Design and Synthesis of 1,3,5-Triazines or Pyrimidines Containing Dithiocarbamate Moiety as PI3Kα Selective Inhibitors

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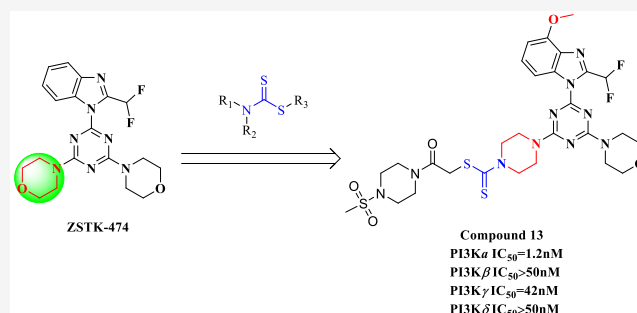Cite This: ACS Med. Chem. Lett. 2023, 14, 1266−1274 | Read Online

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ABSTRACT: Recent studies have shown that phosphoinositide 3-kinase (PI3K) plays a vital role in cell division, and it has become a therapeutic target for many cancers. In this paper, some new 1,3,5-triazine or pyrimidine skeleton derivatives containing dithiocarbamate were designed and synthesized based on the reasonable drug design strategy from the previously effective compound 2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1H-benzimidazole (**ZSTK-474**), in order to get effective selective PI3Kα inhibitors that have not been reported in the literature. In addition, the inhibitory activities of these compounds on PI3Kα and two tumor cell lines in vitro (HCT-116, U87-MG) were evaluated. The representative compound **13** showed a half-maximal inhibitory concentration (IC_{50}) value of 1.2 nM for PI3Kα and an exciting kinase selectivity. Compound **13** displayed strong efficacy in HCT-116 and U87-MG cell lines with IC_{50} values of 0.83 and 1.25 μM, respectively. In addition, compound **13** induced obvious tumor regression in the U87-MG cell line xenografts mouse model, with no obvious signs of toxicity after intraperitoneal injection at a dose of 40 mg/kg. Compound **13** can be an effective selective inhibitor of PI3Kα, and it provides patients with an opportunity to avoid the side effects related to the wider inhibition of the class I PI3K family.

KEYWORDS: PI3, Dithiocarbamate, Antiproliferation, Selectivity inhibitors

ZSTK-474

Compound 13
PI3Kα IC_{50}=1.2nM
PI3Kβ IC_{50}>50nM
PI3Kγ IC_{50}=42nM
PI3Kδ IC_{50}>50nM

Cancer is a major disease that threatens the health of whole people. With the development of the social economy, chronic diseases such as cancer have become the primary factors that endanger the health of our people. In recent years, a variety of drugs for the treatment of malignant tumors have been approved for marketing.[1] The phosphoinositide 3-kinases (PI3K) family is involved in many important cellular functions such as cell growth, proliferation, differentiation, and survival.[2,3] There are three classes (I, II, III) of the PI3K family, with different amino acid sequences, homology, and substrate specificity.[4] Among them, class I PI3Ks are well-known, which are dimers formed by catalytic (p110) and regulatory subunits, further known as PI3Kα, PI3Kβ, PI3Kδ, and PI3Kγ. PI3Kα, PI3Kβ, and PI3Kδ are further classified into class IA, and PI3Kγ is class IB, and they have different regulatory subunits.[5] The PI3K/mTOR signaling pathway is often dysfunctional in tumors, and PI3Kα is one of the most common mutant kinases.[6] PI3Kβ usually controls platelet production in blood cells, which is related to thrombosis.[7] But the PI3K/mTOR pathway is often disordered in cancers, and PI3Kα is widely expressed to regulate the PI3K/AKT/mTOR pathway.[8,9] Therefore, PI3Kα has almost become the most effective subtype to treat solid carcinoma in clinic.[10]
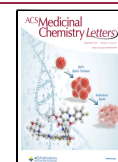
Many inhibitors with different selectivity characteristics targeting PI3K/AKT/mTOR pathway (i.e., PI3K/mTOR dual inhibitors, pan PI3K inhibitors, and PI3Kα-selective inhibitors) have entered clinic trials.[11,12] However, there are still limitations associated with pan-PI3K-mTOR and pan-class I PI3K inhibitors. It is reported that oral PI3K/mTOR dual inhibitors and pan-PI3K inhibitors have limited efficacy, because the extensive targeted toxicity prevents the dosage of inhibiting signal pathway, which may cause significant efficacy.[13−15] It is speculated that selective PI3Kα inhibitors may offer patients with opportunities to avoid side effects related to the wider inhibition of the class I PI3K family, so they can better inhibit the signaling pathway and have a durable antitumor effect.[16]

BYL719 (**I**) (Figure 1) is an effective, selective, and orally active PI3Kα inhibitor. It is targeted to PIK3CA mutant cancer and has been approved by the Food and Drug Administration
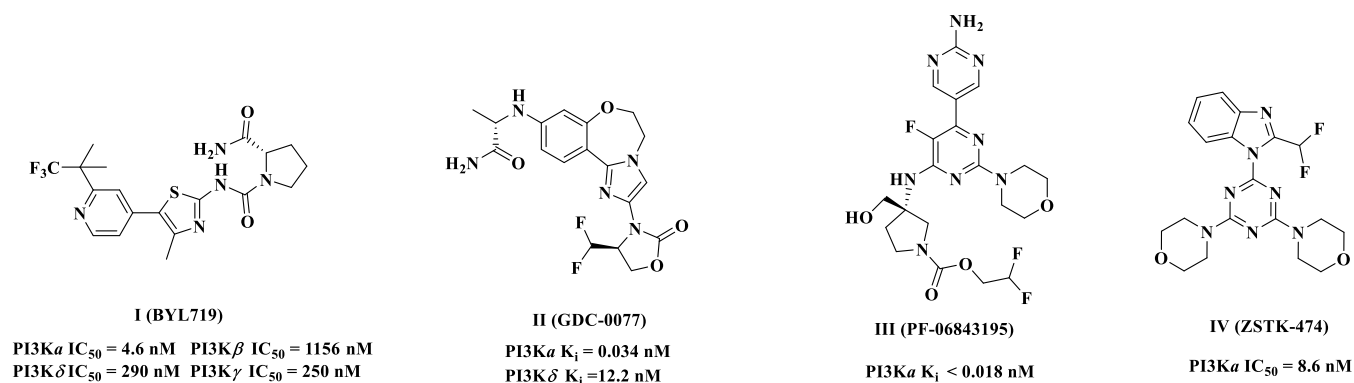
Figure 1. Structures of representative PI3K inhibitors.

I (BYL719)

PI3Ka IC$_{50}$ = 4.6 nM   PI3Kβ IC$_{50}$ = 1156 nM
PI3Kδ IC$_{50}$ = 290 nM   PI3Kγ IC$_{50}$ = 250 nM

II (GDC-0077)

PI3Ka K$_i$ = 0.034 nM
PI3Kδ K$_i$ = 12.2 nM

III (PF-06843195)

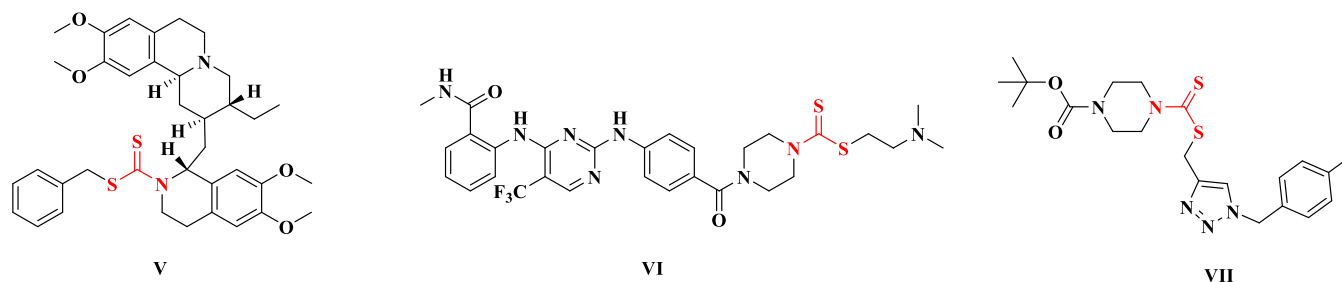PI3Ka K$_i$ < 0.018 nM

IV (ZSTK-474)

PI3Ka IC$_{50}$ = 8.6 nM

Figure 2. Anticancer agents bearing dithiocarbamate fragments.
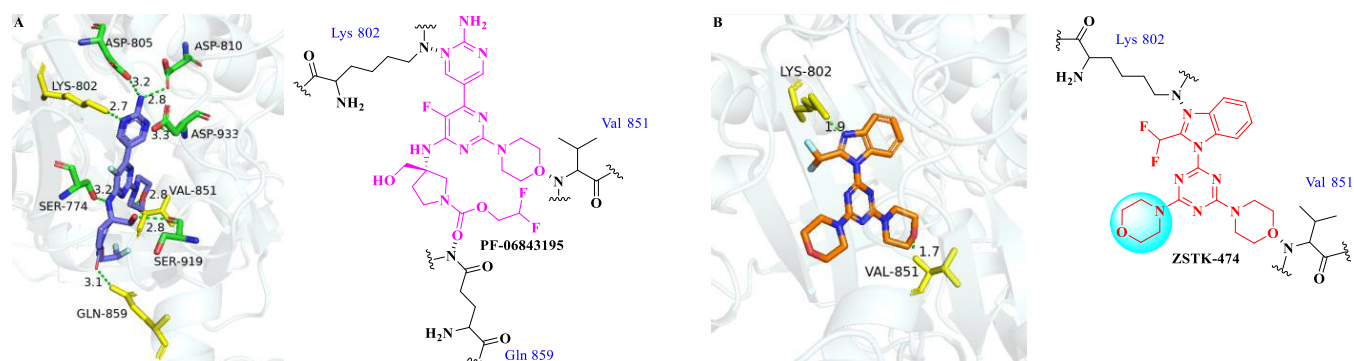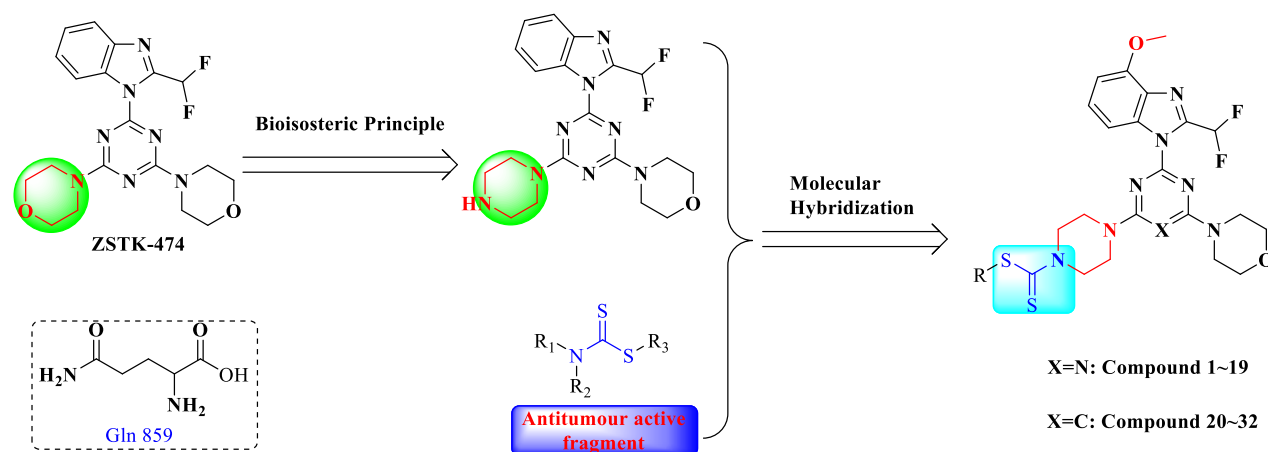
V   VI   VII

Figure 3. (A) Analysis of the reported binding mode of **PF-06843195** (PDB code: 7K6M). (B) Docking analysis of **ZSTK-474** and PI3Kα protein (PDB code: 7K6M).

Figure 4. Design strategy.

(FDA) (May 2019) to treat PIK3CA mutant breast cancer.[17] **GDC-0077** (**II**) (Figure 1) is also a selective inhibitor of

PI3Kα, which is currently in phase III clinical research. It is used to treat patients with breast cancer.[18] **PF-06843195** (**III**)

Scheme 1. Synthetic Route for Compounds 1−19[a]
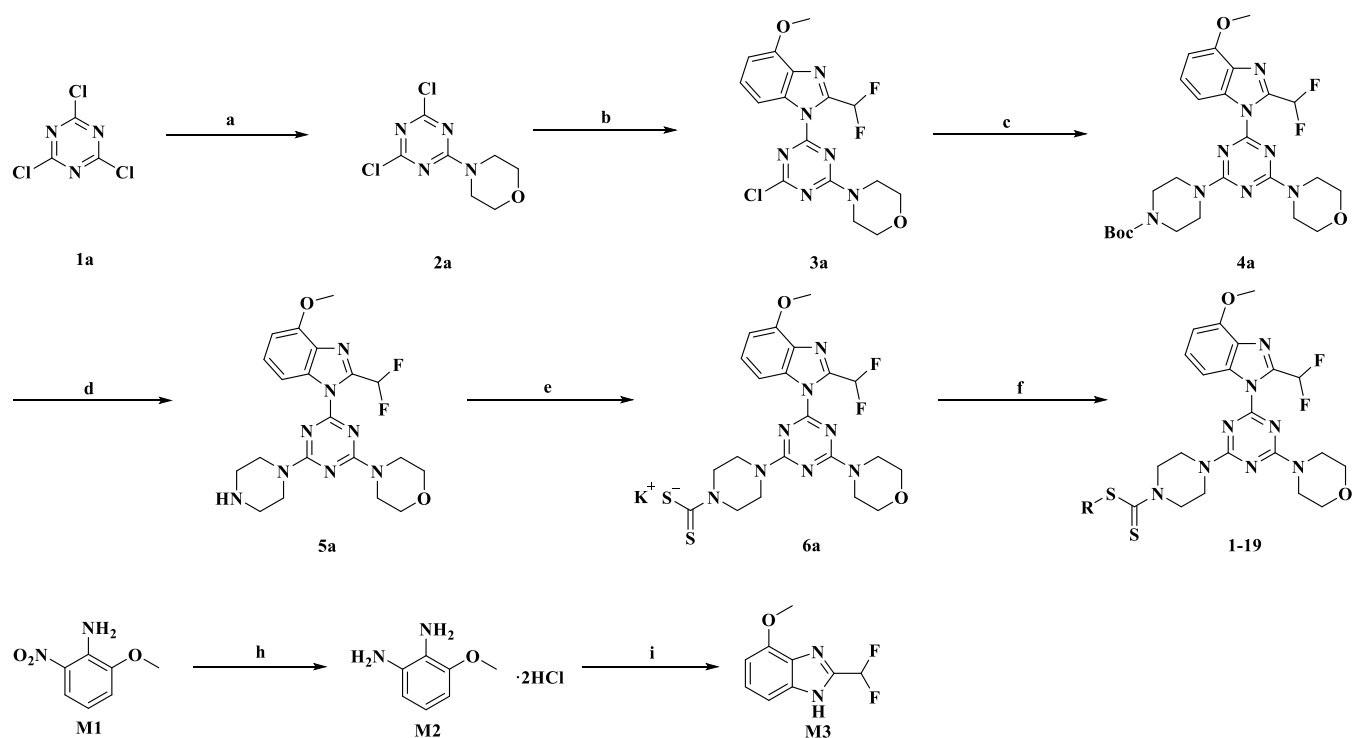
[a]Reagents and conditions: (a) Morpholine, NaHCO$_3$, acetone, −18 °C, 3 h; (b) **M3**, N,N-diisopropylethylamine (DIPEA), N,N-dimethylformamide (DMF), rt, 6 h; (c) N-Boc-piperazine, DIPEA, tetrahydrofuran (THF), rt, 6 h; (d) Trifluoroacetic acid (TFA), dichloromethane (DCM), 0 °C to rt, 4 h; (e) CS$_2$, KOH, MeOH, 0 °C to rt, 4 h; (f) Various chlorinated compounds, DMF, 50 °C, 2 h; (h) (1) Pd/C, H$_2$, MeOH, rt, 12 h; (2) HCl, rt, 2 h; (i) Difluoroacetic acid, polyphosphoric acid, 120 °C, 5 h.
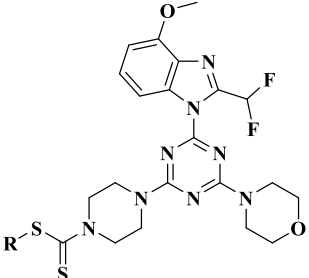
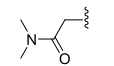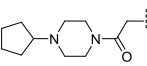## Table 1. Relationship between Structure-Activity and R Group of Compounds 1−8
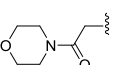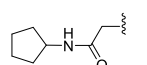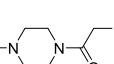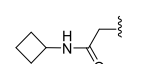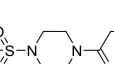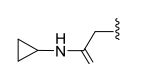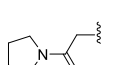
Compd.	R	PI3Kα IC$_{50}$ (nM)	Compd.	R	PI3Kα IC$_{50}$ (nM)
1		21	**6**		29
2		63	**7**		9.1
3		38	**8**		1.6
4		42	**ZSTK-474**	-	6.4
5		20			

(Figure 1) is another selective PI3Kα inhibitor. It has high biological and cellular activity compared to PIKα. From the eutectic structure of **PF-06843195** and PI3Kα protein, it can be seen that the carbonyl group of dithiocarbamate formed a hydrogen bond with the side chain of Gln859, a specific PI3Kα residue (Figure 3A).[19] 2-(Difluoromethyl)-1-[4,6-di(4-mor-

pholinyl)-1,3,5-triazin-2-yl]-1H-benzimidazole (**ZSTK-474**) (**IV**) (Figure 1) is an effective adenosine triphosphate (ATP) competitive pan-class I PI3K inhibitor with better selectivity compared with other kinases, and its half-maximal inhibitory concentration (IC$_{50}$) value for PI3Kα is 8.6 nM.[20,21] It was the first published clinical lead compound based on

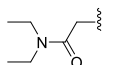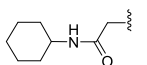**Table 2. Relationship between Structure-Activity and R Group of Compounds 9−19**

Compd.	R	PI3Kα IC$_{50}$ (nM)	Compd.	R	PI3Kα IC$_{50}$ (nM)
9	*(structure)*	0.8	15	*(structure)*	2.5
10	*(structure)*	1.1	16	*(structure)*	5.1
11	*(structure)*	0.7	17	*(structure)*	2.8
12	*(structure)*	1.4	18	*(structure)*	1.6
13	*(structure)*	1.2	19	*(structure)*	1.4
14	*(structure)*	0.9	ZSTK-474	-	6.4

morpholine-substituted triazine cores with pan PI3K activity.[22] However, **ZSTK-474** has been withdrawn from clinic because of its limitations such as resistance, targeted and non-targeted.[23,24] Therefore, we need to optimize the properties of **ZSTK-474** to obtain a higher quality.
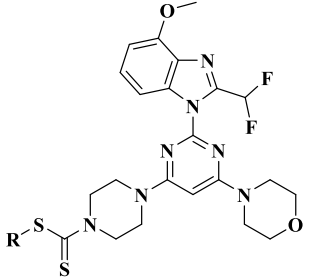
In the past ten years, dithiocarbamates (DTCs) have attracted much attention thanks to their various biological activities such as antibacterial,[25] antifungal,[26] anti-HIV,[27] platelet aggregation inhibitor,[28] anti-inflammatory,[29] antihistaminic,[30] antioxidant,[31] anticholinesterase,[32] etc.[33] Dithiocarbamates have been derivatized with various heterocycles, and their potential anticancer activity has been studied for many years. Compound **V** is an emetine-dithiocarbamate derivative which has shown obvious activity on prostate cancer cell lines with positive and negative androgen receptor.[34] Compound **VI** (Figure 2) is a focal adhesion kinase (FAK) inhibitor with good antiproliferation effects.[35] Compound **VII** (Figure 2) is a lysine specific demethylase (LSD) inhibitor, which can effectively inhibit the growth of gastric cancer cells in human body and has no signs of adverse side effects.[36]

According to the docking results of the **ZSTK-474** reported in the literature, a morpholine oxygen atom could form a hydrogen bond with residue Val851 in the PI3K hinge region, and the 1,3,5-triazine group acts as an adenosine mimic (Figure 3B).[37] However, because the other morpholine group does not have the length to extend into the protein solvent region, it cannot form a hydrogen bond with the specific residue Glu859 of PI3Kα, as shown in Figure 3B. Consequently, **ZSTK-474** displayed no subtype selectivity.

Based on the cocrystal of PI3Kα with **PF-06843195**, the structure of **ZSTK-474** was modified to obtain a PI3Kα selective inhibitor. In this work, the morpholine ring of **ZSTK-474** was replaced with a piperazine ring as a bioisosteric replacement. Then, a dithiocarbamate moiety was introduced to the end of the piperazine ring to study its effect on the side chain of the specific PI3Kα residue Gln859. Finally, in order to further enhance interaction with PI3Kα protein, a methoxy group was introduced at the 4′-position of the 2-(difluoromethyl)-1H-benzo[d]imidazol.[38] Therefore, some new derivatives of 1,3,5-triazine or pyrimidine scaffolds containing the dithiocarbamate moiety have been obtained, in order to expect that they are all highly selective PI3Kα inhibitors, as shown in Figure 4. The design strategies of these new PI3Kα inhibitors are shown, and their preliminary biological studies are disclosed.

Compounds **1−19** were synthesized from the commercially available 2,4,6-trichloro-1,3,5-triazine (**1a**) and 2-methoxy-6-nitroaniline (**M1**) as shown in Scheme 1. The intermediate **M3** was synthesized from **M1** by nitro reduction, salt formation, and a cyclization reaction. Subsequently, the synthesis of intermediate **4a** is based on 2,4,6-trichloro-1,3,5-triazine (**1a**) as the starting material, which was obtained by the successive substitution of morpholine, intermediate **M3**, and N-Boc-piperazine.[38] **4a** is deprotected with trifluoroacetic acid (TFA) to obtain intermediate **5a**. **5a** is further treated with CS$_2$ and converted into key intermediate **6a**. Finally, target compounds **1−19** were successfully obtained via the substitution of **6a** with an appropriate chlorinated compound in dimethylformamide (DMF) at 50 °C.

**Table 3. Relationship between Structure-Activity and R Group of Compounds 20−32**

Compd.	R	PI3Kα IC$_{50}$ (nM)	Compd.	R	PI3Kα IC$_{50}$ (nM)
20		1.8	27		2.7
21		1.3	28		34
22		1.4	29		120
23		1.2	30		81
24		12	31		17
25		34	32		2.5
26		3.3	ZSTK-474	-	6.4

Table 4. Antiproliferative Activity Data *In Vitro*

	IC$_{50}$ (μM)	
Compd.	HCT-116	U87-MG
9	2.78	1.10
11	1.11	0.60
12	0.98	1.67
13	0.83	1.25
20	2.20	2.04
22	1.23	1.99
23	1.53	1.22
ZSTK-474	0.71	0.13

Table 5. Kinase Inhibition Selectivity of Compounds 13, 20, and 23

	IC$_{50}$ (nM)			
Compd.	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
13	1.2	>50	42	>50
20	1.8	>50	33	>50
23	1.2	>50	35	>50
ZSTK-474	6.4	24	3.2	25

Compounds 20−32 were synthesized by replacing triazine with pyrimidine, with reference to the preparation conditions in Scheme 1.

The kinase inhibitory activity of the designed compound on PI3Kα was preliminarily screened by the ADP-Glo Luminescence kinase method. The screening conditions were verified by using ZSTK-474 as a reference, as shown in Table 1. We focused on the properties and length of R substituents in order to explore the structure-activity relation (SAR). According to

the reported literature, if the distance between dithiocarbamate and small molecular amine "tail" is two carbon atoms, the optimal binding effect is better.[39] Therefore, we introduced a two-carbon-atom linker in an attempt to obtain better activity.

First, we tried to introduce methoxy to obtain compound 1, which is about 3-fold less active than ZSTK-474. Subsequently, we introduced a tertiary amine "tail" to obtain compounds 2 and 3. Unfortunately, the activity was further reduced. We also tried to introduce cyclic tertiary amines and found a slight increase in activity (4, 5). When the carbon atom of compound 4 (PI3K IC$_{50}$ = 42 nM) was replaced with an oxygen atom to furnish compound 6 (PI3K IC$_{50}$ = 29 nM), compound 6 had about 2-fold enhanced potency compared to 4. Encouragingly, we introduced *N*-methyl to compound 4 to obtain compound 7, which improved activity by nearly 5-fold with IC$_{50}$ value of 9.1 nM. It is worth noting that the introduction of amide (8) increased the activity by 4-fold compared with ZSTK-474 with IC$_{50}$ value 1.6 nM. Therefore, we speculate that the carbonyl group of amide is helpful for the improvement of activity.

Based on the above findings, we introduced various amides and obtained compounds 9−19 (Table 2). These compounds showed significantly enhanced activity against PI3Kα. Among them, the activities of compounds 9, 10, 11, 12, and 14 were 10-fold higher than those without carbonyl groups. This once again confirmed that amide compounds with carbonyl groups have improved activity compared to compounds without carbonyl groups. On the basis of compound 12, the activity of introducing the electron-withdrawing group methanesulfonyl (13) was slightly increased. However, when the weak electron-donating group cyclopentyl (15) was introduced, the activity decreased by nearly 2-fold. Based on compound 8, compounds

Table 6. Metabolic Stability Data[a] of Compound 13

Compd.	R^2	$t_{1/2}$ (min)	$CL_{int(rat)}$ (μL/min/mg proteins)	$CL_{int(liver)}$ (mL/min/kg)	Remaining (T = 60 min)	Remaining (NCF = 60 min)
13	0.9564	21.5	64	115.8	11.2%	68.2%
ZSTK-474	0.9982	25.7	54	97.0	19.6%	100.1%

[a]R^2: correlation coefficient of the linear regression for the determination of kinetic constant; $T_{1/2}$: half-life; $CL_{int(mic)}$: intrinsic clearance; $CL_{int(liver)}$ = $CL_{int(mic)}$ × mg microsomal protein/g liver weight × g liver weight/kg body weight; NCF: abbreviation of no cofactor. No NADPH is added to NCF samples (replaced by buffer) during the 60 min incubation. If the NCF remaining is less than 60%, then possibly non-NADPH dependent metabolism occurs.
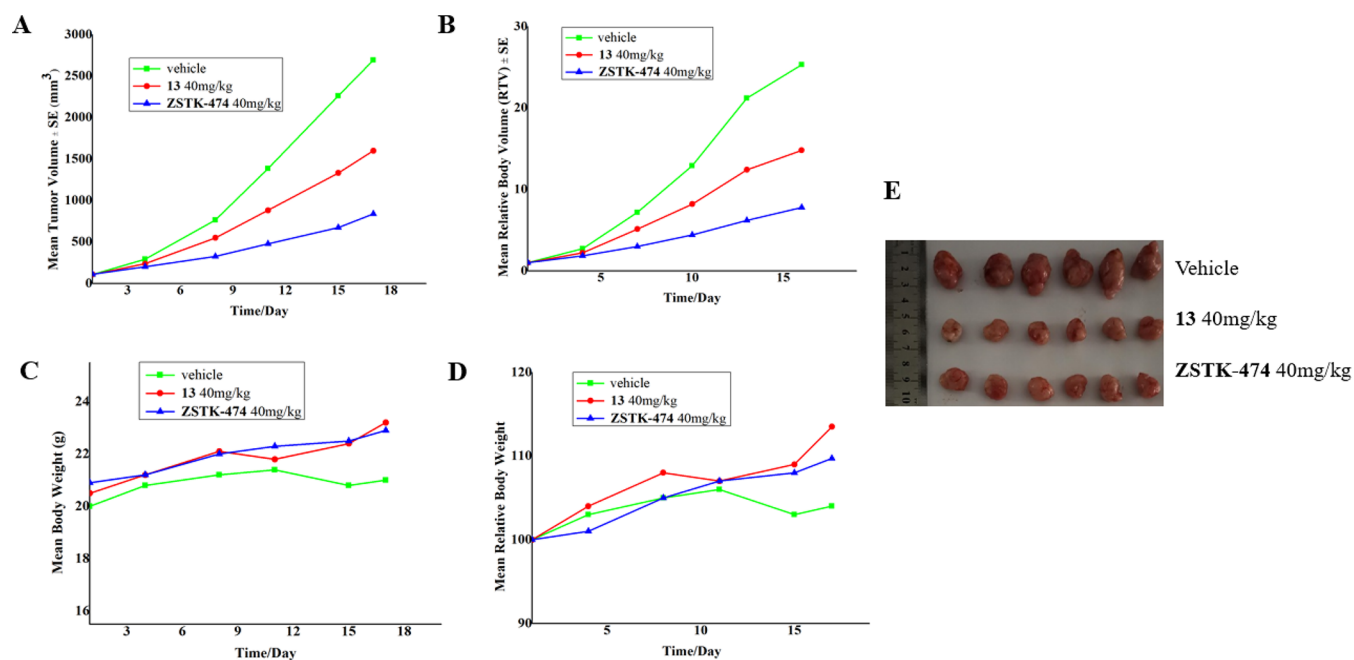
Figure 5. *In vivo* antitumor activity of **13** in the U87-MG mice xenotransplantation model. (A, C) **ZSTK-474** (40 mg/kg) and **13** (40 mg/kg) were given intraperitoneally once a day for 16 days. (B, D) Relative body weight and relative body volume of mice treated with **ZSTK-474** and **13**. (E) Tumor images processed with **ZSTK-474** and **13**.
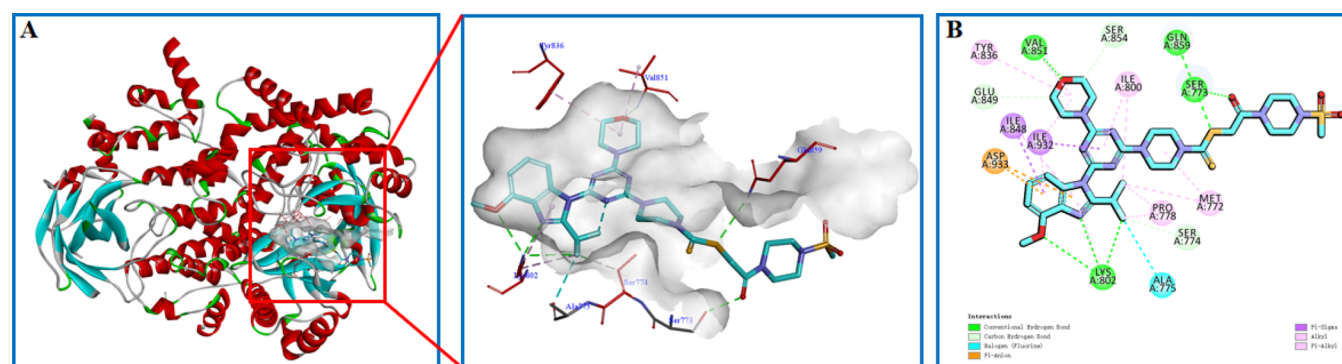
Figure 6. Modeled structure of **13** with PI3Kα. (A) Simulated binding modes of compound **13** with PI3Kα (PDB code: 7K6M). (B) Two-dimensional diagram of the interaction between **13** and PI3K protein.

16−19 were obtained by introducing cyclohexyl, cyclopentyl, cyclobutyl, and cyclopropyl groups, respectively. We found that when a large substituent was introduced, the activity decreased; when a small substituent was introduced, the activity was maintained.

Pyrimidine has been reported as a scaffold for its extensive biological activities.[40] Based on the bioisosteric principle, the 1,3,5-triazine fragment was replaced with pyrimidine in the design and synthesis of compounds **1−13** (Table 3). In compounds **20−32**, the terminal amides were still superior to the amine-containing compounds. The activity of **1−4** was

superior, with an IC$_{50}$ of 1.2−1.8 nM. However, compared with compounds **1−19**, the compounds **20−32** displayed a decreased activity. It is clear from these results that the 1,3,5-triazine fragment can be used as a more suitable scaffold for developing PI3Kα inhibitors than the pyrimidine fragment.

Based on the enzymatic potency analysis, we evaluated the antiproliferation activities of promising compounds on human colon cancer cell line (HCT-116) and human astrocytoma cell line (U87-MG), which have high expression of PI3K.[41,42] We used **ZSTK-474** as a positive control. It can be seen from Table 4 that compounds **1−19** show good inhibitory activity *in*

vitro. It was noticeable that **13** showed the best cellular efficacy against HCT-116 cell lines, with an IC_{50} value of 0.83 μM. Compounds **11**, **12**, and **13** also showed good antitumor activity against two cancer cell lines among all compounds. However, compounds **20**, **22**, and **23** showed slightly reduced activity against the HCT-116 and U87-MG cell lines.

Assuming that targeting PI3Kα and retaining β, γ, and δ subtypes may have stronger inhibition on PI3Kα but have no toxicity to other subtypes, the selectivity of compounds **13**, **20**, and **23** was evaluated. Encouragingly, these compounds have little inhibitory effect on other subtypes of PI3K (Table 5). Specifically, the inhibition of PI3Kα was more than 50-fold higher than that of PI3Kβ and PI3Kδ and 30−50 fold higher than that of PI3Kγ. These findings indicate that by modifying **ZSTK-474**, we have successfully improved the selectivity of PI3Kα. Of these, the PI3Kα selectivity of compound **13** is the most encouraging. Therefore, we intend to conduct further research on this lead.

Based on the above results, compound **13** showed the best activity against PI3Kα and HCT-116/U87-MG cells and showed exciting enzyme selectivity.

The stability test of microsomes was carried out, and the *in vitro* inherent clearance (CL) rate of **13** was determined by observing the ratio of **13** that remained after incubation with rat liver microsomes. As can be seen from Table 6, compound **13** possessed $t_{1/2}$ ($t_{1/2}$ = 21.5 min) and CL values ($CL_{int(liver)}$ = 115.8 mL/min/kg) equivalent to those of **ZSTK-474** in rat liver microsomes.

The antitumor effects of **13** and **ZSTK-474** *in vivo* were further evaluated in the xenotransplantation mouse models with U87-MG. When the mean tumor volume of the mice reached approximately 100 mm^3, **ZSTK-474** (40 mg/kg/day) and **13** (40 mg/kg/day) were injected intraperitoneally once a day for 16 days. The tumor volume and weight of each mouse were measured on the first, fourth, eighth, 11th, 15th, and 17th day. **ZSTK-474** and **13** were capable of significantly reducing tumor load in the mouse model, with tumor growth inhibition (TGI) of 72% and 42%, respectively, as shown in Figure 5. In addition, the animals in the treatment group were well-tolerated, and no obvious mortality or body weight loss was observed. In conclusion, the results demonstrated that compound **13** had an obvious inhibitory effect on tumor growth and has promising potential.

In order to further understand the new PI3K inhibitors, the binding mode of PI3Kα (PDB code: 7K6M) and **13** was simulated. The 2-(difluoromethyl)-4-methoxy-1*H*-benzo[*d*]-imidazole group and the methoxy group introduced at the 4′-position of the 2-(difluoromethyl)-1*H*-benzo[*d*]imidazol formed a hydrogen bond with Lys802, and the former forms a halogen bond with Ala775. The oxygen atom in the morpholine ring formed a hydrogen bond with hinge residue Val851. The sulfur atom of the dithiocarbamate fragment forms a hydrogen bond with Gln859, as shown in Figure 6. In addition, the carbonyl oxygen of the amide tail formed an additional hydrogen bond with Ser773, which helped to explain why compounds containing an amide "tail" have better activity against PI3Kα than compounds with an amine tail.

In conclusion, to discover structurally diverse PI3Kα selective inhibitors, some new 1,3,5-triazine or pyrimidine derivatives containing a dithiocarbamate skeleton were designed, synthesized, and evaluated. All compounds exhibited nanomolar enzymatic activity against PI3Kα, and **13** in particular showed an IC_{50} of 1.2 nM against PI3Ka. At the same time, most of the compounds we designed showed good antiproliferation activity against two cancer cell lines (HCT-116, U87-MG). Selectivity of **13**, **20**, and **23** to class I PI3K was also tested, which showed a good selectivity to PI3Kα. In addition, in the xenotransplantation model of U87-MG mice, **13** showed considerable antiproliferative activity at 40 mg/kg/day *in vivo* as compared to **ZSTK-474** at 40 mg/kg/day by intraperitoneal injection. Taken together, **13** can be further investigated as a promising antitumor PI3Kα inhibitor.

■ ASSOCIATED CONTENT

ⓈI Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.3c00287.

Compound synthesis, characterization, and assay protocols (PDF)

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Notes

The authors declare no competing financial interest.

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