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ORIGINAL ARTICLE

Synthesis and biological evaluation of *N*-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide derivatives as new BET bromodomain inhibitors for anti-hematologic malignancies activities

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Abstract The bromodomain and extra-terminal proteins (BETs), in particular BRD4, has been reported to play important roles in cancer, inflammation, obesity, cardiovascular disease, and neurological disorders. In this paper, a series of benzomorpholinone derivatives were synthesized and biologically evaluated as BETs inhibitors. Detailed structure–activity relationship studies led to the discovery of several new potent compounds, of which **15h** and **15i** displayed IC_{50} values of 2.8 and 4.5 μM against BRD4 (D1), respectively, and showed good anti-proliferation activities against four hematologic malignancies cell lines at low-micromolar concentrations, including MV4-11, OCI-LY10, Pfeifer, and Su-DHL-6 cells. This chemotype could be further optimized with respect to its potency and drug-like properties in the future.

Keywords BET inhibitor · BRD4 · Anti-proliferation activity · Benzomorpholinone · Structure–activity relationship

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Abbreviation

BRDs	Bromodomain-containing proteins
BET	Bromodomain and extra-terminal
AML	Acute myeloid leukemia
SAR	Structure–activity relationship
MTT	3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2-*H*-tetrazolium bromide

Introduction

Bromodomain-containing proteins (BRDs) are a family of epigenetic protein domains that specifically recognize acetylation lysine residues of histone [1]. The bromodomain and extra-terminal (BET) family belongs to the BRDs family which consist of four subtypes in mammals: BRD2, BRD3, BRD4, and BRDT. Each subtype contains two BRD domains (BD1 and BD2). These BRD domains form deep hydrophobic pockets that recognize acetylated lysine residues and an extra-terminal (ET) domain [2].

Extensive research suggests that the abnormal expression of BRDs protein plays important roles in a wide variety of tumor [3,4]. The testis specific isoform BRDT has been found to be highly expressed in lung cancer [5]. A shRNAs screen identified BRD4 as an essential protein in acute myeloid leukemia cells [6]. Genetic knockdown by RNAi or exposure of cells to BET inhibitors resulted in a significant transcriptional down regulation of c-Myc [7], and the pharmacologic inhibition of BET family of BRDs by BET inhibitors could effectively block c-Myc expression in multiple myeloma [8], Burkitt's lymphoma [9], and mixed lineage leukemia [10]. In addition, BETs inhibitors have also exhibited therapeutic potential beyond cancer, such as inflammation [11], obesity [12], cardiovascular disease [13], and neurological disorders [14].

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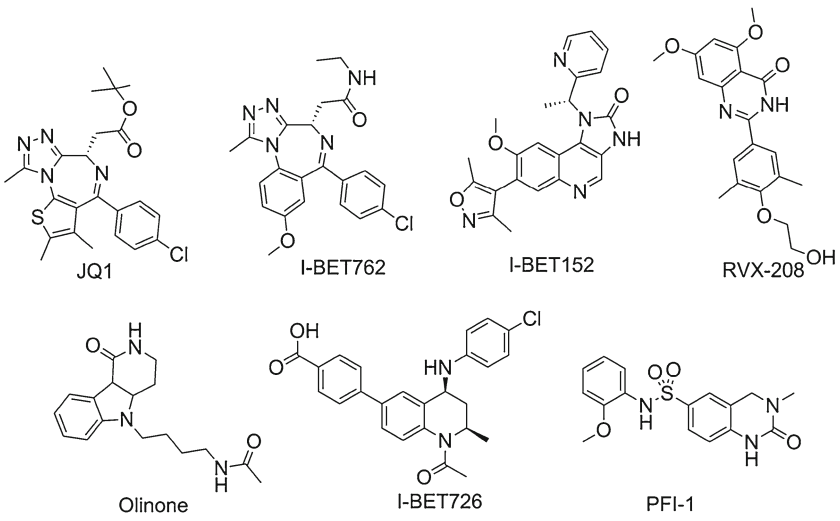


Fig. 1 BET bromodomains inhibitors currently reported

Considering the importance of BET bromodomains as promising targets in the treatment of cancer [10,11], many pharmaceutical research and development institutions have focused their efforts on the development of novel inhibitors of the BET family [15–17]. The main chemical scaffolds of BET inhibitors are shown in Fig. 1. These inhibitors mainly interact with the acetyl-lysine binding site of bromodomain. Even though a number of BET inhibitors have been reported in recent years, the diversity of the chemical scaffolds of these leads is limited, and new BET inhibitors with novel chemotype are worth developing.

PFI-1 (2-methoxy-*N*-(3-methyl-2-oxo-1,2,3,4-tetrahydro -quinazolin-6-yl) -bezenesulfonamide) is a novel lead compound which contains a scaffold of 3,4-dihydro-3-methyl-2(1H)-quinazolinone with an IC_{50} of 0.1–0.5 μM against BRD4 (D1) in a biochemical assay [18,19]. As part of our research program to pursue new scaffolds as BET inhibitors, we first studied the co-crystallization of **PFI-1** with BRD4 (D1) (PDB: 4E96) and found that the cyclic urea of **PFI-1** has a key interaction with Asn140 of BRD4 (D1) and the urea carbonyl also makes a water-bridged hydrogen bond interaction with the phenolic hydroxyl group of Tyr97 of BRD4 (D1) [19]. Then we analyzed the chemical space of **PFI-1** and considered to convert quinazolinone to benzomorpholinone of which the cyclic lactam could mimic the *N*-methyl urea to interact with Asn140, while other parts of the structure of **PFI-1** were kept unchanged. A relatively simple compound **14a**, (2-methyl-*N*-(2-methyl-3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide), was designed and synthesized with an IC_{50} value of 5.4 μM against BRD4 (D1) (Fig. 2). While this compound was less potent than **PFI-1**, we confirmed that this chemotype would be a new compound class for BET inhibition. The synthesis and the structure–activity relationships (SAR) of this new

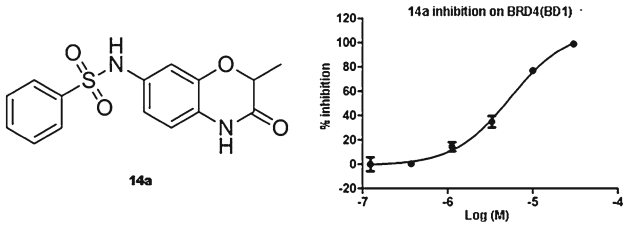


Fig. 2 Structure of the racemic mixture **14a** and the inhibition curve against BRD4 (D1). % inhibition at least two independent titrations

series of benzomorpholinone derivatives will be discussed in this study.

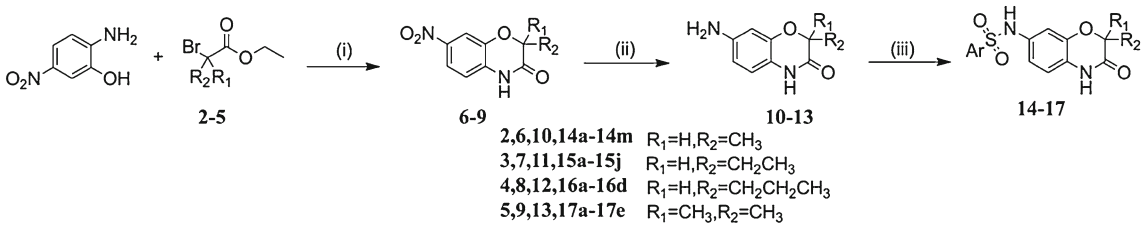
Results and discussion

Synthesis

The general synthesis of *N*-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-7-yl) benzenesulfonamide **14a–14m**, **15a–15j**, **16a–16d**, **17a–17e** are outlined in Scheme 1. 7-Nitro-2*H*-benzo[b][1,4]oxazin-3(4*H*)-ones **6–9** were prepared by cyclization of the α-bromoalkanoic acid ethyl esters **2–5** with 2-amino-5-nitrophenol. Reduction of the nitro group of **6–9** with iron powder and ammonium chloride at reflux temperature provides the corresponding anilines **10–13**, which were further reacted with commercially available aryl sulfonyl chlorides to afford desired final products**14a–14m**, **15a–15j**, **16a–16d**, **17a–17e**.

SAR analysis

Our SAR analysis of the benzomorpholinone derivatives was mainly based on affinity assays results. All of the benzomor-



Scheme 1 Synthesis of benzomorpholinone derivatives. Reagents and conditions: *i* K_2CO_3, DMF, 90 °C, 8–12 h. *ii* Fe, NH_4Cl, CH_3CH_2OH:H_2O = 3:1, reflux. *iii* $ArSO_2Cl$, Triethylamine, Tetrahydrofuran, 0 °C

pholinones were evaluated using the AlphaScreen assay at a single concentration of 10 μM (Table 1). Compounds **14b**–**14g** were synthesized to study the influence of different sulfonyl groups on potency. The replacement of the phenyl group with propyl **14b**, benzyl **14c**, 3-naphthalene **14d**, 2-isoxazole **14e** , and 4-thiophene **14f** did not improve BRD4 inhibitory activity which suggests that a rigid ring with proper size is crucial for potency. The 8-isoquinoline substituted analogue **14g** demonstrated 94% inhibition at 10 μM against BRD4 (D1), which is almost equipotent to **14a**. However, this structure was not selected for further optimization due to its poor water solubility.

Introducing a single substituent on the phenyl ring could in a large part retain the inhibitory activity against BRD4 (D1), as illustrated by **14h**–**14m**. The replacement of methyl on the α-position of the cyclic lactam with an ethyl group could significantly improve the potency as seen in **15e**–**15i**. The introduction of a single substituent at the *ortho*-position of the phenyl ring was favorable for the activity, as demonstrated by **15a**–**15g**. Compounds **15b**–**15g** exhibited comparable potency, suggesting that different electric effect groups on this position were well tolerated. Electron withdrawing groups at the *para*-position of the phenyl ring displayed decreased BRD4 (D1) activity, such as **15h**, **15i** , and **15j**. When a single *n*-propyl group was introduced on the α-position of the cyclic lactam, the potency of these compounds (**16a**–**16d**) decreased significantly. Lastly, a gem-dimethyl substituent also on this position (**17a**–**17e**) was found to be unfavorable for potency. These results suggest that alkyl substituent with two carbon atoms at 2-position of the benzomorpholinone core could be beneficial for BRD4 activity.

BET Bromodomains inhibitory and cytotoxic activity

Compounds which showed inhibition rates of more than 90% at 10 μM against BRD4 (D1) were selected for IC_{50} testing against BRD4 (D1) using the AlphaScreen assay and a cytotoxicity assay against MV4-11, OCI-LY10, Pfeifer, and Su-DHL-6 cell lines using the MTT assay [20]. These results are presented in Table 2. Compounds **15b**, **15c**, **15e**, and **15f**, which contain a single substituent at the *ortho*-position of the phenyl ring, exhibited low-micromolar inhibitory activity against BRD4 (D1) and were better than their *para* substituted analogues **15h** and **15i**. However, compounds **15h** and**15i** displayed better cytotoxicity activities than **15b**, **15c**, **15e**, and **15f**, which showed remarkable inhibitory activities against the above four human cancer cell lines with IC_{50} values ranging from low-micromolar to sub-micromolar values.

The inhibitory activities of the aforementioned compounds against 6 other bromodomains of BETs at the concentration of 10 μM were evaluated subsequently, as shown in Table 3. Almost all the compounds exhibited inhibition rates of more than 90% against BRD2 (D1), BRD2 (D2), BRD3 (D1), and BRD4 (D2), while little inhibitory was observed with respect to BRDT (D1), consistent with **PFI-1** [19], which were also have high selectivity for the BET family of BRDs.

Conclusions

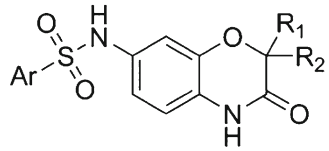
In summary, we have successfully synthesized a series of BET bromodomains inhibitors with potent activities in both molecular and cellular assays based on a benzenesulfonamide scaffold. Our SAR studies revealed that an ethyl group on the α-position of the lactam could significantly improve potency against BRD4. Compounds **15h** and **15i** displayed IC_{50} values of 2.8 and 4.5 μM against BRD4 (D1), respectively, and also showed excellent inhibitory activities against 4 human cancer cell lines at low-micromolar to sub-micromolar concentrations, including MV4-11, OCI-LY10, Pfeifer, and Su-DHL-6 cell lines. Compounds showed strong inhibition at 10 μM against BRD4 (D1) against several human BRDs subtype inhibitory. This work presents the identification of a new chemotype with BRD4 inhibitory activity. Further optimization and mechanism studies on this chemotype are underway.

Materials and methods

General

Chemistry reagents of analytical grade were purchased from Changzheng Chemical Factory, Chengdu, Sichuan, PR China

Table 1 BRD4 (D1) inhibitory profiles of compounds **14a–14m**, **15a–15j**,**16a–16d**, **17a–17e**



No.	Ar	R_1	R_2	Inh% (10 μM)	No.	Ar	R_1	R_2	Inh% (10 μM)
14a	C_6H_5	CH_3	H	96	**15d**	2-NO_2 − 4 − MeO − C_6H_3	CH_2CH_3	H	31
14b	$CH_2CH_2CH_3$	CH_3	H	88	**15e**	2 − Cl − C_6H_4	CH_2CH_3	H	97
14c	$C_6H_5CH_2$	CH_3	H	−1	**15f**	2 − Br − C_6H_4	CH_2CH_3	H	96
14d	2-Naphthalene	CH_3	H	69	**15g**	2 − CF_3 − C_6H_4	CH_2CH_3	H	90
14e	4-3,5-diMeO-Isoxazole	CH_3	H	7	**15h**	4-Me − C_6H_4	CH_2CH_3	H	99
14f	3-2,4-diCl-Thiophene	CH_3	H	3	**15i**	4-Cl − C_6H_4	CH_2CH_3	H	97
14g	8-Isoquinoline	CH_3	H	94	**15j**	4-NO_2 − C_6H_4	CH_2CH_3	H	57
14h	2-Me − C_6H_4	CH_3	H	64	**16a**	2-Cl − C_6H_4	$CH_2CH_2CH_3$	H	13
14i	2-Cl − C_6H_4	CH_3	H	87	**16b**	4-Cl − C_6H_4	$CH_2CH_2CH_3$	H	50
14j	2-Br − C_6H_4	CH_3	H	50	**16c**	2-Br − C_6H_4	$CH_2CH_2CH_3$	H	−4
14k	4-Me − C_6H_4	CH_3	H	90	**16d**	C_6H_5	$CH_2CH_2CH_3$	H	64
14l	4-Cl − C_6H_4	CH_3	H	72	**17a**	4-Me − C_6H_4	CH_3	CH_3	63
14m	4-NO_2-C_6H_4	CH_3	H	31	**17b**	4-NO_2-C_6H_4	CH_3	CH_3	25
15a	C_6H_5	CH_2CH_3	H	21	**17c**	2-NO_2-4-MeO − C_6H_3	CH_3	CH_3	19
15b	2-Me − C_6H_4	CH_2CH_3	H	96	**17d**	2-F − C_6H_4	CH_3	CH_3	83
15c	2-F − C_6H_4	CH_2CH_3	H	97	**17e**	2-CF_3-C_6H_4	CH_3	CH_3	11

All compounds were tested at a concentration of 10 μM. The percent inhibition is an average of at least two independent titrations

Table 2 Bioactivities of selected compounds

No.	IC_{50} (μM)				
	BRD4 (D1) (AlphaScreen™)	MV4-11	OCI-LY10	Pfeifer	Su-DHL-6
14a	5.4 ± 0.2	10.2 ± 0.1	9.4 ± 0.2	7.82 ± 0.2	24.8 ± 0.3
15b	2.1 ± 0.1	7.5 ± 0.1	15.6 ± 0.2	>30	>30
15c	1.5 ± 0.1	9.6 ± 0.2	21 ± 0.3	>30	>30
15e	1.7 ± 0.1	7.5 ± 0.2	17.5 ± 0.2	>30	>30
15f	2.0 ± 0.1	6.3 ± 0.2	18.8 ± 0.1	>30	>30
15h	2.8 ± 0.2	3.7 ± 0.2	2.5 ± 0.1	3.8 ± 0.1	7.4 ± 0.2
15i	4.5 ± 0.3	1.26 ± 0.1	0.56 ± 0.1	0.39 ± 0.1	2.69 ± 0.1
PFI-1	0.3 ± 0.1	0.78 ± 0.1	3.1 ± 0.2	4.4 ± 0.2	12.3 ± 0.2

IC_{50} results are an average of at least two independent titrations

Table 3 BET bromodomains inhibitory profiles of selected compounds

No.	Inh % (10 μM)					
	BRD2 (D1)	BRD2 (D2)	BRD3 (D1)	BRD3 (D2)	BRD4 (D2)	BRDT (D1)
14a	70 ± 2	93 ± 3	94 ± 1	76 ± 1	79 ± 2	−2 ± 2
15b	99 ± 3	98 ± 1	99 ± 2	99 ± 2	98 ± 1	2 ± 3
15c	100 ± 2	99 ± 2	99 ± 2	100 ± 2	99 ± 3	6 ± 3
15e	99 ± 1	97 ± 2	98 ± 3	98 ± 1	95 ± 2	6 ± 3
15f	98 ± 2	96 ± 3	98 ± 3	98 ± 2	95 ± 3	7 ± 2
15h	99 ± 1	95 ± 1	95 ± 1	99 ± 1	86 ± 2	4 ± 3
15i	88 ± 3	81 ± 2	75 ± 2	89 ± 1	54 ± 1	−1 ± 2

All compounds were tested at a concentration of 10 μM. The percent inhibition is an average of at least two independent titrations

and were used without further purification. TLC was performed on 0.20-mm Silica Gel 60 F_{254} plates (Qingdao Ocean Chemical Factory, Shandong, China). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Varian Unity Inova-400 (400/100 MHz) spectrometer using tetramethylsilane (TMS) as internal reference chemical. Chemical shifts (δ) are quoted in ppm relative to TMS as an internal standard, where (δ) TMS = 0.00 ppm. The multiplicity of the signal is indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, defined as all multipeak signals where overlap or complex coupling of signals makes definitive descriptions of peaks difficult. Mass spectra (MS) were measured on a Q-TOF Premier mass spectrometer (Micromass, Manchester, UK) utilizing electrospray ionization (ESI).

Chemistry

2-Methyl-7-nitro-2H-benzo[b][1,4]oxazin-3(4H)-one (**6**)

2-Amino-5-nitrophenol (5 mmol) and potassium carbonate (10 mmol) were mixed in anhydrous dimethylformamide (10 mL). The mixture is stirred at room temperature for 1 h. Then, ethyl 2-bromopropanoate (5 mmol) is slowly added to the reaction mixture and the mixture is stirred at reflux for 8–12 h. After reaction completion (monitored by TLC), it is poured onto ice/water (100 mL), and the resulting precipitate is separated by filtration, washed with water, and dried. Recrystallization from ethanol afforded the desired compound **6** as brown solid (0.65 g, 62.5%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 (s, 1H), 7.88–7.79 (m, 2H), 6.83 (d, J = 8.6 Hz, 1H), 4.69 (q, J = 6.9 Hz, 1H), 1.56 (d, J = 6.8 Hz, 3H); MS (ESI), *m*/*z*: 208.2 $[M+H]^+$.

2-Ethyl-7-nitro-2H-benzo[b][1,4]oxazin-3(4H)-one (**7**)

The title compound was prepared from ethyl 2-bromobutanoate according to the procedure for example **6** and isolated as brown solid (0.82 g, 73.2%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.18 (s, 1H), 7.84 (m, 2H), 6.79 (d, J = 8.6 Hz, 1H), 4.73 (q, J = 6.9 Hz, 1H), 2.14 (m, 2H), 0.94 (d, J = 6.8 Hz, 3H); MS (ESI), *m*/*z*: 246.0 $[M+ Na]^+$.

7-Nitro-2-propyl-2H-benzo[b][1,4]oxazin-3(4H)-one (**8**)

The title compound was prepared from ethyl 2-bromopentanoate according to the procedure for example **6** and isolated as brown solid (0.79 g, 66.6%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 (s, 1H), 7.88–7.79 (m, 2H), 6.83 (d, J = 8.6 Hz, 1H), 4.69 (m, 1H), 1.63 (m, 2H), 1.39 (m, 2H), 0.88 (t, 3H);MS (ESI), *m*/*z*: 259.2 $[M+Na]^+$.

2,2-Dimethyl-7-nitro-2H-benzo[b][1,4]oxazin-3(4H)-one (**9**)

The title compound was prepared from ethyl 2-bromo-2-methylpropanoate according to the procedure for example **6** and isolated as brown solid (0.54 g, 48.7%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.18 (s, 1H), 7.86–7.74 (m, 2H), 6.79 (d, J = 8.6 Hz, 1H), 4.66 (q, 1H), 0.96 (s, 6H);MS (ESI), *m*/*z*: 259.1 $[M+Na]^+$.

7-Amino-2-methyl-2H-benzo[b][1,4]oxazin-3(4H)-one (**10**)

To a solution of **6** (3 mmol) in methanol (20 mL) is added iron powder (15 mmol) followed by an aqueous solution of ammonium chloride (15 mmol) at room temperature. The reaction mixture was stirred and heated at reflux for 1 h and was then allowed to cool to r.t. The solution was filtered and basified with saturated sodium bicarbonate solution. The solution was extracted with ethyl acetate (4 × 100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and the solvent evaporated to give the desired product **10** and isolated as light yellow solid (0.39 g, 73.7%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.16 (s, 1H), 6.54 (d, J = 8.3 Hz, 1H), 6.23–6.12 (m, 2H), 4.85 (s, 2H), 4.36 (dd, J = 8.0, 4.6 Hz, 1H), 1.37 (d, J = 6.8 Hz, 3H).MS (ESI), *m*/*z*: 201.1 $[M+Na]^+$.

7-Amino-2-ethyl-2H-benzo[b][1,4]oxazin-3(4H)-one (**11**)

The title compound was prepared from **7** according to the procedure for example **10** and isolated as light yellow solid (0.56 g, 84.6%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.18 (s, 1H), 6.54 (d, J = 8.3 Hz, 1H), 6.25–6.10 (m, 2H), 4.84 (s, 2H), 4.32 (dd, J = 8.0, 4.6 Hz, 1H), 1.83–1.61 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H); MS (ESI), *m*/*z*: 215.1 $[M+Na]^+$.

7-Amino-2-propyl-2H-benzo[b][1,4]oxazin-3(4H)-one (**12**)

The title compound was prepared from **8** according to the procedure for example **10** and isolated as light yellow solid (0.57 g, 79.9%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.18 (s, 1H), 6.54 (d, J = 8.2 Hz, 1H), 6.20–6.12 (m, 2H), 4.85 (s, 2H), 4.38 (dd, J = 7.8, 5.1 Hz, 1H), 1.72–1.61 (m, 2H), 1.44 (dp, J = 14.4, 7.0 Hz, 2H), 0.90 (t, J = 7.3 Hz, 3H); MS (ESI), *m*/*z*: 207.2 $[M+Na]^+$.

7-Amino-2,2-dimethyl-2H-benzo[b][1,4]oxazin-3(4H)-one (**13**)

The title compound was prepared from **9** according to the procedure for example **10** and isolated as light yellow solid (0.46 g, 68.3%); 1H NMR (400 MHz, DMSO-*d*6) δ 10.20 (s, 1H), 6.52 (d, J = 8.2 Hz, 1H), 6.28–6.05 (m, 2H), 4.84 (s, 2H), 0.95 (d, 6H). MS (ESI); *m*/*z*: 215.1 $[M+H]^+$.

N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide (**14a**)

To a solution of **10** in tetrahydrofuran was added Et_3N (3.0 eq) followed by a benzenesulfonyl chloride (1.0 eq) at 0 °C. The reaction mixture was allowed to warm up to r.t over a period of 1 h and reaction overnight. The reaction mixture was evaporated and dissolved in 10 mL water. 1.0 N HCl was slowly added to the mixture (pH 4), and the resulting solid was filtered and rinsed with water. The crude product was purified on a silica column eluting with petroleum ether/ethyl acetate (7:1) to get **14a** as brown crystals (0.2 g, 62.8%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.54 (s, 1H), 10.11 (s, 1H), 7.72 (d, J = 7.9 Hz, 2H), 7.60 (d, J = 6.7 Hz, 1H), 7.55 (t, J = 7.2 Hz, 2H), 6.74–6.66 (m, 2H), 6.65 (s, 1H), 4.58 (dd, J = 13.4, 6.8 Hz, 1H), 1.35 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.37 (s), 142.96 (s), 139.28 (s), 132.86 (s), 132.63 (s), 129.21 (s), 126.61 (s), 124.33 (s), 115.74 (s), 114.85 (s), 109.24 (s), 72.62 (s), 15.95 (s); MS (ESI), *m*/*z*: 341.3 $[M+Na]^+$.

N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)propane-1-sulfonamide (**14b**)

To a solution of **10** in tetrahydrofuran was added Et_3N (3.0 eq) followed by a benzenesulfonyl chloride (1.0 eq) at 0 °C. The reaction mixture was allowed to warm up to r.t over a period of 1 h and reaction overnight. The reaction mixture was evaporated and dissolved in 10 mL water. 1.0 N HCl was slowly added to the mixture (pH 4), and the resulting solid was filtered and rinsed with water. The crude product was recrystalized from ethanol to obtain desired compound **14b** as brown crystals (0.16 g, 56.7%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.61 (s, 1H), 9.66 (s, 1H), 6.77–6.83 (m, 3H), 4.53 (m, 1H), 3.12 (m, 2H), 1.56–1.68 (m, 5H), 0.96 (m, 3H); ^{13}C NMR(100 MHz, DMSO-*d*6) δ 166.42 (s), 143.21 (s), 133.44 (s), 123.99 (s), 115.92 (s), 114.12 (s), 108.61 (s), 72.69 (s), 51.97 (s), 16.75 (s), 16.02 (s), 12.53 (s). MS (ESI), *m*/*z*: 307.1 $[M+Na]^+$.

N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-1-phenylmethanesulfonamide (**14c**)

The title compound was prepared from **10** and phenylmethanesulfonyl chloride according to the procedure for example **14b** and isolated as brown crystals (0.16 g, 48.7%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.59 (s, 1H), 9.69 (s, 1H), 7.31 (d, J = 32.7 Hz, 5H), 6.81 (s, 3H), 4.64 (s, 1H), 4.41 (s, 2H), 1.41 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.93 (s), 143.68 (s), 134.02 (s), 131.39 (s), 130.01

(s), 128.82 (s), 128.64 (s), 124.20 (s), 116.38 (s), 114.17 (s), 108.75 (s), 73.23 (s), 57.16 (s), 16.55 (s); MS (ESI), *m/z*: 355.2 $[M+Na]^+$.

N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)naphthalene-2-sulfonamide (**14d**)

The title compound was prepared from **10** and naphthalene-2-sulfonyl chloride according to the procedure for example **14a** and isolated as brown solid (0.19 g, 51.6%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.50 (s, 1H), 10.47 (s, 1H), 8.69 (d, J = 8.4 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 7.7 Hz, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.76–7.58 (m, 3H), 6.61 (dd, J = 21.7, 10.7 Hz, 3H), 4.52 (dd, J = 13.5, 6.6 Hz, 1H), 1.30 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.28 (s), 142.91 (s), 134.38 (s), 134.21 (s), 133.68 (s), 132.49 (s), 129.86 (s), 129.05 (s), 128.09 (s), 126.96 (s), 124.46 (s), 124.22 (s), 123.90 (s), 115.72 (s), 113.83 (s), 108.22 (s), 72.58 (s), 15.91 (s); MS (ESI), *m/z*: 391.1 $[M+Na]^+$.

3,5-Dimethyl-N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)isoxazole-4-sulfonamide (**14e**)

The title compound was prepared from **10** and 3,5-dimethylisoxazole-4-sulfonyl chloride according to the procedure for example **14a** and isolated as brown solid (0.2 g, 53.2%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.67 (s, 1H), 10.20 (s, 1H), 6.80 (d, J = 8.9 Hz, 1H), 6.70–6.64 (m, 2H), 4.49 (dd, J = 7.8, 4.4 Hz, 1H), 2.41 (s, 3H), 2.19 (s, 3H), 1.68 (d, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 173.29 (s), 165.93 (s), 157.27 (s), 142.65 (s), 131.54 (s), 125.02 (s), 116.26 (s), 115.80 (s), 115.04 (s), 110.74 (s), 77.13 (s), 23.24 (s), 15.93 (s), 12.05 (s); MS (ESI), *m/z*: 360.2 $[M+Na]^+$.

2,4-Dichloro-N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)thiophene-3-sulfonamide (**14f**)

The title compound was prepared from **10** and 2,4-dichlorothiophene-3-sulfonyl chloride according to the procedure for example **14a** and isolated as brown solid (0.13 g, 33.8%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.64 (s, 1H), 10.52 (s, 1H), 7.29 (s, 1H), 6.80 (d, J = 8.1 Hz, 1H), 6.72 (d, J = 8.0 Hz, 2H), 4.64 (d, J = 6.7 Hz, 1H), 1.38 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.44 (s), 143.00 (s), 135.46 (s), 131.48 (s), 129.65 (s), 126.77 (s), 126.39 (s), 124.93 (s), 115.88 (s), 115.29 (s), 109.76 (s), 72.68 (s), 15.97 (s); MS (ESI), *m/z*: 393.1 $[M+H]^+$.

N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)isoquinoline-8-sulfonamide (**14g**)

The title compound was prepared from **10** and isoquinoline-8-sulfonyl chloride according to the procedure for example **14a** and isolated as brown solid (0.24 g, 64.7%); ^{1}H NMR R (400 MHz, DMSO-*d*6) δ 10.42 (s, 1H), 9.90 (s, 1H), 9.14 (s, 1H), 8.53 (d, J = 7.7 Hz, 1H), 8.30 (dd, J = 19.4, 7.4 Hz, 2H), 7.72 (s, 2H), 6.61 (d, J = 6.2 Hz, 3H), 4.49 (d, J = 6.1 Hz, 1H), 1.29 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.27 (s), 151.45 (s), 142.74 (s), 136.99 (s), 135.02 (s), 134.21 (s), 132.80 (s), 132.08 (s), 128.32 (s), 125.65 (s), 123.91 (s), 122.65 (s), 115.44 (s), 114.30 (s), 108.77 (s), 72.52 (s), 15.88 (s); MS (ESI), *m/z*: 392.1 $[M+Na]^+$.

2-Methyl-N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide (**14h**)

The title compound was prepared from **10** and 2-methylbenzenesulfonyl chloride according to the procedure for example **14a** and isolated as brown crystals (0.24 g, 72.7%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.53 (s, 1H), 10.24 (s, 1H), 7.82 (s, 1H), 7.55 (d, 1H), 7.37 (s, 2H), 6.64–6.70 (d, J = 21.8 Hz, 3H), 4.58 (s, 1H), 2.64(s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.40 (s), 142.92 (s), 136.68 (s), 131.86 (s), 131.55 (s), 129.21 (s), 126.61 (s), 124.33 (s), 115.74 (s), 114.85 (s), 108.40 (s), 72.62 (s), 15.95 (s); MS (ESI), *m/z*: 355.1 $[M+Na]^+$.

2-Chloro-N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide (**14i**)

The title compound was prepared from **10** and 2-chlorobenzenesulfonyl chloride according to the procedure for example **14a** and isolated as brown solid (0.22 g, 58.9%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.52 (s, 1H), 10.44 (s, 1H), 7.99 (d, J = 7.4 Hz, 1H), 7.63 (d, 2H), 7.51 (t, J = 6.2 Hz, 1H), 6.69 (d, J = 11.2 Hz, 3H), 4.62–4.54 (m, 1H), 1.34 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.31 (s), 143.00 (s), 134.64 (s), 131.94 (s), 131.55 (s), 115.77 (s), 108.40 (s), 72.62 (s), 15.95 (s); MS (ESI), *m/z*: 375.5 $[M+Na]^+$.

2-Bromo-N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide (**14j**)

The title compound was prepared from **10** and 2-bromobenzenesulfonyl chloride according to the procedure for example **14a** and isolated as brown solid (0.3 g, 76.3%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.50 (d, J = 30.1 Hz, 2H), 8.03 (s, 1H), 7.83 (s, 1H), 7.54 (s, 2H), 6.70 (s, 3H), 4.59 (s, 1H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, DMSO-

*d*6) δ 166.33 (s), 143.00 (s), 138.09 (s), 135.39 (s), 134.58 (s), 131.99 (s), 131.73 (s), 128.24 (s), 124.10 (s), 119.15 (s), 115.79 (s), 113.88 (s), 108.35 (s), 72.64 (s), 15.95 (s); MS (ESI), *m*/*z*: 419.0 $[M+Na]^+$.

4-Methyl-N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo [b][1,4]oxazin-7-yl)benzenesulfonamide (**14k**)

The title compound was prepared from **10** and 4-methylbenzenesulfonyl chloride according to the procedure for example **14a** and isolated as light brown solid (0.19 g, 57.7%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.55 (s, 1H), 10.06 (s, 1H), 7.60 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 6.75–6.66 (m, 2H), 6.65 (s, 1H), 4.59 (q, J = 6.7 Hz, 1H), 2.34 (s, 3H), 1.36 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6)δ 166.36 (s), 143.16 (s), 142.95 (s), 136.47 (s), 132.82 (s), 129.63 (s), 126.67 (s), 124.19 (s), 115.74 (s), 114.66 (s), 109.06 (s), 72.62 (s), 20.92 (s), 15.95 (s); MS (ESI), *m*/*z*: 355.1 $[M+Na]^+$.

4-Chloro-N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo [b][1,4]oxazin-7-yl)benzenesulfonamide (**14l**)

The title compound was prepared from **10** and 4-chlorobenzenesulfonyl chloride according to the procedure for example **14a** and isolated as brown solid (0.23 g, 64.1%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.57 (s, 1H), 10.18 (s, 1H), 7.70 (d, J = 8.3 Hz, 2H), 7.63 (d, J = 8.3 Hz, 2H), 6.76–6.66 (m, 2H), 6.65 (s, 1H), 4.60 (q, J = 6.8 Hz, 1H), 1.36 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.39 (s), 143.00 (s), 138.08 (s), 137.73 (s), 132.25 (s), 129.40 (s), 128.58 (s), 124.62 (s), 115.82 (s), 115.17 (s), 109.57 (s), 72.64 (s), 15.95 (s); MS (ESI), *m*/*z*: 375.2 $[M+Na]^+$.

N-(2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-4-nitrobenzenesulfonamide (**14m**)

The title compound was prepared from **10** and 4-nitrobenzenesulfonyl chloride according to the procedure for example **14a** and isolated brown solid (0.29 g, 82.1%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.58 (s, 1H), 10.43 (s, 1H), 8.37 (d, J = 8.7 Hz, 2H), 7.95 (d, J = 8.7 Hz, 2H), 6.74 (d, J = 9.0 Hz, 1H), 6.67 (d, 2H), 4.60 (dd, J = 13.6, 6.7 Hz, 1H), 1.09 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.88 (s), 150.27 (s), 145.27 (s), 143.55 (s), 132.34 (s), 128.73 (s), 125.38 (s), 125.09 (s), 116.38 (s), 116.00 (s), 110.38 (s), 73.15 (s), 16.46 (s); MS (ESI), *m*/*z*: 386.1 $[M+Na]^+$.

N-(2-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide (**15a**)

To a solution of **11** in tetrahydrofuran was added Et_3N (3.0 eq) followed by a benzenesulfonyl chloride (1.5 eq) at 0 °C. The reaction mixture was allowed to warm up to r.t over a period of 1.5 h and reaction overnight. The reaction mixture was evaporated and dissolved in 10 mL water. 1.0 N HCl was slowly added to the mixture (pH 4), and the resulting solid was filtered and rinsed with water. The crude product was purified on a silica column to get **15a** as brown solid (0.19 g, 57.2%); ^{1}H NMR (400 MHz, DMSO-*d*6)δ 10.60 (s, 1H), 10.48 (s, 1H), 8.69 (s, 1H), 8.15 (t, 3H), 7.80–7.51 (m, 2H), 6.58 (d, 2H), 4.38 (s, 1H), 1.64 (m, 2H), 0.89 (t, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.81 (s), 142.51 (s), 134.34 (s), 129.87 (s), 129.02 (s), 128.06 (s), 126.93 (s), 124.40 (s), 115.59 (s), 113.87 (s), 108.41 (s), 77.01 (s), 23.10 (s), 8.97 (s); MS (ESI), *m*/*z*: 333.1 $[M+H]^+$.

N-(2-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl) -2-methylbenzenesulfonamide (**15b**)

The title compound was prepared from **11** and 2-methylbenzenesulfonyl chloride according to the procedure for example **15a** and isolated as brown solid (0.25 g, 73.8%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.52 (s, 1H), 10.20 (s, 1H), 7.81 (d, J = 7.7 Hz, 1H), 7.49 (t, J = 7.4 Hz, 1H), 7.35 (dd, J = 13.4, 7.1 Hz, 2H), 6.73–6.60 (m, 3H), 4.43 (dd, J = 7.6, 4.4 Hz, 1H), 2.56 (s, 3H), 1.81–1.59 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.07 (s), 143.12 (s), 135.98 (s), 132.16 (s), 131.55 (s), 129.21 (s), 126.61 (s), 124.33 (s), 115.74 (s), 115.05 (s), 108.40 (s), 72.62 (s), 15.95 (s), 23.10 (s), 8.97 (s); MS (ESI), *m*/*z*: 369.6 $[M+Na]^+$.

N-(2-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-2 -fluorobenzenesulfonamide (**15c**)

The title compound was prepared from **11** and 2-fluorobenzenesulfonyl chloride according to the procedure for example **15a** and isolated as brown solid (0.24 g, 68.6%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.55 (s, 1H), 10.41 (s, 1H), 7.77 (t, J = 6.8 Hz, 1H), 7.68 (dd, J = 13.0, 6.3 Hz, 1H), 7.34 (t, J = 7.7 Hz, 1H), 7.1 (t, 1H), 6.73–6.65 (m, 3H), 4.44 (dd, J = 7.7, 4.5 Hz, 1H), 1.82–1.59 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.43 (s), 143.18 (s), 133.51 (s), 130.89 (s), 129.50 (s), 128.33 (s), 128.14 (s), 123.67 (s), 115.88 (s), 113.62 (s), 108.19 (s), 72.71 (s), 56.60 (s), 16.04 (s); MS (ESI), *m*/*z*: 373.6 $[M+Na]^+$.

N-(2-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-4-methoxy-2-nitrobenzenesulfonamide (**15d**)

The title compound was prepared from **11** and 4-methoxy-2-nitrobenzenesulfonyl chloride according to the procedure for example **15a** and isolated as brown solid (0.13 g, 31.5%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.60 (s, 1H), 10.33 (s, 1H), 7.81 (d, J = 8.9 Hz, 1H), 7.55 (d, J = 2.1 Hz, 1H), 7.40–7.23 (m, 1H), 6.72 (m, J = 23.4, 8.3 Hz, 3H), 4.46 (dd, J = 7.6, 4.5 Hz, 1H), 3.87 (s, 3H), 1.73 (ddt, J = 41.9, 14.4, 7.2 Hz, 2H), 0.93 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.88 (s), 162.86 (s), 149.43 (s), 142.67 (s), 131.71 (d, J = 6.4 Hz), 124.51 (s), 122.24 (s), 117.16 (s), 115.72 (s), 115.19 (s), 109.89 (s), 109.77 (s), 77.10 (s), 56.63 (s), 23.20 (s), 9.01 (s). MS (ESI), *m/z*: 430.4 $[M+Na]^+$.

2-Chloro-N-(2-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide (**15e**)

The title compound was prepared from **11** and 2-chlorobenzenesulfonyl chloride according to the procedure for example **15a** and isolated brown solid (0.16 g, 44.8%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.51 (s, 1H), 10.41 (s, 1H), 8.05 (t, J = 6.8 Hz, 1H), 7.96 (dd, J = 13.0, 6.3 Hz, 1H), 7.57 (t, J = 7.7 Hz, 1H), 7.35 (t, 1H), 6.73–6.65 (m, 3H), 4.41 (d, 1H), 1.82–1.63 (m, 2H), 0.88 (t, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.76 (s), 142.66 (s), 136.28 (s), 134.62 (s), 131.96 (s), 131.78 (s), 131.57 (s), 130.65 (s), 127.68 (s), 123.95 (s), 115.69 (s), 113.97 (s), 108.53 (s), 77.04 (s), 23.16 (s), 9.00 (s); MS (ESI), *m/z*: 389.4 $[M+Na]^+$.

2-bromo-N-(2-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide (**15f**)

The title compound was prepared from **11** and 2-bromobenzenesulfonyl chloride according to the procedure for example **15a** and isolated as brown solid (0.22 g, 54.7%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.53 (s, 1H), 10.43 (s, 1H), 8.02 (dd, J = 7.6, 1.8 Hz, 1H), 7.87–7.77 (m, 1H), 7.53 (m, J = 7.4, 5.9 Hz, 2H), 6.69 (m, J = 9.2, 6.1 Hz, 3H), 4.44 (dd, J = 7.7, 4.5 Hz, 1H), 1.82–1.59 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.78 (s), 142.68 (s), 138.05 (s), 135.38 (s), 134.56 (s), 132.00 (s), 131.75 (s), 128.20 (s), 123.88 (s), 119.16 (s), 115.69 (s), 113.88 (s), 108.46 (s), 77.05 (s), 23.17 (s), 9.01 (s); MS (ESI), *m/z*: 433.6 $[M+Na]^+$.

N-(2-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-2-(trifluoromethyl)benzenesulfonamide (**15g**)

The title compound was prepared from **11** and 2-(trifluoromethyl)benzenesulfonyl chloride according to the procedure for example **15a** and isolated as brown solid (0.13 g, 31.8%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.57 (s, 1H), 10.48 (s, 1H), 8.04 (d, J = 7.2 Hz, 1H), 7.99 (d, J = 7.0 Hz, 1H), 7.85 (dd, J = 13.6, 7.4 Hz, 2H), 6.74 (d, J = 9.1 Hz, 1H), 6.69 (d, J = 6.8 Hz, 2H), 4.45 (dd, J = 7.6, 4.5 Hz, 1H), 1.81–1.71 (m, 1H), 1.71–1.60 (m, 1H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.82 (s), 142.72 (s), 133.41 (s), 133.30 (s), 132.11 (s), 130.65 (s), 124.18 (s), 115.76 (s), 114.53 (s), 109.06 (s), 77.07 (s), 23.18 (s), 8.99 (s); MS (ESI), *m/z*: 423.3 $[M+Na]^+$.

N-(2-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-4-methylbenzenesulfonamide (**15h**)

The title compound was prepared from **11** and 4-methylbenzenesulfonyl chloride according to the procedure for example **15a** and isolated as brown solid (0.2 g, 58.9%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.54 (s, 1H), 10.01 (s, 1H), 7.59 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 6.70 (d, J = 8.5 Hz, 1H), 6.66 (d, J = 2.1 Hz, 1H), 6.64 (s, 1H), 4.43 (dd, J = 7.8, 4.5 Hz, 1H), 2.33 (s, 3H), 1.83–1.65(m, 2H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.81 (s), 143.15 (s), 142.60 (s), 136.38 (s), 132.82 (s), 129.59 (s), 126.67 (s), 123.97 (s), 115.63 (s), 114.69 (s), 109.22 (s), 77.04 (s), 23.17 (s), 20.92 (s), 9.01 (s); MS (ESI), *m/z*: 369.5 $[M+Na]^+$.

4-Chloro-N-(2-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide (**15i**)

The title compound was prepared from **11** and 4-chlorobenzenesulfonyl chloride according to the procedure for example **15a** and isolated as brown solid (0.23 g, 63.2%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.58 (s, 1H), 10.15 (s, 1H), 7.69 (d, J = 8.6 Hz, 2H), 7.63 (d, J = 8.6 Hz, 2H), 6.73 (d, J = 8.4 Hz, 1H), 6.65 (dd, J = 12.3, 3.9 Hz, 2H), 4.45 (dd, J = 7.8, 4.5 Hz, 1H), 1.84–1.59 (m, 2H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.99(s), 142.47 (s), 137.96 (s), 137.72 (s), 132.27 (s), 129.34 (s), 128.60 (s), 124.39 (s), 115.73 (s), 115.29 (s), 109.77 (s), 76.85 (s), 23.18 (s), 8.98 (s); MS (ESI), *m/z*: 389.3 $[M+Na]^+$.

N-(2-ethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-4-nitrobenzenesulfonamide (**15j**)

The title compound was prepared from **11** and 4-nitrobenzenesulfonyl chloride according to the procedure for example **15a** and isolated as brown solid (0.27 g, 73.7%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.60 (s, 1H), 10.40 (s, 1H), 8.37 (d, J = 8.7 Hz, 2H), 7.94 (d, J = 8.7 Hz, 2H), 6.74 (d, J = 8.1 Hz, 1H), 6.71–6.62 (m, 2H), 4.45 (dd, J = 7.7, 4.5 Hz, 1H), 1.72 (m, J = 43.8, 14.5, 7.3 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.85

(s), 149.78 (s), 144.59 (s), 142.72 (s), 131.72 (s), 128.28 (s), 124.74 (s), 124.60 (s), 115.81 (s), 115.55 (s), 110.06 (s), 77.06 (s), 23.18 (s), 8.98 (s); MS (ESI), *m/z*: 400.2 $[M+Na]^+$.

2-Chloro-N-(3-oxo-2-propyl-3,4-dihydro-2H-benzo [b][1,4]oxazin-7-yl)benzenesulfonamide (**16a**)

To a solution of **12** in tetrahydrofuran was added Et_3N (3.0 eq) followed by a 2-chlorobenzenesulfonyl chloride (1.5 eq) at 0 °C. The reaction mixture was allowed to warm up to r.t over a period of 2 h and reaction overnight. The reaction mixture was evaporated and dissolved in 10 mL of water. 1.0 N HCl was slowly added to the mixture (pH 4), and the resulting solid was filtered and rinsed with water. The crude product was purified on a silica column to get **16a** as brown solid (0.3 g, 81.3%); ^{1}H NMR (400MHz, DMSO-d6) δ 10.53 (s, 1H), 10.42 (s, 1H), 7.98 (d, $J = 7.9$ Hz, 1H), 7.67–7.58 (m, 2H), 7.51 (dd, $J = 15.6, 7.3$ Hz, 1H), 6.72–6.62 (m, 3H), 4.48 (dd, $J = 8.3, 4.2$ Hz, 1H), 1.71–1.54 (m, 2H), 1.38 (m, 2H), 0.87 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.03 (s), 142.54 (s), 138.12 (s), 135.37 (s), 134.58 (s), 131.97 (s), 131.83 (s), 128.19 (s), 123.87 (s), 119.17 (s), 115.69 (s), 113.95 (s), 109.52 (s), 75.84 (s), 31.81 (s), 17.60 (s), 13.52 (s); MS (ESI), *m/z*: 403.4 $[M+Na]^+$.

4-Chloro-N-(3-oxo-2-propyl-3,4-dihydro-2H-benzo [b][1,4]oxazin-7-yl)benzenesulfonamide (**16b**)

The title compound was prepared from **12** and 4-chlorobenzenesulfonyl chloride according to the procedure for example **16a** and isolated as brown solid (0.22 g, 56.9%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.58 (s, 1H), 10.15 (s, 1H), 7.76–7.55 (m, 4H), 6.79–6.57 (m, 3H), 4.50 (dd, $J = 8.4, 4.4$ Hz, 1H), 1.80–1.54 (m, 2H), 1.47–1.29 (m, 2H), 0.88 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 166.00 (s), 142.48 (s), 137.98 (s), 137.73 (s), 132.28 (s), 129.35 (s), 128.61 (s), 124.40 (s), 115.74 (s), 115.30 (s), 109.78 (s), 75.86 (s), 31.80 (s), 17.58 (s), 13.52 (s); MS (ESI), *m/z*: 403.3 $[M+Na]^+$.

2-Bromo-N-(3-oxo-2-propyl-3,4-dihydro-2H-benzo [b][1,4]oxazin-7-yl)benzenesulfonamide (**16c**)

The title compound was prepared from **12** and 2-bromobenzenesulfonyl chloride according to the procedure for example **16a** and isolated as brown solid (0.34 g, 79.6%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.55 (s, 1H), 10.45 (s, 1H), 8.02 (d, $J = 7.1$ Hz, 1H), 7.82 (d, $J = 7.5$ Hz, 1H), 7.54 (dd, $J = 14.2, 7.2$ Hz, 2H), 6.68 (d, $J = 11.4$ Hz, 3H), 4.49 (d, $J = 3.5$ Hz, 1H), 1.61 (dd, $J = 20.2, 14.5$ Hz, 2H), 1.37 (d, $J = 7.4$ Hz, 2H), 0.87 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.93 (s), 142.50 (s), 138.01 (s), 135.36 (s), 134.54 (s), 132.01 (s), 131.78 (s), 128.17 (s), 123.87 (s), 119.17 (s), 115.71 (s), 113.95 (s), 108.52 (s), 75.86 (s), 31.79 (s), 17.56 (s), 13.53 (s); MS (ESI), *m/z*: 426.9 $[M+H]^+$.

N-(3-oxo-2-propyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)benzenesulfonamide (**16d**)

The title compound was prepared from **12** and benzenesulfonyl chloride according to the procedure for example **16a** and isolated as brown solid (0.25 g, 74.7%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.56 (s, 1H), 10.08 (s, 1H), 7.71 (d, $J = 7.4$ Hz, 2H), 7.60 (d, $J = 7.1$ Hz, 1H), 7.54 (t, $J = 7.4$ Hz, 2H), 6.74–6.57 (m, 3H), 4.53–4.45 (m, 1H), 1.62 (dd, $J = 20.4, 13.7$ Hz, 2H), 1.37 (d, $J = 10.1$ Hz, 2H), 0.87 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.98 (s), 142.46 (s), 139.24 (s), 132.82 (s), 132.73 (s), 129.16 (s), 126.64 (s), 124.07 (s), 115.65 (s), 114.93 (s), 109.41 (s), 75.85 (s), 31.80 (s), 17.58 (s), 13.53 (s); MS (ESI), *m/z*: 369.1 $[M+Na]^+$.

N-(2,2-dimethyl-3-oxo-3,4-dihydro-2H-benzo [b][1,4]oxazin-7-yl)-4-methylbenzenesulfonamide (**17a**)

To a solution of **10** in tetrahydrofuran was added Et_3N (3.0 eq) followed by a 4-methylbenzenesulfonyl chloride (1.2 eq) at 0 °C. The reaction mixture was allowed to warm up to r.t over a period of 1 h and reaction overnight. The reaction mixture was evaporated and dissolved in 10 mL water. 1.0 N HCl was slowly added to the mixture (pH 4), and the resulting solid was filtered and rinsed with water. The crude product was purified on a silica column eluting with petroleum ether/ethyl acetate (7:1) to get the products **14a** as brown crystals (0.19 g, 55.2%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.54 (s, 1H), 10.15 (s, 1H), 7.75–7.57 (m, 4H), 6.81–6.54 (m, 3H), 2.46 (s, 3H), 1.33 (d, $J = 1.5$ Hz, 6H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 168.59 (s), 142.37 (s), 138.50 (s), 138.21 (s), 132.85 (s), 129.82 (s), 129.09 (s), 125.16 (s), 115.92 (s), 115.74 (s), 110.66 (s), 77.99 (s), 23.70 (s); MS (ESI), *m/z*: 347.4 $[M+H]^+$.

N-(2,2-dimethyl-3-oxo-3,4-dihydro-2H-benzo [b][1,4]oxazin-7-yl)-4-nitrobenzenesulfonamide (**17b**)

The title compound was prepared from **13** and 4-nitrobenzenesulfonyl chloride according to the procedure for example **17a** and isolated as brown solid (0.19 g, 49.5%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.56 (s, 1H), 10.40 (s, 1H), 8.37 (d, $J = 8.9$ Hz, 2H), 7.94 (d, $J = 8.9$ Hz, 2H), 6.75 (d, $J = 8.4$ Hz, 1H), 6.68 (dd, $J = 8.4, 2.2$ Hz, 1H), 6.64 (d, $J = 2.1$ Hz, 1H), 1.33 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 168.59 (s), 150.27 (s), 145.09 (s), 142.43 (s), 132.31 (s), 128.76 (s), 125.47 (s), 125.04 (s), 116.01 (s), 110.92 (s), 78.03 (s), 23.71 (s); MS (ESI), *m/z*: 400.2 $[M+Na]^+$.

N-(2,2-dimethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-4-methoxy-2-nitrobenzenesulfonamide (**17c**)

The title compound was prepared from **13** and 4-methoxy-2-nitrobenzenesulfonyl chloride according to the procedure for example **17a** and isolated as brown solid (0.27 g, 65.7%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.57 (s,1H), 10.33 (s, 1H), 7.81 (s, 1H), 7.56 (s, 1H), 7.32 (s, 1H), 6.69 (s, 3H), 3.87 (s, 3H), 1.35 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.86 (s), 142.65 (s), 138.00 (s), 137.73 (s), 132.27 (s), 129.37 (s), 128.60 (s), 124.42 (s), 115.73 (s), 115.26 (s), 109.77 (s), 77.06 (s), 23.18 (s), 9.00 (s); MS (ESI), *m*/*z*: 430.6 $[M+Na]^+$.

N-(2,2-dimethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-2-fluorobenzenesulfonamide (**17d**)

The title compound was prepared from **13** and 2-fluorobenzenesulfonyl chloride according to the procedure for example **17a** and isolated as brown solid (0.1 g, 28.2%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.50 (s, 1H), 10.40 (s, 1H), 7.77 (t, J = 7.2 Hz, 1H), 7.68 (d, J = 5.6 Hz, 1H), 7.45–7.37 (m, 1H), 7.34 (t, J = 7.5 Hz, 1H), 6.75–6.61 (m, 3H), 1.32 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 168.06 (s), 156.82 (s), 141.86 (s), 135.93 (s), 132.14 (s), 130.45 (s), 124.89 (s), 124.40 (s), 117.29 (s), 117.08 (s), 115.38 (s), 114.45 (s), 109.39 (s), 77.48 (s), 23.18 (s); MS (ESI), *m*/*z*: 373.3 $[M+Na]^+$.

N-(2,2-dimethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-2-(trifluoromethyl)benzenesulfonamide (**17e**)

The title compound was prepared from **13** and 2-(trifluoromethyl)benzenesulfonyl chloride according to the procedure for example **17a** and isolated as brown solid (0.15 g, 37.2%); ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.52 (s, 1H), 10.45 (s, 1H), 8.03 (d, J = 7.4 Hz, 1H), 7.98 (d, J = 7.1 Hz, 1H), 7.84 (td, J = 13.6, 7.7 Hz, 2H), 6.77–6.61 (m, 3H), 1.32 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 168.06 (s), 141.92 (s), 138.18 (s), 133.40 (s), 133.27 (s), 132.16 (s), 130.69 (s), 128.51 (s), 124.44 (s), 115.46 (s), 114.60 (s), 109.54 (s), 77.52 (s), 23.19 (s); MS (ESI), *m*/*z*: 423.5 $[M+Na]^+$.

Cell culture

Cell lines MV4-11, OCI-LY10, Pfeifer, and Su-DHL-6 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). All of them were cultured in RPIM 1640 or Iscove's Modified Dulbecco's Medium supplemented with 10% fetal bovine serum (Gibco, Auckland, N.Z.), penicillin-streptomycin (Life Technologies), and 4 mM L-Glutamine. The cell lines were cultured in a humidified atmosphere with 5% CO_2 at 37 °C.

Cell viability assay

The cell viability treated with the compounds described above was measured by MTT assay. 100 μl of medium containing various concentrations of compound was added to 96-well micro-titer plates, and then, cells were seeded in each well at a density of 5–10 × 10^4 cells per well. After cultured for 72 h, the cells were added 20 μl MTT solution (5 mg/mL) and incubated for another 1–4 h at 37 °C. When reached the appropriate time, the formazan crystal formed by living cells was dissolved with 50 μL of the 20% SDS solution overnight. Then, the optical density was measured using Spectra MAX M5 microplate spectrophotometer (Molecular Devices) at 570 nm and the IC_{50} values were carried out.

AlphaScreen™

Aiphascreen™ assays were performed with minor modifications. All reagents were diluted in the recommended buffer (50 mM HEPES, pH 7.4). BRD4 (D1) protein (Biogenie, Canada) was added to microplate followed by non-biotinylated peptide, solvent, or compound. After incubated at room temperature for 60 min, the plates were added acceptor and donor solution, and then incubated for 60 min at room temperature under subdued light. When reached the appropriate time, the plate was read on a PHERAstar FS plate reader (BMG Labtech, Germany). The data was fit using GraphPad Prism 5 to obtain inhibition values using.

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