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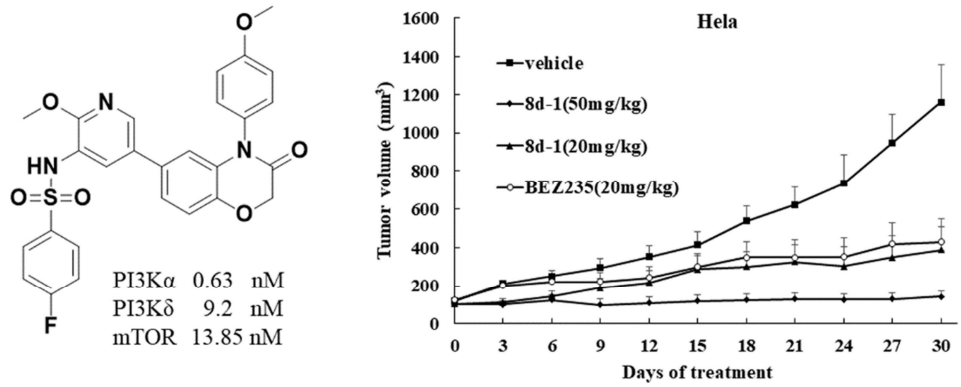

Discovery of 4-phenyl-2H-benzo[*b*][1,4]oxazin-3(4H)-one derivatives as potent and orally active PI3K/mTOR dual inhibitors

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Notes

The authors declare no competing interest.

ABSTRACT

PI3K/Akt/mTOR signaling pathway plays an important role in cancer cell growth and survival. In this study, a new class of molecules with skeleton of 4-phenyl-2H-benzo[*b*][1,4]oxazin-3(4H)-one were designed and synthesized targeting this pathway. Bioassays showed that, among all the molecules, **8d-1** was a pan-class I PI3K/mTOR inhibitor with an IC_{50} of 0.63nM against PI3Kα. In a wide panel of protein kinases assays, no off-target interactions of **8d-1** were identified. **8d-1** was orally available, and displayed favorable pharmacokinetic parameters in mice (oral bioavailability of 24.1%). In addition, **8d-1** demonstrated significant efficiency in Hela/A549 tumor xenograft models (TGI of 87.7% at dose of 50mg/kg in Hela model) without causing significant weight loss and toxicity during 30 days treatment. Based on the bioassays, compound **8d-1** could be used as an anti-cancer drug candidate.

Keywords

PI3K/mTOR; Structural optimization; Dual inhibitor; Cancer treatment

1. INTRODUCTION

PI3K/AKT/mTOR pathway is among the most frequently dysregulated pathways in many human cancers [1-4]. PI3Ks (Phosphatidylinositol 3-kinases) can be subdivided into four classes according to their sequence, homology, and substrate preferences: Class I, II and III are lipid kinases while class IV are Ser/Thr protein kinases [5]. Class IA PI3Ks (PI3Kα, PI3Kβ, and PI3Kδ)

are heterodimers consisting of a p110 catalytic subunit (p110α, p110β, and p110δ) and a regulatory subunit, while Class IB subtype (PI3Kγ) is comprised of a catalytic p110γ and a regulatory p101 subunit [6]. Class I PI3Ks catalyze the phosphorylation of phosphatidylinositol diphosphate (PIP2) to generate its corresponding triphosphate (PIP3), which activate the downstream serine-threonine kinase Akt. Subsequently, the phosphorylation of AKT (pAKT) leads to the activation of the mammalian target of rapamycin (mTOR), a member of the phosphatidylinositol like kinase (PIKK) family with a high degree of active site similarity with PI3Ks, which in turn promotes increased protein synthesis and cell growth. Many molecules targeting this pathway have been reported, compared with individually targeting PI3K or mTOR, dual inhibition of PI3K and mTOR has been proposed to represent a more effective approach for cancer therapy [7]. Several molecules with different scaffolds demonstrated dual inhibition targeting PI3K/mTOR are shown in Figure 1 [8-24].

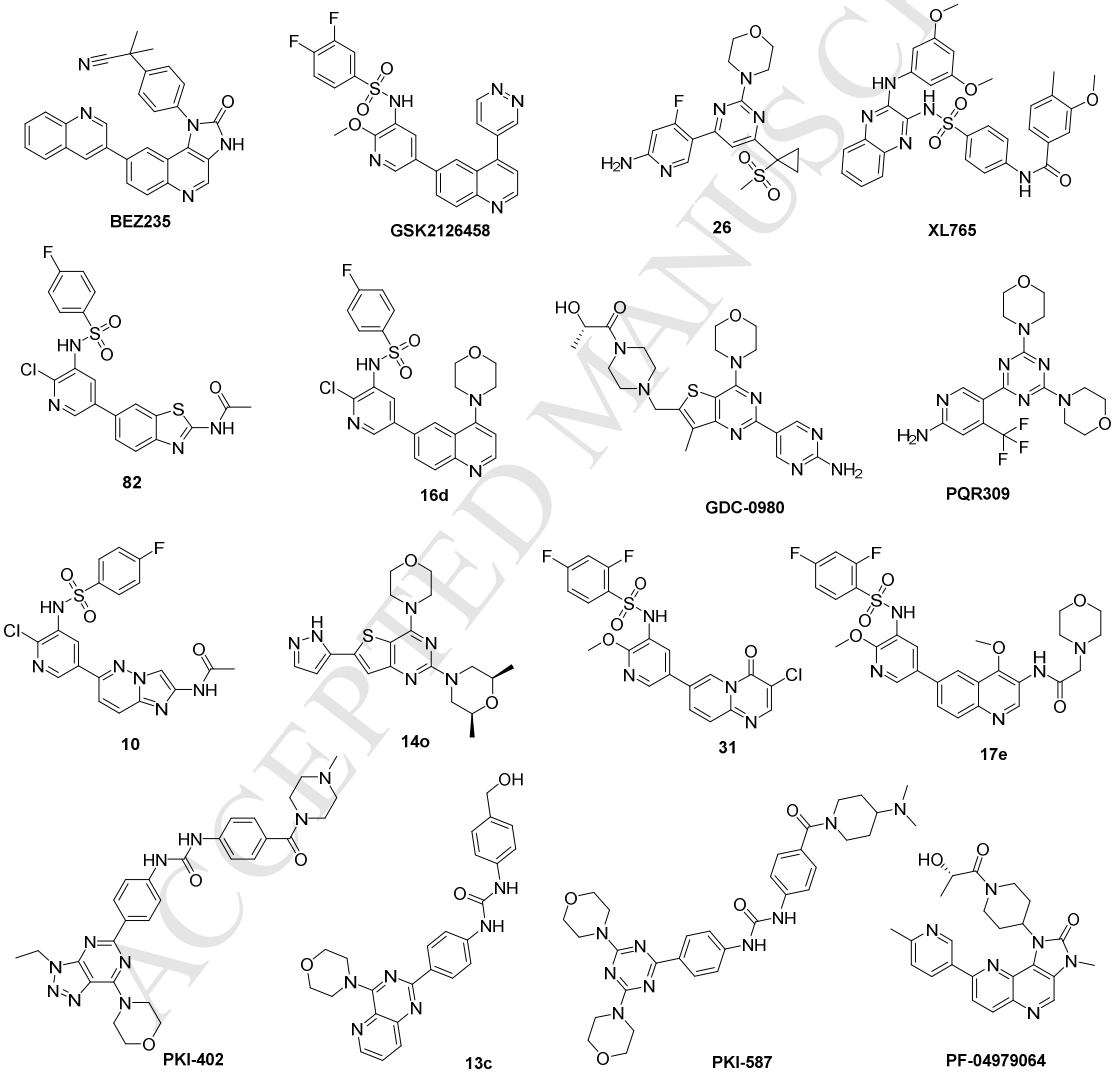
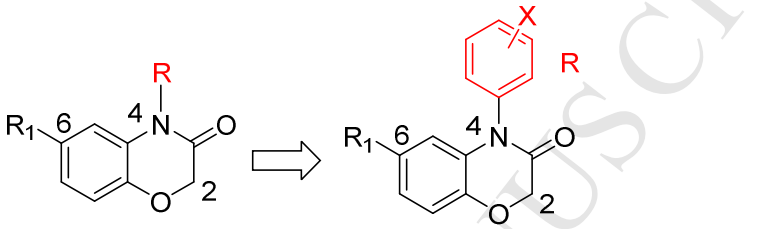


Figure 1. Examples of PI3K/mTOR dual inhibitors.

Among the mentioned molecules, many scaffolds have been employed as core structure, such as quinoline in BEZ235, GSK2126458, 16d and 17e; other structures such as imidazo[1,2-*b*]pyridazine, thieno[3,2-*d*]pyrimidine, 4H-pyrido[1,2-*a*]pyrimidin-4-one,

1,3,5-triazine, 3H-[1,2,3]triazolo[4,5-*d*]pyrimidine, pyrido[3,2-*d*]pyrimidine, and 1,3-dihydro-2H-imidazo[4,5-*c*][1,5]naphthyridin-2-one were also frequently used in PI3K/mTOR dual inhibitors design. As can be concluded, many of the core structures are planar structure, to improve the molecular solubility and get potent inhibitors, here we report a series of 2H-benzo[*b*][1,4]oxazin-3(4H)-one derivatives as PI3K/mTOR dual inhibitors. 2H-benzo[*b*][1,4]oxazin-3(4H)-one demonstrates extensive biological and pharmacological activity, such as antimicrobial, anticancer and antithrombotic activities (Figure S1) [25-28], it was used as core structure in PI3K/mTOR dual inhibitors design; non-planar structure of 2H-benzo[*b*][1,4]oxazin-3(4H)-one may lead to the improvement of molecular solubility and potent inhibition. In this core structure, N-4 and C-6 position are attractive positions for investigation, different fragments were introduced to the two positions for SAR discussion to get potent inhibitors (Figure 2).



R= H, Benzene, Substituted benzenes, Cyclohexyl, Isopropyl, Cyclopropyl
R_1 = Sulfonamide fragments, Quinoline
X = Methyl, Methoxy, Fluorine, Chlorine, H

Figure 2. Design and optimization of molecules based on known PI3K/mTOR inhibitors.

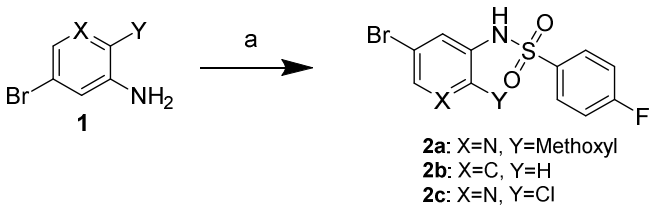
On the C-6 position, sulfonamide fragments were employed for its remarkable potency *in vitro* and *in vivo*. Quinoline is another frequently used moiety in PI3K/mTOR inhibitor design, such as BEZ235, which is a well learned PI3K/mTOR inhibitor [19, 29], quinoline was placed on C-6 position to get better inhibitors. On the N-4 position, chain alkanes have been introduced to get PI3Kα inhibitors [30], based on the activity of reported molecules in Figure 1, we supposed that aromatic rings such as benzene or substituted benzene may be more beneficial, meanwhile, saturated alkanes/cyclanes such as cyclohexyl, isopropyl and cyclopropyl were also introduced for SAR discussion. 2H-benzo[*b*][1,4]oxazin-3(4H)-one derivatives were synthesized and the bioactivities were assayed *in vitro* and *in vivo*. Molecules with scaffold of 4-phenyl-2H-benzo[*b*][1,4]oxazin-3(4H)-one showed advantages over other compounds, and among the molecules, **8d-1** with potent anticancer activity was obtained.

2. RESULTS AND DISCUSSION

2.1 Synthetic Chemistry

The chemistry efforts to obtain **8d-1** involved the preparation of a series of analogues, all of synthetic routes are outlined in the following schemes.

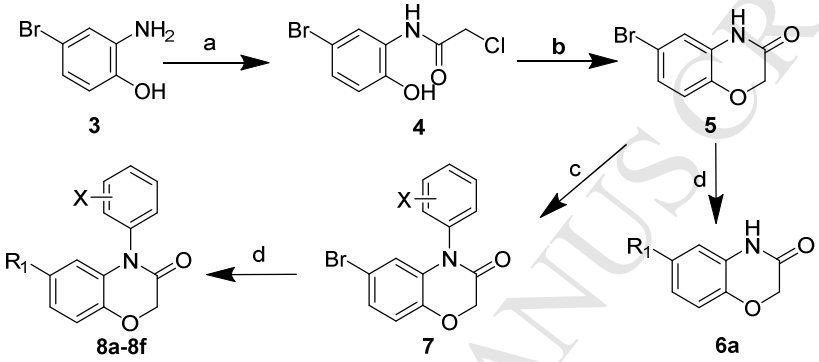
Scheme 1. Synthesis of sulfonamide fragments **2a-2c**



(a) 4-fluorobenzenesulfonyl chloride, DMAP, pyridine, 0□;

Scheme 1 depicted the synthesis of sulfonamide fragment. 4- fluoro sulfonyl chloride and **1** were stirred in pyridine overnight with DMAP as catalyst to afford intermediate molecules **2a-2c**.

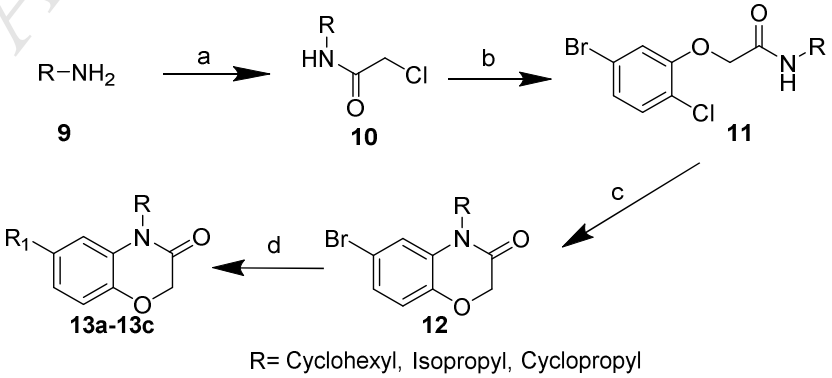
Scheme 2. Synthesis of **6** and **8** series.



(a) Chloroacetyl chloride, $NaHCO_3$, THF,0-4℃; (b) K_2CO_3, THF, ref; (c) $Cu(OAc)_2$, Et_3N, THF, 60℃; (d):Suzuki coupling, boronic acid/ester, $PdCl_2$(dppf), KOAC, dioanxe, 80℃.

In the construction of core structure, firstly, amide condensation reaction was conducted to afford intermediate **4**, subsequently, intramolecular ring forming reaction was conducted to afford 2H-benzo[*b*][1,4]oxazin-3(4H)-one (compound **5**) in 70% yield. Furthermore, **5** was reacted with substituted benzoic boric acids in THF solutions, copper acetate was employed as catalytic agent, Et_3N and 4 Å molecular sieve were added and heated over 60℃ for 5h, the intermediate of N-substituted benzoxazine was obtained (compound **7**), the yield was about 50%. The target compounds (**6a, 8a-8f**) were obtained by the coupling reaction of compound **5**/**7** and intermediate molecules **2a-2c**, 3-bromoquinoline or 4-bromophenol.

Scheme 3. Synthesis of N-4-alkane substituted derivatives



(a) Chloroacetyl chloride, K_2CO_3, CH_3CN, 0□-r.t.; (b) 5-bromo-2-chlorophenol, K_2CO_3, CH_3CN, reflux; (c) Cs_2CO_3, DMF, 150□, microwave; (d) Suzuki coupling, boronic acid/ester, $PdCl_2$(dppf), KOAC, dioanxe, 80□.

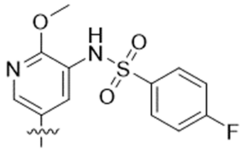
In the construction of core structure, scheme 3 can be employed when the R is cyclohexyl, isopropyl and cyclopropyl. Beginning with alcohol amine or cycloalkane amine in ice cold acetonitrile solution of potassium carbonate, chloroacetyl chloride was added to start amide condensation reaction, intermediate product **10** was obtained. 5- bromine -2 chlorophenol was reacted with **10** under reflux condition, to obtain intermediate **11**. Intramolecular ring forming reaction was conducted by microwave reaction to get **12**. The target compounds(**13a-13c**) were obtained by the coupling reaction.

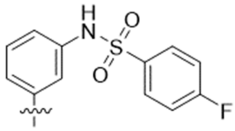
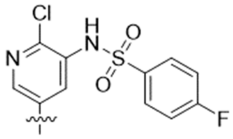
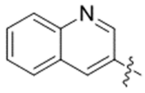
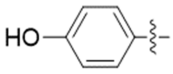
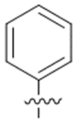
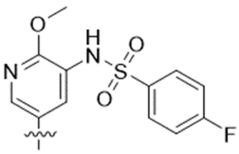
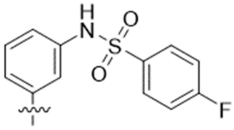
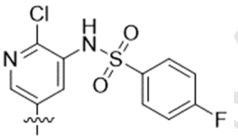
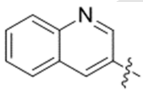
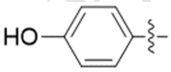
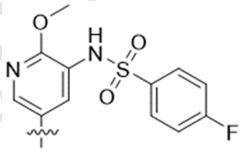
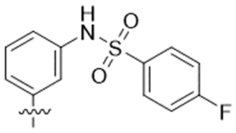
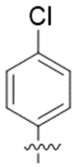
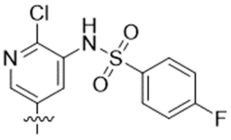
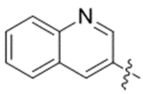
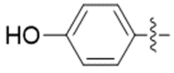
2.2 ***PI3K/mTOR inhibition and SAR study of target compounds***

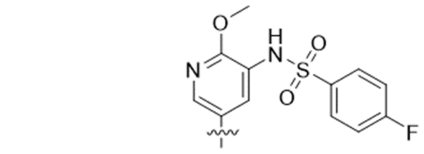
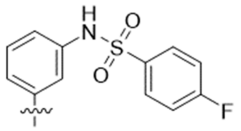
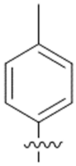
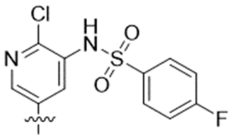
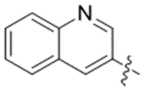
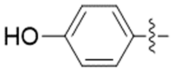
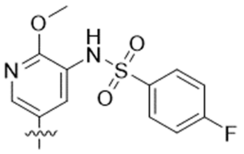
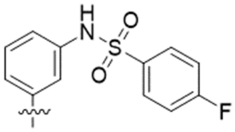
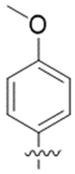
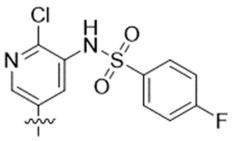
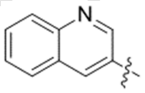
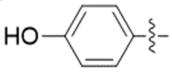
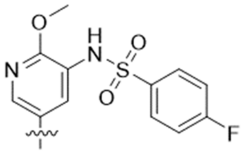
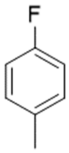
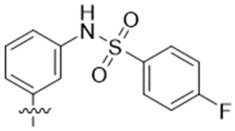
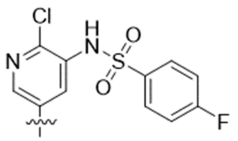
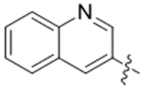
The final products were evaluated for their PI3K/mTOR inhibition potency. In this work, three different kinds of 4- fluoro benzamide sulfonamide fragments and six kinds of substituted benzene rings were employed in molecular design (Scheme 2). Interestingly, the molecules with 4-fluoro-N-(2-methoxypyridin-3-yl)benzenesulfonamide or N-(2-chloro-5-methylpyridin-3-yl)-4-fluorobenzenesulfonamide in C-6 showed good inhibitory activities both against PI3Kα, PI3Kδ and mTOR among all the compounds (Table 1). In addition, the activity of molecules with no substitution in N-4 position (**6a** series) still showed good inhibitory activities, suggesting the importance of sulfonamide fragment in molecular bioactivity. To further validate the importance of sulfonamide fragment for the bioactivity of the molecules, the C-6 position was substituted with 3-quinoline or 4-phenol. The kinases assays turned out that most compounds demonstrated significant decline in contrary with the molecules with sulfonamide fragment in C-6 position. This result makes certain the important role of sulfonamide fragment for the bioactivity of the compounds.

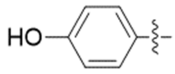
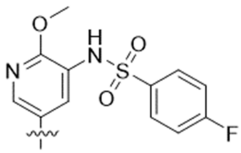
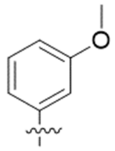
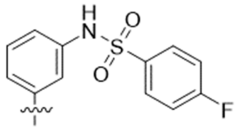
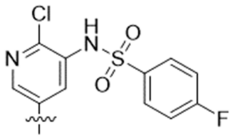
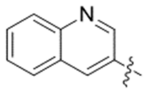
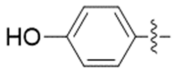
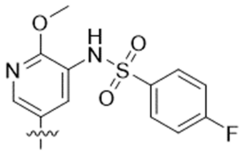
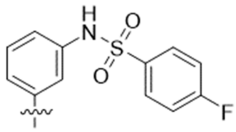
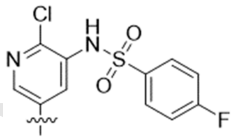
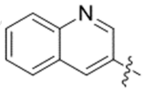
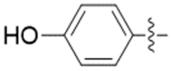
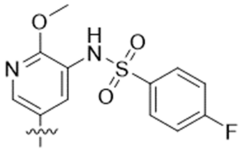
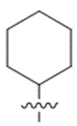
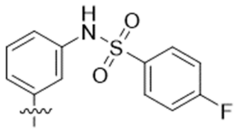
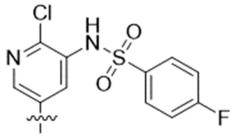
Cyclohexyl, isopropyl and cyclopropyl were introduced to N-4 position to further discuss the SAR. In contrary with benzene ring in N-4 position (**8a** series), the introduced cyclohexyl slightly down-regulated the bioactivities. It can be concluded that the saturated hydrocarbon in N-4 position is not a favorable option. The introduction of isopropyl and cyclopropyl to N-4 position slightly improved the inhibitory activity in contrary with **6a** series, suggesting the size of the substituent in N-4 is critical for the bioactivity of the compounds. A decrease of bioactivity was observed in **8f** series reference to **8d** series, demonstrating the critical influence of the position of substituent to the bioactivities.

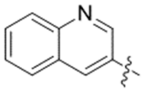
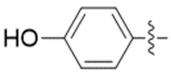
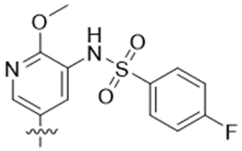
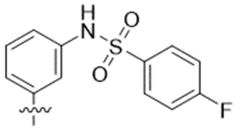
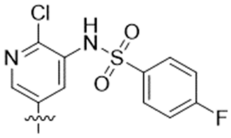
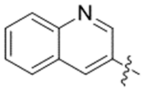
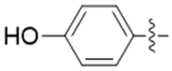
Table 1. IC_{50} Values for Enzymatic Inhibition of PI3Kα and PI3Kδ

Compd	R	R1	IC_{50} (nM)		
			PI3Kα	PI3Kδ	mTOR
6a -1	H		500±12	300±11	635±18

6a -2			>1000	>1000	>1000
6a -3			115±8	92±5	328±7
6a -4			>1000	>1000	>1000
6a -5			>1000	>1000	>1000
8a -1			102±6	80±4	48.6±3
8a -2			>1000	>1000	>1000
8a -3			19±1.1	15±0.4	15.3±0.5
8a -4			>1000	>1000	>1000
8a -5			>1000	>1000	>1000
8b -1			22±1.3	18±0.9	12.6±0.7
8b -2			>1000	>1000	>1000
8b -3			2±0.13	3.2±0.19	14.1±0.52
8b -4			>1000	>1000	>1000
8b -5			>1000	>1000	>1000

8c -1			12±0.56	17±0.62	13.8±0.57
8c -2			>1000	>1000	>1000
8c -3			4.1±0.22	3.7±0.29	19.4±1.2
8c -4			>1000	>1000	>1000
8c -5			>1000	>1000	>1000
8d-1			0.63±0.05	9.2±0.74	13.85±0.86
8d -2			>1000	>1000	>1000
8d -3			0.43±0.02	3.8±0.12	19.8±1.25
8d -4			>1000	>1000	>1000
8d -5			>1000	>1000	>1000
8e-1			21±2.15	19±1.95	34.9±1.53
8e -2			>1000	>10000	>1000
8e -3			5.1±0.23	5±0.31	17.9±0.86
8e -4			>1000	>1000	>1000

8e -5		>1000	>1000	>1000
8f -1		>1000	>1000	>1000
8f -2	 	>1000	>1000	>1000
8f -3		>1000	>1000	>1000
8f -4		>1000	>1000	>1000
8f -5		>1000	>1000	>1000
13a -1		45.3±2.32	18±0.96	3.6±0.21
13a -2		>1000	>1000	>1000
13a -3	 	15.5±0.82	3.8±0.16	4.3±0.13
13a -4		>1000	>1000	>1000
13a -5		>1000	>1000	>1000
13b -1		>1000	406±16	873±21
13b -2	 	>1000	>1000	>1000
13b -3		112±1.16	79±3.32	290.5±3.94

13b -4		>1000	>1000	>1000
13b -5		>10000	>1000	>1000
13c -1		131±0.96	162±1.13	253±2.31
13c -2		>1000	>1000	>1000
13c -3		86±2.21	62±1.32	267±3.32
13c -4		>1000	>1000	>1000
13c -5		>1000	>1000	>1000
BEZ235		80.5±1.37	80±1.56	1.43±0.06

2.3 *In vitro antiproliferative activity*

Inhibition of cell proliferation was measured using MTT with human colon cancer cell line (HCT-116), human lung adenocarcinoma cell line (A549), human breast cancer cell line (MCF-7), human cervical cancer cell line (Hela), human hepatoma cell line (HepG2), human malignant melanoma cell line(A375). Generally, the results were correlated with their effects on kinases activities (Table 2). Different from kinase assays, molecules with 4-fluoro-N-(2-methoxypyridin-3-yl)benzenesulfonamide in C-6 position always showed better anti-cancer activities than N-(2-chloro-5-methylpyridin-3-yl)-4-fluorobenzenesulfonamide. The reason may be that when 4- methoxy was introduced into the sulfonamide fragment, the molecule demonstrated better permeability than chlorine atom in the same position. Among all the molecules, compound **8d-1** showed the most potent anti-proliferation activity. The IC_{50} value of **8d-1** against Hela and A549 are 1.22 and 1.35 μM, with the positive agent BEZ235 with IC_{50} value of 1.17 and 1.08 μM, respectively.

In addition, compound **8d-1** were assessed for toxicities against human normal liver cell line (LO2) and neuronal cell PC12 by the conventional MTT assay *in vitro*. The results are given in Figure 3, in comparation with BEZ235, **8d-1** showed lower toxicities in concentrations from 0.625 μM to 20 μM, demonstrating the safety of **8d-1**.

Table 2. Antiproliferative Activities of Various Cell Lines

Compd	IC_{50} (μM)					
	A549	Hela	HCT-116	HepG2	A375	MCF-7
6a-1	8.07±0.21	7.11±0.16	24.9±0.79	6.59±0.21	26.33±0.81	32.27±1.26
6a -2	32.14±0.95	>40	>40	>40	20.08±0.82	18.34±0.52

6a -3	>40	15.56±0.45	>40	>40	>40	>40
6a -4	>40	>40	>40	26.33±0.79	>40	>40
6a -5	>40	>40	>40	38.15±1.92	>40	>40
8a-1	3.49±0.12	1.53±0.13	18.81±0.76	14.01±0.33	15.17±0.43	8.87±0.31
8a -2	>40	17.69±0.91	>40	30.28±1.22	>40	>40
8a -3	14.7±0.82	10.31±0.51	39.82±1.93	35.67±1.74	36.85±1.83	>40
8a -4	>40	>40	>40	>40	>40	>40
8a -5	>40	>40	>40	>40	>40	29.04±1.64
8b-1	8.35±0.62	4.83±0.31	19.24±1.32	17.93±1.23	24.08±1.42	27.56±1.52
8b -2	31.63±1.79	35.03±2.03	23.89±0.73	27.83±1.88	32.45±1.57	39.81±2.69
8b -3	21.59±1.39	>40	27.91±1.67	33.93±1.33	30.56±1.24	32.77±1.58
8b -4	>40	>40	36.08±1.97	32.72±1.63	>40	>40
8b -5	>40	>40	39.6±2.38	>40	>40	>40
8c-1	5.87±0.32	4.65±0.23	22.68±0.86	28.27±1.12	12.87±0.81	15.41±0.48
8c -2	>40	>40	35.62±1.23	>40	36.19±1.86	32.06±1.28
8c -3	30.9±1.52	32.33±1.23	34.32±1.59	39.58±2.21	38.86±2.21	32.64±2.11
8c -4	>40	>40	>40	>40	>40	>40
8c -5	>40	>40	>40	>40	>40	26.25±1.08
8d-1	1.35±0.09	1.22±0.06	13.44±0.62	13.08±0.59	18.4±1.17	8.26±0.39
8d -2	38.23±1.76	13.16±0.62	28.27±1.37	29.83±1.18	38.52±1.73	32.4±1.17
8d -3	30.35±1.27	8.32±0.45	30.11±1.12	>40	32.72±1.17	36.03±1.38
8d -4	>40	>40	>40	>40	>40	>40
8d -5	>40	16.44±0.87	26.11±1.17	10.82±0.49	>40	17.3±0.74
8e-1	6.5±0.29	10.45±0.48	>40	36.35±1.76	>40	>40
8e -2	>40	>40	>40	>40	>40	>40
8e -3	22.31±1.19	>40	34.2±1.94	>40	>40	32.64±1.39
8e -4	>40	38.78±2.37	>40	37.16±2.15	>40	>40
8e -5	>40	>40	>40	>40	>40	30.2±1.18
8f-1	11.2±0.55	>40	>40	>40	>40	>40
8f -2	>40	>40	>40	>40	>40	>40
8f -3	32.65±1.77	>40	>40	>40	>40	>40
8f -4	>40	11.35±0.43	8.24±0.39	2.08±0.06	23.64±0.83	>40
8f -5	>40	>40	15.33±0.76	26.07±0.94	30.44±1.36	>40
13a-1	4.92±0.18	2.79±0.09	19.21±0.96	37.19±1.13	9.95±0.36	16.95±0.21
13a -2	29.35±1.32	22.65±1.12	22.58±0.52	>40	>40	26.03±0.37
13a -3	5.1±0.22	5.38±0.18	18.92±0.47	21.48±0.19	25.47±0.28	26.59±0.32
13a -4	24.64±1.26	23.12±1.06	23.19±0.86	>40	39.02±2.35	22.91±1.27
13a -5	>40	>40	>40	>40	>40	>40
13b-1	24.64±0.96	6.1±0.29	>40	>40	>40	30.11±1.79
13b -2	14.5±0.79	10.84±0.72	18.28±0.92	35.9±2.76	23.81±0.99	21.37±0.76
13b -3	37.88±1.78	14.32±0.58	>40	>40	>40	>40
13b -4	>40	19.74±0.92	>40	>40	>40	>40
13b -5	>40	>40	>40	>40	>40	>40

13c-1	3.32±0.13	6.03±0.22	24.32±1.38	14.4±0.56	23.86±0.97	35.19±2.77
13c -2	>40	>40	>40	>40	>40	>40
13c -3	4.33±0.24	7.18±0.39	12.41±0.49	8.51±0.42	21.81±0.94	16.74±0.23
13c -4	>40	>40	>40	>40	>40	29.48±0.59
13c -5	>40	>40	>40	32.73±0.75	>40	32.95±0.68
BEZ235	1.08±0.92	1.17±0.11	1.7±0.12	4.04±0.23	0.58±0.05	0.45±0.03

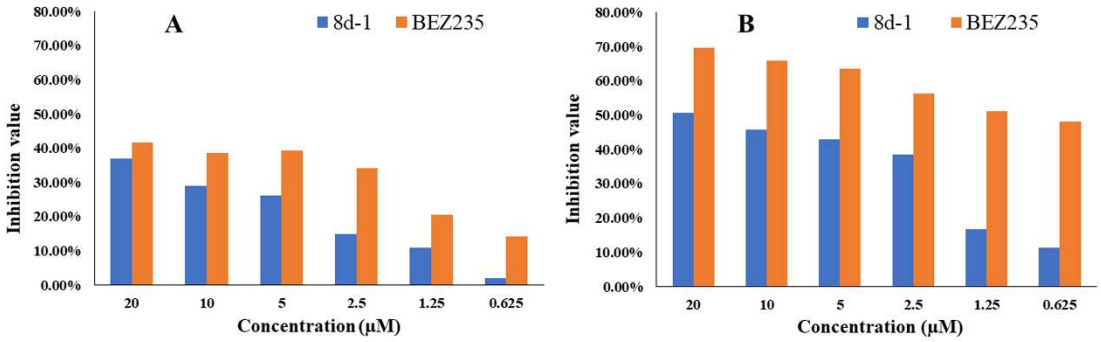


Figure 3. Toxicities of **8d-1** and BEZ235 against LO2 (A) and PC12 (B)

The activities of **8d-1** against the PI3Ks and mTOR were assayed (Table 3). The result demonstrated that **8d-1** is a potent inhibitor against PI3Kα, PI3Kγ, PI3Kδ and mTOR with modest levels of selectivity against PI3Kβ. This result makes **8d-1** a promising Class I PI3K/mTOR dual inhibitor.

To characterize the kinase inhibitory activities and selectivity of compound **8d-1**, kinase inhibition profiling assays with a fixed concentration of 100 nM were carried out against a series of 394 kinases through the kinase profiling provided by Reaction Biology Corporation (Figure 4 and Table S1). Compound **8d-1** displayed almost no inhibitory activity against most of human protein kinases except for mTOR and DNA-PK, which are members of phosphatidylinositol 3-kinase related protein kinases (PIKKs) super family [31].

Table 3. Activities of **8d-1** against Class I PI3Ks and mTOR (IC_{50} Values in nM)

Compd	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ	mTOR
8d-1	0.63	94.54	22	9.2	13.85
PI-103	9.6	11.98	64.03	11	5.3
BEZ235	80.5	703.9	104.2	85.3	1.43

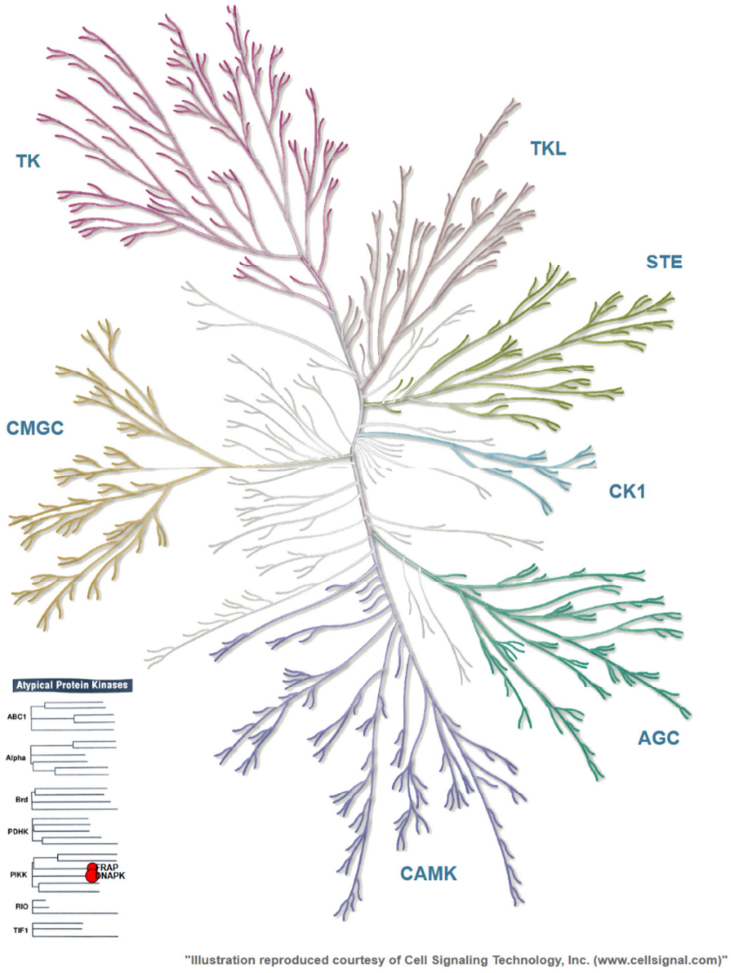


Figure 4. Kinase binding selectivity for **8d-1** shown on the human kinome dendrogram. The inhibition rates were determined using the KinaseProfiler of Reaction Biology Corp. The figure was generated by using an online KinMap program (http://kinhub.org/kinmap/).

Western blot analysis was conducted to evaluate the intracellular PI3K pathway inhibitory activities of **8d-1**. It turned out that **8d-1** could inhibit the PI3K/AKT/mTOR pathway by dose-dependently decrease the level of phosphorylation of AKT and its downstream target S6 in Hela cell line (Figure 5).

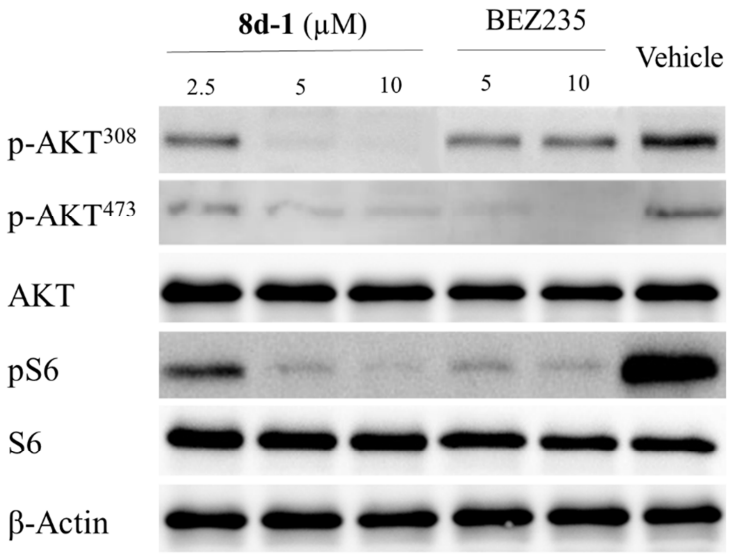


Figure 5. Effects of **8d-1** on pAKT, AKT, pS6, and S6.

Plate clone formation assay was conducted to test the influence of the concentration of **8d-1** on the clone formation of Hela cells. The colony formation rate of Hela cells in the experimental group decreased significantly compared with the control group, in concentration dependent manner. In the concentration above 5 μM, the tumor cells almost did not form clones. At the concentration of 1.25 μM, more than half of the clone formation was inhibited in contrary with the negative control group (Figure 6). This is basically the responding to the MTT results of Hela cells.

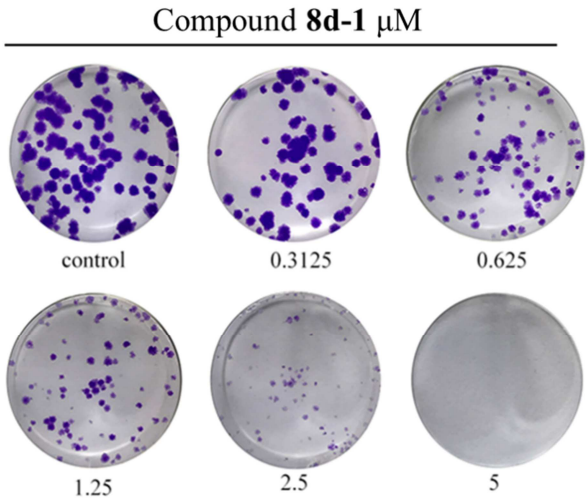


Figure 6. Macroscopic images of colonies formed by Hela treated with different concentrations of **8d-1**.

2.4 *Docking study*

To predict the possible binding mode of **8d-1** with PI3Kα and mTOR, docking analysis was performed by using autodock 4.0 [32]. The X-ray crystal structure of PI3Kα (PDB ID:4L23) [33] and mTOR (PDB ID:4JT5) [34] was obtained as starting point. As demonstrated in Figure 7, five hydrogen bond interactions were formed between **8d-1** and PI3Kα. Three hydrogen bonds were formed between 2H-benzo[*b*][1,4]oxazin-3(4H)-one and PI3Kα, indicating the advantage of the

core structure for the bioactivity of molecules. The fluorine of sulfonamide fragment formed a hydrogen bond with Trp86, another hydrogen bond was formed between the oxygen of 4- methoxy benzene. In addition, 4- methoxy benzene matched very well with the hydrophobic pocket formed by Ile153, Ile105 and Met77, that may be the reason why **8d-1** is a potent PI3Kα inhibitor and it makes 4-phenyl-2H-benzo[*b*][1,4]oxazin-3(4H)-one a reasonable skeleton for further investigation as PI3Kα inhibitors.

The binding mode of **8d-1** with mTOR showed that three hydrogen bonds were formed: two were formed by Lys2187 with the oxygen atom of N-4 substituted 4-methoxy and the oxygen atom of sulfonamide structure, respectively; Asp2357 formed a hydrogen bond with the nitrogen atom of sulfonamide structure. In addition, Lys2187 formed two cation-pi interactions with the benzene ring of N-4 substituted 4-methoxy and the benzene ring of 4-fluoro benzamide sulfonamide fragment, respectively. The binding mode indicated that the 4-fluoro benzamide sulfonamide fragment is a favorable moiety for mTOR inhibitor, and the N-4 substituted 4-methoxy in this molecule scaffold matched very well with PI3Kα and mTOR makes it useful in PI3Kα/mTOR dual inhibitor design.

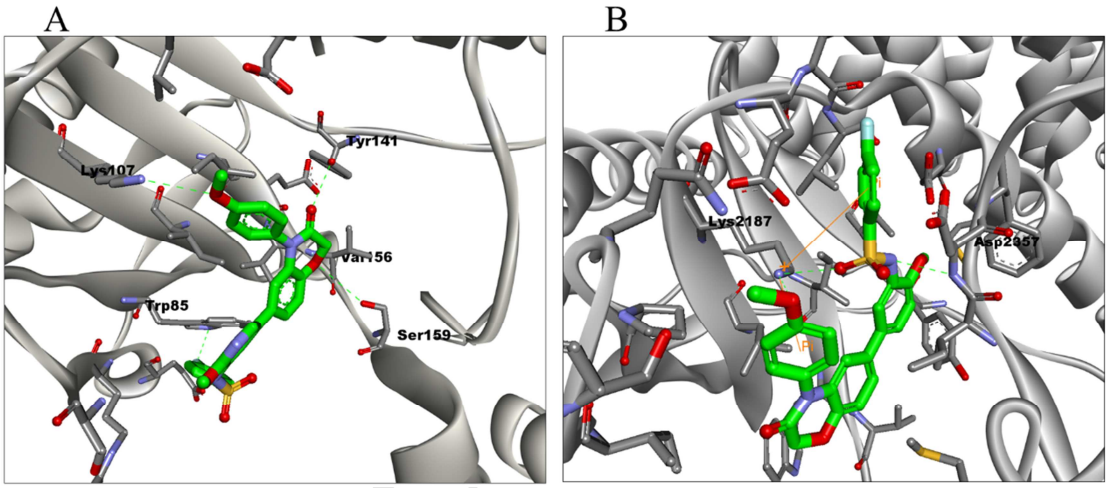


Figure 7. Modeled structure of **8d-1** (green) in PI3Kα (A) and mTOR (B).

2.5 *Pharmacokinetic studies*

The pharmacokinetic properties of **8d-1** were evaluated in Sprague−Dawley rats. After intravenous administration at 1 mg/kg and oral administration at 10 mg/kg, blood samples were taken, and the plasma was detected for the concentrations of **8d-1** by an LC-MS/MS system. As shown in Table 4, **8d-1** showed a plasma clearance of 8.56 $mL \cdot kg^{-1} \cdot min^{-1}$ administered iv in rats, whereas oral administration in rats gave half-time ($T_{1/2}$) of 1.78 h. Acceptable oral bioavailability of 24.1% was achieved, suggesting that **8d-1** could be further investigated as a novel PI3Kα/mTOR dual inhibitor.

Table 4: Pharmacokinetic Parameters for **8d-1** in Rats

IV (1 mg/kg)[a]		PO (10 mg/kg)[b]				
CL (ml/min/kg)	Vss (ml/kg)	T_{max} (h)	C_{max} (ng/ml)	AUCinf (ng*h/ml)	$T_{1/2}$ (h)	F(%)
8.56	1199.81	2.67	886.67	4753.35	1.78	24.1

[a]Vehicle: 5% DMA/10% Solutol HS 15/85 % saline. [b] Vehicle: 0.5% CMC-Na.

2.6 *In vitro antiproliferative activity of compound 8d-1*

The anti-tumor activity in vivo of **8d-1** was conducted based on the promising cellular activity and pharmacokinetic properties. BALB/c nude mice bearing Hela and A549 xenograft tumors were treated with **8d-1** by intragastric administration for 30 days. As can be depicted in Figure 8, the growth of xenograft tumors can be inhibited by **8d-1** in a dose-dependent manner. Tumor growth inhibitions (TGIs) of 87.7%, 66.6% were observed in the Hela xenograft model at dose of 50, and 20 mg/kg, respectively. For the A549 model, administration of **8d-1** at doses of 40 and 20 mg/kg caused TGIs of 70.16% and 57.8%, respectively. BZE235 as positive control in this assay is slightly less active than **8d-1**: at dose of 20 mg/kg leading to TGI of 66.5% in Hela model and 56.08% in A549 model. In addition, **8d-1** did not cause significant weight loss and toxicity during the treatment period in contary with BEZ235 caused little weight loss in this period.

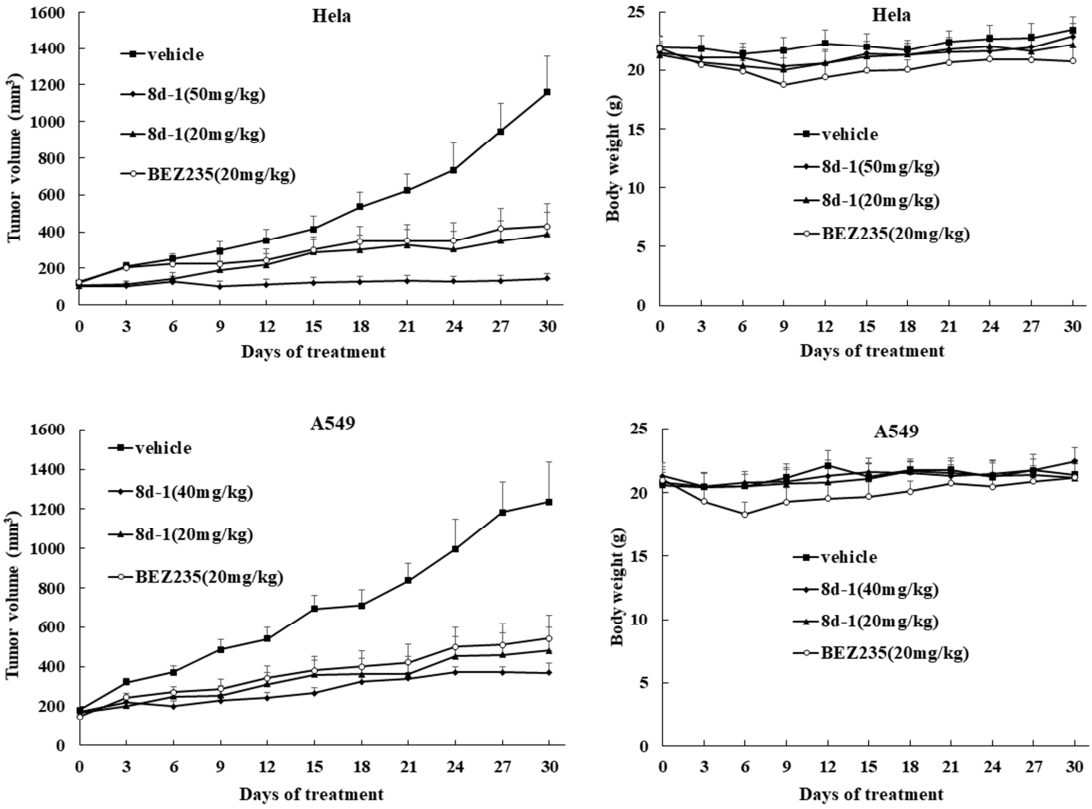


Figure 8. Pharmacodynamic profile of **8d-1** in vivo. Growth inhibitory effect of **8d-1** on established Hela/A549 xenografts in female BALB/c nude mice (N = 6 per group, mean ± SD).

3. CONCLUSIONS

In present study, a series of 2H-benzo[*b*][1,4]oxazin-3(4H)-one derivatives were synthesized and characterized. The discussion of SAR revealed that the benzene or substituted benzene introduced to N-4 position demonstrated advantages over saturated alkane/cyclanes in bioactivities. Based on the SAR, 4-phenyl-2H-benzo[*b*][1,4]oxazin-3(4H)-one as the skeleton of PI3K/mTOR dual inhibitors will be meaningful for further investigation.

Among all the molecules, **8d-1** demonstrated potent anti-proliferation effect on human cancer cell lines Hela and A549 *in vitro* and *in vivo*. In contrary with BEZ235, **8d-1** hold a tenuous advantage in anticancer activity of xenograft model as well as no significant weight loss and toxicity during the treatment period. The existing data indicating that **8d-1** is a promising drug candidate.

4. EXPERIMENTAL SECTION

4.1 chemistry

All solvents and reagents obtained from commercial sources were used without further purification. Flash column chromatography was performed using silica gel from Qingdao Haiyang. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX 400 spectrometer and were calibrated using TMS or residual deuterated solvent as an internal reference ($CDCl_3$: ^{1}H, δ = 7.26 ppm; ^{13}C, δ = 77.16 ppm, DMSO-d_6 2.50 ppm, CD_3OD 3.31 ppm). All of the target compounds were examined by HPLC, and the purity of the biologically tested compounds was ≥ 95%.

*4.1.1 Synthesis of intermediate product **5**:*

To a solution of chloroacetic chloride (4.7 ml, 60 mmol) in 25mL THF was added $NaHCO_3$ (6 g, 72 mmol) and the THF solution of 2- amino -4- bromine phenol (9 g, 48 mmol). The reaction mixture was stirred for 1 h under the ice bath and monitored by TLC. Upon completion, potassium carbonate (10 g, 72 mmol) was added and the reaction solution heated to 80°C to start the next-step reaction for 3h. The reaction was cooled to room temperature, quenched with water, and extracted with EtOAc. The organic layer was collected and washed with diluted hydrochloric acid, saturated $NaHCO_3$ and saturated salt solution, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude material was purified by column chromatography to afford target molecule (6.9g, 63% yield) as brown solid. HRMS (DART-TOF) calculated for $C_8H_6BrNO_2Na$ $[M + Na]^+$ m/z 249.9480, 251.9459, found 249.9448, 251.9419.

4.1.2 General Procedure A for the synthesis of sulfonamide fragment

To a solution of **1** (2.57 mmol) in 8mL pyridine at 0°C was added the pyridine solution of 4-fluoro sulfonyl chloride (4 g, 19.4 mmol) in drops and DMAP (20mg) to start the reaction. The mixture was warmed slowly to room temperature and stirred overnight. After the completion of the reaction (monitored by TLC), the reaction was quenched with water (30mL) and filtered. The filter cake was washed with water and EtOH to obtain white solid which was dissolved in MeOH (10mL) and added with 3 mL saturated potassium carbonate solution. The mixture was stirred at room temperature for 6h and monitored by TLC. The reaction was quenched with water and extracted with EtOAc. The organic layer was collected and washed with water for three times, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude material was purified by column chromatography to afford white solid.

*4.1.2.1 N- (5- bromo -2- methoxy pyridine -3- base) -4- fluoro benzene sulfonamide(**2a**)*

56% isolated yield, white solid. HRMS (DART-TOF) calculated for $C_{12}H_{11}BrFN_2O_3S$ $[M + H]^+$ m/z 360.9658, 362.9637, found 360.9616, 362.9602.

*4.1.2.2 N-(3-bromophenyl)-4-fluorobenzenesulfonamide(**2b**)*

61% isolated yield, white solid. HRMS (DART-TOF) calculated for $C_{12}H_{10}BrFNO_2S$ $[M + H]^+$ m/z 329.9600, 331.9579, found 329.9586, 331.9558.

*4.1.2.3 N- (5- bromine -2- chlorpyridine -3) -4- fluoro sulfonamide(**2c**)*

48% isolated yield, white solid. HRMS (DART-TOF) calculated for $C_{11}H_8BrClFN_2O_2S$ $[M + H]^+$ m/z 364.9162, 366.9142 found 364.9131, 366.9114.

*4.1.3 General Procedure B for the Synthesis of intermediate product **7**.*

To a solution of **5** (350 mg, 1.54 mmol) in 20mL THF was added phenylboronic acid (3.28 mmol),

copper acetate (420 mg, 2.31 mmol), Et_3N (0.5 mL, 4.62 mmol) and 4 Å molecular sieve (50mg). The solution was stirred at 60 °C for 6h. After the completion of the reaction (monitored by TLC), the reaction solution was concentrated, and added with 20mL water. The reaction was filtered to remove solids and extracted with EtOAc, the organic layer was collected and washed with saturated salt solution for three times, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude material was purified by column chromatography to afford target product.

*4.1.3.1 6- bromine -4- phenyl -2H-1,4- benzoxazine -3 (4H) – ketone (**7a**)*

62% isolated yield, white solid. ^{1}H NMR (400 MHz, DMSO) δ 7.63 – 7.58 (m, 2H, Ar-H), 7.53 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.37 (d, *J* = 7.1 Hz, 2H, Ar-H), 7.18 (dd, *J* = 8.5, 2.3 Hz, 1H, Ar-H), 7.06 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.31 (d, *J* = 2.3 Hz, 1H, Ar-H), 4.85 (s, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{14}H_{10}BrNO_2Na$ $[M + Na]^+$ m/z 325.9793, 327.9772 found 325.9771, 327.9762.

*4.1.3.2 6- bromine -4- (4- chlorophenyl) -2H- benzoxazine -3 (4H) - ketone. (**7b**)*

71% isolated yield, grey solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.22 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.11 (dd, *J* = 8.6, 2.2 Hz, 1H, Ar-H), 6.93 (d, *J* = 8.6 Hz, 1H, Ar-H), 6.55 (s, 1H, Ar-H), 4.75 (s, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{14}H_9BrClNO_2Na$ $[M + Na]^+$ m/z 336.9505, 338.9485 found 336.9501, 338.9481.

*4.1.3.3 6- bromine -4- (4- methyl phenyl) -2H- benzoxazine -3 (4H) - ketone. (**7c**)*

53% isolated yield, grey solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.14 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.08 (dd, *J* = 8.5, 2.2 Hz, 1H, Ar-H), 6.91 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.56 (d, *J* = 2.2 Hz, 1H, Ar-H), 4.75 (s, 2H, CH_2), 2.44 (s, 3H, CH_3). HRMS (DART-TOF) calculated for $C_{15}H_{12}BrNO_2Na$ $[M + Na]^+$ m/z 339.9949, 341.9929, found 339.9931, 341.9906.

*4.1.3.4 6- bromine -4- (4- methoxy phenyl) -2H- benzoxazine -3 (4H) - ketone. (**7d**)*

38% isolated yield, grey solid. ^{1}H NMR (400 MHz, DMSO) δ 7.28 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.16 (dd, *J* = 8.5, 2.3 Hz, 1H, Ar-H), 7.12 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.03 (d, *J* = 2.3 Hz, 1H, Ar-H), 6.35 (d, *J* = 2.3 Hz, 1H, Ar-H), 4.82 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3). HRMS (DART-TOF) calculated for $C_{15}H_{12}BrNO_3Na$ $[M + Na]^+$ m/z 355.9898, 357.9878, found 355.9876, 357.9855.

*4.1.3.5 6- bromine -4- (4- fluoro phenyl) -2H- benzoxazine -3 (4H) - ketone. (**7e**)*

66% isolated yield, grey solid. ^{1}H NMR (400 MHz, DMSO) δ 7.47 - 7.40 (m, 4H, Ar-H), 7.19 (dd, *J* = 7.4, 2.3 Hz, 1H, Ar-H), 7.06 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.34 (d, *J* = 2.2 Hz, 1H, Ar-H), 4.84 (s, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{14}H_9BrFNO_2Na$ $[M + Na]^+$ m/z 343.9698, 345.9678, found 343.9612, 345.9655.

*4.1.3.6 6- bromine -4- (3- methoxy phenyl) -2H- benzoxazine -3 (4H) – ketone(**7f**)*

43% isolated yield, grey solid. ^{1}H NMR (400 MHz, DMSO) δ 7.51 (t, *J* = 8.1 Hz, 1H, Ar-H), 7.18 (dd, *J* = 8.5, 2.3 Hz, 1H, Ar-H), 7.11 (dd, *J* = 8.4, 3.4 Hz, 1H, Ar-H), 7.05 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.99 – 6.96 (m, 1H, Ar-H), 6.92 (d, *J* = 8.7 Hz, 1H, Ar-H), 6.36 (d, *J* = 2.3 Hz, 1H, Ar-H), 4.83 (s, 2H, CH_2), 3.79 (s, 3H, OCH_3). HRMS (DART-TOF) calculated for $C_{15}H_{12}BrNO_3Na$ $[M + Na]^+$ m/z 355.9898, 357.9878, found 355.9861, 357.9856.

4.1.4 General Procedure C for the Synthesis of intermediate product 12.

To a solution of **9** (30 mmol) in 25mL THF was added K_2CO_3 (6.2 g, 45 mmol), and stirred for 20 min under the ice bath to make it well mixed. Chloroacetyl chloride (3 mL, 36 mmol) was added in drops and stirred for 3 h under room temperature. The reaction solution was concentrated, water (100 mL) was added, and extracted with EtOAc. The organic layer was collected and washed with saturated salt solution for three times, dried over anhydrous Na_2SO_4 and concentrated in vacuo.

The crude material was purified by column chromatography to afford intermediate product **10**.

To a solution of 5- bromine -2- chlorophenol (2.9 g, 14.3 mmol) in 50mL acetonitrile was added K_2CO_3 (2.5 g， 17.1 mmol), and stirred for 30 min. intermediate product **10** was added and the solution was heated to 80°C for 4h. The reaction solution was concentrated, water (100 mL) was added, and extracted with EtOAc. The organic layer was collected and washed with water and brine for three times, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude material was purified by column chromatography to afford intermediate product **11**.

To a solution of intermediate product **11** (0.64 mmol) in 3mL DMF in microwave reacting tube, Cs_2CO_3 (500 mg, 1.53 mmol) was added. The mixture was heated to 150 ℃ under microwave irradiation for 60 min and monitored by TLC. The reaction was cooled to room temperature, quenched with water, and extracted with EtOAc. The organic layer was collected and washed with water for three times, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude material was purified by column chromatography to afford target molecule.

4.1.4.1 6- bromo -4- isopropyl -2H- benzoxazine -3 (4H) – ketone (12a)

32% isolated yield, grey solid. ^{1}H NMR (400 MHz, DMSO) δ 7.47 (dd, *J* = 7.5, 1.4 Hz, 1H, Ar-H), 7.14 (d, *J* = 1.6 Hz, 1H, Ar-H), 6.99 (d, *J* = 7.5 Hz, 1H, Ar-H), 5.33 (hept, *J* = 5.9 Hz, 1H, CH), 4.81 (s, 2H, CH_2), 1.34 (d, *J* = 5.9 Hz, 6H, CH_3). HRMS (DART-TOF) calculated for $C_{11}H_{13}BrNO_2$ $[M + H]^+$ m/z 270.0130, 272.0109, found 270.0115, 272.0101.

4.1.4.2 6- bromo -4- cyclohexyl -2H- benzoxazine -3 (4H) – ketone (12b)

32% isolated yield, grey solid. ^{1}H NMR (400 MHz, CDCl3) δ 7.47 (dd, *J* = 7.5, 1.4 Hz, 1H, Ar-H), 7.16 (d, *J* = 1.6 Hz, 1H, Ar-H), 6.99 (d, *J* = 7.5 Hz, 1H, Ar-H), 4.81 (s, 2H, CH_2), 4.64 – 4.56 (m, 1H, CH), 2.08 (dt, *J* = 7.1, 6.0 Hz, 2H, CH_2), 1.77 (dd, *J* = 11.1, 5.6 Hz, 2H, CH_2), 1.67 – 1.59 (m, 4H, CH_2), 1.49 (p, *J* = 5.7 Hz, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{14}H_{17}BrNO_2$ $[M + H]^+$ m/z 310.0443, 312.0422, found 310.0436, 312.0415.

4.1.4.3 6- bromo -4- cyclopropyl -2H- benzoxazine -3 (4H) – ketone (12c)

43% isolated yield, grey solid. ^{1}H NMR (400 MHz, CDCl3) δ 7.47 (dd, *J* = 7.5, 1.4 Hz, 1H, Ar-H), 7.22 (d, *J* = 1.4 Hz, 1H, Ar-H), 6.98 (d, *J* = 7.5 Hz, 1H, Ar-H), 4.80 (s, 2H, CH_2), 3.62 (p, *J* = 9.0 Hz, 1H, CH), 1.27 – 0.88 (m, 4H, CH_2). HRMS (DART-TOF) calculated for $C_{11}H_{11}BrNO_2$ $[M + H]^+$ m/z 267.9973, 269.9953, found 267.9952, 269.9932.

4.1.5 General Procedure for the Synthesis of target molecules.

To a solution of **intermediate product 5/7/12 (**0.26 mmol) and **2a-2c,** 3-bromoquinoline or 4-bromophenol (0.26 mmol) in 4 mL dioxane, was added $PdCl_2$(dppf) (14 mg, 0.02 mmol) and potassium acetate (80 mg, 0.8 mmol). Under N_2 atmosphere, the mixture was heated to 80 ℃ for 5 h. Then the reaction mixture was cooled, diluted with ethyl acetate, washed with brine, dried over Na_2SO_4, and the solvent removed in vacuo. Purification on silica yielded the desired compounds**.**

4.1.5.1

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

43% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.81 (s, 1H, CONH), 10.06 (s, 1H, SO_2NH), 8.09 (s, 1H, Ar-H), 7.82 (dd, *J* = 8.8, 5.2 Hz, 2H, Ar-H), 7.71 (s, 1H, Ar-H), 7.40 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.13 (d, *J* = 10.4 Hz, 1H, Ar-H), 7.07 – 7.00 (m, 2H, Ar-H), 4.61 (s, 2H, CH_2), 3.67 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO) δ 164.77, 163.6 (d, J_{C-F} = 250 Hz), 149.56, 148.21, 143.97, 138.30, 136.98, 136.95, 130.89 (d, J_{C-F} = 9 Hz), 130.40, 128.36, 125.25, 124.27, 117.09, 116.94, 116.77,

116.14(d, J_{C-F} = 23 Hz), 68.15, 53.95. HRMS (DART-TOF) calculated for $C_{20}H_{16}FN_3O_5S$ $[M + H]^+$ m/z 429.0795, found 429.0771. Purity 96.3% by HPLC.

*4.1.5.2 4-fluoro-N-(3-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)phenyl) benzenesulfonamide (**6a-2**)*

32% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.82 (s, 1H, CONH), 10.47 (s, 1H, SO_2NH), 7.85 (dd, *J* = 8.8, 5.2 Hz, 2H, Ar-H), 7.40 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.28 (d, *J* = 7.3 Hz, 2H, Ar-H), 7.20 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.09 – 6.99 (m, 4H, Ar-H), 4.61 (s, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO) δ = 165.27, 163.6 (d, J_{C-F} = 262 Hz), 140.99, 138.89, 136.45, 136.42, 134.47, 130.89 (d, J_{C-F} = 6.7 Hz), 128.21, 122.51, 121.72, 119.28, 118.23, 117.08 (d, J_{C-F} = 21 Hz), 116.84, 114.25, 67.26. HRMS (DART-TOF) calculated for $C_{20}H_{15}FN_2O_4S$ $[M + H]^+$ m/z 398.0737, found 398.0721. Purity 98.0% by HPLC.

*4.1.5.3 N-(2-chloro-5-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)-4-fluorobenzenesulfonamide (**6a-3**)*

29% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.86 (s, 1H, CONH), 8.97 (s, 1H, SO_2NH), 8.82 (s, 1H, Ar-H), 8.12 – 7.93 (m, 2H, Ar-H), 7.84 (s, 1H, Ar-H), 7.63 – 7.51 (m, 2H, Ar-H), 7.24 (t, *J* = 8.8 Hz, 1H, Ar-H), 7.12 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.03 (s, 1H, Ar-H), 4.66 (s, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{19}H_{13}ClFN_3O_4S$ $[M + H]^+$ m/z 433.0299, found 433.0291. Purity 96.2% by HPLC.

*4.1.5.4 6-(quinolin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**6a-4**)*

23% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ = 10.86 (s, 1H, CONH), 9.14 (d, *J*=2.3, 1H, Ar-H), 8.51 (d, *J*=2.1, 1H, , Ar-H), 8.06 (dd, *J*=8.0, 3.2, 2H, Ar-H), 7.81 – 7.73 (m, 1H, Ar-H), 7.65 (t, *J*=7.5, 1H, Ar-H), 7.44 (dd, *J*=8.3, 2.2, 1H, Ar-H), 7.33 (d, *J*=2.1, 1H, Ar-H), 7.13 (d, *J*=8.3, 1H, Ar-H), 4.66 (s, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO) δ = 165.21, 149.63, 147.14, 144.01, 132.79, 132.68, 131.89, 129.92, 129.12, 128.80, 128.50, 128.11, 127.56, 122.53, 117.41, 114.83, 67.32, 50.99. HRMS (DART-TOF) calculated for $C_{17}H_{12}N_2O_2$ $[M + H]^+$ m/z 276.0899, found 276.0889. Purity 95.5% by HPLC.

*4.1.5.5 6-(4-hydroxyphenyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**6a-5**)*

29% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ = 10.71 (s, 1H, CONH), 9.48 (d, *J*=7.3, 1H, Ar-H), 7.34 (t, *J*=5.7, 2H, Ar-H), 7.10 (dd, *J*=8.3, 2.2, 1H, Ar-H), 7.05 (d, *J*=2.1, 1H, Ar-H), 6.97 (d, *J*=8.3, 1H, Ar-H), 6.82 (t, *J*=5.8, 2H, Ar-H), 4.57 (s, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{14}H_{11}NO_3$ $[M + H]^+$ m/z 241.0739, found 241.0736. Purity 98.3% by HPLC.

*4.1.5.6 4-fluoro-N-(2-methoxy-5-(3-oxo-4-phenyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl) benzenesulfonamide (**8a-1**)*

41% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.93 (dd, *J* = 8.9, 5.0 Hz, 3H, Ar-H), 7.58 (t, *J* = 7.4 Hz, 2H, Ar-H), 7.52 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.41 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.34 (d, *J* = 7.3 Hz, 2H, Ar-H), 7.20 (t, *J* = 8.5 Hz, 3H, Ar-H), 7.13 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.01 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 6.54 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.83 (s, 2H, CH_2), 3.59 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 167.25, 164.69 (d, J_{C-F} = 262.3 Hz), 164.00, 163.60, 160.70, 146.82, 139.66, 135.48, 131.80 (d, J_{C-F} = 6.7 Hz), 131.70, 130.24, 129.19, 128.72, 122.54, 117.80, 116.14 (d, J_{C-F} = 26.7 Hz), 114.99, 68.21, 53.73. HRMS

(DART-TOF) calculated for $C_{26}H_{20}FN_3O_5S$ $[M + H]^+$ m/z 505.1108, found 505.1101. Purity 97.5% by HPLC.

*4.1.5.7 4-fluoro-N-(3-(3-oxo-4-phenyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)phenyl) benzenesulfonamide (**8a-2**)*

53% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (dd, *J* = 9.0, 5.0 Hz, 2H, Ar-H), 7.57 (t, *J* = 7.5 Hz, 2H, Ar-H), 7.52 – 7.46 (m, 1H, Ar-H), 7.33 (dd, *J* = 8.3, 1.2 Hz, 2H, Ar-H), 7.20 (t, *J* = 7.9 Hz, 1H, Ar-H), 7.10 (dd, *J* = 8.4, 1.8 Hz, 3H, Ar-H), 7.06 (s, 1H, Ar-H), 7.05 – 7.03 (m, 1H, Ar-H), 6.92 (ddd, *J* = 8.0, 2.1, 0.9 Hz, 1H, Ar-H), 6.64 (s, 1H, , Ar-H), 6.57 – 6.51 (m, 1H, Ar-H), 4.81 (s, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 164.14, 144.23(d, J_{C-F} = 320.7 Hz), 136.64, 135.61, 134.90, 130.94, 130.15, 129.96 129.96 (d, J_{C-F} = 9.4 Hz,), 129.80, 129.09, 128.73, 124.01, 122.84, 120.05, 119.76, 117.45, 116.34 (d, J_{C-F} = 22.7 Hz). 115.45, 68.22. HRMS (DART-TOF) calculated for $C_{26}H_{19}FN_2O_4S$ $[M + H]^+$ m/z 474.1050, found 474.1025. Purity 98.1% by HPLC.

4.1.5.8

*N-(2-chloro-5-(3-oxo-4-phenyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)-4-fluorobe nzenesulfonamide (**8a-3**)*

29% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.93 (dd, *J* = 8.9, 5.0 Hz, 3H, Ar-H), 7.58 (t, *J* = 7.4 Hz, 2H, Ar-H), 7.52 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.41 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.34 (d, *J* = 7.3 Hz, 2H, Ar-H), 7.20 (t, *J* = 8.5 Hz, 3H, Ar-H), 7.13 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.01 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 6.54 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.83 (s, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.66 (d, J_{C-F} = 257 Hz), 145.60, 143.20, 136.12, 135.38, 131.99, 131.39, 130.32, 130.29, 130.14, 129.95 (d, J_{C-F} = 9.6 Hz), 129.34, 128.70, 127.72, 122.87, 117.97, 116.76 (d, J_{C-F} = 22.8 Hz). 115.45, 68.16, 29.70. HRMS (DART-TOF) calculated for $C_{25}H_{17}ClFN_3O_4S$ $[M + H]^+$ m/z 509.0612, found 509.0611. Purity 96.3% by HPLC.

*4.1.5.9 4-phenyl-6-(quinolin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8a-4**)*

29% isolated yield, yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.91 (d, *J*=2.2, 1H, Ar-H), 8.08 (dd, *J*=9.3, 5.4, 2H, Ar-H), 7.80 (d, *J*=8.2, 1H, Ar-H), 7.69 (ddd, *J*=8.4, 6.9, 1.4, 1H, Ar-H), 7.61 – 7.52 (m, 3H, Ar-H), 7.49 (ddd, *J*=6.3, 3.9, 1.3, 1H, Ar-H), 7.35 (tt, *J*=6.5, 5.1, 3H, Ar-H), 7.20 (d, *J*=8.3, 1H, Ar-H), 6.73 (d, *J*=2.1, 1H, Ar-H), 4.85 (s, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 164.02, 149.37, 145.09, 135.51, 132.98, 132.88, 132.60, 131.35, 130.24, 129.58, 129.22, 129.05, 128.70, 127.89, 127.20, 123.15, 117.83, 115.78, 68.25. HRMS (DART-TOF) calculated for $C_{23}H_{16}N_2O_2$ $[M + H]^+$ m/z 352.1212, found 352.1210. Purity 98.1% by HPLC.

*4.1.5.10 6-(4-hydroxyphenyl)-4-phenyl-2H-benzo[b][1,4]oxazin-3(4H)-one (**8a-5**)*

21% isolated yield, yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.58 – 7.52 (m, 2H, Ar-H), 7.47 (ddd, *J*=7.4, 3.8, 1.2, 1H, Ar-H), 7.33 (dd, *J*=5.3, 3.2, 2H, Ar-H), 7.23 – 7.17 (m, 2H, Ar-H), 7.15 (dd, *J*=8.3, 2.1, 1H, Ar-H), 7.08 (d, *J*=8.3, 1H, Ar-H), 6.83 – 6.76 (m, 2H, Ar-H), 6.56 (d, *J*=2.0, 1H, Ar-H), 4.80 (s, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 164.35, 155.09, 130.08, 128.97, 128.75, 128.07, 122.45, 117.25, 115.62, 115.20, 68.27. HRMS (DART-TOF) calculated for $C_{20}H_{15}NO_3$ $[M + H]^+$ m/z 317.1052, found 317.1041. Purity 97.3% by HPLC.

4.1.5.11

*N-(5-(4-(4-chlorophenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)-4-fluorobenzenesulfonamide (**8b-1**)*

33% isolated yield, yellow powder. ^{1}H NMR (400 MHz, DMSO) δ 10.04 (s, 1H, SO_2NH), 7.96 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.72 (dd, *J* = 8.9, 5.2 Hz, 2H, Ar-H), 7.69 (d, *J* = 8.7 Hz, 2H), 7.50 (d, *J* =

2.3 Hz, 1H, Ar-H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.38 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.21 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 7.17 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.42 (d, *J* = 1.9 Hz, 1H, Ar-H), 4.85 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3) HRMS (DART-TOF) calculated for $C_{26}H_{19}ClFN_3O_5S$ $[M + H]^+$ m/z 539.0718, found 539.0713. Purity 95.7% by HPLC.

4.1.5.12

*N-(3-(4-(4-chlorophenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)phenyl)-4-fluorobenzenesulfonamide (**8b-2**)*

35% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.42 (s, 1H, SO_2NH), 7.73 (dd, *J* = 8.9, 5.1 Hz, 2H, Ar-H), 7.69 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.47 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.36 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.24 (t, *J* = 7.9 Hz, 1H, Ar-H), 7.17 (d, *J* = 1.1 Hz, 2H, Ar-H), 7.10 – 7.04 (m, 2H, Ar-H), 7.02 – 6.96 (m, 1H, Ar-H), 6.38 (s, 1H, Ar-H), 4.86 (s, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{26}H_{18}ClFN_2O_4S$ $[M + H]^+$ m/z 508.0660, found 508.0645. Purity 98.1% by HPLC.

4.1.5.13

*N-(2-chloro-5-(4-(4-chlorophenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)-4-fluorobenzenesulfonamide (**8b-3**)*

42% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.06 (s, 1H, SO_2NH), 7.94 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.72 (dd, *J* = 8.9, 5.2 Hz, 2H, Ar-H), 7.69 (d, *J* = 8.7 Hz, 2H), 7.50 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.38 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.21 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 7.17 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.42 (d, *J* = 1.9 Hz, 1H, Ar-H), 4.85 (s, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{25}H_{16}C_{l2}FN_3O_4S$ $[M + H]^+$ m/z 543.0223, found 543.0221. Purity 95.9% by HPLC.

*4.1.5.14 4-(4-chlorophenyl)-6-(quinolin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8b-4**)*

23% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.92 (d, *J*=2.3, 1H, Ar-H), 8.08 (dd, *J*=9.5, 5.4, 2H, Ar-H), 7.83 (dd, *J*=8.2, 1.0, 1H, Ar-H), 7.70 (ddd, *J*=8.4, 6.9, 1.4, 1H, Ar-H), 7.61 – 7.49 (m, 2H, Ar-H), 7.35 (dd, *J*=8.3, 2.1, 1H, Ar-H), 7.33 – 7.31 (m, 1H, Ar-H), 7.31 – 7.28 (m, 1H, Ar-H), 7.21 (d, *J*=8.3, 1H, Ar-H), 6.73 (d, *J*=2.1, 1H, Ar-H), 4.84 (s, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 164.06, 149.44, 147.22, 145.11, 135.15, 133.97, 132.89, 132.72, 131.03, 130.52, 130.08, 129.58, 129.19, 127.91, 127.81, 127.20, 123.43, 118.00, 115.64, 68.21. HRMS (DART-TOF) calculated for $C_{23}H_{15}ClN_2O_2$ $[M + H]^+$ m/z 386.0822, found 386.0816. Purity 96.8% by HPLC.

*4.1.5.15 4-(4-chlorophenyl)-6-(4-hydroxyphenyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8b-5**)*

26% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 9.65 (s, 1H, Ar-H), 7.65 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.45 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.17 (ddd, *J* = 22.2, 11.0, 5.2 Hz, 4H, Ar-H), 6.76 (d, *J* = 8.6 Hz, 2H, Ar-H), 6.41 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.83 (s, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{20}H_{14}ClNO_3$ $[M + H]^+$ m/z 351.0662, found 351.0639. Purity 99.1% by HPLC.

4.1.5.16

*4-fluoro-N-(2-methoxy-5-(3-oxo-4-(p-tolyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)benzenesulfonamide (**8c-1**)*

24% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.78 (t, *J* = 4.2 Hz, 1H, Ar-H), 7.71 (dd, *J* = 8.9, 5.0 Hz, 2H, Ar-H), 7.37 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.22 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.11 (d, *J* = 1.5 Hz, 2H, Ar-H), 7.06 (t, *J* = 8.6 Hz, 2H, Ar-H), 6.90 (s, 1H, Ar-H), 6.55 (s, 1H, Ar-H), 4.81 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3). ^{13}C

NMR (101 MHz, $CDCl_3$) δ 165.4(d, J_{C-F} = 255 Hz), 164.13, 153.58, 144.74, 139.64, 139.24, 134.93, 134.89, 132.81, 131.85, 131.24, 130.88, 130.08, 129.91 (d, J_{C-F} = 9.5 Hz), 128.42, 126.08, 122.36, 120.75, 117.59, 116.34 (d, J_{C-F} = 22.7 Hz), 115.15, 68.22, 53.94, 21.29. HRMS (DART-TOF) calculated for $C_{27}H_{22}FN_3O_5S$ $[M + H]^+$ m/z 519.1264, found 519.1243. Purity 98.2% by HPLC.

*4.1.5.17 4-fluoro-N-(3-(3-oxo-4-(p-tolyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)phenyl) benzenesulfonamide (**8c-2**)*

29% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 8.9, 5.0 Hz, 1H, Ar-H), 7.34 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.19 (t, *J* = 8.3 Hz, 2H, Ar-H), 7.10 – 7.04 (m, 3H, Ar-H), 6.93 (ddd, *J* = 7.9, 2.0, 1.1 Hz, 1H, Ar-H), 6.58 – 6.55 (m, 1H, Ar-H), 4.80 (s, 2H, CH_2), 2.41 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.24 (d, J_{C-F} = 255 Hz), 164.34, 141.65, 139.12, 136.76, 135.02, 134.99, 134.94, 132.82, 130.97, 130.84, 129.98 (d, J_{C-F} = 9.4 Hz)., 129.74, 128.40, 123.87, 122.79, 119.80, 119.55, 117.36, 116.32 (d, J_{C-F} = 22.7 Hz,), 115.49, 68.22, 21.29. HRMS (DART-TOF) calculated for $C_{27}H_{21}FN_2O_4S$ $[M + H]^+$ m/z 488.1206, found 488.1206. Purity 97.6% by HPLC.

4.1.5.18

*N-(2-chloro-5-(3-oxo-4-(p-tolyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)-4-fluorob enzenesulfonamide (**8c-3**)*

37% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.93 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.70 (dd, *J* = 9.0, 4.9 Hz, 2H, Ar-H), 7.39 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.22 (d, *J* = 8.3 Hz, 3H, Ar-H), 7.15 (d, *J* = 1.1 Hz, 2H, Ar-H), 7.12 – 7.06 (m, 2H, Ar-H), 6.56 (t, *J* = 1.2 Hz, 1H, Ar-H), 4.84 (s, 2H, CH_2), 2.43 (s, 3H, CH_3). HRMS (DART-TOF) calculated for $C_{26}H_{19}ClFN_3O_4S$ $[M + H]^+$ m/z 523.0769, found 523.0761. Purity 97.3% by HPLC.

*4.1.5.19 6-(quinolin-3-yl)-4-(p-tolyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8c-4**)*

34% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.91 (d, *J*=2.1, 1H, Ar-H), 8.13 – 8.03 (m, 2H, Ar-H), 7.81 (d, *J*=8.1, 1H, Ar-H), 7.69 (ddd, *J*=8.4, 6.9, 1.4, 1H, Ar-H), 7.54 (dt, *J*=11.8, 2.4, 1H, Ar-H), 7.40 – 7.29 (m, 3H, Ar-H), 7.25 – 7.16 (m, 3H, Ar-H), 6.75 (d, *J*=2.1, 1H, Ar-H), 4.84 (s, 2H, CH_2), 2.44 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 164.11, 149.59, 145.03, 139.28, 132.82, 132.73, 132.62, 131.42, 130.94, 129.46, 129.18, 128.36, 127.88, 127.12, 123.03, 117.73, 115.80, 68.24, 21.29. HRMS (DART-TOF) calculated for $C_{24}H_{18}N_2O_2$ $[M + H]^+$ m/z 351.0662, found 351.0649. Purity 96.8% by HPLC.

*4.1.5.20 6-(4-hydroxyphenyl)-4-(p-tolyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8c-5**)*

26% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.34 (d, *J*=8.2, 2H, Ar-H), 7.24 – 7.17 (m, 3H, Ar-H), 7.13 (dd, *J*=8.3, 2.0, 1H, Ar-H), 7.07 (d, *J*=8.3, 1H, Ar-H), 6.80 (d, *J*=8.5, 2H, Ar-H), 6.59 (d, *J*=2.0, 1H, Ar-H), 4.79 (s, 2H, Ar-H), 2.43 (s, 3H, Ar-H). HRMS (DART-TOF) calculated for $C_{21}H_{17}NO_3$ $[M + H]^+$ m/z 331.1208, found 331.1202. Purity 95.3% by HPLC.

*4.1.5.21 4-fluoro-N-(2-methoxy-5-(4-(4-methoxyphenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4] oxazin-6-yl)pyridin-3-yl)benzenesulfonamide (**8d-1**)*

31% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.06 (s, 1H, SO_2NH), 7.92 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.73 (dd, *J* = 8.9, 5.2 Hz, 2H, Ar-H), 7.50 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.38 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.34 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.21 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 7.17 (d, *J* = 0.8 Hz, 2H, Ar-H), 7.15 (s, 1H, Ar-H), 6.42 (d, *J* = 1.9 Hz, 1H, Ar-H), 4.85 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO) δ 164.72(d, J_{C-F} = 250 Hz), 164.45, 159.69, 156.13, 144.70, 140.06, 137.24, 131.95, 131.38, 130.57, 130.04 (d, J_{C-F} = 9 Hz),

129.64, 129.35, 128.44, 122.08, 121.62, 117.81, 116.64(d, J_{C-F} = 22 Hz), 115.64, 114.42, 68.12, 55.86, 53.85. HRMS (DART-TOF) calculated for $C_{27}H_{22}FN_3O_6S$ $[M + H]^+$ m/z 535.1213, found 535.1201. Purity 98.9% by HPLC.

4.1.5.22

*4-fluoro-N-(3-(4-(4-methoxyphenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)phenyl)benzenesulfonamide (**8d-2**)*

39% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.42 (s, 1H, SO_2NH), 7.72 (dd, *J* = 8.9, 5.1 Hz, 2H, Ar-H), 7.38 – 7.33 (m, 2H, Ar-H), 7.31 (d, *J* = 6.9 Hz, 2H, Ar-H), 7.23 (t, *J* = 7.9 Hz, 1H, Ar-H), 7.18 – 7.13 (m, 4H, Ar-H), 7.08 (t, *J* = 1.8 Hz, 1H, Ar-H), 7.03 (d, *J* = 7.8 Hz, 1H, Ar-H), 6.98 (dd, *J* = 8.0, 1.3 Hz, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 4.84 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.25(d, J_{C-F} = 254 Hz), 163.98, 159.77, 144.76, 141.65, 136.73, 135.02, 134.98, 134.91, 131.14, 129.97 (d, J_{C-F} = 9.4 Hz), 129.77, 127.91, 123.90, 122.74, 119.86, 119.58, 117.36, 116.34 (d, J_{C-F} = 22.7 Hz), 115.43, 68.22, 55.54. HRMS (DART-TOF) calculated for $C_{27}H_{21}FN_2O_5S$ $[M + H]^+$ m/z 504.1155, found 504.1142. Purity 97.3% by HPLC.

4.1.5.23

*N-(2-chloro-5-(4-(4-methoxyphenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)-4-fluorobenzenesulfonamide (**8d-3**)*

22% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.53 (s, 1H, SO_2NH), 8.44 (d, *J* = 2.3 Hz, 1H, Ar-H), 8.19 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.73 (dd, *J* = 8.9, 5.2 Hz, 2H, Ar-H), 7.50 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.38 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.34 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.21 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 7.17 (d, *J* = 0.8 Hz, 2H, Ar-H), 7.15 (s, 1H, Ar-H), 6.42 (d, *J* = 1.9 Hz, 1H, Ar-H), 4.85 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3). HRMS (DART-TOF) calculated for $C_{26}H_{19}ClFN_3O_5S$ $[M + H]^+$ m/z 539.0718, found 539.0703. Purity 97.8% by HPLC.

*4.1.5.24 4-(4-methoxyphenyl)-6-(quinolin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8d-4**)*

23% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, *J* = 2.1 Hz, 1H, Ar-H), 8.09 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.82 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.73 – 7.67 (m, 1H, Ar-H), 7.56 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.33 (dd, *J* = 8.3, 2.1 Hz, 1H, Ar-H), 7.28 – 7.24 (m, 2H, Ar-H), 7.19 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.06 (d, *J* = 8.9 Hz, 2H, Ar-H), 6.76 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.85 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3).^{13}C NMR (101 MHz, $CDCl_3$) δ = 164.32, 159.88, 149.42, 146.99, 145.05, 133.00, 132.95, 132.55, 131.58, 129.72, 129.58, 129.01, 127.91, 127.87, 127.78, 127.20, 123.04, 117.76, 115.74, 115.52, 68.24, 55.51. HRMS (DART-TOF) calculated for $C_{24}H_{18}N_2O_3$ $[M + H]^+$ m/z 382.1317, found 382.1309. Purity 97.6% by HPLC.

4.1.5.25 6-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8d-5**)

27% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (t, *J* = 8.5 Hz, 4H, Ar-H), 7.14 (dd, *J* = 8.3, 2.1 Hz, 1H, Ar-H), 7.06 (t, *J* = 8.7 Hz, 3H, Ar-H), 6.80 (d, *J* = 8.7 Hz, 2H, Ar-H), 6.59 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.79 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 164.63, 159.69, 155.13, 143.96, 135.75, 131.03, 129.77, 128.08, 122.32, 117.18, 115.62, 115.37, 115.14, 68.27, 55.53. HRMS (DART-TOF) calculated for $C_{21}H_{17}NO_4$ $[M + H]^+$ m/z 347.1158, found 347.1143. Purity 96.3% by HPLC.

4.1.5.26

*4-fluoro-N-(5-(4-(4-fluorophenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)benzenesulfonamide (**8e-1**)*

22% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (dd, *J* = 6.6, 2.2 Hz, 2H, Ar-H), 7.73 (dd, *J* = 8.9, 5.0 Hz, 2H, Ar-H), 7.55 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.29 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.13 (d, *J* = 1.7 Hz, 2H, Ar-H), 7.08 (t, *J* = 8.5 Hz, 2H, Ar-H), 6.91 (s, 1H, Ar-H), 6.51 (s, 1H, Ar-H), 4.81 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.40 (d, J_{C-F} = 256.3 Hz), 164.08, 153.75, 144.80, 139.74, 134.99, 134.96, 134.55 (d, J_{C-F} = 106 Hz), 132.13, 130.82, 130.44, 130.13, 129.96, 129.84 (d, J_{C-F} = 9.5 Hz), 126.34, 122.80(d, J_{C-F} = 13.5 Hz), 120.78, 117.84, 116.38 (d, J_{C-F} = 22.7 Hz), 115.01, 68.19, 53.99. HRMS (DART-TOF) calculated for $C_{26}H_{19}F_2N_3O_5S$ $[M + H]^+$ m/z 523.1013, found 523.1011. Purity 99.3% by HPLC.

4.1.5.27

*4-fluoro-N-(3-(4-(4-fluorophenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)phenyl)benzenesulfonamide (**8e-2**)*

26% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 7.75 (dd, *J* = 8.8, 5.1 Hz, 2H, Ar-H), 7.69 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.48 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.35 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.24 (t, *J* = 7.9 Hz, 1H, Ar-H), 7.18 (s, 2H, Ar-H), 7.10 (s, 1H, Ar-H), 7.06 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.01 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 4.87 (s, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO) δ 164.73 (d, J_{C-F} = 250 Hz), 164.40, 144.80, 139.80 (d, J_{C-F} = 188.7 Hz), 136.39, 136.36, 135.03, 134.61, 133.90, 131.47, 131.28, 130.56, 130.36, 130.06 (d, J_{C-F} = 9.6 Hz), 122.47 (d, J_{C-F} = 13.5 Hz), 119.13, 117.81 (d, J_{C-F} = 9.4 Hz), 116.89 (d, J_{C-F} = 22.8 Hz), 114.53, 58.26. HRMS (DART-TOF) calculated for $C_{26}H_{18}F_2N_2O_4S$ $[M + H]^+$ m/z 492.0955, found 492.0949. Purity 98.3% by HPLC.

4.1.5.28

*N-(2-chloro-5-(4-(4-fluorophenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)-4-fluorobenzenesulfonamide (**8e-3**)*

23% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 2.2 Hz, 1H, Ar-H), 8.07 – 8.01 (m, 1H, Ar-H), 7.95 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.72 (dd, *J* = 8.9, 4.9 Hz, 2H, Ar-H), 7.57 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.30 (dt, *J* = 11.7, 4.2 Hz, 3H, Ar-H), 7.19 – 7.16 (m, 2H, Ar-H), 7.11 (t, *J* = 8.5 Hz, 2H, Ar-H), 6.53 (d, *J* = 2.8 Hz, 1H, Ar-H), 4.84 (s, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{25}H_{16}ClF_2N_3O_4S$ $[M + H]^+$ m/z 527.0518, found 527.0516. Purity 97.3% by HPLC.

*4.1.5.29 4-(4-fluorophenyl)-6-(quinolin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8e-4**)*

32% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, *J* = 2.2 Hz, 1H, Ar-H), 8.07 (dd, *J* = 9.2, 5.4 Hz, 2H, Ar-H), 7.82 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.73 – 7.66 (m, 1H, Ar-H), 7.59 – 7.51 (m, 3H, Ar-H), 7.39 – 7.28 (m, 3H, Ar-H), 7.20 (d, *J* = 8.3 Hz, 1H, Ar-H), 6.73 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.84 (s, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.07, 149.49, 146.19 (d, J_{C-F} = 220.2 Hz), 135.14, 133.98, 132.84, 132.81 (d, J_{C-F} = 20 Hz), 131.02, 130.52, 130.09, 129.55, 129.23, 127.91, 127.80, 127.18, 123.43, 117.99, 117.34 (d, J_{C-F} = 23.0 Hz), 115.64, 68.21. HRMS (DART-TOF) calculated for $C_{23}H_{15}FN_2O_2$ $[M + H]^+$ m/z 370.1118, found 370.1116. Purity 98.7% by HPLC.

*4.1.5.30 4-(4-fluorophenyl)-6-(4-hydroxyphenyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8e-5**)*

24% isolated yield, white powder. ^{1}H NMR (400 MHz, MeOD) δ 7.60 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.37 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.20 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar-H), 7.16 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.08 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.75 (dd, *J* = 8.8, 2.3 Hz, 1H, Ar-H), 6.53 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.78 (s, 1H, CH_2). HRMS (DART-TOF) calculated for $C_{20}H_{14}FNO_3$ $[M + H]^+$ m/z 335.0958, found

335.0939. Purity 98.5% by HPLC.

*4.1.5.31 4-fluoro-N-(2-methoxy-5-(4-(3-methoxyphenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4] oxazin-6-yl)pyridin-3-yl)benzenesulfonamide (**8f-1**)*

28% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.15 (d, *J*=2.2, 1H, Ar-H), 7.94 (dd, *J*=8.8, 5.0, 4H, Ar-H), 7.48 (t, *J*=8.1, 1H, Ar-H), 7.44 (d, *J*=2.2, 1H, Ar-H), 7.20 (t, *J*=8.5, 4H, Ar-H), 7.12 (d, *J*=8.3, 1H, Ar-H), 7.03 (ddd, *J*=16.9, 8.3, 2.1, 2H, Ar-H), 6.91 (d, *J*=7.8, 1H, Ar-H), 6.86 (s, 1H, Ar-H), 6.58 (d, *J*=1.8, 1H, Ar-H), 4.82 (s, 2H, CH_2), 3.83 (d, *J*=10.0, 3H, OCH_3), 3.59 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.98 (d, J_{C-F} = 257.6 Hz), 164.70, 163.91, 161.02, 160.70, 146.86, 144.85, 139.70, 136.49, 135.49, 135.45, 131.76 (d, J_{C-F} = 9.7 Hz,), 131.15, 130.94, 130.89, 130.32, 122.55, 120.71, 117.75, 117.68, 116.14 (d, J_{C-F} = 22.8 Hz), 115.05, 115.02, 114.36, 68.18, 55.51, 53.72. HRMS (DART-TOF) calculated for $C_{27}H_{22}FN_3O_6S$ $[M + H]^+$ m/z 535.1213, found 535.1211. Purity 97.8% by HPLC.

4.1.5.32

*4-fluoro-N-(3-(4-(3-methoxyphenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)phenyl)benz enesulfonamide (**8f-2**)*

33% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 8.8, 5.0 Hz, 2H, Ar-H), 7.45 (t, *J* = 8.1 Hz, 1H, Ar-H), 7.18 (t, *J* = 7.8 Hz, 1H, Ar-H), 7.12 – 6.98 (m, 7H, Ar-H), 6.96 – 6.89 (m, 2H, Ar-H), 6.85 (t, *J* = 2.1 Hz, 1H, Ar-H), 6.57 (d, *J* = 1.4 Hz, 1H, Ar-H), 4.81 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.21 (d, J_{C-F} = 254 Hz), 164.26, 160.95, 144.67, 141.50, 136.88, 136.59, 135.02, 134.99, 130.86, 130.73, 129.99 (d, J_{C-F} = 9.4 Hz), 129.75, 123.73, 122.92, 120.78, 119.76, 119.39, 117.39, 116.34 (d, J_{C-F} = 22.7 Hz), 115.50, 115.07, 114.29, 68.19, 55.51. HRMS (DART-TOF) calculated for $C_{27}H_{21}FN_2O_5S$ $[M + H]^+$ m/z 504.1155, found 504.1149. Purity 97.9% by HPLC.

4.1.5.33

*N-(2-chloro-5-(4-(3-methoxyphenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)-4-fluorobenzenesulfonamide (**8f-3**)*

23% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.56 (s, 1H, Ar-H), 8.60 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.96 (dd, *J* = 9.0, 5.0 Hz, 2H, Ar-H), 7.69 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.61 – 7.47 (m, 3H, Ar-H), 7.35 (dd, *J* = 8.3, 2.1 Hz, 1H, Ar-H), 7.26 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.10 (dd, *J* = 8.7, 2.9 Hz, 1H, Ar-H), 7.01 (t, *J* = 2.1 Hz, 1H, Ar-H), 6.96 (d, *J* = 10.3 Hz, 1H, Ar-H), 6.62 (d, *J* = 2.1 Hz, 1H, Ar-H), 4.90 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3). HRMS (DART-TOF) calculated for $C_{25}H_{17}ClF_2N_3O_4S$ $[M + H]^+$ m/z 528.0596, found 528.0579. Purity 98.5% by HPLC.

*4.1.5.34 4-(3-methoxyphenyl)-6-(quinolin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8f-4**)*

27% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H, Ar-H), 8.23 – 8.08 (m, 2H, Ar-H), 7.84 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.72 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.58 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.47 (t, *J* = 8.1 Hz, 1H, Ar-H), 7.34 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar-H), 7.20 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.03 (dd, *J* = 8.4, 2.4 Hz, 1H, Ar-H), 6.96 – 6.92 (m, 1H, Ar-H), 6.88 (t, *J* = 2.1 Hz, 1H, Ar-H), 6.77 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.85 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 163.91, 161.05, 145.09, 136.47, 131.28, 130.96, 129.92, 127.93, 127.46, 123.18, 120.68, 117.85, 115.77, 115.00, 114.42, 68.21, 55.49. HRMS (DART-TOF) calculated for $C_{24}H_{18}N_2O_3$ $[M + H]^+$ m/z 382.1317, found 382.1309. Purity 97.5% by HPLC.

*4.1.5.35 6-(4-hydroxyphenyl)-4-(3-methoxyphenyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**8f-5**)*

33% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 9.69 (s, 1H, Ar-H), 7.49 (t, *J* = 8.1 Hz, 1H, Ar-H), 7.20 – 7.07 (m, 5H, Ar-H), 7.00 – 6.92 (m, 2H, Ar-H), 6.75 (d, *J* = 8.6 Hz, 2H,

Ar-H), 6.43 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.82 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3). HRMS (DART-TOF) calculated for $C_{20}H_{15}FNO_3$ $[M + H]^+$ m/z 336.1036, found 336.1029. Purity 97.3% by HPLC.

4.1.5.36

*4-fluoro-N-(5-(4-isopropyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)benzenesulfonamide (**13a-1**)*

22% isolated yield, white powder. 1H NMR (400 MHz, DMSO) δ 10.05 (s, 1H, SO_2NH), 8.27 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.81 (dd, *J* = 9.0, 5.2 Hz, 2H, Ar-H), 7.79 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.42 (t, *J* = 8.9 Hz, 2H, Ar-H), 7.36 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.21 (dd, *J* = 8.3, 1.9 Hz, 1H, Ar-H), 7.12 (d, *J* = 8.3 Hz, 1H, Ar-H), 4.70 (dt, *J* = 13.8, 6.8 Hz, 1H, CH), 4.55 (s, 2H, CH_2), 3.66 (s, 3H, OCH_3), 1.50 (d, *J* = 6.9 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.78, 165.4 (d, J_{C-F} = 254 Hz), 154.00, 146.34, 140.02, 135.18, 135.15, 132.00, 130.43, 129.93 (d, J_{C-F} = 9.5 Hz), 129.83, 126.94, 122.24, 120.83, 117.96, 116.38 (d, J_{C-F} = 22.7 Hz), 114.66, 68.63, 54.00, 47.52, 19.98. HRMS (DART-TOF) calculated for $C_{23}H_{22}FN_3O_5S$ $[M + H]^+$ m/z 471.1264, found 471.1246. Purity 97.2% by HPLC.

*4.1.5.37 4-fluoro-N-(3-(4-isopropyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)phenyl) benzenesulfonamide (**13a-2**)*

33% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (dd, *J* = 8.8, 5.0 Hz, 2H, Ar-H), 7.66 (s, 1H, Ar-H), 7.35 – 7.25 (m, 3H, Ar-H), 7.11 (dt, *J* = 7.8, 5.7 Hz, 4H, Ar-H), 7.04 (d, *J* = 8.3 Hz, 1H, Ar-H), 4.76 (tt, *J* = 9.1, 4.5 Hz, 1H, CH), 4.53 (s, 2H, CH_2), 1.57 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.06, 165.3 (d, J_{C-F} = 254 Hz), 146.34, 141.91, 137.04, 135.15, 135.12, 135.07, 130.09, 129.96 (d, J_{C-F} = 6.7 Hz), 129.57, 124.01, 122.62, 120.22, 120.02, 117.74, 116.39 (d, J_{C-F} = 22.6 Hz), 114.98, 68.63, 47.68, 19.98. HRMS (DART-TOF) calculated for $C_{23}H_{21}FN_2O_4S$ $[M + H]^+$ m/z 440.1206, found 440.1205. Purity 96.3% by HPLC.

4.1.5.38

*N-(2-chloro-5-(4-isopropyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)-4-fluoro benzenesulfonamide (**13a-3**)*

25% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, *J* = 1.5 Hz, 1H, Ar-H), 8.13 (d, *J* = 1.4 Hz, 1H, Ar-H), 7.82 (dd, *J* = 8.8, 4.9 Hz, 2H, Ar-H), 7.21 – 7.10 (m, 4H, Ar-H), 7.03 (s, 1H, Ar-H), 4.81 (dt, *J* = 13.8, 6.9 Hz, 1H, CH), 4.56 (s, 2H, CH_2) 1.60 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.74 (d, J_{C-F} = 256 Hz), 165.56, 147.25, 143.54, 141.03, 136.57, 134.70, 130.43, 130.39, 130.15, 130.00, 129.51 (d, J_{C-F} = 9 Hz), 122.64, 118.30, 116.84 (d, J_{C-F} = 23 Hz), 114.88, 68.59, 47.63, 20.00. HRMS (DART-TOF) calculated for $C_{22}H_{19}ClFN_3O_4S$ $[M + H]^+$ m/z 475.0769, found 475.0757. Purity 99.3% by HPLC.

*4.1.5.39 4-isopropyl-6-(quinolin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**13a-4**)*

22% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ = 9.14 (d, *J*=2.3, 1H, Ar-H), 8.51 (d, *J*=2.1, 1H, , Ar-H), 8.06 (dd, *J*=8.0, 3.2, 2H, Ar-H), 7.81 – 7.73 (m, 1H, Ar-H), 7.65 (t, *J*=7.5, 1H, Ar-H), 7.44 (dd, *J*=8.3, 2.2, 1H, Ar-H), 7.33 (d, *J*=2.1, 1H, Ar-H), 7.13 (d, *J*=8.3, 1H, Ar-H), 4.76 (tt, *J* = 9.1, 4.5 Hz, 1H, CH), 4.66 (s, 2H, CH_2), 1.57 (d, *J* = 7.0 Hz, 6H, CH_3). HRMS (DART-TOF) calculated for $C_{20}H_{18}N_2O_2$ $[M + H]^+$ m/z 318.1368, found 318.1356. Purity 98.3% by HPLC.

*4.1.5.40 6-(4-hydroxyphenyl)-4-isopropyl-2H-benzo[b][1,4]oxazin-3(4H)-one (**13a-5**)*

34% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.14 (s, 1H, Ar-H), 8.24 (d, *J*=2.1, 1H, Ar-H), 8.16 (d, *J*=8.4, 1H, Ar-H), 7.90 (d, *J*=8.1, 1H, Ar-H), 7.74 (t, *J*=7.6, 1H, Ar-H), 7.60 (t, *J*=7.5, 1H, Ar-H), 7.43 (d, *J*=1.8, 1H, Ar-H), 7.33 (dd, *J*=8.2, 1.8, 1H, Ar-H), 7.16 (d, *J*=8.2, 1H, Ar-H), 4.91 – 4.77 (m, 1H, CH), 4.56 (s, 2H, CH_2), 1.62 (d, *J*=7.0, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ

= 165.74, 149.61, 147.27, 146.65, 133.33, 133.01, 132.80, 130.08, 129.56, 129.25, 127.93, 127.25, 122.96, 118.13, 115.23, 68.67, 47.75, 20.01. HRMS (DART-TOF) calculated for $C_{17}H_{17}NO_3$ [M + H]$^+$ m/z 283.1208, found 283.1205. Purity 96.3% by HPLC.

4.1.5.41

*N-(5-(4-cyclohexyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)-4-fluorobenzenesulfonamide (**13b-1**)*

31% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.96 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.83 (dd, *J* = 8.8, 5.0 Hz, 2H, Ar-H), 7.18 – 7.04 (m, 4H, Ar-H), 7.00 (s, 1H, Ar-H, Ar-H), 4.52 (s, 2H, CH_2), 4.27 (ddd, *J* = 15.5, 9.7, 6.1 Hz, 1H, CH), 3.88 (s, 3H, OCH_3), 2.37 (tt, *J* = 12.6, 6.5 Hz, 2H, CH_2), 1.89 (dd, *J* = 18.5, 15.3 Hz, 4H, CH_2), 1.73 (d, *J* = 12.3 Hz, 1H, CH_2), 1.49 – 1.34 (m, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.99, 165.44 (d, $J_{C\text{-}F}$ = 255 Hz), 153.86, 146.50, 139.83, 135.20, 135.17, 131.90, 130.46, 130.43, 129.89 (d, $J_{C\text{-}F}$ = 9.5 Hz), 126.72, 122.21, 120.91, 117.93, 116.53, 116.41 (d, $J_{C\text{-}F}$ = 22.7 Hz), 68.80, 56.95, 54.05, 29.65, 26.42, 25.35. HRMS (DART-TOF) calculated for $C_{26}H_{26}FN_3O_5S$ [M + H]$^+$ m/z 511.1577, found 511.1559. Purity 98.3% by HPLC.

4.1.5.42

*N-(3-(4-cyclohexyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)phenyl)-4-fluorobenzenesulfonamide (**13b-2**)*

28% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (dd, *J* = 8.9, 5.0 Hz, 2H, Ar-H), 7.34 – 7.27 (m, 4H, Ar-H), 7.16 – 7.08 (m, 3H, Ar-H), 7.05 (t, *J* = 7.4 Hz, 2H, Ar-H), 4.52 (s, 2H, CH_2), 4.29 – 4.16 (m, 1H, CH), 2.37 (tt, *J* = 12.7, 6.5 Hz, 2H, CH_2), 1.99 – 1.79 (m, 4H, CH_2), 1.71 (d, *J* = 12.6 Hz, 1H, CH_2), 1.48 – 1.20 (m, 3H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.15, 165.3 (d, $J_{C\text{-}F}$ = 255 Hz), 146.51, 142.02, 136.96, 135.20, 135.17, 134.97, 130.23, 130.04, 129.96 (d, $J_{C\text{-}F}$ = 2.5 Hz), 124.00, 122.57, 120.04, 119.94, 117.70, 116.43 (d, $J_{C\text{-}F}$ = 22.7 Hz), 115.12, 68.81, 57.06, 29.61, 26.39, 25.37. HRMS (DART-TOF) calculated for $C_{26}H_{25}FN_2O_4S$ [M + H]$^+$ m/z 480.1519, found 480.1503. Purity 98.3% by HPLC.

4.1.5.43

*N-(2-chloro-5-(4-cyclohexyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)-4-fluorobenzenesulfonamide (**13b-3**)*

36% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, *J* = 1.5 Hz, 1H, Ar-H), 8.13 (d, *J* = 1.4 Hz, 1H, Ar-H), 7.82 (dd, *J* = 8.8, 4.9 Hz, 2H, Ar-H), 7.21 – 7.10 (m, 4H, Ar-H), 7.03 (s, 1H, Ar-H), 4.56 (s, 2H, CH_2) 4.29 – 4.16 (m, 1H, CH), 2.37 (tt, *J* = 12.7, 6.5 Hz, 2H, CH_2), 1.99 – 1.79 (m, 4H, CH_2), 1.71 (d, *J* = 12.6 Hz, 1H, CH_2), 1.48 – 1.20 (m, 3H, CH_2). HRMS (DART-TOF) calculated for $C_{25}H_{23}ClFN_3O_4S$ [M + H]$^+$ m/z 515.1082, found 515.1065. Purity 97.3% by HPLC.

*4.1.5.44 4-cyclohexyl-6-(quinolin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**13b-4**)*

22% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ = 9.14 (d, *J*=2.3, 1H, Ar-H), 8.51 (d, *J*=2.1, 1H, , Ar-H), 8.06 (dd, *J*=8.0, 3.2, 2H, Ar-H), 7.81 – 7.73 (m, 1H, Ar-H), 7.65 (t, *J*=7.5, 1H, Ar-H), 7.44 (dd, *J*=8.3, 2.2, 1H, Ar-H), 7.33 (d, *J*=2.1, 1H, Ar-H), 7.13 (d, *J*=8.3, 1H, Ar-H), 4.56 (s, 2H, CH_2) 4.29 – 4.16 (m, 1H, CH), 2.37 (tt, *J* = 12.7, 6.5 Hz, 2H, CH_2), 1.99 – 1.79 (m, 4H, CH_2), 1.71 (d, *J* = 12.6 Hz, 1H, CH_2), 1.48 – 1.20 (m, 3H, CH_2). HRMS (DART-TOF) calculated for $C_{23}H_{22}N_2O_2$ [M + H]$^+$ m/z 358.1681, found 358.1663. Purity 99.3% by HPLC.

*4.1.5.45 4-cyclohexyl-6-(4-hydroxyphenyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**13b-5**)*

36% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.14 (d, *J*=2.1, 1H, Ar-H), 8.24 (d,

J=1.9, 1H, Ar-H), 8.15 (d, *J*=8.4, 1H, Ar-H), 7.91 (d, *J*=8.1, 1H, Ar-H), 7.80 – 7.71 (m, 1H, Ar-H), 7.66 – 7.57 (m, 1H, Ar-H), 7.45 (d, *J*=1.7, 1H, Ar-H), 7.32 (dd, *J*=8.2, 1.9, 1H, Ar-H), 7.15 (d, *J*=8.2, 1H, Ar-H), 4.55 (s, 2H, CH_2), 4.28 (ddd, *J*=12.3, 8.0, 3.4, 1H, CH), 2.42 (qd, *J*=12.7, 3.7, 2H, CH_2), 1.91 (dd, *J*=15.4, 6.7, 4H, CH_2), 1.73 (d, *J*=12.5, 1H, CH_2), 1.50 – 1.21 (m, 3H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.96, 149.65, 147.30, 146.79, 133.34, 132.96, 132.77, 130.63, 129.55, 129.28, 127.96, 127.23, 122.98, 118.08, 115.41, 68.83, 57.11, 29.62, 26.40, 25.38. HRMS (DART-TOF) calculated for $C_{20}H_{21}NO_3$ $[M + H]^+$ m/z 323.1521, found 323.1509. Purity 98.1% by HPLC.

4.1.5.46

*N-(5-(4-cyclopropyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-2-methoxypyridin-3-yl)-4-fluorobenzenesulfonamide (**13c-1**)*

23% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.06 (s, 1H, SO_2NH), 8.25 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.83 (dd, *J* = 8.9, 5.2 Hz, 2H, Ar-H), 7.78 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.50 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.42 (t, *J* = 8.9 Hz, 2H, Ar-H), 7.22 (dd, *J* = 8.3, 2.1 Hz, 1H, Ar-H), 7.07 (d, *J* = 8.3 Hz, 1H, Ar-H), 4.62 (s, 2H, CH_2), 3.68 (s, 3H, OCH_3), 2.87 – 2.80 (m, 1H, CH), 1.15 – 1.09 (m, 2H, CH2), 0.71 – 0.61 (m, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{23}H_{20}FN_3O_5S$ $[M + H]^+$ m/z 469.1108, found 469.1105. Purity 95.3% by HPLC.

4.1.5.47

*N-(3-(4-cyclopropyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)phenyl)-4-fluorobenzenesulfonamide (**13c-2**)*

27% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.46 (s, 1H, SO_2NH), 7.86 (dd, *J* = 8.9, 5.1 Hz, 2H, Ar-H), 7.47 – 7.39 (m, 3H, Ar-H), 7.37 – 7.27 (m, 3H, Ar-H), 7.14 (dd, *J* = 8.3, 2.1 Hz, 1H, Ar-H), 7.10 – 7.01 (m, 2H, Ar-H), 4.62 (s, 2H, CH_2), 2.86 – 2.77 (m, 1H, CH), 1.15 – 1.06 (m, 2H, CH_2), 0.72 – 0.61 (m, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 167.33, 165.3 (d, $J_{C\text{-}F}$ = 255 Hz), 145.40, 141.97, 136.80, 135.17, 134.91, 130.45, 130.06 (d, $J_{C\text{-}F}$ = 9.4 Hz), 129.93, 124.19, 122.52, 120.37, 120.30, 117.28, 116.41 (d, $J_{C\text{-}F}$ = 22.7 Hz), 115.07, 68.44, 29.70, 24.17, 9.16. HRMS (DART-TOF) calculated for $C_{23}H_{19}FN_2O_4S$ $[M + H]^+$ m/z 438.1050, found 438.1039. Purity 98.6% by HPLC.

4.1.5.48

*N-(2-chloro-5-(4-cyclopropyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)pyridin-3-yl)-4-fluorobenzenesulfonamide (**13c-3**)*

31% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ 10.06 (s, 1H, SO_2NH), 8.25 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.83 (dd, *J* = 8.9, 5.2 Hz, 2H, Ar-H), 7.78 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.50 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.42 (t, *J* = 8.9 Hz, 2H, Ar-H), 7.22 (dd, *J* = 8.3, 2.1 Hz, 1H, Ar-H), 7.07 (d, *J* = 8.3 Hz, 1H, Ar-H), 4.62 (s, 2H, CH_2), 2.87 – 2.80 (m, 1H, CH), 1.15 – 1.09 (m, 2H, CH2), 0.71 – 0.61 (m, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{22}H_{17}ClFN_3O_4S$ $[M + H]^+$ m/z 473.0612, found 473.0601. Purity 97.9% by HPLC.

*4.1.5.49 4-cyclopropyl-6-(quinolin-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**13c-4**)*

21% isolated yield, white powder. ^{1}H NMR (400 MHz, DMSO) δ = 9.14 (d, *J*=2.3, 1H, Ar-H), 8.51 (d, *J*=2.1, 1H, , Ar-H), 8.06 (dd, *J*=8.0, 3.2, 2H, Ar-H), 7.81 – 7.73 (m, 1H, Ar-H), 7.65 (t, *J*=7.5, 1H, Ar-H), 7.44 (dd, *J*=8.3, 2.2, 1H, Ar-H), 7.33 (d, *J*=2.1, 1H, Ar-H), 7.13 (d, *J*=8.3, 1H, Ar-H), 4.62 (s, 2H, CH_2), 2.87 – 2.80 (m, 1H, CH), 1.15 – 1.09 (m, 2H, CH2), 0.71 – 0.61 (m, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{20}H_{16}N_2O_2$ $[M + H]^+$ m/z 316.1212, found 316.1206. Purity 98.5% by HPLC.

*4.1.5.50 4-cyclopropyl-6-(4-hydroxyphenyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**13c-5**)*

32% isolated yield, white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.16 (d, *J*=2.2, 1H, OH), 8.28 (d, *J*=2.0, 1H, Ar-H), 8.17 (d, *J*=8.5, 1H, Ar-H), 7.90 (d, *J*=7.5, 1H, Ar-H), 7.90 (d, *J*=7.5, 1H, Ar-H), 7.78 – 7.71 (m, 1H, Ar-H), 7.78 – 7.71 (m, 1H, Ar-H), 7.64 (d, *J*=2.0, 1H, Ar-H), 7.61 (dd, *J*=11.0, 4.0, 1H, Ar-H), 7.34 (dd, *J*=8.3, 2.0, 1H, Ar-H), 7.12 (d, *J*=8.3, 1H, Ar-H), 4.63 (s, 2H, CH_2), 2.84 (ddd, *J*=10.7, 7.0, 3.9, 1H, CH), 1.15 – 1.09 (m, 2H, CH2), 0.71 – 0.61 (m, 2H, CH_2). HRMS (DART-TOF) calculated for $C_{17}H_{15}NO_3$ $[M + H]^+$ m/z 281.1052, found 281.1041. Purity 97.8% by HPLC.

4.2 PI3K Inhibition Assays.

The PI3K activity assay was conducted by Sundia MediTech Company, Ltd. (China). Briefly, the compound, PI3K enzyme (PI3Kα，PIK3Cβ，PIK3Cγ，PIK3Cδ from Carna), the PIP2 (Life) substrate, and ATP (Km, Sigma) were diluted in kinase buffer to the indicated concentrations. The assay plate was covered and incubated at room temperature (PI3Kα, for 1 h and PI3Kβ, PI3Kγ，PI3Kδ for 2 h). Then, ADP-Glo reagent (Promega) was added and shaken slowly，Eqilibrate at room temperature for 40 min, followed by the addition of kinase detection reagent, shaken for 1 min, and equilibrated at room temperature for 60 min. The data were collected on PerkinElmer EnVision Reader and presented in Excel. The curves were fitted by Graphpad Prism 5.0.

4.3 mTOR Inhibition Assays.

The mTOR inhibitory activity was evaluated by monitoring the phosphorylation of mTOR's substrate 4EBP1, which was accomplished by Sundia MediTech Company, Ltd. (China). In short, the compound, mTOR (Millipore), ULight-4E-BP1 peptide substrate (PE), and ATP (Km, Sigma) were diluted in kinase buffer to the indicated concentrations. The assay plate was covered and incubated at room temperature for 30 min. Then, the kinase quench buffer (8 mM EDTA) and Eu-antiphospho-4E-BP1 antibody (2 nM) were added. The mixtures were then incubated for 60min at room temperature. The data were collected on PerkinElmer EnVision Reader.

4.4 Kinase Inhibition Assays.

Kinase inhibition profiles were determined using Kinase profiling services provided by Reaction Biology Corporation, and reactions were carried out at 10 μM ATP. The binding affinities of **8d-1** projected on the human kinome tree were generated using the online Kinome Render program.

4.5 Cell Viability Assay.

The human cancer cell lines used were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured at 37 ℃ with 5% CO_2 in RPMI 1640 or DMEM, supplemented with 10% (v/v) fetal bovine serum (Gibco) and 1% (v/v) penicillin− streptomycin (HyClone). Cells in logarithmic phase were seeded in a 96-well plate at 2−5 × 10^3 cells per well for 24 h (37 °C, 5% CO_2). Then, an equal volume of medium containing various concentrations of test compounds was added to each well. After 72 h, MTT was added, and the cells were incubated for an additional 1−4 h. The absorbance values (OD) of the 96-well plate was measured at 450 nm using a Spectra MAX M5 microplate spectrophotometer (Molecular Devices). The IC_{50} values were the means of at least three independent experiments and calculated by GraphPad Prism 5 software.

4.6 Western Blot Analysis.

Hela cells were treated with **8d-1** at the concentrations of 2.5, 5 and 10μM for 24 h at 37 °C, then the cells were harvested, washed in ice-cold PBS, and lysed with RIPA buffer, protease inhibitors, phosphatase cocktails A and B, and PMSF (1 mM). Protein concentration was determined by the BCA Protein Assay Kit (Beyotime#p0012s). The samples were subjected to SDS−PAGE and then transferred onto PVDF membranes (Millpore). The membranes were incubated overnight at 4 ℃ with the primary antibody in 5% BSA/TBST buffer with gentle shaking, then washed with 1 × TBS/T 3 times, followed by incubation for 1 h with a 1/5000 dilution of secondary HRP antibody in 5% nonfat milk/TBST. Primary antibodies to anti pAKTthr308(1/1000 dilution, no. 13038s), pAKTser473 (1/1000 dilution, no. 4058s), S6RP (1/1000 dilution, no. 2217s), pS6RP Ser235/236 (1/1000 dilution, no. 4858p) were from Cell Signaling Technology, and Primary antibodies to β-actin (1/5000 dilution, no. 200068−8F10) from Zen Bio- Science. The target blots were detected with chemiluminescence system.

4.7 Colony formation assay.

To test the survival of Hela treated with **8d-1**, the cells were seeded in a six-well plate (600 cells/well). After 24h incubation, cells were treated with **8d-1** at different concentrations (5 μM, 2.5 μM, 1.25 μM, 0.625 μM, 0.312 μM) and incubated for two weeks. The cell culture was terminated when the cell formed colony was observed, then the supernatant was removed and washed with PBS buffer solution for two times, then it was fixed with 4% polyformaldehyde for 15 min, before the solution was abandoned. Finally, it was stained with 0.5% crystal violet for 20~30 min, then the crystal violet solution was removed and PBS buffer solution was used to scour off the dyeing liquor. Finally, the plates were photographed, and the number of colony formation was analyzed.

4.8 Pharmacokinetic Studies.

Male SD rats (200−220 g, N = 3 per group) were dosed intravenously with 1 mg/kg of **8d-1** prepared in 5% DMA/10% Solutol HS 15/85 % saline, and orally with 10 mg/kg in 0.5% CMC-Na. Blood samples were taken at 0 (prior to dosing), 0.25, 0.5, 1, 2, 4, 6, 12, and 24 h following oral dosing and at 0 (prior to dosing), 0.083, 0.25, 0.5, 1, 2, 4, 8, and 24 h following intravenous dosing. The samples were collected from the jugular vein and stored in ice (0−4 ℃). Plasma was obtained from the blood samples by centrifugation (8000 rpm for 6 min at 2−8 ℃) and stored at −80 ℃. All samples of the compound were determined by LC−MS/MS (Shimadzu; API 4000). The assay lower limit of quantitation (LLOQ) was 1 ng/mL for **8d-1** in plasma. The pharmacokinetic parameters were analyzed by noncompartmental methods using WinNonlin 5.2. (Accomplished by Sichuan XPiscoric Inc.)

4.9 In Vivo Xenograft Studies.

The female BALB/c nude mice were purchased (Beijing HFK Bioscience Co. ltd., Beijing, China). Hela and A549 cells were harvested during the exponential-growth phase and washed twice with serum-free medium. Mice (6−7 weeks old and weighed 18−22 g) were injected subcutaneously with 5×10^6 Hela or A549 cells, which were suspended in 0.1 mL of serum and antibiotic free growth medium. The tumors were allowed to grow to 150−200 mm^3, at which point the mice were divided randomly (6 mice for each group). In the Hela model, the mice were dosed orally with

8d-1 (10, 20, 40, 50 mg/kg/d, dissolved in 10% NMP/90% PEG300, vehicle (10% NMP/90% PEG300), and BEZ235 (positive control, 20 mg/kg/d, dissolved in 10% NMP/90% PEG300). In the A549 model, the mice were dosed orally with **8d-1** (20, 40 mg/kg/d, s dissolved in 10% NMP/90% PEG300), vehicle (10% NMP/90% PEG300), and BEZ235 (positive control, 20 mg/kg/d, dissolved in 10% NMP/90% PEG300). The body weight and tumor volume were measured every 3 days. The tumor volume was determined with Vernier calipers and calculated as follows: tumor volume = $a \times b^2/2$ (a, long diameter; b, short diameter). Percentage of tumor growth inhibition (TGI) was calculated as 100 × {1- [(treated Final day – treated Initial day) / (control Final day – control Initial day)]}. All animal experiments have been approved by Institutional Animal Care and Treatment Committee of Sichuan University in China (IACUC number: 20100318).

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Discovery of 4-phenyl-2H-benzo[*b*][1,4]oxazin-3(4H)-one derivatives as potent and orally active PI3K/mTOR dual inhibitors

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HIGHLIGHTS

1. 50 compounds with 2H-benzo[*b*][1,4]oxazin-3(4H)-one scaffolds were designed and systhesized
2. **8d-1** was potent PI3K/mTOR dual inhibitor with high selectivity
3. Promising antitumor activity in vivo with low toxicity.
4. 4-phenyl-2H-benzo[*b*][1,4]oxazin-3(4H)-one was identified as novel scaffold for developing new PI3K/mTOR dual inhibitors