Article

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# Rational Design and Evaluation of 6-(Pyrimidin-2-ylamino)-3,4-dihydroquinoxalin-2(1H)-ones as Polypharmacological Inhibitors of BET and Kinases

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## ABSTRACT

Cancer exhibits diverse heterogeneity with a complicated molecular basis that usually harbors genetic and epigenetic abnormality, which poses a big challenge for single-target agents. In the current work, we proposed a hybrid strategy by

incorporating pharmacophores that bind to the acetylated lysine binding pocket of BET proteins with typical kinases hinge binder to generate novel polypharmacological inhibitors of BET and kinases. Through elaborating the core structure of 6-(Pyrimidin-2-ylamino)-3,4-dihydroquinoxalin-2(1H)-one, we demonstrated that this rational design can produce high potent inhibitors of CDK9 and BET proteins.In this series, compound **40** was identified as the potential lead compound with balanced activities of BRD4 ($IC_{50}$=12.7 nM) and CDK9 ($IC_{50}$=22.4 nM), as well as good antiproliferative activities on a small cancer cell panel. Together, current study provided a new method for the discovery of bromodomain and kinases dual inhibitors rather than only discovered by serendipity.

**KEYWORD: Polypharmacology; Bromodomain; Kinases; Antitumor; Dihydroquinoxalin-2(1H)-one**

## INTRODUCTION

Complex diseases, such as cancer, can be attributed to the dysregulation of multiple signal pathways and physiological processes, which are hardly cured by the specific modulation of a single target. Polypharmacological inhibitor as an intriguing therapeutic option is gaining a lot of attention in current anticancer drug development[1, 2]. It has several advantages with respect to single-target drugs and combination therapies[3], and the most important aspects can be summarized as follows: 1) a multitarget drug could avoid the drug-drug interactions issues; 2) it may demonstrate a more predictable pharmacokinetic profile compared with combination therapies; 3)

it guarantees the simultaneous presence in the tissues to reduce the acute and delayed toxicities in comparison with a combination therapy; 4) it usually has synergistic effects to reduce the administration dosage and therefore may avoid the associated toxicity issues of a single-target agent.

Started with imatinib, a Bcr-Abl inhibitor for cancer treatment, developing kinases inhibitors for cancer therapies have stimulated tremendous efforts and resulted in magnificent successes with more than 50 kinases inhibitors into the market. However, application of these targeted kinases drugs are impeded by acquired resistance which usually involves drug-induced mutations and epigenetic reprogramming. Based on the recent advance of epigenetic drug development, especially the bromodomain inhibitors, there are a lot of clinical trials are ongoing and the investigations indicated that these epigenetic drugs can rewire the gene expression and regulation network to execute its anticancer effects. Besides, there are many studies indicated that combining bromodomain and extra-terminal domain (BET) inhibitors with kinases inhibitors can enhance the antitumor effects through simultaneous targeting the abnormal signaling kinases pathways and epigenetics-related cellular reprogramming[4-6]. Moreover, the combined use of BET inhibitor could reduce the probability of acquired resistance of kinases drugs. Giving the distinct benefits of combining BET inhibitors with kinases inhibitors, considerable efforts are dedicated to developing the multi-targeted molecules of BET and kinases[7-9]. However, to the best of our knowledge, all of them are discovered by serendipity (compounds **1-3** of Figure 1),[10, 11] as exemplified by the PLK1 and BET dual inhibitor BI-2536[12], or

optimized from these known inhibitors (**4**).

To overcome this limitation, in this work, we presented a rational strategy by hybridising [2]the acetylated lysine (KAc) mimic of BET inhibitors with the hinge binder motif extracted from typical kinases inhibitors. We demonstrated that this design can provide a new opportunity to elegantly develop bromodomain-kinases multi-targeted agents for antitumor treatment.

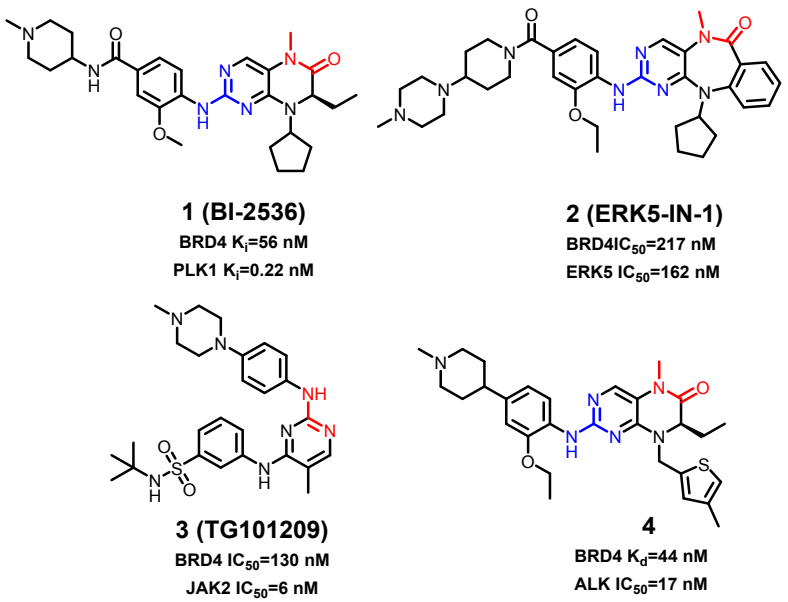



**Figure 1**. The representative dual bromodomain-kinases inhibitors.

## RESULTS AND DISCUSSION

### Inhibitor Design Strategy

In the past two decades, more than 50 kinases inhibitors have been approved by FDA to enter into drug market[13] and most of them are ATP-competitive inhibitors with a conserved hydrogen-bond acceptor motif, usually pyridine or pyrimidine, to interact with the backbone of hinge segment in kinases catalytic domains[14]. Since the ATP binding site of kinases is located at the cleft formed by the N-lobe and the C-lobe, it

also has a large solvent-accessible are that can accommodate diverse and large chemical groups. Based on the binding mode of kinases inhibitors and the biological importance of drug combination of BET and kinases inhibitors, we assumed that hybridize kinases essential hinge binder with the well-characterized acetylated lysine mimic from bromodomain inhibitors may provide new polypharmacological inhibitors with unique biological effects as anticancer drugs. Previously, the dihydropteridinone in BI-2536 was identified as an essential component for developing dual BET-kinase inhibitors. However, this moiety is both a kinase hinger binder and BET protein's pharmacophore, which makes the optimization highly interlocked. Therefore, it limits the target scope and currently only PLK1 and ALK were found to be the targets of this chemotype[11, 12, 15]. We have discovered a series of dihydroquinoxalin-2(1H)-one derivatives as potent BET inhibitors, and found the scaffold dihydroquinoxalin-2(1H)-one is a selective and potent acetylated lysine mimic, which can form several direct and water-bridged hydrogen bonds with BRD4. Also, the 3,5-dimethylisoxazole motif first reported by Hewings et al.,[16, 17] is considered a general pharmacophore for the KAc binding pocket and has been incorporated into many series of BET and non-BET inhibitors. We proposed to use the hybridization strategy ,which will incorporate the acetylated lysine binding moiety with a typical kinases hinge binder to discover novel dual BET-kinases inhibitors. This strategy could enable us to select more biological relevant kinases as BET-kinases ploypharmacological targets. To test the hypothesis of hybrid strategy, we designed four prototype compounds (**5-8** in Table 1) by combining

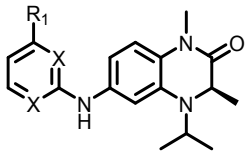
pyrimidin-2-amine group with two acetylated lysine mimic initialized a docking study to check the binding feasibility of them in the first bromodomain of BRD4 (BRD4-BD1). The docking result indicated that the compounds can bind to the acetylated lysine binding pocket of BRD4 and maintain the essential hydrogen-bond interactions between the residue Asn140 and the selected dihydroquinoxalin-2(1H)-one[18-20] or 3,5-dimethylisoxazole motif[21-25] (see the Figure S1 for the calculated binding conformation). Based on this feasibility to maintain the key interactions in the KAc binding site of BRD4-BD1 and the existence of large solvent-accessible subpocket in kinase ATP binding site able to accommodate the BET pharmacophore, we synthesized these four compounds to subject to the biological tests of BRD4 and several kinasess to verify this approach.

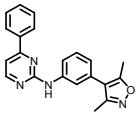
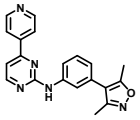
**Structure-Activity Relationship**

As shown in Table 1, two compounds (**5** and **6**) containing 3,5-dimethylisoxazole did not show more than 50% inhibition ratio at the concentration of 1 μM in the BRD4-BD1 fluorescence polarization (FP) assay. However, the compounds (**7** and **8**) with dihydroquinoxalin-2(1H)-one scaffold showed encouraging binding activities towards BRD4, with the $IC_{50}$ values below 200 nM. Based on this initial results, we prepared two derivatives (**9** and **10)** to check the possibility of optimization. Clearly, they demonstrated that the modification at the benzene group could remain a good binding activity to BRD4, especially at the meta-position of the benzene group (**10** with $IC_{50}$=160.5 nM). Previous studies have identified the synergistic effect of BRD4

with kinases such as CDKs[26], PI3Ks[27]and Aurora kinases[28, 29]. Therefore, we tested the inhibition effects of compounds **7-10** on these selected kinases. As listed in Table 1, compounds **7-10** showed differential activities to four selected kinases. In detail, compounds **7-9** have good activities towards CDK9 but with no activity on CDK6, indicating they have the potential to be developed into selective inhibitors. Besides the promising activity on CDK9, compound **8** also showed good activity on PI3Kα and compound **9** showed good activity on Aurora kinase A. For the comparison, we also synthesized the compounds **11-14** by substituting the pyrimidine core with benzene motif, which eliminate the hinge region binding capability and therefore should not inhibit the kinases activity. As expected, compounds **11-14** did not show any activity on these four kinases, further demonstrating that the pyrimidine, a common hinge binder acting as hydrogen-bond acceptor, is very important for the binding to kinases. Contrarily, since the pyrimidine in compounds **7-10** is not essential for binding to BRD4, the substituents (**11-14)** still have good binding activities on BRD4, as indicated by the similar $IC_{50}$ values from BRD4-BD1 FP assay relative to the corresponding compounds **7-10**.

**Table 1. The proof-of-concept of molecular design**[a]



| Compd. | X | $R_1$ | BRD4 BD1 $IC_{50}$(nM) | Inhibition Values(%) CDK9 1μM | CDK9 100nM | CDK6 1μM | CDK6 100nM | PI3Kα 1μM | PI3Kα 100nM | Aurora A 1μM | Aurora A 100nM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **5** | |  | 2.2%@1μM | | | | / | | | | |
| **6** | |  | 37.7%@1μM | | | | | | | | |
| **7** | N |  | 193.5±49.5 | 101.9 | 83.4 | 4.8 | -0.8 | 22.9 | 0.0 | 67.4 | 10.6 |
| **8** | N |  | 83.0±13.0 | 98.0 | 94.5 | 7.7 | 0.2 | 82.4 | 37.5 | 43.7 | 4.0 |
| **9** | N |  | 477.0±61.0 | 103.5 | 67.9 | 8.1 | 2.3 | 0.1 | -2.1 | 86.8 | 37.4 |
| **10** | N |  | 160.5±15.5 | 71.8 | 25.2 | 2.8 | -0.8 | 63.1 | 14.1 | 54.4 | 8.7 |
| **11** | C |  | 357.5±21.5 | 1.3 | -6.1 | 0.9 | 1.8 | 6.1 | -4.8 | -1.5 | -3.4 |
| **12** | C |  | 77.3±2.3 | 0.7 | 2.2 | 0.7 | 2.7 | -1.0 | -4.1 | 14.2 | -6.1 |
| **13** | C |  | 820.5±138.5 | 3.8 | -3.0 | -0.1 | -1.4 | 3.0 | -2.6 | -0.8 | 1.1 |
| **14** | C |  | 666.5±56.5 | -4.1 | 2.0 | -3.3 | 2.3 | 5.2 | -0.1 | -1.8 | -3.0 |

[a]All BRD4-BD1 $IC_{50}$ values and Kinases inhibition values are mean values from at least two independent experiments.

To assist the further optimization, we solved the cocrystal structure of compound **7** bound to the BD1 domain of BRD4. As shown in Figure 2A, the dihydroquinoxalin-2(1H)-one scaffold situated at the same position in the acetylated lysine binding site with respect to our previously reported crystal structure and makes

essential hydrogen-bond interactions with the conserved residue Asn140. The binding conformation of compound **7** in the solved structure adopted an U shape by rotating the 4-phenylpyrimidin-2-amine group to the residue Asp145. Comparison with our previous solved crystal structure, the residue Asp145 slightly receded to eliminate the steric clash with the ligand (Figure 2B).

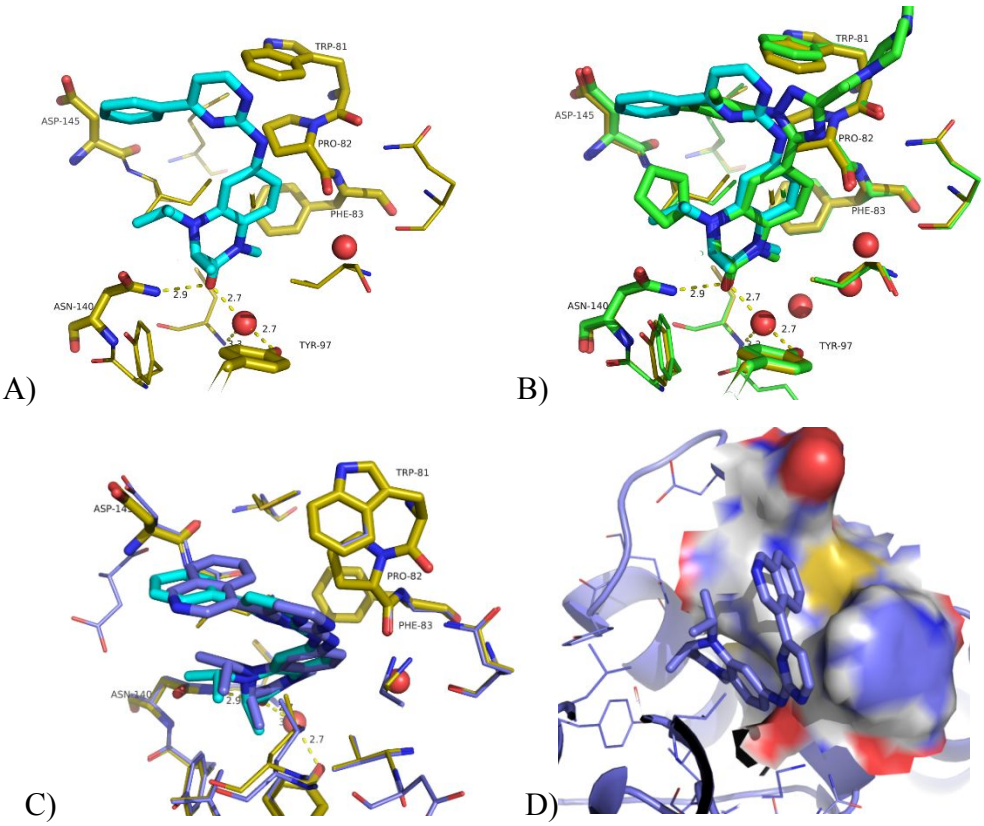



**Figure 2**. The cocrystal structure of compound **7** (cyans) and compound **40** (blue) in complex with BRD4-BD1 (PDB entry number 6LIH and 6LIM, respectively). A) binding mode of compound **7** in the KAc binding pocket. Asn140,Tyr97,Trp81 and Asp145 are represented as stick model and hydrogen bonds are represented by dashed lines. B) Superimposing the structure of compound **7** to our previous crystal structure (PDB entry number : 6JI5) (green). C) Superimposing the structure of compound **7** and compound **40**. D) the interactions between compound **40** and WPF pocket that is

represented as surface diagram.

Based on the cocrystal structure, we found that adding a small group at the 2- or 3-position of the benzene group could extend the ligand into the WPF subpocket, which may enhance the binding affinity with BRD4. Since compounds **7-10** all showed activities on CDK9, as a proof-of-concept project, we decided to simultaneously optimize the compounds as dual inhibitors of BET and CDK9. To probe the binding mode of **7** with CDK9, we docked the compound into the ATP binding site of CDK9 by utilizing the crystal structure 6GZH as the template[30]. From the docking model (Figure 3A), compound **7** adopted similar binding interactions to the ligand A86 ([4-[[4-[5-(cyclopropylmethyl)-1-methyl-pyrazol-4-yl]-5-fluoranyl-pyrimidin-2-yl]amino]cyclohexyl]azanium) in the original crystal structure, forming the essential hydrogen-bond interactions with the hinge residue Cys106 and stretching the benzene group upwards(Figure 3B). The dihydroquinoxalin-2(1H)-one ring extends to the solvent-accessible area with the isopropyl group reverting to the vicinity of the benzene group. According this predicted binding mode, substitutions on benzene group also are feasible to enhance the binding activity on CDK9, which is consistent with the idea proposed for the BRD4 optimization.

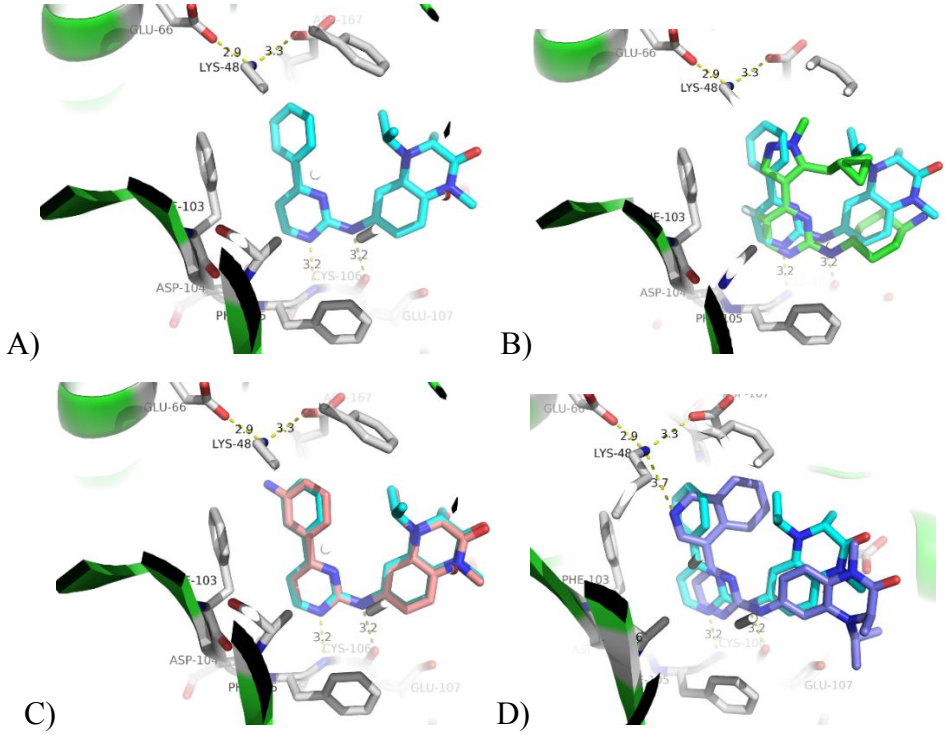


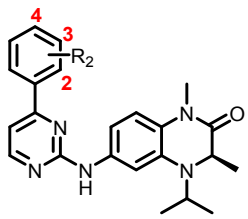

**Figure 3.** The binding conformation of compounds **7**, **21** and **40** in CDK9 ATP binding site from the docking study. The protein was shown in cartoon mode and the residues of binding site were shown in stick mode. A) predicted conformation of compound **7** (cyans) bound to the ATP binding site of CDK9. It can form two hydrogen bonds with hinge residue Cys106. B) superimposition of compound **7** with original ligand A86 (green) in the crystal structure 6GZH. C) predicted conformation of compound **21** (orange) bound to the ATP binding site of CDK9. D) predicted conformation of compound **40** (blue) bound to the ATP binding site of CDK9.

As shown in Table 2, various small functional groups were added to the different position of the benzene group. According to the analysis of the cocrystal structure of compound **7** with BRD4-BD1, the 4-position is at the vicinity of the protein residue Asp145. Therefore, substitutions at the 4-position of the benzene may cause a steric

crash to reduce the binding activities to BRD4, which is consistent with the low activities of compounds **16**, **19** and **22**, especially of compound **19** ($IC_{50}$=5850.0 nM). And compounds with substitutions at 3-position of benzene group also showed decreased activities except compound **21** with a $NH_2$ group that may form electrostatic interaction with the nearby negative-charge residue Asp145. Since the 2-position faces the WPF subpocket, the substitutions at 2-position generally have less detriment effect on the binding with BRD4. Compounds **15-24** were tested in CDK9 enzymatic inhibition assay and all of them except **17** showed moderate to high inhibitory activities. Among them, compound **21** showed very good activity towards CDK9, with an $IC_{50}$ value of 5.9 nM. We docked compound **21** into the ATP binding site of CDK9 (Figure 3C), and found that its binding conformation is very similar to the previous predicted binding mode of compound **7**, only the amino group entering into a hydrophilic subpocket formed by residues Lys48, Glu66 and Asp167, which may create favorable electrostatic interactions.

Considering the promising inhibitory activities of compound **21** on BRD4 and CDK9,we further explore the structure-activity relationship (SAR) around the 3-position of the benzene group in order to obtain potent dual-inhibitors.

**Table 2. The exploration of different substituents on benzene group**[a]



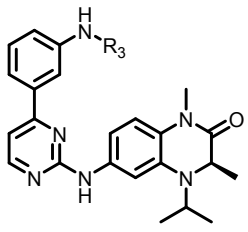
| Compd. | $R_2$ | BRD4 BD1 $IC_{50}$(nM) | CDK9 $IC_{50}$(nM) |
|---|---|---|---|
| **15** | 3-$OCH_3$ | 965.0±135.0 | 126.4 ± 20.1 |
| **16** | 4-$OCH_3$ | 534.5±9.5 | 31.7 ± 0.9 |
| **17** | 2-$CH_3$ | 271.0±12.0 | 1.0%@1μM 1.5%@100nM |
| **18** | 3-$CH_3$ | 759.5±42.5 | 195.8 ± 5.2 |
| **19** | 4-$CH_3$ | 5850.0±150.0 | 488.5± 34.9 |
| **20** | 2-$NH_2$ | 251.5±73.5 | 39.2 ± 2.8 |
| **21** | 3-$NH_2$ | 119.5±30.5 | 5.9±0.5 |
| **22** | 4-$NH_2$ | 480.0±48.0 | 48.9 ±8.7 |
| **23** | 2-$OCH_3$-4-F | 496.5±82.5 | 46.9 ± 6.2 |
| **24** | 2-$CH_3$-4-F | 156.2±34.3 | 578.2 ±97.9 |

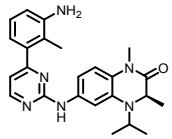
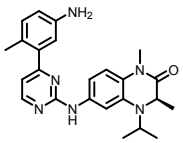
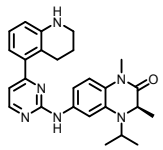
[a]All BRD4-BD1 $IC_{50}$ values and CDK9 $IC_{50}$ values are mean values from at least two independent experiments. The BET selective inhibitor JQ-1 and CDK9 selective inhibitor Dinaciclib were used as the positive control in our enzymatic assay, respectively exhibiting an $IC_{50}$ value of 41.5 nM and 3.0 nM.

On the basis of compound **21**, we synthesized compounds **25-30** by substituted various groups on the 3-$NH_2$ of benzene group. Compound **25** with the added methyl group maintained the binding activity on BRD4 relative to **21**. Compounds with slightly large groups such as ethyl or isopropyl (**26** and **27**) decreased the potency about two folds. the substituents larger than isopropyl (**28-30**) are not tolerable at this position as they showed a dramatic loss in BRD4 binding activities. Since compounds

with 2-methyl group on the benzene (**17** and **24**) also showed good binding activities on BRD4, we prepared compounds **31** and compound **32** to further explore the binding motif around the WPF subpocket. Comparison of compounds **31** and **32,** it was found the methyl at the ortho-position of amino group in compound **31** may extend into WPF pocket and make favorable Van Der Waals (VDW) interactions with the residues lining this subpocket, which slightly increased the binding affinity with BRD4-BD1. Compound **33** with the tetrahydroquinoline motif at this part exhibited high potency to BRD4 ($IC_{50}$=27.0 nM), indicating this added piperidine ring indeed entered into the WPF subpocket. We also investigated the activity of these compounds on CDK9. The result showed that adding the methyl group on the amine ,the potency of compound **25** on CDK9 decreased about 4-fold relative to compound **21**. Substituted with larger groups further reduced the inhibitory activity on CDK9, which generally follows the similar trends in the BRD4 binding assay. Interestingly, although compound **33** coupled a large bicyclic group on the pyrimidine, it still has a good activity to CDK9 ($IC_{50}$=67.0 nM), prompting us that the bicycle motifs could be used to further probe the dual inhibitiors.

**Table 3. The exploration of 3-$NH_2$ on benzene group**[a]



| Compd. | $R_3$ | BRD4 BD1 $IC_{50}$ (nM) | CDK9 $IC_{50}$ (nM) |
|---|---|---|---|
| **25** | Me | 173.4±11.8 | 22.0±1.0 |
| **26** | Et | 319.2±21.2 | 37.8 ± 2.0 |
| **27** | $^{i}$Pr | 268.1±37.1 | 50.9 ± 1.5 |
| **28** |  | 11%@10μM | 121.9± 25.2 |
| **29** |  | 13%@10μM | 168.8± 11.3 |
| **30** | Ph | / | 47.8%@1μM 8.8%@100nM |
| **31** |  | 115.0±14.0 | 182.9 ± 4.4 |
| **32** |  | 236.0±98.0 | 151.2 ± 0.8 |
| **33** |  | 27.0±5.3 | 67.0±3.7 |

[a]All BRD4-BD1 $IC_{50}$ values and CDK9 $IC_{50}$ values are mean values from at least two independent experiments.

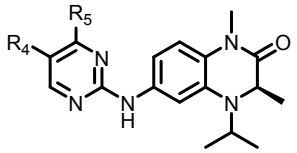
From the above-mentioned SAR study, compounds **34-40** with bicycles coupled on the pyrimidine group were synthesized. From the BRD4 binding assay, it was revealed that compounds containing [6,6]-bicyclic ring system (**37-40**) have better activities than [6,5]-bicycle compounds (**34-36**), especially compounds **38** and **40** with $IC_{50}$ values lower than 20 nM. We checked the CDK9 enzymatic inhibition of

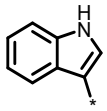
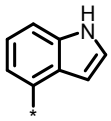
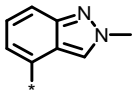
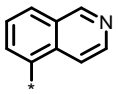
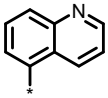
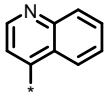
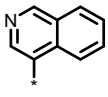
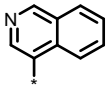
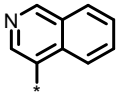
these compounds, and found that compound **35** with NH group at 3-position of benzene group has superior inhibitory activity on CDK9 ($IC_{50}$=13.8 nM). Fortunately, the BRD4-potent compound **40** also showed high activity to CDK9, with an $IC_{50}$ value of 81.6 nM. Comparing compounds **38** with **40**, clearly the nitrogen atom in bicycle played an important role for the binding interactions. We utilized the molecular docking method to examine the possible binding mechanism. Based on the docking model(Figure 3D), the nitrogen atom in isoquinoline ring of compound **40** located at the same position as the 2-nitrogen atom in parazole of the original ligand, which is at the vicinity of the residue Lys48 that participates in the salt bridge interaction with DFG motif. It was noticed that, as comparison with the predicted binding conformation of compounds **7** and **21**, the dihydroquinoxalin-2(1H)-one ring in compound **40** adopted a different orientation to alleviate the possible clash with the larger isoquinoline group.

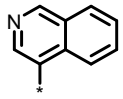
Since the $R_4$ substitutions on pyrimidine are usually utilized to enhance the selectivity on CDK9 (as exemplified in crystal structure 6GZH, the ligand has a fluorine atom at this position, Figure 3B), then we prepared compounds **41-43** and examined them in this two assays. From the Table 4, we can find that although **41** and **42** retained the activity on CDK9, they showed about 5-fold reduction in BRD4 binding activities. While compound **43** remained similar high binding activity on BRD4-BD1 as compound **40**, it did not maintain the inhibitory activity on CDK9. Taking together, compound **40** showed high potency on both BRD4-BD1 and CDK9 proteins, and were selected as a promising dual inhibitor for further profiling the binding mode,

selectivity, and cellular activity.

**Table 4. The exploration of bicycles on the WPF subpocket[a]**



| Compd. | $R_4$ | $R_5$ | BRD4 BD1 $IC_{50}$(nM) | CDK9 $IC_{50}$(nM) |
|---|---|---|---|---|
| **34** | H |  | 329.1±40.9 | 58.6%@1μM<br>7.6%@100nM |
| **35** | H |  | 288.0±26.0 | 13.8±0.9 |
| **36** | H |  | 167.8±35.5 | 110.0±7.0 |
| **37** | H |  | 75.9±13.2 | 79.8±11.2 |
| **38** | H |  | 15.2±2.6 | 81.6±4.5 |
| **39** | H |  | 25.3±3.9 | 19.8±1.5 |
| **40** | H |  | 12.7±4.6 | 22.4±2.3 |
| **41** | F |  | 81.3±15.7 | 23.9±2.5 |
| **42** | Cl |  | 58.1±14.9 | 23.0±1.0 |

| | | | | |
|---|---|---|---|---|
| **43** | Me |  | 19.1±1.3 | 100.0±4.0 |

[a]All BRD4-BD1 $IC_{50}$ values and CDK9 $IC_{50}$ values are mean values from at least two independent experiments.

The cocrystal structure of compound **40** bound to BRD4-BD1 was solved with X-ray crystallography. By superimposing on the cocrystal structure of compound **7**, we can realize that the bicycle ring on the pyrimidine extended into the WPF subpocket,

making favorable VDW interactions with this hydrophobic area as expected. The essential dihydroquinoxalin-2(1H)-one scaffold established the same hydrogen bond interactions with the residues of acetylated lysine binding pocket: one hydrogen bond between the oxygen atom of amide of compound **40** and the side chain of Asn140, one water-bridged hydrogen bond between the same oxygen and the phenol group of residue Tyr97. The notable difference between the binding mode of compound **40** and of compound **7** lies in the pyrimidine ring, which is slightly backward to eliminate the steric clash with the helix around the residue Asp145 and Ile146, and therefore make a new hydrogen bond with the residue Lys91.

**Selectivity profile of compound 40**

Although compound **40** was optimized as a dual inhibitor of BRD4 and CDK9, we examined its selectivity in a diverse kinases panel provided by Eurofins Scientific and in-house purified bromodomains. From the result of DiscoveRx diversity-kinases-panel assay that contains 54 kinases, at 1 μM concentration compound **40** showed the highest percent inhibition of CDK9, although 5 others also displayed more than 80% inhibition (Figure 4). Two other CDK kinases (CDK1 and CDK2) were also strongly inhibited by compound **40** with the inhibition ratio around 90% at 1 μM concentration(Table S1). So we obtained the detailed $IC_{50}$ value(Table S2) of CDK2 (168.9 nM), and showed that compound **40** has about 8-fold selectivity towards CDK9 over CDK2. While for CDK6, compound **40** only showed about 22% inhibition at tested concentration. We also tested the compound on seven in-house

prepared bromodomains, including BRD4-BD1, BRD4-BD2, BRD2-BD1, BRD2-BD2 and non-BET bromomain proteins EP300, CECR2 and BRD9[31]. As demonstrated in Figure 4 and Table S4, it is a potent BET inhibitor and showed less than 12% inhibition activity to three other bromodomains at 1 μM concentration. Overall, the selectivity measurement of compound **40** revealed that the design strategy by hybridising acetylated lysine mimic with hinge binder of the kinases is feasible to provide new options for developing polypharmacological inhibitors of bromodomain and kinases. With the detailed analysis of cocrystal structure of **40**, we could find that although the benzene motif of the bicycle ring extends into the WPF pocket, the other side of bicycle points to the solvent-accessible part. Therefore, we think that it is possible to obtain more selective inhibitors by modifying the bicycle ring motif, as it is the determinant for kinases selectivity.

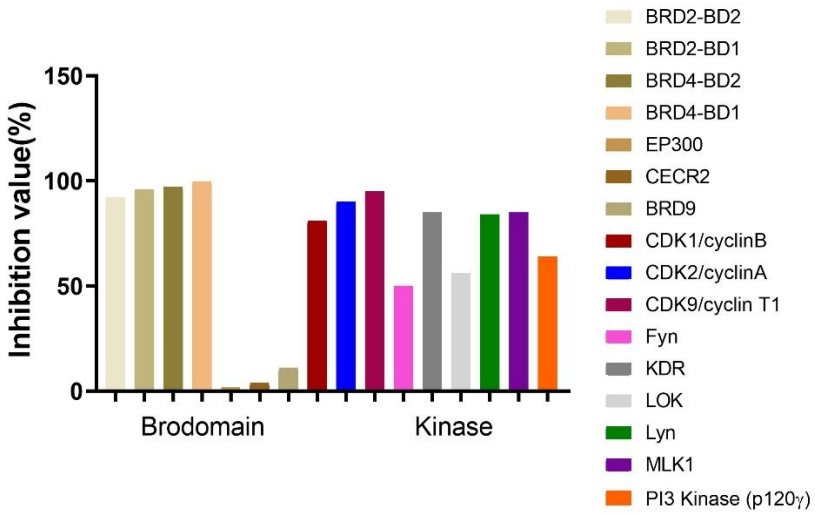



**Figure 4**. the selectivity profile of compound **40** on selected bromodomains and DiscoveRx diversity-kinases-panel. All inhibition values are mean values from at least two independent experiments at the concentration of 1μM. And for kinases selectivity assay, only kinases with inhibition ratio of more than 50% were depicted in

the figure 4 and whole data were listed in supporting material(Table S1).

**Cellular Activity and Western Blot**

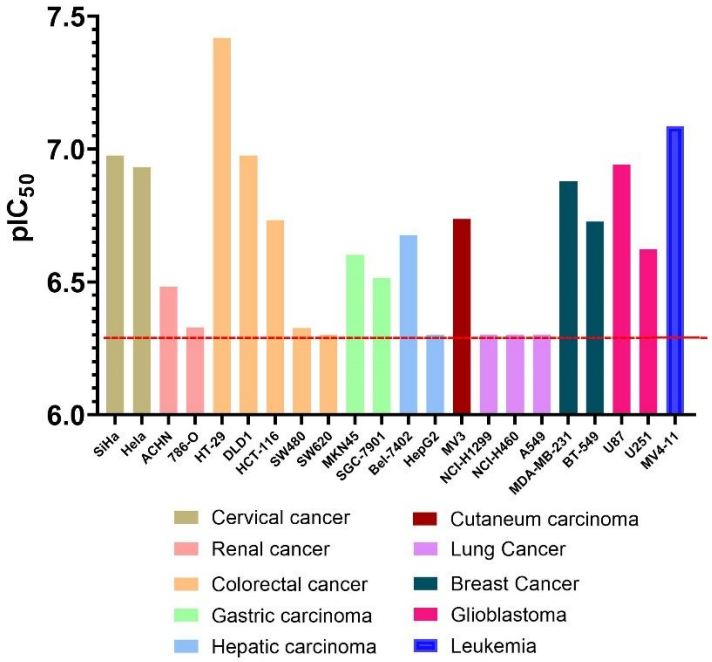



**Figure 5**. Proliferation inhibition of 22 cancer cell lines by Compound **40**.The cutoff line (red dotted-line) represents $IC_{50}$ values were larger than 500 nM. Precise value were listed in SI Table S5.

To further examine the cellular activity of compound **40**, total 22 cancer cell lines were screened to identify the sensitive cancer cells. As shown in Figure 5 and Table S5, compound **40** showed differential effects on the tested cell lines. After 96 hours of treating compound **40**, several cancer cell lines were remarkably sensitive to compound **40**, but others were not. This discrepancy may be due to the heterogeneity of tumor cells. Lars Tögel et al.[32] detected molecular subgroups of colon cancer, which included microsatellite instability (MSI)/ microsatellite stable (MSS) and CpG

island methylator phenotype (CIMP), mutation status of commonly altered oncogenes and tumor suppressor genes in colorectal cell lines; and they found that colorectal cell lines with MSI were significantly more sensitive to JQ1 compared to MSS cell lines. However, no association between JQ1 response and cell lines wild type or mutant for RAS/BRAF, PIK3CA/PTEN or TP53 was observed in their study, nor was there a correlation between JQ1 response and CIMP. The positive control compounds of BRD4 inhibitor JQ-1 and CDK9 inhibitor Atuveciclib were applied to these cell lines, and the results (SI Table S5) indicated the dual inhibitor **40** could enhance the antiproliferative activities, suggesting a synergy effect in the cellular context. Hence, the relatively sensitive solid tumor cell line HCT-116 was selected to detect the cellular activity of drug targets.

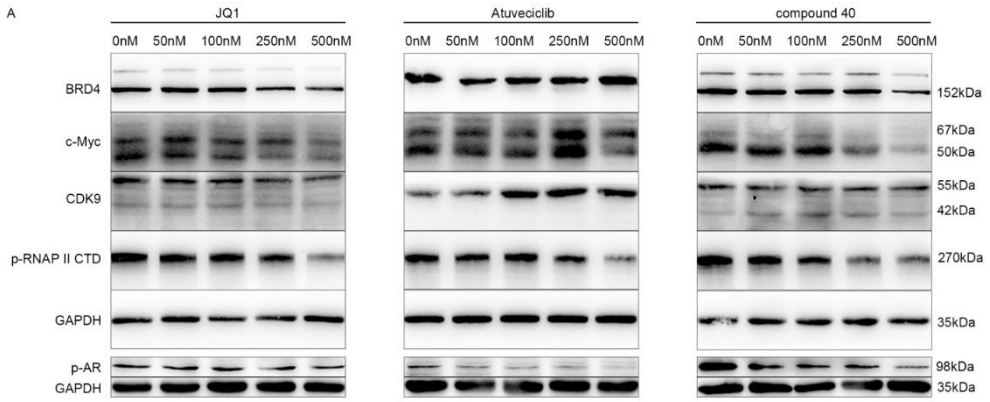



**Figure 6**. HCT-116 cells were treated with the indicated concentration of JQ1, Atuveciclib or compound **40** and examined BRD4, c-Myc, CDK9, p-RNA II CTD, P-AR and GAPDH protein levels by Western Blotting. The molecular weight of different isoform of BRD4, c-Myc and CDK9 were shown at the right of the bands.

To test the effects of the drug on activities of CDK9 and BRD4, HCT-116 cell line

was pretreated with compound JQ1, Atuveciclib or compound **40** (0 nM, 50 nM, 100 nM, 250 nM or 500 nM for 24 h respectively) and then immunoblotted. JQ1 is the positive control of BRD4 and Atuveciclib is the positive control of CDK9. CDK9 and BRD4 facilitate the transition from abortive to productive elongation by phosphorylating the CTD (C-terminal domain) of the large subunit of RNA polymerase II[33]. Regulation of c-Myc gene transcription appears to be the one function of BRD4 targeted by identified cancer therapeutic compounds activates related downstream target[34, 35]. CDK9 also specifically phosphorylates the AR on Ser81 and regulates AR transcriptional activity[33]. Western blot analysis showed that compounds JQ1 and compound **40** caused a dose-dependent reduction in c-Myc protein levels in HCT-116 cell line. Meanwhile, Atuveciclib caused a dose-dependent decrease in p-AR and p-RNAP II CTD but there was no significant trending effect on other proteins (The normalized quantified WB data was shown in SI Figure S4). As shown in Figure 6, compound **40** demonstrated good inhibitory activities on CDK9 and BRD4 similar to JQ1 in vitro and large doses could block CDK9 and BRD4 activities for a short period of time.

**Table 5. Pharmacokinetic parameters of compound 40 in ICR mice** [a]

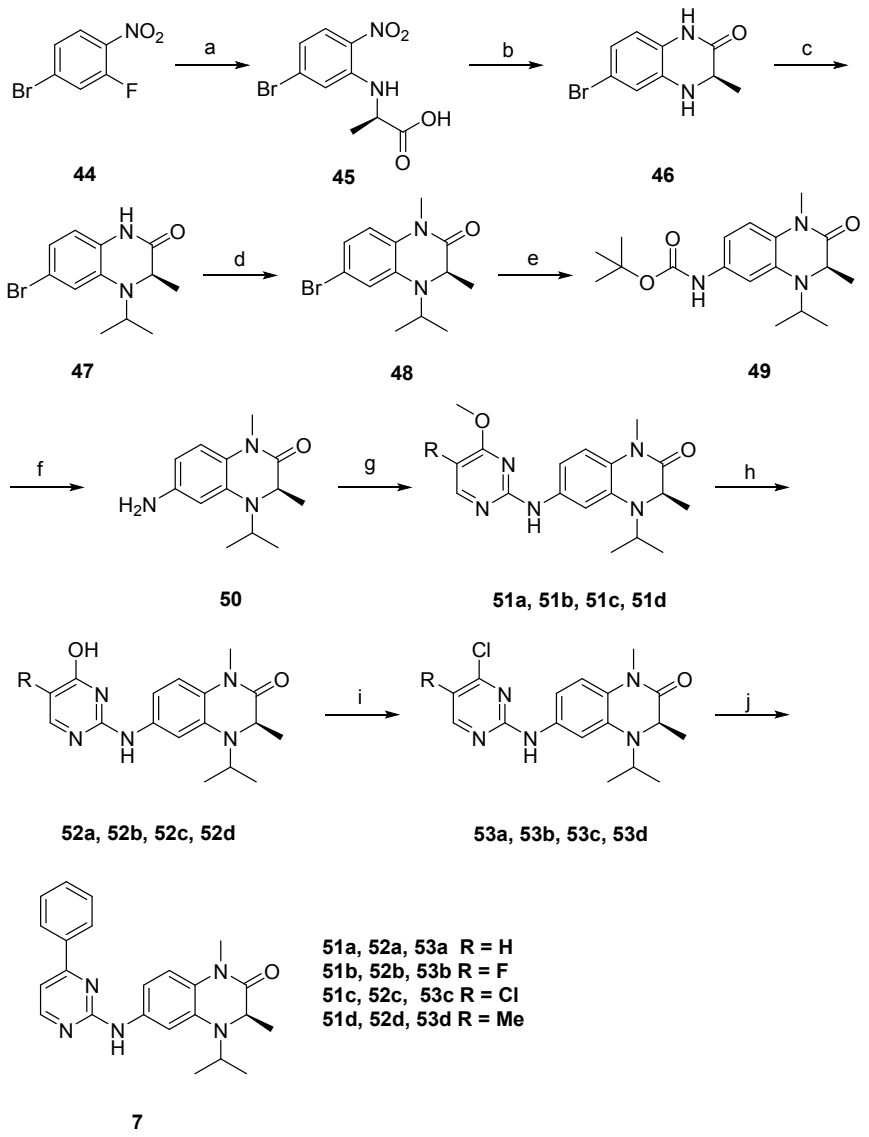
| route | dose | $K_{el}$ | $T_{1/2}$ | $T_{max}$ | $C_{max}$ | $AUC_{0-t}$ | $AUC_{0-inf}$ |
|---|---|---|---|---|---|---|---|
| | | ($h^{-1}$) | (h) | (h) | ($ng \cdot mL^{-1}$) | ($h \cdot ng \cdot mL^{-1}$) | ($h \cdot ng \cdot mL^{-1}$) |
| **IP** | 3 mg/kg | 1.45 | 0.480 | 0.194 | 1366 | 757 | 759 |

[a] Mice (n = 3); Parameters were calculated from composite mean plasma concentration-time data.

To complete the valuation of the lead compound **40**, we also investigated its in vivo pharmacokinetics (PK) parameters in the mice model. From the in vivo PK test, compound **40** dosed at 3 mg/kg showed a moderate plasma exposure with the $AUC_{0\text{-}t}$ value of 757 $h \cdot ng \cdot mL^{-1}$, indicating that the compound could be considered as a potential lead structure for further optimization.

## Chemistry

**Scheme 1. The Synthesis of Compound 7[a]**

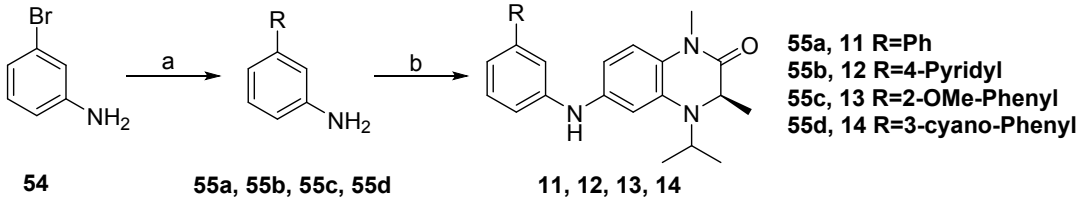



[a]Reagents and conditions: (a) D-alanine, $K_2CO_3$, EtOH: $H_2O$ = 3:1, 80 °C, 8 h; (b) $K_2CO_3$, $Na_2S_2O_4$, $H_2O$, 60 °C, overnight; (c) acetone, phenylsilane, dibutyltindichloride, THF, rt, 10 h; (d) NaH, 0 °C - rt, 30 min and then iodomethane, DMF , rt, 2 h; (e) tert-Butyl carbamate , $CS_2CO_3$ , $Pa(OAc)_2$, Xphos, 1.4-dioxane, 85 °C, 2 d; ( f ) HCl in 1.4-dioxane, rt, 24 h; (g) AcOH, 1.4-dioxane, 100 °C, 8 h; (h) HCl in 1.4-dioxane ,100 °C, 8 h; (i) $POCl_3$, 60 $^0$C, 8 h; (j) $K_2CO_3$, $Pd(dppf)_2Cl_2$, 1.4-dioxane : $H_2O$ = 4 : 1, 80 °C,4 h;

Compound **7** is synthesized according to Scheme 1[18]. **45** is obtained from nucleophilic

substitution of 4-bromo-2-fluoro-1-nitrobenzene with D-alanine, followed by cyclization to afford **46**. **48** was prepared by reductive amination with acetone and then alkylation with iodomethane. Then, palladium catalyzed C-N coupling reaction was utilized to obtain **49**. After removing the Boc protecting group ,a nucleophilic substitution reaction was adopted to give the intermediate **51a-51d**. Sequential acid hydrolysis and chlorination produced the compounds **53a-53d**. A palladium catalyzed Suzuki coupling reaction between aryl chloride (**53a**) and phenylboronic acid yieldes end-product **7**.

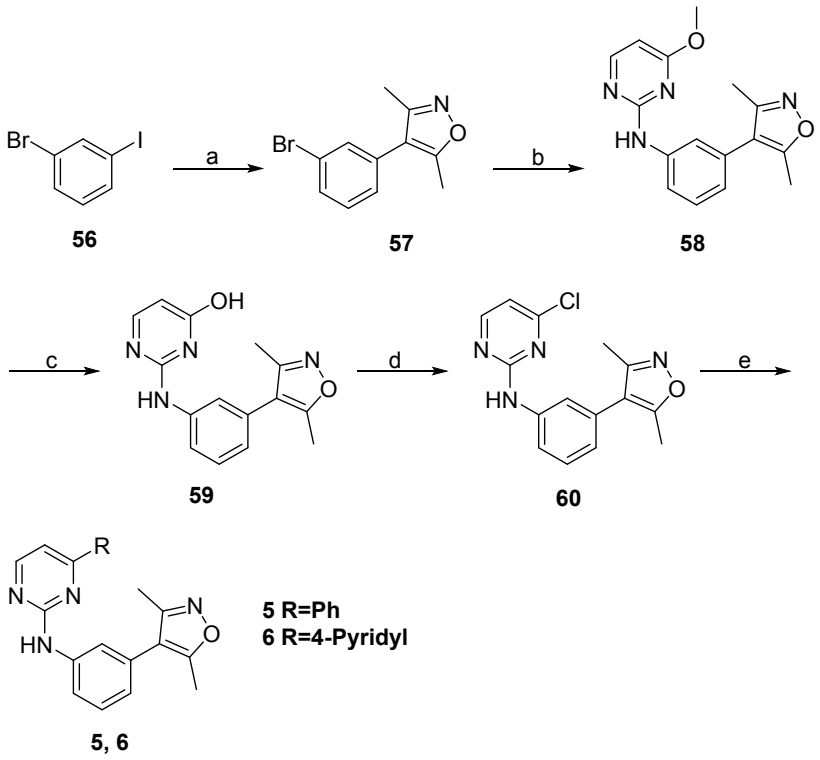
**Scheme 2. The Synthesis of compounds 11-14**[a]




[a]Reagents and conditions:(a) phenylboronic acid, $K_2CO_3$, $Pd(dppf)_2Cl_2$, 1.4-dioxane : $H_2O$ = 4:1, 80 °C, 8 h; (b) **48**, *t*-BuONa, $Pa_2(dba)_3$, Xphos, 1.4-dioxane, 100 °C, overnight;

Compounds **11-14**, as depicted in Scheme 2, were obtained throught palladium catalyzed Suzuki and Buchwald-Hartwig coupling reactions.

**Scheme 3. The Synthesis of compounds 5 and 6**[a]




[a]Reagents and conditions: (a) $NaHCO_3$, $Pa(PPh_3)_4$, 1.4-dioxane : $H_2O$ = 4 : 1, 70 °C, 2 h; (b) 2-Amino-4-methoxypyrimidine , *t*-BuONa, $Pa_2(dba)_3$, Xphos, 1.4-dioxane, 100 °C, 8 h; (c) HCl in 1.4-dioxane, 100 °C, 8 h; (d) $POCl_3$, 60 $^0$C, 8 h; (e) $K_2CO_3$, $Pd(dppf)_2Cl_2$, 1.4-dioxane : $H_2O$ = 4 : 1, 80 °C, 4 h;

The synthetic routes of 3,5-dimethyl-isoxazole derivatives **5** and **6** are outlined in Scheme 3. Like the preparation of compounds **11-14**, Compound **58** was obtained throught two successive palladium catalyzed coupling reactions. Acid hydrolysis ,which could remove the OMe group, and chlorination afford intermediate **60**.As mentioned above,the final step also was Suzuki couploing rection between **60** and the corresponding boronic acid derivatives.

## CONCLUSIONS

Over the past decades, many kinases inhibitors have entered into the market for the

treatment of cancers. However, cancer is notorious for its high heterogeneity and usually harboring genetic and epigenetic abnormality, therefore there is an urgent need of polypharmacological agents to simultaneously targeting multiple dysfunctional pathways. BET inhibitors have entered into clinical trials and demonstrated its antitumor effects and promising synergistic effects by combining with current kinases inhibitors. Although there are several dual inhibitors of BET and kinases have been discovered by serendipity, it remains a challenge for the researchers to rational design dual inhibitors of certain kinases and BET. In the present work, we adopted the hybrid strategy by incorporating typical kinases hinge binder with pharmacophores that bind to the acetylated lysine pocket of BET proteinsand successfully obtained a series of 6-(Pyrimidin-2-ylamino)-3,4-dihydroquinoxalin-2(1H)-ones. Through structure-based drug design, we investigated the structure-activity relationship and demonstrated that it is feasible to discover potent inhibitors of dual BET and kinases, providing not only a lead compound of CDK9 and BET, but also the promising approach for the community to design specific kinase and BET dual inhibitors.

## EXPERIMENTAL SECTION

### General Chemistry

$^{1}$H NMR spectra were recorded by using a Varian Mercury-400 high performance digital FT-NMR spectrometer with tetramethylsilane (TMS) as an internal standard. Low-resolution mass spectra were obtained with a Finnigan LCQ Deca XP mass

spectrometer using a CAPCELL PAK C18 (50 mm × 2.0 mm, 5 μm) or an Agilent ZORBAX Eclipse XDB C18 (50 mm × 2.1 mm, 5 μm) in positive or negative electrospray mode. The purity of compounds was determined by high-performance liquid chromatography (HPLC) and confirmed to be more than 95%, monitored by UV absorption at 214 and 254 nm. TLC analysis was carried out with glass precoated silica gel GF254 plates. TLC spots were visualized under UV light. All solvents and reagents were used directly as obtained commercially unless otherwise noted.

**N-(3-(3,5-dimethylisoxazol-4-yl)phenyl)-4-phenylpyrimidin-2-amine (5).** *Step 1: 4-(3-bromophenyl)-3,5-dimethylisoxazole (**57**).* To a solution of compound **56** (4 g, 14.2 mmol) and 3,5-Dimethylisoxazole-4-boronic acid (1 g ,7.1 mmol) in 1.4-dioxane and $H_2O$ (4:1 50 mL) was added $NaHCO_3$ (1.2 g , 14.2 mmol), the mixture was bubbled with $N_2$ for 5 min, then $Pa(PPh_3)_4$ (820 mg, 0.71 mmol) was added, the mixture was further bubbled with $N_2$ for 5 min, the mixture was heated to 70 °C for 2 h. The reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water and extracted with DCM (3 × 100 mL), The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 10% EtOAc/60-90 °C petroleum ether) to afford compound **57** (1.237 g, 4.906 mmol, 69.1% yield) as a white solid. $^1$H NMR (400 MHz, Chloroform-*d* ) $\delta$ 7.52 (ddt, $J$ = 8.4, 3.6, 1.5 Hz, 1H), 7.43 (dt, $J$ = 3.5, 1.8 Hz, 1H), 7.34 (td, $J$ = 7.8, 3.1 Hz, 1H), 7.24 – 7.15 (m, 1H), 2.43 (d, $J$ = 3.1 Hz, 3H), 2.29 (d, $J$ = 3.1 Hz, 3H);

*Step 2: N-(3-(3,5-dimethylisoxazol-4-yl)phenyl)-4-methoxypyrimidin-2-amine (**58**).* To a solution of compound **57** (1.6 g, 6.4 mmol ) and 2-amine-4-methoxypyrimidine (600 mg , 4.8 mmol) in anhydrous 1.4-dioxane (20 mL) was added *t*-BuONa (921 mg , 9.6 mmol ), the mixture was bubbled with $N_2$ for 5 min ,then $Pa_2(dba)_3$ (446 mg, 0.48 mmol ) and Xphos(458 mg, 0.96 mmol) was added, the mixture was further bubbled with $N_2$ for 5 min, the mixture was heated to 100 °C for 8 h. The reaction was monitored by TLC. Upon completion, the resulting solution was filtered through Celite and concentrated, the residue was purified by silica gel column chromatography (gradient elution, gradient 10 to 33% EtOAc/60-90 °C petroleum ether) to afford compound **58** (510 mg, 4.32 mmol, 90% yield) as a white solid. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 8.17 (d, $J$ = 5.7 Hz, 1H), 7.64 (d, $J$ = 1.8 Hz, 1H), 7.63 – 7.58 (m, 1H), 7.41 (t, $J$ = 7.9 Hz, 1H), 7.16 (s, 1H), 6.98 – 6.88 (m, 1H), 6.29 – 6.18 (m, 1H), 3.96 (s, 3H), 2.46 (s, 3H), 2.33 (s, 3H);

*Step 3: 2-((3-(3,5-dimethylisoxazol-4-yl)phenyl)amino)pyrimidin-4-ol (**59**).* Compound **58** (510 mg, 1.12 mmol) was dissolved in 1.4-dioxane (10 mL) followed by the addition of 4M HCl in 1.4-dioxane (20 mL), the mixture was stirred at 100 $^0$C for 24 h. The reaction was monitored by TLC. Upon completion, the resulting solution was poured into ice water slowly, the pH of this solution was adjusted to 7 with aqueous NaOH and extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 2 to 10% MeOH/DCM)) to afford compound **59** (341 mg, 0.784

mmol, 70% yield) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.02 (d, $J$ = 5.7 Hz, 1H), 7.91 (d, $J$ = 5.7 Hz, 1H), 7.73 (d, $J$ = 1.8 Hz, 1H), 7.67 – 7.63 (m, 1H), 7.41 (t, $J$ = 7.9 Hz, 1H), 7.30 (m, 1H), 6.98 (m, 1H), 2.46 (s, 3H), 2.37 (s, 3H);

*Step 4: 4-chloro-N-(3-(3,5-dimethylisoxazol-4-yl)phenyl)pyrimidin-2-amine (**60**).* Compound **59** (341 mg, 1.2 mmol) was dissolved in $POCl_3$ (10 mL) and then stirred at 60 °C for 8 h. The reaction was monitored by TLC. Upon completion, the reaction mixture was poured into ice water slowly, the pH of this solution was adjusted to 7 with aqueous NaOH and extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 20 to 33% EtOAc/60-90 °C petroleum ether) to afford compound **60** (254 mg, 1.008 mmol, 84% yield) as a white solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.99 (d, $J$ = 5.9 Hz, 1H), 7.80 (d, $J$ = 2.0 Hz, 1H), 7.71 (m, 1H), 7.45 (m, 1H), 7.29 (m, 1H), 7.01 (m, 1H), 2.40 (s, 3H), 2.33 (s, 3H);

*Step 5: N-(3-(3,5-dimethylisoxazol-4-yl)phenyl)-4-phenylpyrimidin-2-amine (**5**).* To a solution of compound **60** (80 mg, 0.266mmol ) and phenylboronic acid (65 mg , 0.532 mmol) in 1.4-dioxane and $H_2O$ (4:1 10 mL) was added $K_2CO_3$ (74 mg , 0.532 mmol ), the mixture was bubbled with $N_2$ for 5 min, then $Pa(dppf)_2Cl_2$ (22 mg, 0.053 mmol) was added, the mixture was further bubbled with $N_2$ for 5 min, the mixture was heated to 80 °C for 8 h. The reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water and extracted with DCM (3 × 50 mL), The combined organic layers were washed with brine, dried over $Na_2SO_4$ and

concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 10 to 33% EtOAc/60-90 °C petroleum ether) to afford compound **5** (30 mg, 0.088 mmol, 33% yield) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.83 (s, 1H), 8.58 (d, $J$ = 5.2 Hz, 1H), 8.21 – 8.07 (m, 2H), 7.94 – 7.87 (m, 2H), 7.55 (dd, $J$ = 5.2, 2.1 Hz, 3H), 7.46 – 7.38 (m, 2H), 6.99 (dt, $J$ = 7.7, 1.3 Hz, 1H), 2.44 (s, 3H), 2.26 (s, 3H). LC-MS (ESI): calcd for $C_{21}H_{18}N_4O$ m/z $[M+H]^+$ 343.15, found 343.16 Retention time 3.211 min, HPLC purity = 100%.

**N-(3-(3,5-dimethylisoxazol-4-yl)phenyl)-4-(pyridin-4-yl)pyrimidin-2-amine (6).** Compound **6** was prepared as a white solid using a method similar to that described for compound **5** in 30% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.97 (s, 1H), 8.84 – 8.74 (m, 2H), 8.69 (d, $J$ = 5.1 Hz, 1H), 8.11 – 8.02 (m, 2H), 7.97 – 7.88 (m, 1H), 7.81 (t, $J$ = 1.9 Hz, 1H), 7.56 (d, $J$ = 5.1 Hz, 1H), 7.44 (t, J = 7.9 Hz, 1H), 7.01 (dt, $J$ = 7.6, 1.3 Hz, 1H), 2.44 (s, 3H), 2.26 (s, 3H).LC-MS (ESI): calcd for $C_{20}H_{17}N_5O$ m/z $[M+H]^+$ 344.14, found 344.12. Retention time 3.230 min, HPLC purity = 99.98%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-phenylpyrimidin-2-yl)amino)-3,4-dihydroquinoxalin-2(1H)-one (7).** *Step 1: (5-bromo-2-nitrophenyl)-D-alanine (**45**).* To a solution of 4-bromo-2-fluoro-1-nitrobenzene (25.0 g,113.6 mmol) in EtOH and $H_2O$ （3:1，500 mL) were added D-alanine (11.1 g,125.0 mmol) and $K_2CO_3$(17.3 g,125.0 mmol),the reaction mixture was heated to reflux for 8h. The reaction was monitored by TLC. Upon completion, the resulting solution were evaporated under reduced pressure, the crude product was used in the next step without further purification. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.35 (d, J = 6.9 Hz, 1H), 8.06 (d, J = 9.1 Hz, 1H), 6.90

(s,1H), 6.85 (d, J = 9.2 Hz, 1H), 4.33 (p, J = 7.0 Hz, 1H), 1.67 (d, J =7.0 Hz, 3H);

*Step 2: (R)-6-bromo-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**46**).* To a solution of compound **45** (28.7 g,99.3 mmol) and $K_2CO_3$ (27.4 g, 198.6 mmol) in $H_2O$ (500 mL) was added $Na_2S_2O_4$ (86.5 g,496.6 mmol) slowly, the reaction mixture was heated to 60 °C overnight. The reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water and extracted with DCM (3 × 250 mL), The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure to afford compound **46** (9 g, 373 mmol, 38% yield) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.42 (s, 1H), 6.89-6.83 (dd, J = 8.2, 2.0 Hz, 1H), 6.81 (s, 1H), 6.68-6.59 (dd, J = 13.1, 7.6 Hz, 1H), 4.02 (q, J = 6.7 Hz, 1H), 3.92 (s, 1H),1.45 (d, J = 6.7 Hz, 3H);

*Step 3: (R)-6-bromo-4-isopropyl-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**47**).* A solution of compound **46** (9.0 g,37.3 mmol), phenylsilane (11.9 g, 113.1 mmol),acetone (8.3 mL, 112.0 mmol) and dibutyltindichloride ( 17.0 g,56.0 mmol ) in THF (100 mL) were stirred at room temperature for 10 h. The reaction was monitored by TLC. Upon completion, the resulting solution was evaporated, the residue was purified by silica gel column chromatography (gradient elution, gradient 10 to 25% EtOAc/60-90 °C petroleum ether) to afford compound **47** (8.8 g, 33.1 mmol, 87% yield) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 10.03 (s, 1H), 6.96 (d, $J$ = 1.9 Hz, 1H), 6.88 (dd, $J$ = 8.3, 2.0 Hz, 1H), 6.73 (d, $J$ = 8.3 Hz, 1H), 4.13 (q, $J$ = 6.8 Hz, 1H), 3.95 – 3.80 (m, 1H), 1.33 (d, $J$ = 6.7 Hz, 3H), 1.26 (d, $J$ = 6.1 Hz, 3H), 1.20 (d, $J$ = 6.7 Hz, 3H);

*Step 4: (R)-6-bromo-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one **(48)***. To a solution of compound **47** (8.8 g,31.1 mmol ) in anhydrous DMF (50 mL) was added NaH (1.3 g, 60% in mineral oil, 46.6 mmol ) portion wise at 0 °C, the reaction mixture were stirred at 0 °C for 2h and then iodomethane(2.9 mL,46.6 mmol) was added at the same temperature, stirred at room temperature for additional 4h. The reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water and extracted with DCM (3 × 100 mL) , The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 10 to 25% EtOAc/60-90 °C petroleum ether) to afford compound **48** (8.8 g, 29.7 mmol, 95% yield) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.04 – 6.93 (m, 3H), 4.15 (q, $J$ = 6.7 Hz, 1H), 3.90 (hept, $J$ = 6.5 Hz, 1H), 3.25 (s, 3H), 1.24 (d, $J$ = 6.6 Hz, 3H), 1.17 (d, $J$ = 6.6 Hz, 3H), 0.97 (d, $J$ = 6.7 Hz, 3H);

Step 5: tert-butyl (R)-(4-isopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl) carbamate ***(49)***. Tert-butyl carbamate (80.0 mg,0.673 mmol) was added to a solution of compound **48** (50.0 mg,0.168 mmol) and $Cs_2CO_3$(110 mg,0.336 mmol) in 1.4-dioxane (5 mL) , the mixture was bubbled with $N_2$ for 5 min, then $Pd(OAc)_2$ (4.0 mg,0.017 mmol) and Xphos(17.0 mg,0.034 mmol ) was added and the mixture was further bubbled with $N_2$ for 5 min. the reaction mixture was heated to 85 °C for 2 d. The reaction was monitored by TLC. Upon completion, the resulting solution was filtered through Celite and concentrated, the residue was purified by silica gel column

chromatography (gradient elution, gradient 20 to 50% EtOAc/60-90 °C petroleum ether) to afford compound **49** (40 mg, 0.120 mmol, 71% yield) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.09 (s, 1H), 6.84 (d, $J$ = 8.6 Hz, 1H), 6.76 (dd, $J$ = 8.5, 2.2 Hz, 1H), 6.69 (s, 1H), 4.15 (q, $J$ = 6.8 Hz, 1H), 3.88 (h, $J$ = 6.6 Hz, 1H), 3.34 (s, 3H), 1.52 (s, 9H), 1.33 (d, $J$ = 6.7 Hz, 3H), 1.20 (d, $J$ = 6.6 Hz, 3H), 1.08 (d, $J$ = 6.8 Hz, 3H);

*Step 6: (R)-6-amino-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**50**)*. Compound **49** (40.0 mg,0.120 mmol) was dissolved in 4M HCl in 1.4-dioxane (5 mL) and then stirred at room temperature for 24 h. The reaction was monitored by TLC. Upon completion, the pH of this solution was adjusted to 7 with aqueous NaOH and extracted with DCM (3 × 200 mL) .The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 20 to 50% EtOAc/60-90 °C petroleum ether) to afford compound **50** (22 mg, 0.094 mmol, 80% yield) as a brown solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.66 (d, $J$ = 8.4 Hz, 1H), 6.21 –6.10 (m, 2H), 4.05 (q, $J$ = 6.8 Hz, 1H), 3.74 (hept, $J$ = 6.7 Hz, 1H), 3.57 (s, 2H), 3.24 (s, 3H), 1.19 (d, $J$ = 6.7 Hz, 3H), 1.13 (d, $J$ = 6.6 Hz, 3H), 1.01 (d, $J$ = 6.8 Hz, 3H);

*Step 7: (R)-4-isopropyl-6-((4-methoxypyrimidin-2-yl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**51a**).* To a solution of compound **50** (2.0 g, 8.7 mmol) in 1.4-dioxane (20 mL) was added 2-chloro-4-methoxypyrimidine (1.6 g, 11.3 mmol) followed by

acetic acid (2 mL), the reaction mixture was heated to 100 °C for 8 h. The reaction was monitored by TLC. Upon completion, the resulting solution was poured into ice water slowly, the pH of this solution was adjusted to 7 with aqueous NaOH and extracted with DCM (3 × 100 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 20 to 50% EtOAc/60-90 °C petroleum ether) to afford compound **51a** (1.1 g, 3.3 mmol, 92% yield) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.40 (s, 1H), 8.18 (d, $J$ = 5.6 Hz, 1H), 7.45 (d, $J$ = 2.2 Hz, 1H), 7.28 (dd, $J$ = 8.7, 2.3 Hz, 1H), 6.97 (d, $J$ = 8.7 Hz, 1H), 6.25 (d, $J$ = 5.6 Hz, 1H), 4.08 (q, $J$ = 6.6 Hz, 1H), 3.92 (s, 3H), 3.83 (p, $J$ = 6.7 Hz, 1H), 3.24 (s, 3H), 1.26 (d, $J$ = 6.6 Hz, 3H), 1.19 (d, $J$ = 6.6 Hz, 3H), 0.98 (d, $J$ = 6.8 Hz, 3H);

*Step 8: (R)-6-((4-hydroxypyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**52a**)*. Compound **51a** (1.1 g,3.3 mmol) was dissolved in 1.4-dioxane (5 mL) followed by the addition of 4M HCl in 1.4-dioxane (15 mL), the mixture was stirred at 100 $^0$C for 24 h. The reaction was monitored by TLC. Upon completion, the resulting solution was poured into ice water slowly, the pH of this solution was adjusted to 7 with aqueous NaOH and extracted with DCM (3 × 100 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 2 to 10% MeOH/DCM) to afford

compound **52a** (700 mg, 2.1 mmol, 65.3% yield) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.77 (s, 1H), 8.80 (s, 1H), 7.72 (d, $J$ =6.5 Hz, 1H), 7.21 (d, $J$ = 2.1 Hz, 1H), 7.13 – 6.95 (m, 2H), 5.80 – 5.73 (m, 1H), 4.11 (q, $J$ = 6.7 Hz, 1H), 3.81 (p, $J$ = 6.6 Hz, 1H), 3.25 (s, 3H), 1.26 (d, $J$ = 6.6 Hz, 3H), 1.19 (d, $J$ = 6.6 Hz, 3H), 0.98 (d, $J$ = 6.8 Hz, 3H);

*Step 9: (R)-6-((4-chloropyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (**53a**)*. Compound **52a** (700.0 mg,2.1 mmol) was dissolved in $POCl_3$ (10 mL) and then stirred at 60 °C for 8 h. The reaction was monitored by TLC. Upon completion, the reaction mixture was poured into ice water slowly, the pH of this solution was adjusted to 7 with aqueous NaOH and extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 20 to 33% EtOAc/60-90 °C petroleum ether) to afford compound **53a** (145 mg, 0.419 mmol, 20% yield) as a red solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.89 (s, 1H), 8.40 (d, $J$ = 5.1 Hz, 1H), 7.41 (d, $J$ = 2.2 Hz, 1H), 7.17 (dd, $J$ = 8.6, 2.2 Hz, 1H), 6.99 (d, $J$ = 8.7 Hz, 1H), 6.91 (d, $J$ = 5.2 Hz, 1H), 4.09 (q, $J$ = 6.5 Hz, 1H), 3.79 (p, $J$ = 6.6 Hz, 1H), 3.24 (s, 3H), 1.28 (d, $J$ = 6.6 Hz, 3H), 1.21 (d, $J$ = 6.8 Hz, 3H), 0.97 (d, $J$ = 6.7 Hz, 3H);

*Step 10: (R)-4-isopropyl-1,3-dimethyl-6-((4-phenylpyrimidin-2-yl)amino)-3,4-dihydroquinoxalin-2(1H)-one (7).* To a solution of compound **53a** (50 mg,0.145mmol ) and

phenylboronic acid (27 mg , 0.217 mmol) in 1.4-dioxane and $H_2O$ (4:1 10 mL) was added $K_2CO_3$ (40 mg ,0.289 mmol ), the mixture was bubbled with $N_2$ for 5 min, then $Pa(dppf)_2Cl_2$ (12 mg, 0.0145 mmol) was added, the mixture was further bubbled with $N_2$ for 5 min, the mixture was heated to 80 °C for 8 h. The reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water and extracted with DCM (3 × 50 mL), The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 1 to 2% MeOH/DCM) to afford compound **7** (34 mg, 0.087 mmol, 60% yield) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.56 (s, 1H), 8.54 (d, $J$ = 5.1 Hz, 1H), 8.18 (dd, $J$ = 6.7, 3.0 Hz, 2H), 7.66 (s, 1H), 7.56 (dd, $J$ = 5.1, 1.9 Hz, 3H), 7.38 (d, $J$ = 5.1 Hz, 1H), 7.28 (dd, $J$ = 8.7, 2.2 Hz, 1H), 7.02 (d, $J$ = 8.7 Hz, 1H), 4.10 (q, $J$ = 6.7 Hz, 1H), 3.91 (p, $J$ = 6.6 Hz, 1H), 3.26 (s, 3H), 1.28 (d, $J$ = 6.6 Hz, 3H), 1.21 (d, $J$ = 6.8 Hz, 3H), 0.97 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{23}H_{25}N_5O$ m/z $[M+H]^+$ 388.21, found 388.19. Retention time 3.208 min, HPLC purity = 97.779%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-(pyridin-4-yl)pyrimidin-2-yl)amino)-3,4-dihydroquinoxalin-2(1H)-one (8).** Compound **8** was prepared as a yellow solid using a method similar to that described for compound **7** in 27% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.56 (s, 1H), 8.54 (d, $J$ = 5.1 Hz, 1H), 8.18 (dd, $J$ = 6.7, 3.0 Hz, 2H), 7.66 (s, 1H), 7.56 (dd, $J$ = 5.1, 1.9 Hz, 3H), 7.38 (d, $J$ = 5.1 Hz, 1H), 7.28 (dd, $J$ = 8.7, 2.2 Hz, 1H), 7.02 (d, $J$ = 8.7 Hz, 1H), 4.10 (q, $J$ = 6.7 Hz, 1H), 3.91 (p, $J$ = 6.6 Hz, 1H), 3.26 (s, 3H), 1.28 (d, $J$ = 6.6 Hz, 3H), 1.21 (d, $J$ = 6.8 Hz, 3H), 0.97 (d, $J$ = 6.7

Hz, 3H); LC-MS (ESI): calcd for $C_{22}H_{24}N_6O$ m/z $[M+H]^+$ 389.20, found 389.12. Retention time 2.508 min, HPLC purity = 98.415%.

**(R)-4-isopropyl-6-((4-(2-methoxyphenyl) pyrimidin-2-yl) amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (9).** Compound **9** was prepared as a yellow solid using a method similar to that described for compound **7** in 29% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.52 (s, 1H), 8.46 (d, $J$ = 5.2 Hz, 1H), 7.93 (dd, $J$ = 7.6, 1.8 Hz, 1H), 7.70 – 7.60 (m, 1H), 7.50 (td, $J$ = 7.9, 7.3, 1.8 Hz, 1H), 7.31 (d, $J$ = 5.2 Hz, 1H), 7.21 (dd, $J$ = 11.1, 7.7 Hz, 2H), 7.10 (t, $J$ = 7.5 Hz, 1H), 6.98 (d, $J$ = 8.7 Hz, 1H), 4.08 (q, $J$ = 6.7 Hz, 1H), 3.88 (s, 3H), 3.87 – 3.78 (m, 1H), 3.25 (s, 3H), 1.22 (d, $J$ = 6.8 Hz, 3H), 1.19 (d, $J$ = 6.5 Hz, 3H), 0.97 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{27}N_5O_2$ m/z $[M+H]^+$ 418.22 found 418.24.LC-MS (ESI): calcd for $C_{26}H_{26}N_6O$ m/z $[M+H]^+$ 439.22, found 439.14. Retention time 3.059 min, HPLC purity = 99.532%.

**(R)-3-(2-((4-isopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)a mino)pyrimidin-4-yl)benzonitrile (10).** Compound **10** was prepared as a yellow solid using a method similar to that described for compound **7** in 34% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.68 (s, 1H), 8.64 – 8.55 (m, 2H), 8.49 (dt, $J$ = 8.0, 1.4 Hz, 1H), 8.04 (dt, $J$ = 7.7, 1.4 Hz, 1H), 7.77 (t, $J$ = 7.9 Hz, 1H), 7.59 (s, 1H), 7.49 (d, $J$ = 5.2 Hz, 1H), 7.24 (dd, $J$ = 8.6, 2.2 Hz, 1H), 7.02 (d, $J$ = 8.7 Hz, 1H), 4.11 (q, $J$ = 6.7 Hz, 1H), 3.88 (p, $J$ = 6.6 Hz, 1H), 3.26 (s, 3H), 1.26 (d, $J$ = 6.6 Hz, 3H), 1.22 (d, $J$ = 6.8 Hz, 3H), 0.99 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{24}N_6O$ m/z $[M+H]^+$ 413.20, found 413.06. Retention time 3.284 min, HPLC purity = 99.103%.

**(R)-4-isopropyl-6-((4-(3-methoxyphenyl)pyrimidin-2-yl)amino)-1,3-dimethyl-3, 4-dihydroquinoxalin-2(1H)-one (15).** Compound **15** was prepared as a yellow solid using a method similar to that described for compound **7** in 25.3% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.52 (s, 1H), 8.46 (d, $J$ = 5.2 Hz, 1H), 7.93 (dd, $J$ = 7.6, 1.8 Hz, 1H), 7.70 – 7.60 (m, 1H), 7.50 (td, $J$ = 7.9, 7.3, 1.8 Hz, 1H), 7.31 (d, $J$ = 5.2 Hz, 1H), 7.21 (dd, $J$ = 11.1, 7.7 Hz, 2H), 7.10 (t, $J$ = 7.5 Hz, 1H), 6.98 (d, $J$ = 8.7 Hz, 1H), 4.08 (q, $J$ = 6.7 Hz, 1H), 3.88 (s, 3H), 3.87 – 3.78 (m, 1H), 3.25 (s, 3H), 1.22 (d, $J$ = 6.8 Hz, 3H), 1.19 (d, $J$ = 6.5 Hz, 3H), 0.97 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{27}N_5O_2$ m/z $[M+H]^+$ 418.22, found 418.21. Retention time 3.211 min, HPLC purity = 99.65%.

**(R)-4-isopropyl-6-((4-(4-methoxyphenyl)pyrimidin-2-yl)amino)-1,3-dimethyl-3, 4-dihydroquinoxalin-2(1H)-one (16).** Compound **16** was prepared as a yellow solid using a method similar to that described for compound **7** in 25.7% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.52 (s, 1H), 8.46 (d, $J$ = 5.2 Hz, 1H), 7.93 (dd, $J$ = 7.6, 1.8 Hz, 1H), 7.70 – 7.60 (m, 1H), 7.50 (td, $J$ = 7.9, 7.3, 1.8 Hz, 1H), 7.31 (d, $J$ = 5.2 Hz, 1H), 7.21 (dd, $J$ = 11.1, 7.7 Hz, 2H), 7.10 (t, $J$ = 7.5 Hz, 1H), 6.98 (d, $J$ = 8.7 Hz, 1H), 4.08 (q, $J$ = 6.7 Hz, 1H), 3.88 (s, 3H), 3.87 – 3.78 (m, 1H), 3.25 (s, 3H), 1.22 (d, $J$ = 6.8 Hz, 3H), 1.19 (d, $J$ = 6.5 Hz, 3H), 0.97 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{27}N_5O_2$ m/z $[M+H]^+$ 418.22, found 418.24. Retention time 3.089 min, HPLC purity = 99.369%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-(o-tolyl)pyrimidin-2-yl)amino)-3,4-dihydro quinoxalin-2(1H)-one (17).** Compound **17** was prepared as a yellow solid using a

method similar to that described for compound **7** in 44% yield.$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.51 (s, 1H), 7.48 (d, $J$ = 3.9 Hz, 2H), 7.38 (t, $J$ = 7.4 Hz, 1H), 7.34 – 7.30 (m, 2H), 7.28 (s, 1H), 7.03 (d, $J$ = 8.6 Hz, 1H), 6.91 (d, $J$ = 8.5 Hz, 1H), 6.89 – 6.83 (m, 1H), 4.24 – 4.09 (m, 1H), 3.90 (p, $J$ = 6.6 Hz, 1H), 3.38 (s, 3H), 2.47 (s, 3H), 1.27 (d, $J$ = 6.7 Hz, 3H), 1.24 (d, $J$ = 6.5 Hz, 3H), 1.13 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{27}N_5O$ m/z $[M+H]^+$ 402.22, found 402.26. Retention time 3.507 min, HPLC purity = 95.934%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-(m-tolyl)pyrimidin-2-yl)amino)-3,4-dihydro quinoxalin-2(1H)-one (18).** Compound 1**8** was prepared as a yellow solid using a method similar to that described for compound **7** in 33% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.56 (s, 1H), 8.55 – 8.49 (m, 1H), 7.96 (d, $J$ = 9.6 Hz, 2H), 7.65 – 7.59 (m, 1H), 7.43 (t, $J$ = 7.5 Hz, 1H), 7.36 (t, $J$ = 6.2 Hz, 2H), 7.31 – 7.18 (m, 1H), 7.02 (d, $J$ = 8.8 Hz, 1H), 4.10 (q, $J$ = 6.7 Hz, 1H), 3.89 (p, $J$ = 6.7 Hz, 1H), 3.26 (s, 3H), 2.41 (s, 3H), 1.26 (d, $J$ = 6.6 Hz, 3H), 1.21 (d, $J$ = 6.5 Hz, 3H), 0.99 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{27}N_5O$ m/z $[M+H]^+$ 402.22, found 402.23. Retention time 3.288 min, HPLC purity = 97.466%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-(p-tolyl)pyrimidin-2-yl)amino)-3,4-dihydro quinoxalin-2(1H)-one (19).** Compound **19** was prepared as a yellow solid using a method similar to that described for compound **7** in 29.6% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.53 (s, 1H), 8.50 (d, $J$ = 5.2 Hz, 1H), 8.08 (d, $J$ = 7.9 Hz, 2H), 7.65 (s, 1H), 7.39 – 7.31 (m, 3H), 7.27 (dd, $J$ = 8.8, 2.1 Hz, 1H), 7.01 (d, $J$ = 8.7 Hz, 1H), 4.10 (q, $J$ = 6.7 Hz, 1H), 3.91 (p, $J$ = 6.7 Hz, 1H), 3.26 (s, 3H), 2.40 (s, 3H), 1.28 (d, $J$ =

6.7 Hz, 3H), 1.22 (d, *J* = 6.7 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 3H);LC-MS (ESI): calcd for $C_{24}H_{27}N_5O$ m/z $[M+H]^+$ 402.22, found 402.23. Retention time 3.232 min, HPLC purity = 99.648%.

**(R)-6-((4-(2-aminophenyl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (20).** Compound **20** was prepared as a yellow solid using a method similar to that described for compound **7** in 15% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.53 (s, 1H), 8.42 (dd, *J* = 5.5, 0.9 Hz, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 2.2 Hz, 1H), 7.31 – 7.24 (m, 1H), 7.24 – 7.11 (m, 2H), 7.00 (d, *J* = 8.8 Hz, 3H), 6.80 (d, *J* = 8.2 Hz, 1H), 6.59 (td, *J* = 7.4, 1.3 Hz, 1H), 4.09 (p, *J* = 7.9, 7.3 Hz, 1H), 3.86 (p, *J* = 6.7 Hz, 1H), 3.26 (s, 3H), 1.27 (d, *J* = 6.6 Hz, 3H), 1.21 (d, *J* = 6.5 Hz, 3H), 0.99 (d, *J* = 6.6 Hz, 3H); LC-MS (ESI): calcd for $C_{23}H_{26}N_6O$ m/z $[M+H]^+$ 403.22, found 403.15. Retention time 2.76 min, HPLC purity = 97.821%.

**(R)-6-((4-(3-aminophenyl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (21).** Compound **21** was prepared as a yellow solid using a method similar to that described for compound **7** in 16.5% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.51 (s, 1H), 8.48 (d, *J* = 5.1 Hz, 1H), 7.63 (d, *J* = 2.3 Hz, 1H), 7.37 – 7.25 (m, 3H), 7.25 – 7.13 (m, 2H), 7.02 (d, *J* = 8.7 Hz, 1H), 6.73 (dt, *J* = 8.1, 1.6 Hz, 1H), 5.29 (s, 2H), 4.10 (q, *J* = 6.7 Hz, 1H), 3.90 (p, *J* = 6.6 Hz, 1H), 3.26 (s, 3H), 1.25 (d, *J* = 6.6 Hz, 3H), 1.21 (d, *J* = 6.5 Hz, 3H), 0.98 (d, *J* = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{23}H_{26}N_6O$ m/z $[M+H]^+$ 403.22, found 403.21. Retention time 2.485 min, HPLC purity = 99.658%.

**(R)-6-((4-(4-aminophenyl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-**

**dihydroquinoxalin-2(1H)-one (22).** Compound **22** was prepared as a yellow solid using a method similar to that described for compound **7** in 14% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.33 (s, 1H), 8.34 (d, $J$ = 5.3 Hz, 1H), 7.92 (d, $J$ = 8.3 Hz, 2H), 7.71 (s, 1H), 7.19 (dd, $J$ = 21.1, 7.3 Hz, 2H), 7.00 (d, $J$ = 8.7 Hz, 1H), 6.65 (d, $J$ = 8.3 Hz, 2H), 5.77 (s, 2H), 4.10 (q, $J$ = 6.7 Hz, 1H), 3.94 (p, $J$ = 6.7 Hz, 1H), 3.26 (s, 3H), 1.29 (d, $J$ = 6.5 Hz, 3H), 1.22 (t, $J$ = 5.3 Hz, 3H), 0.99 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{23}H_{26}N_6O$ m/z $[M+H]^+$ 403.22, found 403.25. Retention time 2.651 min, HPLC purity = 97.661%.

**(R)-6-((4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (23).** Compound **23** was prepared as a yellow solid using a method similar to that described for compound **7** in 10% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.49 (s, 1H), 8.44 (d, $J$ = 5.2 Hz, 1H), 7.97 (dd, $J$ = 8.7, 7.1 Hz, 1H), 7.59 (d, $J$ = 2.3 Hz, 1H), 7.29 – 7.18 (m, 2H), 7.10 (dd, $J$ = 11.5, 2.4 Hz, 1H), 6.99 – 6.87 (m, 2H), 4.06 (q, $J$ = 6.7 Hz, 1H), 3.88 (s, 3H), 3.80 (q, $J$ = 6.6 Hz, 1H), 3.23 (s, 3H), 1.18 (dd, $J$ = 13.5, 6.6 Hz, 6H), 0.95 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{26}FN_6O_2$ m/z $[M+H]^+$ 436.21, found 436.23. Retention time 3.261 min, HPLC purity = 98.539%.

**(R)-6-((4-(4-fluoro-2-methylphenyl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-on (24).** Compound **24** was prepared as a yellow solid using a method similar to that described for compound **7** in 17% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.56 (s, 1H), 8.49 (d, $J$ = 5.0 Hz, 1H), 7.57 – 7.47 (m, 2H), 7.21 (dd, $J$ = 9.7, 6.0, 2.4 Hz, 2H), 7.14 (td, $J$ = 8.6, 2.7 Hz, 1H), 6.98 – 6.89 (m, 2H),

4.05 (q, *J* = 6.7 Hz, 1H), 3.74 (p, *J* = 6.6 Hz, 1H), 3.21 (s, 3H), 2.40 (s, 3H), 1.15 (t, *J* = 6.8 Hz, 6H), 0.94 (d, *J* = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{26}FN_5O$ m/z $[M+H]^+$ 420.21, found 420.23. Retention time 3.765 min, HPLC purity = 99.447%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-(3-(methylamino)phenyl)pyrimidin-2-yl)amino)-3,4-dihydroquinoxalin-2(1H)-one (25).** Compound **25** was prepared as a white solid using a method similar to that described for compound **7** in 9% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.50 (s, 1H), 8.49 (d, *J* = 5.2 Hz, 1H), 7.58 (d, *J* = 2.2 Hz, 1H), 7.37 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.33 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.30 – 7.20 (m, 3H), 7.01 (d, *J* = 8.7 Hz, 1H), 6.72 (dd, *J* = 8.0, 2.4, 1.0 Hz, 1H), 4.09 (q, *J* = 6.7 Hz, 1H), 3.88 (p, *J* = 6.7 Hz, 1H), 3.26 (s, 3H), 2.76 (s, 3H), 1.25 (d, *J* = 6.6 Hz, 3H), 1.20 (d, *J* = 6.5 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{28}N_6O$ m/z $[M+H]^+$ 417.23, found 417.25. Retention time 2.647 min, HPLC purity = 99.204%.

**(R)-6-((4-(3-(ethylamino)phenyl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (26).** Compound **26** was prepared as a white solid using a method similar to that described for compound **7** in 7% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.50 (s, 1H), 8.49 (d, *J* = 5.2 Hz, 1H), 7.59 (d, *J* = 2.2 Hz, 1H), 7.45 – 7.28 (m, 3H), 7.23 (dd, *J* = 8.8, 6.4 Hz, 2H), 7.00 (d, *J* = 8.7 Hz, 1H), 6.78 – 6.66 (m, 1H), 4.10 (q, *J* = 6.7 Hz, 1H), 3.89 (p, *J* = 6.6 Hz, 1H), 3.26 (s, 3H), 3.13 (q, *J* = 7.1 Hz, 2H), 1.28 – 1.14 (m, 9H), 0.99 (d, *J* = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{25}H_{30}N_6O$ m/z $[M+H]^+$ 431.25, found 431.26. Retention time 2.683 min, HPLC purity = 97.197%.

**(R)-4-isopropyl-6-((4-(3-(isopropylamino)phenyl)pyrimidin-2-yl)amino)-1,3-di**

**methyl-3,4-dihydroquinoxalin-2(1H)-one (27).** Compound **27** was prepared as a white solid using a method similar to that described for compound **7** in 12% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.49 (s, 1H), 8.49 (d, $J$ = 5.2 Hz, 1H), 7.57 (d, $J$ = 2.2 Hz, 1H), 7.38 (dd, $J$ = 8.7, 2.2 Hz, 1H), 7.32 – 7.26 (m, 2H), 7.21 (dd, $J$ = 9.3, 6.4 Hz, 2H), 6.99 (d, $J$ = 8.7 Hz, 1H), 6.73 (dt, $J$ = 7.9, 1.5 Hz, 1H), 5.61 (d, $J$ = 8.0 Hz, 1H), 4.10 (q, $J$ = 6.7 Hz, 1H), 3.89 (p, $J$ = 6.6 Hz, 1H), 3.65 (q, $J$ = 6.6 Hz, 1H), 3.26 (s, 3H), 1.26 (d, $J$ = 6.6 Hz, 3H), 1.21 (d, $J$ = 6.6 Hz, 3H), 1.19 – 1.14 (m, 6H), 0.99 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{26}H_{32}N_6O$ m/z $[M+H]^+$ 445.26, found 445.24. Retention time 2.697 min, HPLC purity = 100%.

**(R)-6-((4-(3-(cyclopentylamino)phenyl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (28).** Compound 2**8** was prepared as a white solid using a method similar to that described for compound **7** in 7% yield.$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.45 (d, $J$ = 5.2 Hz, 1H), 7.46 – 7.33 (m, 2H), 7.31 (q, $J$ = 3.8, 3.0 Hz, 2H), 7.29 (m, 1H), 7.19 – 7.16 (m, 1H), 7.14 (d, $J$ = 2.3 Hz, 1H), 7.12 (d, $J$ = 5.2 Hz, 1H), 6.94 (d, $J$ = 8.6 Hz, 1H), 6.75 (dd, $J$ = 7.9, 2.4, 1.0 Hz, 1H), 4.20 (q, $J$ = 6.8 Hz, 1H), 3.98 (p, $J$ = 6.7 Hz, 1H), 3.90 (p, $J$ = 6.2 Hz, 1H), 3.40 (s, 3H), 2.08 (td, $J$ = 14.7, 13.4, 6.8 Hz, 2H), 1.78 (q, $J$ = 6.0, 4.6 Hz, 2H), 1.72 – 1.62 (m, 2H), 1.52 (dt, $J$ = 13.4, 6.7 Hz, 2H), 1.34 (d, $J$ = 6.7 Hz, 3H), 1.28 (d, $J$ = 6.5 Hz, 3H), 1.15 (d, $J$ = 6.8 Hz, 3H); LC-MS (ESI): calcd for $C_{28}H_{34}N_6O$ m/z $[M+H]^+$ 471.28, found 471.20. Retention time 3.032 min, HPLC purity = 98.164%.

**(R)-6-((4-(3-(cyclohexylamino)phenyl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (29).** Compound **29** was prepared as a

white solid using a method similar to that described for compound **7** in 6.6% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.49 (s, 1H), 8.49 (d, $J$ = 5.2 Hz, 1H), 7.53 (d, $J$ = 2.2 Hz, 1H), 7.40 (dd, $J$ = 8.7, 2.2 Hz, 1H), 7.32 (s, 1H), 7.27 (d, $J$ = 7.5 Hz, 1H), 7.24 – 7.15 (m, 2H), 7.00 (d, $J$ = 8.7 Hz, 1H), 6.75 (d, $J$ = 8.0 Hz, 1H), 5.63 (m, 1H), 4.10 (q, $J$ = 6.7 Hz, 1H), 3.88 (p, $J$ = 6.5 Hz, 1H), 3.26 (s, 3H), 1.96 (d, $J$ = 12.6 Hz, 2H), 1.73 (d, $J$ = 12.7 Hz, 2H), 1.61 (d, $J$ = 12.6 Hz, 2H), 1.28 – 1.22 (m, 7H), 1.21 (d, $J$ = 6.6 Hz, 3H), 0.99 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{29}H_{36}N_6O$ m/z $[M+H]^+$ 485.30, found 485.16. Retention time 3.055 min, HPLC purity = 96.265%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-(3-(phenylamino)phenyl)pyrimidin-2-yl)amino)-3,4-dihydroquinoxalin-2(1H)-one (30).** Compound **30** was prepared as a white solid using a method similar to that described for compound **7** in 29% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.53 (s, 1H), 8.52 (d, $J$ = 5.1 Hz, 1H), 8.39 (s, 1H), 7.91 (d, $J$ = 2.0 Hz, 1H), 7.57 (dt, $J$ = 7.7, 1.2 Hz, 1H), 7.52 (d, $J$ = 2.2 Hz, 1H), 7.42 – 7.33 (m, 2H), 7.30 – 7.19 (m, 4H), 7.14 (dq, $J$ = 6.9, 1.4 Hz, 2H), 6.93 – 6.81 (m, 2H), 4.08 (q, $J$ = 6.7 Hz, 1H), 3.86 (p, $J$ = 6.6 Hz, 1H), 3.24 (s, 3H), 1.23 (d, $J$ = 6.6 Hz, 3H), 1.16 (d, $J$ = 6.6 Hz, 3H), 0.96 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{29}H_{30}N_6O$ m/z $[M+H]^+$ 479.25, found 479.19. Retention time 3.565 min, HPLC purity = 97.132%.

**(R)-6-((4-(3-amino-2-methylphenyl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (31).** Compound **31** was prepared as a white solid using a method similar to that described for compound **7** in 22% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.49 (s, 1H), 8.45 (d, $J$ = 5.0 Hz, 1H), 7.62 – 7.56 (m, 1H),

7.24 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.94 (dd, *J* = 8.5, 3.6 Hz, 2H), 6.82 (d, *J* = 5.0 Hz, 1H), 6.68 (d, *J* = 2.4 Hz, 1H), 6.56 (dd, *J* = 8.1, 2.5 Hz, 1H), 5.00 (s, 2H), 4.04 (q, *J* = 6.6 Hz, 1H), 3.77 (p, *J* = 6.6 Hz, 1H), 3.21 (s, 3H), 2.19 (s, 3H), 1.15 (t, *J* = 6.0 Hz, 6H), 0.94 (d, *J* = 6.8 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{28}N_6O$ m/z $[M+H]^+$ 417.23, found 417.19. Retention time 2.519 min, HPLC purity = 95.896%.

**(R)-6-((4-(5-amino-2-methylphenyl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (32).** Compound **32** was prepared as a white solid using a method similar to that described for compound **7** in 25.4% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.50 (s, 1H), 8.44 (d, *J* = 5.0 Hz, 1H), 7.62 – 7.56 (m, 1H), 7.23 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.02 – 6.90 (m, 2H), 6.80 (d, *J* = 5.0 Hz, 1H), 6.71 (dd, *J* = 8.0, 1.3 Hz, 1H), 6.61 (dd, *J* = 7.6, 1.3 Hz, 1H), 5.00 (s, 2H), 4.04 (q, *J* = 6.7 Hz, 1H), 3.75 (p, *J* = 6.6 Hz, 1H), 3.21 (s, 3H), 2.05 (s, 3H), 1.15 (dd, *J* = 9.5, 6.6 Hz, 6H), 0.94 (d, *J* = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{24}H_{28}N_6O$ m/z $[M+H]^+$ 417.23, found 417.20. Retention time 2.537 min, HPLC purity = 95.749%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-(1,2,3,4-tetrahydroquinolin-5-yl)pyrimidin-2-yl)amino)-3,4-dihydroquinoxalin-2(1H)-one (33).** Compound **33** was prepared as a white solid using a method similar to that described for compound **7** in 24.6% yield.$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.42 (d, *J* = 5.1 Hz, 1H), 7.46 (d, *J* = 2.3 Hz, 1H), 7.09 – 6.97 (m, 2H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 5.1 Hz, 1H), 6.74 (d, *J* = 7.4 Hz, 1H), 6.55 (d, *J* = 8.0 Hz, 1H), 4.14 – 4.07 (m, 1H), 3.89 (p, *J* = 6.6 Hz, 1H), 3.35 (s, 3H), 3.32 (t, *J* = 5.6 Hz, 2H), 2.81 (dt, *J* = 6.2, 3.1 Hz, 2H), 1.87 (q, *J* = 5.9 Hz, 2H), 1.26 (d, *J* = 6.9Hz, 3H), 1.22 (d, *J* = 6.6 Hz, 3H), 1.10 (d, *J* = 6.8 Hz, 3H);

LC-MS (ESI): calcd for $C_{26}H_{30}N_6O$ m/z $[M+H]^+$ 443.25, found 443.24. Retention time 2.579 min, HPLC purity = 99.864%.

**(R)-6-((4-(1H-indol-3-yl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (34).** Compound **34** was prepared as a yellow solid using a method similar to that described for compound **7** in 26.3% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.80 (d, $J$ = 2.9 Hz, 1H), 9.27 (s, 1H), 8.60 (d, $J$ = 7.8 Hz, 1H), 8.31 (dd, $J$ = 8.5, 4.1 Hz, 2H), 7.51 – 7.45 (m, 2H), 7.40 (dd, $J$ = 8.7, 2.2 Hz, 1H), 7.27 – 7.11 (m, 3H), 7.01 (d, $J$ = 8.7 Hz, 1H), 4.09 (q, $J$ = 6.7 Hz, 1H), 3.85 (p, $J$ = 6.6 Hz, 1H), 3.28 (s, 3H), 1.23 (d, $J$ = 6.2 Hz, 3H), 1.18 (d, $J$ = 6.5 Hz, 3H), 1.00 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{25}H_{26}N_6O$ m/z $[M+H]^+$ 427.22, found 427.27. Retention time 2.779 min, HPLC purity = 99.724%.

**(R)-6-((4-(1H-indol-4-yl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (35).** Compound **35** was prepared as a yellow solid using a method similar to that described for compound **7** in 29.8% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.40 (s, 1H), 9.52 (s, 1H), 8.52 (d, $J$ = 5.2 Hz, 1H), 7.73 – 7.64 (m, 2H), 7.59 (dd, $J$ = 8.1, 1.0 Hz, 1H), 7.51 (t, $J$ = 2.8 Hz, 1H), 7.36 – 7.28 (m, 2H), 7.24 (t, $J$ = 7.7 Hz, 1H), 7.07 (s, 1H), 6.99 (d, $J$ = 8.7 Hz, 1H), 4.07 (q, $J$ = 6.7 Hz, 1H), 3.85 (p, $J$ = 6.6 Hz, 1H), 3.26 (s, 3H), 1.16 (dd, $J$ = 6.6, 2.6 Hz, 6H), 0.97 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{25}H_{26}N_6O$ m/z $[M+H]^+$ 427.22, found 427.28. Retention time 2.875 min, HPLC purity = 98.975%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-(2-methyl-2H-indazol-4-yl)pyrimidin-2-yl)amino)-3,4-dihydroquinoxalin-2(1H)-one (36).** Compound **36** was prepared as a

yellow solid using a method similar to that described for compound **7** in 36% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.50 (s, 1H), 9.00 (s, 1H), 8.52 (d, $J$ = 5.3 Hz, 1H), 7.86 (d, $J$ = 7.0 Hz, 1H), 7.78 (d, $J$ = 8.5 Hz, 1H), 7.49 – 7.40 (m, 2H), 7.41 – 7.27 (m, 2H), 7.03 (d, $J$ = 8.7 Hz, 1H), 4.20 (s, 3H), 4.08 (q, $J$ = 6.7 Hz, 1H), 3.80 (p, $J$ = 6.7 Hz, 1H), 3.26 (s, 3H), 1.18 (dd, $J$ = 11.0, 6.8 Hz, 6H), 0.97 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{25}H_{27}N_7O$ m/z $[M+H]^+$ 442.23, found 442.25. Retention time 2.836 min, HPLC purity = 98.257%.

**(R)-4-isopropyl-6-((4-(isoquinolin-5-yl)pyrimidin-2-yl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (37).** Compound **37** was prepared as a yellow solid using a method similar to that described for compound **7** in 44% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.72 (s, 1H), 9.45 – 9.39 (m, 1H), 8.63 (d, $J$ = 5.0 Hz, 1H), 8.52 (d, $J$ = 6.0 Hz, 1H), 8.28 (d, $J$ = 8.2 Hz, 1H), 8.19 (d, $J$ = 6.0 Hz, 1H), 8.02 (dd, $J$ = 7.2, 1.2 Hz, 1H), 7.81 (dd, $J$ = 8.2, 7.2 Hz, 1H), 7.70 (s, 1H), 7.13 (d, $J$ = 5.0 Hz, 2H), 6.93 (d, $J$ = 8.7 Hz, 1H), 3.98 (q, $J$ = 6.5 Hz, 1H), 3.55 (m, 1H), 3.20 (s, 3H), 0.99 (d, $J$ = 6.5 Hz, 3H), 0.88 (d, $J$ = 6.7 Hz, 3H), 0.85 – 0.72 (m, 3H); LC-MS (ESI): calcd for $C_{26}H_{26}N_6O$ m/z $[M+H]^+$ 439.22, found 439.12. Retention time 2.598 min, HPLC purity = 100%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-(quinolin-5-yl)pyrimidin-2-yl)amino)-3,4-dihydroquinoxalin-2(1H)-one (38).** Compound **38** was prepared as a yellow solid using a method similar to that described for compound **7** in 47% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.74 (s, 1H), 8.99 (dd, $J$ = 4.1, 1.7 Hz, 1H), 8.77 – 8.70 (m, 1H), 8.64 (d, $J$ = 5.0 Hz, 1H), 8.18 (dt, $J$ = 8.2, 1.2 Hz, 1H), 7.94 – 7.80 (m, 2H), 7.71 (s,

1H), 7.58 (dd, *J* = 8.7, 4.1 Hz, 1H), 7.15 (t, *J* = 6.8 Hz, 2H), 6.94 (d, *J* = 8.7 Hz, 1H), 4.00 (q, *J* = 6.6 Hz, 1H), 3.59 – 3.39 (m, 1H), 3.22 (s, 3H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.90 (d, *J* = 6.7 Hz, 3H), 0.84 (d, *J* = 8.9 Hz, 3H); LC-MS (ESI): calcd for $C_{26}H_{26}N_6O$ m/z $[M+H]^+$ 439.22, found 439.20. Retention time 2.637 min, HPLC purity = 98.598%.

**(R)-4-isopropyl-1,3-dimethyl-6-((4-(quinolin-4-yl)pyrimidin-2-yl)amino)-3,4-dihydroquinoxalin-2(1H)-one (39).** Compound **39** was prepared as a yellow solid using a method similar to that described for compound **7** in 49% yield.$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.05 (d, *J* = 4.4 Hz, 1H), 8.62 (d, *J* = 4.9 Hz, 1H), 8.33 – 8.20 (m, 2H), 7.80 (ddd, *J* = 8.4, 6.8, 1.4 Hz, 1H), 7.62 – 7.54 (m, 3H), 7.45 (s, 1H), 7.05 (d, *J* = 4.9 Hz, 1H), 6.96 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 1H), 4.14 (q, *J* = 6.8 Hz, 1H), 3.75 – 3.64 (m, 1H), 3.36 (s, 3H), 1.12 (d, *J* = 6.5 Hz, 3H), 1.07 (d, *J* = 6.8 Hz, 3H), 0.99 (d, *J* = 6.6 Hz, 3H); LC-MS (ESI): calcd for $C_{26}H_{26}N_6O$ m/z $[M+H]^+$ 439.22, found 439.19. Retention time 2.931 min, HPLC purity = 95.3%.

**(R)-4-isopropyl-6-((4-(isoquinolin-4-yl)pyrimidin-2-yl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (40)**. Compound **40** was prepared as a yellow solid using a method similar to that described for compound **7** in 60% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.73 (s, 1H), 9.44 (s, 1H), 8.70 (s, 1H), 8.64 (d, *J* = 4.9 Hz, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 7.84 (m,, 1H), 7.77 (m, 1H), 7.72 (s, 1H), 7.18 (d, *J* = 4.9 Hz, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 1H), 3.98 (q, *J* = 6.6 Hz, 1H), 3.46 (m, 1H), 3.20 (s, 3H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.88 (d, *J* = 6.7 Hz, 3H), 0.82 (m , 3H).LC-MS (ESI): calcd for $C_{26}H_{26}N_6O$ m/z $[M+H]^+$

439.22, found 439.14. Retention time 2.668 min, HPLC purity = 99.351%.

**(R)-6-((5-fluoro-4-(isoquinolin-4-yl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (41).** Following the procedure for the synthesis of compound **7** from compound **53a**,compound **41** was obtained with **53b** instead of **53a** as a yellow solid with 23% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.82 (s, 1H), 9.49 (s, 1H), 8.73 (dd, $J$ = 16.2, 1.7 Hz, 2H), 8.28 (d, $J$ = 7.9 Hz, 1H), 8.01 (d, $J$ = 8.4 Hz, 1H), 7.81 (dddd, $J$ = 23.5, 8.0, 6.8, 1.3 Hz, 2H), 7.64 (s, 1H), 7.11 – 7.04 (m, 1H), 6.92 (d, $J$ = 8.7 Hz, 1H), 3.97 (q, $J$ = 6.7 Hz, 1H), 3.55 – 3.47 (m, 1H), 3.19 (s, 3H), 0.98 (d, $J$ = 6.5 Hz, 3H), 0.87 (d, $J$ = 6.7 Hz, 3H), 0.82 (d, $J$ = 5.7 Hz, 3H); LC-MS (ESI): calcd for $C_{26}H_{25}FN_6O$ m/z $[M+H]^+$ 457.21, found 457.18. Retention time 2.789 min, HPLC purity = 96.046%.

**(R)-6-((5-chloro-4-(isoquinolin-4-yl)pyrimidin-2-yl)amino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (42).** Following the procedure for the synthesis of compound **7** from compound **53a**,compound **42** was obtained with **53c** instead of **53a** as a yellow solid with 22% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.96 (s, 1H), 9.46 (s, 1H), 8.73 (s, 1H), 8.61 (s, 1H), 8.26 (dd, $J$ = 7.5, 2.0 Hz, 1H), 7.86 – 7.72 (m, 3H), 7.59 (s, 1H), 7.03 (s, 1H), 6.92 (d, $J$ = 8.6 Hz, 1H), 3.96 (d, $J$ = 7.1 Hz, 1H), 3.48 (m, 1H), 3.18 (s, 3H), 0.94 (m, 3H), 0.85 (d, $J$ = 6.7 Hz, 3H), 0.78 (m, 3H); LC-MS (ESI): calcd for $C_{26}H_{25}ClN_6O$ m/z $[M+H]^+$ 473.18, found 473.14. Retention time 2.868 min, HPLC purity = 97.252%.

**(R)-4-isopropyl-6-((4-(isoquinolin-4-yl)-5-methylpyrimidin-2-yl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (43).** Following the procedure for the

synthesis of compound **7** from compound **53a**, compound **43** was obtained with **53d** instead of **53a** as a yellow solid with 26% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.58 (s, 1H), 9.43 (s, 1H), 8.53 (s, 2H), 8.25 (dd, $J$ = 7.3, 1.9 Hz, 1H), 7.83 – 7.72 (m, 2H), 7.69 (s, 2H), 7.05 – 6.98 (m, 1H), 6.88 (d, $J$ = 8.7 Hz, 1H), 3.94 (q, $J$ = 6.7 Hz, 1H), 3.39 (m, 1H), 3.17 (s, 3H), 1.96 (s, 3H), 0.92 (d, $J$ = 6.5 Hz, 3H), 0.85 (d, $J$ = 6.7 Hz, 3H), 0.75 (m 3H); LC-MS (ESI): calcd for $C_{27}H_{28}N_6O$ m/z $[M+H]^+$ 453.23, found 453.19. Retention time 2.679 min, HPLC purity = 98.367%.

**(R)-6-([1,1'-biphenyl]-3-ylamino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (11).** *Step 1： [1,1'-biphenyl]-3-amine (**55a**).* To a solution of 3-bromoaniline (212 mg, 1.2 mmol ) and $K_2CO_3$ (227 mg , 1.6 mmol) in 1.4-dioxane : $H_2O$ = 4:1(10 mL) was added phenylboronic acid( 100 mg , 0.821 mmol), the mixture was bubbled with $N_2$ for 5 min, then $Pd(dppf)_2Cl_2$ (67 mg , 0.082 mmol) was added and the mixture was further bubbled with $N_2$ for other 5 min. the mixture was heated to 80 °C for 8h. The reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water and extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated by evaporation under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 10 to 50% EtOAc/60-90 °C petroleum ether) to afford compound **55a** (87 mg, 0.515 mmol, 62.3% yield) as a brown solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.62 – 7.56 (m, 2H), 7.49 – 7.41 (m, 2H), 7.40 – 7.35 (m, 1H), 7.26 (d, $J$ = 7.8 Hz, 1H), 7.05 – 7.00 (m, 1H), 6.95 (d, $J$ = 2.1 Hz, 1H), 6.75 – 6.68 (m, 1H), 3.69 (s, 2H);

*Step 2: (R)-6-([1,1'-biphenyl]-3-ylamino)-4-isopropyl-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (11).* To a solution of compound **55a** (50 mg,0.295 mmol ) and compound **48** (106 mg , 0.355 mmol) in anhydrous 1.4-dioxane (10 mL) was added *t*-BuONa (57 mg , 0.570 mmol ), the mixture was bubbled with $N_2$ for 5 min ,then $Pa_2(dba)_3$ (28 mg, 0.036 mmol ) and Xphos(29 mg, 0.072 mmol) was added, the mixture was further bubbled with $N_2$ for 5 min, the mixture was heated to 100 °C for 8 h. The reaction was monitored by TLC. Upon completion, the resulting solution was filtered through Celite and concentrated, the residue was purified by silica gel column chromatography (gradient elution, gradient 1 to 5% MeOH/DCM) to afford compound **11** (34 mg, 0.088 mmol, 29.8% yield) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.12 (s, 1H), 7.60 – 7.52 (m, 2H), 7.43 (t, $J$ = 7.7 Hz, 2H), 7.38 – 7.29 (m, 1H), 7.32 – 7.22 (m, 2H), 7.05 – 6.92 (m, 3H), 6.65 (dd, $J$ = 6.5, 2.3 Hz, 2H), 4.07 (q, $J$ = 6.7 Hz, 1H), 3.79 (p, $J$ = 6.6 Hz, 1H), 3.22 (s, 3H), 1.22 (d, $J$ = 6.7 Hz, 3H), 1.18 (d, $J$ = 6.5 Hz, 3H), 0.97 (d, $J$ = 6.7 Hz, 3H); LC-MS (ESI): calcd for $C_{25}H_{27}N_3O$ m/z $[M+H]^+$ 386.22, found 386.07. Retention time 3.98 min, HPLC purity = 95.337%.

**(R)-4-isopropyl-1,3-dimethyl-6-((3-(pyridin-4-yl)phenyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (12)**. Compound **12** was prepared as a white solid using a method similar to that described for compound **11** in 22% yield.$^1$H NMR (400 MHz, Chloroform-*d*) δ 8.66 (d, $J$ = 5.1 Hz, 2H), 7.53 – 7.47 (m, 2H), 7.38 (t, $J$ = 7.8 Hz, 1H), 7.32 – 7.26 (m, 1H), 7.13 (dd, $J$ = 22.4, 7.8, 1.9 Hz, 2H), 6.91 (d, $J$ = 8.3 Hz,

1H), 6.69 (d, *J* = 9.7 Hz, 2H), 5.88 (s, 1H), 4.20 (q, *J* = 6.8 Hz, 1H), 3.79 (dq, *J* = 36.6, 6.8 Hz, 1H), 3.39 (s, 3H), 1.30 (d, *J* = 6.7 Hz, 3H), 1.28 – 1.25 (m, 3H), 1.15 (d, *J* = 6.8 Hz, 3H). LC-MS (ESI): calcd for $C_{24}H_{26}N_4O$ m/z $[M+H]^+$ 387.21, found 387.22. Retention time 2.687 min, HPLC purity = 98.815%.

**(R)-4-isopropyl-6-((2'-methoxy-[1,1'-biphenyl]-3-yl)amino)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one (13).** Compound **13** was prepared as a white solid using a method similar to that described for compound **11** in 15% yield.$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.07 (s, 1H), 7.37 – 7.29 (m, 1H), 7.28 – 7.20 (m, 2H), 7.14 (d, *J* = 1.9 Hz, 1H), 7.10 (d, *J* = 8.3 Hz, 1H), 7.03 – 6.91 (m, 3H), 6.85 (d, *J* = 7.6 Hz, 1H), 6.70 – 6.63 (m, 2H), 4.08 (q, *J* = 6.6 Hz, 1H), 3.78 (m, 1H), 3.77 (s, 3H), 3.23 (s, 3H), 1.24 (d, *J* = 6.7 Hz, 3H), 1.18 (d, *J* = 6.6 Hz, 3H), 0.98 (d, *J* = 6.6 Hz, 3H). LC-MS (ESI): calcd for $C_{26}H_{29}N_3O_2$ m/z $[M+H]^+$ 415.23, found 416.08. Retention time 3.899 min, HPLC purity = 95.415%.

**(R)-3'-((4-isopropyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxalin-6-yl)amino)-[1,1'-biphenyl]-3-carbonitrile (14).** Compound **14** was prepared as a white solid using a method similar to that described for compound **11** in 19% yield .$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.17 (s, 1H), 8.02 (t, *J* = 1.8 Hz, 1H), 7.96 – 7.88 (m, 1H), 7.81 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.64 (t, *J* = 7.8 Hz, 1H), 7.35 – 7.26 (m, 2H), 7.12 – 7.01 (m, 2H), 6.96 (d, *J* = 9.0 Hz, 1H), 6.69 – 6.62 (m, 2H), 4.08 (q, *J* = 6.7 Hz, 1H), 3.79 (p, *J* = 6.6 Hz, 1H), 3.22 (s, 3H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.18 (d, *J* = 6.5 Hz, 3H), 0.97 (d, *J* = 6.7 Hz, 3H). LC-MS (ESI): calcd for $C_{26}H_{26}N_4O$ m/z $[M+H]^+$ 411.21, found 411.08. Retention time 3.836 min, HPLC purity = 95.697%.

**BRD4-BD1 protein expression and crystallization**

The BRD4-BD1 protein expression followed our previous method[18]. Briefly, colonies from freshly transformed plasmid DNA in *E. coli* BL21(DE3)-codon plus-RIL cells were grown overnight at 37 °C in 50 mL of Terrific Broth medium, then cells were grown at 37 °C to an optical density of ~0.8 at OD600 before the temperature was decreased to 16 °C. The system was equilibrated at 16 °C until the optical density was ~1.2 at OD600. Then protein expression was induced overnight at 16 °C with 0.2 mmol isopropyl-β-D-thiogalactopyranoside (IPTG). The bacteria were harvested and cells expressing His6-tagged proteins were purified with previous protocol[18]. Finally, the eluted protein was treated overnight at 4 °C 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 for protein binding assay and crystallization.

The vapor diffusion method was used for crystallization of aliquots of the purified proteins. The complex structure of BRD4-BD1, and the ligands were grown at 18 °C in 1 μL of protein with an equal volume of reservoir solution containing the sodium formate and 10-20% glycerol. Crystals grew to diffracting quality within 1 weeks in all cases.

Data were collected at 100 K on beam line BL17U at the Shanghai Synchrotron Radiation Facility (SSRF) (Shanghai, China) for the co-crystallized structures[36]. The

data were processed with the HKL2000 [37]software packages, and the structures were then solved by molecular replacement using the CCP4 program MOLREP[38]. 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.[39] With the aid of the program Coot[40], compound, water molecules, and others were fitted into the initial $F_o$–$F_c$ maps.

**Docking study**

the BRD4-BD1 structure (PDB code: 6JI5) and CDK9 structure (PDB code: 6GZH) were processed with protein preparation module in Schrödinger software package, and KAc binding site of BRD4-BD1 and ATP binding site of CDK9 were selected for making the grid file with Glide module[41]. Then the ligands were prepared to add the partial charge, minimize to obtain the low-energy conformation. Finally, ligands were docked into the grid files with default parameters at standard precision mode, and the predicted binding modes were depicted with pymol software.

**BRD4-BD1 fluorescence polarization assay**

The binding of compounds to BRD4 was assessed using a fluorescence anisotropy binding assay as reported before[18-20, 42, 43]. Generally, the method involves incubating the bromodomain protein BRD4, fluorescent ligand to reach thermodynamic equilibrium. The test compound or DMSO vehicle (2 ‰ final) in a Corning 384 well Black low volume plate (CLS3575) were added to this reaction mixture and equilibrated in the dark for 4 h at room temperature.The anisotropy of the unbound

fluorescent ligand is measurably different from the bound value. Fluorescence anisotropy was read on a BioTek Synergy2 multimode microplate reader ($\lambda_{ex}$ = 485 nm, $\lambda_{em}$ = 530 nm; dichroism, −505 nM).

**CDK9 enzymatic activity assay**

The mobility Shift assay method was used for testing CDK9 activity. The CDK9 protein was purchased from Carna (batch No. 14CBS-0084G) and the Caliper substrate CTD3 was purchased from GL (batch No. P171207-MJ346885). Generally, the compounds were incubated with the CDK9 protein and substrate CTD3 to reach thermodynamic equilibrium. Then, the activity was measured with mobility Shift method. Dinaciclib was used as the positive control.

**Bromodomain Selectivity**

Bromodomains of BRD4-BD2, BRD2-BD1, BRD2-BD2 and EP300 were expressed similar as BRD4-BD1. And the fluorescence polarization assay protocol was used to test the binding activities of compound **40** towards these bromodomains.

**The kinase Profiling study**

Diversity kinase panel from Eurofin Scientific company (www.eurofins.com) were choosed to assess the kinase selectivity, which included about 60 kinases from all kinases subfamilies. The result was listed in Table S1.

**Cellular antiproliferation assay**

Different cells were cultured in the corresponding medium conditions recommended by the suppliers. Different cells were seeded onto 96-well plates at a suitable density 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, the IC50 was measured with the Cell Counting Kit-8 (CCK-8) assay (Dojindo, Japan) for suspended cells or sulforhodamine B (SRB) (Sigma; MO, USA) for adherent cells.

**Western Blot test**

HCT116 cells were plated into 6-well plates and incubated for 24 h. Then, cells were harvested after 24 h of treatment with compounds at the indicated doses. Western blotting was performed using a previously described method[44].

**In vivo Pharmacokinetic Study**

Compound **40** dissolved in 1%DMSO/0.5%HPMC(5/95, v/v) to a concentration of 1 mg/mL, and was given to ICR mice (Male, 20 – 22 g, n = 3) by intraperitoneal injection administration. Animal procedures were performed according to institutional ethical guidelines of animal care. Blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, and 24 h after administration (anticoagulant: EDTA-$Na_2$). 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 these compounds, 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.

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## ABBREVIATIONS USED

BET, bromodomain and extra-terminal domain ; KAc , acetylated lysine ;VDW, Van Der Waals ;SAR, Structure-Activity Relationship ; Boc, *tert*-butoxycarbonyl ;

## ASSOCIATED CONTENT

### Supporting information

The Supporting Information is available free of charge at ACS, which includes the

kinase selectivity of compound **40**,the CDK2 selectivity result of partial compounds , the BET BDs selectivity of compound **40** , the Non-BET BDs selectivity of compound **40**, Proliferation inhibition of 22 cancer cell lines of compound **40**,the statistical information of crystallography, details of docking study, Superimposition of crystal structures of BI-2536 with compound **7**, The triple experiments of western blot study of compounds **40** and the spectra of typical synthesized compounds.

Table of molecular formula strings.

**Accession Codes**

Cocrystal structures of compounds **7** and **40** bound to BRD4-BD1 were deposited into PDB database with the entry code 6LIH and 6LIM, respectively. Authors will release the atomic coordinates and experimental data upon article publication.

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