Journal Pre-proof

Development of novel chromeno[4,3-*c*]pyrazol-4(2*H*)-one derivates bearing sulfonylpiperazine as antitumor inhibitors targeting PI3Kα

Yong Yin, Shao Sha, Xun Wu, She-Feng Wang, Fang Qiao, Zhong-Cheng Song, Hai-Liang Zhu

PII: S0223-5234(19)30764-0

DOI: https://doi.org/10.1016/j.ejmech.2019.111630

Reference: EJMECH 111630

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 13 June 2019

Revised Date: 14 August 2019

Accepted Date: 16 August 2019

Please cite this article as: Y. Yin, S. Sha, X. Wu, S.-F. Wang, F. Qiao, Z.-C. Song, H.-L. Zhu, Development of novel chromeno[4,3-*c*]pyrazol-4(2*H*)-one derivates bearing sulfonylpiperazine as antitumor inhibitors targeting PI3Kα, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.111630.

Development of novel chromeno[4,3-*c*]pyrazol-4(2*H*)-one derivates bearing sulfonylpiperazine as antitumor inhibitors targeting PI3K*α*

Yong Yin[a,b], Shao Sha[b], Xun Wu[b], She-Feng Wang[b], Fang Qiao[b], Zhong-Cheng Song[*b,c], Hai-Liang Zhu[*b]

[a] Jiangsu Key Laboratory of Bioactive Natural Product Research and State Key Laboratory of Natural Medicines, Department of Natural Medicinal Chemistry, School of Traditional Chinese Pharmacy, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, People' s Republic of China.

[b] State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China.

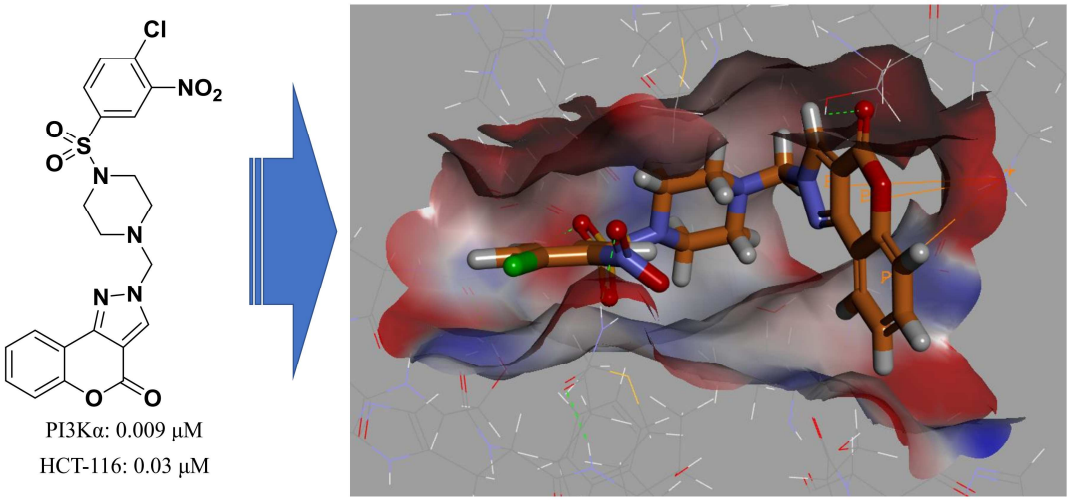
[c] School of Chemistry & Environmental Engineering, Jiangsu University of Technology, 1801 Zhongwu Rd., Changzhou, Jiangsu, 213001 China

* Corresponding authors.

Fax:+86-25-83592672; Tel:+86-25-83592572;

E-mail: zhuhl@nju.edu.cn (Hai-Liang Zhu);

songzhongcheng@jsut.edu.cn(Zhong-Cheng Song)



Development of novel chromeno[4,3-*c*]pyrazol-4(2*H*)-one derivates bearing sulfonylpiperazine as antitumor inhibitors targeting PI3K*α*

Yong Yin[a,b], Shao Sha[b], Xun Wu[b], She-Feng Wang[b], Fang Qiao[b], Zhong-Cheng Song[*b,c], Hai-Liang Zhu[*b]

[a] Jiangsu Key Laboratory of Bioactive Natural Product Research and State Key Laboratory of Natural Medicines, Department of Natural Medicinal Chemistry, School of Traditional Chinese Pharmacy, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, People' s Republic of China.

[b] State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China.

[c] School of Chemistry & Environmental Engineering, Jiangsu University of Technology, 1801 Zhongwu Rd., Changzhou, Jiangsu, 213001 China

* Corresponding authors.

Fax:+86-25-83592672; Tel:+86-25-83592572;

E-mail: zhuhl@nju.edu.cn (Hai-Liang Zhu);

songzhongcheng@jsut.edu.cn(Zhong-Cheng Song)

Abstract

PI3K signal pathway plays a vital role in cellular functions and becomes an attractive approach for cancer therapy. Herein, a new series of novel chromeno[4,3-c]pyrazol-4(2*H*)-one derivatives bearing sulfonylpiperazine based on the PI3K inhibitors and our previous research. They were screened for their PI3K inhibitory activities and anticancer effects *in vitro*. Biological studies indicated that compound **7m** revealed the remarkable antiproliferative activity (IC_{50} ranging from 0.03 to 0.09μM) against four cancer cell lines (A549, Huh7, HL60 and HCT-116). Besides, compound **7m** displayed a certain selective for PI3Kα (IC_{50} = 0.009μM) over PI3Kβ, γ and δ, and meanwhile, it can remarkable decreased the expression level of p-Akt (Ser473) and p-S6K. In addition, compound **7m** could not only induce HCT-116 cell arrest at G1 phase in a dose-dependent manner, but also induce cell apoptosis *via* upregulation of Bax and cleaved-caspase 3/9, and downregulation of Bcl-2. Besides, compound **7m** can remarkably inhibit the growth of tumor *in vivo*. The above results suggested that compound **7m** could be considered as a promising PI3Kα inhibitor.

Keywords: chromeno[4,3-c]pyrazol-4(2*H*)-one derivates; sulfonylpiperazine; antitumor; PI3Kα inhibitor

1. Introduction

As an important signaling pathway, phosphatidylinositol 3-kinases (PI3Ks) play an extremely pivotal role in a large number of cellular functions such as cell motility, differentiation, growth, proliferation, intracellular trafficking and so on [1-3]. The PI3K family is categorized into three classes (classes I, II and III) in terms of their structural features and substrate specificity [4-6]. Out of them, Class I PI3Ks are studied most extensively and have been an attractive approach for cancer therapy over the past decades, and they comprise four different isoforms (PI3Kα, β, γ and δ) and consist of heterodimers between a p110 catalytic subunit and a p85 regulatory subunit, which commonly used to be activated by receptor tyrosine kinases (RTKs) and G-protein coupled receptors [7-10]. Caused by activation of PI3Ks, phosphatidylinositol(4,5)diphosphate (PIP2) is converted into phosphatidylinositol 3,4,5-triphosphate (PIP3), which as one of second messenger, regulating many physiological roles including generation and development of tumor [11-12]. Meanwhile, PIP3 levels are strictly controlled by phosphatase and tensin homologue (PTEN), which convert PIP3 into PIP2 [13-14]. In addition, PI3Kα and PI3Kβ are expressed commonly, nevertheless PI3Kδ and PI3Kγ are present in the central nervous system, epithelial cells and the hematopoietic system [15-18]. Due to the importance in cell functions, PI3K pathway becomes an attractive approach for cancer therapy.

Nowadays many pharmaceutical chemists try to develop PI3K inhibitors cancer therapy, and several drug candidates are in preclinical study [19-22]. Chromeno[4,3-*c*]pyrazol-4(2*H*)-one has been discovered and identified to design new PI3Kα inhibitors in our previous work [23-25]. In fact, chromeno[4,3-*c*]pyrazol-4(2*H*)-one skeleton was designed based on the structural characteristics of LY294002, Wortmannin, GDC-0941 and BEZ235 (Fig. 1), which are all inhibitors of PI3K [26-30]. Subsequently, we designed a novel chromeno[4,3-*c*]pyrazol-4(2*H*)-one derivates and some interesting results were obtained[23-25]. Encouraged by these, continuous research is meaningful to further explore. Based on the molecular structure of PI3K inhibitors, it was observed that piperazine and sulfonamide fragment are employed such as GDC-0941, PI-3065, CH5132799 (Fig. 1) and so on [29, 31-33]. Sulfonylpiperazine fragment is introduced may result in increased binding affinity of derivates and improved biology activity or selectivity. On the basis of this assumption, a novel chromeno[4,3-*c*]pyrazol-4(2*H*)-one derivates bearing sulfonylpiperazine fragment

were designed and synthesized, and biological evaluation was performed to verify their PI3K inhibitory activities and antitumor effects.

2. Results and discussion

2.1. Chemistry

The targeted compounds **7a-7w** were prepared as outlined in Scheme 1. The main core chromeno[4,3-*c*]pyrazol-4(2*H*)-one (**3**) was synthesized by the method in our previous study [23]. Chromeno[4,3-*c*]pyrazol-4(2*H*)-one (**3**) was produced *via* a Vilsmeier-Haack reaction of 4-hydroxycoumarin (**1**) in the presence of dimethylformamide and phosphoryl chloride followed by cyclization with 85% hydrazine hydrate. The yield was up to 85%.

Compounds **7a-7w** were synthesized as outlined in Scheme 1. Compounds **6a-6w** were produced by sulfonylation reaction between piperazine and sulfonyl chloride Subsequently, Compounds **6a-6w** reacted with Chromeno[4,3-*c*]pyrazol-4(2*H*)-one (**3**) in the presence of 40% formaldehyde to get the targeted compounds **7a-7w**.

2.2. Antiproliferative assays in vitro

All synthesized compounds were assessed in terms of anti-proliferative activity against four cancer cell lines (A549, Huh7, HL60 and HCT-116) using MTT assay, and LY294002 and BEZ235 were assigned as positive control. The results were displayed in Table 1. The results manifested that most the designed compounds reveal the better anti-proliferative effects than that of the positive control LY294002. Notably, three tested compounds (**7m**, **7n** and **7o**) showed the significant activities against these four cancer cell lines. Particularly, compound **7m** exhibited the remarkable anti-proliferative effect with the IC_{50} values of 0.03, 0.06, 0.06 and 0.09μM against HCT-116, HL60, A549 and Huh7, respectively.

Anti-proliferative activities of the first series of compounds (**7a-7o**) were compared. As presented in Table 1, most of the compounds showed the potent anti-proliferative activities, indicating that phenylsulfonyl piperazine fragment is beneficial to the improvement of anti-proliferative activity. Compounds **7m-7o** displayed the comparable activities against these cancer cell lines, and it was found that these compounds have two or three substituent group. As can be seen in Table 1, the more substituent groups on the benzene ring, the better anti-proliferative activities they showed. However, compound **7m** (**two substituents** on the benzene ring)

revealed the best anti-proliferative activity, there may be a great possibility caused by the NO_2 group. Furthermore, for the compounds with **mono-substituted** on the benzene ring, it was observed that compounds show the anti-proliferative effects with the tendency of the order is Cl > CF_3 > NO_2 > Br > F > H > CH_3 > OCH_3. Obviously, electron-withdrawing group plays an important role for their anti-proliferative effects against these cancer cell lines.

Meanwhile, compounds (**7p-7w**) with alkylsulfonyl piperazine fragment were synthesized and evaluated their anti-proliferative activities to verify the importance of phenylsulfonyl piperazine fragment. The results were summarized in Table 1, compare to compounds **7a-7o**, compounds **7p-7w** revealed the much poorer anti-proliferative activities against these cancer cell lines. It was further verified that phenylsulfonyl piperazine is the necessary fragment in term of anti-proliferative effects who exhibited.

Furthermore, the top twelve compounds were selected to examine the cytotoxic effects on human normal cells 293T, and the results were shown in Table 2. It was observed that all the tested compounds exhibit the poor anti-proliferative activities against the human normal cells 293T, indicating that there is almost no any effect on human normal cells.

2.3. Inhibition of PI3K kinase

The twelve designed compounds were evaluated for their biological activities against four isoforms of PI3K (α, β, γ and δ) using a competitive fluorescence polarization kinase activity assay (FPA). The results were revealed in Table 2. Notly, compounds **7l-7o** exhibited the potent activities against these four isoforms of PI3K. Interestingly, compound **7m** revealed the prominent inhibition activity comparing to the other compounds, and it displayed an IC_{50} value of 0.009 μM against PI3Kα, with a certain selectivity of 13-fold over β-isozyme, 9-fold over γ-isozyme and 13-fold over δ-isozyme. It was also observed that compound **7m** display an almost 53-fold improvement against PI3Kα comparing to that of LY294002, and comparable activity with BEZ235 to PI3Kα. Furthermore, these compounds were also evaluated for their biological activities against mTOR, and they displayed the poor activities, indicating compound **7m** exhibit the selectivity for PI3Kα. These results suggested compound **7m** could be a promising PI3Kα inhibitor.

2.4. Western blot analysis

The above results indicated that compound **7m** could be a PI3Kα inhibitor, and 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 investigate the mechanism of action, and find out the effects on downstream factors of PI3K pathway, the representative proteins phosphorylated Akt (p-Akt) and phosphorylated S6K (p-S6K) were examined by western blot. The results were displayed in Fig. 2. After treatment by compound **7m** with different concentrations at 0.25, 0.5 and 1 μM in HCT-116 cells, both p-Akt (Ser473) and p-S6K were down-regulated, and the expression level were a remarkably decreased in a dose-dependent manner. These results suggested compound **7m** could also inhibit the PI3K pathway, and it further proved that compound **7m** could act as an antitumor inhibitor targeting PI3Kα.

2.5. Cell cycle arrest

In order to further investigate the mechanism of anti-proliferative effects in cancer cell lines, compound **7m** was selected to test the effect on cell cycle in HCT-116 cells distribution due to its best potency. After treatment by compound **7m** with different concentrations at 0.25, 0.5 and 1 μM for 24 h, the results were displayed in Fig. 3, compound **7m** could induce a remarkable accumulation of HCT-116 cells at G1 phase from 46.87 to 58.13% with a dose-dependent manner. Furthermore, compound **7m** decreased the percentage of cells in S phase (from 42.40 to 33.78%). Besides, there was almost no change in G2 phase (from 10.73 to 8.09%). These results revealed induction of HCT-116 cells arrest at G1 phase may be one of the possible mechanisms of cancer cells death.

2.6. Cell apoptosis

Induce cell apoptosis in HCT-116 was also performed to investigate the mechanism of anti-proliferative effects, and it was examined using Annexin V-FITC/PI FACS assay. HCT-116 cells were treated by compound **7m** at 0.25, 0.5 and 1 μM four different concentrations for 24 h. The results were displayed in Fig. 4**A**, the percentages of late apoptotic population remarkably increased with the concentration increasing, and the percentages ranged from 9.61 to 36.9%. Similarly, the percentages of early apoptotic population ranged from 10.0 to 25.5% with a dose-dependent manner.

To further explore the apoptosis mechanism by compound **7m** in HCT-116 cell

line, apoptotic related proteins was examined in HCT-116 after treatment by **7m** at concentrations of 0.25, 0.5 and 1μM for 24 h. As shown in Fig.4**B**, the proapoptotic protein Bax level was were remarkably upregulated in a dose-dependent manner, in contrast, Bcl-2 level was decreased. What's more, the cleaved caspase-3 and cleaved caspase-9 levels which are considered as bio-markers for cell apoptosis, were increased in a dose-dependent manner. These results indicated that compound **7m** induce cell apoptosis of HCT-116 cells in a dose-dependent manner.

2.7. Molecular modeling

To elucidate the binding model of compound **7m** with PI3Kα, molecular docking was performed by docking **7m** into the active pocket of PI3Kα (PDB: 3HHM). As shown in Fig. 5, compound **7m** could bind well to the active site of 3HHM via three hydrogen bonds and three π-cation interactions. It was observed that chromeno[4,3-*c*]pyrazol-4(2*H*)-one part formed three π-cation interactions with Lys776, one hydrogen bond (distance: 2.05Å) was formed between carbonyl and Ser774. Meanwhile, the sulfonyl formed another hydrogen bond(distance: 2.03Å) with Val851, the third hydrogen bond (distance: 2.36Å) was formed by nitro and Gln859. All these interactions make compound 7m bind well to the active site of PI3Kα, indicating that compound **7m** is a promising PI3Kα inhibitor.

2.9. In vivo antitumor activity evaluations

Due to the potent activity *in vitro*, antitumor activities *in vivo* of compound **7m** were performed. we established the HCT-116 cells xenograft model by subcutaneous inoculation. The mice were divided into three groups once the tumor volume was up to about 100 mm^3, LY294002 was selected as positive drug, and they were treated with compound **7m** (10 mg/kg), LY294002 (10 mg/kg) and control (CMC-Na) by intragastric administration. The results were presented in Fig. 6, it was revealed a distinct effect in inhibiting tumor growth after treatment by compound **7m** at a dose of 10 mg/kg once every three days, what's more, it can decrease the weight of tumor up to 66.4%. By comparison, it showed about 35.2% growth inhibitory rate after treated with LY294002 (10 mg/kg). Overall, these indicated that compound **7m** display the remarkable effect in inhibiting tumor growth and it could be a promising antitumor agent.

3. Conclusion

In summary, we designed and synthesized a series of novel

chromeno[4,3-*c*]pyrazol-4(2*H*)-one derivates bearing sulfonylpiperazine fragment, and their biological evaluation was performed. Most designed compounds revealed the anti-proliferative activities for cancer cell lines (Huh7, A549, HCT-116 and HL60), particularly, compound **7m** displayed the remarkable anti-proliferative effect (IC_{50} ranging from 0.03 to 0.09μM), with the significant improvement in comparison to LY294002. Meanwhile, compound **7m** exhibited the selective activity against PI3Kα (IC_{50} = 0.009μM) which compared to the other three PI3K isoforms (β, γ and δ), and decreased the expression level of p-Akt (Ser473) and p-S6K. Further research indicated that compound **7m** could induce HCT-116 cell arrest at G1 phase in a dose-dependent manner. Not only that, compound **7m** could significantly induce cell apoptosis, and increased the level of cleaved caspase 3, cleaved caspase 9 and Bax, down-regulated the level of Bcl-2, which as one of anti-apoptotic protein. The efficiency in vivo of compound **7m** was also performed on HCT-116 Xenograft nude mice by intragastric administration, indicating that compound **7m** exhibited remarkable antitumor activity *in vivo*, which compared to the positive group (LY294002). All above results suggested compound **7m** may be a promising PI3Kα inhibitor for cancer therapy.

4. Experiments

All chemicals and reagents were purchased from commercial company (*J&K*, China; *Sigma-Aldrich*, China). Melting points were measured by a melting point apparatus (Taike Corp., China). All reactions were monitored by thin layer chromatography (TLC; silica GF_{254} plates) which purchased from Merck. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker DPX400 spectrometer, and TMS as an internal standard. Chemical shifts are described in ppm (δ). ESI mass spectra were determined on a Mariner System 5304 mass spectrometer.

*4.1. Synthesis of chromeno[4,3-c]pyrazol-4(2H)-one (**3**)*

Chromeno[4,3-*c*]pyrazol-4(2*H*)-one was synthesized by described as the reported methods of our previous study [23].

*4.2. General procedure for the preparation of designed compounds **7a-7w***

Compounds **6a-6w** were synthesized by described in the literature [34].

40% formaldehyde (300 μL) was added slowly into the ethanol solution of **6a-6w** (2 mmol) and **3** (2 mmol), the mixtures were stirred at room temperature for

5-7 h. A large quantity of solid was precipitated and then filtered, washed by cold ethanol, and crystallized with ethanol to get the designed compounds **7a-7w**.

4.2.1.

*2-((4-(Phenylsulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7a**)*

White power, yield: 63%. Mp: 199-200 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.04(d, J=7.60 Hz, 1H, ArH), 7.72(d, J=7.28 Hz, 2H, ArH), 7.57-7.47(m, 4H, ArH), 7.38(d, J=8.24 Hz, 1H, ArH), 7.32(t, J=7.48 Hz, 1H, ArH), 5.05(s, 2H, CH_2), 3.07(s, 4H, -CH_2CH_2), 2.74(s, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 157.97, 152.96, 149.35, 135.87, 132.98, 132.33, 130.54, 129.13(2), 127.70(2), 124.57, 122.81, 117.62, 114.79, 108.05, 73.80, 49.26(2), 45.79(2). MS (ESI): 425.33 $[M+H]^+$ (Calcd for 425.48, $C_{21}H_{21}N_4O_4S$).

*4.2.2. 2-((4-Tosylpiperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7b**)*

White power, yield: 57%. Mp: 176-177 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.05(d, J=7.72 Hz, 1H, ArH), 7.61(d, J=7.92 Hz, 2H, ArH), 7.49(t, J=7.70 Hz, 1H, ArH), 7.38(d, J=8.28 Hz, 1H, ArH), 7.31(t, J=9.66 Hz, 3H, ArH), 5.04(s, 2H, -CH_2), 3.05(s, 4H, -CH_2CH_2), 2.75(t, J=4.38 Hz, 4H, -CH_2CH_2), 2.40(s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 157.98, 152.97, 149.37, 143.90, 132.70, 132.27, 130.53, 129.74(2), 127.80(2), 124.58, 122.79, 117.64, 114.78, 108.07, 73.83, 49.37(2), 45.80(2), 21.49. MS (ESI): 439.32 $[M+H]^+$ (Calcd for 439.50, $C_{22}H_{23}N_4O_4S$).

4.2.3.

*2-((4-((4-Methoxyphenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7c**)*

White power, yield: 54%. Mp: 182-184 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.05(d, J=7.64 Hz, 1H, ArH), 7.66(d, J=8.36 Hz, 2H, ArH), 7.48(t, J=5.76 Hz, 1H, ArH), 7.38(d, J=8.24 Hz, 1H, ArH), 7.32(t, J=7.52 Hz, 1H, ArH), 6.96(d, J=8.52 Hz, 2H, ArH), 5.05(s, 2H, -CH_2), 3.85(s, 3H, OCH_3), 3.05(s, 4H, -CH_2CH_2), 2.74(d, J=4.32 Hz, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 163.22, 158.01, 152.98, 149.37, 132.23, 130.54, 129.88(2), 127.31, 124.58, 122.78, 117.64, 114.78, 114.31(2), 108.08, 73.84, 55.64, 49.31(2), 45.79(2). MS (ESI): 455.37 $[M+H]^+$ (Calcd for 455.50, $C_{22}H_{23}N_4O_5S$).

4.2.4.

*2-((4-((4-(Tert-butyl)phenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4 (2H)-one (**7d**)*

White power, yield: 58%. Mp: 264-266 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.22(s, 1H, ArH), 8.05(d, J=7.76 Hz, 1H, ArH), 7.65(d, J=8.44 Hz, 2H, ArH), 7.52(d, J=8.32 Hz, 2H, ArH), 7.47(d, J=7.28 Hz, 1H, ArH), 7.37(d, J=8.24 Hz, 1H, ArH), 7.32(t, J=7.50 Hz, 1H, ArH), 5.05(s, 2H, -CH_2), 3.06(s, 4H, -CH_2CH_2), 2.77(t, J=4.66 Hz, 4H, -CH_2CH_2), 1.33(s, 9H, 3CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 157.98, 156.87, 152.98, 149.41, 132.52, 132.30, 130.54, 127.70(2), 126.11(2), 124.57, 122.80, 117.64, 114.78, 108.10, 73.82, 49.47(2), 45.80(2), 35.20, 31.09(3). MS (ESI): 481.44 $[M+H]^+$ (Calcd for 481.58, $C_{25}H_{29}N_4O_4S$).

4.2.5.

*2-((4-((4-Fluorophenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H) -one (**7e**)*

Yellow power, yield: 55%. Mp: 202-205 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.05(m, 1H, ArH), 7.78(d, J=8.04 Hz, 2H, ArH), 7.31(d, J=8.44 Hz, 2H, ArH), 7.49(m, 1H, ArH), 7.37(d, J=7.88 Hz, 1H, ArH), 7.33(t, J=7.54 Hz, 1H, ArH), 7.31(d, J=8.44 Hz, 2H, ArH), 5.04(s, 2H, CH_2), 3.08(s, 4H, -CH_2CH_2), 2.76(t, J=4.56 Hz, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 161.21(d, J=252.0 Hz), 157.86, 152.91, 149.42, 133.36, 132.34, 130.51, 128.27(d, J=6.8 Hz)(2),124.54, 122.81, 117.56, 114.83, 113.18(d, J=21.6 Hz)(2), 108.01, 73.82, 49.11(2), 45.96(2). MS (ESI): 443.35 $[M+H]^+$ (Calcd for 443.47, $C_{21}H_{20}FN_4O_4S$).

4.2.6.

*2-((4-((4-Chlorophenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7f**)*

Light yellow power, yield: 61%. Mp: 229-230 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.05(d, J=7.72 Hz, 1H, ArH), 7.67(d, J=8.44 Hz, 2H, ArH), 7.48(m, 3H, ArH), 7.38(d, J=8.32 Hz, 1H, ArH), 7.32(t, J=7.48 Hz, 1H, ArH), 5.06(s, 2H, -CH_2), 3.07(s, 4H, -CH_2CH_2), 2.76(t, J=4.52 Hz, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 157.98, 152.98, 149.43, 139.71, 134.28, 132.30, 130.58, 129.50(2), 129.14(2), 124.60, 122.77, 117.67, 114.75, 108.11, 73.76, 49.29(2), 45.78(2). MS (ESI): 459.81 $[M+H]^+$ (Calcd for 459.92, $C_{21}H_{20}ClN_4O_4S$).

4.2.7.

*2-((4-((4-Bromophenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7g**)*

Light yellow power, yield: 57%. Mp: 250-251 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.19(s, 1H, ArH), 8.05(m, 1H, ArH), 7.67(d, J=8.16 Hz, 2H, ArH), 7.59(d, J=8.56 Hz, 2H, ArH), 7.49(m, 1H, ArH), 7.38(d, J=7.92 Hz, 1H, ArH), 7.33(t, J=7.50 Hz, 1H, ArH), 5.06(s, 2H, CH_2), 3.07(s, 4H, -CH_2CH_2), 2.76(t, J=4.84 Hz, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 157.54, 152.87, 148.43, 135.13, 134.90, 132.92(2), 131.02, 129.90(2), 127.71, 125.19, 122.86, 117.81, 115.00, 107.01, 72.97, 48.66(2), 46.12(2). MS (ESI): 504.27 $[M+H]^+$ (Calcd for 504.37, $C_{21}H_{20}BrN_4O_4S$).

4.2.8.

*2-((4-((4-Nitrophenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7h**)*

Yellow power, yield: 59%. Mp: 252-254 ℃. ^{1}H NMR (400 MHz, DMSO): 8.80(s, 1H, ArH), 8.43(d, J=8.76 Hz, 1H, ArH), 8.35(d, J=8.76 Hz, 2H, ArH), 7.97(d, J=8.76 Hz, 2H, ArH), 7.56(m, 1H, ArH), 7.43(d, J=8.12 Hz, 1H, ArH), 7.38(t, J=7.50 Hz, 1H, ArH), 5.19(s, 2H, -CH_2), 3.04(s, 4H, -CH_2CH_2), 2.66(d, J=4.28 Hz, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 157.99, 152.88, 152.24, 149.41, 144.98, 132.32, 130.51, 128.86(2),124.56, 123.87(2), 122.81, 117.59, 114.78, 108.02, 73.78, 48.87(2), 46.12(2). MS (ESI): 470.38 $[M+H]^+$ (Calcd for 470.47, $C_{21}H_{20}N_5O_6S$).

4.2.9.

*2-((4-((3-Nitrophenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7i**)*

Yellow power, yield: 43%. Mp: 231-233 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.11(s, 1H, ArH), 8.05(d, J=7.52 Hz, 1H, ArH), 7.79(m, 2H, ArH), 7.68(d, J=8.48 Hz, 1H, ArH), 7.51(t, J=7.88 Hz, 1H, ArH), 7.37(d, J=8.40 Hz, 1H, ArH), 7.33(t, J=7.42 Hz, 1H, ArH), 5.11(s, 2H, -CH_2), 3.12(s, 4H, -CH_2CH_2), 2.78(s, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 157.94, 152.86, 149.39, 146.14, 133.68, 132.30, 131.41, 130.52, 128.32, 125.87, 124.52, 122.83, 121.94, 117.61, 114.81, 108.06, 73.81, 49.04(2), 45.93(2). MS (ESI): 470.38 $[M+H]^+$ (Calcd for 470.47, $C_{21}H_{20}N_5O_6S$).

4.2.10.

2-((4-((2-Nitrophenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-

*one (**7j**)*

Yellow power, yield: 37%. Mp: 218-219 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.22(s, 1H, ArH), 8.14(m, 1H, ArH), 8.04(d, J=7.60 Hz, 1H, ArH), 7.72(m, 2H, ArH), 7.50(t, J=7.76 Hz, 1H, ArH), 7.42(m, 1H, ArH), 7.39(d, J=8.44 Hz, 1H, ArH), 7.33(t, J=7.46 Hz, 1H, ArH), 5.09(s, 2H, -CH_2), 3.10(s, 4H, -CH_2CH_2), 2.77(s, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 157.96, 152.82, 149.44, 145.32, 132.97, 132.34, 131.14, 130.56, 130.12, 127.21, 124.54, 122.93, 122.81, 117.58, 114.79, 108.02, 73.86, 49.11(2), 45.99(2). MS (ESI): 470.38 $[M+H]^+$ (Calcd for 470.47, $C_{21}H_{20}N_5O_6S$).

4.2.11.

*2-((4-((4-(Trifluoromethyl)phenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7k**)*

Light yellow power, yield: 46%. Mp: 189-291 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.06(s, 1H, ArH), 7.86(d, J=8.20 Hz, 2H, ArH), 7.81(d, J=8.32 Hz, 2H, ArH), 7.49(s, 1H, ArH), 7.37(t, J=7.96 Hz, 2H, ArH), 5.07(s, 2H, CH_2), 3.11(s, 4H, -CH_2CH_2), 2.79(s, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 157.52, 152.84, 148.43, 139.84, 134.88, 133.28(q, J=24.4 Hz), 130.99, 128.91(2), 127.04(q, J=4.5 Hz)(2), 125.14, 123.82(q, J=270 Hz), 122.83, 117.75, 114.96, 107.01, 72.95, 48.75(2), 46.13(2). MS (ESI): 493.31 $[M+H]^+$ (Calcd for 493.47, $C_{22}H_{20}F_3N_4O_4S$).

4.2.12.

*2-((4-((2,5-Dimethylphenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7l**)*

White power, yield: 58%. Mp: 223-225 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.19(s, 1H, ArH), 8.05(m, 1H, ArH), 7.56(d, J=8.44 Hz, 1H, ArH), 7.47(m, 1H, ArH), 7.36(d, J=7.92 Hz, 1H, ArH), 7.33(t, J=7.56 Hz, 1H, ArH), 7.23(m, 2H, ArH), 5.08(s, 2H, CH_2), 3.07(s, 4H, -CH_2CH_2), 2.78(t, J=4.56 Hz, 4H, -CH_2CH_2) 2.60(s, 3H, CH_3), 2.45(s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 157.87, 152.85, 149.38, 135.82, 134.79, 132.31, 131.92, 131.21, 130.52, 128.12, 124.71, 124.57, 122.83, 117.55, 114.81, 108.04, 73.79, 49.12(2), 45.93(2), 23.36, 21.53. MS (ESI): 453.41 $[M+H]^+$ (Calcd for 453.53, $C_{23}H_{25}N_4O_4S$).

4.2.13.

*2-((4-((4-Chloro-3-nitrophenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7m**)*

Light yellow power, yield: 39%. Mp: 247-248 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 2H, ArH), 8.05(d, J=7.56 Hz, 1H, ArH), 7.85(d, J=8.12 Hz, 1H, ArH), 7.74(d, J=8.48 Hz, 1H, ArH), 7.50(t, J=7.76 Hz, 1H, ArH), 7.39(d, J=8.44 Hz, 1H, ArH), 7.33(t, J=7.46 Hz, 1H, ArH), 5.09(s, 2H, -CH_2), 3.14(s, 4H, -CH_2CH_2), 2.80(s, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 157.52, 152.88, 148.44, 148.21, 136.59, 134.91, 133.53, 132.49, 131.03, 130.45, 125.19, 125.05, 122.84, 117.79, 114.97, 107.00, 72.93, 48.60(2), 46.07(2). MS (ESI): 504.73 $[M+H]^+$ (Calcd for 504.91, $C_{21}H_{19}ClN_5O_6S$).

4.2.14.

*2-((4-(Mesitylsulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7n**)*

White power, yield: 67%. Mp: 217-219 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.05(m, 1H, ArH), 7.48(m, 1H, ArH), 7.38(d, J=7.92 Hz, 1H, ArH), 7.32(t, J=7.52 Hz, 1H, ArH), 7.04(m, 2H, ArH), 5.06(s, 2H, CH_2), 3.07(t, J=4.58 Hz, 4H, -CH_2CH_2), 2.76(t, J=4.82 Hz, 4H, -CH_2CH_2), 2.71(s, 6H, 2CH_3), 2.29(s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 157.92, 152.88, 149.36, 144.13, 139.21(2), 135.92, 132.34, 130.50, 128.94(2), 124.54, 122.81, 117.59, 114.84, 108.02, 73.83, 49.08(2), 45.89(2), 21.48(2), 20.29. MS (ESI): 467.39 ($C_{24}H_{27}N_4O_4S$, $[M+H]^+$). (Calcd for 467.56, $C_{24}H_{27}N_4O_4S$).

4.2.15.

*2-((4-((2,4,6-Triisopropylphenyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7o**)*

White power, yield: 65%. Mp: 238-240 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.03(d, J=7.64 Hz, 1H, ArH), 7.48(t, J=7.70 Hz, 1H, ArH), 7.38(d, J=8.36 Hz, 1H, ArH), 7.31(t, J=7.48 Hz, 1H, ArH), 7.09(s, 2H, ArH), 5.12(s, 2H, CH_2), 4.05(m, 2H, 2-CH), 3.24(s, 4H, -CH_2CH_2), 2.85(m, 1H, -CH), 2.71(s, 4H, -CH_2CH_2), 1.21(d, J=6.96 Hz, 6H, 2CH_3), 1.14(d, J=6.68 Hz, 12H, 4CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 157.99, 153.45, 152.96, 151.76(2), 149.28, 132.33, 130.49, 129.47, 124.53, 123.96(2), 122.74, 117.63, 114.78, 107.96, 74.03, 49.48(2), 43.91(2), 34.16, 29.25(2), 24.80(4), 23.52(2). MS (ESI): 551.42 $[M+H]^+$ (Calcd for 551.72, $C_{30}H_{39}N_4O_4S$).

4.2.16.

*2-((4-((Trifluoromethyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7p**)*

Light yellow power, yield: 41%. Mp: 167-169 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.05(d, J=7.76 Hz, 1H, ArH), 7.50(t, J=7.64 Hz, 1H, ArH), 7.37(d, J=8.56 Hz, 1H, ArH), 7.33(t, J=7.64 Hz, 1H, ArH), 5.10(s, 2H, -CH_2), 2.78(t, J=4.18 Hz, 4H, -CH_2CH_2), 2.57(s, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 157.94, 152.93, 149.42, 132.31, 130.48, 124.52, 122.76, 119.94(q, J=274 Hz), 117.61, 114.78, 107.98, 73.82, 49.12(2), 45.86(2). MS (ESI): 417.28 $[M+H]^+$ (Calcd for 417.38, $C_{16}H_{16}F_3N_4O_4S$).

4.2.17.

*2-((4-(Methylsulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7q**)*

White power, yield: 59%. Mp: 191-194 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.04(d, J=7.88 Hz, 1H, ArH), 7.51(t, J=7.60 Hz, 1H, ArH), 7.38(d, J=8.52 Hz, 1H, ArH), 7.33(t, J=7.52 Hz, 1H, ArH), 5.10(s, 2H, -CH_2), 2.97(s, 3H, CH_3), 2.79(t, J=4.22 Hz, 4H, -CH_2CH_2), 2.61(s, 4H, -CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 157.91, 152.89, 149.38, 132.33, 130.54, 124.56, 122.78, 117.63, 114.81, 107.94, 73.80, 49.08(2), 45.82(2), 38.22. MS (ESI): 363.32 $[M+H]^+$ (Calcd for 363.40, $C_{16}H_{19}N_4O_4S$).

*4.2.18. 2-((4-(Ethylsulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7r**)*

White power, yield: 52%. Mp: 201-203 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.05(m, 1H, ArH), 7.50(t, J=7.68 Hz, 1H, ArH), 7.38(d, J=8.44 Hz, 1H, ArH), 7.32(t, J=7.58 Hz, 1H, ArH), 5.11(s, 2H, -CH_2), 3.36(m, 2H, -CH_2), 2.76(t, J=4.30 Hz, 4H, -CH_2CH_2), 2.57(d, J=4.12 Hz, 4H, -CH_2CH_2), 1.22(t, J=7.48 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 157.92, 152.86, 149.41, 132.34, 130.51, 124.54, 122.81, 117.61, 114.82, 108.02, 73.78, 49.14, 49.08(2), 45.82(2), 6.94. MS (ESI): 377.29 $[M+H]^+$ (Calcd for 377.43, $C_{17}H_{21}N_4O_4S$,).

4.2.19.

*2-((4-(Propylsulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one (**7s**)*

White power, yield: 56%. Mp: 208-210 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.21(s, 1H, ArH), 8.05(m, 1H, ArH), 7.51(t, J=7.42 Hz, 1H, ArH), 7.37(d, J=8.32 Hz, 1H, ArH), 7.33(t, J=7.46 Hz, 1H, ArH), 5.08(s, 2H, -CH_2), 3.26(t, J=7.62 Hz, 2H, -CH_2), 2.77(t, J=4.34 Hz, 4H, -CH_2CH_2), 2.54(d, J=4.08 Hz, 4H, -CH_2CH_2), 2.12(m, 2H, -CH_2),1.01(t, J=7.48 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 157.96, 152.91,

149.42, 132.33, 130.57, 124.53, 122.84, 117.66, 114.80, 107.98, 73.79, 58.34, 49.11(2), 45.88(2), 14.53, 12.94. MS (ESI): 391.24 $[M+H]^+$ (Calcd for 391.46, $C_{18}H_{23}N_4O_4S$).

4.2.20. 2-((4-(Butylsulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one ***(7t)***

White power, yield: 51%. Mp: 211-213 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.04(m, 1H, ArH), 7.49(t, J=7.54 Hz, 1H, ArH), 7.38(d, J=8.40 Hz, 1H, ArH), 7.32(t, J=7.34 Hz, 1H, ArH), 5.09(s, 2H, $-CH_2$), 3.29(t, J=7.16 Hz, 2H, $-CH_2$), 2.79(t, J=4.28 Hz, 4H, $-CH_2CH_2$), 2.55(d, J=4.16 Hz, 4H, $-CH_2CH_2$), 1.98(m, 2H, $-CH_2$), 1.34(m, 2H, $-CH_2$), 0.97(m, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 157.98, 152.94, 149.41, 132.32, 130.54, 124.54, 122.87, 117.61, 114.79, 107.97, 73.74, 55.93, 49.08(2), 45.82(2), 20.19, 19.86, 11.89. MS (ESI): 405.26 $[M+H]^+$ (Calcd for 405.49, $C_{19}H_{25}N_4O_4S$).

4.2.21.

2-((4-(Cyclopropylsulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one ***(7u)***

White power, yield: 54%. Mp: 194-196 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.05(m, 1H, ArH), 7.51(t, J=7.48 Hz, 1H, ArH), 7.37(d, J=8.36 Hz, 1H, ArH), 7.34(t, J=7.72 Hz, 1H, ArH), 5.11(s, 2H, $-CH_2$), 3.34(m, 2H, $-CH_2$), 2.81(t, J=4.12 Hz, 4H, $-CH_2CH_2$), 2.57(d, J=4.08 Hz, 4H, $-CH_2CH_2$), 1.94(m, 4H, 2-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 158.01, 152.98, 149.38, 132.34, 130.51, 124.59, 122.82, 117.58, 114.74, 107.99, 73.82, 49.12(2), 45.87(2), 36.15, 3.87(2). MS (ESI): 389.31 $[M+H]^+$ (Calcd for 389.44, $C_{18}H_{21}N_4O_4S$).

4.2.22.

2-((4-((2-Chloroethyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H)-one ***(7v)***

White power, yield: 49%. Mp: 187-189 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.19(s, 1H, ArH), 8.05(m, 1H, ArH), 7.50(t, J=7.56 Hz, 1H, ArH), 7.38(d, J=8.24 Hz, 1H, ArH), 7.32(t, J=7.86 Hz, 1H, ArH), 5.10(s, 2H, $-CH_2$), 3.47(t, J=7.86 Hz, 2H, $-CH_2$), 3.38(t, J=7.78 Hz, 4H, 2-CH_2), 2.79(t, J=4.18 Hz, 4H, $-CH_2CH_2$), 2.58(d, J=4.10 Hz, 4H, $-CH_2CH_2$). ^{13}C NMR (100 MHz, $CDCl_3$): 157.98, 152.91, 149.42, 132.31, 130.53, 124.56, 122.81, 117.54, 114.76, 107.96, 73.81, 59.11, 49.10(2), 45.83(2), 31.82. MS

(ESI): 411.81 $[M+H]^+$ (Calcd for 411.87, $C_{17}H_{20}ClN_4O_4S$).

4.2.23.

*2-((4-((3-Chloropropyl)sulfonyl)piperazin-1-yl)methyl)chromeno[4,3-c]pyrazol-4(2H) -one (**7w**)*

White power, yield: 53%. Mp: 189-192 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 8.20(s, 1H, ArH), 8.04(m, 1H, ArH), 7.49(t, J=7.44 Hz, 1H, ArH), 7.38(d, J=8.36 Hz, 1H, ArH), 7.33(t, J=7.74 Hz, 1H, ArH), 5.09(s, 2H, -CH_2), 3.42(t, J=7.70 Hz, 2H, -CH_2), 3.36(t, J=7.46 Hz, 4H, 2-CH_2), 2.78(t, J=4.34 Hz, 4H, -CH_2CH_2), 2.55(d, J=4.24 Hz, 4H, -CH_2CH_2), 2.11(m, 2H, -CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 157.94, 152.89, 149.36, 132.36, 130.55, 124.52, 122.81, 117.58, 114.81, 107.99, 73.87, 54.31, 49.08(2), 45.80(2), 41.89, 21.04. MS (ESI): 425.79 ($C_{18}H_{22}ClN_4O_4S$, $[M+H]^+$). (Calcd for 425.90, $C_{18}H_{22}ClN_4O_4S$).

4.3. Cell proliferation assay

Four human cell lines HCT-116, Huh7, HL60 and A549 were cultured growth medium (RPMI-1640 medium with 10% FBS, 100 U/mL penicillin and 100 mg/mL streptomycin, and incubated at 37 °C under the atmosphere of 5% CO_2 and 20% O_2.

The antiproliferative activity in vitro was determined by the MTT assay. The tested compounds were dissolved in DMSO and diluted to the different concentrations. Cells in logarithmic phase were harvested and divided into 96-well plates (0.5×10^4 each well), culture medium containing the test compounds were added to each well at different concentrations and incubated for 48 h. Viable cells were examined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay kit (MTT, Sigma) according to the manufacturer's instructions. Triplicate wells were used for each concentration and each assay was carried out at least three times. The cytotoxic activity was expressed as the IC_{50} values.

4.4. PI3K enzyme assay

The inhibition of PI3K activity was examined by competitive fluorescence polarization kinase activity assay as our previous research described [24]. Briefly, 10 μL PI3K (Perkin-Elmer) reactions were performed in pH 7, 2.5 mM $MgCl_2$, 5 mM HEPES, 50 mM ATP and 10 mM DTT using diC_8-PI(4,5)P_2 (Echelon Biosciences) as the substrate. Firstly, tested compounds (ranging from 3.2 nM to 1 mM) were added into per 10 μL reaction volume (prepared with 50 ng of enzyme and 10 mM of substrate). After incubating for 3 h at room temperature, a chelator was added to

quench the reactions. And then a mixture of phosphoinositide binding protein was added and mixed, subsequently, a fluorophorelabeled phosphoinositide tracer was added. Samples were incubated in the dark for 1 h to equilibrate after mixing in 384-well black Corning nonbinding plates. Finally, polarization values were recorded using red fluorophores with appropriate filters to determine the extent of enzyme activity in the reaction.

4.5. Apoptosis analysis

6×10^5 HCT-116 cells in exponential growth was averagely seeded into each well of 6-well plate, and treated with compound **7m** at different concentrations for 24 h, the cells were collected, followed by centrifuged and resuspended in 500 ml AnnexinV binding buffer, and incubated for 15 min in the darkness under the ice-bath. Samples were analyzed using a FACS Calibur flow cytometer (Bectone Dickinson, San Jose, CA, USA).

4.6. Western blotting

HCT-116 cells were incubated in the presence of **7m** for 24 h. Subsequently, trypsinized the cells and collected, the prepared 1 × RIPA lysis buffer (1% NP-40,50 mM Tris-HCl, 150 mM NaCl, pH 7.4, 0.25% deoxycholic acid, 1 mM EDTA containing protease inhibitors PMSF) (Amresco, Solon, USA) was added to extract the total proteins. The proteins was separated by sodium dodecyl sulfate (8% or 10%) polyacrylamide gel electrophoresis (SDS-PAGE, BioRad Laboratories, Hercules, CA), and transferred from the gel onto to PVDF membrane (BioRad Laboratories, Hercules, CA), blotted with primary antibodies, probed with secondary isotype specific antibodies tagged with horseradish peroxidase (Cell Signaling Technology). Bound immunocomplexes were detected using a ChemiDOC™ XRS+system (BioRad Laboratories, Hercules, CA).

4.7. Molecular docking

Docking study was performed by Discovery Studio 3.5 and the PI3Kα protein (PDB:3HHM) was downloaded from RCSB Protein Date Bank (www.rcsb.org). The protein and all ligands were prepared by minimization with CHARMM force field. Molecular docking was carried out using DS-CDOCKER protocol without constraint, all bound water and ligands were eliminated from the protein and the polar hydrogen was added to the proteins.

4.8. Antitumor effect *in vivo*

A mouse tumor xenograft model was established by injecting the HCT-116 cells (5×10^6 cells per animal) into the right flank of nude mice. After the tumor size reach approximately 100 mm^3, the mice were randomly divided into three groups (6 mice per group), they were respectively treated with 5% CMC-Na (control), LY294002 (10 mg/kg, dissolved in 5% CMC-Na) and compound **7m** (10 mg/kg, dissolved in 5% CMC-Na) by intragastric administration every three days in three weeks. The body weight and tumor size were recorded every three days, the tumor volumes were calculated by a formula: tumor volume (mm^3) = 0.5 × length × $width^2$. After treatment for three weeks, the mice were sacrificed and the tumors were separated and weighted.

Acknowledgments

This work was supported by National Natural Science Foundation of China (No. 81602985 and 21202153), the Fundamental Research Funds for the Central Universities (2632018ZD19), It was also supported by Technology research Funds Project of Ocean and financed by National Natural Science Foundation of China (No. 21602103).

References

[1] L.C. Cantley, The phosphoinositide 3-kinase pathway, Science 296 (2002) 1655-1657.

[2] V. Igor, C.L. Sawyers, The phosphatidylinositol 3-Kinase AKT pathway in human cancer, Nat Rev Cancer 2 (2002) 489-501.

[3] F. Janku, T.A. Yap, F. Mericbernstam, Targeting the PI3K pathway in cancer: are we making headway?, Nature Reviews Clinical Oncology 15 (2018) 273-291.

[4] C.L. Carpenter, L.C. Cantley, Phosphoinositide kinases, Biochemistry-Us 29 (1990) 11147-11156.

[5] J.E. Burke, R.L. Williams, Synergy in activating class I PI3Ks, Trends Biochem. Sci. 40 (2015) 88-100.

[6] P. Liu, H. Cheng, T.M. Roberts, J.J. Zhao, Targeting the phosphoinositide 3-kinase pathway in cancer, Nat. Rev. Drug Discov. 8 (2009) 627-644.

[7] W. Zhao, Y. Qiu, D. Kong, Class I phosphatidylinositol 3-kinase inhibitors for cancer therapy, Acta Pharmacol. Sin. B 7 (2017) 27-37.

[8] M.P. Wymann, L. Pirola, Structure and function of phosphoinositide 3-kinases,

Biochim Biophys Acta 1436 (1998) 127-150.

[9] F.I. Raynaud, S. Eccles, P.A. Clarke, A. Hayes, B. Nutley, S. Alix, A. Henley, F. DiStefano, Z. Ahmad, S. Guillard, L.M. Bjerke, L. Kelland, M. Valenti, L. Patterson, S. Gowan, A.d.H. Brandon, M. Hayakawa, H. Kaizawa, T. Koizumi, T. Ohishi, S. Patel, N. Saghir, P. Parker, M. Waterfield, P. Workman, Pharmacologic characterization of a potent inhibitor of class I phosphatidylinositide 3-kinases, Canc. Res. 67 (2007) 5840-5850.

[10] P. Workman, P.A. Clarke, F.I. Raynaud, R.L.M. van Montfort, Drugging the PI3kinome: from chemical tools to drugs in the clinic, Canc. Res. 70 (2010) 2146-2157.

[11] M.P. Czech, PIP2 and PIP3: complex roles at the cell surface, Cell 100 (2000) 603-606.

[12] C.-H. Huang, D. Mandelker, O. Schmidt-Kittler, Y. Samuels, V.E. Velculescu, K.W. Kinzler, B. Vogelstein, S.B. Gabelli, L.M. Amzel, The structure of a human p110 alpha/p85 alpha complex elucidates the effects of oncogenic PI3K alpha mutations, Science 318 (2007) 1744-1748.

[13] N.R. Leslie, C.P. Downes, PTEN function: how normal cells control it and tumor cells lose it, Biochem. J 382 (2004) 1-11.

[14] N. Chalhoub, S.J. Baker, PTEN and the PI3-kinase pathway in cancer, Annu. Rev. Pathol. 4 (2009) 127-150.

[15] Y. Liu, W.Z. Wan, Y. Li, G.L. Zhou, X.G. Liu, Recent development of ATP-competitive small molecule phosphatidylinostitol-3-kinase inhibitors as anticancer agents, Oncotarget 8 (2016) 7181-7200.

[16] K.D. Courtney, R.B. Corcoran, J.A. Engelman, The PI3K pathway as drug target in human cancer, J. Clin. Oncol 28 (2010) 1075-1083.

[17] I.G. Campbell, S.E. Russell, D.Y.H. Choong, K.G. Montgomery, M.L. Ciavarella, C.S.F. Hooi, B.E. Cristiano, R.B. Pearson, W.A. Phillips, Mutation of the PIK3CA gene in ovarian and breast cancer, Cancer Res 64 (2004) 7678-7681.

[18] Y. Samuels, Z.H. Wang, A. Bardelli, N. Silliman, J. Ptak, S. Szabo, H. Yan, A. Gazdar, D.M. Powell, G.J. Riggins, J.K.V. Willson, S. Markowitz, K.W. Kinzler, B. Vogelstein, V.E. Velculescu, High frequency of mutations of the PIK3CA gene in human cancers, Science 304 (2004) 554-554.

[19] A. E Garces, M. Stocks, Class 1 PI3K Clinical Candidates and Recent Inhibitor Design Strategies: A Medicinal Chemistry Perspective, J Med Chem 62 (2018) 4815-4850

[20] M. Falasca, J. Hamilton, M. Selvadurai, K. Sundaram, A. Adamska, P. Thompson, Class II Phosphoinositide 3-Kinases as Novel Drug Targets, J Med Chem 60 (2017) 47-65.

[21] V. Asati, D.K. Mahapatra, S.K. Bharti, PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways inhibitors as anticancer agents: Structural and pharmacological perspectives, Eur J Med Chem 109 (2016) 314-341.

[22] T. Jian-Feng, W. Qing, S. Jian, Z. Wei-Ming, Z. Hai-Liang, Advances in the researches on the biological activities and inhibitors of phosphatidylinositol 3-kinase, Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry - Anti-Cancer Agents) 14 (2014) 673-687.

[23] Y. Yong, W. Xun, H. Hong-Wei, S. Shao, W. She-Feng, Q. Fang, L. Ai-Min, L. Peng-Cheng, Z. Hai-Liang, Discovery and synthesis of a novel series of potent, selective inhibitors of the PI3Kα: 2-alkyl-chromeno[4,3-c]pyrazol-4(2H)-one derivatives, Organic & Biomolecular Chemistry 12 (2014) 9157-9165.

[24] Y. Yin, J. Hu, X. Wu, S. Sha, S. Wang, F. Qiao, Z. Song, H. Zhu, Design, synthesis and biological evaluation of novel chromeno[4,3-c]pyrazol-4(2H)-one derivates containing sulfonamido as potential PI3Kα inhibitors, Bioorg Med Chem 11(2019) 2261-2267.

[25] L. Lu, S. Sha, K. Wang, Y.H. Zhang, Y.D. Liu, G.D. Ju, B. Wang, H.L. Zhu, Discovery of chromeno[4,3-c]pyrazol-4(2H)-one containing carbonyl or oxime derivatives as potential, selective inhibitors PI3Kα, Chem Pharm Bull 64 (2016) 1576-1581.

[26] A. Arcaro, M. Wymann, Wortmannin is a potent phosphatidylinositol 3-kinase inhibitor: The role of phosphatidylinositol 3,4,5-trisphosphate in neutrophil responses, Biochem J 296 (1994) 297-301.

[27] G. Powis, R. Bonjouklian, M.M. Berggren, A. Gallegos, R. Abraham, C. Ashendel, L. Zalkow, W. F Matter, J. Dodge, G. Grindey, Wortmannin, a potent and selective inhibitor of phosphatidylinositol-3-kinase, Cancer Research 54 (1994) 2419-2423

[28] S. Shuho, I. Nanami, I. Masafumi, H. Masaru, Y. Mitsunori, The in vitro and in vivo effects of 2-(4-morpholinyl)-8-phenyl-chromone (LY294002), a specific inhibitor of phosphatidylinositol 3'-kinase, in human colon cancer cells, Clinical Cancer Research An Official Journal of the American Association for Cancer Research 8 (2002) 1957-1963.

[29] A.J. Folkes, K. Ahmadi, W.K. Alderton, S. Alix, S.J. Baker, G. Box, I.S.

Chuckowree, P.A. Clarke, P. Depledge, S.A. Eccles, L.S. Friedman, A. Hayes, T.C. Hancox, A. Kugendradas, L. Lensun, P. Moore, A.G. Olivero, J. Pang, S. Patel, G.H. Pergl-Wilson, F.I. Raynaud, A. Robson, N. Saghir, L. Salphati, S. Sohal, M.H. Ultsch, M. Valenti, H.J.A. Wallweber, N.C. Wan, C. Wiesmann, P. Workman, A. Zhyvoloup, M.J. Zvelebil, S.J. Shuttleworth, The Identification of 2-(1H-Indazol-4-yl)-6-(4-methanesulfonyl-piperazin-1-ylmethyl)-4-morpholin-4-yl-th ieno[3,2-d]pyrimidine (GDC-0941) as a Potent, Selective, Orally Bioavailable Inhibitor of Class I PI3 Kinase for the Treatment of Cancer, J Med Chem 51 (2008) 5522-5532.

[30] S. Maira, F. Stauffer, J. Brueggen, P. Furet, C. Schnell, C. Fritsch, S. Brachmann, P. Chene, A. De Pover, K. Schoemaker, D. Fabbro, D. Gabriel, M. Götte, L. Murphy, P. Finan, W. Sellers, C. García-Echeverría, Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity, Mol Cancer Ther 7 (2008) 1851-1863.

[31] A. Bilancio, B. Rinaldi, M. Antonietta Oliviero, M. Donniacuo, M. Monti, A. Boscaino, I. Marino, L. Friedman, F. Rossi, B. Vanhaesebroeck, A. Migliaccio, Inhibition of p110? PI3K prevents inflammatory response and restenosis after artery injury, Bioscience Reports, 37 (2017) BSR20171112.

[32] C. Garcia-Echeverria, W. Sellers, Drug design approaches targeting the PI3K/Akt pathway in cancer, Oncogene 27(2008) 5511-5526.

[33] O. Mirzoeva, B. Hann, Y. K Hom, J. Debnath, D. Aftab, K. Shokat, W. Michael Korn, Autophagy suppression promotes apoptotic cell death in response to inhibition of the PI3K-mTOR pathway in pancreatic adenocarcinoma, Journal of Molecular Medicine 89 (2011) 877-889.

[34] J. R. Shroff, N4-Substituted N1-tolysulfonylpiperazines, J.med.chem**,** 11 (1968) 196-197.

Captions

Scheme 1. Synthesis of compounds **7a~7w**. Reagents and conditions: (i) DCM/TEA, 0 ℃, 2-4 h. (ii) formaldehyde, EtOH, r.t., 4-6 h.

Table 1. Antiproliferative activities in vitro of target compounds **7a-7w** against four cancer cell lines.

Table 2. Enzymatic activities of selected compounds against PI3K and their cytotoxicity on 293T cell line.

Fig. 1. Various PI3K inhibitors and the design compounds.

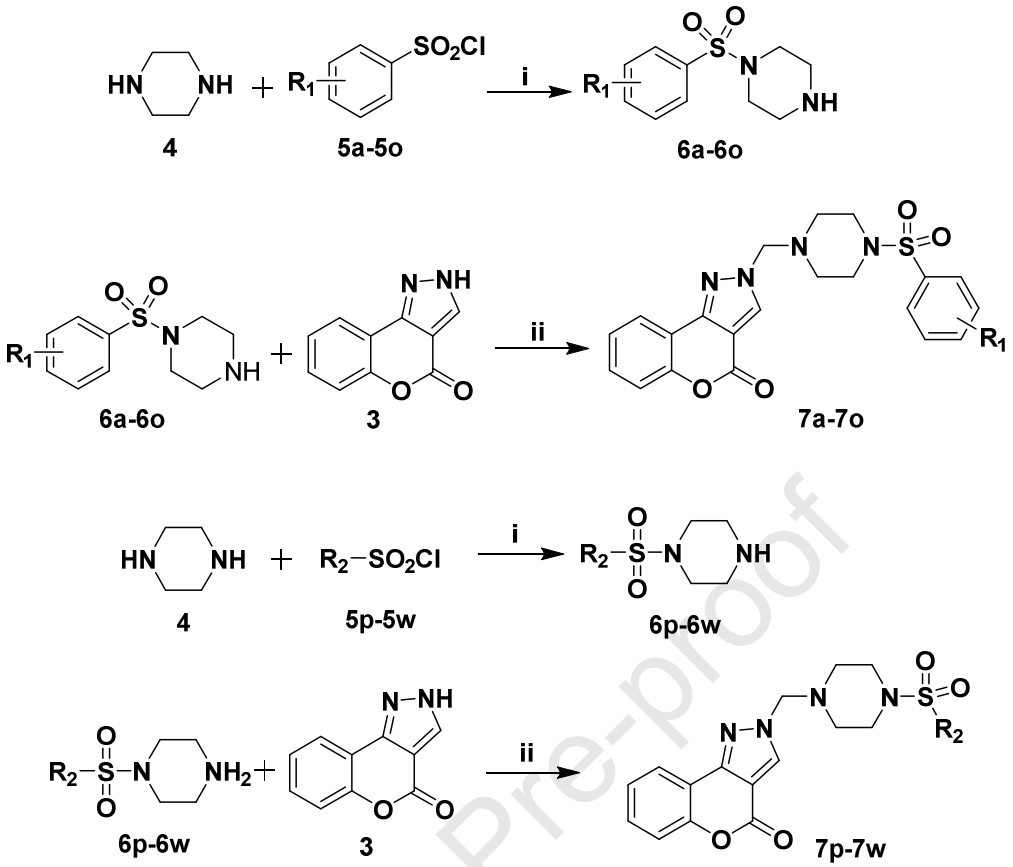
Fig. 2. Effects of compound **7m** on the Akt phosphorylation and S6K phosphorylation. HCT-116 cells were treated with compound **7m** for 24 h, total protein was extracted and subjected to Western blot analysis, β-actin was used as an internal control. Bar graphs represent the expression level of Akt, p-Akt and p-S6K proteins. Data were expressed as Mean±SEM (n = 3). ***p < 0.005 *vs*. the control.

Fig. 3. Compound **7m** induced cell cycle arrest in the HCT-116 cell. Cells were treated with 0.25, 0.5 and 1 μM of **7m** for 24 h. Cell cycle analysis was performed via PI fluorescence and flow cytometer. Data were expressed as Mean ± SEM (n=3). *p < 0.05 vs. the control.

Fig. 4. Induced apoptotic effect of compound **7m** on HCT-116 cell. (A) Apoptotic assay by flow cytometry. HCT-116 cells were treated with compound **7m** at 0, 0.25, 0.5, and 1 μM for 24 h. The cells were stained with Annexin V-FITC/PI, then detected by a flow cytometer. (B) Effects of compound **7m** on Bax, Bcl-2, Cleaved caspase 3 and Cleaved caspase 9 expression. Cells were treated with compound **7m** for 24 h, then total protein was extracted and subjected to Western blot analysis, β-actin was used as an internal control. Bar graphs represent the expression level of Caspase proteins. Data were expressed as Means±SEM (n =3). *p < 0.05, **p < 0.01 and ***p < 0.005 *vs*. the control.

Fig. 5. (A)Molecular docking model of compound **7m** with 3HHM. (B) 3D conformation position of the compound **7m** in the binding pocket.

Fig. 6. Compound **7m** inhibited the growth of HCT-116 xenograft tumors in vivo. (A) Picture of dissected nude mice's tumor tissues untreated or treated with LY294002 (10 mg/kg) and **7m** (10 mg/kg). (B) The growing curves of mice's body weight. (C) The growing curves of mice's tumor volume. (D) Tumor weight results, data were expressed as Means ±SD (n=6). *p < 0.05 and **p < 0.01 *vs*. the control.



Compounds	R_1	Compounds	R_1
7a	H	**7i**	3-NO_2
7b	4-CH_3	**7j**	2-NO_2
7c	4-OCH_3	**7k**	4-CF_3
7d	4-$C(CH_3)_3$	**7l**	2,5-CH_3
7e	4-F	**7m**	4-Cl-3-NO_2
7f	4-Cl	**7n**	2,4,6-CH_3
7g	4-Br	**7o**	2,4,6-$CH(CH_3)_2$
7h	4-NO_2		

Compounds	R_2	Compounds	R_2
7p	CF_3	**7t**	$CH_2CH_2CH_2CH_3$
7q	CH_3	**7u**	cyclopropane
7r	CH_2CH_3	**7v**	CH_2CH_2Cl
7s	$CH_2CH_2CH_3$	**7w**	$CH_2CH_2CH_2Cl$

Scheme 1. Synthesis of compounds **7a~7w**. Reagents and conditions: (i) DCM/TEA, 0 ℃, 2-4 h. (ii) formaldehyde, EtOH, r.t., 4-6 h.

Table 1. Antiproliferative activities in vitro of target compounds **7a-7w** against four cancer cell lines.

compound	IC_{50}±SD [a](μM)			
	HCT-116	A549	Huh7	HL60
7a	7.53±0.68	13.91±1.24	11.35±1.04	22.07±1.91
7b	11.07±1.23	21.52±2.38	28.52±3.09	16.92±1.48
7c	12.14±1.31	27.36±3.05	34.02±2.95	24.51±2.14
7d	0.47±0.04	0.74±0.05	0.90±0.07	0.84±0.07
7e	2.45±0.19	5.11±0.42	6.44±0.52	6.33±0.56
7f	0.81±0.07	1.21±0.11	1.61±0.17	0.64±0.06
7g	1.89±0.15	3.09±0.31	5.08±0.51	4.78±0.43
7h	0.92±0.08	2.10±0.23	2.82±0.29	2.67±0.21
7i	1.29±0.11	3.67±0.31	3.65±0.33	2.01±0.19
7j	4.87±0.37	7.66±0.77	7.92±0.78	8.23±0.78
7k	0.84±0.06	1.15±0.10	2.34±0.13	1.10±0.10
7l	0.13±0.01	0.48±0.05	0.66±0.04	0.22±0.01
7m	0.03±0.002	0.06±0.005	0.09±0.007	0.06±0.005
7n	0.09±0.008	0.11±0.01	0.30±0.02	0.11±0.01
7o	0.08±0.006	0.09±0.006	0.14±0.01	0.09±0.008
7p	>100	>100	>100	>100
7q	>100	>100	97.13±10.06	>100
7r	>100	>100	>100	>100
7s	57.64±5.23	79.71±8.83	87.64±9.17	89.38±9.29
7t	87.93±9.01	>100	>100	>100
7u	69.34±7.22	82.01±8.91	78.99±8.63	71.42±7.28
7v	45.77±4.87	68.74±7.23	62.85±6.78	78.58±6.87
7w	38.04±4.12	52.09±5.14	48.51±4.53	55.10±6.33
LY294002	51.82±4.58	82.32±7.26	67.18±5.64	18.43±2.03
BEZ235	0.24±0.012	0.38±0.017	0.53±0.022	0.91±0.038

[a] Values are average of three independent experimental measurements and expressed as Mean ± SD.

Table 2. Enzymatic activities of selected compounds against PI3K and their cytotoxicity on 293T cell line.

compound	IC_{50}±SD [a](μM)					CC_{50}±SD [b](μM)
	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ	mTOR	
7d	0.95±0.061	2.65±0.32	1.32±0.12	0.75±0.078	>10	231.42±18.94
7e	8.91±0.78	>10	7.51±0.73	9.76±0.89	9.12±0.46	298.13±22.17

7f	1.55±0.13	2.19±0.21	2.65±0.21	4.72±0.47	>10	277.62±26.46
7g	8.30±0.76	9.71±0.87	>10	9.31±0.91	>10	387.43±31.36
7h	2.63±0.21	4.14±0.43	4.01±0.38	5.45±0.52	>10	346.58±29.74
7i	6.18±0.54	>10	9.34±0.81	>10	>10	412.08±37.81
7j	>10	9.14±0.89	8.42±0.78	6.15±0.54	8.37±0.74	372.38±39.35
7k	3.84±0.31	5.75±0.56	2.30±0.22	7.51±0.73	8.12±0.43	334.77±36.27
7l	0.30±0.022	0.85±0.079	0.81±0.075	1.42±0.12	>10	397.15±40.12
7m	0.009±0.001	0.12±0.010	0.081±0.006	0.12±0.011	9.38±0.86	386.07±32.44
7n	0.12±0.011	0.41±0.031	0.14±0.012	0.23±0.018	7.44	354.24±34.81
7o	0.042±0.003	0.21±0.015	0.11±0.009	0.42±0.039	>10	331.53±27.56
LY294002	0.48±0.07	0.98±0.012	0.95±0.082	1.36±0.13	>10	-
BEZ235	0.011±0.002	0.064±0.004	0.027±0.003	0.021±0.003	0.012±0.001	-

[a] Values are average of three independent experimental measurements and expressed as Mean ± SD.

[b] Cytotoxicity on 293T cell line, Values are average of three independent experimental measurements and expressed as Mean ± SD.

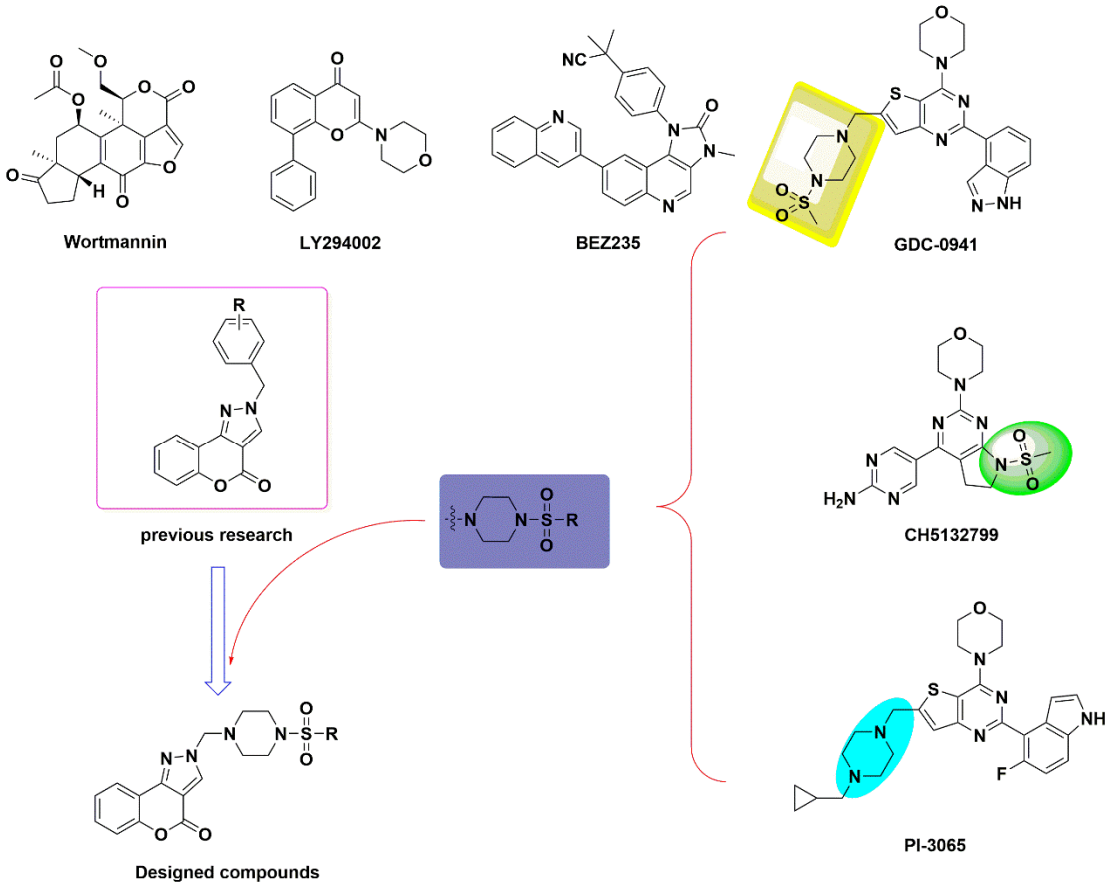


Fig. 1. Various PI3K inhibitors and the design compounds.

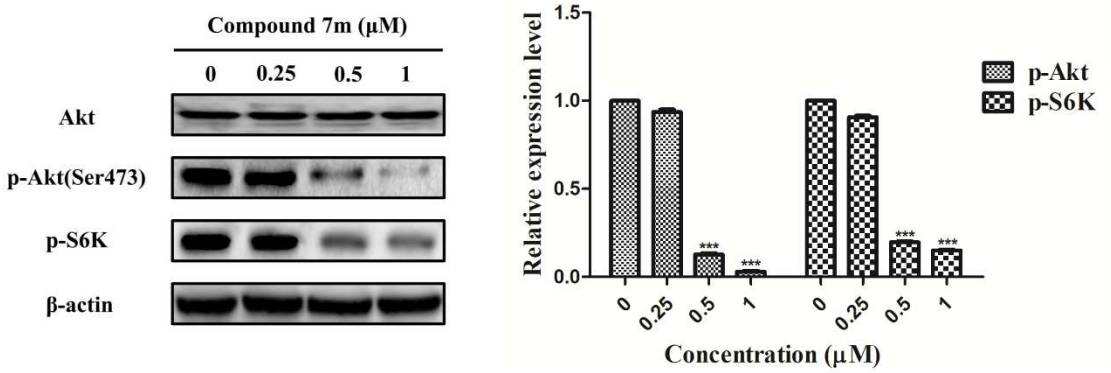


Fig. 2. Effects of compound **7m** on the Akt phosphorylation and S6K phosphorylation. HCT-116 cells were treated with compound **7m** for 24 h, total protein was extracted and subjected to Western blot analysis, β-actin was used as an internal control. Bar graphs represent the expression level of Akt, p-Akt and p-S6K proteins. Data were expressed as Mean±SEM (n = 3). ***$p < 0.005$ *vs*. the control.

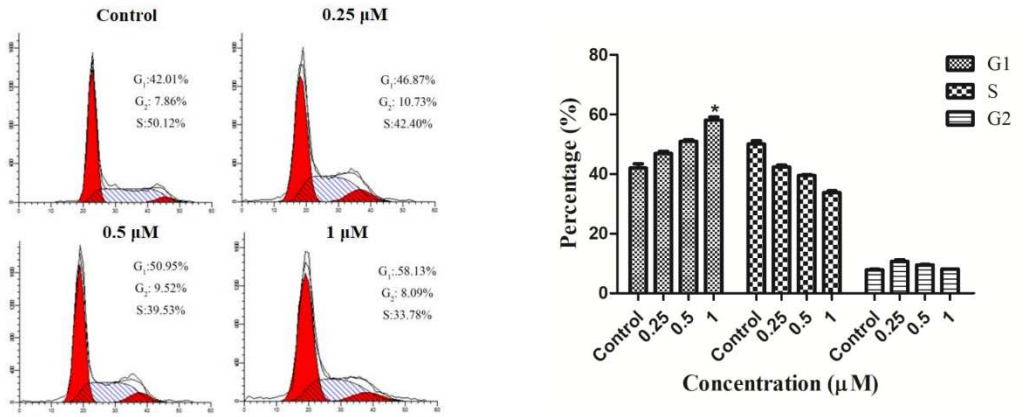


Fig. 3. Compound **7m** induced cell cycle arrest in the HCT-116 cell. Cells were treated with 0.25, 0.5 and 1 µM of **7m** for 24 h. Cell cycle analysis was performed via PI fluorescence and flow cytometer. Data were expressed as Mean ± SEM (n=3). *p < 0.05 vs. the control.

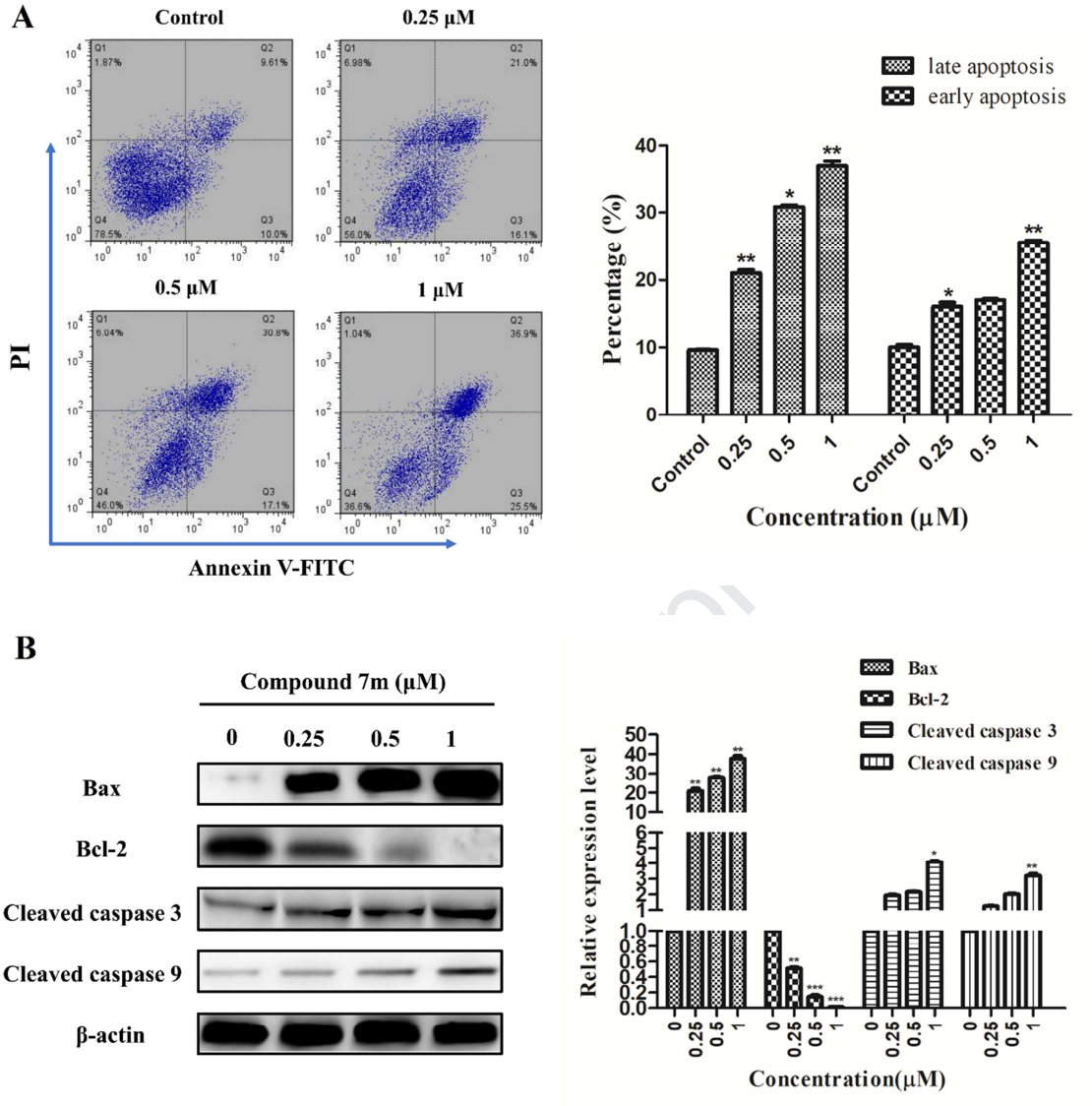


Fig. 4. Induced apoptotic effect of compound **7m** on HCT-116 cell. (A) Apoptotic assay by flow cytometry. HCT-116 cells were treated with compound **7m** at 0, 0.25, 0.5, and 1 μM for 24 h. The cells were stained with Annexin V-FITC/PI, then detected by a flow cytometer. (B) Effects of compound **7m** on Bax, Bcl-2, Cleaved caspase 3 and Cleaved caspase 9 expression. Cells were treated with compound **7m** for 24 h, then total protein was extracted and subjected to Western blot analysis, β-actin was used as an internal control. Bar graphs represent the expression level of Caspase proteins. Data were expressed as Means±SEM (n =3). *p < 0.05, **p < 0.01 and ***p < 0.005 *vs*. the control.

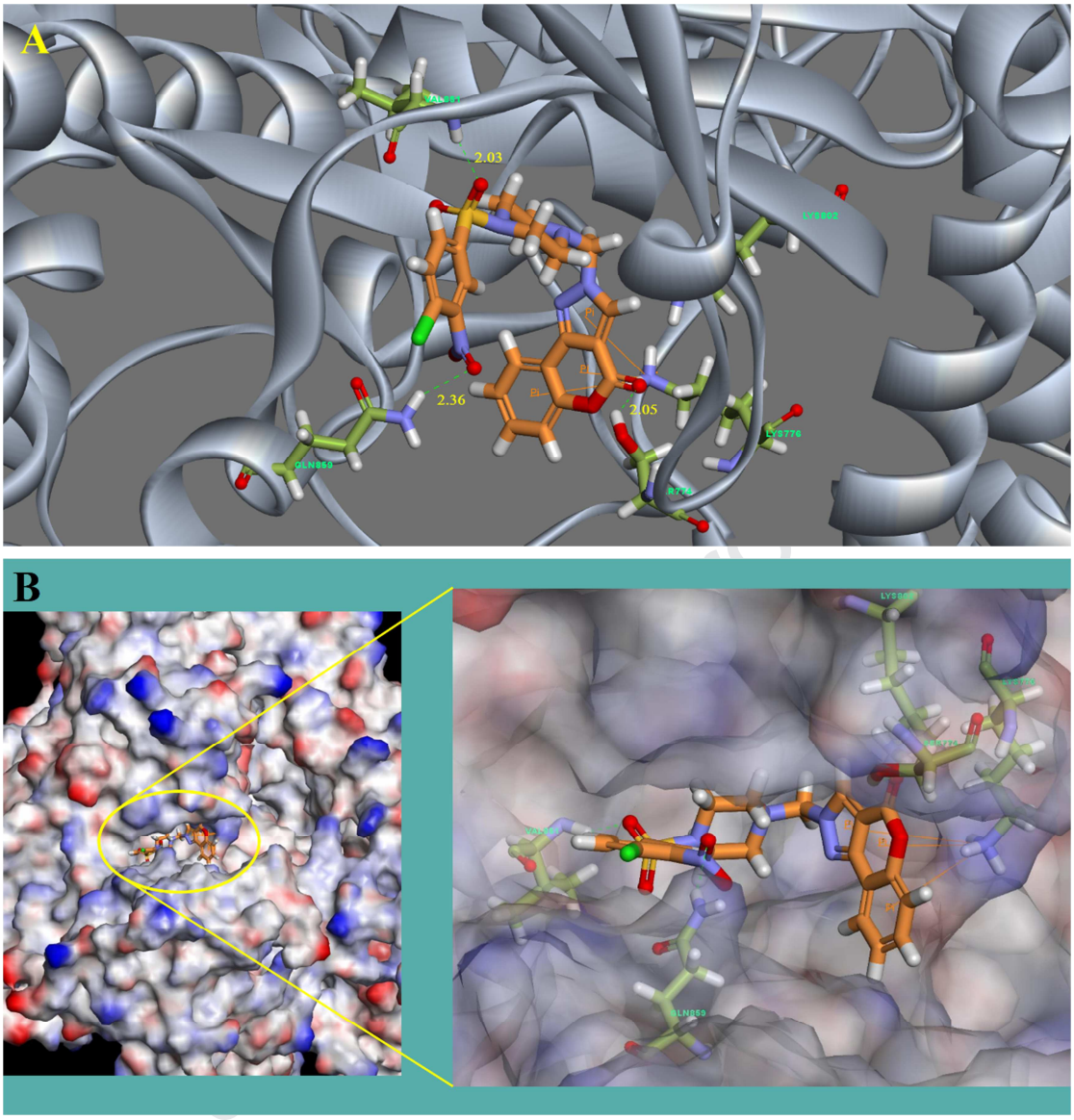


Fig. 5. (A)Molecular docking model of compound **7m** with 3HHM. (B) 3D conformation position of the compound **7m** in the binding pocket.

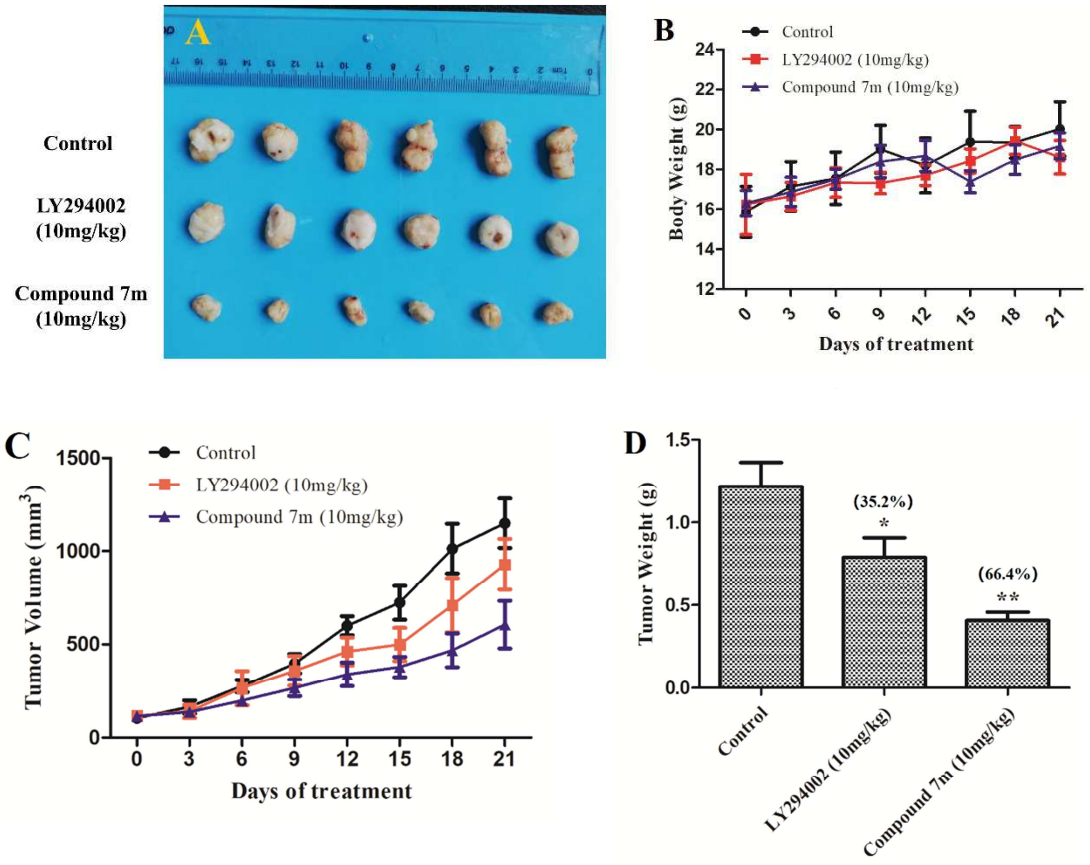


Fig. 6. Compound **7m** inhibited the growth of HCT-116 xenograft tumors in vivo. (A) Picture of dissected nude mice's tumor tissues untreated or treated with LY294002 (10 mg/kg) and **7m** (10 mg/kg). (B) The growing curves of mice's body weight. (C) The growing curves of mice's tumor volume. (D) Tumor weight results, data were expressed as Means ±SD (n=6). *$p < 0.05$ and **$p < 0.01$ *vs*. the control.

Highlights

- a series of novel chromeno[4,3-c]pyrazol-4(2*H*)-one derivatives contained sulfonylpiperazine was synthesized evaluated as potential PI3Kα inhibitors.
- compound **7m** displayed the most potent activity *in vitro* and remarkably inhibition of tumor growth *in vivo*.
- compound **7m** was a promising candidate selective PI3Kα inhibitor for tumor therapy.