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Design and synthesis of novel 7-heterocycle-6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acids bearing a substituted phenyl group as superior AMPA receptor antagonists with good physicochemical properties

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Abstract—We describe the design, synthesis, and physicochemical and biological properties of a novel series of 7-heterocycle-6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acids bearing a substituted phenyl group joined through a urethane or urea linkage to the heterocycle at the 7 position. Introduction of the trifluoromethyl group at the 6 position conferred good biological activity, including neuroprotective effects, as well as good physicochemical properties. In terms of α-amino-3-hydroxy-5-methylisoxazole propionate receptor (AMPA-R) affinity, a urea linkage was equivalent to a urethane linkage and a pyrrole ring at the 7 position reduced affinity in comparison with an imidazole ring. Among this series, compound **14h** (**KRP-199**), which has a 4-carboxyphenyl group joined through a urethane linkage to a 7-imidazolyl heterocycle, was found to possess high potency and selectivity for the AMPA-R in vitro and to exhibit good neuroprotective effects in vivo. Furthermore, the compound showed good physicochemical properties, including stability to light and good solubility in aqueous solutions.

1. Introduction

Glutamic acid, an excitatory amino acid (EAA), is a major excitatory neurotransmitter in the mammalian central nervous system. EAA receptors are mainly postsynaptic and are divided into two types: the ionotropic glutamate receptor subtypes (iGlu-Rs), including those for *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole propionate (AMPA), and kainate (KA), and the metabotropic glutamate receptor subtypes (mGlu-Rs).

The AMPA receptor (AMPA-R) subtype of the iGlu-Rs is an ionotropic glutamate receptor coupled to ion channels that modulate cell excitability by gating the flow of calcium and sodium ions into the cell.[1] However, overstimulation of postsynaptic glutamate receptors by release of EAAs at supraphysiological levels results in neuronal death. Among the glutamate receptor antagonists, AMPA-R antagonists appear to be free from side effects such as schizophrenia[2] and have shown effectiveness against neuronal death, even if administered post-ischemia.[3] In consequence, AMPA-R antagonists have been reported to be effective in the therapy of neurodegenerative disorders such as ischemic stroke, epilepsy, head trauma, and Alzheimer's disease.[3–9]

Since the demonstration of the potent and selective AMPA-R antagonistic activity of NBQX,[3] several research groups have modified the quinoxalinedione structure. The numerous resulting compounds can be categorized as first-generation compounds of substituted simple quinoxalinedione structure, such as NBQX[3] and YM-90K,[10,11] and second-generation compounds with a hydrophilic substituent at the N-1 position of the quinoxalinedione, as exemplified by introduction of acetic acid and phosphonomethyl moieties as the hydrophilic groups to yield YM-872 (zonampanel)[12,13] and

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ZK-200775[14] (Fig. 1), respectively. Among these derivatives, the first-generation compound YM-90K is well established to be a potent and selective AMPA antagonist and has been shown to be neuroprotective in animal models of global and focal cerebral ischemia. However, although this compound has excellent neuroprotective ability, its limited solubility in aqueous solutions has precluded its development as a clinical agent. Furthermore, simple quinoxalinediones such as NBQX have been reported to cause kidney toxicity, probably as a result of their physicochemical properties, particularly poor solubility.[15] The second-generation compounds, which were designed to improve the physicochemical properties of the simple quinoxalinediones, are more soluble and do not appear to cause kidney toxicity. Although the second-generation compounds also appear to be good antagonists, they have yet to be marketed as therapeutic drugs for neuroprotection in acute cerebral ischemia.

In our previous papers,[16,17] we reported the development of a novel third-generation AMPA-R antagonist, the 7-imidazolyl-6-nitro-3-oxoquinoxaline-2-carboxylic acid derivative **1** (GRA-293), which contains a carboxylic acid as a hydrophilic group as well as an imidazole moiety. This compound is characterized by a 4-carboxyphenyl group joined through a urethane linkage onto an imidazole ring at the 7 position on the 3-oxoquinoxaline-2-carboxylic acid nucleus. It shows excellent AMPA-R antagonist activity in vitro and in vivo compared with the known antagonists based on the quinoxalinedione nucleus and is also water-soluble. From these studies, we concluded that introduction of a phenyl group joined through a urethane linkage at the 4 position of a 7-imidazolyl group on the 6-nitro-3-oxoquinoxaline-2-carboxylic acid nucleus confers high affinity and good selectivity for the AMPA-R. However, in continuation studies we found that compound **1** has suboptimal physicochemical properties, particularly instability to fluorescent light in neutral solution. This poor photostability precluded the scaled-up synthesis and pre-formulation studies necessary to develop a manufacturing process. On the other hand, we found that YM-90K and YM-872, which both contain a quinoxalinedione nucleus with a nitro group, also show instability under similar conditions. The fact that these two quinoxalinedione compounds and our reported 3-oxoquinoxaline-2-carboxylic acid show light instability in spite of their different structural nucleus led us to conclude that the problem arises from their common substituents. Namely, we thought that the nitro group at the 6 position and the imidazole ring at the 7 position on each nucleus might be the cause of the photoinstability. In a study of binding to the AMPA-R, Bigge et al. reported that a nitro group at the 6 position of quinoxalinediones is needed for interaction with the AMPA-R because of the increased acidity of the proton of the amide moiety at the 4 position.[18] However, ZK-200775, which has a trifluoromethyl group instead of a nitro group as an electron-withdrawing group, also has good AMPA-R antagonistic activity.[14] Consequently, we studied whether introduction of a trifluoromethyl group instead of a nitro group into the 6 position on our 3-oxoquinoxaline-2-carboxylic acid nucleus could improve photostability. Additionally, during development of 6-nitro-3-oxoquinoxaline-2-carboxylic acids with urethane linkaged substituted phenyl groups, we did not study the consequences for AMPA-R affinity of conversion of the urethane linkage or replacement of the imidazole ring at the 7 position. Therefore, we also decided to test the effect on AMPA-R affinity of changing the urethane linkage to another linkage, such as a urea linkage, and of replacement of the imidazole ring by another five-membered ring, such as a pyrrole ring, at the 7 position of 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid (Fig. 2).

Our research efforts have focused on designing and synthesizing novel 3-oxoquinoxaline-2-carboxylic acids possessing potent selectivity against the AMPA-R and good neuroprotective effects in vivo, as well as good physicochemical properties such as photostability in neutral solution and solubilities as an injection. Moreover, we have also focused on the synthesis of compounds with a urea linkage onto the imidazole or pyrrole groups at the 7 position in the 3-oxoquinoxaline-2-carboxylic acid nucleus. In this paper, we wish to describe the synthesis of novel 7-imidazolyl- and 7-pyrrolyl-6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid derivatives with urethane or urea linkages and their biological properties.

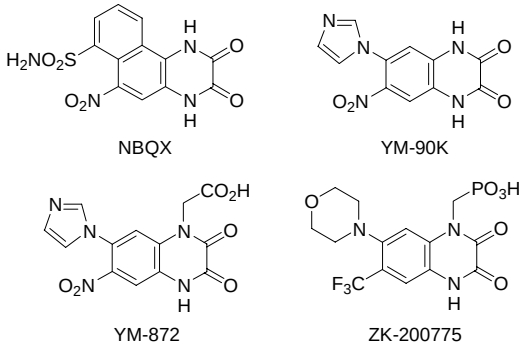


Figure 1.

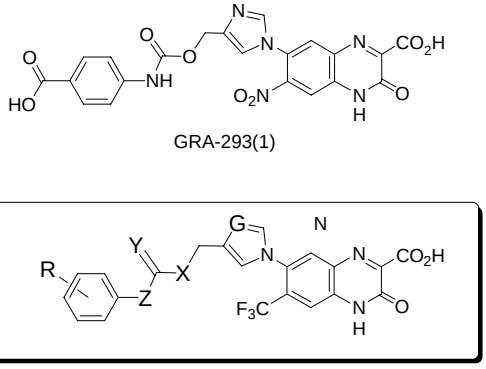
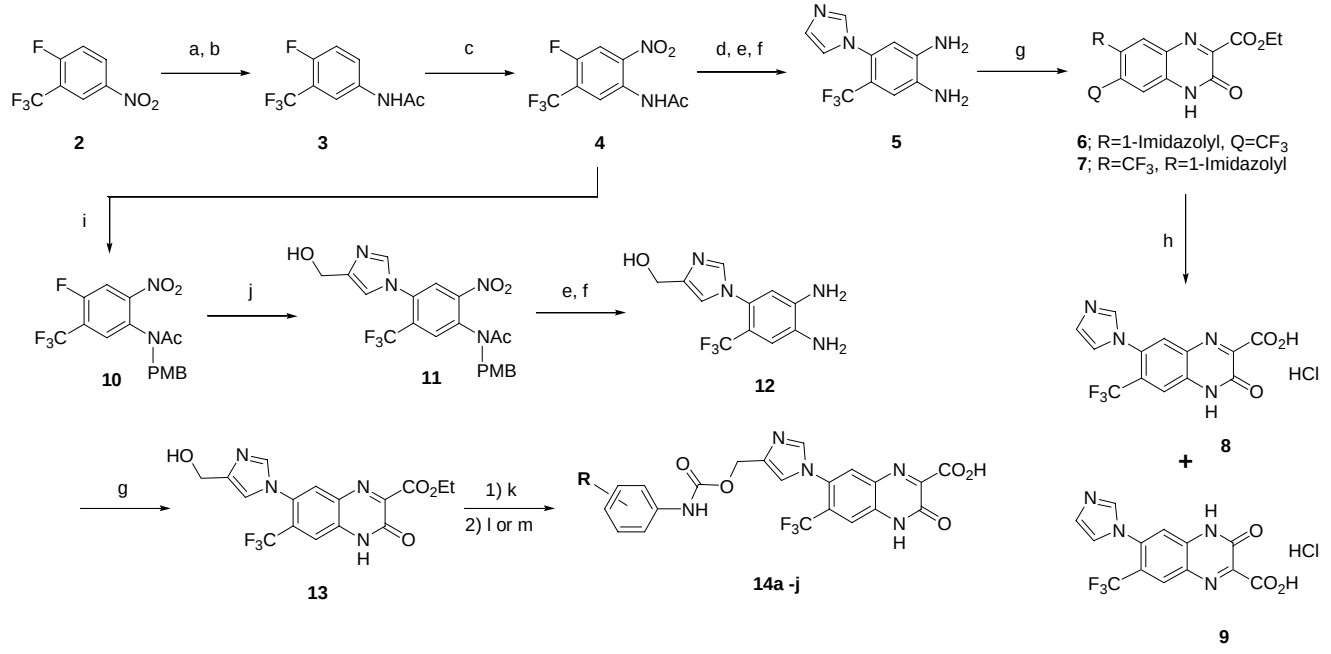


Figure 2.

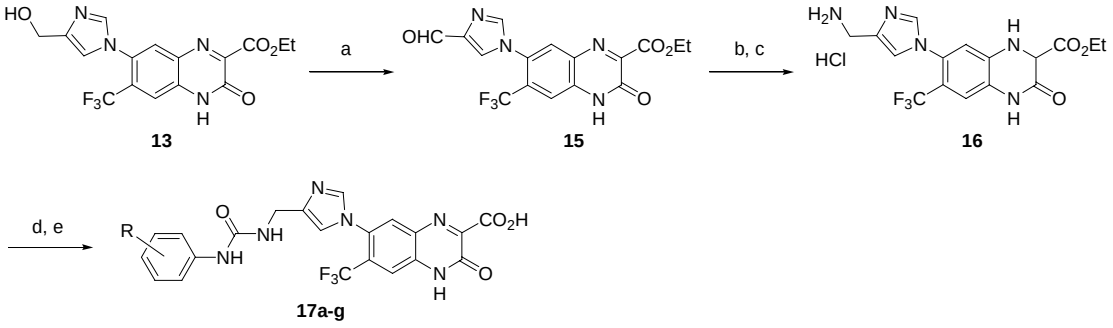
2. Chemistry

The synthesis of 7-imidazolyl-3-oxoquinoxaline-2-carboxylic acid derivatives (**8**, **14a**–**j**, **17a**–**g**) is outlined in Schemes 1 and 2. The starting material for non-substituted 7-imidazolyl quinoxaline-2-carboxylic acid **8** is 4-fluoro-3-trifluoromethylnitrobenzene **2**. After hydrogenation of **2**, acetyl protection of the amino group and selective nitration (fuming HNO_3)[17] at the ortho position were carried out in three steps.[10] Compound **4** was treated with commercially available imidazole, followed by deprotection of the acetyl group and hydrogenation of the nitro group to give phenylenediamine **5**. Compound **5** was cyclized with diethyl ketomalonate, then separated and washed with EtOH to give ethyl 7-imidazolyl-6-trifluoromethyl-3-oxoquinoxaline-2-carboxylate (**6**) and its isomer (**7**). The identities of compound **6** and its isomer **7** were confirmed by the chemical shifts and coupling constants seen on ^1H NMR spectroscopy. Esters **6** and **7** were used to prepare 7-imidazolyl-6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid **8** and its isomer **9**.

Concerning 7-imidazolyl-3-oxoquinoxaline-2-carboxylic acid derivatives (**14a**–**j**) with substituted phenyl groups joined through a urethane linkage to the imidazole at the 7 position, 4-fluoro-2-nitro-5-trifluoromethylacetanilide (**4**) was protected at the amino group by 4-methoxybenzyl chloride (PMBCl), which is needed to increase the reactivity of the nucleophilic displacement in the fluoro group at the 4 position. Compound **10** was treated with commercially available 4-hydroxymethylimidazole, followed by deprotection of the acetyl group and the PMB group with 4 N HCl and hydrogenation of the nitro group to give phenylenediamine **12**. Compound **12** was cyclized with diethyl ketomalonate



Scheme 1. 7-Imidazolyl and 7-urethane linked imidazolyl derivatives. Reagents: (a) H_2 10% Pd-C, AcOH; (b) Ac_2O, Et_3N, $CHCl_3$; (c) *fuming*HNO_3; (d) imidazole, DMF; (e) 4 N HCl; (f) H_2 10% Pd-C, EtOH; (g) diethyl ketomalonate, EtOH; (h) 6 N HCl, AcOH; (i) PMBCl, K_2CO^3, DMF; (j) 4-(hydroxymethyl)imidazole-HCl, MeCN; (k) R-Ph-NCO, DMF; (l) LiOH, EtOH-H_2O; (m) concd HCl, AcOH.



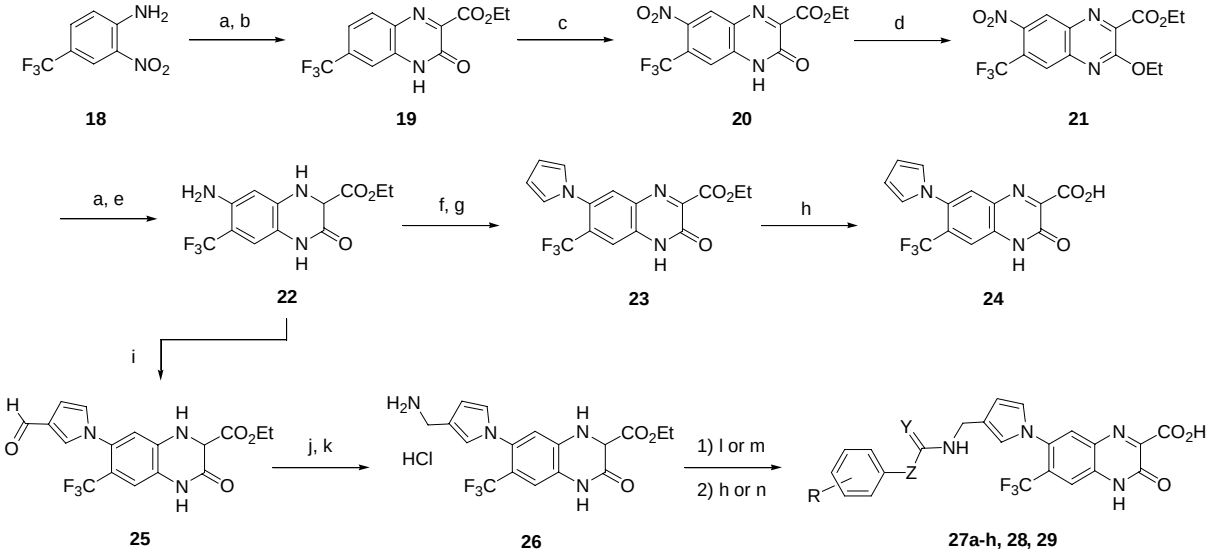
Scheme 2. 7-Urea linked imidazolyl derivatives. Reagents: (a) MnO_2, 1,4-dioxane; (b) NH_2OH-HCl, NaOAc, EtOH; (c) H_2, 10% Pd-C, concd HCl, EtOH; (d) R-Ph-NCO, Et_3N, DMF; (e) NaOH, EtOH–H_2O.

and separated by column chromatography to provide ethyl 7-(4-hydroxymethylimidazolyl)-3-oxoquinoxaline-2-carboxylate **13** as the key intermediate. Treatment with various isocyanates followed by hydrolysis provided the corresponding 4-urethane linked derivatives **14a–j** (Scheme 1).

Concerning 7-imidazolyl-3-oxoquinoxaline-2-carboxylic acid derivatives (**17a–g**) with substituted phenyl groups joined through a urea linkage, the 3-oxoquinoxaline-2-carboxylate **13** was treated with manganese dioxide dissolved in 1,4-dioxane to give the 7-(4-formyl)imidazolyl compound **15**. After compound **15** reacted with hydroxylamine, it was hydrogenated to provide the 7-(4-aminomethyl)imidazolyl compound **16**. The urea linked 3-oxoquinoxaline-2-carboxylic acids **17a–g** were prepared by the reaction of 7-(4-aminomethyl)imidazolyl compound **16** with various isocyanates, whereafter the ethyl ester was hydrolyzed. As the key intermediate **16** reacted with isocyanates, 1,2,3,4-tetrahydroquinoxaline was oxidized to quinoxaline, using the excess triethylamine (Et_3N) as the auxiliary base (Scheme 2).

The synthesis of 7-pyrrolyl-3-oxoquinoxaline-2-carboxylic acid derivatives (**24**, **27a–h**, **28**, and **29**) is outlined in Scheme 3. The starting material for the 7-pyrrolyl derivatives is 2-nitro-4-trifluoromethylaniline. After hydrogenation of compound **18**, the 1,2-phenylenediamine was cyclized with diethyl ketomalonate and recrystallized to provide ethyl 3-oxoquinoxaline-2-carboxylate **19**, which was converted to the nitro compound **20** by selective nitration (KNO_3, concd H_2SO_4)[17] at the 6 position of the 3-oxoquinoxaline-2-carboxylic acid nucleus. Conversion of the nitro group at the 7 position of compound **20** to an amino group by hydrogenation gave a mixture of several reductive compounds, and the main compounds were the desired 7-amino compound and the 7-amino-tetrahydro compound **22**. This hydrogenation brought about reduction of the nitro group, as well as saturation of the quinoxaline ring. We then attempted to isolate the 7-amino-tetrahydro compound **22**. The nitro compound **20** was converted to the imino ether **21** by ethylation of the amide moiety. This was followed by sequential hydrogenation and hydrolysis of the imino ether to give the single 7-amino-tetrahydro compound **22** as the key intermediate. The 7-pyrrolyl compound **24** was prepared by the reaction of the key intermediate compound **22** and commercially available 2,5-dimethoxytetrahydrofuran, followed by oxidation of the quinoxaline ring with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and hydrolysis of the ester.

The 7-(3-formyl)pyrrolyl compound **25** was prepared by the reaction of the key intermediate compound **22** and commercially available 3-formyl-2,5-dimethoxytetrahydrofuran, and compound **25** was then treated with hydroxylamine whereafter it was hydrogenated to provide the 7-(3-aminomethyl)pyrrolyl compound **26**. The desired urea (**27a–h**) or thiourea (**28**) linkage pyrrolyl derivatives were prepared from compound **26** and appropriate isocyanates or isothiocyanate, whereafter the ester was hydrolyzed by the same procedure used for the urea linked 7-imidazolyl compounds (**17a–g**). The amide linkage pyrrolyl derivative **29** was prepared by condensation of compound **26** and 4-fluorophenylacetic acid by 1-ethyl-3-(3′-dimethylaminoisopropyl)carbodiimide hydrochloride (EDCI) whereafter the ester was hydrolyzed.



Scheme 3. 7-Pyrrolyl and 7-urea linked pyrrolyl derivatives. Reagents: (a) H_2, 10% Pd-C, EtOH; (b) diethyl ketomalonate, EtOH; (c) KNO_3, concd H_2SO_4; (d) iodoethane, Ag_2O, toluene; (e) concd HCl, EtOH; (f) 2,5-dimethoxytetrahydrofuran, AcOH; (g) DDQ, 1,4-dioxane; (h) KOH, EtOH–H_2O; (i) 2,5-dimethyoxytetrahydrofuran-3-aldehyde, AcOH; (j) NH_2OH–HCl, NaOAc, EtOH; (k) H_2, 10% Pd-C, concd HCl, EtOH; (l) R-Ph-NCS, Et_3N, DMF; (m) 4-F-PhCH_2CO_2H, EDCl, Et_3N, DMF; (n) NaOH, EtOH–H_2O.

3. Results and discussion

3.1. Study of substituents at the 6 position on 3-oxoquinoxaline-2-carboxylic acid

As a first step, we confirmed the effects of substituents at the 6 position on AMPA-R affinity and photostability. The results are shown in Table 1. Comparison of compounds with a trifluoromethyl group or a nitro group[16,17] at the 6 position on the quinoxaline nucleus confirmed that replacement of the nitro group by a trifluoromethyl group imparted photostability (compounds **A**,[16,17] **B**[17] vs. **8**, **24**) and that introduction of a trifluoromethyl group into a compound with an imidazole ring at the 7 position increased AMPA-R affinity 6-fold compared with a nitro group (compound **A** vs. **8**). Moreover, replacement of the heterocyclic imidazole ring at the 7 position with a pyrrole group decreased AMPA-R affinity 7-fold (compound **8** vs. **24**). On the other hand, 6-imidazolyl-7-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid **9**, which is the anti-substituted compound at the 6 and 7 positions of compound **8**, exhibited loss of AMPA-R affinity. On the basis of these results, we concluded that for 6-nitro-3-oxoquinoxaline-2-carboxylic acids with a heterocyclic group at the 7 position the nitro group is responsible for photoinstability and that a 7-imidazolyl group and a 6-trifluoromethyl group appear to be essential for increased AMPA-R affinity. In summary, for our design of novel AMPA-R antagonists we adopted the 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid nucleus as the core structure instead of the 6-nitro-3-oxoquinoxaline-2-carboxylic acid nucleus.

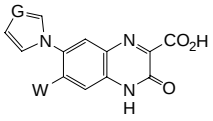
3.2. 7-Urethane linked imidazole derivatives with a 6-trifluoromethyl group

We have already found that introduction of a phenyl group joined through a urethane linkage at the 4 position of the 7-imidazolyl group on 6-nitro-3-oxoquinoxaline-2-carboxylic acid imparts high affinity and good selectivity for the AMPA-R.[16,17] Therefore, on the basis of our knowledge of 6-nitro-3-oxoquinoxaline-2-carboxylic acid, we introduced the same side chain into 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid in order to find an active compound with high AMPA-R affinity and better selectivity for the AMPA-R. The results are shown in Table 2 (**14a**–**j**). Introduction of a phenyl group joined through a urethane linkage led to better AMPA-R affinity than that of the non-linked compound (**8** vs. **14a**). However, introduction of substituents into the terminal phenyl group led to AMPA-R affinity similar to that of the non-substituted phenyl compound **14a**. Among the substituted phenyl compounds, introduction of bromine, methyl, or methoxy substituents at the 4 position of the phenyl group reduced AMPA-R selectivity in spite of retaining good AMPA-R affinity (**14a** vs. **14c**–**14e**). On the other hand, introduction of trifluoromethyl, carboxy or carboxymethyl groups at the 3 or 4 position on the terminal phenyl group led to better selectivity (**14f**–**j**). In particular, substitution by a carboxy group at the 4 position, compound **14h**, led to both high affinity and good selectivity for the AMPA-R. However, conversion of the 4-carboxy group of **14h** to a 4-carboxymethyl group (**14j**) on the terminal phenyl group reduced AMPA-R affinity despite the presence of the same carboxylic acid. In summary, insertion of a urethane linked substituted phenyl group at the 4 position on the 7-imidazolyl group led to better AMPA-R affinity, and the preferred substituent on the terminal phenyl group appears to be a 4-carboxy group.

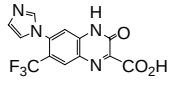
3.3. 7-Urea linked imidazole derivatives with a 6-trifluoromethyl group

As the next step, we investigated the effect of introduction of a urea linkage instead of a urethane linkage at the 4 position of the 7-imidazolyl group on 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid for AMPA-R affinity and selectivity. The results are shown in Table 2 (**17a**–**g**). Introduction of a urea linked phenyl group led to good AMPA-R selectivity, comparable with that of the corresponding compound with a urethane linkage (**14a** vs. **17a**). Moreover, introduction of bromine or methoxy groups at the 4 position of the terminal phenyl group improved AMPA-R selectivity compared with the corresponding urethane compounds (**17b**, **c** vs. **14c**, **e**). In general, it seemed to us that introduction of a urea

Table 1. Activity and stability of 3-oxoquinoxaline-2-carboxylic acid with 7-imidazolyl or 7-pyrrolyl group



A, B, 8, 24

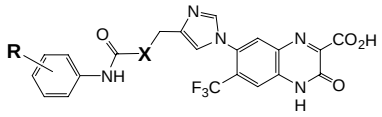


9

Compound	W	G	AMPA-R affinity[21,22] (K_i, nM)	Stability to light[b] (% for control)
A	NO_2	N	560	<10
B	NO_2	CH	920	<35
8[a]	CF_3	N	86	>95
24	CF_3	CH	640	>95
9[a]			880	NT[c]

[a] HC1 salt.
[b] Residual ratios after 7000 Lx, 3 h.
[c] Not tested.

Table 2. 7-Substituted imidazole derivatives



Compound	R	X	AMPA-R affinity[21,22] (K_i, nM)	NMDA-R affinity[23] (K_i, nM)	Selectivity $\left(\frac{\text{NMDA-R}}{\text{AMPA-R}}\right)$
14a	H	O	23	3700	160
14b	3-Br	O	17	4500	260
14c	4-Br	O	20	610	31
14d	4-Me	O	20	1700	85
14e	4-MeO	O	30	1800	60
14f	4-CF_3	O	29	>10,000	>340
14g	3-CO_2H	O	35	>10,000	>290
14h	4-CO_2H	O	16	>10,000	>630
14i	3-CH_2CO_2H	O	28	>10,000	>430
14j	4-CH_2CO_2H	O	23	>10,000	>360
17a	H	NH	39	9800	250
17b	4-Br	NH	37	>10,000	>270
17c	4-MeO	NH	43	>10,000	>230
17d	3-CO_2H	NH	43	>10,000	>230
17e[a]	4-CO_2H	NH	17	>10,000	>590
17f	3-CH_2CO_2H	NH	44	>10,000	>230
17g	4-CH_2CO_2H	NH	43	>10,000	>230

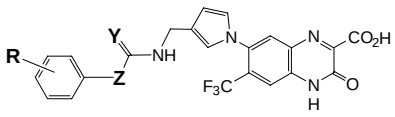
[a] HC1 salt.

linkage conferred good AMPA-R selectivity, although AMPA-R affinity was slightly reduced. Among the urea linked compounds, it was notable that introduction of a carboxy group at the 4 position of the phenyl group (**17e**) led to a marked increase in AMPA-R selectivity together with good AMPA-R affinity. Accordingly, the compound with a 4-carboxyphenyl group joined through a urea linkage to the 7-imidazolyl group on 3-oxoquinoxaline-2-carboxylic acid had AMPA-R affinity and selectivity equal to those of the urethane linkaged compound (**14h** vs. **17e**). In summary, from investigation of the linkage between the substituted terminal phenyl group and the imidazole ring at the 7 position, we concluded that a urea linkage imparts excellent AMPA-R affinity as well as selectivity equivalent to that of the urethane linkage, and that compounds with a urea linkage also represent an important class of development candidates. Furthermore, comparison with the urethane linkage suggests that the NH moiety close to the 4-imidazolylmethyl group in the urea linkage enhances AMPA-R selectivity.

3.4. 7-Urea linked pyrrole derivatives with a 6-trifluoromethyl group

Next, we studied the effect of conversion of the imidazole ring to a pyrrole ring at the 7 position of 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid (**27a**–**h**, **28**, **29**). The non-substituted 7-pyrrolyl compound (**24**) showed reduced AMPA-R affinity compared with that of the corresponding 7-imidazolyl compound (**8**), as shown in Table 1. However, since we have found that a side chain consisting of a substituted phenyl group joined through a urethane or urea linkage to the 7-imidazolyl group on the 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid nucleus enhances AMPA-R affinity, we investigated whether introduction of a side chain into the 7-pyrrolyl compound **24** would also enhance AMPA-R affinity. In general, the urea linkage is more stable than the urethane linkage under chemical synthetic conditions. Therefore, in an attempt to enhance AMPA-R affinity, we introduced a similar substituent group, a phenyl group linked through a urea moiety, into the 7-pyrrolyl compound **24**. The results are shown in Table 3. Insertion of the urea linked phenyl group into the 7-pyrrolyl group improved AMPA-R affinity compared with the non-substituted compound **24**. In particular, introduction of bromine, carboxy or trifluoromethyl substituents at the 4 position of the terminal phenyl group led to better affinity and selectivity for the AMPA-R (**27c**, **f**, **h**), whereas introduction of 4-methoxy or 3-carboxy groups led to slightly weaker affinity (**27e**, **g**). The AMPA-R affinity of **27h**, unfortunately, was inferior to that of **17e**, despite the presence of a 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid nucleus with a 4-carboxyphenyl group joined through a urea linkage. We also converted the urea linkage of selected pyrrolyl compounds to thiourea and amide linkages. However, the AMPA-R affinities of the thiourea and amide linkage compounds were approximately 3- and 10-fold weaker than those of the urea linkage compounds, respectively (**27a** vs. **28**, **27d** vs. **29**). From these results, we believe that an NH moiety close to the terminal phenyl group is an important part of the linkage. In summary, from investigation of the introduction of a urea-linked pyrrole ring at the 7 position in our 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acids, we concluded that the whole of the urea linkage is an important interaction unit that may function as a proton acceptor during interaction with the AMPA-R.

Table 3. 7-Substituted pyrrole derivatives



Compound	R	Y	Z	AMPA-R affinity[21,22] (K_i, nM)	NMDA-R affinity[23] (K_i, nM)	Selectivity ($\frac{NMDA-R}{AMPA-R}$)
17e[a]				17	>10,000	>590
27a	H	O	NH	79	6700	85
27b	3-Br	O	NH	30	>10,000	>330
27c	4-Br	O	NH	36	>10,000	>280
27d	4-F	O	NH	50	>10,000	>200
27e	4-MeO	O	NH	86	>10,000	>120
27f	4-CF_3	O	NH	30	>10,000	>330
27g	3-CO_2H	O	NH	72	>10,000	>140
27h	4-CO_2H	O	NH	37	>10,000	>270
28	H	S	NH	260	NT[b]	NT[b]
29	4-F	O	CH_2	540	NT[b]	NT[b]

[a] HC1 salt.
[b] Not tested.

Table 4. Pharmacological data of substituted 7-heterocycle 3-oxoquinoxaline-2-carboxylic acid derivatives

Compound	AMPA-R affinity[21,22] (K_i, nM)	NMDA-R affinity[23] (K_i, nM)	Selectivity ($\frac{NMDA-R}{AMPA-R}$)	AMPA-R antagonism[24] (D.C. potential)	Stability for Light[a] (% for control)	Protective effects in Focal ischemia Model[19] (Dose; mg/kg/h for 4 h, iv)[b]	
YM-90K	100	43,000	430	(+)	<5	2.8 (15)	$n = 7$
YM-872	62	15,000	240	(+)	<5	0.5 (30)	$n = 6$
1	22	>10,000	>450	(+)	<5	3.0 (2.5)	$n = 3$
14h	16	>10,000	>630	(+)	>95	2.9 (2.5)	$n = 3$
17e	17	>10,000	>590	(+)	>95	2.9 (2.5)	$n = 3$
27h	37	>10,000	>270	(+)	>95	2.5 (2.5)	$n = 2$

[a] Residual ratios after 7000 Lx, 3 h.
[b] Control; <1.0.

3.5. Neuroprotective effects in permanent focal ischemia in rats

The neuroprotective effects of the selected compounds **1**, **14h**, **17e**, and **27h**, and the reference compounds YM-90K and YM-872 were examined with the permanent focal ischemia model in rats, as described by Tamura et al.[19] The results are shown in Table 4 and Fig. 3. The 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid compounds showed better neuroprotective effects in vivo, as well as high AMPA-R affinity and good selectivity in vitro, compared with the previously reported first- and second-generation quinoxalinedione compounds such as the YM series. Moreover, in the 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid series, compound **14h**, with a 4-carboxyphenyl group joined through a urethane linkage onto the imidazole ring at the 7 position, exhibited good neuroprotective effects in vivo, equal to those of compound **17e** with a urea linkage. In particular, the neuroprotective effects of compounds **14h** and **17e** in the rat model were superior to those of the quinoxalinedione compounds, even with a relatively low iv infusion rate of 2.5 mg/kg/h for 4 h, an activity consistent with their high AMPA-R activity in vitro. On the other hand, in comparison of the biological activities of the 7-imidazolyl compound **17e** and the 7-pyrrolyl compound **27h**, both with urea linkages, compound **17e** was more potent in vivo than compound **27h**. However, although compound **17e** had excellent in vitro and in vivo activity, it caused marked sedative side effects in vivo, whereas compounds **14h** and **27h** did not. Moreover, among the selected compounds, the aqueous solubility of **14h** was higher

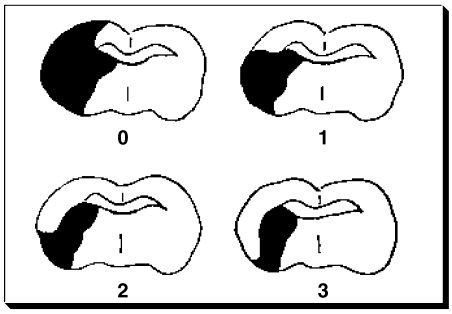


Figure 3. Four-point damage score in focal ischemia model.

than those of **17e**, **27h**, and the 6-nitro compound **1** (compound **14h**, 8.29 mg/mL; compound **17e**, 6.31 mg/mL; compound **27h**, 1.18 mg/mL; compound **1**, 4.9 mg/mL; YM-90 K: <1 mg/mL at pH 7.4).[20] We believe that the good solubility of compounds **14h** and **17e** results from the presence of the nitrogen atom at the 3 position on the 7-imidazolyl ring on the 6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid nucleus. In particular, the high solubility of compound **14h** suggests its suitability as an injectable formulation for the treatment of acute cerebral ischemia. In summary, we believe that compound **14h**, a 7-imidazolyl-6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid with a side chain consisting of a 4-carboxyphenyl group joined through a urethane linkage, is a useful structure for scaled-up chemical synthesis and pre-formulation studies for parenteral delivery and has potential as an injectable therapeutic agent for the treatment of acute cerebral ischemia.

4. Conclusion

Design and synthesis of novel AMPA-R antagonists allowed the identification of the 7-heterocycle-6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid nucleus as a very important core structure and led to compounds with good physicochemical properties and good biological activity. By studying structure–activity relationships and evaluating the properties of the various compounds, we showed that for 3-oxoquinoxaline-2-carboxylic acids with an imidazolyl group at the 7 position a 6-nitro group is responsible for photoinstability and that introduction of a 6-trifluoromethyl group imparts stability to light. Moreover, we found that the urethane or urea linkage is an important moiety that functions as a proton acceptor in the interaction with the AMPA-R, and we identified compounds **14h**, **17e**, and **27h** for further development. In summary, this research allowed us to develop superior AMPA-R antagonist candidates by replacing the 6-nitro group on the 3-oxoquinoxaline-2-carboxylic acid nucleus by a 6-trifluoromethyl group. We established that the 7-imidazolyl-6-trifluoromethyl-3-oxoquinoxaline-2-carboxylic acid is very important for both photostability and solubility, and that superior in vitro activity for AMPA-R and in vivo activity for the treatment of acute cerebral ischemia can be obtained by using a urethane linkage between the 7-imidazolyl group and the terminal phenyl group. In particular, compound **14h** (**KRP-199**), bearing a 4-carboxyphenyl group joined through a urethane linkage onto the imidazole ring, typifies this new strategy for the development of novel third-generation AMPA-R antagonists.

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 standards. 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. 4-Fluoro-3-trifluoromethylacetanilide (3). Compound **2** (100 g, 487 mmol) in AcOH (500 mL) was reduced by hydrogenation with 10% Pd-C (10.0 g) until cessation of H_2 uptake. The catalyst was removed by filtration through Celite®, and the filtrate was concentrated. The residue was dissolved in $CHCl_3$ (500 mL). Ac_2O (48.0 mL, 509 mmol) and Et_3N (140 mL, 1.00 mol) were added to the solution at ice cooling. After standing for 2 days, the reaction was washed with 1 N NaOH, 1 N HCl and brine, dried over Na_2SO_4, and evaporated to give the title compound as light brown oil (116 g, quant.); ^{1}H NMR (DMSO-d_6): δ 7.74–7.72 (m, 2H), 7.43 (br s, 1H), 7.15 (t, J = 9.8 Hz, 1H), 2.19 (s, 3H).

5.1.3. 4-Fluoro-2-nitro-5-trifluoromethylacetanilide (4). Compound **3** (116 g, 487 mmol) was added to fuming HNO_3 (500 mL) at 0 °C and stirred for 30 min 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 to give the title compound as a light brown powder (111 g, 87%); ^{1}H NMR (DMSO-d_6): δ 10.15 (br s, 1H), 9.20 (d, J = 6.8 Hz, 1H), 7.91 (d, J = 9.8 Hz, 1H), 2.32 (s, 3H).

5.1.4. 4-(Imidazol-1-yl)-5-trifluoromethyl-1,2-phenylenediamine (5)

5.1.4.1. Step 1: 4-(Imidazol-1-yl)-2-nitro-5-trifluoromethylaniline. To a solution of compound **4** (3.33 g, 12.5 mmol) in DMF (5 mL) was added imidazole (4.22 g, 62.0 mmol) and heated at 140 °C for 8 h in sealed tube. After cooling, the reaction was concentrated, and 4 N HCl (10 ml) was added to the residue. The solution was heated at 140 °C for 4.5 h and poured into ice water. The solution was made basic with KOH aq, extracted with CH_2Cl_2, dried over Na_2SO_4, and evaporated. AcOEt/i-Pr_2O (1:1) was added to the residue, and the precipitate was collected by filtration, washed with ^{i}Pr_2O, and dried under vacuum to give the title compound as a yellow powder (2.43 g, 71%); ^{1}H NMR (DMSO-d_6): δ 8.04 (s, 1H), 7.97 (s, 1H), 7.75 (s, 1H), 7.60 (s, 1H), 7.31 (s, 1H), 7.05 (s, 1H).

5.1.4.2. Step 2: 4-(Imidazol-1-yl)-5-trifluoromethyl-1,2-phenylenediamine (5). To a solution of 4-(imidazol-1-yl)-2-nitro-5-trifluoromethylaniline (948 mg, 3.48 mmol) in EtOH (50 mL) was added 10% Pd-C (94.8 mg) and stirred for 3.5 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 a brown powder (882 mg, quant.); ^{1}H NMR (DMSO-d_6): δ 7.62 (s, 1H), 7.19 (s, 1H), 6.97 (s, 1H), 6.88 (s, 1H), 6.48 (s, 1H), 5.43 (s, 2H), 5.14 (s, 2H).

5.1.5. Ethyl 3,4-dihydro-7-(4-imidazol-1-yl)-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate (6) and ethyl 3,4-dihydro-6-(4-imidazol-1-yl)-3-oxo-7-trifluoromethylquinoxaline-2-carboxylate (7). To a solution of compound **5** (303 mg, 1.25 mmol) in EtOH (50 mL) was added diethyl ketomalonate (0.21 ml, 1.38 mmol) and refluxed for 6 h. After cooling, the reaction was concentrated, and a small amount of EtOH was added to the residue. The precipitate was collected by filtration, washed with EtOH, and dried under vacuum to give the title compound **7** as a pale yellow powder (193.0 mg, 44%); ^{1}H NMR (DMSO-d_6): δ 13.24 (br s, 1H), 8.39 (s, 1H), 7.93 (s, 1H), 7.49 (s, 1H), 7.33 (s, 1H), 7.14 (s, 1H), 4.41 (q, J = 7.3 Hz, 2H), 1.34 (t, J = 7.3 Hz, 3H).

The filtrate was concentrated, and a small amount of AcOEt/^{i}Pr$_2$O (1:1) was added to the residue. The precipitate was collected by filtration, washed with AcOEt/^{i}Pr$_2$O (1:1), and dried under vacuum to give the title compound **6** as a yellow powder (148 mg, 34%); ^{1}H NMR (DMSO-d_6): δ 13.30 (br s, 1H), 8.09 (s, 1H), 7.83 (s, 1H), 7.78 (s, 1H), 7.39 (s, 1H), 7.09 (s, 1H), 4.40 (q, J = 7.3 Hz, 2H), 1.33 (t, J = 7.3 Hz, 3H).

5.1.6. 3,4-Dihydro-7-(4-imidazol-1-yl)-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid hydrochloride (8). To a solution of compound **6** (141 mg, 400 μmol) in AcOH (5 mL) was added 6 N HCl (1 mL) and stirred for 3.5 h at 80 °C. After cooling, the reaction was concentrated. A small amount of water was added to the residue and stirred for 30 min at ice cooling. The precipitate was collected by filtration and purified by recrystallization (water) to give the title compound as a brown powder (48.7 mg, 38%); mp 232–234 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 8.12 (s, 1H), 8.01 (s, 1H), 7.81 (s, 1H), 7.48 (s, 1H), 7.18 (s, 1H); FAB(−)HRMS 323.0396 (+0.4 mmu); Anal. Calcd for $C_{13}H_7F_3N_4O_3$–HCl: C, 43.29%; H, 2.24%; N, 15.53%. Found: C, 43.20%; H, 2.20%; N, 15.45%.

5.1.7. 3,4-Dihydro-6-(4-imidazol-1-yl)-3-oxo-7-trifluoromethylquinoxaline-2-carboxylic acid hydrochloride (9). To a solution of compound **7** (190 mg, 539 μmol) in AcOH (5 mL) was added 6 N HCl (2 mL) and stirred for 3.5 h at 60 °C. After cooling, the reaction was concentrated. A small amount of water was added to the residue and stirred for 30 min at ice cooling. The solution was concentrated and the residue was purified by synthetic adsorbent Sepabeads® SP850 (water). After concentrating, 4 N HCl was added to the residue and stirred for 30 min at water bath. The reaction was concentrated and CH_3CN was added to the residue. The precipitate was collected by filtration, washed with CH_3CN, and dried under vacuum to give the title compound as brown a powder (48.7 mg, 38%); mp 234–237 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 7.80 (s, 1H), 7.54 (s, 1H), 7.37 (s, 1H), 7.11 (s, 1H), 7.08 (s, 1H); FAB(−)HRMS 323.0418 (+2.6 mmu); Anal. Calcd for $C_{13}H_7F_3N_4O_3$–HCl: C, 43.29%; H, 2.24%; N, 15.53%. Found: C, 43.04%; H, 2.36%; N, 15.38%.

5.1.8. 4-Fluoro-*N*-(4-methoxybenzyl)-2-nitro-5-trifluoromethylacetanilide (10). To a solution of compound **4** (3.00 g, 11.3 mmol) in DMF (20 mL) were added 4-methoxybenzyl chloride (3.54 g, 22.6 mmol) and K_2CO_3 (3.12 g, 22.6 mmol), and stirred for 4 h at 80 °C. After cooling, the reaction was concentrated, and the residue was dissolved in AcOEt. The solution was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by flash chromatography (SiO_2, *n*-hexane/AcOEt, 1:1) to give the title compound as a pale yellow powder (2.38 g, 78%); ^{1}H NMR (DMSO-d_6)* δ 8.26 (br s, 1H), 7.69 (br s, 1H), 7.03 (br s, 2H), 6.81 (br s, 2H), 4.88 (br s, 1H), 4.46 (br s, 1H), 3.71 (s, 3H), 1.84 (br s, 3H). *Measured at 70 °C.

5.1.9. 4-[4-(Hydroxymethyl)imidazol-1-yl]-*N*-(4-methoxybenzyl)-2-nitro-5-trifluoromethylacetanilide (11). To a solution of compound **10** (540 mg, 1.40 mmol) in MeCN (5 mL) was added 4-(hydroxymethyl)imidazole hydrochloride (942 mg, 7.00 mmol) and heated at 140 °C for 16 h in sealed tube. After cooling, the reaction was concentrated, and the residue was dissolved in AcOEt, washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH, 50:1 to 20:1) to give the title compound as a pale yellow powder (590 mg, 91%); ^{1}H NMR (DMSO-d_6)* δ 8.20 (br s, 1H), 7.79 (s, 1H), 7.75 (s, 1H), 7.23 (s, 1H), 7.12 (br s, 2H), 6.86 (br s, 2H), 4.99 (br s, 2H), 4.78 (t, J = 5.9 Hz, 1H), 4.43 (d, J = 5.9 Hz, 2H), 3.72 (s, 3H), 1.90 (br s, 3H). *Measured at 70 °C.

5.1.10. 4-[4-(Hydroxymethyl)imidazol-1-yl]-5-trifluoromethyl-1,2-phenylenediamine (12)

5.1.10.1. Step 1: 4-[4-(Hydroxymethyl)imidazol-1-yl]-2-nitro-5-trifluoromethylaniline. A solution of compound **11** (7.26 g, 15.6 mmol) in 4 N HCl (30 mL) was refluxed overnight. After cooling, the reaction was diluted with AcOEt, washed with satd $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH, 100:1 to 30:1) to give the title compound as a yellow powder (4.19 g, 89%); ^{1}H NMR (DMSO-d_6): δ 8.01 (s, 1H), 7.96 (s, 2H), 7.68 (s, 1H), 7.60 (s, 1H), 7.13 (s, 1H), 4.97 (t, J = 3.9 Hz, 1H), 4.40 (d, J = 3.9 Hz, 2H).

5.1.10.2. Step 2: 4-[4-(Hydroxymethyl)imidazol-1-yl]-5-trifluoromethyl-1,2-phenylenediamine (12). To a solution of 4-[4-(hydroxymethyl)imidazol-1-yl]-2-nitro-5-trifluoromethylaniline (220 mg, 728 μmol) in EtOH (5 mL) was added 10% Pd-C (20.0 mg) and stirred for 3 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 a yellow powder (200 mg, quant.); ^{1}H NMR (DMSO-d_6): δ 7.52 (s, 1H), 6.99 (s, 1H), 6.87 (s, 1H), 6.45 (s, 1H), 5.42 (s, 2H), 5.13 (s, 2H), 4.89 (t, J = 5.9 Hz, 1H), 4.37 (d, J = 5.9 Hz, 2H).

5.1.11. Ethyl 3,4-dihydro-7-[4-(hydroxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate (13). To a solution of compound **12** (200 mg, 782 μmol) in EtOH (10 mL) was added diethyl ketomalonate (163 mg, 937 μmol) and refluxed for 4 h. After cooling, the reaction was concentrated, and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH, 50:1 to 10:1) to give the title compound as a pale yellow powder (129 mg, 43%); ^{1}H NMR (DMSO-d_6): δ 13.26 (br s, 1H), 8.05 (s, 1H), 7.78 (s, 1H), 7.75 (s, 1H), 7.21 (s, 1H), 5.01 (t, J = 5.4 Hz, 1H), 4.43 (d, J = 5.4 Hz, 2H), 4.40 (q, J = 6.8 Hz, 2H), 1.33 (t, J = 6.8 Hz, 3H).

5.1.12. 3,4-Dihydro-3-oxo-7-[4-((phenylamino)carbonyloxymethyl)imidazol-1-yl]-6-trifluoromethylquinoxaline-2-carboxylic acid (14a)

5.1.12.1. Step 1: Ethyl 3,4-dihydro-3-oxo-7-[4-((phenylamino)carbonyloxymethyl)imidazol-1-yl]-6-trifluoromethylquinoxaline-2-carboxylate. To a solution of compound **13** (200 mg, 523 μmol) in DMF (2 mL) was added phenyl isocyanate (114 μL, 1.05 mmol) and stirred for 2 h at 60 °C. After cooling, the reaction was concentrated, and EtOH was added to the residue. The insoluble part was removed by filtration, and the filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, *n*-hexane/AcOEt, 1:1 to 1:3) to give the title compound as ayellow powder (120 mg, 46%); ^{1}H NMR (DMSO-d_6) δ 13.30 (br s, 1H), 9.77 (s, 1H), 8.12 (s, 1H), 7.88 (s, 1H), 7.78 (s, 1H), 7.53 (s, 1H), 7.48 (d, J = 7.3 Hz, 2H), 7.27 (t, J = 7.3 Hz, 2H), 6.98 (t, J = 7.3 Hz, 1H), 5.08 (s, 2H), 4.40 (q, J = 6.8 Hz, 2H), 1.32 (t, J = 6.8 Hz, 3H).

5.1.12.2. Step 2: 3,4-Dihydro-3-oxo-7-[4-((phenylamino)carbonyloxymethyl)imidazol-1-yl]-6-trifluoromethylquinoxaline-2-carboxylic acid (14a). To a solution of ethyl 3,4-dihydro-3-oxo-7-[4-((phenylamino)carbonyloxymethyl)imidazol-1-yl]-6-trifluoromethylquinoxaline-2-carboxylate (100 mg, 199 μmol) in EtOH (4 mL) were added 1 N LiOH (697 μL, 697 μmol) and water (4 mL), and stirred for 1.5 h at 50 °C. After cooling, ice water was added to the reaction, and the insoluble part was removed by filtration. The filtrate was made acidic with 3 N HCl. The precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as a yellow powder (63.0 mg, 64%); mp 193–195 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 13.23 (br s, 1H), 9.77 (s, 1H), 8.10 (s, 1H), 7.89 (s, 1H), 7.80 (s, 1H), 7.54 (s, 1H), 7.48 (d, J = 7.8 Hz, 2H), 7.27 (t, J = 7.3 Hz, 2H), 6.98 (t, J = 7.3 Hz, 1H), 5.08 (s, 2H); FAB(+)HRMS 472.0885 (+1.6 mmu); Anal. Calcd for $C_{21}H_{14}F_3N_5O_5$-$\frac{6}{5}H_2O$: C, 50.95%; H, 3.34%; N, 14.15%. Found: C, 50.95%; H, 3.06%; N, 13.95%.

5.1.13. 7-[4-((3-Bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14b)

5.1.13.1. Step 1: Ethyl 7-[4-((3-bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate. Following the procedure described for compound **14a** (step 1), the title compound was prepared from compound **13** and 3-bromophenyl isocyanate, yellow powder (84%); ^{1}H NMR (DMSO-d_6) δ 13.29 (br s, 1H), 10.01 (s, 1H), 7.88 (s, 1H), 7.78 (s, 1H), 7.76 (s, 1H), 7.54 (s, 1H), 7.43 (d, J = 7.3 Hz, 1H), 7.25 (t, J = 7.3 Hz, 1H), 7.18 (d, J = 7.3 Hz, 1H), 5.09 (s, 2H), 4.40 (q, J = 6.8 Hz, 2H), 1.32 (t, J = 6.8 Hz, 3H).

5.1.13.2. Step 2: 7-[4-((3-Bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14b). Following the procedure described for compound **14a** (step 2), the title compound was prepared from ethyl 7-[4-((3-bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate, colorless powder (77%); mp 219–221 °C; ^{1}H NMR (DMSO-d_6): δ 10.01 (s, 1H), 8.10 (s, 1H), 7.89 (s, 1H), 7.80 (s, 1H), 7.76 (s, 1H), 7.55 (s, 1H), 7.43 (d, J = 9.3 Hz, 1H), 7.25 (t, J = 8.3 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H), 5.09 (s, 2H); FAB(−)HRMS 549.9981 (+0.7 mmu). Anal. Calcd for $C_{21}H_{13}BrF_3N_5O_5$-$\frac{1}{4}H_2O$: C, 44.95%; H, 2.51%; N, 12.47%. Found: C, 44.80%; H, 2.28%; N, 12.21%.

5.1.14. 7-[4-((4-Bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14c)

5.1.14.1. Step 1: Ethyl 7-[4-((4-bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate. Following the procedure described for compound **14a** (step 1), the title compound was prepared from compound **13** and 4-bromophenyl isocyanate, yellow powder (81%); ^{1}H NMR (DMSO-d_6): δ 13.29 (br s, 1H), 9.95 (s, 1H), 8.11 (s, 1H), 7.88 (s, 1H), 7.78 (s, 1H), 7.53 (s, 1H), 7.46 (s, 4H), 5.08 (s, 2H), 4.40 (q, J = 6.8 Hz, 2H), 1.32 (t, J = 6.8 Hz, 3H).

5.1.14.2. Step 2: 7-[4-((4-Bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14c). Following the procedure described for compound **14a** (step 2), the title compound was prepared from ethyl 7-[4-((4-bromophenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate, colorless powder (79%); mp 218–220 °C; ^{1}H NMR (DMSO-d_6): δ 9.94 (s, 1H), 8.09 (s, 1H), 7.89 (s, 1H), 7.80 (s, 1H), 7.54 (s, 1H), 7.46 (s, 4H), 5.08 (s, 2H); FAB(−)HRMS 549.9969 (−0.4 mmu). Anal. Calcd for $C_{21}H_{13}BrF_3N_5O_5$-$\frac{1}{2}H_2O$: C, 44.94%; H, 2.51%; N, 12.47%. Found: C, 45.00%; H, 2.29%; N, 12.23%.

5.1.15. 3,4-Dihydro-7-[4-((4-methylphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14d)

5.1.15.1. Step 1: Ethyl 3,4-dihydro-7-[4-((4-methylphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate. Following the procedure described for compound **14a** (step 1), the title compound was prepared from compound **13** and 4-methylphenyl isocyanate, yellow powder (46%); ^{1}H NMR (DMSO-d_6) δ 13.28 (br s, 1H),

9.65 (s, 1H), 8.11 (s, 1H), 7.87 (s, 1H), 7.78 (s, 1H), 7.51 (s, 1H), 7.35 (d, J = 8.3 Hz, 2H), 7.07 (d, J = 8.3 Hz, 2H), 5.06 (s, 2H), 4.40 (q, J = 7.3 Hz, 2H), 2.23 (s, 3H), 1.32 (t, J = 7.3 Hz, 3H).

5.1.15.2. Step 2: 3,4-Dihydro-7-[4-((4-methylphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14d). Following the procedure described for compound **14a** (step 2), the title compound was prepared from ethyl 3,4-dihydro-7-[4-((4-methylphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate, pale yellow powder (52%); mp 179–181 °C; ^{1}H NMR (DMSO-d_6): δ 9.65 (s, 1H), 8.11 (s, 1H), 7.90 (s, 1H), 7.79 (s, 1H), 7.53 (s, 1H), 7.35 (d, J = 7.3 Hz, 2H), 7.08 (d, J = 7.8 Hz, 2H), 5.07 (s, 2H); FAB(−)HRMS 486.1013 (−1.2 mmu).

5.1.16. 3,4-Dihydro-7-[4-((4-methoxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14e)

5.1.16.1. Step 1: Ethyl 3,4-dihydro-7-[4-((4-methoxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate. Following the procedure described for compound **14a** (step 1), the title compound was prepared from compound **13** and 4-methoxyphenyl isocyanate, yellow powder (56%); ^{1}H NMR (DMSO-d_6): δ 13.27 (br s, 1H), 9.57 (s, 1H), 8.07 (s, 1H), 7.86 (s, 1H), 7.76 (s, 1H), 7.50 (s, 1H), 7.37 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.3 Hz, 2H), 5.05 (s, 2H), 4.39 (q, J = 7.3 Hz, 2H), 3.70 (s, 3H), 1.32 (t, J = 7.3 Hz, 3H).

5.1.16.2. Step 2: 3,4-Dihydro-7-[4-((4-methoxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14e). Following the procedure described for compound **14a** (step 2), the title compound was prepared from ethyl 3,4-dihydro-7-[4-((4-methoxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate, pale yellow powder (89%); mp 210–212 °C; ^{1}H NMR (DMSO-d_6) δ 9.57 (s, 1H), 8.10 (s, 1H), 7.89 (s, 1H), 7.79 (s, 1H), 7.52 (s, 1H), 7.37 (d, J = 8.3 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 5.05 (s, 2H), 3.70 (s, 3H); FAB(−)HRMS 502.0992 (+1.7 mmu). Anal. Calcd for $C_{22}H_{16}F_3N_5O_6$-$\frac{1}{2}H_2O$: C, 51.57%; H, 3.34%; N, 13.67%. Found: C, 51.71%; H, 3.13%; N, 13.43%.

5.1.17. 3,4-Dihydro-3-oxo-6-trifluoromethyl-7-[4-((4-trifluoromethylphenylamino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylic acid (14f)

5.1.17.1. Step 1: Ethyl 3,4-dihydro-3-oxo-6-trifluoromethyl-7-[4-((4-trifluoromethylphenylamino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylate. Following the procedure described for compound **14a** (step 1), the title compound was prepared from compound **13** and 4-trifluoromethylphenyl isocyanate, yellow powder (49%); ^{1}H NMR (DMSO-d_6): δ 13.30 (br s, 1H), 10.23 (s, 1H), 8.10 (s, 1H), 7.88 (s, 1H), 7.78 (s, 1H), 7.69 (d, J = 8.8 Hz, 2H), 7.65 (d, J = 8.8 Hz, 2H), 7.54 (s, 1H), 5.12 (s, 2H), 4.40 (q, J = 6.8 Hz, 2H), 1.32 (t, J = 6.8 Hz, 3H).

5.1.17.2. Step 2: 3,4-Dihydro-3-oxo-6-trifluoromethyl-7-[4-((4-trifluoromethylphenylamino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylic acid (14f). Following the procedure described for compound **14a** (step 2), the title compound was prepared from ethyl 3,4-dihydro-3-oxo-6-trifluoromethyl-7-[4-((4-trifluoromethylphenylamino)carbonyloxymethyl)imidazol-1-yl]quinoxaline-2-carboxylate, colorless powder (75%); mp 194–196 °C; ^{1}H NMR (DMSO-d_6): δ 10.23 (s, 1H), 8.11 (s, 1H), 7.91 (s, 1H), 7.79 (s, 1H), 7.69 (d, J = 9.3 Hz, 2H), 7.65 (d, J = 8.8 Hz, 2H), 7.56 (s, 1H), 5.12 (s, 2H); FAB(−)HRMS 540.0743 (+0.0 mmu).

5.1.18. 7-[4-((3-Carboxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14g)

5.1.18.1. Step 1: Ethyl 3,4-dihydro-7-[4-((3-ethoxycarbonylphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate. Following the procedure described for compound **14a** (step 1), the title compound was prepared from compound **13** and ethyl 3-isocyanatobenzoate, yellow powder (60%); ^{1}H NMR (DMSO-d_6): δ 13.30 (br s, 1H), 10.03 (s, 1H), 8.19 (s, 1H), 8.12 (s, 1H), 7.88 (s, 1H), 7.78 (s, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.54 (s, 1H), 7.43 (t, J = 7.8 Hz, 1H), 5.10 (s, 2H), 4.40 (q, J = 7.3 Hz, 2H), 4.31 (q, J = 6.8 Hz, 2H), 1.32 (t, J = 7.3 Hz, 3H), 1.31 (t, J = 6.8 Hz, 3H).

5.1.18.2. Step 2: 7-[4-((3-Carboxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14g). To a solution of ethyl 3,4-dihydro-7-[4-((3-ethoxycarbonylphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate (180 mg, 314 μmol) in AcOH (5 mL) was added concd HCl (1 mL) and stirred for 2 h at room temperature. The reaction was diluted with water, and the precipitate was collected by filtration, washed with water, and dissolved in 1 N LiOH. The insoluble part was removed by filtration, and the filtrate was adjusted to pH 4 with 3 N HCl. The precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as a brown powder (25.0 mg, 15%); mp 215–217 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 12.94 (br s, 1H), 10.00 (br s, 1H), 8.14 (s, 1H), 8.10 (s, 1H), 7.89 (s, 1H), 7.80 (s, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.58–7.55 (m, 2H), 7.40 (t, J = 8.3 Hz, 1H), 5.10 (s, 2H); FAB(−)HRMS 516.0778 (+1.1 mmu).

5.1.19. 7-[4-((4-Carboxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14h)

5.1.19.1. Step 1: Ethyl 3,4-dihydro-7-[4-((4-ethoxycarbonylphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate. Following the procedure described for compound **14a** (step 1), the title compound was prepared from compound **13** and ethyl 4-isocyanatobenzoate, pale yellow powder (67%); ^{1}H NMR (DMSO-d_6): δ 13.24 (br s, 1H), 10.20 (s, 1H), 8.02 (br s, 1H), 7.89 (d, J = 8.8 Hz, 2H), 7.86 (s, 1H), 7.74 (s, 1H), 7.61 (d, J = 8.8 Hz, 2H), 7.53 (s, 1H), 5.11 (s, 2H), 4.38 (q, J = 6.8 Hz, 2H), 4.28 (q,

J = 7.3 Hz, 2H), 1.32 (t, J = 6.8 Hz, 3H), 1.30 (t, J = 7.3 Hz, 3H).

5.1.19.2. Step 2: 7-[4-((4-Carboxyphenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14h). Following the procedure described for compound **8g** (step 2), the title compound was prepared from ethyl 3,4-dihydro-7-[4-((4-ethoxycarbonylphenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate, colorless powder (57%); mp 278–280 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ 12.62 (br s, 1H), 10.16 (s, 1H), 8.09 (s, 1H), 7.90 (s, 1H), 7.87 (d, J = 8.3 Hz, 2H), 7.81 (s, 1H), 7.59 (d, J = 8.3 Hz, 2H), 7.55 (s, 1H), 5.12 (s, 2H); FAB(−)HRMS 516.0775 (+0.8 mmu). Anal. Calcd for $C_{22}H_{14}F_3N_5O_7$-H_2O: C, 49.35%; H, 2.64%; N, 13.08%. Found: C, 49.36%; H, 3.00%; N, 12.93%.

5.1.20. 7-[4-((3-(Carboxymethyl)phenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14i)

5.1.20.1. Step 1: Ethyl 3,4-dihydro-7-[4-((3-(methoxycarbonylmethyl)phenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate. Following the procedure described for compound **14a** (step 1), the title compound was prepared from compound 13 and methyl (3-isocyanatophenyl)acetate, pale orange powder (75%); ^{1}H NMR (DMSO-d_6)**:** δ 13.29 (br s, 1H), 9.79 (s, 1H), 8.12 (s, 1H), 7.87 (s, 1H), 7.78 (s, 1H), 7.52 (s, 1H), 7.41 (s, 1H), 7.36 (d, J = 7.3 Hz, 1H), 7.22 (t, J = 7.3 Hz, 1H), 6.89 (d, J = 7.3 Hz, 1H), 5.08 (s, 2H), 4.40 (q, J = 6.8 Hz, 2H), 3.62 (s, 2H), 3.61 (s, 3H), 1.32 (t, J = 6.8 Hz, 3H).

5.1.20.2. Step 2: 7-[4-((3-(Carboxymethyl)phenylamino)carbonyloxymethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (14i). To a solution of ethyl 3,4-dihydro-7-[4-((3-(methoxycarbonylmethyl)phenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate (150 mg, 262 μmol) in EtOH (3 mL) were added 1 N LiOH (950 μL, 950 μmol) and water (4 mL), and stirred for 1.5 h at 50 °C. The reaction was poured into ice water, and the insoluble part was removed by filtration. The filtrate was adjusted to pH 4 with 3 N HCl, and the precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as a yellow powder (114 mg, 82%); mp 196–198 °C; ^{1}H NMR (DMSO-d_6): δ 12.33 (br s, 1H), 9.78 (s, 1H), 8.11 (s, 1H), 7.89 (s, 1H), 7.80 (s, 1H), 7.54 (s, 1H), 7.40 (s, 1H), 7.35 (d, J = 8.8 Hz, 1H), 7.21 (t, J = 8.3 Hz, 1H), 6.88 (d, J = 7.3 Hz, 1H), 5.08 (s, 2H), 3.50 (s, 2H); FAB(−)HRMS 530.0925 (+0.2 mmu).

5.1.21. 7-[4-((4-(Carboxymethyl)phenylamino)carbonyloxymethyl)imidazol-3,4-dihydro-3-oxo-6-trifluoromethyl-1-yl]quinoxaline-2-carboxylic acid (14j)

5.1.21.1. Step 1: Ethyl 3,4-dihydro-7-[4-((4-(ethoxycarbonylmethyl)phenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate. Following the procedure described for compound **14a** (step 1), the title compound was prepared from compound **13** and ethyl (4-isocyanatophenyl)acetate, pale orange powder (67%); ^{1}H NMR (DMSO-d_6): δ 13.30 (br s, 1H), 9.76 (s, 1H), 8.11 (s, 1H), 7.87 (s, 1H), 7.78 (s, 1H), 7.52 (s, 1H), 7.41 (d, J = 8.3 Hz, 2H), 7.16 (d, J = 8.3 Hz, 2H), 5.07 (s, 2H), 4.40 (q, J = 7.3 Hz, 2H), 4.06 (q, J = 7.3 Hz, 2H), 3.57 (s, 2H), 1.32 (t, J = 6.8 Hz, 3H), 1.17 (t, J = 6.8 Hz, 3H).

5.1.21.2. Step 2: 7-[4-((4-(Carboxymethyl)phenylamino)carbonyloxymethyl)imidazol-3,4-dihydro-3-oxo-6-trifluoromethyl-1-yl]quinoxaline-2-carboxylic acid (14j). Following the procedure described for compound **14i** (step 2), the title compound was prepared from ethyl 3,4-dihydro-7-[4-((4-(ethoxycarbonylmethyl)phenylamino)carbonyloxymethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate, pale orange powder (88%); mp 210–212 °C; ^{1}H NMR (DMSO-d_6): δ 12.27 (br s, 1H), 9.75 (s, 1H), 8.10 (s, 1H), 7.89 (s, 1H), 7.80 (s, 1H), 7.53 (s, 1H), 7.40 (d, J = 8.8 Hz, 2H), 7.15 (d, J = 8.3 Hz, 2H), 5.07 (s, 2H), 3.48 (s, 2H); FAB(−)HRMS 530.0942 (+1.8 mmu). Anal. Calcd for $C_{23}H_{16}F_3N_5O_7$-$\frac{3}{2}H_2O$: C, 49.47%; H, 3.34%; N, 12.54%. Found: C, 49.67%; H, 3.10%; N, 12.37%.

5.1.22. Ethyl 3,4-dihydro-7-[4-(formyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate (15). To a suspension of compound **13** (1.75 g, 4.58 mmol) in 1,4-dioxane (50 mL) was added manganese dioxide (1.99 g, 22.9 mmol) and refluxed for 24 h. An additional manganese dioxide (1.99 g, 22.9 mmol) was added and refluxed further for 10 h. After cooling, manganese dioxide was removed by filtration through Celite®, and the filtrate was concentrated. ^{i}Pr$_2$O was added to the residue, and the precipitate was collected by filtration, washed with ^{i}Pr$_2$O, and dried under vacuum to give the title compound as a yellow powder (895 mg, 51%); ^{1}H NMR (DMSO-d_6): δ 9.84 (s, 1H), 8.35 (s, 1H), 8.18 (s, 1H), 8.11 (s, 1H), 7.76 (s, 1H), 4.38 (q, J = 7.3 Hz, 2H), 1.32 (t, J = 7.3 Hz, 3H).

5.1.23. Ethyl 7-[4-(aminomethyl)imidazol-1-yl]-3-oxo-1,2,3,4-tetrahydro-6-trifluoromethylquinoxaline-2-carboxylate hydrochloride (16). To a suspension of compound **15** (890 mg, 2.34 mmol) in EtOH (20 mL) were added hydroxylamine hydrochloride (325 mg, 4.68 mmol) and NaOAc (384 mg, 4.68 mmol), and refluxed for 4 h. After cooling, the insoluble part was removed by filtration through Celite®, and the filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2/EtOH, 10:1) and dissolved in EtOH (10 mL). To the solution were added 10% Pd-C (100 mg) and concd HCl (0.5 mL), and stirred for 2 h at room temperature under hydrogen atmosphere (4 atm). The catalyst was removed by filtration through Celite®, and the filtrate was concentrated. AcOEt was added to the residue, and the precipitate was collected by filtration, washed with AcOEt, and dried under vacuum to give the title compound as a pale yellow powder (556 mg, 57%); ^{1}H NMR (DMSO-d_6): δ 11.13 (s, 1H), 8.40–8.20 (br, 4H), 7.83 (s, 1H), 7.59 (s, 1H), 7.21 (s, 1H), 4.86 (s, 2H), 4.15 (q, J = 7.3 Hz, 2H), 1.18 (t, J = 7.3 Hz, 3H).

5.1.24. 3,4-Dihydro-3-oxo-7-[4-(((phenylamino)carbonyl)aminomethyl)imidazol-1-yl]-6-trifluoromethylquinoxaline-2-carboxylic acid (17a). To a solution of compound **16** (110 mg, 262 μmol) in DMF (3 mL) were added phenyl isocyanate (114 μL, 1.05 mmol) and Et_3N (54.8 μL, 393 μmol), and stirred for 1 h at 60 °C. An additional Et_3N (365 μL, 2.62 mmol) was added to the reaction and stirred further for 4 h. After cooling, the reaction was concentrated. The residue was dissolved in AcOEt, washed with water, dried over Na_2SO_4, and evaporated. The residue was dissolved in EtOH (3 mL), and 1 N NaOH (786 μL, 786 μmol) was added. The reaction was refluxed for 1 h. After cooling, the reaction was concentrated, and water was added to the residue. The solution was adjusted to pH 2 with 4 N HCl. The precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as a light brown powder (58.2 mg, 47%); mp 224–226 °C; ^{1}H NMR (DMSO-d_6): δ 8.62 (s, 1H), 8.11 (s, 1H), 7.98 (s, 1H), 7.79 (s, 1H), 7.40–7.35 (m, 3H), 7.22 (t, J = 7.3 Hz, 2H), 6.89 (t, J = 7.3 Hz, 1H), 6.47 (t, J = 5.4 Hz, 1H), 4.27 (d, J = 5.4 Hz, 2H); FAB(−)HRMS 471.1030 (+0.1 mmu).

5.1.25. 7-[4-(((4-Bromophenylamino)carbonyl)aminomethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (17b). Following the procedure described for compound **17a**, the title compound was prepared from compound **16** and 4-bromophenyl isocyanate, light brown powder (49%); mp 220–222 °C; ^{1}H NMR (DMSO-d_6): δ 8.77 (s, 1H), 8.09 (s, 1H), 7.90 (s, 1H), 7.79 (s, 1H), 7.38 (s, 4H), 7.32 (s, 1H), 6.51 (t, J = 5.9 Hz, 1H), 4.25 (d, J = 5.4 Hz, 2H); FAB(−)HRMS 549.0111 (−2.3 mmu).

5.1.26. 3,4-Dihydro-7-[4-(((4-methoxyphenylamino)carbonyl)aminomethyl)imidazol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (17c). Following the procedure described for compound **17a**, the title compound was prepared from compound **16** and 4-methoxyphenyl isocyanate, light brown powder (35%); mp 210–212 °C; ^{1}H NMR (DMSO-d_6): δ 8.41 (s, 1H), 8.10 (s, 1H), 7.92 (s, 1H), 7.79 (s, 1H), 7.31 (s, 1H), 7.29 (d, J = 8.3 Hz, 2H), 6.81 (d, J = 8.3 Hz, 2H), 6.34 (t, J = 5.4 Hz, 1H), 4.24 (d, J = 5.4 Hz, 2H), 3.69 (s, 3H); FAB(−)HRMS 501.1155 (+2.1 mmu).

5.1.27. 7-[4-(((3-Carboxyphenylamino)carbonyl)aminomethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (17d). Following the procedure described for compound **17a**, the title compound was prepared from compound **16** and ethyl 3-isocyanatobenzoate, light brown powder (6%); mp 215–218 °C; ^{1}H NMR (DMSO-d_6) δ 8.88 (s, 1H), 8.14 (s, 1H), 8.06 (s, 1H), 8.20–8.00 (br, 1H), 7.79 (s, 1H), 7.61–7.59 (m, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.39 (s, 1H), 7.34 (t, J = 7.8 Hz, 1H), 6.55 (t, J = 5.4 Hz, 1H), 4.29 (d, J = 4.9 Hz, 2H); FAB(−)HRMS 515.0972 (+4.5 mmu).

5.1.28. 7-[4-(((4-Carboxyphenylamino)carbonyl)aminomethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid hydrochloride (17e). Following the procedure described for compound **17a**, the title compound was prepared from compound **16** and ethyl 4-isocyanatobenzoate, light brown powder (6%); mp 236–238 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 9.01 (s, 1H), 8.10 (s, 1H), 7.91 (s, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.79 (s, 1H), 7.50 (d, J = 8.8 Hz, 2H), 7.34 (s, 1H), 6.62 (t, J = 5.4 Hz, 1H), 4.28 (d, J = 5.4 Hz, 2H); FAB(−)HRMS 515.0908 (−1.9 mmu). Anal. Calcd for $C_{22}H_{15}F_3N_6O_6$–HCl–H_2O: C, 46.29%; H, 3.18%; N, 14.72%. Found: C, 46.56%; H, 3.19%; N, 14.79%.

5.1.29. 7-[4-(((3-Carboxymethylphenylamino)carbonyl)aminomethyl)imidazol-1-yl]-3,4-dihydro- 3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid hydrochloride (17f). Following the procedure described for compound **17a**, the title compound was prepared from compound **16** and methyl (3-isocyanatophenyl)acetate, light brown powder (15%); mp 206–208 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 8.66 (s, 1H), 8.14 (s, 1H), 8.08 (br s, 1H), 7.80 (s, 1H), 7.38 (s, 1H), 7.30 (s, 2H), 7.29 (s, 1H), 7.16–7.13 (m, 1H), 6.79 (d, J = 7.8 Hz, 1H), 6.49 (t, J = 5.4 Hz, 1H), 4.28 (d, J = 4.9 Hz, 2H), 3.48 (s, 2H); FAB(−)HRMS 529.1090 (+0.7 mmu). Anal. Calcd for $C_{23}H_{17}F_3N_6O_6$–HCl–H_2O: C, 47.23%; H, 3.45%; N, 14.37%. Found: C, 47.23%; H, 3.44%; N, 14.19%.

5.1.30. 7-[4-(((4-Carboxymethylphenylamino)carbonyl)aminomethyl)imidazol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid hydrochloride (17g). Following the procedure described for compound **17a**, the title compound was prepared from compound **16** and ethyl (4-isocyanatophenyl)acetate, light brown powder (16%); mp 219–221 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 8.58 (s, 1H), 8.10 (s, 1H), 7.92 (s, 1H), 7.79 (s, 1H), 7.323 (d, J = 8.3 Hz, 2H), 7.321 (d, J = 2.4 Hz, 1H), 7.10 (d, J = 8.3 Hz, 2H), 6.44 (t, J = 5.4 Hz, 1H), 4.26 (d, J = 4.9 Hz, 2H), 3.45 (s, 2H); FAB(−)HRMS 529.1080 (−0.3 mmu). Anal. Calcd for $C_{23}H_{17}F_3N_6O_6$–HCl–H_2O: C, 47.23%; H, 3.45%; N, 14.37%. Found: C, 47.07%; H, 3.59%; N, 14.08%.

5.1.31. Ethyl 3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate (19)

5.1.31.1. Step 1: 4-Trifluoromethyl-1,2-phenylenediamine. To a solution of compound **26** (102 g, 495 mmol) in EtOH (1000 mL) was added 10% Pd-C (5.00 g) and stirred for 4 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 a brown solid (87.2 g, quant.); ^{1}H NMR (DMSO-d_6): δ 6.76 (d, J = 2.0 Hz, 1H), 6.68 (dd, J = 8.3, 1.5 Hz, 1H), 6.57 (d, J = 8.3 Hz, 1H), 5.06 (br s, 2H), 4.84 (br s, 2H).

5.1.31.2. Step 2: Ethyl 3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylate (19). To a solution of 4-trifluoromethyl-1,2-phenylenediamine (15.6 g, 88.6 mmol) in EtOH (200 mL) was added diethyl ketomalonate (16.2 g, 93.0 mmol) and refluxed for 3 h. After cooling, the reaction was concentrated. ^{i}Pr$_2$O was added to the residue, and the precipitate was collected by filtration, washed with ^{i}Pr$_2$O, and dried under vacuum to give the title compound as a light

brown powder (10.4 g, 41%); ^{1}H NMR (DMSO-d_6): δ 13.09 (br s, 1H), 8.05 (d, J = 8.3 Hz, 1H), 7.67 (d, J = 8.8 Hz, 1H), 7.63 (s, 1H), 4.40 (q, J = 7.3 Hz, 2H), 1.33 (t, J = 7.3 Hz, 3H).

5.1.32. Ethyl 3-ethoxy-7-nitro-6-trifluoromethylquinoxaline-2-carboxylate (21). To a solution of compound **19** (500 mg, 1.75 mmol) in concd H_2SO_4 (5 mL) was added KNO_3 (354 mg, 3.50 mmol) at 40 °C and stirred for 3 h at the same temperature. The reaction was poured into ice water and the solution was extracted with AcOEt, dried over Na_2SO_4, and evaporated. Silver (I) oxide (811 mg, 3.50 mmol) and toluene (10 mL) were added to the residue. Iodoethane (280 μL, 3.50 mmol) was added to the mixture at 100 °C and refluxed for 2 h. After cooling, the insoluble part was removed by filtration through Celite®, and the filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, *n*-hexane/AcOEt, 8:1) to give the title compound as brown oil (255 mg, 41%); ^{1}H NMR ($CDCl_3$): δ 8.67 (s, 1H), 8.32 (s, 1H), 4.68 (q, J = 7.3 Hz, 2H), 4.56 (q, J = 7.3 Hz, 2H), 1.53 (t, J = 7.3 Hz, 3H), 1.48 (t, J = 7.3 Hz, 3H).

5.1.33. Ethyl 7-amino-3-oxo-1,2,3,4-tetrahydro-6-trifluoromethylquinoxaline-2-carboxylate (22)

5.1.33.1. Step 1: Ethyl 7-amino-1,2-dihydro-3-ethoxy-6-trifluoromethylquinoxaline-2-carboxylate. To a solution of compound **21** (4.98 g, 13.9 mmol) in EtOH (100 mL) was added 10% Pd-C (500 mg) and stirred for 3 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 a yellow powder (4.01 g, 87%); ^{1}H NMR ($CDCl_3$): δ 7.18 (s, 1H), 6.01 (s, 1H), 4.65 (br s, 1H), 4.49 (d, J = 1.5 Hz, 1H), 4.44–4.31 (m, 2H), 4.18 (q, J = 7.3 Hz, 2H), 3.98 (br s, 2H), 1.36 (t, J = 7.3 Hz, 3H), 1.25 (t, J = 7.3 Hz, 3H).

5.1.33.2. Step 2: Ethyl 7-amino-3-oxo-1,2,3,4-tetrahydro-6-trifluoromethylquinoxaline-2- carboxylate (22). To a solution of ethyl 7-amino-1,2-dihydro-3-ethoxy-6-trifluoromethylquinoxaline-2-carboxylate (3.51 g, 10.6 mmol) in EtOH (35 mL) was added concd HCl (7 mL) and stirred for 20 h at room temperature. The reaction was concentrated, and water was added to the residue, extracted with AcOEt, dried over Na_2SO_4, and evaporated to give the title compound as a light brown powder (2.69 g, 84%); ^{1}H NMR (DMSO-d_6): δ 10.36 (s, 1H), 7.04 (d, J = 1.5 Hz, 1H), 6.70 (s, 1H), 6.18 (s, 1H), 5.10 (br s, 2H), 4.53 (d, J = 2.0 Hz, 1H), 4.13–4.07 (m, 2H), 1.16 (t, J = 7.3 Hz, 3H).

5.1.34. Ethyl 3-oxo-7-(pyrrol-1-yl)-6-trifluoromethylquinoxaline-2-carboxylate (bf 23). To a solution of compound **22** (113 mg, 373 μmol) in AcOH (2 mL) was added 2,5-dimethoxytetrahydrofuran (59.2 μL, 448 μmol) at 50 °C and stirred for 1.5 h at the same temperature. The reaction mixture was poured into water, extracted with AcOEt, dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography (SiO_2, *n*-hexane/AcOEt, 1:1) and concentrated desirable fraction. DDQ (84.7 mg, 373 μmol) and 1,4-dioxane (4 mL) were added to the residue and stirred for 1 h at the room temperature. The reaction was concentrated and the residue was purified by flash chromatography (SiO_2, *n*-hexane/AcOEt, 1:1) to give the title compound as a yellow powder (68.7 mg, 52%); ^{1}H NMR (DMSO-d_6) δ 13.21 (br s, 1H), 7.94 (s, 1H), 7.76 (s, 1H), 6.95 (s, 2H), 6.25 (d, J = 2.4 Hz, 1H), 6.24 (d, J = 1.9 Hz, 1H), 4.40 (q, J = 7.3 Hz, 2H), 1.33 (t, J = 7.3 Hz, 3H).

5.1.35. 3,4-Dihydro-3-oxo-7-(pyrrol-1-yl)-6-trifluoromethylquinoxaline-2-carboxylic acid (24). To a solution of compound **23** (67.0 mg, 191 μmol) in EtOH (2 mL) was added 1 N KOH (382 μL, 382 μmol) and refluxed for 1 h. After cooling, the reaction was concentrated, and water was added to the residue. The solution was adjusted to pH 2 with 4 N HCl, extracted with AcOEt, dried over Na_2SO_4, and evaporated. 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 a brown powder (86%); mp 136–138 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 7.93 (s, 1H), 7.76 (s, 1H), 6.96 (s, 2H), 6.25 (t, J = 1.9 Hz, 1H); FAB(−)HRMS 322.0424 (−1.6 mmu). Anal. Calcd for $C_{22}H_{16}F_3N_5O_4$-$\frac{2}{3}H_2O$: C, 50.16%; H, 2.81%; N, 12.53%. Found: C, 50.18%; H, 3.07%; N, 12.61%.

5.1.36. Ethyl 7-(3-formylpyrrol-1-yl)-3-oxo-1,2,3,4-tetrahydro-6-trifluoromethylquinoxaline-2-carboxylate (25). To a solution of compound **22** (3.60 g, 11.9 mmol) in AcOH (60 mL) was added 2,5-dimethoxytetrahydrofuran-3-aldehyde (2.01 mL, 14.2 mmol) at 50 °C, and stirred for 1.5 h at the same temperature. The reaction was poured into water, extracted with AcOEt, dried over Na_2SO_4, and evaporated. CH_2Cl_2 was added to the residue, and the precipitate was collected by filtration, washed with CH_2Cl_2, and dried under vacuum to give the title compound as yellow powder (2.57 g). The filtrate was concentrated, and the residue was purified by flash column chromatography (SiO_2, *n*-hexane/AcOEt, 1:2) to give further 973 mg. Total yield 3.54 g (78%); ^{1}H NMR (DMSO-d_6): δ 11.02 (s, 1H), 9.74 (s, 1H), 7.79 (s, 1H), 7.61 (d, J = 1.5 Hz, 1H), 7.16 (s, 1H), 7.04 (s, 1H), 6.82 (s, 1H), 6.60 (q, J = 1.5 Hz, 1H), 4.84 (d, J = 2.0 Hz, 1H), 4.17–4.12 (m, 2H), 1.18 (t, J = 7.3 Hz, 3H).

5.1.37. Ethyl 7-[3-(aminomethyl)pyrrol-1-yl]-3-oxo-1,2,3,4-tetrahydro-6-trifluoromethylquinoxaline-2-carboxylate hydrochloride (26). Following the procedure described for compound **16**, the title compound was prepared from compound **25**, colorless powder (93%); ^{1}H NMR (DMSO-d_6): δ 11.00 (s, 1H), 8.06 (br s, 3H), 7.64 (s, 1H), 7.14 (s, 1H), 6.98 (s, 1H), 6.88 (d, J = 2.4 Hz, 1H), 6.69 (s, 1H), 6.33 (t, J = 2.4 Hz, 1H), 4.83 (d, J = 2.0 Hz, 1H), 4.17–4.12 (m, 2H), 3.90 (q, J = 5.4 Hz, 2H), 1.18 (t, J = 7.3 Hz, 3H).

5.1.38. 3,4-Dihydro-3-oxo-7-[3-(((phenylamino)carbonyl)aminomethyl)pyrrol-1-yl]-6-trifluoromethylquinoxaline-2-carboxylic acid (27a). Following the procedure described for compound **17a**, the title compound was prepared from compound **26** and phenyl isocyanate, yel-

low powder (47%); mp >300 °C; ^{1}H NMR (DMSO-d_6): δ 8.45 (s, 1H), 7.89 (s, 1H), 7.76 (s, 1H), 7.39 (dd, J = 8.8, 1.0 Hz, 1H), 7.22 (t, J = 7.8 Hz, 2H), 6.920 (s, 1H), 6.916 (s, 1H), 6.88 (t, J = 7.3 Hz, 1H), 6.30 (t, J = 5.4 Hz, 1H), 6.24 (t, J = 2.0 Hz, 1H), 4.17 (d, J = 5.4 Hz, 2H); FAB(−)HRMS 470.1058 (−1.8 mmu). Anal. Calcd for $C_{22}H_{16}F_3N_5O_4$-1/2H_2O: C, 55.00%; H, 3.57%; N, 14.58%. Found: C, 54.95%; H, 3.69%; N, 14.32%.

5.1.39. 7-[3-(((3-Bromophenylamino)carbonyl)aminomethyl)pyrrol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (27b). Following the procedure described for compound **27a**, the title compound was prepared from compound **26** and 3-bromophenyl isocyanate, yellow powder (22%); mp 207–209 °C; ^{1}H NMR (DMSO-d_6): δ 8.69 (s, 1H), 7.90 (s, 1H), 7.84 (s, 1H), 7.75 (s, 1H), 7.22–7.15 (m, 2H), 7.06 (d, J = 7.3 Hz, 1H), 6.92 (s, 1H), 6.91 (s, 1H), 6.43 (t, J = 4.9 Hz, 1H), 6.24 (t, J = 2.0 Hz, 1H), 4.17 (d, J = 5.4 Hz, 2H); FAB(−)HRMS 548.0226 (+4.5 mmu).

5.1.40. 7-[3-(((4-Bromophenylamino)carbonyl)aminomethyl)pyrrol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (27c). Following the procedure described for compound **27a**, the title compound was prepared from compound **26** and 4-bromophenyl isocyanate, brown powder (49%); mp 196–198 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 8.62 (s, 1H), 7.89 (s, 1H), 7.75 (s, 1H), 7.38 (s, 4H), 6.92 (s, 1H), 6.91 (s, 1H), 6.37 (t, J = 5.4 Hz, 1H), 6.24 (t, J = 2.0 Hz, 1H), 4.17 (d, J = 5.4 Hz, 2H); FAB(−)HRMS 548.0144 (−3.7 mmu). Anal. Calcd for $C_{22}H_{15}BrF_3N_5O_4$-$\frac{4}{3}H_2O$: C, 46.01%; H, 3.10%; N, 12.19%. Found: C, 45.84%; H, 2.82%; N, 12.02%.

5.1.41. 3,4-Dihydro-7-[3-(((4-fluoroophenylamino)carbonyl)aminomethyl)pyrrol-1-yl]-3-oxo-6- trifluoromethylquinoxaline-2-carboxylic acid (27d). Following the procedure described for compound **27a**, the title compound was prepared from compound **26** and 4-fluorophenyl isocyanate, brown powder (43%); mp 168–170 °C; ^{1}H NMR (DMSO-d_6): δ 8.50 (s, 1H), 7.89 (s, 1H), 7.75 (s, 1H), 7.40 (dd, J = 8.8, 4.9 Hz, 2H), 7.08–7.03 (m, 2H), 6.91 (s, 2H), 6.30 (t, J = 5.4 Hz, 1H), 6.23 (s, 1H), 4.17 (d, J = 5.4 Hz, 2H); FAB(−)HRMS 488.0985 (+0.3 mmu).

5.1.42. 3,4-Dihydro-7-[3-(((4-methoxyphenylamino)carbonyl)aminomethyl)pyrrol-1-yl]-3-oxo-6- trifluoromethylquinoxaline-2-carboxylic acid (27e). Following the procedure described for compound **27a**, the title compound was prepared from compound **26** and 4-methoxyphenyl isocyanate, brown powder (46%); mp 198–200 °C; ^{1}H NMR (DMSO-d_6): δ 8.26 (s, 1H), 7.89 (s, 1H), 7.76 (s, 1H), 7.29 (d, J = 9.3 Hz, 2H), 6.91 (s, 2H), 6.81 (d, J = 8.8 Hz, 2H), 6.23 (t, J = 2.0 Hz, 1H), 6.20 (t, J = 5.4 Hz, 1H), 4.16 (d, J = 5.4 Hz, 2H), 3.69 (s, 3H); FAB(−)HRMS 500.1165 (−1.6 mmu). Anal. Calcd for $C_{23}H_{18}F_3N_5O_5$-$\frac{3}{2}H_2O$: C, 52.28%; H, 4.01%; N, 13.25%. Found: C, 51.97%; H, 3.66%; N, 13.07%.

5.1.43. 3,4-Dihydro-3-oxo-6-trifluoromethyl-7-[3-(((4-trifluoromethylphenylamino)carbonyl)aminomethyl)pyrrol-1-yl]quinoxaline-2-carboxylic acid (27f). Following the procedure described for compound **27a**, the title compound was prepared from compound **26** and 4-trifluoromethylphenyl isocyanate, brown powder (42%); mp 194–196 °C; ^{1}H NMR (DMSO-d_6): δ 8.91 (s, 1H), 7.89 (s, 1H), 7.77 (s, 1H), 7.60 (d, J = 9.3 Hz, 2H), 7.57 (d, J = 9.3 Hz, 2H), 6.93 (s, 2H), 6.49 (t, J = 5.4 Hz, 1H), 6.25 (t, J = 2.0 Hz, 1H), 4.20 (d, J = 4.9 Hz, 2H); FAB(−)HRMS 538.0938 (−1.2 mmu).

5.1.44. 7-[3-(((3-Carboxyphenylamino)carbonyl)aminomethyl)pyrrol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (27g). Following the procedure described for compound **27a**, the title compound was prepared from compound **26** and ethyl 3-isocyanatobenzoate, yellow powder (20%); mp >300 °C; ^{1}H NMR (DMSO-d_6): δ 8.70 (s, 1H), 8.05 (t, J = 1.5 Hz, 1H), 7.89 (s, 1H), 7.79 (s, 1H), 7.60 (dd, J = 8.3, 1.0 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.34 (t, J = 7.8 Hz, 1H), 6.93 (s, 2H), 6.38 (t, J = 5.4 Hz, 1H), 6.25 (t, J = 2.0 Hz, 1H), 4.19 (d, J = 5.4 Hz, 2H); FAB(−)HRMS 514.1017 (+4.3 mmu). Anal. Calcd for $C_{23}H_{16}F_3N_5O_6$-2H_2O: C, 50.10%; H, 3.66%; N, 12.70%. Found: C, 50.21%; H, 3.67%; N, 12.71%.

5.1.45. 7-[3-(((4-Carboxyphenylamino)carbonyl)aminomethyl)pyrrol-1-yl]-3,4-dihydro-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (27h). Following the procedure described for compound **27a**, the title compound was prepared from compound **26** and ethyl 4-isocyanatobenzoate, brown powder (20%); mp 234–236 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 8.86 (s, 1H), 7.89 (s, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.77 (s, 1H), 7.50 (d, J = 8.8 Hz, 2H), 6.93 (s, 2H), 6.47 (t, J = 5.4 Hz, 1H), 6.25 (t, J = 2.0 Hz, 1H), 4.19 (d, J = 4.9 Hz, 2H); FAB(−)HRMS 514.0968 (−0.6 mmu). Anal. Calcd for $C_{23}H_{16}F_3N_5O_6$-H_2O: C, 51.79%; H, 3.40%; N, 13.13%. Found: C, 51.91%; H, 3.43%; N, 12.82%.

5.1.46. 3,4-Dihydro-3-oxo-7-[3-(((phenylamino)thiocarbonyl)aminomethyl)pyrrol-1-yl]-6-trifluoromethylquinoxaline-2-carboxylic acid (28). Following the procedure described for compound **27a**, the title compound was prepared from compound **26** and phenyl thioisocyanate, yellow powder (47%); mp 198–200 °C; ^{1}H NMR (DMSO-d_6): δ 9.54 (s, 1H), 7.90 (s, 1H), 7.86 (t, J = 2.0 Hz, 1H), 7.77 (s, 1H), 7.47–7.44 (m, 2H), 7.33–7.29 (m, 2H), 7.09 (t, J = 7.3 Hz, 1H), 7.00 (s, 1H), 6.94 (s, 1H), 6.32 (t, J = 1.5 Hz, 1H), 4.56 (d, J = 4.4 Hz, 2H); FAB(−)HRMS 486.0858 (+1.1 mmu).

5.1.47. 3,4-Dihydro-7-[3-((4-fluorophenylacetyl)aminomethyl)pyrrol-1-yl]-3-oxo-6-trifluoromethylquinoxaline-2-carboxylic acid (29). To a solution of compound **26** (200 mg, 478 μmol) and 4-fluorophenylacetic acid (88.3 mg, 573 μmol) in DMF (5 mL) were added EDCI (274 mg, 1.43 mmol) and Et_3N (266 μL, 1.91 mmol), and stirred for 18 h at room temperature. The reaction was diluted with AcOEt, washed with water, dried over Na_2SO_4, and evaporated. The residue was dissolved in EtOH (5 mL) and 1 N KOH (600 μL, 600 μmol), and

refluxed for 1 h. After cooling, the reaction was concentrated, and water was added to the residue. The insoluble part was removed by filtration, and the filtrate was adjusted to pH 2 with 4 N HCl. The precipitate was collected by filtration, washed with water, and dried under vacuum to give the title compound as a yellow powder (43.3 mg, 18%); ^{1}H NMR (DMSO-d_6) δ 8.36 (t, J = 4.9 Hz, 1H), 7.85 (s, 1H), 7.75 (s, 1H), 7.32–7.28 (m, 2H), 7.13–7.08 (m, 2H), 6.89 (d, J = 2.4 Hz, 1H), 6.84 (s, 1H), 6.16 (dd, J = 2.4, 1.5 Hz, 1H), 4.15 (d, J = 5.4 Hz, 2H), 3.44 (s, 2H); FAB(−)HRMS 487.1067 (+3.8 mmu). Anal. Calcd for $C_{23}H_{16}F_4N_4O_4$-$\frac{5}{4}H_2O$: C, 54.07%; H, 3.62%; N, 10.97%. Found: C, 54.09%; H, 3.59%; N, 11.14%.

5.2. Light stability measurements

The light stability of test compounds was determined in 0.1 M phosphate buffer (pH 7.4) at ambient temperature. The test compounds were dissolved to pH 7.4-phosphate buffer (0.1 M) to achieve a sample concentration of 0.01 mg/ml. The solution was transferred to a sealed clear glass vial and the solution was irradiated 3 h with 7000 Lx fluorescent lamp at ambient temperature. The light stability of the test compounds in the solution was determined by high-performance liquid chromatography (HPLC) using a C18 reversed-phase column (4.6 × 250 mm; J'shpere ODS-120T, TOSO, Japan), with a mobile phase consisting of 30% or 50% MeOH in 50 mM phosphate buffer (pH 6) run at a flow rate of 1.0 ml/min, and detection at a wavelength of 275 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).

5.3. 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 times 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.[21–23] 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 liquid scintillation counter 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 et al.[24] 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 5 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. 3). 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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