Bioorganic Chemistry 68 (2016) 236–244

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Design, synthesis and biological evaluation of dihydroquinoxalinone derivatives as BRD4 inhibitors

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ARTICLE INFO

Article history:
Received 17 June 2016
Revised 22 August 2016
Accepted 23 August 2016
Available online 24 August 2016

Keywords:
BRD4 inhibitors
Cancer
Molecular docking
Dihydroquinoxalinone derivatives

ABSTRACT

BRD4 plays a key role in transcriptional regulation. Recent biological and pharmacological studies have demonstrated that bromodomain-containing protein 4 (BRD4) is a viable drug target for cancer treatment. In this study, we synthesized a series of dihydroquinoxalinone derivatives and evaluated their BRD4 inhibitory activities, obtaining compound **5i** with IC_{50} value of **73 nM** of binding activity in BRD4 (1) and **258 nM** of cellular activity in MV-4-11 cancer cell lines. Docking studies were performed to explain the structure-activity relationship. Based on its potent biochemical and anti-proliferative activity, the novel BRD4 inhibitor compound **5i**, is a promising lead compound for further investigation.

1. Introduction

Bromodomain-containing protein 4 (BRD4) is a member of the bromodomain and extra-terminal domain (BET) family of proteins which can activate transcription through recognizing specific ε-*N*-acetyl modified lysine residues found within histone tails [1–3]. As known, acetylation of histones lysine residues plays an essential role in the epigenetic regulation of gene expression [4]. Lots of studies have revealed the conserved roles of the BET protein in gene expression regulation [5–8]. Regulating function of BET bromodomains to modulate the gene expression has become a hot research field [9]. To date, there are plenty of crystal structures of BRDs for BET family protein uncovered in the Protein Data Bank (http://www.rcsb.org.pdb) [10–14]. The BET family includes BRD2, BRD3, BRD4, and BRDT, each of which contains two bromodomain modules [15,16]. There are obvious structural similarities among the BRDs, such as a left-handed four-helix bundle (αA, αB, αC, αZ) [17]. The BET proteins have emerged as promising drug targets and play important roles in human disease, including viral infections and cancers [18–20].

Bromodomain protein 4 (BRD4) is considered as the best studied member of the BET family [21]. It is a nuclear protein that plays a vital role in maintaining chromatin architecture [22]. Importantly, BRD4 remains related with chromatin throughout the cell cycle. Therefore BRD4 could directly keep higher-order structure of chromatin [17]. Interestingly, BRD4 interacts with P-TEFb through a conserved P-TEFb-interacting domain and promotes phosphorylation of RNA Pol II to resume transcription elongation [21,23,24]. In addition, BRD4 mediates transcriptional activation of target genes such as the MYC oncogene [25–27]. Above all, BRD4 bromodomain protein has developed to be an interesting drug target for the treatment of cancer [28–30], obesity [31], kidney disease [32], lung fibrosis [33] and other inflammatory diseases [34].

However, JQ1, a potent BRD4 inhibitor, has serious preclinical toxicity in vivo and was used as a tool compound [35]. Therefore, novel BRD4 inhibitors with excellent ADMET properties were needed. Recently several reports described 3,5-dimethylisoxazoles as potent inhibitors of BRD4 [36–47], including compound **28a** with IC_{50} value of **180 nM** of binding activity [48]. In this study, compound **28a** was docked into BRD4 crystal complex (PDB id: 3P5O) and the docking conformation was analyzed in detail. Its 3,5-dimethylisoxazole as an acetylated lysine mimic anchored into Kac pocket through a directed hydrogen bond interaction formed with the conserved Asn140 at the bottom of the pocket (Fig. 1A). From the docking conformation, we also found that the 4-cyano benzyl of compound 28a was able to interact with WPF shelf composed of W81, P82 and F83. However, the compound **28a** fails to interact with the waters around ZA channel region and the 1-bromobenzyl substitution was far from the WPF region. In addition, Rooney et al. employ a dihydroquinoxalinone scaffold as the KAc mimic in a series of CREBBP bromodomain ligands. The amide of the dihydroquinoxalinone formed two

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http://dx.doi.org/10.1016/j.bioorg.2016.08.009
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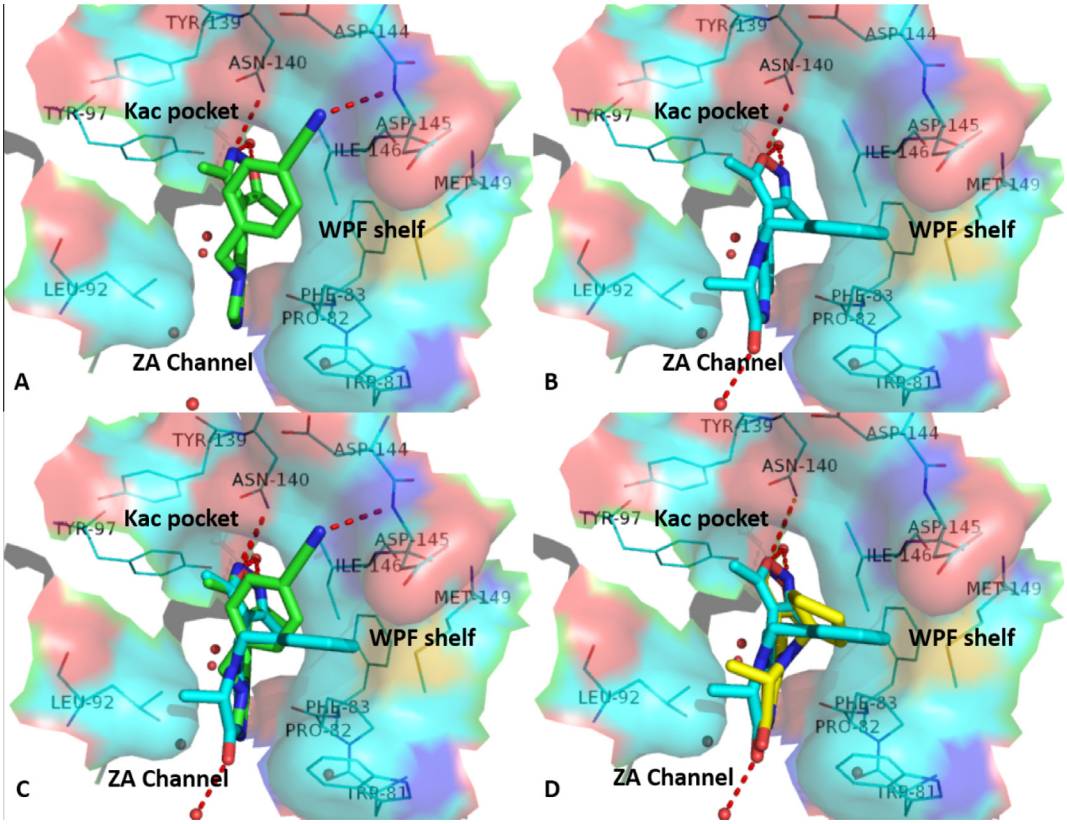


Fig. 1. (A) Docking conformation of compound **28a** in BRD4 protein (PDB id: 3P5O). (B) Docking conformation of compound **5a** in BRD4 protein (PDB id: 3P5O). (C) Superimposition docking conformation of compound **28a** (Green) and compound **5a** (Blue) in BRD4 protein (PDB id: 3P5O). (D) Superimposition docking conformation of compound **5a** (Blue) and compound **5d** (Yellow) in BRD4 protein (PDB id: 3P5O).

hydrogen bonds with N1168, and the methyl group resided at the base of the Kac binding pocket [49]. On the basis of that analysis, the 2,3-dihydro-1*H*-benzo[*d*]imidazole core was replaced by dihydroquinoxalinone skeleton to build interaction with ZA Channel region (Fig. 1B). Meanwhile the interaction between the 1-bromobenzyl substitution of dihydroquinoxalinone and WPF shelf was strengthened. Most of these dihydroquinoxalinone analogues were proved to be novel BRD4 inhibitors by biochemical activity and cellular activity in vitro. Among all the synthesized compounds, compound **5i** with excellent BRD4 inhibition activity (**70 nM**) and anti-proliferative activity (**258 nM**) was considered to be a promising lead compound worthy of further investigation.

2. Results and discussion

2.1. Chemistry

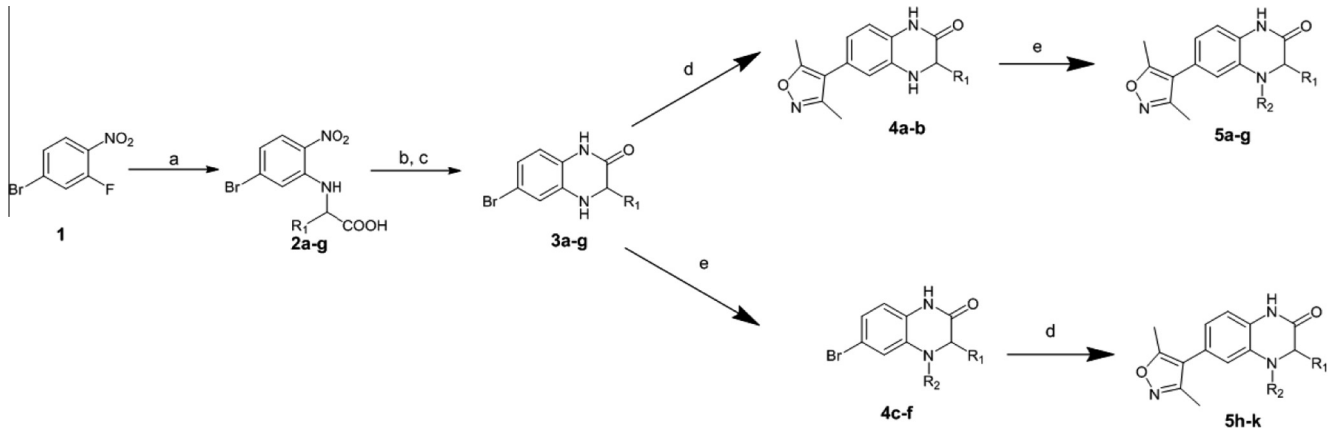
The general schemes for synthesis of dihydroquinoxalinone derivatives **5a**–**k** and **8** are summarized in Scheme 1 and 2. The structures of dihydroquinoxalinone derivatives were confirmed by ^{1}H NMR and ^{13}C NMR spectrum, mass spectrometry, and IR spectrum. Purification by column chromatography was carried out over silica gel (200–300 mesh) and checked for purity using HPLC before being tested in biological evaluation (purity was >97%).

As shown in Scheme 1, the commercially starting material compound **1** was treated with different amino acid to afford the desired intermediates **2a**–**g** by a substitution reaction. Then the intermediates **2a**–**g** were further converted to intermediates **3a**–**g** by reduction and cyclization with sodium hydrosulfite in water at room temperature. The obtained **3a**–**g** were treated by reductive amination and Suzuki coupling reaction to give the target compound **5a**–**k**.

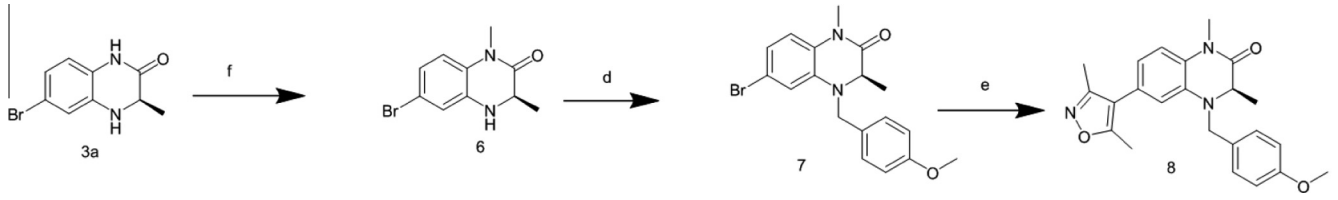
The dihydroquinoxalinone derivative **8** was synthesized starting from intermediate **3a** as shown in Scheme 2. The intermediate **6** was obtained by methylation reaction using starting material **3a** and iodomethane. The intermediate **6** was further treated with *para*-methoxybenzaldehyde in tetrahydrofuran at room temperature to prepare the key intermediate **7**. Then compound **8** was obtained by Suzuki coupling reaction using 3,5-dimethylisoxazole-4-boronic acid pinacol ester.

2.2. Biological activity and SAR study

In order to improve the BRD4 inhibitory activity, the dihydroquinoxalinone skeleton was designed and synthesized to replace the benzimidazolone core of compound **28a**. The binding activity was investigated by an AlphaScreen assay to prove the rational structure-activity relationship (Table 1). Compound **28a** was used as positive control. The binding activities are presented in Table 1. The initially benzimidazolone core of compound **28a** was replaced by dihydroquinoxalinone skeleton to afford compound **5a** with binding activity (IC_{50} = **75 nM**). This demonstrated that dihydroquinoxalinone skeleton was feasible as a core of BRD4 inhibitors. Then various bromobenzyl or naphthene substituents were adopted to explore structure-activity relationship at WPF shelf of BRD4. The result showed that benzyl group gives a relatively better activity than aliphatic ring when interacting with BRD4 WPF shelf. Meanwhile, benzyl group with electron-donating substituents (**5b**) exhibited a better effect on inhibition than benzyl group with electron-withdrawing substituents (**5c**). This series was docked into BRD4(1) protein (PDB id: 3P5O) by Glide docking protocol in

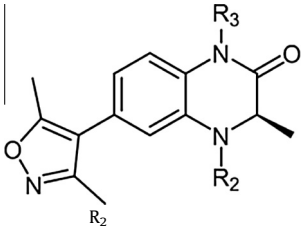


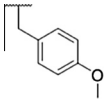
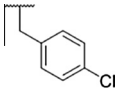
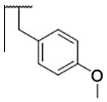
Scheme 1. Reagents and conditions: (a) K_2CO_3, amino acid, H_2O, EtOH, reflux; (b) K_2CO_3, H_2O, RT; (c) $Na_2S_2O_4$, H_2O, RT; (d) dibutyltin dichloride, Phenylsilane, benzaldehydes/cyclic ketones, THF, RT; (e) phenylboronic acids, $Pd(PPh_3)_4$, Na_2CO_3, N_2, H_2O/EtOH/Toluene, 80 °C



Scheme 2. Reagents and conditions: (f) NaH, DMF, CH_3I, 0 °C; (d) dibutyltin dichloride, phenylsilane, p-methoxybenzaldehyde, THF, RT; (e) phenylboronic acids, $Pd(P(Ph)_3)_4$, Na_2CO_3, N_2, H_2O/EtOH/Toluene, 80 °C.

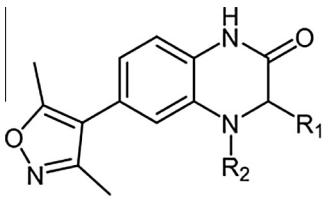
Table 1
Structures and BRD4-BD1 inhibitory effects of compounds **5a–e** and compound **8**.

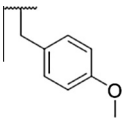
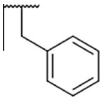
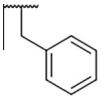
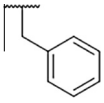
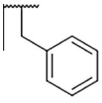
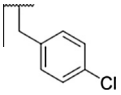


Compound	Conformation	R_2	R_3	BRD4(1) IC50 (μmol/L)[b]
28a[c]				0.190 ± 0.013
5a	R		H	0.075 ± 0.021
5b	R		H	0.160 ± 0.011
5c	R		H	0.440 ± 0.024
5d	R		H	0.370 ± 0.017
5e	R		H	NA[a]
8	R			0.220 ± 0.015

[a] Mean of the BRD4-BD1 inhibitory activity $IC_{50} > 1$ μm.
[b] IC_{50} values for BRD4-BD1 activities presented are the mean ± SD values of three independent determinations.
[c] Used as positive control.

Table 2
Structures and BRD4-BD1 inhibitory effects of compounds **5f–k**.



Compound	Conformation	R_1	R_2	BRD4(1) IC_{50} (μmol/L)[b]
5f	S			NA[a]
5g	S			NA[a]
5h	R			0.650 ± 0.021
5i	R			0.073 ± 0.012
5j	R			0.151 ± 0.015
5k	R	H		0.500 ± 0.026

[a] Mean of the BRD4-BD1 inhibitory activity IC_{50} > 1 μm.
[b] IC_{50} values for BRD4-BD1 activities presented is the mean ± SD value of three independent determinations.

Schrodinger 10.2. Compared to other substituent at R_1 region, benzyl group was considered as lead candidate with the best biological activity. The docking conformations of compounds **5a** and **5d** are shown in Fig. 1D. The docking conformation shows that the cyclopentane group of compound **5d** was 7.65 Å from the Pro82. This distance from cyclopentane group to Pro82 indicates compound 5d cannot stretch into WPF hydrophobic pocket. On the contrary, the benzyl group of compound **5a** could occupy WPF region. This subtle difference may lead to the decrease of biology value (IC_{50}). In addition, compared to compound **5b**, the introduction of methyl group at R_3 in compound **8** also weakens the binding efficiency.

Based on these results above, we explored various substituent groups into the R_2 position of the lead compound **5a** to obtain compound **5f–i** (Table 2). Gratifyingly, compound **5i** exhibited a slight improvement on potency compared with compound **5a** when a hydrophobic ethyl group at R_2 position was introduced. Compound **5h** with a hydrophilic substituent (methylol) at R_2 position exhibited a markedly reduced inhibitory activity in the dihydroquinoxalinone core. The superimposition docking conformation of **5h** and **5i** is shown in Fig. 2B. Therefore, a hydrophobic group was necessary to increase the lipophilic effects with the hydrophobic WPF shelf, but the introduction of hydrophobic group (benzyl group) exhibited unfavorable steric to decrease the binding efficiency. For this reason, compound **5j** has a weaker inhibitory activity with IC_{50} value of **151 nM**. Compounds **5a** and **5j** were docking into BRD4(1) protein to explain the structure-activity relationship. The superimposition of compound **5a** and **5j** in BRD4(1) protein is shown in Fig. 2C. In addition, the "S" configuration of the stereogenic center at R_1 position decreased the binding efficiency. According to the overlap docking conformation, a small hydrophobic group (ethyl group) at R_2 position was necessary for the improvement of binding efficiency.

2.3. In-vitro anticancer evaluation against cancer cell line MV-4-11

BRD4 inhibitors showed potent effects in cellular systems, including inhibition of proliferation. We further tested whether the compounds **5a** and **5i** could inhibit cancer cell lines MV-4-11. Then, we assessed the effect of compounds **5a** and **5i** on cell proliferation in abroad panel of human cancer cell lines, MV-4-11 [50–52]. As shown in Table 3, compound **5i** significantly inhibited the proliferation of MV-4-11 cell lines with IC_{50} value of **258 nM**. These results established that compound **5i** could be a promising lead compound for anti-cancer.

Table 3
Anti-proliferative activities of compound **5i** and **5a** on MV-4-11 cell lines.

Compound no.	mw	IC_{50} (MV-4-11 cell lines) (μmol/L)[b]	clogP[c]
28a[a]	330.39	0.632 ± 0.013	2.809
5a	347.42	0.378 ± 0.021	3.540
5i	361.45	0.258 ± 0.014	4.069
(+)-JQ1	456.99	4.136 ± 0.028	4.820

[a] Used as positive control.
[b] IC_{50} values for BRD4-BD1 activities presented is the mean ± SD value of three independent determinations.
[c] clogP values were estimated with ChemDraw Ultra, version 14.0.

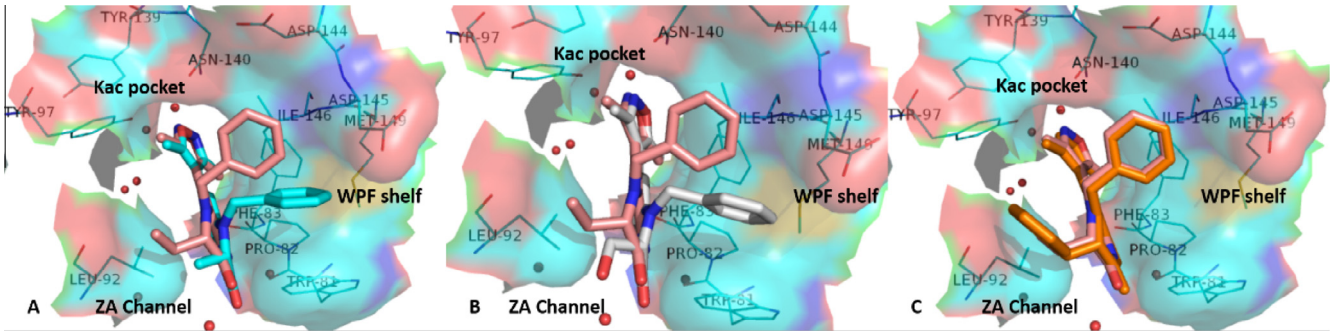


Fig. 2. (A) Superimposition docking conformation of compound **5a** (Blue) and compound **5i** (Pink) in BRD4 protein (PDB id: 3P5O). (B) Superimposition docking conformation of compound **5h** (Gray) and compound **5i** (Pink) in BRD4 protein (PDB id: 3P5O). (C) Superimposition docking conformation of compound **5j** (Orange) and compound **5i** (Pink) in BRD4 protein (PDB id: 3P5O).

3. Conclusion

In summary, we designed and synthesized a series of novel dihydroquinoxalinone derivatives as BRD4 inhibitors. Most of these compounds were evaluated as excellent BRD4 suppressant in vitro. Especially compound **5i** had excellent BRD4 inhibition activity (IC_{50} **73 nM**) better than the compound **28a** (IC_{50} **180 nM**). Meanwhile, compound **5a** and compound **5i** exhibited potency of anti-proliferative activity in MV-4-11 cancer cell lines. In addition, docking studies were performed to claim the structure-activity relationship. All of these results demonstrated that compound **5i** was a potent BRD4 inhibitor and worthy for further investigation.

4. Experiment

4.1. Chemistry

All chemicals (reagent grade) used were purchased from Sinopharm Chemical Reagent Co., Ltd. (China) and treated with standard methods before use. Purifications by column chromatography were carried out over silica gel (200–300 mesh). Melting points were measured on capillary tube and were uncorrected. IR spectra (in KBr pellets) were taken using Shimadzu FT-IR-8400S spectrophotometer. ^1H and ^{13}C NMR spectra (DMSO-d_6) were measured on Bruker AV-300 spectrometer at 25 °C and referenced to TMS. Chemical shifts were reported in ppm (δ) using the residual solvent line as internal standard. Analytical thin-layer chromatography (TLC) was performed on the glass-backed silica gel sheets (silica gel 60 Å GF 254). High-resolution mass spectra were recorded using an Agilent QTOF 6520 (Beijing, China).

4.1.1. General procedure for the preparation of ***2a–f***

To a solution of 4-bromo-2-fluoro-1-nitrobenzene (1 g, 4.55 mmol), K_2CO_3 (0.78 g, 5.68 mmol) and different amino acid (5.45 mmol) in ethanol (5 ml) and water (4 ml) were refluxed at 100 °C for 10 h. After cooling to the room temperature, pH value was adjusted to 2 with 1 M HCl solution. The mixture was filtered and the precipitate was collected to afford **2a–g** as golden yellow solid.

*4.1.1.1. (5-Bromo-2-nitrophenyl)-d-alanine (**2a**).* Golden yellow solid, yield 98.2%; ESI-MS *m/z* 287.9 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 8.39 (d, *J* = 6.8 Hz, 1H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.23 (s, 1H), 6.90 (dd, *J* = 9.1, 1.7 Hz, 1H), 4.62–4.46 (m, 1H), 1.46 (d, *J* = 6.9 Hz, 3H).

*4.1.1.2. (R)-2-((5-bromo-2-nitrophenyl)amino)butanoic acid (**2b**).* Sandy solid, yield 94%; ESI-MS *m/z* 301.9 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 8.44 (d, *J* = 7.3 Hz, 1H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.25 (d, *J* = 1.9 Hz, 1H), 6.88 (dd, *J* = 9.1, 1.9 Hz, 1H), 4.62–4.43 (m, 1H), 1.98–1.77 (m, 2H), 0.87 (t, *J* = 7.3 Hz, 3H).

*4.1.1.3. (5-Bromo-2-nitrophenyl)-d-serin (**2c**).* Yellow solid, yield 91%; ESI-MS *m/z* 303.9 $[M+H]^+$.

*4.1.1.4. (5-Bromo-2-nitrophenyl)-l-alanine (**2d**).* Golden yellow solid, yield 94%; ESI-MS *m/z* 287.9 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 13.33 (bs, 1H), 8.39 (d, *J* = 7.1 Hz, 1H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.24 (s, 1H), 7.00–6.88 (m, 1H), 4.63–4.50 (m, 1H), 1.45 (d, *J* = 7.0 Hz, 3H).

*4.1.1.5. (5-Bromo-2-nitrophenyl)glycine (**2e**).* Brown solid, yield 92%; ESI-MS *m/z* 273.9 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 13.23 (bs, 1H), 8.42 (s, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.15 (s, 1H), 6.88 (d, *J* = 7.0 Hz, 1H), 4.19 (s, 2H).

*4.1.1.6. (5-Bromo-2-nitrophenyl)-d-phenylalanine (**2f**).* Yellow solid, 96%;ESI-MS *m/z* 364.0 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 13.41 (s, 1H), 8.24 (d, *J* = 7.9 Hz, 1H), 7.98 (d, *J* = 9.1 Hz, 1H), 7.33–7.11 (m, 6H), 6.92–6.82 (m, 1H), 4.91 (m, 1H), 3.26–3.11 (m, 2H).

4.1.2. General procedure for the preparation of ***3a–f***

To a solution of 2a–g (1 g, 3.46 mmol) and K_2CO_3 (0.96 g, 6.92 mmol) in water was added sodium hydrosulfite (3.01 g, 17.3 mmol) in portions. Then the mixture was stirred for overnight. The white precipitate was collected by filtration to afford **3a–g** without further purification.

*4.1.2.1. (R)-6-bromo-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**3a**).* White solid, yield 45%; ESI-MS *m/z* 239.9 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.27 (s, 1H), 6.81 (d, *J* = 2.0 Hz, 1H), 6.73 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.64 (d, *J* = 8.2 Hz, 1H), 6.26 (s, 1H), 3.81 (q, *J* = 6.3 Hz, 1H), 1.24 (d, *J* = 6.6 Hz, 3H).

*4.1.2.2. (S)-6-bromo-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**3b**).* Off white solid, yield 40%; ESI-MS *m/z* 239.9 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.29 (s, 1H), 6.81 (d, *J* = 2.0 Hz, 1H), 6.73 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.63 (d, *J* = 8.2 Hz, 1H), 6.27 (s, 1H), 3.80 (m, 1H), 1.23 (d, *J* = 6.5 Hz, 3H).

*4.1.2.3. (R)-6-bromo-3-(hydroxymethyl)-3,4-dihydroquinoxalin-2(1H)-one (**3c**).* White solid, yield 40%; ESI-MS *m/z* 255.9 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.36 (s, 1H), 6.87 (s, 1H), 6.78–6.49 (m, 2H), 6.31 (s, 1H), 4.98 (s, 1H), 3.83 (s, 1H), 3.59 (s, 2H).

*4.1.2.4. (R)-6-bromo-3-ethyl-3,4-dihydroquinoxalin-2(1H)-one (**3d**).* White solid, yield 40%; ESI-MS *m/z* 254.0 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.31 (s, 1H), 6.85 (d, *J* = 2.1 Hz, 1H), 6.70 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.61 (d, *J* = 8.2 Hz, 1H), 6.29 (s, 1H), 3.76–3.66 (m, 1H), 1.72–1.52 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 3H).

*4.1.2.5. 6-Bromo-3,4-dihydroquinoxalin-2(1H)-one (**3e**).* Off white solid, yield 43%; ESI-MS *m/z* 225.9 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.33 (s, 1H), 8.19 (s, 1H), 7.97 (d, *J* = 2.2 Hz, 1H), 7.70 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.24 (d, *J* = 8.8 Hz, 1H), 3.78 (s, 2H).

*4.1.2.6. (R)-3-benzyl-6-bromo-3,4-dihydroquinoxalin-2(1H)-one (**3f**).* White solid, yield 45%; ESI-MS *m/z* 316.0 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.34 (s, 1H), 7.33–7.10 (m, 5H), 6.83 (s, 1H), 6.67 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.57 (d, *J* = 8.2 Hz, 1H), 6.19 (s, 1H), 4.08 (t, *J* = 4.8 Hz, 1H), 2.99–2.78 (m, 2H).

4.1.3. General procedure for the preparation of ***4a–b***

A mixture of **3a–b** (0.5 mmol),3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole (0.5 mmol), sodium carbonate (1.5 mmol), Tetrakis (triphenylphosphine) palladium (0.05 mmol) in a mixed solvent of water (3 mL), ethanol (1 mL) and toluene (3 mL) was refluxed under N_2 for 12 h. After the reaction was completed, the mixture was diluted with water (20 mL) and filtered with diatomite. Then the filtrate was extracted with ethyl acetate (10 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give brown oil. The resulting crude oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 3:1) to afford **4a–b**.

*4.1.3.1. (R)-6-(3,5-dimethylisoxazol-4-yl)-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**4a**).* Light yellow solid, yield 65%; ESI-MS *m/z* 257.1 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.29 (s, 1H),

6.80 (d, J = 7.9 Hz, 1H), 6.66 (s, 1H), 6.59 (d, J = 7.9 Hz, 1H), 6.13 (s, 1H), 3.83 (q, J = 6.1 Hz, 1H), 2.36 (s, 3H), 2.19 (s, 3H), 1.27 (d, J = 6.1 Hz, 3H).

*4.1.3.2. (S)-6-(3,5-dimethylisoxazol-4-yl)-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**4b**).* Light yellow solid, yield 74%; ESI-MS m/z 257.1 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.30 (s, 1H), 6.80 (d, J = 7.9 Hz, 1H), 6.65 (s, 1H), 6.62–6.54 (m, 1H), 6.13 (s, 1H), 3.83 (q, J = 7.0 Hz, 1H), 2.36 (s, 3H), 2.19 (s, 3H), 1.27 (d, J = 7.0 Hz, 3H).

*4.1.4. General procedure for the preparation of **4c–f***

A mixture of **3c**–**f** (2.07 mmol), benzaldehydes (6.22 mmol), phenylsilane (0.67 g, 6.22 mmol) and dibutyltin dichloride (0.69 g, 2.28 mmol) in THF (10 mL) was stirred for 8 h at room temperature. Then the mixture was quenched with water and extracted with EtOAc (3 × 20 ml). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give yellow oil. The resulting crude oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 3:1) to afford **4c**–**f**.

*4.1.4.1. (R)-4-benzyl-3-(hydroxymethyl)-6-methyl-3,4-dihydroquinoxalin-2(1H)-one (**4c**).* Yellow solid, yield 81%; ESI-MS m/z 347.2 $[M+H]^+$.

*4.1.4.2. (R)-4-benzyl-6-bromo-3-ethyl-3,4-dihydroquinoxalin-2(1H)-one (**4d**).* Light yellow solid, yield 80%; ESI-MS m/z 344.1 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.56 (s, 1H), 7.35–7.25 (m, 5H), 6.84–6.66 (m, 3H), 4.69 (d, J = 15.6 Hz, 1H), 4.36 (d, J = 15.6 Hz, 1H), 3.84–3.73 (m, 1H), 1.70–1.43 (m, 2H), 0.81 (t, J = 7.5 Hz, 3H).

*4.1.4.3. (R)-3,4-dibenzyl-6-bromo-3,4-dihydroquinoxalin-2(1H)-one (**4e**).* White solid, yield 83%; ESI-MS m/z 406.1 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.57 (s, 1H), 7.26 (m, 8H), 7.07 (d, J = 6.1 Hz, 2H), 6.77 (d, J = 5.9 Hz, 2H), 6.63 (d, J = 8.8 Hz, 1H), 4.57 (d, J = 15.4 Hz, 1H), 4.16 (d, J = 15.4 Hz, 1H), 4.08 (t, J = 6.2 Hz, 1H), 2.80 (m, 2H).

*4.1.4.4. 6-Bromo-4-(4-chlorobenzyl)-3,4-dihydroquinoxalin-2(1H)-one (**4f**).* Light brown solid, yield 81%; ESI-MS m/z 350.0 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.57 (s, 1H), 7.42 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.3 Hz, 2H), 6.85–6.70 (m, 3H), 4.45 (s, 2H), 3.78 (s, 2H).

*4.1.5. General procedure for the preparation of **5a–g***

A mixture of **4a**–**b** (2.07 mmol), benzaldehydes/cyclic ketones (6.22 mmol), phenylsilane (0.67 g, 6.22 mmol) and dibutyltin dichloride (0.69 g, 2.28 mmol) in THF (10 mL) was stirred for 8 h at room temperature. Then the mixture was quenched with water and extracted with EtOAc (3 × 20 ml). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give yellow oil. The resulting crude oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 3:1) to afford target products **5a**–**g**.

*4.1.5.1. 1(R)-4-benzyl-6-(3,5-dimethylisoxazol-4-yl)-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**5a**).* Light yellow solid, yield 70%; m.p.: 112–114 °C; IR (KBr, cm^{-1}): 3185.84, 2920.3, 1689.57, 1522.49, 1444.04, 1393.57, 739.46, 514.83; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.54 (s, 1H), 7.40–7.20 (m, 5H), 6.84 (d, J = 8.2 Hz, 1H), 6.79–6.66 (m, 2H), 4.58 (d, J = 15.3 Hz, 1H), 4.27 (d, J = 15.3 Hz, 1H), 3.84 (q, J = 6.7 Hz, 1H), 1.07 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.1, 164.3, 157.9, 137.7, 133.0, 128.5, 127.0, 126.4, 124.1, 118.8, 116.0, 114.9, 114.0, 57.8, 51.1, 40.3, 40.0, 39.7, 39.5, 39.2, 38.9, 38.6, 13.3, 10.9, 10.0. MS (ESI, m/z): 370.1 $[M+Na]^+$; Anal. calcd. for $C_{21}H_{21}N_3O_2$: C, 72.60; H, 6.09; N, 12.10. Found: C, 72.56; H, 6.07; N, 12.06.

*4.1.5.2. 2(R)-6-(3,5-dimethylisoxazol-4-yl)-4-(4-methoxybenzyl)-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**5b**).* Light yellow solid, yield 78%; m.p.: 107–109 °C; IR (KBr, cm^{-1}): 3451.33, 1684.65, 1512., 1245.82, 1033.52, 815.47; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.52 (s, 1H), 7.28 (d, J = 8.5 Hz, 2H), 6.95–6.80 (m, 3H), 6.67 (d, J = 7.9 Hz, 1H), 6.50 (s, 1H), 4.50 (d, J = 15.1 Hz, 1H), 4.24 (d, J = 15.1 Hz, 1H), 3.91 (d, J = 6.7 Hz, 1H), 3.72 (s, 3H), 2.17 (s, 3H), 2.00 (s, 3H), 1.09 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.1, 164.4, 159.0, 158.4, 158.0, 133.2, 129.2, 128.5, 126.4, 124.1, 118.8, 116.0, 114.9, 114.0, 57.3, 55.0, 50.3, 40.0, 39.7, 39.4, 39.2, 38.9, 13.0, 11.0, 10.1. MS (ESI, m/z): 400.1 $[M+Na]^+$; Anal. calcd. for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 70.01; H, 6.12; N, 11.16.

*4.1.5.3. 3(R)-4-(4-chlorobenzyl)-6-(3,5-dimethylisoxazol-4-yl)-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**5c**).* Light yellow solid, yield 70%; m.p.: 104–106 °C; IR (KBr, cm^{-1}): 3445.43, 1685.47, 1522.60, 1393.00, 815.47; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.55 (s, 1H), 7.39 (s, 4H), 6.89 (d, J = 7.9 Hz, 1H), 6.67 (d, J = 7.9 Hz, 1H), 6.39 (s, 1H), 4.56 (d, J = 15.9 Hz, 1H), 4.36 (d, J = 15.9 Hz, 1H), 4.00 (q, J = 6.8 Hz, 1H), 2.12 (s, 3H), 1.94 (s, 3H), 1.12 (d, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.0, 164.3, 157.9, 136.8, 132.8, 131.4, 128.9, 128.4, 126.4, 124.1, 119.0, 115.9, 115.0, 114.0, 112.9, 58.0, 50.3, 40.0, 39.7, 39.4, 39.2, 38.9, 24.9, 13.4, 10.9, 10.0. MS (ESI, m/z): 404.0 $[M+Na]^+$; Anal. calcd. for $C_{21}H_2{}^{\circ}ClN_3O_2$: C, 66.05; H, 5.28; N, 11.00. Found: C, 66.03; H, 5.25; N, 11.06.

*4.1.5.4. 4(R)-4-cyclopentyl-6-(3,5-dimethylisoxazol-4-yl)-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**5d**).* Light yellow solid, yield 71%; m.p.: 111–113 °C; IR (KBr, cm^{-1}): 3457.23, 2938.05, 1692.34, 1520.44, 1392.90, 815.47; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.47 (s, 1H), 6.89 (d, J = 8.1 Hz, 1H), 6.79–6.64 (m, 2H), 3.95 (q, J = 6.7 Hz, 1H), 3.90–3.78 (m, 1H), 2.39 (s, 3H), 2.22 (s, 3H), 1.97 (s, 2H), 1.60 (s, 6H), 0.99 (d, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.5, 164.4, 158.1, 133.6, 127.2, 124.1, 119.2, 116.2, 115.5, 115.0, 58.6, 53.6, 40.0, 39.7, 39.4, 39.2, 38.9, 29.9, 23.7, 23.2, 14.3, 11.3, 10.5. MS (ESI, m/z): 348.1 $[M+Na]^+$; Anal. calcd. for $C_{19}H_{23}N_3O_2$: C, 70.13; H, 7.12; N, 12.91. Found: C, 70.11; H, 7.14; N, 12.87.

*4.1.5.5. 5(R)-4-cyclohexyl-6-(3,5-dimethylisoxazol-4-yl)-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**5e**).* Light yellow solid, yield 75%; m.p.: 116–118 °C; IR (KBr, cm^{-1}): 3433.63, 2926.25, 1683.08, 1516.76, 1440.15, 1301.66, 1024.68; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.42 (s, 1H), 6.88 (d, J = 7.9 Hz, 1H), 6.76 (s, 1H), 6.71 (dd, J = 7.9, 1.6 Hz, 1H), 4.02–3.92 (m, 1H), 3.50–3.38 (m, 1H), 2.39 (s, 3H), 2.22 (s, 3H), 2.01–1.92 (m, 1H), 1.81–1.69 (m, 2H), 1.67–1.24 (m, 7H), 1.02 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.0, 164.4, 157.8, 133.3, 126.9, 124.3, 118.8, 116.1, 115.2, 114.9, 58.3, 51.4, 40.0, 39.7, 39.4, 39.2, 38.9, 31.2, 30.9, 25.6, 25.4, 25.2, 24.9, 17.0, 11.3, 10.5. MS (ESI, m/z): 362.2 $[M+Na]^+$; Anal. calcd. for $C_{20}H_{25}N_3O_2$: C, 70.77; H, 7.42; N, 12.38. Found: C, 70.71; H, 7.44; N, 12.37.

*4.1.5.6. 6(S)-6-(3,5-dimethylisoxazol-4-yl)-4-(4-methoxybenzyl)-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**5f**).* Light yellow solid, yield 57%; m.p.: 107–109 °C; IR (KBr, cm^{-1}): 3445.43, 1685.40, 1512.58, 1246.17, 1033.52, 815.47; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.55 (s, 1H), 7.28 (d, J = 8.1 Hz, 2H), 6.96–6.85 (m, 3H), 6.67 (d, J = 7.8 Hz, 1H), 6.50 (s, 1H), 4.49 (d, J = 15.2 Hz, 1H), 4.24 (d, J = 15.2 Hz, 1H), 4.00–3.85 (m, 1H), 3.72 (s, 3H), 2.16 (s, 3H), 1.99 (s, 3H), 1.09 (d, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ:

168.1, 164.4, 163.2, 158.4, 157.9, 133.2, 130.7, 129.2, 128.4, 126.4, 124.1, 118.8, 116.0, 114.9, 114.0, 57.3, 55.0, 50.3, 40.0, 39.7, 39.5, 39.2, 13.0, 11.0, 10.1. MS (ESI, *m*/*z*): 400.1 $[M+Na]^+$; Anal. calcd. for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 70.01; H, 6.17; N, 11.21.

*4.1.5.7. 7(S)-4-cyclopentyl-6-(3,5-dimethylisoxazol-4-yl)-3-methyl-3,4-dihydroquinoxalin-2(1H)-one (**5g**).* Light yellow solid, yield 75%; m.p.: 110–113 °C; IR (KBr, cm^{-1}): 3457.23, 2943.95, 1692.60, 1520.70, 1393.06, 815.47; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.47 (s, 1H), 6.89 (d, *J* = 8.3 Hz, 1H), 6.73 (d, *J* = 3.7 Hz, 2H), 4.04–3.90 (m, 1H), 3.90–3.76 (m, 1H), 2.38 (s, 3H), 2.21 (s, 3H), 1.97 (s, 2H), 1.59 (s, 6H), 0.99 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.5, 164.3, 158.0, 133.6, 127.2, 124.1, 119.2, 116.1, 115.4, 115.0, 58.5, 53.6, 39.7, 39.4, 39.2, 38.9, 29.9, 23.7, 23.2, 14.3, 11.3, 10.5. MS (ESI, *m*/*z*): 348.1 $[M+Na]^+$; Anal. calcd. for $C_{19}H_{23}N_3O_2$: C, 70.13; H, 7.12; N, 12.91. Found: C, 70.11; H, 7.17; N, 12.85.

*4.1.6. General procedure for the preparation of **5h–k***

A mixture of **4c-f** (0.5 mmol),3,5-dimethyl-4-(4,4,5,5-tetrame thyl-1,3,2-dioxaborolan-2-yl) isoxazole (0.5 mmol), sodium carbonate (1.5 mmol), Tetrakis (triphenylphosphine) palladium (0.05 mmol) in a mixed solvent of water (3 mL), ethanol (1 mL) and toluene (3 mL) was refluxed under N_2 for 12 h. After the reaction was completed, the mixture was diluted with water (20 mL) and filtered with diatomite. Then the filtrate was extracted with ethyl acetate (10 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give brown oil. The resulting crude oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 3:1) to afford target products.

*4.1.6.1. 8(R)-4-benzyl-6-(3,5-dimethylisoxazol-4-yl)-3-(hydroxymethyl)-3,4-dihydroquinoxalin-2(1H)-one (**5h**).* Light brown solid, yield 66%; m.p.: 122–124 °C; IR (KBr, cm^{-1}): 3410.03, 2926.25, 1681.77, 1524.00, 1409.74, 1065.93, 694.66. ^1H NMR (300 MHz, DMSO-d_6) δ: 10.62 (s, 1H), 7.48–7.09 (m, 5H), 6.81 (d, *J* = 7.5 Hz, 1H), 6.55 (d, *J* = 7.5 Hz, 1H), 6.32 (s, 1H), 5.03 (s, 1H), 4.70 (d, *J* = 16.1 Hz, 1H), 4.53 (d, *J* = 16.1 Hz, 1H), 4.07 (s, 1H), 3.66 (s, 2H), 2.06 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 166.0, 138.1, 133.8, 132.0, 131.5, 131.3, 128.8, 128.6, 128.5, 126.7, 125.9, 123.7, 117.6, 114.6, 112.8, 65.2, 60.9, 52.1, 40.0, 39.7, 39.4, 39.2, 38.9, 10.9, 10.0. MS (ESI, *m*/*z*): 386.1 $[M+Na]^+$; Anal. calcd. for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.40; H, 5.81; N, 11.58.

*4.1.6.2. (R)-4-benzyl-6-(3,5-dimethylisoxazol-4-yl)-3-ethyl-3,4-dihydroquinoxalin-2(1H)-one (**5i**).* Light yellow solid, yield 52%; m.p.: 112–114 °C; IR (KBr, cm^{-1}): 3439.53, 2967.55, 1688.83, 1381.22, 1230.94, 1151.38, 821.36, 732.97; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.54 (s, 1H), 7.33 (d, *J* = 4.3 Hz, 4H), 7.27–7.16 (m, 1H), 6.85 (d, *J* = 7.9 Hz, 1H), 6.62 (d, *J* = 7.9 Hz, 1H), 6.43 (s, 1H), 4.67 (d, *J* = 15.7 Hz, 1H), 4.44 (d, *J* = 15.7 Hz, 1H), 3.92–3.85 (m, 1H), 2.10 (s, 3H), 1.93 (s, 3H), 1.73–1.61 (m, 1H), 1.59–1.48 (m, 1H), 0.85 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 157.8, 137.6, 135.5, 128.6, 128.5, 126.9, 126.5, 124.0, 118.4, 116.1, 114.7, 113.3, 73.2, 63.3, 51.4, 45.2, 40.0, 39.7, 39.4, 39.2, 38.9, 24.9, 22.5, 10.9, 10.1, 9.9. MS (ESI, *m*/*z*): 384.1 $[M+Na]^+$; Anal. calcd. for $C_{22}H_{23}N_3O_2$: C, 73.11; H, 6.41; N, 11.63. Found: C, 73.14; H, 6.41; N, 11.58.

*4.1.6.3. (R)-3,4-dibenzyl-6-(3,5-dimethylisoxazol-4-yl)-3,4-dihydroquinoxalin-2(1H)-one (**5j**).* Light brown solid, yield 52%; m.p.: 106–108 °C; IR (KBr, cm^{-1}): 3427.73, 1681.93, 1522.49, 1394.39, 1230.94, 744.75, 691.71; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.57 (s, 1H), 7.37–6.98 (m, 10*H*), 6.76 (d, *J* = 8.0 Hz, 1H), 6.58 (d, *J* = 7.0 Hz, 1H), 6.42 (s, 1H), 4.54 (d, *J* = 15.5 Hz, 1H), 4.20 (s, 2H), 2.83 (s, 2H), 2.15 (s, 3H), 1.99 (s, 3H); MS (ESI, *m*/*z*): 446.1 $[M+Na]^+$; Anal. calcd. for $C_{27}H_{25}N_3O_2$: C, 76.57; H, 5.95; N, 9.92. Found: C, 76.55; H, 5.90; N, 9.92.

*4.1.6.4. 4-(4-Chlorobenzyl)-6-(3,5-dimethylisoxazol-4-yl)-3,4-dihydroquinoxalin-2(1H)-one (**5k**).* Light yellow solid, yield 62%; m.p.: 150–152 °C; IR (KBr, cm^{-1}): 3427.73, 2922.92, 2849.56, 2353.98, 1680.99, 1403.61, 794.84; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.58 (s, 1H), 7.44–7.34 (m, 4H), 6.89 (d, *J* = 7.9 Hz, 1H), 6.70–6.62 (m, 1H), 6.47 (s, 1H), 4.46 (s, 2H), 3.86 (s, 2H), 2.16 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 165.4, 164.3, 157.8, 136.3, 134.7, 131.3, 129.1, 128.5, 126.4, 123.9, 118.6, 115.9, 115.2, 114.2, 112.5, 70.2, 52.5, 52.0. MS (ESI, *m*/*z*): 390.0 $[M+Na]^+$; Anal. calcd. for $C_{20}H_{18}ClN_3O_2$: C, 65.31; H, 4.93; N, 11.42. Found: C, 65.30; H, 4.91; N, 11.44.

*4.1.7. Preparation of (R)-6-bromo-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one **(6)***

To a solution of **3a** (1.52 g, 6.30 mmol) in Dimethyl Formamide was added 60% NaH (0.38 g, 9.64 mmol) in portions at ice bath. Then the mixture was stirred for 20 min. Methyl iodide (0.59 mL, 9.46 mmol) was added dropwise and then was stirred for another one hour at room temperature. The mixture was quenched with saturated $NaHCO_3$ solution and extracted with 20 ml × 3. The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and evaporated in vacuo to give brown oil. The resulting crude oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 3:1) to afford 6 (1.04 g, 64%) as a light yellow solid. ESI-MS *m*/*z* 254.0 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6): δ: 6.89–6.86 (m, 3H), 6.36 (s, 1H), 3.85 (q, *J* = 6.6 Hz, 1H), 3.21 (s, 3H), 1.25 (d, *J* = 6.6 Hz, 3H).

*4.1.8. Preparation of (R)-6-bromo-4-(4-methoxybenzyl)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one **(7)***

A mixture of **6** (0.85 g, 3.30 mmol), p-methoxybenzaldehyde (1.22 mL, 10.00 mL), phenylsilane (1.23 mL, 10.00 mmol) and dibutyltin dichloride (1.11 g, 3.67 mmol) in THF (4 mL) was stirred for 8 h at room temperature. Then the mixture was quenched with water and extracted with EtOAc (3 × 20 ml). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give yellow oil. The resulting crude oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 3:1) to afford 7 (1.02 g, 81%). ESI-MS *m*/*z* 344.0 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d_6) δ: 7.27 (d, *J* = 8.5 Hz, 2H), 6.98 (s, 2H), 6.93 (s, 1H), 6.90 (s, 1H), 6.87 (s, 1H), 4.51 (d, *J* = 14.6 Hz, 1H), 4.15 (d, *J* = 14.6 Hz, 1H), 3.90 (q, *J* = 6.7 Hz, 1H), 3.74 (s, 3H), 3.26 (s, 3H), 0.99 (d, *J* = 6.7 Hz, 3H).

*4.1.9. Preparation of (R)-6-(3,5-dimethylisoxazol-4-yl)-4-(4-methoxybenzyl)-1,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-one **(8)***

A mixture of **7** (0.20 g, 0.53 mmol), 3,5-dimethyl-4-(4,4,5,5-tet ramethyl-1,3,2-dioxaborolan-2-yl) isoxazole (0.12 g, 0.53 mmol), sodium carbonate (0.17 g, 1.60 mmol), Tetrakis (triphenylphosphine) palladium (0.06 g, 0.053 mmol) in a mixed solvent of water (3 mL), ethanol (1 mL) and toluene (3 mL) was refluxed under N_2 for 12 h. After the reaction was completed, the mixture was diluted with water (20 mL) and filtered with diatomite. Then the filtrate was extracted with ethyl acetate (10 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give brown oil. The resulting crude oil was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 3:1) to afford target product **8** (light yellow solid, yield 52%). m.p.: 118–120 °C; IR (KBr, cm^{-1}): 3436.73, 2959.18, 1677.19, 1512.41, 1243.83, 816.33; ^1H NMR (300 MHz,

DMSO-d_6) δ: 7.29 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 7.9 Hz, 1H), 6.90 (d, J = 8.3 Hz, 2H), 6.81 (d, J = 7.9 Hz, 1H), 6.56 (s, 1H), 4.50 (d, J = 14.7 Hz, 1H), 4.24 (d, J = 14.7 Hz, 1H), 4.06–4.01 (m, 1H), 3.72 (s, 3H), 3.33 (s, 3H), 2.18 (s, 3H), 2.01 (s, 3H), 1.04 (d, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 167.5, 164.6, 158.4, 158.0, 134.7, 128.9, 128.7, 128.6, 124.6, 119.0, 114.8, 114.1, 114.0, 58.7, 57.3, 55.0, 50.4, 40.0, 39.7, 39.4, 39.2, 38.9, 28.5, 12.3, 11.0, 10.1. MS (ESI, m/z):414.1 $[M+Na]^+$; Anal. calcd. for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73. Found: C, 70.55; H, 6.51; N, 10.68.

4.2. Docking studies

All ligand molecules were drawn in ChemDraw 2014, and saved as sdf style. Then ligands were processed at a simulated pH of 7.4 ± 1.0 to generate all possible tautomers, stereoisomers, and protonation states and were finally minimized at the OPLS 2005 force field with Ligand preparation protocol of Maestro 10.2 [53]. The crystal complex (PDB id: 3P5O) was selected as the BRD4-BD1 docking protein. The protein was opened with Glide generation protocol of Maestro 10.2, seven molecules of water around the binding site were saved (id: HOH18, HOH27, HOH33, HOH256, HOH267, HOH268, HOH358), and the bond orders were assigned. Hydrogen atoms were added. Then a 10- Å box centered on the geometrical center of the ligand binding site was generated for grid calculation. The docking studies were processed with the Glide docking protocol. After docking finished, only one docking conformation was saved for every compound.

4.3. Biological evaluation

4.3.1. BRD4-BD1 binding activity

11 compounds were evaluated against BRD4-BD1 in vitro with compound **28a** as the reference compound. BRD4 (44–168 aa) was bought from Active Motif. After preparing 1× assay buffer (modified HEPES Buffer), target compounds were transferred to assay plate by Echo, and the final concentration of DMSO solution was 0.1%. Then the preparation of protein solution and substrate solution in 1× assay buffer was performed. After that, 5 μL of protein solution was transferred to assay plate and incubated at room temperature for 15 min. The protein concentration was 5 nM. Then 5 μL of substrate solution was added to each well to start reaction with an incubation period for 60 min at room temperature. Finally, 15 μL acceptor and donor solution were added, followed by another incubation period for 60 min at room temperature under subdued light. The IC_{50} values were calculated from the endpoints of EnSpire with Alpha mode.

4.3.2. Cell proliferation assay

MV-4-11 cells were seeded in 96-well tissue culture plates without test compound and incubated at 37 °C and an atmosphere of 5% CO_2 for 24 h. On the next day, various concentrations of compounds **5i**, **5a** and reference compound **28a** were prepared and added into the wells. After that, cells were further incubated for 72 h at 37 °C and an atmosphere of 5% CO_2. Cell proliferation was then determined using CellTiter-Glo agents that were added into every well and shaken for 10 min [54]. Then optical signal was stabilized at room temperature for two minutes and tested by Envision. The data were processed in XL fit. The cell proliferation assay was processed to obtain the IC_{50} for the compounds **5i**, **5a** and reference compound **28a** reported in Table 3.

Acknowledgments

This research was supported by the Natural Science Foundation of Jiangsu Province (No. BK20141349) and the China National Key Hi-Tech Innovation Project for the R&D of Novel Drugs (No. 2013ZX09301303-002).

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