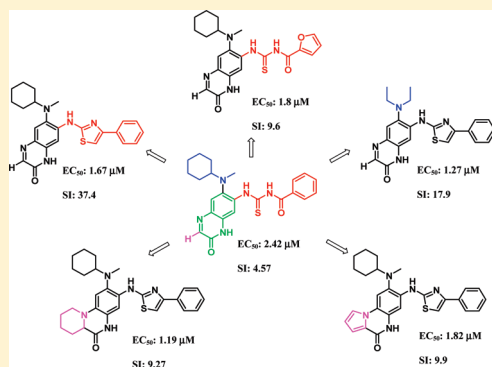


Quinoxalin-2(1H)-One Derivatives As Inhibitors Against Hepatitis C Virus

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Supporting Information

ABSTRACT: Hepatitis C virus (HCV) infection is a serious problem worldwide, but no effective drugs are currently available. Through screening of our privileged structure library, quinoxalin-2(1H)-one derivative *N*-(7-(cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamothioyl)benzamide (compound **1**) was identified as potent HCV inhibitor in vitro. Subsequently, a structure–activity relationship analysis was carried out that showed *N*-(7-(cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamothioyl)furan-2-carboxamide (compound **11**, EC₅₀ = 1.8 μM, SI = 9.6), 6-(cyclohexyl(methyl)amino)-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (compound **33**, EC₅₀ = 1.67 μM, SI = 37.4), 2-(cyclohexyl(methyl)amino)-3-(4-phenylthiazol-2-ylamino)-7,8,9,10-tetrahydro-5H-pyrido[1,2-*a*]quinoxalin-6(6*a*H)-one (compound **60**, EC₅₀ = 1.19 μM, SI = 9.27), 8-(cyclohexyl(methyl)amino)-7-(4-phenylthiazol-2-ylamino)pyrrolo[1,2-*a*]quinoxalin-4(5H)-one (compound **65**, EC₅₀ = 1.82 μM, SI = 9.9), and 6-(diethylamino)-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (compound **78**, EC₅₀ = 1.27 μM, SI = 17.9) acted against HCV. The data from the structure–activity relationship study suggests that quinoxalin-2(1H)-one derivatives exhibited potent activity against HCV.



INTRODUCTION

Hepatitis C virus (HCV) belongs to the *flaviviridae* family and is a positive single-stranded RNA virus.¹ It was first identified in 1989 as the pathogen responsible for non-A and non-B hepatitis.² Currently, HCV infection is a significant health problem worldwide. An estimated 3% of the world's population (i.e., more than 170 million people) is infected by HCV, with 3–4 million new cases being reported each year.³ Approximately 60–80% of these infections lead to the chronic form of hepatitis C virus that may lead to fibrosis, cirrhosis, and hepatocellular carcinoma. Unfortunately, there is no vaccine or specific antiviral drugs available against HCV. Interferon-based therapy was the main strategy for treatment of this disease for more than two decades.⁴ Treatment with interferon α or PEG-interferon α , either alone or in combination with a broad spectrum antiviral ribavirin, is the current standard of care. However, this treatment regimen is only effective for 40–60% of people infected with HCV genotype-1, which accounts for the majority of infections in the U.S., Europe, and Asia.^{5,6} Meanwhile, serious adverse effects, such as depression and flu-like symptoms, also limit its application. Recently, much effort has been devoted toward developing drugs that inhibit viral replication. The most promising agents under development are viral protease and polymerase inhibitors.

Several small-molecule inhibitors of NS3/4A protease are currently in preclinical or clinical studies, such as ITMN-191, telaprevir, and boceprevir^a (Figure 1).⁷ HCV NSSB polymerase is another attractive target for antiviral therapy. The inhibitors of NSSB polymerase include nucleoside (such as NM-283 and R7128) and nonnucleoside polymerase inhibitors (such as PF-00868554 and VCH-916) (Figure 1).⁸ However, the emergence of drug resistance is the major limitation for anti-HCV therapies targeted at viral protease or viral polymerase.⁹ Therefore, there is an urgent need to develop new classes of antiviral agents for the treatment of HCV infection.

Antiviral screening of our privileged structure library was carried out, and compound **1**, *N*-(7-(cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamothioyl)benzamide (Figure 2), was identified as a potent HCV inhibitor in vitro. Quinoxalinone is an important subunit in drugs with many biological activities, such as antitumor, antimicrobial, and antithrombotic functions.¹⁰ However, as one type of small molecular heterocyclic compound, quinoxalin-2(1H)-one has not been reported for its anti-HCV activity. As the compound

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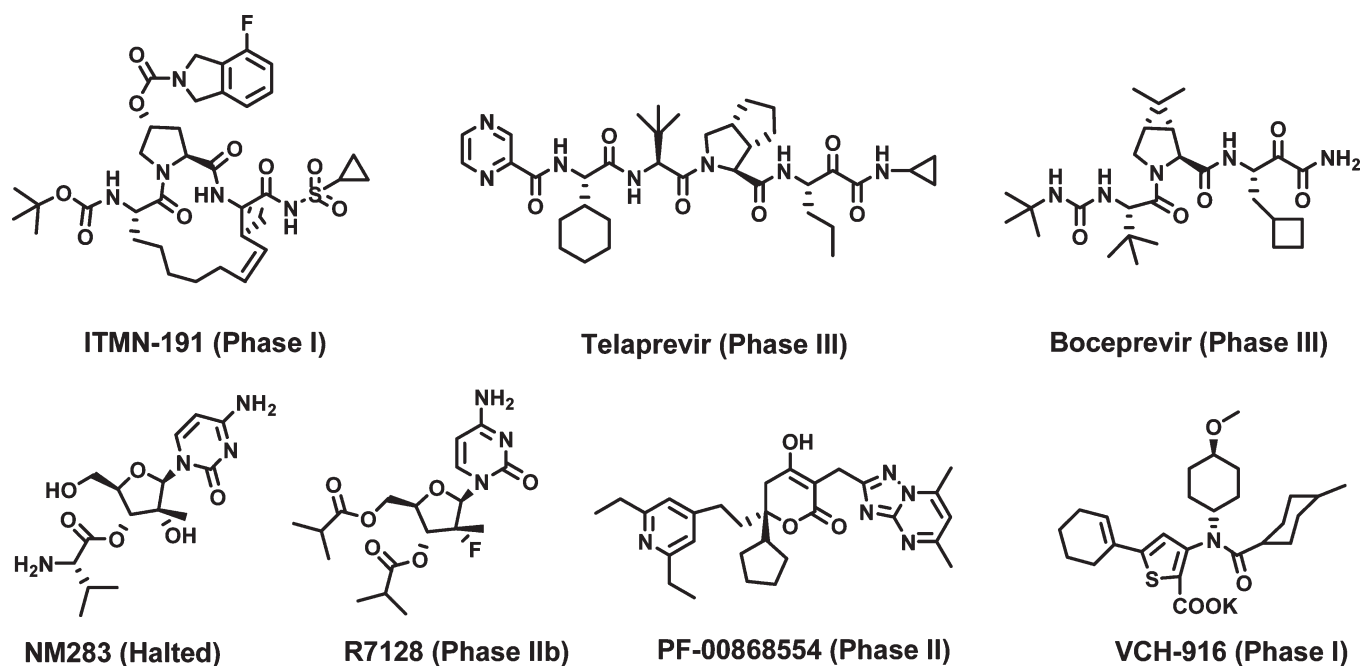


Figure 1. Candidate drugs under development for treatment of hepatitis C virus infection.

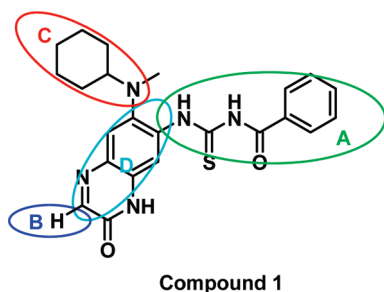


Figure 2. Structure of compound 1.

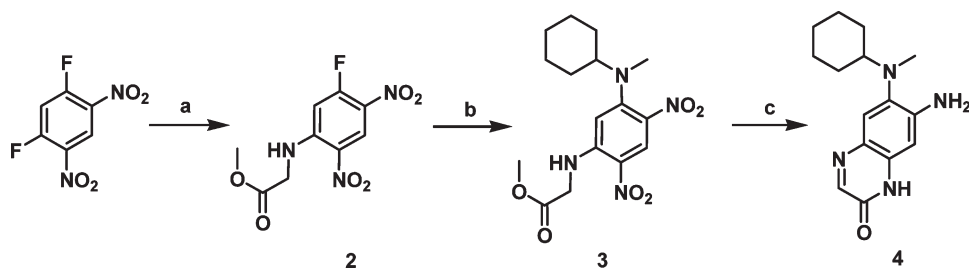
contains a unique quinoxalin-2(1*H*)-one scaffold amenable to chemical modification, we carried out a structure–activity relationship (SAR) investigation of compound 1 with the goal to develop compounds with improved efficacy against HCV and lower cytotoxicity properties. The results are reported in this article.

RESULTS AND DISCUSSION

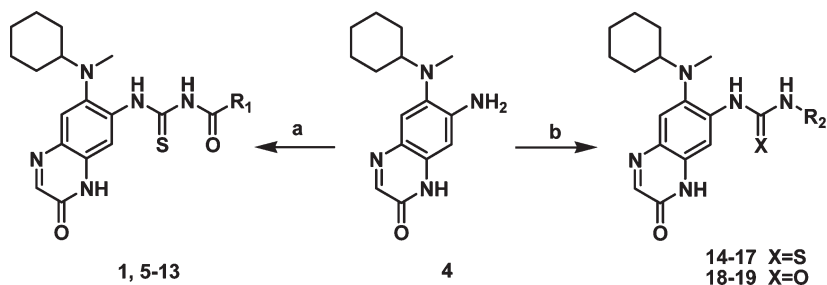
Chemistry. To facilitate the synthesis and subsequent SAR analysis, compound 1 was characterized from the four major segments indicated in Figure 2. To modify segment A, the core structure 4 was synthesized according to our previous report (Scheme 1).¹¹ In the presence of diisopropylethylamine (DIPEA), two fluoro atoms of 1,5-difluoro-2,4-dinitrobenzene (DFDNB) were substituted by an equivalent methyl 2-aminoacetate hydrochlorate and subsequently *N*-methylcyclohexanamine to give compounds 2 and 3. After reduction of the two nitro groups using 10% palladium on activated carbon, followed by a self-cyclization, the key intermediate 4 was obtained in total yield of 68%.

Segment A Modifications (Schemes 2–5). To investigate the medicinal potential, a series of compounds with modifications

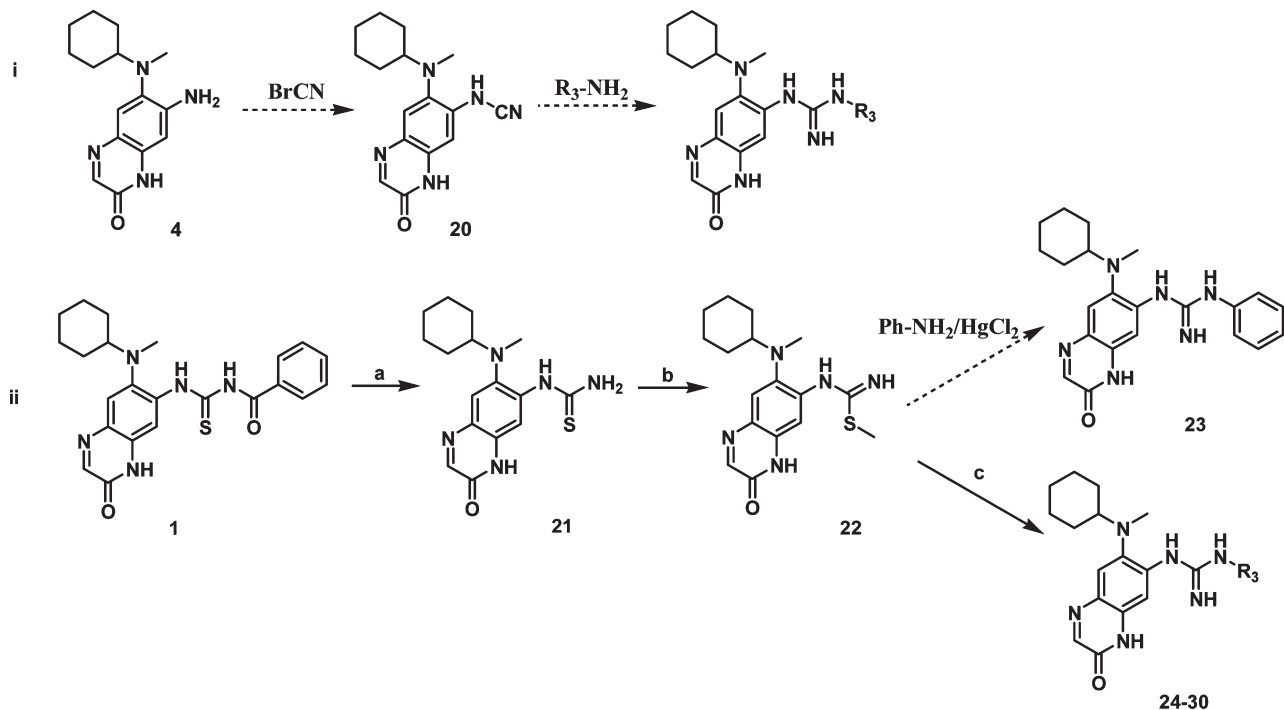
of segment A were considered from its three pharmacophores, including phenyl, thiourea, and acyl groups. To examine the effect of the phenyl group for antiviral activity, compound 4 was reacted with various acyl isothiocyanates with different R_1 groups, including electron-withdrawing, electron-donating, and large aromatic hydrocarbon groups, to provide compounds 5–13 (Scheme 2). The selection of various acyl isothiocyanates was further considered, including halogenations, aromatic heterocycles, and double bonds, for their substituent influence on the benzoyl group of segment A. Second, the acyl thiourea was replaced by thiourea (compounds 14–17) or urea (compounds 18–19) through the reaction of 4 with isothiocyanates or isocyanates to simplify the acyl thiourea group. The guanidino group, a classic bioisostere of the thiourea group, is an important pharmacophore in many drugs. Therefore, the acyl thiourea group was replaced by a guanidino group mimicking thiourea and urea. As illustrated in Scheme 3i, the key intermediate cyanamide 20 was predicted to form from the reaction of cyanogen bromide with compound 4. However, the reactivity of the 7-amino group of compound 4 was too weak to obtain the target compound, so the alternative Scheme 3ii was designed and was successfully performed. Compound 1 was first converted into the free thiourea compound 21 in the presence of K_2CO_3 . The reaction of 21 with CH_3I in ethanol afforded the methylated compound 22 that offered a route to compounds 24–30 by refluxing 22 with primary amines in pyridine.¹² However, when intermediate 22 was reacted with aniline, compound 23 did not form, even in the presence of $HgCl_2$.¹³ A thiohydantoin group was introduced to block the NH group H-bonding donor of the thiourea moiety and to fix the steric configuration. As shown in Scheme 4i, from the reaction of compound 14 with 2-chloroacetyl chloride in the presence of Et_3N , a compound with four possible structures, 31, 31', 31'', or 31''', was obtained.¹⁴ From the 1H NMR spectra obtained, the two H atoms of the methylene group of thiohydantoin showed geminal coupling 4.11 (d, 1H, $J = 17.4$ Hz) and 4.19 (d, 1H, $J = 17.4$ Hz), indicating that the

Scheme 1^a

^a Reagents and conditions used: (a) $\text{NH}_2\text{CH}_2\text{COOCH}_3 \cdot \text{HCl}$, DIPEA, THF, room temperature; (b) *N*-methylcyclohexylamine, DIPEA, THF, reflux; (c) 10% Pd/C, NH_4COOH , THF/ $\text{CH}_3\text{CH}_2\text{OH}$, room temperature.

Scheme 2^a

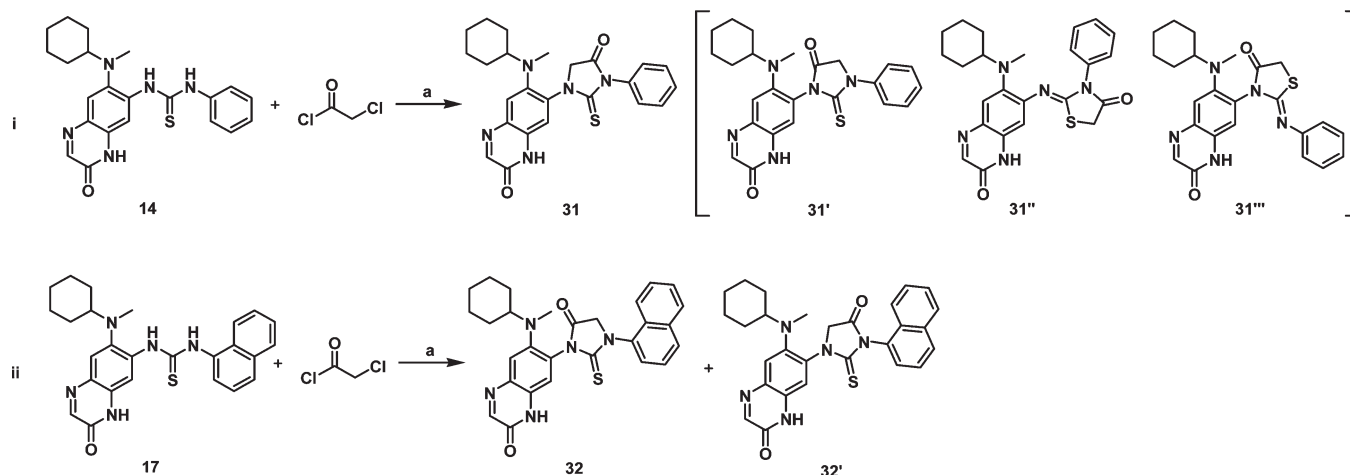
^a Reagents and conditions used: (a) R_1CONCS , acetone, reflux; (b) R_2NCX , THF, reflux.

Scheme 3^a

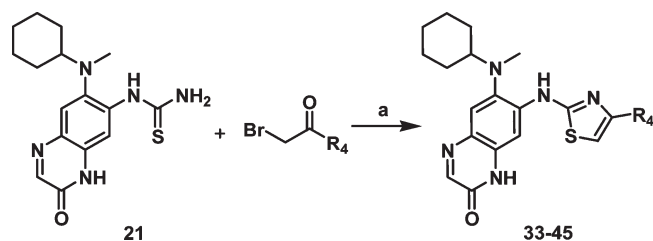
^a Reagents and conditions used: (a) K_2CO_3 , $\text{CH}_3\text{OH}/\text{H}_2\text{O}$, 75 °C; (b) CH_3I , $\text{CH}_3\text{CH}_2\text{OH}$, 50 °C; (c) $\text{R}_3\text{-NH}_2$, pyridine, reflux.

methylene group of the thiohydantoin was adjacent to a stereo 7-*N* atom. This observation excluded the possibility of compounds **31''** and **31'''**. Further structural identification using

nuclear Overhauser effect spectroscopy (NOESY) revealed that the methylene group correlated with 6-*N*-methylcyclohexylamine, but not with a phenyl group (Figure 3, **31**, the NOESY is

Scheme 4^a

^a Reagents and conditions used: (a) Et₃N, 1,4-dioxane, reflux.

Scheme 5^a

^a Reagents and conditions used: (a) CH₃CH₂OH, reflux.

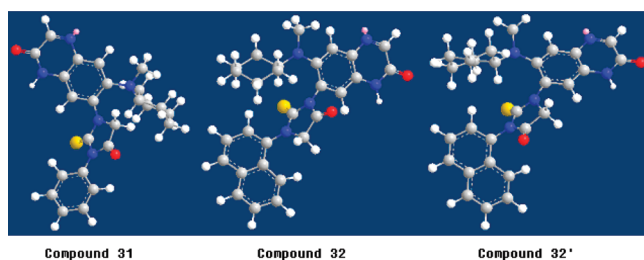


Figure 3. Three dimensional structures of compounds 31, 32, and 32' plotted using the Chem3D software package.

shown in the Supporting Information). Taking all this information together, the final compound was determined to be compound 31. However, in the reaction of compound 17 with 2-chloroacetyl chloride, two final products were obtained in yields of 59% (32) and 34% (32') (Scheme 4ii). Interestingly, NOE data was not indicated in NOESY experiments on 32 and 32'. The difference in the ¹H NMR spectra arises from the chemical shift of the methylene group of thiohydantoin 32 4.31 (d, 1H, *J* = 17.1 Hz) and 4.51 (d, 1H, *J* = 17.1 Hz), which is shifted to a lower field compared to 32' 4.14 (d, 1H, *J* = 17.1 Hz) and 4.23 (d, 1H, *J* = 17.1 Hz). It is thought that the naphthalene group deshields the methylene group of thiohydantoin 32, and so it is concluded that the methylene group of compound 32, but not compound 32', is closed to the naphthalene group (Figure 3).

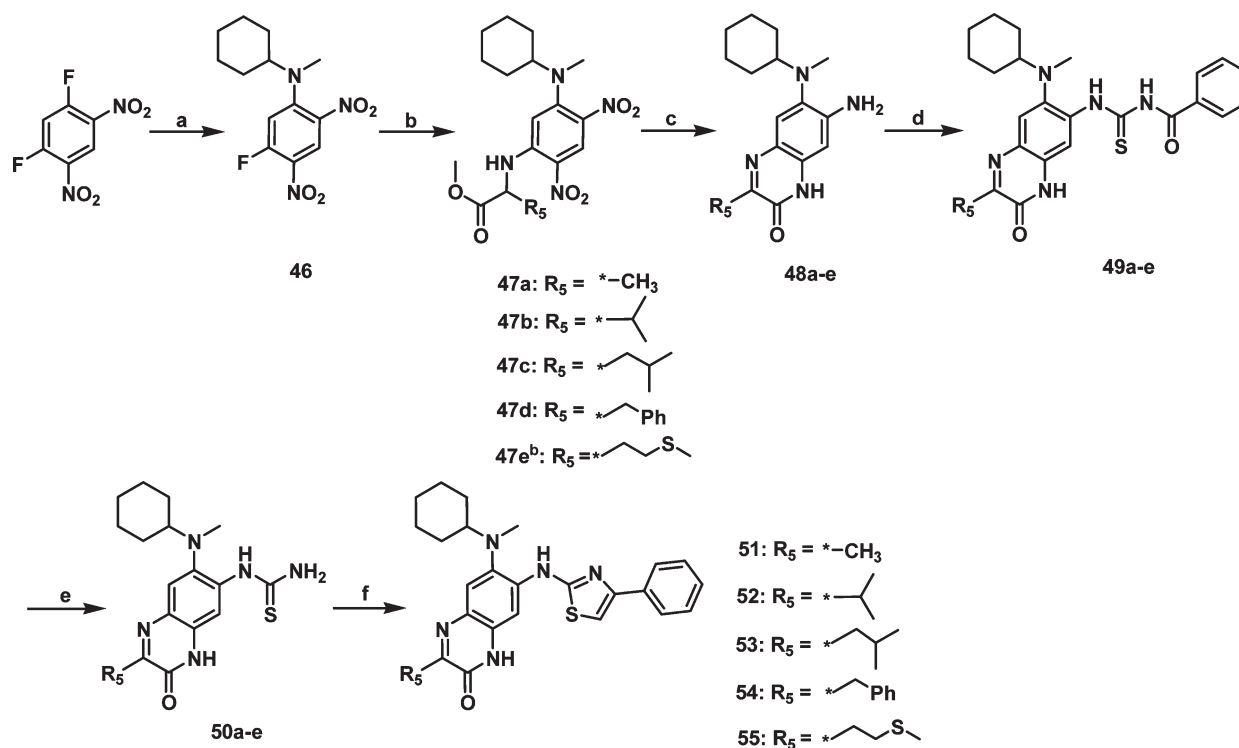
The thiocarbonyl group is a reactive group that can have potential adverse effects on patients who take the drug over a long period of time. Therefore, we further cyclized it into a thiazole moiety. Compounds 33–45 were prepared from the reaction of 21 with various commercially available α-bromo substituted ketones that offer thiazole analogues of compound 1 (Scheme 5).

Segment B Modifications (Schemes 6–8). Segment B was studied because it is located directly in the quinoxalin-2(1*H*)-one core structure. Alkyl, aryl, or thioether groups were introduced to examine the effect of steric hindrance, π–π interaction, and the change in hydrophobicity (compounds 51–55, Scheme 6). Intermediates 48a–48d were synthesized according to a procedure similar to Scheme 1, where the R₅ group was introduced using different amino acid alkyl esters. The difference was that compound 48e was obtained from a reduction reaction via SnCl₂/HCl. Accordingly, 49a–49e were obtained after reaction of benzoyl isothiocyanate, which further afforded free thiourea compounds 50a–50e after treatment with K₂CO₃. On cyclization of 50a–50e with 2-bromo-1-phenylethanone, compounds 51–55 were obtained.

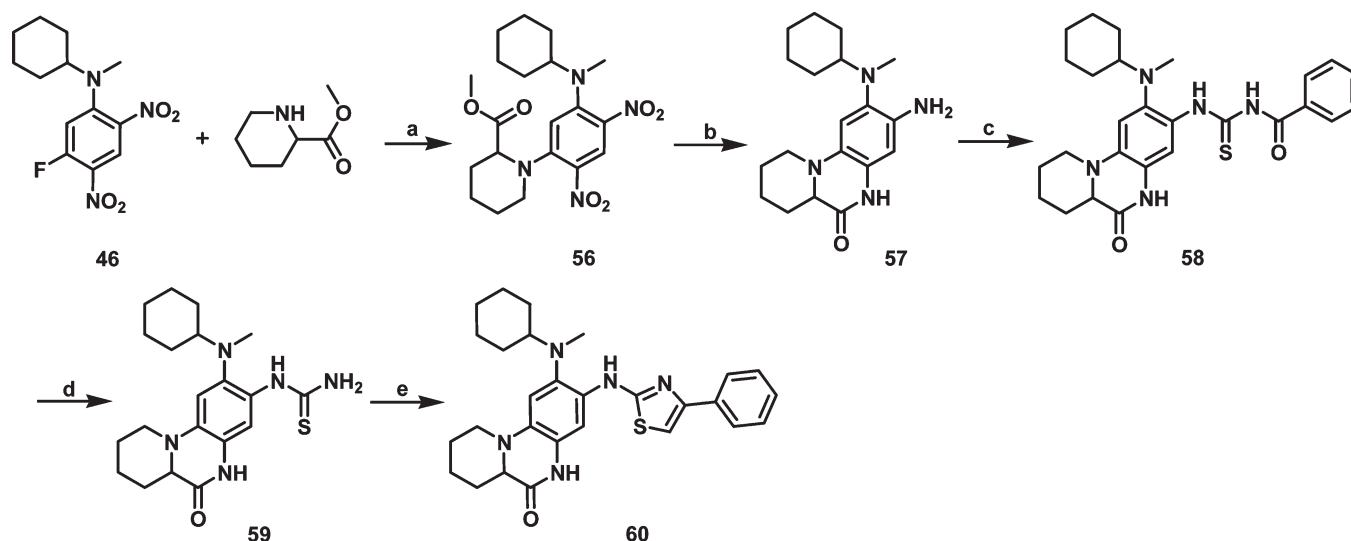
Notably, two tricyclic compounds 60 and 65 were constructed (Schemes 7 and 8), which provided a large hydrophobic region and a mimic of the 3,4-double bond of segment B. As illustrated in Scheme 7, starting from 46, methyl piperidine-2-carboxylate was used to prepare the disubstituted compound 56, which was subsequently reduced via catalytic hydrogenation to obtain compound 57. Condensation of 57 with benzoyl isothiocyanate was followed by hydrolysis of the resulting compound 58 and subsequent cyclization of 59 with 2-bromo-1-phenylethanone to give the desired compound 60.

While attempting to prepare the anticipated compound 65', intermediate 62' could not be obtained. Further investigation showed that 62' was converted into 62 under the experimental conditions used,¹⁵ which allowed the synthesis of compound 65 (Scheme 8).

Segment C Modifications (Scheme 9). The significance of the *N*-methylcyclohexanamine moiety for anti-HCV activity was examined using segment C. The role of any electronic effect was explained by introducing an *N*-methylbenzenamine group (70). Steric hindrance and the necessity of the *N*-methyl moiety on the

Scheme 6^a

^a Reagents and conditions used: (a) *N*-methylcyclohexanamine, DIPEA, THF, room temperature; (b) $R_5CH(NH_2)COOCH_3 \cdot HCl$, DIPEA, THF, reflux; (c) 10% Pd/C, NH_4COOH , THF/ CH_3CH_2OH , room temperature; (d) PhCONCS, acetone, reflux; (e) K_2CO_3 , CH_3OH/H_2O , 75 °C; (f) PhCOCH₂Br, CH_3CH_2OH , reflux. ^b Reagents and conditions used: (c) $SnCl_2 \cdot 2H_2O$, 36% HCl, CH_3CH_2OH , reflux.

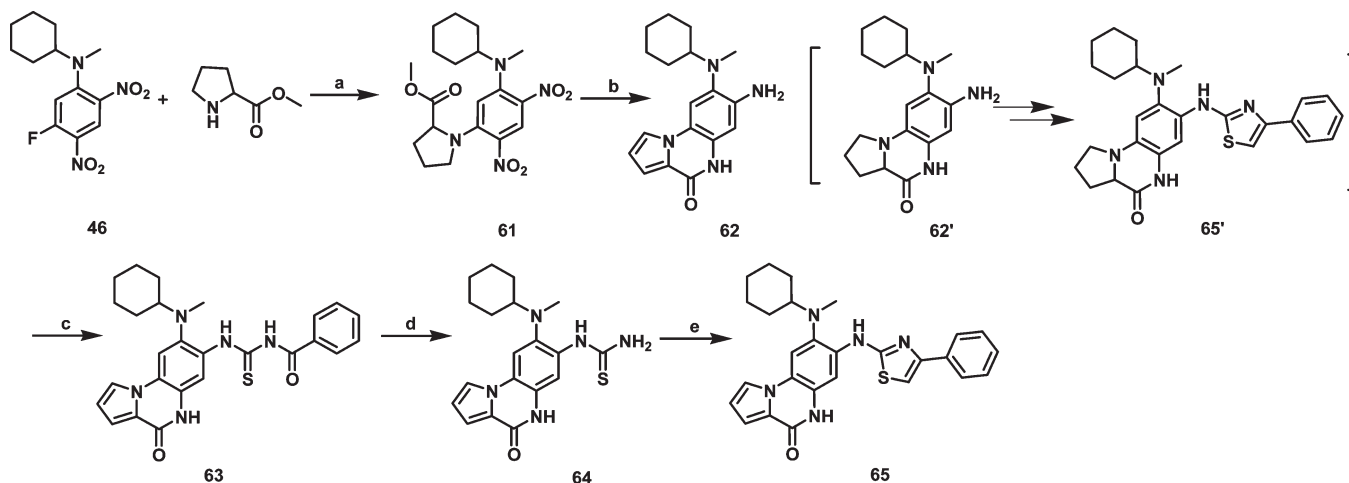
Scheme 7^a

^a Reagents and conditions used: (a) DIPEA, THF, reflux; (b) 10% Pd/C, NH_4COOH , THF/ CH_3CH_2OH , room temperature; (c) PhCONCS, acetone, reflux; (d) K_2CO_3 , CH_3OH/H_2O , 75 °C; (e) PhCOCH₂Br, CH_3CH_2OH , reflux.

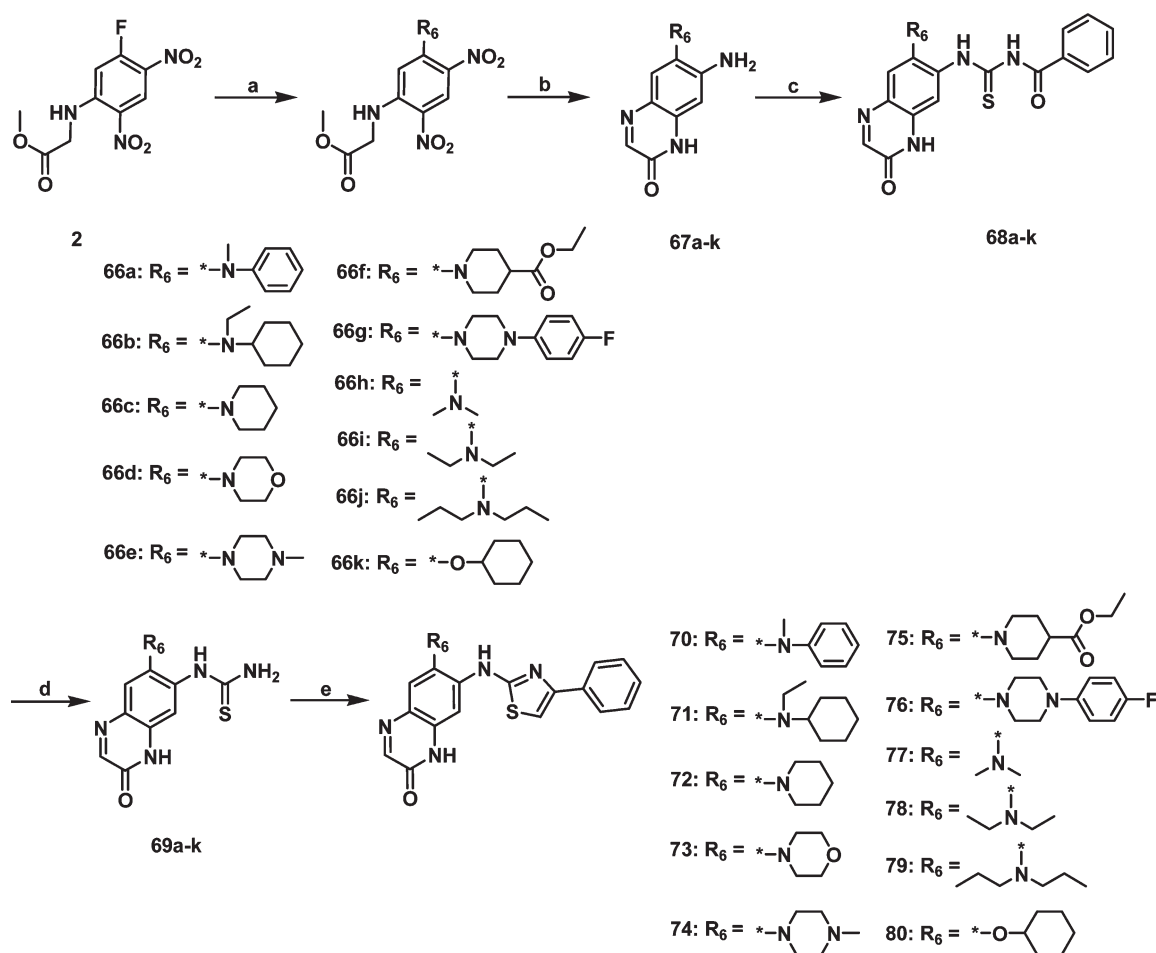
N-methylcyclohexanamine group were investigated by introducing an *N*-ethylcyclohexanamine group (71). Considering the drug-like properties of the entire molecule, the *N*-methylcyclohexanamine group was replaced by various six-member rings, such as piperidine, morpholine, and 1-methylpiperazine (72–76). Mimicking

N-cyclohexanamine using a chain alkyl amine was also investigated (77–79). When the N atom was replaced by an O atom, a substituent of cyclohexanol could be introduced (80).

As shown in Scheme 9, the reaction of methyl-2-(5-fluoro-2,4-dinitrophenylamino)acetate (2) with various commercially

Scheme 8^a

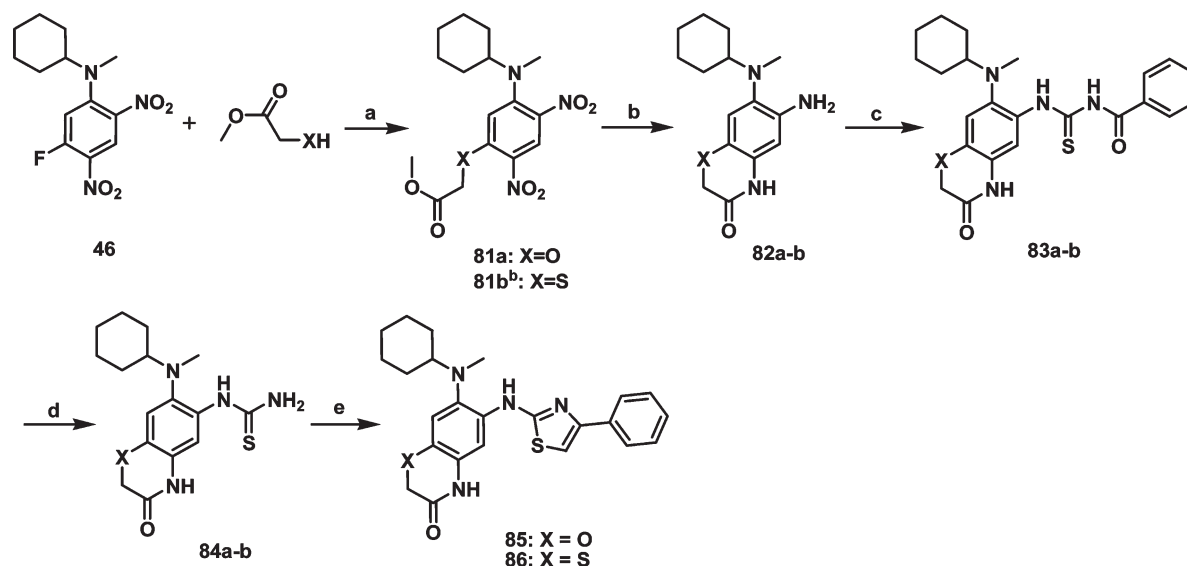
^a Reagents and conditions used: (a) DIPEA, THF, reflux; (b) 10% Pd/C, cyclohexene, reflux; (c) PhCONCS, acetone, reflux; (d) K₂CO₃, CH₃OH/H₂O, 75 °C; (e) PhCOCH₂Br, CH₃CH₂OH, reflux.

Scheme 9^a

^a Reagents and conditions used: (a) secondary amine or sodium cyclohexanolate, DIPEA, THF, reflux; (b) 10% Pd/C, NH₄COOH, THF/CH₃CH₂OH, room temperature; (c) PhCONCS, acetone, reflux; (d) K₂CO₃, CH₃OH/H₂O, 75 °C; (e) PhCOCH₂Br, CH₃CH₂OH, reflux.

available secondary amines or sodium alcoholate in the presence of a base gave the corresponding intermediates **66a–66k**,

followed by a reduction and then self-cyclization to afford the key structures **67a–67k**. Similar to the previous schemes shown,

Scheme 10^a

^a Reagents and conditions used: (a) HOCH₂COOCH₃ or HSCH₂COOCH₃, DIPEA, THF, reflux; (b) 10% Pd/C, NH₄COOH, THF/CH₃CH₂OH, room temperature; (c) PhCONCS, acetone, reflux; (d) K₂CO₃, CH₃OH/H₂O, 75 °C; (e) PhCOCH₂Br, CH₃CH₂OH, reflux. ^b Reagents and conditions used: (b) SnCl₂·2H₂O, 36% HCl, CH₃CH₂OH, reflux.

67a–67k were condensed with benzoyl isothiocyanate, followed by hydrolysis and subsequent cyclization with 2-bromo-1-phenylethanone, to prepare the target compounds 70–80.

Segment D Modifications (Scheme 10). When we fixed the substituent on segments A–C, the role of the quinoxalin-2(1H)-one core structure was then elucidated by replacing it with other structures. In the presence of DIPEA in THF, the intermediates 81a and 81b were prepared by coupling 46 with methyl 2-hydroxyacetate or methyl 2-mercaptoacetate, respectively. The reduction of the nitro groups of 81a and 81b with Pd/C or SnCl₂, and then self-cyclization, gave compounds 82a and 82b, respectively. The target compounds 85 and 86 were obtained following reaction steps c–e in Scheme 9.

Anti-HCV Activity in Vitro. All the synthesized compounds were evaluated for anti-HCV activity and cytotoxicity in the HCV RNA replicon assay in Huh 7 ET cells, as described in a previous publication.¹⁶ The results are summarized in Tables 1–8. Briefly, the concentration of compound inhibiting HCV RNA replication activity by 50% (EC₅₀), the concentration of compound decreasing cell viability by 50% (IC₅₀), and the selective index (SI) is presented.

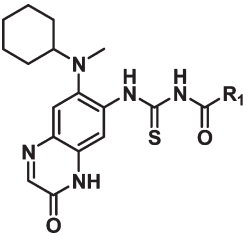
Our initial effort to outline the SAR values of segment A focused on the replacement of substituents of the phenyl group (Table 1). On replacement of the phenyl group by a 4-fluorophenyl group (5, EC₅₀ = 2.97 μM), the potency against the HCV replicon was retained, compared to compound 1. Similar results were observed for a 4-CF₃O-phenyl group (6) with an EC₅₀ value of 2.33 μM. In the case of a 3-CF₃-phenyl group (7), a significant reduction in cytotoxicity was observed, however, anti-HCV activity was lost. Introduction of an electron-donating substituent (CH₃O–, 8) or π–π interactions (9 and 10) also decreased the antiviral activity. Mimicking the phenyl group using different heterocycles gave variable results. The furan analogue 11 exhibited the most potent antiviral activity in the series, with an EC₅₀ value of 1.8 μM and low cytotoxicity (17.28 μM), resulting in a selectivity index approaching 10-fold (SI = 9.6).

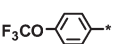
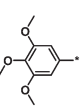
Other mimicking analogues 12 and 13 did not show an improved antiviral inhibitory potency compared to compound 1. None of the other compounds had a significant selection index.

Further modifications of segment A are summarized in Tables 2–5. Removal of the carbonyl group from 1 gave the thiourea analogue 14, which showed a greatly reduced activity against HCV. Introduction of the electron-withdrawing group 4-fluoro-phenyl 15, 3-CF₃-phenyl 16, or large aryl group naphthyl 17 also resulted in inactive compounds (Table 2). These results imply that simplifying the acyl thiourea moiety into a thiourea group is not appropriate for anti-HCV activity. Changing the thiourea moiety to a bioisostere urea group gave compounds 18 and 19, which had a higher toxicity to the virus host cells. The effect on the anti-HCV activity of the guanidino group is shown in Table 3. Compound 24, which changed the benzoylthiourea group to a benzylguanidino group, exhibited an EC₅₀ value of 11.44 μM. Furthermore, neither the introduction of an electron-withdrawing group (F[–] 25, Cl[–] 26, CF₃[–] 27, and CF₃O[–] 28) nor an electron-donating group (CH₃O[–] 29) provided an obvious potency or improvement in the selectivity index. A pyridine mimic of the phenyl moiety was also disadvantageous (30). All of these suboptimal results suggest that the introduction of a urea moiety or an H-bond donor (i.e., the NH group of the guanidino group) contributed a reduction in potency. Introduction of a thiohydantoin group (31 and 32) was also unfavorable for the antiviral potency (Table 4).

Cyclization of the thiourea moiety to a thiazole ring provided compound 33, which had a significantly reduced cytotoxicity compared to compound 1 (Table 5, 33, EC₅₀ = 1.67 μM, IC₅₀ = 62.47 μM, SI = 37.4). Various groups were tested to identify the optimal substituent for the R₄ group. The 4-fluoro-phenyl substitutive compound 34 had an EC₅₀ value of 5.26 μM, and the 4-chloro-phenyl substitutive compound 35 (EC₅₀ = 5.39 μM) had a reduced antiviral activity and selection index. These results show that halogen substituents for R₄ may be harmful to the anti-HCV potency. The same trend was observed for a cyano group,

Table 1. Inhibitory Effects of Quinoxalin-2(1H)-one Derivatives on HCV Replication in Huh 7 Cells (Modification of the Phenyl Group)



Compound	R ₁	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity IC ₅₀ (μM)	SI ^a
1		2.42	11.05	4.57
5		2.97	11.38	3.8
6		2.33	6.47	2.78
7		4.08	31.51	7.5
8		5.16	15.89	3.1
9		6.77	10.96	1.6
10		5.48	9.98	1.82
11		1.8	17.28	9.6
12		3.27	12.56	3.84
13		16.4	34.49	2.1
rIFNα-2b		0.06 IU/mL	> 2 IU/mL	> 33.3

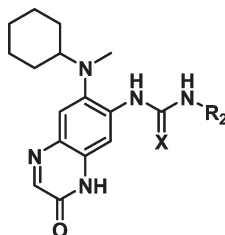
^a SI calculated as IC₅₀/EC₅₀.

which is the bioisostere of the chloro atom (36, EC₅₀ = 6.07 μM). Adding electron density to the phenyl group by the addition of an electron-donating moiety yielded compounds 37 and 38. Both of these compounds were devoid of antiviral activity. Furthermore, the replacement of the phenyl group of 33 with a naphthyl moiety resulted in 39, which exhibited comparable cytotoxicity but had a lower selectivity index than compound 33. The biphenyl analogue 40 had no measurable potency against HCV but with lower cell toxicity. We hypothesized that the larger aryl substituent may reduce the inhibitory activity by increasing the π - π interactions between the compound and the target. Therefore, the compounds were tested with further modification of the phenyl group into its bioisosteres, such as furan 41, thiophene 42, and pyridine 43.

None of these analogues showed an improved potency. The necessity of a phenyl group in R₄ was further supported by changing it into a CF₃ group, which also led to a loss in anti-HCV activity (44). Fixation of the steric configuration between the thiazole group and the phenyl moiety on 33 by a methylene group yielded compound 45, which exhibited a marked cytotoxicity. All of the data in Table 5 supports the conclusion that the thiazole-phenyl moiety was favorable for anti-HCV potency as well as having lower cytotoxicity.

Next, modifications of segment B (R₅) were investigated in detail (Table 6) when segment A was maintained as the thiazole-phenyl moiety. In principle, lower alkyl groups can improve the liposolubility of small molecules and block

Table 2. Inhibitory Effects of Quinoxalin-2(1*H*)-one Derivatives on HCV Replication in Huh 7 Cells (Replacement of the Aromatic Acyl Thiourea Group by a Thiourea and Urea Group)



Compound	X	R ₂	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity IC ₅₀ (μM)	SI ^a
14	S		> 20	49.56	<2.8
15	S		> 20	64.31	<3.2
16	S		> 20	78.43	<3.9
17	S		> 20	43.64	<2.2
18	O		11.38	5.52	0.5
19	O		9.84	5.33	0.5
rIFNα-2b			0.06 IU/mL	> 2 IU/mL	>33.3

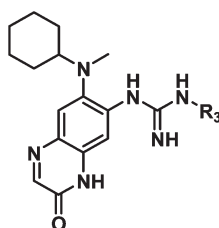
^a SI calculated as IC₅₀/EC₅₀.

metabolism sites. However, methyl (**51**), isopropyl (**52**), and isobutyl (**53**) group R₅ substituents demonstrated lower in vitro potency than an H atom did (**33**). The replacement of the R₅ group with an aromatic group (benzyl, **54**) also made the compound inactive. Similarly, introducing a thioether group on R₅ also completely removed the activity (compound **55**). Cyclization at the 3- and 4- positions of **33** with a six-member ring yielded compound **60**, which improved the anti-HCV activity and had a good selectivity index (EC₅₀ = 1.19 μM, IC₅₀ = 11.03 μM). Another tricyclic compound **65** also had a good antiviral potency and an acceptable cytotoxicity (EC₅₀ = 1.82 μM, IC₅₀ = 18.01 μM). From multiple modifications and the corresponding anti-HCV results, we concluded that substitution of the R₅ group may be prevented by steric limitations, but the 3,4-double bond could be mimicked by a cycloalkyl group.

In an effort to obtain an increased potency, we studied the effect of R₆ substitutions (segment C, Table 7). Compounds **70–80** were initially assessed at a single concentration of 20 μM. All potentially active or cytotoxic compounds in the primary assay were then evaluated further in a dose–response assay. Replacement of R₆ with *N*-methylbenzenamine (**70**) reduced the compound's activity. We proposed that introducing π–π interactions from an aromatic group may be harmful for the anti-HCV

potency. Changing the *N*-methylcyclohexanamine group of compound **33** to *N*-ethylcyclohexanamine provided compound **71**, which showed no activity in the initial single dose screening. This may be because of steric hindrance at this position. Moving the N atom of the R₆ substituent to various heterocyclic aliphatics also did not improve the activity against HCV. For example, compound **72**, with a piperidine substitution on R₆, showed only a 9% inhibition under the test conditions. Introducing an ethyl ester (**75**) to compound **72** did not significantly change the activity of the compound. Morpholine substitution (**73**) resulted in a cytotoxicity value (IC₅₀) of 58.32 μM but resulted in a near 3-fold loss of inhibitory activity against HCV compared with compound **33**. Moreover, introduction of piperazine analogues yielded a lower activity of **74** and an inactive **76** under the test conditions. An alternative strategy to modify R₆ with a chain alkyl amine gave different results. Replacement of R₆ with dimethylamine (**77**) led to a complete loss of anti-HCV activity. It is interesting to note that similar antiviral potency but higher cytotoxicity of compound **78** (EC₅₀ = 1.27 μM, IC₅₀ = 22.78 μM, SI = 17.9) with a diethylamine group at R₆ was achieved compared to the R₆ with *N*-methylcyclohexanamine of compound **33**. However, further extension of the alkyl chain of a dipropylamine group (**79**) was disadvantageous with EC₅₀ = 3.81 μM. Taking all

Table 3. Inhibitory Effects of Quinoxalin-2(1H)-one Derivatives on HCV Replication in Huh 7 Cells (Replacement of the Aromatic Acyl Thiourea Group by a Guanidino Group)



Compound	R ₃	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity IC ₅₀ (μM)	SI ^a
24		11.44	9.94	0.87
25		12.75	10.22	0.8
26		3.94	4.4	1.12
27		> 20	53.98	<2.7
28		3.97	3.70	0.9
29		> 20	19.54	<0.98
30		> 20	48.62	<2.4
rIFNα-2b		0.08 IU/mL	> 2 IU/mL	> 25

^a SI calculated as IC₅₀/EC₅₀.

these results into account, mimicking the *N*-methylcyclohexanamine group at the R₆ position with a proper length alkyl chain and the steric conformation of a lipophilic group may be promising for improved anti-HCV activity. Further investigation of substitution of the N atom at the R₆ position was also carried out. Replacement of R₆ with cyclohexanol (**80**) resulted in a loss of activity.

Finally, two mimics of the quinoxalin-2(1H)-one core structure were synthesized and evaluated for their anti-HCV activity (segment D, Table 8). Changing the N atom at the 4- position of compound **33** by its bioisostere O or S atom gave compounds **85** and **86**, respectively. However, both of these compounds had no potency against HCV. As a result, quinoxalin-2(1H)-one was considered as the optimal core structure for anti-HCV activity.

CONCLUSIONS

This study has identified new quinoxalin-2(1H)-one derivatives as inhibitors of HCV. Compound **1** was first identified for its anti-HCV activity by screening a library of compounds using the HCV replicon and cytotoxicity assays. A series of modifications of compound **1** were carried out in an effort to improve anti-HCV

potency and decrease cytotoxic effects. Five compounds, **11**, **33**, **60**, **65**, and **78**, showed high antiviral potency and an acceptable selectivity index, with EC₅₀ = 1.8 μM and SI = 9.6, EC₅₀ = 1.67 μM and SI = 37.4, EC₅₀ = 1.19 μM and SI = 9.27, EC₅₀ = 1.82 μM and SI = 9.9 μM, and EC₅₀ = 1.27 μM and SI = 17.9, respectively.

To elucidate the action mechanism of the compound, biochemical assays were carried out essentially as previously described.¹⁷ The results indicated that the compound **33** did not exhibit any inhibitory effects on HCV protease, helicase, and NSSB polymerase (data not shown herein). It was concluded that **33** are acting on other virus-encoded and/or cellular targets that need to be further investigated.

In conclusion, quinoxalin-2(1H)-one with a thiazole-phenyl moiety is optimal for anti-HCV activity; there may be a steric limitation at 3- position of quinoxalin-2(1H)-one, however, the 3,4-double bond was mimicked by a cycloalkyl group and a hydrophobic group with proper alkyl chain and steric conformation at 6- position is necessary for the anti-HCV activity. Although the mechanism underlying how these compounds inhibit HCV is unknown, further SAR analysis and mechanistic studies on this class of compounds against HCV are currently being investigated.

Table 4. Inhibitory Effects of Quinoxalin-2(1*H*)-one Derivatives on HCV Replication in Huh 7 Cells (Replacement of the Aromatic Acyl Thiourea Group by a Thiohydantoin Group)

Compound	Structure	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity IC ₅₀ (μM)	SI ^a
31		2.21	2.99	1.4
32		13.08	11.15	0.85
rIFNα-2b		0.08 IU/mL	> 2 IU/mL	> 25

^a SI calculated as IC₅₀/EC₅₀.

EXPERIMENTAL SECTION

Chemistry. Unless otherwise noted, all materials were obtained from commercial suppliers and used without purification. All NMR experiments were carried out on a Varian Mercury 300 or 400 or 500 MHz NMR spectrometer using DMSO-*d*₆ as the solvent. 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 × 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 (v/v) 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 LC-MS was carried out by Agilent LC/MSD TOF using a column of Agilent ZORBAX SB-C18 (rapid resolution, 3.5 μm, 2.1 mm × 30 mm) at a flow of 0.40 mL/min. The solvent is methanol/water = 75:25 (v/v) containing 5 mmol/L ammonium formate. The ion source is electrospray ionization (ESI). Flash column chromatography was performed with silica gel 60 (200–300 mesh) from Qingdao Haiyang Chemical Factory. All tested compounds were purified until the purity was ≥95%, detected by HPLC under UV 254 nm wavelength, NMR, melting point, and HRLC-MS.

Preparation of the Key Intermediate Compound 4. To a stirred solution of 1,5-difluoro-2,4-dinitrobenzene (DFDNB, 2.04 g, 10 mmol) in THF (50 mL) was added DIPEA (20 mmol) and NH₂CH₂COOCH₃·HCl (10 mmol). After vigorously stirring at room temperature until the total disappearance of DFDNB, compound 2 was obtained without purification. Then, *N*-methylcyclohexanamine (10 mmol) and DIPEA (10 mmol) were added and stirred under reflux for 8 h. The solvent was removed under reduced pressure to give compound 3, which was used directly. The above two reactions were

traced by a fast LC-MS system until all of the starting material was changed to the anticipated compound. Compound 3 (1 g) was dissolved in a mixed solvent of THF (30 mL) and EtOH (30 mL), followed by the addition of 10% Pd/C (1 g) and HCOONH₄ (2 g). The mixture was stirred at room temperature for 3 h. The residue solid was filtered off, and the filtrate was concentrated in vacuo. Water (50 mL) was added to the resulting product and was further extracted by CH₂Cl₂ (3 × 50 mL). The organic layers were combined, dried over anhydrous MgSO₄, and evaporated in vacuo. Intermediate 4 was obtained as a yellow solid after chromatography through silica gel eluting with CHCl₃–CH₃OH (30:1, v/v). Yield 68%. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.03–1.26 (m, 3H), 1.29–1.37 (m, 2H), 1.54 (m, 1H), 1.68–1.77 (m, 4H), 2.55 (s, 3H), 2.65–2.72 (m, 1H), 5.71 (s, 2H), 6.45 (s, 1H), 7.22 (s, 1H), 7.67 (s, 1H), 11.97 (s, 1H). HRMS calcd for C₁₅H₂₁N₄O (M + H⁺) 273.1710; found 273.1704.

General Procedure for the Synthesis of Compounds 1, 5–13. To a stirred solution of 4 (0.5 mmol) in 20 mL of dried acetone, various aroyl isothiocyanates (0.5 mmol) were added. The reaction mixture was refluxed for 4 h. After the reaction was completed, the solvent was evaporated in vacuo. The final products were characterized after purification by silica gel column chromatography.

***N*-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamoithiyl)benzamide (1).** Yellow powder in 88% yield; mp 190–191 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.00–1.36 (m, 5H), 1.49–1.52 (m, 1H), 1.65–1.69 (m, 2H), 1.76–1.80 (m, 2H), 2.65 (s, 3H), 2.78 (m, 1H), 7.51–7.56 (m, 2H), 7.63–7.69 (m, 2H), 7.95 (d, 2H, *J* = 7.2 Hz), 8.09 (s, 1H), 8.93 (s, 1H), 11.60 (s, 1H), 12.42 (s, 1H), 13.44 (s, 1H). ¹³C NMR (150 MHz, DMSO-*d*₆): δ 24.8, 25.4, 29.1, 37.5, 61.7, 108.2, 122.6, 128.4, 128.6, 129.6, 132.1, 133.1, 137.0, 140.3, 150.6, 154.9, 167.7, 177.6. HRMS calcd for C₂₃H₂₆N₅O₂S (M + H⁺) 436.1801; found 436.1815.

***N*-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamoithiyl)-4-fluorobenzamide (5).** Light-yellow powder in 85% yield; mp 214–215 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.02–1.14 (m, 3H), 1.19–1.33 (m, 2H), 1.50–1.53 (m, 1H), 1.67–1.70 (m, 2H), 1.77–1.81 (m, 2H), 2.65 (s, 3H), 2.75–2.82 (m, 1H), 7.36–7.41 (m, 2H), 7.65 (s, 1H), 8.05–8.10 (m, 3H),

Table 5. Inhibitory Effects of Quinoxalin-2(1H)-one Derivatives on HCV Replication in Huh 7 Cells (Cyclization of the Thiourea Moiety to a Thiazole Ring)

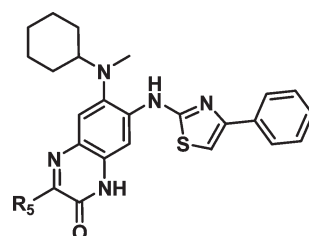
Compound	R ₄	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity IC ₅₀ (μM)	SI ^a
33		1.67	62.47	37.4
34		5.26	9.92	1.9
35		5.39	16.71	3.1
36		6.07	18.10	2.98
37		> 20	15	<0.75
38		> 20	61.17	<3.1
39		6.80	57.44	8.4
40		> 20	82.04	<4.1
41		12.18	11.91	1
42		> 20	71.26	<3.6
43		5.49	11.46	2.09
44	CF ₃	8.12	37.38	4.6
45 ^b		4.57	10.94	2.39
rIFNα-2b		0.08 IU/mL	> 2 IU/mL	> 25

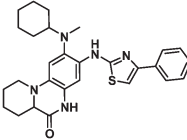
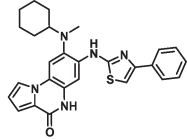
^a SI calculated as IC₅₀/EC₅₀. ^b The structure of compound 45.

8.96 (s, 1H), 11.67 (s, 1H), 12.43 (s, 1H), 13.43 (s, 1H). HRMS calcd for C₂₃H₂₅N₅O₂FS (M + H⁺) 454.1707; found 454.1723.

N-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamothioyl)-4-(trifluoromethoxy)benzamide (6). Yellow powder in 87% yield; mp 176–178 °C. ¹H NMR (300 MHz,

DMSO-*d*₆): δ 0.99–1.33 (m, 5H), 1.47–1.50 (m, 1H), 1.64–1.67 (m, 2H), 1.75–1.79 (m, 2H), 2.63 (s, 3H), 2.77 (m, 1H), 7.50 (d, 2H, *J* = 8.1 Hz), 7.63 (s, 1H), 8.07–8.10 (m, 3H), 8.97 (s, 1H), 11.75 (s, 1H), 12.43 (s, 1H), 13.40 (s, 1H). HRMS calcd for C₂₄H₂₅N₅O₃F₃S (M + H⁺) 520.1624; found 520.1645.

Table 6. Inhibitory Effects of Quinoxalin-2(1*H*)-one Derivatives on HCV Replication in Huh 7 Cells (Modification of Segment B)


Compound	R ₅	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity IC ₅₀ (μM)	SI ^a
51	CH ₃ -*	3.91	12.32	3.15
52		8.62	67.42	7.8
53		4.19	33.28	7.9
54		> 20	41.98	<2.1
55		> 20	85.97	<4.3
60 ^b		1.19	11.03	9.27
65 ^b		1.82	18.01	9.9
rIFNα-2b		0.14 IU/mL	> 2 IU/mL	> 14.3

^a SI calculated as IC₅₀/EC₅₀. ^b The structure of compounds 60 and 65.

N-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamoithiyl)-3-(trifluoromethyl)benzamide (7). Pale-yellow solid in 85% yield; mp 187–189 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.07–1.28 (m, 5H), 1.51 (m, 1H), 1.66–1.77 (m, 4H), 2.64 (s, 3H), 2.78 (m, 1H), 7.65 (s, 1H), 7.78 (m, 1H), 8.00–8.09 (m, 2H), 8.21–8.31 (m, 2H), 8.98 (s, 1H), 11.96 (s, 1H), 12.43 (s, 1H), 13.40 (s, 1H). HRMS calcd for C₂₄H₂₅N₅O₂F₃S (M + H⁺) 504.1687; found 504.1675.

N-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamoithiyl)-3,4,5-trimethoxybenzamide (8). Pale-yellow powder in 74% yield; mp 110–111 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.09–1.32 (m, 5H), 1.52–1.79 (m, 5H), 2.66 (s, 3H), 2.76 (m, 1H), 3.71–3.88 (m, 9H), 7.22 (s, 1H), 7.35 (s, 1H), 7.62 (s, 1H), 8.10 (s, 1H), 8.83 (s, 1H), 11.61 (s, 1H), 12.41 (s, 1H), 13.39 (s, 1H). ¹³C NMR (150 MHz, DMSO-*d*₆): δ 24.9, 25.4, 28.9, 37.1, 55.8, 56.1, 60.1, 61.8, 106.3, 106.5, 108.8, 122.4, 125.8, 126.7, 128.2, 129.6, 136.7, 140.5, 141.6, 150.7, 152.5, 154.8, 166.8, 177.8. HRMS calcd for C₂₆H₃₂N₅O₅S (M + H⁺) 526.2118; found 526.2140.

N-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamoithiyl)-3-phenylacrylamide (9). Light-yellow powder in 89% yield; mp 232–233 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.07–1.34 (m, 5H), 1.53–1.81 (m, 5H), 2.64 (s, 3H), 2.72–2.74 (m, 1H), 7.08 (d, 1H, *J* = 15.9 Hz), 7.46–7.48 (m, 3H), 7.63–7.67 (m, 3H), 7.76 (d, 1H, *J* = 15.9 Hz), 8.08 (d, 1H, *J* = 1.8 Hz), 8.92 (s, 1H), 11.57 (s, 1H), 12.42 (s, 1H), 13.38 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 24.9, 25.4, 29.1, 37.5, 61.9, 108.0, 119.9, 122.7, 128.2, 128.4, 129.1, 130.7, 134.0, 137.0, 140.2, 144.4, 150.6, 154.9, 165.3, 177.5. HRMS calcd for C₂₅H₂₈N₅O₂S (M + H⁺) 462.1958; found 462.1949.

N-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamoithiyl)-1-naphthamide (10). Yellow powder in 80% yield; mp 169–170 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.06–1.21 (m, 3H), 1.33–1.43 (m, 2H), 1.53–1.57 (m, 1H), 1.71–1.75 (m, 2H), 1.82–1.86 (m, 2H), 2.69 (s, 3H), 2.84–2.87 (m, 1H), 7.59–7.66 (m, 4H), 7.80–7.82 (m, 1H), 8.04–8.06 (m, 1H), 8.10–8.15 (m, 3H), 8.96 (s, 1H), 12.03 (s, 1H), 12.45 (s, 1H), 13.49

Table 7. Inhibitory Effects of Quinoxalin-2(1H)-one Derivatives on HCV Replication in Huh 7 Cells (Modification of Segment C)

Compound	R ₆	Anti-HCV activity EC ₅₀ (μM)	Cytotoxicity IC ₅₀ (μM)	SI ^a
70		4.61	15.89	3.4
71 ^b		35%	58%	ND ^c
72 ^b		9%	95.1%	ND ^c
73		5.19	58.32	11.2
74		4.59	10.22	2.2
75 ^b		37.6%	59.6%	ND ^c
76 ^b		35%	84.3%	ND ^c
77		> 20	57.48	<2.9
78		1.27	22.78	17.9
79		3.81	31.63	8.3
80 ^b		4.9%	74.4%	ND ^c
rIFNα-2b		0.14 IU/mL	2 IU/mL	> 14.3

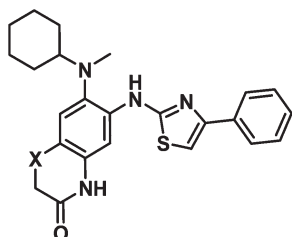
^a SI calculated as IC₅₀/EC₅₀. ^b Compounds assessed at a single-concentration of 20 μM with the percentage donating inhibition level compared to the control cell. ^c ND = not detected.

(s, 1H). HRMS calcd for C₂₇H₂₈N₅O₂S (M + H⁺) 486.1958; found 486.1968.

N-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamothioyl)furan-2-carboxamide (11). Yellow powder in 81% yield; mp 202–204 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.00–1.32 (m, 5H), 1.49–1.52 (m, 1H), 1.65–1.69 (m, 2H), 1.76–1.80 (m, 2H), 2.63 (s, 3H), 2.72–2.80 (m, 1H), 6.75–6.77 (m, 1H), 7.64 (s, 1H), 7.87–7.88 (m, 1H), 8.07–8.09 (m, 2H), 8.96 (s, 1H), 11.28 (s, 1H), 12.42 (s, 1H), 13.29 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 24.3, 24.9, 28.7, 37.4, 61.2, 107.3, 112.1, 118.3, 122.3, 128.0, 129.0, 136.6, 139.6, 144.0, 147.9, 150.1, 154.4, 156.4, 176.6. HRMS calcd for C₂₁H₂₄N₅O₃S (M + H⁺) 426.1594; found 426.1605.

N-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamothioyl)-3-methylthiophene-2-carboxamide (12). Pale-yellow powder in 85% yield; mp 182–184 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.07–1.32 (m, 5H), 1.52–1.78 (m, 5H), 2.51 (s, 3H), 2.63 (s, 3H), 2.76 (m, 1H), 7.07 (d, 1H, *J* = 4.8 Hz), 7.63 (s, 1H), 7.84 (d, 1H, *J* = 4.8 Hz), 8.09 (s, 1H), 8.89 (s, 1H), 11.06 (s, 1H), 12.42 (s, 1H), 13.17 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 15.5, 24.8, 25.4, 29.0, 37.5, 61.7, 108.1, 122.6, 128.4, 128.9, 129.6, 131.1, 131.9, 136.9, 140.3, 143.9, 150.7, 154.9, 162.5, 177.0. HRMS calcd for C₂₂H₂₆N₅O₂S₂ (M + H⁺) 456.1522; found 456.1523.

6-Chloro-N-(7-(cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylcarbamothioyl)nicotinamide (13). Yellow powder in 84% yield; mp 215–217 °C. ¹H NMR (300 MHz,

Table 8. Inhibitory Effects of Quinoxalin-2(1H)-one Derivatives on HCV Replication in Huh 7 Cells (Modification of Segment D)

compd	X	anti-HCV activity		cytotoxicity	SI ^a
		EC ₅₀ (μM)	IC ₅₀ (μM)		
85	O	15.36	17.53		1.1
86	S	>20	13.85		<0.69
rIFNα-2b		0.14 IU/mL	>2 IU/mL		>14.3

^a SI calculated as IC₅₀/EC₅₀.

DMSO-*d*₆): δ 1.07–1.24 (m, 5H), 1.49–1.51 (m, 1H), 1.65–1.68 (m, 2H), 1.77–1.80 (m, 2H), 2.63 (s, 3H), 2.77 (m, 1H), 7.65–7.70 (m, 2H), 8.09 (s, 1H), 8.31–8.34 (m, 1H), 8.90 (s, 1H), 9.01 (s, 1H), 11.96 (s, 1H), 12.44 (s, 1H), 13.36 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 24.8, 25.4, 29.2, 38.0, 61.8, 107.7, 122.9, 124.0, 127.9, 128.5, 129.6, 137.0, 140.0, 140.1, 150.1, 150.7, 153.8, 154.9, 165.4, 177.1. HRMS calcd for C₂₂H₂₄N₆O₂SCl (M + H⁺) 471.1364; found 471.1369.

General Procedure for the Synthesis of Compounds 14–17.

Intermediate **4** (0.2 mmol) was dissolved in dried THF (5 mL). Various isothiocyanates (1 mmol) were added and the solution was heated at 65 °C for 24 h. After the reaction was completed, the solvent was evaporated in vacuo. The final products were characterized after purification by silica gel column chromatography.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-phenylthiourea (14). Light-yellow powder in 84% yield; mp 131–132 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.82–1.02 (m, 5H), 1.22 (m, 1H), 1.48–1.58 (m, 4H), 2.49 (s, 3H), 2.58 (m, 1H), 7.26–7.30 (m, 1H), 7.44–7.45 (m, 4H), 7.54 (s, 1H), 8.01 (d, 1H, *J* = 2.4 Hz), 8.74 (s, 1H), 9.74 (s, 1H), 10.44 (s, 1H), 12.35 (s, 1H). HRMS calcd for C₂₂H₂₆N₅OS (M + H⁺) 408.1853; found 408.1859.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(4-fluorophenyl)thiourea (15). Yellow powder in 85% yield; mp 137–138 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.01–1.09 (m, 5H), 1.48–1.60 (m, 5H), 2.51 (s, 3H), 2.61 (m, 1H), 7.25–7.30 (m, 2H), 7.45–7.50 (m, 2H), 7.54 (m, 1H), 8.02 (s, 1H), 8.69 (s, 1H), 9.67 (s, 1H), 10.40 (s, 1H), 12.35 (s, 1H). HRMS calcd for C₂₂H₂₅FN₅OS (M + H⁺) 426.1758; found 426.1762.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(3-(trifluoromethyl)phenyl)thiourea (16). Yellow powder in 79% yield; mp 127–129 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.01–1.34 (m, 5H), 1.47–1.66 (m, 5H), 2.58 (s, 3H), 2.64 (m, 1H), 7.54–7.58 (m, 2H), 7.62–7.67 (m, 1H), 7.78–7.81 (m, 1H), 7.98 (s, 1H), 8.04 (s, 1H), 8.44 (s, 1H), 9.70 (s, 1H), 10.66 (s, 1H), 12.35 (s, 1H). HRMS calcd for C₂₃H₂₅F₃N₅OS (M + H⁺) 476.1726; found 476.1731.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(naphthalen-1-yl)thiourea (17). Yellow powder in 82% yield; mp 233–235 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.20 (m, 2H), 0.67 (m, 1H), 0.82–0.88 (m, 2H), 1.23–1.35 (m, 5H), 2.00 (s, 3H), 2.32 (m, 1H), 7.45 (s, 1H), 7.59–7.65 (m, 4H), 7.88–7.91 (m, 1H), 7.97 (s, 1H), 8.01–8.04 (m, 2H), 9.12 (s, 1H), 9.72 (s, 1H), 10.57

(s, 1H), 12.39 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 24.3, 24.9, 29.2, 38.4, 61.1, 122.5, 122.8, 125.9, 126.0, 126.8, 127.0, 128.1, 129.3, 130.0, 133.1, 134.3, 138.2, 138.3, 149.1, 156.0, 178.8. HRMS calcd for C₂₆H₂₈N₅OS (M + H⁺) 458.2009; found 458.1993.

General Procedure for the Synthesis of Compounds 18 and 19. To a stirred solution of **4** (0.2 mmol) in 5 mL of dried THF, various isocyanates (1.0 mmol) were added and the reaction mixture was refluxed for 10 h. After the reaction was completed, the solvent was evaporated in vacuo. The final products were characterized after purification by silica gel column chromatography.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-phenylurea (18). Pale-yellow powder in 89% yield; mp 150–152 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.02–1.23 (m, 5H), 1.51–1.55 (m, 1H), 1.67–1.70 (m, 2H), 1.87–1.90 (m, 2H), 2.64 (m, 4H), 6.97–7.02 (m, 1H), 7.27–7.33 (m, 2H), 7.49–7.52 (m, 2H), 7.57 (s, 1H), 7.95 (s, 1H), 8.24 (s, 1H), 8.81 (s, 1H), 9.76 (s, 1H), 12.26 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 24.9, 25.4, 29.2, 38.8, 62.2, 102.6, 118.1, 118.4, 122.1, 123.5, 127.1, 128.7, 128.8, 130.0, 136.8, 139.3, 139.5, 148.0, 152.0, 155.2. HRMS calcd for C₂₂H₂₆N₅O₂ (M + H⁺) 392.2081; found 392.2090.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(naphthalen-1-yl)urea (19). Pale-yellow powder in 79% yield; mp 226–228 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.97–1.22 (m, 5H), 1.46–1.50 (m, 1H), 1.60–1.77 (m, 4H), 2.55 (s, 3H), 2.63 (m, 1H), 7.47–7.63 (m, 4H), 7.74 (d, 1H, *J* = 8.1 Hz), 7.80 (d, 1H, *J* = 7.2 Hz), 7.94 (d, 1H, *J* = 1.8 Hz), 7.97 (s, 1H), 8.13 (d, 1H, *J* = 8.1 Hz), 8.21 (s, 1H), 8.98 (s, 1H), 9.61 (s, 1H), 12.27 (s, 1H). HRMS calcd for C₂₆H₂₈N₅O₂ (M + H⁺) 442.2243; found 442.2240.

General Procedure for the Synthesis of Compounds 24–30.

Compound **1** (1 mmol) and K₂CO₃ (1.5 mmol) were dissolved in a mixed solvent of CH₃OH (20 mL) and H₂O (5 mL). The reaction mixture was refluxed with stirring for 1 h. After evaporation of the solvent in vacuo, compound **21** was obtained as a yellow solid after chromatography through silica gel. The yield was 84%. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.01–1.18 (m, 3H), 1.22–1.31 (m, 2H), 1.55 (m, 1H), 1.68–1.79 (m, 4H), 2.62 (s, 3H), 2.65 (s, 1H), 7.47 (s, 1H), 7.99–8.01 (m, 3H), 8.25 (s, 1H), 9.14 (s, 1H), 12.29 (s, 1H). HRMS calcd for C₁₆H₂₂N₅OS (M + H⁺) 332.1540; found 332.1542.

To a solution of compound **21** (1 mmol) in ethanol (30 mL) was added methyl iodide (2 mmol). The mixture was heated at 45 °C for 5 h and then purified by silica gel column chromatography. Compound **22** was obtained as a yellow powder in 88% yield. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.03–1.22 (m, 4H), 1.31–1.38 (m, 2H), 1.52–1.61 (m, 2H), 1.69–1.72 (m, 2H), 2.36 (s, 3H), 2.60 (s, 3H), 3.11 (m, 1H), 6.39 (s, 2H), 6.53 (s, 1H), 7.13 (s, 1H), 7.92 (s, 1H), 12.11 (s, 1H). HRMS calcd for C₁₇H₂₄N₅OS (M + H⁺) 346.1696; found 346.1684.

Compound **22** (0.5 mmol) was dissolved in pyridine (20 mL). Various amines (0.75 mmol) were added, and the solution was refluxed. After the reaction was completed, the solution was evaporated in vacuo. The residue was neutralized with 10% HCl. The mixture was partitioned between CHCl₃ and water. The organic phase was then dried with anhydrous magnesium sulfate and evaporated in vacuo. The final products **24–30** were characterized after purification by silica gel column chromatography.

1-Benzyl-3-(7-(cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)guanidine (24). Orange powder in 43% yield; mp 235–237 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.06–1.36 (m, 5H), 1.55–1.69 (m, 5H), 2.63 (s, 3H), 2.70 (m, 1H), 4.49 (d, 2H, *J* = 5.4 Hz), 7.16 (s, 1H), 7.32–7.43 (m, 5H), 7.53 (s, 1H), 7.99 (s, 1H), 8.14 (s, 1H), 8.39 (m, 1H), 9.02 (s, 1H), 12.45 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 25.4, 28.5, 34.2, 44.4, 61.8, 111.7, 121.9, 127.2, 127.6, 128.0, 128.5, 130.5, 133.1, 136.7, 142.8, 151.2, 154.5, 154.6. HRMS calcd for C₂₃H₂₉N₆O (M + H⁺) 405.2397; found 405.2402.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(4-fluorobenzyl)guanidine (25). Yellow powder in

48% yield; mp 151–153 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.04–1.22 (m, 3H), 1.30–1.34 (m, 3H), 1.52–1.64 (m, 4H), 2.59 (s, 3H), 3.00 (m, 1H), 3.94 (s, 2H), 4.43 (s, 2H), 6.87 (s, 1H), 7.16–7.29 (m, 4H), 7.37–7.49 (m, 3H), 7.98 (s, 1H). HRMS calcd for $\text{C}_{23}\text{H}_{28}\text{N}_6\text{OF}$ ($\text{M} + \text{H}^+$) 423.2303; found 423.2310.

1-(4-Chlorobenzyl)-3-(7-(cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)guanidine (26). Yellow powder in 43% yield; mp 165–167 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.04–1.13 (m, 3H), 1.22–1.34 (m, 3H), 1.54–1.75 (m, 4H), 2.62 (s, 3H), 2.68 (m, 1H), 4.47 (d, 2H, $J = 6.0$ Hz), 7.14 (s, 1H), 7.37 (d, 2H, $J = 8.4$ Hz), 7.45 (d, 2H, $J = 8.4$ Hz), 7.52 (s, 1H), 7.98 (s, 1H), 8.14 (s, 1H), 8.36 (m, 1H), 9.07 (s, 1H), 12.45 (s, 1H). HRMS calcd for $\text{C}_{23}\text{H}_{28}\text{N}_6\text{OCl}$ ($\text{M} + \text{H}^+$) 439.2007; found 439.2006.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(3-(trifluoromethyl)benzyl)guanidine (27). Yellow powder in 47% yield; mp 199–200 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.01–1.13 (m, 3H), 1.22–1.28 (m, 3H), 1.50–1.64 (m, 4H), 2.60 (s, 3H), 2.71 (m, 1H), 4.58 (d, 2H, $J = 5.1$ Hz), 7.15 (s, 1H), 7.49 (s, 1H), 7.61–7.73 (m, 4H), 8.00 (s, 1H), 8.14 (s, 1H), 8.39 (m, 1H), 9.08 (s, 1H), 12.45 (s, 1H). HRMS calcd for $\text{C}_{24}\text{H}_{28}\text{N}_6\text{OF}_3$ ($\text{M} + \text{H}^+$) 473.2271; found 473.2279.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(4-(trifluoromethoxy)benzyl)guanidine (28). Yellow powder in 47% yield; mp 199–200 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.03–1.13 (m, 3H), 1.22–1.33 (m, 3H), 1.51–1.65 (m, 4H), 2.61 (s, 3H), 2.71 (m, 1H), 4.51 (d, 2H, $J = 5.4$ Hz), 7.15 (s, 1H), 7.39 (d, 2H, $J = 8.4$ Hz), 7.48–7.52 (m, 3H), 7.98 (s, 1H), 8.14 (s, 1H), 8.38 (m, 1H), 9.08 (s, 1H), 12.46 (s, 1H). HRMS calcd for $\text{C}_{24}\text{H}_{28}\text{N}_6\text{O}_2\text{F}_3$ ($\text{M} + \text{H}^+$) 489.2220; found 489.2223.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(4-methoxybenzyl)guanidine (29). Light-yellow powder in 53% yield; mp 177–179 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.03–1.05 (m, 3H), 1.22–1.34 (m, 3H), 1.53–1.67 (m, 4H), 2.61 (s, 3H), 2.69 (m, 1H), 3.74 (s, 3H), 4.41 (d, 2H, $J = 3.6$ Hz), 6.93 (d, 2H, $J = 8.4$ Hz), 7.14 (s, 1H), 7.28 (d, 2H, $J = 8.4$ Hz), 7.51 (s, 1H), 7.99 (s, 1H), 8.13 (s, 1H), 8.39 (s, 1H), 9.05 (s, 1H), 12.46 (s, 1H). HRMS calcd for $\text{C}_{24}\text{H}_{31}\text{N}_6\text{O}_2$ ($\text{M} + \text{H}^+$) 435.2503; found 435.2515.

1-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(pyridin-2-ylmethyl)guanidine (30). Yellow powder in 44% yield; mp 199–201 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.06–1.11 (m, 3H), 1.22–1.35 (m, 2H), 1.54–1.70 (m, 5H), 2.64 (s, 3H), 2.73 (m, 1H), 4.61 (d, 2H, $J = 5.1$ Hz), 7.20 (s, 1H), 7.36–7.42 (m, 2H), 7.54 (s, 1H), 7.83–7.89 (m, 1H), 8.05 (s, 1H), 8.13 (s, 1H), 8.44 (m, 1H), 8.59 (d, 1H, $J = 4.8$ Hz), 9.27 (s, 1H), 12.43 (s, 1H). HRMS calcd for $\text{C}_{22}\text{H}_{28}\text{N}_7\text{O}$ ($\text{M} + \text{H}^+$) 406.2349; found 406.2354.

6-(Cyclohexyl(methyl)amino)-7-(4-oxo-3-phenyl-2-thioxoimidazolidin-1-yl)quinoxalin-2(1H)-one (31). To a stirred solution of compound 14 (1 mmol) in 1,4-dioxane (50 mL) was added Et_3N (2 mmol) and 2-chloroacetyl chloride (1 mmol). The mixture was refluxed for 8 h. After the reaction was completed, the solution was evaporated in vacuo. The final product 31 was obtained as a yellow powder after purification by silica gel column chromatography eluting with CHCl_3 – CH_3OH (40:1, v/v). Yield 51%; mp 140–142 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.07–1.23 (m, 5H), 1.56–1.70 (m, 5H), 2.60 (s, 3H), 2.74 (m, 1H), 4.11 (d, 1H, $J = 17.4$ Hz), 4.19 (d, 1H, $J = 17.4$ Hz), 6.83–6.85 (m, 2H), 7.07–7.12 (m, 1H), 7.25 (s, 1H), 7.32–7.37 (m, 2H), 7.57 (s, 1H), 8.19 (s, 1H), 12.47 (s, 1H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ 25.3, 25.6, 25.7, 28.6, 29.8, 32.9, 33.5, 62.2, 117.2, 120.4, 121.9, 124.2, 127.3, 129.3, 132.9, 145.9, 147.9, 152.6, 154.4, 155.2, 170.9. HRMS calcd for $\text{C}_{24}\text{H}_{26}\text{N}_5\text{O}_2\text{S}$ ($\text{M} + \text{H}^+$) 448.1802; found 448.1804.

6-(Cyclohexyl(methyl)amino)-7-(3-(naphthalen-1-yl)-5-oxo-2-thioxoimidazolidin-1-yl)quinoxalin-2(1H)-one (32). To a stirred solution of compound 17 (1 mmol) in 1,4-dioxane (50 mL) was added

Et_3N (2 mmol) and 2-chloroacetyl chloride (1 mmol). The mixture was refluxed for 8 h. After the reaction was completed, the solution was evaporated in vacuo. The final product 32 and 32' were obtained as yellow powders after purification by silica gel column chromatography eluting with CHCl_3 – CH_3OH (40:1, v/v).

32: Yield 59%; mp 210–211 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 0.53–0.86 (m, 3H), 1.09–1.22 (m, 4H), 1.37–1.62 (m, 3H), 2.39 (s, 3H), 2.66 (m, 1H), 4.31 (d, 1H, $J = 17.1$ Hz), 4.51 (d, 1H, $J = 17.1$ Hz), 6.68 (s, 1H), 7.19 (s, 1H), 7.55–7.70 (m, 4H), 7.79–7.82 (m, 1H), 8.00 (s, 1H), 8.05–8.11 (m, 2H), 12.22 (s, 1H). ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$): δ 25.2, 25.4, 25.5, 28.0, 28.6, 33.0, 33.1, 41.4, 59.5, 106.5, 119.5, 122.3, 125.7, 126.5, 127.0, 127.2, 128.4, 129.3, 129.5, 131.7, 133.9, 141.3, 145.4, 149.4, 154.7, 156.0, 168.5, 171.9. HRMS calcd for $\text{C}_{28}\text{H}_{28}\text{N}_5\text{O}_2\text{S}$ ($\text{M} + \text{H}^+$) 498.1963; found 498.1955.

32': Yield 34%; mp 207–209 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.10–1.34 (m, 5H), 1.41–1.76 (m, 5H), 2.70 (s, 3H), 2.82 (m, 1H), 4.14 (d, 1H, $J = 17.1$ Hz), 4.23 (d, 1H, $J = 17.1$ Hz), 6.94 (d, 1H, $J = 7.5$ Hz), 7.42–7.54 (m, 4H), 7.60 (s, 1H), 7.65 (d, 1H, $J = 8.4$ Hz), 7.87–7.92 (m, 2H), 8.20 (s, 1H), 12.58 (s, 1H). HRMS calcd for $\text{C}_{28}\text{H}_{28}\text{N}_5\text{O}_2\text{S}$ ($\text{M} + \text{H}^+$) 498.1963; found 498.1953.

General Procedure for the Synthesis of Compounds 33–45.

Compound 21 (1 mmol) and various α -bromo substituted ketones (1 mmol) were dissolved in EtOH (30 mL) and refluxed for 2 h. The mixture was concentrated and the final products 33–45 were characterized after purification by silica gel column chromatography.

6-(Cyclohexyl(methyl)amino)-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (33). Yellow powder in 80% yield; mp 226–228 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.08–1.31 (m, 5H), 1.50–1.54 (m, 1H), 1.66–1.70 (m, 2H), 1.84–1.88 (m, 2H), 2.65 (s, 3H), 2.72 (m, 1H), 7.31–7.36 (m, 1H), 7.43–7.48 (m, 3H), 7.58 (s, 1H), 7.96 (s, 1H), 8.08 (d, 2H, $J = 7.2$ Hz), 8.58 (s, 1H), 9.75 (s, 1H), 12.72 (s, 1H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 24.9, 25.4, 29.1, 38.4, 62.0, 101.4, 104.8, 123.3, 126.0, 127.0, 127.6, 128.5, 130.2, 134.2, 136.8, 139.8, 147.4, 150.1, 155.5, 162.0. HRMS calcd for $\text{C}_{24}\text{H}_{26}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 432.1858; found 432.1841.

6-(Cyclohexyl(methyl)amino)-7-(4-(4-fluorophenyl)thiazol-2-ylamino)quinoxalin-2(1H)-one (34). Pale-yellow powder in 83% yield; mp 245–247 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.08–1.26 (m, 5H), 1.50–1.53 (m, 1H), 1.66–1.69 (m, 2H), 1.84–1.88 (m, 2H), 2.65 (s, 3H), 2.71 (m, 1H), 7.23–7.29 (m, 2H), 7.46 (s, 1H), 7.57 (s, 1H), 7.96 (s, 1H), 8.12–8.16 (m, 2H), 8.59 (s, 1H), 9.78 (s, 1H), 12.73 (s, 1H). HRMS calcd for $\text{C}_{24}\text{H}_{25}\text{FN}_5\text{OS}$ ($\text{M} + \text{H}^+$) 450.1758; found 450.1759.

7-(4-(4-Chlorophenyl)thiazol-2-ylamino)-6-(cyclohexyl(methyl)amino)quinoxalin-2(1H)-one (35). Pale-yellow powder in 87% yield; mp 278–280 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.08–1.27 (m, 5H), 1.50–1.54 (m, 1H), 1.66–1.70 (m, 2H), 1.85–1.88 (m, 2H), 2.65 (s, 3H), 2.72 (m, 1H), 7.48 (d, 2H, $J = 8.4$ Hz), 7.55 (m, 2H), 7.57 (s, 1H), 8.11 (d, 2H, $J = 8.4$ Hz), 8.58 (s, 1H), 9.81 (s, 1H), 12.74 (s, 1H). HRMS calcd for $\text{C}_{24}\text{H}_{25}\text{ClN}_5\text{OS}$ ($\text{M} + \text{H}^+$) 466.1463; found 466.1455.

4-(2-(7-(Cyclohexyl(methyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-ylamino)thiazol-4-yl)benzonitrile (36). Light-yellow powder in 85% yield; mp 261–263 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.07–1.26 (m, 5H), 1.53 (m, 1H), 1.66–1.69 (m, 2H), 1.85–1.88 (m, 2H), 2.65 (s, 3H), 2.72 (m, 1H), 7.58 (s, 1H), 7.78 (s, 1H), 7.88 (d, 2H, $J = 8.4$ Hz), 7.97 (s, 1H), 8.28 (d, 2H, $J = 8.4$ Hz), 8.61 (s, 1H), 9.91 (s, 1H), 12.77 (s, 1H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 24.9, 25.5, 29.1, 38.5, 62.1, 101.7, 108.9, 109.6, 119.0, 123.4, 126.6, 127.1, 130.1, 132.6, 136.9, 138.3, 139.7, 147.6, 148.3, 155.5, 162.4. HRMS calcd for $\text{C}_{25}\text{H}_{25}\text{N}_6\text{OS}$ ($\text{M} + \text{H}^+$) 457.1810; found 457.1792.

6-(Cyclohexyl(methyl)amino)-7-(4-(4-methoxyphenyl)thiazol-2-ylamino)quinoxalin-2(1H)-one (37). Yellow powder in 83% yield; mp 226–228 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.09–1.27 (m, 5H), 1.50–1.54 (m, 1H), 1.66–1.88 (m, 4H), 2.65 (s, 3H), 2.72

(m, 1H), 3.80 (s, 3H), 6.99 (d, 2H, $J = 8.4$ Hz), 7.30 (s, 1H), 7.57 (s, 1H), 7.95 (s, 1H), 8.01 (d, 2H, $J = 8.4$ Hz), 8.57 (s, 1H), 9.71 (s, 1H), 12.71 (s, 1H). HRMS calcd for $C_{25}H_{28}N_5O_2S$ ($M + H^+$) 462.1958; found 492.1961.

7-(4-(Benzo[d][1,3]dioxol-5-yl)thiazol-2-ylamino)-6-(cyclohexyl(methyl)amino)quinoxalin-2(1H)-one (38). Yellow powder in 70% yield; mp 209–210 °C. 1H NMR (300 MHz, DMSO- d_6): δ 1.08–1.27 (m, 5H), 1.50–1.53 (m, 1H), 1.66–1.88 (m, 4H), 2.65 (s, 3H), 2.71 (m, 1H), 6.06 (s, 2H), 6.96 (d, 1H, $J = 7.8$ Hz), 7.34 (s, 1H), 7.57 (s, 1H), 7.63–7.66 (m, 2H), 7.95 (d, 1H, $J = 2.1$ Hz), 8.53 (s, 1H), 9.71 (s, 1H), 12.74 (s, 1H). HRMS calcd for $C_{25}H_{26}N_5O_3S$ ($M + H^+$) 476.1751; found 476.1769.

6-(Cyclohexyl(methyl)amino)-7-(4-(naphthalen-1-yl)thiazol-2-ylamino)quinoxalin-2(1H)-one (39). Yellow powder in 82% yield; mp 247–249 °C. 1H NMR (300 MHz, DMSO- d_6): δ 1.10–1.31 (m, 5H), 1.52–1.55 (m, 1H), 1.69–1.72 (m, 2H), 1.86–1.90 (m, 2H), 2.67 (s, 3H), 2.76 (m, 1H), 7.27 (s, 1H), 7.54–7.63 (m, 4H), 7.87–7.98 (m, 4H), 8.29 (s, 1H), 8.37–8.40 (m, 1H), 9.73 (s, 1H), 12.43 (s, 1H). HRMS calcd for $C_{28}H_{28}N_5OS$ ($M + H^+$) 482.2009; found 482.1955.

7-(4-(Biphenyl-4-yl)thiazol-2-ylamino)-6-(cyclohexyl(methyl)amino)quinoxalin-2(1H)-one (40). Yellow powder in 85% yield; mp 232–234 °C. 1H NMR (300 MHz, DMSO- d_6): δ 1.06–1.28 (m, 5H), 1.50–1.54 (m, 1H), 1.67–1.71 (m, 2H), 1.86–1.90 (m, 2H), 2.66 (s, 3H), 2.73 (m, 1H), 7.35–7.40 (m, 1H), 7.47–7.52 (m, 2H), 7.55 (s, 1H), 7.58 (s, 1H), 7.72–7.77 (m, 4H), 7.96 (d, 1H, $J = 1.5$ Hz), 8.18 (d, 2H, $J = 8.7$ Hz), 8.63 (s, 1H), 9.79 (s, 1H), 12.76 (s, 1H). HRMS calcd for $C_{30}H_{30}N_5OS$ ($M + H^+$) 508.2165; found 508.2188.

6-(Cyclohexyl(methyl)amino)-7-(4-(furan-2-yl)thiazol-2-ylamino)quinoxalin-2(1H)-one (41). Yellow powder in 77% yield; mp 196–197 °C. 1H NMR (300 MHz, DMSO- d_6): δ 1.08–1.25 (m, 5H), 1.50–1.53 (m, 1H), 1.66–1.70 (m, 2H), 1.84–1.88 (m, 2H), 2.64 (s, 3H), 2.72 (m, 1H), 6.63 (dd, 1H, $J_1 = 1.5$ Hz, $J_2 = 2.7$ Hz), 7.11 (s, 1H), 7.14 (d, 1H, $J = 2.7$ Hz), 7.57 (s, 1H), 7.72 (s, 1H), 7.95 (d, 1H, $J = 1.5$ Hz), 8.63 (s, 1H), 9.85 (s, 1H), 12.61 (s, 1H). HRMS calcd for $C_{22}H_{24}N_5O_2S$ ($M + H^+$) 422.1645; found 422.1666.

6-(Cyclohexyl(methyl)amino)-7-(4-(thiophen-2-yl)thiazol-2-ylamino)quinoxalin-2(1H)-one (42). Yellow powder in 75% yield; mp 196–197 °C. 1H NMR (300 MHz, DMSO- d_6): δ 1.08–1.26 (m, 5H), 1.50–1.54 (m, 1H), 1.66–1.70 (m, 2H), 1.84–1.88 (m, 2H), 2.65 (s, 3H), 2.72 (m, 1H), 7.11–7.14 (m, 1H), 7.22 (s, 1H), 7.49–7.51 (m, 1H), 7.57 (s, 1H), 7.70–7.71 (m, 1H), 7.95 (s, 1H), 8.43 (s, 1H), 9.75 (s, 1H), 12.63 (s, 1H). HRMS calcd for $C_{22}H_{24}N_5OS_2$ ($M + H^+$) 438.1417; found 438.1408.

6-(Cyclohexyl(methyl)amino)-7-(4-(pyridin-3-yl)thiazol-2-ylamino)quinoxalin-2(1H)-one (43). Yellow powder in 73% yield; mp 156–158 °C. 1H NMR (300 MHz, DMSO- d_6): δ 1.01–1.27 (m, 5H), 1.50–1.53 (m, 1H), 1.66–1.70 (m, 2H), 1.85–1.88 (m, 2H), 2.65 (s, 3H), 2.72 (m, 1H), 7.46–7.50 (m, 1H), 7.58 (s, 1H), 7.66 (s, 1H), 7.96 (s, 1H), 8.43–8.45 (m, 1H), 8.54–8.55 (m, 1H), 8.63 (s, 1H), 9.32 (d, 1H, $J = 1.5$ Hz), 9.87 (s, 1H), 12.74 (s, 1H). HRMS calcd for $C_{23}H_{25}N_6OS$ ($M + H^+$) 433.1805; found 433.1819.

6-(Cyclohexyl(methyl)amino)-7-(4-(trifluoromethyl)thiazol-2-ylamino)quinoxalin-2(1H)-one (44). Yellow powder in 81% yield; mp 225–227 °C. 1H NMR (300 MHz, DMSO- d_6): δ 1.06–1.25 (m, 5H), 1.52–1.84 (m, 5H), 2.63 (s, 3H), 2.70 (m, 1H), 7.56 (s, 1H), 7.74 (s, 1H), 7.96 (s, 1H), 8.25 (s, 1H), 10.04 (s, 1H), 12.56 (s, 1H). HRMS calcd for $C_{19}H_{21}N_5OF_3S$ ($M + H^+$) 424.1418; found 424.1424.

7-(8H-Indeno[1,2-d]thiazol-2-ylamino)-6-(cyclohexyl(methyl)amino)quinoxalin-2(1H)-one (45). Light-yellow powder in 73% yield; mp 262–264 °C. 1H NMR (300 MHz, DMSO- d_6): δ 1.01–1.31 (m, 5H), 1.50–1.53 (m, 1H), 1.66–1.70 (m, 2H), 1.86–1.89 (m, 2H), 2.66 (s, 3H), 2.73 (m, 1H), 3.86 (s, 2H), 7.20–7.25 (m, 1H), 7.36–7.40 (m, 1H), 7.54 (d, 1H, $J = 7.5$ Hz), 7.57 (s, 1H), 7.67 (d, 1H, $J = 7.5$ Hz), 7.95 (s, 1H), 8.67 (s, 1H), 9.81 (s, 1H),

12.46 (s, 1H). HRMS calcd for $C_{25}H_{26}N_5OS$ ($M + H^+$) 444.1853; found 444.1851.

6-(Cyclohexyl(methyl)amino)-3-methyl-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (51). To a stirred solution of 1,5-difluoro-2,4-dinitrobenzene (DFDNB, 2.04 g, 10 mmol) in THF (50 mL) was added DIPEA (10 mmol) and *N*-methylcyclohexanamine (10 mmol). After vigorously stirring at room temperature until the total disappearance of DFDNB, compound **46** was obtained without purification. Then, $NH_2CH(CH_3)COOCH_3 \cdot HCl$ (10 mmol) and DIPEA (20 mmol) were added and stirred under reflux for 8 h. The solvent was removed under reduced pressure to give compound **47a** that was used directly. The above two reactions were traced by a fast LC-MS system until all of the starting material was changed into the anticipated compound. Compound **47a** (1 g) was dissolved in a mixed solvent of THF (30 mL) and EtOH (30 mL), followed by the addition of 10% Pd/C (1 g) and $HCOONH_4$ (2 g). The mixture was stirred at room temperature for 3 h. The residue solid was filtered off, and the filtrate was concentrated in vacuo. Water (50 mL) was added to the resulting product and then was extracted by CH_2Cl_2 (3×50 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Intermediate **48a** was obtained as a yellow solid after chromatography through silica gel eluting with $CHCl_3-CH_3OH$ (30:1, v/v). Yield 70%. 1H NMR (300 MHz, DMSO- d_6): δ 1.03–1.25 (m, 3H), 1.28–1.36 (m, 2H), 1.54 (m, 1H), 1.67–1.74 (m, 4H), 2.26 (s, 3H), 2.54 (s, 3H), 2.65–2.72 (m, 1H), 5.49 (s, 2H), 6.44 (s, 1H), 7.25 (s, 1H), 11.88 (s, 1H). HRMS calcd for $C_{16}H_{23}N_4O$ ($M + H^+$) 287.1866; found 287.1856.

Intermediate **48a** (1 mmol) and benzoyl isothiocyanate (1 mmol) were dissolved in dried acetone (30 mL). The reaction mixture was refluxed with stirring for 4 h and then concentrated in vacuo to give compound **49a** without purification. Intermediate **49a** (1 mmol) and K_2CO_3 (1.5 mmol) in CH_3OH (20 mL)/ H_2O (5 mL) were heated to reflux for 1 h. After evaporation of the solvent in vacuo, compound **50a** was obtained as a yellow solid after chromatography through silica gel eluting with $CHCl_3-CH_3OH$ (30:1, v/v). Yield 75%. 1H NMR (300 MHz, DMSO- d_6): δ 1.07–1.34 (m, 3H), 1.22–1.30 (m, 2H), 1.55 (m, 1H), 1.68–1.79 (m, 4H), 2.35 (s, 3H), 2.61 (s, 3H), 2.65–2.68 (m, 1H), 7.40 (s, 1H), 7.91 (s, 2H), 8.14 (s, 1H), 9.08 (s, 1H), 12.16 (s, 1H). HRMS calcd for $C_{17}H_{24}N_5OS$ ($M + H^+$) 346.1696; found 346.1680.

50a (0.5 mmol) and 2-bromo-1-phenylethanone (0.5 mmol) were dissolved in EtOH (30 mL) and refluxed for 2 h. After the reaction was completed, the solvent was evaporated in vacuo. The final product **51** was obtained as a yellow powder after purification by silica gel column chromatography eluting with $CHCl_3-CH_3OH$ (80:1, v/v). Yield 84%; mp 229–231 °C. 1H NMR (300 MHz, DMSO- d_6): δ 1.08–1.26 (m, 5H), 1.50–1.53 (m, 1H), 1.66–1.70 (m, 2H), 1.84–1.88 (m, 2H), 2.36 (s, 3H), 2.64 (s, 3H), 2.71 (m, 1H), 7.33–7.35 (m, 1H), 7.43–7.48 (m, 3H), 7.51 (s, 1H), 8.08 (d, 2H, $J = 7.2$ Hz), 8.55 (s, 1H), 9.68 (s, 1H), 12.62 (s, 1H). HRMS calcd for $C_{25}H_{28}N_5OS$ ($M + H^+$) 446.2009; found 446.1987.

6-(Cyclohexyl(methyl)amino)-3-isopropyl-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (52). Compound **52** was prepared in a similar manner to the synthesis of compound **51**, substituting $NH_2CH(CH_3)COOCH_3 \cdot HCl$ for $NH_2CH(CH_3)COOCH_3 \cdot HCl$. The final product **52** was obtained as a yellow solid after purification by silica gel column chromatography. Yield 86% (the last step); mp 208–210 °C. 1H NMR (300 MHz, DMSO- d_6): δ 1.05–1.27 (m, 11H), 1.50–1.53 (m, 1H), 1.66–1.69 (m, 2H), 1.84–1.88 (m, 2H), 2.65 (s, 3H), 2.72 (m, 1H), 3.41–3.46 (m, 1H), 7.33–7.35 (m, 1H), 7.43–7.48 (m, 3H), 7.52 (s, 1H), 8.08 (d, 2H, $J = 7.2$ Hz), 8.55 (s, 1H), 9.68 (s, 1H), 12.62 (s, 1H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 20.1, 24.9, 25.5, 29.2, 29.5, 38.6, 62.1, 101.3, 104.5, 122.8, 126.0, 126.4, 127.5, 128.5, 129.8, 134.2, 136.5, 138.6, 150.1, 154.7, 161.8, 162.1. HRMS calcd for $C_{27}H_{32}N_5OS$ ($M + H^+$) 474.2322; found 474.2349.

6-(Cyclohexyl(methyl)amino)-3-isobutyl-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (53). Compound **53** was prepared in a similar manner to the synthesis of compound **51**, substituting $\text{NH}_2\text{CH}(\text{CH}_2\text{CH}(\text{CH}_3)_2)\text{COOCH}_3 \cdot \text{HCl}$ for $\text{NH}_2\text{CH}(\text{CH}_3)\text{COOCH}_3 \cdot \text{HCl}$. The final product **53** was obtained as a pale-yellow powder after purification by silica gel column chromatography. Yield 77% (the last step); mp 223–224 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 0.92 (d, 6H, $J = 6.3$ Hz), 1.08–1.27 (m, 5H), 1.49–1.53 (m, 1H), 1.66–1.70 (m, 2H), 1.84–1.88 (m, 2H), 2.19–2.24 (m, 1H), 2.61 (d, 2H, $J = 7.5$ Hz), 2.65 (s, 3H), 2.71 (m, 1H), 7.30–7.35 (m, 1H), 7.42–7.48 (m, 3H), 7.53 (s, 1H), 8.07 (d, 2H, $J = 7.2$ Hz), 8.54 (s, 1H), 9.67 (s, 1H), 12.61 (s, 1H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 22.6, 24.9, 25.5, 26.3, 29.2, 38.4, 41.3, 62.0, 101.3, 104.5, 122.7, 126.0, 126.6, 127.5, 128.5, 129.8, 134.2, 136.5, 138.6, 150.1, 155.4, 157.4, 162.1. HRMS calcd for $\text{C}_{28}\text{H}_{34}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 488.2479; found 488.2502.

3-Benzyl-6-(cyclohexyl(methyl)amino)-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (54). Compound **54** was prepared in a similar manner to the synthesis of compound **51**, substituting $\text{NH}_2\text{CH}(\text{CH}_2\text{Ph})\text{COOCH}_3 \cdot \text{HCl}$ for $\text{NH}_2\text{CH}(\text{CH}_3)\text{COOCH}_3 \cdot \text{HCl}$. The final product **54** was obtained as a pale-yellow powder after purification by silica gel column chromatography. Yield 71% (the last step); mp 239–241 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.07–1.26 (m, 5H), 1.49–1.52 (m, 1H), 1.65–1.87 (m, 4H), 2.64 (s, 3H), 2.70 (m, 1H), 4.08 (s, 2H), 7.19–7.22 (m, 1H), 7.26–7.35 (m, 5H), 7.42–7.47 (m, 3H), 7.51 (s, 1H), 8.07 (d, 2H, $J = 7.2$ Hz), 8.55 (s, 1H), 9.70 (s, 1H), 12.70 (s, 1H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 24.9, 25.4, 29.2, 38.4, 62.0, 101.3, 104.5, 122.8, 126.0, 126.1, 126.5, 127.5, 128.2, 128.5, 129.0, 130.1, 134.2, 136.7, 138.0, 138.9, 150.1, 155.1, 156.4, 162.1. HRMS calcd for $\text{C}_{31}\text{H}_{32}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 522.2322; found 522.2345.

6-(Cyclohexyl(methyl)amino)-3-(2-(methylthio)ethyl)-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (55). To a stirred solution of compound **46** (10 mmol) in THF (50 mL) was added DIPEA (20 mmol) and $\text{NH}_2\text{CH}((\text{CH}_2)_2\text{SCH}_3)\text{COOCH}_3 \cdot \text{HCl}$ (10 mmol). After vigorously stirring at 65 °C until the total disappearance of the starting materials, compound **47e** was obtained without purification. Compound **47e** (5 mmol) and $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (75 mmol) were dissolved in ethanol (75 mL). Then 15 equiv of 12 mol/L hydrochloric acid was added and the mixture was stirred under reflux for 3 h. The solvent was then evaporated. The residue was neutralized with 40% NaOH until the pH reached 8. The mixture was filtered, and the filtrate was extracted with 100 mL of CHCl_3 twice. The organic solvent was washed with brine, dried over anhydrous sodium sulfate, and evaporated in vacuo. The intermediate **48e** was obtained as a yellow solid after purification by silica gel column chromatography. Yield 51%. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.03–1.19 (m, 3H), 1.26–1.36 (m, 2H), 1.54 (m, 1H), 1.67–1.76 (m, 4H), 2.08 (s, 3H), 2.55 (s, 3H), 2.65–2.72 (m, 1H), 2.78–2.84 (m, 2H), 2.89–2.95 (m, 2H), 5.56 (s, 2H), 6.44 (s, 1H), 7.20 (s, 1H), 11.92 (s, 1H). HRMS calcd for $\text{C}_{18}\text{H}_{27}\text{N}_4\text{OS}$ ($\text{M} + \text{H}^+$) 347.1900; found 347.1889.

Intermediate **48e** (1 mmol) and benzoyl isothiocyanate (1 mmol) were dissolved in dried acetone (30 mL). The reaction mixture was refluxed with stirring for 4 h and then concentrated to give compound **49e** without purification. Intermediate **49e** (1 mmol) and K_2CO_3 (1.5 mmol) in CH_3OH (20 mL)/ H_2O (5 mL) were heated to reflux for 1 h. After evaporation of the solvent in vacuo, compound **50e** was obtained as a yellow solid after chromatography through silica gel eluting with CHCl_3 – CH_3OH (10:1, v/v). Yield 66%. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.07–1.13 (m, 3H), 1.27–1.31 (m, 2H), 1.55 (m, 1H), 1.68–1.79 (m, 4H), 2.09 (s, 3H), 2.59 (s, 3H), 2.62–2.65 (m, 1H), 2.83–2.88 (m, 2H), 3.00–3.04 (m, 2H), 7.42 (s, 1H), 7.92 (s, 2H), 8.17 (s, 1H), 9.10 (s, 1H), 12.22 (s, 1H). HRMS calcd for $\text{C}_{19}\text{H}_{28}\text{N}_5\text{OS}_2$ ($\text{M} + \text{H}^+$) 406.1730; found 406.1716.

50e (0.5 mmol) and 2-bromo-1-phenylethanone (0.5 mmol) were dissolved in EtOH (30 mL) and refluxed for 2 h. After the reaction was completed, the solvent was evaporated in vacuo. The final product **55**

was obtained as a yellow powder after purification by silica gel column chromatography eluting with CHCl_3 – CH_3OH (50:1, v/v). Yield 75%; mp 130–131 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.15–1.31 (m, 5H), 1.59–1.63 (m, 1H), 1.71–1.91 (m, 4H), 2.09 (s, 3H), 2.80–2.85 (m, 2H), 2.94–2.99 (m, 2H), 3.23 (m, 1H), 3.40 (s, 3H), 5.58 (d, 1H, $J = 8.1$ Hz), 6.52 (s, 1H), 7.17 (s, 1H), 7.26–7.31 (m, 1H), 7.36–7.43 (m, 2H), 7.57 (s, 1H), 7.88 (d, 2H, $J = 7.2$ Hz), 11.97 (s, 1H). HRMS calcd for $\text{C}_{27}\text{H}_{32}\text{N}_5\text{OS}_2$ ($\text{M} + \text{H}^+$) 506.2043; found 506.2064.

2-(Cyclohexyl(methyl)amino)-3-(4-phenylthiazol-2-ylamino)-7,8,9,10-tetrahydro-5H-pyrido[1,2-*a*]quinoxalin-6(6aH)-one (60). To a stirred solution of compound **46** (10 mmol) in THF (50 mL), DIPEA (10 mmol) and ethyl piperidine-2-carboxylate (10 mmol) was added. After vigorously stirring at 65 °C until the total disappearance of the starting materials, compound **56** was obtained without purification. Compound **56** (1 g) was dissolved in a mixed solvent of THF (30 mL) and EtOH (30 mL), followed by the addition of 10% Pd/C (1 g) and HCOONH_4 (2 g). The mixture was stirred at room temperature for 3 h. The residue solid was filtered off, and the filtrate was concentrated in vacuo. Water (50 mL) was added to the resulting product and then was extracted by CH_2Cl_2 (3 $\times$ 50 mL). The organic layers were combined, dried over anhydrous MgSO_4 , and evaporated in vacuo. The intermediate **57** was obtained as a white solid after purification by silica gel column chromatography with a yield of 68%. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.00–1.53 (m, 9H), 1.66–1.79 (m, 6H), 2.00–2.03 (m, 1H), 2.40–2.43 (m, 1H), 2.53 (s, 3H), 2.60–2.66 (m, 1H), 3.09–3.13 (m, 1H), 3.50–3.54 (m, 1H), 4.35 (s, 2H), 6.22 (s, 1H), 6.46 (s, 1H), 10.08 (s, 1H). HRMS calcd for $\text{C}_{19}\text{H}_{29}\text{N}_4\text{O}$ ($\text{M} + \text{H}^+$) 329.2336; found 329.2320.

Intermediate **57** (1 mmol) and benzoyl isothiocyanate (1 mmol) were dissolved in dried acetone (30 mL). The reaction mixture was refluxed with stirring for 4 h and then concentrated to give compound **58** without purification. Intermediate **58** (1 mmol) and K_2CO_3 (1.5 mmol) in CH_3OH (20 mL)/ H_2O (5 mL) were heated to reflux for 1 h. Compound **59** was obtained as a white solid after evaporation of the solvent in vacuo and chromatography through silica gel eluting with CHCl_3 – CH_3OH (40:1, v/v). Yield 69%. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 0.99–1.17 (m, 4H), 1.29–1.54 (m, 6H), 1.67–1.70 (m, 4H), 1.76–1.80 (m, 2H), 2.00–2.03 (m, 1H), 2.55 (s, 3H), 2.67–2.74 (m, 1H), 3.35–3.39 (m, 1H), 3.68–3.72 (m, 1H), 6.51 (s, 1H), 7.06 (s, 1H), 7.34 (s, 2H), 8.61 (s, 1H), 10.24 (s, 1H). HRMS calcd for $\text{C}_{20}\text{H}_{30}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 388.2166; found 388.2157.

Compound **59** (0.5 mmol) and 2-bromo-1-phenylethanone (0.5 mmol) were dissolved in ethanol (30 mL) and refluxed for 2 h. After the reaction was completed, the solvent was evaporated in vacuo. The final product **60** was obtained as a white solid after purification by silica gel column chromatography eluting with CHCl_3 – CH_3OH (50:1, v/v). Yield 73%; mp 200–202 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.07–1.24 (m, 6H), 1.41–1.48 (m, 4H), 1.64–1.68 (m, 2H), 1.74–1.79 (m, 3H), 2.05 (m, 1H), 2.58 (s, 3H), 2.68 (m, 1H), 3.30 (s, 1H), 3.31–3.35 (m, 1H), 3.67–3.71 (m, 1H), 6.67 (s, 1H), 7.23 (s, 1H), 7.26–7.31 (m, 1H), 7.38–7.43 (m, 2H), 7.80 (s, 1H), 7.96 (d, 2H, $J = 7.8$ Hz), 8.94 (s, 1H), 10.59 (s, 1H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 23.0, 23.3, 25.0, 25.5, 26.5, 29.3, 29.6, 37.6, 46.3, 58.7, 61.9, 102.4, 106.1, 107.5, 123.5, 125.9, 127.3, 128.4, 129.3, 130.7, 134.6, 136.3, 150.0, 163.9, 168.0. HRMS calcd for $\text{C}_{28}\text{H}_{34}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 488.2479; found 488.2495.

8-(Cyclohexyl(methyl)amino)-7-(4-phenylthiazol-2-ylamino)-pyrrolo[1,2-*a*]quinoxalin-4(5H)-one (65). To a stirred solution of compound **46** (10 mmol) in THF (50 mL) was added DIPEA (10 mmol) and methyl pyrrolidine-2-carboxylate (10 mmol). After vigorously stirring at 65 °C until the total disappearance of the starting materials, compound **61** was obtained without purification. Compound **61** (5 mmol) and 10% Pd/C (5 g) were dissolved in ethanol (50 mL). Then cyclohexene (5 mmol) was added, and the mixture was stirred under reflux for 2 h. The mixture was filtered, and the filtrate was evaporated in vacuo. The intermediate **62** was obtained as a white solid after purification by silica gel

column chromatography. Yield 68%. ^1H NMR (300 MHz, DMSO- d_6): δ 1.12–1.21 (m, 3H), 1.27–1.37 (m, 2H), 1.55 (m, 1H), 1.68–1.78 (m, 4H), 2.60 (s, 3H), 2.72–2.79 (m, 1H), 4.98 (s, 2H), 6.53–6.55 (m, 2H), 6.87–6.88 (m, 1H), 7.57 (s, 1H), 8.04 (m, 1H), 10.89 (s, 1H). HRMS calcd for $\text{C}_{18}\text{H}_{23}\text{N}_4\text{O}$ ($\text{M} + \text{H}^+$) 311.1866; found 311.1864.

Intermediate **62** (1 mmol) and benzoyl isothiocyanate (1 mmol) were dissolved in dried acetone (30 mL). The reaction mixture was refluxed with stirring for 4 h and then concentrated to give compound **63** without purification. Intermediate **63** (1 mmol) and K_2CO_3 (1.5 mmol) in CH_3OH (20 mL)/ H_2O (5 mL) were heated to reflux for 1 h. Compound **64** was obtained as a white solid after evaporation of the solvent and chromatography through silica gel eluting with CHCl_3 – CH_3OH (40:1, v/v). Yield 69%. ^1H NMR (300 MHz, DMSO- d_6): δ 1.08–1.16 (m, 3H), 1.31–1.35 (m, 2H), 1.55 (m, 1H), 1.68–1.79 (m, 4H), 2.66 (s, 3H), 2.77 (m, 1H), 6.64–6.65 (m, 1H), 6.97–6.98 (m, 1H), 7.69 (m, 3H), 7.78 (s, 1H), 8.21 (s, 1H), 8.89 (s, 1H), 11.10 (s, 1H). HRMS calcd for $\text{C}_{19}\text{H}_{24}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 370.1696; found 370.1693.

Compound **64** (0.5 mmol) and 2-bromo-1-phenylethanone (0.5 mmol) were dissolved in ethanol (30 mL) and refluxed for 2 h. After the reaction was completed, the solvent was evaporated in vacuo. The final product **65** was obtained as a white powder after purification by silica gel column chromatography eluting with CHCl_3 – CH_3OH (50:1, v/v). Yield 70%; mp 248–250 °C. ^1H NMR (300 MHz, DMSO- d_6): δ 1.02–1.32 (m, 5H), 1.50–1.53 (m, 1H), 1.67–1.71 (m, 2H), 1.85–1.88 (m, 2H), 2.69 (s, 3H), 2.74–2.81 (m, 1H), 6.63–6.65 (m, 1H), 6.97–6.99 (m, 1H), 7.29–7.34 (m, 1H), 7.37 (s, 1H), 7.42–7.47 (m, 2H), 7.92 (s, 1H), 8.07–8.09 (m, 2H), 8.20–8.21 (m, 1H), 8.48 (s, 1H), 9.39 (s, 1H), 11.55 (s, 1H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 24.9, 25.5, 29.4, 38.3, 62.0, 103.7, 104.5, 110.6, 110.7, 112.1, 116.8, 117.7, 122.8, 125.4, 126.0, 127.4, 128.5, 134.4, 135.2, 136.0, 150.1, 155.3, 162.7. HRMS calcd for $\text{C}_{27}\text{H}_{28}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 470.2009; found 470.2028.

6-(Methyl(phenyl)amino)-7-(4-phenylthiazol-2-ylamino)-quinoxalin-2(1H)-one (70). To a stirred solution of compound **2** (10 mmol) in THF (50 mL) was added K_2CO_3 (10 mmol) and *N*-methylaniline (10 mmol). The mixture was stirred under reflux for 8 h. The solvent was removed under reduced pressure to give compound **66a** that was used directly. Compound **66a** (1 g) was dissolved in a mixed solvent of THF (30 mL) and ethanol (30 mL), followed by the addition of 10% Pd/C (1 g) and HCOONH_4 (2 g). The mixture was stirred at room temperature for 3 h. The residue solid was filtered off, and the filtrate was concentrated in vacuo. Water (50 mL) was added to the resulting product and then was extracted by CH_2Cl_2 (3 $\times$ 50 mL). The organic layers were combined, dried over anhydrous MgSO_4 , and evaporated in vacuo. Intermediate **67a** was obtained as a yellow solid after chromatography through silica gel eluting with CHCl_3 – CH_3OH (30:1, v/v). Yield 68%. ^1H NMR (300 MHz, DMSO- d_6): δ 3.11 (s, 3H), 5.85 (s, 2H), 6.56–6.58 (m, 3H), 6.64–6.69 (m, 1H), 7.10–7.15 (m, 2H), 7.25 (s, 1H), 7.69 (s, 1H), 12.08 (s, 1H). HRMS calcd for $\text{C}_{15}\text{H}_{15}\text{N}_4\text{O}$ ($\text{M} + \text{H}^+$) 267.1240; found 267.1230.

Intermediate **67a** (1 mmol) and benzoyl isothiocyanate (1 mmol) were dissolved in dried acetone (30 mL). The reaction mixture was refluxed with stirring for 4 h and then concentrated to give compound **68a** without purification. Intermediate **68a** (1 mmol) and K_2CO_3 (1.5 mmol) in CH_3OH (20 mL)/ H_2O (5 mL) were heated to reflux for 1 h. Compound **69a** was obtained as a yellow solid after evaporation of the solvent and chromatography through silica gel eluting with CHCl_3 – CH_3OH (40:1, v/v). Yield 84%. ^1H NMR (300 MHz, DMSO- d_6): δ 2.07 (s, 3H), 6.65–6.67 (m, 2H), 6.73–6.78 (m, 1H), 7.14–7.19 (m, 2H), 7.47 (s, 1H), 7.87 (s, 2H), 8.02 (s, 1H), 8.52 (s, 1H), 9.23 (s, 1H), 12.42 (s, 1H). HRMS calcd for $\text{C}_{16}\text{H}_{16}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 326.1070; found 326.1055.

Compound **69a** (0.5 mmol) and 2-bromo-1-phenylethanone (0.5 mmol) were dissolved in ethanol (30 mL) and refluxed for 2 h.

After the reaction was completed, the solvent was evaporated in vacuo. The final product **70** was obtained as a light-yellow powder after purification by silica gel column chromatography eluting with CHCl_3 – CH_3OH (50:1, v/v). Yield 70%; mp 262–264 °C. ^1H NMR (300 MHz, DMSO- d_6): δ 3.15 (s, 3H), 6.62 (d, 2H, $J = 7.8$ Hz), 6.71–6.76 (m, 1H), 7.14–7.20 (m, 2H), 7.31–7.36 (m, 1H), 7.44–7.49 (m, 4H), 7.96 (d, 1H, $J = 1.8$ Hz), 8.11 (d, 2H, $J = 7.2$ Hz), 8.89 (s, 1H), 10.14 (s, 1H), 12.89 (s, 1H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 39.3, 102.3, 105.3, 114.0, 117.6, 126.1, 127.5, 127.6, 128.6, 128.7, 131.8, 133.4, 134.2, 140.7, 147.9, 149.5, 149.9, 155.6, 162.2. HRMS calcd for $\text{C}_{24}\text{H}_{20}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 426.1383; found 426.1361.

6-(Cyclohexyl(ethyl)amino)-7-(4-phenylthiazol-2-ylamino)-quinoxalin-2(1H)-one (71). Compound **71** was prepared in a similar manner to the synthesis of compound **70**, substituting *N*-ethylcyclohexanamine for *N*-methylaniline and DIPEA for K_2CO_3 , respectively. The final product **71** was obtained as a yellow powder after purification by silica gel column chromatography. Yield 82% (the last step); mp 247–249 °C. ^1H NMR (300 MHz, DMSO- d_6): δ 0.85 (t, 3H, $J = 6.6$ Hz), 1.00–1.19 (m, 5H), 1.49–1.52 (m, 1H), 1.66 (m, 2H), 1.91 (m, 2H), 2.80 (m, 1H), 3.07 (q, 2H, $J = 6.6$ Hz), 7.31–7.36 (m, 1H), 7.43–7.49 (m, 3H), 7.58 (s, 1H), 7.96 (s, 1H), 8.09 (d, 2H, $J = 7.8$ Hz), 8.60 (s, 1H), 9.84 (s, 1H), 12.77 (s, 1H). HRMS calcd for $\text{C}_{25}\text{H}_{28}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 446.2009; found 446.2026.

7-(4-Phenylthiazol-2-ylamino)-6-(piperidin-1-yl)quinoxalin-2(1H)-one (72). Compound **72** was prepared in a similar manner to the synthesis of compound **71**, substituting piperidine for *N*-ethylcyclohexanamine. The final product **72** was obtained as a yellow powder after purification by silica gel column chromatography. Yield 79% (the last step); mp 248–250 °C. ^1H NMR (300 MHz, DMSO- d_6): δ 1.55 (m, 2H), 1.78 (m, 4H), 2.81 (m, 4H), 7.33–7.35 (m, 1H), 7.43–7.49 (m, 4H), 7.96 (s, 1H), 8.06 (d, 2H, $J = 7.2$ Hz), 8.54 (s, 1H), 9.52 (s, 1H), 12.71 (s, 1H). HRMS calcd for $\text{C}_{22}\text{H}_{22}\text{N}_5\text{OS}$ ($\text{M} + \text{H}^+$) 404.1540; found 404.1549.

6-Morpholino-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (73). Compound **73** was prepared in a similar manner to the synthesis of compound **71**, substituting morpholine for *N*-ethylcyclohexanamine. The final product **73** was obtained as a yellow powder after purification by silica gel column chromatography. Yield 75% (the last step); mp 234–236 °C. ^1H NMR (300 MHz, DMSO- d_6): δ 2.86 (br s, 4H), 3.87 (br s, 4H), 7.30–7.35 (m, 1H), 7.43–7.48 (m, 2H), 7.50 (s, 1H), 7.54 (s, 1H), 7.97 (s, 1H), 8.07 (d, 2H, $J = 7.2$ Hz), 8.60 (s, 1H), 9.66 (s, 1H), 12.73 (s, 1H). HRMS calcd for $\text{C}_{21}\text{H}_{20}\text{N}_5\text{O}_2\text{S}$ ($\text{M} + \text{H}^+$) 406.1332; found 406.1329.

6-(4-Methylpiperazin-1-yl)-7-(4-phenylthiazol-2-ylamino)-quinoxalin-2(1H)-one (74). Compound **74** was prepared in a similar manner to the synthesis of compound **71**, substituting 1-methylpiperazine for *N*-ethylcyclohexanamine. The final product **74** was obtained as a yellow powder after purification by silica gel column chromatography. Yield 75% (the last step); mp 223–225 °C. ^1H NMR (300 MHz, DMSO- d_6): δ 2.89 (s, 3H), 3.15 (br s, 4H), 3.47 (br s, 4H), 7.34–7.36 (m, 1H), 7.43–7.48 (m, 2H), 7.53 (s, 1H), 7.58 (s, 1H), 7.99 (s, 1H), 8.08 (d, 2H, $J = 7.2$ Hz), 8.64 (s, 1H), 9.59 (s, 1H), 12.79 (s, 1H). HRMS calcd for $\text{C}_{22}\text{H}_{23}\text{N}_6\text{OS}$ ($\text{M} + \text{H}^+$) 419.1649; found 419.1670.

Ethyl-1-(2-oxo-7-(4-phenylthiazol-2-ylamino)-1,2-dihydroquinoxalin-6-yl)-piperidine-4-carboxylate (75). Compound **75** was prepared in a similar manner to the synthesis of compound **71**, substituting ethyl piperidine-4-carboxylate for *N*-ethylcyclohexanamine. The final product **75** was obtained as a yellow powder after purification by silica gel column chromatography. Yield 71% (the last step); mp 259–261 °C. ^1H NMR (300 MHz, DMSO- d_6): δ 1.21 (t, 3H, $J = 7.5$ Hz), 1.89–1.93 (m, 2H), 2.01–2.12 (m, 2H), 2.43 (m, 1H), 2.68–2.75 (m, 2H), 2.98–3.01 (m, 2H), 4.10 (q, 2H, $J = 7.5$ Hz), 7.30–7.35 (m, 1H), 7.42–7.45 (m, 2H), 7.48–7.49 (m, 2H), 7.96 (s, 1H), 8.06 (d, 2H, $J = 7.5$ Hz), 8.59 (s, 1H), 9.61 (s, 1H), 12.72 (s, 1H). HRMS calcd for $\text{C}_{25}\text{H}_{26}\text{N}_5\text{O}_3\text{S}$ ($\text{M} + \text{H}^+$) 476.1751; found 476.1772.

6-(4-(4-Fluorophenyl)piperazin-1-yl)-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (76). Compound 76 was prepared in a similar manner to the synthesis of compound 71, substituting 1-(4-fluorophenyl)piperazine for *N*-ethylcyclohexanamine. The final product 76 was obtained as a yellow powder after purification by silica gel column chromatography. Yield 77% (the last step); mp 245–247 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.02 (br s, 4H), 3.39 (br s, 4H), 7.00–7.08 (m, 4H), 7.33–7.36 (m, 1H), 7.43–7.48 (m, 2H), 7.50 (s, 1H), 7.57 (s, 1H), 7.98 (s, 1H), 8.07 (d, 2H, *J* = 7.5 Hz), 8.61 (s, 1H), 9.65 (s, 1H), 12.75 (s, 1H). HRMS calcd for C₂₇H₂₄FN₄OS (M + H⁺) 499.1711; found 499.1735.

6-(Dimethylamino)-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (77). Compound 77 was prepared in a similar manner to the synthesis of compound 71, substituting dimethylamine for *N*-ethylcyclohexanamine. The final product 77 was obtained as a yellow powder after purification by silica gel column chromatography. Yield 80% (the last step); mp 221–223 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.66 (s, 6H), 7.30–7.35 (m, 1H), 7.43–7.47 (m, 3H), 7.51 (s, 1H), 7.95 (s, 1H), 8.08 (d, 2H, *J* = 7.5 Hz), 8.68 (s, 1H), 9.99 (s, 1H), 12.74 (s, 1H). HRMS calcd for C₁₉H₁₈N₅OS (M + H⁺) 364.1227; found 364.1225.

6-(Diethylamino)-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (78). Compound 78 was prepared in a similar manner to the synthesis of compound 71, substituting diethylamine for *N*-ethylcyclohexanamine. The final product 78 was obtained as a yellow powder after purification by silica gel column chromatography. Yield 82% (the last step); mp 238–240 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.92 (t, 6H, *J* = 6.9 Hz), 2.98 (q, 4H, *J* = 6.9 Hz), 7.31–7.36 (m, 1H), 7.43–7.47 (m, 3H), 7.57 (s, 1H), 7.95 (d, 1H, *J* = 1.8 Hz), 8.09 (d, 2H, *J* = 7.5 Hz), 8.64 (s, 1H), 9.95 (s, 1H), 12.77 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 11.8, 48.7, 101.2, 104.9, 123.0, 126.0, 127.1, 127.5, 128.5, 130.4, 134.2, 134.3, 141.0, 147.4, 150.0, 155.6, 162.0. HRMS calcd for C₂₁H₂₂N₅OS (M + H⁺) 392.1540; found 392.1557.

6-(Dipropylamino)-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (79). Compound 79 was prepared in a similar manner to the synthesis of compound 71, substituting dipropylamine for *N*-ethylcyclohexanamine. The final product 79 was obtained as a yellow powder after purification by silica gel column chromatography. Yield 69% (the last step); mp 215–217 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.81 (t, 6H, *J* = 7.5 Hz), 1.30–1.42 (m, 4H), 2.89 (t, 4H, *J* = 7.5 Hz), 7.31–7.36 (m, 1H), 7.43–7.49 (m, 3H), 7.61 (s, 1H), 7.95 (d, 1H, *J* = 2.1 Hz), 8.08 (d, 2H, *J* = 7.2 Hz), 8.59 (s, 1H), 9.79 (s, 1H), 12.75 (s, 1H). HRMS calcd for C₂₃H₂₆N₅OS (M + H⁺) 420.1853; found 420.1872.

6-(Cyclohexyloxy)-7-(4-phenylthiazol-2-ylamino)quinoxalin-2(1H)-one (80). Compound 80 was prepared in a similar manner to the synthesis of compound 71, substituting sodium cyclohexanolate for *N*-ethylcyclohexanamine. The final product 80 was obtained as a pale-yellow solid after purification by silica gel column chromatography. Yield 78% (the last step); mp 215–216 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.22–1.44 (m, 4H), 1.53–1.60 (m, 2H), 1.76–1.80 (m, 2H), 2.02–2.05 (m, 2H), 4.47 (m, 1H), 7.30–7.36 (m, 2H), 7.43–7.51 (m, 2H), 7.85–7.87 (m, 1H), 7.96 (s, 1H), 8.08 (d, 2H, *J* = 7.5 Hz), 8.73 (s, 1H), 9.76 (s, 1H), 12.75 (s, 1H). HRMS calcd for C₂₃H₂₃N₄O₂S (M + H⁺) 419.1536; found 419.1553.

7-(Cyclohexyl(methyl)amino)-6-(4-phenylthiazol-2-ylamino)-2H-benzo[b][1,4]oxazin-3(4H)-one (85). Compound 85 was prepared in a similar manner to the synthesis of compound 51, substituting HOCH₂COOCH₃ for NH₂CH(CH₃)COOCH₃·HCl. The final product 85 was obtained as a light-yellow powder after purification by silica gel column chromatography. Yield 86% (the last step); mp 205–206 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.07–1.24 (m, 5H), 1.53–1.80 (m, 5H), 2.54 (s, 3H), 2.61 (m, 1H), 4.52 (s, 2H), 6.84 (s, 1H), 7.28–7.32 (m, 2H), 7.39–7.44 (m, 2H), 7.97–8.01 (m, 3H), 9.09 (s, 1H), 10.90 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 25.0, 25.5, 29.3, 37.6, 61.8, 66.8, 103.0, 106.0, 111.3, 123.4, 125.9, 127.4, 128.4, 131.9, 134.5, 136.0, 137.7,

149.9, 163.4, 165.0. HRMS calcd for C₂₄H₂₇N₄O₂S (M + H⁺) 435.1849; found 435.1868.

7-(Cyclohexyl(methyl)amino)-6-(4-phenylthiazol-2-ylamino)-2H-benzo[b][1,4]thiazin-3(4H)-one (86). To a stirred solution of compound 46 (10 mmol) in THF (50 mL) was added DIPEA (10 mmol) and HSCH₂COOCH₃ (10 mmol). After vigorously stirring at 65 °C until the total disappearance of the starting materials, compound 81b was obtained without purification. Compound 81b (5 mmol) and SnCl₂·2H₂O (75 mmol) were dissolved in ethanol (75 mL). Then 15 equiv of 12 mol/L hydrochloric acid was added and the mixture was stirred under reflux for 3 h. The solvent was then evaporated. The residue was neutralized with 40% NaOH until the pH reached 8. The mixture was filtered, and the filtrate was extracted with 100 mL of CHCl₃ twice. The organic solvent was washed with brine, dried over anhydrous sodium sulfate, and evaporated in vacuo. The intermediate 82b was obtained as a white solid after purification by silica gel column chromatography. Yield 49%. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.11–1.18 (m, 3H), 1.21–1.28 (m, 2H), 1.53 (m, 1H), 1.67–1.70 (m, 4H), 2.48 (s, 3H), 2.61–2.64 (m, 1H), 4.86 (s, 2H), 6.32 (s, 1H), 6.81 (s, 1H), 10.20 (s, 1H). HRMS calcd for C₁₅H₂₂N₃OS (M + H⁺) 292.1478; found 292.1472.

Intermediate 82b (1 mmol) and benzoyl isothiocyanate (1 mmol) were dissolved in dried acetone (30 mL). The reaction mixture was refluxed with stirring for 4 h and then concentrated to give compound 83b without purification. Intermediate 83b (1 mmol) and K₂CO₃ (1.5 mmol) in CH₃OH (20 mL)/H₂O (5 mL) were heated to reflux for 1 h. After evaporation of the solvent in vacuo, compound 84b was obtained as a white solid after chromatography through silica gel eluting with CHCl₃–CH₃OH (20:1, v/v). Yield 60%. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.06–1.18 (m, 3H), 1.23–1.34 (m, 2H), 1.51–1.54 (m, 1H), 1.68–1.71 (m, 4H), 2.53 (s, 3H), 2.62–2.65 (m, 1H), 3.42 (s, 2H), 7.00 (s, 1H), 7.46 (s, 1H), 7.60 (s, 2H), 8.78 (s, 1H), 10.40 (s, 1H). HRMS calcd for C₁₆H₂₃N₄O₂S (M + H⁺) 351.1308; found 292.1294.

84b (0.5 mmol) and 2-bromo-1-phenylethanone (0.5 mmol) were dissolved in ethanol (30 mL) and refluxed for 2 h. After the reaction was completed, the solvent was evaporated in vacuo. The final product 86 was obtained as a yellow powder after purification by silica gel column chromatography eluting with CHCl₃–CH₃OH (40:1, v/v). Yield 81%; mp 249–250 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.06–1.24 (m, 5H), 1.49–1.52 (m, 1H), 1.65–1.69 (m, 2H), 1.78–1.82 (m, 2H), 2.56 (s, 3H), 2.64 (m, 1H), 3.43 (s, 2H), 7.15 (s, 1H), 7.28–7.34 (m, 2H), 7.40–7.45 (m, 2H), 7.99 (d, 2H, *J* = 7.5 Hz), 8.13 (s, 1H), 9.24 (s, 1H), 10.75 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 24.9, 25.4, 29.2, 29.3, 37.9, 61.8, 103.6, 106.5, 110.3, 122.2, 125.9, 127.4, 128.4, 134.3, 134.4, 135.7, 136.1, 150.1, 162.7, 165.4. HRMS calcd for C₂₄H₂₇N₄O₂S (M + H⁺) 451.1621; found 451.1643.

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.¹⁸ The activity of the luciferase reporter is proportional to HCV RNA levels. Briefly, the replicon cells were seeded into two identical sets of 96-well plates at a density of 5000/well in 100 μL of DMEM without G418 overnight. Compounds were solubilized in DMSO and then dilutions prepared in DMEM and added to the wells. Following 72 h 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 cytotoxicity 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 inhibiting HCV RNA replication activity by 50%), IC₅₀ (concentration decreasing cell viability by 50%), and SI₅₀ (selective index which is calculated by IC₅₀/EC₅₀) values.

■ ASSOCIATED CONTENT

S Supporting Information. The general procedure for the synthesis of intermediates **50b–d**, **69b–k**, and **84a**. NMR spectra and HPLC analysis results of all the final compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ ABBREVIATIONS USED

HCV, hepatitis C virus; DFDNB, 1,5-difluoro-2,4-dinitrobenzene; DIPEA, diisopropylethylamine; NOESY, nuclear Overhauser effect spectroscopy; THF, tetrahydrofuran; EC_{50} , concentration of compound inhibiting HCV replicon by 50%; IC_{50} , concentration of compound decreasing cell viability by 50%; SI, selectivity index; SAR, structure–activity relationship

■ ADDITIONAL NOTE

^a Telaprevir and boceprevir were approved by USA FDA recently for treatment of HCV infection.

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