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Synthesis of Diaminobutane Derivatives as Potent Ca^{2+}-Permeable AMPA Receptor Antagonists

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Abstract—We synthesized diaminobutane derivatives as potent Ca^{2+}-permeable AMPA receptor antagonists with non-hypotensive activity. Compound **10c** showed selective Ca^{2+}-permeable AMPA receptor antagonist activity and neuroprotective effects in transient global ischemia models in gerbils.

Introduction

L-Glutamate is a major excitatory neurotransmitter in the mammalian central nervous system.[1] Excessive amounts of glutamate, which are released in pathophysiological conditions such as cerebral ischemia and epilepsy, overstimulate glutamate receptors, leading to the delayed neuronal cell death after global ischemia through an increase in Ca^{2+} influx. We focused on Ca^{2+}-permeable AMPA receptors, one of the glutamate receptors modulating Ca^{2+} influx,[2] and studied antagonists against the receptors.

Spider toxin JSTX-3 (**1**) and its analogue NAS (**2**) are well known Ca^{2+}-permeable AMPA receptor antagonists (Fig. 1).[3] However, compound **2** and its derivatives[5] show strong hypotensive activity which may deteriorate cerebral blood flow in patients with cerebral ischemia.[4] In a previous paper, we investigated non-hypotensive Ca^{2+}-permeable AMPA receptor antagonists and found a series of polyamine derivatives including **3** (Fig. 1).[5] However, compound **3**, as well as **1** and **2**, did not exhibit neuroprotective effects in transient global ischemia models in gerbils after intraperitoneal (ip) or intravenous (iv) administration. We therefore focused on an increase in lipophilicity of newly synthesized compounds, expecting that they show neuroprotective effects in in vivo ischemia model. As a result, we found that diamine compounds showed potent Ca^{2+}-permeable AMPA receptor antagonist activity with non-hypotensive activity, and that one of the diamine compounds exhibited neuroprotective effects in an in vivo ischemia model after ip administration. In this paper, we describe the synthesis and biological activity of a novel series of diaminobutane derivatives.

Chemistry

The synthetic pathways of compounds **9**, **10a–c**, **13** and **15a–d** are shown in Scheme 1. Commercially available 4-aminomethylpiperidine **4** was converted to phthalimide **5**, which was coupled with 1-naphthylacetic acid and then treated with hydrazine to give amine **6**. Amine **6** was alkylated with 4-bromobutylphthalimide and then protected with a *tert*-butoxycarbonyl (Boc) group to give **7**, the phthaloyl group of which was removed to yield amine **8**. Amine **8** was converted to compound **9** by treatment with concd HCl. Compounds **10a–c** were prepared by reductive alkylation using the corresponding aldehydes and successive deprotection of the Boc group with concd HCl. Alcohol **12** was synthesized from amine **6** by alkylation with 4-bromobutyl acetate followed by protection with a Boc group and alkali hydrolysis. Alcohol **12** was mesylated with mesyl chloride, coupled with piperidine and then treated with concd HCl to afford compound **13**. Swern oxidation of alcohol **12** gave aldehyde **14**, which was converted to compounds **15a–d** by reductive alkylation using the corresponding amines and treatment with concd HCl.

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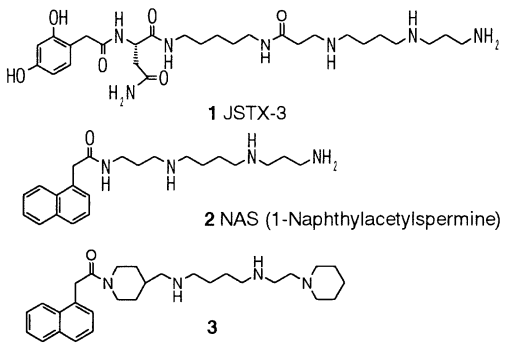
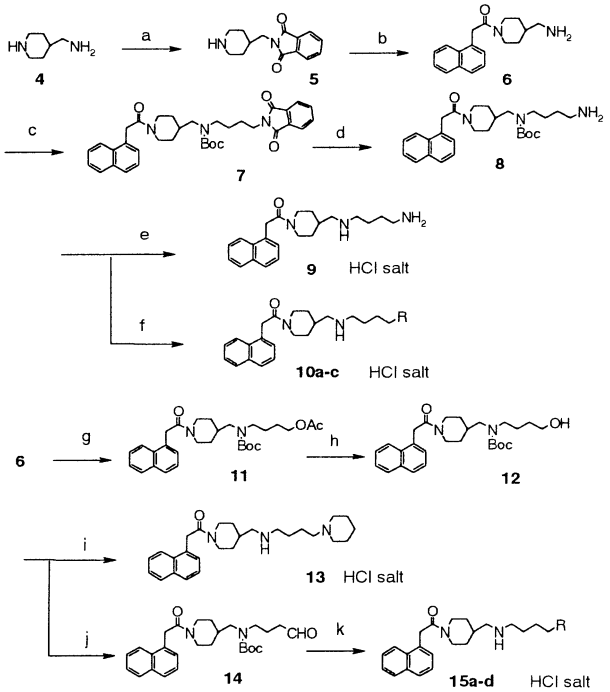


Figure 1.

Compounds **21** and **25** were synthesized as shown in Scheme 2. Ethyl isonipecotate **16** was protected with a benzyloxycarbonyl (Z) group, and the resulting compound was converted to aldehyde **18** by reduction of its ester group and successive Swern oxidation. Wittig reaction of **18** with 4-carboxybutyltriphenylphosphonium bromide followed by esterification, catalytic reduction and condensation with 1-naphthylacetic acid afforded **19**, which was converted to aldehyde **20** in 3 steps. Aldehyde **20** was reductively alkylated with cyclohexylmethylamine and then treated with concd HCl to give compound **21**. Compound **25** was synthesized from cyclohexylaldehyde **22** in 8 steps in a similar procedure.



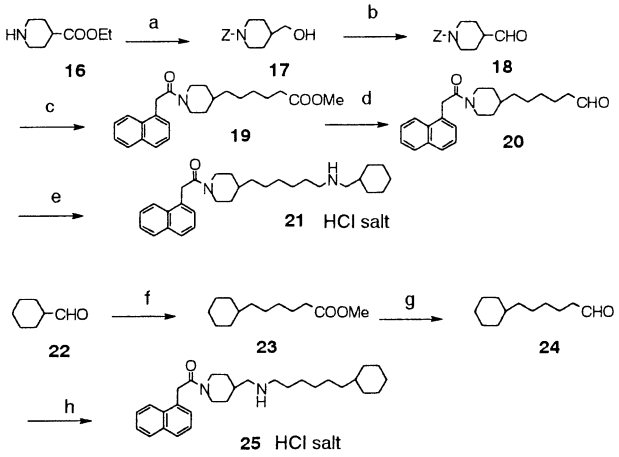
Scheme 1. Synthesis of compounds **9**, **10a–c**, **13** and **15a–d**. Reagents and conditions: (a) phthalic anhydride, Δ (61%); (b) (i) (1-naphthyl)-acetic acid, EDC·HCl, TEA, CH_2Cl_2 (69%), (ii) $NH_2NH_2{\cdot}H_2O$, EtOH (quant.); (c) (i) 4-bromobutylphthalimide, KF–Celite, MeCN, (ii) Boc_2O, CH_2Cl_2; (d) $NH_2NH_2{\cdot}H_2O$, EtOH (3 steps 55%); (e) c HCl, EtOH (62%); (f) (i) aldehyde, $NaBH_4$, MeOH, (ii) Boc_2O, CH_2Cl_2, (iii) c HCl, EtOH (3 steps 25–57%); (g) (i) 4-bromobutyl acetate, KF–Celite, MeCN, (ii) Boc_2O, CH_2Cl_2; (h) K_2CO_3, MeOH (3 steps 48%); (i) (i) MsCl, Py, (ii) piperidine, KF–Celite, MeCN, (iii) c HCl, EtOH (3 steps 58%); (j) Swern oxidation (71%); (k) (i) amine, $NaBH_4$, MeOH, (ii) Boc_2O, CH_2Cl_2, (iii) c HCl, EtOH (3 steps 24–50%).

Results and Discussion

Antagonist activity of test compounds against Ca^{2+}-permeable AMPA receptors (IC_{50}) was measured using a two-electrode voltage clamp method:[6] kainate (KA) was used as an agonist for the receptors expressed in *Xenopus* oocytes by injection of rat brain mRNA. However, since KA non-selectively induces inward currents via stimulation of KA receptors, Ca^{2+}-permeable and Ca^{2+}-impermeable receptors, antagonist activity of test compounds for Ca^{2+}-permeable AMPA receptors was calculated by offsetting the inward currents due to blockade of the other two receptors.[7] Hypotensive activity was evaluated after iv administration to Wistar rats. These activities are shown in Table 1.

In this paper, we planned to modify compound **3** to match those with higher lipophilicity. The design was carried out based on the speculation that a decrease in the number of basic nitrogen atoms of compound **3** would result in an increase in lipophilicity. At first, we evaluated whether simple diaminobutane derivative **9** and monoamine derivatives **21** and **25** had Ca^{2+}-permeable AMPA receptor antagonist activity. Among these compounds, diaminobutane **9** and monoamine **21** showed considerable activity, but not monoamine **25**. Based on these results, we selected the diaminobutane skeleton for further modification because of its easiness and higher possibility in modification.

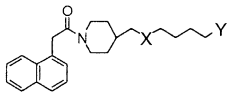
Alkylation of the terminal amino group of compound **9** caused improvement of potency. Cyclohexylmethylamino derivative **10c** exhibited the highest potency in the compounds synthesized here. On the other hand, introduction of an oxygen atom into the terminal alkyl group

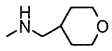
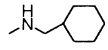
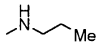
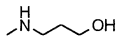
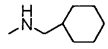
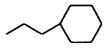
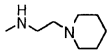


Scheme 2. Synthesis of compounds **21** and **25**. Reagents and conditions: (a) (i) Z-Cl, aq $NaHCO_3$, CH_2Cl_2, (ii) $LiBH_4$, EtOH (2 steps 85%); (b) Swern oxidation (quant.); (c) (i) 4-carboxybutyl triphenylphosphonium bromide, NaHMDS, THF, (ii) c H_2SO_4, MeOH (2 steps 46%), (iii) H_2, Pd–C, MeOH, (iv) (1-naphthyl)acetic acid, EDC·HCl, HOBt, NMM, CH_2Cl_2 (2 steps 79%); (d) (i) LiOH, MeOH, (ii), BH_3–THF, THF (2 steps 34%), (iii) Swern oxidation (quant.); (e) (i) cyclohexanemethylamine, $NaBH_4$, MeOH, (ii) Boc_2O, CH_2Cl_2 (2 steps 58%), (iii) c HCl, EtOH (quant); (f) (i) 4-carboxybutyl triphenylphosphonium bromide, NaHMDS, (ii) c H_2SO_4, MeOH (2 steps 83%), (iii) H_2, Pd–C, MeOH (quant.); (g) (i) LAH, THF (85%), (ii) Swern oxidation (quant.); (h) (i) **6**, $NaBH_4$, MeOH, (ii) Boc_2O, CH_2Cl_2 (2 steps 89%), (iii) c HCl, EtOH (quant.).

resulted in a slight decrease in potency (**10b** and **15b**), suggesting that a polar group, such as an oxygen atom, in this position is unfavorable for interactions with the receptor. Dimethylamino analogue **10a** seemed to have somewhat higher potency compared to those of secondary amino derivatives **15a**,**c**,**d**. Piperidino derivative **13** showed potency almost comparable to that of **10c**.

Table 1. Inhibitory effect on the Ca^{2+}-permeable AMPA receptor and effect on blood pressure



Compd	X	Y	IC_{50} (μM)[a]	ΔSBP (mmHg)[c]	Predicted LogP[f]
9			54%[b]	−53	2.44
10a			0.53	12	3.21
10b			35%[b]	−22	3.45
10c			0.25	11 (17[d])	5.32
13			0.39	4	4.22
15a			0.74	9	3.89
15b			44%[b]	−6	2.47
15c			0.73	10	3.91
15d			0.79	7	4.18
21			42%[b]	−35	7.51
25			1%[b]	−50	7.51
3			0.19	5	3.72
NAS (**2**)	—	—	0.69	−55[e]	1.74

[a]IC_{50} (μM) was defined as the concentration of a compound that reduces the inward current induced by kainate by 50%. The compound was co-administered with kainate to *Xenopus* oocytes and the inward current was measured by the two-electrode voltage clamp method. The values represent the means of results from 6 *Xenopus* oocytes.
[b]Because some compounds could not exert 50% inhibition, the amount of inhibition (%) exerted at a concentration of 1 μM is shown.
[c]The systolic blood pressure (mmHg) of rats after intravenous (iv) administration of a 3 mg/kg dose was compared with the systolic blood pressure before administration ($n = 1$–2).
[d]The dose of 10 mg/kg was used.
[e]The dose of 1 mg/kg was used.
[f]The values were calculated by PROLOGP(ver. 3.0).[12]

With respect to hypotensive activity, compound **9** with a primary amino group showed potent activity. Mono- or di-alkylation of the terminal amino group of **9** eliminated the hypotensive activity (**10a**,**c**, **13** and **15a**–**d**), except for compound **10b**. Contrary to non-hypotensive compound **10c**, monoamine derivatives **21** and **25** with a similar cyclohexylalkylamino group showed strong hypotensive activity. It is uncertain why compounds **9**, **10b**, **21** and **25** have hypotensive activity and also whether all the compounds interact with the same receptors mediating hypotensive activity.

We selected compounds **10c** and **13**, which showed both potent Ca^{2+}-permeable AMPA receptor antagonist activity in vitro and non-hypotensive activity in vivo, for evaluation of neuroprotective effects using a transient global ischemia models in gerbils.[8] Compound **10c** was effective in the models, but not **13**. A 4-time repeated dose of 2.5, 5.0 or 10 mg/kg of **10c** was intraperitoneally administered at 0, 2, 4 and 6 h after 5-min ischemia: compound **10c** significantly protected neurons from cell death at a repeated dose of 10 mg/kg (Fig. 2). Compound **10c** had higher predicted log P than compound **3** or **13**, suggesting that the high lipophilicity of **10c** may contribute to the in vivo neuroprotective effects. In addition, compound **10c** would not show hypotensive effects in the gerbil transient global ischemia models, since the compound did not reduce blood pressure in rats up to at least 10 mg/kg iv administration.

Selectivity of **10c** between Ca^{2+}-permeable and Ca^{2+}-impermeable AMPA receptors was determined by using recombinant AMPA receptors according to the described method (Table 2).[9] Ca^{2+}-Permeable AMPA receptors are assembled from only GluR3 subunits, while Ca^{2+}-impermeable AMPA receptors are assembled from GluR3 and GluR2 subunits.[10] Compound **10c** as well as JSTX-3[11] showed selective inhibition for Ca^{2+}-permeable AMPA receptors.

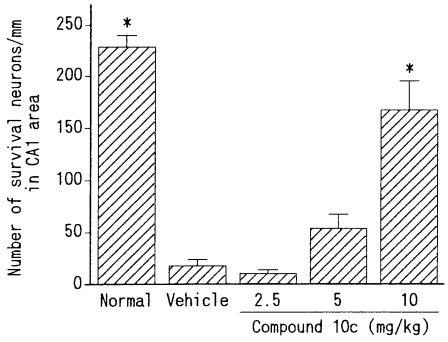


Figure 2. Effect of compound **10c** on number of surviving neurons in the hippocampal CA1 area in gerbils 5 days after global ischemia. Values are means ± SEM of neuronal density in individual hippocampi in each group ($n = 6$–20). Compound **10c** was intraperitoneally administered 4 times after ischemia. *: $p < 0.01$ versus vehicle.

Table 2. Blocking effects of JSTX-3 and **10c** on Ca^{2+}-permeable (GluR3 only) or Ca^{2+}-impermeable AMPA receptors (GluR3 + GluR2)

Sample	%inhibition[a] (GluR3 only)	%inhibition[a] (GluR3 + GluR2)
JSTX-3 (**1**)	79.7	4.3
10c	65.8	4.4

[a]*Xenopous* oocytes that had been injected with GluR3cRNA (50 ng) have Ca^{2+}-permeable AMPA receptors. On the other hand, *Xenopous* oocytes that had been injected with both GluR3cRNA and GluR2cRNA (1:9, total 100 ng) had Ca^{2+}-impermeable AMPA receptors. Both of these compounds strongly decreased the inward current via Ca^{2+}-permeable AMPA receptors but had little effect on the current via Ca^{2+}-impermeable AMPA receptors. JSTX-3 or **10c** was administered at the dose of 3 μM with 300 μM KA. Each value is the mean of results from 2–3 *Xenopous* oocytes.

Consequently, diamine derivative **10c** showed selective antagonist activity for Ca^{2+}-permeable AMPA receptors, non-hypotensive effects in rats and neuroprotective effects in transient global ischemia models in gerbils.

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