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Design, synthesis, and AMPA receptor antagonistic activity of a novel 6-nitro-3-oxoquinoxaline-2-carboxylic acid with a substituted phenyl group at the 7 position

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Abstract—We describe the design, synthesis, and biological properties of a novel series of 7-substituted 6-nitro-3-oxoquinoxaline-2-carboxylic acids. After designing, studying the structure–activity relationships, and evaluating the properties of various compounds, we found that 7-heterocyclic-6-nitro-3-oxoquinoxaline-2-carboxylic acids that contain a substituted phenyl group linked through urethane at the 7 position possess good α-amino-3-hydroxy-5-methylisoxazole propionate receptor (AMPA-R) antagonistic activity. Among the compounds tested, compound **29p** (**GRA-293**), which has a 4-carboxy group on the terminal phenyl moiety, exhibited high potency and selectivity for the AMPA-R in vitro and good neuroprotective efficacy in vivo. It also showed good aqueous solubility.

1. Introduction

Glutamic acid, an excitatory amino acid (EAA), is a major excitatory neurotransmitter in the mammalian central nervous system. Postsynaptic EAA receptors can be divided into two main subtypes, namely ionotropic glutamate receptors (iGlu-Rs), such as *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole propionate (AMPA) and kinate (KA), and metabotropic glutamate receptors (mGlu-Rs). The iGlu-Rs are associated with integral cation-specific ion channels, which modulate cell excitability by gating the flow of calcium and sodium ions into the cell.[1] However, overstimulation of postsynaptic receptors by EAA is known to worsen neurodegeneration in conditions such as ischemic stroke, epilepsy, Huntington's disease, head trauma, Parkinson's disease, and Alzheimer's disease.[2–8]

Since the discovery of NBQX,[2] a potent and selective AMPA receptor (AMPA-R) antagonist, many quinoxalinedione compounds with competitive AMPA-R antagonistic activity have been synthesized and tested against each of the EAA receptor subtypes. Although the AMPA-R antagonists do not produce side effects such as schizophrenia,[9] and effectively protect against neuronal death even in postischemic animal models,[2] none of these compounds have yet been marketed as therapeutic agents for conditions such as acute cerebral ischemia. The previously described AMPA-R antagonistic quinoxalinedione derivatives can be divided into first generation agents (e.g., NBQX and YM-90K[10,11]) and second generation agents (e.g., YM-872 [zonampanel][12,13]). Both the first and second generation agents are potent, selective AMPA-R antagonists, and have been shown to exhibit good neuroprotective effects in animal models of global and focal cerebral ischemia (Fig. 1).[2,11,13] Unfortunately, the first-generation compounds caused renal toxicity as a result of their physicochemical properties, particularly very low solubility in water,[14] and were thus rejected after reaching clinical trials. In second generation agents such as YM-872, these undesirable physicochemical properties have been ameliorated by introducing a hydrophilic functional group (such as acetic acid) into the quinoxalinedione skeleton. Because of its good aqueous solubility, YM-872 has not caused renal toxicity and, together with tissue plasminogen

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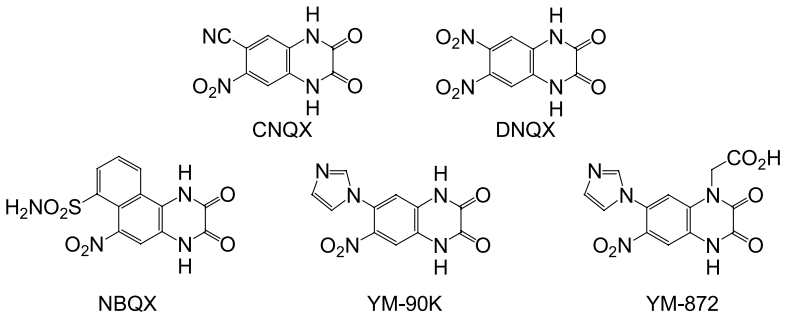


Figure 1. Known competitive AMPA-R antagonists.

activator (t-PA), is the only agent for the treatment of cerebral ischemia to have reached the clinical trial stage. When we started to search for AMPA-R antagonists, other researchers had already advanced the development of several improved quinoxalinediones with good solubility, and therefore there appeared to be little scope for developing new second generation AMPA-R antagonists based on chemical modifications of the quinoxalinedione structure. Thus, we refocused our research efforts on designing and synthesizing novel third generation compounds, which would not have a quinoxalinedione skeleton, but would have potent AMPA-R affinity, strong neuroprotective effects in vivo and be water soluble, thus allowing them to be administered by injection.

In an AMPA-R pharmacophore model, three important interaction units have already been identified for the binding of quinoxalinediones, namely (1) the 2,3-dione moiety of quinoxalinedione is needed to form a coulombic interaction between the tautomeric 2-oxo moiety and the AMPA-R, (2) a hydrogen bond donor at 4 position of the quinoxalinedione molecule is needed to interact with the AMPA-R, and (3) the nitro group at 6 position undergoes a specific interaction with the AMPA-R.[15] However, it also appeared to us that the acetic acid group introduced into the quinoxalinedione skeleton of YM-872 resulted in improved affinity for the AMPA-R compared with YM-90K. We therefore decided that an acetic acid group on the quinoxaline skeleton would affect the ability of the compound to interact with the AMPA-R as well as improve the water solubility. With these attributes in mind, we concluded that 3-oxoquinoxaline-2-carboxylic acids bearing a hydrophilic group on a quinoxaline nucleus, instead of a quinoxalinedione structure with a hydrophilic group, would provide a good nucleus for a new generation of AMPA-R antagonists with both good AMPA-R activity and solubility. Consequently, we designed and synthesized a series of new compounds based on 7-substituted-6-nitro-3-oxo-quinoxaline-2-carboxylic acids (Fig. 2). In this paper, we describe the design and synthesis of these novel 7-substituted 6-nitro-3-oxoquinoxaline-2-carboxylic acid derivatives and their biological effects.

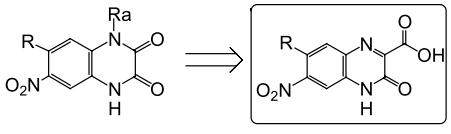
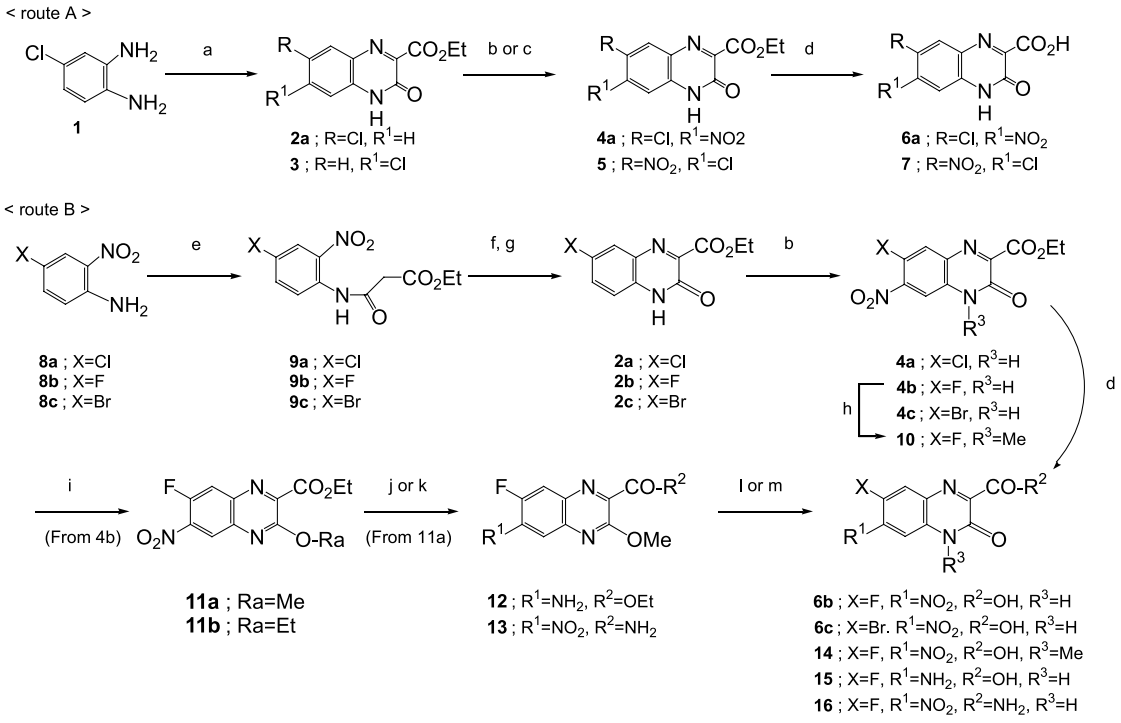


Figure 2. Synthetic targets for a new generation of AMPA-R antagonists.

2. Chemistry

The 7-halogeno-6-nitro-3-oxoquinoxaline-2-carboxylic acid derivatives were prepared using two methods (routes A and B), as shown in Scheme 1. In the case of the 7-chloro compound produced via route A, 4-chloro-1,2-phenylenediamine (**1**) was condensed with diethyl ketomalonate, then separated by column chromatography to give ethyl 7-chloro-3-oxoquinoxaline-2-carboxylate (**2a**) and its isomer (**3**). The esters **2a** and **3** were converted to the 6- and 7-nitro compounds (**4a** and **5**) by selective nitration (**2a**, fuming HNO_3, AcOH; **3**, KNO_3, concd H_2SO_4) followed by hydrolysis to give 6- and 7-nitro carboxylic acids (**6a** and **7**, respectively). Compound **6a** could also be synthesized from 4-chloro-2-nitroaniline (**8a**) using an improvement of the standard procedure,[16] as shown in route B. The nitroaniline **8a** was treated with ethyl 3-chloro-3-oxopropionate, followed by intramolecular cyclization and deoxygenation to give ethyl 7-chloro-3-oxoquinoxaline-2-carboxylate (**2a**); the chemical shift and coupling constants seen on ^1H NMR spectroscopy indicated that this product was consistent with compound **2a**, as obtained from 4-chloro-1,2-phenylenediamine (**1**) and diethyl ketomalonate via route A. In the case of the 7-fluoro and 7-bromo derivatives, quinoxalines **2b** and **c** were prepared from 4-fluoro- and 4-bromo-2-nitroaniline (**8b** and **c**) as shown, following the same procedures used to produce ethyl 7-chloro-3-oxoquinoxaline-2-carboxylate (**2a**) from **8a**. These intermediates were then converted to the nitro compounds **4b** and **c** by selective nitration (fuming HNO_3, AcOH) at the 6 position, followed by hydrolysis to give the 7-fluoro and 7-bromo carboxylic acids (**6b** and **c**, respectively).

A quinoxaline methylated at the 4 position (**14**) was prepared from the 6-nitro compound **4b** by methylation with iodomethane, followed by hydrolysis of the ester group. The 6-amino compound **15** and 2-carboxamide compound **16** were prepared from the imino ether **11a**, which reacted with the 7-F ester **4b** and iodomethane in the presence of silver(I) oxide. Hydrogenation or amidation of the imino ether **11a** followed by hydrolysis

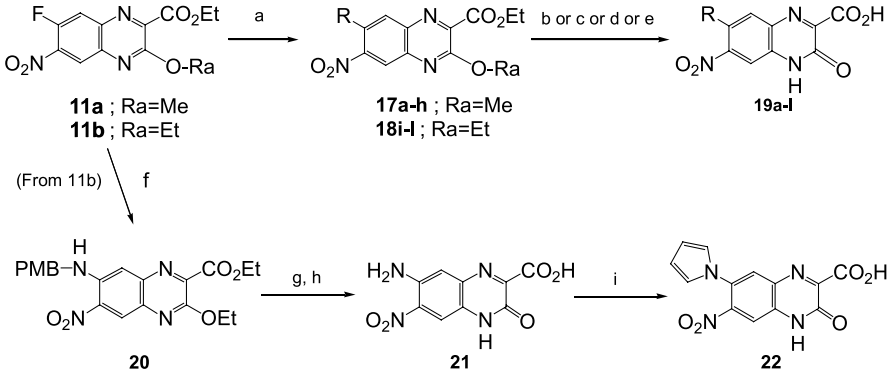


Scheme 1. 7-Halogeno-6-nitro-3-oxoquinoxaline-2-carboxylic acid derivatives. Reagents: (a) Diethyl ketomalonate, EtOH and then separation by chromatography; (b) fuming HNO_3, AcOH; (c) KNO_3, concd H_2SO_4; (d) 1 N KOH, EtOH; (e) ethyl 3-chloro-3-oxopropionate, toluene or Et_3N-DMF; (f) KO^{t}Bu, DMF; (g) PBr_3, DMF; (h) NaH, MeI, DMF; (i) MeI or EtBr, Ag_2O, toluene; (j) H_2, 10% Pd–C, EtOH; (k) NH_4OH, MeOH; (l) 1 N NaOH, EtOH followed by 48% HBr, AcOH; (m) 48% HBr or concd HCl, AcOH.

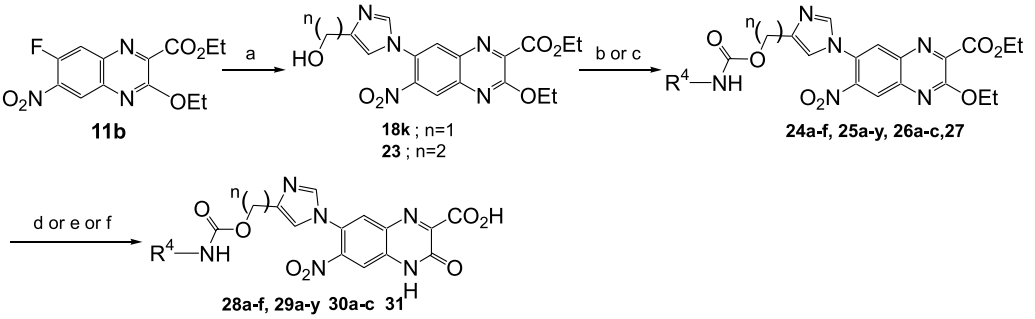
gave compounds **15** and **16**. Treatment of the 7-F ester **4b** with bromoethane instead of iodomethane in the presence of silver(I) oxide yielded the imino ether **11b**.

The syntheses of the novel 7-heterocyclic-6-nitro-3-oxoquinoxaline-2-carboxylic acid derivatives are outlined in Scheme 2. The imino ethers **11a** or **11b**, derived from the 7-fluoro ester **4b**, were reacted with commercially available amines (R'_2-NH) such as dialkyl, alicyclic, and heterocyclic amines in THF, MeCN, or DMF to produce nucleophilic substitution of the fluoro group at the 7 position, followed by hydrolysis to form the 7-dialkyl, alicyclic, and heterocyclic amino substituted derivatives **19a**–**l**. The imino ether **11b** was also treated with 4-methoxybenzylamine in THF, followed by deprotection of both the 4-methoxybenzyl (PMB) group and the imino ether, then hydrolyzed to give the 7-amino compound **21**. The 7-pyrrolyl compound **22** was prepared from the 7-amino compound **21** using 2,5-dimethoxytetrahydrofuran in AcOH.

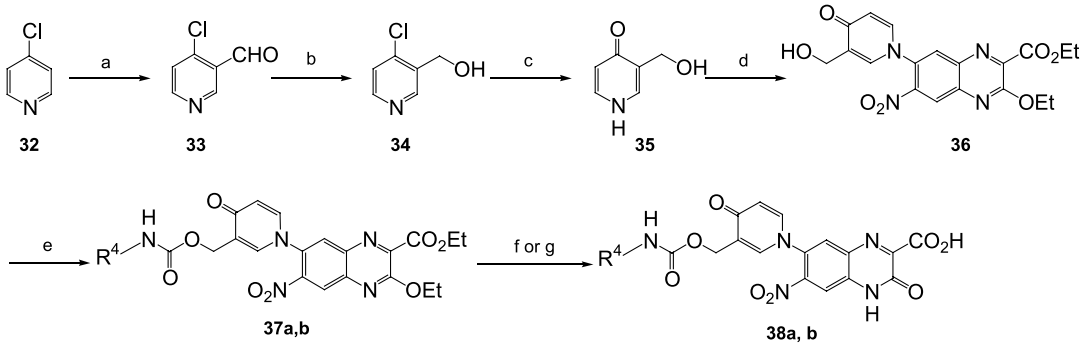
Next, 4-urethane linked imidazole derivatives (**28a**–**f**, **29a**–**y**, and **30a**–**c**) were prepared from the key intermediate 7-[4-(hydroxymethyl)imidazolyl]quinoxaline (**18k**),



Scheme 2. 7-Heterocyclic-6-nitro-3-oxoquinoxaline-2-carboxylic acid derivatives. Reagents: (a) (R'_2-NH), Et_3N or none, THF or MeCN or DMF; (b) concd HCl; (c) KOH or NaOH, EtOH–H_2O followed by 3 N HCl or 48% HBr; (d) concd HCl or 48% HBr, AcOH; (e) concd HCl, AcOH followed by LiOH, H_2O; (f) 4-methoxybenzyl amine, Et_3N, THF; (g) CF_3CO_2H, anisole; (h) 1 N NaOH, EtOH followed by concd HCl, EtOH; (i) 2,5-dimethoxytetrahydrofuran, AcOH.



Scheme 3. 4-Urethane linked imidazole derivatives. (a) 4-[HO(CH$_2$)$_n$]imidazole, Et$_3$N, MeCN or DMA; (b) R^4-NCO, CH$_2$Cl$_2$ or benzene or MeCN; (c) R^{4}CO$_2$H, DPPA, Et$_3$N, benzene; (d) 48% HBr or concd HCl, AcOH; (e) concd HCl, AcOH followed by 1 N LiOH or 2 N NaOH; (f) 1 N KOH, EtOH followed by 48% HBr.



Scheme 4. 3-Urethane linked pyridone derivatives. (a) *n*-BuLi, ^{i}Pr$_2$NH, THE followed by DMF; (b) NaBH$_4$, EtOH; (c) NaOH, H$_2$O; (d) compound **11b**, DMF; (e) R^4-NCO, CH$_2$Cl$_2$; (f) consd HCl, AcOH; (g) concd HCl, AcOH followed by LiOH, H$_2$O.

which was synthesized from imino ether **11b** and 4-(hydroxymethyl)imidazole. Treatment with various isocyanates followed by hydrolysis provided the corresponding 4-urethane linked derivatives shown in Scheme 3. Compound **31** was also prepared from 7-[4-(2-hydroxyethyl)imidazolyl]quinoxaline (**23**), which was obtained from imino ether **11b** using 4-(2-hydroxyethyl)imidazole[17] instead of 4-(hydroxymethyl)imidazole in the above procedure.

The 3-urethane linked pyridone derivatives **38a** and **b** were prepared from 7-[3-(hydroxymethyl)-4-pyridon-1-yl]quinoxaline (**36**) as the key intermediate following the same procedure as for the imidazole derivatives. 3-(Hydroxymethyl)-4-pyridone (**35**), the starting material for the heterocycle at the 7 position, was synthesized by hydroxylation of 3-(hydroxymethyl)-4-chloropyridine (**34**) with NaOH solution. This was in turn prepared by lithiation of 4-chloropyridine (**32**) followed by treatment with *N*,*N*-dimethylformamide and reduction with sodium borohydride (Scheme 4).

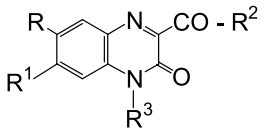
3. Results and discussion

The structures and characteristics of the 7-substituted-6-nitro-3-oxoquinoxaline-2-carboxylic acid derivatives are shown in Tables 1–6.

3.1. 7-Halogeno derivatives

The structures and AMPA-R affinities of the 7-halogeno compounds are shown in Table 1. Previous studies on quinoxalinedione compounds with known AMPA-R antagonistic activity[10,13] have indicated that a nitro group at the 6 position on the quinoxalinedione nucleus confers high affinity for the AMPA-R. To provide initial confirmation that the 3-oxoquinoxaline-2-

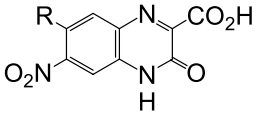
Table 1. 7-Halogenated derivatives

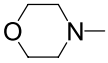
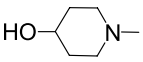
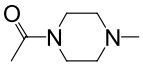
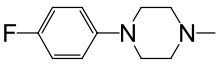
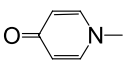


Compound	R	R^1	R^2	R^3	AMPA-R affinity[25,26] K_i (nM)
6a	Cl	NO$_2$	OH	H	1300
7	NO$_2$	Cl	OH	H	6700
4a	Cl	NO$_2$	OEt	H	>10,000
6b	F	NO$_2$	OH	H	2000
6c	Br	NO$_2$	OH	H	690
14	F	NO$_2$	OH	Me	>10,000
15	F	NH$_2$	OH	H	>10,000
16	F	NO$_2$	NH$_2$	H	>10,000

carboxylic acid nucleus would be suitable for the generation of AMPA-R antagonists, we introduced a nitro group by carrying out electron-withdrawing at the 6 or 7 position in the 3-oxoquinoxaline-2-carboxylic acid. Accordingly, 3-oxoquinoxaline-2-carboxylic acid, which has a nitro group at the 6 position (**6a**), exhibited good AMPA-R affinity. However, 6-chloro-7-nitro-3-oxoquinoxaline-2-carboxylic acid (**7**), which contains anti-substituted compounds at the 6 and 7 positions, was approximately 5-fold less potent than the parent compound **6a**. These binding-affinity results can be ascribed to the presence of a chloro group, a weak electron-withdrawing group, rather than a nitro group at the 6 position. On the other hand, replacing the halogen atoms at the 7 position (fluorine [**6b**] or bromine [**6c**]) produced nearly the same activity as in **6a**. An electron-donating group such as an amino group (**15**) at the 6 position in the 3-oxoquinoxaline-2-carboxylic acid also resulted in loss of AMPA-R affinity. In particular, in the 3-oxoquinoxaline-2-carboxylic acids with a nitro group at the 6 position, conversion of the carboxyl group to an ester group (**4a**) or an amide group (**16**) and replacement of the intramolecular amide moiety by a methyl group (**14**) resulted in markedly decreased AMPA-R affinity. These results confirmed that potent AMPA-R affinity depended on the presence of a strong electron-withdrawing group at the 6 position, a carboxyl group at the 2 position, and a hydrogen atom at the 4 position of the 3-oxoquinoxaline-2-carboxylic acid.

Table 2. 7-Substituted amino and 7-heterocyclic 2-quinoxalinecarboxylic acid derivatives



Compound	R	AMPA-R affinity[25,26] K_i (nM)
19a	$(CH_3)_2N$–	910
19b		3800
19c		2000
19d		2500
19e		3700
19f		2600
19g		330
19h		560
22		920

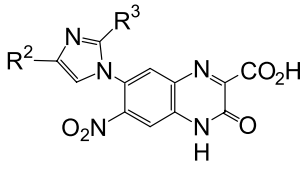
3.2. 7-Dialkyl, alicyclic, and heterocyclic amino derivatives

As the next approach, based on the results obtained with the 7-halogeno-3-oxoquinoxaline-2-carboxylic derivatives, we selected a compound with a nitro group at the 6 position and performed chemical modifications which introduced a variety of amines (such as dialkyl, alicyclic, and heterocyclic amines) at the 7 position. The structures and AMPA-R affinities of these 7-(substituted)amino compounds are shown in Table 2. The dialkyl and alicyclic amino derivatives (**19a–f**) showed similar AMPA-R affinity to the 7-halogeno derivatives. However, introduction of a heterocyclic amine, as in the 4-pyridon-1-yl (**19g**), imidazol-1-yl (**19h**), and pyrrol-1-yl (**22**) compounds considerably enhanced AMPA-R binding affinity. Consequently, substitution of a flat heterocyclic structure bearing π-electrons at the 7 position on the 6-nitro-3-oxoquinoxaline-2-carboxylic acid appears to be essential for increasing AMPA-R affinity.

3.3. 7-(Substituted)imidazole derivatives and their molecular modeling

Next, we compared the effects of substitution onto the imidazole moiety on AMPA-R affinity using commercially available imidazoles. Compared to a nonsubstituted imidazole, AMPA-R affinity was reduced by the introduction of substituents at the 2 or 4 position on the imidazole moiety (**19h** vs **19i–19l**). Substitution at the 2 position produced a particularly marked decrease in AMPA-R affinity. Among the compounds with substituents at the 4 position, however, the carboxymethyl compound **19l** maintained AMPA-R affinity equal to that of the nonsubstituted imidazole compound **19h.** Compounds **19j** and **19k** showed decreased activity compared to **19h**. Interestingly, the AMPA-R affinity of compound **19j** was increased by introducing a carboxy group onto the 4-methyl group on the imidazole ring. From these results, we concluded that a carboxymethyl group on the imidazole moiety may facilitate interaction

Table 3. 7-Substituted imidazole derivatives



Compound	R^2	R^3	AMPA-R affinity[25,26] K_i (nM)
19h	H	H	560
19i	H	CH_3	>10,000
19j	CH_3	H	1300
19k	CH_2OH	H	2600
19l	CH_2CO_2H	H	530

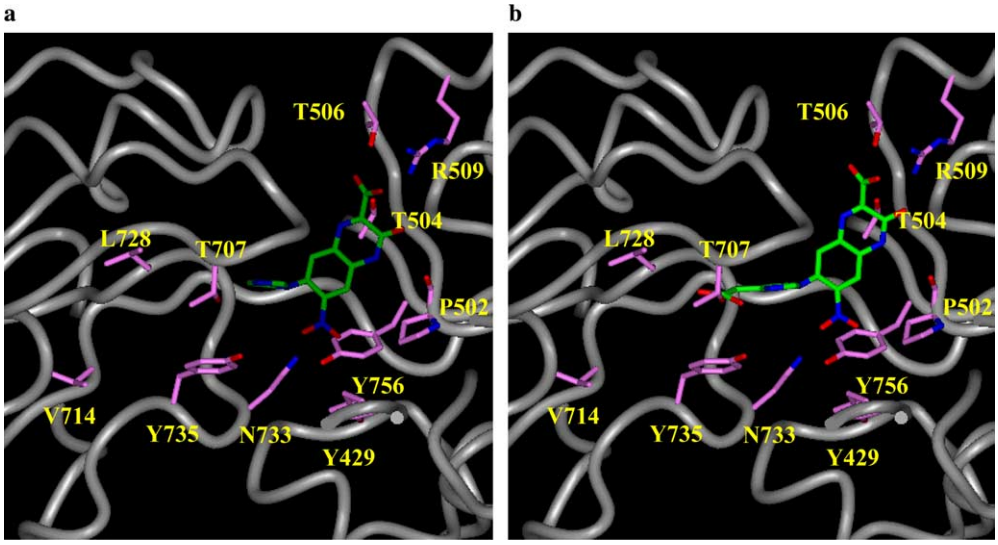


Figure 3. Modeled structure of AMPA-R bound to ligands. (a) The bound conformation of **19h** in the active site of the modeled AMPA-R. (b) The bound conformation of compound **19l** in the active site of modeled AMPA-R.

with the AMPA-R, and that a carboxyl group would be needed as a proton acceptor and/or donor to interact with the AMPA-R (Table 3).

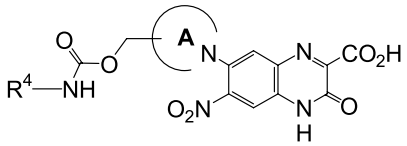
Based on the binding-affinity results, we studied the impact of structural conformation on molecular modeling. We divided the structure of compound **19h** into three parts (Fig. 3a). The first part was the 3-oxo-2-carboxylic acid moiety of the 6-nitro-3-oxoquinoxaline-2-carboxylic acid. This interacts with the AMPA-R through two potential hydrogen bonds to Thr504 and Thr506, and an ion pair to Arg509. The second part consists of the amide nitrogen of **19h**, which forms a hydrogen bond with the backbone carbonyl group of Pro502. The third part consists of the 6-nitro group on the quinoxaline ring, which forms a hydrogen bond with the side chains of Tyr756 and Asn733. These modeling results convinced us that the three individual parts of the molecule were essential for interaction of the AMPA-R with 6-nitro-3-oxoquinoxaline-2-carboxylic acids. In the model, the 7-imidazole ring of compound **19h** lies near the side chain of Thr707, and the nitrogen atom at the 3 position on the 7-imidazole ring could form a weak hydrogen bond with the hydroxyl group of Thr707.

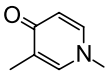
We next attempted a docking study, in which compound **19l** (which possesses a carboxymethyl group at the 4 position on the imidazole ring) was superimposed on the modeled AMPA-R structure (Fig. 3b). Because compound **19l** had greater AMPA-R affinity compared to compounds **19k** and **19j**, we concluded that the carbonyl oxygen of its carboxymethyl group produced the most favorable interaction with the side chain of Thr707. Moreover, we believed that it would be possible to increase the AMPA-R affinity of compound **19l** by introducing substituents at the 4 position on the imidazole ring directed toward another interaction site, which includes Val714, Leu728, and Tyr735. Because this region is predominantly hydrophobic in nature, lipophilic groups joined through various links with hydrogen acceptors would be the most favorable substituents at the 4 position on the imidazole group.

3.4. 7-Urethane linked imidazole derivatives

Based on the information yielded by molecular modeling, we synthesized a series of carbonyl compounds with urethane-containing groups attached through the hydroxymethyl group on the imidazole moiety of **19k**, which could in turn be readily synthesized. The structures and affinities of these 7-urethane linked imidazole derivatives for the AMPA-R and NMDA-R are shown in Table 4. We found that compounds with the urethane linkage exhibited more potent AMPA-R affinity than the nonsubstituted compound **19h** and alcohol and the carboxylic compounds **19k** and **l**. In particular, urethane compounds with a terminal lipophilic ring, such as a cyclohexyl (**28c**) or phenyl ring (**28d**) exhibited good AMPA-R affinity and selectivity. However, when the methylene chain between the urethane linkage and the terminal phenyl group was extended, AMPA-R affinity was reduced (**28e** and **f**). We also attempted the introduction of a similar substituent group, a phenyl group linked through a urethane moiety, to a 4-pyridone compound (**19g**), which exhibited good AMPA-R affinity. Despite having the same urethane-linked phenyl group as a substituent at the 7 position on the quinoxaline ring, the 4-pyridone compound **38a** showed weaker AMPA-R affinity than the imidazole compound **28d**. Therefore, we concluded that an imidazole group at the 7 position on the quinoxaline ring is optimal, and that the imidazole group is needed to confer functional activity through the urethane linkage to enhance AMPA-R affinity. We further concluded that the urethane moiety would function in a similar way to the carboxyl group of compound **19l**, and that a terminal lipophilic ring (such as phenyl ring) would be needed to allow good interaction with the AMPA-R, as suggested by molecular modeling.

Table 4. 7-Urethane linked imidazole/pyridone derivatives



Compound	Ring A	R^4	AMPA-R affinity[25,26] K_i (nM)	NMDA-R affinity[27] K_i (nM)	Selectivity $\left(\frac{\text{NMDA-R}}{\text{AMPA-R}}\right)$
28a		n-Bu	82	8900	110
28b		iso-Pr	100	NT	NT
28c		cycl.Hex	39	>10,000	>260
28d		Ph	86	>10,000	>120
28e[a]		$PhCH_2$	67	7200	110
28f		$PhCH_2CH_2$	170	NT	NT
38a		Ph	170	>10,000	>59

NT, not tested.
[a] Na salt.

3.5. Confirmation of AMPA-R binding of an imidazole with a 7-urethane linked phenyl group (28d) by molecular modeling

A model of the binding between compound **28d** and the AMPA-R is shown in Figure 4a. The quinoxaline nucleus of compound **28d** exhibited the same interactions as in the three essential parts of compound **19h**. Moreover, we confirmed that the ether oxygen on the urethane linkage of compound **28d** formed a hydrogen bond with the side chain of Thr707, and that the terminal phenyl moiety interacted with the side chains of Val714, Leu728, and Tyr735. A substituent introduced at the 4 position on the imidazole group produced a significant increase in AMPA-R affinity when a urethane linkage was present. Indeed, AMPA-R affinity increased to a subnanomolar level, 7-fold stronger than that of compound **19h**. Therefore, it became clear that a urethane linkage and a terminal lipophilic ring, such as a phenyl ring, are needed for good interaction with AMPA-R. Our structural modeling experiments on the binding of the AMPA-R to essential components of compound **19h**, thus, eventually led to the synthesis of compound **28d**, which shows high AMPA-R affinity. This implies that rational drug design using homology modeling and docking strategies is useful.

Recently, the soluble ligand binding core of GluR2 (GluR2-S1S2J) was created and the X-ray crystallography structure of the S1S2J-DNQX complex (PDB entry: 1FTL) was determined as shown in Figure 4b.[18] When we compared our model structure with the X-ray structure, several differences were apparent in some of the loop regions, which were generally difficult to model correctly. However, the amino acid residues near the ligand-binding regions in our model structure were con-

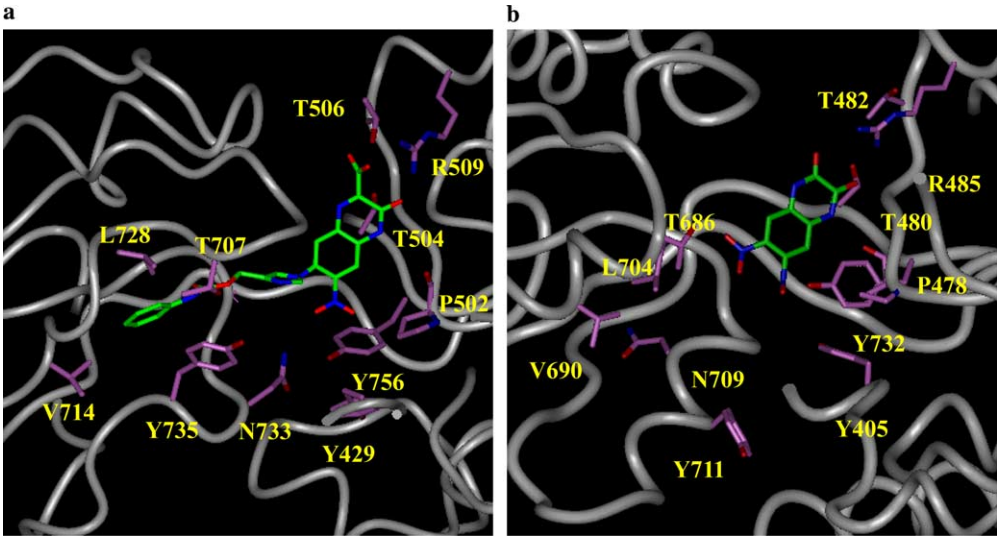


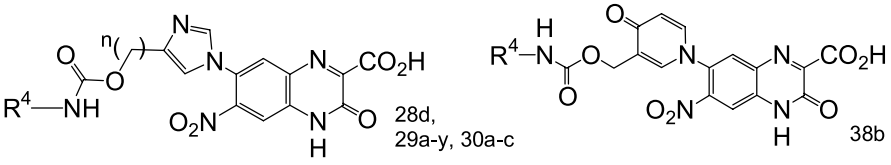
Figure 4. Modeled and X-ray structures of AMPA-R bound to ligands. (a) The bound conformation of **28d** in the active site of the modeled AMPA-R. (b) The bound conformation of DNQX in the X-ray structure of AMPA-R active site.

sistent with those seen in the X-ray structure. This confirmed that our AMPA-R model structure was useful for the design of novel, highly potent inhibitors of the AMPA-R.

3.6. 7-Urethane linked imidazole derivatives with a substituted phenyl group

Next, we investigated the effects of substituents in the terminal phenyl group attached through the urethane linkage in order to identify an active compound with high AMPA-R affinity and better selectivity for the AMPA-R than the NMDA-R (Table 5). There was a significant increase in AMPA-R affinity after the introduction of various substituents into the terminal phenyl ring. Among these modifications, mono-substitution at the 2 or 3 position resulted in significantly better selectivity, together with good AMPA-R affinity, except for the 2-carboxylic compound **29n**, which was inferior to the other compounds. Although the reason for this is not clear, we assume that the interaction of terminal phenyl group on the compound **29n** against the AMPA-R might be different from other carboxylic compounds (**29o**, **p**) because of intramolecular hydrogen bonding formed between 2-carboxy group and urethane moiety. In particular, compounds with strong electron-withdrawing groups substituted at the 3 or 4 position exhibited excellent AMPA-R affinity and selectivity (**29l**, **m**, **o**, **p**). On the other hand, except when a carboxylic acid or trifluoromethyl group was introduced to the phenyl ring (**29m** and **p**), compounds with substituents at the 4 position showed low selectivity (**29c**, **f**, **i**, **j**). Moreover, di-substituted compounds with substituents at the 4 position also showed low selectivity, even if the substituents introduced at the 2 or 3 position were associated with good selectivity (**29s**, **v**, **y** vs **29r**, **t**, **u**, **w**, **x**). Although a non-substituted benzyl compound (**28e**) exhibited slightly less selectivity for the AMPA-R

Table 5. 7-Imidazole/pyridone derivatives with a urethane linkage



Compound	R^4	n	AMPA-R affinity[25,26] K_i (nM)	NMDA-R affinity[27] K_i (nM)	Selectivity $\left(\frac{\text{NMDA-R}}{\text{AMPA-R}}\right)$
28d	Ph	1	86	>10,000	>120
29a	2-Cl-Ph	1	20	4100	210
29b	3-Cl-Ph	1	18	6600	370
29c	4-Cl-Ph	1	30	1300	43
29d	2-Br-Ph	1	30	8300	280
29e[a]	3-Br-Ph	1	16	7000	440
29f	4-Br-Ph	1	32	1200	38
29g[a]	2-Me-Ph	1	16	5900	370
29h	3-Me-Ph	1	22	6300	290
29i	4-Me-Ph	1	30	2100	70
29j[b]	4-MeO-Ph	1	30	2000	67
29k	2-CF_3-Ph	1	32	9300	290
29l	3-CF_3-Ph	1	20	>10,000	>500
29m	4-CF_3-Ph	1	27	>10,000	>370
29n	2-CO_2H-Ph	1	>1000	>10,000	>10
29o	3-CO_2H-Ph	1	27	>10,000	>370
29p	4-CO_2H-Ph	1	22	>10,000	>450
29q	4-CO_2Et-Ph	1	60	>10,000	>170
29r	2,3-Cl_2-Ph	1	17	4400	260
29s	2,4-Cl_2-Ph	1	32	1300	41
29t	2,5-Cl_2-Ph	1	15	4100	270
29u	2,6-Cl_2-Ph	1	17	3200	190
29v[a]	3,4-Cl_2-Ph	1	31	2400	77
29w	3,5-Cl_2-Ph	1	14	3200	230
29x	1-Naphtyl	1	14	8600	610
29y	2-Naphtyl	1	32	2000	63
31	4-CO_2H-Ph	2	270	NT	NT
30a	2-Br-PhCH2	1	29	4700	160
30b	3-Br-PhCH2	1	100	6900	69
30c	4-Br-PhCH2	1	47	2900	62
38b	4-CO_2H-Ph	—	120	>10,000	>83

NT, not tested.
[a] HCl salt.
[b] Na salt

than the phenyl compound **28d**, we investigated the influence of the length of the methylene chain between the terminal phenyl ring and the urethane group on the side chain at the 7 position in an attempt to enhance AMPA-R affinity and selectivity. Unfortunately, benzyl compounds with bromine substituents on the terminal phenyl group exhibited reduced AMPA-R selectivity or affinity (**29d–f** vs **30a–c**). Moreover, when a methylene group was inserted between the imidazole group and the urethane group there was also significantly lower AMPA-R affinity (**29p** vs **31**). Consequently, we consider that extending the methylene chain between the urethane linkage and the terminal phenyl group, or between the imidazole ring and the urethane linkage, adversely influences AMPA-R affinity and selectivity. Therefore, we concluded that the best side chain to attach to the 4 position of the imidazole ring is a 'phenylaminocarbonyloxymethyl group', which forms a very important core for interaction with the AMPA-R. In addition, a urethane linkage and a terminal phenyl group are both important interaction units that may function as proton acceptors during interaction with the AMPA-R. On the other hand, we attempted the introduction of carboxy group at the 4 position on the terminal phenyl group of pyridine **38a**, because the 4-carboxylic compound **29p** exhibited more potent AMPA-R affinity than the nonsubstituted compound **28d**. However, the 4-pyridone **38b** with 4-carboxyphenyl group linked through a urethane also showed weak AMPA-R affinity, and the result fell short of our expectation.

3.7. Neuroprotective effects in permanent focal ischemia in rats

The neuroprotective effects of the selected compounds, **19g**, **19h**, **29o**, and **29p**, and those of the reference compounds, NBQX, YM-90K, and YM-872, were examined using the rat permanent focal ischemia model described by Tamura et al.[19] The neuroprotective effects, as assessed using a four-point damage score, are shown in Table 6 and Figure 5. Among our selected compounds, the 4-pyridone compound **19g** was highly soluble in an aqueous solution (8.1 mg/mL at pH 7.4),[20] and showed good, dose-dependent neuroprotective effects when administered at an iv infusion rate of 20–40 mg/kg/h for 4 h. However, the nonsubstituted imidazole **19h** did not show neuroprotective effects at the same iv infusion rate. Compounds **29o** and **29p** showed better neuroprotective effects in vivo, as well as AMPA-R activity in vitro, than NBQX, YM-90K, and YM-872. It was noteworthy that the AMPA-R affinity and neuroprotective effects of **19h** were inferior to those of **29o** and **29p**, despite the presence of a 7-imidazolyl-6-nitro-3-oxoquinoxaline-2-carboxylic acid nucleus. Compound **19h** lacked the urethane-linked phenyl ring substituent at the 7 position on the imidazole group, indicating that this novel substituent not only confers potent AMPA-R affinity but also contributes to therapeutic efficacy in animal models. In a comparison of the biological activities of compounds **29o** and **29p**, both of which have a carboxyl group on the terminal phenyl group, the 4-carboxyphenyl compound **29p** exhibited markedly more potent activity in vivo than the 3-carboxyphenyl compound **29o**. In particular, **29p** showed neuroprotective effects superior to those of any of the previously reported quinoxalinedione compounds, and this was achieved with a relatively low iv infusion rate of at least 2.5 mg/kg/h for 4 h. Furthermore, compound **29p** showed the best aqueous solubility among the 4-urethane linked imidazole derivatives, which was higher than aqueous solubility of the first generation compounds (compound **29p**: 4.9 mg/mL, NBQX and YM-90K: <1 mg/mL at pH 7.4).[20] These characteristics warrant further investigation of this compound for use

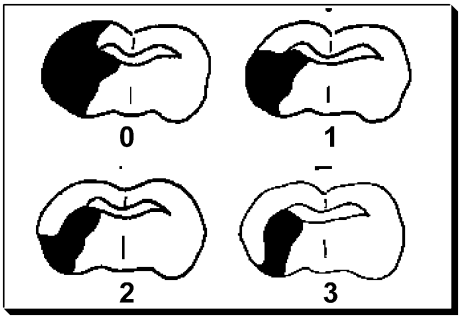


Figure 5. Four-point damage score in focal ischemia model.

Table 6. Pharmacological data for 7-heterocyclic quinoxalinecarboxylic acid derivatives

Compound	AMPA-R affinity[25,26] K_i (nM)	NMDA-R affinity[27] K_i (nM)	Selectivity $\left(\frac{\text{NMDA}-\text{R}}{\text{AMPA}-\text{R}}\right)$	AMPA-R antagonism[28] (D.C. potential)	Protective effects in focal ischemia model[19] (Dose:mg/kg/h for 4 h, iv)	
NBQX	65	>84,500	>1300	NT	2.3 (30)	$n = 4$
YM-90K	100	43,000	430	(+)	2.8 (15)	$n = 7$
YM-872	62	15,000	240	(+)	0.5 (30)	$n = 6$
19g	330	27,000	190	(+)	2.2 (20)	$n = 6$
					2.8 (40)	$n = 5$
19h	560	63,000	480	(+)	0.2 (20)	$n = 6$
29o	27	>10,000	>370	(+)	2.0 (2.5)	$n = 3$
					2.3 (5.0)	$n = 3$
29p	22	>10,000	>450	(+)	1.6 (1.25)	$n = 5$
					3.0 (2.5)	$n = 3$
					3.0 (3.0)	$n = 2$

NT, not tested.
Control; <1.0.

as an injectable formulation in the treatment of acute cerebral ischemia.

4. Conclusion

Molecular design and synthesis of novel third generation AMPA-R antagonists based on the 6-nitro-3-oxoquinoxaline-2-carboxylic acid nucleus led to the creation of novel quinoxaline compounds with good neuroprotective activities in vivo. In particular, we found that 3-oxoquinoxaline-2-carboxylic acids with both a nitro group at the 6 position (as an electron-withdrawing group) and a flat heterocyclic group bearing π-electrons at the 7 position formed a very important scaffold for AMPA-R antagonistic activity, and that a urethane linkage played an important role as a proton acceptor in interactions with the AMPA-R. After investigating the structure–activity relationships and evaluating the properties of various compounds, we identified compound **29p** (**GRA-293**) as a novel AMPA-R antagonist that possesses high potency and good selectivity in vitro as well as more potent neuroprotective effects in an animal model than known quinoxalinedione compounds.

5. Experimental section

5.1. Chemistry

5.1.1. General. All reagents, starting materials, and solvents were purchased from commercial suppliers and used as received. Evaporation was carried out on a rotary evaporator at bath temperatures <45 °C and reduced pressure. Column chromatography was performed with silica gel (Merck: silica gel 60 with particle size 0.040–0.063 mm). Reactions were monitored by TLC on silica gel 60 F_{254} (Merck). Melting points were determined with a YANAKO MP-500D and are uncorrected. Proton NMR spectra were recorded on a JEOL JNM-EX400 and JEOL JNM-ECA400 with tetramethylsilane as an internal standard. Chemical shifts are given in parts per million (δ) and splitting patterns are designated as follows: s, singlet; d, doublet; dd, double doublet; dt, double triplet; t, triplet; td, triple doublet; q, quartet; m, multiplet; br, broad; and br s, broad like singlet. HRMS and FABHRMS data were recorded on a JEOL JMS-SX102A. Elemental analyses were carried out on a YANAKO CHN CORDER MT-5.

5.1.2. Ethyl 7-chloro-3,4-dihydro-3-oxoquinoxaline-2-carboxylate (2a) and ethyl 6-chloro-3,4-dihydro-3-oxoquinoxaline-2-carboxylate (3) prepared from compound 1 (route A). To a solution of compound **1** (1.00 g, 7.01 mmol) in EtOH (20 mL), diethyl ketomalonate (1.12 mL, 7.36 mmol), was added and the solution was refluxed for 5 h. After cooling, the reaction was concentrated and the residue was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 2:1) to give compound **2a** (610 mg, 34%) as yellow powder and compound **3** (500 mg, 28%) as yellow powder. Compound **2a**: ^{1}H NMR (DMSO-d_6) δ: 12.69 (br s, 1H), 7.97 (d, J = 2.4 Hz, 1H), 7.59 (dd, J = 8.6, 2.4 Hz, 1H), 7.41 (d, J = 8.6 Hz, 1H), 4.55 (q, J = 7.3 Hz, 2H), 1.48 (t, J = 7.3 Hz, 3H). Compound **3**: ^{1}H NMR (DMSO-d_6) δ: 12.23 (br s, 1H), 7.90 (d, J = 8.6 Hz, 1H), 7.44 (d, J = 1.8 Hz, 1H), 7.38 (dd, J = 8.6, 2.4 Hz, 1H), 4.56 (q, J = 7.3 Hz, 2H), 1.49 (t, J = 7.3 Hz, 3H).

5.1.3. Ethyl 7-chloro-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylate (4a). To a solution of compound **2a** (200 mg, 792 μmol) in AcOH (2 mL), fuming HNO_3 (65.5 μL, 1.58 mmol), was added at 80 °C and the solution was stirred for 2 h at the same temperature. The reaction was poured into ice water and the precipitate was collected by filtration, washed with water, and dried under vacuum. The precipitate was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 2:1) to give the title compound as yellow powder (119 mg, 50%); ^{1}H NMR (DMSO-d_6) δ: 13.24 (br s, 1H), 8.29 (s, 1H), 7.91 (s, 1H), 4.40 (q, J = 7.3 Hz, 2H), 1.33 (t, J = 7.3 Hz, 3H).

5.1.4. Ethyl 6-chloro-3,4-dihydro-7-nitro-3-oxoquinoxaline-2-carboxylate (5). To a solution of compound **3** (200 mg, 792 μmol) in concd H_2SO_4 (4 mL), a solution of KNO_3 (160 mg, 1.58 mmol) in concd H_2SO_4 (2 mL) was added, and the solution was stirred for 2 h at room temperature. The reaction was poured into ice water and the precipitate was collected by filtration, washed with water, and dried under vacuum. The precipitate was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 2:1) to give the title compound as pale yellow powder (102 mg, 43%); ^{1}H NMR (DMSO-d_6) δ: 13.26 (br s, 1H), 8.62 (s, 1H), 7.49 (s, 1H), 4.39 (q, J = 7.3 Hz, 2H), 1.32 (t, J = 7.3 Hz, 3H).

5.1.5. 7-Chloro-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (6a). To a suspension of compound **4a** (108 mg, 363 μmol) in EtOH (2 mL), 1 N KOH (1.09 mL, 1.09 mmol) was added and the solution was refluxed for 3 h. After cooling, the reaction was diluted with water and adjusted to pH 4 with AcOH, and concentrated. A small amount of water was added to the residue and the precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as yellow powder (55.8 mg, 57%); mp 227–229 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.28 (s, 1H), 7.92 (s, 1H); HRMS 268.9824 (−1.5 mmu).

5.1.6. 6-Chloro-3,4-dihydro-7-nitro-3-oxoquinoxaline-2-carboxylic acid (7). Following the procedure described for compound **6a**, the title compound was prepared from compound **5**, pale yellow powder (92%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ: 8.61 (s, 1H), 7.73 (s, 1H); FAB(−)HRMS 267.9721 (−4.0 mmu).

5.1.7. Ethyl 3-[(4-chloro-2-nitrophenyl)amino]-3-oxopropionate (9a). A solution of compound **8b** (20.0 g, 116 mmol) and ethyl 3-chloro-3-oxopropionate (15.7 mL, 122 mmol) in toluene (200 mL) was refluxed for 24 h. After cooling, the reaction was concentrated and ^{i}Pr$_2$O was added to the residue. The precipitate was collected by filtration, washed with ^{i}Pr$_2$O, and dried

under vacuum to give the title compound as brown powder (63%); ^{1}H NMR ($CDCl_3$) δ: 8.71 (d, J = 9.0 Hz, 1H), 8.19 (d, J = 2.4 Hz, 1H), 7.59 (dd, J = 9.0, 2.4 Hz, 1H), 4.30 (q, J = 7.3 Hz, 2H), 3.56 (s, 2H), 1.21 (t, J = 7.3Hz, 3H).

5.1.8. Ethyl 3-[(4-fluoro-2-nitrophenyl)amino]-3-oxopropionate (9b). Following the procedure described for compound **9a**, the title compound was prepared from compound **8b**, yellow powder (88%); ^{1}H NMR ($CDCl_3$) δ: 8.71 (dd, J = 9.7, 4.8 Hz, 1H), 7.91 (dd, J = 8.4, 3.1 Hz, 1H), 7.49–7.27 (m, 1H), 4.30 (q, J = 7.0 Hz, 2H), 3.56 (s, 2H), 1.33 (t, J = 7.0 Hz, 3H).

5.1.9. Ethyl 3-[(4-bromo-2-nitrophenyl)amino]-3-oxopropionate (9c). To a solution of compound **8c** (82.7 g, 381 mmol) in DMF (500 mL), ethyl 3-chloro-3-oxopropionate (51.2 mL, 400 mmol) and Et_3N (55.8 mL, 400 mmol) were added at ice cooling, and the solution was stirred for 30 min at the same temperature followed by for 2 h at room temperature. The reaction was poured into ice water and the precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as brown powder (121 g, 96%); ^{1}H NMR (DMSO-d_6) δ: 10.63 (br s, 1H), 8.18 (d, J = 2.5 Hz, 1H), 7.93 (dd, J = 8.8, 2.4 Hz, 1H), 7.67 (d, J = 8.8 Hz, 1H), 4.13 (q, J = 6.9 Hz, 2H), 3.53 (s, 2H), 1.21 (t, J = 6.8 Hz, 3H).

5.1.10. Ethyl 7-chloro-3,4-dihydro-3-oxoquinoxaline-2-carboxylate (2a) prepared from compound 9a (route B)

5.1.10.1. Step 1: Ethyl 7-chloro-3,4-dihydro-3-oxoquinoxaline-2-carboxylate 1-oxide. To a solution of compound **9a** (6.00 g, 21.0 mmol) in DMF (30 mL), a suspension of KO^{t}Bu (4.70 g, 41.8 mmol) in DMF (20 mL) was added at 0 °C and the solution was stirred for 1.5 h at the same temperature. The reaction was adjusted to pH 4 with 1 N HCl, extracted with CH_2Cl_2, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (SiO_2, CH_2Cl_2:MeOH, 4:1) to give the title compound (1.59 g, 28%) as pale yellow powder; ^{1}H NMR ($CDCl_3$) δ: 8.33 (d, J = 2.2 Hz, 1H), 7.65 (dd, J = 8.8, 2.2 Hz, 1H), 7.39 (d, J = 8.8 Hz, 1H), 4.56 (q, J = 6.9 Hz, 2H), 1.46 (t, J = 6.9 Hz, 3H).

5.1.10.2. Step 2: Ethyl 7-chloro-3,4-dihydro-3-oxoquinoxaline-2-carboxylate (2a). To a solution of ethyl 7-chloro-3,4-dihydro-3-oxoquinoxaline-2-carboxylate 1-oxide (1.80 g, 6.70 mmol) in DMF (20 mL), PBr_3 (1.20 mL, 13.4 mmol) was added, and the solution was stirred for 1.5 h at room temperature. The reaction was poured into ice water, extracted with $CHCl_3$, dried over Na_2SO_4, and evaporated. The residue was washed with ^{i}Pr$_2$O, and dried under vacuum to give the title compound (1.55 g, 92%) as pale yellow powder; ^{1}H NMR ($CDCl_3$) δ: 7.94 (d, J = 2.0 Hz, 1H), 7.58 (dd, J = 8.8, 2.0 Hz, 1H), 7.41 (d, J = 8.8 Hz, 1H), 4.40 (q, J = 7.1 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H).

5.1.11. Ethyl 3,4-dihydro-7-fluoro-3-oxoquinoxaline-2-carboxylate (2b)

5.1.11.1. Step 1: Ethyl 3,4-dihydro-7-fluoro-3-oxoquinoxaline-2-carboxylate 1-oxide. Following the procedure described for compound **2a** (route B, step 1), the title compound was prepared from compound **9b**, brown powder (31%); ^{1}H NMR ($CDCl_3$) δ: 8.09–7.96 (m, 1H), 7.51–7.42 (m, 2H), 4.56 (q, J = 7.0 Hz, 2H), 1.46 (t, J = 7.0 Hz, 3H).

5.1.11.2. Step 2: Ethyl 3,4-dihydro-7-fluoro-3-oxoquinoxaline-2-carboxylate (2b). Following the procedure described for compound **2a** (route B, step 2), the title compound was prepared from ethyl 3,4-dihydro-7-fluoro-3-oxoquinoxaline-2-carboxylate 1-oxide, pale yellow powder (75%); ^{1}H NMR ($CDCl_3$) δ: 7.70–7.59 (m, 1H), 7.48–7.38 (m, 2H), 4.55 (q, J = 7.0 Hz, 2H), 1.48 (t, J = 7.0 Hz, 3H).

5.1.12. Ethyl 7-bromo-3,4-dihydro-3-oxoquinoxaline-2-carboxylate (2c)

5.1.12.1. Step 1: Ethyl 7-bromo-3,4-dihydro-3-oxoquinoxaline-2-carboxylate 1-oxide. Following the procedure described for compound **2a** (route B, step 1), the title compound was prepared from compound **9c**, brown powder (51%); ^{1}H NMR (DMSO-d_6) δ: 8.22 (d, J = 2.5 Hz, 1H), 7.91 (dd, J = 8.8, 2.0 Hz, 1H), 7.36 (d, J = 8.8 Hz, 1H), 4.39 (q, J = 6.9 Hz, 2H), 1.31 (t, J = 6.9 Hz, 3H).

5.1.12.2. Step 2: Ethyl 7-bromo-3,4-dihydro-3-oxoquinoxaline-2-carboxylate (2c). Following the procedure described for compound **2a** (route B, step 2), the title compound was prepared from ethyl 7-bromo-3,4-dihydro-3-oxoquinoxaline-2-carboxylate 1-oxide, yellow powder (65%); ^{1}H NMR (DMSO-d_6) δ: 8.06 (d, J = 2.0 Hz, 1H), 7.80 (dd, J = 8.8, 2.0 Hz, 1H), 7.30 (d, J = 8.8 Hz, 1H), 4.37 (q, J = 7.3 Hz, 2H), 1.32 (t, J = 7.3 Hz, 3H).

5.1.13. Ethyl 3,4-dihydro-7-fluoro-6-nitro-3-oxoquinoxaline-2-carboxylate (4b). Following the procedure described for compound **4a**, the title compound was prepared from compound **2b**, yellow powder (45%); ^{1}H NMR ($CDCl_3$) δ: 8.16 (d, J = 6.2 Hz, 1H), 7.89 (d, J = 10.6 Hz, 1H), 4.58 (q, J = 7.0 Hz, 2H), 1.49 (t, J = 7.0 Hz, 3H).

5.1.14. Ethyl 7-bromo-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylate (4c). Following the procedure described for compound **4a**, the title compound was prepared from compound **2c**, yellow powder (93%); ^{1}H NMR (DMSO-d_6) δ: 13.24 (br s, 1H), 8.40 (s, 1H), 7.86 (s 1H), 4.40 (q, J = 7.3 Hz, 2H), 1.33 (t, J = 7.3 Hz, 3H).

5.1.15. Ethyl 3,4-dihydro-7-fluoro-4-methyl-6-nitro-3-oxoquinoxaline-2-carboxylate (10). To a solution of compound **4c** (345 mg, 1.23 mmol) in DMF (10 mL), sodium hydride (50% in oil, 61.3 mg, 1.54 mmol) was added, and the solution was stirred for 30 min at room temperature. Iodomethane (95.5 μL, 1.54 mmol) was added to the reaction and the solution was stirred for 2 h at room temperature. The reaction was poured into ice water, extracted with AcOEt, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 5:2) to

give the title compound as pale yellow powder (272 mg, 75%); ^{1}H NMR (DMSO-d_6) δ: 8.04 (d, J = 6.3 Hz, 1H), 7.87 (d, J = 10.3 Hz, 1H), 4.53 (q, J = 7.3 Hz, 2H), 3.77 (s, 3H), 1.45 (t, J = 7.3 Hz, 3H).

5.1.16. Ethyl 7-fluoro-3-methoxy-6-nitroquinoxaline-2-carboxylate (11a). A suspension of compound **4b** (1.00 g, 3.56 mmol), iodomethane (440 μL, 7.07 mmol), and silver(I) oxide (990 mg, 4.31 mmol) in toluene (100 mL) was stirred for 2 h at 100 °C. After cooling, the reaction was filtered through Celite®, and the filtrate was concentrated. The residue was purified by flash column chromatography (SiO_2, CH_2Cl_2:AcOEt, 4:1) to give the title compound as pale yellow powder (580 mg, 55%); ^{1}H NMR ($CDCl_3$) δ: 8.57 (d, J = 7.3 Hz, 1H), 7.95 (d, J = 10.8 Hz, 1H), 4.55 (q, J = 7.3 Hz, 2H), 4.18 (s, 3H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.17. Ethyl 7-fluoro-3-methoxy-6-nitroquinoxaline-2-carboxylate (11b). Following the procedure described for compound **11a**, the title compound was prepared from compound **4b** and bromoethane, pale yellow powder (68%); ^{1}H NMR ($CDCl_3$) δ: 8.63 (d, J = 7.8 Hz, 1H), 8.33 (d, J = 11.7 Hz, 1H), 4.57 (q, J = 7.3 Hz, 2H), 4.47 (q, J = 6.8 Hz, 2H), 1.41 (t, J = 7.3 Hz, 2H), 1.36 (t, J = 6.8 Hz, 3H).

5.1.18. Ethyl 6-amino-7-fluoro-3-methoxyquinoxaline-2-carboxylate (12). To a solution of compound **11a** (300 mg, 1.02 mmol) in EtOH (50 mL), 10% Pd–C (60 mg) was added, and the solution was stirred for 2 h at room temperature under hydrogen atmosphere. The catalyst was removed by filtration through Celite® and the filtrate was concentrated to give the title compound as yellow needles (260 mg, 96%); ^{1}H NMR ($CDCl_3$) δ: 7.65 (d, J = 11.2 Hz, 1H), 7.03 (d, J = 8.8 Hz, 1H), 4.50 (q, J = 7.3 Hz, 2H), 4.45 (br s, 2H), 4.10 (s, 3H), 1.45 (t, J = 7.3 Hz, 3H).

5.1.19. 7-Fluoro-3-methoxy-6-nitroquinoxaline-2-carboxamide (13). To a suspension of compound **11a** (542 mg, 1.84 mmol) in MeOH (20 mL), 28% NH_3 aq (1.5 mL) was added, and the solution was refluxed for 3 h. After cooling, the reaction was concentrated and water was added to the residue. The precipitate was collected by filtration, dissolved in AcOEt, dried over Na_2SO_4, and evaporated. The residue was washed with $^iPr_2O$, and dried under vacuum to give the title compound as reddish brown powder (369 mg, 76%); ^{1}H NMR (DMSO-d_6) δ: 8.56 (d, J = 7.3 Hz, 1H), 7.97 (d, J = 10.7 Hz, 1H), 4.18 (s, 3H).

5.1.20. 3,4-Dihydro-7-fluoro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (6b). Following the procedure described for compound **6a**, the title compound was prepared from compound **4b**, yellow powder (77%); mp 213–215 °C (decomp.); ^{1}H NMR ($CDCl_3$) δ: 8.10 (d, J = 11.7 Hz, 1H), 8.07 (d, J = 7.3 Hz, 1H); HRMS 253.0162 (+2.7 mmu).

5.1.21. 7-Bromo-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (6c). Following the procedure described for compound **6a**, the title compound was prepared from compound **4c**, yellow powder (64%); mp 218–220 °C (decomp.); ^{1}H NMR ($CDCl_3$) δ: 8.38 (s, 1H), 7.88 (s, 1H); HRMS 312.9358 (+2.4 mmu). Anal. Calcd for $C_9H_4BrN_3O_5 \cdot \frac{2}{3}H_2O$: C, 33.15%; H, 1.65%; N, 12.89%. Found: C, 33.04%; H, 1.89%; N, 12.83%.

5.1.22. 3,4-Dihydro-7-fluoro-4-methyl-6-nitro-3-oxoquinoxaline-2-carboxylic acid (14). To a solution of compound **10** (207 mg, 701 μmol) in AcOH (15 mL), concd HCl (1 mL) and water (1 mL) were added, and the solution was stirred for 1 h at room temperature followed by 3 h at 80 °C. After cooling, the reaction was concentrated. The residue was washed with MeCN and dried under vacuum to give the title compound as brown powder (78.4 mg, 41%); mp 173–175 °C; ^{1}H NMR (DMSO-d_6) δ: 8.32 (d, J = 6.8 Hz, 1H), 8.16 (d, J = 11.2 Hz, 1H), 3.67 (s, 3H); FAB(+)HRMS 268.0366 (−0.4 mmu). Anal. Calcd for $C_{10}H_6FN_3O_5 \cdot \frac{1}{5}H_2O$: C, 44.36%; H, 2.38%; N, 15.52%. Found: C, 44.33%; H, 2.25%; N, 15.79%.

5.1.23. 6-Amino-3,4-dihydro-7-fluoro-3-oxoquinoxaline-2-carboxylic acid (15). To a solution of compound **12** (50.0 mg, 189 μmol) in MeOH (1 mL), 1 N NaOH (500 μL) was added, and the solution was stirred for 1 h at room temperature. The reaction was concentrated and the residue was dissolved in AcOH (3 mL). Forty-seven percent of HBr (1 mL) was added to the solution and stirred overnight. The reaction was concentrated, and the residue was dissolved in 1 N NaOH and eluted through synthetic adsorbent Sepabeads® SP850 (water). The elution was concentrated and made acidic with 1 N HCl. The precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as brown powder (10.2 mg, 23%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ: 7.59 (d, J = 11.7 Hz, 1H), 6.91 (br s, 2H), 6.63 (d, J = 8.3 Hz, 1H); FAB(+)HRMS 224.0516 (+4.4 mmu). Anal. Calcd for $C_9H_6FN_3O_3 \cdot \frac{3}{5}H_2O$: C, 46.20%; H, 3.10%; N, 17.96%. Found: C, 46.32%; H, 3.02%; N, 17.77%.

5.1.24. 3,4-Dihydro-6-nitro-7-fluoro-3-oxoquinoxaline-2-carboxamide (16). To a solution of compound **13** (108 mg, 406 μmol) in AcOH (3 mL), 48% HBr (0.6 mL) was added at 0 °C, and the reaction was stirred for 1 h at room temperature followed by for 1.5 h at 60 °C. The reaction was poured into ice water, and the solution was stirred for 20 min. The precipitate was collected by filtration and dried under vacuum to give the title compound as brown powder (69.7 mg, 68%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ: 13.01 (s, 1H), 8.22 (br s, 1H), 8.09 (d, J = 11.7 Hz, 1H), 8.03 (d, J = 6.8 Hz, 1H), 7.99 (br s, 1H); FAB(+)HRMS 251.0221 (+0.5 mmu). Anal. Calcd for $C_9H_5FN_4O_4$: C, 42.87%; H, 2.00%; N, 22.22%. Found: C, 42.89%; H, 2.03%, N, 21.96%.

5.1.25. 3,4-Dihydro-6-nitro-3-oxo-7-(4-pyridon-1-yl)quinoxaline-2-carboxylic acid (19g)

5.1.25.1. Step 1: Ethyl 3-methoxy-6-nitro-7-(4-pyridon-1-yl)quinoxaline-2-carboxylate (17g). To a solution of compound **11a** (180 mg, 610 μmol) in THF (20 mL), 4-pyridone (290 mg, 3.05 mmol) was added, and the

solution stirred for 4 h at 100 °C followed by 18 h at 90 °C in a sealed tube. After cooling, the reaction was concentrated and the residue was purified by flash column chromatography (SiO_2, $CHCl_3$:EtOH, 40:1 to 20:1) to give the title compound as pale yellow oil (70.0 mg, 31%); ^{1}H NMR ($CDCl_3$) δ: 8.60 (s, 1H), 8.22 (s, 1H), 7.38 (d, J = 7.8 Hz, 2H), 6.52 (d, J = 7.8 Hz, 2H), 4.56 (q, J = 7.2 Hz, 2H), 4.24 (s, 3H), 1.47 (t, J = 7.2 Hz, 3H).

5.1.25.2. Step 2: 3,4-Dihydro-6-nitro-3-oxo-7-(4-pyridon-1-yl)quinoxaline-2-carboxylic acid (19g). To a solution of compound **17g** (1.34 g, 3.62 mmol) in EtOH (40 mL), water (10 mL) and 1 N KOH (10.9 mL) were added, and the reaction was refluxed for 4 h. After cooling, cation-exchange resin Dowex® XFS43279.00 was added to neutralize. The resin was removed by filtration, and the filtrate was concentrated. The residue was dissolved into 3 N HCl (70 mL) and the solution was stirred for 4 h at room temperature. The solution was concentrated, and the residue was washed with water and dried under vacuum to give the title compound as yellow powder (1.00 g, 81%); mp 283–285 °C; ^{1}H NMR (DMSO-d_6) δ: 8.35 (s, 1H), 8.11 (s, 1H), 7.83 (d, J = 7.3 Hz, 2H), 6.24 (d, J = 7.3 Hz, 2H); FAB(+)HRMS 329.0542 (+ 2.0 mmu). Anal. Calcd for $C_{14}H_9N_4O_6 \cdot \frac{4}{5}H_2O$: C, 49.07%, H, 2.82%; N, 16.35%. Found: C, 48.84%; H, 2.62%; N, 16.05%.

5.1.26. 3,4-Dihydro-7-dimethylamino-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19a)

5.1.26.1. Step 1: Ethyl 7-dimethylamino-3-methoxy-6-nitroquinoxaline-2-carboxylate (17a). Following the procedure described for compound **19g** (step 1), the title compound was prepared from compound **11a** and dimethylamine, brown oil (36%); ^{1}H NMR ($CDCl_3$) δ: 8.29 (s, 1H), 7.66 (s, 1H), 4.43 (q, J = 7.3 Hz, 2H), 4.05 (s, 3H), 2.86 (s, 6H), 1.35 (t, J = 7.3 Hz, 3H).

5.1.26.2. Step 2: 3,4-Dihydro-7-dimethylamino-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19a). Following the procedure described for compound **19g** (step 2), the title compound was prepared from compound **17a**, dark brown powder (15%); mp 194.5–196.5 °C; ^{1}H NMR (DMSO-d_6) δ: 7.72 (s, 1H), 7.62 (s, 1H), 2.79 (s, 6H); HRMS 278.0641 (−1.0 mmu).

5.1.27. 3,4-Dihydro-6-nitro-3-oxo-7-piperidinoquinoxaline-2-carboxylic acid (19b)

5.1.27.1. Step 1: Ethyl 3-methoxy-6-nitro-7-piperidinoquinoxaline-2-carboxylate (17b). Following the procedure described for compound **19g** (step 1), the title compound was prepared from compound **11a** and piperidine, red oil (88%); ^{1}H NMR ($CDCl_3$) δ: 8.13 (s, 1H), 7.69 (s, 1H), 4.53 (q, J = 7.3 Hz, 2H), 4.13 (s, 3H), 3.05 (br t, J = 4.8 Hz, 2H), 1.77–1.71 (m, 4H), 1.64–1.58 (m, 2H), 1.46 (t, J = 7.3 Hz, 3H).

5.1.27.2. Step 2: 3,4-Dihydro-6-nitro-3-oxo-7-piperidinoquinoxaline-2-carboxylic acid (19b). Following the procedure described for compound **19g** (step 2), the title compound was prepared from compound **17b**, purple powder (50%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ: 7.72 (s, 1H), 7.70 (s, 1H), 2.93 (br t, J = 4.9 Hz, 2H), 1.66–1.57 (m, 4H), 1.56–1.48 (m, 2H); HRMS 318.0977 (+1.3 mmu).

5.1.28. 3,4-Dihydro-7-morpholino-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19c). A solution of compound **11a** (506 mg, 1.71 mmol) and morpholine (749 μL, 8.56 mmol) in MeCN (2 mL) was stirred for 6 h at 80 °C. After cooling, the reaction was concentrated and the residue was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 2:1) to give crude compound **17c** as red oil. The obtained compound **17c** was dissolved into MeOH (2 mL) and 5% NaOH aq (5 mL) was added. After stirring for 24 h at room temperature, the reaction was adjusted to pH 3 with 3 N HCl, extracted with CH_2Cl_2, dried over Na_2SO_4, and evaporated. HCl (3 N, 5 mL) was added to the residue and stirred for 65 h. The precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as red powder (275 mg, 48%); mp 213.5–214.5 °C; ^{1}H NMR (DMSO-d_6) δ: 7.82 (s, 1H), 7.73 (s, 1H), 3.69 (t, J = 4.9 Hz, 2H), 2.98 (t, J = 4.9 Hz, 2H); FAB(−)HRMS 319.0688 (+0.9 mmu). Anal. Calcd for $C_{13}H_{12}N_4O_6 \cdot \frac{9}{10}H_2O$: C, 46.41%; H, 4.13%; N, 16.65%. Found: C, 46.66%; H, 4.00%; N, 16.32%.

5.1.29. 3,4-Dihydro-7-(4-hydoxypiperidino)-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19d)

5.1.29.1. Step 1: Ethyl 7-(4-hydoxypiperidino)-3-methoxy-6-nitroquinoxaline-2-carboxylate (17d). Following the procedure described for compound **19g** (step 1), the title compound was prepared from compound **11a** and 4-hydroxypiperidine, red gum (83%); ^{1}H NMR ($CDCl_3$) δ: 8.15 (s, 1H), 7.73 (s, 1H), 4.54 (q, J = 7.3 Hz, 2H), 4.14 (s, 3H), 3.97–3.88 (m, 1H), 3.38–3.28 (m, 2H), 2.99–2.91 (m, 2H), 2.09–2.00 (m, 2H), 1.82–1.72 (m, 2H), 1.46 (t, J = 7.3 Hz, 3H).

5.1.29.2. Step 2: 3,4-Dihydro-6-nitro-3-oxo-7-piperidinoquinoxaline-2-carboxylic acid (19d). Following the procedure described for compound **19g** (step 2), the title compound was prepared from compound **17d**, brown powder (38%); mp 253–255 °C; ^{1}H NMR (DMSO-d_6) δ: 7.71 (s, 2H), 4.72 (br s, 1H), 3.20–3.00 (m, 2H), 2.85–2.75 (m, 2H), 1.80–1.90 (m, 2H), 1.55–1.45 (m, 2H); FAB(+)HRMS 334.0894 (−1.9 mmu). Anal. Calcd for $C_{14}H_{14}N_4O_6 \cdot \frac{3}{10}H_2O$: C, 49.50%; H, 4.33%; N, 16.49%. Found: C, 49.76%; H, 4.19%; N, 16.32%.

5.1.30. 7-(4-Acetylpiperazin-1-yl)-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19e)

5.1.30.1. Step 1: Ethyl 7-(4-acetylpiperazin-1-yl)-3-methoxy-6-nitroquinoxaline-2-carboxylate (17e). Following the procedure described for compound **19g** (step 1), the title compound was prepared from compound **11a** and 1-acetylpiperazine, red gum (69%); ^{1}H NMR ($CDCl_3$) δ: 8.20 (s, 1H), 7.76 (s, 1H), 4.54 (q, J = 7.3 Hz, 2H), 4.15 (s, 3H), 3.83–3.77 (m, 2H), 3.67–3.62 (m, 2H), 3.13–3.06 (m, 4H), 2.15 (s, 3H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.30.2. Step 2: 7-(4-Acetylpiperazin-1-yl)-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19e). Following the procedure described for compound **19g**

(step 2), the title compound was prepared from compound **17e**, with 47% HBr instead of 3 N HCl, brown powder (57%); mp 212–214 °C; ^{1}H NMR (DMSO-d_6) δ: 7.81 (s, 1H), 7.76 (s, 1H), 3.60–3.50 (m, 4H), 3.10–2.90 (m, 4H), 2.03 (s, 3H); FAB(+)HRMS 362.1153 (+5.2 mmu).

5.1.31. 3,4-Dihydro-7-[4-(4-fluorophenyl)piperazin-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19f)

5.1.31.1. Step 1: Ethyl 7-[4-(4-fluorophenyl)piperazin-1-yl]-3-methoxy-6-nitroquinoxaline-2-carboxylate (17f). Following the procedure described for compound **19g** (step 1), the title compound was prepared from compound **11a** and 1-(4-fluorophenyl)piperazine, red gum (87%); ^{1}H NMR ($CDCl_3$) δ: 8.18 (s, 1H), 7.80 (s, 1H), 7.00 (t, J = 8.8 Hz, 2H), 6.94 (dd, J = 8.8, 4.4 Hz, 2H), 4.55 (q, J = 7.3 Hz, 2H), 4.15 (s, 3H), 3.27 (s, 8H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.31.2. Step 2: 3,4-Dihydro-7-[4-(4-fluorophenyl)piperazin-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19f). Following the procedure described for compound **19e** (step 2), the title compound was prepared from compound **17f**, pale yellow powder (55%); mp 235.5–237.5 °C; ^{1}H NMR (DMSO-d_6) δ: 7.86 (s, 1H), 8.75 (s, 1H), 7.07 (t, J = 9.3 Hz, 2H), 7.01 (dd, J = 9.3, 4.9 Hz, 2H), 3.19 (d, J = 5.4 Hz, 4H), 3.15 (d, J = 5.4 Hz, 4H); FAB(+)HRMS 414.1188 (−2.6 mmu). Anal. Calcd for $C_{19}H_{16}FN_5O_5 \cdot \frac{7}{10}H_2O$: C, 53.57%; H, 4.12%; N, 16.44%. Found: C, 53.74%; H, 3.77%; N, 16.15%.

5.1.32. 3,4-Dihydro-7-(imidazol-1-yl)-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19h)

5.1.32.1. Step 1: Ethyl 7-(imidazol-1-yl)-3-methoxy-6-nitroquinoxaline-2-carboxylate (17h). A solution of compound **11a** (1.41 g, 4.78 mmol) and imidazole (1.63 g, 23.9 mmol) in MeCN (10 mL) was stirred for 9 h at 50 °C. The reaction was diluted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography [SiO_2, *n*-hexane:AcOEt (1:1) to AcOEt] to give the title compound as orange oil (423 mg, 26%); ^{1}H NMR ($CDCl_3$) δ: 8.46 (s, 1H), 8.18 (s, 1H), 7.72 (s, 1H), 7.27 (s, 1H), 7.15 (t, J = 1.5 Hz, 1H), 4.56 (q, J = 7.3 Hz, 2H), 4.23 (s, 3H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.32.2. Step 2: 3,4-Dihydro-7-(imidazol-1-yl)-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19h). A solution of compound **17h** (423 mg, 1.23 mmol) in 3 N HCl (20 mL) was stirred for 6 h at 80 °C. To the reaction, concd HCl (2 mL) was added, and the reaction was stirred for 10 h at 80 °C. After cooling, the precipitate was collected by filtration, washed with water and MeOH, and dried under vacuum to give the title compound as brown powder (166 mg, 44%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ: 8.24 (s, 1H), 8.22 (s, 1H), 8.06 (s, 1H), 7.56 (s, 1H), 7.25 (s, 1H); FAB(−)HRMS 300.0347 (−2.2 mmu). Anal. Calcd for $C_{12}H_7N_5O_5 \cdot \frac{1}{2}H_2O$: C, 46.46%; H, 2.60%; N, 22.58%. Found: C, 46.17%; H, 2.44%; N, 22.61%.

5.1.33. 3,4-Dihydro-7-(2-methylimidazol-1-yl)-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19i)

5.1.33.1. Step 1: Ethyl 3-ethoxy-7-(2-methylimidazol-1-yl)-6-nitroquinoxaline-2-carboxylate (18i). 2-Methylimidazole (438 mg, 5.33 mmol) and Et_3N (223 μL, 1.60 mmol) were added, to a solution of compound **11b** (330 mg, 1.07 mmol) in DMF (2 mL) and stirred for 12 h at 110 °C. After cooling, the reaction was poured into ice water, extracted with AcOEt, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 1:2) to give the title compound as light brown amorphous solid (268 mg, 68%); ^{1}H NMR ($CDCl_3$) δ: 8.42 (s, 1H), 8.12 (s, 1H), 7.01 (d, J = 1.5 Hz, 1H), 6.97 (d, J = 1.5 Hz, 1H), 4.67 (q, J = 7.3 Hz, 2H), 4.55 (q, J = 7.3 Hz, 2H), 2.28 (s, 3H), 1.53 (t, J = 7.3 Hz, 3H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.33.2. Step 2: 3,4-Dihydro-7-(2-methylimidazol-1-yl)-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19i). To a solution of compound **18i** (218 mg, 587 μmol) in AcOH (10 mL), concd HCl (700 μL) was added, and the reaction was stirred for 24 h at room temperature. The reaction was concentrated, and the residue was washed with water and dried under vacuum to give the title compound as brown powder (97.4 mg, 53%); mp 268–271 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.31 (s, 1H), 8.17 (s, 1H), 7.50 (s, 1H), 7.27 (s, 1H), 2.30 (s, 3H); FAB(−)HRMS 314.0514 (−1.2 mmu).

5.1.34. 3,4-Dihydro-7-(4-methylimidazol-1-yl)-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19j)

5.1.34.1. Step 1: Ethyl 3-ethoxy-7-(4-methylimidazol-1-yl)-6-nitroquinoxaline-2-carboxylate (18j). A solution of compound **11b** (2.00 g, 6.47 mmol) and 4-methylimidazole (2.66 g, 32.4 mmol) in MeCN (15 mL) was stirred for 8 h at 100 °C in a sealed tube. After cooling, the reaction was diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 1:1) to give the title compound as yellow powder (1.20 g, 50%); ^{1}H NMR ($CDCl_3$) δ: 8.38 (s, 1H), 8.12 (s, 1H), 7.60 (d, J = 1.5 Hz, 1H), 6.83 (s, 1H), 4.66 (q, J = 7.0 Hz, 2H), 4.55 (q, J = 7.2 Hz, 2H), 2.31 (s, 3H), 1.52 (t, J = 7.1 Hz, 3H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.34.2. Step 2: 3,4-Dihydro-7-(4-methylimidazol-1-yl)-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19j). Following the procedure described for compound **19e** (step 2), the title compound was prepared from compound **18j**, brown powder (69%); mp 210–212 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.19 (s, 1H), 8.08 (s, 2H), 7.23 (s, 1H), 2.19 (s, 3H); FAB(+)HRMS 316.0688 (−1.6 mmu).

5.1.35. 3,4-Dihydro-7-[4-(hydroxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19k)

5.1.35.1. Step 1: Ethyl 3-ethoxy-7-[4-(hydroxymethyl)imidazol-1-yl]-6-nitroquinoxaline-2-carboxylate (18k). To a solution of compound **11b** (6.90 g, 22.3 mmol) in MeCN (70 mL), 4-(hydroxymethyl)imidazole hydrochloride (15.1 g, 112 mmol) and Et_3N (23.4 mL,

168 mmol) were added, and the solution was refluxed for 16 h. After cooling, the reaction was diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 1:1) to give the title compound as brown power (3.69 g, 43%); ^{1}H NMR ($CDCl_3$) δ: 8.43 (s, 1H), 8.15 (s, 1H), 7.68 (d, J = 1.5 Hz, 1H), 7.09 (s, 1H), 4.71 (s, 2H), 4.66 (q, J = 7.2 Hz, 2H), 4.55 (q, J = 7.2 Hz, 2H), 1.53 (t, J = 7.1 Hz, 3H), 1.47 (t, J = 7.1 Hz, 3H).

5.1.35.2. Step 2: 3,4-Dihydro-7-[4-(hydroxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19k). A solution of compound **18k** (100 mg, 258 μmol) in 3 N HCl (2 mL) was stirred for 2 h at room temperature. The reaction was concentrated, and the residue was purified by synthetic adsorbent Sepabeads® SP850 (water) to give the title compound as orange powder (36.7 mg, 43%); mp 240–242 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.16 (s, 1H), 8.05 (s, 1H), 7.97 (s, 1H), 7.28 (s, 1H), 5.07 (br s, 1H), 4.42 (s, 2H); FAB(−)HRMS 330.0511 (+3.6 mmu).

5.1.36. 7-[4-(Carboxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (19l). A solution of compound **11b** (450 mg, 1.46 mmol) and methyl imidazole-4-acetate (617 mg, 4.40 mmol) in MeCN (5 mL) was stirred for 15 h at 110 °C in a sealed tube. After cooling, the reaction was concentrated and the residue was purified by flash column chromatography (SiO_2, CH_2Cl_2) to give crude compound **18l**. The obtained compound **18l** was dissolved into AcOH–concd HCl (5:1, 3 ml) and stirred overnight. The reaction was concentrated and the residue was dissolved into MeOH. A solution of LiOH monohydrate (119 mg, 2.84 mmol) in water (1 mL) was added to the reaction, and stirred overnight. The reaction was concentrated, made acidic with 0.5 N HCl, and concentrated again. Water was added to the residue, the precipitate was collected by filtration, washed with water and AcOEt, and dried under vacuum to give the title compound as brown powder (149 mg, 27%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ: 8.19 (s, 1H), 8.04 (s, 1H), 8.01 (s, 1H), 7.33 (s, 1H), 3.56 (s, 2H); FAB(−)HRMS 358.0425 (+0.2 mmu). Anal. Calcd for $C_{14}H_9N_5O_7 \cdot H_2O$: C, 44.57%; H, 2.94%; N, 18.56%. Found: C, 44.64%; H, 3.11%; N, 18.64%.

5.1.37. Ethyl 3-ethoxy-7-(4-methoxybenzyl)amino-6-nitroquinoxaline-2-carboxylate (20). To a solution of compound **11b** (2.00 g, 6.47 mmol) in THF (15 mL), 4-methoxybenzylamine (1.06 g, 7.76 mmol) and Et_3N (785 mg, 7.76 mmol) were added, and the solution was refluxed for 24 h. After cooling, the reaction was diluted with AcOEt, washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by flash column chromatography [SiO_2, CH_2Cl_2 to CH_2Cl_2:AcOEt (1:1)] to give the title compound as purple powder (2.09 g, 76%); ^{1}H NMR (DMSO-d_6) δ: 8.49 (s, 1H), 8.08 (t, J = 6.3 Hz, 1H), 7.37 (d, J = 8.8 Hz, 2H), 7.22 (s, 1H), 6.91 (d, J = 8.8 Hz, 2H), 4.77 (d, J = 6.3 Hz, 2H), 4.58 (q, J = 7.3 Hz, 2H), 4.41 (q, J = 7.3 Hz, 2H), 3.72 (s, 3H), 1.37 (t, J = 7.3 Hz, 3H), 1.32 (t, J = 7.3 Hz, 3H).

5.1.38. 7-Amino-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (21)

5.1.38.1. Step 1: Ethyl 7-amino-3-ethoxy-6-nitroquinoxaline-2-carboxylate. To a solution of compound **20** (2.09 g, 4.90 mmol) in anisole (5 mL) was added CF_3CO_2H (5 mL). After stirring for 6 h at room temperature, the reaction was concentrated. The residue was purified by flash column chromatography (SiO_2, CH_2Cl_2) to give the title compound as purple powder (1.20 g, 80%); ^{1}H NMR (DMSO-d_6) δ: 8.41 (s, 1H), 8.49 (s, 1H), 7.12 (s, 2H), 4.47 (d, J = 6.8 Hz, 2H), 4.43 (q, J = 7.3 Hz, 2H), 1.37 (t, J = 7.3 Hz, 3H), 1.35 (t, J = 6.8 Hz, 3H).

5.1.38.2. Step 2: 7-Amino-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (21). To a solution of ethyl 7-amino-3-ethoxy-6-nitroquinoxaline-2-carboxylate (200 mg, 653 μmol) in EtOH (10 mL), 1 N NaOH (1.96 mL, 1.96 mmol) and water (2 mL) were added, and the solution was refluxed for 30 min. After cooling, the reaction was adjusted to pH 4 with 10% HCl, diluted with brine, extracted with $CHCl_3$, dried over $MgSO_4$, and evaporated. The residue was dissolved into EtOH (10 mL), and concd HCl (2 mL) was added. After stirring for 24 h at room temperature, the reaction was concentrated, washed with ^{i}Pr$_2$O, and dried under vacuum to give the title compound as brown powder (156 mg, 95%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ: 7.94 (s, 1H), 7.43 (s, 1H), 7.17 (s, 2H); HRMS 250.0311 (−2.7 mmu).

5.1.39. 3,4-Dihydro-6-nitro-3-oxo-7-(pyrrole-1-yl)quinoxaline-2-carboxylic acid (22). To a solution of compound **21** (50.0 mg, 200 μmol) in AcOH (5 mL), 2,5-dimethoxytetrahydrofuran (31.7 mg, 240 μmol) was added, and stirred for 4 h at 80 °C. The reaction was concentrated, washed with water and ^{i}Pr$_2$O, and dried under vacuum to give the title compound as brown powder (28.0 mg, 47%); mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ: 8.07 (s, 1H), 7.91 (s, 1H), 6.99 (t, J = 2.0 Hz, 2H), 6.27 (t, J = 2.0 Hz, 2H); HRMS 300.0502 (+0.7 mmu).

5.1.40. Ethyl 3-ethoxy-7-[4-(2-hydroxyethyl)imidazolyl]-6-nitroquinoxaline-2-carboxylate (23). To a solution of compound **11b** (309 mg, 999 μmol) in DMA (10 mL), 4-(2-hydroxyethyl)imidazole (270 mg, 2.41 mmol) and Et_3N (1 mL) were added. After stirring for 15 h at 120 °C, the reaction was concentrated. The residue was purified by flash column chromatography [SiO_2, CH_2Cl_2 to CH_2Cl_2:MeOH (10:1)] to give the title compound as brown powder (114 mg, 28%); ^{1}H NMR ($CDCl_3$) δ: 8.42 (s, 1H), 8.15 (s, 1H), 7.66 (d, J = 1.0 Hz, 1H), 6.94 (d, J = 1.0 Hz, 1H), 4.66 (q, J = 7.3 Hz, 2H), 4.55 (q, J = 6.8 Hz, 2H), 3.97 (t, J = 5.9 Hz, 2H), 2.92–2.87 (m, 2H), 2.81 (d, J = 4.4 Hz, 1H), 1.53 (t, J = 7.3 Hz, 3H), 1.47 (t, J = 6.8 Hz, 3H).

5.1.41. 7-[4-((*n*-Butylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (28a). To a solution of compound **18k** (100 mg, 258 μmol) in CH_2Cl_2 (1 mL), *n*-butyl isocyanate (43.6 μL, 387 μmol) was added . After stirring for 8 h at room temperature, the reaction was concentrated to

give crude compound **24a**. The obtained compound **24a** was dissolved into AcOH (3 mL) and concd HCl (0.6 mL). After stirring for 24 h at room temperature, the reaction was concentrated. The residue was dissolved into 2 N NaOH, and the precipitate was removed by filtration. The solution was neutralized with concd HCl. The precipitate was collected by filtration, washed with water and AcOEt, and dried under vacuum to give the title compound as yellow powder (31.2 mg, 28%); mp 194–196 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.19 (s, 1H), 8.03 (s, 1H), 8.00 (s, 1H), 7.48 (s, 1H), 7.20 (t, J = 5.6 Hz, 1H), 4.91 (s, 2H), 2.98 (q, J = 6.5 Hz, 2H), 1.41–1.34 (m, 2H), 1.31–1.23 (m, 2H), 0.86 (t, J = 7.3 Hz, 3H); FAB(−)HRMS 429.1161 (+0.2 mmu). Anal. Calcd for $C_{18}H_{18}BrN_6O_7 \cdot \frac{1}{2}H_2O$: C, 49.20%; H, 4.36%; N, 19.13%. Found: C, 49.06%; H, 4.23%; N, 18.92%.

5.1.42. 3,4-Dihydro-7-[4-((isopropylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (28b). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and isopropyl isocyanate, yellow powder (64%); mp 199–201 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.17 (s, 1H), 8.03 (s, 1H), 7.94 (s, 1H), 7.45 (s, 1H), 7.13 (d, J = 7.3 Hz, 1H), 4.90 (s, 2H), 3.63–3.57 (m, 1H), 1.05 (d, J = 6.8 Hz, 6H); FAB(−)HRMS 415.1001 (−0.2 mmu). Anal. Calcd for $C_{17}H_{16}N_6O_7 \cdot H_2O$: C, 47.01%; H, 4.18%; N, 19.35%. Found: C, 47.19%; H, 3.91%; N, 19.40%.

5.1.43. 7-[4-((Cyclohexylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (28c)

5.1.43.1. Step 1: Ethyl 7-[4-((cyclohexylamino)carbonyloxymethyl)imidazol-1-yl]-3-ethoxy-6-nitroquinoxaline-2-carboxylate (24c). To a solution of compound **18k** (100 mg, 258 μmol) in benzene (10 mL), cyclohexyl isocyanate (65.9 μL, 516 μmol) was added, and the solution was refluxed for 2 h. After cooling, the reaction was concentrated and the residue was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 1:1) to give the title compound as brown amorphous solid (61.0 mg, 46%); ^{1}H NMR ($CDCl_3$) δ: 8.43 (s, 1H), 8.14 (s, 1H), 7.68 (d, J = 1.5 Hz, 1H), 7.18 (d, J = 1.0 Hz, 1H), 5.10 (s, 2H), 4.66 (q, J = 7.3 Hz, 2H), 4.55 (q, J = 7.3 Hz, 2H), 3.52–3.48 (m, 1H), 1.95–1.91 (m, 2H), 1.72–1.67 (m, 2H), 1.60–1.55 (m, 2H), 1.53 (t, J = 7.3 Hz, 3H), 1.47 (t, J = 7.3 Hz, 3H), 1.44–1.30 (m, 2H), 1.25–1.08 (m, 2H).

5.1.43.2. Step 2: 7-[4-((Cyclohexylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (28c). Following the procedure described for compound **19i**, the title compound was prepared from compound **24c**, brown powder (81%); mp 237–239 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.19 (s, 1H), 8.03 (s, 1H), 8.02 (s, 1H), 7.49 (s, 1H), 7.17 (d, J = 7.8 Hz, 1H), 4.91 (s, 2H), 1.76–1.65 (m, 4H), 1.25–1.05 (m, 6H); FAB(−)HRMS 455.1324 (−2.0 mmu). Anal. Calcd for $C_{20}H_{20}N_6O_7 \cdot \frac{3}{4}H_2O$: C, 51.12%; H, 4.61%; N, 17.88%. Found: C, 51.35%; H, 4.62%; N, 17.44%.

5.1.44. 3,4-Dihydro-6-nitro-3-oxo-7-[4-((phenylamino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylic acid (28d). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and phenyl isocyanate, dark brown powder (52%); mp 241–243 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.81 (s, 1H), 8.37 (s, 1H), 8.27 (s, 1H), 8.08 (s, 1H), 7.72 (s, 1H), 7.47 (d, J = 7.8 Hz, 2H), 7.28 (t, J = 8.1 Hz, 2H), 6.99 (t, J = 7.3 Hz, 1H), 5.12 (s, 2H); FAB(+)HRMS 451.1008 (+0.5 mmu).

5.1.45. Sodium 7-[4-((Benylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylate (28e). To a solution of compound **18k** (100 mg, 258 μmol) in CH_2Cl_2 (3 mL), benzyl isocyanate (47.8 μl, 387 μmol) was added. After stirring for 6 h at room temperature, the reaction was concentrated to give crude compound **24e**. The obtained compound **24e** was dissolved into AcOH (3 mL) and concd HCl (0.6 mL). After stirring for 36 h at room temperature, the reaction was concentrated. The residue was dissolved into 2 N NaOH, and washed with AcOEt. The aqueous layer was concentrated, and the precipitate was collected by filtration, washed with water and $CHCl_3$, and dried under vacuum to give the title compound as yellow powder (49.0 mg, 36%); mp 222–224 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.21 (s, 1H), 8.13 (s, 1H), 7.95 (s, 1H), 7.81 (t, J = 6.6 Hz, 1H), 7.48 (s, 1H), 7.34–7.21 (m, 5H), 4.95 (s, 2H), 4.10 (d, J = 6.4 Hz, 1H); FAB(+)HRMS 487.0998 (+2.0 mmu).

5.1.46. 3,4-Dihydro-6-nitro-3-oxo-7-[4-(((2-phenylethyl)amino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylic acid (28f)

5.1.46.1. Step 1: Ethyl 3-ethoxy-6-nitro-7-[4-(((2-phenylethyl)amino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylate (24f). To a solution of compound **18k** (150 mg, 387 μmol) and 3-phenylpropionic acid (116 mg, 774 μmol) in benzene (12 mL), diphenylphosphoryl azide (167 μL, 774 μmol) and Et_3N (108 μL, 774 rmumol) were added, and the solution was refluxed for 3 h. After cooling, the reaction was concentrated and the residue was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 1:1) to give the title compound as light brown amorphous solid (123 mg, 59%); ^{1}H NMR ($CDCl_3$) δ: 8.43 (s, 1H), 8.14 (s, 1H), 7.67 (d, J = 1.5 Hz, 1H), 7.31–7.27 (m, 2H), 7.22–7.17 (m, 3H), 5.12 (s, 2H), 4.85 (br s, 1H), 4.66 (q, J = 6.9 Hz, 2H), 4.55 (q, J = 7.3 Hz, 2H), 3.47 (q, J = 6.9 Hz, 2H), 2.82 (t, J = 6.9 Hz, 2H), 1.53 (t, J = 6.9 Hz, 3H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.46.2. Step 2: 3,4-Dihydro-6-nitro-3-oxo-7-[4-(((2-phenylethyl)amino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylic acid (28f). Following the procedure described for compound **19i**, the title compound was prepared from compound **24f**, light brown powder (30%); mp 212–214 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.20 (s, 1H), 8.03 (s, 1H), 8.00 (s, 1H), 7.45 (s, 1H), 7.32–7.16 (m, 6H), 4.92 (s, 2H), 3.17 (t, J = 7.3 Hz, 2H), 2.72 (t, J = 7.3 Hz, 2H); FAB(−)HRMS 477.1151 (−0.8 mmu). Anal. Calcd for $C_{22}H_{18}N_6O_7 \cdot \frac{1}{2}H_2O$: C, 54.21%; H, 3.93%; N, 17.24%. Found: C, 54.22%; H, 3.78%; N, 17.21%.

5.1.47. 7-[4-((2-Chlorophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29a). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 2-chlorophenyl isocyanate, yellow powder (64%); mp 205–207 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.20 (s, 1H), 8.18 (s, 1H), 8.15 (s, 1H), 7.98 (d, J = 1.0 Hz, 1H), 7.59 (d, J = 6.8 Hz, 1H), 7.56 (s, 1H), 7.47 (dd, J = 8.1 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.18 (td, J = 7.3, 1.4 Hz, 1H), 5.07 (s, 2H); FAB(−)HRMS 483.0466 (+1.0 mmu).

5.1.48. 7-[4-((3-Chlorophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29b). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 3-chlorophenyl isocyanate, yellow powder (36%); mp 215–217 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.02 (s, 1H), 8.19 (s, 1H), 8.05 (s, 1H), 7.98 (d, J = 1.5 Hz, 1H), 7.62 (s, 1H), 7.57 (s, 1H), 7.39 (d, J = 9.3 Hz, 1H), 7.31 (t, J = 8.1 Hz, 1H), 7.06–7.03 (m, 1H), 5.08 (s, 2H); FAB(−)HRMS 483.0476 (+2.0 mmu). Anal. Calcd for $C_{20}H_{13}ClN_6O_7 \cdot HCl \cdot \frac{1}{2}H_2O$: C, 45.30%; H, 2.85%; N, 15.85%. Found: C, 45.23%; H, 2.95%; N, 15.84%.

5.1.49. 7-[4-((4-Chlorophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29c). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 4-chlorophenyl isocyanate, yellow powder (63%); mp 250–252 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.94 (s, 1H), 8.20 (s, 1H), 8.04 (s, 1H), 8.00 (s, 1H), 7.57 (s, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.34 (d, J = 9.3 Hz, 2H), 5.07 (s, 2H); FAB(−)HRMS 483.0451 (−0.5 mmu).

5.1.50. 7-[4-((2-Bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29d). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 2-bromophenyl isocyanate, brown powder (66%); mp 260–262 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.12 (s, 1H), 8.23 (s, 1H), 8.08 (s, 1H), 8.04 (s, 1H), 7.64 (dd, J = 8.3, 1.5 Hz, 1H), 7.58 (s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 7.13 (td, J = 7.8, 1.5 Hz, 1H), 5.07 (s, 2H); FAB(+)HRMS 529.0084 (−2.3 mmu). Anal. Calcd for $C_{20}H_{13}BrN_6O_7 \cdot H_2O$: C, 43.89%; H, 2.76%; N, 15.36%. Found: C, 44.24%; H, 2.66%; N, 15.03%.

5.1.51. 7-[4-((3-Bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid hydrochloride (29e). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 3-bromophenyl isocyanate, yellow powder (50%); mp 266–268 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.03 (s, 1H), 8.22 (s, 1H), 8.16 (s, 1H), 7.99 (s, 1H), 7.77 (s, 1H), 7.59 (s, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.25 (t, J = 8.1 Hz, 1H), 7.17 (d, J = 8.3 Hz, 1H), 5.08 (s, 2H); FAB(+)HRMS 529.0140 (+3.3 mmu). Anal. Calcd for $C_{20}H_{13}BrN_6O_7 \cdot HCl \cdot H_2O$: C, 41.15%; H, 2.76%; N, 14.40%. Found: C, 41.07%; H, 2.67%; N, 14.35%.

5.1.52. 7-[4-((4-Bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29f). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 4-bromophenyl isocyanate, yellow powder (60%); mp 270–272 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.95 (s, 1H), 8.20 (s, 1H), 8.03 (s, 1H), 7.99 (s, 1H), 7.57 (d, J = 1.0 Hz, 1H), 7.48–7.43 (m, 4H), 5.07 (s, 2H); FAB(+)HRMS 529.0123 (+1.5 mmu). Anal. Calcd for $C_{20}H_{13}BrN_6O_7 \cdot \frac{1}{2}H_2O$: C, 44.62%; H, 2.62%; N, 15.61%. Found: C, 44.97%; H, 2.51%; N, 15.26%.

5.1.53. 3,4-Dihydro-7-[4-((2-methylphenylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid hydrochloride (29g). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 2-methylphenyl isocyanate, brown powder (12%); mp 252–254 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.96 (s, 1H), 8.20 (s, 1H), 8.04 (s, 1H), 8.00 (s, 1H), 7.54 (s, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.19–7.13 (m, 2H), 7.05 (d, J = 7.3 Hz, 1H), 5.04 (s, 2H), 2.20 (s, 3H); FAB(−)HRMS 463.1009 (+0.7 mmu). Anal. Calcd for $C_{21}H_{16}BrN_6O_7 \cdot HCl$: C, 50.36%; H, 3.42%; N, 16.78%. Found: C, 50.38%; H, 3.64%; N, 16.80%.

5.1.54. 3,4-Dihydro-7-[4-((3-methylphenylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29h). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 3-methylphenyl isocyanate, yellow powder (25%); mp 223–225 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.69 (s, 1H), 8.20 (s, 1H), 8.04 (s, 1H), 7.98 (s, 1H), 7.56 (s, 1H), 7.29 (s, 1H), 7.27 (d, J = 7.8 Hz, 1H), 7.15 (d, J = 7.8 Hz, 1H), 6.80 (d, J = 7.3 Hz, 1H), 5.06 (s, 2H), 2.25 (s, 3H); FAB(−)HRMS 463.0996 (−0.6 mmu). Anal. Calcd for $C_{21}H_{16}N_6O_7 \cdot \frac{1}{2}H_2O$: C, 53.28%; H, 3.62%; N, 17.75%. Found: C, 53.27%; H, 3.51%; N, 17.61%.

5.1.55. 3,4-Dihydro-7-[4-((4-methylphenylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29i). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 4-methylphenyl isocyanate, yellow powder (49%); mp 265–267 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.65 (s, 1H), 8.19 (s, 1H), 8.05 (s, 1H), 7.98 (d, J = 1.5 Hz, 1H), 7.55 (d, J = 1.0 Hz, 1H), 7.35 (d, J = 8.3 Hz, 2H), 7.07 (d, J = 8.3 Hz, 2H), 5.05 (s, 2H), 2.23 (s, 3H); FAB(+)HRMS 465.1156 (−0.3 mmu).

5.1.56. Sodium 3,4-dihydro-7-[4-((4-methoxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylate (29j). Following the procedure described for compound **28e**, the title compound was prepared from compound **18k** and 4-methoxylphenyl isocyanate, yellow powder (53%); mp 265–267 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.58 (s, 1H), 8.22 (s,

1H), 8.15 (s, 1H), 7.98 (d, J = 1.0 Hz, 1H), 7.56 (s, 1H), 7.37 (d, J = 8.8 Hz, 1H), 6.86 (d, J = 8.8 Hz, 2H), 5.04 (s, 2H), 3.70 (s, 3H); FAB(+)HRMS 503.0913 (−1.4 mmu). Anal. Calcd for $C_{21}H_{15}N_6O_8Na \cdot \frac{1}{2}H_2O$: C, 49.32%; H, 3.15%; N, 16.43%. Found: C, 49.51%; H, 3.08%; N, 16.58%.

5.1.57. 3,4-Dihydro-6-nitro-3-oxo-7-[4-((2-trifluoromethylphenylamino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylic acid (29k). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 2-trifluoromethylphenyl isocyanate, yellow powder (72%); mp 230–232 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.21 (s, 1H), 8.20 (s, 1H), 8.04 (s, 1H), 8.00 (s, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.68 (t, J = 7.8 Hz, 1H), 7.53 (s, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.45 (t, J = 7.8 Hz, 1H), 5.04 (s, 2H); FAB(−)HRMS 517.0704 (−1.5 mmu). Anal. Calcd for $C_{21}H_{13}F_3N_6O_7 \cdot \frac{1}{4}H_2O$: C, 48.23%; H, 2.60%; N, 16.07%. Found: C, 47.93%; H, 2.52%; N, 16.09%.

5.1.58. 3,4-Dihydro-6-nitro-3-oxo-7-[4-((3-trifluoromethylphenylamino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylic acid (29l). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 3-trifluoromethylphenyl isocyanate, yellow powder (38%); mp 256–258 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.18 (s, 1H), 8.21 (s, 1H), 8.04 (s, 1H), 8.01 (s, 1H), 7.93 (s, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.59 (s, 1H), 7.52 (t, J = 7.8 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 5.10 (s, 2H); FAB(−)HRMS 517.0723 (+0.3 mmu). Anal. Calcd for $C_{21}H_{13}F_3N_6O_7 \cdot H_2O$: C, 45.46%; H, 2.54%; N, 15.15%. Found: C, 46.13%; H, 2.54%; N, 15.44%.

5.1.59. 3,4-Dihydro-6-nitro-3-oxo-7-[4-((4-trifluoromethylphenylamino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylic acid (29m). Following the procedure described for compound **28f**, the title compound was prepared from compound **18k** and 4-trifluoromethylbenzoic acid, yellow powder (11%); mp 276–278 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.22 (s, 1H), 8.21 (s, 1H), 8.03 (s, 1H), 8.01 (s, 1H), 7.68 (d, J = 9.3 Hz, 2H), 7.65 (d, J = 9.3 Hz, 2H), 7.59 (s, 1H), 5.11(s, 2H); FAB(−)HRMS 517.0703 (−1.6 mmu).

5.1.60. 7-[4-((2-Carboxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29n)

5.1.60.1. Step 1: Ethyl 3-ethoxy-7-[4-((2-ethoxycarbonylphenylamino)carbonyloxymethyl)imidazol-1- yl]-6-nitroquinoxaline-2-carboxylate (25n). To a solution of compound **18k** (200 mg, 516 μmol) in CH_2Cl_2 (10 mL), ethyl 2-isocyanatobenzoate (455 mg, 2.38 mmol) was added. After stirring for 18 h at room temperature, the reaction was concentrated. The residue was purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 2:1 to 1:3) to give the title compound as pale yellow powder (199 mg, 67%); ^{1}H NMR ($CDCl_3$) δ: 10.33 (s, 1H), 8.66 (s, 1H), 8.49 (s, 1H), 8.18 (d, J = 8.8 Hz, 1H), 8.05 (s, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.634 (t, J = 8.8 Hz, 1H), 7.625 (s, 1H), 7.16 (t, J = 8.8 Hz, 1H), 5.12 (s, 2H), 4.62 (q, J = 7.3 Hz, 2H), 4.48 (q, J = 7.3 Hz, 2H), 4.32 (q, J = 7.3 Hz, 2H), 1.43 (t, J = 7.3 Hz, 3H), 1.37 (t, J = 7.3 Hz, 3H), 1.32 (t, J = 7.3 Hz, 3H).

5.1.60.2. Step 2: 7-[4-((2-Carboxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29n). To a suspension of compound **25n** (97.0 mg, 197 μmol) in EtOH (5 mL), 1 N KOH (592 μL, 592 μmol) and water (1 mL) were added, and the solution was refluxed for 1 h. After cooling, the reaction was concentrated, and the residue was dissolved into 47% HBr (1.6 mL). After stirring for 24 h at room temperature, the reaction was concentrated. The residue was washed with water and dried under vacuum to give the title compound as brown powder (81.0 mg, quant.); mp 216–218 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.75 (s, 1H), 8.31 (d, J = 8.3 Hz, 1H), 8.24 (s, 1H), 8.05 (s, 1H), 7.99–7.97 (m, 2H), 7.62 (t, J = 7.8 Hz, 1H), 7.57 (s, 1H), 7.12 (t, J = 7.8 Hz, 1H), 5.11 (s, 2H); FAB(−)HRMS 493.0752 (+0.8 mmu).

5.1.61. 7-[4-((3-Carboxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29o). A solution of compound **18k** (500 mg, 1.29 mmol) and ethyl 3-isocyanatobenzoate (321 μL, 1.94 mmol) in benzene (15 mL) was refluxed for 2 h. After cooling, the reaction was concentrated and purified by flash column chromatography (SiO_2, *n*-hexane:AcOEt, 1:1) to give compound **25o** as yellow oil. The obtained compound **25o** was dissolved into AcOH (5 mL) and concd HCl (1 mL), and stirred for 24 h at room temperature. The reaction was concentrated, and water was added. The precipitate was collected by filtration, washed with water and AcOEt, and dried under vacuum. LiOH (1 N, 15 mL) was added and the solution was stirred for 3 h at room temperature. The insoluble part was removed by filtration, and the filtrate was made acidic with concd HCl. The precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as brown powder (297 mg, 44%); mp 272–274 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.00 (s, 1H), 8.22 (s, 1H), 8.13 (s, 1H), 8.08 (s, 1H), 8.04 (s, 1H), 7.69 (t, J = 9.3 Hz, 1H), 7.61 (s, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.40 (t, J = 7.8 Hz, 1H), 5.10 (s, 2H); FAB(−)HRMS 493.0739 (−0.5 mmu). Anal. Calcd for $C_{21}H_14N_6O_9 \cdot \frac{3}{2}H_2O$: C, 48.47%; H, 3.29%; N, 16.15%. Found: C, 48.62%; H, 3.13%; N, 16.27%.

5.1.62. 7-[4-((4-Carboxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29p). Following the procedure described for compound **29o**, the title compound was prepared from compound **18k** and ethyl 4-isocyanatobenzoate, brown powder (37%); mp 268–270 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.18 (s, 1H), 8.23 (s, 1H), 8.13 (s, 1H), 8.05 (s, 1H), 7.87 (d, J = 8.8 Hz, 2H), 7.63 (s, 1H), 7.58 (d, J = 8.8 Hz, 2H), 5.12 (s, 2H); FAB(−)HRMS 493.0769 (+2.5 mmu).

5.1.63. 3,4-Dihydro-7-[4-((4-ethoxycarbonylphenylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitro-3- oxoquinoxaline-2-carboxylic acid (29q)

5.1.63.1. Step 1: Ethyl 3-ethoxy-7-[4-((4-ethoxycarbonylphenylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitroquinoxaline-2-carboxylate (25p). Following the procedure described for compound **28c** (step 1), the title compound was prepared from compound **18k** and ethyl 4-isocyanatobenzoate, yellow amorphous solid (quant.); ^{1}H NMR ($CDCl_3$) δ: 8.45 (s, 1H), 8.15 (s, 1H), 7.99 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 1.5 Hz, 1H), 7.47 (d, J = 8.8 Hz, 2H), 7.25 (d, J = 1.0 Hz, 1H), 7.17 (s, 1H), 5.25 (s, 2H), 4.66 (q, J = 6.9 Hz, 2H), 4.55 (q, J = 7.3 Hz, 2H), 4.35 (q, J = 6.9 Hz, 2H), 1.53 (t, J = 6.9 Hz, 3H), 1.46 (t, J = 7.3 Hz, 3H), 1.38 (t, J = 6.9 Hz, 3H).

5.1.63.2. Step 2: 3,4-Dihydro-7-[4-((4-ethoxycarbonylphenylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29q). Following the procedure described for compound **19i** (step 2), the title compound was prepared from compound **25p**, brown powder (80%); mp 207–209 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.23 (s, 1H), 8.24 (s, 1H), 8.06 (s, 1H), 7.89 (d, J = 8.8 Hz, 2H), 7.66 (s, 1H), 7.61 (d, J = 8.8 Hz, 2H), 5.13 (s, 2H), 4.28 (q, J = 7.3 Hz, 2H), 1.30 (t, J = 7.3 Hz, 3H); FAB(−)HRMS 521.1057 (−0.8 mmu). Anal. Calcd for $C_{23}H_{18}N_6O_9 \cdot \frac{3}{2}H_2O$: C, 50.28%; H, 3.85%; N, 15.30%. Found: C, 50.02%; H, 3.62%; N, 14.94%.

5.1.64. 7-[4-((2,3-Dichlorophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29r). Following the procedure described for compound **28c**, the title compound was prepared from compound **18k** and 2,3-dichlorophenyl isocyanate, brown powder (68%); mp 234–236 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.43 (s, 1H), 8.22 (s, 1H), 8.05 (s, 1H), 7.59 (dd, J = 8.5, 1.5 Hz, 1H), 7.58 (s, 1H), 7.45 (dd, J = 7.8, 1.5 Hz, 1H), 7.35 (t, J = 8.3 Hz, 1H), 5.09 (s, 2H); FAB(−)HRMS 517.0043 (−0.5 mmu). Anal. Calcd for $C_{20}H_{12}Cl_2N_6O_7$: C, 46.26%; H, 2.33%; N, 16.18%. Found: C, 46.12%; H, 2.38%; N, 15.90%.

5.1.65. 7-[4-((2,4-Dichlorophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29s). Following the procedure described for compound **28c**, the title compound was prepared from compound **18k** and 2,4-dichlorophenyl isocyanate, brown powder (68%); mp 272–274 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.33 (s, 1H), 8.23 (s, 1H), 8.10 (s, 1H), 8.04 (s, 1H), 7.64–7.60 (m, 3H), 7.41 (dd, J = 8.8, 2.4 Hz, 1H), 5.09 (s, 2H); FAB(−)HRMS 517.0090 (+2.4 mmu).

5.1.66. 7-[4-((2,5-Dichlorophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29t). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 2,5-dichlorophenyl isocyanate, yellow powder (53%); mp 203–205 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.41 (s, 1H), 8.22 (s, 1H), 8.09 (s, 1H), 8.04 (s, 1H), 7.75 (d, J = 2.4 Hz, 1H), 7.61 (s, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.25 (dd, J = 8.3, 2.4 Hz, 1H), 5.11 (s, 2H); FAB(−)HRMS 517.0046 (−2.0 mmu). Anal. Calcd for $C_{20}H_{12}Cl_2N_6O_7 \cdot H_2O$: C, 44.71%; H, 2.63%; N, 15.64%. Found: C, 44.39%; H, 2.40%; N, 15.34%.

5.1.67. 7-[4-((2,6-Dichlorophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29u). Following the procedure described for compound **28c**, the title compound was prepared from compound **18k** and 2,6-dichlorophenyl isocyanate, brown powder (7%); mp 253–255 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.46 (s, 1H), 8.18 (s, 1H), 8.05 (s, 1H), 7.98 (s, 1H), 7.54 (d, J = 8.3 Hz, 2H), 7.53 (s, 1H), 7.34 (t, J = 8.3 Hz, 1H), 5.03 (s, 2H); FAB(−)HRMS 517.0087 (+2.1 mmu).

5.1.68. 7-[4-((3,4-Dichlorophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid hydrochloride (29v). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 3,4-dichlorophenyl isocyanate, yellow powder (36%); mp 218–220 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.15 (s, 1H), 8.19 (s, 1H), 8.06 (s, 1H), 7.98 (s, 1H), 7.79 (d, J = 2.4 Hz, 1H), 7.58 (s, 1H), 7.55 (d, J = 8.8 Hz, 1H), 7.42 (dd, J = 9.0, 2.7 Hz, 1H), 5.09 (s, 2H); FAB(−)HRMS 517.0062 (−0.5 mmu). Anal. Calcd for $C_{20}H_{12}Cl_2N_6O_7 \cdot HCl \cdot \frac{1}{2}H_2O$: C, 42.54%; H, 2.50%; N, 14.88%. Found: C, 42.79%; H, 2.54%; N, 14.95%.

5.1.69. 7-[4-((3,5-Dichlorophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29w). Following the procedure described for compound **28a**, the title compound was prepared from compound **18k** and 3,5-dichlorophenyl isocyanate, yellow powder (81%); mp 246–248 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.23 (s, 1H), 8.21 (s, 1H), 8.03 (s, 1H), 8.01 (s, 1H), 7.59 (s, 1H), 7.53 (d, J = 2.0 Hz, 2H), 7.21 (t, J = 2.0 Hz, 1H), 5.10 (s, 2H); FAB(−)HRMS 517.0065 (−0.2 mmu). Anal. Calcd for $C_{20}H_{12}Cl_2N_6O_7 \cdot \frac{1}{2}H_2O$: C, 45.47%; H, 2.48%; N, 15.91%. Found: C, 45.43%; H, 2.28%; N, 15.95%.

5.1.70. 3,4-Dihydro-7-[4-((1-naphthylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline- 2-carboxylic acid (29x)

5.1.70.1. Step 1: Ethyl 3-ethoxy-7-[4-((1-naphthylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitroquinoxaline-2-carboxylate (25x). Following the procedure described for compound **28c** (step 1), the title compound was prepared from compound **18k** and 1-naphthyl isocyanate, yellow amorphous (quant.); ^{1}H NMR ($CDCl_3$) δ: 8.45 (s, 1H), 8.16 (s, 1H), 7.90–7.72 (m, 3H), 7.66 (d, J = 8.3 Hz, 1H), 7.53 (dd, J = 6.9, 1.5 Hz, 1H), 7.52–7.45 (m, 3H), 7.13 (s, 1H), 5.30 (d, J = 1.5 Hz, 2H), 4.66 (q, J = 7.3 Hz, 2H), 4.55 (q, J = 7.3 Hz, 2H), 1.53 (t, J = 7.3 Hz, 3H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.70.2. Step 2: 3,4-Dihydro-7-[4-((1-naphthylamino) carbonyloxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29x). Following the procedure described for compound **19i**, the title compound was

prepared from compound **25x**, brown powder (70%); mp 224–226 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.70 (s, 1H), 8.33 (s, 1H), 8.29 (s, 1H), 8.28 (s, 1H), 8.083 (s, 1H), 8.077–8.05 (m, 1H), 7.93 (dd, J = 6.4, 3.4 Hz, 1H), 7.75 (d, J = 8.3 Hz, 1H), 7.70 (d, J = 5.4 Hz, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.55–7.47 (m, 3H), 5.16 (s, 2H); FAB(−)HRMS 499.1031 (+2.9 mmu). Anal. Calcd for $C_{24}H_{16}N_6O_7 \cdot H_2O$: C, 55.60%; H, 3.50%; N, 16.21%. Found: C, 55.67%; H, 3.39%; N, 15.88%.

5.1.71. 3,4-Dihydro-7-[4-((2-naphthylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline- 2-carboxylic acid (29y)

5.1.71.1. Step 1: Ethyl 3-ethoxy-7-[4-((2-naphthylamino)carbonyloxymethyl)imidazol-1-yl]-6-nitroquinoxaline-2-carboxylate (25y). Following the procedure described for compound **28f** (step 1), the title compound was prepared from compound **18k** and naphthalene-2-carboxylic acid, brown amorphous (quant.); ^{1}H NMR ($CDCl_3$) δ: 8.44 (s, 1H), 8.15 (s, 1H), 7.99 (s, 1H), 7.76 (d, J = 8.8 Hz, 1H), 7.75 (d, J = 7.3 Hz, 1H), 7.71 (d, J = 1.0 Hz, 1H), 7.46–7.35 (m, 3H), 7.13 (s, 1H), 7.27 (d, J = 1.0 Hz, 1H), 7.01 (s, 1H), 5.28 (s, 2H), 4.66 (q, J = 6.9 Hz, 2H), 4.54 (q, J = 7.3 Hz, 2H), 1.52 (t, J = 6.9 Hz, 3H), 1.46 (t, J = 7.3 Hz, 3H).

5.1.71.2. Step 2: 3,4-Dihydro-7-[4-((2-naphthylamino)-carbonyloxymethyl)imidazol-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (29y). Following the procedure described for compound **19i**, the title compound was prepared from compound **25y**, brown powder (58%); mp 275–277 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.01 (s, 1H), 8.22 (s, 1H), 8.09 (s, 1H), 8.05–8.03 (m, 2H), 7.84–7.76 (m, 3H), 7.61 (s, 1H), 7.54 (dd, J = 8.8, 2.0 Hz, 1H), 7.47–7.43 (m, 1H), 7.39–7.35 (m, 1H), 5.13 (s, 2H); FAB(−)HRMS 499.1021 (+1.9 mmu). Anal. Calcd for $C_{24}H_{16}N_6O_7 \cdot \frac{3}{4}H_2O$: C, 56.09%; H, 3.43%; N, 16.18%. Found: C, 56.25%; H, 3.36%; N, 15.94%.

5.1.72. 7-[4-((2-Bromobenylamino)carbonyloxymethyl)imidazol-1-yl]-3-ethoxy-6-nitroquinoxaline-2-carboxylic acid (30a)

5.1.72.1. Step 1: Ethyl 7-[4-((2-Bromobenylamino)carbonyloxymethyl)imidazol-1-yl]-3-ethoxy-6-nitroquinoxaline-2-carboxylate (26a). Following the procedure described for compound **28f** (step 1), the title compound was prepared from compound **18k** and 2-bromophenylacetic acid, brown powder (34%); ^{1}H NMR ($CDCl_3$) δ: 8.44 (s, 1H), 8.14 (s, 1H), 7.68 (s, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.41 (d, J = 6.9 Hz, 1H), 7.33–7.12 (m, 2H), 5.40–5.30 (br, 1H), 5.15 (s, 2H), 4.67 (q, J = 6.9 Hz, 2H), 4.55 (q, J = 7.3 Hz, 2H), 4.46 (d, J = 6.4 Hz, 2H), 1.53 (t, J = 6.9 Hz, 3H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.72.2. Step 2: 7-[4-((2-Bromobenylamino)carbonyloxymethyl)imidazol-1-yl]-3-ethoxy-6- nitroquinoxaline-2-carboxylic acid (30a). Following the procedure described for compound **19i**, the title compound was prepared from compound **26a**, brown powder (45%); mp 187–189 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.18 (s, 1H), 8.07 (s, 1H), 7.96 (s, 1H), 7.86 (t, J = 5.9 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.49 (s, 1H), 7.21 (t, J = 7.8 Hz, 1H), 4.97 (s, 2H), 4.24 (d, J = 5.9 Hz, 2H); FAB(−)HRMS 541.0113 (+0.6 mmu). Anal. Calcd for $C_{21}H_{15}BrN_6O_7$: C, 46.43%; H, 2.78%; N, 15.47. Found: C, 46.13%; H, 2.80%; N, 15.34.

5.1.73. 7-[4-((3-Bromobenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (30b)

5.1.73.1. Step 1: Ethyl 7-[4-((3-bromobenylamino)carbonyloxymethyl)imidazol-1-yl]-3-ethoxy-6-nitroquinoxaline-2-carboxylate (26b). Following the procedure described for compound **28f** (step 1), the title compound was prepared from compound **18k** and 3-bromophenylacetic acid, brown gum (18%); ^{1}H NMR ($CDCl_3$) δ: 8.44 (s, 1H), 8.15 (s, 1H), 7.68 (d, J = 1.0 Hz, 1H), 7.44–7.38 (m, 2H), 7.24–7.18 (m, 3H), 5.17 (s, 2H), 4.67 (q, J = 7.3 Hz, 2H), 4.55 (q, J = 7.3 Hz, 2H), 4.37 (d, J = 5.9 Hz, 2H), 1.53 (t, J = 7.3 Hz, 3H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.73.2. Step 2: 7-[4-((3-Bromobenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (30b). Following the procedure described for compound **19i**, the title compound was prepared from compound **26b**, yellow powder (41%); mp 159–161 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.16 (s, 1H), 8.08 (s, 1H), 7.95 (s, 1H), 7.84 (t, J = 5.9 Hz, 1H), 7.47–7.42 (m, 3H), 7.29–7.26 (m, 2H), 4.96 (s, 2H), 4.20 (d, J = 5.9 Hz, 2H); FAB(−)HRMS 541.0085 (−2.2 mmu). Anal. Calcd for $C_{21}H_{15}BrN_6O^7$: C, 46.43%; H, 2.78%; N, 15.47%. Found: C, 46.55%; H, 2.87%; N, 14.92%.

5.1.74. 7-[4-((4-Bromobenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (30c)

5.1.74.1. Step 1: Ethyl 7-[4-((4-bromobenylamino)carbonyloxymethyl)imidazol-1-yl]-3-ethoxy-6-nitroquinoxaline-2-carboxylate (26c). Following the procedure described for compound **28f** (step 1), the title compound was prepared from compound **18k** and 4-bromophenylacetic acid, yellow amorphous solid (52%); ^{1}H NMR ($CDCl_3$) δ: 8.44 (s, 1H), 8.14 (s, 1H), 7.68 (s, 1H), 7.45 (d, J = 8.3 Hz, 2H), 7.19 (s, 1H), 7.17 (d, J = 8.3 Hz, 2H), 5.16 (s, 2H), 4.67 (q, J = 7.3 Hz, 2H), 4.55 (q, J = 7.3 Hz, 2H), 4.34 (d, J = 6.4 Hz, 2H), 1.53 (t, J = 7.3 Hz, 3H), 1.47 (t, J = 7.3 Hz, 3H).

5.1.74.2. Step 2: 7-[4-((4-Bromobenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (30c). Following the procedure described for compound **19i**, the title compound was prepared from compound **26c**, yellow powder (20%); mp 217–219 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 8.18 (s, 1H), 8.05 (s, 1H), 7.95 (s, 1H), 7.83 (t, J = 5.9 Hz, 1H), 7.51 (d, J = 8.3 Hz, 2H), 7.47 (s, 1H), 7.22 (d, J = 8.3 Hz, 2H), 4.95 (s, 2H), 4.17 (d, J = 5.9 Hz, 2H); FAB(−)HRMS 541.0099 (−0.8 mmu). Anal. Calcd for $C_{21}H_{15}BrN_6O_7 \cdot \frac{1}{2}H_2O$: C, 45.67%; H, 2.92%; N, 15.22%. Found: C, 45.71%; H, 2.79%; N, 15.20%.

5.1.75. 7-[4-(2-((4-Carboxyphenylamino)carbonyloxy)ethyl)imidazol-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (31).

To a solution of compound **23**

(110 mg, 274 μmol) in MeCN (5 mL), ethyl 4-isocyanatobenzoate (57.0 mg, 298 μmol) was added, and the solution was stirred overnight. The reaction was concentrated and purified by flash column chromatography [SiO_2, CH_2Cl_2 to CH_2Cl_2:MeOH (50:1)] to give crude compound **27**. The obtained compound **27** was dissolved into AcOH–concd HCl (5:1, 6 mL), and stirred for 20 min at 80 °C followed by 16 h at room temperature. The reaction was concentrated, and the residue was dissolved into MeOH (5 mL). LiOH monohydrate (60.0 mg, 1.43 mmol) in water (1 mL) was added to the solution, and stirred for 2 h at 50 °C. The reaction was concentrated, adjusted to pH 2 with concd HCl, and concentrated again. Water was added to the residue, and the precipitate was collected by filtration, washed with water and AcOEt, and dried under vacuum to give the title compound as brown powder (70.5 mg, 51%); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ: 12.65 (s, 1H), 10.05 (s, 1H), 8.19 (s, 1H), 8.03 (s, 1H), 8.02 (s, 1H), 7.86 (d, J = 8.6 Hz, 2H), 7.57 (d, J = 9.2 Hz, 2H), 7.35 (s, 1H), 4.38 (t, J = 7.3 Hz, 2H), 2.95 (t, J = 7.3 Hz, 2H); FAB(−)HRMS 507.0902 (+0.1 mmu).

5.1.76. 4-Chloropyridine-3-methanol (34). To a solution of ^{i}Pr$_2$NH (18.7 mL, 133 mmol) in THF (100 mL) 1.55 M *n*-BuLi in *n*-hexane (103 mL, 160 mmol) was added dropwise at −78 °C under Ar atmosphere. After stirring for 15 min at the same temperature, compound **32** (10.0 g, 66.7 mmol) was added to the solution, and stirred for 30 min. DMF (6.19 mL, 80.0 mmol) was added dropwise to the solution at −78 °C. The reaction was warmed to room temperature slowly, and stirred overnight. The reaction was quenched with 3 N HCl and stirred for 2 h at room temperature. The solution was neutralized with $NaHCO_3$, extracted with AcOEt, dried over Na_2SO_4, and evaporated to give crude compound **33** as brown oil. The obtained compound **33** was dissolved into EtOH (100 mL), and $NaBH_4$ (3.03 g, 80.0 mmol) was added to the solution. After stirring for 3 h at room temperature, the reaction was concentrated. Water was added to the residue, extracted with AcOEt, dried over Na_2SO_4, and evaporated to give the title compound as pale yellow powder (7.02 g, 73%); ^{1}H NMR ($CDCl_3$) δ: 8.68 (s, 1H), 8.46 (d, J = 5.4 Hz, 1H), 7.33 (d, J = 5.4 Hz, 1H), 4.84 (s, 2H), 2.23 (s, 1H).

5.1.77. Ethyl 3-ethoxy-7-[3-(hydroxymethyl)-4-pyridon-1-yl]-6-nitroquinoxaline-2-carboxylate (36). To a solution of compound **34** (2.33 g, 16.2 mmol) in water (25 mL), NaOH (5.20 g, 130 mmol) was added, and the solution was refluxed for 24 h. After cooling, the reaction was neutralized with concd HCl, and concentrated to give crude compound **35**. The obtained compound **35** was dissolved into DMF (20 mL), and compound **11b** (500 mg, 1.62 mmol) was added. After stirring for 4 h at 110 °C, the reaction was poured into ice water, extracted with AcOEt, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (SiO_2, AcOEt) to give the title compound as yellow powder (410 mg, 61%); ^{1}H NMR ($CDCl_3$) δ: 8.57 (s, 1H), 8.20 (s, 1H), 7.45 (d, J = 2.4 Hz, 1H), 7.41 (dd, J = 7.3, 2.4 Hz, 1H), 6.52 (d, J = 8.0 Hz, 1H), 4.68 (q, J = 7.2 Hz, 2H), 4.59 (s, 2H), 4.56 (q, J = 7.2 Hz, 2H), 1.54 (t, J = 7.1 Hz, 3H), 1.47 (t, J = 7.1 Hz, 3H).

5.1.78. 3,4-Dihydro-7-[3-((phenylamino)carbonyloxymethyl)-4-pyridon-1-yl]-6-nitro-3-oxoquinoxaline-2-carboxylic acid (38a). Following the procedure described for compound **28a**, the title compound was prepared from compound **36** and phenyl isocyanate, yellow powder (51%); mp 230–232 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 9.71 (s, 1H), 8.38 (s, 1H), 8.13 (s, 1H), 8.06 (s, 1H), 7.92 (dd, J = 7.3, 2.0 Hz, 1H), 7.45 (d, J = 7.8 Hz, 2H), 7.26 (t, J = 7.8 Hz, 2H), 6.97 (t, J = 7.3 Hz, 1H), 6.34 (d, J = 7.8 Hz, 1H), 4.91 (s, 2H); FAB(−)HRMS 476.0837 (−0.6 mmu). Anal. Calcd for $C_{22}H_{15}N_5O_8 \cdot \frac{3}{2}H_2O$: C, 52.39%; H, 3.60%; N, 13.88%. Found: C 52.70%; H, 3.41%; N, 13.81%.

5.1.79. 7-[3-((4-Carboxyphenylamino)carbonyloxymethyl)-4-pyridon-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (38b)

5.1.79.1. Step 1: Ethyl 3-ethoxy-7-[3-((4-ethoxycarbonylphenylamino)carbonyloxymethyl)-4-pyridon- 1-yl]-6-nitroquinoxaline-2-carboxylate (37b). Following the procedure described for compound **28c** (step 1), the title compound was prepared from compound **36** and ethyl 4-isocyanatobenzoate, yellow powder (94%); ^{1}H NMR ($CDCl_3$) δ: 8.57 (s, 1H), 8.22 (s, 1H), 7.97 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 2.5 Hz, 1H), 7.43 (d, J = 8.8 Hz, 2H), 7.39 (dd, J = 7.8, 2.5 Hz, 1H), 6.56 (d, J = 7.8 Hz, 1H), 5.15 (s, 2H), 4.68 (q, J = 6.9 Hz, 2H), 4.55 (q, J = 7.3 Hz, 2H), 4.34 (q, J = 7.3 Hz, 2H), 1.54 (t, J = 6.9 Hz, 3H), 1.47 (t, J = 7.3 Hz, 3H), 1.37 (t, J = 7.3 Hz, 3H).

5.1.79.2. Step 2: 7-[3-((4-Ethoxycarbonylphenylamino)carbonyloxymethyl)-4-pyridon-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid. To a solution of compound **37b** (692 mg, 1.14 mmol) in AcOH (12 mL), concd HCl (3 mL) was added, and stirred for 18 h at room temperature. Water was added to the reaction, and the precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as yellow powder (548 mg, 86%); mp 201–203 °C (decomp.); ^{1}**H** NMR (DMSO-d_6) δ: 10.15 (s, 1H), 8.38 (s, 1H), 8.12 (s, 1H), 8.05 (d, J = 2.0 Hz, 1H), 7.91–7.86 (m, 3H), 7.58 (d, J = 8.3 Hz, 2H), 6.30 (d, J = 7.8 Hz, 1H), 4.94 (s, 2H), 4.27 (q, J = 7.3 Hz, 2H), 1.30 (t, J = 7.3 Hz, 3H); FAB(−)HRMS 548.1053 (−0.1 mmu). Anal. Calcd for $C_{25}H_{19}N_5O_{10} \cdot \frac{1}{2}H_2O$: C, 53.78%; H, 3.61%; N, 12.54%. Found: C, 53.82%; H, 3.69%; N, 12.60%.

5.1.79.3. Step 3:7-[3-((4-Carboxyphenylamino)carbonyloxymethyl)-4-pyridon-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (38b). To a suspension of 7-[3-((4-ethoxycarbonylphenylamino)carbonyloxymethyl)-4-pyridon-1-yl]-3,4-dihydro-6-nitro-3-oxoquinoxaline-2-carboxylic acid (383 mg, 697 μmol) in water (4 mL), 1 N LiOH (6.97 mL, 6.97 mmol) was added, and the suspension was stirred for 3 h at room temperature. After purification by synthetic adsorbent Sepabeads® SP850 (water–MeCN, 20:1), water was added and adjusted to pH 2 with concd HCl. The precipitate

was collected by filtration, washed with water, and dried under vacuum to give the title compound as yellow powder (253 mg, 65%); mp 231–233 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ: 10.10 (s, 1H), 8.38 (s, 1H), 8.13 (s, 1H), 8.06 (d, J = 2.5 Hz, 1H), 7.90 (dd, J = 7.3, 2.5 Hz, 1H), 7.85 (d, J = 8.8 Hz, 2H), 7.56 (d, J = 8.8 Hz, 2H), 6.32 (d, J = 7.3 Hz, 1H), 4.94 (s, 2H); FAB(−)HRMS 520.0740 (+0.0 mmu). Anal. Calcd for $C_{23}H_{15}N_5O_{10}\cdot 2H_2O$: C, 49.56%; H, 3.44%; N, 12.56%. Found: C, 49.32%; H, 3.41%; N, 12.47%.

5.2. Modeling

5.2.1. Molecular modeling. The crystal structure of bacterial lysine/arginine/ornithine-binding protein (LAOBP) solved at 1.9 Å resolution was chosen as the template for the AMPA-R homology model (Protein Data Bank entry: 2LAO[21]) because bacterial LAOBP is homologous with glutamate receptors of known three-dimensional structure. The amino acid sequences of the GluR3 ligand binding core of the AMPA-R and LAOBP were reported by O'Hara et al.[22] The three-dimensional structure of the GluR3 ligand binding core was modeled using the procedures and strategies outlined by Blundell et al.[23] The processes employed were essentially the same as those used by O'Hara et al.[22,24] The docking experiments were carried out by manually docking compound **19h**, a quinoxaline-2-carboxylic acid derivative, into our AMPA-R model. Several docking orientations of the ligand in the binding site of AMPA-R were obtained and all possible docking model structures were minimized using the conjugate gradient method of the CVFF force field until a gradient convergence of 0.1 kcal/mol Å was reached (Cα carbon atoms were fixed, and the dielectric constant 78 was used). The docking model structure with the lowest interaction energy between the ligand and the AMPA-R model was selected as the AMPA-R complex model among docking model structures with the low total energy.

5.2.2. Hardware and software. Molecular modeling studies were carried out using the Insight II/Discover version 98.0 molecular modeling package (Accelrys Inc., San Diego, CA, USA) on a Silicon Graphics O2 R10K workstation.

5.3. Solubility measurements

The solubilities of the test compounds were determined in 0.1 M phosphate buffer (pH 7.4) at ambient temperature as described by Higuchi et al.[20] About 2 mg of each compound was mixed with 200 mL phosphate buffer and sonicated for 10 min. After centrifugation, 10 mL of the supernatant was diluted with 100 mL 50% MeOH and 10 mL DMSO, in the case of reference samples containing 10 mg/mL of the test compounds. The concentration of the test compound in the solution was determined by high-performance liquid chromatography (HPLC) using a C18 reversed-phase column (4.6 × 75 mm; J'shpere ODS-H80, YMC, Japan), with a mobile phase consisting of 30% or 50% MeOH in 50 mM NaH_2PO_4 run at a flow rate of 1 mL/min, and detection at a wavelength of 254 nm. The HPLC analyses were performed with a Hitachi L-6200 pump, a Hitachi L-4000 ultraviolet wavelength detector (Hitachi, Japan) and a Tosoh AS-8010 automatic sample injection system (Tosoh, Japan).

5.4. Biology

5.4.1. Radioligand receptor-binding assay. Receptor binding was measured as the percentage displacement of 5 nM [^{3}H]AMPA and 10 nM [^{3}H]CGS-19755 from extensively washed rat cortical synaptosomal membranes, as described by Honore et al., Johansen et al., and Murphy et al.[25–27] Using 50 mM Tris–HCl buffer (pH 7.4), tubes containing the membranes, 5 nM [^{3}H]AMPA, 2.5 mM $CaCl_2$, 100 mM KSCN, and the test compounds were incubated for 30 min at 0 °C, while other tubes containing 10 nM [^{3}H]CGS-19755 (pH 8.0) and the test compounds were incubated for 15 min at 4 °C. Nonspecific binding was determined in the presence of 0.1 or 1 mM glutamic acid. After stopping the reaction by suction filtration, the radioactivity on the filter was measured with a liquid scintillation counter TR2300 (Packard, Tokyo, Japan). IC_{50} values were calculated and converted to K_i values using the Cheng–Prosoff equation.

5.4.2. AMPA-evoked depolarization in rat cortical slices (DC potential). Coronal sections of rat brain (400 μm thick) were trimmed to form 'wedges' of tissue containing cerebral cortex and corpus callosum as described by Harrison and Simmonds.[28] After 3 h of incubation in oxygenated Krebs medium, each slice was placed in a two-compartment recording chamber. This was arranged so that the cortical tissue was contained almost entirely in one compartment and the ventral margin of the cortex passed through a greased slot so that the corpus callosum was entirely in the other compartment. The cortical tissue was depolarized by superfusion of AMPA (5 μM) for 2 min. The DC potential between the two compartments was monitored via Ag/AgCl electrodes and a high input impedance amplifier. AMPA-induced deviations from this baseline DC potential were measured at peak amplitude. Each test compound was applied to the cortical end of the preparation 10 min before exposure to AMPA, and the ability of the test compound to inhibit AMPA-induced DC potentials was assessed.

5.4.3. Rat focal ischemia model. Male Wistar rats (300–350 g) were subjected to permanent occlusion of the right middle cerebral artery (MCA) under halothane anesthesia as described by Tamura et al.[19] Rectal temperature was maintained at 37 ± 1 °C during the experiment. After 24 h, the brains were removed and sliced into five coronal sections (2 mm thick) with the aid of a rat brain matrix (a manual slicer). The slices were placed in 2% (w/v) triphenyltetrazolium chloride (TTC) solution, followed by 10% (v/v) phosphate-buffered formalin. Tissue damage (the area not stained with TTC) was scored on a four-point damage score as the infarct volumes (see Fig. 5). On evaluation of four-point damage score in focal ischemia model, gray areas are infarct damage area, then grade 1 damage is '0' and grade

4 damage is '3' in four-point scores. The '3' of grade 4 damage is most effective score against focal ischemia model. Each test compound was administered by continuous iv infusion for 4 h, starting immediately after occlusion of the MCA. Control rats received saline only, and their four-point damage scores were less than 1.0.

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