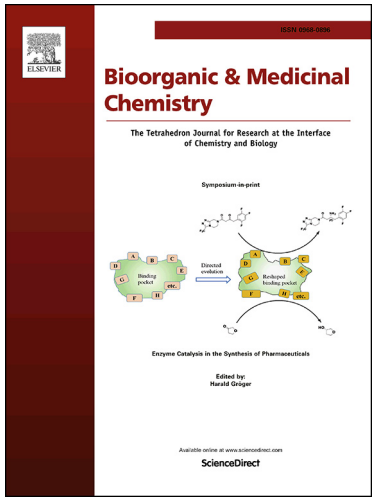

Design, synthesis and biological evaluation of novel chromeno[4,3-c]pyrazol-4(2*H*)-one derivates containing sulfonamido as potential PI3Kα inhibitors

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PII: S0968-0896(19)30423-7
DOI: https://doi.org/10.1016/j.bmc.2019.04.021
Reference: BMC 14875

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 13 March 2019
Revised Date: 10 April 2019
Accepted Date: 14 April 2019

Please cite this article as: Yin, Y., Hu, J-Q., Wu, X., Sha, S., Wang, S-F., Qiao, F., Song, Z-C., Zhu, H-L., Design, synthesis and biological evaluation of novel chromeno[4,3-c]pyrazol-4(2*H*)-one derivates containing sulfonamido as potential PI3Kα inhibitors, *Bioorganic & Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.bmc.2019.04.021

Design, synthesis and biological evaluation of novel chromeno[4,3-c]pyrazol-4(2*H*)-one derivates containing sulfonamido as potential PI3Kα inhibitors

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Abstract

A series of novel chromeno[4,3-c]pyrazol-4(2*H*)-one derivates contained sulfonamido were designed and synthesized, and their anticancer effects in vitro was evaluated to develop some new PI3Kα inhibitors. Most of desired compounds exhibited the better antiproliferative activities against four cancer cell lines than that

of LY294002. Out of them, compound **4o** displayed the potent antiproliferative activity and high selectivity against the PI3Kα protein and it can induce apoptosis of HCT116 in a dose-dependent manner. Western blot assay indicated that compound **4o** obviously down-regulated expression of p-Akt (S473). Molecular docking was performed to clarify the possible binding mode between compound **4o** and PI3Kα. All these results indicated that compound **4o** could be a potential inhibitor of PI3Kα.

Keywords: chromeno[4,3-c]pyrazol-4(2*H*)-one derivates, anticancer, PI3Kα, Molecular docking

1. Introduction

PI3K/Akt/mTOR signaling pathway is an important component in cellular signaling pathways and it plays a critical role in occurrence, development, treatment and transformation of tumors.[1-3] As an upstream molecule, PI3K (phosphatidylinositol 3-kinases) involved in many cellular functions such as cell growth, survival, proliferation, motility, differentiation and intracellular trafficking.[4-9]

As a family of lipid kinases, based on the sequence homology and substrate specificity, PI3Ks are categorized into four different classes: class I, class II, class III and Class IV. In particular, the class I is the most common studied and it is divided into PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ, which are heterodimers composed of a p110 catalytic subunit and a p85 regulatory subunit.[10-13] Of these, PI3Ks α and β are ubiquitously expressed, whereas the expression of δ and γ is more restricted, mostly to leukocytes.[14-17] The main function of class I PI3Ks is to phosphorylate phosphatidylinositol at the 3-position hydroxyl group of the inositol ring, and the important second messenger phosphatidylinositol 3,4,5-triphosphate (PIP3) which regulate many cellular functions is converted from 4,5-phosphatidylinositolbisphosphate (PIP2) by phosphorylation. Meanwhile, the process is strictly controlled and the high PIP3 levels resulting in negatively regulate by phosphatase and tensin homologue (PTEN), which catalyzes the dephosphorylation of PIP3 back to PIP2.[18-21] The abnormalities which show in

functions of both the phosphatase and kinase are commonly discovered in tumors, thus the PI3K pathway plays an important role in cancer and it becomes one of the most promising target for cancer treatment. [22-24]

In the past decades, many PI3K inhibitors have been discovered and identified, such as Wortmannin, LY294002, BEZ235, GDC0941 and so on.[25-27] Out of them, Wortmannin and LY294002 were recognized as the first-generation PI3K inhibitors. In our previous research, chromeno[4,3-c]pyrazol-4(2*H*)-one has been designed as the so-called core region which based on the structure of these PI3K inhibitors, and as we expected, chromeno[4,3-c]pyrazol-4(2*H*)-one derivates exhibited the potent antiproliferative activity against cancer cell lines.[28] Meanwhile, sulfonamido fragment was observed in some PI3K inhibitors such as GDC0941, XL147, CH5132799, GSK2126458, PIK75 and so on,[27,29-32] sulfonamido fragment is used to employ in drug design and it is a common strategy for structural modification. So it may be a valid method that sulfonamido fragment is employed to optimize the chromeno[4,3-c]pyrazol-4(2*H*)-one derivates (Figure 1).

In this study, a series of novel chromeno[4,3-c]pyrazol-4(2*H*)-one

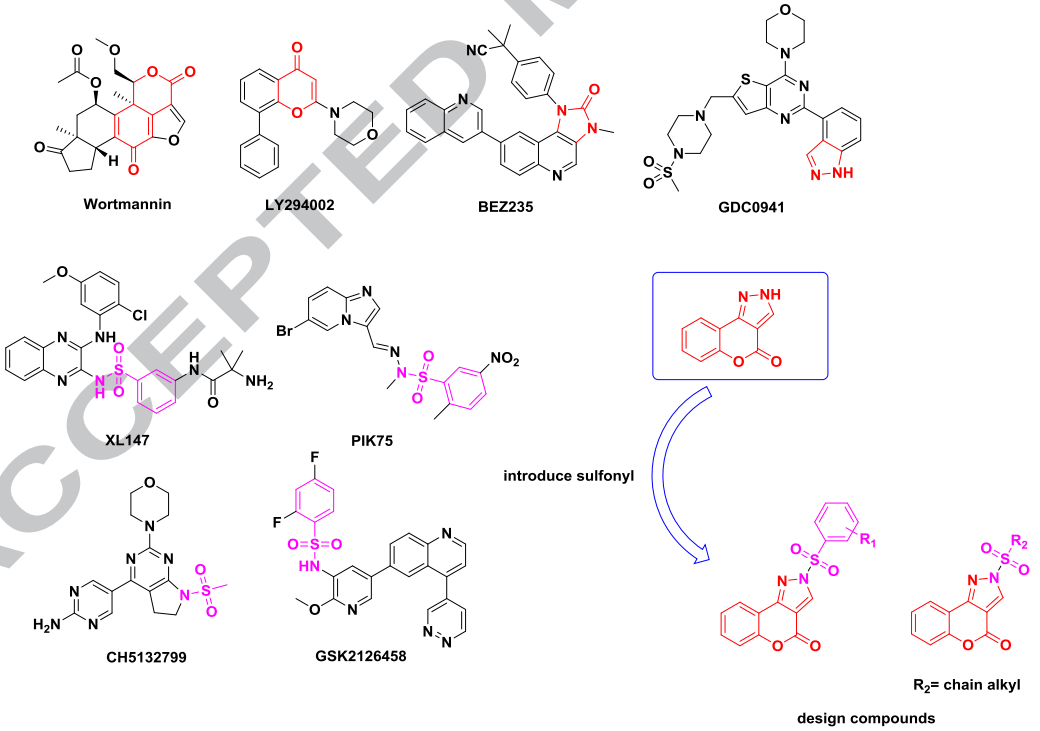


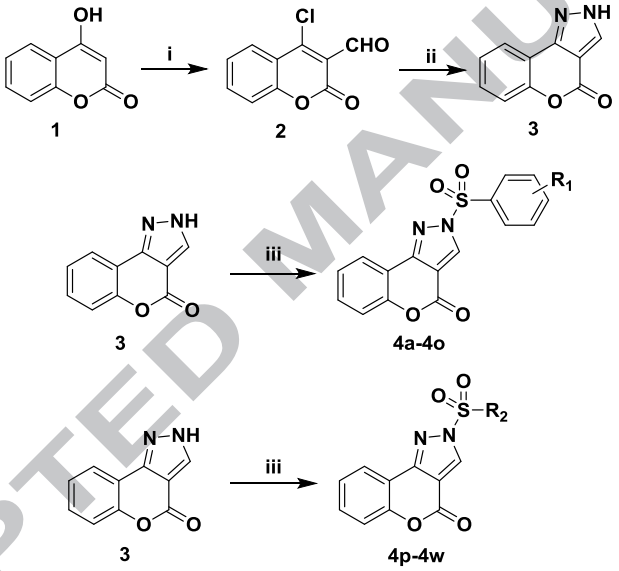
Figure 1. Design novel 2-alkyl-chromeno[4,3-*c*]pyrazol-4(2*H*)-one containing sulfonyl derivatives.

derivates contained sulfonamido were designed and synthesized, and their anticancer effects in vitro was evaluated to develop some new PI3K inhibitors.

2. Results and discussion

2.1. Chemistry

The desired compounds (**4a-4w**) was synthesized followed the general pathway outlined in Scheme 1. Chromeno[4,3-c]pyrazol-4(2*H*)-one was prepared according to the previous research.[28] Firstly, 3-formyl-4-chlorocoumarin (**2**) was prepared by Vilsmeier-Haack reaction ($POCl_3$/DMF) form 4-hydroxycoumarin (**1**). Subsequently, cyclization reaction using 85% hydrazine hydrate to obtain chromeno[4,3-c]pyrazol-4(*2H*)-one (**3**) in high yields (87%). Lastly, chromeno[4,3-c]pyrazol-4(2H)-one (**3**) and various sulfonyl chloride were reacted to obtain the targeted compounds (**4a**–**4w**).



compound	R_1	compound	R_1
4a	H	**4i**	3-NO_2
4b	4-CH_3	**4j**	4-NO_2
4c	4-OCH_3	**4k**	4-CF_3
4d	4-$C(CH_3)_3$	**4l**	2,5-CH_3
4e	4-F	**4m**	4-Cl-3-NO_2
4f	4-Cl	**4n**	2,4,6-CH_3
4g	4-Br	**4o**	2,4,6-$CH(CH_3)_2$
4h	2-NO_2		
compound	R_2	compound	R_2
4p	CF_3	**4t**	$CH_2CH_2CH_2CH_3$

4q	CH_3	**4u**	cyclopropane
4r	CH_2CH_3	**4v**	CH_2CH_2Cl
4s	$CH_2CH_2CH_3$	**4w**	$CH_2CH_2CH_2Cl$

Scheme 1. Synthesis of compounds **4a-4w**. Reagents and conditions: (i) DMF, $POCl_3$, 60 °C, 6 h. (ii) Ethanol, Et_3N, 85% hydrazine hydrate, r.t.,14 h. (iii) benzenesulfonyl chloride/ alkylsulfonyl chloride, DCM/TEA, 0 ℃, 6-8 h.

2.2. Antiproliferative activity

All the desired compounds (**4a-4w**) were evaluated for the anticancer activity *in vitro* against four cell lines: A549, HCT116, HL60 and Huh7. The results are summarized in Table 1. The results indicated that most desired compounds exhibit the potent antiproliferative activity against the four cancer cell lines and they were better than the positive control LY294002. In addition, the compounds containing the phenylsulfonyl displayed the antiproliferative activity were superior to that of compounds containing alkylsulfonyl. Out of them, compound **4o** exhibited the most potent antiproliferative activity against four cancer cell lines (IC_{50} = 0.07, 0.12, 0.09, and 0.09 μM for HCT116, A549, Huh7 and HL60, respectively).

Table 1. Antiproliferative activities in vitro of target compounds **4a-4w** against four cancer cell lines.

compound	IC_{50} ± SD(μM)			
	HCT116	A549	Huh7	HL60
4a	42.64±4.12	60.31±5.58	58.14±4.82	50.13±4.49
4b	8.03±0.73	9.78±9.07	14.68±1.23	11.65±1.07
4c	6.18±0.58	9.30±8.62	11.12±1.03	7.65±7.93
4d	6.74±0.61	7.75±6.86	11.37±0.98	9.02±9.27
4e	32.46±2.84	49.85±4.21	39.29±3.41	21.44±1.99
4f	27.40±2.11	32.04±2.86	47.06±4.34	38.73±4.03
4g	19.08±1.78	21.99±1.95	32.88±2.73	44.15±4.18
4h	11.50±1.02	13.65±1.17	10.24±1.09	14.01±1.26
4i	4.11±0.37	4.45±3.88	3.21±0.25	4.22±0.38
4j	1.04±0.11	0.97±0.08	1.49±0.17	5.03±0.44
4k	2.64±0.27	1.90±0.14	3.47±0.26	3.98±0.36
4l	0.36±0.24	0.35±0.03	0.19±0.02	0.65±0.04
4m	0.12±0.01	0.48±0.04	0.19±0.02	0.54±0.05
4n	0.08±0.01	0.17±0.01	0.12±0.01	0.12±0.01
4o	0.07±0.003	0.12±0.01	0.09±0.01	0.09±0.01

4p	>100	>100	>100	>100
4q	>100	>100	89.64±8.15	92.08±8.83
4r	>100	>100	>100±	87.19±8.14
4s	82.14±7.94	89.45±8.28	76.83±6.74	>100
4t	67.11±6.13	78.66±7.23	92.34±8.31	>100
4u	97.42±9.43	>100	>100	>100
4v	>100	>100	>100	74.68±6.71
4w	83.94±7.87	>100	96.44±8.97	>100
LY294002	51.82 ±4.58	82.32 ±7.26	67.18 ±5.64	18.43 ±2.03

As showed in Table 1, the compounds containing phenylsulfonyl showed the most antiproliferative activity, and there was a significant improvement in comparison with LY294002. In particular, compound **4o** exhibited an 200-740 fold improvement versus the positive control LY294002 in the inhibition of four cancer cells proliferation. It demonstrated that phenylsulfonyl fragment in desired compounds play a critical role in the antiproliferative effect.

Among the compound **4a-4o**, it was observed that the compounds with trisubstituted benzene ring showed the interesting activities, such as compounds **4n** and **4o**. What's more, comparing the mono-substituted groups on the benzene ring, the compounds containing the double substituent on the benzene ring displayed the better antiproliferative activities. It was indicated that the more substituent on the phenyl group, the better activity it exhibited. In addition, the compounds with mono-substituted benzene ring exhibited the antiproliferative activities in the order of electro-withdrawing substituents >electro-donating substituents >halogen substituents, and the substituted position on the benzene ring exhibited the antiproliferative activities in the order of *para* > *meta* > *ortho*. For the mono-substituted benzene ring, electronic effects played relevant roles in the antiproliferative profiles, derivatives with positive Hammett σ constants, displayed the best profiles, for example, σ_p=0.78 for -NO_2; σ_p=0.54 for -CF_3 and σ_p= -0.27 for -OCH_3[33]. Among the compound **4p-4w** containing the alkylsulfonyl group, the poor activities were showed. As can be seen in Table 1, The longer the alkyl chain, the less activity it showed. In a word, the result indicated that the phenylsulfonyl group is necessary to show the potent antiproliferative activity.

2.3. PI3K enzymatic activity

Due to the compounds (**4a-4o**) containing the phenylsulfonyl group show the

better antiproliferative activities, they were selected to evaluate for their PI3K enzymatic activities. The results are displayed in Table 2. As a whole, most of compounds exhibited the certain inhibitory activities against the four protein kinases PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ, and the tendency of inhibition was roughly similar to their inhibition for cancer cells. Besides, compare to another three protein kinases, the compounds showed a certain selectivity against protein PI3Kα. Specifically, compound **4o** exhibited the high selectivity to inhibit the protein PI3Kα with IC_{50} value up to 0.021 μM, which has an approximately 20-fold improvement for LY294002. Meanwhile, it has an increased potency up to approximately 50-fold for another three protein kinases. It was concluded that compound **4o** has a great potential to be a new PI3Kα inhibitor for antitumor.

Table 2. Enzymatic activities of compounds **4a-4o** against PI3K.

compound	IC_{50} ± SD (μM)			
	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
4a	52.87±4.82	32.65±2.32	38.29±2.34	56.19±3.12
4b	8.12±1.01	4.64±0.22	10.53±1.03	23.68±1.99
4c	6.48±0.78	22.65±1.77	18.24±1.46	44.74±2.76
4d	9.68±0.93	20.84±1.91	17.81±1.52	23.68±2.37
4e	44.66±0.68	67.92±3.78	42.57±2.47	67.81±4.43
4f	40.78±0.56	54.87±3.44	24.64±1.83	38.16±2.11
4g	27.67±0.44	56.74±2.98	32.18±2.14	47.92±2.83
4h	12.54±0.21	22.84±1.71	19.03±1.07	28.61±1.98
4i	3.69±0.32	18.66±1.13	9.47±0.95	11.16±0.94
4j	1.44±0.18	9.58±0.89	5.10±0.44	8.14±0.77
4k	1.17±0.13	11.32±0.97	3.48±0.41	9.49±0.82
4l	0.73±0.04	1.68±0.12	1.90±0.11	3.91±.34
4m	0.45±0.02	2.49±0.17	1.87±0.12	4.62±0.29
4n	0.10±0.01	1.13±0.093	0.80±0.02	1.78±0.11
4o	0.02±0.0003	0.90±0.014	0.28±0.01	0.99±0.04
LY294002	0.48±0.17	0.98±0.012	0.94±0.01	1.36±0.07

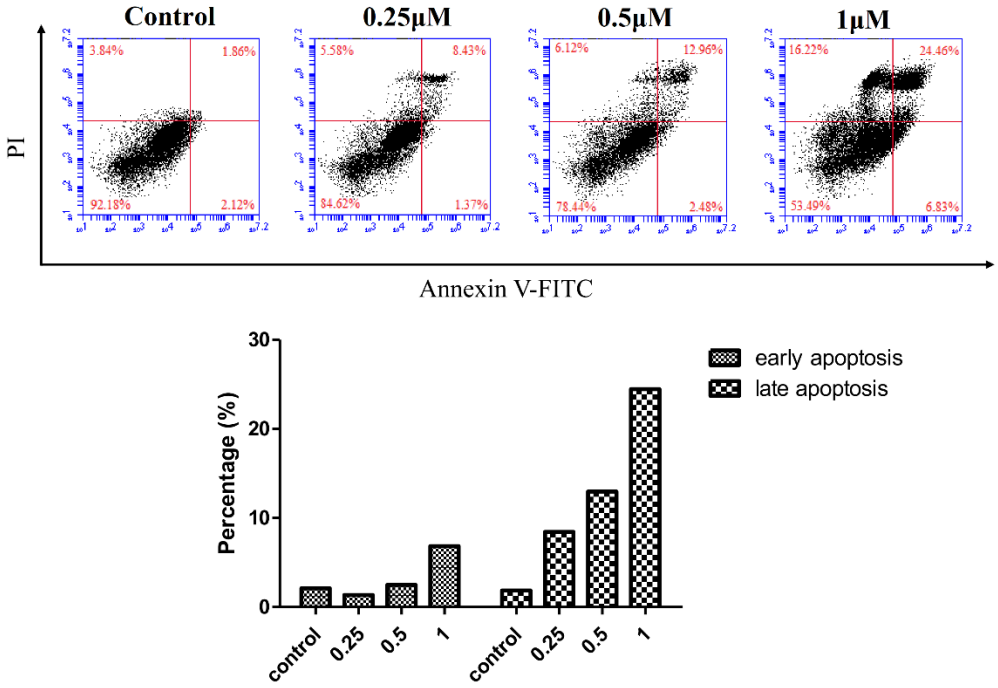


Figure 2. Compound **4o** induced apoptosis in HCT116 cells with the concentrations of 0.25, 0.5 and 1 μM. HCT-116 cells were treated for 24 h. Values represent the mean ± S.D., n = 3. $P < 0.05$ versus the control. The percentage of cells in each part is indicated.

2.4. Cells apoptosis assay

In order to explore the mechanism of cancer cells death, apoptosis induced in HCT116 cell was performed with Annexin V-FITC/PI FACS assay. The HCT116 cells were treated with compound **4o** in different concentration (0.25, 0.5 and 1μM) for 24 hours. The result was showed in Figure 2. The percentage of late apoptotic cells from 8.43 to 24.46% was significant influence in a dose-dependent manner by compound **4o**. It was indicated that compound **4o** killed the cancer cell by induction of apoptosis.

2.5. Western blot assay

As an upstream molecule, activation of the PI3K can phosphorylation of Akt directly and then regulated the downstream molecule, inhibition of PI3K leads to suppression of Akt phosphorylation. To further determine whether compound **4o** affect phosphorylation of Akt after inhibition the PI3K, western blot was performed to examine the related protein including Akt and p-Akt (S473) in HCT-116 cells by treatment of compound **4o**. As can be seen in Figure 3. compound **4o** obviously down-regulated expression of p-Akt (S473) in a dose-dependent manner, and the Akt expression level unchanged. It was indicated that compound may be a promising

PI3K inhibitor.

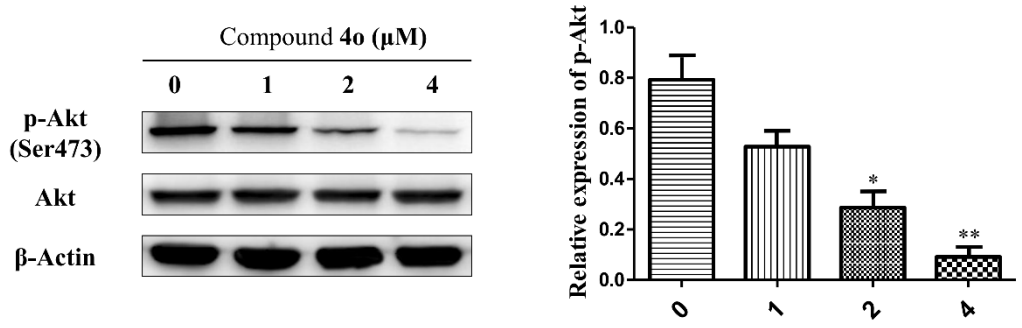


Figure 3. Western blot analysis of compound **4o**. The inhibition effects of compound **4o** (1μM, 2μM and 4μM) on the expression of p-Akt, Akt in HCT116 cells are depicted. β-Actin was used as internal control. Bar graphs showed the quantitative results of clonogenicity. Data are presented as the mean for three independent experiments. *P < 0.05, **P < 0.01 compared with control.

2.6. Molecular docking

Molecular docking was performed to clarify the possible binding mode between Ligands and proteins, compound **4o** was fitted into the active center of the PI3Kα protein. The results are showed in Figure 4(A and B). As can be seen, the binding model of ligand and protein mainly focus on chroman-2-one moiety of compound and PI3Kα protein. There are two hydrogen bonds, One hydrogen bond was formed between the amino hydrogen of Lys802 and the carboxyl oxygen atoms of compound **4o** (distance :1.83 Å, Angle: 160.47°), and the other hydrogen bond was caused by the oxygen atom of chroman-2-one interaction with Lys776 (distance :2.02 Å, Angle: 145.59°). It was indicated that the two amino acids Lys802 and Lys776 play an important in the pocket of protein. Besides, in the Figure 4B, the phenylsulfonyl fragment was located into the hydrophobic pocket, it might be the reason that the compound **4o** has the high selectivity against the PI3Kα protein.

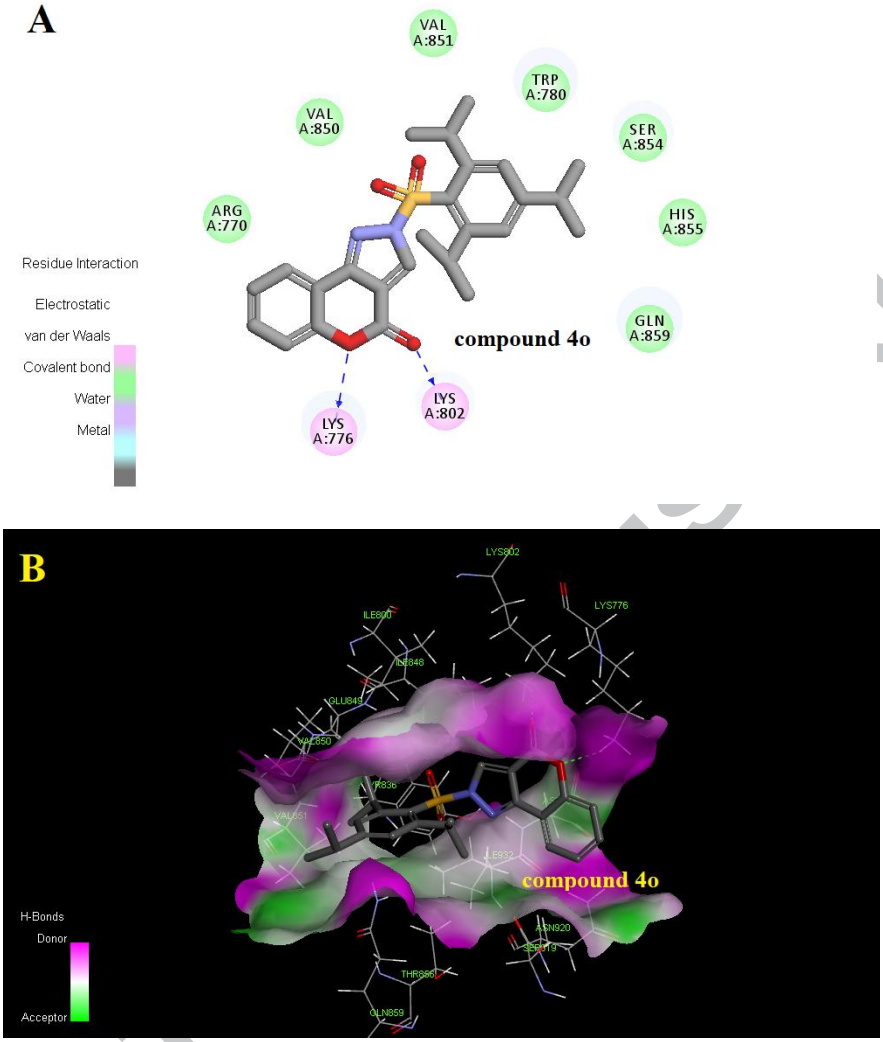


Figure 4. (A) 2D molecular docking model of compound **4o** with 3HHM. (B) 3D interaction map between compound **4o** and active pocket of PI3Kα protein.

3. Conclusion

In summary, a series of novel chromeno[4,3-c]pyrazol-4(2*H*)-one derivates contained sulfonamido were designed and synthesized, and their anticancer effects in vitro was evaluated to develop some new PI3Kα inhibitors. Most of desired compounds exhibited the better antiproliferative activities against four cancer cell lines than that of LY294002. Out of them, compound **4o** displayed the potent antiproliferative activity and high selectivity against the PI3Kα protein and it can induce apoptosis of HCT116 in a dose-dependent manner. Western blot assay indicated that compound **4o** obviously down-regulated expression of p-Akt (S473). Molecular docking was performed to clarify the possible binding mode between compound **4o** and PI3Kα. In a word, it can be concluded that compound **4o** was a potential PI3K inhibitor for cancer treatment.

4. Experimental section

All chemicals and reagents used in the current study were of analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points (uncorrected) were determined on an XT4MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, ^{1}H NMR spectra were collected on a Bruker DPX400 spectrometer and ^{13}C NMR spectra were collected on a Bruker Ascend 600 spectrometer at room temperature with TMS and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument, and were within ±0.4% of the theoretical values.

*4.1. General procedure for the preparation of compound **3***

Chromeno[4,3-*c*]pyrazol-4(2*H*)-one (**3**) was prepared according to the previous research.[28]

*4.2. General procedure for the preparation of compound **4a-4w***

To the solution of **3** (1 mmol) and TEA (10 μL) in dry DCM (15 mL), various sulfonyl chloride was added in the ice-bath and the reaction mixture was stirred at 0 °C for 6-8 h. After the reaction was complete, the mixture was concentrated in vacuo. The residue was subjected to crystallization to afford the desired compounds **4a-4w**.

*4.2.1. 2-(Phenylsulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4a**)*

White power, yield: 49%. Mp: 213-214 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.90(s, 1H, ArH), 8.14(d, J=7.92 Hz, 2H, ArH), 8.10(d, J=7.76 Hz, 1H, ArH), 7.73(t, J=7.38 Hz, 1H, ArH), 7.61(t, J=7.68 Hz, 2H, ArH), 7.51(t, J=7.78 Hz, 1H, ArH), 7.32(m, 2H, ArH). MS(ESI): 327.18 ($C_{16}H_{11}N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{16}H_{10}N_2O_4S$: C, 58.89; H, 3.09; N, 8.58%. Found: C, 58.90; H, 3.11; N, 8.59%.

*4.2.2. 2-Tosylchromeno[4,3-c]pyrazol-4(2H)-one (**4b**)*

White power, yield: 51%. Mp: 221-223 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.88(s, 1H,

ArH), 8.09(m, 1H, ArH), 8.01(d, J=8.40 Hz, 2H, ArH), 7.50(m, 1H, ArH), 7.39(d, J=8.12 Hz, 2H, ArH), 7.31(m, 2H, ArH), 2.44(s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): 156.91, 153.34, 151.93, 147.34, 134.15, 132.53, 131.86, 130.45(2), 128.92(2), 124.83, 123.72, 117.72, 113.64, 109.74, 21.88. MS(ESI): 341.42 ($C_{17}H_{13}N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{17}H_{12}N_2O_4S$: C, 59.99; H, 3.55; N, 8.23%. Found: C, 59.97; H, 3.56; N, 8.25%.

*4.2.3. 2-((4-Methoxyphenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4c**)*

White power, yield: 58%. Mp: 207-208 ℃. ^1H NMR (400 MHz, $CDCl_3$): 8.87(s, 1H, ArH), 8.08(t, J=8.92 Hz, 3H, ArH), 7.50(t, J=7.78 Hz, 1H, ArH), 7.31(m, 2H, ArH), 7.04(d, J=8.84 Hz, 2H, ArH), 3.88(s, 3H, OCH_3). MS(ESI): 357.25 ($C_{17}H_{13}N_2O_5S$, $[M+H]^+$). Anal.Calcd for $C_{17}H_{12}N_2O_5S$: C, 57.30; H, 3.39; N, 7.86%. Found: C, 57.32; H, 3.41; N, 7.85%.

*4.2.4. 2-((4-(Tert-butyl)phenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4d**)*

White power, yield: 48%. Mp: 223-224 ℃. ^1H NMR (400 MHz, $CDCl_3$): 8.88(s, 1H, ArH), 8.12(d, J=7.72 Hz, 1H, ArH), 8.05(d, J=8.24 Hz, 2H, ArH), 7.60(d, J=8.24 Hz, 2H, ArH), 7.50(t, J=7.84 Hz, 1H, ArH), 7.32(m, 2H, ArH), 1.32(s, 9H, $3CH_3$). ^{13}C NMR (150 MHz, $CDCl_3$): 160.11, 153.36, 151.91, 134.16, 132.44, 131.85, 128.75(3), 126.91(2), 124.83, 123.74, 117.73, 113.68, 109.78, 35.57, 30.90. MS (ESI): 383.29 ($C_{20}H_{19}N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{20}H_{18}N_2O_4S$: C, 62.81; H, 4.74; N, 7.33%. Found: C, 62.83; H, 4.75; N, 7.35%.

*4.2.5. 2-((4-Fluorophenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4e**)*

Light yellow power, yield: 39%. Mp: 198-201 ℃. ^1H NMR (400 MHz, $CDCl_3$): 8.89(s, 1H, ArH), 8.10(d, J=7.68 Hz, 1H, ArH), 8.00(d, J=8.04 Hz, 2H, ArH), 7.69(d, J=7.92 Hz, 2H, ArH), 7.55(t, J=7.72 Hz, 1H, ArH), 7.31(m, 2H, ArH). MS (ESI): 345.19 ($C_{16}H_{10}FN_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{16}H_9FN_2O_4S$: C, 55.81; H, 2.63; N, 8.14%. Found: C, 55.83; H, 2.64; N, 8.13%.

*4.2.6. 2-((4-Chlorophenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4f**)*

Light yellow power, yield: 42%. Mp: 207-209 ℃. ^1H NMR (400 MHz, $CDCl_3$): 8.89(d, J=3.08 Hz, 1H, ArH), 8.08(t, J=4.20 Hz, 3H, ArH), 7.59(m, 2H, ArH), 7.52(d, J=7.16 Hz, 1H, ArH), 7.33(m, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): 156.67, 153.39, 152.27, 142.84, 134.32, 133.92, 132.09, 130.29(2), 130.23(2), 124.93, 123.72,

117.79, 113.43, 110.03. MS (ESI): 361.62 ($C_{16}H_{10}ClN_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{16}H_9ClN_2O_4S$: C, 53.27; H, 2.51; N, 7.76%. Found: C, 53.29; H, 2.52; N, 7.75%.

*4.2.7. 2-((4-Bromophenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4g**)*

Light yellow power, yield: 45%. Mp: 216-217 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.88(s, 1H, ArH), 8.08(d, J=7.76 Hz, 1H, ArH), 8.00(d, J=8.40 Hz, 2H, ArH), 7.75(d, J=8.40 Hz, 2H, ArH), 7.52(t, J=7.86 Hz, 1H, ArH), 7.33(m, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): 156.66, 153.78, 152.27, 134.47, 134.34, 133.23(2), 132.09, 131.57, 130.24(2), 124.93, 123.72, 117.78, 113.41, 110.03. MS (ESI): 406.37 ($C_{16}H_{10}BrN_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{16}H_9BrN_2O_4S$: C, 47.42; H, 2.24; N, 6.91%. Found: C, 47.44; H, 2.23; N, 6.93%.

*4.2.8. 2-((2-Nitrophenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4h**)*

Yellow power, yield: 48%. Mp: 197-199 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.89(s, 1H, ArH), 8.57(d, J=7.36 Hz, 1H, ArH), 8.06(m, 3H, ArH), 7.67(m, 1H, ArH), 7.54(m, 1H, ArH), 7.30(m, 2H, ArH). MS (ESI): 372.28 ($C_{16}H_{10}N_3O_6S$, $[M+H]^+$). Anal.Calcd for $C_{16}H_9N_3O_6S$: C, 51.75; H, 2.44; N, 11.32%. Found: C, 51.78; H, 2.45; N, 11.34%.

*4.2.9. 2-((3-Nitrophenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4i**)*

Yellow power, yield: 44%. Mp: 224-227 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.92(s, 1H, ArH), 8.81(s, 1H, ArH), 8.54(d, J=7.76 Hz, 1H, ArH), 8.24(m, 1H, ArH), 8.00(m, 2H, ArH), 7.52(d, J=7.72 Hz, 1H, ArH), 7.30(m, 2H, ArH). MS (ESI): 372.28 ($C_{16}H_{10}N_3O_6S$, $[M+H]^+$). Anal.Calcd for $C_{16}H_9N_3O_6S$: C, 51.75; H, 2.44; N, 11.32%. Found: C, 51.76; H, 2.45; N, 11.30%.

*4.2.10. 2-((4-Nitrophenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4j**)*

Yellow power, yield: 42%. Mp: 237-239 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.90(s, 1H, ArH), 8.35(d, J=7.76 Hz, 2H, ArH), 8.12(d, J=7.92 Hz, 2H, ArH), 8.00(m, 1H, ArH), 7.51(m, 1H, ArH), 7.32(m, 2H, ArH). MS (ESI): 372.28 ($C_{16}H_{10}N_3O_6S$, $[M+H]^+$). Anal.Calcd for $C_{16}H_9N_3O_6S$: C, 51.75; H, 2.44; N, 11.32%. Found: C, 51.77; H, 2.42; N, 11.33%.

*4.2.11. 2-((4-(Trifluoromethyl)phenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4k**)*

Light yellow power, yield: 47%. Mp: 214-216 ℃. ^{1}H NMR (400 MHz, $CDCl_3$):

8.89(s, 1H, ArH), 8.08(d, J=8.04 Hz, 1H, ArH), 8.02(d, J=8.28 Hz, 2H, ArH), 7.79(d, J=8.40 Hz, 2H, ArH), 7.51(t, J=7.92 Hz, 1H, ArH), 7.33(m, 2H, ArH). MS (ESI): 395.26 ($C_{17}H_{10}F_3N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{17}H_9F_3N_2O_4S$: C, 51.78; H, 2.30; N, 7.10%. Found: C, 51.80; H, 2.31; N, 7.08%.

*4.2.12. 2-((2,5-Dimethylphenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4l**)*

White power, yield: 52%. Mp: 221-223 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.94(s, 1H, ArH), 8.05(d, J=7.92 Hz, 2H, ArH), 7.50(t, J=7.82 Hz, 1H, ArH), 7.40(d, J=7.68 Hz, 1H, ArH), 7.34(d, J=8.36 Hz, 1H, ArH), 7.29(t, J=7.60 Hz, 1H, ArH), 7.23(d, J=7.76 Hz, 1H, ArH), 2.62(s, 3H, CH_3), 2.44(s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): 156.96, 153.35, 151.64, 137.41, 136.62, 134.29, 133.41, 133.29, 131.84, 131.52, 124.82, 123.72, 118.07, 117.73, 113.65, 109.38, 20.86, 20.24. MS (ESI): 355.24 ($C_{18}H_{15}N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{18}H_{14}N_2O_4S$: C, 61.01; H, 3.98; N, 7.90%. Found: C, 61.03; H, 3.99; N, 3.97%.

*4.2.13. 2-((4-Chloro-3-nitrophenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4m**)*

Yellow power, yield: 33%. Mp: 229-231 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.90(s, 1H, ArH), 8.00(m, 1H, ArH), 7.87(d, J=8.08 Hz, 1H, ArH), 7.78(d, J=8.32 Hz, 1H, ArH), 7.52(m, 1H, ArH), 7.44(d, J=8.40 Hz, 1H, ArH), 7.33(m, 2H, ArH). MS (ESI): 406.67 ($C_{16}H_9ClN_3O_6S$, $[M+H]^+$). Anal.Calcd for $C_{16}H_8ClN_3O_6S$: C, 47.36; H, 1.99; N, 10.36%. Found: C, 47.38; H, 2.01; N, 10.34%.

*4.2.14. 2-(Mesitylsulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4n**)*

White power, yield: 51%. Mp: 242-244 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.92(s, 1H, ArH), 8.00(dd, J1=7.80 Hz, J2=1.56 Hz, 1H, ArH), 7.48(m, 1H, ArH), 7.33(d, J=7.76 Hz, 1H, ArH), 7.28(d, J=6.72 Hz, 1H, ArH), 7.03(s, 2H, ArH), 2.73(s, 6H, $2CH_3$), 2.32(s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): 157.13, 153.30, 151.28, 146.11, 142.08(2), 133.46, 132.74(2), 131.68, 129.58, 124.74, 123.58, 117.68, 113.76, 108.77, 23.27(2), 21.27. MS (ESI): 369.34 ($C_{19}H_{17}N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{19}H_{16}N_2O_4S$: C, 61.94; H, 4.38; N, 7.60%. Found: C, 61.97; H, 4.40; N, 7.58%.

*4.2.15. 2-((2,4,6-Triisopropylphenyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4o**)*

White power, yield: 48%. Mp: 231-234 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.94(s, 1H, ArH), 8.00(d, J=3.72 Hz, 1H, ArH), 7.50(t, J=7.82 Hz, 1H, ArH), 7.36(d, J=8.28 Hz, 1H, ArH), 7.30(t, J=7.60 Hz, 1H, ArH), 7.26(s, 2H, ArH), 4.29(m, 2H, 2-CH-),

2.93(m, 1H, -CH-), 1.26(t, J=6.02 Hz, 18H, $6CH_3$). ^{13}C NMR (150 MHz, $CDCl_3$): 157.10, 156.23, 153.35, 153.23(2), 151.01, 132.88, 131.66, 128.54, 124.82(3), 123.44, 117.74, 113.82, 108.99, 34.39, 30.27(2), 24.69(4), 23.40(2). MS (ESI): 453.36 ($C_{25}H_{29}N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{25}H_{28}N_2O_4S$: C, 66.35; H, 6.24; N, 6.19%. Found: C, 66.36; H, 6.23; N, 6.21%.

*4.2.16. 2-((Trifluoromethyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4p**)*

Yellow power, yield: 41%. Mp: 167-169 ℃. ^1H NMR (400 MHz, $CDCl_3$): 8.92(s, 1H, ArH), 8.01(m, 1H, ArH), 7.50(m, 1H, ArH), 7.34(d, J=7.96 Hz, 1H, ArH), 7.26(d, J=7.52 Hz, 1H, ArH). MS (ESI): 319.17 ($C_{11}H_6F_3N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{11}H_5F_3N_2O_4S$: C, 41.52; H, 1.58; N, 8.80%. Found: C, 41.53; H, 1.60; N, 8.79%.

*4.2.17. 2-(Methylsulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4q**)*

White power, yield: 43%. Mp: 173-174 ℃.^1H NMR (400 MHz, $CDCl_3$): 8.92(s, 1H, ArH), 8.00(dd, J1=7.84 Hz, J2=1.60 Hz, 1H, ArH), 7.50(m, 1H, ArH), 7.35(d, J=7.84 Hz, 1H, ArH), 7.29(d, J=7.12 Hz, 1H, ArH), 2.97(s, 3H, CH_3). MS (ESI): 265.21 ($C_{11}H_9N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{11}H_8N_2O_4S$: C, 50.00; H, 3.05; N, 10.60%. Found: C, 50.02; H, 3.03; N, 10.58%.

*4.2.18. 2-(Ethylsulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4r**)*

White power, yield: 40%. Mp: 179-181 ℃. ^1H NMR (400 MHz, $CDCl_3$): 8.94(s, 1H, ArH), 8.00(m, 1H, ArH), 7.48(m, 1H, ArH), 7.36(d, J=8.04 Hz, 1H, ArH), 7.28(d, J=7.44 Hz, 1H, ArH), 3.38(m, 2H, $-CH_2$), 1.26(t, J=7.32 Hz, 3H, CH_3). MS (ESI): 279.31 ($C_{12}H_{11}N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{12}H_{10}N_2O_4S$: C, 51.79; H, 3.62; N, 10.07%. Found: C, 51.82; H, 3.63; N, 10.06%.

*4.2.19. 2-(Propylsulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4s**)*

White power, yield: 39%. Mp: 181-283 ℃. ^1H NMR (400 MHz, $CDCl_3$): 8.94(s, 1H, ArH), 8.00(d, J=7.86 Hz, 1H, ArH), 7.49(m, 1H, ArH), 7.33(d, J=7.92 Hz, 1H, ArH), 7.28(d, J=7.48 Hz, 1H, ArH), 3.38(t, J=7.06 Hz, 2H, $-CH_2$), 1.92(m, 2H, $-CH_2$), 0.95(t, J=7.38 Hz, 3H, CH_3). MS (ESI): 293.21 ($C_{13}H_{13}N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{13}H_{12}N_2O_4S$: C, 53.42; H, 4.14; N, 9.58%. Found: C, 53.44; H, 4.16; N, 9.57%.

*4.2.20. 2-(Butylsulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4t**)*

White power, yield: 41%. Mp: 177-179 ℃. ^1H NMR (400 MHz, $CDCl_3$): 8.92(s, 1H,

ArH), 8.00(m, 1H, ArH), 7.49(m, 1H, ArH), 7.33(d, J=7.96 Hz, 1H, ArH), 7.28(d, J=7.52 Hz, 1H, ArH), 3.41(t, J=7.28 Hz, 2H, -CH2), 1.94(m, 2H, -CH_2), 1.73(m, 2H, -CH_2), 0.98(t, J=7.14 Hz, 3H, CH_3). MS (ESI): 307.31 ($C_{14}H_{15}N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{14}H_{14}N_2O_4S$: C, 54.89; H, 4.61; N, 9.14%. Found: C, 54.92; H, 4.60; N, 9.15%.

*4.2.21. 2-(Cyclopropylsulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4u**)*

White power, yield: 37%. Mp: 168-171 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.92(s, 1H, ArH), 8.00(dd, J1=8.04 Hz, J2=1.56 Hz, 1H, ArH), 7.51(m, 1H, ArH), 7.38(d, J=7.92 Hz, 1H, ArH), 7.30(d, J=7.32 Hz, 1H, ArH), 3.42(m, 1H, -CH-), 1.97(m, 4H, 2-CH_2). MS (ESI): 291.32 ($C_{13}H_{11}N_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{13}H_{10}N_2O_4S$: C, 53.79; H, 3.47; N, 9.65%. Found: C, 53.78; H, 3.48; N, 9.67%.

*4.2.22. 2-((2-Chloroethyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4v**)*

Light yellow power, yield: 41%. Mp: 164-166 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.92(s, 1H, ArH), 8.00(m, 1H, ArH), 7.51(m, 1H, ArH), 7.36(d, J=7.84 Hz, 1H, ArH), 7.28(d, J=7.36 Hz, 1H, ArH), 3.48(t, J=7.14 Hz, 2H, -CH_2), 3.42(t, J=7.18 Hz, 2H, -CH_2). MS (ESI): 313.67 ($C_{12}H_{10}ClN_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{12}H_9ClN_2O_4S$: C, 46.09; H, 2.90; N, 8.96%. Found: C, 46.11; H, 2.92; N, 8.95%.

*4.2.23. 2-((3-Chloropropyl)sulfonyl)chromeno[4,3-c]pyrazol-4(2H)-one (**4w**)*

Light yellow power, yield: 38%. Mp: 170-172 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.94(s, 1H, ArH), 8.00(m, 1H, ArH), 7.48(m, 1H, ArH), 7.34(d, J=7.92 Hz, 1H, ArH), 7.30(d, J=7.28 Hz, 1H, ArH), 3.44(t, J=7.22 Hz, 2H, -CH_2), 3.41(t, J=7.08 Hz, 2H, -CH_2), 2.21(m, 2H, -CH_2). MS (ESI): 327.71 ($C_{13}H_{12}ClN_2O_4S$, $[M+H]^+$). Anal.Calcd for $C_{13}H_{11}ClN_2O_4S$: C, 47.78; H, 3.39; N, 8.57%. Found: C, 47.80; H, 3.40; N, 8.55%.

4.3. Cell proliferation assay

Four human cell lines HCT116, A549, Huh7 and HL60 were cultured in RPMI-1640 (Invitrogen Corp., Carlsbad, CA) medium with heat-inactivated 10% fetal bovine serum, penicillin (100 units mL^{-1}) and streptomycin (100 mg mL^{-1}) and incubated under an atmosphere with 20% O_2, 5% CO_2 at 37 °C.

The inhibitory activity in vitro was measured using the MTT assay. All the compounds were dissolved in DMSO at a concentration of 100 μM and were then diluted to appropriate concentrations. Cells were plated in 96-well plates for 24 h and

subsequently treated with different concentrations of all the tested compounds for 72 h. Viable cells were determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay kit (MTT, Sigma) according to the manufacturer's instructions. The concentration of drug causing 50% inhibition of absorbance compared with control cells (IC_{50}) was calculated using the software of dose-effect analysis with microcomputers.

4.4. PI3K enzyme assay

The inhibition of PI3K activity was determined using a competitive fluorescence polarization kinase activity assay based on the principle that PI3K phosphorylates $PI(3,4)P_2$ and converts it into $PI(3,4,5)P_3$. PI3K isoforms were purchased from Perkin-Elmer or were expressed and purified as heterodimeric recombinant proteins. Tetramethylrhodamine-labeled PIP_3 (TAMRA-PIP_3), diC_8-PIP_2, and PIP_3 detection reagents were purchased from Echelon Biosciences. PI3K reactions were performed in 5 mM HEPES, pH 7, 2.5 mM $MgCl_2$, 10 mM DTT and 50 mM ATP using diC_8-$PI(4,5)P_2$ as the substrate, and the final reaction volumes were 10 μL. For the evaluation of PI3K inhibitors, 50 ng of enzyme and 10 mM of substrate were used per 10 μL reaction volume with inhibitor concentrations ranging from 3.2 nM to 1 mM. After incubating for 3 h at room temperature, reactions were quenched by the addition of a chelator. A mixture of phosphoinositide binding protein was added and mixed, followed by the addition of a fluorophorelabeled phosphoinositide tracer. Samples were then mixed in 384-well black Corning nonbinding plates and incubated in the dark for 1 h to equilibrate. Finally, polarization values were measured using red fluorophores with appropriate filters to determine the extent of enzyme activity in the reaction.[34]

4.5. Apoptosis analysis

5×10^5 HCT116 cells in exponential growth was seeded into each well of 6-well plate. After incubation for 12 h, they were treated with various concentrations of the test compounds for 24 h. Then the cells were collected totally, centrifuged and resuspended in 500 mL of staining solution (containing 5 mL Annexin V-FITC and 5 mL PI in Binding Buffer) and incubated for 15 min on the ice with avoiding light. Samples were analyzed on FACScalibur flow cytometer (BD, USA).

4.6. Western blotting

Total cell lysates were prepared in RIPA buffer (50mM Tris-HCl, Ph 8.0, 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS). The protein concentration was determined, and equal amounts of protein samples were subjected to 10% SDSPAGE. Separated proteins were transferred to PVDF membranes (Millipore, Bedford, MA, USA) and probed with the indicated antibodies. Exposures were obtained using ChemiDoc Touch biomolecular imager (Bio-Rad, USA).

4.7. Molecular docking

The crystal structures of the protein complexes were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The three-dimensional structures of the aforementioned compounds were constructed using Chem. 3D ultra 12.0 software [Chemical Structure Drawing Standard; Cambridge Soft Corporation, USA (2010)]; then they were energetically minimized using MMFF94 with 5000 iterations and a minimum RMS gradient of 0.10. Molecular docking of compound 4o into the three-dimensional PI3Kα complex structure (3HHM.pdb, downloaded from the PDB) was carried out using the Discovery Studio (version 3.5) as implemented through the graphical user interface DS-CDOCKER protocol, all bound water and ligands were eliminated from the protein and the polar hydrogen was added to the proteins.

Acknowledgment

This work was supported by National Natural Science Foundation of China (No. 81602985), the Fundamental Research Funds for the Central Universities (No. 2632018ZD19), a grant (No. 201505023) from Public Science, Technology research Funds Project of Ocean and financed by National Natural Science Foundation of China (No. 21602103). It was also supported by the National Undergraduate Innovation Program and the National Natural Science Foundation of China (No. J1210026).

References

1. Johnson SM, Gulhati PRampy BA, Han Y, Rychahou PG, Doan, HQ, Weiss, HL, Evers BM. *J Am Coll Surgeons.* 2010, 210:767-776.
2. Wu P, Y-Z H. *Curr Med Chem.* 2010, 17:4326-4341.
3. Nandini D, de Pradip; Brian, LJ. *Pharmacol Therapeut.* 2017, 175, 91-106.

4. Vivanco I, Sawyers CL. *Nat Rev Cancer.* 2002;2:489-501.
5. Engelman JA, Luo J, Cantley LC. *Nat Rev Genet.* 2006;7:606-619.
6. Liu PX, Cheng HL, Roberts TM, Zhao JJ. *Nat Rev Drug Discov.* 2009;8:627-644.
7. Cantley LC. Science 2002;296: 1655-1657.
8. Thorpe LM, Haluk Y, Zhao JJ. *Nat Rev Cancer*. 2015, 15:7-24.
9. Burke JE, Williams RL. *Trends Biochem Sci.* 2015, 40:88-100.
10. Wymann MP, Pirola L. *Biochim Biophys Acta*. 1998**,** *1436*, 127-150.
11. Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD. *Annu. Rev. Cell Dev. Biol.* 2001, 17, 615−675.
12. Fruman DA, Meyers RE, Cantley LC. *Annu. Rev. Biochem.* 1998, 67, 481−507.
13. Vanhaesebroeck B, Leevers SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ, Waterfield MD. *Annu. Rev. Biochem.* 2001, 70, 535−602.
14. Knight ZA, Beatriz G, Feldman ME, Zunder ER, Goldenberg DD, Olusegun W, Robbie L, David S, Andras B, Balazs, T. *Cell.* **2006,** *125*, 733-717.
15. Jackson, S. P.; Schoenwaelder, S. M.; Goncalves, I.; Nesbitt, W. S.; Yap, C. L.; Wright, C. E.; Kenche, V.; Anderson, K. E.; Dopheide, S. M.; Yuan, Y.; Sturgeon, S. A.; Prabaharan, H.; Thompson, P. E.; Smith, G. D.; Shepherd, P. R.; Daniele, N.; Kulkarni, S.; Abbott, B.; Saylik, D.; Jones, C.; Lu, L.; Giuliano, S.; Hughan, S. C.; Angus, J. A.; Robertson, A. D.; Salem, H. H. *Nat. Med.* 2005, 11 (5), 507−514.
16. SP, J.; SM, S. *Journal of Thrombosis & Haemostasis.* **2012,** *10*, 2123-2116.
17. Yoo, E. J.; Ojiaku, C. A.; Sunder, K.; Jr, P. R. *American Journal of Respiratory Cell & Molecular Biology.* 2016, 56: 700−707.
18. Min, S. S.; Salmena, L.; Pandolfi, P. P. *Nat Rev Mol Cell Biol* **2012,** *13*, 283-296.
19. Brachmann, S. M.; Yballe, C. M.; Metello, I.; Deane, J. A.; Fruman, D. A.; Thomas, S. M.; Cantley, L. C. *Molecular & Cellular Biology.* **2005,** *25*, 2593-2606.
20. Czech, M. P. *Cell.* **2000,** *100*, 603-606.
21. Chuan-Hsiang, H.; Diana, M.; Oleg, S. K.; Yardena, S.; Velculescu, V. E.; Kinzler, K. W.; Bert, V.; Gabelli, S. B.; L Mario, A. *Science.* **2007,** *318*, 1744-1748.
22. Burke, J. E.; Olga, P.; Masson, G. R.; Oscar, V.; Williams, R. L. *P Natl Acad Sci Usa.* **2012,** *109*, 15259.
23. Stephen, J.; Flanagan, J. U.; Sharada, K.; Christina, B.; Kendall, J. D.; Woo-Jeong, L.; Rewcastle, G. W.; Denny, W. A.; Ripudaman, S.; James, D. *Biochem J.* **2011,** *438*, 53-62.

24. Carson, J. D.; van Glenn, A.; Ruth, L.; Sinnamon, R. H.; Kirkpatrick, R. B.; Auger, K. R.; Dashyant, D.; Copeland, R. A.; Gontarek, R. R.; Tummino, P. J. *Biochem J.* **2008,** *409*, 519-524.
25. Semba, S.; Itoh, N.; Ito, M.; Harada, M.; Yamakawa, M. *Clin Cancer Res.* 2002, 8, 1957-1963.
26. Maira, S.; Stauffer, F. J.; Furet, P.; Schnell, C.; Fritsch, C.; Brachmann, S.; Chene, P.; De-Pover, A.; Schoemaker, K.; Fabbro, D. *Mol Cancer Ther.* **2008,** *7*, 1851-1863.
27. Folkes, A. J.; Khatereh, A.; Alderton, W. K.; Sonia, A.; Baker, S. J.; Gary, B.; Chuckowree, I. S.; Clarke, P. A.; Paul, D.; Eccles, S. A. *J Med Chem.* **2008,** *51*, 5522-5532.
28. Yong, Y.; Xun, W.; Hong-Wei, H.; Shao, S.; She-Feng, W.; Fang, Q.; Ai-Min, L.; Peng-Cheng, L.; Hai-Liang, Z. *Org & Biomol Chem.* **2014,** *12*, 9157-9165.
29. Anindita, C.; Violeta, S.; Kuba, M. G.; Cammie, R.; Arteaga, C. L. *Proc Natl Acad Sci USA.* **2012,** *109*, 2718-2723.
30. Ohwada, J.; Ebiike, H.; Kawada, H.; Tsukazaki, M.; Nakamura, M.; Miyazaki, T.; Morikami, K.; Yoshinari, K.; Yoshida, M.; Kondoh, O. *Bioorg Med Chem Lett.* **2011,** *21*, 1767-1772.
31. Kong D, Yamori T. *Acta Pharmacol Sin.* 2010;31:1189-1197
32. Frederick, R.; Denny, W. A. *Cheminform.* 2008;48:629-638.
33. Hansch, C.; Leo, A.; Taft, R.W.. *Chem. Rev.* 1991, 91:165-195.
34. Wu P, Su Y, Liu X, Zhang L, Ye Y, Xu J, Weng S, Li Y, Liu T, Huang S, Yang B, He Q, Hu Y. *Eur J Med Chem.* 2011, 46: 5540-5548.

Captions

Scheme 1. Synthesis of compounds **4a-4w**. Reagents and conditions: (i) DMF, $POCl_3$, 60 °C, 6 h. (ii) Ethanol, Et3N, 85% hydrazine hydrate, r.t.,14 h. (iii) benzenesulfonyl chloride/ alkylsulfonyl chloride, DCM/TEA, 0 ℃, 6-8 h.

Table 1. Antiproliferative activities in vitro of target compounds **4a-4w** against four cancer cell lines.

Table 2. Enzymatic activities of compounds **4a-4o** against PI3K.

Figure 1. Design novel 2-alkyl-chromeno[4,3-*c*]pyrazol-4(2*H*)-one containing sulfonyl derivatives.

Figure 2. Compound **4o** induced apoptosis in HCT116 cells with the concentrations of 0.25, 0.5 and 1 μM. HCT-116 cells were treated for 24 h. Values represent the mean ± S.D., n = 3. $P < 0.05$ versus the control. The percentage of cells in each part is indicated.

Figure 3. Western blot analysis of compound **4o**. The inhibition effects of compound **4o** (1μM, 2μM and 4μM) on the expression of p-Akt, Akt in HCT116 cells are depicted. β-Actin was used as internal control. Bar graphs showed the quantitative results of clonogenicity. Data are presented as the mean for three independent experiments. *$P < 0.05$, **$P < 0.01$ compared with control.

Figure 4. (A) 2D molecular docking model of compound **4o** with 3HHM. (B) 3D interaction map between compound **4o** and 3HHM binding sites of PI3Kα protein.

Design, synthesis and biological evaluation of novel chromeno[4,3-*c*]pyrazol-4(2*H*)-one derivates containing sulfonamido as potential PI3Kα inhibitors

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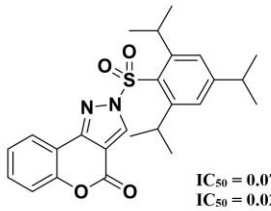

IC_{50} = 0.07 μM for HCT116
IC_{50} = 0.021 μM for PI3Kα

compound 4o