Accepted Manuscript

Discovery of a series of dihydroquinoxalin-2(1*H*)-ones as selective BET inhibitors from a dual PLK1-BRD4 inhibitor

Jianping Hu, Yingqing Wang, Yanlian Li, Lin Xu, Danyan Cao, ShanShan Song, Mohammadali Soleimani Damaneh, Xin Wang, Tao Meng, Yue-Lei Chen, Jingkang Shen, Zehong Miao, Bing Xiong

PII: S0223-5234(17)30418-X

DOI: 10.1016/j.ejmech.2017.05.049

Reference: EJMECH 9479

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 31 January 2017

Revised Date: 19 May 2017

Accepted Date: 23 May 2017

Please cite this article as: J. Hu, Y. Wang, Y. Li, L. Xu, D. Cao, S. Song, M.S. Damaneh, X. Wang, T. Meng, Y.-L. Chen, J. Shen, Z. Miao, B. Xiong, Discovery of a series of dihydroquinoxalin-2(1*H*)-ones as selective BET inhibitors from a dual PLK1-BRD4 inhibitor, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.05.049.

Discovery of a Series of Dihydroquinoxalin-2(1*H*)-ones as Selective BET Inhibitors from a Dual PLK1-BRD4 inhibitor

Jianping Hu[a,c,#], Yingqing Wang[b,#], Yanlian Li[a,#] , Lin Xu[b,c], Danyan Cao[a], ShanShan Song[b], Mohammadali Soleimani Damaneh [b,c], Xin Wang[a], Tao Meng[a], Yue-Lei Chen[a], Jingkang Shen[a,*], Zehong Miao[b,*], Bing Xiong[a,*]

[a]*Department of Medicinal Chemistry, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China*

[b]*Division of Anti-tumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China*

[c]*University of Chinese Academy of Sciences, NO.19A Yuquan Road, Beijing 100049, China*

[#]*These authors contributed equally*

[*]Corresponding authors. Tel: +86 21 50806600 ext. 5412 fax: +86 21 50807088.

Email: (B. X.) bxiong@simm.ac.cn; (Z. M.) zhmiao@simm.ac.cn; (J. S.) jkshen@simm.ac.cn

Abbreviations

PLK1, Polo-like Kinase 1;

JAK2, Janus kinase 2;

PTMs, post-translational modifications

KAc, acetylated lysine;

BET, Bromodomain and Extra-Terminal;

P-TEFb, positive transcription elongation factor B;

BRD4-BD1, first bromodomain of BRD4;

SAR, structure- activity relationship;

FA, fluorescence anisotropy

SSRF, Shanghai Synchrotron Radiation Facility.

Abstract

Recent years have seen much effort to discover new chemotypes of BRD4 inhibitors. Interestingly, some kinase inhibitors have been demonstrated to be potent bromodomain inhibitors, especially the PLK1 inhibitor BI-2536 and the JAK2 inhibitor TG101209, which can bind to BRD4 with IC_{50} values of 0.025 μM and 0.13 μM, respectively. Although the concept of dual inhibition is intriguing, selective BRD4 inhibitors are preferred as they may diminish off-target effects and provide more flexibility in anticancer drug combination therapy. Inspired by BI-2536, we designed and prepared a series of dihydroquinoxalin-2(1*H*)-one derivatives as selective bromodomain inhibitors. We found compound **54** had slightly higher activity than (+)-JQ1 in the fluorescence anisotropy assay and potent antiproliferative cellular activity in the MM.1S cell line. We have successfully solved the cocrystal structure of **52** in complex with BRD4-BD1, providing a solid structural basis for the binding mode of compounds of this series. Compound **54** exhibited high selectivity over most non-BET subfamily members and did not show bioactivity towards the PLK1 kinase at 10 or 1 μM. From in vivo studies, compound **54** demonstrated a good PK profile, and the results from in vivo pharmacological studies clearly showed the efficacy of **54** in the mouse MM.1S xenograft model.

Keywords: BRD4 inhibitor; kinase; BI-2536; dihydroquinoxalin-2(1*H*)-one.

Introduction

Acetylation of histone lysine residues is one of the most widely studied post-translational modifications (PTMs) that regulate chromatin structure and gene expression in the cell [1],[2]. Acetylated histones are recognized by "readers", which are typically found in chromatin- and transcription-associated proteins that partake in many protein−protein interactions, facilitating the formation of the protein complexes that drive active transcription [3],[4]. So far, three readers (bromodomain [5],[6], double PHD finger [7],[8], and pleckstrin homology domain) [9] were identified to recognize acetylated lysine (KAc) and among them the bromodomain is the most thoroughly characterized [10]. Totally there are 61 bromodomains distributed in 46 different proteins encoded in human genome. These bromodomains can be divided into eight distinct families [11]. Of the 61 human bromodomains known, the bromo and extra-terminal domain (BET) proteins have recently emerged as druggable targets for the development of new anticancer agents owing to their roles in the transcriptional regulation of genes involved in tumor development and survival [12],[13]. The BET family is characterized by double bromodomains and consists of BRD2, BRD3, BRD4, and BRDT [14]. Particularly, given that BRD4 regulate the transcription of oncogene c-Myc, the inhibition of BRD4 provides an alternative and indirect strategy to curing disease related to the c-Myc abnormality [15], which is urgently needed since c-Myc itself is an undruggable transcription factor with no suitable small molecule binding pocket identified yet [16],[17]. In addition, BRD4 inhibition leads to good efficacy in xenograft models representing multiple cancer types [18],[19].

Since the discovery of BRD4 inhibitor (+)-JQ1 (**1**) [20], some BET bromodomain inhibitors, including representative molecules I-BET762 (**2**) [21], PFi-1(**3**) [22], **4**

[23] and RVX-208 (**5**) [24] (Figure 1), have been investigated as bromodomain inhibitors [6].

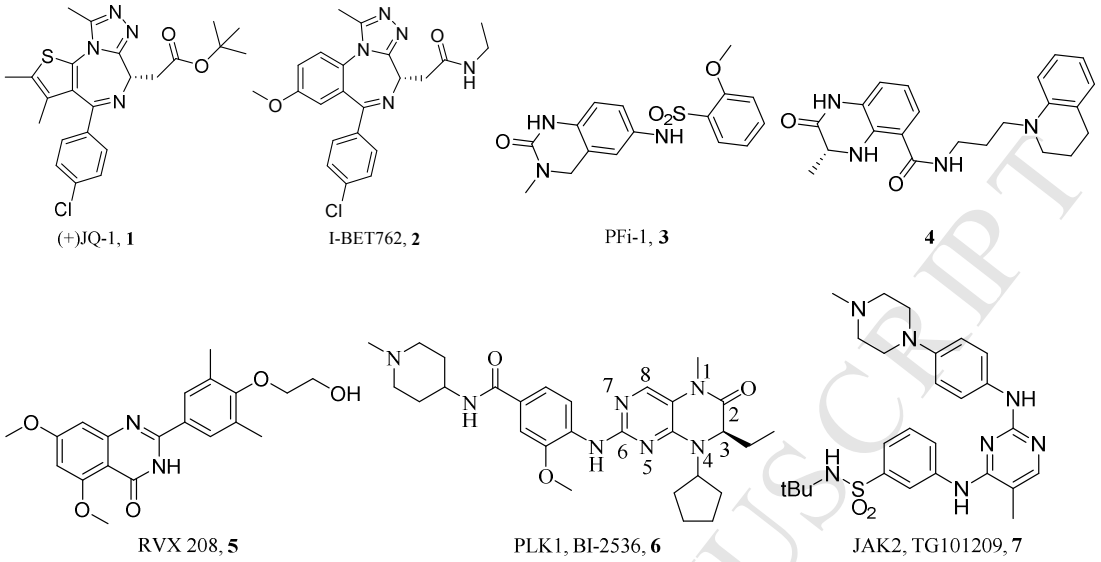


Figure 1. Structures of clinical BET bromodomain inhibitors (+)-JQ1 (**1**), I-BET762 (**2**), PFi-1 (**3**), **4**, RVX-208 (**5**) and dual kinase-bromodomain inhibitors BI-2536 (**6**) and TG101209 (**7**).

Since BRD4 has been shown to be an atypical kinase that phosphorylates Ser2 of the RNA Pol II carboxy-terminal domain [25], the concept of kinase-bromodomain dual inhibitors has been explored in recent years. A number of kinase inhibitors have been identified as bromodomain inhibitors by binding to the KAc binding pocket [26]. Among them, the PLK1 inhibitor BI-2536 (**6**) and the JAK2 inhibitor TG101209 (**7**) [26] (Figure 1) are very potent inhibitors of the BET bromodomains, with IC_{50} values of 0.025 μM and 0.13 μM against BRD4, respectively. On the other side, we hope to utilize the information of kinase-bromodomain dual inhibitors and obtain more selective BRD4 inhibitors, as they could offer more flexibility in anticancer drug combination treatments. Therefore, we designed, synthesized and evaluated a series of novel dihydroquinoxalin-2(1*H*)-one derivatives as selective bromodomain inhibitors. By exploring the structure-activity relationship (SAR) of the new dihydroquinoxalin-

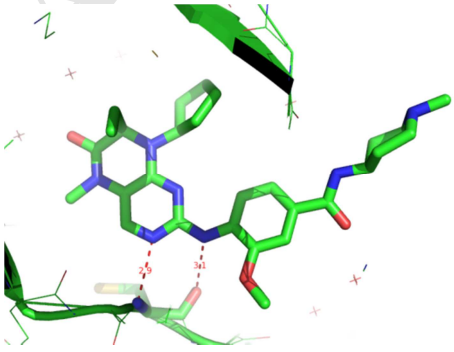
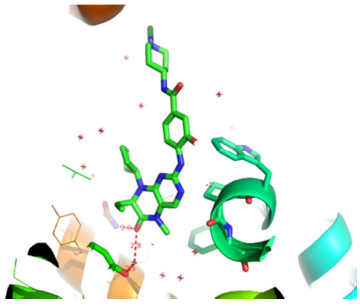
2(1*H*)-one structures, we were able to obtain several potent BRD4 inhibitors in the fluorescence anisotropy (FA) assay and in the antiproliferation cell based assay. Comparing with the similar bromodomain inhibitors PFi-1 (**3**) and **4**, we can find that the scaffold dihydroquinoxalin-2(1*H*)-one only use the oxygen atom in carbonyl group to interact with the conserved residue Asn140 of BRD4 and form one hydrogen bond. While the ring systems of PFi-1 (**3**) and **4** are flipped, and interact with Asn140 with the amide group to form two hydrogen bonds. Therefore, these chemical similar inhibitors have very different interaction pattern and distinct binding mode. Finally, compound **54** was found to be the most potent BRD4 inhibitor with similar activity of (+)-JQ1 in our FA assay and in the MM.1S cell assay. By solving a cocrystal structure of BRD4-BD1 complexed with **52**, which is very similar to **54**, we provided a solid structure basis for the binding mode of compounds of this series. Compound **54** also exhibited high selectivity toward most non-BET subfamily members and did not show bioactivity towards the PLK1 kinase at 10 or 1μM. In in vivo pharmacokinetic studies, compound **54** demonstrated a good half-life and a sufficient plasma exposure after oral administration at 10 mg/kg. Additionally, in an in vivo MM.1S cell derived xenograft model, it demonstrated clear efficacy in inhibiting the tumor growth. Together, we demonstrated a new direction to obtain selective BRD4 inhibitors through original dual kinase-bromodomain inhibitors.

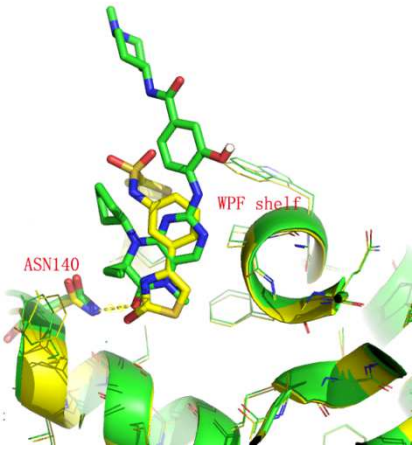
Results and Discussion

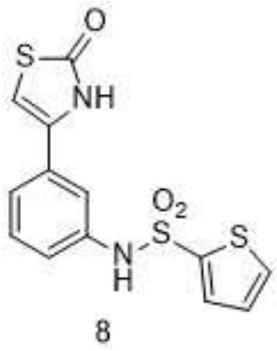
Comparison of the binding mode of BI-2536 (**6**) and our previously-reported 2-thiazolidinone compound **8** [27] in the BRD4-BD1 binding site showed that both **6** and **8** forms conserved hydrogen bonds with the asparagine residue (N140) that interacts with the acetylated lysine in histone proteins (Figure 2B, 2C). Further

analysis of the crystal structure of **6** bound to BRD4-BD1[28] revealed that the methylated amide of **6** functions as the *ε*-*N*-acetylated lysine group and forms a direct hydrogen bond with the side chain amide of N140 and a water-mediated hydrogen bond with Y97, while the methyl group is extended into a hydrophobic subpocket formed from F83, M132, and C136. These interactions are dominant forces for **6** binding to BRD4-BD1. Additionally, the ethyl group protrudes into a small hydrophobic subpocket (V87/L92/L94/Y97), and the cyclopentyl moiety and the *N*-methylpiperidine point to the solvent, forming no hydrogen bonding with residues nearby. Analysis of the cocrystal structure of PLK1 bound with **6** (Figure 2A) shows that one of the pyrimidine nitrogens and the aniline NH form critical hydrogen bonds with the hinge part of PLK1[29].

To obtain selective bromodomain inhibitors, one could preserve lactam structure and remove at least one of nitrogens that made critical hydrogen bonds with hinge part of PLK1. For example, we used dihydroquinoxalin-2(1*H*)-one to replace the 7,8-dihydropteridin-6(5*H*)-one in BI-2536. Meanwhile, from the superimposed crystal structures of **6** and **8** with BRD4-BD1, we speculated that substitution at C6 position of the scaffold may introduce similar interactions with WPF subpocket as compound **8** does. Along this line, we explored R^4 substitution in dihydroquinoxalin-2(1*H*)-one in order to find a functional group targeting the WPF shelf to enhance its binding affinity against BRD4.

A.  B. 

C. 

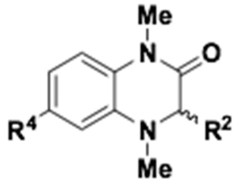
D. 

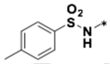
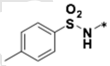
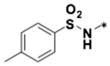
8

Figure 2. Analyses of crystal structures of BI-2536 bound to PLK1 and BRD4-BD1. (A) crystal structure of compound BI-2536 (**6**) bound to PLK1 kinase domain (PDB entry: 2RKU); (B) crystal structure of compound BI-2536 bound to BRD4-BD1 (PDB entry: 4OGI); (C) Superimpose crystal structure of BI-2536 (**6**) and **8** bound to BRD4-BD1; (D) Chemical Structures of **8.**

Shown in Table 1 are the 1,4-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-one derivatives with various R^2 and R^4 substituents and their BRD4 binding activities. For R^4 group, 4-methylbenzenesulfonamide substitution (compound **12**) showed better potency than other groups (compounds **9-11**). For R^2 group, replacement of (*S*)-Me with (*S*)-ethyl reduced the binding activity by almost 2-fold (compare **12** with **13**), while inversion of the stereochemistry of C3 position did not affect the binding affinity to BRD4 significantly (compare **13** with **14**).

Table 1. Effects of R^2, R^4-substituted 1,4-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-one derivatives on inhibition of BRD4-BD1 by fluorescence anisotropy assay [a]



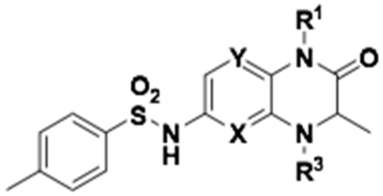
Cmpd	R^2	R^4	BRD4-BD1 IC_{50} (μM)	Cmpd	R^2	R^4	BRD4-BD1 IC_{50} (μM)
(+)-JQ1	-	-	0.07±0.001	**11**			1.53±0.22
I-BET762	-	-	0.26±0.01	**12**			0.77±0.14
BI-2536	-	-	0.25±0.01	**13**			1.35±0.05
9			>1.0	**14**			1.47±0.32
10			41.89%@1μM				

[a]The IC_{50} in the table was calculated from two independent experimental measurements and expressed as mean ± SE. The fluorescence compound used in the assay was JQ1-FITC (The synthesis route was provided in our group previous work) [27].

Next, we studied the SAR of R^1 and R^3 substitutions and the scaffold modification of dihydroquinoxalin-2(1*H*)-one. Initially, we explored R^1 group while preserving the dihydroquinoxalin-2(1*H*)-one skeleton and N4-cyclopentyl. Exploring substitution on the R^1 position with H, Me or Ethyl, we found that R^1 as methyl group had better potency than others (compare **16** with **15** or **17**). Secondly, we preserved scaffold as dihydroquinoxalin-2(1*H*)-one and R^1 as Me to explore R^3 group. Comparison of **16** and **18-24** indicated that better binding activity could be obtained when R^3 was cyclopropyl (compound **19**), as larger R^3, including isobutyl, 2-

methoxyethyl, 2-morpholinoethyl, (tetrahydrofuran-2-yl)methyl, 2-methoxybenzyl (compounds **20-24**) all reduced the inhibitory activity. Thirdly, we preserved N1-Me and N4-cyclopropyl to modify scaffold. We changed dihydroquinoxalin-2(1*H*)-one to dihydropyrido[2,3-b]pyrazin-2(1*H*)-one or dihydropyrazino[2,3-b]pyrazin-2(1*H*)-one (compare **19** with **25** or **26**), and found that **25** had similar potency to **19,** while **26** deceased the potency remarkably. Although the scaffold dihydropyrido[2,3-b]pyrazin-2(1*H*)-one is very promising, SAR study on this new core is challenging. In current work, we focus on the scaffold of dihydroquinoxalin-2(1*H*)-one.

Table 2. Effects of R^1 and R^3 substitutions and scaffold modification on dihydroquinoxalin-2(1*H*)-one derivatives in the BRD4-BD1 fluorescence anisotropy assay[a]



Cmpd	X	Y	R^1	R^3	BRD4-BD1 IC_{50} (μM)
(+)-JQ1	-	-	-	-	0.07±0.001
I-BET762	-	-	-	-	0.26±0.01
BI-2536	-	-	-	-	0.25±0.01
15	C	C	H		>1.0
16	C	C	Me		0.38±0.04

17	C	C			0.76±0.21
18	C	C	Me		0.35±0.05
19	C	C	Me		0.25±0.01
20	C	C	Me		0.73±0.42
21	C	C	Me		0.77±0.02
22	C	C	Me		1.03±0.40
23	C	C	Me		1.04±0.10
24	C	C	Me		>1.0
25	N	C	Me		0.27±0.02
26	N	N	Me		>1.0

[a]The IC_{50} in the table was calculated from two independent experimental measurements and expressed as mean ± SE.

Before further exploring the SAR of this series, we need to confirm that the ligand contacts with the WPF subpocket, and we solved a co-crystal structure of BRD4-BD1 bound with compound **19** (Figure 3A). As expected, the sulfonamide group makes a turn to enable the 4-methylbenzene go into the WPF subpocket. This showed a distinct binding conformation as comparing BI-2536 in BRD4-BD1 binding site.

Except this remarkable difference, the dihydroquin-oxalin-2(1*H*)-one scaffold preserved the essential hydrogen bonding interactions as BI-2536 and located at almost exact position in the binding site. By comparing the solved structure with PFi-1 (**3**) and **4** (PDB entry: 4E96 and 4NYW), we can find that the scaffold dihydroquinoxalin-2(1*H*)-one only uses the oxygen atom in carbonyl group to interact with the conserved residue Asn140 of BRD4 and forms one hydrogen bond. While the ring systems of PFi-1 (**3**) and **4** are flipped, and interact with Asn140 with the amide group to form two hydrogen bonds. Therefore, the comparison indicated that the binding mode is different from PFi-1 or **4**, and represented as an interesting scaffold for bromodomain inhibitor development.

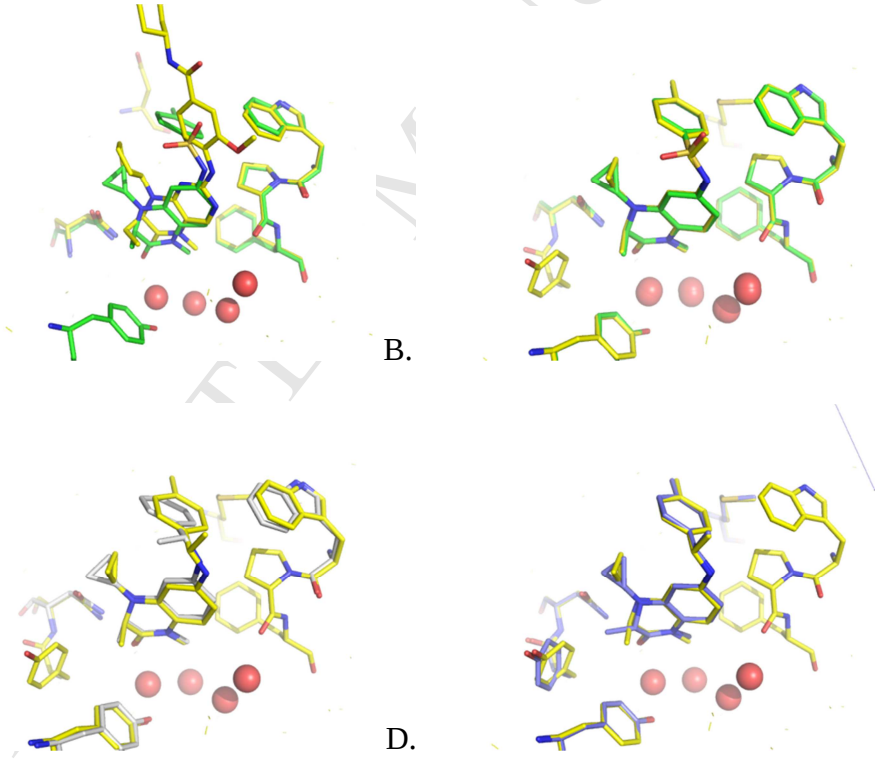


Figure 3. Crystal structures of compounds **19, 35, 52, 53** bound to BRD4-BD1. (A) superimposed structures of **19** (green, PDB entry: 5XHY) and BI-2536 (yellow) bound to BRD4-BD1; (B) superimposed structures of **52** (yellow, 5XI2) and **19** (green) bound to BRD4-BD1; (C) superimposed structures of **52** (yellow) and **35**

(gray, PDB entry: 5XI3) bound to BRD4-BD1; (D) superimposed structures of **52** (yellow) and **53** (blue, PDB entry: 5XI4) bound to BRD4-BD1.

To further probe the SAR of R^4 group, we synthesized various sulfonamides, carbamates and amine groups to attach the core phenyl ring (Table 3). From compounds **27**-**36**, it was found that when R^4 was (*S*)-1-phenylethan-1-amine (**36**) it showed better potency than those having R^4 as sulfonamides or carbamates. Replacement of 4-methylbenzenesulfonamide group with a 4-methoxybenzenesulfonamide group reduced the inhibitory activity by almost 2.3-fold (compare **19** with **27**), and replacing the 4-methylbenzenesulfonamide group with a 4-methylbenzamide group maintained binding affinity (comparing **19** with **31**). Changing the sulfonamides to carbamates reduced potency by almost 3.3-fold (comparing **19** with **29**). Further investigation found that adding an ethyl group to the nitrogen atom of 4-methylbenzenesulfonamide diminish potency significantly (compare **19** with **28**). In addition, adding an ethyl group to the nitrogen atom of benzylamine reduced the inhibitory activity by almost 1.7-fold (compare **32** with **33**). These results indicated that adding another alkyl group to the nitrogen atom which attached to the core phenyl ring was detrimental to potency.

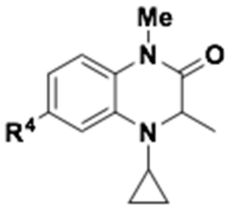
Using commercial available building blocks, we further explored the SAR of R^4 on the basis of 1-phenylethan-1-amine. As shown in Table 3, we found that compounds with methyl or ethyl substitution at the methylene of benzylamine (**34** or **37**) had better potency that the unsubstituted one (**32**), larger *n*-propyl-substituted compound **38** demonstrated marginally reduced potency, and *tert*-butyl-substituted compound **39** and dimethyl-substituted compound **40** showed significantly decreased potency.

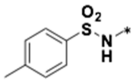
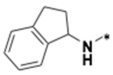
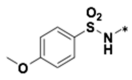
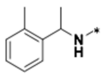
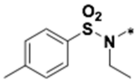
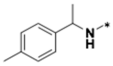
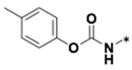
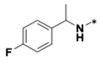
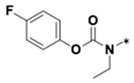
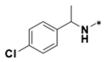
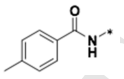
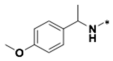
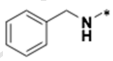
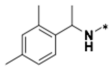
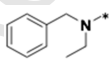
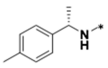
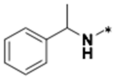
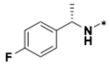
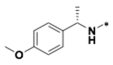
Cyclizing methyl to the benzene group decreased activity remarkably (comparing **34** with **41**).

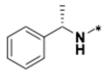
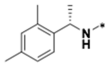
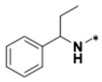
Next, we turned our attention to the substitutions on benzene of benzylamine. Comparing **43-46** with **34**, we found that Me, F, Cl or OMe substituents at the para position of benzene improved binding affinity slightly, and the electronic nature of the substituents on this position was not critical for the binding. As demonstrated by compounds **42** and **43**, the position of the methyl substituent at the benzene ring was important for the inhibitory activity: the *para*-methyl substituted compound (**43**) was more potent than the *ortho*-methyl substituted compound (**42**). Moreover, 2,4-dimethyl substituted compound **47** had better potency than the 4-methyl substituted **43**. These indicated that WPF subpokcet is critical for the binding interaction.

Furthermore, comparing **35** with **36**, better potency of (*S*)-1-phenylethan-1-amine substituted **36** than (*R*)-1-phenylethan-1-amine substituted **35** indicated stereochemistry of the benzylamine is important. We preserved (*S*)-1-phenylethan-1-amine and synthesized compounds **48-51**. As expected, (*S*)-compounds are more potent than their racemic analogues (**43**, **44**, **46** and **47**), confirmed the (*S*)-1-phenylethan-1-amine was the preferred configuration for binding at WPF shelf.

Table 3. Effects of R^4 position in 4-cyclopropyl-1,3-dimethyl-3,4-dihydroquinoxalin -2(1*H*)-one derivatives on inhibition of BRD4-BD1 from fluorescence anisotropy assays.[a]



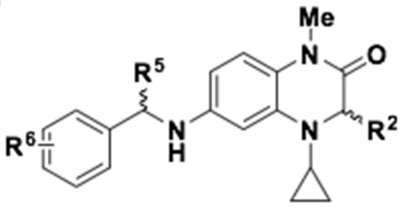
Cmpd	R^4	BRD4-BD1 IC_{50} (μM)	Cmpd	R^4	BRD4-BD1 IC_{50} (μM)
(+)-JQ1	-	0.07±0.001	**38**		0.61±0
I-BET762	-	0.26±0.01	**39**		20.08%@1μM
BI-2536	-	0.25±0.01	**40**		19.31%@1μM
19		0.25±0.01	**41**		43.79%@1μM
27		0.58±0.07	**42**		0.27±0.02
28		13.16%@1μM	**43**		0.17±0.02
29		0.83±0.05	**44**		0.27±0.04
30		0.81±0.11	**45**		0.21±0.01
31		0.24±0.003	**46**		0.28±0.001
32		0.46±0.01	**47**		0.12±0.01
33		0.79±0.05	**48**		0.087±0.01
34		0.34±0.04	**49**		0.11±0.01
35		38.35%@1μM	**50**		0.23±0.004

36		0.18±0.01	**51**		0.10±0.001
37		0.41±0.01			

[a]The IC_{50} in the table was calculated from two independent experimental measurements and expressed as mean ± SE.

Although compounds **48** and **51** had the best activities than others, both demonstrated as a pair of diastereomers due to the chirality of C3 of scaffold. To assess the SAR of individual diastereomers, we resolved **48** and **51** by means of chromatography (supporting information Table S1, Figure S1 and Figure S2) and obtained compounds **52-55** in Table 4. Surprisingly, we found (3*R*)-enantiomer had better potency than (+)-JQ-1, I-BET762 and BI-2536 in FA assays (**52** and **54**), while (3*S*)-enantiomer diminished potency dramatically (**53** and **55**), implying that the chirality of R^2 was very important for this type of compounds.

Table 4. Effects of Compounds **36**, **43**, **47-49** and **51-55** on Inhibition of BRD4-BD1 in FA Assay and Antiproliferation Effects against Cell Lines MM.1S and TY-82



Cmpd	R^2	R^5	R^6	BRD4-BD1 IC_{50} (μM) [a]	MM.1S IC_{50} (μM) [a]	TY-82 IC_{50} (μM) [a]
(+)-JQ1	-	-	-	0.07±0.001	0.019±0.009	0.018±0.007

I-BET762	-	-	-	0.26±0.01	0.23±0.027	0.20±0.015
BI-2536	-	-	-	0.25±0.01	N.T. [b]	0.001±0.0
36	Me		H	0.18±0.01	0.74±0.013	0.50±0.071
43	Me	Me	4-Me	0.17±0.02	0.38±0.010	0.31±0.065
47	Me	Me	2,4-dimethyl	0.12±0.01	0.10±0.036	0.095±0.032
48	Me		4-Me	0.087±0.01	0.081±0.005	0.058±0.006
49	Me		4-F	0.11±0.01	0.33±0.019	0.35±0.026
51	Me		2,4-dimethyl	0.10±0.001	0.10±0.018	0.23±0.031
52			4-Me	0.052±0.007	0.065±0.016	0.047±0.016
53			4-Me	16.98%@1μM	N.T.	N.T.
54			2,4-dimethyl	0.045±0.002	0.016±0.008	0.063±0.020
55			2,4-dimethyl	16.08%@1μM	N.T.	N.T.

[a]The IC_{50} in the table was calculated from two independent experimental measurements and expressed as mean ± SE, [b] N.T. represents not tested.

To visualize the stereochemistry effect on the binding activity, we solved three crystal structures of BRD4-BD1 bound with the representative compounds **35**, **52** and **53**. From chemical structure point of view, compound **35** contains ((*R*)-1-phenylethyl)amino group and a racemic scaffold; compound **52** contains ((*S*)-1-phenylethyl)amino group and (*R*)-4-cyclopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-one scaffold; while compound **53** differed from **52** only at the scaffold,

containing (*S*)-4-cyclopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-one. By comparing the crystal structures of **35** and **52** (Figure 3C), we found that different chiral phenylethyl-amino groups showed almost no difference in binding conformation, except for the rotation of cyclopropyl of the scaffold. The notable difference in BRD4-BD1 around the stereocenter was downward of the residue TRP81 on WPF shelf, which implies the Van der Waals interactions between **35** and WPF were attenuated. This is not surprising because this series of BRD4 inhibitors are mainly exploring the SAR around the WPF shelf and the binding affinity is very sensitive around this subpocket as demonstrated by several compounds shown in Table 3. To understand the remarkable difference between compounds **52** and **53**, we compared their cocrystal structures (Figure 3D) and identified the major difference lies at the dihydroquinoxalin-2(1*H*)-one scaffold. Binding interactions of **53** with BRD4-BD1 showed the methyl group stays almost in the plane of scaffold, forcing the surrounding residues TYR139 slightly shifted. The distance of the methyl carbon atoms of **53** to the nearest atom of TYR139 is 3.3 Å, which is not in the favorable range of carbon-carbon interaction. By utilizing Schrödinger program to minimize compound **53** in water, we also found the methyl group significantly deviated away from the scaffold plane (see supporting information Figure S4). Both evidences implied that the ligand **53** must adopted a high-energy conformation to interact with the binding site of BRD4-BD1. These may account for the diminished binding affinity of **53** when compared to compound **52**.

Bromodomain Selectivity Screening

Bromodomain is the conserved module in evolution, and they share a common 3D structure pattern of one short helix (helix Z) and three long helices (helix A−C). The acetylated lysine can bind to the top of the bromodomains by forming several

hydrogen bonds with the conserved residue asparagine (Asn140 in BRD4). As selectivity is critical for the success of drug discovery, we selected the most potent BET inhibitor **54** to profile the binding specificity in 32 representative bromodomain modules by using the DiscoveRx BROMOScan platform at 1 μM concentration. As showed in Figure 4, it was clearly shown that compound **54** was generally selective inhibitors for BET subfamily over other non-BET family except its moderate inhibition on EP300/CREBBP (The detail number was provided as Table S3 in supporting material).

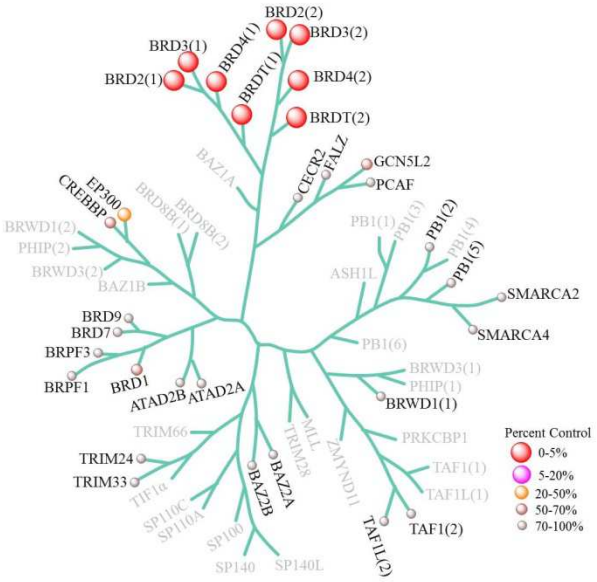


Figure 4. Bromodomain selectivity profile of compound **54**. Red, Purple red, Orange, Purple gray and Gray dots indicate the assay ctrl% range: 0-5%, 5−20%, 20-50%, 50-70% and 70-100%, respectively, in the DiscoveRX assay at 1 μM concentration. The ctrl%=(test compound signal – positive control signal)/(negative control signal – positive control signal) ∗100%.

PLK1 Kinase Selectivity Profile

We hope to discover a new series of dihydroquinoxalin-2(1*H*)-one as selective BRD4 inhibitors by using PLK1-BRD4 dual inhibitor BI-2536 as the starting point. Thence, on the basis of molecular activity and structural diversity, we chose three compounds to profile their PLK1 kinase activity, along with BI-2536 and a highly active ATP competitive kinase inhibitor staurosporine as positive control. From Table 5, we found that staurosporine and BI-2536 showed significant inhibitory effects on PLK1 kinase at 10 μM or 1 μM. However, compounds **19**, **25** and **54** demonstrated only negligible inhibitory activity for PLK1 kinase at 10 μM or 1 μM as we excepted. Thus, removing pyrimidine ring nitrogen in BI-2536 as a critical hydrogen acceptor for kinase hinge binding successfully reduced the inhibitory activity for PLK1 kinase.

Table 5. PLK1 kinase activity of compounds **19**, **25** and **54**

Cmpd	Inhibition (%, 10 μM)	Inhibition (%, 1 μM)	Cmpd	Inhibition (%, 10 μM)	Inhibition (%, 1 μM)
Staurosporine	95	58	**25**	5	7
BI-2536	100	101	**54**	2	5
19	3	7			

Cellular activity

The compounds with better enzymatic activity (IC_{50} values $<$0.2 μM) were further assessed in the cellular antiproliferation assay (**36**, **43**, **47-49**, **51-52** and **54**). The graphs of cell viability for representative compounds **36**, **48**, **51**, **52**, **54**, I-BET762 and (+)-JQ-1 were shown in supporting information Figure S5. The results (Table 4) indicated these compounds had good cellular activities with IC_{50} below 1 μM both in MM.1S and TY-82 cell lines, and among them compounds **48, 52** and **54** showed strong proliferation inhibition activity with IC_{50} below 0.1 μM. Particularly,

compound **54** even had similar cellular activities with (+)-JQ1 in MM.1S cell line. From table 4, we also found BI-2536 showed extremely inhibition on TY-82 cell line, however, it exhibited moderate binding affinity on BRD4-BD1, so we conjectured its high cell activity was not only due to its inhibition on BRD4-BD1 but also because of its inhibition on PLK1 kinase in cell.

Effects on c-Myc Protein and mRNA Expression.

The BRD4 inhibitor (+)-JQ1 induces an antiproliferative effect associated with the down-regulation of c-Myc transcription. In order to study whether they went through the BRD4-dependent pathway in the cell, we performed the Western blotting experiment and quantitative real-time PCR (RT-qPCR) to study the cellular effect related to c-Myc. On the basis of the protein binding and cellular antiproliferation assays, we chose **52**, **54** and the positive controls (+)-JQ-1, I-BET762, OTX-015, and BI-2536 to evaluate their effects on c-Myc protein and mRNA expression. As showed in Figure 5A, both compounds **52** and **54** displayed significant inhibition on the expression of c-Myc protein both at 0.2 μM and 1 μM. In particular, compound **54** remarkably suppressed the expression of c-Myc protein at 1 μM.

As indicated by RT-qPCR assays in Figure 5B, compounds **52** and **54** strongly down-regulated the expression of c-myc mRNA in a dose-dependent manner, showing more than 60% inhibition at 1 μM. Taking the selectivity profiles together, these data implied that the antiproliferation effects of dihydroquinoxalin-2(1*H*)-one series of compounds attributed to the BRD4-dependent pathway.

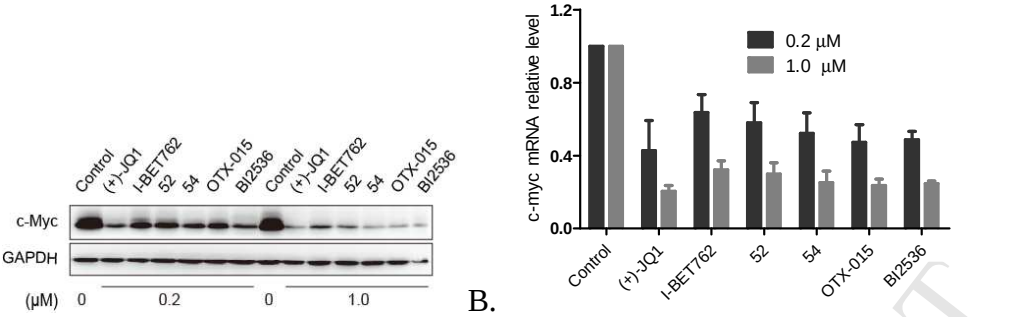


Figure 5. (A) Inhibition of compounds **52** and **54** on the expression of c-Myc protein. TY-82 cells were treated with compounds **52** and **54**, I-BET762, OTX-015, BI-2536 and (+)-JQ1 (0.2 μM or 1 μM) for 24 h, respectively. Cells were collected and lyzed for Western blotting. The level of c-Myc protein was detected and GAPDH protein was chosen as the loading control. The experiments were repeated three times. (B) Inhibition of compounds **52** and **54** on the expression of c-Myc mRNA. TY-82 cells were treated as in (A). Then, total RNA was isolated and reverse-transcribed for RT-qPCR analyses. The data were expressed as mean ± SD, representing the relative levels of c-myc mRNA from three independent experiments.

In Vivo Study of Compound 54

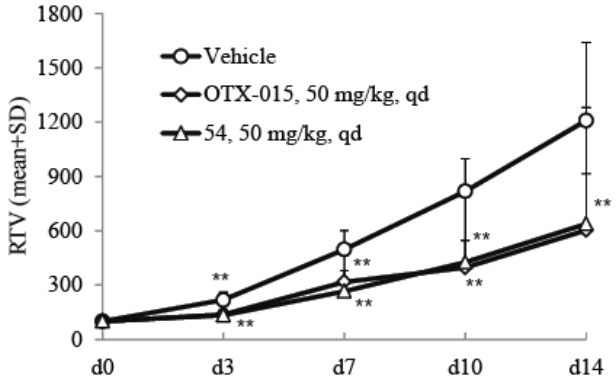
Based on aforementioned data, compound **54** was selected for further in vivo PK study. After single oral administration of 10 mg/kg of compound **54** (DMAC/HPMC formulation) to male ICR (CD-1) mice, the pharmacokinetic parameters of **54** in plasma samples were summarized in Table 6. The maximum plasma concentration of **54** (869 ng/mL) was observed at 0.33 h after drug administration. At 24 h after administration, the plasma concentration was below the lower limit of quantity (LLOQ = 0.3 ng/mL). From Table 6, it showed that compound **54** has a moderate half-life (2.23 h) and a reasonable plasma exposure of 1689 ng × h/mL, indicating **54** could be advanced for in vivo pharmacological study.

Table 6. In Vivo PK Data for Compound **54**[a]

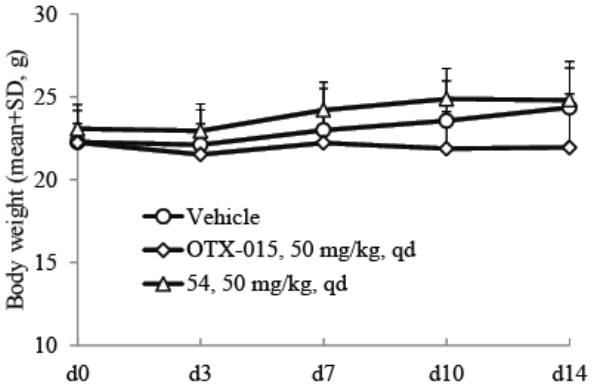
Cpd.	Route of administration	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)	AUC_{0-inf} (ng·h/mL)	$T_{1/2}$ (h)
54	p.o.	10	0.33	869	1683	1689	2.23

[a] 5% dimethylacetamide and 0.5% hydroxypropyl methyl cellulose were used as vehicle.

To evaluate the therapeutic effect of compound **54** in vivo, we established human MM.1S xenograft model in Balb/c nude mice. Tumor-bearing mice were treated with **54** by intraperitoneal injection (50 mg/kg daily). The result showed that treatment of compound **54** significantly decreased the growth of xenografts measured by tumor volume (Figure 6A) but did not cause loss of the body weight (Figure 6B). No animal death occurred during the experiments. By comparing with the positive control BRD4 inhibitor OTX-015 (oral dosage 50mg/kg daily), **54** and OTX-015 showed similar inhibition effects about RTV 638% and 603% respectively, although they are administrated via different route. These data illustrate the therapeutic potential of compound **54** for tumor treatments.



（A）



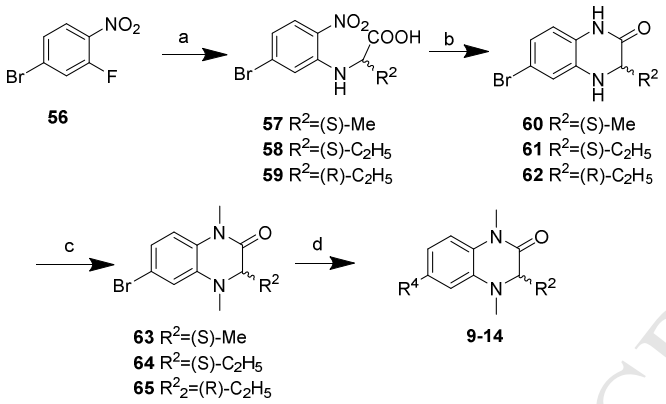
（B）

Figure 6. (A) Relative tumor volume (RTV) of human MM.1S xenografts in Balb/c nude mice after treatments. **, OTX-015 or compound **54** group versus vehicle group, *p* value < 0.01, Formulation: **54**: 0.5% Tween80 and 0.5% Methylcellulose aqueous solution, OTX-015: 5% dimethylacetamide and 0.5% hydroxypropyl methyl cellulose; (B) Body weight changes of mice.

Chemistry

The syntheses of novel dihydroquinoxalin-2(1*H*)-onederivatives were depicted in schemes 1-3. In schemes 1, the reaction of commercially available **56** with different amino acids through aromatic nucleophilic substitutions (S_NAr) furnished anilines **57-59**, wherein the nitro group directed ortho attack. The dihydroquinoxalin-2(1*H*)-one scaffold was next constructed by reductive heterocyclizations of **57-59** into **60-62**, respectively, with tin(II) chloride dihydrate ($SnCl_2 \cdot 2H_2O$) and con.HCl. Introduction of the methyl group was achieved by deprotonation of the amide NH followed by quenching with iodomethane to yield **63-65**. The 3,6-substituted 1,4-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-one derivatives **9-14** were obtained through compounds **63-65** coupling with different amines or sulfonamides.

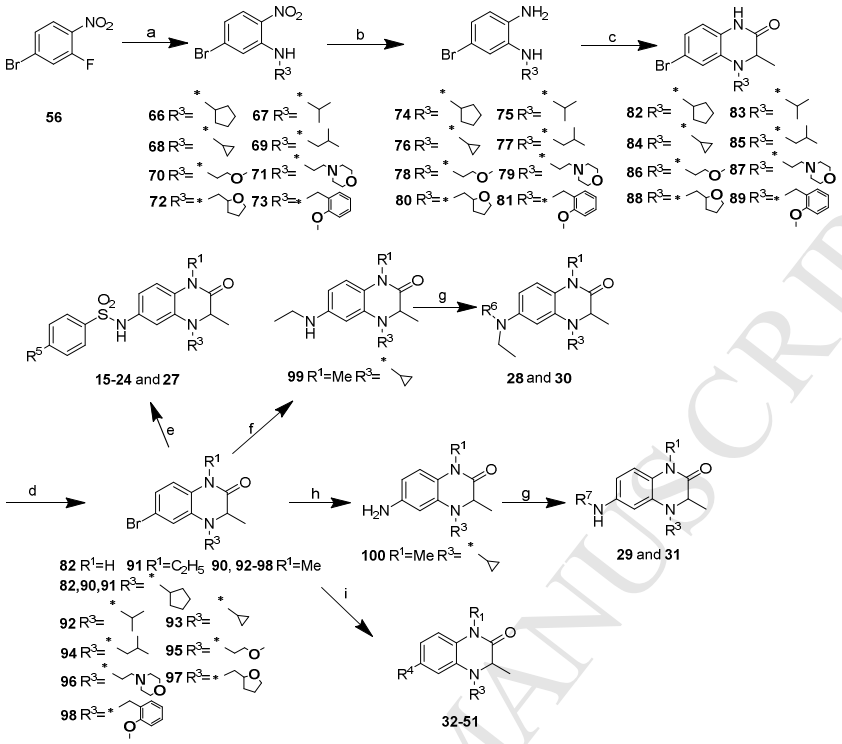
Scheme 1 Syntheses of the 3,6-substituted 1,4-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-one derivatives **9-14**.



Reagents and conditions: a) $R^2CH(NH_2)COOH$, K_2CO_3, EtOH, reflux, 4 h; b) tin(II) chloride dihydrate, con.HCl, EtOH, reflux, 5 h; c) NaH, 0 °C, 30 min, iodomethane, rt, 2 h; d) **9** and **10**: different amines, Cs_2CO_3, DMF, Pd_2dba_3, Xphos, 110 °C, 1h; **11-14**: different sulfonamides, K_2CO_3, 2-metyhtetrahydrofuran, allylpalladium chloride dimer, *t*BuXPhos, 85 °C, overnight.

As depicted in Scheme 2, S_NAr of different amines to **56** afforded compounds **66-73**. Then reduction with Fe powder and NH_4Cl provided amines **74-81**. Compounds **82-89** were obtained through two steps, including treatment of **74-81** with 2-bromopropanoyl bromide to afford corresponding amides, and the ring formation through intramolecular nucleophilic substitution reaction. For compounds **90-98**, introduction of the R^1 group was achieved by deprotonation of the amide NH followed by quenching with iodomethane or bromoethane. Coupling of **93** and diverse amines via Buchwald-Hartwig reaction gave compounds **32-51** and **99**. Compounds **15-24** and **27** were obtained through the reaction between intermediates **82** and **90-98** and 4-methylbenzenesulfonamide or 4-methoxybenzenesulfonamide. Treatment of **93** with *tert*-butyl carbamate via coupling reaction and subsequent acidic deprotection afforded compound **100**. Condensation of **99** with R^6Cl yielded **28** or **30** in a similar fashion used for producing compound **29** or **31**.

Scheme 2. Syntheses of the 1,4,6-substituted 3-methyl-3,4-dihydroquinoxalin-2(1*H*)-one derivatives **15-24** and **27-51**.

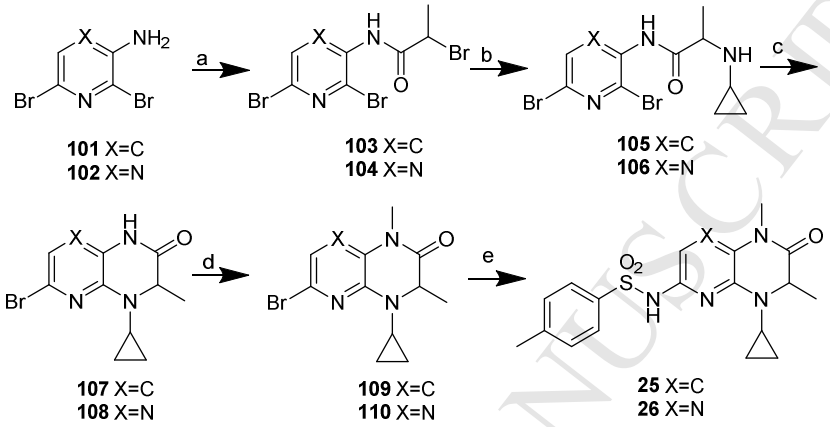


Reagents and conditions: a) R^3NH_2, $ClCH_2CH_2Cl$, 80 °C, 12 h; b) Fe, NH_4Cl, EtOH, 80 °C, 1 h; c) 1. 2-bromopropanoyl bromide, DIPEA, DCM, 0 °C - rt, 2 h; 2. CH_3CN, DIPEA, 80 °C, overnight; d) iodomethane or bromoethane, NaH, DMF, 0 °C - rt, 2 h; e) 4-methylbenzenesulfonamide or 4-methoxybenzenesulfonamide, K_2CO_3, 2-metyhtetrahydrofuran, allylpalladium chloride dimer, *t*BuXPhos, 85 °C, overnight; f) sodium tert-butoxide, ethylamine hydrochloride, toluene, palladium acetate, tBu_3P, 85 °C, overnight; g) R^6Cl or R^7Cl, Et_3N, DCM, 0 °C - rt, overnight; h) 1. *tert*-butyl carbamate, Cs_2CO_3, dioxane, palladium acetate, Xphos, 85 °C, overnight; 2. TFA, CH_2Cl_2, rt, overnight; i) sodium tert-butoxide, toluene, palladium acetate, tBu_3P, 85 °C, overnight.

In Scheme 3, treatment of commercially available **101** and **102** with 2-bromopropanoyl bromide formed amides **103** and **104**. The reaction of **103** and **104** with cyclopropylamine gave **105** and **106** through intermolecular nucleophilic substitution reaction. Compounds **107** and **108** were constructed through

intramolecular S_NAr reaction. Deprotonation of the amide NH followed by quenching with iodomethane yielded **109** and **110.** Compounds **25** and **26** were obtained through coupling **109** and **110** with 4-methylbenzenesulfonamide, respectively.

Scheme 3. Synthesis of the dihydropyrido[2,3-b]pyrazin-2(1*H*)-one or dihydropyrazino[2,3-b]pyrazin-2(1*H*)-one derivative **25** or **26**.



Reagents and conditions: a) 2-bromopropanoyl bromide, Na_2CO_3, CH_2Cl_2, 0 °C - rt, overnight; b) cyclopropylamine, CH_3CN, 80 °C, overnight; c) DIPEA, DMF, 150 °C, overnight; d) NaH, 0 °C, 30 min, iodomethane, rt, 2 h; e) 4-methylbenzenesulfonamide, K_2CO_3, 2-metyhtetrahydrofuran, allylpalladium chloride dimer, *t*BuXPhos, 85 °C, overnight.

Conclusion

Inspired by PLK1-BET bromodomain dual inhibitor BI-2536 (**6**), we rationally designed, synthesized, and evaluated a series of novel dihydroquinoxalin-2(1*H*)-one derivatives as selective bromodomain inhibitors. Through iterative structure-based designs, we obtained several potent BRD4 inhibitors with IC_{50} below 0.1 μM both in FA assays and antiproliferation cell based assays. Compound **54** with good potency in FA and cell assays displayed concentration-dependent inhibition on the expression of c-Myc protein and c-Myc mRNA, and demonstrated good pharmacokinetic and

pharmacodynamic properties in mice. Our study demonstrated a practice for designing selective bromodomain inhibitors starting from the kinase inhibitors.

Experimental Section

Chemistry

General:

^{1}H NMR (400 MHz) spectra were recorded by using a Varian Mercury-400 High Performance Digital FT-NMR spectrometer with tetramethylsilane (TMS) as an internal standard. ^{13}C NMR (126 MHz) spectra were recorded by using a Varian Mercury-500 High Performance Digital FT-NMR spectrometer. Abbreviations for peak patterns in NMR spectra: br = broadened, s = singlet, d = doublet, t = triplet, dd = doublet of doublets and m = multiplet. Low-resolution mass spectra were obtained with a Finnigan LCQ Deca XP mass spectrometer using a CAPCELL PAK C18 (50mm × 2.0mm, 5 μM) or an Agilent ZORBAX Eclipse XDB C18 (50mm × 2.1m, 5 μM) in positive or negative electrospray mode. High-resolution mass spectra were recorded by using a Finnigan MAT-95 mass spectrometer. Purity of all compounds was determined by analytical Gilson high-performance liquid chromatography (HPLC) using an YMC ODS3 column (50 mm × 4.6 mm, 5 μM). Conditions were as follows: CH_3CN/H_2O eluent at 2.5 $mLmin^{-1}$ flow [containing 0.1% trifluoroacetic acid (TFA)] at 35 °C, 8 min, gradient 5% CH_3CN to 95% CH_3CN, monitored by UV absorption at 214 nm and 254 nm. TLC analysis was carried out with glass precoated silica gel GF254 plates. TLC spots were visualized under UV light. Flash column chromatography was performed with a Teledyne ISCO CombiFlash R_f system. All solvents and reagents were used directly as obtained commercially unless otherwise noted. All air and moisture sensitive reactions were carried out under an atmosphere

of dry argon with heat-dried glassware and standard syringe techniques. Melting points were determined using a SGW X-4 hot stage microscope and are uncorrected. (spectra data of the synthesized compounds were provided as supporting material)

Synthetic Procedures:

Compounds **56** and **101-102** were purchased. Other compounds were prepared by one of four schemes.

Scheme 1. *(5-bromo-2-nitrophenyl)-L-alanine* (**57**). To a solution of compound **56** (2 g, 9.09 mmol) and L-alanine (0.81 g, 9.09 mmol) in EtOH (10 mL) was added K_2CO_3 (1.51 g, 10.91 mmol) in water (3 mL). The mixture was heated to reflux for 4 hours and monitored by TLC. Upon completion, EtOH was evaporated. The residue was acidified with 1 N aq. HCl to pH 3 – 4, diluted with water and extracted with EtOAc (3 × 50 mL). Combined organic layers were washed with brine, and dried with Na_2SO_4 to afford compound **57** (2.48 g, 8.58 mmol, 94% yield) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, J = 7.0 Hz, 1H), 8.02 (d, J = 9.1 Hz, 1H), 7.24 (s, 1H), 6.90 (d, J = 9.5 Hz, 1H), 4.62 – 4.52 (m, 1H), 1.45 (d, J = 6.9 Hz, 3H).

(S)-6-bromo-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**60**). To a solution of compound **57** (2.48 g, 8.58 mmol) in EtOH (20 mL) was mixed with a solution of tin(II) chloride dihydrate (7.74 g, 34.3 mmol) in 10 mL of EtOH and 2.7 mL of con. HCl. The mixture was heated to reflux for 5 hours and monitored through TLC. Upon completion, the reaction mixture was cooled to room temperature and filtered through Celite. The filtrate was basified with saturated $NaHCO_3$ solution to pH 6 – 7, diluted with water, and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 30% EtOAc/60–90 °C petroleum ether) gave compound **60** (1.80 g, 7.47 mmol, 87% yield) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (s, 1H), 6.85 (dd, J = 8.2, 2.0 Hz, 1H), 6.80 (d, J = 1.8 Hz, 1H), 6.64 (d, J = 8.2 Hz, 1H), 4.06 – 3.99 (m, 1H), 3.92 (s, 1H), 1.45 (d, J = 6.7 Hz, 3H); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 241.05; retention time 2.66 min, > 99% pure.

(S)-6-bromo-1,3,4-trimethyl-3,4-dihydroquinoxalin-2(1H)-one (**63**). To a solution of compound **60** (1.5 g, 6.22 mmol) in anhydrous DMF (5 mL) was added NaH (1.0 g, 24.88 mmol) portionwise at

0 °C. The mixture was stirred at 0 °C for 30 min, then iodomethane (1.55 mL, 24.88 mmol) was added and stirred at room temperature for another 2 h. The reaction was monitored by TLC. Upon completion, the mixture was poured to 100 mL ice water slowly, then the white solid was filtered, washed with water, dried, and offered the white solid compound **63** (1.37 g, 5.10 mmol, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.74 (d, *J* = 2.1 Hz, 1H), 3.98 (q, *J* = 6.9 Hz, 1H), 3.34 (s, 3H), 2.84 (s, 3H), 1.12 (d, *J* = 6.8 Hz, 3H).

(S)-6-((2-methoxyphenyl)amino)-1,3,4-trimethyl-3,4-dihydroquinoxalin-2(1H)-one (**9**). To a solution of compound **63** (220 mg, 0.82 mmol), 2-methoxyaniline (0.14 mL, 1.23 mmol), Cs_2CO_3 (534 mg, 1.64 mmol) in anhydrous DMF (30 mL) at room temperature were added Pd_2dba_3 (23 mg, 0.025 mmol) and XPhos (23 mg, 0.049 mmol). The mixture was sealed in a microwave tube and heated to 110 °C for 1 h. The reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water, and extracted with EtOAc (3 × 10 mL). Combined organic layers were washed with brine, then dried with Na_2SO_4. The crude proudct was purified by flash column chromatography (gradient elution, gradient 0 to 40% EtOAc/60–90 °C petroleum ether) to give compound **9** (159 mg, 0.51 mmol, 62% yield) as a light white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.23 (m, 1H), 6.91 – 6.88 (m, 1H), 6.88 – 6.86 (m, 1H), 6.86 – 6.84 (m, 1H), 6.84 – 6.82 (s, 1H), 6.67 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.46 (d, *J* = 2.3 Hz, 1H), 3.98 (q, *J* = 6.8 Hz, 1H), 3.90 (s, 3H), 3.36 (s, 3H), 2.82 (s, 3H), 1.13 (d, *J* = 6.8 Hz, 3H) (One NH was not seen); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 312.20; retention time 3.39 min, > 96% pure.

Following the similar procedures as for compound **9** gave compound **10**.

(S)-1,3,4-trimethyl-6-(pyridin-2-ylamino)-3,4-dihydroquinoxalin-2(1H)-one (**10**). Light yellow solid, 70% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 4.1 Hz, 1H), 7.48 (t, *J* = 6.9 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.85 – 6.80 (m, 2H), 6.73 – 6.69 (m, 1H), 6.66 – 6.62 (m, 2H), 3.99 (q, *J* = 6.8 Hz, 1H), 3.37 (s, 3H), 2.84 (s, 3H), 1.14 (d, *J* = 6.8 Hz, 3H); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 283.27; retention time 2.11 min, > 97% pure.

(S)-2-chloro-N-(1,3,4-trimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)benzenesulfonamide (**11**). To a solution of compound **63** (150 mg, 0.56 mmol), 2-chlorobenzenesulfonamide (129 mg, 0.67 mmol), K_2CO_3 (155 mg, 1.12 mmol) in 2-metyhtetrahydrofuran (5 mL) at room temperature were added allylpalladium chloride dimer (4 mg, 0.011 mmol) and *t*BuXPhos (5 mg, 0.011

mmol). The mixture was sealed in a microwave tube and heated to 85 °C overnight. The reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water, and extracted with EtOAc (3 × 10 mL). Combined organic layers were washed with brine, then dried with Na_2SO_4. The crude proudct was purified by flash column chromatography (gradient elution, gradient 0 to 40% EtOAc/60–90 °C petroleum ether) to give compound **11** (133 mg, 0.35 mmol, 63% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.65 – 7.55 (m, 2H), 7.49 (t, J = 6.3 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.53 (d, J = 8.0 Hz, 1H), 6.43 (s, 1H), 4.01 – 3.85 (m, 1H), 3.15 (s, 3H), 2.66 (s, 3H), 0.87 (d, J = 6.5 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.80, 136.62, 136.17, 134.53, 132.84, 131.77, 131.73, 130.83, 127.60, 125.66, 114.64, 109.72, 104.40, 58.61, 34.51, 28.33, 11.41; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{17}H_{19}ClN_3O_3S^+$) 380.083, found 380.0828; retention time 3.12 min, > 96% pure.

Following the similar procedures as for compound **11** gave compounds **12**-**14**.

(S)-4-methyl-N-(1,3,4-trimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)benzenesulfonamide (**12**). Light yellow solid, 69% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.3 Hz, 2H), 7.46 (s, 1H), 7.20 (d, J = 8.1 Hz, 2H), 6.71 (d, J = 8.5 Hz, 1H), 6.53 (dd, J = 8.5, 2.2 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 3.95 (q, J = 6.8 Hz, 1H), 3.28 (s, 3H), 2.73 (s, 3H), 2.36 (s, 3H), 1.05 (d, J = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.01, 143.80, 136.75, 136.21, 133.02, 129.62 (2 × C), 127.48 (2 × C), 127.04, 114.60, 112.17, 107.03, 59.92, 35.28, 29.08, 21.62, 11.95; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{18}H_{22}N_3O_3S^+$) 360.1376, found 360.1381; retention time 3.16 min, > 95% pure.

(S)-N-(3-ethyl-1,4-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**13**). Light yellow solid, 67% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 8.2 Hz, 2H), 7.24 – 7.17 (m, 3H), 6.69 (d, J = 8.4 Hz, 1H), 6.48 (dd, J = 8.4, 2.1 Hz, 1H), 6.39 (d, J = 2.0 Hz, 1H), 3.83 (dd, J = 7.5, 5.2 Hz, 1H), 3.30 (s, 3H), 2.81 (s, 3H), 2.36 (s, 3H), 1.70 – 1.56 (m, 1H), 1.55 – 1.41 (m, 1H), 0.79 (t, J = 7.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.62, 143.62, 137.20, 136.15, 133.11, 129.50 (2 × C), 127.42 (2 × C), 126.56, 114.48, 111.52, 105.96, 65.25, 36.14, 28.99, 21.95, 21.53, 10.15; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{19}H_{24}N_3O_3S^+$) 374.1533, found 374.154; retention time 3.29 min, > 99% pure.

(R)-N-(3-ethyl-1,4-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**14**). Light yellow solid, 59% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.3 Hz, 2H), 7.37 (s, 1H), 7.20 (d, *J* = 8.1 Hz, 2H), 6.68 (d, *J* = 8.5 Hz, 1H), 6.49 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.40 (d, *J* = 2.3 Hz, 1H), 3.83 (dd, *J* = 7.5, 5.1 Hz, 1H), 3.29 (s, 3H), 2.81 (s, 3H), 2.35 (s, 3H), 1.69 – 1.55 (m, 1H), 1.53 – 1.39 (m, 1H), 0.78 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.62, 143.62, 137.20, 136.15, 133.11, 129.49 (2 × C), 127.42 (2 × C), 126.55, 114.48, 111.51, 105.95, 65.24, 36.13, 28.99, 21.95, 21.53, 10.15; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{19}H_{24}N_3O_3S^+$) 374.1533, found 374.1535; retention time 3.29 min, > 95% pure.

Scheme 2 *5-bromo-N-cyclopropyl-2-nitroaniline* (**68**). A solution of compound **56** (10 g, 45.45 mmol) and cyclopropanamine (6.3 mL, 90.91 mmol) in 1,2-dichloroethane (20 mL) was heated to 80 °C for 12 h. The reaction was monitored by TLC. Upon completion, the reaction mixture was cooled to room temperature and poured into 50 mL of water and extracted with dichloromethane (3 × 50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered and excess solvent removed via rotary evaporator to give compound **68** (11.57 g, 45.0 mmol, 99% yield) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.96 (d, *J* = 9.1 Hz, 1H), 7.45 (d, *J* = 2.0 Hz, 1H), 6.77 (dd, *J* = 9.1, 2.0 Hz, 1H), 2.60 – 2.50 (m, 1H), 0.97 – 0.88 (m, 2H), 0.69 – 0.61 (m, 2H).

5-bromo-N^1-cyclopropylbenzene-1,2-diamine (**76**). To a solution of compound **68** (11.5 g, 44.75 mmol) in EtOH (25 mL) and iron (10.02 g, 179.0 mmol) was added ammonium chloride (9.58 g, 179.0 mmol) in 5 mL water at 50 – 55 °C. The reaction mixture was heated to 80 °C for 1 hour, then cooled to room temperature, and filtered through Celite. The filtrate was basified with saturated $NaHCO_3$ solution to pH 7 – 8, diluted with water, and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude proudct was purified by flash column chromatography (gradient elution, gradient 0 to 20% EtOAc/60–90 °C petroleum ether) to give compound **76** (8.17 g, 35.98 mmol, 80% yield) as brown liquid.

6-bromo-4-cyclopropyl-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**84**). To a stirred solution of compound **76** (8.17 g, 35.98 mmol) in anhydrous dichloromethane (20 mL) at 0 °C were slowly

added DIPEA (12.54 mL, 71.96 mmol) and 2-bromopropanoyl bromide (4.52 mL, 43.18 mmol). After the addition was completed the cooling bath was removed and the reaction stirred for 2 h at rt. The reaction mixture was cooled again to 0 °C, diluted with water, and extracted with EtOAc (3 × 50 mL). The combined organic fractions were washed with brine, dried (Na_2SO_4), concentrated by evaporation under reduced pressure. The residue and DIPEA (12.54 mL, 71.96 mmol) in acetonitrile (20 mL) were heated to reflux overnight. The reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water, and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 25% EtOAc/60–90 °C petroleum ether) gave compound **84** as a white soild (4.75 g, 16.9 mmol, 47% yield, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 7.18 (d, *J* = 1.8 Hz, 1H), 6.91 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.64 (d, *J* = 8.2 Hz, 1H), 4.04 (q, *J* = 6.8 Hz, 1H), 2.46 – 2.36 (m, 1H), 1.24 (d, *J* = 6.9 Hz, 3H), 1.04 – 0.96 (m, 1H), 0.85 – 0.77 (m, 1H), 0.68 – 0.60 (m, 1H), 0.60 – 0.52 (m, 1H); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 281.17; retention time 3.48 min, > 98% pure.

6-bromo-4-cyclopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**93**). To a solution of compound **84** (4.75 g, 16.89 mmol) in anhydrous DMF (10 mL) was added NaH (2.03 g, 50.67 mmol) at 0 °C, the mixture was stirred at 0 °C for 30 min, then iodomethane (1.37 mL, 21.96 mmol) was added and stirred at room temperature for another 2 h. The reaction was monitored by TLC. Upon completion, the reaction mixture was acidified with 1 N aq. HCl to pH 7 – 8, diluted with water and extracted with EtOAc (3 × 50 mL). The combined organic fractions were washed with brine, dried over Na_2SO_4, concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 25% EtOAc/60–90 °C petroleum ether) gave compound **93** as a white soild (3.86 g, 13.1 mmol, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, *J* = 2.2 Hz, 1H), 6.91 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.70 (d, *J* = 8.5 Hz, 1H), 4.03 (q, *J* = 6.9 Hz, 1H), 3.24 (s, 3H), 2.37 – 2.28 (m, 1H), 1.10 (d, *J* = 6.9 Hz, 3H), 0.97 – 0.90 (m, 1H), 0.79 – 0.68 (m, 1H), 0.62 – 0.52 (m, 1H), 0.50 – 0.42 (m, 1H); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 295.15; retention time 3.83 min, > 98% pure.

N-(4-cyclopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**19**). To a solution of compound **93** (630 mg, 2.13 mmol), 4-methylbenzenesulfonamide (548 mg, 3.20 mmol), K_2CO_3 (588 mg, 4.26 mmol) in 2-metyhtetrahydrofuran (5 mL) at room temperature were added allylpalladium chloride dimer (16 mg, 0.043 mmol) and *t*BuXPhos (18 mg, 0.043 mmol). The mixture was sealed in a microwave tube and heated to 85 °C overnight. The reaction was monitored by TLC. Upon completion, the mixture was diluted with water, and extracted with EtOAc (3 × 10 mL). The combined organic fractions were washed with brine, then dried (Na_2SO_4). The crude proudct was purified by flash column chromatography (gradient elution, gradient 0 to 5% MeOH/DCM) to give compound **19** as a white soild (501 mg, 1.3 mmol, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.2 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 6.90 (s, 1H), 6.83 (d, *J* = 2.2 Hz, 1H), 6.73 (d, *J* = 8.5 Hz, 1H), 6.56 (dd, *J* = 8.4, 2.3 Hz, 1H), 4.05 (q, *J* = 6.8 Hz, 1H), 3.27 (s, 3H), 2.38 (s, 3H), 2.30 – 2.23 (m, 1H), 1.11 (d, *J* = 6.8 Hz, 3H), 0.88 – 0.79 (m, 1H), 0.79 – 0.69 (m, 1H), 0.61 – 0.52 (m, 1H), 0.36 – 0.28 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.52, 143.64, 136.48, 136.18, 132.88, 129.51 (2 × C), 127.44 (2 × C), 126.89, 114.63, 112.81, 108.29, 58.00, 28.99, 27.79, 21.53, 12.12, 9.26, 6.65; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{20}H_{24}N_3O_3S^+$) 386.1533, found 386.1535; retention time 3.36 min, > 97% pure.

Following the similar procedures as for compound **19** gave compounds **15-18, 20-24** and **27**.

N-(4-cyclopentyl-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**15**). White soild, 71% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 9.77 (s, 1H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 6.63 (d, *J* = 8.2 Hz, 1H), 6.48 – 6.40 (m, 2H), 3.83 (q, *J* = 6.7 Hz, 1H), 3.50 (p, *J* = 7.4 Hz, 1H), 2.31 (s, 3H), 1.87 – 1.73 (m, 2H), 1.69 – 1.48 (m, 5H), 1.41 – 1.27 (m, 1H), 0.88 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.29, 142.95, 136.60, 133.47, 132.46, 129.44 (2 × C), 126.80 (2 × C), 125.03, 114.92, 112.36, 109.42, 58.94, 54.49, 29.64, 29.56, 23.59, 23.29, 20.91, 13.93; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{21}H_{26}N_3O_3S^+$) 400.1689, found 400.1692; retention time 3.35 min, > 97% pure.

N-(4-cyclopentyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**16**). White soild, 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.08 (s, 1H), 6.75 (d, *J* = 8.4 Hz, 1H), 6.57 (d, *J* = 2.2 Hz, 1H), 6.54 (dd, *J* = 8.4, 2.3 Hz, 1H), 4.15 (q, *J* = 6.8 Hz, 1H), 3.59 (p, *J* = 7.3 Hz, 1H), 3.29 (s, 3H), 2.36 (s, 3H), 1.97

– 1.84 (m, 2H), 1.78 – 1.71 (m, 1H), 1.66 – 1.54 (m, 4H), 1.47 – 1.36 (m, 1H), 0.97 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.00, 143.68, 136.28, 136.17, 132.53, 129.56 (2 × C), 128.34, 127.44 (2 × C), 114.82, 113.19, 110.55, 58.82, 54.83, 30.78, 30.68, 29.17, 24.20, 23.74, 21.57, 13.67; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{22}H_{28}N_3O_3S^+$) 414.1846, found 414.1846; retention time 3.62 min, > 99% pure.

N-(4-cyclopentyl-1-ethyl-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**17**). White soild, 69% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 8.3 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.80 – 6.75 (m, 2H), 6.54 (d, *J* = 2.3 Hz, 1H), 6.51 (dd, *J* = 8.4, 2.3 Hz, 1H), 4.10 (q, *J* = 6.7 Hz, 1H), 4.02 (dq, *J* = 14.4, 7.3 Hz, 1H), 3.78 (dq, *J* = 14.1, 7.0 Hz, 1H), 3.59 (p, *J* = 7.3 Hz, 1H), 2.37 (s, 3H), 1.94 – 1.83 (m, 2H), 1.79 – 1.69 (m, 1H), 1.66 – 1.54 (m, 4H), 1.47 – 1.38 (m, 1H), 1.21 (t, *J* = 7.1 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.18, 143.74, 136.53, 136.25, 132.25, 129.60 (2 × C), 127.48 (2 × C), 127.24, 114.60, 113.36, 110.99, 59.12, 54.64, 37.17, 30.77, 30.66, 24.20, 23.76, 21.61, 13.66, 12.70; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{23}H_{30}N_3O_3S^+$) 428.2002, found 428.2008; retention time 3.76 min, > 95% pure.

N-(4-isopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**18**). White soild, 74% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 8.3 Hz, 2H), 7.51 (s, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 6.74 (d, *J* = 8.5 Hz, 1H), 6.60 (d, *J* = 2.2 Hz, 1H), 6.55 (dd, *J* = 8.5, 2.2 Hz, 1H), 4.18 – 4.13 (m, 1H), 3.75 – 3.63 (m, 1H), 3.28 (s, 3H), 2.35 (s, 3H), 1.17 (dd, *J* = 8.0, 6.8 Hz, 6H), 1.01 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.29, 143.78, 136.26, 135.84, 132.69, 129.60 (2 × C), 128.00, 127.49 (2 × C), 115.13, 112.81, 109.78, 51.69, 50.01, 29.29, 21.60, 21.12, 20.99, 16.72; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{20}H_{26}N_3O_3S^+$) 388.1689, found 388.1693; retention time 3.10 min, > 99% pure

N-(4-isobutyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**20**). White soild, 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.2 Hz, 2H), 7.48 (s, 1H), 7.21 (d, *J* = 8.1 Hz, 2H), 6.73 (d, *J* = 8.5 Hz, 1H), 6.53 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.39 (d, *J* = 2.0 Hz, 1H), 3.95 (q, *J* = 6.8 Hz, 1H), 3.30 (s, 3H), 3.07 (dd, *J* = 13.7, 5.5 Hz, 1H), 2.49 (dd, *J* = 13.7, 8.8 Hz, 1H), 2.36 (s, 3H), 1.82 – 1.65 (m, 1H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.86 (dd, *J* = 8.3, 6.8 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.08, 143.72, 136.27, 135.97, 132.84, 129.59 (2 × C),

127.46 (2 × C), 127.15, 114.96, 112.16, 107.46, 59.14, 55.88, 29.17, 25.94, 21.62, 20.57, 20.09, 13.07; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{21}H_{28}N_3O_3S^+$) 402.1846, found 402.1846; retention time 3.61 min, > 99% pure.

N-(4-(2-methoxyethyl)-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**21**). White soild, 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 7.9 Hz, 2H), 6.68 (d, *J* = 8.5 Hz, 1H), 6.56 (d, *J* = 8.5 Hz, 1H), 6.51 (s, 1H), 4.05 (q, *J* = 6.5 Hz, 1H), 3.48 – 3.32 (m, 3H), 3.24 (s, 3H), 3.21 (s, 3H), 3.14 – 3.04 (m, 1H), 2.28 (s, 3H), 0.99 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.00, 143.41, 136.04, 135.45, 133.16, 129.34 (2 × C), 127.19 (2 × C), 126.67, 114.90, 111.92, 106.89, 69.30, 58.72, 57.92, 47.57, 29.00, 21.34, 13.17; LCMS *m/z* (ESI, negative) found $[M - H]^+$ 402.3; retention time 2.92 min, > 98% pure.

N-(1,3-dimethyl-4-(2-morpholinoethyl)-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**22**). White soild, 67% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.3 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 6.73 (d, *J* = 8.5 Hz, 1H), 6.54 (d, *J* = 2.2 Hz, 1H), 6.49 (dd, *J* = 8.4, 2.2 Hz, 1H), 4.03 (q, *J* = 6.8 Hz, 1H), 3.73 – 3.67 (m, 4H), 3.45 – 3.35 (m, 1H), 3.29 (s, 3H), 3.15 – 3.03 (m, 1H), 2.59 – 2.45 (m, 6H), 2.37 (s, 3H), 1.07 (d, *J* = 6.8 Hz, 3H) (One NH was not seen); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.91, 143.82, 136.32, 135.59, 132.94, 129.68 (2 × C), 127.41 (2 × C), 127.37, 115.07, 112.31, 107.52, 66.92 (2 × C), 58.76, 55.77, 53.93 (2 × C), 45.88, 29.20, 21.66, 13.76; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{23}H_{31}N_4O_4S^+$) 459.2061, found 459.2058; retention time 2.59 min, > 95% pure.

N-(1,3-dimethyl-2-oxo-4-((tetrahydrofuran-2-yl)methyl)-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzenesulfonamide (**23**). White soild, 66% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, *J* = 8.3, 6.6 Hz, 2H), 7.29 (d, *J* = 3.0 Hz, 1H), 7.20 (d, *J* = 8.1 Hz, 2H), 6.72 (t, *J* = 8.1 Hz, 1H), 6.57 – 6.50 (m, 1H), 6.47 (dd, *J* = 8.4, 2.2 Hz, 1H), 4.16 (q, *J* = 6.8 Hz, 1H), 4.03 – 3.95 (m, 1H), 3.91 – 3.79 (m, 1H), 3.75 – 3.68 (m, 1H), 3.34 (dd, *J* = 14.2, 3.3 Hz, 1H), 3.27 (d, *J* = 3.7 Hz, 3H), 2.99 – 2.90 (m, 1H), 2.36 (s, 3H), 2.05 – 1.93 (m, 1H), 1.91 – 1.79 (m, 2H), 1.60 – 1.46 (m, 1H), 1.05 (dd, *J* = 6.8, 2.6 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.12, 143.58, 136.17, 135.76, 132.90, 129.49 (2 × C), 127.39, 127.34, 127.10, 114.93, 112.15, 107.37, 76.84, 68.12, 58.51,

52.43, 29.49, 29.12, 25.46, 21.52, 13.28; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{22}H_{28}N_3O_4S^+$) 430.1795, found 430.1801; retention time 3.24 min, > 95% pure.

N-(4-(2-methoxybenzyl)-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzene-sulfonamide (**24**). White soild, 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.3 Hz, 2H), 7.39 (s, 1H), 7.26 – 7.20 (m, 1H), 7.18 (d, *J* = 7.5 Hz, 1H), 7.11 (d, *J* = 8.1 Hz, 2H), 6.88 (d, *J* = 8.1 Hz, 1H), 6.84 (t, *J* = 7.5 Hz, 1H), 6.70 (d, *J* = 8.5 Hz, 1H), 6.55 (d, *J* = 2.2 Hz, 1H), 6.46 (dd, *J* = 8.5, 2.2 Hz, 1H), 4.45 (d, *J* = 15.4 Hz, 1H), 4.13 – 4.01 (m, 2H), 3.86 (s, 3H), 3.28 (s, 3H), 2.33 (s, 3H), 1.11 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.24, 157.41, 143.48, 136.10, 135.97, 133.02, 129.49 (2 × C), 128.93, 128.59, 127.31 (2 × C), 126.92, 124.33, 120.49, 114.76, 111.59, 110.51, 107.28, 58.39, 55.41, 46.32, 29.10, 21.55, 13.31; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{25}H_{28}N_3O_4S^+$) 466.1795, found 466.1784; retention time 3.65 min, > 95% pure.

N-(4-cyclopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methoxy-benzenesulfo-namide (**27**). White soild, 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.9 Hz, 2H), 6.90 (d, *J* = 8.9 Hz, 2H), 6.84 (d, *J* = 2.3 Hz, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 6.66 (s, 1H), 6.53 (dd, *J* = 8.5, 2.4 Hz, 1H), 4.06 (q, *J* = 6.9 Hz, 1H), 3.83 (s, 3H), 3.27 (s, 3H), 2.28 (td, *J* = 6.6, 3.3 Hz, 1H), 1.12 (d, *J* = 6.8 Hz, 3H), 0.89 – 0.82 (m, 1H), 0.80 – 0.71 (m, 1H), 0.61 – 0.54 (m, 1H), 0.39 – 0.31 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.54, 163.10, 136.58, 132.85, 130.73, 129.62 (2 × C), 127.06, 114.67, 114.13 (2 × C), 112.92, 108.44, 58.07, 55.67, 29.04, 27.87, 12.21, 9.35, 6.73; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{20}H_{24}N_3O_4S^+$) 402.1482, found 402.1489; retention time 3.12 min, > 97% pure.

4-cyclopropyl-6-(ethylamino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**99**). To a solution of compound **93** (0.5 g, 1.69 mmol), sodium tert-butoxide (0.6 g, 6.27 mmol), ethylamine hydrochloride (0.39 g, 4.78 mmol) in toluene (5 mL) at room temperature were added palladium acetate (23 mg, 0.1 mmol) and *t*Bu_3P (0.068 mL, 0.29 mmol). The mixture was sealed in a microwave tube and heated to 85 °C overnight. The reaction was monitored by TLC. Upon completion, the mixture was diluted with water, and extracted with EtOAc (3 × 10 mL). The combined organic fractions were washed with brine, then dried (Na_2SO_4). The crude proudct was purified by flash column chromatography (gradient elution, gradient 0 to 35% EtOAc/60–90 °C petroleum ether) to give compound **99** (0.3 g, 1.16 mmol, 69% yield) as a white soild.

6-amino-4-cyclopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**100**). To a solution of compound **93** (0.5 g, 1.69 mmol), *tert*-butyl carbamate (0.302 g, 2.57 mmol), Cs_2CO_3 (0.784 g, 2.41 mmol) in dioxane (5 mL) at room temperature was added palladium acetate (38 mg, 0.17 mmol) and XPhos (129 mg, 0.27 mmol). The mixture was sealed in a microwave tube and heated to 85 °C overnight. The reaction was monitored by TLC. Upon completion, the mixture was diluted with water and extracted with EtOAc (3 × 50 mL). The combined organic fractions were washed with brine, dried with Na_2SO_4, then concentrated by evaporation under reduced pressure. The residue and TFA (2 mL) in dichloromethane (10 mL) was stirred at room temperature overnight. The reaction was monitored by TLC. Upon completion, the reaction was poured into 10 mL cold water and basified with saturated $NaHCO_3$ solution to pH 7 – 8, then extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 25% EtOAc/60–90 °C petroleum ether) gave compound **100** (0.15 g, 0.65 mmol, 38% yield, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, *J* = 2.2 Hz, 1H), 6.97 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 4.08 (q, *J* = 6.8 Hz, 1H), 3.29 (s, 3H), 2.42 – 2.32 (m, 1H), 1.15 (d, *J* = 6.9 Hz, 3H), 1.00 – 0.92 (m, 1H), 0.81 – 0.74 (m, 1H), 0.65 – 0.57 (m, 1H), 0.55 – 0.47 (m, 1H).

N-(4-cyclopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-N-ethyl-4-methylbenzen-esulfonamide (**28**). A solution of compound **99** (0.055 g, 0.212 mmol) and triethylamine (0.089 mL, 0.636 mmol) in dichloromethane (5 mL) was added toluenesulfonyl chloride (0.081 g, 0.424 mmol) at 0 °C, the mixture stirred under room temperature overnight. The reaction was monitored by TLC. Upon completion, the mixture was diluted with water, and extracted with EtOAc (3 × 20 mL). The combined organic fractions were washed with brine, dried with Na_2SO_4, then concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 35% EtOAc/60–90 °C petroleum ether) gave compound **28** (0.041 g, 0.098 mmol, 46% yield) as a white soild. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.2 Hz, 2H), 7.24 (d, *J* = 8.3 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.67 (d, *J* = 2.1 Hz, 1H), 6.60 (dd, *J* = 8.4, 2.2 Hz, 1H), 4.06 (q, *J* = 6.8 Hz, 1H), 3.64 (dq, *J* = 14.3, 7.2 Hz, 1H), 3.55 (dq, *J* = 13.9, 7.1 Hz, 1H), 3.31 (s, 3H), 2.41 (s, 3H), 2.23 – 2.13 (m, 1H), 1.14 (d, *J* = 6.8 Hz,

3H), 1.10 (t, *J* = 7.1 Hz, 3H), 0.76 – 0.63 (m, 2H), 0.60 – 0.53 (m, 1H), 0.37 – 0.28 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.68, 143.28, 136.22, 135.86, 134.56, 129.43 (2 × C), 129.16, 127.89 (2 × C), 120.14, 114.61, 114.31, 58.12, 45.90, 29.11, 27.85, 21.63, 14.29, 12.32, 9.29, 6.74; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{22}H_{28}N_3O_3S^+$) 414.1846, found 414.1846; retention time 3.66 min, > 99% pure.

Following the similar procedures as for compound **28** gave compounds **29**-**31**.

p-tolyl(4-cyclopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)carbamate (**29**). White soild, 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 1H), 7.19 (d, *J* = 8.3 Hz, 2H), 7.06 (d, *J* = 8.3 Hz, 2H), 6.98 (s, 1H), 6.85 (s, 2H), 4.10 (q, *J* = 6.7 Hz, 1H), 3.33 (s, 3H), 2.42 – 2.32 (m, 4H), 1.17 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 4.7 Hz, 1H), 0.77 (dd, *J* = 12.8, 7.7 Hz, 1H), 0.66 – 0.57 (m, 1H), 0.57 – 0.48 (m, 1H); ^{13}C NMR (126 MHz, CDCl3) δ 168.53, 152.09, 148.46, 136.79, 135.56, 133.81, 130.10 (2 × C), 125.92, 121.54 (2 × C), 114.64, 109.31, 105.23, 58.35, 29.10, 28.07, 21.01, 12.32, 9.61, 6.78; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{21}H_{24}N_3O_3^+$) 366.1812, found 366.1814;retention time 3.69 min, > 98% pure.

4-fluorophenyl(4-cyclopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-(ethyl)carbamate (**30**). White soild, 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 – 6.97 (m, 5H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.3 Hz, 1H), 4.13 (q, *J* = 6.8 Hz, 1H), 3.81 (s, 2H), 3.35 (s, 3H), 2.44 – 2.37 (m, 1H), 1.28 – 1.18 (m, 6H), 1.01 – 0.91 (m, 1H), 0.86 – 0.76 (m, 1H), 0.68 – 0.61 (m, 1H), 0.58 – 0.50 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.57, 159.93, 157.99, 152.85, 146.34, 135.45, 127.43, 122.05 (d, *J* = 8.4 Hz), 116.88, 114.90, 114.72, 113.47 (2 × C), 112.17, 57.14, 45.11, 28.05, 26.96, 11.45 (2 × C), 8.37, 5.84; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{22}H_{25}FN_3O_3^+$) 398.1874, found 398.1874; retention time 3.65 min, > 99% pure.

N-(4-cyclopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)-4-methylbenzamide (**31**). White soild, 54% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.60 (s, 1H), 7.27 (d, *J* = 7.4 Hz, 2H), 7.08 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.87 (d, *J* = 8.6 Hz, 1H), 4.10 (q, *J* = 6.8 Hz, 1H), 3.33 (s, 3H), 2.46 – 2.35 (m, 4H), 1.16 (d, *J* = 6.8 Hz, 3H), 1.06 – 0.96 (m, 1H), 0.82 – 0.71 (m, 1H), 0.67 – 0.50 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.59, 165.83, 142.43, 136.58, 134.49, 132.32, 129.53 (2 × C), 127.15 (2 × C), 126.27, 114.50, 110.95, 106.67, 58.35,

29.09, 28.08, 21.62, 12.27, 9.62, 6.75; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{21}H_{24}N_3O_2^+$) 350.1863, found 350.1869; retention time 3.23 min, > 99% pure.

6-(benzylamino)-4-cyclopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**32**). To a solution of compound **93** (0.12 g, 0.41 mmol), sodium tert-butoxide (0.145 g, 1.51 mmol), phenylmethanamine (0.13 mL, 1.16 mmol) in toluene (5 mL) at room temperature were added palladium acetate (6 mg, 0.025 mmol) and tBu$_3$P (0.017 mL, 0.07 mmol). The mixture was sealed in a microwave tube and heated to 85 °C overnight. The reaction was monitored by TLC. Upon completion, the mixture was extracted with EtOAc (3 × 20 mL). The combined organic fractions were washed with brine, dried with Na_2SO_4, then concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 25% EtOAc/60–90 °C petroleum ether) gave compound **32** as a yellow soild (0.077 g, 0.24 mmol, 59% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 7.1 Hz, 2H), 7.35 (t, J = 7.4 Hz, 2H), 7.31 – 7.24 (m, 1H), 6.74 (d, J = 8.5 Hz, 1H), 6.44 (d, J = 2.2 Hz, 1H), 6.18 (dd, J = 8.5, 2.3 Hz, 1H), 4.34 (s, 2H), 4.05 (q, J = 6.8 Hz, 1H), 3.28 (s, 3H), 2.35 – 2.22 (m, 1H), 1.16 (d, J = 6.8 Hz, 3H), 0.82 – 0.67 (m, 2H), 0.62 – 0.54 (m, 1H), 0.50 – 0.41 (m, 1H) (One NH was not seen); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.11, 145.12, 139.64, 137.11, 128.72 (2 × C), 127.64 (2 × C), 127.34, 121.41, 115.20, 103.09, 100.01, 58.45, 48.92, 29.01, 27.87, 12.04, 9.48, 6.61; HRMS (ESI) m/z $[M + H]^+$, calcd for ($C_{20}H_{24}N_3O^+$) 322.1914, found 322.1917; retention time 2.69 min, > 98% pure.

Following the similar procedures as for compound **32** gave compounds **33-51**.

6-(benzyl(ethyl)amino)-4-cyclopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**33**). White soild, 52% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.24 (m, 5H), 6.78 (d, J = 8.7 Hz, 1H), 6.50 (d, J = 2.5 Hz, 1H), 6.27 (dd, J = 8.7, 2.5 Hz, 1H), 4.55 (d, J = 2.6 Hz, 2H), 4.08 (q, J = 6.7 Hz, 1H), 3.63 – 3.45 (m, 2H), 3.31 (s, 3H), 2.32 – 2.20 (m, 1H), 1.28 (t, J = 7.0 Hz, 3H), 1.20 (d, J = 6.8 Hz, 3H), 0.75 – 0.62 (m, 2H), 0.61 – 0.53 (m, 1H), 0.42-0.34 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.96, 145.47, 139.54, 136.72, 128.52 (2 × C), 126.73, 126.54 (2 × C), 120.03, 114.98, 102.78, 99.54, 58.38, 54.68, 45.80, 28.81, 27.62, 12.38, 11.99, 9.14, 6.46; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{22}H_{28}N_3O^+$) 350.2227, found 350.2229; retention time 2.72 min, > 98% pure.

4-cyclopropyl-1,3-dimethyl-6-((1-phenylethyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (**34**). White soild, 52% yield. ^{1}H NMR (a mixture of rotamers was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.43 – 7.36 (m, 4H), 7.35 – 7.29 (m, 4H), 7.26 – 7.20 (m, 2H), 6.69 – 6.62 (m, 2H), 6.38 (d, *J* = 2.1 Hz, 1H), 6.25 (d, *J* = 2.0 Hz, 1H), 6.10 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.01 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.52 – 4.43 (m, 2H), 4.08 – 3.96 (m, 3H), 3.27 – 3.22 (m, 6H), 2.26 – 2.13 (m, 2H), 1.57 – 1.50 (m, 6H), 1.16 – 1.09 (m, 6H), 0.90 - 0.83 (m, 1H), 0.75 – 0.68 (m, 1H), 0.67 – 0.60 (m, 1H), 0.60 – 0.52 (m, 2H), 0.52 – 0.41 (m, 2H), 0.18 – 0.09 (m, 1H) (One NH was not seen); ^{13}C NMR (mixture of rotamers, 126 MHz, $CDCl_3$) δ 168.08, 167.99, 145.63, 145.60, 144.37, 144.21, 136.98, 136.77, 128.73 (4 × C), 126.97, 126.93, 125.96 (2 × C), 125.88 (2 × C), 121.14, 120.83, 115.05, 115.02, 104.05, 103.53, 100.48, 100.21, 58.42, 58.38, 54.29, 54.12, 28.93, 28.91, 27.84, 27.71, 25.42, 25.14, 12.00, 11.96, 9.56, 9.12, 6.52, 6.49; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{21}H_{26}N_3O^+$) 336.207, found 336.2071; retention time 2.72 min, > 42% pure; retention time 2.75 min, > 57% pure.

4-cyclopropyl-1,3-dimethyl-6-(((R)-1-phenylethyl)amino)-3,4-dihydroquinoxalin-2-(1H)-one (**35**). White soild, 58% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.44 – 7.36 (m, 4H), 7.36 – 7.29 (m, 4H), 7.26 – 7.19 (m, 2H), 6.70 – 6.61 (m, 2H), 6.40 (d, *J* = 2.3 Hz, 1H), 6.26 (d, *J* = 2.3 Hz, 1H), 6.12 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.02 (dd, *J* = 8.5, 2.3 Hz, 1H), 4.53 – 4.43 (m, 2H), 4.11 (s, 2H), 4.06 – 3.98 (m, 2H), 3.25 (s, 3H), 3.24 (s, 3H), 2.27 – 2.14 (m, 2H), 1.57 – 1.49 (m, 6H), 1.17 – 1.10 (m, 6H), 0.91 – 0.84 (m, 1H), 0.75 – 0.67 (m, 1H), 0.67 – 0.59 (m, 1H), 0.59 – 0.53 (m, 2H), 0.52 – 0.43 (m, 2H), 0.18 – 0.10 (m, 1H); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 336.05; retention time 2.71 min, > 45% pure; retention time 2.74 min, > 54% pure.

4-cyclopropyl-1,3-dimethyl-6-(((S)-1-phenylethyl)amino)-3,4-dihydroquinoxalin-2-(1H)-one (**36**). White soild, 53% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.44 – 7.36 (m, 4H), 7.36 – 7.29 (m, 4H), 7.26 – 7.19 (m, 2H), 6.69 – 6.62 (m, 2H), 6.40 (d, *J* = 2.3 Hz, 1H), 6.26 (d, *J* = 2.3 Hz, 1H), 6.11 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.02 (dd, *J* = 8.5, 2.3 Hz, 1H), 4.53 – 4.44 (m, 2H), 4.11 (s, 2H), 4.07 – 3.98 (m, 2H), 3.25 (s, 3H), 3.24 (s, 3H), 2.25 – 2.20 (m, 1H), 2.20 – 2.14 (m, 1H), 1.56 – 1.49 (m, 6H), 1.17 – 1.09 (m, 6H), 0.92 – 0.85 (m, 1H), 0.75 – 0.67 (m, 1H), 0.67 – 0.59 (m, 1H), 0.58-0.54 (m,

2H), 0.51 – 0.43 (m, 2H), 0.18 – 0.10 (m, 1H); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 336.04; retention time 2.71 min, > 46% pure; retention time 2.74 min, > 53% pure.

4-cyclopropyl-1,3-dimethyl-6-((1-phenylpropyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (**37**). White soild, 52% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.40 – 7.28 (m, 8H), 7.25 – 7.19 (m, 2H), 6.69 – 6.61 (m, 2H), 6.39 (d, *J* = 2.2 Hz, 1H), 6.27 (d, *J* = 2.1 Hz, 1H), 6.12 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.03 (dd, *J* = 8.5, 2.2 Hz, 1H), 4.29 – 4.18 (m, 2H), 4.12 (s, 1H), 4.06 – 3.97 (m, 2H), 3.27 – 3.22 (m, 6H), 2.26 – 2.20 (m, 1H), 2.20 – 2.14 (m, 1H), 1.94 – 1.77 (m, 4H), 1.17 – 1.08 (m, 6H), 1.03 –0.94 (m, 6H), 0.92 – 0.86 (m, 1H), 0.76 – 0.68 (m, 1H), 0.67 – 0.60 (m, 1H), 0.59 – 0.44 (m, 4H), 0.18 – 0.10 (m, 1H) (one NH was not seen);^{13}C NMR (mixture, 126 MHz, $CDCl_3$) δ 167.94, 167.86, 144.55, 144.36, 144.21, 144.14, 136.85, 136.66, 128.46 (4 × C), 126.87, 126.84, 126.54 (2 × C), 126.49 (2 × C), 120.89, 120.58, 114.94, 114.92, 104.02, 103.43, 100.32, 99.98, 60.51, 60.18, 58.32, 58.29, 31.81, 31.62, 28.81, 28.80, 27.74, 27.62, 11.87, 11.84, 10.83 (2 × C), 9.49, 9.09, 6.41, 6.38; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{22}H_{28}N_3O^+$) 350.2227, found 350.2229; retention time 2.89 min, > 46% pure; retention time 2.96 min,> 53% pure.

4-cyclopropyl-1,3-dimethyl-6-((1-phenylbutyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (**38**). White soild, 56% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.39 – 7.28 (m, 8H), 7.25 – 7.18 (m, 2H), 6.68 – 6.60 (m, 2H), 6.37 (s, 1H), 6.25 (s, 1H), 6.10 (d, *J* = 8.3 Hz, 1H), 6.02 (d, *J* = 8.4 Hz, 1H), 4.35 – 4.25 (m, 2H), 4.14 – 3.95 (m, 4H), 3.27 – 3.21 (m, 6H), 2.28 – 2.13 (m, 2H), 1.89 – 1.69 (m, 4H), 1.48 – 1.35 (m, 4H), 1.16 – 1.08 (m, 6H), 0.99 – 0.92 (m, 6H), 0.91 – 0.85 (m, 1H), 0.75 – 0.68 (m, 1H), 0.67 – 0.60 (m, 1H), 0.59 – 0.43 (m, 4H), 0.18 – 0.11 (m, 1H); ^{13}C NMR (mixture, 151 MHz, $CDCl_3$) δ 168.02, 167.93, 144.64, 144.57 (2 × C), 144.38, 136.92, 136.73, 128.56 (4 × C), 126.91, 126.87, 126.48 (2 × C), 126.43 (2 × C), 120.94, 120.64, 115.01, 114.98, 103.99, 103.40, 100.32, 99.99, 58.85, 58.53, 58.38, 58.35, 41.33, 41.12, 28.88, 28.87, 27.78, 27.67, 19.59, 19.55, 14.07 (2 × C), 11.96, 11.92, 9.54, 9.14, 6.48, 6.45; LCMS *m/z* (ESI, positive) found $[M + H]^+$ 364.05; retention time 3.06 min, > 49% pure; retention time 3.14 min, > 50% pure.

4-cyclopropyl-6-((2,2-dimethyl-1-phenylpropyl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**39**). White soild, 57% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.36 – 7.27 (m, 8H), 7.24 – 7.18 (m, 2H), 6.63 (d, *J* = 8.5 Hz, 1H), 6.60 (d, *J* = 8.5 Hz, 1H), 6.31 (d, *J* = 2.4 Hz, 1H), 6.21 (d, *J* = 2.4 Hz, 1H), 6.10 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.00 (dd, *J* = 8.5, 2.4 Hz, 1H), 4.23 (s, 1H), 4.10 – 3.96 (m, 4H), 3.26 – 3.19 (m, 6H), 2.23 (ddd, *J* = 9.8, 6.6, 3.6 Hz, 1H), 2.11 (ddd, *J* = 10.1, 6.7, 3.7 Hz, 1H), 1.12 (d, *J* = 6.8 Hz, 3H), 1.08 (d, *J* = 6.8 Hz, 3H), 1.02 (s, 18H), 0.89 – 0.86 (m, 1H), 0.74 – 0.67 (m, 1H), 0.66 – 0.60 (m, 1H), 0.59 – 0.50 (m, 3H), 0.50 – 0.42 (m, 1H), 0.13 – 0.04 (m, 1H) (One NH was not seen); ^{13}C NMR (mixture, 151 MHz, $CDCl_3$) δ 167.95, 167.85, 144.92, 144.62, 141.56, 141.31, 136.83, 136.67, 128.51 (4 × C), 127.73 (2 × C), 127.70 (2 × C), 126.82, 126.78, 120.71, 120.38, 114.91 (2 × C), 104.12, 103.40, 100.28, 99.69, 68.16, 67.61, 58.33 (2 × C), 34.94, 34.82, 28.81 (2 × C), 27.73, 27.61, 27.13 (6 × C), 11.92, 11.86, 9.55, 9.19, 6.40 (2 × C); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 378.13; retention time 3.99 min, > 56% pure; retention time 4.14 min, > 41% pure.

4-cyclopropyl-1,3-dimethyl-6-((2-phenylpropan-2-yl)amino)-3,4-dihydroquinoxalin-2(1H)-one (**40**). White soild, 58% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.9 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 2H), 7.21 (t, *J* = 7.3 Hz, 1H), 6.59 (d, *J* = 8.5 Hz, 1H), 6.09 (d, *J* = 1.7 Hz, 1H), 5.96 (dd, *J* = 8.5, 1.8 Hz, 1H), 3.97 (q, *J* = 6.7 Hz, 1H), 3.23 (s, 3H), 2.01 – 1.89 (m, 1H), 1.67 (s, 3H), 1.63 (s, 3H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.58 (q, *J* = 10.7 Hz, 1H), 0.50 – 0.35 (m, 2H), 0.26 – 0.15 (m, 1H) (One NH was not seen); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.87, 147.79, 142.89, 136.20, 128.46 (2 × C), 126.26, 125.63 (2 × C), 120.68, 114.56, 106.15, 102.10, 58.23, 55.87, 31.13, 30.22, 28.70, 27.57, 11.77, 9.07, 6.17; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{22}H_{28}N_3O^+$) 350.2227, found 350.2231; retention time 2.73 min, > 99% pure.

4-cyclopropyl-6-((2,3-dihydro-1H-inden-1-yl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**41**). White soild, 59% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.42 (d, *J* = 7.1 Hz, 2H), 7.29 – 7.20 (m, 6H), 6.79 (d, *J* = 2.7 Hz, 1H), 6.77 (d, *J* = 2.7 Hz, 1H), 6.54 (s, 2H), 6.31 – 6.25 (m, 2H), 5.06 – 4.99 (m, 2H), 4.08 (q, *J* = 6.8 Hz, 2H), 3.31 (s, 6H), 3.11 – 3.00 (m, 2H), 2.97 – 2.86 (m, 2H),

2.66 – 2.54 (m, 2H), 2.39 – 2.30 (m, 2H), 2.03 – 1.91 (m, 2H), 1.20 (d, *J* = 6.8 Hz, 6H), 0.94 – 0.84 (m, 2H), 0.80 – 0.71 (m, 2H), 0.66 – 0.53 (m, 4H) (Two NH was not seen); ^{13}C NMR (mixture, 126 MHz, $CDCl_3$) δ 168.00 (2 × C), 144.67, 144.62, 144.56, 144.54, 143.61, 143.52, 137.15, 137.10, 127.93, 127.89, 126.64, 126.63, 124.89, 124.88, 124.29 (2 × C), 121.24, 121.13, 115.21, 115.18, 103.48, 103.21, 100.29, 100.10, 59.02 (2 × C), 58.39 (2 × C), 33.89, 33.77, 30.29, 30.26, 28.93 (2 × C), 27.82 (2 × C), 11.96 (2 × C), 9.54, 9.46, 6.58, 6.54; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{22}H_{26}N_3O^+$) 348.207, found 348.207; retention time 2.72 min, > 95% pure.

4-cyclopropyl-1,3-dimethyl-6-((1-(o-tolyl)ethyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (**42**). White soild, 54% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.49 (d, *J* = 7.0 Hz, 1H), 7.44 (d, *J* = 7.2 Hz, 1H), 7.21 – 7.11 (m, 6H), 6.70 – 6.63 (m, 2H), 6.31 (d, *J* = 2.2 Hz, 1H), 6.17 (d, *J* = 2.2 Hz, 1H), 6.07 (dd, *J* = 8.5, 2.3 Hz, 1H), 5.96 (dd, *J* = 8.5, 2.3 Hz, 1H), 4.73 – 4.64 (m, 2H), 4.06 – 3.98 (m, 3H), 3.29 – 3.21 (m, 6H), 2.47 (s, 6H), 2.25 – 2.18 (m, 1H), 2.18 – 2.12 (m, 1H), 1.53 – 1.46 (m, 6H), 1.18-1.08 (m, 6H), 0.87 – 0.80 (m, 1H), 0.75 – 0.62 (m, 2H), 0.61 – 0.52 (m, 2H), 0.51 – 0.43 (m, 2H), 0.16 – 0.06 (m, 1H) (One NH was not seen); ^{13}C NMR (mixture, 151 MHz, $CDCl_3$) δ 167.97, 167.84, 144.36, 144.15, 143.06, 143.02, 136.91, 136.65, 134.30, 134.18, 130.54, 130.50, 126.69, 126.63 (2 × C), 126.60, 124.76 (2 × C), 120.94, 120.59, 115.02, 115.00, 103.81, 103.20, 100.15, 99.65, 58.34, 58.30, 50.40, 50.24, 28.84, 28.81, 27.75, 27.63, 23.28, 23.13, 19.01, 18.98, 11.90, 11.83, 9.53, 8.98, 6.41 (2 × C); HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{22}H_{28}N_3O^+$) 350.2227, found 350.2229; retention time 2.87 min, > 99% pure.

4-cyclopropyl-1,3-dimethyl-6-((1-(p-tolyl)ethyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (**43**). White soild, 55% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.32 – 7.24 (m, 4H), 7.18 – 7.11 (m, 4H), 6.69 – 6.62 (m, 2H), 6.41 (s, 1H), 6.28 (s, 1H), 6.10 (d, *J* = 8.6 Hz, 1H), 6.02 (d, *J* = 8.4 Hz, 1H), 4.50 – 4.41 (m, 2H), 4.07 – 3.98 (m, 3H), 3.28 – 3.22 (m, 6H), 2.33 (s, 6H), 2.27 – 2.18 (m, 2H), 1.56 – 1.48 (m, 6H), 1.18 – 1.10 (m, 6H), 0.92 – 0.86 (m, 1H), 0.77 – 0.70 (m, 1H), 0.69 – 0.61 (m, 1H), 0.60 – 0.47 (m, 4H), 0.24 – 0.16 (m, 1H) (One NH was not seen); ^{13}C NMR (mixture, 151 MHz, $CDCl_3$) δ 167.99, 167.91, 144.38, 144.24, 142.53, 142.50, 136.87, 136.68, 136.39, 136.32, 129.31

(2 × C), 129.29 (2 × C), 125.79 (2 × C), 125.74 (2 × C), 120.97, 120.68, 114.99, 114.94, 103.91, 103.35, 100.39, 100.18, 58.35, 58.32, 53.83, 53.70, 28.85, 28.83, 27.77, 27.66, 25.33, 25.04, 21.09, 21.06, 11.89 (2 × C), 9.50, 9.10, 6.46, 6.42; LCMS *m/z* (ESI, positive) found $[M + H]^+$ 350.00; retention time 2.87 min, > 99% pure.

4-cyclopropyl-6-((1-(4-fluorophenyl)ethyl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**44**). White soild, 55% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.40 – 7.30 (m, 4H), 7.04 – 6.95 (m, 4H), 6.69 – 6.60 (m, 2H), 6.36 (d, *J* = 1.8 Hz, 1H), 6.23 (d, *J* = 1.8 Hz, 1H), 6.07 (d, *J* = 8.5 Hz, 1H), 6.00 – 5.94 (m, 1H), 4.52 – 4.38 (m, 2H), 4.15 – 3.94 (m, 4H), 3.29 – 3.20 (m, 6H), 2.25 – 2.13 (m, 2H), 1.53 – 1.46 (m, 6H), 1.16 – 1.08 (m, 6H), 0.92 – 0.80 (m, 2H), 0.74 – 0.67 (m, 1H), 0.67 – 0.61 (m, 1H), 0.59 – 0.45 (m, 4H); ^{13}C NMR (mixture, 126 MHz, $CDCl_3$) δ 167.97, 167.88, 162.70, 160.76, 144.15, 143.97, 141.30 (d, *J* = 2.52 Hz), 141.26 (d, *J* = 2.52 Hz), 136.91, 136.70, 127.38, 127.33, 127.32, 127.27, 121.14, 120.85, 115.48 (2 × C), 115.31 (2 × C), 114.97 (2 × C), 104.01, 103.47, 100.39, 100.10, 58.33, 58.30, 53.59, 53.38, 28.84, 28.82, 27.76, 27.65, 25.41, 25.20, 11.91, 11.87, 9.48, 9.09, 6.44 (2 × C); HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{21}H_{25}FN_3O^+$) 354.1976, found 354.1976; retention time 2.78 min, > 49% pure; retention time 2.83 min, > 50% pure.

6-((1-(4-chlorophenyl)ethyl)amino)-4-cyclopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**45**). White powder, 51% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.37 – 7.26 (m, 8H), 6.68 – 6.61 (m, 2H), 6.35 (d, *J* = 2.2 Hz, 1H), 6.21 (d, *J* = 2.2 Hz, 1H), 6.05 (dd, *J* = 8.5, 2.3 Hz, 1H), 5.94 (dd, *J* = 8.5, 2.3 Hz, 1H), 4.48 – 4.39 (m, 2H), 4.06 – 3.92 (m, 3H), 3.29 – 3.20 (m, 6H), 2.26 – 2.14 (m, 2H), 1.54 – 1.47 (m, 6H), 1.15 – 1.08 (m, 6H), 0.90 – 0.82 (m, 1H), 0.77 – 0.62 (m, 2H), 0.61 – 0.44 (m, 4H), 0.22 – 0.14 (m, 1H) (One NH was not seen); ^{13}C NMR (mixture, 151 MHz, $CDCl_3$) δ 168.08, 168.00, 144.27, 144.22, 144.07, 143.89, 137.05, 136.85, 132.52, 132.49, 128.89 (2 × C), 128.87 (2 × C), 127.37 (2 × C), 127.34 (2 × C), 121.39, 121.11, 115.07 (2 × C), 104.01, 103.47, 100.46, 100.21, 58.43, 58.39, 53.79, 53.60, 28.96, 28.94, 27.86, 27.76, 25.39, 25.23, 12.07, 12.04,

9.57, 9.18, 6.58 (2 × C); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 369.98; retention time 2.97min, > 49% pure; retention time 3.04 min, > 50% pure.

4-cyclopropyl-6-((1-(4-methoxyphenyl)ethyl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**46**). White powder, 59% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.34 – 7.27 (m, 4H), 6.91 – 6.83 (m, 4H), 6.69 – 6.62 (m, 2H), 6.38 (s, 1H), 6.27 (s, 1H), 6.08 (d, *J* = 8.6 Hz, 1H), 6.01 (d, *J* = 8.6 Hz, 1H), 4.48 – 4.39 (m, 2H), 4.11 – 3.89 (m, 3H), 3.81 – 3.76 (m, 6H), 3.26 – 3.22 (m, 6H), 2.28 – 2.15 (m, 2H), 1.54 – 1.47 (m, 6H), 1.15 – 1.09 (m, 6H), 0.90 – 0.84 (m, 1H), 0.76 – 0.68 (m, 1H), 0.68 – 0.61 (m, 1H), 0.60 – 0.46 (m, 4H), 0.25 – 0.17 (m, 1H) (One NH was not seen); ^{13}C NMR (mixture, 151 MHz, $CDCl_3$) δ 168.09, 168.02, 158.58 (2 × C), 144.43, 144.29, 137.66, 137.63, 136.99, 136.80, 127.01 (2 × C), 126.94 (2 × C), 121.10, 120.83, 115.07, 115.03, 114.10 (2 × C), 114.08 (2 × C), 103.97, 103.48, 100.45, 100.26, 58.43, 58.40, 55.40, 55.36, 53.62, 53.46, 28.95, 28.93, 27.85, 27.76, 25.43, 25.14, 12.00 (2 × C), 9.58, 9.25, 6.55, 6.53; LCMS *m/z* (ESI, positive) found $[M + H]^+$ 365.92; retention time 2.70 min, > 99% pure

4-cyclopropyl-6-((1-(2,4-dimethylphenyl)ethyl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**47**). White soild, 57% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:0.4 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.38 (d, *J* = 8.3 Hz, 0.4H), 7.34 (d, *J* = 7.7 Hz, 1H), 7.05 – 6.98 (m, 2.8H), 6.71 – 6.66 (m, 1.4H), 6.36 (d, *J* = 2.4 Hz, 0.4H), 6.22 (d, *J* = 2.4 Hz, 1H), 6.08 (dd, *J* = 8.5, 2.4 Hz, 1H), 5.98 (dd, *J* = 8.5, 2.4 Hz, 0.4H), 4.74 – 4.63 (m, 1.4H), 4.11 – 3.98 (m, 2.4H), 3.29 – 3.25 (m, 4.2H), 2.45 (s, 4.2H), 2.35 – 2.30 (m, 4.2H), 2.29 – 2.20 (m, 1.4H), 1.54 – 1.48 (m, 4.2H), 1.20 – 1.12 (m, 4.2H), 0.94 – 0.87 (m, 1.4H), 0.79 – 0.72 (m, 0.4H), 0.72 – 0.65 (m, 1H), 0.63 – 0.47 (m, 2.8H) (the minor one NH was not seen); ^{13}C NMR (mixture, 126 MHz, $CDCl_3$) δ 168.04, 167.93, 144.45, 144.27, 140.08, 140.03, 136.97, 136.73, 136.13, 136.08, 134.27, 134.13, 131.37 (2 × C), 127.37, 127.33, 124.79 (2 × C), 120.99, 120.67, 115.08, 115.04, 103.74, 103.09, 100.23, 99.79, 58.40, 58.37, 50.17, 50.05, 28.87 (2 × C), 27.81, 27.71, 23.40, 23.22, 20.99, 20.97, 18.99, 18.96, 11.95, 11.91, 9.58, 9.06, 6.48 (2 × C); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 363.97; retention time 2.96 min, > 98% pure.

4-cyclopropyl-1,3-dimethyl-6-(((S)-1-(p-tolyl)ethyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (**48**). White soild, 56% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.32 – 7.24 (m, 4H), 7.17 – 7.11 (m, 4H), 6.69 – 6.62 (m, 2H), 6.40 (d, *J* = 2.4 Hz, 1H), 6.27 (d, *J* = 2.4 Hz, 1H), 6.09 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.01 (dd, *J* = 8.5, 2.4 Hz, 1H), 4.50 – 4.41 (m, 2H), 4.06 – 3.97 (m, 2H), 3.25 (s, 3H), 3.24 (s, 3H), 2.36 – 2.31 (s, 6H), 2.25 – 2.18 (m, 2H), 1.55 – 1.49 (m, 6H), 1.17 – 1.10 (m, 6H), 0.91 – 0.85 (m, 1H), 0.76 – 0.69 (m, 1H), 0.68 – 0.62 (m, 1H), 0.59 – 0.47 (m, 4H), 0.24 – 0.16 (m, 1H) (Two NH was not seen); ^{13}C NMR (mixture, 126 MHz, $CDCl_3$) δ 168.10, 168.02, 144.44, 144.30, 142.59, 142.57, 136.99, 136.80, 136.52, 136.45, 129.39 (4 × C), 125.88 (2 × C), 125.82 (2 × C), 121.12, 120.84, 115.07, 115.03, 103.97, 103.44, 100.49, 100.28, 58.44, 58.41, 53.93, 53.81, 28.94, 28.93, 27.86, 27.75, 25.40, 25.10, 21.17, 21.14, 11.99 (2 × C), 9.58, 9.18, 6.55, 6.51; HRMS (ESI) m/z $[M + H]^+$ calcd for ($C_{22}H_{28}N_3O^+$) 350.2227, found 350.2227; retention time 2.87 min, > 99% pure.

4-cyclopropyl-6-(((S)-1-(4-fluorophenyl)ethyl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**49**). White powder, 57% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.41 – 7.30 (m, 4H), 7.05 – 6.94 (m, 4H), 6.69 – 6.60 (m, 2H), 6.36 (d, *J* = 2.5 Hz, 1H), 6.23 (d, *J* = 2.5 Hz, 1H), 6.07 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.98 (dd, *J* = 8.5, 2.5 Hz, 1H), 4.51 – 4.40 (m, 2H), 4.05 – 3.96 (m, 2H), 3.24 (s, 3H), 3.23 (s, 3H), 2.25 – 2.20 (m, 1H), 2.19 – 2.14 (m, 1H), 1.53 – 1.47 (m, 6H), 1.16 – 1.07 (m, 6H), 0.91 – 0.80 (m, 1H), 0.75 – 0.67 (m, 1H), 0.67 – 0.61 (m, 1H), 0.58 – 0.52 (m, 2H), 0.51 – 0.44 (m, 2H), 0.21 – 0.14 (m, 1H) (Two NH was not seen); ^{13}C NMR (mixture, 126 MHz, $CDCl_3$) δ 167.95, 167.86, 162.66, 160.72, 144.14, 143.96, 141.29 (d, *J* = 2.52 Hz), 141.25 (d, *J* = 3.78 Hz), 136.87, 136.67, 127.36, 127.31, 127.30, 127.25, 121.08, 120.79, 115.44 (2 × C), 115.27 (2 × C), 114.95 (2 × C), 103.99, 103.45, 100.36, 100.08, 58.30, 58.27, 53.55, 53.34, 28.81, 28.79, 27.73, 27.63, 25.38, 25.17, 11.87, 11.84, 9.45, 9.07, 6.41 (2 × C); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 354.05; retention time 2.76 min, > 46% pure; retention time 2.81 min, > 53% pure.

4-cyclopropyl-6-(((S)-1-(4-methoxyphenyl)ethyl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**50**). White soild, 53% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.36 – 7.27 (m, 4H), 6.91 – 6.82 (m, 4H), 6.69 – 6.61 (m, 2H), 6.39 (d, *J* = 2.4 Hz, 1H), 6.28 (d, *J* = 2.4 Hz, 1H), 6.09 (dd, *J* = 8.5, 2.4

Hz, 1H), 6.01 (dd, *J* = 8.5, 2.4 Hz, 1H), 4.48 – 4.38 (m, 2H), 4.13 – 3.95 (m, 4H), 3.78 (s, 3H), 3.77 (s, 3H), 3.24 (s, 3H), 3.24 (s, 3H), 2.27 – 2.16 (m, 2H), 1.53 – 1.47 (m, 6H), 1.17 – 1.08 (m, 6H), 0.90 – 0.84 (m, 1H), 0.75 – 0.68 (m, 1H), 0.67 – 0.60 (m, 1H), 0.59 – 0.45 (m, 4H), 0.24 – 0.17 (m, 1H); ^{13}C NMR (mixture, 151 MHz, $CDCl_3$) δ 168.02, 167.95, 158.54 (2 × C), 144.41, 144.26, 137.64, 137.61, 136.92, 136.74, 126.96 (2 × C), 126.89 (2 × C), 121.04, 120.77, 115.01, 114.97, 114.06 (2 × C), 114.03 (2 × C), 103.97, 103.48, 100.42, 100.23, 58.39, 58.36, 55.33, 55.29, 53.56, 53.40, 28.89, 28.87, 27.81, 27.72, 25.36, 25.07, 11.93 (2 × C), 9.53, 9.21, 6.48 (2 × C); HRMS (ESI) m/z $[M + H]^+$, calcd for ($C_{22}H_{28}N_3O_2^+$) 366.2176, found 366.2177; retention time 2.70 min, > 99% pure.

4-cyclopropyl-6-(((S)-1-(2,4-dimethylphenyl)ethyl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**51**). White soild, 58% yield. ^{1}H NMR (a mixture was observed at RT and the ratio was 1:1 by proton 400 MHz NMR integration at $CDCl_3$ solvent) δ 7.35 (d, *J* = 8.3 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 7.02 – 6.95 (m, 4H), 6.69 – 6.62 (m, 2H), 6.32 (d, *J* = 2.1 Hz, 1H), 6.18 (d, *J* = 2.1 Hz, 1H), 6.04 (dd, *J* = 8.5, 2.0 Hz, 1H), 5.93 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.68 – 4.60 (m, 2H), 4.07 – 3.88 (m, 4H), 3.28 – 3.22 (m, 6H), 2.41 (s, 6H), 2.32 – 2.27 (m, 6H), 2.25 – 2.17 (m, 2H), 1.51 – 1.44 (m, 6H), 1.16 – 1.09 (m, 6H), 0.89 – 0.82 (m, 1H), 0.77 – 0.69 (m, 1H), 0.68 – 0.62 (m, 1H), 0.61 – 0.54 (m, 2H), 0.53 – 0.44 (m, 2H), 0.20 – 0.12 (m, 1H); ^{13}C NMR (mixture, 126 MHz, $CDCl_3$) δ 168.10, 167.99, 144.48, 144.30, 140.12, 140.07, 137.05, 136.81, 136.23, 136.17, 134.36, 134.21, 131.44 (2 × C), 127.44, 127.40, 124.85 (2 × C), 121.11, 120.79, 115.13, 115.10, 103.76, 103.10, 100.30, 99.85, 58.47, 58.44, 50.23, 50.13, 28.95, 28.93, 27.87, 27.77, 23.48, 23.28, 21.06, 21.03, 19.05, 19.02, 12.03, 12.00, 9.64, 9.12, 6.54 (2 × C); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 363.95; retention time 2.96 min, > 99% pure.

Compounds **52-55** were obtained by chiral separation.

(R)-4-cyclopropyl-1,3-dimethyl-6-(((S)-1-(p-tolyl)ethyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (**52**). White soild. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 7.9 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 1H), 6.28 (d, *J* = 2.4 Hz, 1H), 6.09 (dd, *J* = 8.5, 2.4 Hz, 1H), 4.46 (q, *J* = 6.6 Hz, 1H), 4.01 (q, *J* = 6.8 Hz, 1H), 3.25 (s, 3H), 2.33 (s, 3H), 2.23 (ddd, *J* = 9.9, 6.5, 3.7 Hz, 1H), 1.52 (d, *J* = 6.7 Hz, 3H), 1.13 (d, *J* = 6.8 Hz, 3H), 0.69 – 0.61 (m, 1H), 0.58 – 0.45 (m, 2H), 0.23 – 0.16 (m, 1H) (One NH was not seen); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.97, 143.28,

141.55, 135.77, 135.42, 128.36 (2 × C), 124.80 (2 × C), 119.81, 113.99, 102.96, 99.26, 57.39, 52.78, 27.89, 26.73, 24.38, 20.12, 10.98, 8.16, 5.49; HRMS (ESI) m/z $[M + H]^+$, calcd for ($C_{22}H_{28}N_3O^+$) 350.2227, found 350.2235; retention time 2.85 min, > 99% pure.

(S)-4-cyclopropyl-1,3-dimethyl-6-(((S)-1-(p-tolyl)ethyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (**53**). White soild. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 7.9 Hz, 2H), 7.15 (d, *J* = 7.8 Hz, 2H), 6.64 (d, *J* = 8.5 Hz, 1H), 6.40 (d, *J* = 2.2 Hz, 1H), 6.01 (dd, *J* = 8.5, 2.2 Hz, 1H), 4.45 (q, *J* = 6.6 Hz, 1H), 4.03 (q, *J* = 6.8 Hz, 1H), 3.24 (s, 3H), 2.33 (s, 3H), 2.26 – 2.15 (m, 1H), 1.52 (d, *J* = 6.7 Hz, 3H), 1.13 (d, *J* = 6.8 Hz, 3H), 0.93 - 0.84 (m, 1H), 0.77 – 0.68 (m, 1H), 0.64 – 0.50 (m, 2H) (One NH was not seen); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.05, 143.41, 141.57, 135.95, 135.47, 128.37 (2 × C), 124.86 (2 × C), 120.09, 114.03, 102.42, 99.47, 57.42, 52.90, 27.90, 26.83, 24.08, 20.14, 10.96, 8.55, 5.52; LCMS *m/z* (ESI, positive) found $[M + H]^+$ 350.00; retention time 2.82 min, > 99% pure.

(R)-4-cyclopropyl-6-(((S)-1-(2,4-dimethylphenyl)ethyl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**54**). White soild. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, *J* = 7.7 Hz, 1H), 6.99 (m, 2H), 6.68 (d, *J* = 8.5 Hz, 1H), 6.22 (d, *J* = 2.5 Hz, 1H), 6.07 (dd, *J* = 8.5, 2.5 Hz, 1H), 4.67 (q, *J* = 6.6 Hz, 1H), 4.03 (q, *J* = 6.8 Hz, 1H), 3.26 (s, 3H), 2.44 (s, 3H), 2.30 (s, 3H), 2.27 – 2.21 (m, 1H), 1.49 (d, *J* = 6.6 Hz, 3H), 1.16 (d, *J* = 6.8 Hz, 3H), 0.71 – 0.63 (m, 1H), 0.59 – 0.46 (m, 2H), 0.22 – 0.11 (m, 1H) (One NH was not seen); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.81, 144.17, 139.94, 136.61, 135.95, 134.04, 131.27, 127.28, 124.73, 120.56, 114.95, 103.74, 99.73, 58.29, 49.99, 28.77, 27.63, 23.30, 20.89, 18.91, 11.85, 8.98, 6.39; HRMS (ESI) m/z $[M + H]^+$, calcd for ($C_{23}H_{30}N_3O^+$) 364.2383, found 364.2386; retention time 2.88 min, > 99% pure.

(S)-4-cyclopropyl-6-(((S)-1-(2,4-dimethylphenyl)ethyl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**55**). White soild. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.3 Hz, 1H), 7.00 (m, 2H), 6.65 (d, *J* = 8.5 Hz, 1H), 6.34 (d, *J* = 2.5 Hz, 1H), 5.95 (dd, *J* = 8.5, 2.5 Hz, 1H), 4.65 (q, *J* = 6.6 Hz, 1H), 4.03 (q, *J* = 6.8 Hz, 1H), 3.25 (s, 3H), 2.42 (s, 3H), 2.30 (s, 3H), 2.24 – 2.16 (m, 1H), 1.49 (d, *J* = 6.6 Hz, 3H), 1.13 (d, *J* = 6.8 Hz, 3H), 0.89 – 0.82 (m, 1H), 0.77 – 0.69 (m, 1H), 0.63 – 0.51 (m, 2H) (One NH was not seen); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.02, 144.43, 140.06, 136.95, 136.12, 134.26, 131.35, 127.32, 124.79, 120.99, 115.06, 103.08, 100.23, 58.40, 50.16,

28.87, 27.80, 23.21, 20.99, 18.95, 11.91, 9.58, 6.47; LCMS *m/z* (ESI, positive) found $[M + H]^+$ 363.94; retention time 2.96 min, > 99% pure.

Scheme 3 *2-bromo-N-(2,6-dibromopyridin-3-yl)propanamide* (**103**). To a stirred solution of compound **101** (1.0 g, 3.97 mmol) in dichloromethane (20 mL) at 0 °C were slowly added Na_2CO_3 (0.42 g, 3.97 mmol) and 2-bromopropanoyl bromide (0.46 mL, 4.37 mmol). After the addition was completed the cooling bath was removed and the reaction stirred overnight at rt. The reaction was monitored by TLC. Upon completion, the reaction mixture was cooled again to 0°C, diluted with water, and extracted with EtOAc (3 × 50 mL). The combined organic fractions were washed with brine, dried over Na_2SO_4, concentrated by evaporation under reduced pressure. The crude proudct was purified by flash column chromatography (gradient elution, gradient 0 to 20% EtOAc/60–90 °C petroleum ether) to give compound **103** (1.2 g, 3.10 mmol, 78% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.54 (d, *J* = 8.5 Hz, 1H), 7.46 (d, *J* = 8.5 Hz, 1H), 4.59 (q, *J* = 7.1 Hz, 1H), 1.99 (d, *J* = 7.1 Hz, 3H).

2-(cyclopropylamino)-N-(2,6-dibromopyridin-3-yl)propanamide (**105**). A solution of compound **103** (1.0 g, 2.59 mmol) and cyclopropanamine (0.18 mL, 2.59 mmol) and DIPEA (1.13 mL, 6.48 mmol) in 20 mL of acetonitrile is heated to 80 °C overnight. The reaction was monitored by TLC. Upon completion, the reaction cooled to room temperature, the mixture was poured into 50 mL of water, and extracted with dichloromethane (3 × 50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered and excess solvent removed via rotary evaporator. The crude proudct was purified by flash column chromatography (gradient elution, gradient 0 to 10% EtOAc/60–90 °C petroleum ether) to give compound **105** as a white solid (0.8 g, 2.22 mmol, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1H), 8.67 (dd, *J* = 9.3, 8.5 Hz, 1H), 7.39 (t, *J* = 8.8 Hz, 1H), 3.43 (p, *J* = 7.2 Hz, 1H), 2.39 – 2.26 (m, 1H), 1.46 – 1.39 (m, 3H), 0.59 – 0.40 (m, 4H); LCMS *m/z* (ESI, positive) found $[M + H]^+$ 362.02; retention time 2.09 min, > 98% pure.

6-bromo-4-cyclopropyl-3-methyl-3,4-dihydropyrido[2,3-b]pyrazin-2(1H)-one (**107**). A solution of compound **105** (0.8 g, 2.22 mmol) and DIPEA (0.77 mL, 4.44 mmol) in DMF (8 mL) was heated to 150 °C overnight. The reaction was monitored by TLC. Upon completion, the reaction cooled to room temperature, the mixture was poured into 50 mL of water and extracted with

dichloromethane (3 × 50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered and excess solvent removed via rotary evaporator. The crude proudct was purified by flash column chromatography (gradient elution, gradient 0 to 20% EtOAc/60– 90 °C petroleum ether) to give compound **107** as a white solid (0.5 g, 1.77 mmol, 80% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 6.93 (d, *J* = 7.9 Hz, 1H), 6.89 (d, *J* = 7.8 Hz, 1H), 4.00 (q, *J* = 6.9 Hz, 1H), 2.65 – 2.58 (m, 1H), 1.21 (d, *J* = 6.9 Hz, 3H), 0.98 – 0.90 (m, 1H), 0.73 – 0.65 (m, 1H), 0.64 – 0.57 (m, 1H), 0.49 –0.41 (m, 1H).

6-bromo-4-cyclopropyl-1,3-dimethyl-3,4-dihydropyrido[2,3-b]pyrazin-2(1H)-one (**109**). A solution of compound **107** (0.5 g, 1.77 mmol) in anhydrous DMF (4 mL) was added NaH (0.21 g, 5.31 mmol) at 0 °C, the mixture stirred at 0 °C for 0.5 h. Then iodomethane (0.22 mL, 3.54 mmol) was added and stirred at room temperature for another 2 h. The reaction was monitored by TLC. Upon completion, the reaction cooled to room temperature, the reaction mixture was diluted with water, and extracted with EtOAc (3 × 20 mL). The combined organic fractions were washed with brine, dried over Na_2SO_4, concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 25% EtOAc/60–90 °C petroleum ether) gave compound **109** as colorless transparent liquid (0.4 g, 1.35 mmol, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (d, *J* = 8.1 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 4.08 (q, *J* = 6.9 Hz, 1H), 3.17 (s, 3H), 2.62 – 2.52 (m, 1H), 1.16 (d, *J* = 6.9 Hz, 3H), 0.99 – 0.90 (m, 1H), 0.69 – 0.58 (m, 1H), 0.52 – 0.37 (m, 2H); LCMS *m/z* (ESI, positive) found $[M+H]^+$ 296.18; retention time 3.41 min, > 98% pure.

N-(4-cyclopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-6-yl)-4-methylbenz-enesulfonamide (**25**). To a solution of compound **109** (0.2 g, 0.68 mmol), 4-methylbenzenesulfonamide (0.175 g, 1.02 mmol), K_2CO_3 (0.19 g, 1.36 mmol) in 2-metyhtetrahydrofuran (5 mL) at room temperature were added allylpalladium chloride dimer (6 mg, 0.014 mmol) and *t*BuXPhos (6 mg, 0.014 mmol). The mixture was sealed in a microwave tube and heated to 85 °C overnight. The reaction was monitored by TLC. Upon completion, the mixture was diluted with water, and extracted with EtOAc (3 × 10 mL). The combined organic fractions were washed with brine, then dried. The crude proudct was purified by flash column chromatography (gradient elution, gradient 0 to 30% EtOAc/60–90 °C petroleum ether) to give

compound **25** as a white soild (0.15 g, 0.39 mmol, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.3 Hz, 2H), 7.28 (s, 2H), 7.04 (s, 1H), 6.98 (d, *J* = 8.3 Hz, 1H), 6.75 (d, *J* = 8.3 Hz, 1H), 4.14 (q, *J* = 6.8 Hz, 1H), 3.25 (s, 3H), 2.51 – 2.44 (m, 1H), 2.40 (s, 3H), 1.21 (d, *J* = 6.9 Hz, 3H), 0.94 – 0.83 (m, 1H), 0.75 – 0.64 (m, 1H), 0.54 – 0.45 (m, 1H), 0.40 – 0.32 (d, *J* = 4.9 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.18, 146.64, 144.08, 143.78, 136.79, 129.54 (2 × C), 127.28 (2 × C), 122.15, 120.93, 101.87, 57.86, 28.64, 27.21, 21.52, 14.56, 10.00, 5.76; HRMS (ESI) m/z [M + H]$^{+}$ calcd for ($C_{19}H_{23}N_4O_3S^{+}$) 387.1485, found 387.1489; retention time 3.10 min, > 99% pure.

Following the similar procedures as for compound **25** gave compound **26**.

N-(8-cyclopropyl-5,7-dimethyl-6-oxo-5,6,7,8-tetrahydropyrazino[2,3-b]pyrazin-2-yl)-4-methylbe-nzenesulfonamide (**26**). White soild, 57% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.2 Hz, 2H), 7.69 (s, 1H), 7.30 – 7.23 (m, 3H), 4.19 (q, *J* = 6.8 Hz, 1H), 3.34 (s, 3H), 2.53 – 2.46 (m, 1H), 2.39 (s, 3H), 1.31 (d, *J* = 6.9 Hz, 3H), 0.95 – 0.83 (m, 1H), 0.80 – 0.68 (m, 1H), 0.58 – 0.47 (m, 1H), 0.41 – 0.32 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.70, 144.31, 142.46, 140.18, 136.54, 134.59, 129.82 (2 × C), 127.58 (2 × C), 119.48, 58.10, 27.50, 27.03, 21.70, 16.65, 9.87, 5.76; HRMS (ESI) m/z [M + H]$^{+}$ calcd for ($C_{18}H_{22}N_5O_3S^{+}$) 388.1438, found 388.144; retention time 3.10 min, > 97% pure.

Protein expression

The BRD4 (I) protein expression followed the protocol of Filippakopoulos et al. Colonies from freshly transformed plasmid DNA in *E. coli* BL21(DE3)-condon plus-RIL cells, were grown overnight at 37 ºC in 50 mL of Terrific Broth medium with 50 μg/mL kanamycin and 34 μg/mL chloramphenicol (start-up culture). Then start-up culture was diluted 100 fold in 1 L of fresh TB medium and cell was growth at 37 ºC to an optical density of about 0.8 at OD600 before the temperature was decreased to 16 ºC. When the system equilibrated at 16 ºC the optical density was about 1.2 at OD600 and protein expression was induced over night at 16 ºC with 0.2 mmol isopropyl-β-D-thiogalactopyranoside (IPTG). The bacteria were harvested by

centrifugation (4000 × g for 20 min at 4 ºC) and were frozen at -80 ºC as pellets for storage. Cells expressing His6-tagged proteins were re-suspended in lysis buffer (50 mmol HEPES, pH 7.5 at 25 ºC, 500 mmol NaCl, 10 mmol imidazole, 5 % glycerol with freshly added 0.5 mmol TCEP (Tris(2-carboxyethyl)phosphine hydrochloride) and 1 mmol PMSF (phenylmethanesulfonyl fluoride) and lysed with an JN 3000 PLUS high pressure homogenizer (JNBIO - Guangzhou, China) at 4 ºC. The lysate was cleared by centrifugation (12,000 × g for 1 h at 4 ºC) and was applied to a nickel-nitrilotiacetic acid agarose column. The column was washed once with 50 mL of wash buffer containing 30 mmol imidazole. The protein was eluted using a step elution of imidazole in elution buffer (100-250 mmol imidazole in 50 mmol HEPES, pH 7.5 at 25 ºC, 500 mmol NaCl, 5% glycerol). All fractions were collected and monitored by SDS-polyacrylamide gel electrophoresis (Bio-Rad Criterion™ Precast Gels, 4-12% Bis-Tris, 1.0 mm, from Bio-Rad, CA.). After the addition of 1 mmol dithiothreitol (DTT), the eluted protein was treated overnight at 4 ºC with Tobacco Etch Virus (TEV) protease to remove the His6 tag. The protein was concentrated and further purified with size exclusion chromatography on a Superdex 75 16/60 HiLoad gel filtration column. Samples were monitored by SDS-polyacrylamide gel electrophoresis and concentrated to 8-10 mg/mL in the gel filtration buffer, 10 mmol Hepes pH 7.5, 500 mmol NaCl, 1 mmol DTT and were used for protein binding assay and crystallization.

Other four bromodomain proteins (BRD2 (aa 67-200), EP300 (aa 1040-1161), BRG1 (aa 1448-1569), ATAD2 (aa 981-1108)) were prepared as BRD4 (I), with same protocol for expression and protein purification. These bromodomain proteins were used in thermal shift assay for ligand selectivity test.

Crystallization and Data collection

Aliquots of the purified proteins were set up for crystallization using the vapour diffusion method. BRD4 (I) Crystals were grown by mixing 1 μL of the protein (9 mg/mL) with an equal volume of reservoir solution containing 6 M sodium formate and 10 % glycerol. Its complex with 41 fragments was grown at 4 °C in 1 μL protein (10 mg/mL + 5 mmol fragment) with an equal volume of reservoir solution containing 6 M sodium formate and 10 % glycerol. Crystals grew to diffracting quality within 1−3 weeks in all cases.

Data were collected at 100 K on beamLine BL17U (at wavelength 0.9793 Å) at the Shanghai Synchrotron Radiation Facility (SSRF) (Shanghai, China) for the co-crystallized structures. The data were processed with the HKL2000,[30] software packages, and the structures were then solved by molecular replacement, using the CCP4 program MOLREP.[31] The search model used for the crystals was the BRD4-JQ1 complex structure (PDB code 3mXF). The structures were refined using the CCP4 program REFMAC5 combined with the simulated-annealing protocol implemented in the program PHENIX.[32] With the aid of the program Coot,[33] compound, water molecules, and others were fitted into to the initial F_o-F_c maps.

Fluorescence anisotropy binding assay

The binding of compounds to BRD4 was assessed using a Fluorescence Anisotropy Binding Assay. The fluorescent ligand was prepared by attaching a fluorescent fragment (Fluorescein isothiocyanate isomer I, 5-FITC) to the (+)-JQ1.[20],[27] Generally the method involves incubating the Bromodomain protein BRD4, fluorescent ligand and a variable concentration of test compound together to reach thermodynamic equilibrium under conditions such that in the absence of test compound the fluorescent ligand is significantly (> 50%) bound and in the presence

of a sufficient concentration of a potent inhibitor the anisotropy of the unbound fluorescent ligand is measurably different from the bound value.

Detailedly, all components were dissolved in buffer of composition 50 mmol HEPES pH 7.4, 150 mmol NaCl and 0.5 mmol CHAPS with final concentrations of BRD4 (I) 40 nM, fluorescent ligand 5 nM. This reaction mixture was added various concentrations of test compound or DMSO vehicle (5‰ final) in Corning 384 well Black low volume plate (CLS3575) and equilibrated in dark for 4 hours at room temperature. Fluorescence anisotropy was read on BioTek Synergy2 Multi-Mode Microplate Reader (ex= 485 nm, EM = 530 nm; Dichroic -505 nM).

Bromodomain Selectivity Assay

T7 phage strains displaying bromodomains were grown in parallel in 24-well blocks in an E. coli host derived from the BL21 strain. E. coli were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (5,000 x g) and filtered (0.2µm) to remove cell debris. Streptavidin-coated magnetic beads were treated with biotinylated small molecule or acetylated peptide ligands for 30 minutes at room temperature to generate affinity resins for bromodomain assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining bromodomains, liganded affinity beads, and test compounds in 1x binding buffer (16 % SeaBlock, 0.32x PBS, 0.02%BSA, 0.04 % Tween 20, 0.004% Sodium azide, 7.9 mM DTT). Test compounds were prepared as 1000X stocks in 100% DMSO and subsequently diluted 1:25 in monoethylene glycol (MEG). The compounds were then diluted directly into the assays such that the final

concentrations of DMSO and MEG were 0.1% and 2.4%, respectively. All reactions were performed in polypropylene 384-well plates in a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 2 µM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The bromodomain concentration in the eluates was measured by qPCR.

Percent Control (%Ctrl): The compound(s) were screened at the concentration(s) requested, and results for primary screen binding interactions are reported as '% Ctrl', where lower numbers indicate stronger hits.

%Ctrl Calculation: (test compound signal - positive control signal)/ (negative control signal - positive control signal) × 100%

PLK1 Kinase Assay: Z´-LYTE™ Kinase Assay

1. Kinase Reaction

□ Assay buffer: 50 mM Hepes pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 0.01% Brij-35

□ 10 nM PLK1

□ 2 mM Ser/Thr 16 Peptide, 13 mM ATP

□ 60 minutes @ 23 □

2. Development Reaction

□ 1 : 16 dilution of Development Reagent B with Development buffer

□ 60 minutes @ 23 □

3. Detection Equipment

□ Envision (PerkinElmer # 2104)

Cell Viability Assays

MM.1S cells were purchased from the American Type Culture Collection and TY-82 cells were purchased from the JCRB Cell Bank. Cells were cultured in RPMI-1640 medium supplemented with 10% FBS (Life Technologies) at 37 °C in a humidified atmosphere containing 5% CO_2. MM.1S cells were seeded onto 96-well plates at a

density of 10,000 cells/well (8000 for TY-82 cells) in a volume of 100 μL medium. After incubation overnight, compounds dissolved in DMSO stock solutions were thawed at room temperature and diluted to the desired concentrations with normal saline. The compounds were added to the assay plate and after 72 h of incubation, 10 μL CCK-8 solution (Dojindo Laboratories) was added. After another 4 h incubation at 37°C, the signal from the viable cells was measured on spectra-MAX190 (Molecular Devices) at the absorbance of 450 nm.

Expression Analysis

TY-82 cells were treated with compounds for 24 h. RNA extraction was done with TRIzol Reagent (Invitrogen). cDNA was reverse-transcribed (Takara) and subsequently underwent quantitative real time PCR with SYBR Green (Takara) on an Applied Biosystems 7500 fast Real-Time PCR system. The following primers (synthesized by Sanggon Corporation) were used: β-actin primer, 5′-TCTACAATGAGCTGCGTGTG-3′ (forward), 5′-GGTGAGGATCTTCATGAGGT-3′ (backward); c-myc primer, 5′-GCCCAGTGAGGATATCTGGA-3′ (forward), 5′-ATCGCAGATGAAGCTCTGGT-3′ (backward). Analysis was performed on triplicate PCR data for each biological duplicate (normalized to β-actin).

Western Blotting

TY-82 cells were seeded onto 6-well plates and incubated overnight. The cells were treated with compounds at the indicated concentration for 24 h. Then, the cells were lysed in 1 × SDS lysis buffer [50 mmol/L Tris-HCl (pH 6.8), 100 mmol/L DTT, 2% SDS, 0.1% bromphenol blue, and 10% glycerol] and then boiled for 10 min. Western blotting analyses were conducted as previously described[34] using c-Myc (Cell Signaling Technology) and GAPDH (Beyotime z Biotechnology) antibodies, and the

levels of cellular proteins were visualized with peroxidase-coupled secondary antibodies (Jackson ImmunoResearch) using an ECL Kit from Millipore company.

In Vivo Study

All the animal experiments were performed according to the institutional ethical guide-lines of animal care.

In Vivo PK Study

Compound **54** (10 mg/kg) dissolved in Dimethylacetamide: 0.5% HPMC (Hydroxypropyl methylcellulose) (5 : 95, v/v) to a concentration of 1 mg/mL, and was given to ICR mice (Male, 18 – 22 g, n = 3) by gavage administration. Blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, and 24 h after administration (anticoagulant: EDTA-Na2). 100 μL of solvent of methanol: acetonitrile (1:1, v/v) with internal standard was added to 10 μL of plasma and vortexed thoroughly. It was centrifuged for 5 min, then 20 μL of the supernatant was mixed with 20 μL of water for analysis. Samples were analyzed by Xevo TQ-S triple quadrupole mass spectrometer (Waters, USA). The ACQUITY UPLC BEH C18 (1.7 μm, 2.0 mm × 50 mm, Waters, USA) was used for the analysis. Gradient elution was applied consisting of 5 mM ammonium acetate aqueous solution containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. After analyzing the concentrations of compound **54**, the value of AUClast, AUCINF_obs and MRTINF_obs was calculated from time - concentration curves in each animal using Phoenix WinNonlin (CERTARA, USA). Cmax was determined as the maximum plasma concentration, and Tmax was the time to reach the maximum concentration.

In Vivo pharmacological Study

Six week old female Balb/c nude mice were obtained from BEIJING HFK BIOSCIENCE CO., LTD (Beijing, China), and were acclimated one week prior to tumor cell inoculation. A total of 8×10^6 human multiple myeloma cells MM.1S were injected subcutaneously. Twenty seven days after inoculation, mice with established xenografts were randomized into different groups for treatments of daily OTX-015 at 50 mg/kg PO, vehicle and compound **54** at 50 mg/kg IP, respectively (n = 6 per group). The maximum width (X) and length (Y) of the subcutaneous xenograft were measured with a caliper twice a week and the volume (V) was calculated using the formula: $V = (X^2Y)/2$. Then, relative tumor volume (RTV) was calculated as follows: $RTV = V_t/V_0$, where V_0 was the tumor volume at the beginning of the treatments, and V_t was the tumor volume at the end of treatments. The animal body weight was also measured at the same time.

Acknowledgements

We are grateful for financial supports from the National Natural Science Foundation of China (Grant No. 81330076), the National Science & Technology Major Project "Key New Drug Creation and Manufacturing Program" of China (Grant No. 2014ZX09507-002) and the "Personalized Medicines——Molecular Signature-based Drug Discovery and Development", Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA12020318).

Supporting Information Available: The statistics of crystal structures, the minimized conformation of 53 and the spectra data and figures of potent inhibitors were provided. This material is available free of charge via the Internet.

Authors will release the atomic coordinates and experimental data upon article publication.

Reference

[1] T. Kouzarides, Chromatin modifications and their function, Cell, 128 (2007) 693-705.

[2] B.D. Strahl, C.D. Allis, The language of covalent histone modifications, Nature, 403 (2000) 41-45.

[3] C.M. Chiang, Brd4 engagement from chromatin targeting to transcriptional regulation: selective contact with acetylated histone H3 and H4, F1000 biology reports, 1 (2009) 98.

[4] C.H. Arrowsmith, C. Bountra, P.V. Fish, K. Lee, M. Schapira, Epigenetic protein families: a new frontier for drug discovery, Nature reviews. Drug discovery, 11 (2012) 384-400.

[5] C. Dhalluin, J.E. Carlson, L. Zeng, C. He, A.K. Aggarwal, M.M. Zhou, Structure and ligand of a histone acetyltransferase bromodomain, Nature, 399 (1999) 491-496.

[6] P. Filippakopoulos, S. Knapp, Targeting bromodomains: epigenetic readers of lysine acetylation, Nature reviews. Drug discovery, 13 (2014) 337-356.

[7] M. Lange, B. Kaynak, U.B. Forster, M. Tonjes, J.J. Fischer, C. Grimm, J. Schlesinger, S. Just, I. Dunkel, T. Krueger, S. Mebus, H. Lehrach, R. Lurz, J. Gobom, W. Rottbauer, S. Abdelilah-Seyfried, S. Sperling, Regulation of muscle development by DPF3, a novel histone acetylation and methylation reader of the BAF chromatin remodeling complex, Gene Dev, 22 (2008) 2370-2384.

[8] L. Zeng, Q. Zhang, S. Li, A.N. Plotnikov, M.J. Walsh, M.M. Zhou, Mechanism and regulation of acetylated histone binding by the tandem PHD finger of DPF3b, Nature, 466 (2010) 258-262.

[9] D. Su, Q. Hu, Q. Li, J.R. Thompson, G.F. Cui, A. Fazly, B.A. Davies, M.V. Botuyan, Z.G. Zhang, G. Mer, Structural basis for recognition of H3K56-acetylated histone H3-H4 by the chaperone Rtt106, Nature, 483 (2012) 104-107.

[10] P. Filippakopoulos, S. Picaud, M. Mangos, T. Keates, J.P. Lambert, D. Barsyte-Lovejoy, I. Felletar, R. Volkmer, S. Muller, T. Pawson, A.C. Gingras, C.H. Arrowsmith, S. Knapp, Histone recognition and large-scale structural analysis of the human bromodomain family, Cell, 149 (2012) 214-231.

[11] R. Sanchez, J. Meslamani, M.M. Zhou, The bromodomain: From epigenome reader to druggable target, Bba-Gene Regul Mech, 1839 (2014) 676-685.

[12] J.E. Delmore, G.C. Issa, M.E. Lemieux, P.B. Rahl, J.W. Shi, H.M. Jacobs, E. Kastritis, T. Gilpatrick, R.M. Paranal, J. Qi, M. Chesi, A.C. Schinzel, M.R. McKeown, T.P. Heffernan, C.R. Vakoc, P.L. Bergsagel, I.M. Ghobrial, P.G. Richardson, R.A. Young, W.C. Hahn, K.C. Anderson, A.L. Kung, J.E. Bradner, C.S. Mitsiades, BET Bromodomain Inhibition as a Therapeutic Strategy to Target c-Myc, Cell, 146 (2011) 904-917.

[13] A. Wyce, G. Ganji, K.N. Smitheman, C.W. Chung, S. Korenchuk, Y. Bai, O. Barbash, B. Le, P.D. Craggs, M.T. McCabe, K.M. Kennedy-Wilson, L.V. Sanchez, R.L. Gosmini, N. Parr, C.F. McHugh, D. Dhanak, R.K. Prinjha, K.R. Auger, P.J. Tummino, BET inhibition silences expression of MYCN and BCL2 and induces cytotoxicity in neuroblastoma tumor models, PloS one, 8 (2013) e72967.

[14] S.Y. Wu, C.M. Chiang, The double bromodomain-containing chromatin adaptor Brd4 and transcriptional regulation, J Biol Chem, 282 (2007) 13141-13145.

[15] J.A. Mertz, A.R. Conery, B.M. Bryant, P. Sandy, S. Balasubramanian, D.A. Mele, L. Bergeron, R.J. Sims, 3rd, Targeting MYC dependence in cancer by inhibiting BET bromodomains, Proc Natl Acad Sci U S A, 108 (2011) 16669-16674.

[16] S. Fletcher, E.V. Prochownik, Small-molecule inhibitors of the Myc oncoprotein, Biochimica et biophysica acta, 1849 (2015) 525-543.

[17] K.Y. Jung, H.B. Wang, P. Teriete, J.L. Yap, L.J. Chen, M.E. Lanning, A. Hu, L.J. Lambert, T. Holien, A. Sundan, N.D.P. Cosford, E.V. Prochownik, S. Fletcher, Perturbation of the c-Myc-Max Protein-Protein Interaction via Synthetic alpha-Helix Mimetics, Journal of medicinal chemistry, 58 (2015) 3002-3024.

[18] D.S. Hewings, T.P. Rooney, L.E. Jennings, D.A. Hay, C.J. Schofield, P.E. Brennan, S. Knapp, S.J. Conway, Progress in the development and application of small molecule inhibitors of bromodomain-acetyl-lysine interactions, Journal of medicinal chemistry, 55 (2012) 9393-9413.

[19] D.S. Hewings, M. Wang, M. Philpott, O. Fedorov, S. Uttarkar, P. Filippakopoulos, S. Picaud, C. Vuppusetty, B. Marsden, S. Knapp, S.J. Conway, T.D. Heightman, 3,5-dimethylisoxazoles act

as acetyl-lysine-mimetic bromodomain ligands, Journal of medicinal chemistry, 54 (2011) 6761-6770.

[20] P. Filippakopoulos, J. Qi, S. Picaud, Y. Shen, W.B. Smith, O. Fedorov, E.M. Morse, T. Keates, T.T. Hickman, I. Felletar, M. Philpott, S. Munro, M.R. McKeown, Y. Wang, A.L. Christie, N. West, M.J. Cameron, B. Schwartz, T.D. Heightman, N. La Thangue, C.A. French, O. Wiest, A.L. Kung, S. Knapp, J.E. Bradner, Selective inhibition of BET bromodomains, Nature, 468 (2010) 1067-1073.

[21] E. Nicodeme, K.L. Jeffrey, U. Schaefer, S. Beinke, S. Dewell, C.W. Chung, R. Chandwani, I. Marazzi, P. Wilson, H. Coste, J. White, J. Kirilovsky, C.M. Rice, J.M. Lora, R.K. Prinjha, K. Lee, A. Tarakhovsky, Suppression of inflammation by a synthetic histone mimic, Nature, 468 (2010) 1119-1123.

[22] P.V. Fish, P. Filippakopoulos, G. Bish, P.E. Brennan, M.E. Bunnage, A.S. Cook, O. Federov, B.S. Gerstenberger, H. Jones, S. Knapp, B. Marsden, K. Nocka, D.R. Owen, M. Philpott, S. Picaud, M.J. Primiano, M.J. Ralph, N. Sciammetta, J.D. Trzupek, Identification of a chemical probe for bromo and extra C-terminal bromodomain inhibition through optimization of a fragment-derived hit, Journal of medicinal chemistry, 55 (2012) 9831-9837.

[23] T.P. Rooney, P. Filippakopoulos, O. Fedorov, S. Picaud, W.A. Cortopassi, D.A. Hay, S. Martin, A. Tumber, C.M. Rogers, M. Philpott, M. Wang, A.L. Thompson, T.D. Heightman, D.C. Pryde, A. Cook, R.S. Paton, S. Muller, S. Knapp, P.E. Brennan, S.J. Conway, A series of potent CREBBP bromodomain ligands reveals an induced-fit pocket stabilized by a cation-pi interaction, Angewandte Chemie (International ed. in English), 53 (2014) 6126-6130.

[24] K.G. McLure, E.M. Gesner, L. Tsujikawa, O.A. Kharenko, S. Attwell, E. Campeau, S. Wasiak, A. Stein, A. White, E. Fontano, R.K. Suto, N.C. Wong, G.S. Wagner, H.C. Hansen, P.R. Young, RVX-208, an inducer of ApoA-I in humans, is a BET bromodomain antagonist, PloS one, 8 (2013) e83190.

[25] B.N. Devaiah, B.A. Lewis, N. Cherman, M.C. Hewitt, B.K. Albrecht, P.G. Robey, K. Ozato, R.J. Sims, D.S. Singer, BRD4 is an atypical kinase that phosphorylates Serine2 of the RNA Polymerase II carboxy-terminal domain, P Natl Acad Sci USA, 109 (2012) 6927-6932.

[26] S.W. Ember, J.Y. Zhu, S.H. Olesen, M.P. Martin, A. Becker, N. Berndt, G.I. Georg, E. Schonbrunn, Acetyl-lysine binding site of bromodomain-containing protein 4 (BRD4) interacts with diverse kinase inhibitors, ACS chemical biology, 9 (2014) 1160-1171.

[27] L.L. Zhao, D.Y. Cao, T.T. Chen, Y.Q. Wang, Z.H. Miao, Y.C. Xu, W.Y. Chen, X. Wang, Y.D. Li, Z.Y. Du, B. Xiong, J. Li, C.Y. Xu, N.X. Zhang, J.H. He, J.K. Shen, Fragment-Based Drug Discovery of 2-Thiazolidinones as Inhibitors of the Histone Reader BRD4 Bromodomain, Journal of medicinal chemistry, 56 (2013) 3833-3851.

[28] P. Ciceri, S. Muller, A. O'Mahony, O. Fedorov, P. Filippakopoulos, J.P. Hunt, E.A. Lasater, G. Pallares, S. Picaud, C. Wells, S. Martin, L.M. Wodicka, N.P. Shah, D.K. Treiber, S. Knapp, Dual kinase-bromodomain inhibitors for rationally designed polypharmacology, Nat Chem Biol, 10 (2014) 305-312.

[29] L.J. Chen, J.L. Yap, M. Yoshioka, M.E. Lanning, R.N. Fountain, M. Raje, J.A. Scheenstra, J.W. Strovel, S. Fletcher, BRD4 Structure-Activity Relationships of Dual PLK1 Kinase/BRD4 Bromodomain Inhibitor BI-2536, Acs Med Chem Lett, 6 (2015) 764-769.

[30] Z. Otwinowski, W. Minor, Processing of X-ray diffraction data collected in oscillation mode, Method Enzymol, 276 (1997) 307-326.

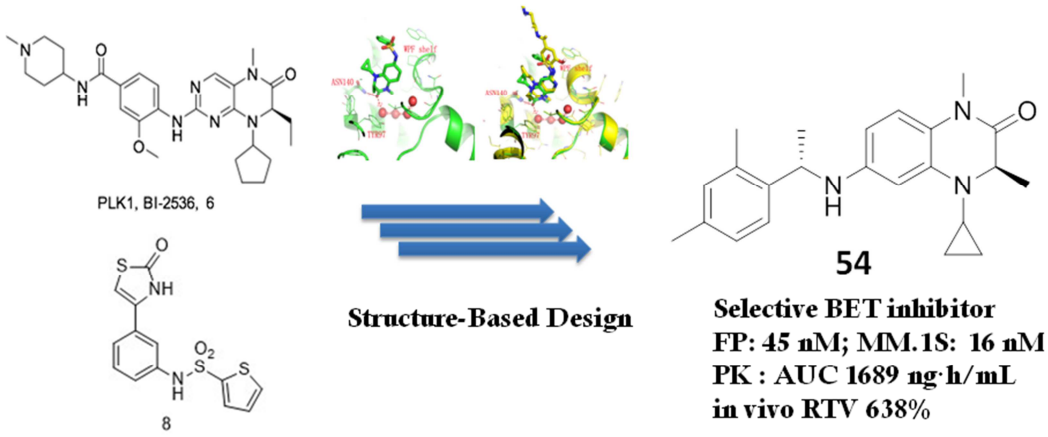
[31] G.N. Murshudov, A.A. Vagin, E.J. Dodson, Refinement of macromolecular structures by the maximum-likelihood method, Acta Crystallogr D, 53 (1997) 240-255.

[32] P.D. Adams, R.W. Grosse-Kunstleve, L.W. Hung, T.R. Ioerger, A.J. McCoy, N.W. Moriarty, R.J. Read, J.C. Sacchettini, N.K. Sauter, T.C. Terwilliger, PHENIX: building new software for automated crystallographic structure determination, Acta Crystallogr D, 58 (2002) 1948-1954.

[33] P. Emsley, B. Lohkamp, W.G. Scott, K. Cowtan, Features and development of Coot, Acta Crystallogr D, 66 (2010) 486-501.

[34] J.X. Li, J.M. Feng, Y. Wang, X.H. Li, X.X. Chen, Y. Su, Y.Y. Shen, Y. Chen, B. Xiong, C.H. Yang, J. Ding, Z.H. Miao, The B-Raf(V600E) inhibitor dabrafenib selectively inhibits RIP3 and alleviates acetaminophen-induced liver injury, Cell Death Dis, 5 (2014).

Graphical Abstract



Highlights:

- Using scaffold modification to design BRD4 inhibitors based on the PLK1-BRD4 dual inhibitor BI-2536
- Structure-based lead optimization and identify potent BRD4 inhibitors
- Selectivity profile on 32 bromodomains showed that this series is selective BET inhibitors
- In vitro study confirmed the compounds have profound effects on down-regulation of c-Myc
- In vivo study confirmed the efficacy on MM.1S CDX model.