Design, Synthesis, and Biological Evaluation of Dual c-Met/HDAC Inhibitors Bearing 2-Aminopyrimidine Scaffold

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Abstract

A series of c-Met/histone deacetylase (HDAC) bifunctional inhibitors was designed and synthesized by merging pharmacophores of c-Met and HDAC inhibitors. Among them, the most potent compound, **2o**, inhibited c-Met kinase and HDACs, with IC_{50} values of 9.0 and 31.6 nM, respectively, and showed efficient antiproliferative activities against both A549 and HCT-116 cancer cell lines with greater potency than an equimolar mixture of the respective inhibitors of the two enzymes: crizotinib and vorinostat (SAHA). Our study provided an efficient strategy for the discovery of multitargeted antitumor drugs.

Keywords

- c-Met/HDAC inhibitor
- dual inhibitor
- hybrid
- antitumor drugs
- multipharmacology

Introduction

c-Mesenchymal–epithelial transition factor (c-Met), which is a prototype member of a subfamily of heterodimeric receptor tyrosine kinases (RTKs) binding to hepatocyte growth factor, plays important roles in cancer formation, progression, dissemination, and drug resistance. Aberrant c-Met activation has been identified in various human cancers.[1–3] Moreover, the overexpression of c-Met was demonstrated to correlate with poor prognosis or metastatic progression in several major human cancers.[4,5] So, c-Met has emerged as an attractive target for cancer treatment.

crizotinib (►**Fig. 1**), a dual inhibitor of c-Met/ALK kinase, was approved by Food and Drug Administration as the first launched c-Met inhibitor in 2011 for the treatment of patients with ALK-positive advanced or metastatic nonsmall cell lung cancer (NSCLC).[6] crizotinib on-target c-Met mutations including Y1230H/C, D1228N/H, and D1231Y single-point alterations have emerged among upon administration of crizotinib in NSCLC, and led to the subsequent chemoresistance.[7–9] Moreover, similar to other RTK inhibitors, due to complex factors, such as network complexity and compensatory activities, c-Met inhibition alone is usually not sufficient to block tumor progression, exhibiting low efficacy or acquired resistance in clinical trials.

Epigenetic aberrations contribute to tumor generation and development. Among the numerous epigenetic enzymes, human histone deacetylases (HDACs) play a crucial role in the regulation of multiple processes of life, from gene expressions, transcription, cell proliferation, and differentiation to protein activities.[10] More importantly, HDACs are also found to be overexpressed in a variety of human cancers, so HDAC inhibitors (HDACi) have emerged as promising new therapeutic agents for treating cancer.[11–13] Thus far, five HDACi have been launched on the market for cancer treatment, namely, vorinostat (SAHA), Romidepsin (FK-228), Belinostat (PXD-101), Panobinostat (LBH-589), and Chidamide (CS-055) (►**Fig. 1**). Although HDACi and other epigenetic agents showed efficacy in malignancies, challenges still remain in treating against solid tumors.[14,15]

HDACs influence over c-Met and its downstream signaling pathways both directly and indirectly, and considerable studies have demonstrated that HDACi exert antiproliferative effects against c-Met-dependent cells.[16] Given the above evidence, we hypothesized that the development of a single

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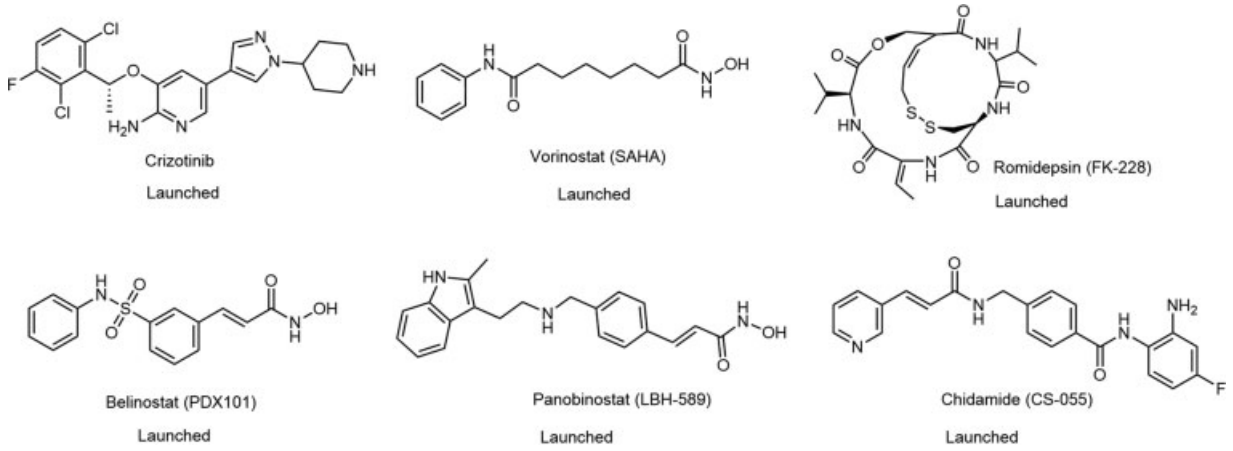


Fig. 1 Structures of crizotinib and representative approved histone deacetylase inhibitor.

molecule that concurrently inhibits c-Met and HDAC activities would serve as a promising strategy for related cancer treatments. Herein we decide to design and synthesize a series of dual c-Met/HDAC inhibitors by incorporating the pharmacophores of HDACi and c-Met inhibitor into a single molecule.

Our previous structure–activity relationship (SAR) study and modeling work showed that the substituted 2-aminopyridine moiety of crizotinib occupies the hinge region of the ATP-binding site to form two hydrogen bonds with the residues of c-Met protein, and the substituent at C5 of the pyridine moiety extends into the solvent-exposed region of the protein. The SAR results revealed that the modification of side chains had no significant effect on c-Met inhibitory activity. Most HDACi share the basic pharmacophore model, as depicted by

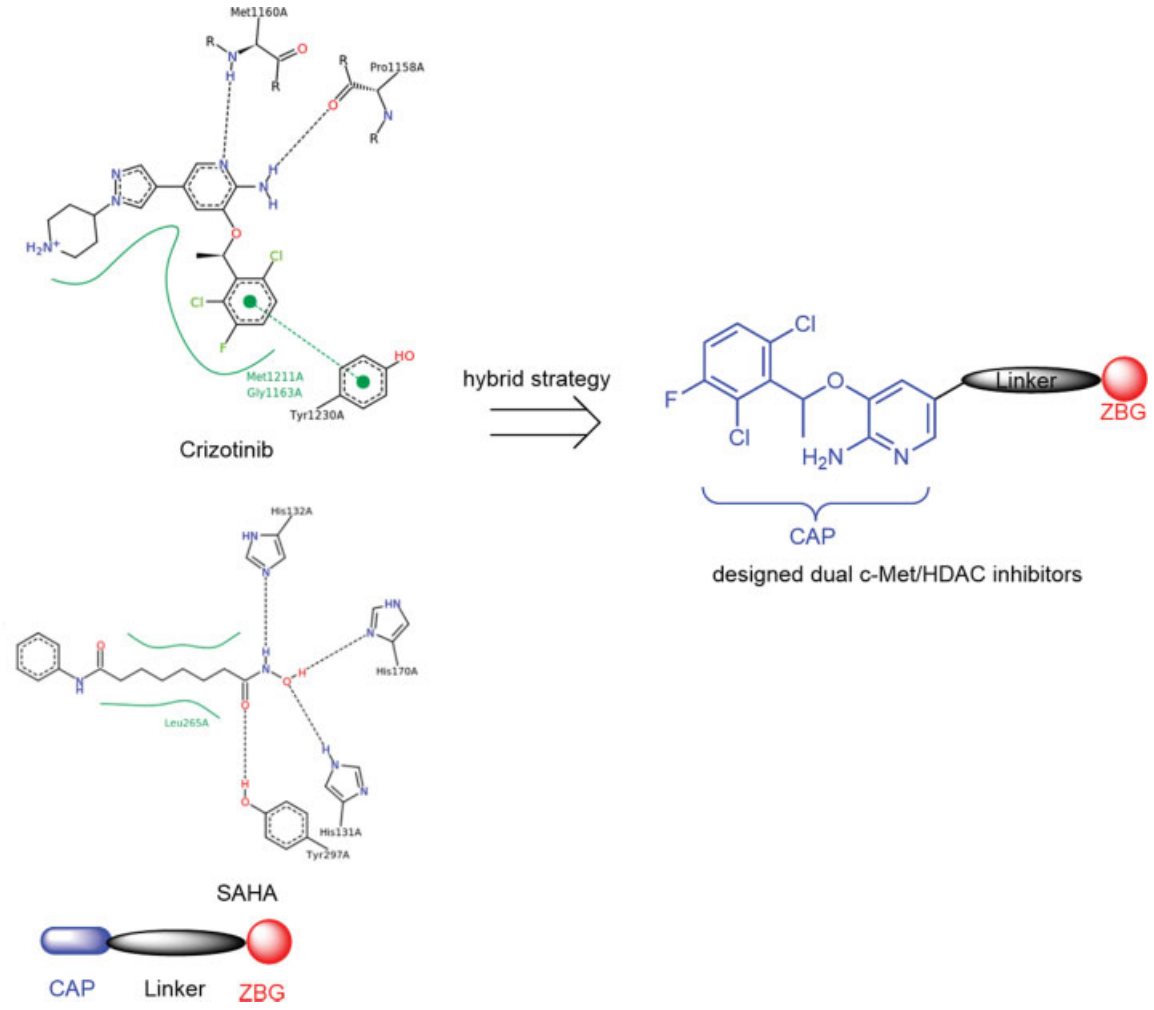
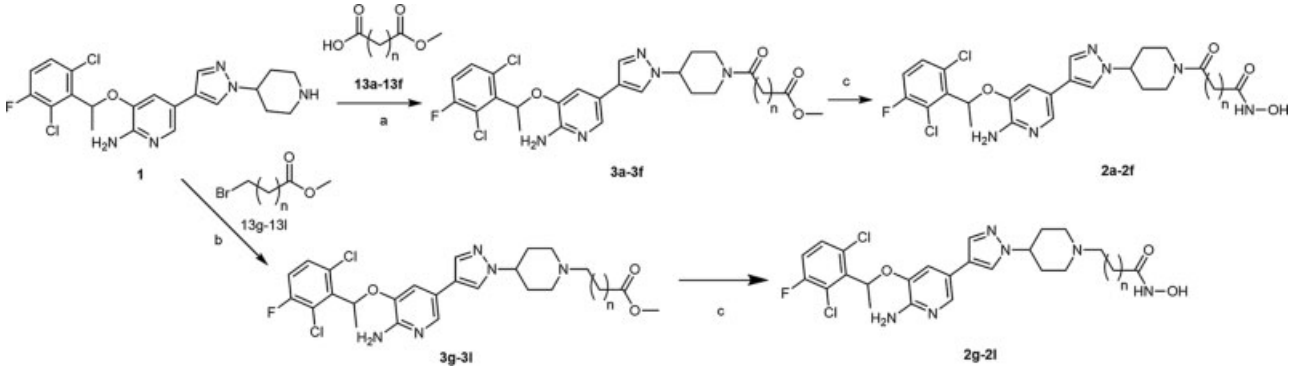
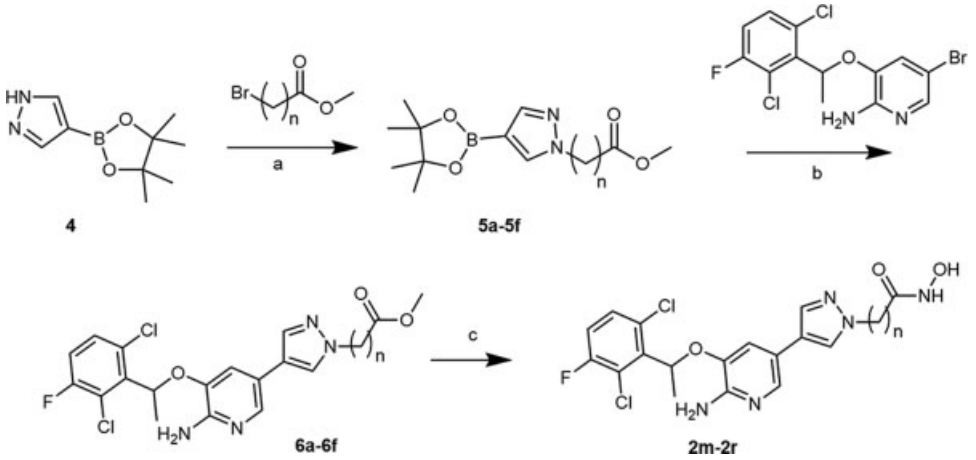


Fig. 2 Structure–activity relationship (SAR) study suggested design strategy of dual c-Met/HDAC inhibitors. HDAC, histone deacetylase.



Scheme 1 Reagents and conditions: (a) HBTU, TEA, DMF, rt; (b) K_2CO_3, DMF, 90°C; (c) NH_2OH/MeOH, rt.

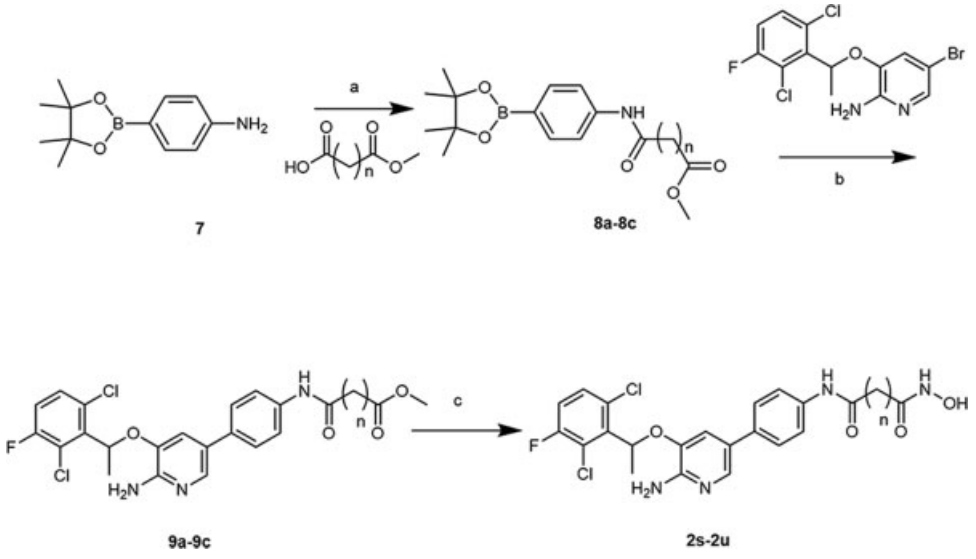


Scheme 2 Reagents and conditions: (a) NaH, DMF, 0°C; (b) $Pd(dppf)_2Cl_2.CH_2Cl_2$, Cs_2CO_3, DME/H_2O, 90°C; (c) NH_2OH, MeOH, 0°C to rt.

SAHA, which is composed of a zinc-binding group (ZBG), a linker, and a cap group (CAP) (►**Fig. 2**). Among them, ZBG plays an essential role in HDAC inhibitory activity. Therefore, we incorporated a ZBG at the C5 position of the 2-aminopyrimidine moiety of crizotinib with a proper linker to design dual c-Met/HDAC inhibitors. Herein we report the synthesis and biological activities of these hybrid c-Met/HDAC inhibitors.

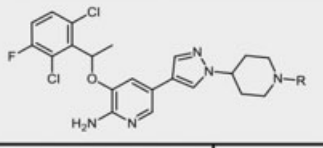
Results and Discussion

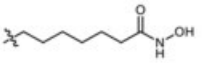
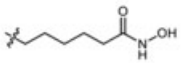
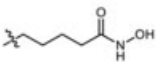
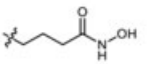
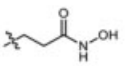
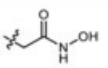
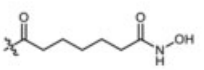
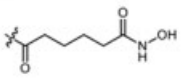
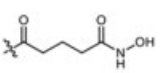
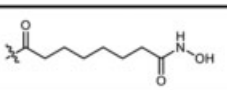
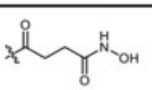
The route for the synthesis of piperidine-replaced compounds 2a–2l is outlined in **Scheme 1**. The starting material crizotinib (racemate) **1** was treated with intermediates **13a–13f** or **13g–13l** to give condensed ester products **3a–3l**, respectively, which were treated with freshly prepared hydroxylamine methanol solution to give target compounds **2a–2l**.



Scheme 3 Reagents and conditions: (a) HBTU, TEA, DMF, rt; (b) $Pd(dppf)_2Cl_2.CH_2Cl_2$, Cs_2CO_3, DME/H_2O, 90°C; (c) NH_2OH, MeOH, 0°C to rt.

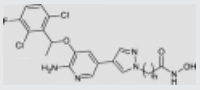
Table 1 c-Met and HDAC inhibitory activities of target compounds 2a–2l



Compd.	R	c-Met inhibition (IC_{50} nM)[a]	HDACs inhibition @1 μM[b]
2a		66	< 10%
2b		62	< 10%
2c		70	< 10%
2d		65	< 10%
2e		61	< 10%
2f		17	< 10%
2g		20	< 10%
2h		70.2	< 10%
2i		62.3	< 10%
2j		70	< 10%
2k		61	< 10%
2l		68	< 10%
crizotinib[c]		12	NT
SAHA[c]		NT	58 nM (IC_{50})

Abbreviations: HDAC, histone deacetylase; NT, not tested.
Note: For a description of the assay conditions, please see Huang et al[17] and Zhang et al[18].
[a]IC_{50} values are reported as the mean of at least two independent determinations with eight concentrations each. All compounds were assayed at least twice, and the inhibitory values were averaged.
[c]Used as a positive control.

Table 2 c-Met and HDAC inhibitory activities of target compounds 2m–2r



Compd.	*n*	c-Met inhibition (IC_{50} nM)[a]	HDAC inhibition (IC_{50} nM)[a]
2m	6	63	50
2n	5	92	>20,000
2o	4	9	31.6
2p	3	101	>20,000
2q	2	65	>20,000
2r	1	86	>20,000
crizotinib[b]		12	NT
SAHA		NT	58 nM

Abbreviations: HDAC, histone deacetylase; NT, not tested.
Note: For a description of the assay conditions, please see Huang et al[17] and Zhang et al[18].
[a]IC_{50} values are reported as the mean of at least two independent determinations with eight concentrations each.
[b]Used as a positive control.

Further pyrazolyl-replaced analogs **2m–2r** were synthesized from 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole **4**, as depicted in **Scheme 2**. Compound **4** was reacted with bromo-intermediates to produce the corresponding ester derivatives **5a–5f**, which were further transformed to **6a–6f** after Suzuki reactions. Next, hydroxamic acids **2m–2r** were obtained from **6a–6f** via reactions with NH_2OH in MeOH.

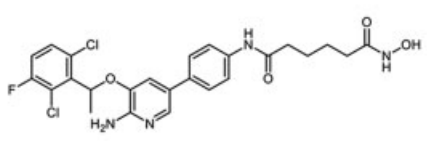
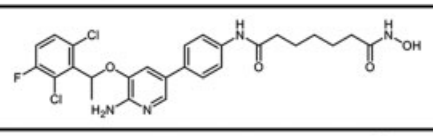
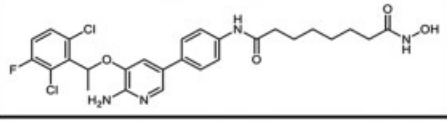
The construction of the 5-phenyl-2-aminopyrimidine scaffold is described in **Scheme 3**. The starting material **7** was coupled with a series of acids to afford the corresponding ester derivatives **8a–8c**, respectively, which were further transformed to the target compounds **2s–2u** via Suzuki reactions and reactions with NH_2OH in MeOH.

c-Met and HDAC enzyme inhibitory activity were conducted as described in Huang et al and Zhang et al to evaluate the final compounds using compounds crizotinib and SAHA as reference drugs.[17,18] The results are summarized in ►**Tables 1–2** to **3**.

The biological data listed in ►**Table 1** showed that most of the piperidine-replaced compounds **2a–2l** exhibited excellent inhibitory activity against c-Met, with IC_{50} values in the low nanomolar range, similar to crizotinib. These results showed that the c-Met kinase inhibition was unaffected upon changing the carbon length of the side chain. However, for HDAC inhibition, the target compounds **2a–2l** did not exhibit apparent inhibitory activities at 1 μM (HDACs: inhibition rate < 10%). We presume that compared with benzamide-based ligands of SAHA, the chair conformation of piperidine based ligands maybe not oriented in the correct direction to enable favorable interactions with the HDAC residues on the entrance of the narrow lipophilic pocket. Thus, the piperidine group was considered an unbefitting CAP for the intended dual-action inhibitors.

Subsequently, by removing the piperidine group, we directly introduced a ZBG at the N-1 position of the pyrazole

Table 3 c-Met and HDAC inhibitory activities of target compounds 2s–2u

Compd.	Structure	c-Met inhibition (IC_{50} nM)[a]	HDACs inhibition (IC_{50} nM)[a]
2s		323	2,716
2t		416	2,213
2u		679	998
crizotinib[b]		12	NT
SAHA[b]		NT	58 nM

Abbreviations: HDAC, histone deacetylase; NT, not tested.
Note: For a description of the assay conditions, please see Huang et al[17] and Zhang et al[18].
[a]IC_{50} values are reported as the mean of at least two independent determinations with eight concentrations each.
[b]Used as a positive control.

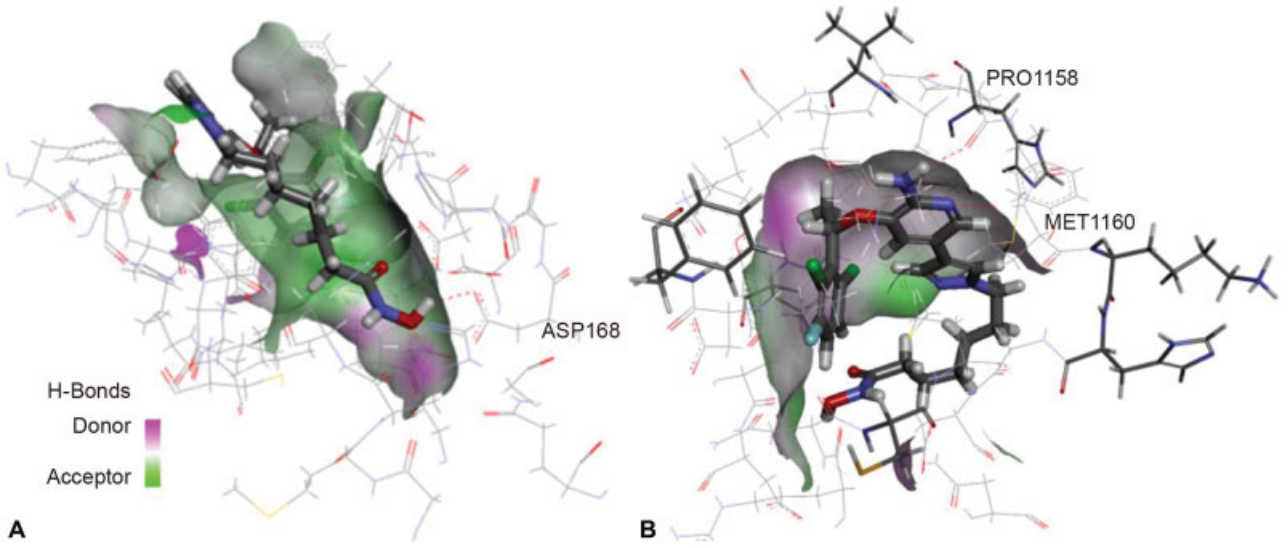


Fig. 3 (**A**) Proposed 3D binding mode of compound **2o** in the active site of HDAC (PDB:1C3S). (**B**) Proposed 3D binding mode of compound **2o** in the active site of c-Met (PDB:2WGJ). The figure was generated using Discovery Studio 4.0. 3D, three-dimensional; HDAC, histone deacetylase.

Table 4 The antiproliferative activities of 2o against A549, HCT116, and MCF-7 cell lines in vitro

Compd.	IC_{50} (μM)[a]		
	A549	HCT 116	MCF-7
2o	0.78	0.90	2.41
SAHA[b]	10.2	2.91	3.11
crizotinib[b]	2.10	3.57	1.65
SAHA + crizotinib (1:1)[b]	5.73	4.01	2.77

[a]Inhibition values represent the average of at least three independent experiments.
[b]Used as a positive control.

moiety to arrive at compounds **2m–2r**. As shown in ►**Table 2**, the tested compounds exhibited excellent c-Met enzymatic potency with IC_{50} values in the nanomolar range, which suggested removing the piperidine group had notable impact on c-Met binding. The potency of **2o** against c-Met ($IC_{50} = 9$ nM) had been slightly improved compared to that of crizotinib ($IC_{50} = 12$ nM). Notably, compounds **2m** and **2o** showed potent inhibition against HDACs with IC_{50} values of 50 and 31.6 nM, respectively, representing a modest improvement over that of the reference drug SAHA (58 nm).

Meanwhile, a SAR study and structural modifications of the pyrazole moiety were performed, as shown in ►**Table 3**. It seemed that the replacement of the pyrazole ring at position C-5 by a phenyl group (**2s–2u**) resulted in a loss of their c-Met inhibitory activity. For HDAC inhibition, analogues **2s–2u** similarly abolished HDAC inhibitory activity, indicating the important functions of the pyrazole ring in the HDAC kinase binding.

Docking simulations were performed for the most potent compound **2o** to investigate whether it can form favorable interactions with the targets. For the binding mode of **2o** with HDAC (PDB ID:1C3S; ►**Fig. 3A**), the hydroxamic acid coordinated to the catalytic Zn^{2+} of HDAC and formed a hydrogen bond with Asp168. The molecular docking with **2o** in c-Met (PDB ID:2WGJ) was also performed; and result is shown in ►**Fig. 3B**. The 2-aminopyrimidine scaffold formed a hydrogen bond with the residues of Met1160 and Pro1158 in the hinge region. Meanwhile, the hydroxamic acid tail was exposed as designed to the solvent accessible region.

Encouraged by the potent inhibitory activity of c-Met/HDAC, compound **2o** was further tested against three solid tumor cell lines selected as representatives of hard-to-treat solid tumors, which consisted of A549 (human lung adenocarcinoma), HCT-116 (human colon cancer), and MCF-7 (human breast cancer) using the MTT assay.[17] Data in ►**Table 4** demonstrate that compound **2o** showed significant growth inhibition with IC_{50} values of 0.78 and 0.90 μM against A549 and HCT116 cells respectively, which was obviously superior to that of SAHA or crizotinib. Most notably, compound **2o** outperformed a equimolar mixture of SAHA and crizotinib (IC_{50} values of 5.73 and 4.01 μM for A549 and HCT116, respectively), indicating that simpleco-administering SAHA and crizotinib does not appear to be synergistic and deserve further study in the treatment of cancer.

Conclusions

In conclusion, a series of dual c-Met/HDAC inhibitors bearing 2-aminopyrimidine scaffold were designed, synthesized, and evaluated by merging the pharmacophores of the c-Met inhibitor crizotinib and the HDACi SAHA. Among all the target compounds, compound **2o** exhibited nanomolar potency against c-Met and HDACs as well as potent antiproliferative activity in three tested cancer cell lines. Especially, we could observe that **2o** showing clearly synergistic antiproliferation activities against both A549 and HCT-116 cancer cell lines. This study indicates that **2o** as a potent "lead" compound targeting c-Met/HDAC and deserves further

investigation and development. Further evaluation of compound **2o** is ongoing and will be reported in due time.

Conflict of Interest

None.

Acknowledgments

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