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Communications to the Editor

Synthesis and Pharmacology of a New AMPA−Kainate Receptor Agonist with Potent Convulsant Activity

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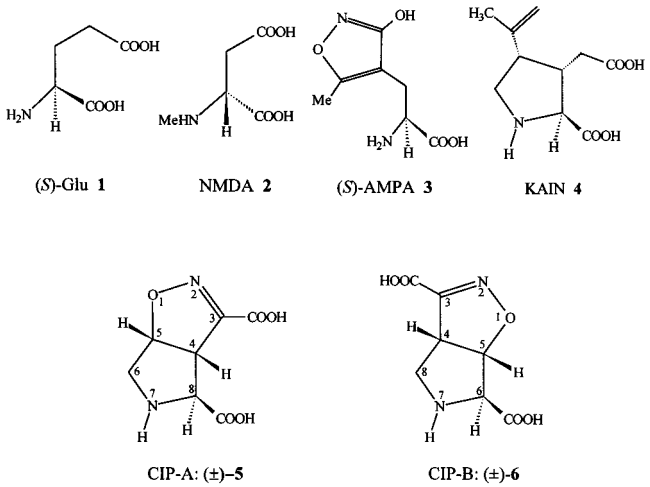
Introduction. Excitatory neuronal transmission within the central nervous system (CNS) is mediated predominantly by the amino acid (*S*)-glutamate (Glu, **1**) which plays a role of utmost importance in many physiological processes such as neural plasticity, memory, and learning.[1−3] An imbalance of excitatory pathways seems to be implicated in the pathogenesis of a number of acute and chronic neurological disorders, i.e., epilepsy, cerebral ischemia, stroke, hypoxia, and schizophrenia, as well as chronic neurodegenerative pathologies, i.e., neuropathic pain, amyotrophic lateral sclerosis, and Huntington's, Parkinson's, and Alzheimer's diseases.[4]

(*S*)-Glutamic acid activates two families of receptors: the ionotropic (iGlu)[5,6] and metabotropic (mGlu)[7,8] receptors. The iGluRs are multimeric Glu-gated channels which control the flux of cations (Na^+, K^+, and Ca^{2+}) across the postsynaptic membrane. They are responsible for the fast depolarization of postsynaptic cells. On the basis of the pharmacological and functional properties of selective agonists, they have been classified into NMDA (*N*-methyl-D-aspartic acid, **2**), AMPA [(±)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid, **3**], and KAIN (kainic acid, **4**) receptors (Chart 1). On the other hand, the mGluRs belong to the superfamily of G protein-coupled receptors and modulate the activity of phospholipase C (PLC) or adenylyl cyclase (AC). To date, eight distinct metabotropic glutamate ($mGlu_{1-8}$) receptors have been cloned and classified based on their amino acid sequence homology, signal transduction mechanism, and pharmacology.[9−11] The eight mGlu receptors have been grouped into three subsets termed group I ($mGlu_{1,5}$), linked to PLC stimulation, and group II ($mGlu_{2,3}$) and group III ($mGlu_{4,6,7,8}$), both negatively coupled to adenylyl cyclase.

Chart 1



A prerequisite for the determination of the physiological role and pharmacological relevance of the subgroups of iGlu receptors as well as the subtypes of mGlu receptors is the availability of highly selective agonists and antagonists.[12] In previous studies a number of agonists and antagonists were developed, which allowed the pharmacological characterization of iGlu receptor subtypes, notably the AMPA receptors, and to delineate a relationship between structure and activity.[13−15] This paper deals with the synthesis of the bicyclic Glu analogues CIP-A [3a,5,6,6a-tetrahydro-4*H*-pyrrolo[3,4-*d*]isoxazole-3,4-dicarboxylic acid, (±)-**5**] and

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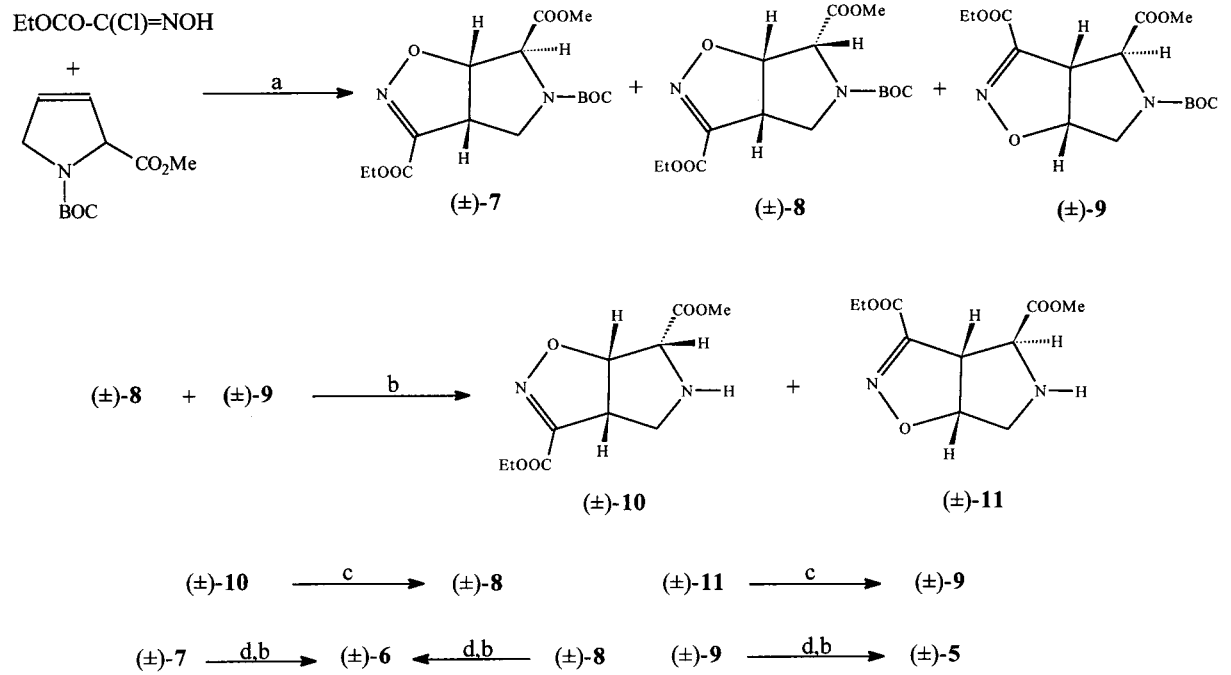
Scheme 1[a]



[a] (a) $NaHCO_3$/EtOAc; (b) CF_3COOH/CH_2Cl_2; (c) BOC_2O-NEt_3/CH_2Cl_2; (d) $NaOH/H_2O$−MeOH.

CIP-B [3a,5,6,6a-tetrahydro-4*H*-pyrrolo[3,4-*d*]isoxazole-3,6-dicarboxylic acid, (±)-**6**] and the evaluation of their biological profile at glutamate receptors.

Chemistry. The key step in the synthesis of target compounds (±)-**5** and (±)-**6** is represented by the 1,3-dipolar cycloaddition of ethoxycarbonylformonitrile oxide, generated in situ by treatment of ethyl 2-chloro-2-(hydroxyimino)acetate with a base, to the suitably protected racemic 3,4-didehydroproline.[16] As shown in Scheme 1, the pericyclic reaction allows the characterization of three out of the four possible stereoisomers. Column chromatography of the reaction mixture yielded two fractions containing pure (±)-**7** and an inseparable mixture of (±)-**8** and (±)-**9**, respectively. By treating the mixture of (±)-**8** and (±)-**9** with excess trifluoroacetic acid, we obtained the corresponding secondary amines (±)-**10** and (±)-**11** which could be separated by column chromatography and were then reconverted into *N*-*tert*-butyl carbamates (±)-**8** and (±)-**9** under standard conditions. The final compounds (±)-**5** and (±)-**6** were obtained through the alkaline hydrolysis of the two ester groups of cycloadducts (±)-**7**, (±)-**8**, and (±)-**9** followed by treatment of the intermediates with a 30% dichloromethane solution of trifluoroacetic acid. Noteworthy, the alkaline hydrolysis of cycloadducts (±)-**7** and (±)-**8** produced the same final derivative (±)-**6**, since the hydrolysis of derivative (±)-**8** with sodium hydroxide is accompanied by an inversion of the chiral center at C-6. This result can be rationalized on the basis of the higher thermodynamic stability of the 5,6-trans isomer [(±)-**7**] versus the 5,6-cis one [(±)-**8**]. The assignment of the structure to all the synthesized compounds is based on the ^1H NMR spectra. The ^1H NMR resonances of cycloadducts (±)-**7**, (±)-**8**, and (±)-**9** were assigned by standard methods that rely on correlation through chemical bonds (COSY). The coupling constants of H-5 (see Chart 1 for numbering) are highly diagnostic in assigning the structure to the cycloadducts. As a matter of fact, such a proton appears as a duplet in cycloadduct (±)-**7**, as a double duplet in (±)-**8**, and as an eight-line signal (double duplet of duplets) in (±)-**9**.

Results and Discussion. The two regioisomeric 3-carboxyisoxazolinyl prolines [CIP-A, (±)-**5**, and CIP-B, (±)-**6**] were assayed in vitro by means of receptor binding techniques, second-messenger assays, and the rat cortical wedge preparation. The convulsant activity of the compounds was evaluated in vivo on DBA/2 mice. The receptor affinity of the products (±)-**5** and (±)-**6** for NMDA, AMPA, and KAIN receptors was determined by using the radioligands [^3H]CPP, [^3H]AMPA, and [^3H]-KAIN, respectively.[17−19] The activity of the same compounds at metabotropic Glu receptors was evaluated at $mGlu_{1\alpha}$, $mGlu_2$, and $mGlu_{4a}$, expressed in CHO cells, as representatives for group I, II, and III metabotropic receptors, respectively.[20] None of the compounds tested showed significant activity at the above-mentioned metabotropic receptors.

Amino acids CIP-A [(±)-**5**] and CIP-B [(±)-**6**] were also tested in vivo on DBA/2 mice, a suitable animal model to study the tonic−clonic seizures induced by agonists acting at AMPA and KAIN receptors.[21,22]

As shown in Table 1, both compounds under investigation display affinity for AMPA and KAIN receptors; CIP-B [(±)-**6**] also binds to the NMDA receptor complex, though, due to the agonist activity at AMPA receptors, it could not be determined in the electrophysiological experiments whether this affinity is related to agonist or antagonist activity at NMDA receptors. The excitatory activity observed for compound (±)-**6** in the cortical slice model[23] is antagonized by NBQX (5 μM) and not by CPP (10 μM). For comparison AMPA (5 μM) is fully antagonized (>80% reduction) by 5 μM NBQX, whereas KAIN (10 μM) requires 20 μM NBQX for full blockade. NMDA (10 μM) are not antagonized by NBQX in these doses but can be fully antagonized by CPP (10 μM). The binding data collected for derivative CIP-A show a quite

Table 1. Receptor Binding and Electropharmacological Data (Values ± SEM, $n = 3-4$)

compd	receptor binding, IC_{50} (μM)			electropharmacology, EC_{50} (μM)
	[^{3}H]AMPA	[^{3}H]KAIN	[^{3}H]CPP	
CIP-A	1.3 ± 0.5	0.48 ± 0.11	>100	5.4 ± 0.6[a]
CIP-B	24.7 ± 6.2	48.0 ± 4.4	23.0 ± 3.6	284 ± 30[b]
AMPA	0.040 ± 0.014	>100	>100	3.5 ± 0.2[b]
KAIN	4.0 ± 1.2	0.007 ± 0.002	>100	25 ± 3[c]

[a] CIP-A (10 μM) is partially antagonized by 5 μM NBQX and fully antagonized by 20 μM NBQX. [b] CIP-B (500 μM) or AMPA (5 μM) is fully antagonized by 5 μM NBQX. [c] KAIN (10 μM) is not antagonized by 5 μM NBQX (<20% reduction) but fully antagonized by 20 μM NBQX (>80% reduction).

Table 2. CD_{50} Values of CIP-A, CIP-B, KAIN, and (±)-AMPA in the Absence and Presence of CPP, GYKI 52466, and NBQX

treatment	CD_{50} values[a] (±95% confidence limits)		potency ratio
	clonus	tonus	
CIP-A	0.027 (0.016−0.04)	0.16 (0.087−0.296)	
CIP-A + CPP	0.039 (0.013−0.12)	0.19 (0.130−0.296)	1.2−1.4
CIP-A + GYKI 52466	0.50 (0.16−2.27)	1.26 (0.61−2.62)	7.9−18.5
CIP-A + NBQX	0.43 (0.19 + 1.0)	0.99 (0.43−1.53)	6.2−15.9
CIP-B	8.48 (4.85−18.84)	39.9 (18.64−85.45)	
CIP-B + CPP	24.82 (18.5−33.3)	134.12 (88.8−202.57)	2.9−3.4
CIP-B + GYKI 52466	17.62 (12.14−25.57)	54.28 (33.31−88.47)	1.4−2.1
CIP-B + NBQX	9.27 (4.23−20.35)	53.95 (35.42−81.11)	1.1−1.3
KAIN	0.015 (0.010−0.024)	0.032 (0.017−0.060)	
KAIN + CPP	0.024 (0.016−0.036)	0.071 (0.038−0.133)	1.6−2.2
KAIN + GYKI 52466	0.071 (0.053−0.095)	0.232 (0.184−0.292)	4.7−7.2
KAIN + NBQX	0.094 (0.066−0.134)	0.468 (0.382−0.573)	6.3−14.6
AMPA	1.76 (1.06−3.07)	2.90 (1.83−4.58)	
AMPA + CPP	2.69 (1.45−5.0)	3.18 (2.48−4.06)	1.1−1.5
AMPA + GYKI 52466	7.61 (4.75−12.2)	16.1 (8.9−29.3)	4.3−5.5
AMPA + NBQX	6.70 (4.0−11.1)	15.7 (11.4−21.8)	3.8−5.4

[a] All the data are expressed as nmol/mouse. The CD_{50} values are related to icv administration of the convulsant. The potency ratio is the ratio between the CD_{50} value of the drug in the presence of an antagonist versus its CD_{50} value

high affinity for both AMPA and KAIN receptors. These results are confirmed in the cortical slice model where CIP-A displays an EC_{50} value very close to that of AMPA, the model compound (EC_{50} 5.4 versus 3.5 μM). The depolarization evoked by CIP-A is only partially antagonized by 5 μM NBQX, whereas virtually full antagonism is observed with 20 μM NBQX, further evidencing the involvement of KAIN receptors.

Even more striking are the data collected on in vivo assays where an icv injection of CIP-A [(±)-**5**] shows convulsant properties, measured as tonus and clonus seizures, 18−65 times higher than those produced by AMPA and only 2−5 times lower than those induced by KAIN (Table 2). The convulsions induced by CIP-A [(±)-**5**] are partially antagonized by classical AMPA receptor antagonists, i.e., GYKI 52466 and NBQX, and are unaffected by CPP, a selective NMDA antagonist. On the contrary, the convulsions induced by CIP-B [(±)-**6**] are antagonized by CPP and marginally by GYKI 52466 and NBQX (Table 2) confirming, in part, the results obtained in binding experiments where it showed a moderate affinity for both AMPA−KAIN and NMDA receptors. A preliminary investigation indicates that CIP-A [(±)-**5**] is also quite active by ip administration since it is able to induce seizures in mice at doses as low as 3.2 nmol/mouse. At the moment it is rather difficult to account for the discepancy between the moderate binding affinity of CIP-A at AMPA and KAIN receptors and its remarkable in vivo convulsant activity. Such an activity is likely due to synergistic activity at AMPA and KAIN receptors as evident from the binding data showing CIP-A to have an affinity profile somewhat similar to that of KAIN. A further possibility is that CIP-A interferes with the transporter mechanism for the endogenous neurotransmitter.

The present results suggest the following considerations. Since AMPA and its bicyclic analogues are characterized by the presence in their structure of a 3-hydroxyisoxazole nucleus, we can deduce that such a structural feature is of utmost importance for a selective interaction with the AMPA receptor complex. The replacement of the 3-hydroxyisoxazole nucleus of AMPA-selective ligands with the 3-carboxyisoxazolinyl moiety gives compounds, i.e., CIP-A [(±)-**5**], in which the spatial arrangement of the pharmacophoric groups is suitable for an additional interaction with the KAIN receptor subsites. As shown in Table 1 bicyclic isoxazolinyl derivatives CIP-A and CIP-B bind to both AMPA and KAIN receptors. The affinity of CIP-A [(±)-**5**] for the KAIN receptor complex is noteworthy.

To establish if the eutomer of CIP-A shares the configuration of the chiral centers with natural kainic acid, we have undertaken the synthesis of its enantiomers. This achievement will make it possible to uncover the impact of chirality on the selectivity among AMPA and KAIN receptors. Moreover, the measurement of parameters such as pK_a and log P along with a comparative study of the conformational profile of the derivatives under investigation with that of model compounds will give new clues to design new potent and selective ligands.

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Supporting Information Available: Experimental details for the preparation of compounds in this paper, their physical property data, and the procedures for in vitro and in vivo biological assays (5 pages). Ordering information is given on any current masthead page.

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