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Synthesis and biological evaluation of N^4-(hetero)arylsulfonylquinoxalinones as HIV-1 reverse transcriptase inhibitors

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ABSTRACT

A series of novel N^4-(hetero)arylsulfonylquinoxalinone derivatives were prepared in a straight and efficient way. Of all the synthesized compounds, five compounds exhibited potent anti-HIV-1 replication activities with IC_{50} value at the level of 10^{-7} mol/L. Preliminary structure–activity relationships were studied in details and that will shed light on the discovery of more potent non-nucleoside reverse-transcriptase inhibitors.

1. Introduction

Since the human immunodeficiency virus 1 (HIV-1) was first confirmed as the causative agent of acquired immunodeficiency syndrome (AIDS), there had been an intensive investigation aimed at developing anti-HIV drugs. Up to now, more than 20 anti-HIV drugs, classified into seven groups, respectively, had been approved for clinical treatment.[1] Among these clinic drugs, non-nucleoside reverse-transcriptase inhibitors (NNRTIs), which interact with a specific allosteric non-substrate binding site on HIV-1 reverse transcriptase, have proved to be effective anti-HIV drugs because of their high potency, low toxicity and improved pharmacokinetics.[2,3] To date, three NNRTIs, including Nevirapine, efavirenz and delavirdine, have been approved for clinical treatment of HIV infection (Fig. 1). Efavirenz acted as an essential component of the highly active anti-retroviral therapy (HAART).[4,5] Nevertheless, HIV has a low genetic barrier to generate resistance to NNRTIs, which accounts for the rapid emergence of drug resistance.[6] Therefore, an enormous effort is continued to develop novel chemical entities with improved sensitivity and resistance profiles.

In our ongoing study to search for new chemical entities of NNRTIs by screening our in-house library, 6-fluoro-N^4-(quinoline-8-sulfonyl)-3,4-dihydroquinoxalin-2(1*H*)-one **1** was identified with anti-HIV activity at micromolar level (Fig. 1). The lead compound **1** is a quinoxaline-based chemical entity, and this subunit also was presented in the potent NNRTIs such as GW420867x[7,8] and HBY097.[9] With a purpose to improve the anti-HIV activity of the lead compound **1**, a wide array of N^4-(hetero)arylsulfonylquinoxalinone analogs were designed and synthesized, and their antiviral activities were evaluated. Herein, the detailed synthetic procedure, anti-HIV-1 activity and preliminary structure–activity relationships (SAR) studies of these new quinoxalinone chemical entities are described as follows.

2. Chemistry

The compound **1** and its analogs **5–22** were synthesized straightforward as shown in Scheme 1. In the presence of bases (DIEA or K_2CO_3), the aromatic nucleophilic substitution reaction of the structurally diverse *o*-halo nitrobenzene **2a–c** with natural or unnatural amino acids or their esters provided the key intermediates **3a–i** in the yield of 20–80%. The quinoxalinone scaffold **4** was achieved by the reductive cyclization of intermediates **3** in situ in moderate to high yield (37–98%). In the course of optimizing reaction condition, a wide range of reducing reagents such as $Na_2S_2O_4/K_2CO_3$, Pd–C/H_2, Pd–C/$HCOONH_4$ or Fe/HOAc has been attempted. As Pd–C/$HCOONH_4$ was employed in the cyclization reaction, compounds **4a**, **4e** and **4i** were generated in high yield (88–96%); while moderate to good yield (37–98%) was obtained when $Na_2S_2O_4/K_2CO_3$ was chosen as the reducing reagent (e.g., **4b**, **4d**, **4f** and **4g**). Compound **4c** was afforded in 66% yield via the catalytic hydrogenation of compound **3c** in the presence of 10% Pd–C. After attempting several reducing reagents, Fe/HOAc was used to reduce compound **3h** to give compound **4h** in 49% yield smoothly. Finally, sulfonylation of quinoxalinones **4** with various aryl or heteroaryl sulfonyl chloride in the presence of pyridine provided the desired quinoxalinone derivatives **1**, **5–22**.

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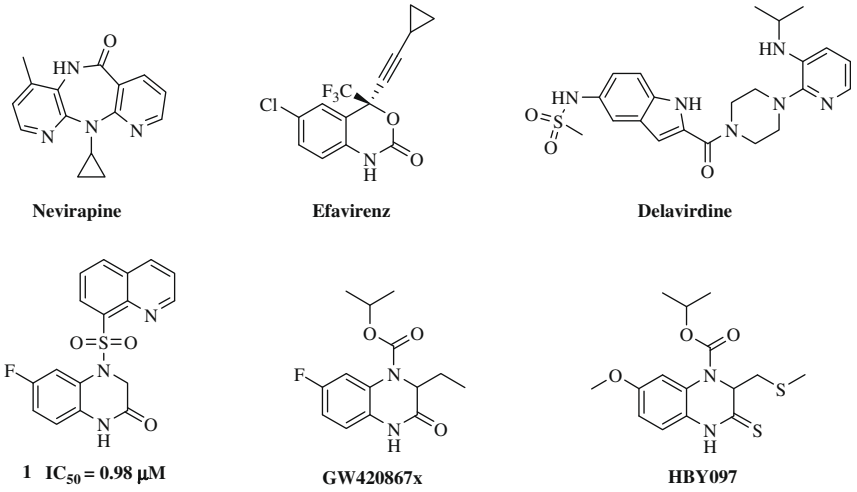
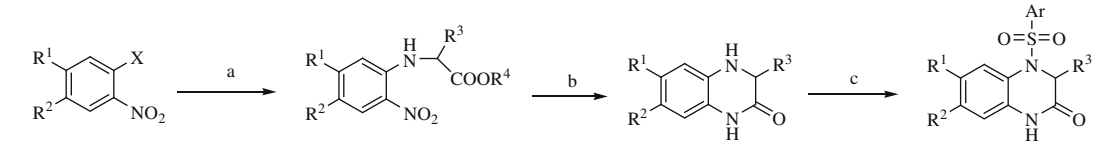


Figure 1. Chemical structures of FDA-approved NNRTIs and quinoxaline-based NNRTIs.



2a: R^1 = F, R^2 = H, X = F
2b: R^1 = Cl, R^2 = H, X = Cl
2c: R^1 = H, R^2 = CF_3, X = Cl

3a: R^1 = F, R^2 = H, R^3 = H, R^4 = C_2H_5
3b: R^1 = F, R^2 = H, R^3 = CH_3, R^4 = H
3c: R^1 = F, R^2 = H, R^3 = C_2H_5, R^4 = H
3d: R^1 = F, R^2 = H, R^3 = $CH_2CH_2SCH_3$, R^4 = CH_3
3e: R^1 = F, R^2 = H, R^3 = CH_2COOCH_3, R^4 = CH_3
3f: R^1 = Cl, R^2 = H, R^3 = H, R^4 = C_2H_5
3g: R^1 = Cl, R^2 = H, R^3 = CH_3, R^4 = H
3h: R^1 = Cl, R^2 = H, R^3 = C_2H_5, R^4 = H
3i: R^1 = H, R^2 = CF_3, R^3 = H, R^4 = C_2H_5

4a: R^1 = F, R^2 = H, R^3 = H
4b: R^1 = F, R^2 = H, R^3 = CH_3
4c: R^1 = F, R^2 = H, R^3 = C_2H_5
4d: R^1 = F, R^2 = H, R^3 = $CH_2CH_2SCH_3$
4e: R^1 = F, R^2 = H, R^3 = CH_2COOCH_3
4f: R^1 = Cl, R^2 = H, R^3 = H
4g: R^1 = Cl, R^2 = H, R^3 = CH_3
4h: R^1 = Cl, R^2 = H, R^3 = C_2H_5
4i: R^1 = H, R^2 = CF_3, R^3 = H

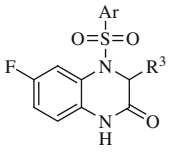
Scheme 1. Reagents and conditions: (a) amino acid/K_2CO_3, ethanol/water or amino acid ester/DIEA, CH_3CN; (b) $Na_2S_2O_4$/K_2CO_3/ethanol/water or H_2/Pd–C/ethanol; Pd–C/$HCOONH_4$; Fe/HOAc; (c) $ArSO_2Cl$ or heteroaryl sulfonyl chloride, pyridine, CH_2Cl_2.

3. Biological results and discussion

The anti-HIV-1 activities of all target compounds **5–22** were evaluated by a cell-based HIV-1 replication pharmacological model which was set up by HIV-1(pNL4-3) core packed with vesicular stomatitis virus glycoprotein. The level of HIV-1 replication was presented by a reporter gene expression (i.e., luciferase activity) in infected cells. The results are expressed as IC_{50} values. Azidothymidine (AZT) and Efavirenz were used as reference molecules (Tables 1–3).

The quinoxaline derivatives GW420867x and HBY097 were reported with potent anti-HIV activities.[7–9] Both GW420867x and HBY097 have a common *iso*-propyloxy carbonyl group on *N*-4 position; while within our lead compound **1**, a heteroaryl sulfonyl functionality is installed on *N*-4 instead of an *iso*-propyloxy carbonyl group. Hence, our initial investigation was performed on the modification of the substituents on *N*-4 position. As shown in Table 1, compound **6** exhibited a comparable anti-HIV-1 replication activity to lead compound **1**. Meanwhile, compounds **7–11** were designed and synthesized as shown in Scheme 1 on the basis of the following rationales: (1) the known SAR studies on quinoxalines[8] showed that the advantageous effect of small alkyl group on C-3 to the anti-HIV activity; (2) the installment of methyl group on C-3 could be easily completed using alanine as nucleophilic reagent. As expected, compounds **9** and **10** exhibited increased anti-HIV-1 activity in comparison with compounds **5** and **6**, respectively; while compound **7** showed sevenfold less anti-HIV-1 activity than that of lead compound **1**, probably due to the more bulky size of the quinoline-8-sulfonyl group.

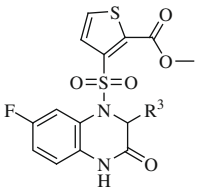
Table 1
The chemical structures and anti-HIV replication activities of compounds **1**, **5–11**[a]



Compound	R^3	$ArSO_2$	IC_{50}[b] (μM)
1	H	Quinoline-8-sulfonyl	0.98
5	H	3-Cyanobenzene-1-sulfonyl	>100
6	H	2-Methyloxycarbonylthiophene-3-sulfonyl	2.02
7	CH_3	Quinoline-8-sulfonyl	7.0
8	CH_3	4-Nitrobenzene-1-sulfonyl	>100
9	CH_3	3-Cyanobenzene-1-sulfonyl	55
10	CH_3	2-Methyloxycarbonylthiophene-3-sulfonyl	0.2
11	CH_3	2,5-Dichlorothiophene-3-sulfonyl	>100

[a] AZT and Efavirenz were used as reference molecules; IC_{50} for AZT was 48 nM; IC_{50} for Efavirenz was 1.3 nM.
[b] Compound dose (μM) required to inhibit the HIV activity by 50%.

Table 2
The chemical structures and anti-HIV-1 replication activities of compounds **6**, **10** and **12–14**[a]



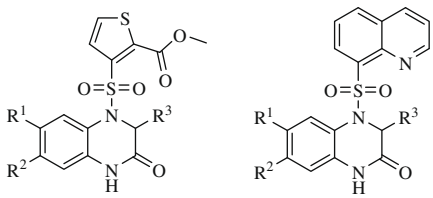
Compound	R^3	IC_{50}[b] (μM)
6	H	2.02
10	CH_3	0.2
12	C_2H_5	5.0
13	$CH_2CH_2SCH_3$	>100
14	CH_2COOCH_3	>100

[a] AZT and Efavirenz were used as reference molecules; IC_{50} for AZT was 48 nM; IC_{50} for Efavirenz was 1.3 nM.
[b] Compound dose (μM) required to inhibit the HIV activity by 50%.

Studies of a series of known crystal structures of the complexes of NNRTIs and HIV-1 RT indicated that a small hydrophobic group incorporated in NNRTIs played a key role for triggering the conformational switch of Tyr181 and lead to the increased binding affinity.[8,10] As shown in Table 1, compound **10** exhibited the most potent inhibitory activity with an IC_{50} value of 0.2 μM, so we preserved the 2-methoxylcarbonyl-3-sulfonylthiophene and further explored the effect of various 3-substitutent. As depicted in Table 2, the bulky group on C-3 position has a significant impact on the anti-HIV potency of the synthesized compounds. As R^3 group increase in bulky size, the activity of the corresponding compound tends to decrease. For example, the larger R^3 group such as $CH_2CH_2SCH_3$ and CH_2COOCH_3 lead to a complete loss of the anti-HIV activity (e.g., compounds **13** and **14**); In contrast, the smaller alkyl group such as methyl and ethyl are tolerated.

The role of the substitutes of the phenyl ring on the biological activities was also investigated as shown in Table 3. Compounds (**I**) and (**II**) were chosen as template due to their potent anti-HIV-1 activity. The replacement of 6-fluoro with chloro give rise to an advantageous effect on the activity in moderate degree (**6** vs **15**, **1** vs **19** and **7** vs **20**). However, the introduction of CF_3 group on C-7 position is detrimental to the anti-HIV-1 activity (**15** vs **18**, **19** vs **22**). The most potent compounds **10** and **16** were discovered with IC_{50} value of 0.2 μM with either fluoro or chloro substituent on C-6 position.

Table 3
The chemical structures and anti-HIV-1 replication activities of compounds **15–22**[a]



(**I**) (**II**)

Compound	Structure	R^1	R^2	R^3	IC_{50}[b] (μM)
15	I	Cl	H	H	0.83
16	I	Cl	H	CH_3	0.2
17	I	Cl	H	C_2H_5	6.3
18	I	H	CF_3	H	>100
19	II	Cl	H	H	0.33
20	II	Cl	H	CH_3	0.65
21	II	Cl	H	C_2H_5	>100
22	II	H	CF_3	H	>100

[a] AZT and Efavirenz were used as reference molecules; IC_{50} for AZT was 48 nM; IC_{50} for Efavirenz was 1.3 nM.
[b] Compound dose (μM) required to inhibit the HIV replication activity by 50%.

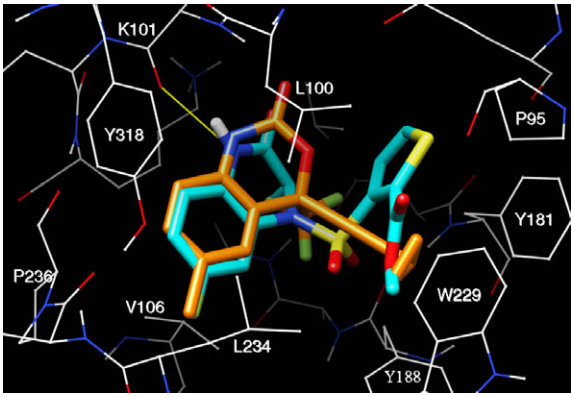


Figure 2. FlexX-modeled binding mode of **10** (carbon atoms colored cyan) in comparison with the crystal structure (1FK9 in PDB) of Efavirenz (carbon atoms colored orange). The key hydrogen bond is illustrated with yellow line. Molecular image was generated with UCSF Chimera.[13]

To investigate the binding mode of the synthesized arylsulfonylquinoxalinones, computational modeling was performed using FlexX algorithm implemented in SYBYL 6.9.[11] The coordinates of the RT–Efavirenz complex (pdb code: 1FK9)[12] was downloaded, and the molecular structure of the most potent compound **10** was docked into its active site. As shown in Figure 2, the orientation and position of compound **10** is quite similar to that of Efavirenz. The quinoxalinone ring has a hydrophobic contact with the surrounding residues such as Leu100, Val106, Leu234, Pro236 and Tyr318. The crucial hydrogen bonding interaction, which is also frequently used for some other typical NNRTIs to bind to RT, was formed between the carbonyl oxygen of Lys101 and NH of the quinoxalinone compound **10**. The 2-methoxylcarbonyl-3-sulfonylthiophene fragment lies in a hydrophobic pocket consisting of Pro95, Tyr181, Tyr188 and Trp229, which is occupied by the cyclopropyl-propynyl of Efavirenz in the complex. As expected, the methyl substituent of compound **10** takes a similar orientation to the trifluoromethyl group of Efavirenz. In consistent with the above SAR studies, the larger C-3 substituents resulted in decreased activity probably due to their steric interference with the residues Val106, Val179 and Gly190 in the binding site.

4. Conclusions

In summary, a wide array of novel heteroarylsulfonylquinoxaline-based chemical entities was synthesized as potent NNRTIs with IC_{50} at submicromolar level. The SAR and molecular modeling studies of the titled compounds were analyzed, and that will serve as guidance to further development of more potent quioxalines NNRTIs.

5. Experimetal

5.1. Chemistry

5.1.1. General

Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H NMR (300 MHz) on a Varian Mercury 300 spectrometer was recorded in DMSO-d_6 or $CDCl_3$. Chemical shifts are reported in δ (ppm) units relative to the inter-

nal standard tetramethylsilane (TMS). High resolution mass spectra (HRMS) were obtained on an Agilent Technologies LC/MSD TOF spectrometer. All chemicals and solvents used were of reagent grade without purified or dried before use. All the reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV lamp. Column chromatography separations were performed with silica gel (200–300 mesh).

5.1.2. 6-Fluoro-4-(quinoline-8-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (1)

5.1.2.1. Ethyl 2-(5-fluoro-2-nitrophenyl)-acetate (3a). To a stirred solution of 2,4-difluoronitro-benzene **2a** (6.36 g, 40 mmol) in CH_3CN (20 mL) was added glycine ethyl ester hydrochloride (6.70 g, 48 mmol) followed by addition of a solution of DIEA (12.41 g, 96 mmol) in CH_3CN (10 mL). The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated to afford the crude product which was purified by recrystalization in 95% ethanol. Compound **3a** was obtained as yellow needle crystal (7.67 g,79% yield): mp 101–104 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.55 (br s, 1H), 8.26 (dd, J_1 = 9.3 Hz, J_2 = 6.3 Hz, 1H), 6.44 (ddd, J_1 = 9.6 Hz, J_2 = 7.2 Hz, J_3 = 2.1 Hz, 1H), 6.34 (dd, J_1 = 11.1 Hz, J_2 = 2.4 Hz, 1H), 4.30 (q, J = 7.2 Hz, 2H), 4.04 (d, J = 4.8 Hz, 2H), 1.33 (t, J = 6.9 Hz, 3H).

5.1.2.2. 6-Fluoro-3,4-dihydroquinoxalin-2-(1*H*)-one (4a). The reaction mixture of compound **3a** (3.91 g, 16.14 mmol), $HCOONH_4$ (15.26 g, 242.15 mmol) and 10% Pd–C (6.85 g, 3.23 mmol) in ethanol (300 mL) was stirred for 20 h. The solid was removed by filtration and the filtrate was concentrated to afford the yellow solid which was purified by column chromatography on silica gel with petroleum ether–EtOAc (v/v = 2:1). Compound **4a** (2.57 g) was obtained as yellow solid (2.57 g, 96% yield); mp 165–169 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) δ 10.22 (s, 1H), 6.66 (dd, J_1 = 8.4 Hz, J_2 = 5.6 Hz, 1H), 6.43 (dd, J_1 = 10.4 Hz, J_2 = 2.8 Hz, 1H), 6.34 (td, J_1 = 8.8 Hz, J_2 = 2.8 Hz, 1H), 6.20 (s, 1H), 3.72 (s, 2H).

5.1.2.3. 6-Fluoro-4-(quinoline-8-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (1). To a well stirred solution of compound **4a** (0.12 g, 0.70 mmol) in pyridine (10 mL) was added quinoline-8-sulfonyl chloride (0.19 g, 0.84 mmol). The reaction mixture was stirred at room temperature for 12 h and then heated at 50 °C for 24 h. The saturated $CuSO_4$ aqueous solution was added to quench the reaction. The reaction mixture was extracted with EtOAc (30 mL × 3) and the organic layer was washed with saturated $CuSO_{4(aq)}$ (30 mL × 2), dried over anhydrous $MgSO_4$, concentrated to give the crude product which was purified by column chromatography on silica gel to afford compound **1** as white solid (0.12 g, 48%); mp 195–196 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ *8.92 (dd,* J_1 = 4.2 Hz, J_2 = 1.8 Hz, 1H), 8.46 (dd, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 1H), 8.18 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H), 8.14 (br s, 1H), 8.00 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H), 7.66 (dd, J_1 = 10.2 Hz, J_2 = 2.7 Hz, 1H), 7.57 (t, J = 7.8 Hz, 3H), 7.44 (dd, J_1 = 8.4 Hz, J_2 = 4.2 Hz, 1H), 6.70 (td, J_1 = 8.4 Hz, J_2 = 2.7 Hz, 1H), 6.51 (dd, J_1 = 8.7 Hz, J_2 = 5.1 Hz, 1H), 4.89 (s, 2H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{12}N_2O_4F_2$ [M+H$^+$]: 358.0759, found 358.0793.

5.1.3. 6-Fluoro-4-(3-cyanobenzene-1-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (5)

Following the preparation protocol of Section 5.1.2.3, the mixture of compound **4a** (0.083 g, 0.5 mmol) and 3-cyanobenzene-1-sulfonyl chloride (0.12 g, 0.6 mmol) in pyridine was stirred at room temperature to provide the title compound as light yellow solid (0.08 g, 48% yield); mp 258–262 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.32 (s, 1H), 8.20 (dt, J_1 = 7.2 Hz, J_2 = 1.5 Hz 1H), 7.93 (s, 1H), 7.66–7.75 (m, 2H), 7.37 (dd, J_1 = 9.6 Hz, J_2 = 2.7 Hz, 1H), 7.20 (td, J_1 = 8.4 Hz, J_2 = 2.7 Hz, 1H), 6.79 (dd, J_1 = 9.0 Hz, J_2 = 5.1 Hz, 1H), 4.34 (s,2H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{11}N_3O_3FS$ [M+H$^+$]: 332.0505, found 332.0504.

5.1.4. 6-Fluoro-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (6)

Following the preparation protocol of Section 5.1.2.3, the mixture of compound **4a** (0.083 g, 0.5 mmol) and 2-methyoxycarbonylthiophene-3-sulfonyl chloride (0.14 g, 0.6 mmol) in pyridine was stirred at room temperature for 48 h to provide the title compound as light yellow solid (0.04 g, 22% yield); mp 182–184 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.53 (s, 1H), 7.95 (d, J = 5.4 Hz, 1H), 7.27 (d, J = 5.1 Hz,1H), 7.24 (dd, J_1 = 9.6 Hz, J_2 = 2.7 Hz, 1H), 7.09 (td, J_1 = 8.7 Hz, J_2 = 2.7 Hz, 1H), 6.86 (dd, J_1 = 8.7 Hz, J_2 = 5.4 Hz, 1H), 4.41 (s,2H), 3.72 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{14}H_{12}N_2O_5FS_2$ [M+H$^+$]: 371.0166, found 371.0166.

5.1.5. 6-Fluoro-3-methyl-4-(quinoline-8-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (7)

5.1.5.1. 2-(5-Fluoro-2-nitrophenylamino)propanoic acid (3b). To a stirred solution of K_2CO_3 (5.53 g, 40 mmol) and alanine (1.78 g, 20 mmol) in water (50 mL) was added dropwise 2,4-difluoronitrobenzene **2a** (3.82 g, 24 mmol) in ethanol (30 mL). The reaction mixture was stirred at room temperature for 12 h. The solvent was evaporated in vacuo. The residue was dissolved in water (100 mL) and washed with ether (30 mL × 3). The aqueous phase was adjusted to pH 2 with 2 N HCl aqueous solution, and then extracted with EtOAc (50 mL × 4). The organic layer was dried over anhydrous $MgSO_4$, concentrated and purified with column chromatography on silica gel to afford compound **3b** as yellow solid (3.0 g, 66% yield); mp 118–120 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.44 (d, J = 6.3 Hz, 1H), 8.26 (dd, J_1 = 9.3 Hz, J_2 = 6.0 Hz, 1H), 6.49–6.37 (m, 2H), 4.27 (m, J = 6.9 Hz, 1H), 1.68 (d, J = 7.2 Hz, 3H).

5.1.5.2. 6-Fluoro-3-methyl-3,4-dihydroquinoxalin-2-(1*H*)-one (4b). To a stirred solution of K_2CO_3 (2.76 g, 20 mmol) and $Na_2S_2O_4$ (10.69 g, 60 mmol) in water (60 mL) was added dropwise compound **3b** (2.28 g, 10 mmol) in ethanol (80 mL). The reaction mixture was stirred at room temperature until TLC indicated the reaction completed. The solvent was evaporated in vacuo. The residue was dissolved in water (100 mL) and extracted with EtOAc (50 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, concentrated and purified with column chromatography on silica gel to afford compound **4b** as yellow solid (1.3 g, 74% yield); mp 111–113 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 9.20 (s, 1H), 6.70 (dd, J_1 = 8.7 Hz, J_2 = 5.1 Hz, 1H), 6.38–6.47 (m, 2H), 4.02 (q, J = 6.6 Hz, 1H), 3.96 (br s, 1H), 1.46 (d, J = 6.9 Hz, 3H).

5.1.5.3. 6-Fluoro-3-methyl-4-(quinoline-8-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (7). Following the preparation protocol of Section 5.1.2.3, the mixture of compound **4b** (0.13 g, 0.70 mmol) and quinoline-8-sulfonyl chloride (0.19 g, 0.84 mmol) in pyridine was stirred to provide the title compound as yellow oil (0.094 g, 36% yield); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.20 (s, 1H), 8.83 (dd, J_1 = 4.2 Hz, J_2 = 1.5 Hz, 1H), 8.48 (dd, J_1 = 8.4 Hz, J_2 = 1.5 Hz, 1H), 8.39 (d, J = 7.2 Hz, 1H), 8.27 (d, J = 7.8 Hz, 1H), 7.68 (m, 2H), 7.49 (dd, J_1 = 10.2 Hz, J_2 = 2.7 Hz, 1H), 6.85 (td, J_1 = 8.4 Hz, J_2 = 2.7 Hz, 1H), 6.63 (dd, J_1 = 8.7 Hz, J_2 = 5.7 Hz, 1H), 5.35 (q, J = 7.5 Hz, 1H), 1.29 (d, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{15}N_3O_3FS$ [M+H$^+$]: 372.0813, found 372.0826.

5.1.6. 6-Fluoro-3-methyl-4-(4-nitrobenzene-1-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (8)

Following the preparation protocol of Section 5.1.2.3, the mixture of compound **4b** (0.25 g, 1.40 mmol) and 4-nitrobenzene-1-sulfonyl chloride (0.37 g, 1.68 mmol) in pyridine was stirred to provide the title compound as yellow solid (0.34 g, 67% yield);

mp 240–241 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.19(s, 1H), 8.32(d, J = 8.7 Hz, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.25–7.54(m, 2H), 6.66 (dd, J_1 = 8.7 Hz, J_2 = 5.7 Hz, 1H), 6.46 (dd, J_1 = 10.2 Hz, J_2 = 2.7 Hz, 1H), 6.36 (td, J_1 = 8.7 Hz, J_2 = 2.7 Hz, 1H), 3.78(q, J_1 = 6.6 Hz, 1H), 1.23 (d, J = 6.6 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{13}N_3O_5FS$ [M+H$^+$]: 366.0554, found 366.0573.

5.1.7. 6-Fluoro-3-methyl-4-(3-cyanobenzene-1-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (9)

The mixture of compound **4b** (0.05 g, 0.3 mmol) in CH_2Cl_2 (6 mL) and 3-cyanobenzene-1-sulfonyl chloride (0.18 g, 0.9 mmol) was added pyridine (0.12 g, 1.5 mmol) in CH_2Cl_2 (4 mL). The reaction mixture was stirred at room temperature to provide the title compound as yellow solid (0.09 g, 88% yield); mp 213–215 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.38 (s, 1H), 8.19 (dt, J_1 = 7.8 Hz, J_2 = 1.5 Hz, 1H), 7.92 (s, 1H), 7.63–7.74 (m, 2H), 7.40 (dd, J_1 = 9.3 Hz, J_2 = 2.7 Hz, 1H), 7.22 (td, J_1 = 8.7 Hz, J_2 = 3.0 Hz, 1H), 6.81(dd, J_1 = 9.0 Hz, J_2 = 5.4 Hz, 1H), 4.62 (q, J = 6.6 Hz, 1H), 1.15 (d, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{13}N_3O_3FS$ [M+H$^+$]: 346.0662, found 346.0688.

5.1.8. 6-Fluoro-3-methyl-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxa-lin-2-(1*H*)-one (10)

Following the preparation protocol of Section 5.1.7, the mixture of compound **4b** (0.05 g, 0.3 mmol), 2-methyoxycarbonylthiophene-3-sulfonyl chloride (0.22 g, 0.9 mmol) and pyridine (0.12 g, 1.5 mmol) in CH_2Cl_2 was stirred to provide the title compound as yellow solid (0.05 g, 44% yield); mp 184–186 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.58 (s, 1H), 7.95 (d, J = 5.1 Hz, 1H), 7.31 (dd, J_1 = 7.5 Hz, J_2 = 2.7 Hz, 1H), 7.29 (d, J = 5.1 Hz, 1H), 7.10 (td, J_1 = 8.7 Hz, J_2 = 2.7 Hz, 1H), 6.88 (dd, J_1 = 9.0 Hz, J_2 = 5.7 Hz, 1H), 4.80 (q, J = 7.5 Hz, 1H), 3.69 (s, 3H), 1.18 (d, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{15}H_{14}N_2O_5FS_2$ [M+H$^+$]: 385.0322, found 385.0313.

5.1.9. 6-Fluoro-3-methyl-4-(2,5-dichlorothiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (11)

Following the preparation protocol of Section 5.1.7, the mixture of compound **4b** (0.045 g, 0.25 mmol), 2,5-dichlorothiophene-3-sulfonyl chloride (0.19 g, 0.75 mmol) and pyridine (0.10 g, 1.25 mmol) in CH_2Cl_2 was stirred to provide the title compound as yellow solid (0.075 g, 76%); mp 203–206 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ 8.24 (s, 1H), 7.49 (dd, J_1 = 9.2 Hz, J_2 = 2.8 Hz, 1H), 7.01 (td, J_1 = 8.4 Hz, J_2 = 2.8 Hz, 1H), 6.83 (s, 1H), 6.77 (dd, J_1 = 8.8 Hz, J_2 = 4.8 Hz, 1H), 4.92 (q, J = 7.2 Hz, 1H), 1.37 (d, J_1 = 7.2 Hz, 1H); HRMS (ESI): *m/z*, calcd for $C_{13}H_{10}N_2O_3FS_2Cl_2$ [M+H$^+$]: 394.9494, found 394.9531.

5.1.10. 6-Fluoro-3-ethyl-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxa-lin-2-(1*H*)-one (12)

5.1.10.1. 2-(5-fluoro-2-nitrophenylamino)butanoic acid (3c). The reaction mixture of K_2CO_3 (0.28 g, 2.0 mmol) and 2-aminobutanoic acid (0.10 g, 1.0 mmol) in water (2 mL) and 2,4-difluoronitrobenzene **2a** (0.19 g, 1.2 mmol) in ethanol (0.5 mL) was heated by microwave (power 50 W, temperature 120 °C) for 30 min. The reaction mixture was concentrated in vacuo. The residue was dissolved in water (40 mL) and washed with ether (10 mL × 3). The aqueous phase was adjusted to pH 2 with 5% HCl aqueous solution, and then extracted with EtOAc (20 mL × 2). The organic layer was dried over anhydrous $MgSO_4$, concentrated and purified with column chromatography on silica gel to afford compound **3c** as yellow oil (0.075 g, yield 31.0%); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.47 (d, J = 6.6 Hz, 1H), 8.26 (dd, J_1 = 9.0 Hz, J_2 = 6.3 Hz, 1H), 6.44 (m, 2H), 4.16 (dd, J_1 = 12.0 Hz, J_2 = 5.1 Hz, 1H), 2.05 (m, 2H), 1.10 (t, J = 7.2 Hz,, 3H).

5.1.10.2. 6-Fluoro-3-ethyl-3,4-dihydroquinoxalin-2-(1*H*)-one (4c). The mixture of compound **3c** (0.073 g, 0.3 mmol) and 10% Pd–C (0.127 g, 0.06 mmol) in ethanol (20 mL) was subjected to hydrogenation under the pressure of 60 psi for 30 min. The reaction mixture was filtered and the filtrate was concentrated and purified with column chromatography to afford the compound **4c** as brown solid (38 mg, 66% yield); mp 133–135 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.46 (s, 1H), 6.63 (dd, J_1 = 9.6 Hz, J_2 = 6.9 Hz, 1H), 6.43 (d, J = 7.8 Hz, 1H), 6.40 (d, J = 9.6 Hz, 1H), 4.02 (s, 1H), 3.87 (t, J_1 = 6.0 Hz, 1H), 1.84 (m, 2H), 1.03 (t, J = 7.2 Hz,, 3H).

5.1.10.3. 6-Fluoro-3-ethyl-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (12). Following the preparation protocol ofSection 5.1.7, the mixture of compound **4c** (19 mg, 0.1 mmol), 2-methyoxycarbonylthiophene-3-sulfonyl chloride (48 mg, 0.2 mmol) and pyridine (40 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (2 mL) was stirred to provide the title compound as light yellow oil (15 mg, 38% yield); ^{1}H NMR (300 MHz, acetone-d_6, δ ppm) δ 9.43 (s, 1H), 7.80 (d, J = 5.4 Hz, 1H), 7.48 (dd, J_1 = 9.9 Hz, J_2 = 2.7 Hz, 1H), 7.33 (d, J = 5.1 Hz, 1H), 6.96–7.02 (m, 2H), 4.77 (dd, J_1 = 10.2 Hz, J_2 = 5.1 Hz, 1H), 3.76 (s, 3H), 1.68 (m, 1H), 1.44 (m, 1H), 1.00 (t, J_1 = 7.5 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{15}NO_4F_2S_2$ [M+H$^+$]: 399.0410, found 399.0417.

5.1.11. 6-Fluoro-3-((2-methylthio)-ethyl)-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (13)

5.1.11.1. Methyl 2-(5-fluoro-2-nitrophenylamino)-4-methylthiobutanoate (3d). Following the preparation protocol of Section 5.1.2.1, the mixture of 2,4-difluoronitrobenzene **2a** (0.79 g, 5.0 mmol), methionine methyl ester hydrochloride (1.20 g, 6.0 mmol) and DIEA (1.55 g, 12 mmol) in CH_3CN was stirred at room temperature for 12 h and then refluxed to afford compound **3d** (1.44 g) as yellow oil in yield 95%.

5.1.11.2. 6-Fluoro-3-((2-methylthio)-ethyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (4d). Following the preparation protocol of Section 5.1.5.2, a solution of K_2CO_3 (553 mg g, 4.0 mmol), $Na_2S_2O_4$ (2.09 g, 12 mmol) and compound **3d** (605 mg, 2.0 mmol) in the mixture of water and ethanol was stirred at room temperature for 30 min to afford compound **4d** (475 mg) as light yellow oil in yield 99%. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.69 (s, 1H), 6.66 (dd, J_1 = 7.5 Hz, J_2 = 5.1 Hz, 1H), 6.44 (t, J = 8.4 Hz, 1H), 6.43 (d, J = 8.7 Hz, 1H), 4.38 (s, 1H), 4.09 (m, 1H), 2.74–2.62 (m, 2H), 2.22–2.16 (m, 1H), 2.13 (s, 3H), 2.08–1.99 (m, 1H).

5.1.11.3. 6-Fluoro-3-((2-methylthio)-ethyl)-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (13). Following the preparation protocol of Section 5.1.7, the mixture of compound **4d** (48 mg, 0.2 mmol), 2-methyoxycarbonylthiophene-3-sulfonyl chloride (144 mg, 0.6 mmol) and pyridine (79 mg, 1.0 mmol) in anhydrous CH_2Cl_2 was stirred to provide the title compound (20 mg) as yellow oil in yield 22%. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 9.05 (s, 1H), 7.56 (d, J = 9.3 Hz, 1H), 7.41 (d, J = 5.1 Hz, 1H), 7.34 (d, J = 5.1 Hz, 1H), 6.89 (t, J = 7.8 Hz, 1H), 6.78 (m, 1H), 5.15 (dd, J_1 = 9.9 Hz, J_2 = 3.9 Hz, 1H), 3.76 (s, 3H), 2.64 (m, 2H), 2.06 (s, 3H), 1.96 (m, 2H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{17}N_2O_5FS_3Na$ [M+Na$^+$]: 467.0181, found 467.0180.

5.1.12. 6-Fluoro-3-((2-methoxycarbonyl)-methyl)-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (14)

5.1.12.1. Dimethyl 2-(5-fluoro-2-nitrophenylamino)-succinate (3e). Following the preparation protocol of Section 5.1.2.1, the mixture of 2,4-difluoronitrobenzene **2a** (0.79 g, 5.0 mmol), glutamic acid dimethyl hydrochloride (1.19 g, 6.0 mmol) and DIEA (1.55 g, 12 mmol) in CH_3CN was stirred at room temperature and then refluxed for 24 h to afford compound **3e** (735 mg) as yellow

oil in yield 49%. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 9.26 (br s, 1H), 8.25 (dd, J_1 = 9.3 Hz, J_2 = 6.0 Hz, 1H), 6.42–6.53 (m, 2H), 4.57 (m, 1H), 3.81 (s, 3H), 3.74 (s, 3H), 2.98 (d, J = 6.0 Hz, 2H).

5.1.12.2. 6-Fluoro-3-((2-methoxycarbonyl)-methyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (4e). Following the preparation protocol of Section 5.1.2.2, a reaction mixture of compound **3e** (735 mg, 2.45 mmol), $HCOONH_4$ (2.32 g, 36.75 mmol) and 10% Pd–C (1.04 g, 0.49 mmol) in ethanol (40 mL) was stirred to afford the yellow solid 0.56 g, yield 95%, mp 165–169 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.68 (d, J = 7.8 Hz, 1H), 8.25 (dd, J_1 = 9.3 Hz, J_2 = 6.0 Hz, 1H), 6.42–6.53 (m, 2H), 4.57 (m, 1H), 3.81 (s, 3H), 3.74 (s, 3H), 2.98 (d, J = 6.0 Hz, 2H).

5.1.12.3. 6-Fluoro-3-((2-methoxycarbonyl)-methyl)-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (14). Following the preparation protocol of Section 5.1.2.3, the mixture of compound **4e** (48 mg, 0.2 mmol) and 2-methyoxycarbonylthiophene-3-sulfonyl chloride (144 mg, 0.6 mmol) in pyridine (5 mL) was stirred to provide the title compound (70 mg) as yellow oil in yield 80%. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.52 (br s, 1H), 7.55 (dd, J_1 = 9.6 Hz, J_2 = 2.7 Hz, 1H), 7.41 (d, J = 5.4 Hz, 1H), 7.38 (d, J = 5.4 Hz, 1H), 6.87 (dd, J_1 = 7.8 Hz, J_2 = 2.7 Hz, 1H), 6.73 (dd, J_1 = 8.7 Hz, J_2 = 5.1 Hz, 1H), 5.60 (dd, J_1 = 8.4 Hz, J_2 = 5.1 Hz, 1H), 3.81 (s, 3H), 3.66 (s, 3H), 2.71 (m, 2H); HRMS (ESI): m/z, calcd for $C_{17}H_{16}N_2O_7FS_2$ [M+H$^+$]: 443.0377, found 443.0380.

5.1.13. 6-Chloro-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (15)

5.1.13.1. Ethyl 2-(5-chloro-2-nitrophenyl)-acetate (3f). Following the preparation protocol of Section 5.1.2.1, a solution of 2,4-dichloronitrobenzene **2b** (15.36 g, 80 mmol), glycine ethyl ester hydrochloride (13.40 g, 96 mmol) and DIEA (24.81 g, 192 mmol) in CH_3CN (60 mL) was stirred at room temperature to afford compound **3f** (4.85 g) as yellow solid, yield 23%, mp 96–99 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.47 (br s, 1H), 8.16 (d, J = 9.6 Hz, 1H), 6.71 (m, 2H), 4.30 (q, J = 7.2 Hz, 2H), 4.06 (d, J = 5.4 Hz, 2H), 1.33 (t, J = 6.9 Hz, 3H).

5.1.13.2. 6-Chloro-3,4-dihydroquinoxalin-2-(1*H*)-one (4f). Following the preparation protocol of Section 5.1.5.2, a solution of K_2CO_3 (5.19 g, 37.52 mmol), $Na_2S_2O_4$ (19.60 g, 112.56 mmol) and compound **3f** (4.85 g, 18.76 mmol) in the mixture of water and ethanol was stirred at room temperature to afford compound **4f** (1.92 g) as light red solid in yield 56%, mp 179–182 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.32 (s, 1H), 6.67 (d, J = 8.4 Hz, 1H), 6.65 (d, J = 2.1 Hz, 1H), 6.56 (dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 1H), 6.20 (s, 1H), 3.73 (s, 2H).

5.1.13.3. 6-Chloro-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (15). Following the preparation protocol of Section 5.1.7. the mixture of compound **4f** (55 mg, 0.30 mmol), 2-methyoxycarbonylthiophene-3-sulfonyl chloride (24 mg, 0.1 mmol) and pyridine (40 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (2 mL) was stirred to provide the title compound (23 mg) in yield 59%, white solid, mp 158–159 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.62 (s, 1H), 7.96 (d, J = 5.4 Hz, 1H), 7.42 (d, J = 2.4 Hz, 1H), 7.28 (dd, J_1 = 9.6 Hz, J_2 = 2.4 Hz, 1H), 7.25 (d, J = 5.4 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 4.41 (s, 2H), 3.71 (s, 3H); HRMS (ESI): m/z, calcd for $C_{13}H_{10}N_2O_6NaS_3$ [M+Na$^+$]: 408.9599, found 408.9607.

5.1.14. 6-Chloro-3-methyl-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxa-lin-2-(1*H*)-one (16)

5.1.14.1. 2-(5-Chloro-2-nitrophenylamino)propanoic acid (3g). Following the preparation protocol of Section 5.1.10.1, a reaction mixture of K_2CO_3 (1.38 g, 10.0 mmol) and alanine (445 mg, 5.0 mmol) in water and 2,4-dichloronitrobenzene **2b** (1.15 g, 6.0 mmol) in ethanol was heated by microwave (power 50 W, temperature 150 °C) for 30 min. The reaction mixture was concentrated in vacuo. The residue was dissolved in water (60 mL) and washed with ether (10 mL × 3). The aqueous phase was adjusted to pH 2 with 5% HCl aqueous solution, and the solid (380 mg) was precipitated in yield 31%, mp 138–141 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 8.42 (d, J = 7.2 Hz, 1H), 8.10 (d, J = 9.0 Hz, 1H), 7.09 (d, J = 2.1 Hz, 1H), 6.76 (dd, J_1 = 9.0 Hz, J_2 = 2.4 Hz, 1H), 4.56 (m, J_1 = 6.9 Hz, 1H), 1.47 (d, J = 6.9 Hz, 3H).

5.1.14.2. 6-Chloro-3-methyl-3,4-dihydroquinoxalin-2-(1*H*)-one (4g). The preparation method is same as that of compound **3b**. A solution of K_2CO_3 (276 mg, 2.0 mmol), $Na_2S_2O_4$ (1.04 g, 6.0 mmol) and compound **3g** (245 mg, 1.0 mmol) in the mixture of water and ethanol was stirred at room temperature to afford compound **4g** (73 mg) as light red solid in yield 37%, mp 119–121 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.28 (s, 1H), 6.58–6.69 (m, 3H), 6.28 (s, 1H), 3.80 (q, J =6.6 Hz, 1H), 1.23 (d, J = 6.6 Hz, 3H).

5.1.14.3. 6-Chloro-3-methyl-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (16). Following the preparation protocol of Section 5.1.7, the mixture of compound **4g** (39 mg, 0.20 mmol), 2-methyoxycarbonylthiophene-3-sulfonyl chloride (24 mg, 0.1 mmol) and pyridine (40 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (2 mL) was stirred to provide the title compound (22 mg) in yield 55%, light yellow solid, mp 193–194 °C. ^{1}H NMR (300 MHz, acetone-d_6, δ ppm) δ 9.51 (s, 1H), 7.83 (d, J = 5.1 Hz, 1H), 7.63 (d, J = 2.4 Hz, 1H), 7.32 (d, J = 5.4 Hz, 1H), 7.25 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H), 6.99 (d, J =8.7 Hz, 1H), 5.00 (q, J = 6.9 Hz, 1H), 3.76 (s, 3H), 1.29 (d, J = 7.2 Hz, 3H); HRMS (ESI): m/z, calcd for $C_{15}H_{14}N_2O_5S_2Cl$ [M+H$^+$]: 401.0027, found 401.0012.

5.1.15. 6-Chloro-3-ethyl-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (17)

5.1.15. 1. 2-(5-Chloro-2-nitrophenylamino)butanoic acid (3h). Following the preparation protocol of Section 5.1.10.1, a reaction mixture of K_2CO_3 (553 mg, 4.0 mmol) and 2-aminobutanoic acid (206 mg, 2.0 mmol) in water and 2,4-dichloronitrobenzene (461 mg, 2.4 mmol) in ethanol was heated by microwave (power 50 W, temperature 180 °C) for 30 min. Compound **3h** (108 mg) was obtained as brown oil in yield 21%. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 8.41 (d, J = 7.5 Hz, 1H), 8.11 (d, J = 9.0 Hz, 1H), 7.12 (d, J = 2.1 Hz,1H), 6.76 (dd, J_1 = 9.3 Hz, J_2 = 2.1 Hz, 1H), 4.58 (m, 1H), 1.89 (m, 2H), 0.87 (t, J = 7.5 Hz, 3H).

5.1.15.2. 6-Chloro-3-ethyl-3,4-dihydroquinoxalin-2-(1*H*)-one (4h). Fe power (247 mg, 4.2 mmol) was added to a solution of compound **3h** (108 mg, 0.42 mmol) in acetic acid (2 mL). The reaction mixtur was refluxed for 1 h and cooled to room temperature. Fe powder was filtered off and the filtrate was concentrated. The residue was dissolved in EtOAc (20 mL) and washed with saturated $NaHCO_3$ (20 mL × 2), dried over anhydrous $MgSO_4$ and concentrated to give the crude product which was purified by column chromatography. Compound **4h** (43 mg) was obtained as light brown solid in yield 49%. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.29 (s, 1H), 6.62 (m, 3H), 6.29 (s, 1H), 3.70 (m, 1H), 1.61 (m, 2H), 0.90 (t, J = 7.5 Hz, 3H).

5.1.15.3. 6-Chloro-3-ethyl-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (17). Following the preparation protocol of Section 5.1.7, the mixture of compound **4h** (42 mg, 0.20 mmol), 2-methyoxycarbonylthiophene-3-sulfonyl chloride (24 mg, 0.1 mmol) and pyridine (40 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (2 mL) was stirred to provide the title compound (21 mg) in yield 51%, light yellow solid, mp 93–95 °C. ^{1}H NMR

(300 MHz, acetone-d_6, δ ppm) δ 9.48 (br s, 1H), 7.82 (d, 1H, J = 5.1 Hz), 7.71 (d, 1H, J = 2.4 Hz), 7.31 (d, 1H, J = 5.4 Hz), 7.24 (dd, J_1 = 8.7 Hz, J_2 = 2.4 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 4.76 (dd, J_1 = 10.2 Hz, J_2 = 4.8 Hz, 1H), 3.87 (s, 3H), 1.67 (m, 1H), 1.45 (m, 1H), 1.01 (t, J = 7.5 Hz, 3H) ; HRMS (ESI): *m/z*, calcd for $C_{16}H_{16}N_2O_5S_2Cl$ [M+H$^+$]: 415.0183, found 415.0183.

5.1.16. 7-Trifluoromethyl-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxa-lin-2-(1*H*)-one (18)

5.1.16.1. Ethyl2-(4-trifluoromethyl-2-nitrophenyl)-acetate(3i). Following the preparation protocol of Section 5.1.2.1, a solution of 2-chloro-5-trifluoromethylnitrobenzene **2c** (9.02 g, 40 mmol), glycine ethyl ester hydrochloride (6.70 g, 48 mmol) and DIEA (12.4 g, 96 mmol) in CH_3CN (60 mL) was stirred at room temperature for 24 h and then refluxed for 48 h to afford compound **3i** (8.81 g) as yellow needle crystal, yield 75%, mp 109–111 °C.

5.1.16.2. 7-Trifluoromethyl-3,4-dihydroquinoxalin-2-(1*H*)-one (4i). Following the preparation protocol of Section 5.1.2.2, a reaction mixture of compound **3j** (10.69 g, 36.58 mmol), $HCOONH_4$ (34.60 g, 548.75 mmol) and 10% Pd–C (15.51 g, 7.32 mmol) in ethanol (600 mL) was stirred to afford the yellow solid 6.94 g, yield 88%, mp 150–152 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) δ 10.43 (s, 1H), 7.05 (d, J = 8.4 Hz, 1H), 6.96 (s, 1H), 6.72 (d, J = 8.4 Hz, 1H), 6.61 (s, 1H), 3.84 (s, 2H).

5.1.16.3. 7-Trifluoromethyl-4-(2-methyoxycarbonylthiophene-3-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (18). Following the preparation protocol of Section 5.1.7, the mixture of compound **4i** (65 mg, 0.30 mmol), 2-methyoxycarbonylthiophene-3-sulfonyl chloride (24 mg, 0.1 mmol) and pyridine (40 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (2 mL) was stirred to provide the title compound (28 mg) in yield 66%, light yellow solid, mp 161–162 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) δ 10.77 (s, 1H), 7.98 (d, J = 5.4 Hz, 1H), 7.61 (d, J = 5.4 Hz,1H), 7.38 (dd, J_1 = 8.7 Hz, J_2 = 1.2 Hz, 1H), 7.33 (d, J = 5.1 Hz, 1H), 7.16 (d, J = 1.5 Hz, 1H), 4.48 (s, 2H), 3.69 (s, 3H); HRMS (ESI): *m/z*, calcd for $C_{16}H_{11}NO_4F_4S_2$ [M+H$^+$]: 421.0060, found 421.0062.

5.1.17. 6-Chloro-4-(quinoline-8-sulfonyl)-3,4-dihydro-quinoxalin-2-(1*H*)-one (19)

Following the preparation protocol of Section 5.1.7, the mixture of compound **4f** (37 mg, 0.20 mmol), quinoline-8-sulfonyl chloride (137 mg, 0.6 mmol) and pyridine (79 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (2 mL) was stirred to provide the title compound (31 mg) in yield 41%, light yellow solid, mp 220–221 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.94 (dd, J_1 = 4.2 Hz, J_2 = 1.5 Hz, 1H), 8.46 (dd, J_1 = 8.1 Hz, J_2 = 1.2 Hz, 1H), 8.18 (dd, J_1 = 8.1 Hz, J_2 = 1.8 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.57 (t, J_1 = 8.1 Hz, 1H), 7.49 (dd, J_1 = 12.9 Hz, J_2 = 8.7 Hz, 1H), 7.46 (s, 1H), 6.93 (m, 1H), 6.43 (d, J = 8.4 Hz, 1H), 4.89 (s, 2H); HRMS (ESI): *m/z*, calcd for $C_{17}H_{13}N_3O_3SCl$ [M+H$^+$]: 374.0366, found 374.0383.

5.1.18. 6-Chloro-3-methyl-4-(quinoline-8-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (20)

Following the preparation protocol of Section 5.1.7, the mixture of compound **4g** (20 mg, 0.10 mmol), quinoline-8-sulfonyl chloride (68 mg, 0.3 mmol) and pyridine (40 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (2 mL) was stirred to provide the title compound (32 mg) in yield 82%, white solid, mp 214–215 °C. ^{1}H NMR (300 MHz, acetone-d_6, δ ppm) δ 9.18 (s, 1H), 8.88 (dd, J_1 = 4.2 Hz, J_2 = 1.8 Hz, 1H), 8.45 (m, 2H), 8.23 (dd, J_1 = 8.1 Hz, J_2 = 1.5 Hz, 1H), 7.88 (d, J = 2.1 Hz, 1H), 7,70 (dd, J_1 = 8.4 Hz, J_2 = 7.5 Hz, 1H), 7.63 (dd, J_1 = 8.4 Hz, J_2 = 4.2 Hz, 1H), 6.99 (dd, J_1 = 8.7 Hz, J_2 = 2.1 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 5.57 (q, J = 7.2 Hz, 1H), 1.40 (d, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{15}N_3O_3SCl$ [M+H$^+$]: 388.0517, found 388.0518.

5.1.19. 6-Chloro-3-ethyl-4-(quinoline-8-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (21)

Following the preparation protocol of Section 5.1.7, the mixture of compound **4h** (42 mg, 0.20 mmol), quinoline-8-sulfonyl chloride (137 mg, 0.6 mmol) and pyridine (79 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (2 mL) was stirred to provide the title compound (31 mg) in yield 39%, white solid, mp 107–108 °C. ^{1}H NMR (300 MHz, acetone-d_6, δ ppm) δ 9.16 (s, 1H), 8.88 (dd, J_1 = 4.2 Hz, J_2 = 1.8 Hz, 1H), 8.41–8.48 (m, 2H), 8.22 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.70 (dd, J_1 = 8.4 Hz, J_2 = 7.5 Hz, 1H), 7.63 (dd, J_1 = 8.4 Hz, J_2 = 4.2 Hz, 1H), 6.97 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H), 6.69 (d, J = 8.4 Hz, 1H), 5.32 (dd, J_1 = 10.8 Hz, J_2 = 4.8 Hz, 1H), 1.83 (m, 1H), 1.53 (m, 1H), 1.11 (t, J = 7.2 Hz, 3H); HRMS (ESI): *m/z*, calcd for $C_{19}H_{17}N_3O_3SCl$ [M+H$^+$]: 402.0673, found 402.0675.

5.1.20. 7-Trifluoromethyl-4-(quinoline-8-sulfonyl)-3,4-dihydroquinoxalin-2-(1*H*)-one (22)

Following the preparation protocol of Section 5.1.7, the mixture of compound **4i** (32 mg, 0.15 mmol), quinoline-8-sulfonyl chloride (102 mg, 0.45 mmol) and pyridine (59 mg, 0.75 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred to provide the title compound (60 mg) in yield 97%, light yellow solid, mp 205–206 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) δ 8.92 (dd, J_1 = 4.2 Hz, J_2 = 1.5 Hz, 1H), 8.45 (dd, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 1H), 8.19 (dd, J_1 = 8.1 Hz, J_2 = 1.8 Hz, 1H), 8.01 (d, J = 8.1 Hz, 2H), 7.64 (br s, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.49 (dd, J_1 = 8.4 Hz, J_2 = 4.2 Hz, 1H), 7.21 (d, J = 8.7 Hz, 1H), 6.76 (s, 1H), 4.94 (s, 2H); HRMS (ESI): *m/z*, calcd for $C_{18}H_{13}N_3O_3F_3S$ [M+H$^+$]: 408.0624, found 408.0604.

5.2. Anti-HIV activity assay

Vesicular stomatitis virus glycoprotein (VSV-G) plasmid was co-transfected with env-deficient HIV-1 vector, pNL4-3.luc.R$^-$E$^-$,[14,15] into 293 cells by using modified $Ca_3(PO_4)_2$ method.[16] Briefly, 293 cells (100 mm plate) were co-transfected with 8 μg HIV-1 vector and 3 μg VSVG DNA. After 16 h, plates were washed by PBS, and fresh media DMEM with 10% FBS was added into the plates. Forty eight hours post-transfection, supernatant was harvested and filtered through a 0.45 μm filter. The supernatant contained VSVG/HIV-1 pseudotyped virions which were quantified by p24 concentrations which were detected by ELISA (ZeptoMetrix, Cat.:0801111). VSVG/HIV-1 viral solution was diluted to 0.2 ng p24/mL and can be used directly or stored at −80 °C.

One day prior to infection, 293ET cells were plated on 24-well plates at the density of 6×10^4 cells per well. Compound was incubated with target cells for 15 min prior to adding VSVG/HIV-1(0.5 mL/well) for infection. The same amount of solvent alone was used as control. After post-infection for 48 h, cells were lysed in 50 μL cell lysis reagent (Promega). Luciferase activity of the cell lysate was measured by a FB15 luminometer (Berthold Detection System) according to the manufacture's instructions.

5.3. Computational studies

All calculations and manipulations were performed using FlexX software package integrated in SYBYL 6.9[11], running on SGI Fuel workstation. The X-ray crystal structure of reverse transcriptase complexed with efavirenz was retrieved from PDB (PDB code: 1FK9).[12] In FlexX docking, the Receptor Description File (RDF) described the active site environment. It contains the information about the protein, its amino acids, the active site, non-amino acid residues, and specific torsion angles. The active site was defined as all residues within 6.5 Å radius of the cocrystallized efavirenz.

The default SYBYL/FlexX parameters were used. The newly synthesized compound **10** was built using the SYBYL Sketcher model and fully minimized with the Powell method (Tripos force field and Gasteiger-Huckel charges) to an energy gradient of 0.05 kcal/(mol* Å). The 30 final docked conformations were ranked according to their binding free energy. The docking mode was chosen on the basis of binding affinity rank.

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References and notes

1. De Clercq, E. *Nat. Rev. Drug discov.* **2007**, *6*, 1001.
2. Young, S. D.; Britcher, S. F.; Tran, L. O.; Payne, L. S.; Lumma, W. C.; Lyle, T. A.; Huff, J. R.; Anderson, P. S.; Olsen, D. B.; Carroll, S. S.; Pettibone, D. J.; O'brien, J. A.; Ball, R. G.; Balani, S. K.; Lin, J. H.; Chen, I.-W.; Schleif, W. A.; Sardana, V. V.; Long, W. J.; Byrnes, V. W.; Emini, E. A. *Antimicrob. Agents Chemother.* **1995**, *39*, 2602.
3. Spence, R. A.; Kati, W. M.; Anderson, K. S.; Johnson, K. A. *Science* **1995**, *267*, 988.
4. Gallant, J. E.; DeJesus, E.; Arribas, J. R.; Pozniak, A. L.; Gazzard, B.; Campo, R. E.; Lu, B.; McColl, D.; Chunk, S.; Enejosa, J.; Toole, J. J.; Cheng, A. K. *N. Eng. J. Med.* **2006**, *354*, 251.
5. Staszewski, S.; Morates-Ramirez, J.; Tashima, K. T.; Rachlis, A.; Skiest, D.; Stanford, J.; Stryker, R.; Johnson, P.; Labriola, D. F.; Farina, D.; Manion, D. J.; Ruiz, N. M. *N. Eng. J. Med.* **1999**, *341*, 1865.
6. De Clercq, E. *Biochem. Pharmacol.* **1994**, *47*, 155.
7. Arasteh, K.; Wood, R.; Muller, M.; Prince, W.; Cass, L.; Moore, K. H.; Dallow, N.; Jones, A.; Klein, A.; Burt, V.; Kleim, J. P. *HIV Clin. Trial* **2001**, *2*, 307.
8. Ren, J.; Nichols, C. E.; Chamberlain, P. P.; Weaver, K. L.; Short, S. A.; Chan, J. H.; Kleim, J. P.; Stammers, D. K. *J. Med. Chem.* **2007**, *50*, 2301.
9. Kleim, J. P.; Bender, R.; Kirsch, R.; Meichsner, C.; Paessens, A.; Rosner, M.; Rubsamen-Waigmann, H.; Kaiser, R.; Wichers, M.; Schneweis, K. E.; Winkler, I.; Riess, G. *Antimicrob. Agents Chemother.* **1995**, *39*, 2253.
10. Hopkins, A. L.; Ren, J.; Esnouf, R. M.; Willcox, B. E.; Jones, Y.; Ross, C.; Miyasaka, T.; Walker, R. T.; Tanaka, H.; Stammers, D. K.; Stuart, D. I. *J. Med. Chem.* **1996**, *39*, 1589.
11. SYBYL 6.9, Tripos Inc., 1699 South Hanley Road, St. Louis, MO 631444, USA.
12. Ren, J.; Milton, J.; Weaver, K. L.; Short, S. A.; Stuart, D. I. *Structure* **2000**, *8*, 1089.
13. Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605.
14. He, J.; Choe, S.; Walker, R.; Di Marzio, P.; Morgan, D. O.; Landau, N. R. *J. Virol.* **1995**, *69*, 6705.
15. Connor, R. I.; Chen, B. K.; Choe, S.; Landau, N. R. *Virology* **1995**, *206*, 935.
16. Rong, L.; Bates, P. *J. Virol.* **1995**, *69*, 4847.