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Discovery of (S)-N^1-(thiazol-2-yl) pyrrolidine-1,2-dicarboxamide derivatives targeting PI3Ka/HDAC6 for the treatment of cancer

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ABSTRACT

Recently, PI3K and HDAC have been considered as promising targets for the cancer therapy. A couple of pan-PI3K/HDAC dual inhibitors have been developed as a new class of anticancer agents. Herein, we discovered a new series of (*S*)-N^1-(thiazol-2-yl) pyrrolidine-1,2-dicarboxamide derivatives targeting PI3Kα/HDAC6. All the derivatives exerted dual-target inhibitory activities. Particularly, in the enzymatic selectivity assay, compound **21j** was identified as a subtype-selective PI3Kα/HDAC6 dual inhibitor (IC_{50} = 2.9 and 26 nM against PI3Kα and HDAC6, respectively), which displayed high potency against L-363 cell line with IC_{50} value of 0.17 μM. In addition, **21j** significantly inhibited phosphorylation of pAkt(Ser473) and induced accumulation of acetylated α-tubulin while having a negligible effect on the levels of acetylated Histone H3 and H4 at nanomolar level. Attributed to its favorable *in vitro* performance, **21j** has the potential to alleviate the adverse effects resulted from pan-PI3K inhibition and pan-HDAC inhibition. It is valuable for further functional investigation as an anti-cancer agent.

Phosphoinositide 3-kinases (PI3Ks) are lipid kinases categorized into three classes (known as class I, class II, and class III PI3Ks) according to their structural characteristics, activation mechanisms and selective types of lipid substrates [1,2]. Among which, class I PI3Ks are further divided into four subtypes including PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ [2]. Of these isoforms, PI3Kα is the most commonly associated with human cancers. There are three hotspot mutations in PI3Kα helical (E542K and E545K) and kinase catalytic (H1047R) domains, which lead to the occurrence of various malignant tumors, such as colorectal cancer, glioblastoma, gastric cancer, hepatocellular carcinoma, and breast cancer [3,4]. In contrast, PI3Kβ has been a target for treating the formation of blood clots, and phosphatase and tensin homologue (PTEN) deficient cancers. The PI3Kδ and PI3Kγ isoforms are primarily expressed in leukocytes and play crucial roles in immune response, inflammation, and respiratory indications. Additionally, the recent approval of PI3Kδ inhibitors for the treatment of chronic lymphocytic leukemia highlights their significance [4,5].

Despite the implicated importance of inhibiting PI3Kα in cancer, achieving selective inhibition of PI3Kα isoform has proved challenging and there are only few inhibitors under clinical trials exemplified by Alpelisib (**1**), Inavolisib (**2**) and Serabelisib (**3**) (Figure 1) [5]. In particular, Alpelisib, the first and only one approved PI3Kα-selective inhibitor, is used for the treatment of patients with *PIK3CA* gene (code for catalytic subunit of PI3Kα, termed P110α) mutation, HR-positive, HER2-negative advanced breast cancer who had received endocrine therapy previously [6–8]. However, Alpelisib shows less efficacy as a single therapy, and it is used in combination with fluvastatin in clinical practice [8].

Histone deacetylases (HDACs) are a class of epigenetic enzymes that catalyze the removal of an acetyl group from ε-amino lysine residues on histones to regulate chromatin structure and transcriptional activity [9,10]. HDAC superfamily, comprising 18 members, is divided into four classes based on the phylogenetic analysis, including Zn^{2+}-dependent class I HDACs (HDAC1, 2, 3 and 8), class II HDACs (IIa HDAC4, 5, 7 and 9; IIb HDAC 6 and 10), HDAC IV (HDAC11), and NAD^{+}-dependent class III HDACs (SIRT1-7) [11,12]. Numerous investigations have revealed that HDACs are considered to be promising targets for cancer therapy, and several pan-HDAC inhibitors have been approved by FDA for treating hematological malignancies, such as Vorinostat (SAHA, **4**), Romidepsin (**5**), Beliinostat (**6**), and Chidamide (**7**) [13]. However, due

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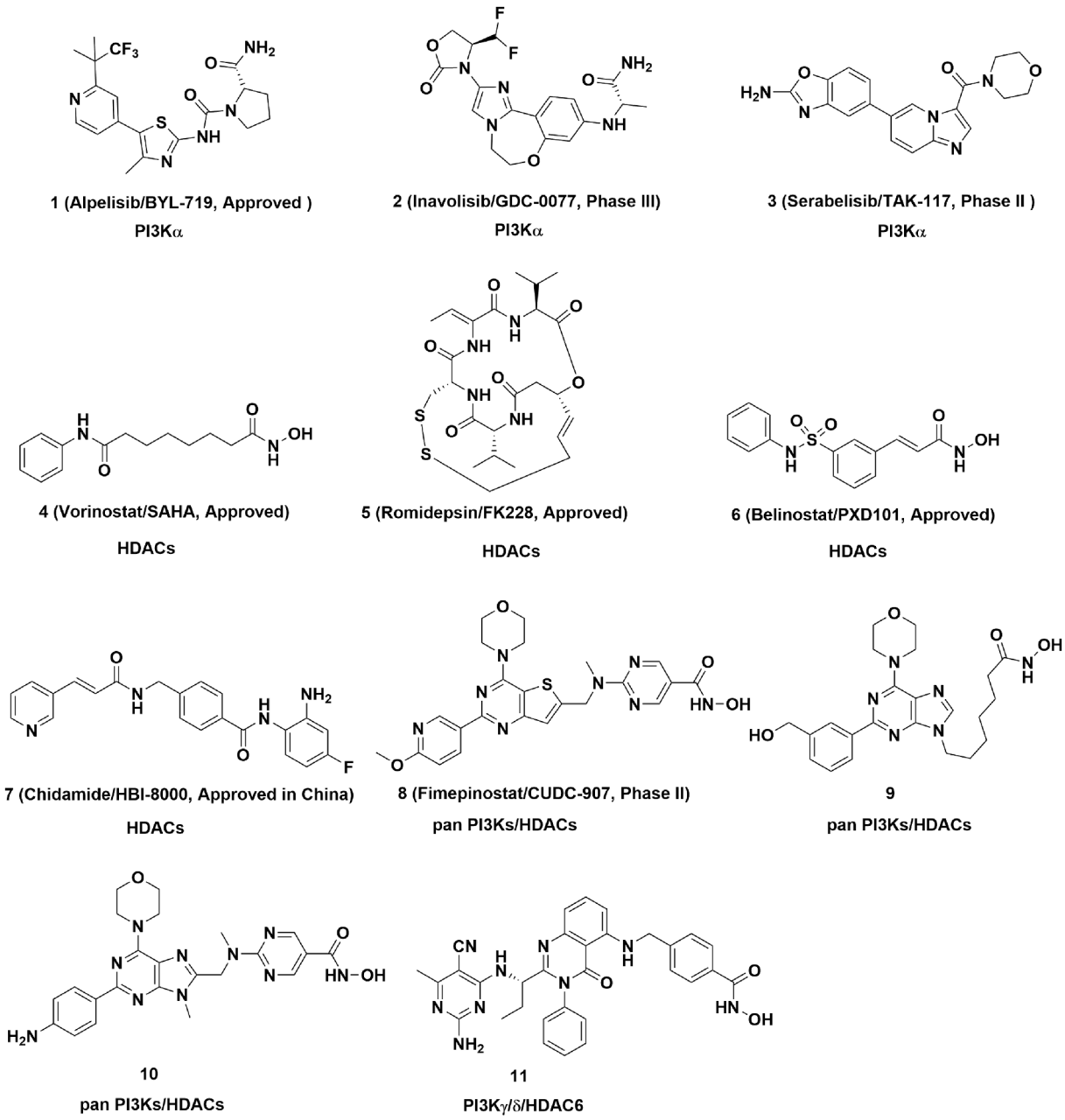


Figure 1. Selected chemical structures of PI3Kα inhibitors (**1**–**3**), HDAC inhibitors (**4**–**7**), PI3K/HDAC dual inhibitors (**8**–**11**).

to the lack of subtype specificity, pan-HDAC inhibitors also have undesirable side-effects, such as hematological, gastrointestinal, and cardiac toxicity [14,15]. It is worth noting that cytoplasm localized HDAC6 is unique in its capability to regulate the acetylation state of various non-histone substrates (α-tubulin, HSP-90, HSF-1 et al.) and thereby is involved in oncogenic cell transformation, stress response, metastasis and drug resistance.[15]Additionally, distinct from the severe defects or lethal effects after genetically ablating class I HDACs, HDAC6 knockout mice are viable and healthy without obvious phenotype abnormality [16]. Thus, as a wealth of research revealed, targeting HDAC6 is more promising in showing better tolerance and efficacy against cancer than other subtypes [17]. Presently, subtype selective HDAC6 inhibitors exemplified by Ricolinostat (**12**), Tubastain (**13**) and **14** have been considerated as the next-generation HDAC-targeted drugs (Figure 2) [15].

A series of studies show that combination of PI3K inhibitor and HDAC inhibitor can not only inhibit tumor growth, but also improve the efficacy, reduce drug resistance and provide a better treatment window than a single inhibitor [18,19]. Based on this, a couple of PI3K/HDAC dual inhibitors (**8**–**11**) have developed as a new class of anticancer agents (Figure 1) [20–23]. Currently, CUDC-907 (**8**) as a pan-PI3K/HDAC inhibitor has been investigated into phase II clinical trials (ClinicalTrials.gov Identifier: NCT03002623) for the treatment of metastatic and locally advanced thyroid cancer. However, lack of selective inhibition of PI3K and HDAC subtypes, pan-PI3K/HDAC dual inhibitors are considered to have inevitable toxicity and tolerance problems [20]. Thus, development of specific subtypes inhibitor of HDAC and PI3K may have lower toxicity and better tolerance. On the other hand, considering the anti-cancer effects of PI3Kα and HDAC6 as narrated above, we have recently initiated a medicinal chemistry project to identify polypharmacological agents with PI3Kα/HDAC6 bi-functional inhibitory activities.

HDAC inhibitors are commonly comprised of three structural elements for targeting binding sites, including surface recognition moiety

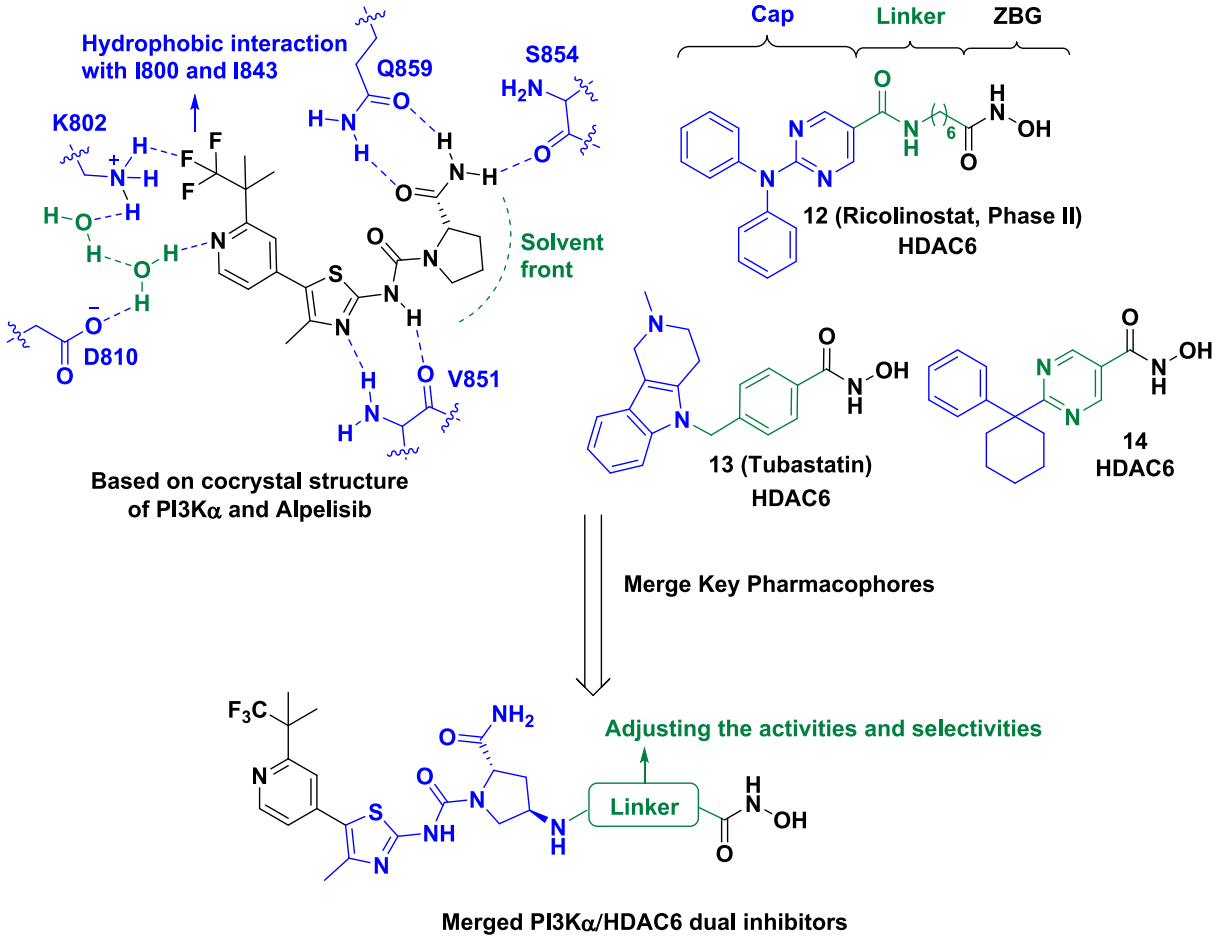
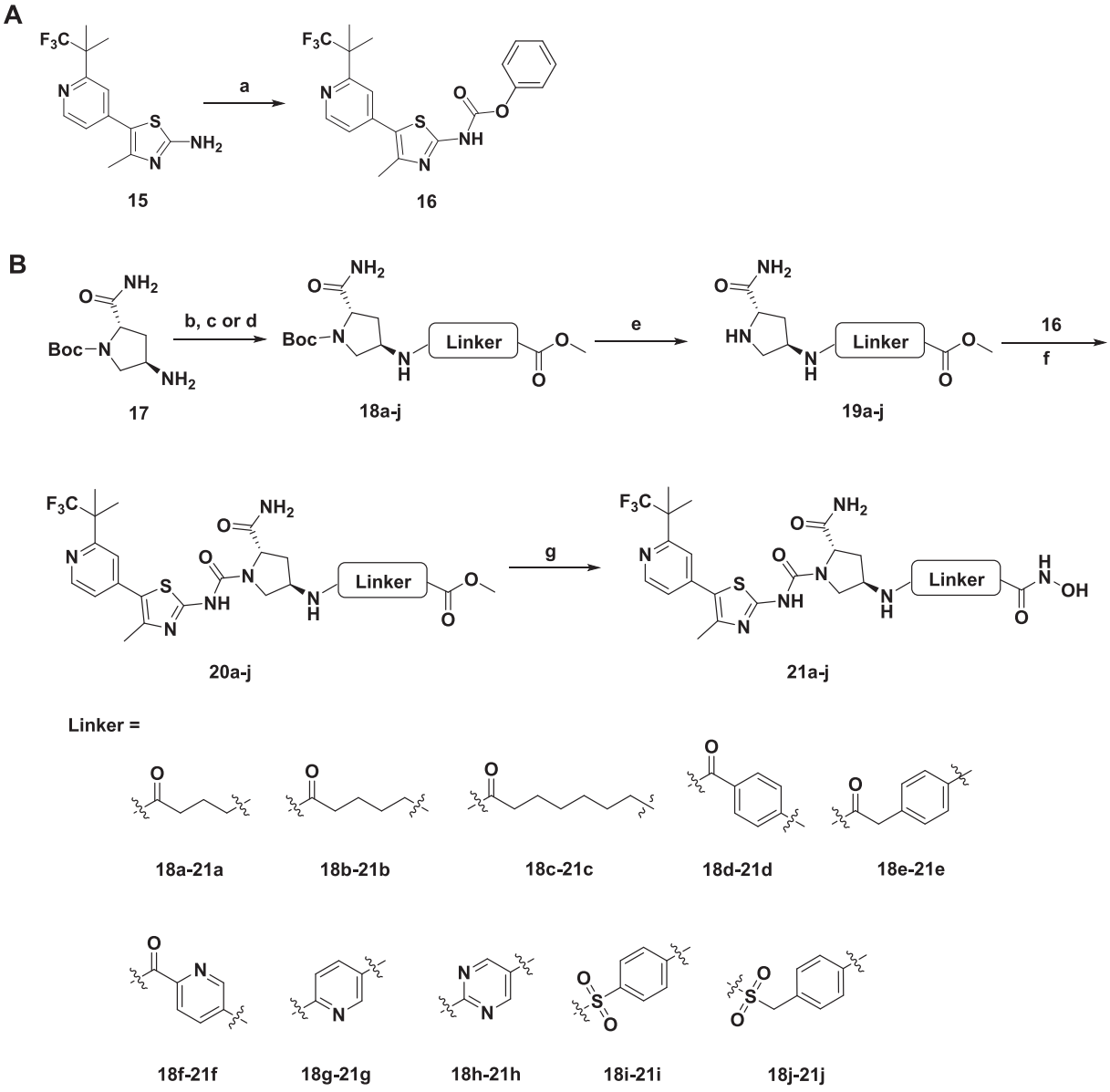
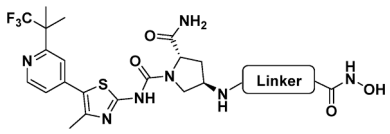


Figure 2. Design strategy of PI3Kα/HDAC6 dual inhibitors.



Scheme 1. The synthetic route for target compounds **21a-j**. Reagents and conditions: (a) Phenyl chloroformate, DIPEA, 1,4-dioxane, 40 °C, 20 h; (b) Corresponding carboxylic-substituted alkyl or aryl linker, HOBT, EDCI, DCM, 25 °C, 4 h for **18a-f**; (c) 2-chloropyridine, DIPEA, Isopropyl alcohol, 135 °C, 20 h for **18 g**; (d) 2-chloropyrimidine or corresponding sulfonyl chloride, DIPEA, DCM, 25 °C, 4 h for **18 h-j**; (e) CF_3COOH, DCM, 0 °C to rt, 2 h; (f) DIPEA, 1,4-dioxane, 60 °C 12 h; (g) NH_2OH (50 wt% in water), NaOH, MeOH, THF, 0 °C to rt, 1 h.

Table 1
IC_{50} values for enzymatic inhibition of PI3Kα and HDAC6.



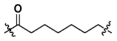
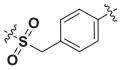
Cpd.	Linker	IC_{50} (nM) PI3Kα	HDAC6
21a		42	152
21b		56	680
21c		39	28
21d		48	45
21e		140	130
21f		13	9.8
21 g		8.1	53
21 h		15	39
21i		13	38
21j		2.9	26
Vorinostat	–	–	12
Alpelisib	–	5.0	–

Table 2
The anti-proliferative activities of selected compounds.

Cpd.	IC_{50} (μM)[a]			Cpd.	IC_{50} (μM)[a]		
	T47D	MCF-7	L-363		T47D	MCF-7	L-363
21c	>10	>10	6.6 ± 0.35	**21j**	>10	1.23 ± 0.31	0.17 ± 0.024
21f	>10	1.7 ± 0.41	1.4 ± 0.26	Vorinostat	1.5 ± 0.26	3.56 ± 0.37	0.48 ± 0.035
21 h	>10	>10	4.7 ± 0.37	Alpelisib	2.3 ± 0.34	0.25 ± 0.042	0.26 ± 0.028
21i	>10	>10	8.1 ± 0.22				

[a] . Data, shown as mean ± SD, were calculated based on the results of three biological replicates.

(Cap), chemical linker, and the terminal zinc binding group (ZBG) [13]. Compared with other HDACs, the substrate channel in HDAC6 is wider and shallower, favoring compounds with shorter linkers and larger, extended Caps [24]. Built upon these considerations, our design strategy of PI3Kα/HDAC6 dual inhibitor as showed in Figure 2. 1) as revealed by the cocrystal structure(4JPS) of PI3Kα and Alpelisib, the 4-position of the L-prolinamide moiety in Alpelisib orients towards the solvent-accessible surface in PI3Kα, and offers a synthetic handle for incorporating structural elements of an HDAC6 inhibitor. 2) target compounds featuring the L-prolinamide derivative Cap were constructed via tethering the hydroxamic acid ZBG to the 4-amino of L-prolinamide moiety in Alpelisib through chemical linkage. 3) target compounds were introduced diverse linkers that mimic those of the investigated HDAC6 inhibitors to optimize their activities and selectivities. Therefore, a series of (*S*)-N^1-(thiazol-2-yl) pyrrolidine-1,2-dicarboxamide derivatives targeting PI3Kα and HDAC6 were designed (Figure 2).

The synthetic route for target compounds **21a-j** is outlined in Scheme 1. Firstly, 2-aminothiazole derivative **15** was treated with phenyl chloroformate to provide intermediate **16**. Reaction of (2*S*,4*R*)-1-Boc-2-carbamoyl-4-aminopyrrolidine **17** with corresponding carboxylic-substituted alkyl or aryl linker in the presence of EDCI and HOBt generated intermediates **18a-f**. In addition, compound **17** was subjected to reaction with 2-chloropyridine, 2-chloropyrimidine and corresponding sulfonyl chloride to form intermediates **18 g-j**, respectively. Followed by Boc-deprotection of **18a-j** with TFA, the newly formed (2*S*,4*R*) − 4-amino-L-prolinamide **19a-j** was treated with carbamate derivative **16**, thereby leading to the generation of (*S*)-N^1-(thiazol-2-yl) pyrrolidine-1,2-dicarboxamide derivatives **20a-j**. Subsequently, **20a-j** were ultimately transformed into target Compounds **21a-j** via treatment with aqueous hydroxylamine.

According to the data of enzymatic inhibition test presented in Table 1, most of target compounds displayed potent inhibitory activities with the IC_{50} values < 100 nM against both PI3Kα and HDAC6. In particular, compounds **21f** and **21j** exhibited remarkably potent inhibitory activities against both PI3Kα and HDAC6 with IC_{50} values at low nanomolar level, and their inhibitory activities were comparable to the reference compounds Alpelisib and Vorinostat, respectively.

As showed in Table 1, despite the structural variation as the substructure (linker and ZBG) attached to 4-aminio position of pyrrolidine in Alpelisib, compounds **21f-j** retain the PI3Kα inhibitory activities, even surpass Alpelisib. This highlighted the 4-amino position of pyrrolidine in Alpelisib was tolerable for diverse structural derivatizations without significantly abolishing the PI3Kα inhibitory activity. Except for 2-amidopyridine **21f**, compounds linked Alpelisib with amide bond (**21a-e**) led to an obvious decrease of PI3Kα inhibition. By contrast, linked Alpelisib with amino-group or sulfonamide group (**21 g-j**), the level of PI3Kα inhibition was maintained (IC_{50} = 2.9–15 nM). Interestingly, introduction of phenylmethanesulfonamino group (**21j**) to the Alpelisib resulted in a dramatic improvement in PI3Kα inhibitory activity compared with benzenesulfonamino group **21i**.

As for HDAC6 inhibitory activities, compounds bearing aliphatic linkers of -C_3H_6- (**21a**), -C_4H_8- (**21b**) and -C_6H_{12}- (**21c**) showed significantly different activities in inhibition of HDAC6 (IC_{50} = 152 nM, 680 and 28 nM for **21a-c**, respectively). This result gave strong support to the length of the linker served as a prominent factor influence on HDAC6 inhibitory activity. Additionally, compounds with aromatic linker (**21d-j**) displayed moderate to potent HDAC6 inhibitory activities (IC_{50} = 9.8–130 nM). Among these compunds, **21f** was optimal, and the corresponding IC_{50} value of 9.8 nM against HDAC6.

Compounds **21c**, **21f**, **21 h**, **21i** and **21j** with potent PI3Kα/HDAC6 dual inhibitory activities were biologically evaluated for their anti-proliferative activities against the human breast cancer cell lines T47D and MCF-7, and the human multiple myeloma cell line L-363 (Table 2).

Table 3
The inhibitory activities of **21f** and **21j** against HDACs and PI3Ks.

Cpd.	IC_{50} (nM) HDAC1(Class I)	HDAC6(Class II)	HDAC8(Class I)	HDAC11(Class IV)	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
21f	471	9.8	>2000	>2000	13	>1000	318	214
21j	>2000	26	>2000	586	2.9	880	285	42
Vorinostat	29	12	814	–	–	–	–	–
CUDC907	–	–	–	177	–	–	–	–
Alpelisib	–	–	–	–	5.0	>1000	102	69

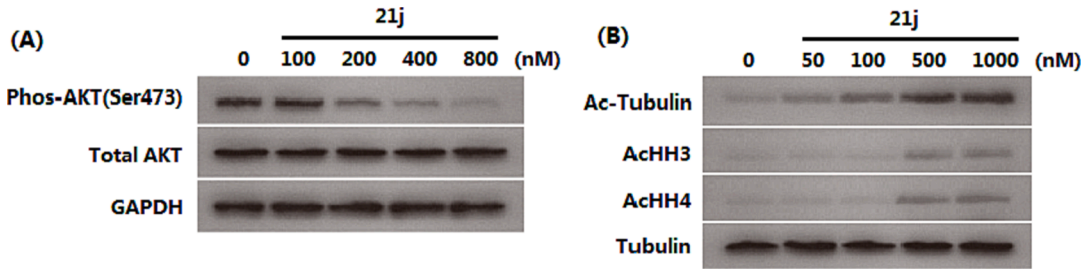


Figure 3. Western Blot analysis of effect on Phos-AKT (Ser473) and AcHH3, AcHH4 and Ac-tubulin levels in L-363 cells after treatment with **21j**.

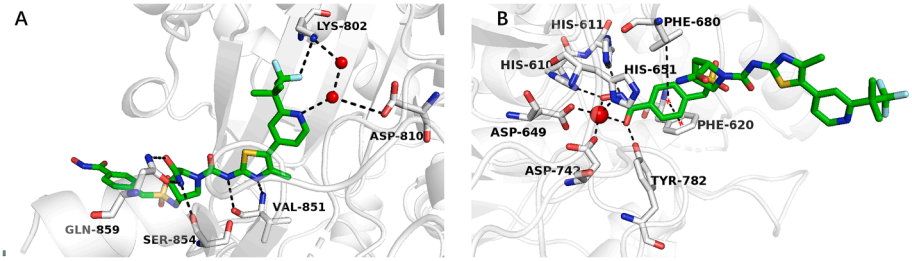


Figure 4. Molecular docking of **21j** with PI3Kα (A) and HDAC6 (B).

Among the tested compounds, **21j** displayed the most attractive potency against L-363 with the IC_{50} value of 0.17 μM, which was superior to that of Vorinostat and Alpelisib. Besides, **21f** and **21j** displayed distinguished efficacy against MCF-7 cell line with IC_{50} values at low micromolar level. While the other compounds showed moderate potency or even lost anti-proliferative activities against these three cell lines.

21f and **21j** were selected to identify their PI3K and HDAC subtype-selectivity against PI3Kα, PI3Kβ, PI3Kγ, PI3Kδ, HDAC1/8 (representative for class I HDACs), HDAC6 (representative for class II HDACs) and HDAC11 (representative for class IV HDACs). As demonstrated by the experimental results (Table 3), **21f** and **21j** exhibited excellent selectivity for PI3Kα and HDAC6. In detail, the PI3Kα selectivity fold based on the ratios of IC_{50} values (PI3Kβ/PI3Kα, PI3Kγ/PI3Kα and PI3Kγ/PI3Kα) were > 77, 24, 16 for **21f**, and 303, 98, 14 for **21j**, respectively. It is comparable to the reference compound Alpelisib. Similarly, the HDAC6 selectivity fold based on the ratios of IC_{50} values (HDAC1/HDAC6, HDAC8/HDAC6 and HDAC11/HDAC6) were 48, >204, >204 for **21f**, and > 76, >76, 22 for **21j**, respectively.

21j was further selected for Western blot analysis to investigate its capability to interfere with PI3K signaling and HDAC-mediated histone/nonhistone deacetylation in L-363 cells. Phos-AKT (Ser473) represented the biomarker for PI3K signaling, while AcHH3/4 and Ac-tubulin are considered as the biomarkers of class I HDACs and HDAC6 in cells, respectively. At the concentration of 200 nM, **21j** significantly down-regulated the level of Phos-AKT (Ser473) (Figure 3A), indicating the inhibition of PI3K signaling. Western blot results also showed that **21j** was able to significantly increase the level of acetylated α-tubulin in a concentration-dependent manner. Meanwhile, **21j** induced a slight increase in histone H3 and H4 acetylation (Figure 3B). These results showed that **21j** had markedly selective effect on HDAC6.

To investigate the possible binding modes, docking analysis of **21j** interactions with PI3Kα (PDB code 4JPS) and HDAC6 (PDB code 5EDU) were performed. As showed in Figure 4A, the amino acid residues Val851, Ser854 and Gln859 in PI3Kα form key hydrogen bond interactions with compound **21j**. The pyridine nitrogen atom of **21j** is part of a hydrogen bond network involving two water molecules and the residues Lys802 and Asp810. In addition, Lys802 also forms a hydrogen bond with one of the fluorine atoms of the trifluoromethyl group. In the case of **21j** docked with HDAC6 (Figure 4B), the hydroxamic acid substituted phenyl inserts into the catalytic channel of HDAC6, and its hydroxamic acid moiety coordinates with the zinc ion and interacts with the key amino acids His610, His611 and Tyr782. Simultaneously, the phenyl forms a π-π stack with Phe680 and Phe620.

In conclusion, we have discovered a new series of (*S*)-N^1-(thiazol-2-yl) pyrrolidine-1,2-dicarboxamide derivatives targeting PI3Kα/HDAC6. In particular, **21j** was identified as a subtype-selective PI3Kα/HDAC6 dual inhibitor with potent enzymatic (IC_{50} = 2.9 and 26 nM against PI3Kα and HDAC6, respectively) and cellular activities (IC_{50} = 0.17 μM against L-363). Furthermore, **21j** significantly inhibited phosphorylation of pAkt(Ser473) and increased acetylation of α-tubulin while having a negligible effect on the levels of acetylated Histone H3 and H4 at the nanomolar level. The biological profile of **21j** validated this compound as a means of anti-cancer cell growth by targeting PI3Kα and HDAC6.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.bmcl.2023.129462.

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