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3-Hydroxyisoindolin-1-one derivates: Synthesis by palladium-catalyzed C—H activation as BRD4 inhibitors against human acute myeloid leukemia (AML) cells

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ABSTRACT

Bromodomain protein 4 (BRD4) is a member of the bromodomain and extra-terminal domain (BET) protein family, which plays a key role in transcriptional regulation. Recent biological and pharmacological studies have enabled linking of the BET bromodomains with diseases, including inflammation and cancer, suggesting that bromodomains are druggable targets. In this study, we made further structural modifications of our previously reported BRD4 inhibitors, to develop new chemical scaffold 3-Hydroxyisoindolin-1-One. Then a series of compounds (**10a-q**) were synthesized via palladium-catalyzed C—H activation and BRD4-inhibitory activities and anti-proliferative effects of these compounds were evaluated. Compound **10e** exhibited excellent BRD4-inhibitory activity with IC_{50} value of **80 nM** and anti-proliferation potency with IC_{50} value of **365 nM** in HL-60 (human promyelocytic leukemia) cancer cell lines. We have demonstrated compound **10e** modulated the intrinsic apoptotic pathway. In conclusion, these results suggested that compound **10e** could be utilized as a BRD4 inhibitor for further leukemia treatment.

1. Introduction

Epigenetic marks on histones are associated with transcriptional processes [1]. Proteins of this target class are classified into readers, writers, and erasers of marks on histones or other nuclear proteins and DNA [2,3]. Context-specific molecular recognition of lysine residues (Kac) on histone tails is principally mediated by bromodomains [4].

The bromodomain and extra-terminal domain (BET) family proteins [5], including BRD2, BRD3, BRD4 and BRDT, shares a common domain architecture featuring two amino-terminal bromodomains, which interact with acetylated histones [6–8]. Bromodomain protein 4 (BRD4) is the best studied member of the BET family and is a critical mediator of transcriptional elongation by recruiting the positive transcription elongation factor complex (P-TEFb) [1]. Through its direct interaction with the acetylated cyclin T1 subunit of the positive transcription elongation factor b, BRD4 furthermore controls transcription elongation via phosphorylation of RNA polymerase II by CDK9 [9–12]. BRD4 also binds to acetylated RelA, thus stabilizing nuclear NF-B and controlling the expression of downstream target genes [13,14]. Above all, BRD4 bromodomain protein has developed to be an interesting drug target for the treatment of cancer [1,15,16], obesity [17], kidney disease [13], lung fibrosis [18] and other inflammatory diseases [19].

The potential of targeting BET family proteins for cancer therapy has been substantiated using small-molecule inhibitors, such as JQ1, Abbv-075, I-BET151 and OTX015 etc. (Fig. 1). JQ1 was the first uncovered BRD4 inhibitor [7] and I-BET762 was acquired from optimization of a hit which was designed to enhance ApoA1 expression [20]. Gratifyingly, I-BET762 has entered clinical trials for NUT midline carcinoma and other cancers. OTX015 has completed Phase I trials for hematologic malignancies and NUT midline carcinoma, and the results exhibited clinically meaningful activity with nontoxic doses [21–23]. ABBV-075 is in phase I clinical trials for the treatment of patients with solid tumors, acute myeloid leukemia or multiple myeloma on the basis of the strong biochemical potency for BRD4 (Ki = 15 nM) [24–27].

Upon the basis of the previously disclosed BRD4 inhibitors, we herein report a rapid synthesis of 3-Hydroxyisoindolin-1-One derivates by a Palladium-Catalyzed C—H Activation strategy, which enabled the investigation of their SAR, and the biological activities of BRD4

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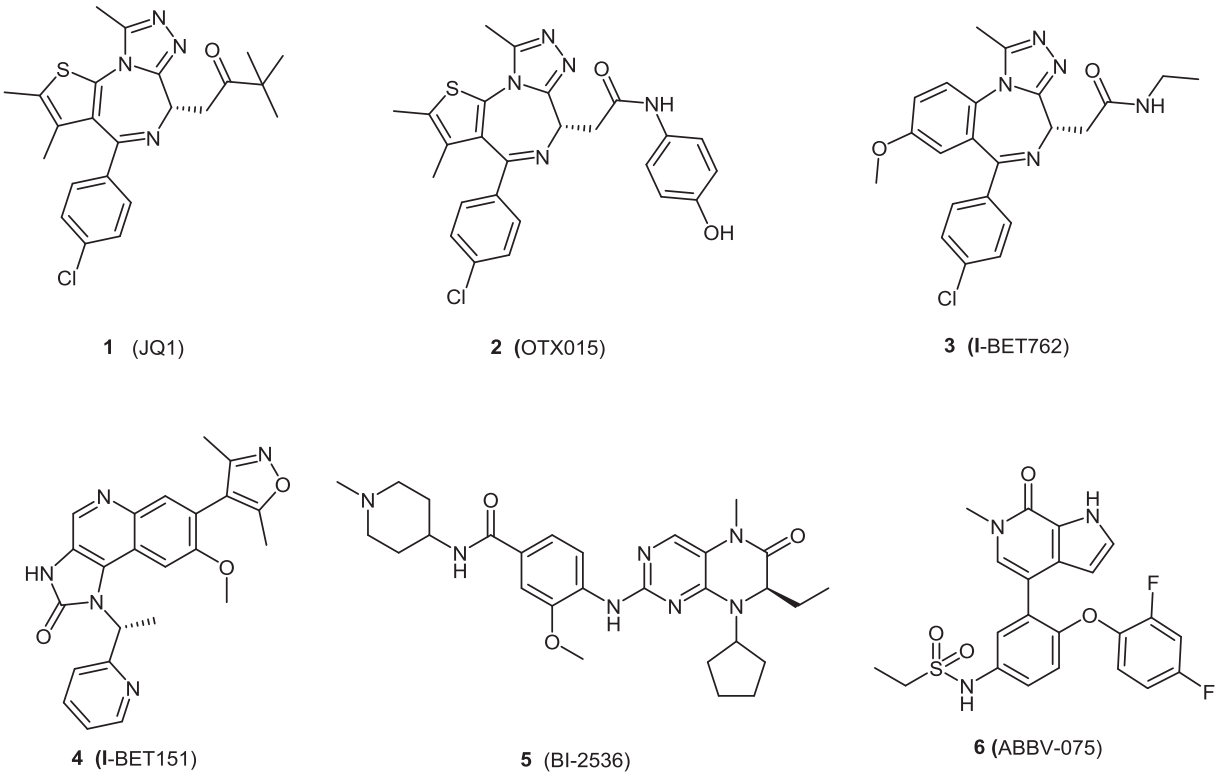


Fig. 1. Structures of known BET bromodomain inhibitors.

inhibitors for further leukemia treatment.

2. Results and discussion

2.1. Chemistry

The general synthetic routes of 3-hydroxyisoindolin-1-one derivates (**3a-3p**) were outlined in Scheme 1. All the target products and key intermediates were confirmed by ^{1}H NMR and ^{13}C NMR spectrum, mass spectra. Purifications by column chromatography were carried out over silica gel (200–300 mesh) and purity was checked by using HPLC before being tested in biological evaluation (purity was > 98%).

As shown in Scheme1, the target compounds **10a-p** were prepared from the commercially starting material 4-bromobenzoic acid (**7**). Then 4-bromobenzoic acid was treated with $MeONH_2{\cdot}HCl$ or $BnONH_2{\cdot}HCl$ by acylation to obtain the intermediates **8a-b**. As recently reported, a palladiumcatalyzed oxidative acylation was employed as an efficient synthesis for hydroxyl isoindolones [28,29]. Therefore, the key intermediates **9a-p** could be synthesized via palladium-catalyzed C−H activation (Fig. 2) avoiding the tedious multistep processes. A series of different substituted benzaldehydes were employed under mild reaction conditions via C−H activation to get 3-hydroxyisoindolin-1-one derivates **9a-p**. The target **10a-p** were obtained by Suzuki coupling reaction with 3, 5-dimethylisoxazole-4-boronic acid pinacol ester.

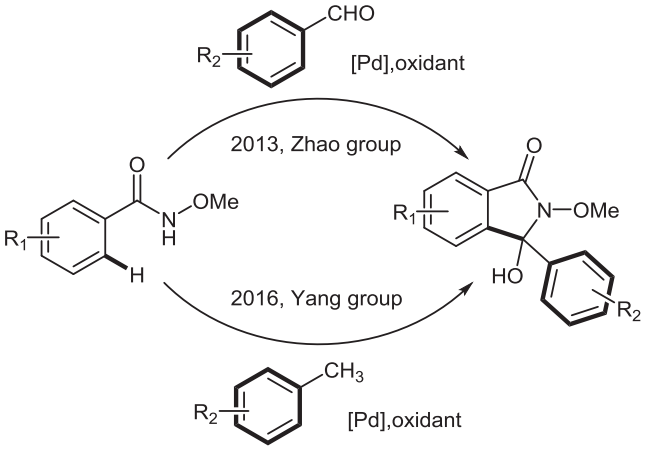
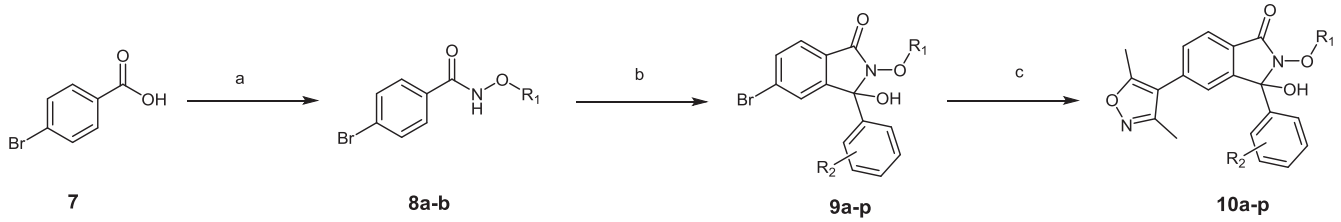


Fig. 2. Synthesis of 3-hydroxyisoindolin-1-one via C−H activation.

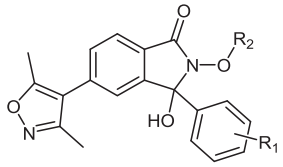
2.2. Palladium-Catalyzed C−H activation

Phthalimides were used as starting materials for the synthesis of 3-hydroxyisoindolin-1-ones in traditional methods. The Grignard reaction of the carbonyl groups of phthalimides [30] or the photochemical



Scheme 1. Reagents and conditions: (a) (1) ClCOCOCl, DMF, DCM, 0 ℃ to room temperature;(2) $MeONH_2{\cdot}HCl$ or $BnONH_2{\cdot}HCl$, K_2CO_3, EtOAc, H_2O, 0 ℃ to r.t; (b) benzaldehyde, $Pd(OAc)_2$, TBHP, Dioxane, 80 ℃; (c) phenylboronic acids, $Pd(P(Ph)_3)_4$, Na_2CO_3, N_2, H_2O/EtOH/Toluene, 80 ℃.

Table 1
Structures and BRD4 inhibitory effects of compounds **10a-p**.



Compound No.	R_1	R_2	BRD4(1) IC_{50}[a] (μmol/L)	BRD4(2) IC_{50} (μmol/L)
10a	2,4-di-F-phenyl	Me	0.224 ± 0.012	4.260 ± 0.024
10b	4-Me-phenyl	Me	0.245 ± 0.014	4.580 ± 0.028
10c	3-F-phenyl	Me	0.090 ± 0.012	2.140 ± 0.014
10d	4-F-phenyl	Me	0.077 ± 0.018	1.700 ± 0.027
10e	phenyl	Me	0.080 ± 0.011	0.290 ± 0.011
10f	3-CF_3-phenyl	Me	2.560 ± 0.023	NA[b]
10g	2- F-phenyl	Me	0.300 ± 0.015	1.300 ± 0.018
10h	2-Cl-phenyl	Me	2.100 ± 0.022	NA
10i	3-Cl-phenyl	Me	0.330 ± 0.014	1.400 ± 0.024
10j	3-F-phenyl	benzyl	0.532 ± 0.012	NA
10k	2-F-phenyl	benzyl	0.780 ± 0.019	NA
10l	3-CN- phenyl	benzyl	1.500 ± 0.021	NA
10m	4-F-phenyl	benzyl	0.390 ± 0.016	1.560 ± 0.023
10n	4-Me-phenyl	benzyl	1.800 ± 0.022	NA
10o	3-OCH_3- phenyl	benzyl	1.680 ± 0.025	NA
10p	phenyl	benzyl	0.320 ± 0.012	1.240 ± 0.026
JQ1[c]	–	–	0.078 ± 0.009	0.093 ± 0.012

[a] IC_{50} values for BRD4-BD1 activities presented are the mean ± SD values of three independent determinations
[b] Mean of the BRD4 inhibitory activity IC_{50} > 5 μM.
[c] Employed as positive control.

reaction of phthalimides to afford 3-benzyl-3-hydroxyisoindol-1-ones commonly require harsh reaction conditions and complicated reagents. Kim et al. first reported secondary benzamides and aryl aldehydes via the rhodium-catalyzed C—H bond activation with an intramolecular cyclization [31]. Then, palladiumcatalyzed oxidative acylation was demonstrated as an efficient synthesis for hydroxyl isoindolones (Fig. 2). Recently, Yang et al. disclosed the synthesis of 3-hydroxyisoindolin-1-ones through the palladium-catalyzed C—H bond ortho acylation/annulation with toluene derivatives [32].

2.3. Biological activity and SAR study

In our previous research, dihydroquinoxalinone compound **5i** exhibited IC_{50} value of 73 nM of binding activity in BRD4 (1) and 258 nM of cellular activity in MV4-11 cancer cell lines. Rational scaffold hopping was employed to increase the type of BRD4 inhibitors and the protein binding potency. Then, a series of compounds (**10a-q**) containing 3-hydroxyisoindolin-1-one skeleton were designed and synthesized for biological and pharmacodynamics evaluation (Table 1). The initially dihydroquinoxalinone core of compound **5i** was replaced by 3-hydroxyisoindolin-1-one to obtain compound **10e** with binding potency (IC_{50} = **80 nM**). Subsequently, compound **10e** was docked into BRD4BD1 protein (PDB id: 3P5O) via Glide docking protocol of Maestro 10.2 and overlapped with compound **5i** (Fig. 3). The docking conformations showed the isoxazole group of compound **10e** behaving as the KAc mimetic. The amine formed hydrogen bonds to Asn140 and a through-water interaction to Tyr97. The phenyl group is placed on the WPF stack (W81, P82, and F83) in a similar manner to compound 5i. This result showed that 3-hydroxyisoindolin-1-one module was feasible as a core of BRD4 inhibitors.

Then various substituents phenyl were introduced to explore structure-activity relationship for the improvement of binding potency and druggable profile (Table 1). Initially, it was pleasing to observe increase selectivity in BD1 potency with the addition of single F substituent to the shelf group (**10c, d** vs **10e**) without significant increase in BD1 potency but decreasing BD2 potency. The di-F-phenyl group (**10a**) caused both decrease in BD1 and BD2 potency. The compound **10b** displayed similar potency and selectivity as **10a**. Disappointingly, compound **10f** showed poor selectivity and reduced potency, indicating that strong electron-withdrawing group changes the electronic cloud density in phenyl leading to evident decrease in potency. The m-position and p-position substituents showed higher potency in BRD4BD1 than o-position (compound **10c, d** vs **10 g**). Compound **10j-p** containing benzyl replacement with methoxy group at the 2-position

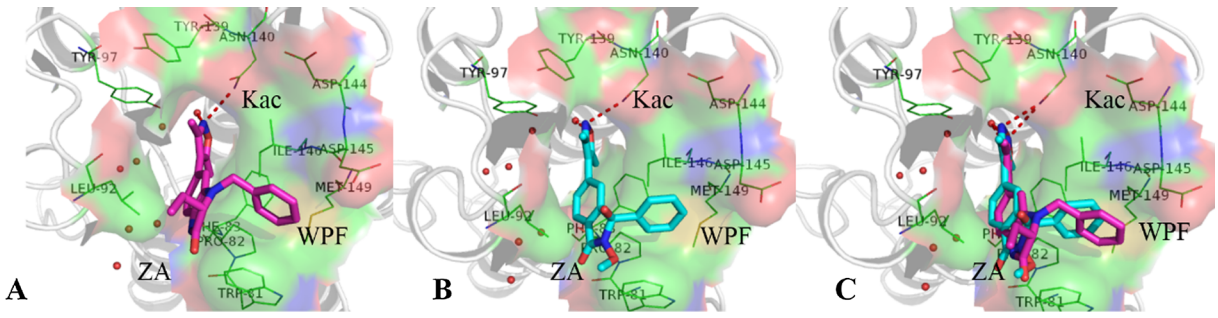


Fig. 3. (A) Docking conformation of compound 5i in BRD4 protein (PDB id: 3P5O). (B) Docking conformation of compound 10e in BRD4 protein (PDB id: 3P5O). (C) Superimposition docking conformation of compound 5i (Pink) and compound 10e (Blue) in BRD4 protein (PDB id: 3P5O).

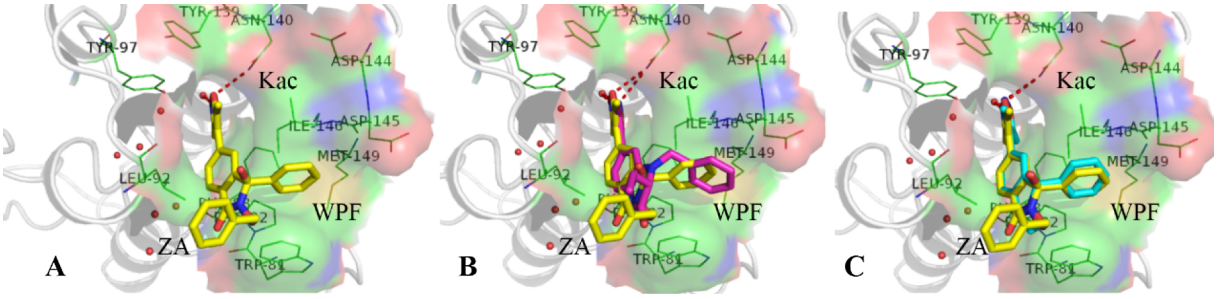


Fig. 4. (A) Docking conformation of compound 10p in BRD4 protein (PDB id: 3P5O). (B) Superimposition docking conformation of compound 5i (Pink) and compound 10p (Yellow) in BRD4 protein (PDB id: 3P5O). (C) Superimposition docking conformation of compound 10p (Yellow) and compound 10e (Blue) in BRD4 protein (PDB id: 3P5O).

exhibited poor selectivity and reduced potency, suggesting that the 2-position toward a small lipophilic pocket, as occupied by the methoxy group of **10e** (Fig. 3). The docking conformation of compound **10p** demonstrated that the small pocket was not taken up by big benzyl substituent (Fig. 4).

2.4. Anti-proliferative activities across cancer cell lines

Subsequently, the compounds (**10a-e**, **10 g**, **10i**, **10 m** and **10p**) with excellent BRD4 inhibitory potency were further evaluated in LNcap-1(human prostatic cells), MV4-11(Human acute monocytic leukemia cells) and HL-60 (human promyelocytic leukemia) for their anti-proliferation activities (Table 2). Most compounds exhibited favorable anti-proliferation activities in HL-60 and MV4-11 cell lines. Gratifyingly, compound **10e** showed remarkable biological activities with IC50 value of **365 nM** in HL-60 and **420 nM** in MV4-11 cell lines. These results indicated that compound **10e** was worthy for further investigation in leukemia therapy. In addition, activities of compounds on BRD4-independent LNcap-1 cells was weaker than on BRD4-sensitive lines and this result is consistent with our previous reports [24].

2.5. Induce apoptosis via the mitochondrial pathways in leukemia cells

Western blot was employed to acquire more insight on how compound **10e** trigger apoptosis in HL-60 cancer cells. Then varied concentrations of compound **10e** were utilized to evaluate the expression of several key proteins in intrinsic apoptotic pathway (Fig. 5). These results showed that compound **10e** downregulated c-Myc at 24 h, consistent with JQ1. Early apoptotic events such as caspase activation (caspase 3) and PARP cleavage were already apparent at the low concentration of compound **10e** (Fig. 5). These data suggest that compound **10e** induced apoptosis via the mitochondrial pathways in leukemia cells.

Table 2
Anti-proliferation evaluation against cancer cell lines LNcap-1, HL-60 and MV4-11.

Compound No.	LNcap-1 IC_{50} (μM) [a]	HL-60 IC_{50} (μM)	MV4-11 IC_{50} (μM)
10a	6.850 ± 0.023	6.537 ± 0.021	5.970 ± 0.021
10b	NA	7.340 ± 0.016	5.134 ± 0.022
10c	NA	1.223 ± 0.013	0.895 ± 0.012
10d	NA	NA	NA [b]
10e	NA	0.365 ± 0.018	0.420 ± 0.011
10g	NA	1.399 ± 0.019	1.250 ± 0.014
10i	NA	NA	6.530 ± 0.024
10m	NA	NA	NA
10p	NA	2.983 ± 0.022	2.460 ± 0.026
JQ1 [c]	4.720 ± 0.024	0.686 ± 0.013	1.380 ± 0.023

[a] IC_{50} values for BRD4-BD1 activities presented are the mean ± SD values of three independent determinations
[b] Mean of the BRD4 inhibitory activity IC_{50} > 5 μM.
[c] Employed as positive control.

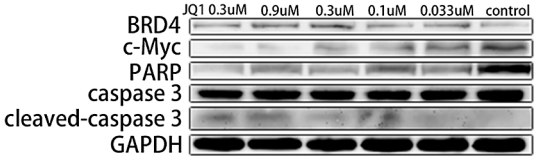


Fig. 5. BRD4 inhibitor compound 10e induces apoptosis in HL-60 cancer cells.

3. Conclusion

In summary, we report our structural modification chemical synthesis and evaluation of a new series of BRD4 inhibitors. Upon the basis of the previously disclosed BRD4 inhibitors, a novel chemical scaffold 3-hydroxyisoindolin-1-one was synthesized by Palladium-Catalyzed C—H Activation. The BRD4-inhibitory activities and anti-proliferative effects of these compounds were evaluated. Meanwhile, we found compound **10e** has remarkable anti-proliferative activities in HL-60 cancer cells and could induce apoptosis via mitochondrial pathways. All of these results demonstrated compound **10e** could be utilized as a BRD4 inhibitor for further investigation in leukemia therapy.

4. Experiment section

4.1. Chemistry

All chemicals (reagent grade) used were purchased from Sinopharm Chemical Reagent Co., Ltd. (China) and treated with standard methods. Melting points were measured on capillary tube and were uncorrected. ^1H and ^{13}C NMR spectra (DMSO-d_6) were measured on Bruker AV-300 spectrometer at 25 °C and referenced to TMS. Chemical shifts were reported in ppm (d) using the residual solvent line as internal standard. High-resolution mass spectra were recorded using an Agilent QTOF 6520 (Beijing, China)

4.1.1. General procedure for the preparation of 8a-b

To a solution of the 4-bromobenzoic acid (2 g, 9.95 mmol) and DMF (cat.) in DCM (20 mL), oxalyl chloride (1.27 mL, 1.89 mmol) was added dropwise at 0 °C and stirred at room temperature for another 3 h. The solution was concentrated under reduced pressure to give yellow oil. Then the residue was quenched with EtOAc (30 mL) and K_2CO_3 (2.75 g, 19.9 mmol), $MeONH_2$·HCl or $BnOBH_2$·HCl (11.94 mmol), water (15 mL) were added subsequently. The reaction mixture was stirred at room temperature until the reaction was completed. The organic layer was separated and washed with saturated sodium bicarbonate solution (3 × 20 mL) and brine (20 mL) and the organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give 8a-

b without further purification.

*4.1.1.1. 4-Bromo-N-methoxybenzamide (**8a**).* White solid, yield 78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.84 (s, 1H), 8.02 – 7.32 (m, 4H), 3.71 (s, 3H). MS (ESI, *m/z*): 230.3[M + H]$^+$.

*4.1.1.2. N-Benzyl-O-(4-bromobenzoyl)hydroxylamine (**8b**).* White solid, yield 89%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 – 7.64 (m, 4H), 7.39 (ddd, *J* = 22.2, 14.0, 7.4 Hz, 6H), 4.93 (s, 2H). MS (ESI, *m/z*): 306.3[M + H]$^+$.

*4.1.2. General procedure for the preparation of **9a-p***

To a solution of compound 8a, b (0.87 mmol), varied substituent benzaldehyde (1.3 mmol) in toluene, $Pd(OAc)_2$ (19.52 mg, 0.08 mmol) and TBHP (1 mL, 10.43 mmol) were added respectively in sealed tube. Then the reaction mixture was stirred at 80 °C under air for 12 h. After cooling to the room temperature, the reaction mixture was diluted with saturated sodium bicarbonate solution (10 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with and brine (30 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (petroleum ether/ethyl acetate, 5/1 to 3/1) to give 9a-p.

*4.1.2.1. 5-Bromo-3-(2, 4-difluorophenyl)-3-hydroxy-2-methoxyisoindolin-1-one (**9a**).* Offwhite solid, yield 81%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.13 – 7.99 (m, 1H), 7.90 (s, 1H), 7.76 (ddd, *J* = 20.6, 11.0, 4.7 Hz, 2H), 7.52 (d, *J* = 1.2 Hz, 1H), 7.27 – 7.06 (m, 2H), 3.81 (d, *J* = 1.2 Hz, 3H). MS (ESI, *m/z*): 370.4[M + H]$^+$.

*4.1.2.2. 5-Bromo-3-hydroxy-2-methoxy-3-(p-tolyl)isoindolin-1-one (**9b**).* White solid, yield 73%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.77 – 7.66 (m, 2H), 7.60 (s, 1H), 7.47 (d, *J* = 1.5 Hz, 1H), 7.20 (dd, *J* = 20.8, 8.2 Hz, 4H), 3.82 (s, 3H), 2.28 (s, 3H). MS (ESI, *m/z*): 348.4[M + H]$^+$.

*4.1.2.3. 5-Bromo-3-(3-fluorophenyl)-3-hydroxy-2-methoxyisoindolin-1-one (**9c**).* White solid, yield 68%. MS (ESI, *m/z*): 352.1[M + H]$^+$.

*4.1.2.4. 5-Bromo-3-(4-fluorophenyl)-3-hydroxy-2-methoxyisoindolin-1-one (**9d**).* Yellow solid, yield 79%.^{1}H NMR (300 MHz, DMSO-d_6) δ 7.81 – 7.66 (m, 3H), 7.54 (s, 1H), 7.46 – 7.34 (m, 2H), 7.20 (t, *J* = 8.6 Hz, 2H), 3.83 (s, 3H). MS (ESI, *m/z*): 352.3[M + H]$^+$.

*4.1.2.5. 5-Bromo-3-hydroxy-2-methoxy-3-phenylisoindolin-1-one (**9e**).* Yellow solid, yield 73%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82 – 7.59 (m, 3H), 7.52 (s, 1H), 7.37 (s, 5H), 3.83 (d, *J* = 4.3 Hz, 3H). MS (ESI, *m/z*): 334.3[M + H]$^+$.

*4.1.2.6. 5-Bromo-3-hydroxy-2-methoxy-3-(3-(trifluoromethyl)phenyl) isoindolin-1-one (**9f**).* White solid, yield 78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (s, 1H), 7.85 – 7.69 (m, 4H), 7.58 (d, *J* = 24.3 Hz, 3H), 3.86 (d, *J* = 3.3 Hz, 3H). MS (ESI, *m/z*): 402.1[M + H]$^+$.

*4.1.2.7. 5-Bromo-3-(2-fluorophenyl)-3-hydroxy-2-methoxyisoindolin-1-one (**9 g**).* White solid, yield 84%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.02 (td, *J* = 8.1, 1.8 Hz, 1H), 7.83 – 7.68 (m, 3H), 7.51 – 7.29 (m, 3H), 7.11 – 7.01 (m, 1H), 3.81 (s, 3H). MS (ESI, *m/z*): 352.1[M + H]$^+$.

*4.1.2.8. 5-Bromo-3-(3-chlorophenyl)-3-hydroxy-2-methoxyisoindolin-1-one (**9i**).* White solid, yield 88%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 – 7.53 (m, 3H), 7.43 (d, *J* = 1.0 Hz, 1H), 7.38 – 7.23 (m, 3H), 5.26 (s, 1H), 3.87 (d, *J* = 13.8 Hz, 3H). MS (ESI, *m/z*): 368.2[M + H]$^+$.

*4.1.2.9. 2-(Benzyloxy)-5-bromo-3-hydroxy-3-(p-tolyl)isoindolin-1-one (**9n**).* White solid, yield 81%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.80 – 7.67 (m, 3H), 7.52 (d, *J* = 1.2 Hz, 1H), 7.41 – 7.31 (m, 5H), 7.28 (d, *J* = 8.2 Hz, 2H), 7.17 (d, *J* = 8.2 Hz, 2H), 5.12 (d, *J* = 10.0 Hz, 1H), 4.99 (d, *J* = 10.0 Hz, 1H), 2.27 (s, 3H). MS (ESI, *m/z*): 424.3[M + H]$^+$.

*4.1.2.10. 2-(Benzyloxy)-5-bromo-3-hydroxy-3-phenylisoindolin-1-one (**9p**).* White solid, yield 79%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.83 – 7.68 (m, 3H), 7.55 (d, *J* = 1.0 Hz, 1H), 7.46 – 7.28 (m, 10*H*), 5.17 – 5.09 (m, 1H), 4.99 (d, *J* = 10.0 Hz, 1H). MS (ESI, *m/z*): 411.1[M + H]$^+$.

*4.1.3. General procedure for the preparation of **10a-p***

A mixture of 9a-p (0.36 mmol), 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) isoxazole (0.08 g, 0.36 mmol), sodium carbonate (0.14 g, 1.08 mmol), Tetrakis (triphenylphosphine) palladium (0.04 g, 0.036 mmol) in a mixed solvent of water (3 mL), ethanol (1 mL) and toluene (3 mL) was refluxed under N_2 for 12 h. After the reaction was completed, the mixture was diluted with water (20 mL) and filtered with diatomite. Then the filtrate was extracted with ethyl acetate (10 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give brown oil. The resulting crude oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 3:1) to afford target product 10a-p.

*4.1.3.1. 3-(2, 4-Difluorophenyl)-5-(3, 5-dimethylisoxazol-4-yl)-3-hydroxy-2-methoxyisoindolin-1-one (**10a**).* White solid, yield 78%. m.p.: 128–130 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.05 (d, *J* = 6.9 Hz, 1H), 7.93 – 7.76 (m, 2H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.29 (s, 1H), 7.14 (dd, *J* = 20.7, 8.7 Hz, 2H), 3.82 (s, 3H), 2.35 (s, 3H), 2.16 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.38, 162.96, 158.28, 146.41, 135.40, 131.55, 131.40, 130.80, 127.93, 123.63, 123.05, 115.77, 111.95, 111.71, 105.13, 104.79, 104.44, 87.80, 65.48, 40.89, 40.62, 40.35, 40.07, 39.80, 39.52, 39.24, 11.81, 10.80. MS (ESI, *m/z*): 387.4[M + H]$^+$; Anal. calcd. for $C_{20}H_{16}F_2N_2O_4$: C, 62.18; H, 4.17; N, 7.25. Found: C, 62.48; H, 4.21; N, 7.38.

*4.1.3.2. 5-(3, 5-Dimethylisoxazol-4-yl)-3-hydroxy-2-methoxy-3-(p-tolyl) isoindolin-1-one (**10b**).* Yellow solid, yield 83%. m.p.: 133–135 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.83 (d, *J* = 7.8 Hz, 1H), 7.61 – 7.50 (m, 2H), 7.34 – 7.21 (m, 3H), 7.16 (d, *J* = 8.3 Hz, 2H), 3.85 (s, 3H), 2.36 (s, 3H), 2.27 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.38, 162.62, 158.30, 147.86, 138.03, 136.50, 135.32, 130.48, 129.36, 127.35, 126.49, 123.67, 123.37, 115.82, 90.83, 65.34, 40.89, 40.63, 40.35, 40.07, 39.79, 39.51, 39.24, 21.09, 11.82, 10.83. MS (ESI, *m/z*): 365.2[M + H]$^+$; Anal. calcd. for $C_{21}H_{20}N_2O_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.48; H, 5.51; N, 7.71.

*4.1.3.3. 5-(3,5-Dimethylisoxazol-4-yl)-3-(3-fluorophenyl)-3-hydroxy-2-methoxyisoindolin-1-one (**10c**).* Yellow solid, yield 91%. m.p.: 147–149 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.90 – 7.75 (m, 2H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.37 (d, *J* = 8.3 Hz, 2H), 7.29 (d, *J* = 10.4 Hz, 1H), 7.21 – 7.05 (m, 2H), 3.88 (s, 3H), 2.36 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.45, 164.25, 162.68, 158.33, 147.17, 142.53, 142.44, 135.57, 130.87, 130.79, 127.24, 123.84, 123.48, 122.54, 115.78, 113.91, 113.59, 90.30, 65.53, 40.91, 40.63, 40.35, 40.07, 39.79, 39.51, 39.23, 11.81, 10.80. MS (ESI, *m/z*): 369.2[M + H]$^+$; Anal. calcd. for $C_{20}H_{17}FN_2O_4$: C, 65.21; H, 6.65; N, 7.60. Found: C, 65.24; H, 6.51; N, 7.83.

*4.1.3.4. 5-(3,5-Dimethylisoxazol-4-yl)-3-(4-fluorophenyl)-3-hydroxy-2-methoxyisoindolin-1-one (**10d**).* Yellow solid, yield 81%. m.p.: 143–145 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.85 (d, *J* = 7.8 Hz, 1H), 7.65 (d, *J* = 6.0 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.44 (dd, *J* = 8.7, 5.5 Hz, 2H), 7.34 (s, 1H), 7.19 (t, *J* = 8.9 Hz, 2H), 3.88 (s, 3H), 2.37 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.42, 162.58, 158.31, 147.49, 135.66, 135.50, 130.67, 128.90, 128.78, 127.28, 123.79, 123.43, 115.79, 115.50, 90.45, 65.47, 40.90, 40.62, 40.34, 40.06,

39.78, 39.50, 39.23, 11.81, 10.81. MS (ESI, *m/z*): 369.2[M + H]$^+$; Anal. calcd. for $C_{20}H_{17}FN_2O_4$: C, 65.21; H, 6.65; N, 7.60. Found: C, 65.14; H, 6.61; N, 7.63.

*4.1.3.5. 5-(3, 5-Dimethylisoxazol-4-yl)-3-hydroxy-2-methoxy-3-phenylisoindolin-1-one (**10e**)*. Yellow solid, yield 71%. m.p.: 139–141 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84 (d, J = 7.8 Hz, 1H), 7.66 (s, 1H), 7.56 (d, J = 7.7 Hz, 1H), 7.49 – 7.24 (m, 6H), 3.86 (s, 3H), 2.35 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.40, 162.65, 158.30, 147.73, 139.46, 135.38, 130.56, 128.83, 128.73, 127.34, 126.54, 123.72, 123.42, 115.81, 90.85, 65.40, 40.91, 40.63, 40.35, 40.08, 39.80, 39.52, 39.24, 11.80, 10.81. MS (ESI, *m/z*): 351.2[M + H]$^+$; Anal. calcd. for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.18; N, 8.00. Found: C, 65.44; H, 5.31; N, 7.93.

*4.1.3.6. 5-(3,5-Dimethylisoxazol-4-yl)-3-hydroxy-2-methoxy-3-(3-(trifluoromethyl)phenyl)isoindolin −1-one (**10f**)*. White solid, yield 78%. m.p.: 143–145 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (s, 1H), 7.88 (d, J = 7.6 Hz, 1H), 7.82 (s, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.62 – 7.56 (m, 3H), 7.42 (s, 1H), 3.89 (s, 3H), 2.36 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.47, 162.69, 158.33, 146.90, 143.63, 141.04, 135.71, 134.32, 132.40, 130.97, 130.77, 130.23, 127.23, 123.95, 123.57, 123.09, 115.76, 90.31, 65.64, 40.92, 40.64, 40.36, 40.08, 39.80, 39.52, 39.24, 11.78, 10.76. MS (ESI, *m/z*): 419.2[M + H]$^+$; Anal. calcd. for $C_{21}H_{17}F_3N_2O_4$: C, 60.29; H, 4.10; N, 6.70. Found: C, 60.24; H, 4.21; N, 6.73.

*4.1.3.7. 5-(3,5-Dimethylisoxazol-4-yl)-3-(2-fluorophenyl)-3-hydroxy-2-methoxyisoindolin-1-one (**10g**)*. White solid, yield 88%. m.p.: 149–151 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.03 (t, J = 8.0 Hz, 1H), 7.84 (d, J = 7.5 Hz, 1H), 7.76 (s, 1H), 7.60 – 7.55 (m, 1H), 7.42 (d, J = 5.5 Hz, 1H), 7.35 – 7.25 (m, 2H), 7.05 (dd, J = 11.8, 8.5 Hz, 1H), 3.83 (s, 3H), 2.35 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.35, 158.27, 146.66, 135.27, 131.37, 130.69, 130.06, 128.06, 126.06, 125.93, 124.86, 123.58, 123.02, 116.39, 116.09, 115.79, 88.04, 65.40, 40.91, 40.63, 40.35, 40.08, 39.80, 39.52, 39.24, 11.80, 10.80. MS (ESI, *m/z*): 369.4[M + H]$^+$; Anal. calcd. for $C_{20}H_{17}FN_2O_4$: C, 65.21; H, 4.65; N, 7.60. Found: C, 65.27; H, 4.61; N, 7.57.

*4.1.3.8. 3-(2-Chlorophenyl)-5-(3,5-dimethylisoxazol-4-yl)-3-hydroxy-2-methoxyisoindolin-1-one (**10h**)*. White solid, yield 84%. m.p.: 148–150 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.28 (d, J = 8.3 Hz, 1H), 7.83 (d, J = 7.6 Hz, 1H), 7.69 (s, 1H), 7.61 – 7.30 (m, 4H), 7.13 (s, 1H), 3.76 (s, 3H), 2.33 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.27, 162.99, 158.27, 146.12, 135.09, 134.94, 131.31, 131.13, 131.08, 130.73, 129.32, 127.73, 123.55, 122.96, 115.84, 88.67, 64.94, 40.81, 40.53, 40.26, 39.98, 39.70, 39.42, 39.14, 11.75, 10.78. MS (ESI, *m/z*): 385.3[M + H]$^+$; Anal. calcd. for $C_{20}H_{17}ClN_2O_4$: C, 62.42; H, 4.45; N, 7.28. Found: C, 62.41; H, 4.41; N, 7.27.

*4.1.3.9. 3-(3-Chlorophenyl)-5-(3,5-dimethylisoxazol-4-yl)-3-hydroxy-2-methoxyisoindolin-1-one (**10i**)*. White solid, yield 78%. m.p.: 151–153 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.93 – 7.75 (m, 2H), 7.52 (dd, J = 41.3, 17.7 Hz, 5H), 7.25 (s, 1H), 3.89 (s, 3H), 2.37 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.00, 162.16, 157.86, 146.53, 141.45, 135.11, 133.11, 130.40, 130.35, 128.33, 126.65, 126.01, 124.80, 123.39, 122.98, 115.24, 113.14, 89.79, 65.12, 40.30, 40.04, 39.75, 39.48, 39.20, 38.92, 38.65, 11.33, 10.32. MS (ESI, *m/z*): 385.4[M + H]$^+$; Anal. calcd. for $C_{20}H_{17}ClN_2O_4$: C, 62.42; H, 4.45; N, 7.28. Found: C, 62.31; H, 4.51; N, 7.34.

*4.1.3.10. 2-(Benzyloxy)-5-(3,5-dimethylisoxazol-4-yl)-3-(3-fluorophenyl)-3-hydroxyisoindolin-1-one (**10j**)*. Yellow solid, yield 81%. m.p.: 156–158 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.86 (d, J = 11.0 Hz, 2H), 7.58 (d, J = 7.9 Hz, 1H), 7.37 (dd, J = 16.4, 6.3 Hz, 8H), 7.13 (dd, J = 22.3, 8.0 Hz, 2H), 5.09 (dd, J = 34.0, 10.0 Hz, 2H), 2.36 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.47, 162.95, 158.34, 147.20, 142.58, 142.48, 135.67, 135.63, 130.98, 130.87, 129.47, 129.02, 128.76, 127.31, 123.89, 123.59, 122.62, 115.80, 113.99, 113.68, 90.50, 79.61, 40.91, 40.63, 40.35, 40.07, 39.79, 39.52, 39.24, 11.82, 10.81. MS (ESI, *m/z*): 445.4[M + H]$^+$; Anal. calcd. for $C_{26}H_{21}FN_2O_4$: C, 70.26; H, 4.76; N, 6.30. Found: C, 70.31; H, 4.81; N, 6.24.

2-(Benzyloxy)-5-(3,5-dimethylisoxazol-4-yl)-3-(2-fluorophenyl)-3-hydroxyisoindolin-1-one (**10*k***)

Yellow solid, yield 79%. m.p.: 146–148 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.84 (s, 2H), 7.59 (s, 1H), 7.34 (s, 8H), 7.05 (s, 1H), 5.23 – 4.90 (m, 2H), 2.37 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 165.89, 162.87, 157.80, 146.20, 135.15, 134.81, 131.05, 131.01, 130.95, 130.93, 130.21, 129.53, 128.89, 128.47, 128.21, 127.53, 124.39, 123.14, 122.59, 115.92, 115.63, 115.27, 87.85, 78.92, 40.32, 40.04, 39.77, 39.49, 39.21, 38.93, 38.65, 11.32, 10.33. MS (ESI, *m/z*): 445.5[M + H]$^+$; Anal. calcd. for $C_{26}H_{21}FN_2O_4$: C, 70.26; H, 4.76; N, 6.30. Found: C, 70.21; H, 4.74; N, 6.33.

*4.1.3.11. 3-(2-(Benzyloxy)-6-(3,5-dimethylisoxazol-4-yl)-1-hydroxy-3-oxoisoindolin-1-yl)benzonitrile (**10l**)*. Yellow solid, yield 81%. m.p.: 151–152 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, J = 8.5 Hz, 2H), 7.89 (d, J = 7.9 Hz, 1H), 7.82 (d, J = 7.0 Hz, 1H), 7.59 (dd, J = 11.7, 9.2 Hz, 3H), 7.46 (s, 1H), 7.43 – 7.32 (m, 5H), 5.12 (dd, J = 31.6, 10.1 Hz, 2H), 2.38 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 170.02, 166.02, 162.52, 146.27, 140.67, 135.24, 135.06, 132.27, 132.02, 131.99, 131.51, 131.38, 130.91, 130.50, 130.06, 129.77, 129.02, 128.80, 128.64, 128.58, 128.27, 126.73, 123.51, 123.20, 79.24, 40.31, 39.76, 39.47, 39.20, 38.92, 38.63, 11.34, 10.33. MS (ESI, *m/z*): 452.5[M + H]$^+$; Anal. calcd. for $C_{27}H_{21}N_3O_4$: C, 71.83; H, 4.69; N, 9.31. Found: C, 71.23; H, 4.63; N, 9.35.

*4.1.3.12. 2-(Benzyloxy)-5-(3,5-dimethylisoxazol-4-yl)-3-(4-fluorophenyl)-3-hydroxyisoindolin-1-one (**10m**)*. Yellow solid, yield 79%. m.p.: 149–151 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.87 (d, J = 7.8 Hz, 1H), 7.77 (s, 1H), 7.59 (d, J = 7.9 Hz, 1H), 7.50 – 7.32 (m, 8H), 7.19 (t, J = 8.8 Hz, 2H), 5.10 (dd, J = 38.5, 10.1 Hz, 2H), 2.38 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.45, 162.84, 160.83, 158.33, 147.52, 135.68, 135.55, 130.74, 129.46, 129.00, 128.97, 128.85, 128.75, 127.33, 123.83, 123.53, 115.81, 115.51, 90.65, 79.55, 40.91, 40.63, 40.35, 40.08, 39.80, 39.52, 39.24, 11.83, 10.83. MS (ESI, *m/z*): 445.5[M + H]$^+$; Anal. calcd. for $C_{26}H_{21}FN_2O_4$: C, 70.26; H, 4.76; N, 6.30. Found: C, 70.23; H, 4.73; N, 6.31.

2-(Benzyloxy)-5-(3,5-dimethylisoxazol-4-yl)-3-hydroxy-3-(p-tolyl) isoindolin-1-one (**10*n***)

White solid, yield 78%. m.p.: 149–151 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.85 (d, J = 7.7 Hz, 1H), 7.64 (s, 1H), 7.58 (dd, J = 7.8, 1.4 Hz, 1H), 7.46 – 7.27 (m, 8H), 7.16 (d, J = 8.0 Hz, 2H), 2.37 (s, 3H), 2.26 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 165.92, 162.33, 157.82, 147.37, 137.56, 136.04, 135.21, 134.87, 131.99, 131.51, 131.38, 130.04, 129.00, 128.89, 128.79, 128.63, 128.49, 128.24, 126.87, 126.05, 123.25, 122.97, 115.32, 90.55, 78.97, 40.32, 40.03, 39.76, 39.48, 39.21, 38.93, 38.66, 20.60, 11.33, 10.36. MS (ESI, *m/z*): 441.3[M + H]$^+$; Anal. calcd. for $C_{27}H_{24}N_2O_4$: C, 73.62; H, 5.49; N, 6.36. Found: C, 73.53; H, 5.51; N, 6.32.

*4.1.3.13. 2-(Benzyloxy)-5-(3, 5-dimethylisoxazol-4-yl)-3-hydroxy-3-(3-methoxyphenyl)isoindolin-1-o ne (**10o**)*. White solid, yield 85%. m.p.: 138–140 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.86 (d, J = 8.1 Hz, 1H), 7.72 (s, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.48 – 7.21 (m, 7H), 7.08 (s, 1H), 6.97 – 6.79 (m, 2H), 5.10 (dd, J = 38.3, 10.1 Hz, 2H), 3.73 (s, 3H), 2.38 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 165.91, 162.39, 159.23, 157.85, 147.11, 140.63, 135.18, 134.91, 130.14, 129.51, 129.01, 128.52, 128.25, 126.81, 123.29, 123.01, 118.15, 115.31, 113.42, 112.22, 90.39, 79.01, 55.04, 40.28, 39.98, 39.73, 39.45, 39.17, 38.90, 38.60, 11.33, 10.35. MS (ESI, *m/z*): 457.3[M + H]$^+$;

Anal. calcd. for $C_{27}H_{24}N_2O_5$: C, 71.04; H, 5.30; N, 6.14. Found: C, 71.03; H, 5.41; N, 6.12.

*4.1.3.14. 2-(Benzyloxy)-5-(3, 5-dimethylisoxazol-4-yl)-3-hydroxy-3-phenylisoindolin-1-one (**10p**)*. White solid, yield 88%. m.p.: 142–143 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.87 (d, J = 7.6 Hz, 1H), 7.73 (s, 1H), 7.59 (d, J = 7.3 Hz, 1H), 7.53 – 7.23 (m, 11*H*), 5.11 (dd, J = 41.3, 10.1 Hz, 2H), 2.38 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 166.42, 162.85, 158.31, 147.73, 139.51, 135.72, 135.43, 130.62, 129.45, 128.99, 128.84, 128.78, 128.74, 127.41, 126.61, 123.77, 123.53, 91.04, 79.47, 40.91, 40.64, 40.36, 40.08, 39.80, 39.52, 39.25, 11.82, 10.82. MS (ESI, m/z): 427.3[M + H]$^+$; Anal. calcd. for $C_{26}H_{22}N_2O_4$: C, 73.23; H, 5.20; N, 6.57. Found: C, 73.26; H, 5.21; N, 6.62.

4.2. Docking studies

All compounds were sketched in ChemDraw 2014, and saved as sdf style. Then ligands were finally minimized at the OPLS 2005 force field with Ligand preparation protocol of Maestro 10.2. The crystal complex (PDB id: 3P5O) was selected as the BRD4-BD1 docking protein. Several molecules of water around the Kac-pocket were saved (id: HOH18, HOH27, HOH33, HOH256, HOH267, HOH268, HOH358). The procedure and conditions were described in our previous work [33]. After docking finished, only one best docking conformation was exported.

4.3. Biological evaluation

4.3.1. AlphaScreen bromodomain binding assay

The AlphaScreen bromodomain binding assay was performed by Shanghai ChemPartner Co. Firstly 1x assay buffer (modified HEPES Buffer) was prepared for this experiment. Small molecules were transferred to assay plates with Echo. DMSO's final concentration is 0.1% following protein solution prepared in 1x assay buffer (5 nM). The procedure and conditions were described in our previous work [24].

4.3.2. Cell proliferation assay

LNcap-1, HL-60, MV4-11 cells were seeded in 96-well tissue culture plates without test compound and incubated at 37 °C and an atmosphere of 5% CO_2 for 24 h. On the next day, various concentrations of compounds were prepared and added into the wells. After that, cells were further incubated for 72 h at 37 °C and an atmosphere of 5% CO_2. MTT colorimetric assay was performed following standard protocols.

4.3.3. Western blot analysis

Cell lysates were prepared in cell lysis buffer (RIPA Lysis Buffer, Beyotime, P0013B) with protease inhibitor cocktail (Roche, 5892970001). 30 μg of total protein was resolved on a 12% SDS polyacrylamide gel and probed with anti-cMyc (Abcam Ab32072), anti-PARP (Cell Signaling #9542), anti-BRD4 (Cell Signaling #13440), anti-cleaved caspase3 (Cell Signaling # 9664), anti-caspase3 (Cell Signaling # 9665), GAPDH loading control Cell Signaling#5174. Western blotting was performed following standard protocols.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.034.

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