as a pale yellow solid. ESI-MS *m/z* 552.3 $[M+H]^+$ calc. for $C_{30}H_{41}N_5O_5$.

3-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6H-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-N-tetrahydropyran-2-yloxy-cyclopentanecarboxamide (47j)

To a solution of compound **46j** (500 mg, 1.14 mmol) in DMF (30 mL) were added EDC·HCl (438 mg, 2.3 mmol), HOBt (310 mg, 2.3 mmol), $THPONH_2$ (269 mg, 2.3 mmol) and NMM (345 mg, 3.4 mmol) and the mixture was stirred at room temperature overnight. Then, the solution was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give the desired **47j** (300 mg, 50%) as a pale yellow solid. ESI-MS *m/z* 538.3 $[M+H]^+$ calc. for $C_{29}H_{39}N_5O_5$. This intermediate was used in the next step without further purification.

2-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-*N*-tetrahydropyran-2-yloxy-cyclopropanecarboxamide (47k)

To a solution of compound **46k** (125 mg, 0.3 mmol) in DMF (20 mL) were added EDC·HCl (97 mg, 0.5 mmol), HOBt (68 mg, 0.5 mmol), $THPONH_2$ (59 mg, 0.5 mmol) and NMM (101 mg, 1.0 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the desired **47k** (93 mg, 61%). ESI-MS *m/z* 510.2 $[M+H]^+$ calc. for $C_{27}H_{35}N_5O_5$. This intermediate was used in the next step without further purification.

1-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-*N*-tetrahydropyran-2-yloxy-piperidine-4-carboxamide (47l)

To a solution of compound **46l** (130 mg, 0.287 mmol) in DMF (10 mL) were added EDC·HCl (110 mg, 0.57 mmol), HOBt (77 mg, 0.57 mmol), $THPONH_2$ (67 mg, 0.57 mmol) and NMM (87 mg, 0.86 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give the compound **47l** (110 mg, 70%) as a yellow solid. ESI-MS *m/z* 553 $[M+H]^+$ calc. for $C_{29}H_{40}N_6O_5$. This intermediate was used in the next step without further characterization.

1-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-*N*-tetrahydropyran-2-yloxy-azetidine-3-carboxamide (47m)

To a solution of compound **46m** (400 mg, 0.94 mmol) in DMF (20 mL) were added EDC·HCl (360 mg, 1.88 mmol), HOBt (254 mg, 1.88 mmol), $THPONH_2$ (220 mg, 1.88 mmol) and NMM (300 mg, 2.97 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **47m** (300 mg, 60%) as a yellow solid. ESI-MS *m/z* 525.3 $[M+H]^+$ calc. for $C_{27}H_{36}N_6O_5$. This intermediate was used in the next step without further characterization.

2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-*N*-tetrahydropyran-2-yloxy-cyclopropanecarboxamide (47o)

To a solution of compound **46o** (1.62 g, 4.0 mmol) in DMF (60 mL) were added EDC·HCl (1.54 g, 8 mmol), HOBt (1.08 g, 8 mmol), $THPONH_2$ (940 mg, 8 mmol) and NMM (1.2 g, 12 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give compound **47o** (1.75 g, 88%). ESI-MS *m/z* 496.3 $[M+H]^+$ calc. for $C_{26}H_{33}N_5O_5$. This intermediate was used in the next step without further characterization.

2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]ethanehydroxamic acid (48a)

Compound **47a** (116 mg, 0.25 mmol) was dissolved in HCl/EtOAc (4.0 M, 5 mL) and stirred at room temperature for 1 hour. Then, the mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **48a** (12.2 mg, 13%) as a white solid; m.p.: 159-160 ^oC. ^1H NMR (MeOD, 400 MHz): δ 7.84 (s, 1H), 7.46-7.43 (m, 1H), 7.13-7.10 (d, *J* = 8.4 Hz, 1H), 4.22-4.18 (m, 5H), 3.43 (s, 2H), 2.89-2.85 (m, 2H), 1.85-1.76 (m, 2H), 1.46-1.43 (m, 3H), 1.02-0.98 (m, 3H). ESI-MS *m/z* 386.2 $[M+H]^+$ calc. for $C_{19}H_{23}N_5O_4$

3-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]propanehydroxamic acid (48b)

Compound **47b** (60 mg, 0.124 mmol) was dissolved in HCl/EtOAc (4.0 M, 5 mL) and stirred at room temperature for 1 hour. Then, the mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in

supporting information) to obtain pure compound **48b** (20 mg, 40%) as a red solid; m.p.: 166-167 ^{o}C. ^{1}H NMR (MeOD, 400 MHz) : δ 7.75 (s, 1H), 7.37-7.36 (d, J = 6.8 Hz, 1H), 7.10-7.08 (d, J = 8.8 Hz, 1H), 4.23 (s, 3H), 4.20-4.17 (m, 2H), 2.96-2.87 (m, 4H), 2.42-2.39 (m, 2H), 1.86-1.77 (m, 2H), 1.46-1.42 (m, 3H), 1.03-0.99 (m, 3H). ESI-MS *m/z* 400.1 $[M+H]^+$ calc. for $C_{20}H_{25}N_5O_4$

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]piperazin-1-yl]pyrimidine-5-carbohydroxamic acid (48c)

A solution of compound **47c** (120 mg, 0.19 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then, the mixture was concentrated to give the crude compound which was purified by preparative HPLC (Method 1 described in supporting information) to obtain pure compound **48c** (26.2 mg, 25%) as a white solid; m.p.: 173-174 ^{o}C. ESI-MS *m/z* 548.3 $[M+H]^+$ calc. for $C_{27}H_{33}N_9O_4$. ^{1}H NMR (MeOD, 400 MHz): δ 8.74 (s, 2H), 8.03 (s, 1H), 7.68-7.66 (d, J = 8.4 Hz, 1H), 7.31-7.29 (d, J = 8.8 Hz, 1H), 5.05-4.91 (m, 4H), 4.42 (s, 2H), 4.30-4.27 (m, 2H), 4.24 (s, 3H), 3.39-3.35 (m, 4H), 2.90-2.86 (m, 2H), 1.86-1.77 (m, 2H), 1.49-1.46 (m, 3H), 1.02-0.98 (m, 3H).

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]pyrimidine-5-carbohydroxamic acid (48d)

A solution of compound **47d** (400 mg, 0.635 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then, the mixture was concentrated to give the crude compound which was purified by preparative HPLC (Method 1 described in supporting information) to obtain pure compound **48d** (150 mg, 43%) as a white solid; m.p.: 185.5-186.5 ^{o}C. ESI-MS *m/z* 547.4 $[M+H]^+$ calc. for $C_{28}H_{34}N_8O_4$. ^{1}H NMR (DMSO, 400 MHz): δ 11.93 (s, 1H), 11.06 (s, 1H), 8.63 (s, 2H), 7.46 (s, 1H), 7.28-7.26

(d, *J* = 8.4 Hz, 1H), 7.07-7.05 (d, *J* = 8.0 Hz, 1H), 4.70-4.67 (m, 2H), 4.14 (s, 3H), 4.11-4.08 (m, 2H), 2.90-2.87 (m, 2H), 2.78-2.74 (m, 2H), 2.53-2.50 (m, 2H), 1.74-1.65 (m, 5H), 1.32-1.29 (m, 3H), 1.12-1.00 (m, 2H), 0.94-0.91 (m, 3H).

6-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]pyridine-3-carbohydroxamic acid (48e)

A solution of compound **47e** (100 mg, 0.159 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then, the mixture was concentrated to give the crude compound which was purified by preparative HPLC (Method 1 described in supporting information) to obtain pure compound **48e** (22.2 mg, 25%) as a white solid; m.p.: 156.5-157.5 ^{o}C. ESI-MS *m/z* 546.3 $[M+H]^+$ calc. for $C_{29}H_{35}N_7O_4$. ^{1}H NMR (MeOD, 400 MHz): δ 8.34 (s, 1H), 8.08-8.06 (d, *J* = 9.2 Hz, 1H), 7.74 (s, 1H), 7.35-7.34 (d, *J* = 6.8 Hz, 1H), 7.21-7.18 (d, *J* = 9.6 Hz, 1H), 7.12-7.10 (d, *J* = 8.8 Hz, 1H), 4.32-4.28 (m, 2H), 4.23 (s, 3H), 4.22-4.18 (m, 2H), 3.18-3.12 (m, 2H), 2.91-2.87 (m, 2H), 2.65-2.63 (m, 2H), 2.13-1.79 (m, 5H), 1.47-1.44 (m, 3H), 1.40-1.35 (m, 2H), 1.02-0.99 (m, 3H).

4-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]benzenecarbohydroxamic acid (48f)

A solution of compound **47f** (60 mg, 0.09 mmol) in HCl/EtOAc (2.0 M, 10 mL) was stirred at room temperature for 1 hour. Then, the mixture was concentrated to give the crude product which was purified by preparative HPLC (Method 1 described in supporting information) to obtain pure compound **48f** (8.3 mg, 17%) as a white solid; m.p.: 203-204 ^{o}C. ESI-MS *m/z* 545.2 $[M+H]^+$ calc. for $C_{30}H_{36}N_6O_4$. ^{1}H NMR (MeOD, 400 MHz): δ 7.80-7.50 (m, 1H), 7.50-7.45 (m, 2H), 7.40-7.30 (m, 1H), 7.15-7.00 (m,

3H), 4.30-4.15 (m, 5H), 3.90-3.75 (m, 2H), 2.95-2.85 (m, 4H), 2.65-2.55 (m, 2H), 1.90-1.70 (m, 5H), 1.50-1.30 (m, 5H), 1.05-0.95 (m, 3H).

3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-1-piperidyl]propanehydroxamic acid (48g)

Compound **47g** (80 mg, 0.13 mmol) was dissolved in HCl/EtOAc (2.0 M, 10 mL) and stirred at room temperature for 1 hour. Then the mixture was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **48g** (36.1 mg, 56%) as a yellow solid; m.p.: 144-145 ^{o}C. ^{1}H NMR (MeOD, 400 MHz): δ 7.75-7.70 (m, 1H), 7.40-7.30 (m, 1H), 7.15-7.05 (m, 1H), 4.35-4.15 (m, 5H), 3.60-3.45 (m, 2H), 3.40-3.30 (m, 2H), 3.00-2.75 (m, 4H), 2.65-2.50 (m, 4H), 1.95-1.65 (m, 5H), 1.60-1.40 (m, 5H), 1.05-0.95 (m, 3H). ESI-MS *m/z* 497.2 $[M+H]^+$ calc. for $C_{26}H_{36}N_6O_4$

2-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclohexyl]ethanehydroxamic acid (48h)

Compound **47h** (200 mg, 0.35 mmol) was dissolved in HCl/EtOAc (2.0 M, 10 mL) and the mixture was stirred at room temperature for 1 hour. Then, the solution was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **48h** (45 mg, 27%) as a white solid; m.p.: 165-166 ^{o}C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15-8.14 (m, 1H), 7.25-7.15 (m, 1H), 6.95-6.91 (m, 1H), 4.35-4.15 (m, 5H), 3.05-2.85 (m, 2H), 2.63-2.55 (m, 1H), 2.55-2.48 (m, 2H), 2.30-2.20 (m, 1H), 2.20-1.95 (m, 2H), 1.95-1.80 (m, 2H), 1.80-1.65 (m, 4H), 1.60-1.35 (m, 6H), 1.35-1.20 (m, 1H), 1.10-0.85 (m, 6H). ESI-MS *m/z* 482.2 $[M+H]^+$ calc. for $C_{26}H_{35}N_5O_4$

4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclohexanecarbohydroxamic acid (48i1 and 48i2)

Compound **47i** (100 mg, 0.18 mmol) was dissolved in HCl/EtOAc (2.0 M, 10 mL) and stirred at room temperature for 1 hour. Then the solution was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compounds **48i1** (5.1 mg, 6.2%) and **48i2** (10.2 mg, 12%) as a white solids; m.p.: 176.5-177.5 ^{o}C and 209-210 ^{o}C. **48i1**: ^{1}H NMR (DMSO, 400 MHz): δ 11.92 (s, 1H), 10.33 (s, 1H), 8.60 (s, 1H), 7.44-7.43 (m, 1H), 7.28-7.25 (m, 1H), 7.07-7.05 (m, 1H), 4.15 (s, 3H), 4.10-4.08 (m, 2H), 2.80-2.70 (m, 2H), 2.60-2.50 (m, 3H), 1.80-1.60 (m, 5H), 1.50-1.30 (m, 6H), 1.30-1.20 (m, 3H), 1.00-0.80 (m, 3H). ESI-MS *m/z* 468.2 $[M+H]^{+}$ calc. for $C_{25}H_{33}N_5O_4$. Purity: 99.40%. **48i2**: ^{1}H NMR (DMSO, 400 MHz): δ 11.92 (s, 1H), 10.33 (s, 1H), 8.60 (s, 1H), 7.44-7.43 (m, 1H), 7.28-7.25 (m, 1H), 7.07-7.05 (m, 1H), 4.15 (s, 3H), 4.10-4.08 (m, 2H), 2.80-2.70 (m, 2H), 2.50-2.40 (m, 3H), 1.80-1.55 (m, 6H), 1.55-1.40 (m, 2H), 1.40-1.25 (m, 4H), 1.00-0.85 (m, 5H). ESI-MS *m/z* 468.2 $[M+H]^{+}$ calc. for $C_{25}H_{33}N_5O_4$. Purity: 95.47%.

3-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclopentanecarbohydroxamic acid (48j)

Compound **47j** (300 mg, 0.56 mmol) was dissolved in HCl/EtOAc (2.0 M, 10 mL) and stirred at room temperature for 1 hour. Then, the solution was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **48j** (16.4 mg, 7%) as a white solid; m.p.: 102-103 ^{o}C. ^{1}H NMR (DMSO, 400 MHz): δ 11.95 (s, 1H), 10.36 (s, 1H), 7.45-7.43 (m, 1H), 7.30-7.28 (m, 1H), 7.06-7.04 (m, 1H), 4.10-4.08 (m, 5H), 3.51-3.41 (m,

2H), 3.36-3.35 (m, 1H), 2.79-2.75 (m, 2H), 2.60-2.50 (m, 2H), 1.76-1.71 (m, 6H), 1.33-1.29 (m, 4H), 0.95-0.91 (m, 3H). ESI-MS *m/z* 454.2 $[M+H]^+$ calc. for $C_{24}H_{31}N_5O_4$. Purity 98.89%

2-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]cyclopropanecarbohydroxamic acid (48k)

Compound **47k** (93 mg, 0.183 mmol) was dissolved in HCl/EtOAc (1.0 M, 20 mL) and stirred at room temperature for 2 hours. Then, the mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain the desired compound **48k** (32 mg, 41%). ^{1}H NMR (MeOD, 400 MHz): δ 7.74 (s, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.11 (d, *J* = 8.8 Hz, 1H), 4.23-4.17 (m, 5H), 2.91-2.87 (m, 2H), 2.71-2.65 (m, 2H), 1.84-1.79 (m, 2H), 1.65 (m, 1H), 1.46-1.43 (m, 3H), 1.40-1.38 (m, 1H), 1.14 (m, 1H), 1.03-0.99 (m, 3H), 0.81 (m, 1H). ESI-MS *m/z* 426.2 $[M+H]^+$ calc. for $C_{22}H_{27}N_5O_4$

1-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]piperidine-4-carbohydroxamic acid (48l)

A solution of compound **47l** (110 mg, 0.199 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then, the solution was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **48l** (26.7 mg, 29%) as a white solid; m.p.: 167-168 ^{o}C. ^{1}H NMR (MeOD, 400 MHz): δ 8.04 (s, 1H), 7.66-7.64 (d, *J* = 8.0 Hz, 1H), 7.29-7.27 (d, *J* = 9.2 Hz, 1H), 4.35 (s, 2H), 4.29-4.26 (m, 2H), 4.23 (s, 3H), 3.69-3.57 (m, 2H), 3.06 (s, 2H), 2.89-2.80 (m, 2H), 2.42 (s, 1H), 2.00-1.94 (m, 4H), 1.85-

1.76 (m, 2H), 1.49-1.45 (m, 3H), 1.02-0.98 (m, 3H). ESI-MS *m/z* 469.2 $[M+H]^+$ calc. for $C_{24}H_{32}N_6O_4$

1-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]azetidine-3-carbohydroxamic acid (48m)

A solution of compound **47m** (300 mg, 0.57 mmol) in HCl/EtOAc (4.0 M, 15 mL) was stirred at room temperature for 1 hour. Then, the reaction mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **48m** (14.3 mg, 6%) as a white solid; m.p.: 132.5-133.5 ^oC. ^1H NMR (DMSO, 400 MHz): δ 12.07 (s, 1H), 10.81 (s, 1H), 10.54-10.40 (m, 1H), 7.73-7.61 (m, 1H), 7.60-7.58 (m, 1H), 7.22-7.20 (m, 1H), 4.39-4.14 (m, 2H), 4.14-4.01 (m, 9H), 3.41-3.37 (m, 1H), 2.79-2.75 (m, 2H), 1.77-1.70 (m, 2H), 1.33-1.30 (m, 3H), 0.96-0.92 (m, 3H). ESI-MS *m/z* 441.2 $[M+H]^+$ calc. for $C_{22}H_{28}N_6O_4$

3-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]cyclobutanecarbohydroxamic acid (48n)

To a solution of compound **46n** (512 mg, 1.25 mmol) in DMF (40 mL) were added BOP (995 mg, 2.25 mmol), DIEA (413 mg, 3.2 mmol) and $NH_2OH{\cdot}HCl$ (152 mg, 2.2 mmol) and the resulting mixture was stirred at 80 ^oC overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **48n** (320 mg, 60%) as a yellow solid; m.p.: 152.5-153.5 ^oC. ^1H NMR (MeOD, 400 MHz): δ 7.81-7.77 (m, 1H), 7.48-7.43 (m, 1H),

7.15-7.12 (m, 1H), 4.23-4.18 (m, 5H), 3.80-3.48 (m, 1H), 3.02-2.96 (m, 1H), 2.91-2.87 (m, 2H), 2.50-2.44 (m, 2H), 2.41-2.39 (m, 2H), 1.85-1.79 (m, 2H), 1.47-1.42 (m, 3H), 1.03-0.99 (m, 3H). ESI-MS *m/z* 426.2 $[M+H]^+$ calc. for $C_{22}H_{27}N_5O_4$

2-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]cyclopropanecarbohydroxamic acid (48o)

A solution of compound **47o** (1.35 g, 2.7 mmol) in HCl/EtOAc (0.2 N, 50 mL) was stirred at 0 ^oC for 3 hours. Then, the solution was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **48o** (400 mg, 36%). ^1H NMR (MeOD, 400 MHz): δ 7.63 (s, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 1H), 4.23-4.16 (m, 5H), 2.89 (t, *J* = 7.6 Hz, 2H), 2.44 (m, 1H), 1.84-1.79 (m, 2H), 1.72-1.70 (m, 1H), 1.51 (m, 1H), 1.46-1.42 (m, 3H), 1.29 (m, 1H), 1.01 (t, *J* = 7.2 Hz, 3H). ESI-MS *m/z* 412.1 $[M+H]^+$ calc. for $C_{21}H_{25}N_5O_4$

Ethyl 3-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]phenyl]propanoate (49a)

To a solution of compound **25** (1.20 g, 2.74 mmol) in 1,4-dioxane (30 mL) were added ethyl 3-[4-(bromomethyl)phenyl]propanoate (**Int. 18**, synthesis described in supporting information) (670 mg, 2.48 mmol), K_2CO_3 (1.13 g, 8.18 mmol in 2.0 mL water) and $Pd(PPh_3)_4$ (287 mg, 0.25 mmol) and the mixture was stirred at 80 ^oC overnight under N_2 protection. Then, the mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by column chromatography to give pure compound

49a (830 mg, 67%) as a yellow oil. ESI-MS *m/z* 503 $[M+H]^+$ calc. for $C_{29}H_{34}N_4O_4$. This intermediate was used in the next step without further characterization.

Methyl (*E*)-3-[4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]phenyl]prop-2-enoate (49b)

To a solution of compound **25** (300 mg, 0.685 mmol) in 1,4-dioxane/H_2O (5:2, 28 mL) were added methyl (*E*)-3-[4-(bromomethyl)phenyl]prop-2-enoate (190 mg, 0.75 mmol), $Pd(PPh_3)_4$ (79 mg, 0.067 mmol) and K_2CO_3 (284 mg, 2.06 mmol) and the mixture was stirred at 85 ^oC for 1 hour under MW. Then, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **49b** (200 mg, 60%) as a white solid. ESI-MS *m/z* 487.2 $[M+H]^+$ calc. for $C_{28}H_{30}N_4O_4$. This intermediate was used in the next step without further characterization.

Ethyl 2-[4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]cyclohexyl]acetate (49c)

To a solution of compound **25** (500 mg, 1.14 mmol) in 1,4-dioxane (20 mL) were added ethyl 2-[4-(trifluoromethylsulfonyloxy)cyclohex-3-en-1-yl]acetate (**Int. 19**, synthesis described in supporting information) (384 mg, 1.25 mmol), K_2CO_3 (473 mg, 3.42 mmol in 2 mL water) and $Pd(PPh_3)_4$ (132 mg, 0.11 mmol) and the mixture was stirred at 80 ^oC overnight under N_2 protection. Then, the reaction mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude compound which was purified by column chromatography to give pure intermediate ethyl 2-[4-[4-ethoxy-3-(1-methyl-7-oxo-3-

propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]cyclohex-3-en-1-yl]acetate (385 mg, 70%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.09 (s, 1H), 8.44 (s, 1H), 7.47-7.45 (d, *J* = 8.8 Hz, 1H), 6.99-6.97 (d, *J* = 8.8 Hz, 1H), 6.10 (s, 1H), 4.29-4.15 (m, 7H), 2.98-2.94 (m, 3H), 2.52 (s, 2H), 2.36-2.35 (d, *J* = 7.2 Hz, 1H), 2.23-2.14 (m, 1H), 2.01-1.82 (m, 5H), 1.61-1.57 (m, 3H), 1.31-1.27 (m, 3H), 1.07-1.03 (m, 3H). ESI-MS *m/z* 479 $[M+H]^+$ calc. for $C_{27}H_{34}N_4O_4$. To a solution of this intermediate (245 mg, 0.513 mmol) in MeOH (20 mL) was added Pd/C (150 mg) at H_2 atmosphere (1 atm) and the mixture was stirred at room temperature for 1 hour until LC-MS showed the starting material was consumed completely. Then the reaction mixture was filtered and the filtrate was concentrated to give compound **49c** (150 mg, 61%) as a white solid. ESI-MS *m/z* 481 $[M+H]^+$ calc. for $C_{27}H_{36}N_4O_4$. This intermediate was used in the next step without further characterization.

Methyl 6-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]pyridine-3-carboxylate (49d)

To a solution of compound **25** (300 mg, 0.68 mmol) in 1,4-dioxane/H_2O (5:2, 28 mL) were added methyl 6-chloropyridine-3-carboxylate (129 mg, 0.75 mmol), $Pd(PPh_3)_4$ (79 mg, 0.068 mmol) and K_2CO_3 (284 mg, 2.06 mmol) and the solution was stirred at 85 ^oC for 1 hour under MW. Then, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **49d** (150 mg, 49%) as a white solid. ESI-MS *m/z* 448.2 $[M+H]^+$ calc. for $C_{24}H_{25}N_5O_4$. This intermediate was used in the next step without further characterization.

Methyl 5-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]pyridine-2-carboxylate (49e)

To a solution of compound **25** (300 mg, 0.68 mmol) in 1,4-dioxane /H_2O (5:2, 28 mL) were added methyl 5-bromopyridine-2-carboxylate (162 mg, 0.75 mmol), $Pd(PPh_3)_4$ (79 mg, 0.068 mmol) and K_2CO_3 (284 mg, 2.06 mmol) and the mixture was stirred at 85 ^oC for 1 hour under MW. Then the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **49e** (170 mg, 56%) as a white solid. ESI-MS *m/z* 448 $[M+H]^+$ calc. for $C_{24}H_{25}N_5O_4$. This intermediate was used in the next step without further characterization.

Methyl 5-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]furan-2-carboxylate (49f)

To a solution of compound **25** (300 mg, 0.68 mmol) in 1,4-dioxane/H_2O (5:2, 28 mL) were added methyl 5-bromofuran-2-carboxylate (**Int. 20**, synthesis described in supporting information) (153 mg, 0.75 mmol), $Pd(PPh_3)_4$ (79 mg, 0.068 mmol) and K_2CO_3 (284 mg, 2.06 mmol) and the mixture was stirred at 85 ^oC for 1 hour under MW. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography to give compound **49f** (140 mg, 48%) as a white solid. ESI-MS *m/z* 437.2 $[M+H]^+$ calc. for $C_{28}H_{24}N_4O_5$. This intermediate was used in the next step without further characterization.

3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]phenyl]propanoic acid (50a)

To a solution of compound **49a** (830 mg, 1.65 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (694 mg, 16.54 mmol) and the reaction mixture was stirred at 40 ^oC overnight until LC-MS showed the starting material was consumed completely. Then, the solution was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **50a** (780 mg, 99%) as a white solid. ESI-MS *m/z* 475 $[M+H]^+$ calc. for $C_{27}H_{30}N_4O_4$. This intermediate was used in the next step without further characterization.

(*E*)-3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]phenyl]prop-2-enoic acid (50b)

To a solution of compound **49b** (200 mg, 0.41 mmol) in THF/MeOH/H_2O (3:3:2, 16 mL) was added LiOH·H_2O (172 mg, 4.1 mmol) and the resulting mixture was stirred at room temperature overnight. Then the mixture was diluted with water and adjusted pH to 6~7 with 1 N HCl. The solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the desired product **50b** (180 mg, 93%). ESI-MS *m/z* 473.2 $[M+H]^+$ calc. for $C_{27}H_{28}N_4O_4$. This intermediate was used in the next step without further characterization.

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]cyclohexyl]acetic acid (50c)

To a solution of compound **49c** (150 mg, 0.31 mmol) in MeOH/THF/H_2O (1:3:1, 15 mL) was added LiOH·H_2O (130 mg, 3.10 mmol) and the reaction mixture was stirred at

room temperature overnight. Then, the solution was concentrated, diluted with H_2O and adjusted pH to 1-2 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **50c** (130 mg, 92%) as a white solid. ESI-MS *m/z* 453 $[M+H]^+$ calc. for $C_{25}H_{32}N_4O_4$. This intermediate was used in the next step without further characterization.

6-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]pyridine-3-carboxylic acid (50d)

To a solution of compound **49d** (150 mg, 0.34 mmol) in THF/MeOH/H_2O (3:3:2, 16 mL) was added LiOH·H_2O (143 mg, 3.4 mmol) and the resulting mixture was stirred at room temperature overnight. Then the mixture was diluted with water and adjusted pH to 6~7 with 1 N HCl. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the desired product **50d** (110 mg, 75%). ESI-MS *m/z* 434.2 $[M+H]^+$ calc. for $C_{23}H_{23}N_5O_4$. This intermediate was used in the next step without further characterization.

5-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]pyridine-2-carboxylic acid (50e)

To a solution of compound **49e** (170 mg, 0.38 mmol) in THF/MeOH/H_2O (3:3:2, 16 mL) was added LiOH·H_2O (160 mg, 3.8 mmol) and the resulting mixture was stirred at room temperature overnight. Then, mixture was diluted with water and adjusted pH to 6~7 with 1 N HCl. The solution was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the

desired product **50e** (120 mg, 72%). ESI-MS *m/z* 434.2 $[M+H]^+$ calc. for $C_{23}H_{23}N_5O_4$. This intermediate was used in the next step without further characterization.

5-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]furan-2-carboxylic acid (50f)

To a solution of compound **49f** (140 mg, 0.32 mmol) in THF/MeOH/H_2O (3:3:2, 16 mL) was added LiOH·H_2O (134 mg, 3.2 mmol) and the resulting mixture was stirred at room temperature overnight. Then the solution was diluted with water and adjusted pH to 6~7 with 1 N HCl. Then the mixture was extracted with EtOAc and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the desired product **50f** (100 mg, 74%). ESI-MS *m/z* 423.2 $[M+H]^+$ calc. for $C_{22}H_{22}N_4O_5$. This intermediate was used in the next step without further characterization.

3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]phenyl]-*N*-tetrahydropyran-2-yloxy-propanamide (51a)

To a solution of compound **50a** (780 mg, 1.64 mmol) in DMF (10 mL) were added EDC·HCl (634 mg, 3.30 mmol), HOBt (446 mg, 3.30 mmol), $THPONH_2$ (385mg, 3.30 mmol) and NMM (500 mg, 4.95 mmol) and the mixture was stirred at room temperature overnight. Then the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **51a** (350 mg, 37%) as a white solid. ESI-MS *m/z* 574 $[M+H]^+$ calc. for $C_{32}H_{39}N_5O_5$. This intermediate was used in the next step without further characterization.

(*E*)-3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]phenyl]-*N*-tetrahydropyran-2-yloxy-prop-2-enamide (51b)

To a solution of compound **50b** (180 mg, 0.38 mmol) in DMF (20 mL) were added EDC·HCl (150 mg, 0.76 mmol), HOBt (100 mg, 0.76 mmol), $THPONH_2$ (95 mg, 0.81 mmol) and NMM (120 mg, 1.18 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give compound **51b** (120 mg, 55%) as a pale yellow solid. ESI-MS *m/z* 572.2 $[M+H]^+$ calc. for $C_{32}H_{37}N_5O_5$. This intermediate was used in the next step without further characterization.

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]cyclohexyl]-*N*-tetrahydropyran-2-yloxy-acetamide (51c)

To a solution of compound **50c** (130 mg, 0.288 mmol) in DMF (10 mL) were added EDC·HCl (110 mg, 0.57 mmol), HOBt (78 mg, 0.57 mmol), $THPONH_2$ (68 mg, 0.57 mmol) and NMM (87 mg, 0.86 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give pure compound **51c** (141 mg, 89%) as a white solid. ESI-MS *m/z* 552 $[M+H]^+$ calc. for $C_{30}H_{41}N_5O_5$. This intermediate was used in the next step without further characterization.

6-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-*N*-tetrahydropyran-2-yloxy-pyridine-3-carboxamide (51d)

To a solution of compound **50d** (110 mg, 0.25 mmol) in DMF (20 mL) were added EDC·HCl (96 mg, 0.5 mmol), HOBt (68 mg, 0.5 mmol), $THPONH_2$ (60 mg, 0.5 mmol) and NMM (80 mg, 0.79 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give compound **51d** (100 mg, 75%) as a pale yellow solid. ESI-MS *m/z* 533.2 $[M+H]^+$ calc. for $C_{28}H_{32}N_6O_5$. This intermediate was used in the next step without further characterization.

5-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-*N*-tetrahydropyran-2-yloxy-pyridine-2-carboxamide (51e)

To a solution of compound **50e** (120 mg, 0.28 mmol) in DMF (20 mL) were added EDC·HCl (107 mg, 0.56 mmol), HOBt (76 mg, 0.56 mmol), $THPONH_2$ (66 mg, 0.56 mmol) and NMM (85 mg, 0.84 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product which was purified by preparative TLC to give compound **51e** (100 mg, 68%) as a pale yellow solid. ESI-MS *m/z* 533.2 $[M+H]^+$ calc. for $C_{28}H_{32}N_6O_5$. This intermediate was used in the next step without further characterization.

5-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]-*N*-tetrahydropyran-2-yloxy-furan-2-carboxamide (51f)

To a solution of compound **50f** (100 mg, 0.24 mmol) in DMF (20 mL) were added EDC·HCl (92 mg, 0.48 mmol), HOBt (65 mg, 0.48 mmol), $THPONH_2$ (56 mg, 0.48 mmol) and NMM (73 mg, 0.72 mmol) and the mixture was stirred at room temperature overnight. Then, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 filtered and concentrated to give the crude product which was purified by preparative TLC to give compound **51f** (80 mg, 64%) as a pale yellow solid. ESI-MS *m/z* 522.2 $[M+H]^+$ calc. for $C_{27}H_{31}N_5O_6$. This intermediate was used in the next step without further characterization.

3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]phenyl]propanehydroxamic acid (52a)

A solution of compound **51a** (350 mg, 0.61 mmol) in HCl/1,4-dioxane (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then, the mixture was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **52a** (88 mg, 29%) as a white solid; m.p.: 190-191 ^oC. ^1H NMR (DMSO, 400 MHz): δ 11.94 (s, 1H), 10.36 (s, 1H), 8.69 (s, 1H), 7.48 (s, 1H), 7.30-7.29 (m, 1H), 7.16-7.07 (m, 5H), 4.14 (s, 3H), 4.08-4.06 (d, 2H), 3.89 (s, 2H), 2.77-2.74 (m, 4H), 2.23-2.20 (m, 2H), 1.75-1.70 (m, 2H), 1.31-1.28 (m, 3H), 0.95-0.91 (m, 3H). ESI-MS *m/z* 490.2 $[M+H]^+$ calc. for $C_{27}H_{31}N_5O_4$

(*E*)-3-[4-[[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]phenyl]prop-2-enehydroxamic acid (52b)

A solution of compound **51b** (120 mg, 0.21 mmol) in HCl/EtOAc (2.0 M, 10 mL) was stirred at room temperature for 1 hour. Then the mixture was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **52b** (24.3 mg, 23%) as a white solid; m.p.: 185-186 ^{o}C. ^{1}H NMR (DMSO, 400 MHz): δ 11.92 (s, 1H), 10.71 (s, 1H), 8.99 (s, 1H), 7.51-7.49 (m, 3H), 7.49-7.47 (m, 1H), 7.47-7.28 (m, 3H), 7.09-7.07 (m, 1H), 6.42-6.38 (m, 1H), 4.14-4.06 (m, 5H), 3.97 (s, 2H), 2.77-2.74 (m, 2H), 1.75-1.70 (m, 2H), 1.32-1.29 (m, 3H), 0.94-0.90 (m, 3H). ESI-MS *m/z* 488.1 $[M+H]^+$ calc. for $C_{27}H_{29}N_5O_4$

2-[4-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]cyclohexyl]ethanehydroxamic acid (52c)

A solution of compound **51c** (141 mg, 0.256 mmol) in HCl/EtOAc (4.0 M, 5 mL) was stirred at room temperature for 1 hour. Then the solution was concentrated to give the crude compound which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **52c** (11.0 mg, 9%) as a red solid; m.p.: 156-157 ^{o}C. ^{1}H NMR (DMSO, 400 MHz): δ 11.94 (s, 1H), 10.38 (s, 1H), 7.49 (s, 1H), 7.35 (m, 1H), 7.08 (m, 1H), 4.15-4.00 (m, 6H), 3.47 (m, 1H), 2.77 (m, 2H), 2.15-2.00 (m, 2H), 1.75-1.50 (m, 10H), 1.30 (m, 3H), 0.94 (m, 3H). ESI-MS *m/z* 468.3 $[M+H]^+$ calc. for $C_{25}H_{33}N_5O_4$. Purity 98.64%.

6-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]pyridine-3-carbohydroxamic acid (52d)

A solution of compound **51d** (100 mg, 0.19 mmol) in HCl/EtOAc (2.0 M, 10 mL) was stirred at room temperature for 1 hour. Then, the solution was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in

supporting information) to obtain pure compound **52d** (11.1 mg, 13%) as a white solid; m.p.: 210-211 ^{o}C. ^{1}H NMR (DMSO, 400 MHz): δ 12.16 (s, 1H), 11.41 (s, 1H), 8.97 (s, 1H), 8.36-8.06 (m, 4H), 7.29-7.27 (m, 1H), 4.22-4.17 (m, 5H), 2.81-2.77 (m, 2H), 1.78-1.72 (m, 2H), 1.36-1.32 (m, 3H), 0.96-0.92 (m, 3H). ESI-MS *m/z* 449.1 $[M+H]^+$ calc. for $C_{23}H_{24}N_6O_4$

5-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]pyridine-2-carbohydroxamic acid (52e)

A solution of compound **51e** (100 mg, 0.19 mmol) in HCl/EtOAc (2.0 M, 10 mL) was stirred at room temperature for 1 hour. Then, the solution was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **52e** (14 mg, 16%) as a white solid; m.p.: 182-183 ^{o}C. ^{1}H NMR (DMSO, 400 MHz): δ 12.18 (s, 1H), 11.45 (s, 1H), 9.10 (s, 1H), 8.90 (s, 1H), 8.28-8.25 (m, 1H), 8.05-7.92 (m, 3H), 7.32-7.30 (m, 1H), 4.22-4.17 (m, 5H), 2.80-2.77 (m, 2H), 1.78-1.72 (m, 2H), 1.36-1.32 (m, 3H), 0.96-0.92 (m, 3H). ESI-MS *m/z* 449.1 $[M+H]^+$ calc. for $C_{23}H_{24}N_6O_4$. Purity 94.62%.

5-[4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]furan-2-carbohydroxamic acid (52f)

A solution of compound **51f** (80 mg, 0.15 mmol) in HCl/EtOAc (2.0 M, 10 mL) was stirred at room temperature for 1 hour. Then, the mixture was concentrated to give the crude product which was purified by preparative HPLC (method 1 described in supporting information) to obtain pure compound **52f** (13 mg, 20%) as a white solid; m.p.: 126-127 ^{o}C. ^{1}H NMR (DMSO, 400 MHz): δ 12.20 (s, 1H), 11.27 (s, 1H), 9.12 (s, 1H), 8.03-7.97 (m, 2H), 7.25-7.01 (m, 3H), 4.17-4.13 (m, 5H), 2.81-2.77 (m, 2H) , 1.77-

1.71 (m, 2H), 1.32-1.29 (m, 3H), 0.96-0.92 (m, 3H). ESI-MS *m/z* 438.1 $[M+H]^+$ calc. for $C_{22}H_{23}N_5O_5$

Biological Test Methods. *In-vitro* studies

Acetyl-Histone H3 Lysine 9 (H3K9ac) cellular detection assay (AlphaLisa technology)

Briefly, 2000 cells (SH-SY5Y) were plated in a poly-D-lysine- treated 384-well plate. Cells were incubated with different concentrations of compounds **4** and **1** during 2 h. After incubation, the medium was removed and cells were lysed, histones were extracted and histone carrying the acetylation mark was detected following the manufacturer's instructions (PerkinElmer; Cat number AL714 A/C kit assay). Signal of acetylation mark was obtained after 18 h of dark incubation at room temperature and was normalized by the unmodified histone signal and calculated as folds over basal levels, considered as those obtained in the absence of assayed compounds.

HDACs and PDEs enzyme activity assays

HDACs enzyme activities were measured with a specific fluorescence-labelled substrate (BPS Biosciences, Cat # 50037) after its deacetylation by HDACs. The fluorogenic substrate, containing an acetylated lysine side chain, can be deacetylated and then sensitized to subsequent treatment with the lysine developer, which produces a fluorophore that can be measured with a fluorescence plate reader. Human HDAC1 (GenBank Accession No. NM_004964), full length, with C-terminal His-tag and C-terminal Flag-tag, was obtained from BPS Biosciences (Cat. # 50051). Human HDAC2 (GenBank Accession No. NM_001527), full length, with C-terminal His-tag was

obtained from BPS Biosciences (Cat. # 50002). Human HDAC3 (GenBank Accession No. NM_003883), full length, with C-terminal His-tag and human NCOR2, N-terminal GST-tag was obtained from BPS Biosciences (Cat. # 50003). Human HDAC6 (GenBank Accession number No. BC069243), full length with N-terminal GST tag was obtained from BPS Biosciences (Cat. # 50006). 5 µL of vehicle or tested compound 10 x concentrated prepared in assay buffer (BPS Biosciences, Cat # 50031) were added in black 96 well plates (final volume of 100 µL). The final percentage of DMSO was 1%. 5 µL of HDAC1 (4 µg/mL) or HDAC2 (15 µg/mL) or HDAC3 (10 µg/mL) or HDAC6 (36 µg/mL) enzyme in assay buffer was added (final HDAC1, HDAC2, HDAC3 and HDAC6 concentration of 0.4 µg/mL, 1.5 µg/mL, 0.1 µg/mL and 3.6 µg/mL respectively) and the reaction was started by the addition of 40 µL of reaction mixture containing 0.125 mg/mL BSA (final concentration of 0.1 mg/mL) and 12.5 µM of fluorogenic HDACs substrate (final concentration of 10 µM). The reaction was incubated for 30 min at 37ºC. After incubation, the reaction was stopped with 50 µL of lysine assay developer (BPS Biosciences, Cat # 50030). After incubation during 20 minutes at room temperature, the fluorescence of each well was measured at 355nm excitation and 460nm emission in a Mithras plate reader (Berthold). Positive control was obtained in the presence of the vehicle of the compounds. Negative control was obtained in the absence of HDAC enzyme activity. A best fit curve was fitted using GraphPad Prism 5 to derive the half maximal inhibitory concentration (IC_{50}) from this curve.

PDE5A and PDE9A enzyme activity was measured with the HTRF cGMP assay kit from CisBio (CisBio, Cat.#62GM2PEB), which determines the amount of cGMP present in the reaction. Human PDE5A1 (GenBank Accession No. NM_001083) or human PDE9A isoform b (GenBank Accession No. NM_001083), full length, with N-

terminal GST tag was obtained from BPS Biosciences (Cat. # 60050 or # 60090). 2.5 µL of vehicle or tested compound 4 x concentrated prepared in assay buffer (50 mM Tris-HCl, 6 mM $MgCl_2$, pH 7.4) were added in 384 well plates (final volume of 20 µL). The final percentage of DMSO was 0.5%; 2.5 µL of PDE5A (7 µg/mL) or of PDE9A (0.2 µg/mL) enzyme in assay buffer was added (final PDE5A concentration 1.75 µg/mL or final PDE9A concentration 0.05 µg/mL) and the reaction was started by the addition of 5 µL of substrate cGMP (4 x concentrated) to a final concentration of 100 nM cGMP. The reaction was incubated for 30 min at 37ºC. After incubation, the reaction was stopped with 5 µL of cGMP-D2 (cGMP labelled with the dye D2) and 5 µL of Mab anti-cGMP labelled with cryptate (cGMP-cryptate). After incubation during 1 hour at room temperature, the fluorescence of each well was measured at 665nm excitation and 620nm emission in an Envision plate reader (PerkinElmer) and the results were expressed as the 665 nm/ 620nm ratio. Positive control was obtained in the presence of the vehicle of the compounds. Negative control was obtained in the absence of cGMP and labelled cGMP-D2 cyclic nucleotide. A best fit curve was fitted using GraphPad Prism 5 to derive the half maximal inhibitory concentration (IC_{50}) from this curve.

PDE3A and PDE6C enzyme activity assays were carried out at BPS Bioscience (https://bpsbioscience.com/)

Cytotoxicity in THLE-2 cells

Cytotoxic effects of assayed compounds were tested using the immortalized human liver cell line THLE-2 (ATCC CRL-2706), cultured in BEGM medium (Clonetics #CC-4175). Medium was completed by adding 0.7µg/mL phosphoethanolamine, 0.5 ng/mL epidermal growth factor, antibiotics (penicillin and streptomycin) and 10% fetal bovine serum (FBS). Cells were plated in 96-well black microplates at 10,000 cells/well and

incubated at 37 ºC (5% CO_2, 95% humidity) for 24 h. Test compounds were solubilized in 100% DMSO and then diluted with cell culture medium containing 10% DMSO. The final concentrations of the test compounds (1% DMSO) ranged from 0-100µM in a final volume of 200 µL. After 72 h, cell viability in each well was determined by measuring the concentration of cellular adenosine triphosphate (ATP) using the VialightTM Plus Cell Proliferation/Cytotoxicity Kit as described by the manufacturer (Cambrex, East Rutherford, NJ). After addition of cell lysis buffer, test plate was incubated for 45 min at room temperature (orbital shaker). ATP monitoring solution was added and ATP concentration determined by reading luminescence using a Envision plate reader (PerkinElmer). The percentage of viable cells relative to the non-drug treated controls was determined for each well and LC_{50} values were calculated as concentrations projected to kill 50% of the cells following a 72 h exposure.

Cytotoxicity in Neurons Glia Cells

Cytotoxic effects of assayed compounds were tested using primary cultures of mice brain embryo tissue. Cells growth in 96-well black microplates were incubated at 37 ºC (5% CO_2, 95% humidity) for 5 days to permit neurons formation. After that, 100µL/well of medium and studied compounds was added. Test compounds were solubilized in 100% DMSO at a concentration curve way and then diluted with cell culture medium containing 10% DMSO. The final concentrations of the test compounds (1% DMSO) ranged from 0-100µM in a final volume of 200µL. Microplates were maintained at 37ºC (5% CO_2, 95% humidity) during 3 days. Following this 72 h exposure to test compounds, cell viability in each well was determined by measuring the concentration of cellular adenosine triphosphate (ATP) using the ATP1Step Kit as described by the manufacturer (Perkin-Elmer). In a typical procedure, 50µL of cell

reagent is added to all wells of each test plate followed by incubation for 10 min at room temperature on an orbital shaker. ATP concentration was determined by reading chemical luminescence using the Envision plate reader (PerkinElmer). The percentage of viable cells relative to the non-drug treated controls was determined for each well and LC_{50} values were calculated as concentrations projected to kill 50% of the cells following a 72 h exposure.

PAMPA Permeability

The permeability of compounds was evaluated with the parallel artificial membrane permeation assay (PAMPA) as an in vitro model of passive diffusion. Donor solutions of test compounds (180 µL. 50 µM in PBS/ETOH 70:30) were added to each well of the donor plate, whose PVDF membrane was precoated with 4 µL of a 20 $mg \times mL^{-1}$ PBL/dodecane mixture. PBS/EtOH (180 µL) was added to each well of the PTFE acceptor plate. The donor and acceptor plates were combined together and incubated for 18 h at 20 ºC without shaking. In each plate, compounds and controls were tested in duplicate. Drug concentration in the acceptor, the donor, and the reference wells was determined using the UV plate reader with 130 µL of acceptor and donor samples. Permeability rates (Pe in nm s^{-1}) were calculated with Equation (1). The permeability rate of each compound is the averaged value of three independent measurements.

Equation (1) $P_e = C \times \left(-ln\left(1 - \frac{[drug]_{acceptor}}{[drug]_{equilibrium}}\right)\right) \times 10^7$;

where $C = \frac{V_D \times V_A}{(V_D + V_A) \times Area \times time}$; V_D = 0.18 mL; V_A = 0.18 mL; Area = 0.32 cm^2; time = 64800 s; D_F = 180/130; $[drug]_{equilibrium} = ([drug]_{donor} \times V_D + [drug]_{acceptor} \times V_A) / (V_D + V_A)$; $[drug]_{donor} = (A_a/A_i * D_F)_{donor}$; $[drug]_{acceptor} = (A_a/A_i * D_F)_{acceptor}$; $A_{a\ donor} = Abs_{donor} - Abs_{vehicle}$; $A_{a\ acceptor} = Abs_{acceptor} - Abs_{vehicle}$, $Ai = Abs_{withoutPBL} - Abs_{vehicle}$.

PDE and HDAC functional response in vitro

To analyze the functional activity of the different compounds we used primary neuronal cultures and human neuroblastoma SH-SY5Y cell line. Primary neuronal cultures were obtained from the hippocampus and cortex of embryonic day 16 (E16) wild type (WT) mice and used at 15 days *in vitro* (DIV).[54] Cells were incubated with the different compounds and after incubation (30 min or 2h), medium was removed and cells were lysed in a buffer containing Tris HCl 10 mM, NaF 1 mM, $NaVO_4$ 0.1 mM , sodium dodecyl sulfate (SDS) 2% and protease inhibitors.

Biological Test Methods. *In-vivo* studies

Determination of brain to plasma concentration ratio

Compound **37** was measured in plasma and brain samples using an Acquity UPLC system (Waters, Manchester, UK) coupled to a Xevo-TQ MS triple quadrupole mass spectrometer with electrospray ionization (ESI) source. Plasma and brain samples were collected at different times (0.25, 0.5 and 1 hour). Compound **37** was injected (40 mg/kg, i.p.) to mice (n=3 per time point). Three control mice were sacrificed 15 min after the administration of vehicle solution. Compound solutions were prepared by dissolving the solid in DMSO and this solution was diluted with a mixture of Tween 20 and 0.9% NaCl up to a final composition of 1:1:8 (v:v:v, DMSO/Tween 20/saline). Blood was collected at the different time points in EDTA-coated tubes and centrifuged at 2500 rpm for 5 min at 4˚ C to obtain the plasma. The brain was removed following whole body perfusion with saline. All plasma and brain samples were stored at -80˚ C until further analysis.

Chromatographic separation was performed by gradient elution at 0.45 mL/min using an Acquity UPLC BEH C18 column (50 x 2.1 mm, 1.7 um; Waters). The mobile phase consisted of A: water with 0.1% formic acid, B: methanol with 0.1% formic acid. The autosampler temperature was set at 10˚ C and column temperature at 40˚ C. For detection and quantification, the electrospray ionization operated in the positive mode was set up for multiple reaction monitoring (MRM). The collision gas used was ultra-pure argon at a flow rate of 0.15 mL min^{-1}.

At the time of analysis, frozen plasma samples were thawed at room temperature, vortex-mixed thoroughly and 50 µL were subjected to the sample preparation procedure described below. Brain samples were thawed unassisted at room temperature, homogenized using a Branson 250 ultrasonic probe sonicator (Branson, Danbury, Connecticut, USA) and 75 mg of the homogenate were subjected to the sample preparation procedure described below. Quantification was achieved by external calibration using matrix-matched standards. Concentrations were calculated using a weighted least-squares linear regression (W = 1/x). Calibration standards were prepared by adding the appropriate volume of diluted solutions of the compound (made in a mixture of methanol and water, 50:50, v:v) to either aliquots of 50 µL of blank plasma or 75 mg of the blank brain homogenate. The calibration standard and sample preparation is as follows: 450 µL of 2% formic acid in acetonitrile was added to precipitate the proteins (approx. vol. ratio 1:10). The mixture was then vortex-mixed for 5 min and centrifuged at 13200 rpm for 10 min at 4˚ C. The resulting supernatants were transferred to an Ostro plate (Waters, Manchester, UK), designed to remove phospholipids. The resulting eluents were evaporated at 37˚ C under a stream of nitrogen. Plasma and brain residues were dissolved in 100 µL of a mixture of methanol

and water with 0.1% formic acid (50:50, v:v). A 10 µL aliquot of the resulting solution was injected into the LC-MS/MS system for analysis.

PDE and HDAC functional response in vivo

To confirm the ability of **37** to inhibit HDAC and PDE in the brain, the compound (40 mg/kg) was administered to WT mice (n=3). One hour later, mice were sacrificed and their hippocampus was quickly dissected from the brains. Total tissue homogenates were obtained by homogenizing the hippocampus in a lysis buffer containing Tris HCl 10 mM, NaF 1 mM, $NaVO_4$ 0.1 mM, sodium dodecyl sulfate (SDS) 2% and protease inhibitors. Western blot was carried out to analyze AcH3K9 and pCREB-Ser133.

Western blot analysis of brain samples

For western blot analysis of histones, pCREB and tubulin, protein samples were mixed with 6X Laemmli sample buffer and resolved onto SDS-polyacrylamide gels and transferred to nitrocellulose membrane. In all cases, the membranes were blocked with 5% milk, 0.05% Tween-20 in tris-buffered saline (TBS) followed by overnight incubation with the following primary antibodies: rabbit monoclonal anti-acetylated H3 (Lys9), rabbit monoclonal anti-pCREB (Ser133), mouse monoclonal anti-actin, mouse monoclonal anti-acetylated-tubulin (1:20 000, Sigma-Aldrich, St. Louis, MO, USA) in the corresponding buffer. Following two washes in PBS/Tween-20 or TBS/Tween-20 and one PBS or TBS alone, immunolabelled protein bands were detected by using HRP-conjugated anti-rabbit or anti-mouse antibody (1:5000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-goat (1:1500, Dako) antibody following an enhanced chemiluminescence system (ECL, GE Healthcare Bioscience, Buckinghamshire, UK),

and autoradiographic exposure to Hyperfilm ECL (GE Healthcare Bioscience). Quantity One software v.4.6.3 (Bio-Rad, Hercules, CA, USA) was used for quantification.

ASSOCIATED CONTENT

Supporting Information

Details about purification methods, SFC methods, synthesis of intermediates, purities, HRMS data and HPLC traces for final compounds, superposition of PDE5 inhibitors extracted from crystal complexes as well as biochemical activities as pIC_{50} values. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes

These authors declare no competing financial interest.

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ABBREVIATIONS

ADME, absorption, distribution, metabolism and excretion; THP, Tetrahydropyranyl; PAMPA, parallel artificial membrane permeability assay; BPL; Brain Polar Lipid; BOC, *tert*-butoxycarbonyl; DMF, dimethylformamide; Et_3N, triethylamine; TLC, thin layer chromatography; HPLC, High-performance liquid chromatography; rt, room temperature; Rt, retention time; THF, tetrahydrofuran; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBt, Hydroxybenzotriazole; $THPONH_2$, *N*-(tetrahydro-2*H*-pyran-2-yloxy)amine; MeOH, methanol; EtOH, ethanol; NMM, N-Methylmorpholine; DMSO, dimethylsulfoxide; EtOAc, ethyl acetate; TFA, trifluoroacetic acid; AcOH, acetic acid; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DEAD, diisopropyl azodicarboxylate; MsCl, methanesulfonyl chloride; xantphos, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene; m.p., melting point; NMR, nuclear magnetic resonance; NIS, N-iodosuccinimide; NBS, N-bromosuccinimide; DIEA, diethanolamine, BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; CAN, ceric ammonium nitrate; MW, microwave; DCC, *N*,*N*'-dicyclohexylcarbodiimide; LHMDS, lithium bis(trimethylsilyl)amide; TMSCl, trimethylsilyl chloride; 9-BBN-H, 9-borabicyclo[3.3.1]nonane; POT, tri-o-tolylphosphine; ESI-MS, electrospray ionisation mass spectrometry, LCMS, liquid chromatography–mass spectrometry, *t*BuOK, potassium *tert*-butoxide, SFC, supercritical fluid chromatography; DIBAL-H, diisobutylaluminium hydride.

REFERENCES

(1) Zheng, H.; Fridkin, M.; Youdim, M. New Approaches to Treating Alzheimer's Disease. *Perspect. Med. Chem.* **2015**, *7*, 1–8.

(2) García-Osta, A.; Cuadrado-Tejedor, M.; García-Barroso, C.; Oyarzábal, J.; Franco, R. Phosphodiesterases as Therapeutic Targets for Alzheimer's Disease. *ACS Chem. Neurosci.* **2012**, *3*, 832–844.

(3) Cuadrado-Tejedor, M.; Oyarzabal, J.; Lucas, M. P.; Franco, R.; García-Osta, A. Epigenetic Drugs in Alzheimer's Disease. *Biomol. Concepts* **2013**, *4*, 433–445.

(4) Gräff, J.; Tsai, L.-H. The Potential of HDAC Inhibitors as Cognitive Enhancers. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 311–330.

(5) Benito, E.; Urbanke, H.; Ramachandran, B.; Barth, J.; Halder, R.; Awasthi, A.; Jain, G.; Capece, V.; Burkhardt, S.; Navarro-Sala, M.; Nagarajan, S.; Schütz, A.-L.; Johnsen, S. A.; Bonn, S.; Lührmann, R.; Dean, C.; Fischer, A. HDAC Inhibitor-Dependent Transcriptome and Memory Reinstatement in Cognitive Decline Models. *J. Clin. Invest.* **2015**, *125*, 3572–3584.

(6) Kilgore, M.; Miller, C. A.; Fass, D. M.; Hennig, K. M.; Haggarty, S. J.; Sweatt, J. D.; Rumbaugh, G. Inhibitors of Class 1 Histone Deacetylases Reverse Contextual Memory Deficits in a Mouse Model of Alzheimer's Disease. *Neuropsychopharmacology* **2010**, *35*, 870–880.

(7) Maurice, D. H.; Ke, H.; Ahmad, F.; Wang, Y.; Chung, J.; Manganiello, V. C. Advances in Targeting Cyclic Nucleotide Phosphodiesterases. *Nat. Rev. Drug Discovery* **2014**, *13*, 290–314.

(8) Ugarte, A.; Gil-Bea, F.; García-Barroso, C.; Cedazo-Minguez, Á.; Ramírez, M. J.; Franco, R.; García-Osta, A.; Oyarzabal, J.; Cuadrado-Tejedor, M. Decreased

Levels of Guanosine 3', 5'-monophosphate (cGMP) in Cerebrospinal Fluid (CSF) Are Associated with Cognitive Decline and Amyloid Pathology in Alzheimer's Disease. *Neuropathol. Appl. Neurobiol.* **2015**, *41*, 471–482.

(9) Cuadrado-Tejedor, M.; Hervias, I.; Ricobaraza, A.; Puerta, E.; Pérez-Roldán, J. M.; García-Barroso, C.; Franco, R.; Aguirre, N.; García-Osta, A. Sildenafil Restores Cognitive Function without Affecting β-Amyloid Burden in a Mouse Model of Alzheimer's Disease. *Br. J. Pharmacol.* **2011**, *164*, 2029–2041.

(10) García-Barroso, C.; Ricobaraza, A.; Pascual-Lucas, M.; Unceta, N.; Rico, A. J.; Goicolea, M. A.; Sallés, J.; Lanciego, J. L.; Oyarzabal, J.; Franco, R.; Cuadrado-Tejedor, M.; García-Osta, A. Tadalafil Crosses the Blood-Brain Barrier and Reverses Cognitive Dysfunction in a Mouse Model of AD. *Neuropharmacology* **2013**, *64*, 114–123.

(11) Reneerkens, O. A. H.; Rutten, K.; Akkerman, S.; Blokland, A.; Shaffer, C. L.; Menniti, F. S.; Steinbusch, H. W. M.; Prickaerts, J. Phosphodiesterase Type 5 (PDE5) Inhibition Improves Object Recognition Memory: Indications for Central and Peripheral Mechanisms. *Neurobiol. Learn. Mem.* **2012**, *97*, 370–379.

(12) Falkenberg, K. J.; Johnstone, R. W. Histone Deacetylases and Their Inhibitors in Cancer, Neurological Diseases and Immune Disorders. *Nat. Rev. Drug Discovery* **2014**, *13*, 673–691.

(13) Guan, J.-S.; Haggarty, S. J.; Giacometti, E.; Dannenberg, J.-H.; Joseph, N.; Gao, J.; Nieland, T. J. F.; Zhou, Y.; Wang, X.; Mazitschek, R.; Bradner, J. E.; DePinho, R. A.; Jaenisch, R.; Tsai, L.-H. HDAC2 Negatively Regulates Memory Formation and Synaptic Plasticity. *Nature* **2009**, *459*, 55–60.

(14) Gräff, J.; Rei, D.; Guan, J.-S.; Wang, W.-Y.; Seo, J.; Hennig, K. M.; Nieland, T. J. F.; Fass, D. M.; Kao, P. F.; Kahn, M.; Su, S. C.; Samiei, A.; Joseph, N.;

Haggarty, S. J.; Delalle, I.; Tsai, L.-H. An Epigenetic Blockade of Cognitive Functions in the Neurodegenerating Brain. *Nature* **2012**, *483*, 222–226.

(15) Kim, D.; Frank, C. L.; Dobbin, M. M.; Tsunemoto, R. K.; Tu, W.; Peng, P. L.; Guan, J.-S.; Lee, B.-H.; Moy, L. Y.; Giusti, P.; Broodie, N.; Mazitschek, R.; Delalle, I.; Haggarty, S. J.; Neve, R. L.; Lu, Y.; Tsai, L.-H. Deregulation of HDAC1 by p25/Cdk5 in Neurotoxicity. *Neuron* **2008**, *60*, 803–817.

(16) Ding, H.; Dolan, P. J.; Johnson, G. V. W. Histone Deacetylase 6 Interacts with the Microtubule-Associated Protein Tau. *J. Neurochem.* **2008**, *106*, 2119–2130.

(17) Selenica, M.-L.; Benner, L.; Housley, S. B.; Manchec, B.; Lee, D. C.; Nash, K. R.; Kalin, J.; Bergman, J. A.; Kozikowski, A.; Gordon, M. N.; Morgan, D. Histone Deacetylase 6 Inhibition Improves Memory and Reduces Total Tau Levels in a Mouse Model of Tau Deposition. *Alzheimer's Res. Ther.* **2014**, *6*, 12.

(18) Xiong, Y.; Zhao, K.; Wu, J.; Xu, Z.; Jin, S.; Zhang, Y. Q. HDAC6 Mutations Rescue Human Tau-Induced Microtubule Defects in Drosophila. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 4604–4609.

(19) Sung, Y. M.; Lee, T.; Yoon, H.; DiBattista, A. M.; Song, J. M.; Sohn, Y.; Moffat, E. I.; Turner, R. S.; Jung, M.; Kim, J.; Hoe, H.-S. Mercaptoacetamide-Based Class II HDAC Inhibitor Lowers Aβ Levels and Improves Learning and Memory in a Mouse Model of Alzheimer's Disease. *Exp. Neurol.* **2013**, *239*, 192–201.

(20) Zhang, L.; Liu, C.; Wu, J.; Tao, J.-J.; Sui, X.-L.; Yao, Z.-G.; Xu, Y.-F.; Huang, L.; Zhu, H.; Sheng, S.-L.; Qin, C. Tubastatin A/ACY-1215 Improves Cognition in Alzheimer's Disease Transgenic Mice. *J. Alzheimer's Dis.* **2014**, *41*, 1193–1205.

(21) Cuadrado-Tejedor, M.; Garcia-Barroso, C.; Sanzhez-Arias, J.; Mederos, S.; Rabal, O.; Ugarte, A.; Franco, R.; Pascual-Lucas, M.; Segura, V.; Perea, G.;

Oyarzabal, J.; Garcia-Osta, A. Concomitant Histone Deacetylase and Phosphodiesterase 5 Inhibition Synergistically Prevents the Disruption in Synaptic Plasticity and It Reverses Cognitive Impairment in a Mouse Model of Alzheimer's Disease. *Clin. Epigenet.* **2015**, *7*, 108.

(22) Robers, M. B.; Dart, M. L.; Woodroofe, C. C.; Zimprich, C. A.; Kirkland, T. A.; Machleidt, T.; Kupcho, K. R.; Levin, S.; Hartnett, J. R.; Zimmerman, K.; Niles, A. L.; Ohana, R. F.; Daniels, D. L.; Slater, M.; Wood, M. G.; Cong, M.; Cheng, Y.-Q.; Wood, K. V. Target Engagement and Drug Residence Time Can Be Observed in Living Cells with BRET. *Nat. Commun.* **2015**, *6*, 10091.

(23) Cuadrado-Tejedor, M.; Garcia-Barroso, C.; Sánchez-Arias, J. A.; Rabal, O.; Mederos, S.; Ugarte, A.; Franco, R.; Segura, V.; Perea, G.; Oyarzabal, J.; Garcia-Osta, A. A First-in-Class Small-Molecule That Acts as a Dual Inhibitor of HDAC and PDE5, and That Rescues Hippocampal Synaptic Impairment in Alzheimer's Disease Mice. *Neuropsychopharmacology* **2016**, in press, doi: 10.1038/npp.2016.163.

(24) Konsoula, R.; Jung, M. In Vitro Plasma Stability, Permeability and Solubility of Mercaptoacetamide Histone Deacetylase Inhibitors. *Int. J. Pharm.* **2008**, *361*, 19–25.

(25) Li, X.; Inks, E. S.; Li, X.; Hou, J.; Chou, C. J.; Zhang, J.; Jiang, Y.; Zhang, Y.; Xu, W. Discovery of the First N-Hydroxycinnamamide-Based Histone Deacetylase 1/3 Dual Inhibitors with Potent Oral Antitumor Activity. *J. Med. Chem.* **2014**, *57*, 3324–3341.

(26) Palmieri, D.; Lockman, P. R.; Thomas, F. C.; Hua, E.; Herring, J.; Hargrave, E.; Johnson, M.; Flores, N.; Qian, Y.; Vega-Valle, E.; Taskar, K. S.; Rudraraju, V.; Mittapalli, R. K.; Gaasch, J. A.; Bohn, K. A.; Thorsheim, H. R.; Liewehr, D. J.;

Davis, S.; Reilly, J. F.; Walker, R.; Bronder, J. L.; Feigenbaum, L.; Steinberg, S. M.; Camphausen, K.; Meltzer, P. S.; Richon, V. M.; Smith, Q. R.; Steeg, P. S. Vorinostat Inhibits Brain Metastatic Colonization in a Model of Triple-Negative Breast Cancer and Induces DNA Double-Strand Breaks. *Clin. Cancer Res.* **2009**, *15*, 6148–6157.

(27) Zimmermann, G. R.; Lehár, J.; Keith, C. T. Multi-Target Therapeutics: When the Whole Is Greater than the Sum of the Parts. *Drug Discovery Today* **2007**, *12*, 34–42.

(28) Bischoff, E. Potency, Selectivity, and Consequences of Nonselectivity of PDE Inhibition. *Int. J. Impotence Res.* **2004**, *16 Suppl 1*, S11–S14.

(29) Cuadrado-Tejedor, M.; García-Osta, A.; Ricobaraza, A.; Oyarzabal, J.; Franco, R. Defining the Mechanism of Action of 4-Phenylbutyrate to Develop a Small-Molecule-Based Therapy for Alzheimer's Disease. *Curr. Med. Chem.* **2011**, *18*, 5545–5553.

(30) Atadja, P. Development of the Pan-DAC Inhibitor Panobinostat (LBH589): Successes and Challenges. *Cancer Lett.* **2009**, *280*, 233–241.

(31) Arts, J.; King, P.; Mariën, A.; Floren, W.; Beliën, A.; Janssen, L.; Pilatte, I.; Roux, B.; Decrane, L.; Gilissen, R.; Hickson, I.; Vreys, V.; Cox, E.; Bol, K.; Talloen, W.; Goris, I.; Andries, L.; Du Jardin, M.; Janicot, M.; Page, M.; van Emelen, K.; Angibaud, P. JNJ-26481585, a Novel "second-Generation" oral Histone Deacetylase Inhibitor, Shows Broad-Spectrum Preclinical Antitumoral Activity. *Clin. Cancer Res.* **2009**, *15*, 6841–6851.

(32) Papavassiliou, K. A.; Papavassiliou, A. G. Histone Deacetylases Inhibitors: Conjugation to Other Anti-Tumour Pharmacophores Provides Novel Tools for Cancer Treatment. *Expert Opin. Invest. Drugs* **2014**, *23*, 291–294.

(33) Paris, M.; Porcelloni, M.; Binaschi, M.; Fattori, D. Histone Deacetylase Inhibitors: From Bench to Clinic. *J. Med. Chem.* **2008**, *51*, 1505–1529.

(34) Thaler, F.; Mercurio, C. Towards Selective Inhibition of Histone Deacetylase Isoforms: What Has Been Achieved, Where We Are and What Will Be Next. *ChemMedChem* **2014**, *9*, 523–526.

(35) Zhang, K. Y. J.; Card, G. L.; Suzuki, Y.; Artis, D. R.; Fong, D.; Gillette, S.; Hsieh, D.; Neiman, J.; West, B. L.; Zhang, C.; Milburn, M. V; Kim, S.-H.; Schlessinger, J.; Bollag, G. A Glutamine Switch Mechanism for Nucleotide Selectivity by Phosphodiesterases. *Mol. Cell* **2004**, *15*, 279–286.

(36) Lauffer, B. E. L.; Mintzer, R.; Fong, R.; Mukund, S.; Tam, C.; Zilberleyb, I.; Flicke, B.; Ritscher, A.; Fedorowicz, G.; Vallero, R.; Ortwine, D. F.; Gunzner, J.; Modrusan, Z.; Neumann, L.; Koth, C. M.; Lupardus, P. J.; Kaminker, J. S.; Heise, C. E.; Steiner, P. Histone Deacetylase (HDAC) Inhibitor Kinetic Rate Constants Correlate with Cellular Histone Acetylation but Not Transcription and Cell Viability. *J. Biol. Chem.* **2013**, *288*, 26926–26943.

(37) Angibaud, P.; Van Emelen, K.; Decrane, L.; van Brandt, S.; Ten Holte, P.; Pilatte, I.; Roux, B.; Poncelet, V.; Speybrouck, D.; Queguiner, L.; Gaurrand, S.; Mariën, A.; Floren, W.; Janssen, L.; Verdonck, M.; van Dun, J.; van Gompel, J.; Gilissen, R.; Mackie, C.; Du Jardin, M.; Peeters, J.; Noppe, M.; Van Hijfte, L.; Freyne, E.; Page, M.; Janicot, M.; Arts, J. Identification of a Series of Substituted 2-Piperazinyl-5-Pyrimidylhydroxamic Acids as Potent Histone Deacetylase Inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 294–298.

(38) BPS Bioscience Home Page. https://bpsbioscience.com (accessed July 1, 2016).

(39) Rankovic, Z. CNS Drug Design: Balancing Physicochemical Properties for Optimal Brain Exposure. *J. Med. Chem.* **2015**, *58*, 2584–2608.

(40) Mensch, J.; Oyarzabal, J.; Mackie, C.; Augustijns, P. In Vivo, in Vitro and in Silico Methods for Small Molecule Transfer across the BBB. *J. Pharm. Sci.* **2009**, *98*, 4429–4468.

(41) Terrett, N. K.; Bell, A. S.; Brown, D.; Ellis, P. Sildenafil (Viagra TM), a Potent and Selective Inhibitor of Type 5 CGMP Phosphodiesterase with Utility for the Treatment of Male Erectile Dysfunction. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1819–1824.

(42) Sung, B.-J.; Hwang, K. Y.; Jeon, Y. H.; Lee, J. I.; Heo, Y.-S.; Kim, J. H.; Moon, J.; Yoon, J. M.; Hyun, Y.-L.; Kim, E.; Eum, S. J.; Park, S.-Y.; Lee, J.-O.; Lee, T. G.; Ro, S.; Cho, J. M. Structure of the Catalytic Domain of Human Phosphodiesterase 5 with Bound Drug Molecules. *Nature* **2003**, *425*, 98–102.

(43) Wang, H.; Liu, Y.; Huai, Q.; Cai, J.; Zoraghi, R.; Francis, S. H.; Corbin, J. D.; Robinson, H.; Xin, Z.; Lin, G.; Ke, H. Multiple Conformations of Phosphodiesterase-5: Implications for Enzyme Function and Drug Development. *J. Biol. Chem.* **2006**, *281*, 21469–21479.

(44) Wang, H.; Ye, M.; Robinson, H.; Francis, S. H.; Ke, H. Conformational Variations of Both Phosphodiesterase-5 and Inhibitors Provide the Structural Basis for the Physiological Effects of Vardenafil and Sildenafil. *Mol. Pharmacol.* **2008**, *73*, 104–110.

(45) *Pipeline Pilot*, Version 8.5. Accelrys. San Diego, CA 2011.

(46) Wang, D.; Helquist, P.; Wiest, O. Zinc Binding in HDAC Inhibitors: A DFT Study. *J. Org. Chem.* **2007**, *72*, 5446–5449.

(47) Butler, K. V; Kalin, J.; Brochier, C.; Vistoli, G.; Langley, B.; Kozikowski, A. P. Rational Design and Simple Chemistry Yield a Superior, Neuroprotective HDAC6 Inhibitor, Tubastatin A. *J. Am. Chem. Soc.* **2010**, *132*, 10842–10846.

(48) Witt, O.; Deubzer, H. E.; Milde, T.; Oehme, I. HDAC Family: What Are the Cancer Relevant Targets? *Cancer Lett.* **2009**, *277*, 8–21.

(49) Gómez-Vallejo, V.; Ugarte, A.; García-Barroso, C.; Cuadrado-Tejedor, M.; Szczupak, B.; Dopeso-Reyes, I. G.; Lanciego, J. L.; García-Osta, A.; Llop, J.; Oyarzabal, J.; Franco, R. Pharmacokinetic Investigation of Sildenafil Using Positron Emission Tomography and Determination of Its Effect on Cerebrospinal Fluid cGMP Levels. *J. Neurochem.* **2016**, *136*, 403–415.

(50) Di, L.; Kerns, E. H.; Fan, K.; McConnell, O. J.; Carter, G. T. High Throughput Artificial Membrane Permeability Assay for Blood-Brain Barrier. *Eur. J. Med. Chem.* **2003**, *38*, 223–232.

(51) Gleeson, M. P. Generation of a Set of Simple, Interpretable ADMET Rules of Thumb. *J. Med. Chem.* **2008**, *51*, 817–834.

(52) Beca, S.; Ahmad, F.; Shen, W.; Liu, J.; Makary, S.; Polidovitch, N.; Sun, J.; Hockman, S.; Chung, Y. W.; Movsesian, M.; Murphy, E.; Manganiello, V.; Backx, P. H. Phosphodiesterase Type 3A Regulates Basal Myocardial Contractility through Interacting with Sarcoplasmic Reticulum Calcium ATPase Type 2a Signaling Complexes in Mouse Heart. *Circ. Res.* **2013**, *112*, 289–297.

(53) Wager, T. T.; Hou, X.; Verhoest, P. R.; Villalobos, A. Central Nervous System Multiparameter Optimization Desirability: Application in Drug Discovery. *ACS Chem. Neurosci.* **2016**, *7*, 767–775.

(54) Ricobaraza, A.; Cuadrado-Tejedor, M.; Pérez-Mediavilla, A.; Frechilla, D.; Del Río, J.; García-Osta, A. Phenylbutyrate Ameliorates Cognitive Deficit and Reduces Tau Pathology in an Alzheimer's Disease Mouse Model. *Neuropsychopharmacology* **2009**, *34*, 1721–1732.

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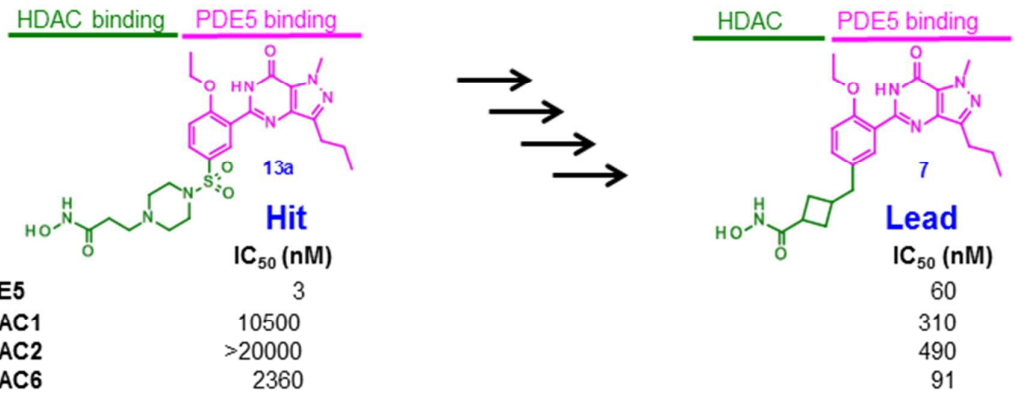

	Hit IC_{50} (nM)	Lead IC_{50} (nM)
PDE5	3	60
HDAC1	10500	310
HDAC2	>20000	490
HDAC6	2360	91