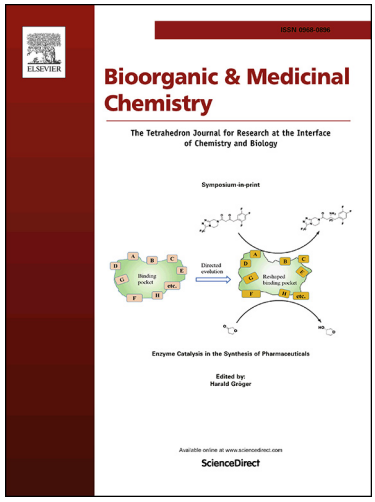
Accepted Manuscript



Design, synthesis and biological evaluation of novel series of 2*H*-benzo[b][1,4]oxazin-3(4H)-one and 2*H*-benzo[b][1,4]oxazine scaffold derivatives as PI3Kα inhibitors

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Design, synthesis and biological evaluation of novel series of 2*H*-benzo[b][1,4]oxazin-3(4H)-one and 2*H*-benzo[b][1,4]oxazine scaffold derivatives as PI3Kα inhibitors

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Abstract: The abnormal activation of PI3K signaling pathway leads to the occurrence of various cancers. The PI3Kα is frequently mutated and overexpressed in many human cancers. Therefore, the PI3Kα was considered as a promising target in therapeutic treatment of cancer. In this study, two series of compounds containing 2*H*-benzo[b][1,4]oxazin-3(4H)-one and 2*H*-benzo[b][1,4]oxazine scaffold were synthesized and evaluated antiproliferative activities against three cancer cell lines, including HCT-116, MDA-MB-231 and SNU638. Compound **7f** with the most potent antiproliferative activity was selected for further evaluation on normal cells and PI3K kinase. Studies indicated that compound **7f** could decrease the phospho-Akt (T308) in a dose-dependent manner. Four key hydrogen bonding interactions were found in the docking of **7f** with PI3K enzyme. All the results suggested that **7f** was a potent PI3Kα inhibitor.

Keywords: PI3Kα, 2*H*-benzo[b][1,4]oxazin-3(4H)-one; 2*H*-benzo[b][1,4]oxazine; anticancer.

1. Introduction

The phosphatidylinositol 3-kinases (PI3Ks) are members of a unique group of intracellular lipid kinases [1]. Based on the primary structure, regulation and in vitro lipid substrate specificity, the PI3K family is divided into four different classes: Class I, Class II, Class III, and Class IV. Of these, the most commonly studied is the Class I which is further divided into PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ [2-3]. The PI3K/Akt signaling pathway is frequently activated, amplified and mutated in human cancers [4-7] and regulates various cellular processes, such as proliferation, growth and apoptosis [8].

Given the important role of PI3K/Akt signaling pathway in cancers and inhibition of this pathway is a promising approach for novel chemotherapeutic agents [9-11]. Several inhibitors targeting PI3K have been developed and are being evaluated in preclinical studies and early clinical trials [12-14]. Among them, there are some PI3K inhibitors which containing morpholine group being developed, such as BKM120 [15], GDC0941 [16-17], GDC0980 [18], GSK2636771 [19-20], LY294002 (Fig.1) [21]. What's more, Omipalisib (GSK2126458) has been identified as a highly potent PI3K inhibitor with picomolar activity and is currently under evaluation in clinical trials for oncology applications [22].

Based on the reported corresponding X-ray structure, Omipalisib uses the position-1 nitrogen as a hinge binder [22]. The research has shown that a tremendous number of PI3K inhibitors which containing morpholine group using the oxidation of morpholine interact with the hinge region of the ATP [23]. Thus, we try to replace the pyridine ring of quinoline with the morpholine ring to get compounds with new skeleton on the basis of Omipalisib. Considering to reduce the difficulty of synthesis, we replace the quinoline moiety with 2*H*-benzo[b][1,4]oxazin-3(4H)-one which also has the morpholine ring scaffold. Not only that, similar to the cyano group of Dactolisib (NVP-BEZ235) as a hydrophilic group [24], benzoxazine can easily introduce hydrophilic substituents on the position-4 nitrogen atom or position-3 to increase the solubility of the molecules. As a result, we designed two series of derivatives containing benzoxazine moiety. They were prepared and evaluated for their anticancer effects and PI3K inhibitory activities *in vitro*. The results suggested that compound **7f** could be as a PI3Kα inhibitor.

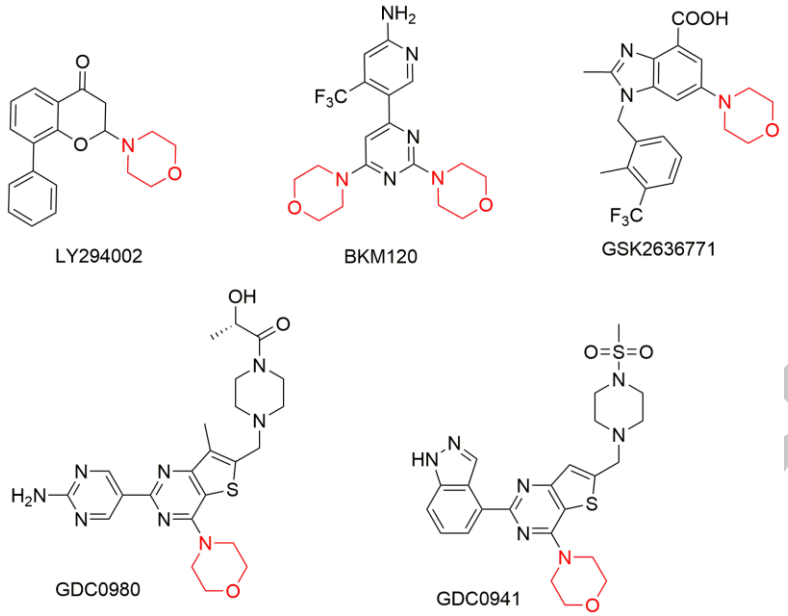


Fig.1. The structures of some PI3K inhibitors which have the morpholine ring.

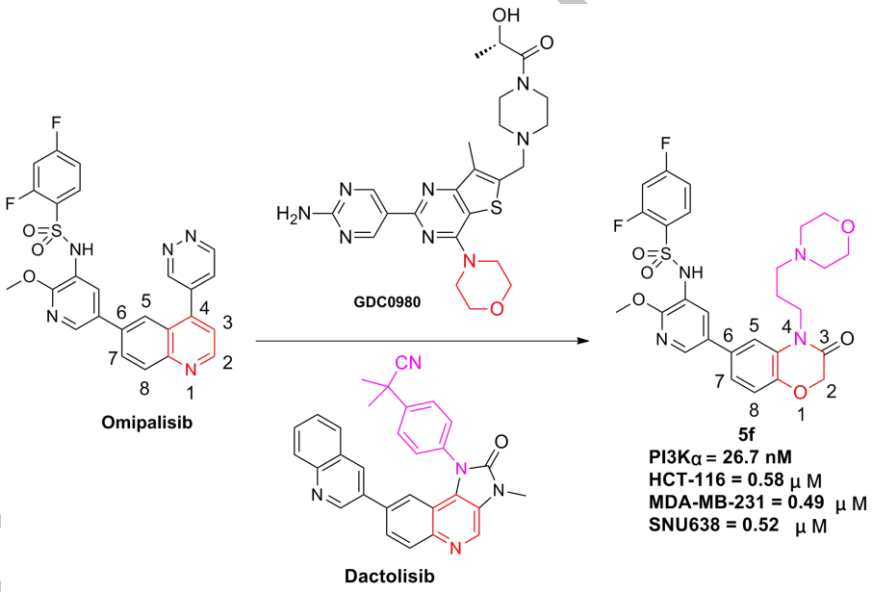


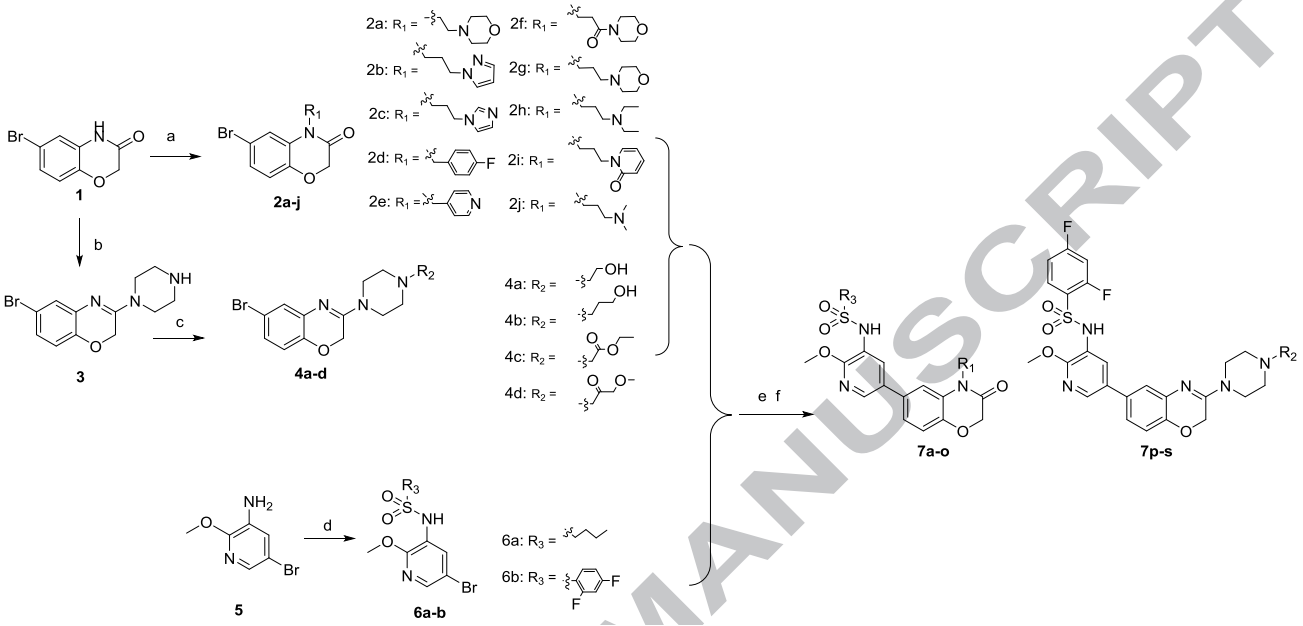
Fig.2. The design strategy based on Omipalisib, PI3K inhibitors which containing morpholine group and Dactolisib.

2. Results and discussion

2.1 Chemistry

The general synthetic routes for the target compounds are summarized in Scheme 1. Coupling reaction of **1** with ten chlorinated compounds to give **2a-j**. Compound **1** was treated with P_2S_5 and piperazine to get **3**. Coupling reaction of **3** with four chlorinated compounds to give **4a-d**. Sulfonylation of **5** with two sulfonyl chlorides to give **6a-b**, which were then subjected to Suzuki coupling with bis(pinacolato)diborane to get arylboronic esters. Then, the arylboronic esters which

were not purified coupling with **2a-j** and **4a-d** via adding the catalyst required for the Suzuki coupling reaction to yield the target compounds **7a-s**. The structure of the new analogs were characterized by ^{1}H NMR, ^{13}C NMR and HRMS.



Scheme 1. (a) chlorinated compounds, K_2CO_3, DMF, 60 ºC, 6 h; (b) i) THF, P_2S_5, 90 ºC, 8 h ii) THF, Piperazine, 90 ºC, 6 h; (c) chlorinated compounds, K_2CO_3, DMF, 60 ºC, 6 h; (d) sulfonyl chlorides, pyrid ine, 25 ºC, 24 h; (e) $Pd(dppf)_2Cl_2$, AcOK, bis(pinacolato)diborane, DMF, 100 ºC, 8 h; (f) $Pd(dppf)_2Cl_2$, C s_2CO_3, DMF, 90 ºC, 4 h.

2.2 Antiproliferative assays in vitro

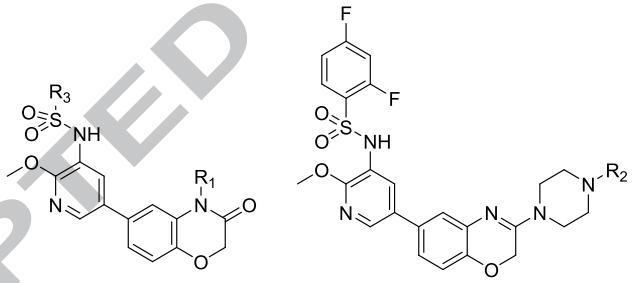
All synthesized compounds were evaluated for their cytotoxicities *in vitro* against three human cancer cell lines including HCT-116, MDA-MB-231 and SNU638 cells. The antiproliferative results of all the compounds were summarized in Table 1. The results of antiproliferative effect assay showed that most of the derivatives exhibited high antiproliferative effects, especially against HCT-116. Compound **7a** (IC_{50} = 2.40 μM against HCT-116) was more potent than compound **7l** (IC_{50} = 15.8 μM against HCT-116) and compound **7f** (IC_{50} = 0.58 μM against HCT-116) was more potent than compound **7k** (IC_{50} = 4.62 μM against HCT-116) suggesting that 2,4-difluorophenyl compared to N-butyl at position of R_3 can significantly increase the antiproliferative activity. This conclusion was also verified by **7c** vs. **7n** and **7e** vs. **7o**. Compound **7f** was more potent than compound **7a** also suggesting that the activity could be increased with the increased length of alkyl group and this conclusion could also be confirmed by compounds **7k** vs. **7l**. From compounds **7i** (IC_{50} = 33.6 μM against HCT-116), **7k**, **7m** (IC_{50} = 32.9 μM against HCT-116) and **7o** (IC_{50} = 42.6 μM against

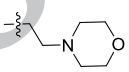
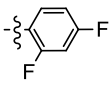
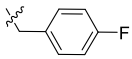
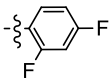
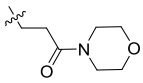
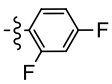
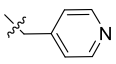
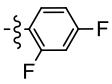
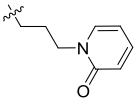
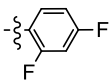
HCT-116) suggesting that the activity of the compounds with unsaturated group was weaker than the compounds with saturated group on R_1 position. Among them, compound **7f** showed the best anticancer activities with IC_{50} value of about 0.5 μM against three cancer cell lines and the activity was better than the positive control Dactolisib.

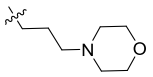
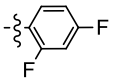
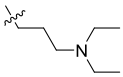
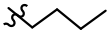
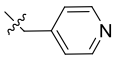
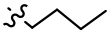
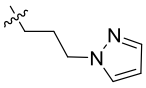
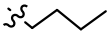
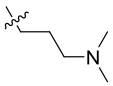
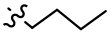
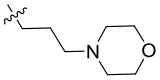
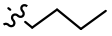
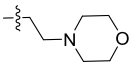
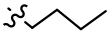
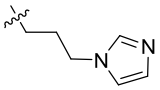
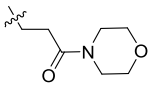
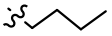
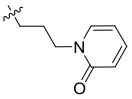
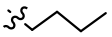
To develop more core structures, we synthesized another series of compounds **7p** to **7s** which substituted at position-3 of benzoxazine scaffold. However, the activities of this series were no significant improvement as good compared to the first series derivatives. However, very similar to **7a-o**, the introduction of hydrophilic groups at position 3 could also improve their biological activity which could be verified by **7p** vs. **7r** and **7q** vs. **7s**.

We also test the cytotoxic effects of **7f** on normal human cells. Compound **7f** showed much less inhibitory activity against MRC5 (IC_{50} = 32.8 μM against MRC5 and IC_{50} = 15.6 μM against HUVEC) (Table 2). These indicated that our compounds could inhibit the growth of cancer cell in effective concentration *in vitro* and no effect on normal cells.

Table 1 Cytotoxicity *in vitro* of target compounds against three cancer cell lines (IC_{50} Values[a] in μM).



Compd	R_1	R_2	R_3	HCT-116	MDA-MB-231	SNU638
7a				2.40±0.12	1.82±0.06	2.38±0.11
7b				1.24±0.04	4.98±0.26	7.84±0.32
7c				3.18±0.22	3.26±0.13	5.77±0.16
7d				2.22±0.09	5.49±0.15	6.96±0.12
7e				4.23±0.32	12.4±0.58	6.76±0.31

7f			0.58±0.03	0.49±0.02	0.52±0.04
7g			14.6±0.62	13.8±0.71	17.6±0.89
7h			41.4±3.13	37.9±2.71	46.5±1.42
7i			33.6±1.12	37.9±2.06	36.2±3.46
7j			8.43±0.82	9.35±0.74	8.97±0.66
7k			4.62±0.42	4.23±0.36	5.01±0.60
7l			15.8±0.76	19.5±0.92	20.4±1.14
7m			32.9±2.30	40.4±3.16	32.7±2.42
7n			23.5±1.72	43.8±2.86	41.9±1.96
7o			42.6±1.42	47.9±3.18	44.3±2.54
7p			6.18±0.32	8.26±0.60	7.39±0.54
7q			3.26±0.22	4.78±0.16	4.97±0.22
7r			14.5±0.62	`17.2±0.72	21.4±0.68
7s			18.6±0.60	22.8±0.48	24.9±0.94
Dactolisib			0.84±0.12	0.56±0.09	1.24±0.13

[a]IC_{50} values are the mean of triplicate measurements.

Table 2 Cytotoxicity of **7f** to normal human cells (IC_{50} Values in μM)

Cells	MRC5	HUVEC
7f	32.8	15.6

2.3 PI3K enzymatic activity assay

To elucidate the mechanism of antiproliferative activities of these active compounds, we selected the most potent compound **7f** according to the antiproliferative results for the further PI3K enzymatic activity assays. Dactolisib was selected as the positive drug. As shown in Table 3, the inhibitory activity of **7f** was 26.7 nM for PI3Kα. The activity against PI3Kα is seven-fold higher than the other class I PI3Ks (PI3Kβ, PI3Kγ and PI3Kδ). These results suggest that compound **7f** was a selective PI3Kα inhibitor.

Table 3 Activities of **7f** and Dactolisib against Class I PI3K (IC_{50} Values in nM)

	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
7f	26.7	382.3	184.7	425.2
Dactolisib	16.4	35.9	23.6	78.4

2.4 Western blot assay

Activation of the PI3K pathway leads to phosphorylation of Akt and because of compound **7f** suppressed PI3Kα activity, therefore, we evaluated the effects of **7f** on the related protein levels of Akt by western blot analysis. As shown in Fig.3, compound **7f** could decrease the phospho-Akt (T308) in a dose-dependent manner, indicating that **7f** might act as a potential PI3K inhibitor.

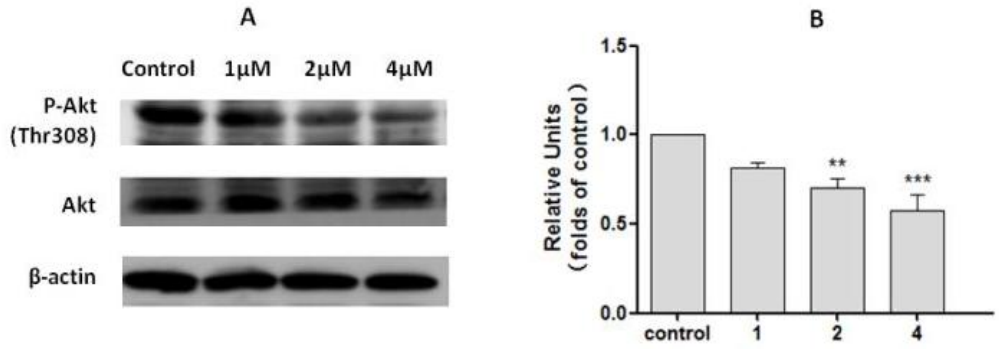


Fig.3. Western blot analysis of **7f**. (A) The inhibition effects of compound **7f** (1 μM, 2 μM and 4 μM) on the expression of p-Akt, Akt in HCT-116 cells are depicted. β-actin was used as internal control. (B) Bar graphs showed the quantitative results of clonogenicity. Data are presented as the mean for three independent experiments. ** $P < 0.01$, *** $P < 0.001$ compared with control.

2.5 Molecular docking

Molecular docking study was performed to elucidate the binding model of **7f** in the binding site of PI3K. As showed in Fig 4 (A), the oxygen atom of morpholine ring of **7f** forms hydrogen bond interaction with Val882 in the hinge binder region of PI3K. Besides, the oxygen of sulfonamide

group interaction with Lys833 and nitrogen of pyridyl group formed a hydrogen bond with the conserved water molecule. In addition, the morpholinyl formed an additional hydrogen bond with Tyr887. As showed in Fig 4 (B), the folded form of compound **7f** in the protein pocket was very similar to that of Omipalisib. The formation of hydrogen bonds suggested that **7f** can exactly interact with the catalytic domain of PI3K and also indicated that **7f** might be a potent PI3K inhibitor.

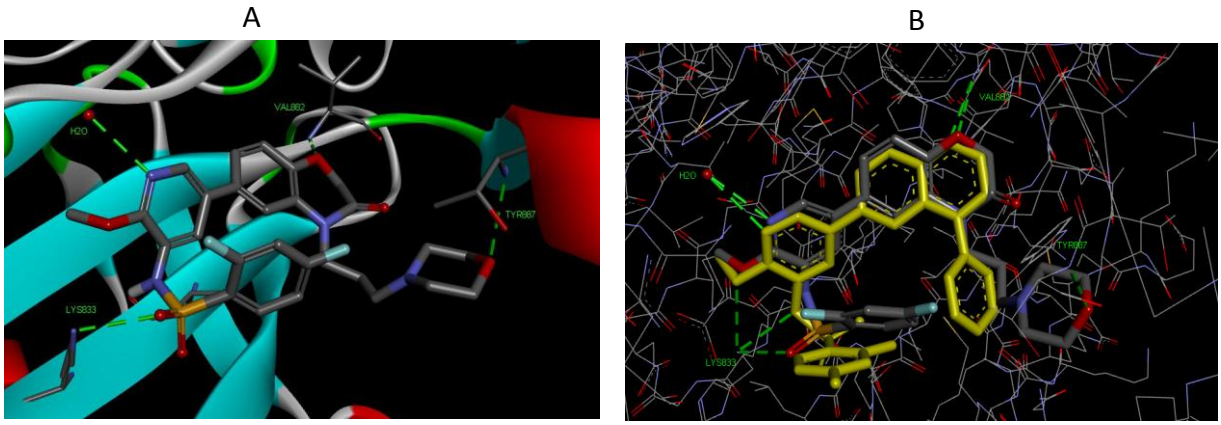


Fig.4. Docking mode of **7f** with protein crystal structure of PI3K. (A) Key interactions of compound **5f** in the active site of PI3K (PDB: 3L08). (B) The binding pose of **7f** and Omipalisib in the active site of PI3K. Omipalisib was highlighted with yellow.

3. Conclusion

In summary, two series of 2*H*-benzo[b][1,4]oxazin derivatives containing sulfonamide substituted pyridyl group were designed and synthesized based on Omipalisib, PI3K inhibitors which containing morpholine group and Dactolisib. Their antiproliferative activities against three cancer cell lines, two normal human cell lines were evaluated *in vitro* which indicating that **7f** selectively inhibits the proliferation of cancer cells compared to normal cells. The structure activity relationships (SARs) of the title compounds was discussed. The PI3K inhibitory activities and Western blot assay proved that **7f** could be considered as a PI3Kα inhibitor. According to these results, it can be concluded that 2*H*-benzo[b][1,4]oxazin core via adding a hydrophilic group was a feasible way to get PI3K inhibitor with high activities.

4. Experimental section

4.1 Chemistry and chemical methods

All reagents and solvents were commercially available without further purification. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on 400 and 600 Bruker NMR spectrometer with

tetramethylsilane (TMS) as an internal standard. All chemical shifts are reported in ppm (δ) and coupling constants (*J*) are in hertz (Hz). High-resolution exact mass measurements were performed using electrospray ionization (positive mode) on a quadrupole time-of-flight (QTOF) mass spectrometer (microTOF-Q, Bruker Inc).

*4.1.1 6-bromo-4-(2-morpholinoethyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**2a**)*

To a 25 mL flask was added 6-bromo-2*H*-benzo[b][1,4]oxazin-3(4*H*)-one (0.46 g, 2 mmol), 4-(2-chloroethyl)morpholine (0.30 g, 2 mmol), potassium carbonate (0.42 g, 3 mmol) and DMF (8 mL), the reaction mixture was heated at 60 ºC and stirred for 6 h. After the reaction was completed, the DMF was removed at reduced pressure and the residue was purified on silica gel with chloroform/methanol (V:V 100:1) to give the title compound as a white powder. Yield 94.1%, m.p. 80-82 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.51 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.17 (dd, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 6.97 (d, *J* = 8.5 Hz, 1H, Ar-H), 4.65 (s, 2H, Ar-H), 4.02 (t, *J* = 6.4 Hz, 2H, CH_2), 3.53 (s, 4H, CH_2×2), 2.47 (t, *J* = 6.4 Hz, 2H, CH_2), 2.43 (s, 4H, CH_2×2). ESI-MS: m/z 341.0 $[M+H]^+$.

Compounds **2b**-**j** were synthesized according to the procedure described in **2a**.

*4.1.2 4-(3-(1H-pyrazol-1-yl)propyl)-6-bromo-2H-benzo[b][1,4]oxazin-3(4H)-one (**2b**)*

Yield 80.2%, m.p. 99-102 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.75 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.50-7.47 (m, 1H, Ar-H), 7.25 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.16 (dd, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 6.96 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.26 (t, *J* = 2.0 Hz, 1H, Ar-H), 4.65 (s, 2H, CH_2), 4.20 (t, *J* = 6.7 Hz, 2H, CH_2), 3.87-3.83 (m, 2H, CH_2), 2.07-2.03 (m, 2H, CH_2). ESI-MS: m/z 336.0 $[M+H]^+$.

*4.1.3 4-(3-(1H-imidazol-1-yl)propyl)-6-bromo-2H-benzo[b][1,4]oxazin-3(4H)-one (**2c**)*

Yield 75.6%, m.p. 116-120 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.71 (s, 1H, Ar-H), 7.29 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.24 (s, 1H, Ar-H), 7.18 (dd, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 6.97 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.94 (s, 1H, Ar-H), 4.66 (s, 2H, CH_2), 4.06 (t, *J* = 7.0 Hz, 2H, CH_2), 3.89-3.85 (m, 2H, CH_2), 2.02-1.96 (m, 2H, CH_2). ESI-MS: m/z 336.0 $[M+H]^+$.

*4.1.4 6-bromo-4-(4-fluorobenzyl)-2H-benzo[b][1,4]oxazin-3(4H)-one **(2d)***

Yield 95.1%, m.p. 118-120 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.36-7.31 (m, 2H, Ar-H×2), 7.21 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.18 (t, *J* = 8.9 Hz, 2H, Ar-H×2), 7.15 (dd, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 6.98 (d, *J* = 8.5 Hz, 1H, Ar-H), 5.17 (s, 2H, CH_2), 4.82 (s, 2H, CH_2). ESI-MS: m/z 336.0 $[M+H]^+$.

*4.1.5 6-bromo-4-(pyridin-4-ylmethyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**2e**)*

Yield 93.3%, m.p. 120-124 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.53 (d, *J* = 6.0 Hz, 2H, Ar-H×2), 7.28 (d, *J* = 6.0 Hz, 2H, Ar-H×2), 7.17 (dd, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 7.11 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.01 (d, *J* = 8.5 Hz, 1H, Ar-H), 5.21 (s, 2H, CH_2), 4.86 (s, 2H, CH_2). ESI-MS: m/z 319.0 $[M+H]^+$.

*4.1.6 6-bromo-4-(3-morpholino-3-oxopropyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**2f**)*

Yield 81.5%, m.p. 118-120ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.42 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.17 (dd, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 6.97 (d, *J* = 8.5 Hz, 1H, Ar-H), 4.64 (s, 2H, CH_2), 4.10-4.07 (m, 2H, CH_2), 3.54 (q, *J* = 5.0, 4.6 Hz, 4H, CH_2×2), 3.40 (dt, *J* = 22.1, 4.9 Hz, 4H, CH_2×2), 2.65-2.62 (m, 2H, CH_2). ESI-MS: m/z 369.0 $[M+H]^+$.

*4.1.7 6-bromo-4-(3-morpholinopropyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**2g**)*

Yield 78.4%, m.p. 136-140 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.47 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.17 (dd, *J* = 8.5, 2.1 Hz, 1H, Ar-H), 6.97 (d, *J* = 8.5 Hz, 1H, Ar-H), 4.65 (s, 2H, CH_2), 3.92 (t, *J* = 7.0 Hz, 2H, CH_2), 3.60 (s, 4H, CH_2×2), 2.31 (s, 6H, CH_2×3), 1.74-1.67 (m, 2H, CH_2). ESI-MS: m/z 355.1 $[M+H]^+$.

*4.1.8 6-bromo-4-(3-(diethylamino)propyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**2h**)*

Yield 76.6%, m.p. 76-78 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.44 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.17 (dd, *J* = 8.5, 2.1 Hz, 1H, Ar-H), 6.97 (d, *J* = 8.5 Hz, 1H, Ar-H), 4.65 (s, 2H, CH_2), 3.94-3.90 (m, 2H, CH_2), 2.56 (dq, *J* = 13.7, 6.9 Hz, 6H, CH_2×3), 1.68 (p, *J* = 7.1 Hz, 2H, CH_2), 0.99 (t, *J* = 7.1 Hz, 6H, CH_2×3). ESI-MS: m/z 341.1 $[M+H]^+$.

*4.1.9 6-bromo-4-(3-(2-oxopyridin-1(2H)-yl)propyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**2i**)*

Yield 80.2%, m.p. 132-135 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.72 (dd, *J* = 6.7, 1.8 Hz, 1H, Ar-H), 7.41 (ddd, *J* = 8.9, 6.6, 2.0 Hz, 1H, Ar-H), 7.30 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.17 (dd, *J* = 8.5, 2.1 Hz, 1H, Ar-H), 6.97 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.37 (d, *J* = 9.1 Hz, 1H, Ar-H), 6.23 (t, *J* = 6.6 Hz, 1H, Ar-H), 4.65 (s, 2H, CH_2), 3.93 (dt, *J* = 16.2, 7.3 Hz, 4H, CH_2×2), 1.91 (q, *J* = 7.3 Hz, 2H, CH_2). ESI-MS: m/z 363.0 $[M+H]^+$.

*4.1.10 6-bromo-4-(3-(dimethylamino)propyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**2j**)*

Yield 80.2%, m.p. 172-178ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.44 (d, *J* = 1.7 Hz, 1H, Ar-H), 7.20 (dd, *J* = 8.5, 1.8 Hz, 1H, Ar- H), 6.99 (d, *J* = 8.5 Hz, 1H, Ar-H), 4.69 (s, 2H, CH_2), 3.96 (t, *J* = 7.1 Hz, 2H, CH_2), 3.13 (s, 2H, CH_2), 2.77 (s, 6H, CH_2×3), 1.95-1.90 (m, 2H, CH_2). ESI-MS: m/z 313.0 $[M+H]^+$.

*4.1.11 6-bromo-3-(piperazin-1-yl)-2H-benzo[b][1,4]oxazine (**3**)*

To a 25 mL flask was added 6-bromo-2*H*-benzo[b][1,4]oxazin-3(4*H*)-one (0.46 g, 2 mmol), P_2S_5 (0.89 g, 4 mmol) and THF (8 mL), the reaction mixture was heated at 90 ºC and stirred for 8 h. After the reaction was completed, the THF was removed at reduced pressure and pour into water, filtered dry and directly reacted with piperazine (0.34g, 4 mmol) at 90 ºC in THF without purification. Then the THF was removed at reduced pressure and the residue was purified on silica gel with chloroform/methanol (V:V 10:1) to give the title compound as a white powder. Yield 56.2%, oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.99 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.92 (dd, *J* = 8.4, 2.4 Hz, 1H, Ar-H), 6.74 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.76 (s, 2H, Ar-H), 3.50 – 3.39 (m, 4H, CH_2×2), 3.19 (m, 4H, CH_2×2). ESI-MS: m/z 296.0 $[M+H]^+$.

*4.1.12 2-(4-(6-bromo-2H-benzo[b][1,4]oxazin-3-yl)piperazin-1-yl)ethanol (**4a**)*

To a solution of 4-nitro-1H-pyrazole (0.29 g, 1 mmol) in DMF (10 mL) was added K_2CO_3 (0.14 g, 1 mmol) and 2-Bromoethanol (0.15 g, 1.2 mmol). Then the mixture was stirred at 60 ºC for 12 h. DMF was removed at reduced pressure and add water (100 mL), extracted with ethyl acetate (3 × 100 mL), dried with Na_2SO_4 and evaporated to give and the residue which was purified on silica gel with chloroform/methanol (V:V 10:1) to give the title compound as a white powder. Yield 82.6%, oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.01 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.93 (dd, *J* = 8.4, 2.4 Hz, 1H, Ar-H), 6.75 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.77 (s, 2H, CH_2), 4.45 (s, 1H, OH), 3.56 – 3.50 (m, 4H, CH_2×2), 3.38 (d, *J* = 15.4 Hz, 2H, CH_2), 2.47 – 2.44 (m, 4H, CH_2×2), 2.42 (t, *J* = 6.2 Hz, 2H, CH_2). ESI-MS: *m/z* 340.1 $[M+H]^+$.

Compounds **4b-d** was synthesized according to the procedure described in **4a**.

*4.1.13 3-(4-(6-bromo-2H-benzo[b][1,4]oxazin-3-yl)piperazin-1-yl)propan-1-ol (**4b**)*

Yield 81.6%, oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.01 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.93 (dd, *J* = 8.4, 2.4 Hz, 1H, Ar-H), 6.75 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.77 (s, 2H, CH_2), 3.52 (s, 2H, CH_2), 3.45 (t, *J* = 6.3 Hz, 4H, CH_2×2), 2.42 – 2.38 (m, 4H, CH_2×2), 2.36 (d, *J* = 7.4 Hz, 2H, CH_2), 1.62 – 1.57 (m, 2H, CH_2). ESI-MS: m/z 354.1 $[M-H]^+$.

*4.1.14 ethyl 2-(4-(6-bromo-2H-benzo[b][1,4]oxazin-3-yl)piperazin-1-yl)acetate (**4c**)*

Yield 78.3%, m.p. 133-105 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.01 (d, *J* = 2.3 Hz, 1H, Ar-H), 6.94 (dd, *J* = 8.4, 2.4 Hz, 1H, Ar-H), 6.75 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.78 (s, 2H, CH_2), 4.11 – 4.06

(m, 2H, CH_2), 3.53 (s, 4H, CH_2×2), 3.28 (s, 2H, CH_2), 2.60 – 2.55 (m, 4H, CH_2×2), 1.19 (d, *J* = 7.2 Hz, 3H, CH_3). ESI-MS: m/z 382.1 $[M-H]^+$.

*4.1.15 methyl 3-(4-(6-bromo-2H-benzo[b][1,4]oxazin-3-yl)piperazin-1-yl)propanoate (**4d**)*

Yield 71.7%, m.p. oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.02 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.93 (dd, *J* = 8.4, 2.4 Hz, 1H, Ar-H), 6.74 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.77 (s, 2H, CH_2), 3.61 (s, 4H, CH_2×2), 3.51 (s, 3H, CH_3), 2.61 (t, *J* = 6.9 Hz, 2H, CH_2), 2.51 (d, *J* = 6.6 Hz, 2H, CH_2), 2.44 – 2.40 (m, 4H, CH_2×2). ESI-MS: m/z 382.1 $[M-H]^+$.

*4.1.16 N-(5-bromo-2-methoxypyridin-3-yl)butane-1-sulfonamide (**6a**)*

To a solution of 5-bromo-2-methoxypyridin-3-amine (2.01 g, 10 mmol) in pyridine (50 mL) at 0 ºC was added methanesulfonyl chloride (1.72 g, 11 mmol). Then the mixture was stirred at 25 ºC for 24 h. Pyridine was removed at reduced pressure and add water (100 mL), extracted with ethyl acetate (3×100 mL), the organic layer was washed with water (50 mL), dried with Na_2SO_4 and evaporated to give compound **6a** as a white solid. Yield 78.4%, m.p. 150-151 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) 9.51 (s, 1H, NH), 8.09 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.77 (d, *J* = 2.4 Hz, 1H, Ar-H), 3.91 (s, 3H, OCH_3), 3.16 (t, *J* = 7.6 Hz, 2H, CH_2), 1.73-1.65 (m, 2H, CH_2), 1.42-1.33 (m, 2H, CH_2), 0.87 (t, *J* = 7.2 Hz, 3H, CH_3). ESI-MS: m/z 384.1 $[M+H]^+$.

Compounds **6b** was synthesized according to the procedure described in **6a**.

*4.1.17 N-(5-bromo-2-methoxypyridin-3-yl)-2,4-difluorobenzenesulfonamide (**6b**)*

Yield 85.6%, m.p. 163-165 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1H, NH), 8.13 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.83-7.74 (m, 2H, Ar-H×2), 7.62-7.52 (m, 1H, Ar-H), 7.24 (td, *J* = 2.0, 8.5 Hz, 1H, Ar-H), 3.62 (s, 3H, OCH_3). ESI-MS: m/z 376.9 $[M-H]^+$.

*4.1.18 2,4-difluoro-N-(2-methoxy-5-(4-(2-morpholinoethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)benzenesulfonamide (**7a**)*

A solution of the *N*-(5-bromo-2-methoxypyridin-3-yl)-2,4-difluorobenzenesulfonamide (0.19 g, 0.5 mmol), bis(pinacolato)diboron (0.13 g, 0.5 mmol), potassium acetate (0.15 g, 1.5 mmol), $Pd(dppf)_2Cl_2$ (18 mg, 0.025 mmol) and anhydrous DMF (30 mL) was reflux under an atmosphere of N_2 atmosphere for 4 h. To the resulted residue was added $Pd(dppf)_2Cl_2$ (0.018 g, 0.025 mmol), 6-bromo-4-(2-morpholinoethyl)-2*H*-benzo[b][1,4]oxazin-3(4*H*)-one (0.14 g, 0.4 mmol), Cs_2CO_3 (0.33 g, 0.56 mmol) and water (0.5 mL). The obtained mixture was refluxed under an atmosphere of N_2 at 90 ºC for another 4 h. DMF was removed at reduced pressure and the residue was purified

through a column chromatography on silica gel with chloroform/methanol (V:V 50:1) as a white solid. Yield 50.5%, m.p. 185-188 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.29 (s, 1H, NH), 8.32 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.89 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.76 (dd, *J* = 8.3, 6.5 Hz, 1H, Ar-H), 7.61-7.55 (m, 1H, Ar-H), 7.44 (d, *J* = 1.7 Hz, 1H, Ar-H), 7.26-7.23 (m, 1H, Ar-H), 7.23-7.19 (m, 1H, Ar-H), 7.10 (d, *J* = 7.5 Hz, 1H, Ar-H), 4.68 (s, 2H, CH_2), 4.15 (t, *J* = 6.7 Hz, 2H, CH_2), 3.64 (s, 3H, OCH_3), 3.53 (t, *J* = 4.2 Hz, 4H, CH_2×2), 2.53 (t, *J* = 6.8 Hz, 2H, CH_2), 2.47 (s, 4H, CH_2×2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.40 (dd, *J* = 253.9, 11.8 Hz), 163.33, 158.77 (dd, *J* = 257.6, 13.5 Hz), 156.63, 144.16, 141.43, 133.43, 131.16 (d, *J* = 10.7 Hz), 130.45, 128.65, 128.47, 124.65 (dd, *J* =15, 4.5 Hz), 120.97, 119.00, 116.64, 113.02, 111.18 (dd, *J* = 22.2, 3.2 Hz), 105.17 (t, *J* = 26.1 Hz), 66.49, 65.55, 54.43, 52.83, 52.70, 37.22. HRMS m/z calc for $C_{26}H_{26}F_2N_4O_6S$ $[M+Na]^+$: 583.1541; found: 583.1433.

Compounds **7b**-**s** were synthesized according to the procedure described in **7a**.

*4.1.19 2,4-difluoro-N-(5-(4-(4-fluorobenzyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)benzenesulfonamide (**7b**)*

Yield 53.2%, m.p. 177-179 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.27 (s, 1H, NH), 8.17 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.79 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.71 (td, *J* = 8.5, 6.2 Hz, 1H, Ar-H), 7.56 (ddd, *J* = 10.4, 9.1, 2.5 Hz, 1H, Ar-H), 7.41 (dd, *J* = 8.6, 5.5 Hz, 2H, Ar-H×2), 7.30 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.22 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 7.20 (dd, *J* = 8.5, 2.4 Hz, 1H, Ar-H), 7.19-7.15 (m, 2H, Ar-H×2), 7.11 (s, 1H, Ar-H), 5.29 (s, 2H, CH_2), 4.84 (s, 2H, CH_2), 3.62 (s, 3H, OCH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.5 (dd, *J* = 252.0, 10.5 Hz), 161.51 (d, *J* = 3.0 Hz), 159.90, 158.76 (dd, *J* = 257.7, 13.4 Hz), 156.59, 144.17, 141.27, 133.39, 132.07 (d, *J* = 2.9 Hz), 131.15 (d, *J* = 10.8 Hz), 130.15, 129.41 (t, *J* = 4.5 Hz), 129.11, 124.46 (dd, *J* = 13.5, 3.0 Hz), 121.09, 118.85, 116.65, 114.97, 114.83, 113.23, 111.17 (dd, *J* = 22.2, 3.1 Hz), 105.17 (t, *J* = 26.0 Hz), 66.50, 52.73, 41.91. HRMS m/z calc for $C_{27}H_20F_3N_3O_5S$ $[M+H]^+$: 556.1076; found: 556.1149.

*4.1.20 2,4-difluoro-N-(2-methoxy-5-(4-(3-morpholino-3-oxopropyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)benzenesulfonamide (**7c**)*

Yield 52.4%, m.p. 154-156 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.28 (s, 1H, NH), 8.35 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.89 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.65-7.55 (m, 1H, Ar-H), 7.43 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.24 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 7.23-7.18 (m, 1H, Ar-H), 7.10 (d, *J* = 8.2 Hz, 1H, Ar-H), 4.67 (s, 2H, CH_2), 4.27-4.21 (m, 2H, CH_2), 3.63 (s, 3H, OCH_3), 3.51 (dt, *J* = 7.9,

5.0 Hz, 4H, CH_2×2), 3.45-3.41 (m, 2H, CH_2), 3.41-3.37 (m, 2H, CH_2), 2.72-2.67 (m, 2H, CH_2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.27, 164.44 (dd, *J* = 253.9, 11.8 Hz), 163.32, 158.77(dd, *J* = 257.6, 13.4 Hz), 156.62, 144.03, 141.74, 133.64, 131.20 (d, *J* = 10.7 Hz), 130.60, 128.68, 128.33, 124.51 (dd, *J* = 14.4, 3.5 Hz), 121.07, 118.75, 116.65, 112.80, 111.19 (dd, *J* = 22.2, 3.2 Hz), 105.18 (t, *J* = 26.1 Hz), 66.45, 65.34, 52.69, 44.68, 40.78, 29.39. HRMS m/z calc for $C_{27}H_{26}F_2N_4O_7S$ $[M+H]^+$: 589.1490; found: 589.1563.

*4.1.21 2,4-difluoro-N-(2-methoxy-5-(3-oxo-4-(pyridin-4-ylmethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)benzenesulfonamide (**7d**)*

Yield 50.4%, m.p. 102-105 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.28 (s, 1H, NH), 8.53 (d, *J* = 5.0 Hz, 2H, Ar-H×2), 8.14 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.78 (d, *J* = 3.1 Hz, 1H, Ar-H), 7.73-7.67 (m, 1H, Ar-H), 7.60-7.53 (m, 1H, Ar-H), 7.34 (d, *J* = 6.3 Hz, 2H, Ar-H×2), 7.27-7.22 (m, 1H, Ar-H), 7.22-7.16 (m, 2H, Ar-H×2), 7.14 (d, *J* = 8.3 Hz, 1H, Ar-H), 5.34 (s, 2H, CH_2), 4.88 (s, 2H, CH_2), 3.61 (s, 3H, OCH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.44 (dd, *J* = 253.9, 11.8 Hz), 163.89, 158.75 (dd, *J* = 257.7, 13.5 Hz), 156.61, 149.27, 145.07, 144.09, 141.31, 133.36, 131.14 (d, *J* = 10.8 Hz), 130.27, 128.37, 128.19, 124.47 (dd, *J* = 14.4, 3.5 Hz), 121.27, 121.22, 118.85, 116.75, 112.97, 111.16 (dd, *J* = 22.1, 3.2 Hz), 105.16 (t, *J* = 26.1 Hz), 66.48, 52.71, 42.05. HRMS m/z calc for $C_{26}H_{20}F_2N_4O_5S$ $[M+H]^+$: 539.1122; found: 539.1195.

*4.1.22 2,4-difluoro-N-(2-methoxy-5-(3-oxo-4-(3-(2-oxopyridin-1(2H)-yl)propyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)benzenesulfonamide (**7e**)*

Yield 53.1%, m.p. 83-85 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.30 (s, 1H, NH), 8.31 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.86 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.73 (dd, *J* = 6.7, 2.0 Hz, 2H, Ar-H×2), 7.57 (s, 1H, Ar-H), 7.38 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 7.20 (s, 2H, Ar-H×2), 7.10 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.37 (d, *J* = 9.1 Hz, 1H, Ar-H), 6.22-6.19 (m, 1H, Ar-H), 4.68 (s, 2H, CH_2), 4.07-3.96 (m, 4H, CH_2×2), 3.64 (s, 3H, OCH_3), 1.99 (s, 2H, CH_2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.42 (dd, *J* = 254.0, 11.8 Hz), 163.31, 160.82, 158.78 (dd, *J* = 257.5, 13.5 Hz), 156.70, 144.09, 141.74, 139.30, 138.42, 133.76, 131.19 (d, *J* = 10.8 Hz), 130.56, 128.62, 128.12, 124.56 (dd, *J* = 14.4, 3.5 Hz), 121.10, 119.01, 118.76, 116.74, 112.37, 111.17 (dd, *J* = 22.1, 3.1 Hz), 105.17 (t, *J* = 25.5 Hz), 104.72, 72.90, 66.37, 52.70, 45.84, 24.36. HRMS m/z calc for $C_{28}H_{24}F_2N_4O_6S$ $[M+H]^+$: 583.1385; found: 583.1457.

4.1.23 2,4-difluoro-N-(2-methoxy-5-(4-(3-morpholinopropyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]o

*xazin-6-yl)pyridin-3-yl)benzenesulfonamide (**7f**)*

Yield 54.6%, m.p. 151-153 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.28 (s, 1H, NH), 8.29 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.85 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.76 (dd, *J* = 8.4, 6.4 Hz, 1H, Ar-H), 7.63-7.50 (m, 1H, Ar-H), 7.39 (d, *J* = 1.3 Hz, 1H, Ar-H), 7.24-7.21 (m, 1H, Ar-H), 7.21-7.18 (m, 1H, Ar-H), 7.10 (d, *J* = 8.2 Hz, 1H, Ar-H), 4.67 (s, 2H, CH_2), 4.05 (t, *J* = 7.1 Hz, 2H, CH_2), 3.64 (s, 3H, OCH_3), 3.49 (s, 4H, CH_2×2), 2.36 (d, *J* = 6.8 Hz, 2H, CH_2), 2.33 (d, *J* = 11.5 Hz, 4H, CH_2×2), 1.79-1.73 (m, 2H, CH_2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.36 (dd, *J* = 253.7, 11.7 Hz), 163.22, 158.76 (dd, *J* = 257.5, 13.4 Hz), 156.58, 144.09, 141.21, 133.12, 131.17 (d, *J* = 10.7 Hz), 130.62, 128.74, 128.46, 124.72 (dd, *J* = 14.5, 3.3 Hz), 120.99, 119.33, 116.63, 112.81, 111.13 (dd, *J* = 22.1, 3.2 Hz), 105.15 (t, *J* = 26.1 Hz), 66.46, 65.39, 54.67, 52.68, 52.62, 37.93, 22.93. HRMS m/z calc for $C_{27}H_{28}F_2N_4O_6S$ $[M+H]^+$: 575.1698; found: 575.1770.

*4.1.24 N-(5-(4-(3-(diethylamino)propyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)butane-1-sulfonamide (**7g**)*

Yield 49.5%, m.p. 68-70 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.33 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.87 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.44 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.26 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 7.12 (d, *J* = 8.3 Hz, 1H, Ar-H), 4.70 (s, 2H, CH_2), 4.08 (t, *J* = 7.3 Hz, 2H, CH_2), 3.96 (s, 3H, OCH_3), 3.15-3.08 (m, 2H, CH_2), 3.02-2.89 (m, 4H, CH_2×2), 2.85 (s, 2H, CH_2), 1.88 (d, *J* = 10.3 Hz, 2H, CH_2), 1.77-1.69 (m, 2H, CH_2), 1.39 (h, *J* = 7.4 Hz, 2H, CH_2), 1.09 (t, *J* = 6.8 Hz, 6H, CH_3×2), 0.88 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.46, 155.54, 144.06, 140.21, 131.06, 130.99, 128.74, 128.12, 121.26, 120.44, 116.70, 112.77, 66.43, 53.11, 51.43, 48.03, 45.61, 37.33, 25.73, 24.58, 20.48, 20.21, 12.93. HRMS m/z calc for $C_{25}H_{36}N_4O_5S$ $[M+H]^+$: 505.2406; found: 505.2479.

*4.1.25 N-(2-methoxy-5-(3-oxo-4-(pyridin-4-ylmethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)butane-1-sulfonamide (**7h**)*

Yield 48.5%, m.p. 65-68 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.35 (s, 1H, NH), 8.64 (d, *J* = 6.1 Hz, 2H, Ar-H×2), 8.14 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.72 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.55 (d, *J* = 5.4 Hz, 2H, Ar-H×2), 7.26 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 7.17-7.13 (m, 2H, Ar-H×2), 5.41 (s, 2H, CH_2), 4.89 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3), 3.62 (t, *J* = 6.6 Hz, 2H, CH_2), 1.72-1.66 (m, 2H, CH_2), 1.37 (q, *J* = 7.4 Hz, 2H, CH_2), 0.86 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.00, 155.36, 144.01, 139.71, 130.73, 130.32, 128.39, 128.11, 122.15, 121.44, 120.53, 117.24, 116.75,

115.26, 113.00, 66.49, 58.94, 53.08, 45.01, 20.18, 12.92, 7.94. HRMS m/z calc for $C_{24}H_{26}N_4O_5S$ $[M+H]^+$: 483.1624; found: 483.1679.

*4.1.26 N-(5-(4-(3-(1H-pyrazol-1-yl)propyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)butane-1-sulfonamide (**7i**)*

Yield 51.1%, m.p. 63-65 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.40 (s, 1H, NH), 8.29 (d, J = 2.3 Hz, 1H, Ar-H), 7.83 (d, J = 2.2 Hz, 1H, Ar-H), 7.76 (d, J = 1.9 Hz, 1H, Ar-H), 7.45 (d, J = 1.3 Hz, 1H, Ar-H), 7.26 (d, J = 1.7 Hz, 1H, Ar-H), 7.24 (d, J = 1.9 Hz, 1H, Ar-H), 7.10 (d, J = 8.2 Hz, 1H, Ar-H), 6.23 (t, J = 2.0 Hz, 1H, Ar-H), 4.68 (s, 2H, CH_2), 4.23 (t, J = 6.6 Hz, 2H, CH_2), 3.97 (s, 3H, OCH_3), 3.97-3.93 (m, 2H, CH_2), 3.16-3.09 (m, 2H, CH_2), 2.13 (p, J = 6.8 Hz, 2H, CH_2), 1.73 (p, J = 7.7 Hz, 2H, CH_2), 1.39 (q, J = 7.5 Hz, 2H, CH_2), 0.88 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.40, 156.70, 145.04, 141.34, 139.26, 132.22, 132.00, 130.56, 129.73, 129.22, 122.20, 121.45, 117.74, 113.51, 105.46, 67.46, 54.18, 52.55, 49.01, 38.45, 27.68, 25.66, 21.28, 14.00. HRMS m/z calc for $C_{24}H_{29}N_5O_5S$ $[M+H]^+$: 500.1889; found: 500.1902.

*4.1.27 N-(5-(4-(3-(dimethylamino)propyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)butane-1-sulfonamide (**7j**)*

Yield 50.5%, m.p. 79-85 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.34 (d, J = 2.1 Hz, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 7.44 (s, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 4.69 (s, 2H, CH_2), 4.07 (s, 2H, CH_2), 3.96 (s, 3H, OCH_3), 3.14-3.10 (m, 2H, CH_2), 2.89 (s, 6H, CH_3×2), 1.94 (s, 2H, CH_2), 1.91 (s, 2H, CH_2), 1.72 (p, J = 7.7 Hz, 2H, CH_2), 1.40 (t, J = 7.4 Hz, 2H, CH_2), 0.88 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.41, 155.51, 143.98, 140.17, 130.96, 128.71, 128.16, 125.78, 121.20, 120.43, 116.67, 112.73, 66.39, 54.22, 53.09, 51.42, 42.51, 37.36, 25.73, 24.58, 20.20, 12.93. HRMS m/z calc for $C_{23}H_{32}N_4O_5S$ $[M+H]^+$: 477.2093; found: 477.2166.

*4.1.28 N-(2-methoxy-5-(4-(3-morpholinopropyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)butane-1-sulfonamide (**7k**)*

Yield 50.6%, m.p. 54-58 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.38 (s, 1H, NH), 8.29 (d, J = 2.3 Hz, 1H, Ar-H), 7.84 (d, J = 2.3 Hz, 1H, Ar-H), 7.40 (d, J = 1.9 Hz, 1H, Ar-H), 7.23 (dd, J = 8.3, 1.9 Hz, 1H, Ar-H), 7.10 (d, J = 8.3 Hz, 1H, Ar-H), 4.67 (s, 2H, CH_2), 4.04 (t, J = 7.2 Hz, 2H, CH_2), 3.96 (s, 3H, OCH_3), 3.50-3.46 (m, 4H, CH_2×2), 3.13-3.10 (m, 2H, CH_2), 2.33 (d, J = 6.8 Hz, 2H, CH_2), 2.32 (s, 4H, CH_2×2), 1.76 (d, J = 7.2 Hz, 2H, CH_2), 1.72 (s, 2H, CH_2), 1.40-1.36 (m, 2H, CH_2), 0.87 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.20, 155.40, 144.04, 140.02, 130.91,

130.72, 127.79, 126.47, 121.07, 120.48, 116.59, 112.90, 66.45, 65.44, 54.70, 53.09, 52.66, 51.37, 37.98, 24.56, 22.96, 20.19, 12.91. HRMS m/z calc for $C_{25}H_{34}N_4O_6S$ $[M+H]^+$: 519.2199; found: 519.2272.

*4.1.29 N-(2-methoxy-5-(4-(2-morpholinoethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)butane-1-sulfonamide (**7l**)*

Yield 50.6%, m.p. 115-118 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.40 (s, 1H, NH), 8.38 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.90 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.44 (d, *J* = 1.8 Hz, 1H, Ar-H), 7.29 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.14 (d, *J* = 8.3 Hz, 1H, Ar-H), 4.73 (s, 2H, CH_2), 4.44 (t, *J* = 6.7 Hz, 2H, CH_2), 3.96 (s, 3H, OCH_3), 3.67 (s, 4H, CH_2×2), 3.43 (t, *J* = 6.7 Hz, 2H, CH_2), 3.22-3.10 (m, 4H, CH_2×2), 1.77-1.70 (m, 2H, CH_2), 1.41-1.37 (m, 2H, CH_2), 1.27-1.16 (m, 2H, CH_2), 0.88 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.25, 162.76, 156.67, 145.00, 141.54, 132.31, 132.10, 129.72, 128.57, 121.44, 117.90, 113.68, 67.40, 63.80, 54.15, 52.88, 52.53, 51.84, 46.09, 25.65, 21.29, 14.00. HRMS m/z calc for $C_{24}H_{32}N_4O_6S$ $[M+Na]^+$: 527.2043; found: 527.1935.

*4.1.30 N-(5-(4-(3-(1H-imidazol-1-yl)propyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)butane-1-sulfonamide (**7m**)*

Yield 49.2%, m.p. 69-71 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.43 (s, 1H, NH), 8.30 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.83 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.27 (d, *J* = 8.3 Hz, 2H, Ar-H×2), 7.24 (dd, *J* = 8.1, 2.0 Hz, 1H, Ar-H), 7.11 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.93 (s, 1H, Ar-H), 4.68 (s, 2H, CH_2), 4.09 (t, *J* = 7.0 Hz, 2H, CH_2), 3.96 (s, 3H, OCH_3), 3.37 (q, *J* = 7.2 Hz, 2H, CH_2), 3.16-3.10 (m, 2H, CH_2), 2.06 (p, *J* = 7.1 Hz, 2H, CH_2), 1.73 (p, *J* = 7.8 Hz, 2H, CH_2), 1.39 (q, *J* = 7.5 Hz, 2H, CH_2), 0.88 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.48, 156.58, 145.09, 141.22, 137.69, 132.03, 131.98, 129.74, 129.17, 128.55, 122.27, 121.52, 119.80, 117.77, 113.61, 67.47, 54.17, 52.51, 52.45, 44.27, 38.21, 25.66, 21.28, 14.00. HRMS m/z calc for $C_{24}H_{29}N_5O_5S$ $[M+Na]^+$: 522.1889; found: 522.1815.

*4.1.31 N-(2-methoxy-5-(4-(3-morpholino-3-oxopropyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)butane-1-sulfonamide (**7n**)*

Yield 51.1%, m.p. 56-59 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.36 (s, 1H, NH), 8.33 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.86 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.43 (d, *J* = 1.8 Hz, 1H, Ar-H), 7.25 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.11 (d, *J* = 8.3 Hz, 1H, Ar-H), 4.67 (s, 2H, CH_2), 4.22 (t, *J* = 7.5 Hz, 2H, CH_2), 3.95 (s, 3H, OCH_3), 3.50 (t, *J* = 8.4 Hz, 4H, CH_2×2), 3.44-3.40 (m, 2H, CH_2), 3.40-3.36 (m, 2H, CH_2),

3.15-3.10 (m, 2H, CH_2), 2.69 (t, *J* = 7.5 Hz, 2H, CH_2), 1.72 (d, *J* = 7.6 Hz, 2H), 1.44-1.34 (m, 2H), 0.88 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.31, 164.39, 156.56, 145.04, 141.26, 132.06, 131.97, 129.84, 129.35, 122.23, 121.50, 117.69, 113.95, 67.53, 66.41, 54.16, 52.51, 45.75, 41.84, 30.44, 25.66, 21.28, 14.00. HRMS m/z calc for $C_{25}H_{32}N_4O_7S$ $[M+Na]^+$: 555.1992; found: 555.1884.

*4.1.32 N-(2-methoxy-5-(3-oxo-4-(3-(2-oxopyridin-1(2H)-yl)propyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)butane-1-sulfonamide (**7o**)*

Yield 50.6%, m.p. 80-83 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.39 (s, 1H, NH), 8.30 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.84 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.72 (dd, *J* = 6.9, 2.0 Hz, 1H,Ar-H), 7.42-7.36 (m, 1H, Ar-H), 7.30 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.24 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 7.11 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.36 (ddd, *J* = 9.1, 1.4, 0.6 Hz, 1H, Ar-H), 6.20 (td, *J* = 6.7, 1.4 Hz, 1H, Ar-H), 4.68 (s, 2H, CH_2), 4.03 (t, *J* = 7.5 Hz, 2H, CH_2), 3.98 (s, 2H, CH_2), 3.97 (s, 3H, OCH_3), 3.16-3.09 (m, 2H, CH_2), 2.00 (d, *J* = 7.3 Hz, 2H, CH_2), 1.72 (tt, *J* = 8.7, 6.9 Hz, 2H, CH_2), 1.42-1.35 (m, 2H, CH_2), 0.87 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.39, 161.88, 156.60, 145.10, 141.22, 140.38, 139.49, 132.03, 129.78, 129.15, 122.24, 121.49, 120.07, 117.77, 113.54, 105.79, 67.45, 54.17, 52.52, 46.91, 38.19, 26.89, 25.66, 21.28, 14.00. HRMS m/z calc for $C_{26}H_{30}N_4O_6S$ $[M+H]^+$: 527.1886; found: 527.1959.

4.1.33

*2,4-difluoro-N-(5-(3-(4-(2-hydroxyethyl)piperazin-1-yl)-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)benzenesulfonamide (**7p**)*

Yield 54.3%, oil. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.22 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.76 (d, *J* = 6.5 Hz, 1H, Ar-H), 7.75 (d, *J* = 6.0 Hz, 1H, Ar-H), 7.58 – 7.53 (m, 1H, Ar-H), 7.21 (td, *J* = 8.5, 2.2 Hz, 1H), 7.09 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.05 (dd, *J* = 8.2, 2.2 Hz, 1H, Ar-H), 6.89 (d, *J* = 8.1 Hz, 1H, Ar-H), 4.80 (s, 2H, CH_2), 4.03 (t, *J* = 7.1 Hz, 1H, OH), 3.65 (s, 3H, CH_3), 3.56 (t, *J* = 6.0 Hz, 4H, CH_2×2), 2.55 – 2.53 (m, 4H, CH_2×2), 2.49 (t, *J* = 6.1 Hz, 2H, CH_2), 2.28 (s, 2H, CH_2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.43 (dd, *J* = 253.7, 11.9 Hz), 162.77, 159.79 (dd, *J* = 257.5, 13.3 Hz), 157.21, 155.75, 144.73, 141.54, 136.21, 133.46, 132.27 (d, *J* = 10.5 Hz), 130.74, 130.24, 125.68 (dd, *J* = 15.0, 3.0 Hz), 121.53, 121.03, 120.30, 115.67, 112.24 (d, *J* = 20.1 Hz), 106.21 (t, *J* = 26.0 Hz), 60.65, 60.44, 58.70, 53.72 (2C), 53.16 (2C). HRMS m/z calc for $C_{26}H_{28}F_2N_5O_5S$ $[M+H]^+$: 560.1774; found: 560.1748.

4.1.33

*2,4-difluoro-N-(5-(3-(4-(3-hydroxypropyl)piperazin-1-yl)-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)benzenesulfonamide (**7q**)*

Yield 56.4%, oil. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.22 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.76 (d, *J* = 10.9 Hz, 1H, Ar-H), 7.73 (d, *J* = 10.1 Hz, 1H, Ar-H), 7.58 – 7.54 (m, 1H, Ar-H), 7.20 (td, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 7.09 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.04 (dd, *J* = 8.2, 2.3 Hz, 1H, Ar-H), 6.88 (d, *J* = 8.1 Hz, 1H, Ar-H), 4.80 (s, 2H, CH_2), 4.31 (t, *J* = 4.8 Hz, 4H, CH_2×2), 3.64 (s, 3H, CH_3), 3.56 (s, 2H, CH_2), 3.39 (d, *J* = 6.5 Hz, 1H, OH), 2.42 (brs, 2H, CH_2), 2.47 – 2.35 (m, 4H, CH_2×2), 1.64 – 1.61 (m, 2H, CH_2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.47(dd, *J* = 252.3, 12.0 Hz), 163.51, 159.19(dd, *J* = 242.0, 11.9 Hz), 157.23, 155.74, 144.75, 141.71, 136.20, 133.62, 132.28(d, *J* = 10.5 Hz), 130.71, 130.24, 125.59 (d, *J* = 15.0 Hz), 121.54, 121.06, 120.10, 115.68, 112.27 (d, *J* = 24.2 Hz), 106.24 (t, *J* = 25.5 Hz), 67.65, 60.66, 59.58, 55.34, 53.74, 52.84 (2C), 43.85(2C). HRMS m/z calc for $C_{27}H_{30}F_2N_5O_5S$ $[M+H]^+$: 574.1930; found: 574.1907.

4.1.33 ethyl

*2-(4-(6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)-2H-benzo[b][1,4]oxazin-3-yl)piperazin-1-yl)acetate (**7r**)*

Yield 48.2%, oil. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.25 (s, 1H, NH), 8.24 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.77 (d, *J* = 5.8 Hz, 1H, Ar-H), 7.76 (d, *J* = 6.6 Hz, 1H, Ar-H), 7.61 – 7.54 (m, 1H, Ar-H), 7.21 (td, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 7.10 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.07 – 7.04 (m, 1H, Ar-H), 6.89 (d, *J* = 8.1 Hz, 1H, Ar-H), 4.10 (q, *J* = 7.1 Hz, 2H, CH_2), 3.65 (s, 3H, CH_3), 3.56 (s, 4H, CH_2×2), 3.35 (s, 2H, CH_2), 3.30 (s, 2H, CH_2),2.62 – 2.58 (m, 4H, CH_2×2), 1.20 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.27, 165.50 (dd, *J* = 254.2, 11.7 Hz), 159.80 (dd, *J* = 257.8, 13.3 Hz), 157.24, 155.75, 144.75, 141.93, 136.17, 133.81, 132.30 (d, *J* = 10.5 Hz), 130.67, 130.26, 125.48 (dd, *J* = 14.0, 2.7 Hz), 121.53, 121.07, 119.83, 115.68, 112.28 (d, *J* = 21.8 Hz), 106.24 (t, *J* = 26.2 Hz), 60.66, 60.35, 58.61, 53.74 (2C), 52.00, 44.02(2C), 14.58. HRMS m/z calc for $C_{28}H_{30}F_2N_5O_6S$ $[M+H]^+$: 602.1807; found: 602.1854.

4.1.33 methyl

*3-(4-(6-(5-(2,4-difluorophenylsulfonamido)-6-methoxypyridin-3-yl)-2H-benzo[b][1,4]oxazin-3-yl)piperazin-1-yl)propanoate (**7s**)*

Yield 48.2%, oil. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.22 (s, 1H, NH), 8.23 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.77 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.76 – 7.73 (m, 1H, Ar-H), 7.57 (t, *J* = 8.6 Hz, 1H, Ar-H), 7.21 (td, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 7.09 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.05 (dd, *J* = 8.2, 2.3 Hz, 1H, Ar-H), 6.88 (d, *J* = 8.1 Hz, 1H, Ar-H), 4.80 (s, 2H, CH_2), 3.94 (s, 2H, CH_2), 3.65 (s, 3H, CH_3), 3.61 (s, 3H, CH_3), 3.53 (s, 4H, CH_2×2), 2.63 (t, *J* = 7.1 Hz, 2H, CH_2), 2.47 – 2.44 (m, 4H, CH_2×2). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.77, 165.48 (dd, *J* = 254.0, 11.9 Hz), 159.80 (dd, *J* = 257.5, 13.4 Hz), 157.23, 155.71, 144.74, 141.82, 136.22, 133.72, 132.29 (d, *J* = 11.1 Hz), 130.68, 130.26, 125.54 (dd, *J* = 15.0, 3.0 Hz), 121.54, 121.03, 119.96, 115.66, 112.28 (d, *J* = 22.1 Hz), 106.23 (t, *J* = 26.2 Hz), 60.65 (2C), 53.73, 53.48, 52.58, 51.77 (2C), 43.92 (2C), 31.88. HRMS m/z calc for $C_{28}H_{30}F_2N_5O_6S$ $[M+H]^+$: 602.1807; found: 602.1819.

4.2 Biological assay methods

4.2.1 Cell culture

Cancer cells were grown in DMEM (SNU-638) or RPMI 1640 (HCT-116 and MDA-MB-231 cells) supplemented with 10% FBS and antibiotics-antimycotics (PSF; 100 units/mL penicillin G sodium, 100 μg/mL streptomycin, and 250 ng/mL amphotericin B) in a humidified incubator containing 5% CO_2 at 37 °C.

4.2.2 Antiproliferative activity

The cell viability was evaluated using the sulforhodamine B (SRB) cellular protein-staining method with minor modifications. Briefly, cells were treated with various concentrations of compounds in 96-well plates and incubated at 37 ºC in a humidified atmosphere with 5% CO_2 for 72 h. After treatment, the cells were fixed with 10% TCA solution, and cell viability was determined with a sulforhodamine B (SRB) assay. The percentage of cell-growth inhibition was calculated using the formulae below. The IC_{50} values were calculated using a non-linear regression analysis (percent growth versus concentration).

4.2.3 PI3K enzymatic activity assay

The PI3K kinase assay was determined by PI3K-GloTM Class I Profiling Kit (Promega, #V1690) measuring the amount of ADP produced during the kinase reaction. Transfer 1 μL of the test compound serial dilutions to the 384 well low volume assay plate. Dilute Lipid Kinase Enzyme ((PI3K (p110α/p85α), PI3K (p110β/p85α), PI3K (p120γ), PI3K (p110δ/p85α)) into prepared PI3K Reaction Buffer/Lipid Substrate (combining equal volumes of 2.5X PI3K Reaction buffer with 2.5X

Lipid Substrate working solution).Transfer 8 μL of prepared working kinase solution to the wells containing 1 μL of test compound. Incubate at room temperature for 15 minutes to allow inhibitor binding to kinase. Start reaction by adding 1 μL of 250 μM ATP. After incubation at room temperature for 1 h, 10 μL of ADP-GloTM reagent was added per well. The plates were incubated at room temperature for 40 minutes and then 20uL of kinase detection reagent was added. After incubation at room temperature for 40 minutes, the luminescence was recorded using Bio-Tek SYENRGY2. The IC_{50} values were calculated using nonlinear regression with normalized dose-response fitting using Prism software (GraphPad Software, San Diego, CA, USA).

4.2.4 Western blot assay

Total cell lysates were prepared in RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM 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 ImageQuant LAS 4000 biomolecular imager (GE Healthcare).

4.2.5 Molecular docking studies

The crystal structure of PI3K (PDB entry code: 3L08) in complex with Omipalisib was used for molecular modeling. The molecular docking procedure was performed by using C-DOCKER protocol within Accelrys Discovery Studio Visualizer 4.0. For enzyme preparation, the hydrogen atoms were added. The whole PI3K enzyme was defined as a receptor and the site sphere was selected on the basis of the ligand binding location of Omipalisib. Compound Omipalisib was removed and compound **7f** was placed. Accelrys Discovery Studio Visualizer 4.0 was used for graphic display.

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Highlights

- Two series of novel 2*H*-benzo[b][1,4]oxazin derivatives were synthesized and characterized.
- Their antiproliferative activities against cancer cell lines were evaluated.
- **7f** exhibited PI3Kα inhibitory activity with an IC_{50} value of 26.7 nM.
- **7f** exhibited potent antiproliferative effects via PI3K/Akt signaling pathway inhibition.