Synthesis of 3,4-Dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide Derivatives as Potential Allosteric Modulators of AMPA/Kainate Receptors

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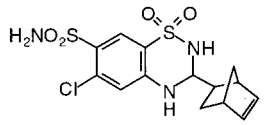
Abstract: A series of 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide derivatives were synthesized and evaluated for their activity as allosteric modulators of kainate-activated currents in primary cultures of cerebellar granule neurons. Substitution of different groups at the 3-position of the benzothiadiazine ring distinguished between positive and negative allosteric modulatory properties.

Introduction. Recent studies indicate that compounds that reduce the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor desensitization could improve impaired synaptic functions associated with learning and cognition pathology and can be useful in the treatment of attention disorders in children as well as in senile dementias, including early stages of Alzheimer disease.[1,2] Among these compounds are two benzothiadiazine derivatives: cyclothiazide (**1**) and IDRA 21 (**2**).



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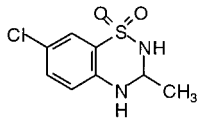

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Cyclothiazide (**1**) has been demonstrated to be one of the most potent compounds in vitro that by removal of AMPA receptors desensitization enhances synaptic transmission.[3−6] Since cyclothiazide does not cross the blood−brain barrier, it is important to develop analogues of this compound that manage to reach the central nervous system and do not have peripheral effects. IDRA 21 (**2**)[7] fulfills these requirements, and it has gained strong interest because of its potency in abating pharmacologically induced cognitive impairments in patas monkey,[8,9] in improving cognition in rats,[10] and in promoting the induction of long-term potentiation.[11] Recent reports showed that **2** worsens neuronal ischemic injury[12] probably because of excessive AMPA receptor activation, although the concentrations used were higher than those needed for the nootropic effect. Thus, the development of analogues of **1** or **2** with putative AMPA receptor modulatory activity for the treatment of cognitive disorders gains importance.

As part of an ongoing project in this area, 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide derivatives (**3**−**12**) were synthesized and studied for their activity as allosteric modulators of kainate-activated currents in primary cultures of cerebellar granule neurons, since kainic acid (KA) elicits a nondesensitizing current mediated mainly by AMPA receptor.[13,14] Particular attention was paid in analyzing the influence on the modulatory activity played by the nature and size of the side chains in the 3-position of the benzothiadiazine ring.

Chemistry. The 3,4-dihydro-2*H*-1,2,4-benzothiadiazine derivatives **3**−**12** were prepared from the corresponding 2-aminobenzensulfonamides **13** and **14** by condensation with a suitable aldehyde or the corresponding ethyl hemiacetal in acidic medium, as outlined in Scheme 1.

Synthesized compounds (**3**−**12**) possess a stereogenic carbon atom in the 3-position of benzothiadiazine ring, and as stereisomers often shown different pharmacological activities, it seemed advisable to resolve the racemic mixture to investigate the biological properties of each enantiomer. In a previous study[15] an enantioseparation method employing tris(3,5-dimethylphenylcarbamate) as a chiral stationary phase (Chiralcel OD and Chiralcel OD-R) was developed for the separation of the enantiomers of **2**, and subsequent enantiomerization studies[16,17] revealed the rapid interconversion of enantiomers in aqueous solvents. The HPLC method has been applied to the structurally related chiral compounds synthesized (**3**−**12**), and the enantiomeric nature of eluates was confirmed by circular dichroism spectra (Table 1). However, like for compound **2**, up evaporation of mobile phases of collected peak fractions corresponding to the single enantiomers, racemization occurred. Furthermore, the individual enantiomers of the studied compounds were isolated by preparative chromatography using poly(*N*-acryloyl-*S*-phenylalanine ethyl ester) as chiral stationary phase, employing conditions where no enantiomerization took place, followed by dissolution at a concentration of 1 mM in the extracellular medium used in the electrophysiological tests. Rate constants of enantiomerization of individual enantiomers were determined at room temperature, revealing an enantiomerization half-life that is intractably short, averting efforts to measure stereospecific or stereoselective biological activities of synthesized compounds. Hence, racemates were used in pharmacological tests.

Pharmacology. The pharmacological tests were performed using the patch-clamp technique on primary cultures of cerebellar granule neurons.[18−20]

The synthesized compounds were dissolved in DMSO and diluted at the final concentration in extracellular solution (DMSO at the final concentration was less than 1%) and were applied directly by gravity through a Y-tube perfusion system.[21] The low solubility of compound **8** in DMSO and in other tested solvents precluded

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Scheme 1

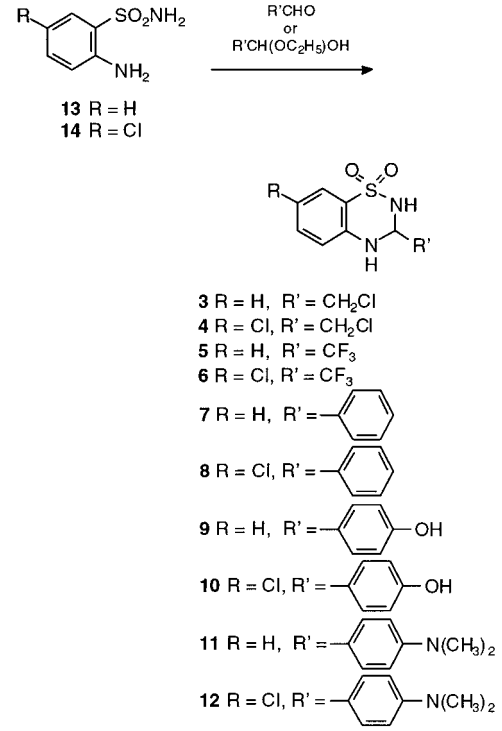

Table 1. Chromatographic Enantioseparation of Racemic Benzothiadiazine Derivatives **2**−**12**

	CHIRALCEL OD[a]				CHIRALCEL OD-R[b]			
compd	$k'_{(-)}$	$k'_{(+)}$	α	R_S	$k'_{(-)}$	$k'_{(+)}$	α	R_S
3	3.47	5.00	1.44	3.73	1.29	1.44	1.12	1.11
4	2.40	3.09	1.29	2.13	2.54	2.88	1.13	1.13
5	3.88	11.88	3.06	9.10	1.18	2.44	2.07	3.00
6	3.60	5.09	1.41	2.94	*c*	*c*	*c*	*c*
7	1.74	3.05	1.75	1.37	*c*	*c*	*c*	*c*
8	1.32	1.70	1.29	1.2	*c*	*c*	*c*	*c*
9	4.35	5.42	1.25	2.31	4.47	5.88	1.31	1.94
10	3.79	4.72	1.24	2.53	*c*	*c*	*c*	*c*
11	8.44	11.47	1.36	3.70	*c*	*c*	*c*	*c*
12	1.74	2.94	1.69	1.38	1.41	1.78	1.26	1.83

[a] Column, Chiralcel OD; mobile phase, hexane/2-propanol 60:40 (v/v); temperature, 25 °C; flow, 0.5 mL/min. [b] Column, Chiralcel OD-R; mobile phase, water/acetonitrile 60:40 (v/v); temperature, 25 °C; flow, 1 mL/min. [c] No enantiomeric separation.

performing the pharmacological test. KA was dissolved in the extracellular solution.

Results and Discussion. We tested the activity of **2** and of synthesized derivatives (**3**−**12**) as allosteric modulators of KA-activated currents in primary cultures of cerebellar granule neurons. Application of KA (100 μM) evoked a nondesensitizing current that is mediated by both AMPA and KA receptors activation. Compounds **1** (100 μM) and **2** (1 mM) potentiate the amplitude of the KA current by 111 ± 21% (n = 9) and 115 ± 24% (n = 17), respectively.

In the series of synthesized 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide derivatives, **5** and **6** are the only compounds that, similar to **2**, act as positive modulators of KA-evoked currents, even though to a lesser extent. These compounds indeed have in position 3 a trifluoromethyl group that has a size similar to the size of the methyl group present in the parent compound. The lower modulatory activity could be ascribed to the electron-withdrawing effect of fluorine atoms.

Table 2. Variation of KA-Evoked Currents Induced by Benzothiadiazine Derivatives **2**−**7** and **9**−**12**[a]

compd	variation of KA current, %	compd	variation of KA current, %
1	111 ± 21	**7**	−34 ± 9
2	115 ± 24	**9**	−60 ± 8
3	−22 ± 10	**10**	−51 ± 3
4	−26 ± 13	**11**	−1 ± 3
5	70 ± 9	**12**	−1 ± 3
6	72 ± 19		

[a] KA and **1** were tested at 100 μM, compounds **2**−**12** were tested at 1 mM. Each value is the mean ± SEM of at least six cells.

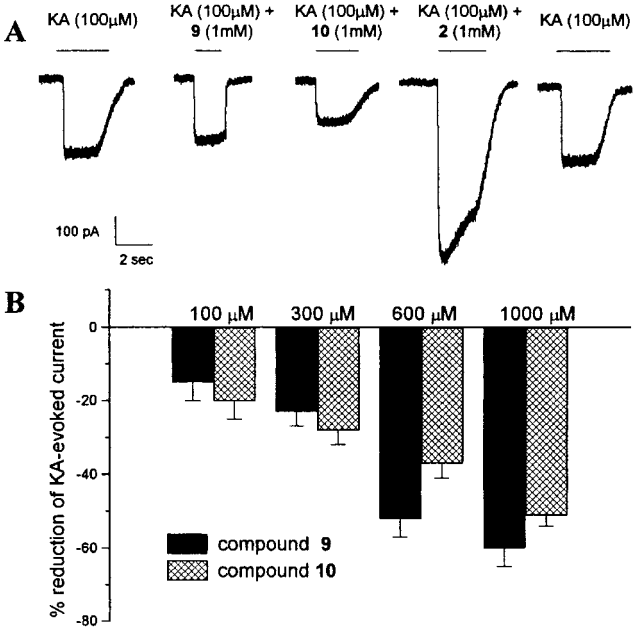

Figure 1. (A) Representative recording of inward currents evoked by the application of KA and modulation by compounds **2**, **9**, and **10**. The cells were voltage-clamped at −60 mV. Bars at the top of the current traces show the duration of the drug application. (B) Dose-dependent effect of compounds **9** and **10** on KA (100 μM)-evoked currents. Each bar is the mean ± SEM of the percent reduction of KA-evoked currents measured on at least five cells.

Substitution in position 3 of the methyl group of **2** with a bulkier substituent (compounds **3**, **4**, **7**, **9**, and **10**) changes the pharmacological activity of the molecule, which becomes a negative modulator of KA-activated currents (see Table 2 and Figure 1A). The current evoked by application of 100 μM KA was reduced in a dose-dependent fashion by increasing the concentrations (see the histograms of Figure 1B for the compounds **9** and **10**). A further increase in the steric hindrance of substituents as in **11** and **12** abolished the pharmacological activity.

The presence of the chlorine atom in position 7 does not seem to be an essential requisite for the studied activity.

All the compounds were tested for their capability of eliciting a response by itself. None of them were active when applied alone.

We can surmise that these changes in the pharmacological activity of compounds **3**, **4**, **7**, **9**, and **10** could be attributed to the addition of a substituent able to interact with another recognition site within the receptor structure. This interaction could lead to some

conformational changes of the receptor protein that ultimately resolve in a negative modulatory activity of the compounds. When a dimethylamino group (compounds **11** and **12**) substitutes a phenolic hydroxyl group (compounds **9** and **10**), we could not measure any type of modulatory activity either positive or negative. The increase in the steric hindrance, the different spatial arrangement, and the ability of this functional group to act mainly as hydrogen bond acceptor are likely responsible for the different activity of compounds **11** and **12**. We are currently investigating this hypothesis.

These compounds represent novel tools for a better understanding of the structural requirements for positive and negative modulation of the AMPA receptor. Furthermore, they could be prototypes for a novel series of compounds that may be useful in the treatment of learning and cognition pathology.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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