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Synthesis and AMPA Receptor Antagonistic Activity of a Novel Class of Quinoxalinecarboxylic Acid with a Substituted Phenyl Group at the C-7 Position

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Abstract—The synthesis and biological properties of a novel class of 7-heterocycle-substituted quinoxalinecarboxylic acids, which bear a substituted phenyl group through a urethane linkage at the C-7 position, are described. One of the synthesized compounds, **15l**, which has a 4-carboxyphenyl carbamoyloxymethyl imidazole group at the C-7 position and is water-soluble, was found to possess high potency in vitro and showed excellent neuroprotective efficacy in vivo.

Glutamic acid, an excitatory amino acid (EAA), is a major excitatory neurotransmitter in the mammalian central nervous system. However, overstimulation of the postsynaptic glutamate receptors by release of EAA at greater-than-normal physiological levels results in neuronal death. Consequently, it is thought that this process may induce neurodegenerative disorders such as stroke, epilepsy,[1] Huntington's disease, and Alzheimer's disease.[2]

Recent studies of EAA receptors have shown that α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPA-R) antagonists have no side effects such as schizophrenia[3] and protect effectively against neuronal death even in post-ischemia animal models.[4] Additionally, many quinoxalinedione derivatives with competitive AMPA-R antagonistic activity have been synthesized and tested against the EAA receptor. However, there have been no conclusive clinical trials showing that compounds with a quinoxalinedione structure are therapeutic in settings such as acute cerebral ischemia. These quinoxalinedione compounds can be divided into first-generation compounds, such as NBQX[4] and YM-90K,[5] and second-generation compounds, such as YM-872[6] (Fig. 1), and have been shown to exhibit good AMPA-R antagonistic activity. However, among these compounds the first-generation agents have been shown to cause kidney toxicity as a result of their physicochemical properties (particularly, low water solubility),[7] and have been limited to use in clinical trials. On the other hand, in second-generation agents such as YM-872 these undesirable properties have been ameliorated by introducing a hydrophilic functional group, for example acetic acid, into the quinoxalinedione skeleton by medicinal chemistry, and this compound has been shown to have neuroprotective effects in animal models of focal cerebral ischemia.[6b] Therefore, it seemed to us that little scope remained for development of new second-generation AMPA-R antagonists based on chemical

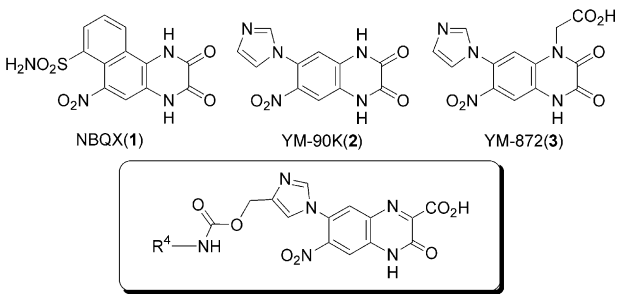

Figure 1.

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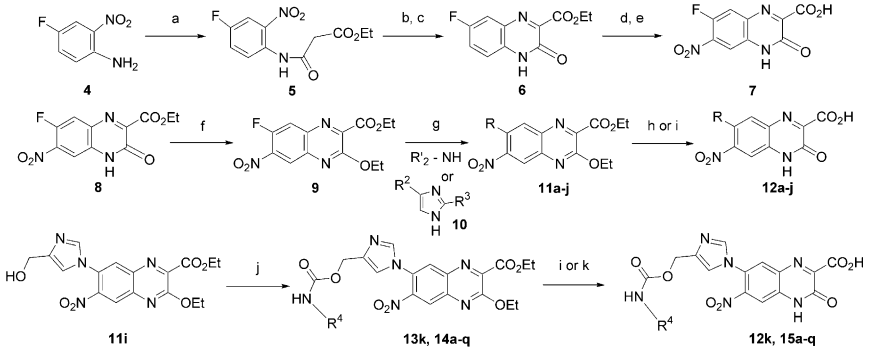
modification of the quinoxalinedione structure. Thus, our research efforts have focused on designing and synthesizing novel third-generation compounds with no quinoxalinedione skeleton that have potent AMPA-R affinities and potent neuroprotective effects in vivo and that in addition are water-soluble, allowing their use as injections.

It has been reported that the 2,3-dione moiety on the quinoxalinedione skeleton is required to form a coulombic interaction with the AMPA-R as an essential part of the proton acceptor.[8a] On the other hand, it seemed to us that introduction of an acetic acid group onto the quinoxalinedione skeleton, for example YM-872, results in improved affinity for the AMPA-R as compared with YM-90K. We considered, therefore, that the acetic acid group on the quinoxaline skeleton affects the ability of the compound to interact with the AMPA-R as well as improving water solubility. Thus, we designed and synthesized new compounds, 3-oxo-2-quinoxalinecarboxylic acids, with the hydrophilic group on the quinoxaline nucleus instead of a 2,3-dione moiety with an acetic acid group. As a result, we have discovered a novel compound with a 4-carboxyphenyl carbamoyloxymethyl imidazole substitution of the C-7 side chain of quinoxalinecarboxylic acid (Fig. 1). In this communication, we wish to describe the synthesis of novel quinoxalinecarboxylic acid derivatives and their biological effects.

The syntheses of novel quinoxalinecarboxylic acid derivatives are outlined in Scheme 1. The 7-fluoroquinoxaline (**7**) was prepared from 4-fluoroaniline using an improvement of the standard procedure.[9] The nitroaniline **4** was treated with ethyl malonyl chloride (Et_3N, DMF), followed by intermolecular cyclization (*t*-BuOK, EtOH) and deoxygenation (PBr_3, DMF) to provide 7-fluoroquinoxalinecarboxylate **6**, and then, converted to the nitro compound by selective nitrozation (*f*. HNO_3, AcOH) at the C-6 position and hydrolysis of an ester group (KOH, EtOH–H_2O) with an overall yield of 29% from **4**. The conversion of the 7-fluoro ester **8** to the imino ether **9** was performed by ethylation of the amide moiety with a 78% yield, followed by sequential nucleophilic substitution of the fluoro group and conversion to **12a–j** by hydrolysis with a 5–81% yield from **9**. Next, 4-urethane substituted imidazole derivatives **12k–l**, **15a–l**, **15n–q** were prepared from key intermediate **11i**; 4-hydroxymethyl imidazole **11i** was treated with various isocyanates followed by hydrolyses to provide corresponding 4-urethane derivatives with a 11–81% yield from **11i** as shown in Scheme 1. Furthermore, the 7-pyridone compound **12l** was prepared from 3-hydroxymethyl-4-pyridone[10] instead of 4-hydroxymethylimidazole **11i**. Then, **15m** was also prepared from 4-hydroxyethylimidazole[11] instead of 4-hydroxymethylimidazole **11i**.

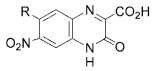
The affinities of the synthesized compounds for the AMPA and NMDA receptors were assessed by measuring their ability to displace, respectively, ^{3}H-AMPA (5 nM) and ^{3}H-CGS-19755 (10 nM) bound to crude synaptic membranes prepared from rat cerebral cortex.[12a,13]

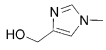
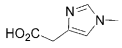
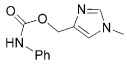
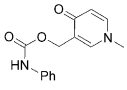
We selected a nitro group at the C-6 position because this structure has been shown to confer high AMPA-R affinity on quinoxalinedione derivatives,[8b] and performed chemical modification at the C-7 position of the 6-nitro-3-oxo-2-quinoxalinecarboxylic acid compound (Table 1). From these results, except for the imidazole group and 4-pyridone group, none of the amino derivatives was significantly different in its AMPA-R affinity at the C-7 position. The AMPA-R affinity of the heterocycle derivatives (**12e**, **f**) was generally better than that of the alkyl amine derivatives (**12a–d**). Next, we compared AMPA-R affinity between various substituents on the imidazole moiety. Unfortunately, AMPA-R affinity was reduced by the introduction of substituents into the imidazole moiety (**12g–i**). However, the carboxymethyl compound (**12j**) maintained an AMPA-R affinity equal to that of the non-substituted imidazole compound (**12f**). From these results, we considered that the carbonyl group on the imidazole moiety may facilitate interaction with AMPA-R, and thus we synthesized a series of carbonyl compounds based on the hydroxymethyl group of **12i**.



Scheme 1. (a) Ethyl malonyl–Cl, Et_3N, DMF; (b) ^{t}BuO-K, EtOH; (c) PBr_3, DMF; (d) *f*. HNO_3, AcOH; (e) KOH, EtOH–H_2O; (f) EtBr, Ag_2O, toluene; (g) R′$_2$-NH or imidazole **10**, Et_3N, THF or CH_2Cl_2 or benzene; (h) KOH, EtOH–H_2O followed by aq HCl; (i) 48% HBr or c.HCl, AcOH; (j) R^4-NCO, Et_3N, CH_2Cl_2 or benzene; (k) c.HCl, AcOH followed by 1N-LiOH.

Table 1. 7-Halogenated and 7-heterocycle derivatives



Compd	R	AMPA-R affinity K_i (nM)[12a]
7	F	2000
12a	$(CH_3)_2N$–	910
12b		3800
12c		2000
12d		2500
12e		330
12f		560
12g		1300
12h		> 10,000
12i		2600
12j		530
12k		86
12l		170

We found that compound **12k**, with the urethane linkage, exhibited more potent AMPA-R affinity than did the alcohol compound (**12i**) and the carboxylic compound (**12j**) (Table 1). On the other hand, we attempted the introduction of a similar substituent group, a phenyl group through a urethane linkage, to a 4-pyridone compound (**12e**). Despite having the same *N*-phenyl urethane group as a substituent group at the C-7 position of the quinoxaline nucleus, the 4-pyridone compound (**12l**) showed weaker AMPA-R affinity than the imidazole compound (**12k**). From these results, we concluded that an imidazole group is optimal at the 7-position of the quinoxaline ring, and that the imidazole group is needed to confer functional activity through the urethane linkage for enhancement of AMPA-R affinity. Moreover, we concluded that the urethane moiety has the same proton acceptor effect as the acid moiety of compound **12j**.

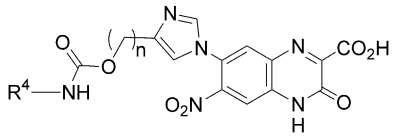
In order to find an active compound with high AMPA-R affinity and better selectivity for the AMPA-R than the NMDA-R (*N*-methyl-D-aspartate receptor), we investigated the effects of substituents in the terminal benzene moiety joined through a urethane linkage (Table 2). There was a significant increase in AMPA-R affinity after introduction of various substituents into the terminal benzene ring, except for the 2-carboxylic derivatives (**15j**), which were inferior to the other compounds. On the other hand, the introduction of various substituents into the 2- or 3-position of the terminal benzene ring resulted in significantly better selectivity. However, compounds substituted at the 4-position showed low selectivity, except when a carboxylic acid group was introduced to the benzene ring. Furthermore, we investigated the influence of the methylene length of the C-7 side chain with respect to AMPA-R affinity and selectivity. When the methylene group was inserted between the imidazole group and the urethane group there was significantly lower AMPA-R affinity (**15l** vs **15m**). On the other hand, when the methylene group was extended at the terminal substituted benzene moiety the AMPA-R affinity or the selectivity were reduced (**15p** vs **15e** and **15o** vs **15d**, respectively), although a non-substituted phenyl compound exhibited the same AMPA-R affinity and selectivity (**12k** vs **15n**). Consequently, we concluded that methylene length between the imidazole ring and the urethane linkage, and extension between the urethane linkage and the terminal substituted benzene, influence AMPA-R affinity and selectivity.

The neuroprotective effects of the selected compounds, and those of NBQX, YM-90K and YM-872, were examined using the permanent focal ischemia model in rats described by Tamura et al.[14] The results are shown in Table 3.[15] The compounds showed better neuroprotective effects in vivo, as well as AMPA-R antagonistic activity in vitro, than NBQX, YM-90K and YM-872. The AMPA-R antagonistic activity in vitro confirmed the inhibitory effect on AMPA-induced DC potential in rat cortical slices.[12b] In a comparison of the biological activities of the non-substituted imidazole **12f** and the imidazole urethane-linked at the C-7 position **15l**, compound **15l** had markedly more potent activity in vivo than compound **12f**.

It was noteworthy that the AMPA-R affinity and neuroprotective effects of **12f** were inferior to those of **15k** and **15l**, despite the presence of a quinoxalinecarboxylic acid nucleus. Compound **12f** lacked the urethane linkage on the imidazole group. Based on these results, this novel substituent at the C-7 position, namely a substituted benzene ring with a urethane linkage to imidazole, not only confers potent AMPA-R affinity but also contributes to therapeutic efficacy in animal models. In particular, the neuroprotective effect of **15l** in the rat model was superior to that of any previously reported compounds, with a relatively low iv infusion rate of 2.5 mg/kg/h for 4 h, and its aqueous solubility was higher than that of the first-generation compounds.[16] These characteristics qualify this compound, in an injectable formulation, for use in the treatment of acute cerebral ischemia.

In conclusion, the molecular design of novel AMPA-R antagonists based on the quinoxalinecarboxylic acid

Table 2. 7-Substituted imidazole derivatives



Compd	R^4	*n*	AMPA-R affinity[12a] (K_i, nM)	Selectivity[13] ($\frac{NMDA\text{-}R}{AMPA\text{-}R}$)
3	YM-872	—	62	240
12k	Ph	1	86	>100
15a[a]	2-Cl–Ph	1	20	210
15b[a]	3-Cl–Ph	1	18	370
15c	4-Cl–Ph	1	30	43
15d	2-Br–Ph	1	30	280
15e[a]	3-Br–Ph	1	16	440
15f	4-Br–Ph	1	32	38
15g	2-Me–Ph	1	16	370
15h	3-Me–Ph	1	22	290
15i[a]	4-Me–Ph	1	30	70
15j	2-CO_2H–Ph	1	>900	>9.9
15k	3-CO_2H–Ph	1	27	>330
15l	4-CO_2H–Ph	1	22	>400
15m	4-CO_2H–Ph	2	270	—
15n[b]	$PhCH_2$	1	67	>110
15o	2-Br–$PhCH_2$	1	29	160
15p	3-Br–$PhCH_2$	1	100	69
15q	4-Br–$PhCH_2$	1	47	62

[a]HCl salt.
[b]Sodium salt.

Table 3. Pharmacological data of 7-heterocycle-substituted quinoxalinecarboxylic acid derivatives

Compd	AMPR-R affinity[12a] (K_i, nM)	Selectivity[13] ($\frac{NMDA\text{-}R}{AMPA\text{-}R}$)	AMPA-R antagonism[12b] (DC potential)	Protective effects in focal ischemia model[14,15] (dose; mg/kg/h for 4 h, iv)
NBQX (**1**)	65	>1300	N.T.	2.3 (30)
YM-90K (**2**)	100	430	(+)	2.8 (15)
YM-872 (**3**)	62	240	(+)	0.5 (30)
12f	560	480	(+)	0.2 (20)
15k	27	>330	(+)	2.3 (5.0)
15l	22	>400	(+)	3.0 (2.5)

N.T., not tested.

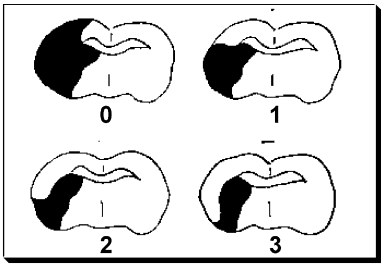


Figure 2. Four-point scale.

nucleus has created novel quinoxaline compounds with good biological activities. By studying structure–activity relationships and evaluating the properties of various compounds, we identified compound **15l** (GRA-293)[17] 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 in vivo than known quinoxalinedione compounds.

Acknowledgements

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10. This compound, 3-hydroxymethyl-4-pyridone, was synthesized from reduction by sodium borohydride of 3-formyl-4-hydroxypyridine, which was prepared by lithiation of 4-chloropyridine followed by treatment of *N*,*N*-dimethylformamide, and hydroxylation with NaOH solution.
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15. **Evaluation of the rat focal ischemia model**: After 24 h of MCA occlusion as described by Tamura et al.,[14] brains were removed and sliced into five coronal (2 mm thick) sections with the use of a rat brain matrix (a manual slicer). Slices were placed in 2% (w/v) triphenyltetrazolium chloride (TTC) solution, and then in 10% (v/v) phosphate-buffered formalin. Tissue damage (areas not stained with TTC) was scored on a four-point scale (see Fig. 2). 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 its four-point scale value was less than 1.0.
16. **Solubility test**: The solubility of test compounds was determined in 0.1 M phosphate buffer (pH 7.4) at room temperature. After centrifugation, the concentrations in the supernatant were assayed by HPLC. The results at pH 7.4 were as follows: compounds **1** and **2**, <1 mg/mL; compound **3**, >5 mg/mL; compound **15l**, 4.86 mg/mL: Takeru, H.; Fong-Mei, L. S.; Toshikiro, K.; Ryttingj, J. H. *J. Pharm. Sci*. **1979**, *68*, 1267.
17. The newly-synthesized compound GRA-293 has the following ^{1}H NMR and MS spectral characteristics: 7-[4-[*N*-[4-carboxyphenyl]carbamoyloxy]methyl]imidazolyl]-3,4-dihydro-6-nitro-3-oxo-quinoxaline-2-carboxylic acid (**15l**): pale-brown solid; mp 268–270 °C; ^{1}H NMR (DMSO-d_6) δ 5.10 (2H, s), 7.58 (2H, d, $J=8.8$ Hz), 7.58 (1H, s), 7.86 (2H, d, $J=8.8$ Hz), 7.99 (1H, s), 8.03 (1H, s), 8.20 (1H, s), 10.2 (1H, s), 12.6 (1H, bs); LRMS (FAB$^-$) *m*/*z* 493 (MH$^-$); HRMS (FAB$^-$) *m*/*z* calcd for $C_{21}H_{13}O_9N_6$ (MH$^-$) 493.3690, Found 493.0744. Anal. calcd for $C_{21}H_{13}O_9N_6$–$2H_2O$: C, 47.55; H, 3.42; N, 15.65. Found: C, 47.46; H, 3.33; N, 15.65.