

Structure–activity relationship studies on quinoxalin-2(1*H*)-one derivatives containing thiazol-2-amine against hepatitis C virus leading to the discovery of BH6870

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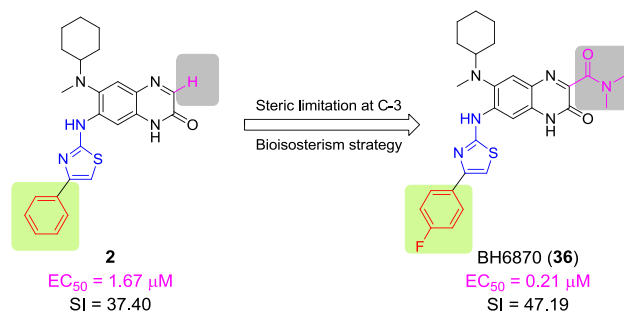
Abstract Chronic hepatitis C virus infection represents a serious global public health problem, typically resulting in fibrosis, cirrhosis, and ultimately hepatocellular carcinoma. Based on our previous discovery of lead compound **2** (Liu et al. *J Med Chem* 54:5747–5768, 2011), 35 new quinoxalinone derivatives were explored in this study. Outline of the structure–activity relationships (SARs) revealed that compound BH6870 (**36**) showed high anti-HCV potency ($EC_{50} = 0.21 \mu\text{M}$) and a good cell safety index ($SI = 47.19$). SARs analysis indicated that quinoxalin-2(1*H*)-one containing a 4-aryl-substituted thiazol-2-amine moiety was optimal for antiviral activity. Introducing a hydrogen-bond acceptor (such as ester or amide group) at the C-3 position of quinoxalin-2(1*H*)-one was beneficial for the antiviral potency, and especially, *N,N*-disubstituted amide was far superior to *N*-monosubstituted amide. Incorporation of more than one halogen (fluorine or chlorine atom) or a strong electron-withdrawing group on the benzene ring of the thiazole-phenyl moiety might reduce electron atmosphere density further and resulted in a dramatical loss of activity. The NH-group of the lactam moiety was clearly required for anti-HCV activity.

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Graphical Abstract Design and synthesis of quinoxalin-2(1*H*)-one derivatives as new non-nucleoside small-molecule HCV inhibitors. BH6870 (**36**), showing higher antiviral potency and a good cell safety index, was identified.



Keywords Antiviral activity · Hepatitis C virus · Quinoxalinone · Structure–activity relationships

Introduction

In 1989, the virus responsible for most transfusion-associated non-A and non-B hepatitis was identified and cloned, and named hepatitis C virus (HCV) [1]. HCV belongs to the Flaviviridae family and is described as having positive-sense, single-stranded RNA genomes [2]. HCV infection typically progresses to a chronic state, may resulting in fibrosis, cirrhosis, and hepatocellular carcinoma, and has emerged as a leading cause for liver transplantation [3]. There are at least six distinct HCV genotypes (genotypes 1–6), of which genotype 1 accounts for the majority of infections in the U.S., Europe, and Asia and has been particularly difficult to treat [4,5]. Currently, chronic HCV infection represents a serious global public health problem. It has been estimated that

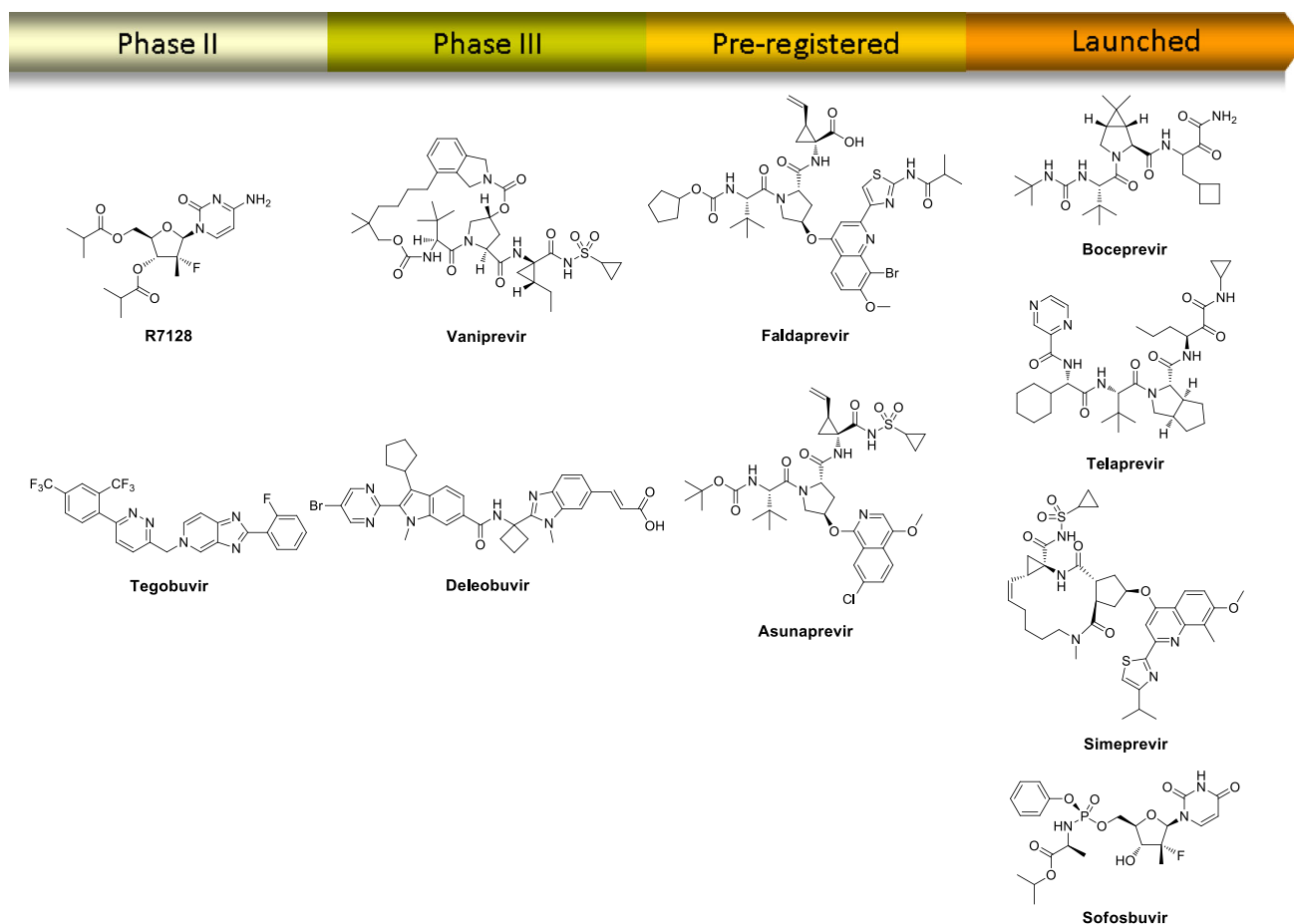


Fig. 1 Anti-HCV agents in late-stage clinical development or launched

150 million people worldwide are chronically infected with HCV and that approximately 3–4 million people are newly infected each year, with more than 350,000 deaths annually due to hepatitis C-related liver diseases [6].

HCV has a small genome encoding six nonstructural proteins, including NS2, NS3, NS4A, NS4B, NS5A, and NS5B. It is believed that the nonstructural (NS) proteins provide the catalytic machinery for viral replication [7]. Until recently, the standard of care (SOC) for HCV infection consists in the combination of nonspecific immune modulatory agent pegylated interferon (PEG-IFN) plus the antiviral agent ribavirin, which have limited efficacy particularly against major genotype 1 [8] and severe side effects. To address these deficiencies, many molecular targets have been pursued in an effort to identify direct-acting antiviral agents (DAAs) as anti-HCV drugs [9–13]. In 2011, the massive efforts devoted to developing new HCV DAAs were rewarded by the approval of the first-generation of HCV NS3/4A protease inhibitors, boceprevir [14–16] and telaprevir (Fig. 1) [17–22]. Consequently, the new SOC for patients infected with genotype 1a or 1b has been put into practice by using a combination of the NS3/4A protease

inhibitor boceprevir or telaprevir with PEG-IFN and ribavirin, increasing the rate of sustained virologic response (SVR) to nearly 70% [23]. Two years later, the second-generation NS3/4A protease inhibitor simeprevir launched in 2013 for use in combination with PEG-IFN and ribavirin for the treatment of genotype 1 chronic HCV patients (Fig. 1) [24–28]. The other small-molecule inhibitors of NS3/4A protease will be available in clinics in the near future (Fig. 1) [29]. For example, faldaprevir [30,31], vaniprevir [32–34], and asunaprevir [35,36] are currently in phase III trials. In addition to the NS3/4A protease, the RNA-dependent RNA polymerase NS5B is another attractive target for antiviral therapy. Several classes of inhibitors for NS5B polymerase are in late-stage clinical trials or have been recently approved. The inhibitors of NS5B polymerase include nucleoside (such as sofosbuvir [37,38] and R7128 [39,40]) and non-nucleoside polymerase inhibitors (e.g., deleobuvir [41,42], tegobuvir [43–45]) (Fig. 1). However, the high mutation rate of HCV and the rapid emergence of drug resistance have prompted continuous efforts to discover and develop drugs with high efficacy and novel mechanisms of action [46–48].

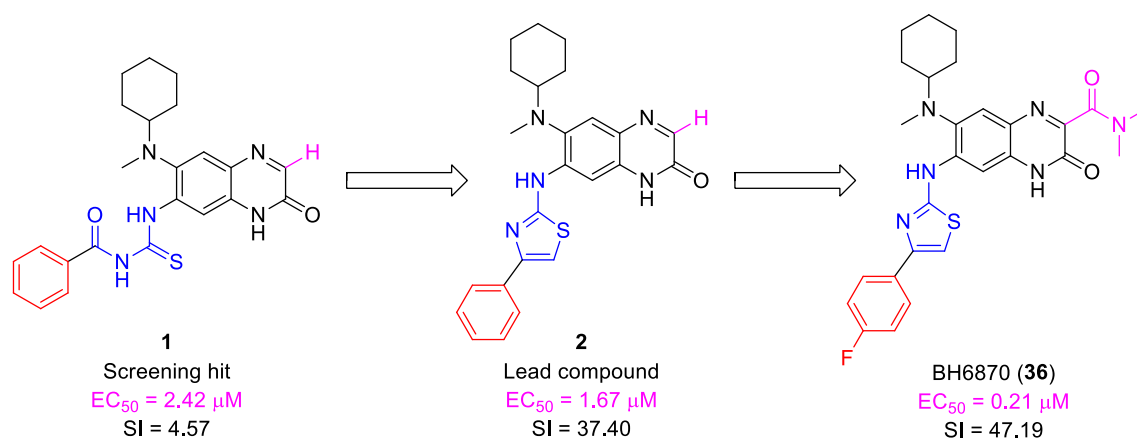


Fig. 2 Key lead series discovered as small-molecule HCV inhibitors

Quinoxalin-2(1*H*)-one derivatives have been first reported by our group [49] to be potent inhibitors against HCV. Considering the potential cytotoxicity of the analogues with a thiourea group such as compound **1**, cyclization strategy was carried out to identify **2** as a potent inhibitor of the HCV with lower cell toxicity properties *in vitro* (Fig. 2). We demonstrated that the thiazole-phenyl moiety was optimal for anti-HCV activity, and steric conformation in the position C-6 was necessary for inhibition of the HCV replicon system. Moreover, there may be a steric limitation at the C-3 position of quinoxalin-2(1*H*)-one. Consequently, further work aimed at optimization of moieties at the C-3 position and exploration of optimal substituents on the benzene ring of the thiazole-phenyl moiety was undertaken. Herein, we report our findings that led to the discovery of BH6870.

Results and discussion

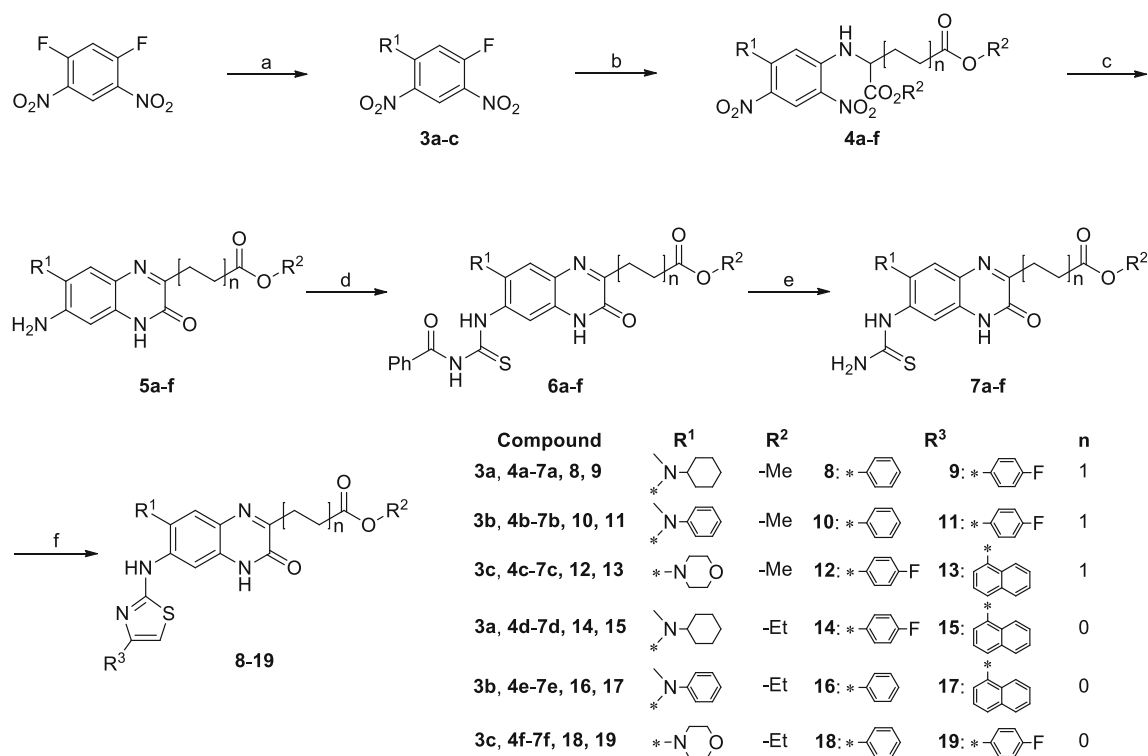
Chemistry

Preparation of the ester-substituted quinoxalinone analogues is depicted in Scheme 1. The core structure **5** was constructed according to our previous report [49]. In the first step, secondary amines were used as nucleophiles to replace quantitatively one fluorine atom of 1,5-difluoro-2,4-dinitrobenzene (DFDNB) in the presence of *N,N*-diisopropylethylamine (DIPEA) or potassium carbonate (K_2CO_3). Subsequently, the remaining fluorine was substituted by primary amines to give **4a–f**. Then 10% palladium on activated carbon was utilized to convert the dinitro substrates to diamino intermediates followed by a self-cyclization to obtain key intermediates **5a–f**. Accordingly, subsequent nucleophilic addition with benzoyl isothiocyanate in acetone, followed by deprotection of the benzoyl group provided thiourea compounds **7a–f** after

treatment with potassium carbonate. Finally, for the construction of the thiazole ring, thiourea substrates were utilized to react with suitably substituted 2-bromoacetophenones to afford target compounds **8–19**.

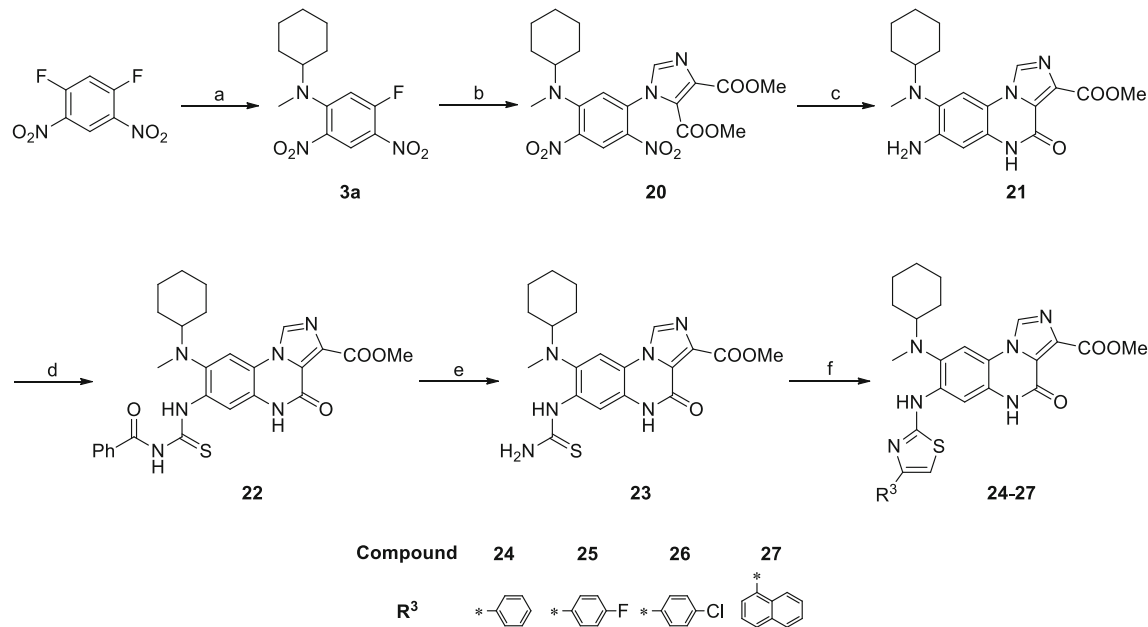
Tricyclic quinoxalinone compounds were effectively prepared in a 6-step procedure from DFDNB (Scheme 2). Reaction of monosubstituted intermediate **3a** with dimethyl 1*H*-imidazole-4,5-dicarboxylate in acetone and with potassium carbonate under reflux for 6 h gave smoothly the key 4,6-dinitro intermediate **20**. Reductive cyclization of **20** to the corresponding imidazo[1,5-*a*]quinoxalin-4-one **21** was initially attempted according to the hydrogenation procedures used above. Unfortunately, it was found that the products were a complex mixture of several components, of which only a small amount of the desired compound **21** was observed by HPLC-MS, but the major product was its hydroxylamine intermediate. Finally, it was found that the combination of sodium dithionite [50] in concentrated hydrochloric acid was the effective condition of choice for conducting the reductive cyclization of **20** to **21** and its product of ester hydrolysis, which was next converted conveniently to the desired product **21** after the reaction mixture was filtered and concentrated *in vacuo* followed by an esterification reaction with methanol in the presence of concentrated sulfuric acid. Finally, the target compounds **24–27** could easily be obtained by the introduction of a thiourea moiety into the free amino group of intermediate **21** and then performing a cyclization in 3 steps following reaction steps d–f in Scheme 1.

Amide-substituted quinoxalinones were obtained according to the synthetic route shown in Scheme 3. Treatment of **5** with lithium hydroxide (LiOH) easily gave the corresponding carboxylic acid intermediates. And then, the intermediate was incorporated to commercially available methylamine or dimethylamine solution in tetrahydrofuran using EDCI and HOBt as condensation agents to deliver the amide-substituted



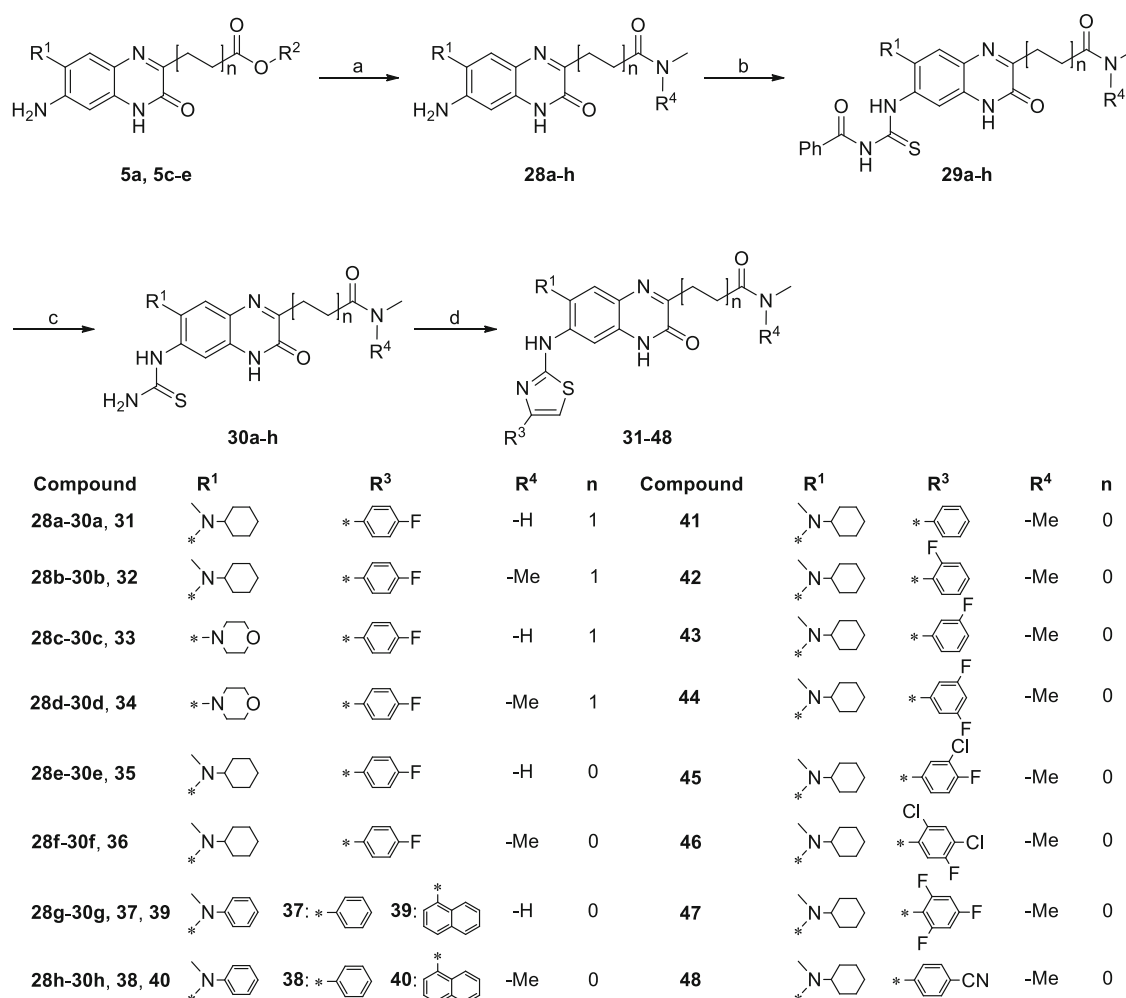
Scheme 1 Synthesis of ester-substituted quinoxalinone analogues **8–19**. Reagents and conditions: **a** R¹H, DIPEA, THF, rt or R¹H, K₂CO₃, THF, reflux; **b** NH₂CHCO₂R²(CH₂CH₂)_nCO₂R²·HCl, DIPEA, THF,

reflux; **c** 10% Pd/C, NH₄COOH, THF/EtOH (1:1, v/v), rt; **d** PhCONCS, acetone, reflux; **e** K₂CO₃, EtOH/H₂O (4:1, v/v), 75 °C; **f** R³COCH₂Br, EtOH, reflux



Scheme 2 Synthesis of tricyclic quinoxalinone analogues **24–27**. Reagents and conditions: **a** *N*-methylcyclohexylamine, DIPEA, THF, rt; **b** dimethyl 1*H*-imidazole-4,5-dicarboxylate, K₂CO₃, acetone,

reflux; **c** Na₂S₂O₄, concd. HCl, MeOH/H₂O (1:1, v/v), 70 °C; then concd. H₂SO₄, MeOH, reflux; **d** PhCONCS, acetone, reflux; **e** NaOMe, MeOH, 50 °C; **f** R³COCH₂Br, MeOH, reflux



Scheme 3 Synthesis of amide-substituted quinoxalinone analogues **31–48**. Reagents and conditions: **a** (i) LiOH·H₂O, EtOH/H₂O (5:1, v/v), 50 °C; (ii) 2M MeNHR⁴ in THF, EDCI, HOBt, THF, 60 °C; **b** PhCONCS, acetone, reflux; **c** K₂CO₃, EtOH/H₂O (4:1, v/v), 75 °C; **d** R³COCH₂Br, EtOH, reflux

intermediates **28a–h**. Subsequently, the target compounds **31–48** could be smoothly provided using the aforementioned introduction of the thiourea moiety and cyclization conditions.

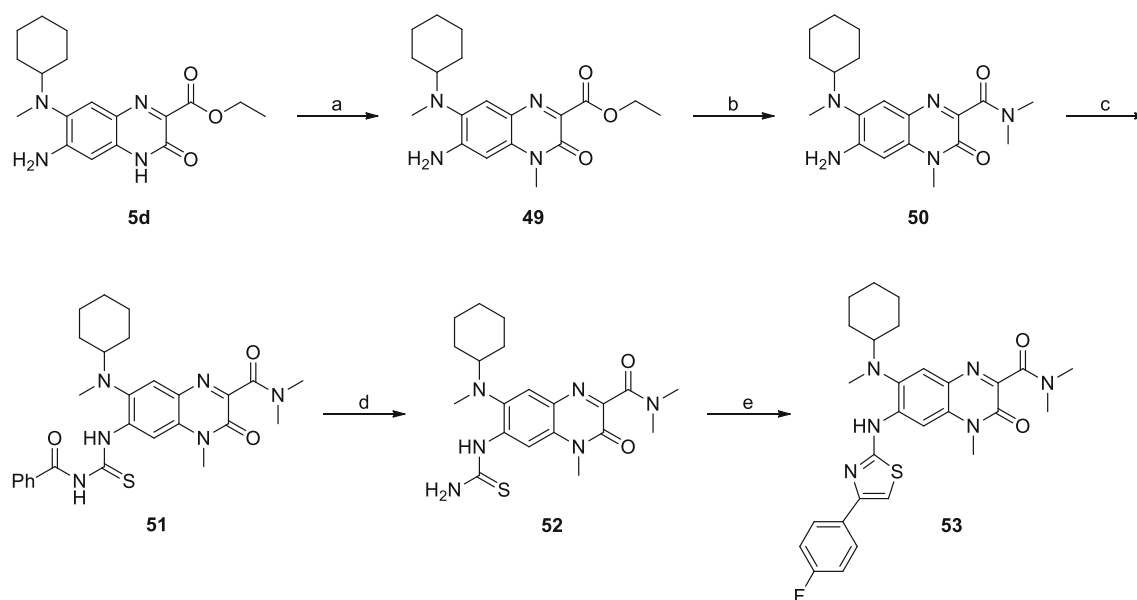
Synthesis of 1-*N*-substituted quinoxalinone **53** was accomplished as outlined in Scheme 4. Treatment of lactam **5d** with methyl iodide using potassium carbonate as a base resulted in the 1-*N*-substituted quinoxalinone intermediate **49** followed by an ester hydrolysis and coupling with dimethylamine to yield amide **50**. Accordingly, target compound **53** was readily obtained following the same protocol shown in Scheme 1.

Anti-HCV activity in vitro and SAR studies

The anti-HCV activity and cytotoxicity of synthesized quinoxalinone derivatives were evaluated in the HCV RNA replicon system in Huh7-ET cells, as previously described

[49,51]. The results are currently summarized in Tables 1, 2, 3, 4. rIFN α -2b (recombinant human interferon alfa-2b) is a fused form of IFN α -2b with human serum albumin used as a positive control for inhibition of HCV with an approximate EC₅₀ of 0.08 IU/mL [49]. Briefly, the concentration of compound to inhibit HCV RNA replication activity by 50% (EC₅₀), the concentration of compound to decrease cell viability by 50% (CC₅₀), and the selective index (SI) calculated as the CC₅₀/EC₅₀ ratio are presented.

Previous studies on structure–activity relationships (SARs) indicated that there may be a steric limitation at the C-3 position of quinoxalin-2(1*H*)-one [49]. Initially, the hydrogen atom at the C-3 position was replaced by an ester group. These modifications were intended to introduce a hydrogen-bond acceptor and change the distance between the ester group and quinoxalinone skeleton, thus making the resulting compounds show an improved anti-HCV activity. Results of these modifications are summarized in Table 1.



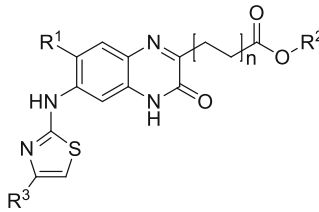
Scheme 4 Synthesis of 1-*N*-substituted quinoxalinone **53**. Reagents and conditions: **a** CH₃I, K₂CO₃, acetone, 50 °C; **b** (i) LiOH · H₂O, EtOH/H₂O (5:1, v/v), 50 °C; (ii) 2M dimethylamine in THF, EDCl,

HOBt, THF, 60 °C; **c** PhCONCS, acetone, reflux; **d** K₂CO₃, EtOH/H₂O (4:1, v/v), 75 °C; **e** 4-FC₆H₄COCH₂Br, MeOH, reflux

Thus, replacement of hydrogen atom at the C-3 position of **2** with propionate side chain resulted in **8** with EC₅₀ = 7.13 μM, fourfold less active than **2** (EC₅₀ = 1.67 μM). Incorporation of a fluorine atom on the phenyl ring of **8** resulted in compound **9** exerting no antiviral activity against HCV. Replacement of *N*-methylcyclohexylamine at the C-6 position of **8** with *N*-methylaniline side chain resulted in **10** and **11**. Interestingly, fluorine-substituted compound **11** exhibited potent activity against HCV with EC₅₀ = 1.30 μM similar to that of inhibitor **2**. However, introduction of a morpholine group (**12** and **13**) in the position C-6 was unfavorable for the antiviral potency. This could have been due to the fact that a compound containing hydrophobic groups may effectively improve its binding to the target protein. When the substituent at the C-6 position was kept constant as *N*-methylcyclohexylamine, decreasing the distance between the ester group and quinoxalinone skeleton resulted in **14** and **15**. Similarly, compound **14** bearing a fluorine atom had an excellent cellular potency against HCV with EC₅₀ = 1.14 μM. However, enhancing π – π interactions (**15**) significantly decreased the antiviral activity. Furthermore, neither the introduction of *N*-methylaniline side chain at the C-6 position (**16** and **17**) nor morpholine group (**18** and **19**) provided an obvious potency against HCV or improvement in the selectivity index. Consequently, although no single compound displayed significantly increased potency against the HCV replicon compared to **2**, it was encouraging to see this kind of activity with ester-substituted compounds.

Previous SARs from our group indicated that the mimic of 3,4-double bond of quinoxalinone by a five-membered ring provided a slight improvement in binding potency [49]. Thus, in order to balance the lipid/water partition coefficient, we selected the hydrophilic imidazole ring to mimic the 3,4-double bond of quinoxalinone and incorporated an ester group on the five-membered ring to further study the effect of steric limitation. Cellular data of compounds with double bond mimics are tabulated in Table 2. Preparation of inhibitor **25**, bearing a fluorine atom in the *para* position, resulted in a slightly lower potency (EC₅₀ = 4.14 μM) than that of **14** (EC₅₀ = 1.14 μM). Attempts to replace the fluorine atom with a chlorine atom (**26**, EC₅₀ = 4.94 μM) or directly remove the halogen (**24**, EC₅₀ = 3.94 μM) failed to provide improvement in binding potency. Moreover, enhancing π – π interactions (**27**, EC₅₀ = 6.14 μM) did not show appreciable binding affinity with the target either. It can be concluded from the data in Table 2 that increase of steric hindrance and hydrophilicity is unfavorable for the anti-HCV activity, but the 3,4-double bond can be to some extent mimicked by a five-membered ring.

Bioisosteres modulate biological activity by virtue of subtle differences in their physicochemical properties. A classical divalent bioisosteric replacement involving two single bonds is –O– with –NH–. This replacement has been widely used in the drug discovery process [52]. On the basis of the observed SARs, it was encouraging to know that replacement of the hydrogen atom at the C-3 posi-

Table 1 Inhibitory effects of ester-substituted quinoxalin-2(1*H*)-one derivatives on HCV replication in Huh7


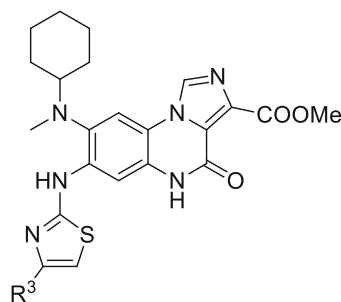
Compound	R ¹	R ²	R ³	n	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity CC ₅₀ (μM)	SI ^a
8		Me		1	7.13	8.78	1.23
9		Me		1	>10	>10	1.00
10		Me		1	>10	>10	1.00
11		Me		1	1.30	2.98	2.29
12		Me		1	>10	>10	1.00
13		Me		1	>10	>10	1.00
14		Et		0	1.14	>10	>8.77
15		Et		0	>10	>10	1.00
16		Et		0	>10	7.59	<0.76
17		Et		0	>10	>10	1.00
18		Et		0	9.59	>10	>1.04
19		Et		0	9.85	9.60	0.97
rIFNα-2b					0.06 IU/mL	>2 IU/mL	>33.3

^a The selectivity index (SI) is calculated as CC₅₀/EC₅₀

tion with an ester group preserved antiviral activity against HCV. Consequently, we decided to further explore optimal substituents for the hydrogen atom at the C-3 position by introducing an amide group as ester bioisostere. The effect on the anti-HCV activity of the bioisostere derivatives is shown in Table 3. Initially, retaining *N*-methylcyclohexylamine as R¹ group, incorporation of *N*-substituted propionamide side chain resulted in **31** and **32** with EC₅₀ = 11.62 and 3.16 μM, respectively. Introduction of a morpholine

group as a replacement of the *N*-methylcyclohexylamine yielded *N*-monosubstituted amide **33** and *N,N*-disubstituted amide **34** with lower binding affinity (EC₅₀ > 20 μM and EC₅₀ = 6.03 μM, respectively). Interestingly, it was obviously found that amide types of **32** (EC₅₀ = 3.16 μM) and **34** (EC₅₀ = 6.03 μM) exhibited significant enhancement in activity compared to the corresponding ester types of **9** (EC₅₀ > 10 μM) and **12** (EC₅₀ > 10 μM). Further, the *N,N*-disubstituted amide inhibitor was far superior to

Table 2 Inhibitory effects of tricyclic quinoxalinone derivatives on HCV replication in Huh7 cells



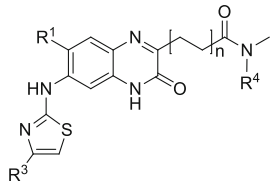
Compound	R ³	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity CC ₅₀ (μM)	SI ^a
24		3.94	4.54	1.15
25		4.14	3.99	0.96
26		4.94	4.85	0.98
27		6.14	5.11	0.83
rIFNα-2b		0.08 IU/mL	>2 IU/mL	>25

^a The selectivity index (SI) is calculated as CC₅₀/EC₅₀

the *N*-monosubstituted amide compound in antiviral activity against HCV. Similarly, the rules deduced from further results of incorporation of a formamide group were consistent with the data obtained from the introduction of propionamide side chain above. Thus, it was encouraging to see that the antiviral potency of inhibitors **35** and **36** was greatly improved with *N*-methylcyclohexylamine as R¹ group with EC₅₀ = 1.12 μM and 0.21 μM, a fivefold improvement in potency compared to the respective analogues of the corresponding ester type **14**. In addition, it was evident that the application of bioisostere provided better selectivity (**36**, SI = 47.19). When the *N*-methylaniline moiety was introduced to C-6 position, removal of the fluorine atom from the benzene ring resulted in compounds **37** (EC₅₀ > 20 μM) and **38** (EC₅₀ = 1.25 μM), which also demonstrated that *N,N*-disubstituted amide inhibitor did appreciably improve target binding or cellular activity. However, replacement of phenyl with the naphthalene group yielded compounds **39** and **40** with EC₅₀ > 20 μM and EC₅₀ = 7.78 μM, respectively. This result clearly reinforced the fact that enhancing π – π interactions resulted in loss of potency.

In view of the observed SARs above, it was clear that incorporation of the *N,N*-disubstituted formamide moiety

did appreciably improve antiviral activity against HCV. Thus, to further explore amide analogues with high potency and excellent selectivity, compounds bearing a wide variety of halogen substituents attached to the benzene ring were synthesized. *N*-methylcyclohexylamine was retained as R¹ side chain because it demonstrated improved activity against HCV in comparison to other R¹ moieties. Data for the resulting compounds are summarized in Table 4. Moving the fluorine atom to *ortho* or *meta* position of phenyl resulted in **42** and **43** with EC₅₀ = 3.58 μM and 3.08 μM, respectively, a 19- to 22-fold loss in activity compared to inhibitor **36** which exhibited potent activity against HCV (EC₅₀ = 0.16 μM). However, removal of the fluorine atom in **42** gave **41**, a slightly more active compound with EC₅₀ = 1.58 μM. The same effect was observed when incorporating more than one fluorine or chlorine atom on the benzene ring (compounds **44–47**). On the other hand, replacement of the hydrogen atom in **41** with a cyano group in the *para* position of phenyl yielded **48** with similar potency (EC₅₀ = 2.48 μM). All the above data suggested that introduction of more than one halogen (fluorine or chlorine atom) or a strong electron-withdrawing group on the benzene ring might reduce electron atmosphere density further and affect the π – π interactions, and this may

Table 3 Inhibitory effects of amide-substituted quinoxalin-2(1*H*)-one derivatives on HCV replication in Huh7 cells


Compound	R ¹	R ⁴	R ³	n	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity CC ₅₀ (μM)	SI ^a
31		H		1	11.62	11.88	1.02
32		Me		1	3.16	5.58	1.77
33		H		1	>20	>20	1.00
34		Me		1	6.03	>20	>3.32
35		H		0	1.12	12.85	11.47
36 (BH6870)		Me		0	0.21	9.91	47.19
37		H		0	>20	>20	1.00
38		Me		0	1.25	11.25	9.00
39		H		0	>20	>20	1.00
40		Me		0	7.78	10.02	1.29
rIFNα-2b					0.04 IU/mL	>2 IU/mL	>50

^a The selectivity index (SI) is calculated as CC₅₀/EC₅₀

contribute to the difference in potency and cytotoxicity. Moreover, based on inhibitor **36**, modification by blocking the lactam NH-group of quinoxalin-2(1*H*)-one was performed to result in **53** with EC₅₀ = 3.43 μM, with a 21-fold loss in activity, which demonstrated that the NH-group on the lactam moiety was definitely required for anti-HCV activity.

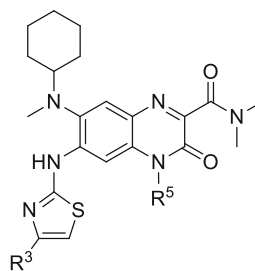
Conclusions

This study has identified new quinoxalin-2(1*H*)-one derivatives as potent inhibitors against HCV. On the basis of lead compound **2**, 35 new quinoxalinone derivatives were designed and synthesized. The anti-HCV activity and a further SARs study of these compounds were carried out in an effort to lead to the discovery of BH6870 (**36**) as a new non-nucleoside small-molecule HCV inhibitor showing high

antiviral potency (EC₅₀ = 0.21 μM) and a good cell safety index (SI = 47.19).

In conclusion, quinoxalin-2(1*H*)-one containing a 4-aryl-substituted thiazol-2-amine moiety was optimal for antiviral activity. Increase of steric hindrance and hydrophilicity was unfavorable for the anti-HCV activity although the 3,4-double bond could be to some extent mimicked by a five-membered ring. Introducing a hydrogen-bond acceptor (such as ester or amide group) at the C-3 position of quinoxalin-2(1*H*)-one was beneficial for the antiviral potency, especially when *N,N*-disubstituted amide was far superior to *N*-monosubstituted amide. Moreover, incorporation of more than one halogen (fluorine or chlorine atom) or a strong electron-withdrawing group on the benzene ring of thiazole-phenyl moiety might reduce electron atmosphere density further and resulted in a dramatical loss of potency, and the NH-group of the lactam moiety was clearly required for anti-HCV activity.

Table 4 Inhibitory effects of *N,N*-dimethyl formamide substituted quinoxalin-2-one derivatives on HCV replication in Huh7 cells



Compound	R ³	R ⁵	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity CC ₅₀ (μM)	SI ^a
36 (BH6870) ^b		H	0.16	9.88	61.75
41		H	1.58	7.17	4.54
42		H	3.58	7.78	2.17
43		H	3.08	6.90	2.24
44		H	2.97	6.89	2.32
45		H	3.26	9.14	2.80
46		H	3.11	10.40	3.34
47		H	3.38	6.62	1.96
48		H	2.48	4.48	1.81
53		Me	3.43	6.85	2.00
rIFNα-2b			0.09 IU/mL	>2 IU/mL	>22.2

^a The selectivity index (SI) is calculated as CC₅₀/EC₅₀

^b The repeated data confirmed in the same assays

Experimental

Chemistry

General All of the chemicals used were obtained from commercial sources (Acros Organics, Alfa Aesar, and Sigma-Aldrich), and were used without further purification. Solvents were used without purification or drying, unless other-

wise noted. ¹H NMR and ¹³C NMR spectra were recorded at 300 and 150 MHz, respectively, on Varian Mercury and Varian NMR System spectrometers in acetone-*d*₆ or DMSO-*d*₆ with TMS as the internal standard. Chemical shifts were reported in ppm (δ) relative to the solvent, and coupling constants (*J*) were reported in Hz. Melting points were determined without correction with a Yanaco micromelting point apparatus. Automatic HPLC-MS analysis was performed

on a Thermo Finnigan LCQ Advantage mass spectrometer equipped with an Agilent pump, an Agilent detector, an Agilent liquid handler, and a fluent splitter. The column used was a Kromasil C18 column (4.6 μm , 4.6 mm \times 50 mm) from DIKMA for analysis. The eluent was a mixture of acetonitrile and water containing 0.05 % HCOOH with a linear gradient from 5:95 to 95:5 (v/v) of acetonitrile–H₂O within 5 min at a 1 mL/min flow rate for analysis. The UV detection was carried out at a UV wavelength of 254 nm. The 5 % of the eluent was split into the MS system. Mass spectra were recorded in either positive or negative ion mode using electrospray ionization (ESI). High-resolution mass spectra (HRMS) were recorded on Thermo Scientific Exactive Plus System. The ion source is ESI. Flash column chromatography was performed with silica gel 60 (200–300 mesh) from Qingdao Haiyang Chemical Co., Ltd. All tested compounds were purified until the purity was $\geq 95\%$, detected by HPLC-MS under UV 254 nm wavelength, NMR, melting point, and HRMS.

General procedure A for the synthesis of compounds 4a, 4c, 4d, and 4f

To a stirred solution of 1,5-difluoro-2,4-dinitrobenzene (DFDNB; 2.04 g, 10 mmol) in THF (50 mL) was added DIPEA (1.75 mL, 10 mmol) and *N*-methylcyclohexylamine (1.30 mL, 10 mmol) or morpholine (0.87 mL, 10 mmol). After vigorously stirring at room temperature until the total disappearance of DFDNB monitored by HPLC-MS analysis, intermediate (**3a** or **3c**) was obtained without purification. Then, DIPEA (3.50 mL, 20 mmol) and L-glutamic acid dimethyl ester hydrochloride (2.12 g, 10 mmol) or diethyl aminomalonate hydrochloride (2.12 g, 10 mmol) were added and stirred under reflux for 6–36 h. After the reaction was complete (monitored by HPLC-MS analysis), the solvent was evaporated in vacuo. Saturated ammonium chloride solution (50 mL) was added to the resulting product and was further extracted by CH₂Cl₂ (2 \times 50 mL). The organic layers were combined, dried over anhydrous Na₂SO₄, and evaporated in vacuo. Intermediate compound (**4a**, **4c**, **4d** or **4f**) was obtained after purification by silica gel column chromatography.

Dimethyl 2-((5-(cyclohexyl(methyl)amino)-2,4-dinitrophenyl)amino)pentanedioate (4a)

Following general procedure A starting from *N*-methylcyclohexylamine (1.30 mL, 10 mmol) yielded an orange oil (3.75 g, 83 % over two steps); ¹H NMR (300 MHz, Acetone-*d*₆): δ = 8.70–8.67 (m, 2H), 6.23 (s, 1H), 4.65 (q, *J* = 6.9 Hz, 1H), 3.80 (s, 3H), 3.62 (s, 3H), 3.49–3.41 (m, 1H), 2.80 (s, 3H), 2.54 (t, *J* = 7.2 Hz, 2H), 2.29 (q, *J* = 6.9 Hz, 2H), 1.86–1.82 (m, 4H), 1.72–1.59 (m, 3H), 1.49–1.08 ppm (m, 3H);

HRMS-ESI *m/z* [M+H]⁺ calcd for C₂₀H₂₉N₄O₈: 453.1980, found: 453.1976.

Dimethyl 2-((5-morpholino-2,4-dinitrophenyl)amino)pentanedioate (4c)

Following general procedure A starting from morpholine (0.87 mL, 10 mmol) yielded a dark-yellow powder (3.28 g, 77 % over two steps); mp: 142–144 °C; ¹H NMR (300 MHz, Acetone-*d*₆): δ = 8.87 (d, *J* = 7.2 Hz, 1H), 8.83 (s, 1H), 6.46 (s, 1H), 4.78 (q, *J* = 7.2 Hz, 1H), 3.81 (s, 3H), 3.78 (t, *J* = 4.5 Hz, 4H), 3.62 (s, 3H), 3.24 (t, *J* = 4.5 Hz, 4H), 2.53 (t, *J* = 7.2 Hz, 2H), 2.28 ppm (q, *J* = 7.2 Hz, 2H); HRMS-ESI *m/z* [M+H]⁺ calcd for C₁₇H₂₃N₄O₉: 427.1460, found: 427.1456.

Diethyl 2-((5-(cyclohexyl(methyl)amino)-2,4-dinitrophenyl)amino)malonate (4d)

Following general procedure A starting from *N*-methylcyclohexylamine (1.30 mL, 10 mmol) yielded a dark-yellow powder (3.62 g, 80 % over two steps); mp: 123–124 °C; ¹H NMR (300 MHz, Acetone-*d*₆): δ = 9.13 (d, *J* = 6.0 Hz, 1H), 8.71 (s, 1H), 6.19 (s, 1H), 5.31 (d, *J* = 6.0 Hz, 1H), 4.42–4.25 (m, 4H), 3.53–3.43 (m, 1H), 2.78 (s, 3H), 1.88–1.84 (m, 4H), 1.72–1.60 (m, 3H), 1.52–1.36 (m, 2H), 1.29 (t, *J* = 7.2 Hz, 6H), 1.24–1.06 ppm (m, 1H); HRMS-ESI *m/z* [M+H]⁺ calcd for C₂₀H₂₉N₄O₈: 453.1980, found: 453.1978.

Diethyl 2-((5-morpholino-2,4-dinitrophenyl)amino)malonate (4f)

Following general procedure A starting from morpholine (0.87 mL, 10 mmol) yielded a dark-yellow powder (3.07 g, 72 % over two steps); mp: 121–122 °C; ¹H NMR (300 MHz, Acetone-*d*₆): δ = 9.20–9.18 (m, 1H), 8.84 (s, 1H), 6.34 (s, 1H), 5.41–5.39 (m, 1H), 4.38–4.28 (m, 4H), 3.79 (t, *J* = 4.5 Hz, 4H), 3.23 (t, *J* = 4.5 Hz, 4H), 1.29 ppm (t, *J* = 7.2 Hz, 6H); HRMS-ESI *m/z* [M+H]⁺ calcd for C₁₇H₂₃N₄O₉: 427.1460, found: 427.1458.

General procedure B for the synthesis of compounds 4b and 4e

To a stirred solution of 1,5-difluoro-2,4-dinitrobenzene (DFDNB; 2.04 g, 10 mmol) in THF (50 mL) was added K₂CO₃ (1.38 g, 10 mmol) and *N*-methylaniline (1.08 mL, 10 mmol). The mixture was stirred under reflux for 36 h. The suspension was cooled and filtered. Then, the filtrate was concentrated under reduced pressure. The residue was dissolved in saturated ammonium chloride solution (50 mL) and extracted by CH₂Cl₂ (2 \times 50 mL). The organic phases were combined, dried over anhydrous Na₂SO₄, and evap-

orated in vacuo to give crude intermediate **3b**. Then, the residue was dissolved in THF (50 mL). DIPEA (3.50 mL, 20 mmol) and L-glutamic acid dimethyl ester hydrochloride (2.12 g, 10 mmol) or diethyl aminomalonate hydrochloride (2.12 g, 10 mmol) were added and stirred under reflux for 12–24 h. After the reaction was completed (monitored by HPLC-MS analysis), the solvent was evaporated in vacuo. Saturated ammonium chloride solution (50 mL) was added to the resulting product and was further extracted by CH₂Cl₂ (2 × 50 mL). The organic layers were combined, dried over anhydrous Na₂SO₄, and evaporated in vacuo. Intermediate compound (**4b** or **4e**) was obtained after purification by silica gel column chromatography.

Dimethyl 2-((5-(methyl(phenyl)amino)-2,4-dinitrophenyl)amino)pentanedioate (4b)

Following general procedure B yielded an orange oil (3.35 g, 75 % over two steps); ¹H NMR (300 MHz, Acetone-*d*₆): δ = 8.90 (d, *J* = 6.9 Hz, 1H), 8.70 (s, 1H), 7.32–7.26 (m, 2H), 7.09–7.04 (m, 3H), 6.76 (s, 1H), 4.82–4.76 (m, 1H), 3.82 (s, 3H), 3.62 (s, 3H), 3.52 (s, 3H), 2.54 (t, *J* = 6.9 Hz, 2H), 2.32 ppm (q, *J* = 6.9 Hz, 2H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₀H₂₃N₄O₈: 447.1510, found: 447.1508.

Diethyl 2-((5-(methyl(phenyl)amino)-2,4-dinitrophenyl)amino)malonate (4e)

Following general procedure B yielded an orange powder (3.03 g, 68 % over two steps); mp: 118–119 °C; ¹H NMR (300 MHz, Acetone-*d*₆): δ = 9.24 (d, *J* = 6.3 Hz, 1H), 8.69 (s, 1H), 7.32–7.27 (m, 2H), 7.12–7.07 (m, 3H), 6.60 (s, 1H), 5.39 (d, *J* = 6.3 Hz, 1H), 4.41–4.25 (m, 4H), 3.49 (s, 3H), 1.29 ppm (t, *J* = 7.2 Hz, 6H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₀H₂₃N₄O₈: 447.1510, found: 447.1507.

General procedure C for the synthesis of compounds 5a–f

Compound **4** (3 g) was dissolved in a mixed solvent of THF (50 mL) and EtOH (50 mL), followed by the addition of 10 % Pd/C (3 g) and NH₄COOH (6 g). The mixture was stirred at room temperature for 2 h. After the reaction was complete (monitored by HPLC-MS analysis), the residue solid was filtered off, and the filtrate was concentrated in vacuo. Key intermediate **5** was obtained after purification by silica gel column chromatography.

Methyl 3-(6-amino-7-(cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-2-yl) propanoate (5a)

Following general procedure C starting from **4a** (3 g, 6.64 mmol) yielded a brown-yellow powder (2.07 g, 87 %); mp: 207–208 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 11.94

(s, 1H), 7.16 (s, 1H), 6.46 (s, 1H), 5.55 (s, 2H), 3.60 (s, 3H), 2.93 (t, *J* = 7.2 Hz, 2H), 2.72 (t, *J* = 7.2 Hz, 2H), 2.69–2.66 (m, 1H), 2.56 (s, 3H), 1.77–1.69 (m, 4H), 1.56–1.52 (m, 1H), 1.38–1.04 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₉H₂₇N₄O₃: 359.2078, found: 359.2074.

Methyl 3-(6-amino-7-(methyl(phenyl)amino)-3-oxo-3,4-dihydroquinoxalin-2-yl) propanoate (5b)

Following general procedure C starting from **4b** (3 g, 6.73 mmol) yielded a light-yellow powder (1.99 g, 84 %); mp: 183–185 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.04 (s, 1H), 7.16–7.11 (m, 3H), 6.68 (t, *J* = 7.5 Hz, 1H), 6.58–6.56 (m, 3H), 5.67 (s, 2H), 3.56 (s, 3H), 3.11 (s, 3H), 2.92 (t, *J* = 6.9 Hz, 2H), 2.69 ppm (t, *J* = 6.9 Hz, 2H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₉H₂₁N₄O₃: 353.1608, found: 353.1606.

Methyl 3-(6-amino-7-morpholino-3-oxo-3,4-dihydroquinoxalin-2-yl)propanoate (5c)

Following general procedure C starting from **4c** (3 g, 7.04 mmol) yielded a brown-yellow powder (1.80 g, 77 %); mp: >300 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 11.97 (s, 1H), 7.11 (s, 1H), 6.48 (s, 1H), 5.64 (s, 2H), 3.76 (t, *J* = 4.2 Hz, 4H), 3.60 (s, 3H), 2.93 (t, *J* = 7.2 Hz, 2H), 2.80 (t, *J* = 4.2 Hz, 4H), 2.72 ppm (t, *J* = 7.2 Hz, 2H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₆H₂₁N₄O₄: 333.1557, found: 333.1558.

Ethyl 6-amino-7-(cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxaline-2-carboxylate (5d)

Following general procedure C starting from **4d** (3 g, 6.64 mmol) yielded an orange powder (1.96 g, 86 %); mp: 214–216 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.30 (s, 1H), 7.28 (s, 1H), 6.46 (s, 1H), 6.15 (s, 2H), 4.27 (q, *J* = 7.2 Hz, 2H), 2.74–2.68 (m, 1H), 2.57 (s, 3H), 1.79–1.69 (m, 4H), 1.56–1.52 (m, 1H), 1.38–1.05 ppm (m, 8H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₈H₂₅N₄O₃: 345.1921, found: 345.1917.

Ethyl 6-amino-7-(methyl(phenyl)amino)-3-oxo-3,4-dihydroquinoxaline-2-carboxylate (5e)

Following general procedure C starting from **4e** (3 g, 6.73 mmol) yielded an orange powder (1.82 g, 80 %); mp: 132–134 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.40 (s, 1H), 7.32 (s, 1H), 7.15 (t, *J* = 7.8 Hz, 2H), 6.70 (t, *J* = 7.2 Hz, 1H), 6.60 (d, *J* = 8.1 Hz, 2H), 6.56 (s, 1H), 6.26 (s, 2H), 4.27 (q, *J* = 7.2 Hz, 2H), 3.12 (s, 3H), 1.27 ppm (t, *J* = 7.2 Hz, 3H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₈H₁₉N₄O₃: 339.1452, found: 339.1446.

General procedure D for the synthesis of compounds 7a–f, 30a–h, and 52

To a stirred solution of amine intermediate (**5**, **28** or **50**, 2.0 mmol) in dry acetone (30 mL), benzoyl isothiocyanate (0.32 mL, 2.4 mmol) was added. The reaction mixture was refluxed for 3 h. After the reaction was completed, the solvent was evaporated in vacuo to give crude compound (**6**, **29** or **51**) without further purification. Then, the residue and K₂CO₃ (415 mg, 3.0 mmol) were dissolved in a mixed solvent of EtOH (40 mL) and H₂O (10 mL). The reaction mixture was heated at 75 °C for 1 h. After the reaction was complete (monitored by HPLC-MS analysis), the solvent was evaporated carefully under reduced pressure. The thiourea intermediate (**7**, **30** or **52**) was characterized after purification by silica gel column chromatography.

Methyl 3-(7-(cyclohexyl(methyl)amino)-3-oxo-6-thioureido-3,4-dihydroquinoxalin-2-yl)propanoate (7a)

Following general procedure D starting from **5a** (716 mg, 2.0 mmol) yielded a dark-yellow powder (584 mg, 70% over two steps); mp: 117–119 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.23 (s, 1H), 9.12 (s, 1H), 8.17 (s, 1H), 7.93 (s, 2H), 7.37 (s, 1H), 3.60 (s, 3H), 3.02 (t, *J* = 7.2 Hz, 2H), 2.77 (t, *J* = 7.2 Hz, 2H), 2.72–2.68 (m, 1H), 2.63 (s, 3H), 1.80–1.69 (m, 4H), 1.56–1.53 (m, 1H), 1.36–1.01 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₀H₂₈N₅O₃S: 418.1907, found: 418.1902.

Methyl 3-(7-(methyl(phenyl)amino)-3-oxo-6-thioureido-3,4-dihydroquinoxalin-2-yl) propanoate (7b)

Following general procedure D starting from **5b** (704 mg, 2.0 mmol) yielded a yellow powder (534 mg, 65% over two steps); mp: 125–127 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.37 (s, 1H), 9.21 (s, 1H), 8.43 (s, 1H), 7.86 (br. s, 2H), 7.36 (s, 1H), 7.18 (t, *J* = 7.8 Hz, 2H), 6.76 (t, *J* = 7.2 Hz, 1H), 6.67 (d, *J* = 8.1 Hz, 2H), 3.56 (s, 3H), 3.11 (s, 3H), 3.01 (t, *J* = 6.9 Hz, 2H), 2.73 ppm (t, *J* = 6.9 Hz, 2H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₀H₂₂N₅O₃S: 412.1438, found: 412.1437.

General procedure E for the synthesis of compounds 8–19, 24–27, 31–48, and 53

Thiourea intermediate (**7**, **23**, **30** or **52**, 0.20 mmol) and various α-bromo-substituted ketones (0.24 mmol) were dissolved in EtOH (6 mL) and refluxed until the total disappearance of thiourea intermediate monitored by HPLC-MS analysis. The mixture was concentrated in vacuo, and the final products **8–19**, **24–27**, **31–48**, and **53** were characterized after purification by silica gel column chromatography.

Methyl 3-(7-(cyclohexyl(methyl)amino)-3-oxo-6-((4-phenylthiazol-2-yl)amino)-3,4-dihydroquinoxalin-2-yl)propanoate (8)

Following general procedure E starting from **7a** (83 mg, 0.20 mmol) and 2-bromo-1-phenylethanone (48 mg, 0.24 mmol) yielded a yellow powder (74 mg, 72%); mp: 234–236 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.68 (s, 1H), 9.69 (s, 1H), 8.58 (s, 1H), 8.10 (d, *J* = 7.5 Hz, 2H), 7.49–7.44 (m, 4H), 7.34 (t, *J* = 7.5 Hz, 1H), 3.62 (s, 3H), 3.03 (t, *J* = 7.2 Hz, 2H), 2.79 (t, *J* = 7.2 Hz, 2H), 2.73 (br. s, 1H), 2.66 (s, 3H), 1.89–1.85 (m, 2H), 1.71–1.67 (m, 2H), 1.54–1.51 (m, 1H), 1.31–1.02 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₈H₃₂N₅O₃S: 518.2220, found: 518.2218.

Methyl 3-(7-(cyclohexyl(methyl)amino)-6-((4-(4-fluorophenyl)thiazol-2-yl)amino)-3-oxo-3,4-dihydroquinoxalin-2-yl)propanoate (9)

Following general procedure E starting from **7a** (83 mg, 0.20 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded a yellow powder (80 mg, 75%); mp: 247–249 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.66 (s, 1H), 9.71 (s, 1H), 8.56 (s, 1H), 8.13 (dd, *J* = 8.7 Hz, 5.7 Hz, 2H), 7.47 (s, 1H), 7.43 (s, 1H), 7.26 (t, *J* = 9.0 Hz, 2H), 3.61 (s, 3H), 3.01 (t, *J* = 7.2 Hz, 2H), 2.77 (t, *J* = 7.2 Hz, 2H), 2.71 (br. s, 1H), 2.65 (s, 3H), 1.87–1.83 (m, 2H), 1.69–1.66 (m, 2H), 1.53–1.50 (m, 1H), 1.29–1.00 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₈H₃₁FN₅O₃S: 536.2126, found: 536.2122.

Methyl 3-(7-(methyl(phenyl)amino)-3-oxo-6-((4-phenylthiazol-2-yl)amino)-3,4-dihydroquinoxalin-2-yl)propanoate (10)

Following general procedure E starting from **7b** (82 mg, 0.20 mmol) and 2-bromo-1-phenylethanone (48 mg, 0.24 mmol) yielded a brown-yellow powder (85 mg, 83%); mp: 234–236 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.80 (s, 1H), 10.06 (s, 1H), 8.87 (s, 1H), 8.11 (d, *J* = 7.2 Hz, 2H), 7.49–7.43 (m, 3H), 7.35–7.31 (m, 2H), 7.16 (t, *J* = 7.8 Hz, 2H), 6.73 (t, *J* = 7.2 Hz, 1H), 6.62 (d, *J* = 7.8 Hz, 2H), 3.56 (s, 3H), 3.14 (s, 3H), 3.00 (t, *J* = 6.9 Hz, 2H), 2.73 ppm (t, *J* = 6.9 Hz, 2H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₈H₂₆N₅O₃S: 512.1751, found: 512.1746.

Methyl 3-(6-((4-(4-fluorophenyl)thiazol-2-yl)amino)-7-(methyl(phenyl)amino)-3-oxo-3,4-dihydroquinoxalin-2-yl)propanoate (11)

Following general procedure E starting from **7b** (82 mg, 0.20 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded a brown-yellow powder (84 mg,

79 %); mp: 253–254 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*₆): δ = 12.80 (s, 1H), 10.08 (s, 1H), 8.86 (s, 1H), 8.16 (dd, J = 8.7 Hz, 5.7 Hz, 2H), 7.42 (s, 1H), 7.35 (s, 1H), 7.27 (t, J = 9.0 Hz, 2H), 7.16 (t, J = 7.8 Hz, 2H), 6.73 (t, J = 7.2 Hz, 1H), 6.62 (d, J = 8.1 Hz, 2H), 3.56 (s, 3H), 3.13 (s, 3H), 3.01 (t, J = 6.9 Hz, 2H), 2.73 ppm (t, J = 6.9 Hz, 2H); HRMS-ESI m/z [M + H]⁺ calcd for C₂₈H₂₅FN₅O₃S: 530.1657, found: 530.1653.

Methyl 3-(6-((4-(4-fluorophenyl)thiazol-2-yl)amino)-7-morpholino-3-oxo-3,4-dihydroquinoxalin-2-yl)propanoate (12)

Following general procedure E starting from **7c** (78 mg, 0.20 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded a yellow powder (90 mg, 88 %); mp: 254–256 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*₆): δ = 12.65 (s, 1H), 9.59 (s, 1H), 8.57 (s, 1H), 8.12 (dd, J = 8.7 Hz, 5.7 Hz, 2H), 7.45 (s, 1H), 7.42 (s, 1H), 7.26 (t, J = 9.0 Hz, 2H), 3.86 (br. s, 4H), 3.61 (s, 3H), 3.02 (t, J = 6.9 Hz, 2H), 2.85 (br. s, 4H), 2.77 ppm (t, J = 6.9 Hz, 2H); HRMS-ESI m/z [M + H]⁺ calcd for C₂₅H₂₅FN₅O₄S: 510.1606, found: 510.1604.

Methyl 3-(7-morpholino-6-((4-(naphthalen-1-yl)thiazol-2-yl)amino)-3-oxo-3,4-dihydroquinoxalin-2-yl)propanoate (13)

Following general procedure E starting from **7c** (78 mg, 0.20 mmol) and 2-bromo-1-(naphthalen-1-yl)ethanone (60 mg, 0.24 mmol) yielded a brown-yellow powder (76 mg, 70 %); mp: 236–237 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*₆): δ = 12.35 (s, 1H), 9.57 (s, 1H), 8.42–8.38 (m, 1H), 8.26 (s, 1H), 8.00–7.94 (m, 2H), 7.88 (d, J = 6.3 Hz, 1H), 7.62–7.53 (m, 3H), 7.41 (s, 1H), 7.26 (s, 1H), 3.87 (br. s, 4H), 3.60 (s, 3H), 2.98 (t, J = 7.2 Hz, 2H), 2.88 (br. s, 4H), 2.75 ppm (t, J = 7.2 Hz, 2H); HRMS-ESI m/z [M + H]⁺ calcd for C₂₉H₂₈N₅O₄S: 542.1856, found: 542.1853.

Ethyl 7-(cyclohexyl(methyl)amino)-6-((4-(4-fluorophenyl)thiazol-2-yl)amino)-3-oxo-3,4-dihydroquinoxaline-2-carboxylate (14)

Following general procedure E starting from **7d** (81 mg, 0.20 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded an orange powder (84 mg, 81 %); mp: 184–186 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*₆): δ = 13.14 (s, 1H), 9.96 (s, 1H), 8.66 (s, 1H), 8.16 (dd, J = 8.7 Hz, 5.7 Hz, 2H), 7.65 (s, 1H), 7.52 (s, 1H), 7.29 (t, J = 9.0 Hz, 2H), 4.34 (q, J = 7.2 Hz, 2H), 2.78–2.70 (m, 1H), 2.66 (s, 3H), 1.90–1.87 (m, 2H), 1.71–1.68 (m, 2H), 1.55–1.52 (m, 1H), 1.32 (t, J = 7.2 Hz, 3H), 1.24–1.06 ppm (m, 5H); $^{13}\text{C NMR}$ (150 MHz, DMSO-*d*₆): δ

= 163.85, 161.92, 161.72 (d, $^1J_{\text{C-F}}$ = 243.2 Hz), 153.07, 149.10, 145.24, 141.37, 137.73, 131.88, 130.81 (d, $^4J_{\text{C-F}}$ = 2.4 Hz), 128.10 (d, $^3J_{\text{C-F}}$ = 7.6 Hz), 125.69, 123.92, 115.37 (d, $^2J_{\text{C-F}}$ = 21.2 Hz), 105.26, 100.76, 62.02, 61.28, 38.46, 29.11, 25.50, 24.99, 14.05 ppm; HRMS-ESI m/z [M + H]⁺ calcd for C₂₇H₂₉FN₅O₃S: 522.1970, found: 522.1971.

Ethyl 7-(cyclohexyl(methyl)amino)-6-((4-(naphthalen-1-yl)thiazol-2-yl)amino)-3-oxo-3,4-dihydroquinoxaline-2-carboxylate (15)

Following general procedure E starting from **7d** (81 mg, 0.20 mmol) and 2-bromo-1-(naphthalen-1-yl)ethanone (60 mg, 0.24 mmol) yielded an orange powder (83 mg, 75 %); mp: 132–134 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*₆): δ = 12.85 (s, 1H), 9.92 (s, 1H), 8.40–8.38 (m, 2H), 8.03–7.90 (m, 3H), 7.65–7.55 (m, 4H), 7.33 (s, 1H), 4.32 (q, J = 7.2 Hz, 2H), 2.82–2.75 (m, 1H), 2.69 (s, 3H), 1.93–1.89 (m, 2H), 1.74–1.70 (m, 2H), 1.57–1.54 (m, 1H), 1.33–1.05 ppm (m, 8H); $^{13}\text{C NMR}$ (150 MHz, DMSO-*d*₆): δ = 163.82, 161.59, 152.82, 149.45, 145.29, 141.40, 137.80, 133.49, 132.68, 131.87, 130.64, 128.31, 128.23, 127.46, 126.48, 125.87, 125.68, 125.51, 125.48, 123.87, 109.64, 100.59, 61.91, 61.24, 38.27, 29.14, 25.50, 25.01, 14.03 ppm; HRMS-ESI m/z [M + H]⁺ calcd for C₃₁H₃₂N₅O₃S: 554.2220, found: 554.2219.

Ethyl 7-(methyl(phenyl)amino)-3-oxo-6-((4-phenylthiazol-2-yl)amino)-3,4-dihydroquinoxaline-2-carboxylate (16)

Following general procedure E starting from **7e** (79 mg, 0.20 mmol) and 2-bromo-1-phenylethanone (48 mg, 0.24 mmol) yielded a dark-yellow powder (62 mg, 62 %); mp: 255–257 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*₆): δ = 13.26 (s, 1H), 10.26 (s, 1H), 8.92 (s, 1H), 8.12 (d, J = 7.2 Hz, 2H), 7.54–7.45 (m, 4H), 7.34 (t, J = 7.2 Hz, 1H), 7.17 (t, J = 7.8 Hz, 2H), 6.75 (t, J = 7.2 Hz, 1H), 6.65 (d, J = 7.8 Hz, 2H), 4.31 (q, J = 7.2 Hz, 2H), 3.15 (s, 3H), 1.29 ppm (t, J = 7.2 Hz, 3H); HRMS-ESI m/z [M + H]⁺ calcd for C₂₇H₂₄N₅O₃S: 498.1594, found: 498.1590.

Ethyl 7-(methyl(phenyl)amino)-6-((4-(naphthalen-1-yl)thiazol-2-yl)amino)-3-oxo-3,4-dihydroquinoxaline-2-carboxylate (17)

Following general procedure E starting from **7e** (79 mg, 0.20 mmol) and 2-bromo-1-(naphthalen-1-yl)ethanone (60 mg, 0.24 mmol) yielded an orange powder (83 mg, 76 %); mp: 135–137 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*₆): δ = 12.95 (s, 1H), 10.27 (s, 1H), 8.73 (s, 1H), 8.39–8.36 (m, 1H), 8.02–7.92 (m, 3H), 7.63 (t, J = 7.5 Hz, 1H), 7.57–7.53 (m, 3H), 7.28 (s, 1H), 7.19 (t, J = 7.8 Hz, 2H), 6.76 (t, J = 7.2 Hz, 1H), 6.68 (d, J = 8.1 Hz, 2H), 4.29 (q, J = 7.2 Hz, 2H), 3.18 (s,

3H), 1.27 ppm (t, $J = 7.2$ Hz, 3H); HRMS-ESI m/z [M+H]⁺ calcd for C₃₁H₂₆N₅O₃S: 548.1751, found: 548.1749.

Ethyl 7-morpholino-3-oxo-6-((4-phenylthiazol-2-yl)amino)-3,4-dihydroquinoxaline-2-carboxylate (18)

Following general procedure E starting from **7f** (75 mg, 0.20 mmol) and 2-bromo-1-phenylethanone (48 mg, 0.24 mmol) yielded an amber powder (67 mg, 70%); mp: 263–265 °C; ¹H NMR (300 MHz, DMSO-*d*₆): $\delta = 13.13$ (s, 1H), 9.80 (s, 1H), 8.67 (s, 1H), 8.10 (d, $J = 7.5$ Hz, 2H), 7.60 (s, 1H), 7.55 (s, 1H), 7.47 (t, $J = 7.5$ Hz, 2H), 7.35 (t, $J = 7.5$ Hz, 1H), 4.34 (q, $J = 7.2$ Hz, 2H), 3.89 (br. s, 4H), 2.88 (br. s, 4H), 1.32 ppm (t, $J = 7.2$ Hz, 3H); HRMS-ESI m/z [M + H]⁺ calcd for C₂₄H₂₄N₅O₄S: 478.1544, found: 478.1542.

Ethyl 6-((4-(4-fluorophenyl)thiazol-2-yl)amino)-7-morpholino-3-oxo-3,4-dihydroquinoxaline-2-carboxylate (19)

Following general procedure E starting from **7f** (75 mg, 0.20 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded an orange powder (77 mg, 78%); mp: 264–266 °C; ¹H NMR (300 MHz, DMSO-*d*₆): $\delta = 13.13$ (s, 1H), 9.82 (s, 1H), 8.67 (s, 1H), 8.15 (dd, $J = 8.4$ Hz, 5.7 Hz, 2H), 7.60 (s, 1H), 7.53 (s, 1H), 7.28 (t, $J = 8.7$ Hz, 2H), 4.34 (q, $J = 7.2$ Hz, 2H), 3.89 (br. s, 4H), 2.88 (br. s, 4H), 1.32 ppm (t, $J = 7.2$ Hz, 3H); HRMS-ESI m/z [M + H]⁺ calcd for C₂₄H₂₃FN₅O₄S: 496.1449, found: 496.1445.

Dimethyl 1-(5-(cyclohexyl(methyl)amino)-2,4-dinitrophenyl)-1H-imidazole-4,5-dicarboxylate (20)

To a stirred solution of 1,5-difluoro-2,4-dinitrobenzene (DFDNB; 2.04 g, 10 mmol) in THF (50 mL) was added DIPEA (1.75 mL, 10 mmol) and *N*-methylcyclohexylamine (1.30 mL, 10 mmol). The reaction mixture was stirred at room temperature until the total disappearance of DFDNB monitored by HPLC-MS analysis. The solvent was evaporated under reduced pressure, and the oily residue was dissolved in saturated ammonium chloride solution (50 mL) and extracted by CH₂Cl₂ (2 × 50 mL). The organic phases were combined, dried over anhydrous Na₂SO₄, and evaporated in vacuo to give crude intermediate **3a**. Then, the residue was dissolved in acetone (70 mL). Dimethyl 1H-imidazole-4,5-dicarboxylate (1.84 g, 10 mmol) and K₂CO₃ (2.76 g, 20 mmol) were added and stirred under reflux for 6 h. After the reaction was complete (monitored by HPLC-MS analysis), the solvent was evaporated in vacuo. Saturated ammonium chloride solution (50 mL) was added to the resulting product and was further extracted by CH₂Cl₂ (2 × 50 mL). The organic layers were combined, dried over anhydrous Na₂SO₄, and evaporated in vacuo. Intermediate compound

20 was obtained after purification by silica gel column chromatography as a dark-yellow powder (3.32 g, 72% over two steps); mp: 165–167 °C; ¹H NMR (300 MHz, DMSO-*d*₆): $\delta = 8.72$ (s, 1H), 8.22 (s, 1H), 7.60 (s, 1H), 3.86 (s, 3H), 3.82–3.75 (m, 1H), 3.68 (s, 3H), 2.74 (s, 3H), 1.78–1.56 (m, 7H), 1.44–1.06 ppm (m, 3H); HRMS-ESI m/z [M + H]⁺ calcd for C₂₀H₂₄N₅O₈: 462.1619, found: 462.1618.

*Methyl 7-amino-8-(cyclohexyl(methyl)amino)-4-oxo-4,5-dihydroimidazo[1,5-*a*]quinoxaline-3-carboxylate (21)*

To a stirred solution of intermediate **20** (4.61 g, 10 mmol) in a mixed solvent of MeOH (150 mL) and H₂O (150 mL) was added sodium dithionite (17.41 g, 100 mmol), and the mixture was heated at 70 °C. Concentrated hydrochloric acid (10 mL) was added drop-wise, and the reaction mixture was stirred at 70 °C for 1 h. After the reaction was complete (monitored by HPLC-MS analysis), the reaction mixture was cooled and filtered. The filtrate was concentrated under reduced pressure to give a yellow solid residue, which was then dissolved in MeOH (300 mL), acidified to pH 1–2 with concentrated sulfuric acid, and stirred under reflux for 4 h. The reaction mixture was cooled, neutralized using 2 M sodium hydroxide solution with good stirring, and concentrated in vacuo to give a brown-green solid residue, which was purified by silica gel column chromatography to give the key intermediate **21** as a brown-green powder (3.21 g, 87%); mp: 258–260 °C; ¹H NMR (300 MHz, DMSO-*d*₆): $\delta = 11.32$ (s, 1H), 9.02 (s, 1H), 7.82 (s, 1H), 6.59 (s, 1H), 5.26 (br. s, 2H), 3.82 (s, 3H), 2.80–2.73 (m, 1H), 2.62 (s, 3H), 1.80–1.70 (m, 4H), 1.56–1.51 (m, 1H), 1.40–1.06 ppm (m, 5H); HRMS-ESI m/z [M + H]⁺ calcd for C₁₉H₂₄N₅O₃: 370.1874, found: 370.1875.

General procedure F for the synthesis of compounds 24–27

To a stirred solution of **21** (1.85 g, 5 mmol) in 80 mL of dry acetone, benzoyl isothiocyanate (0.81 mL, 6 mmol) was added. The reaction mixture was refluxed for 3 h. After the reaction was completed, the solvent was evaporated in vacuo to give crude compound **22** without further purification. Then, the residue was dissolved in MeOH (30 mL), and the mixture was heated at 50 °C. Sodium methoxide (0.54 g, 10 mmol) in methanol (50 mL) was added drop-wise, and the reaction mixture was stirred at 50 °C for 1 h. The reaction mixture was cooled, neutralized using 5% hydrochloric acid, and concentrated under reduced pressure to give a yellow solid residue, which was purified by silica gel column chromatography. Intermediate compound **23** was obtained as a yellow powder in 76% yield over two steps. Finally, thiourea compound **23** (86 mg, 0.20 mmol) and various α -bromo-substituted ketones (0.24 mmol) were dissolved in MeOH (6 mL) and refluxed until the total disappearance of

23 monitored by HPLC-MS analysis. The reaction mixture was concentrated in vacuo, and the final products **24–27** were characterized after purification by silica gel column chromatography.

Methyl 8-(cyclohexyl(methyl)amino)-4-oxo-7-((4-phenylthiazol-2-yl)amino)-4,5-dihydroimidazo[1,5-a]quinoxaline-3-carboxylate (24)

Following general procedure F starting from 2-bromo-1-phenylethanone (48 mg, 0.24 mmol) yielded a yellow powder (86 mg, 81 %); mp: 248–250 °C; ^1H NMR (300 MHz, DMSO-*d*6): δ = 11.96 (s, 1H), 9.53 (s, 1H), 9.16 (s, 1H), 8.56 (s, 1H), 8.15 (s, 1H), 8.10 (d, J = 7.5 Hz, 2H), 7.46 (t, J = 7.5 Hz, 2H), 7.43 (s, 1H), 7.34 (t, J = 7.2 Hz, 1H), 3.85 (s, 3H), 2.82–2.75 (m, 1H), 2.71 (s, 3H), 1.91–1.87 (m, 2H), 1.72–1.69 (m, 2H), 1.55–1.52 (m, 1H), 1.36–1.04 ppm (m, 5H); ^{13}C NMR (150 MHz, DMSO-*d*6): δ = 162.62, 162.46, 153.17, 150.15, 136.98, 136.37, 134.33, 133.31, 131.96, 128.54, 127.53, 126.29, 126.05, 121.64, 113.54, 111.79, 104.25, 103.99, 62.03, 51.82, 38.15, 29.27, 25.48, 25.02 ppm; HRMS-ESI m/z [$\text{M} + \text{H}$] $^+$ calcd for $\text{C}_{28}\text{H}_{29}\text{N}_6\text{O}_3\text{S}$: 529.2016, found: 529.2014.

Methyl 8-(cyclohexyl(methyl)amino)-7-((4-(4-fluorophenyl)thiazol-2-yl)amino)-4-oxo-4,5-dihydroimidazo[1,5-a]quinoxaline-3-carboxylate (25)

Following general procedure F starting from 2-bromo-1-(4-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded a light-yellow powder (85 mg, 78 %); mp: 262–264 °C; ^1H NMR (300 MHz, DMSO-*d*6): δ = 11.95 (s, 1H), 9.55 (s, 1H), 9.16 (s, 1H), 8.57 (s, 1H), 8.17–8.12 (m, 3H), 7.41 (s, 1H), 7.28 (t, J = 9.0 Hz, 2H), 3.85 (s, 3H), 2.81–2.74 (m, 1H), 2.70 (s, 3H), 1.91–1.87 (m, 2H), 1.73–1.69 (m, 2H), 1.55–1.52 (m, 1H), 1.36–1.10 ppm (m, 5H); ^{13}C NMR (150 MHz, DMSO-*d*6): δ = 162.61, 162.58, 161.65 (d, $^1J_{\text{C}-\text{F}}$ = 243.2 Hz), 153.17, 149.05, 136.96, 136.38, 133.32, 131.96, 130.97 (d, $^4J_{\text{C}-\text{F}}$ = 2.7 Hz), 128.08 (d, $^3J_{\text{C}-\text{F}}$ = 7.8 Hz), 126.26, 121.63, 115.30 (d, $^2J_{\text{C}-\text{F}}$ = 21.3 Hz), 113.55, 111.81, 104.00, 103.96, 62.03, 51.83, 38.18, 29.27, 25.48, 25.02 ppm; HRMS-ESI m/z [$\text{M} + \text{H}$] $^+$ calcd for $\text{C}_{28}\text{H}_{28}\text{FN}_6\text{O}_3\text{S}$: 547.1922, found: 547.1920.

Methyl 7-((4-(4-chlorophenyl)thiazol-2-yl)amino)-8-(cyclohexyl(methyl)amino)-4-oxo-4,5-dihydroimidazo[1,5-a]quinoxaline-3-carboxylate (26)

Following general procedure F starting from 2-bromo-1-(4-chlorophenyl)ethanone (56 mg, 0.24 mmol) yielded a light-yellow powder (81 mg, 72 %); mp: 261–263 °C; ^1H NMR (300 MHz, DMSO-*d*6): δ = 11.95 (s, 1H), 9.59 (s, 1H), 9.16 (s, 1H), 8.56 (s, 1H), 8.16–8.11 (m, 3H), 7.52–7.49 (m, 3H), 3.85 (s, 3H), 2.82–2.74 (m, 1H), 2.70 (s, 3H), 1.91–1.87

(m, 2H), 1.73–1.69 (m, 2H), 1.55–1.51 (m, 1H), 1.36–1.04 ppm (m, 5H); ^{13}C NMR (150 MHz, DMSO-*d*6): δ = 162.65, 162.60, 153.18, 148.82, 136.90, 133.32, 133.21, 131.97, 131.92, 128.50, 127.78, 126.28, 121.63, 113.60, 111.82, 105.11, 104.02, 62.07, 51.83, 38.20, 29.24, 25.47, 25.01 ppm; HRMS-ESI m/z [$\text{M} + \text{H}$] $^+$ calcd for $\text{C}_{28}\text{H}_{28}\text{ClN}_6\text{O}_3\text{S}$: 563.1627, found: 563.1624.

Methyl 8-(cyclohexyl(methyl)amino)-7-((4-(naphthalen-1-yl)thiazol-2-yl)amino)-4-oxo-4,5-dihydroimidazo[1,5-a]quinoxaline-3-carboxylate (27)

Following general procedure F starting from 2-bromo-1-(naphthalen-1-yl)ethanone (60 mg, 0.24 mmol) yielded a light-yellow powder (87 mg, 75 %); mp: 257–258 °C; ^1H NMR (300 MHz, DMSO-*d*6): δ = 11.68 (s, 1H), 9.52 (s, 1H), 9.16 (s, 1H), 8.42–8.39 (m, 1H), 8.25 (s, 1H), 8.14 (s, 1H), 8.01–7.95 (m, 2H), 7.90–7.86 (m, 1H), 7.64–7.54 (m, 3H), 7.20 (s, 1H), 3.83 (s, 3H), 2.89–2.81 (m, 1H), 2.73 (s, 3H), 1.92–1.88 (m, 2H), 1.75–1.71 (m, 2H), 1.57–1.53 (m, 1H), 1.40–1.06 ppm (m, 5H); ^{13}C NMR (150 MHz, DMSO-*d*6): δ = 162.66, 162.61, 152.90, 149.38, 136.69, 133.48, 133.32, 132.82, 132.02, 130.66, 128.27, 128.14, 127.39, 127.34, 126.34, 126.13, 125.82, 125.60, 125.49, 121.62, 113.93, 111.52, 108.12, 104.75, 61.99, 51.80, 37.66, 29.24, 25.47, 25.10 ppm; HRMS-ESI m/z [$\text{M} + \text{H}$] $^+$ calcd for $\text{C}_{32}\text{H}_{31}\text{N}_6\text{O}_3\text{S}$: 579.2173, found: 579.2170.

General procedure G for the synthesis of compounds 28a–h and 50

To a stirred solution of ester intermediate (**5a**, **5c**, **5d**, **5e** or **49**, 5 mmol) in a mixed solvent of EtOH (25 mL) and H₂O (5 mL), lithium hydroxide monohydrate (629 mg, 15 mmol) was added. The reaction mixture was stirred at 50 °C for 1 h. After the reaction was complete (monitored by HPLC-MS analysis), a partial volume of the solvent was evaporated in vacuo, and the remaining mixture was acidified to pH 5–6 with 5 % hydrochloric acid. The formed precipitate was collected by filtration to give the corresponding crude carboxylic acid intermediate as a yellow solid. The intermediate (3.0 mmol) was dissolved in THF (50 mL), and then, HOBt (486 mg, 3.6 mmol) and EDCI (690 mg, 3.6 mmol) were added, followed by the addition of methylamine or dimethylamine (4.5 mmol, 2 M in THF) drop-wise. The reaction mixture was stirred at 60 °C. After the reaction was complete (monitored by HPLC-MS analysis), the solvent was evaporated carefully under reduced pressure to give a black oily residue, which was purified by silica gel column chromatography to give the amide intermediate (**28** or **50**).

3-(6-Amino-7-(cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-2-yl)-N-methylpropanamide (28a)

Following general procedure G starting from carboxylic acid type of **5a** (1.03 g, 3.0 mmol) yielded a yellow powder (760 mg, 71 %); mp: 125–127 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 11.91 (s, 1H), 7.78 (q, *J* = 4.5 Hz, 1H), 7.18 (s, 1H), 6.46 (s, 1H), 5.54 (s, 2H), 2.88 (t, *J* = 7.2 Hz, 2H), 2.74–2.67 (m, 1H), 2.58–2.56 (m, 6H), 2.47 (t, *J* = 7.2 Hz, 2H), 1.78–1.69 (m, 4H), 1.56–1.52 (m, 1H), 1.38–1.04 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₉H₂₈N₅O₂: 358.2238, found: 358.2237.

3-(6-Amino-7-(cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-2-yl)-N,N-dimethylpropanamide (28b)

Following general procedure G starting from carboxylic acid type of **5a** (1.03 g, 3.0 mmol) yielded an orange powder (790 mg, 71 %); mp: 190–192 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 11.90 (s, 1H), 7.18 (s, 1H), 6.46 (s, 1H), 5.53 (s, 2H), 3.02 (s, 3H), 2.88 (t, *J* = 7.2 Hz, 2H), 2.82 (s, 3H), 2.74–2.66 (m, 3H), 2.56 (s, 3H), 1.77–1.69 (m, 4H), 1.55–1.52 (m, 1H), 1.37–1.03 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₀H₃₀N₅O₂: 372.2394, found: 372.2394.

3-(6-Amino-7-morpholino-3-oxo-3,4-dihydroquinoxalin-2-yl)-N-methylpropanamide (28c)

Following general procedure G starting from carboxylic acid type of **5c** (954 mg, 3.0 mmol) yielded a brown-yellow powder (705 mg, 71 %); mp: 248–250 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 11.93 (s, 1H), 7.78 (q, *J* = 4.5 Hz, 1H), 7.13 (s, 1H), 6.48 (s, 1H), 5.62 (s, 2H), 3.77 (t, *J* = 4.2 Hz, 4H), 2.88 (t, *J* = 7.2 Hz, 2H), 2.80 (t, *J* = 4.2 Hz, 4H), 2.57 (d, *J* = 4.5 Hz, 3H), 2.47 ppm (t, *J* = 7.2 Hz, 2H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₆H₂₂N₅O₃: 332.1717, found: 332.1715.

3-(6-Amino-7-morpholino-3-oxo-3,4-dihydroquinoxalin-2-yl)-N,N-dimethylpropanamide (28d)

Following general procedure G starting from carboxylic acid type of **5c** (954 mg, 3.0 mmol) yielded a tan powder (725 mg, 70 %); mp: >300 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 11.93 (s, 1H), 7.13 (s, 1H), 6.48 (s, 1H), 5.61 (s, 2H), 3.76 (t, *J* = 4.2 Hz, 4H), 3.03 (s, 3H), 2.89 (t, *J* = 7.2 Hz, 2H), 2.82 (s, 3H), 2.80 (t, *J* = 4.2 Hz, 4H), 2.68 ppm (t, *J* = 7.2 Hz, 2H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₇H₂₄N₅O₃: 346.1874, found: 346.1868.

6-Amino-7-(cyclohexyl(methyl)amino)-N-methyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (28e)

Following general procedure G starting from carboxylic acid type of **5d** (948 mg, 3.0 mmol) yielded a dark-yellow powder (622 mg, 63 %); mp: 277–278 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.53 (s, 1H), 9.37 (q, *J* = 4.8 Hz, 1H), 7.34 (s, 1H), 6.50 (s, 1H), 6.19 (s, 2H), 2.83 (d, *J* = 4.8 Hz, 3H), 2.76–2.70 (m, 1H), 2.58 (s, 3H), 1.80–1.69 (m, 4H), 1.56–1.53 (m, 1H), 1.39–1.06 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₇H₂₄N₅O₂: 330.1924, found: 330.1922.

6-Amino-7-(cyclohexyl(methyl)amino)-N,N-dimethyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (28f)

Following general procedure G starting from carboxylic acid type of **5d** (948 mg, 3.0 mmol) yielded a yellow powder (731 mg, 71 %); mp: 253–255 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.24 (s, 1H), 7.24 (s, 1H), 6.49 (s, 1H), 5.85 (s, 2H), 2.96 (s, 3H), 2.85 (s, 3H), 2.74–2.68 (m, 1H), 2.56 (s, 3H), 1.79–1.69 (m, 4H), 1.56–1.53 (m, 1H), 1.38–1.05 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₈H₂₆N₅O₂: 344.2081, found: 344.2076.

6-Amino-N-methyl-7-(methyl(phenyl)amino)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (28g)

Following general procedure G starting from carboxylic acid type of **5e** (930 mg, 3.0 mmol) yielded an orange powder (795 mg, 82 %); mp: 270–272 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.57 (s, 1H), 9.20 (q, *J* = 4.8 Hz, 1H), 7.38 (s, 1H), 7.16 (t, *J* = 7.8 Hz, 2H), 6.71 (t, *J* = 7.2 Hz, 1H), 6.61 (d, *J* = 7.8 Hz, 2H), 6.60 (s, 1H), 6.27 (s, 2H), 3.14 (s, 3H), 2.81 ppm (d, *J* = 4.8 Hz, 3H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₇H₁₈N₅O₂: 324.1455, found: 324.1452.

6-Amino-N,N-dimethyl-7-(methyl(phenyl)amino)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (28h)

Following general procedure G starting from carboxylic acid type of **5e** (930 mg, 3.0 mmol) yielded a yellow powder (728 mg, 72 %); mp: 265–267 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.34 (s, 1H), 7.27 (s, 1H), 7.14 (t, *J* = 7.8 Hz, 2H), 6.69 (t, *J* = 7.2 Hz, 1H), 6.60–6.58 (m, 3H), 5.98 (s, 2H), 3.12 (s, 3H), 2.95 (s, 3H), 2.86 ppm (s, 3H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₈H₂₀N₅O₂: 338.1612, found: 338.1612.

3-(7-(Cyclohexyl(methyl)amino)-3-oxo-6-thioureido-3,4-dihydroquinoxalin-2-yl)-N-methylpropanamide (**30a**)

Following general procedure D starting from **28a** (714 mg, 2.0 mmol) yielded a dark-yellow powder (607 mg, 73 % over two steps); mp: >300 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.18 (s, 1H), 9.10 (s, 1H), 8.16 (s, 1H), 7.92 (s, 2H), 7.80 (q, *J* = 4.5 Hz, 1H), 7.38 (s, 1H), 2.96 (t, *J* = 7.5 Hz, 2H), 2.72–2.67 (m, 1H), 2.63 (s, 3H), 2.57 (d, *J* = 4.5 Hz, 3H), 2.52 (t, *J* = 7.5 Hz, 2H), 1.80–1.69 (m, 4H), 1.56–1.53 (m, 1H), 1.36–1.02 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₀H₂₉N₆O₂S: 417.2067, found: 417.2065.

3-(7-(Cyclohexyl(methyl)amino)-3-oxo-6-thioureido-3,4-dihydroquinoxalin-2-yl)-N,N-dimethylpropanamide (**30b**)

Following general procedure D starting from **28b** (742 mg, 2.0 mmol) yielded a yellow powder (611 mg, 71 % over two steps); mp: >300 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.18 (s, 1H), 9.12 (s, 1H), 8.17 (s, 1H), 7.92 (s, 2H), 7.39 (s, 1H), 3.03 (s, 3H), 2.97 (t, *J* = 7.2 Hz, 2H), 2.82 (s, 3H), 2.75 (t, *J* = 7.2 Hz, 2H), 2.68–2.59 (m, 4H), 1.80–1.69 (m, 4H), 1.56–1.53 (m, 1H), 1.36–1.01 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₁H₃₁N₆O₂S: 431.2224, found: 431.2224.

N-methyl-3-(7-morpholino-3-oxo-6-thioureido-3,4-dihydroquinoxalin-2-yl)propanamide (**30c**)

Following general procedure D starting from **28c** (662 mg, 2.0 mmol) yielded a yellow powder (585 mg, 75 % over two steps); mp: 192–194 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.21 (s, 1H), 9.06 (s, 1H), 8.03 (s, 1H), 7.94 (br. s, 2H), 7.81 (q, *J* = 4.5 Hz, 1H), 7.34 (s, 1H), 3.81 (br. s, 4H), 2.97 (t, *J* = 7.2 Hz, 2H), 2.83 (br. s, 4H), 2.57 (d, *J* = 4.5 Hz, 3H), 2.52 ppm (t, *J* = 7.2 Hz, 2H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₇H₂₃N₆O₃S: 391.1547, found: 391.1541.

N,N-dimethyl-3-(7-morpholino-3-oxo-6-thioureido-3,4-dihydroquinoxalin-2-yl)propanamide (**30d**)

Following general procedure D starting from **28d** (690 mg, 2.0 mmol) yielded a yellow powder (614 mg, 76 % over two steps); mp: 184–186 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.21 (s, 1H), 9.06 (s, 1H), 8.03 (s, 1H), 7.93 (br. s, 2H), 7.35 (s, 1H), 3.80 (br. s, 4H), 3.04 (s, 3H), 2.97 (t, *J* = 7.2 Hz, 2H), 2.84 (br. s, 4H), 2.82 (s, 3H), 2.75 ppm (t, *J* = 7.2 Hz, 2H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₈H₂₅N₆O₃S: 405.1703, found: 405.1703.

7-(Cyclohexyl(methyl)amino)-N-methyl-3-oxo-6-thioureido-3,4-dihydroquinoxaline-2-carboxamide (**30e**)

Following general procedure D starting from **28e** (658 mg, 2.0 mmol) yielded an orange powder (566 mg, 73 % over two steps); mp: > 300 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.77 (s, 1H), 9.28 (s, 1H), 9.09 (q, *J* = 4.8 Hz, 1H), 8.45 (s, 1H), 8.12 (s, 2H), 7.59 (s, 1H), 2.82 (d, *J* = 4.8 Hz, 3H), 2.71–2.67 (m, 1H), 2.64 (s, 3H), 1.82–1.70 (m, 4H), 1.56–1.53 (m, 1H), 1.36–1.02 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₈H₂₅N₆O₂S: 389.1754, found: 389.1748.

7-(Cyclohexyl(methyl)amino)-N,N-dimethyl-3-oxo-6-thioureido-3,4-dihydroquinoxaline-2-carboxamide (**30f**)

Following general procedure D starting from **28f** (686 mg, 2.0 mmol) yielded a yellow powder (659 mg, 82 % over two steps); mp: 230–232 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.58 (br. s, 1H), 9.19 (s, 1H), 8.26 (s, 1H), 8.01 (s, 2H), 7.46 (s, 1H), 2.98 (s, 3H), 2.87 (s, 3H), 2.70–2.66 (m, 1H), 2.62 (s, 3H), 1.81–1.69 (m, 4H), 1.56–1.53 (m, 1H), 1.36–1.01 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₉H₂₇N₆O₂S: 403.1911, found: 403.1907.

N-methyl-7-(methyl(phenyl)amino)-3-oxo-6-thioureido-3,4-dihydroquinoxaline-2-carboxamide (**30g**)

Following general procedure D starting from **28g** (646 mg, 2.0 mmol) yielded an orange powder (573 mg, 75 % over two steps); mp: 240–242 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.85 (s, 1H), 9.34 (s, 1H), 8.96 (q, *J* = 4.8 Hz, 1H), 8.70 (s, 1H), 8.04 (s, 2H), 7.56 (s, 1H), 7.18 (t, *J* = 7.8 Hz, 2H), 6.78 (t, *J* = 7.2 Hz, 1H), 6.68 (d, *J* = 8.1 Hz, 2H), 3.14 (s, 3H), 2.80 ppm (d, *J* = 4.8 Hz, 3H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₈H₁₉N₆O₂S: 383.1285, found: 383.1282.

N,N-dimethyl-7-(methyl(phenyl)amino)-3-oxo-6-thioureido-3,4-dihydroquinoxaline-2-carboxamide (**30h**)

Following general procedure D starting from **28h** (674 mg, 2.0 mmol) yielded an orange powder (578 mg, 73 % over two steps); mp: 184–186 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.73 (s, 1H), 9.27 (s, 1H), 8.62 (s, 1H), 7.96 (br. s, 2H), 7.49 (s, 1H), 7.18 (t, *J* = 7.8 Hz, 2H), 6.77 (t, *J* = 7.2 Hz, 1H), 6.68 (d, *J* = 8.1 Hz, 2H), 3.13 (s, 3H), 2.97 (s, 3H), 2.88 ppm (s, 3H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₁₉H₂₁N₆O₂S: 397.1441, found: 397.1438.

3-(7-(Cyclohexyl(methyl)amino)-6-((4-(4-fluorophenyl)thiazol-2-yl)amino)-3-oxo-3,4-dihydroquinoxalin-2-yl)-N-methylpropanamide (**31**)

Following general procedure E starting from **30a** (83 mg, 0.2 mmol) and 2-bromo-1-(4-fluorophenyl) ethanone (52 mg, 0.24 mmol) yielded a light-yellow powder (69 mg, 65 %); mp: 120–122 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.63 (s, 1H), 9.71 (s, 1H), 8.57 (s, 1H), 8.15 (dd, *J* = 8.7 Hz, 5.7 Hz, 2H), 7.82 (q, *J* = 4.5 Hz, 1H), 7.50 (s, 1H), 7.45 (s, 1H), 7.27 (t, *J* = 9.0 Hz, 2H), 2.97 (t, *J* = 7.8 Hz, 2H), 2.76–2.68 (m, 1H), 2.66 (s, 3H), 2.59 (d, *J* = 4.5 Hz, 3H), 2.54 (t, *J* = 7.8 Hz, 2H), 1.89–1.85 (m, 2H), 1.71–1.67 (m, 2H), 1.55–1.51 (m, 1H), 1.31–1.02 ppm (m, 5H); ¹³C NMR (150 MHz, DMSO-*d*₆): δ = 171.86, 162.29, 161.67 (d, ¹*J*_{C–F} = 243.2 Hz), 157.04, 155.13, 149.02, 138.67, 136.54, 130.93 (d, ⁴*J*_{C–F} = 2.7 Hz), 129.84, 128.07 (d, ³*J*_{C–F} = 8.1 Hz), 126.40, 122.68, 115.34 (d, ²*J*_{C–F} = 21.2 Hz), 104.35, 101.39, 62.14, 38.65, 31.64, 29.23, 28.37, 25.52, 25.00, 24.97 ppm; HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₈H₃₂FN₆O₂S: 535.2286, found: 535.2285.

3-(7-(Cyclohexyl(methyl)amino)-6-((4-(4-fluorophenyl)thiazol-2-yl)amino)-3-oxo-3,4-dihydroquinoxalin-2-yl)-N,N-dimethylpropanamide (**32**)

Following general procedure E starting from **30b** (86 mg, 0.2 mmol) and 2-bromo-1-(4-fluorophenyl) ethanone (52 mg, 0.24 mmol) yielded a yellow powder (89 mg, 81 %); mp: 151–153 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.63 (s, 1H), 9.71 (s, 1H), 8.56 (s, 1H), 8.15 (dd, *J* = 8.7 Hz, 5.7 Hz, 2H), 7.51 (s, 1H), 7.45 (s, 1H), 7.28 (t, *J* = 9.0 Hz, 2H), 3.05 (s, 3H), 2.98 (t, *J* = 7.2 Hz, 2H), 2.84 (s, 3H), 2.78–2.74 (m, 3H), 2.66 (s, 3H), 1.89–1.85 (m, 2H), 1.71–1.67 (m, 2H), 1.55–1.52 (m, 1H), 1.31–1.02 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₉H₃₄FN₆O₂S: 549.2442, found: 549.2441.

3-(6-((4-(4-Fluorophenyl)thiazol-2-yl)amino)-7-morpholino-3-oxo-3,4-dihydroquinoxalin-2-yl)-N-methylpropanamide (**33**)

Following general procedure E starting from **30c** (78 mg, 0.2 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded a flavo-green powder (65 mg, 64 %); mp: 261–263 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.62 (s, 1H), 9.60 (s, 1H), 8.57 (s, 1H), 8.13 (dd, *J* = 8.7, 5.7 Hz, 2H), 7.82 (q, *J* = 4.5 Hz, 1H), 7.46 (s, 1H), 7.45 (s, 1H), 7.27 (t, *J* = 9.0 Hz, 2H), 3.88 (br. s, 4H), 2.98 (t, *J* = 7.5 Hz, 2H), 2.86 (br. s, 4H), 2.59 (d, *J* = 4.5 Hz, 3H), 2.54 ppm (t, *J* = 7.5 Hz, 2H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₅H₂₆FN₆O₃S: 509.1766, found: 509.1768.

3-(6-((4-(4-Fluorophenyl)thiazol-2-yl)amino)-7-morpholino-3-oxo-3,4-dihydroquinoxalin-2-yl)-N,N-dimethylpropanamide (**34**)

Following general procedure E starting from **30d** (81 mg, 0.2 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded a yellow powder (74 mg, 71 %); mp: 257–259 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 12.61 (s, 1H), 9.59 (s, 1H), 8.57 (s, 1H), 8.13 (dd, *J* = 8.7 Hz, 5.7 Hz, 2H), 7.46 (s, 2H), 7.27 (t, *J* = 9.0 Hz, 2H), 3.88 (br. s, 4H), 3.05 (s, 3H), 2.98 (t, *J* = 7.2 Hz, 2H), 2.86 (br. s, 4H), 2.84 (s, 3H), 2.76 ppm (t, *J* = 7.2 Hz, 2H); ¹³C NMR (150 MHz, DMSO-*d*₆): δ = 171.27, 162.46, 161.66 (d, ¹*J*_{C–F} = 243.2 Hz), 157.48, 155.13, 148.92, 137.45, 136.76, 130.94 (d, ⁴*J*_{C–F} = 2.6 Hz), 129.52, 128.06 (d, ³*J*_{C–F} = 8.0 Hz), 126.68, 119.28, 115.33 (d, ²*J*_{C–F} = 21.2 Hz), 104.59, 102.31, 65.99, 52.39, 36.72, 34.89, 28.86, 27.94 ppm; HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₆H₂₈FN₆O₃S: 523.1922, found: 523.1926.

7-(Cyclohexyl(methyl)amino)-6-((4-(4-fluorophenyl)thiazol-2-yl)amino)-N-methyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**35**)

Following general procedure E starting from **30e** (78 mg, 0.2 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded an orange powder (69 mg, 68 %); mp: 252–254 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 13.23 (s, 1H), 9.97 (s, 1H), 9.20 (q, *J* = 4.8 Hz, 1H), 8.68 (s, 1H), 8.16 (dd, *J* = 8.7 Hz, 5.7 Hz, 2H), 7.69 (s, 1H), 7.52 (s, 1H), 7.29 (t, *J* = 9.0 Hz, 2H), 2.85 (d, *J* = 4.8 Hz, 3H), 2.79–2.73 (m, 1H), 2.68 (s, 3H), 1.91–1.87 (m, 2H), 1.72–1.68 (m, 2H), 1.55–1.52 (m, 1H), 1.32–1.02 ppm (m, 5H); HRMS-ESI *m/z* [M + H]⁺ calcd for C₂₆H₂₈FN₆O₂S: 507.1973, found: 507.1972.

7-(Cyclohexyl(methyl)amino)-6-((4-(4-fluorophenyl)thiazol-2-yl)amino)-N,N-dimethyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**36**)

Following general procedure E starting from **30f** (80 mg, 0.2 mmol) and 2-bromo-1-(4-fluorophenyl) ethanone (52 mg, 0.24 mmol) yielded a dark-yellow powder (84 mg, 81 %); mp: 163–165 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ = 13.02 (s, 1H), 9.85 (s, 1H), 8.65 (s, 1H), 8.16 (dd, *J* = 8.4 Hz, 5.7 Hz, 2H), 7.59 (s, 1H), 7.49 (s, 1H), 7.28 (t, *J* = 8.7 Hz, 2H), 3.00 (s, 3H), 2.91 (s, 3H), 2.77–2.70 (m, 1H), 2.66 (s, 3H), 1.90–1.86 (m, 2H), 1.71–1.68 (m, 2H), 1.55–1.52 (m, 1H), 1.29–0.98 ppm (m, 5H); ¹³C NMR (150 MHz, DMSO-*d*₆): δ = 165.24, 162.12, 161.71 (d, ¹*J*_{C–F} = 243.4 Hz), 153.22, 151.21, 149.04, 140.22, 137.36, 130.87 (d, ⁴*J*_{C–F} = 2.6 Hz), 130.75, 128.09 (d, ³*J*_{C–F} = 8.0 Hz), 126.14, 123.31, 115.37 (d, ²*J*_{C–F} = 21.3 Hz), 104.87, 101.37, 62.07, 38.49, 36.94,

33.74, 29.16, 25.50, 24.99 ppm; HRMS-ESI m/z [$M + H$]⁺ calcd for C₂₇H₃₀FN₆O₂S: 521.2130, found: 521.2128.

N-methyl-7-(methyl(phenylamino)-3-oxo-6-((4-phenylthiazol-2-yl)amino)-3,4-dihydroquinoxaline-2-carboxamide (**37**)

Following general procedure E starting from **30g** (76 mg, 0.2 mmol) and 2-bromo-1-phenylethanone (48 mg, 0.24 mmol) yielded an orange powder (79 mg, 82 %); mp: 171–173 °C; ¹H NMR (300 MHz, DMSO-*d*6): δ = 13.31 (s, 1H), 10.28 (s, 1H), 9.06 (q, *J* = 4.8 Hz, 1H), 8.95 (s, 1H), 8.14 (d, *J* = 7.5 Hz, 2H), 7.57 (s, 1H), 7.51–7.46 (m, 3H), 7.36 (t, *J* = 7.5 Hz, 1H), 7.19 (t, *J* = 7.8 Hz, 2H), 6.76 (t, *J* = 7.5 Hz, 1H), 6.66 (d, *J* = 8.1 Hz, 2H), 3.17 (s, 3H), 2.82 ppm (d, *J* = 4.8 Hz, 3H); ¹³C NMR (150 MHz, DMSO-*d*6): δ = 163.27, 162.03, 154.99, 149.96, 149.47, 146.00, 141.81, 134.60, 134.17, 132.80, 128.75, 128.62, 128.17, 127.63, 126.84, 126.09, 117.89, 114.20, 105.77, 101.52, 39.43, 25.73 ppm; HRMS-ESI m/z [$M + H$]⁺ calcd for C₂₆H₂₃N₆O₂S: 483.1598, found: 483.1597.

N,N-dimethyl-7-(methyl(phenylamino)-3-oxo-6-((4-phenylthiazol-2-yl)amino)-3,4-dihydroquinoxaline-2-carboxamide (**38**)

Following general procedure E starting from **30h** (79 mg, 0.2 mmol) and 2-bromo-1-phenylethanone (48 mg, 0.24 mmol) yielded a dark-yellow powder (73 mg, 74 %); mp: 165–167 °C; ¹H NMR (300 MHz, DMSO-*d*6): δ = 13.17 (s, 1H), 10.19 (s, 1H), 8.94 (s, 1H), 8.13 (d, *J* = 7.5 Hz, 2H), 7.51–7.46 (m, 4H), 7.35 (t, *J* = 7.2 Hz, 1H), 7.18 (t, *J* = 7.8 Hz, 2H), 6.75 (t, *J* = 7.2 Hz, 1H), 6.66 (d, *J* = 8.1 Hz, 2H), 3.17 (s, 3H), 2.98 (s, 3H), 2.90 ppm (s, 3H); ¹³C NMR (150 MHz, DMSO-*d*6): δ = 165.06, 162.16, 153.34, 151.59, 149.89, 149.45, 141.08, 134.21, 133.92, 132.30, 128.76, 128.60, 127.60, 127.50, 126.57, 126.09, 117.74, 114.06, 105.46, 102.18, 36.92, 33.72 ppm; HRMS-ESI m/z [$M + H$]⁺ calcd for C₂₇H₂₅N₆O₂S: 497.1754, found: 497.1752.

N-methyl-7-(methyl(phenylamino)-6-((4-(naphthalen-1-yl)thiazol-2-yl)amino)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**39**)

Following general procedure E starting from **30g** (76 mg, 0.2 mmol) and 2-bromo-1-(naphthalen-1-yl)ethanone (60 mg, 0.24 mmol) yielded a dark-yellow powder (78 mg, 73 %); mp: 282–284 °C; ¹H NMR (300 MHz, DMSO-*d*6): δ = 13.00 (s, 1H), 10.28 (s, 1H), 9.03 (q, *J* = 4.8 Hz, 1H), 8.75 (s, 1H), 8.41–8.38 (m, 1H), 8.03–7.94 (m, 3H), 7.64 (t, *J* = 7.5 Hz, 1H), 7.60–7.54 (m, 3H), 7.29 (s, 1H), 7.21 (t, *J* = 7.8 Hz, 2H), 6.77 (t, *J* = 7.2 Hz, 1H), 6.69 (d, *J* = 8.1 Hz, 2H), 3.20 (s, 3H), 2.79 ppm (d, *J* = 4.8 Hz, 3H); ¹³C NMR (150 MHz,

DMSO-*d*6): δ = 163.22, 161.85, 154.78, 149.47, 149.09, 145.92, 141.95, 134.63, 133.52, 132.77, 132.74, 130.66, 128.77, 128.32, 128.18, 127.49, 126.85, 126.49, 125.87, 125.55, 125.46, 117.91, 114.23, 109.96, 101.53, 39.45, 25.70 ppm; HRMS-ESI m/z [$M + H$]⁺ calcd for C₃₀H₂₅N₆O₂S: 533.1754, found: 533.1756.

N,N-dimethyl-7-(methyl(phenylamino)-6-((4-(naphthalen-1-yl)thiazol-2-yl)amino)-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**40**)

Following general procedure E starting from **30h** (79 mg, 0.2 mmol) and 2-bromo-1-(naphthalen-1-yl)ethanone (60 mg, 0.24 mmol) yielded a dark-yellow powder (81 mg, 74 %); mp: 162–164 °C; ¹H NMR (300 MHz, DMSO-*d*6): δ = 12.85 (s, 1H), 10.19 (s, 1H), 8.73 (s, 1H), 8.42–8.39 (m, 1H), 8.03–7.93 (m, 3H), 7.64 (t, *J* = 7.5 Hz, 1H), 7.60–7.54 (m, 2H), 7.49 (s, 1H), 7.27 (s, 1H), 7.20 (t, *J* = 7.8 Hz, 2H), 6.76 (t, *J* = 7.2 Hz, 1H), 6.68 (d, *J* = 8.1 Hz, 2H), 3.19 (s, 3H), 2.96 (s, 3H), 2.86 ppm (s, 3H); ¹³C NMR (150 MHz, DMSO-*d*6): δ = 165.03, 162.00, 153.10, 151.60, 149.43, 149.07, 141.20, 133.96, 133.51, 132.77, 132.28, 130.65, 128.77, 128.31, 128.15, 127.50, 127.47, 126.57, 126.48, 125.85, 125.54, 125.48, 117.76, 114.08, 109.64, 102.20, 39.41, 36.89, 33.70 ppm; HRMS-ESI m/z [$M + H$]⁺ calcd for C₃₁H₂₇N₆O₂S: 547.1911, found: 547.1908.

7-(Cyclohexyl(methylamino)-*N,N*-dimethyl-3-oxo-6-((4-phenylthiazol-2-yl)amino)-3,4-dihydroquinoxaline-2-carboxamide (**41**)

Following general procedure E starting from **30f** (80 mg, 0.2 mmol) and 2-bromo-1-phenylethanone (48 mg, 0.24 mmol) yielded an orange powder (86 mg, 86 %); mp: 165–167 °C; ¹H NMR (300 MHz, DMSO-*d*6): δ = 13.02 (s, 1H), 9.84 (s, 1H), 8.65 (s, 1H), 8.11 (d, *J* = 7.2 Hz, 2H), 7.60 (s, 1H), 7.51 (s, 1H), 7.47 (t, *J* = 7.8 Hz, 2H), 7.35 (t, *J* = 7.5 Hz, 1H), 3.00 (s, 3H), 2.91 (s, 3H), 2.78–2.71 (m, 1H), 2.67 (s, 3H), 1.91–1.87 (m, 2H), 1.72–1.68 (m, 2H), 1.55–1.52 (m, 1H), 1.33–1.03 ppm (m, 5H); ¹³C NMR (150 MHz, DMSO-*d*6): δ = 165.23, 161.96, 153.21, 151.18, 150.13, 140.22, 137.31, 134.21, 130.77, 128.58, 127.62, 126.11, 126.04, 123.28, 105.10, 101.33, 62.06, 38.46, 36.92, 33.73, 29.15, 25.49, 24.98 ppm; HRMS-ESI m/z [$M + H$]⁺ calcd for C₂₇H₃₁N₆O₂S: 503.2224, found: 503.2223.

7-(Cyclohexyl(methylamino)-6-((4-(2-fluorophenyl)thiazol-2-yl)amino)-*N,N*-dimethyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**42**)

Following general procedure E starting from **30f** (80 mg, 0.2 mmol) and 2-bromo-1-(2-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded a dark-yellow powder (76 mg, 73 %);

mp: 153–155 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*6): δ = 13.02 (s, 1H), 9.89 (s, 1H), 8.61 (s, 1H), 8.49–8.43 (m, 1H), 7.60 (s, 1H), 7.42–7.28 (m, 4H), 3.00 (s, 3H), 2.91 (s, 3H), 2.77–2.70 (m, 1H), 2.67 (s, 3H), 1.91–1.87 (m, 2H), 1.72–1.68 (m, 2H), 1.55–1.52 (m, 1H), 1.33–1.02 ppm (m, 5H); $^{13}\text{C NMR}$ (150 MHz, DMSO-*d*6): δ = 165.21, 161.40, 159.60 (d, $^1J_{\text{C-F}}$ = 247.4 Hz), 153.20, 151.27, 143.67, 140.14, 137.42, 130.70, 130.42 (d, $^4J_{\text{C-F}}$ = 2.4 Hz), 129.14 (d, $^3J_{\text{C-F}}$ = 8.4 Hz), 126.18, 124.83, 123.30, 121.74 (d, $^3J_{\text{C-F}}$ = 10.5 Hz), 115.80 (d, $^2J_{\text{C-F}}$ = 22.2 Hz), 109.91 (d, $^2J_{\text{C-F}}$ = 15.9 Hz), 101.41, 62.05, 38.40, 36.92, 33.73, 29.13, 25.49, 24.99 ppm; HRMS-ESI m/z [M + H] $^+$ calcd for C₂₇H₃₀FN₆O₂S: 521.2129, found: 521.2131.

7-(Cyclohexyl(methyl)amino)-6-((4-(3-fluorophenyl)thiazol-2-yl)amino)-*N,N*-dimethyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**43**)

Following general procedure E starting from **30f** (80 mg, 0.2 mmol) and 2-bromo-1-(3-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded a dark-yellow powder (88 mg, 85 %); mp: 155–157 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*6): δ = 13.05 (s, 1H), 9.88 (s, 1H), 8.63 (s, 1H), 7.97–7.89 (m, 2H), 7.63 (s, 1H), 7.60 (s, 1H), 7.54–7.47 (m, 1H), 7.17 (td, J = 8.7 Hz, 2.1 Hz, 1H), 3.00 (s, 3H), 2.91 (s, 3H), 2.77–2.70 (m, 1H), 2.66 (s, 3H), 1.91–1.87 (m, 2H), 1.72–1.68 (m, 2H), 1.55–1.52 (m, 1H), 1.32–1.02 ppm (m, 5H); HRMS-ESI m/z [M + H] $^+$ calcd for C₂₇H₃₀FN₆O₂S: 521.2129, found: 521.2130.

7-(Cyclohexyl(methyl)amino)-6-((4-(3,5-difluorophenyl)thiazol-2-yl)amino)-*N,N*-dimethyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**44**)

Following general procedure E starting from **30f** (80 mg, 0.2 mmol) and 2-bromo-1-(3,5-difluorophenyl)ethanone (56 mg, 0.24 mmol) yielded a dark-yellow powder (87 mg, 81 %); mp: 261–263 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*6): δ = 13.08 (s, 1H), 9.92 (s, 1H), 8.61 (s, 1H), 7.81 (dd, J = 8.7 Hz, 1.8 Hz, 2H), 7.75 (s, 1H), 7.60 (s, 1H), 7.20 (tt, J = 9.3 Hz, 1.8 Hz, 1H), 3.00 (s, 3H), 2.91 (s, 3H), 2.77–2.70 (m, 1H), 2.66 (s, 3H), 1.90–1.87 (m, 2H), 1.71–1.68 (m, 2H), 1.55–1.51 (m, 1H), 1.32–1.02 ppm (m, 5H); $^{13}\text{C NMR}$ (150 MHz, DMSO-*d*6): δ = 165.21, 162.80 (dd, $^{1,3}J_{\text{C-F}}$ = 243.4 Hz, 13.6 Hz), 162.31, 153.16, 151.34, 147.83, 140.01, 137.71 (t, $^3J_{\text{C-F}}$ = 10.2 Hz), 137.46, 130.67, 126.24, 123.28, 108.88 (dd, $^{2,4}J_{\text{C-F}}$ = 20.7 Hz, 5.2 Hz), 108.12, 102.78 (t, $^2J_{\text{C-F}}$ = 25.8 Hz), 101.58, 62.05, 38.40, 36.92, 33.73, 29.11, 25.49, 25.00 ppm; HRMS-ESI m/z [M + H] $^+$ calcd for C₂₇H₂₉F₂N₆O₂S: 539.2035, found: 539.2034.

6-((4-(3-Chloro-4-fluorophenyl)thiazol-2-yl)amino)-7-(cyclohexyl(methyl)amino)-*N,N*-dimethyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**45**)

Following general procedure E starting from **30f** (80 mg, 0.2 mmol) and 2-bromo-1-(3-chloro-4-fluorophenyl)ethanone (60 mg, 0.24 mmol) yielded a dark-yellow powder (78 mg, 70 %); mp: 164–166 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*6): δ = 13.03 (s, 1H), 9.89 (s, 1H), 8.60 (s, 1H), 8.24–7.97 (m, 2H), 7.64 (s, 1H), 7.59 (s, 1H), 7.49 (t, J = 9.0 Hz, 1H), 3.00 (s, 3H), 2.91 (s, 3H), 2.77–2.70 (m, 1H), 2.66 (s, 3H), 1.90–1.86 (m, 2H), 1.71–1.68 (m, 2H), 1.55–1.52 (m, 1H), 1.32–1.02 ppm (m, 5H); $^{13}\text{C NMR}$ (150 MHz, DMSO-*d*6): δ = 165.21, 162.35, 156.61 (d, $^1J_{\text{C-F}}$ = 245.8 Hz), 153.16, 151.30, 147.68, 140.08, 137.45, 132.16 (d, $^4J_{\text{C-F}}$ = 3.2 Hz), 130.68, 127.50, 126.82 (d, $^3J_{\text{C-F}}$ = 7.0 Hz), 126.22, 123.26, 119.85 (d, $^2J_{\text{C-F}}$ = 17.7 Hz), 117.00 (d, $^2J_{\text{C-F}}$ = 20.8 Hz), 106.41, 101.47, 62.03, 38.38, 36.92, 33.73, 29.12, 25.49, 25.00 ppm; HRMS-ESI m/z [M + H] $^+$ calcd for C₂₇H₂₉ClFN₆O₂S: 555.1740, found: 555.1741.

7-(Cyclohexyl(methyl)amino)-6-((4-(2,4-dichloro-5-fluorophenyl)thiazol-2-yl)amino)-*N,N*-dimethyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**46**)

Following general procedure E starting from **30f** (80 mg, 0.2 mmol) and 2-bromo-1-(2,4-dichloro-5-fluorophenyl)ethanone (69 mg, 0.24 mmol) yielded a dark-yellow powder (102 mg, 87 %); mp: 157–159 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*6): δ = 12.96 (s, 1H), 9.92 (s, 1H), 8.47 (s, 1H), 8.17 (d, J = 10.5 Hz, 1H), 7.93 (d, J = 6.9 Hz, 1H), 7.76 (s, 1H), 7.58 (s, 1H), 3.00 (s, 3H), 2.90 (s, 3H), 2.76–2.70 (m, 1H), 2.66 (s, 3H), 1.90–1.86 (m, 2H), 1.71–1.68 (m, 2H), 1.55–1.52 (m, 1H), 1.33–1.02 ppm (m, 5H); $^{13}\text{C NMR}$ (150 MHz, DMSO-*d*6): δ = 165.18, 161.46, 156.14 (d, $^1J_{\text{C-F}}$ = 245.2 Hz), 153.09, 151.39, 144.13, 139.95, 137.59, 133.15 (d, $^3J_{\text{C-F}}$ = 7.2 Hz), 131.76, 130.57, 126.30, 126.13 (d, $^4J_{\text{C-F}}$ = 2.6 Hz), 123.20, 119.14 (d, $^2J_{\text{C-F}}$ = 19.2 Hz), 118.70 (d, $^2J_{\text{C-F}}$ = 23.7 Hz), 112.18, 101.58, 61.98, 38.20, 36.91, 33.72, 29.06, 25.50, 25.02 ppm; HRMS-ESI m/z [M + H] $^+$ calcd for C₂₇H₂₈Cl₂FN₆O₂S: 589.1350, found: 589.1349.

7-(Cyclohexyl(methyl)amino)-*N,N*-dimethyl-3-oxo-6-((4-(2,4,6-trifluorophenyl)thiazol-2-yl)amino)-3,4-dihydroquinoxaline-2-carboxamide (**47**)

Following general procedure E starting from **30f** (80 mg, 0.2 mmol) and 2-bromo-1-(2,4,6-trifluorophenyl)ethanone (61 mg, 0.24 mmol) yielded a dark-yellow powder (90 mg, 81 %); mp: 234–235 °C; $^1\text{H NMR}$ (300 MHz, DMSO-*d*6): δ = 12.70 (s, 1H), 9.84 (s, 1H), 8.37 (s, 1H), 7.58 (s, 1H), 7.33 (t, J = 8.7 Hz, 2H), 7.29 (s, 1H), 2.98 (s, 3H), 2.88 (s, 3H), 2.78–2.71 (m, 1H), 2.66 (s, 3H), 1.90–1.86 (m, 2H),

1.72–1.68 (m, 2H), 1.56–1.52 (m, 1H), 1.33–1.03 ppm (m, 5H); HRMS-ESI m/z $[M + H]^+$ calcd for $C_{27}H_{28}F_3N_6O_2S$: 557.1941, found: 557.1939.

6-((4-(4-Cyanophenyl)thiazol-2-yl)amino)-7-(cyclohexyl(methyl)amino)-*N,N*-dimethyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**48**)

Following general procedure E starting from **30f** (80 mg, 0.2 mmol) and 4-(2-bromoacetyl)benzotrile (54 mg, 0.24 mmol) yielded an orange powder (80 mg, 76%); mp: 249–251 °C; 1H NMR (300 MHz, DMSO-*d*₆): δ = 13.07 (s, 1H), 9.98 (s, 1H), 8.68 (s, 1H), 8.31 (d, J = 8.7 Hz, 2H), 7.92 (d, J = 8.7 Hz, 2H), 7.81 (s, 1H), 7.60 (s, 1H), 3.00 (s, 3H), 2.91 (s, 3H), 2.77–2.70 (m, 1H), 2.66 (s, 3H), 1.91–1.87 (m, 2H), 1.71–1.68 (m, 2H), 1.55–1.52 (m, 1H), 1.32–1.02 ppm (m, 5H); ^{13}C NMR (150 MHz, DMSO-*d*₆): δ = 165.20, 162.41, 153.21, 151.34, 148.27, 140.10, 138.33, 137.45, 132.60, 130.67, 126.64, 126.22, 123.35, 119.06, 109.67, 109.07, 101.57, 62.08, 38.51, 36.93, 33.73, 29.13, 25.50, 24.99 ppm; HRMS-ESI m/z $[M + H]^+$ calcd for $C_{28}H_{30}N_7O_2S$: 528.2176, found: 528.2175.

Ethyl 6-amino-7-(cyclohexyl(methyl)amino)-4-methyl-3-oxo-3,4-dihydroquinoxaline-2-carboxylate (**49**)

To a stirred solution of intermediate **5d** (2.75 g, 8.0 mmol) in acetone (150 mL) was added K_2CO_3 (1.66 g, 12 mmol) and methyl iodide (0.60 mL, 9.6 mmol), and the mixture was stirred at 50 °C for 6 h. The suspension was cooled and filtered. Then, the filtrate was concentrated under reduced pressure. The residue was dissolved in saturated ammonium chloride solution (100 mL) and extracted by CH_2Cl_2 (2 × 100 mL). The organic phases were combined, dried over anhydrous Na_2SO_4 , and evaporated in vacuo to give a dark-yellow solid residue, which was purified by silica gel column chromatography (CH_2Cl_2 /MeOH, 80:1) to give the title compound as a dark-yellow powder (2.21 g, 77%); mp: 187–188 °C; 1H NMR (300 MHz, DMSO-*d*₆): δ = 7.32 (s, 1H), 6.62 (s, 1H), 6.20 (s, 2H), 4.28 (q, J = 7.2 Hz, 2H), 3.50 (s, 3H), 2.78–2.71 (m, 1H), 2.59 (s, 3H), 1.79–1.70 (m, 4H), 1.56–1.52 (m, 1H), 1.40–1.05 ppm (m, 8H); HRMS-ESI m/z $[M + H]^+$ calcd for $C_{19}H_{27}N_4O_3$: 359.2078, found: 359.2077.

6-Amino-7-(cyclohexyl(methyl)amino)-*N,N*,4-trimethyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**50**)

Following general procedure G starting from carboxylic acid type of **49** (991 mg, 3.0 mmol) yielded a yellow powder (890 mg, 83%); mp: 215–216 °C; 1H NMR (300 MHz, DMSO-*d*₆): δ = 7.29 (s, 1H), 6.66 (s, 1H), 5.91 (s, 2H), 3.51 (s, 3H), 2.97 (s, 3H), 2.84 (s, 3H), 2.78–2.71 (m, 1H), 2.58 (s,

3H), 1.79–1.70 (m, 4H), 1.56–1.52 (m, 1H), 1.40–1.07 ppm (m, 5H); HRMS-ESI m/z $[M + H]^+$ calcd for $C_{19}H_{28}N_5O_2$: 358.2238, found: 358.2238.

7-(Cyclohexyl(methyl)amino)-*N,N*,4-trimethyl-3-oxo-6-thioureido-3,4-dihydroquinoxaline-2-carboxamide (**52**)

Following general procedure D starting from **50** (714 mg, 2.0 mmol) yielded a dark-yellow powder (632 mg, 76% over two steps); mp: 192–194 °C; 1H NMR (300 MHz, DMSO-*d*₆): δ = 9.32 (s, 1H), 8.76 (s, 1H), 8.15 (s, 2H), 7.56 (s, 1H), 3.58 (s, 3H), 2.99 (s, 3H), 2.87 (s, 3H), 2.73–2.67 (m, 1H), 2.65 (s, 3H), 1.83–1.70 (m, 4H), 1.56–1.53 (m, 1H), 1.36–1.02 ppm (m, 5H); HRMS-ESI m/z $[M + H]^+$ calcd for $C_{20}H_{29}N_6O_2S$: 417.2067, found: 417.2066.

7-(Cyclohexyl(methyl)amino)-6-((4-(4-fluorophenyl)thiazol-2-yl)amino)-*N,N*,4-trimethyl-3-oxo-3,4-dihydroquinoxaline-2-carboxamide (**53**)

Following general procedure E starting from **52** (83 mg, 0.2 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (52 mg, 0.24 mmol) yielded a dark-yellow powder (90 mg, 84%); mp: 223–224 °C; 1H NMR (300 MHz, DMSO-*d*₆): δ = 10.02 (s, 1H), 8.97 (s, 1H), 8.03 (dd, J = 8.7 Hz, 5.7 Hz, 2H), 7.65 (s, 1H), 7.51 (s, 1H), 7.31 (t, J = 8.7 Hz, 2H), 3.73 (s, 3H), 3.01 (s, 3H), 2.89 (s, 3H), 2.81–2.74 (m, 1H), 2.68 (s, 3H), 1.92–1.88 (m, 2H), 1.72–1.68 (m, 2H), 1.55–1.52 (m, 1H), 1.34–1.03 ppm (m, 5H); ^{13}C NMR (150 MHz, DMSO-*d*₆): δ = 165.16, 162.33, 161.66 (d, $^1J_{C-F}$ = 243.4 Hz), 152.38, 149.76, 148.59, 140.46, 137.27, 131.78, 130.96, 127.51 (d, $^3J_{C-F}$ = 8.0 Hz), 126.46, 124.05, 115.63 (d, $^2J_{C-F}$ = 21.4 Hz), 105.12, 101.15, 61.94, 38.36, 36.94, 33.72, 29.10, 28.92, 25.50, 24.99 ppm; HRMS-ESI m/z $[M + H]^+$ calcd for $C_{28}H_{32}FN_6O_2S$: 535.2286, found: 535.2286.

Biology

HCV antiviral assay The Huh7-ET (luc-ubi-neo/ET) cell line which harbors a dicistronic self-replicating HCV RNA replicon with a firefly luciferase gene was used for antiviral evaluation [51]. The activity of the luciferase reporter is directly proportional to HCV RNA levels. Briefly, the ET cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 2 mM L-glutamine, nonessential amino acids, 10% fetal bovine serum (FBS), and 0.25 mg/mL G418 (Invitrogen, Carlsbad, CA). The cells were seeded in two identical sets of 96-well plates used for analysis of antiviral activity or cell numbers (cytotoxicity) at a density of 5000/well in 100 μ L of DMEM without G418 overnight. Serial dilutions of compounds solubilized in DMSO were prepared in DMEM and then added to the appropriate wells. After 72 h of incubation, one set of the cells was processed

to assess the replicon-derived luciferase activity with the Steady-Glo luciferase assay system (Promega, Madison, WI) according to manufacturer's instruction. Another set of the plates was used to determine cytotoxic effect of the compounds using a tetrazolium-based CytoTox-1 cell proliferation assay (Promega, Madison WI). Each data point represents an average of four replicates to derive EC₅₀ (concentration of compound to inhibit HCV RNA replication activity by 50%), CC₅₀ (concentration of compound to decrease cell viability by 50%), and SI₅₀ (selective index calculated as the CC₅₀/EC₅₀ ratio) values.

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