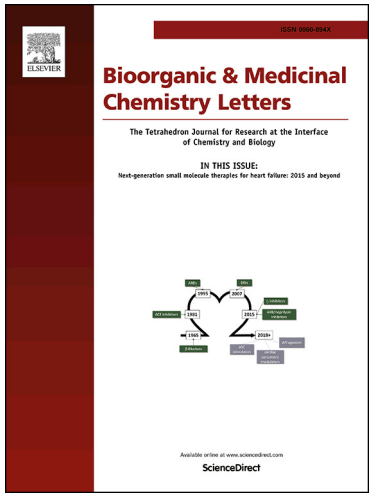

Design, synthesis and biological evaluation of 3,5-dimethylisoxazole and pyridone derivatives as BRD4 inhibitors

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Graphical Abstract

Design, synthesis and biological evaluation of 3,5-dimethylisoxazole and pyridone derivatives as BRD4 inhibitors

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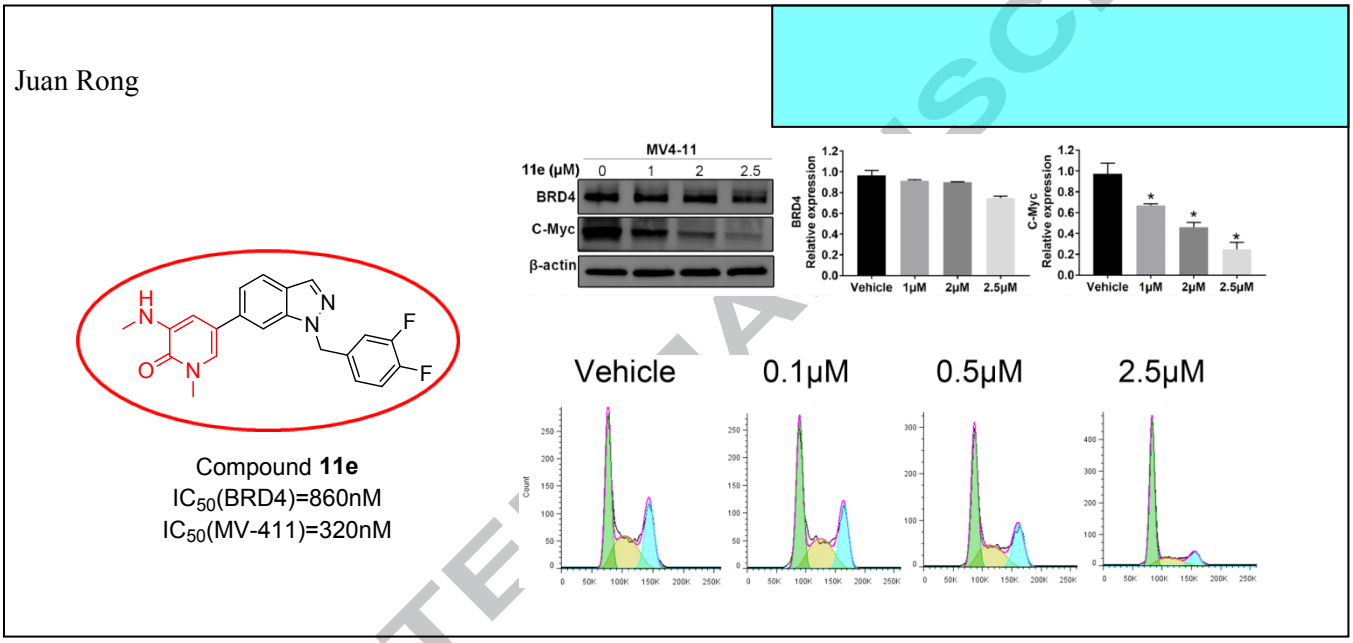

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ABSTRACT

Bromodomain-containing protein 4 (BRD4), a member of the bromodomain and extra-terminal (BET) family, has been recognized as an attractive candidate target for the treatment targeting gene transcription in several types of cancers. In this study, two types of novel compounds were designed, synthesized and evaluated as BRD4 inhibitors. Therein, pyridone derivatives were more effective against BRD4 protein and human leukemia cell lines MV4-11. Among them, compounds **11d**, **11e** and **11f** were the most potential ones with IC_{50} values of 0.55 μM, 0.86 μM and 0.80 μM against BRD4, and exhibited remarkable antiproliferative activities against MV4-11 cells with IC_{50} values of 0.19 μM, 0.32 μM and 0.12 μM, respectively. Moreover, in western blot assay, compound **11e** induced down-regulation of C-Myc, which is a significant downstream gene of BRD4. Cell cycle analysis assay also showed that compound **11e** could block MV4-11 cells at G0/G1 phase. Taken together, our results suggested that compound **11e** and its derivatives were a class of novel structural potential BRD4 inhibitors and could serve as lead compounds for further exploration.

Lysine acetylation in histones is one of the most important epigenetic post-translational modifications,[1] the disorder of which leads to aberrant transcription, promoting the occurrence of cancers and other diseases. Bromodomains (BRDs) protein can recognize the specific acetylated lysine (KAc) residues, and then mediate signaling transduction to regulate specific gene expression.[2,3] Bromodomain and extra terminal (BET) family (BRD2, BRD3, BRD4 and BRDT), is most extensively investigated. BRD4 is a well-studied member of the BET family, it plays an essential role in gene epigenetics by binding KAc on chromatin structures as the 'readers' of lysine acetylation state. BRD4 can recruit P-TEFb, one positive transcriptional elongation factor complex and essential in the regulation of transcription by RNA polymerase II in eukaryotes.[4] In addition, BRD4 was suggested to stimulate G1 gene transcription and promote cell cycle progression to S phase.[5] And it was able to regulate the expression of specific cancer-related genes, for instance, C-Myc and Bcl-2,[6,7] which are key regulators for the proliferation of tumor cells. Therefore, the inhibition against BRD4 can down-regulate the expression of corresponding oncogenes. Furthermore, it was reported that BRD4 played a key roles in the proliferation of many types of cancers, such as activated B-cell-like subtype of diffuse large B-cell lymphoma, lung adenocarcinoma.[8] Therefore, BRD4 represents a novel therapeutic target for antineoplastic drug development. BRD4 consists of two N-terminal tandem bromodomain (BD1 and BD2) and an extra-terminal recruitment domain (ET). Each bromodomain comprises a four-helix bundle (helices αZ, αA, αB, and αC) and two loop regions (ZA and BC loops).[9] Helices αZ and αA, helices αB and αC form two hydrophobic loop, called ZA Loop and BC Loop. Then ZA Loop, BC Loop and αZ together constitute a hydrophobic structure area, which is " WPF shelf ".[10,11] The entire spatial structure is called KAc binding site.

At present, a mass of small molecule BET bromodomain inhibitors undergoing clinical trials with encouraging emerging data.[12] Particularly, multiple BRD4 inhibitors with different scaffolds have been reported, and studied in clinical trials for cancer treatment.[13,14,15] As shown in Fig.1. (+)-JQ1(Compound $\mathbf{X_1}$), with a triazolothienodiazepine scaffold, was the first reported potent BRD4 inhibitor with an IC_{50} value of 77 nM in the Alpha-Screen assay,[16] and was widely used to study the physiology function of BET proteins in cancer.[17] I-BET151 (Fig.1, compound $\mathbf{X_2}$) was the earliest BRD4 inhibitor bearing with 3,5-dimethylisoxazole scaffold discovered by GSK, and expressed well efficacy in vivo against MLL-fusion leukemia.[18] ABBV-075 (Fig.1, compound $\mathbf{X_3}$), another potent BET family bromodomain inhibitor, was proved to have anti-proliferative activity in multiple cancer cell lines.[19] Meanwhile, RVX-208 (Fig.1, compound $\mathbf{X_4}$), as a specific BET bromodomain inhibitor for BD2, was under phase-Ⅱ clinical trial for the treatment of coronary syndrome and atheros-clerosis.[20] I-BET762 (Fig.1, compound $\mathbf{X_5}$), also recognized as a selective BET inhibitor, had entered phase II clinical trial.[21,22] Besides, PFI-1 (compound $\mathbf{X_6}$) was another BRD4 inhibitor with IC_{50} of 220nM.[23]

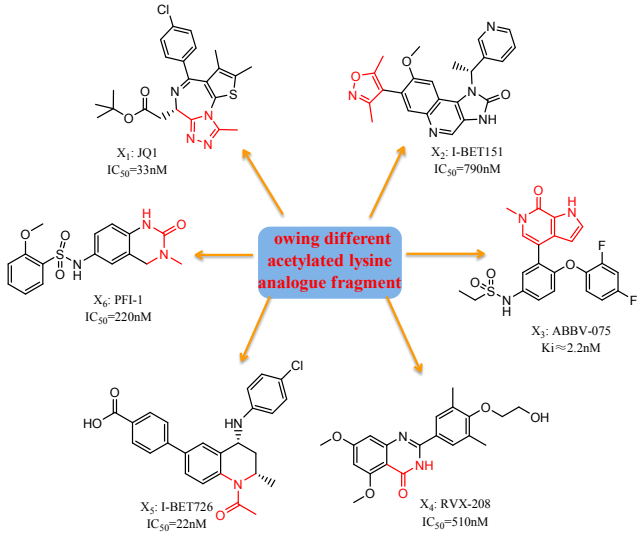


Figure 1. The structures of reported BRD4 inhibitors (Compound $\mathbf{X_1}$-$\mathbf{X_6}$)

Although many reported effective inhibitors have made great progress, the pace of exploration will not stop. Novel inhibitors with therapeutic potential still need to be discovered to provide more pharmacological tools in the investigation of BRD4's functions and selection of optimal structure.

I-BET151 and many other inhibitors contained a 3,5-dimethylisoxazole fragment, as an acetyl-lysine binding mimic,[24] and the Hewings team also verified that 3,5-dimethylisoxazole was an efficient acetylated lysine analogue fragment.[25] Some BRD4 inhibitors containing 3,5-dimethylisoxazole were listed in Fig.2A (left), and the IC_{50} of which were all at a low level. Fig.2A (right) showed that, 3,5-dimethylisoxazole fragment of compound Y_4 interacted with KAc binding site in BRD4 protein. According to above-mentioned, we retained 3,5-dimethylisoxazole fragment in the first structure.

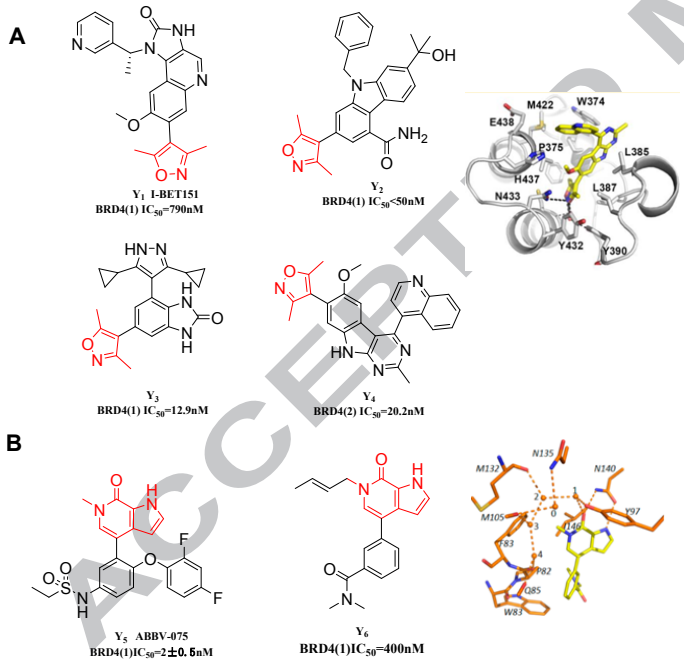


Figure 2. (A) The structures of reported BRD4 inhibitors containing 3,5-dimethylisoxazole fragment, BRD4 (BD2) is shown to interact with Compound Y_4. (carbon atom in gray, oxygen in red, nitrogen in blue, sulfur in gold; BRD4 is present in a gray cartoon)[26]; (B) The structures of reported BRD4 inhibitors containing 1-methylpyridin-2(1H)-one fragment, Inhibitor Y_6 bound to BRD4 (BD1).[27]

At the same time, we found that pyridone substructure was widely used in designs of BRD4 inhibitors (Fig.2B). The oxygen and nitrogen atoms in pyridone fragment of compound Y_6 bound to aspartic acid residue in KAc site as shown in Fig.2B (right). So we decided to introduce 1-methyl-3-(methylamino)pyridin-2(1H)-one fragment, as acetyl-lysine binding mimic in the second structure.

In the design of the first type (Fig.3A), we retained the acetylated lysine analogue fragment, 3,5-dimethylisoxazole, and replaced scaffold with an indazole according to scaffold hopping, one side of the chain was jointed with a substituted benzene ring by an N-H bridge to increased its hydrophilicity and was expected to reach the ZA channel of KAc in BRD4. In the design of the second type, the pyridone fragment was reserved, pyrrol ring in pyridone substructure was transformed into aliphatic hydrocarbon, N-hydrogen was still reserved because of its function to form additional hydrogen bonds (Fig.3B).

A series of compounds containing 3,5-dimethylisoxazole fragment were synthesized, and the main steps were exhibited in Scheme 1. All synthesized compounds were evaluated inhibitory activities against BRD4 protein and human leukemia cell line MV4-11.[28] (Table 1)

Firstly, comparing **5a**-**5m** with **5n**-**5p**, when 3,5-dimethylisoxazole was in R^1 position of the indazole ring , the activity potency was relatively superior. A reasonable explanation was that R^1 position was essential for the binding to KAc recognition site. Removing R^4 substituent group from the indazole ring caused the reducing of inhibitory activity with 3~4 fold (compare **5a** with **5b** and **5m**), indicating that R^4 substitution was important for inhibitory activity. Then, we fixed R^4 position with a tetrahydropyrane ring, compounds **5c**-**5f** were prepared to explore the effects of the type and position of the substituent at side chain phenyl group in R^3 position, the results indicated that poly-substitution or mono-substitution make no contribution to activity.

When tetrahydropyran ring in R^4 position was replaced with benzene ring, there was no significant change for inhibitory activity. Overall, substituents in R^1 and R^4 position were necessary for inhibitory activity. Noticeably, when 5-position of the indazole ring was occupied by a methoxy group, compound **5m** with 3,5-dimethylisoxanthine at the 6-position of indazole gained multiplied inhibitory activity, which could be inferred that methoxy group was likely to facilitate the formation of hydrogen bonds between the acetylated lysine residue mimic and BRD4 protein.

As shown in table 2, when the core scaffold was replaced with a pyrimidine ring, the inhibition rate of those compounds at 1μM was less than 20%, so the activity of this type was not improved.

In summary, isoxazole derivatives (**5a**-**5r**) all showed poor activity both in protein and cell levels. Therefore, we gave up the further research on this kind of structure but focused on the second type of structure, pyridone derivatives.

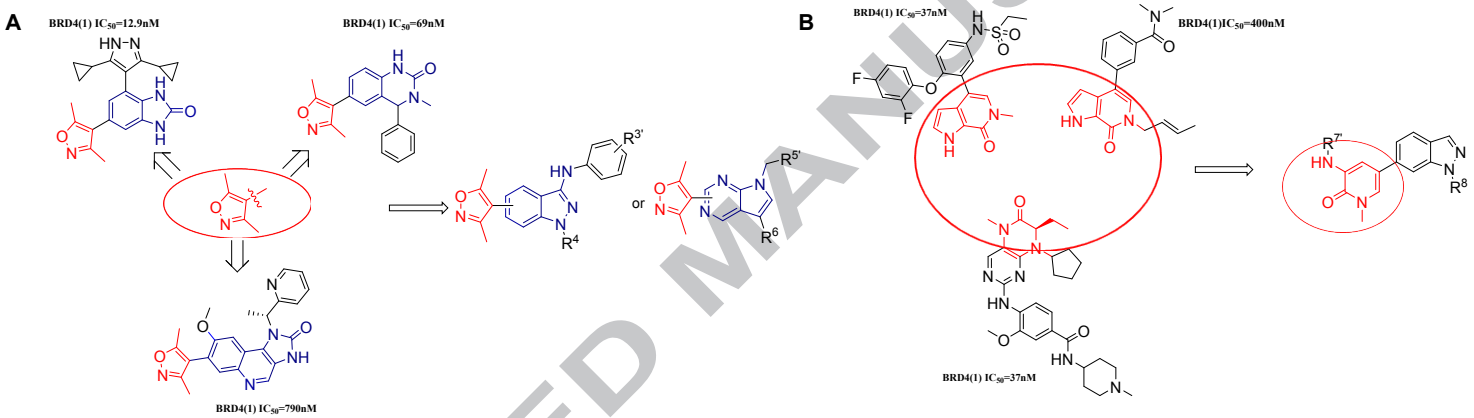
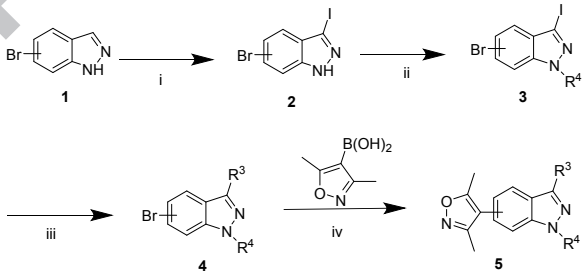
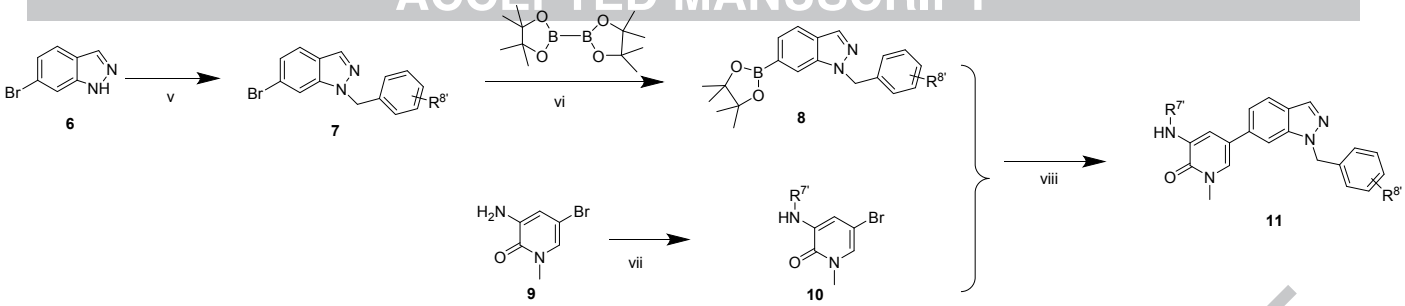


Figure 3. (A) Design of 3,5-dimethylisoxazole derivatives; (B) Design of pyridone derivatives.[29]

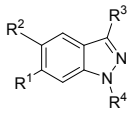


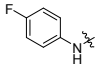
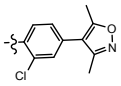
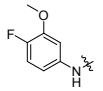
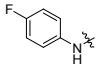
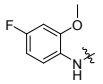
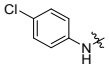
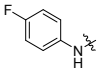
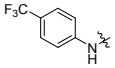
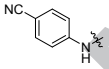
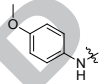
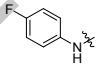
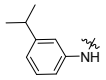
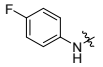
Scheme 1. Synthesis of 3,5-dimethylisoxazole derivatives. (i) I_2, KOH, DMF; rt, 1~2h. 85%. (ii) 3,4-Dihydropyran or Benzyl bromide, *p*-toluenesulfonic acid, DCM, rt, overnight. 75%. (iii) $Pd(AcO)_2$, Xantphos, dry DMF, phenylamine, N_2, 60℃, 6~7h. 40%. (iv) 3,5-dimethylisoxanthraquinone, Na_2CO_3, dioxane and water, $Pd(dppf)Cl_2$, N_2, 90℃, 6~9h. 50%.



Scheme 2. Synthesis of pyridone derivatives. (v) Benzyl bromide or benzyl chloride, *p*-toluenesulfonic acid, DCM, rt, overnight. 75%. (vi) Potassium acetate, $Pd(dppf)Cl_2$, Bis(pinacolato)diboron, dioxane, N_2, 95℃, 12h, 50%. (vii) HCHO or CH_3CHO, methanol, acetic acid, DCM, rt, 0.5h, 65%. (viii) Na_2CO_3, dioxane and water, $Pd(dppf)Cl_2$, N_2, 90℃, 6~7h. 50%.

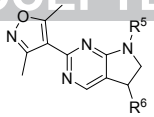
Table 1. Structure-activity relationship analysis of 3,5-dimethylisoxazole derivatives.

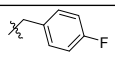
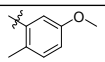
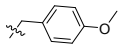


Compound	R^1	R^2	R^3	R^4	[a]Inhibition at 1μM	MV4-11,IC_{50} (μM)[b]
5a		H		H	< 20%	7.80
5b		H	H		33%	2.80
5c		H			< 20%	9.30
5d		H			< 20%	9.90
5e		H			< 20%	8.50
5f		H			< 20%	7.40
5g		H			< 20%	ND
5h		H			< 20%	-10.30
5i		H			< 20%	5.80
5j		H			< 20%	11.90
5k		H			< 20%	9.10
5l		H			< 20%	8.10
5m			H		45%	1.90
5n	H				< 20%	ND
5o	H				< 20%	17.50
5p	H				ND	ND
JQ1					0.09	0.06

[a] represent inhibitory rate against BRD4 protein at 1μM; [b] Values are the average of three distinct runs.
ND represent the inhibitory activities are not performed.

a
b
le 2.



Compound	R^5	R^6	[a]Inhibition at 1 μM	MV4-11 IC_{50} (μM)
5q			< 20%	ND
5r			< 20%	ND

[a] represent inhibitory rate against BRD4 protein at 1μM; ND represent the inhibitory activities are not performed.

Then, thirteen compounds with 1-methyl-3-(methylamino) pyridin-2(1H)-one fragment were designed and synthesized (Fig.3B, Scheme 2). Obviously, the inhibitory activity of pyridone derivatives were markedly improved compared with 3,5-dimethylisoxazole derivatives.

As shown in Table 3. Firstly, to investigate the impact of 1-methyl-3-(methylamino)pyridin-2(1H)-one substructure, we replaced N-methyl in amidogen of pyridine ring with N-ethyl, leading to 2~10 fold reduction in potency (compare **11i**, **11j** to **11a**, **11d**). It was possible that N-methyl was necessary to anchor into the Kac binding pocket of BRD4. Then, we investigated the impact of R^8 position. When R^8 position was mono-substitution phenyl, there was no significant difference among compounds **11a**-**11c** with IC_{50} values of 1.4~1.6 μM, indicating that electron-withdrawing group or electron-donating group had no effect on the activity. when R^8 position was disubstituted (3,5-dimethoxybenzyl, 3,4-difluoro-benzyl and 4-fluoro-3-nitrobenzyl), compounds (**11d**, **11e** and **11f)** showed leap-type improvement of inhibitory activity, with IC_{50} of 0.55, 0.86 and 0.8 μM against BRD4 protein., suggesting R^8 preferred to disubstituted benzene and m-substitution. Finally, when the substituents in R^8 position were cyclohexyl, aliphatic chain hydrocarbon and cyclopropyl, the inhibition rates of compounds **11k**, **11l** and **11m** decreased slightly, so the position of R^8 preferred rigid flat phenyl.

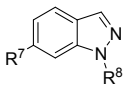
Then, multiple tumor cell lines were utilized to further validate the cellular proliferation inhibition effects of pyridone derivatives. Compounds **11d**, **11e** and **11f** exhibited reasonable anti-proliferative activity, especially to cell line MV4-11 with IC_{50} value of 0.19, 0.32 and 0.13 μM, respectively (Table 4 and fig.5A), illustrating that activity of pyridone derivatives on BRD4-sensitive cells(MV4-11) was more significant than BRD4-independent cells (HCT-116, SW480, DU145, Capan-1, BT474).

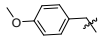
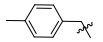
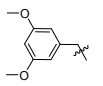
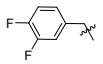
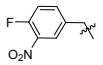
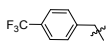
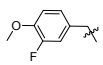
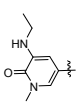
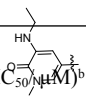
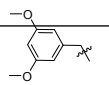
Table 4. cellular proliferation inhibition effects of compound **11d**, **11e** and **11f** in multiple tumor cell lines

Cpd	Tumor cell inhibitory activity (IC_{50}/μM)[b]				
	MV4-11	HCT116	SW480	DU145	Capan-1	BT474
11d	**0.19**	2.90	7.40	4.80	3.70	8.80
11e	**0.32**	3.00	8.40	3.40	4.00	6.50
11f	**0.13**	2.60	10.10	0.20	0.80	1.70
JQ1	0.09	0.40	14.10	0.09	0.06	0.06

[b] Values are the average of three distinct runs.

Table 3. Structure-activity relationship analysis of pyridone derivatives.



Compound	R^7	R^8	BRD4 (BD1,2) IC_{50}(μM)[a]	MV4-11 IC_{50} (μM)[b]
11a			1.40	2.39
11b			1.60	2.70
11c			1.60	3.28
11d			**0.55**	**0.20**
11e			**0.86**	**0..32**
11f			**0.80**	**0.13**
11g			2.40	4.37
11h			1.40	4.98
11i			2.25	5.65
11j			1.81	2.59
11k			1.81	2.69
11m			1.96	3.45

[a] AlphaScreen assay.
[b] Values are the average of three distinct runs.

Then we selected compound **11e**, which was least cytotoxic to human normal hepatocytes LO2 (Fig.4B), with relatively higher

activity against cancer cell lines, to further evaluate its biological properties.

The anti-proliferative mechanism of **11e** was investigated in MV4-11 cells , and C-Myc was selected to determine the inhibition of **11e** against the transcription of BRD4 downstream genes. As shown in Fig.5, the expression of C-Myc was measured by western blot. Results showed that the expression of C-Myc were decreased in a dose-dependent manner, indicating that compound **11e** could affect the transcription and expression of some downstream target genes.

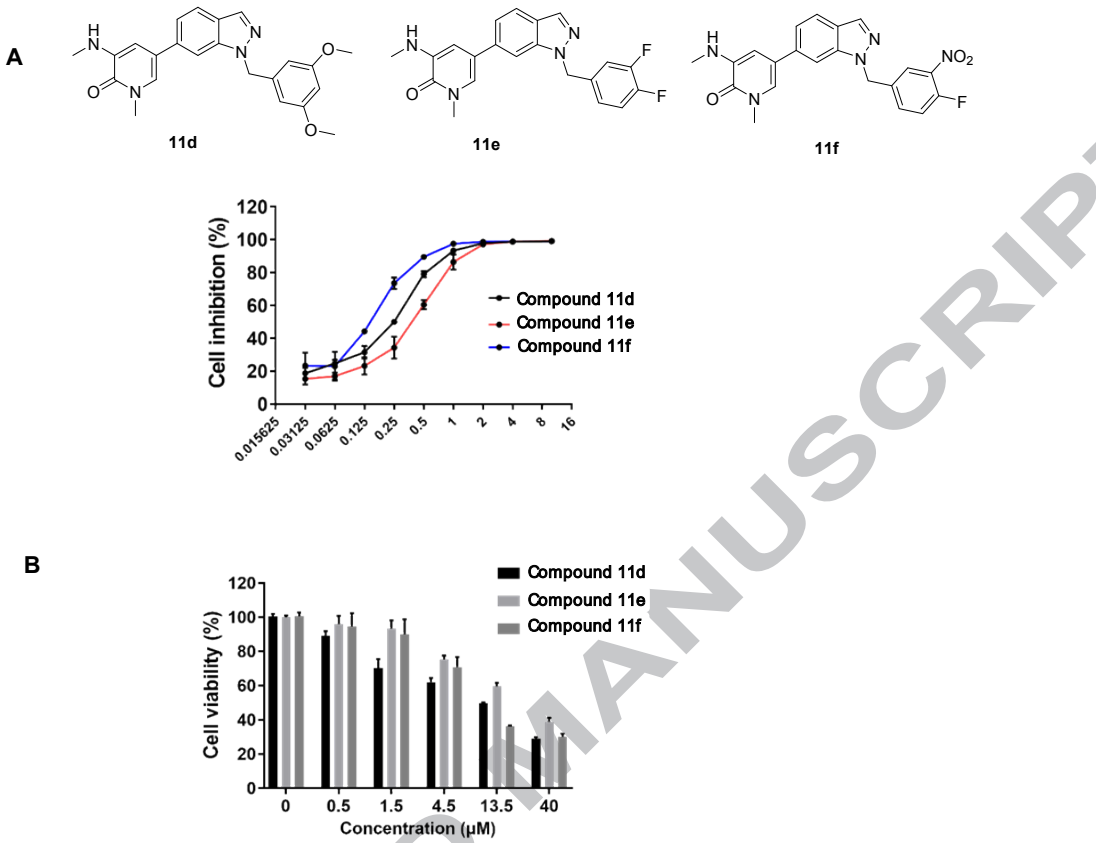


Figure 4. (A) Inhibition to MV4-11 cells by compounds **11d-11f**. (B) The effects of **11d-11f** on the normal human liver cell line LO2. MTT assays were used after the treatment of drugs for 72h.

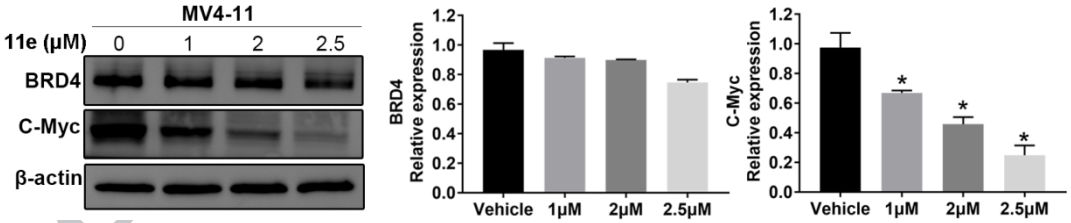


Figure 5. The expression of BRD4 and C-Myc was determined by Western blot assay. MV4-11 cells were treated with DMSO or the indicated concentrations of compound **11e** (0.1, 0.5 and 2.5 μM) for 48 hours and the expression of BRD4 and C-Myc were detected with the specific antibody. The expression of β-actin was used as the internal control.

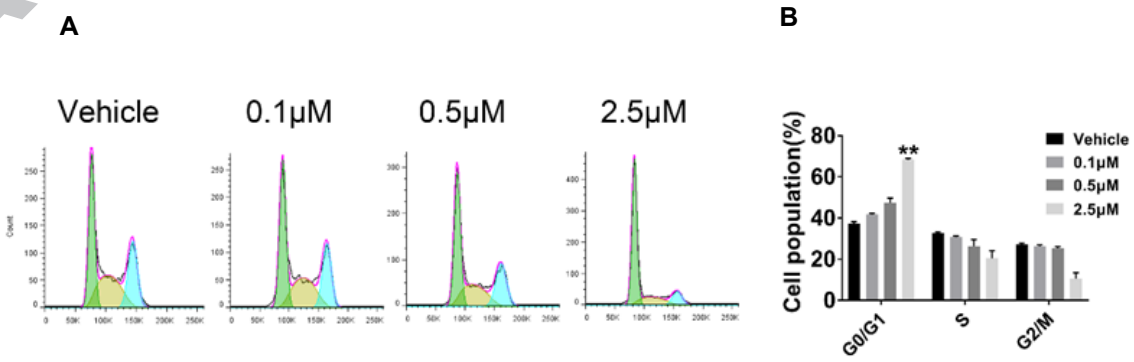


Figure 6. (A) MV4-11 cells were treated with the indicated concentrations of compound **11e** (0.1, 0.5 and 2.5 μM) for 48 hours. The distribution of cell cycle was analyzed by flow cytometry. (B) The quantification of cell cycle phase is shown in right panel (*P<0.05), the treatment group and the control group were compared by t-test.

In the cell cycle arrest assay, MV4-11 cells were treated with different concentrations of compound **11e**. As shown in Fig.6A and 6B, flow cytometry analysis results indicated that the number of cells in the G0/G1 phase obviously increased in a dose dependent manner, showing that G0/G1 phase arrest might be associated with the effects of compound **11e** in MV4-11 cells.

In this study, a new series of BRD4 inhibitors have been designed and synthesized through fragment-based strategy. Firstly, we designed two kinds of new structure, isoxazole derivatives on the basis of reported BRD4 inhibitors owing 3,5-dimethylisoxazole fragment, and pyridone derivatives based on pyridone substructure. Unfortunately, isoxazole derivatives showed an inferior inhibitory efficacy against BRD4 protein and leukemia cell MV4-11. By contrast, pyridone derivatives with 1-methyl-3-(methylamino)pyridin-2(1H)-one fragment could inhibit BRD4 protein with relatively higher efficiency, and exhibited remarkable anti-proliferative activities toward MV4-11 cell at the same time. Structure-activity relationship study was performed and resulted in substantial improvement of the inhibitory potency against BRD4 protein. Among them, compounds **11d**, **11e** and **11f** were the most potent inhibitors against BRD4 with IC_{50} values of 0.55 μM, 0.86 μM and 0.80 μM, respectively. And compound **11e** exhibited effective and selective inhibitory activities in MV4-11 cell with IC_{50} value of 0.32 μM, and relatively lower toxicity to human hepatocytes LO2. In addition, **11e** was also verified to block cell cycle at G0/G1 phase. In the western blot assay, compound **11e** could decrease the protein expressions of C-Myc in MV4-11 cell. Further evaluation of these pyridone derivatives as BRD4 inhibitors and deeper SAR exploration will be completed in due course to seek optimal inhibitor. In brief, these results indicated that this type of structure owing novel scaffold was promising to become superior lead compounds, to explore potent BRD4 inhibitor as drug candidate in the therapy of BRD4 protein-related cancers.

Acknowledgments

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References

1. Verdone L, Caserta M, Mauro ED. Role of histone acetylation in the control of gene expression. Biochemistry and cell biology. 2005, 83(3):344-53.
2. Marushige K . Activation of chromatin by acetylation of histone side chains. Proceedings of the National Academy of Sciences of the United States of America. 1976, 73(11):3937-3941.
3. Shogren KM. Histone H4-K16 Acetylation controls chromatin structure and protein interactions. Science. 2006, 311(5762): 844-847.
4. Donato E, Croci O, Sabò A, et al. Compensatory RNA polymerase 2 loading determines the efficacy and transcriptional selectivity of JQ1 in Myc-driven tumors. Leukemia. 2016, 31(2): 479–490.
5. Asangani I, Dommeti V, Wang X, et al. Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer. Nature. 2014, 510(7504):278-282.
6. Delmore JE, Issa GC, Lemieux ME, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell. 2011, 146(6): 904–917.
7. Wyce A., Ganji G, Smitheman KN, et al. BET inhibition silences expression of MYCN and BCL2 and induces cytotoxicity in neuroblastoma tumor models. PLOS ONE. 2013, 8(8), e72967.
8. Lockwood WW, Zejnullahu K, Bradner JE, et al. Sensitivity of human lung adenocarcinoma cell lines to targeted inhibition of BET epigenetic signaling proteins. Proceedings of the National Academy of Sciences. 2012, 109(47):19408-19413.
9. Wu SY, Chiang CM. The double bromodomain-containing chromatin adaptor brd4 and transcriptional regulation. Journal of Biological Chemistry. 2007, 282(18):13141-13145.
10. Romero FA, Taylor AM, Crawford TD, et al. Disrupting acetyl-lysine recognition: Progress in the development of bromodomain inhibitors. Journal of Medicinal Chemistry. 2015, 59(4): 1271-1298.
11. Belkina AC, Denis GV. BET domain co-regulators in obesity, inflammation and cancer. Nature Reviews Cancer. 2012, 12(7): 465-477.
12. Theodoulou NH, Tomkinson NC, Prinjha RK, et al. Clinical progress and pharmacology of small molecule bromodomain inhibitors. Current Opinion in Chemical Biology. 2016, 33:58-66.
13. Zhang Z, Gu L, Wang B, et al. Discovery of novel coumarin derivatives as potent and orally bioavailable BRD4 inhibitors based on scaffold hopping. Journal of Enzyme Inhibition and Medicinal Chemistry. 2019, 34(1):808–817.
14. Xing J, Zhang R, Jiang X, et al. Rational design of 5-((1H-imidazol-1-yl)methyl)quinolin-8-ol derivatives as novel bromodomain-containing protein 4 inhibitors. European Journal of Medicinal Chemistry. 2019, 163:281-294.
15. Shen Z, Wang X, Yang Y, et al. An Overview on Small Molecule Inhibitors of BRD4. Mini Reviews in Medicinal Chemistry. 2016, 16(17): 1403-1414.
16. Filippakopoulos P, Qi J, Picaud S, et al. Selective inhibition of BET bromodomains. Nature. 2010, 468(7327): 1067-1073.
17. Jiménez, Irene, Baruchel, et al. Bromodomain and extraterminal protein inhibitors in pediatrics: A review of the literature. Pediatric Blood & Cancer. 2016, 64, e26334.
18. Seal J, Lamotte, Y, Donche, F, et al. Identification of a novel series of BET family bromodomain inhibitors: Binding mode and profile of I-BET151 (GSK1210151A). Bioorganic & Medicinal Chemistry Letters. 2012, 22(8): 2968–2972.
19. Mcdaniel KF, Wang L, Soltwedel T, et al. Discovery of N-(4-(2,4-difluorophenoxy)-3-(6-methyl-7-oxo-6,7-dihydro-1H-pyrrolo[2,3-c]pyridin-4-yl)phenyl)ethanesulfonamide(ABBV-075/mivebresib), a potent and orally available bromodomain and extra-terminal domain (BET) family bromodomain inhibitor[J]. Journal of Medicinal Chemistry. 2017:acs.jmedchem.7b00746.
20. Picaud S, Wells C, Felletar I, et al. RVX-208, an inhibitor of BET transcriptional regulators with selectivity for the second bromo-domain. Proceedings of the National Academy of Sciences. 2013, 110(49): 19754–19759.
21. Mirguet O, Gosmini R, Toum J, et al. Discovery of epigenetic regulator I-BET762: Lead optimization to afford a clinical can-didate inhibitor of the BET bromodomains. Journal of Medicinal Chemistry. 2013, 56(19):7501-7515.
22. Romero FA, Taylor AM, Crawford TD, et al. Disrupting Acetyl-Lysine Recognition: Progress in the Development of Bromodomain Inhibitors. Journal of Medicinal Chemistry. 2015, 59(4):1271-1298.
23. Cheng Z, Gong Y, Ma Y, et al. Inhibition of BET Bromodomain Targets Genetically Diverse Glioblastoma. Clinical Cancer Research. 2013, 19(7):1748-1759.
24. Ying-Chao D, Yuanyuan G, Wenping Q, et al. Targeting Brd4 for Cancer therapy: Inhibitors and Degraders. Med Chem Comm, 2018, 9:1779-1802.
25. Hewings DS, Fedorov O, Filippakopoulos P, et al. Optimization of 3,5-Dimethylisoxazole derivatives as potent bromodomain ligands. Journal of Medicinal Chemistry. 2013, 56(8):3217–3227.

26. Zhao Y, Bai L, Liu L, et al. Structure-based discovery of 4-(6-Methoxy-2-methyl-4-(quinolin-4-yl)-9H-pyrimido[4,5-b]indol-7-yl)-3,5-dimethylisoxazole (CD161) as a potent and orally bioavailable BET bromodomain inhibitor. Journal of Medicinal Chemistry. 2017, 60(9):3887–3901.
27. Crawford TD, Tsui V, Flynn EM, et al. Diving into the water: inducible binding conformations for BRD4, TAF1(2), BRD9, and CECR2 bromodomains. Journal of Medicinal Chemistry. 2016, 59(11):5391–5402.
28. Zuber J, Shi J, Wang E, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukemia. Nature. 2011, 478(7370):524–528.
29. Watts E, Heidenreich D, Tucker E, et al. Designing dual inhibitors of anaplastic lymphoma kinase (ALK) and bromodomain-4 (BRD4) by tuning kinase selectivity. Journal of Medicinal Chemistry. 2019:acs.jmedchem.8b01947.

Graphical Abstract

Design, synthesis and biological evaluation of 3,5-dimethylisoxazole and pyridone derivatives as BRD4 inhibitors

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