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A novel class of allosteric modulators of AMPA/Kainate receptors

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ABSTRACT

The rapid hydrolysis in vivo of IDRA21 to 2-amino-5-chlorobenzensulfonamide has been demonstrated by microdialysis experiments. The IDRA21 metabolite possess in vitro a biological activity similar to that of IDRA21 itself. Taking 2-amino-5-chlorobenzensulfonamide as lead compound, a novel class of AMPAR positive allosteric modulators has been prepared.

L-Glutamate is the principal excitatory neurotransmitter in the mammalian central nervous system (CNS), which can activate ionotropic and metabotropic specific receptors.[1–3] There is evidence from animal studies that an excessive excitation mediated by glutamate receptors may lead to neuronal damages produced by the excessive entry of Ca^{2+} inside the cell. However, there is also data suggesting that a reduced function of such receptors seems to be involved in the learning and memory deficits observed in Alzheimer's disease.[4–6]

It has been demonstrated that a clinically used cognition-enhancing (nootropic) drug, aniracetam, selectively inhibits the spontaneous rapid desensitization of AMPA receptors. The potential therapeutic benefit of compounds able to activate AMPA receptors has lead to the search for new AMPA receptor modulators.[7]

Pyrrolidinone derivatives, such as aniracetam, benzoylpiperidine derivatives, such as 1-BCP [1-(1,3-benzodioxol-5-yl carbonyl)-piperidine], and benzothiadiazine derivatives such as diazoxide, cyclothiadiazide and (±)IDRA21 [7-chloro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide] (±)(**1**) (Fig. 1), attenuate the spontaneous rapid desensitization rate of AMPA receptors.[8–19] Among the benzothiadiazine derivatives, (±)IDRA21 has attracted particular interest due to its ability to act as cognitive enhancing agent in normal young and aged rhesus monkeys when given orally in low (0.5–10 mg/kg) doses.[20]

The observed cognition-enhancing effect of (±)IDRA21, which is one of the most effective compound studied, suggests that the compound may be useful in the treatment of learning, memory and attention disorders resulting from aging, central nervous system trauma, stroke and neurodegenerative disorders like Alzheimer's disease.[18]

Despite the high potency in vivo, (±)IDRA21 potency in modulating AMPA receptor function in vitro is low with an EC_{50} close to 100 μM. At least two hypotheses could be formulated to explain the differences in (±)IDRA21 potency between in vitro and in vivo experiments.[15,19]

It is possible that (±)IDRA21 elicits its action on AMPA receptor desensitization by preferential metabolism of one of the enantiomeric forms, since it has been demonstrated that only one enantiomer is the active one.[19] However, recently work has demonstrated that a rapid interconversion of enantiomers of (±)IDRA21 occurred in saline solution.[21–23]

The other hypothesis is that (±)IDRA21 could elicit its modification of drug-induced cognitive deficit in vivo via a metabolite more active than (±)IDRA21 itself.[13] Although a great number of studies on the pharmacological actions of (±)IDRA21 have been published, no information has been performed on a possible pharmacodynamic mechanism of the compound.[24]

Since (±)IDRA21 has been administered orally in the in vivo pharmacological tests, studies on the stability of the drug in acidic condition similar to that of stomach are clearly matter of interest. In a recent work the rapid hydrolysis of (±)IDRA21 (**1**) (Scheme 1)

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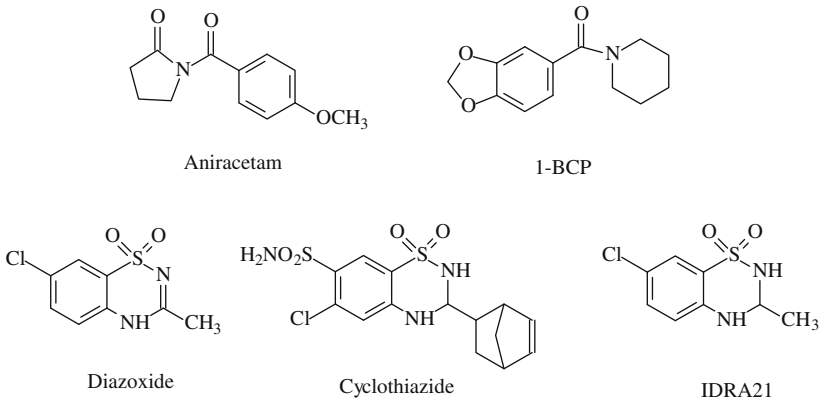
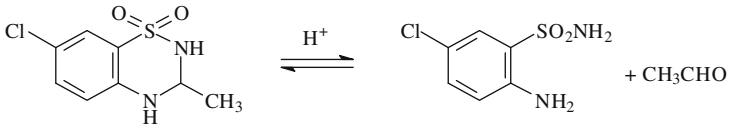


Figure 1. Allosteric AMPA positive modulators.



Scheme 1.

to acetaldehyde and 2-amino-5-chlorobenzenesulfonamide in simulated gastric fluid has been demonstrated.[23] In the present work, microdialysis studies have been performed in the rat in order to determine whether oral administration of IDRA21 (**1**) produced **2** in hippocampus.

Figure 2 shows the extracellular levels of (−)IDRA21 ((−)**1**), (+)IDRA21 ((+)**1**) and **2** in rat hippocampus after oral administration of racemic IDRA21 (100 mg/kg). The concentrations of both enantiomers increased in the first 60 min after administration to reach the concentration of 2 μg/ml and then gradually decreased to be undetectable in the following 3 h. On the contrary levels of **2** increased in the first 4 h to reach the concentration of 12 μg/ml, 3 times the level that of single enantiomers of **1** and decreased slowly for the next 2 h to a concentration of 8 μg/ml.

We tested the activity of (±)IDRA21 and of **2** as allosteric modulators of KA-activated currents in primary cultures of cerebellar granule neurons. Application of KA (100 μM) evoked a nondesensitizing current that was mediated by both AMPA and KA receptors activation. IDRA21 (200 μM), used as reference compound, and **2** potentiated the amplitude of the KA current by 80 ± 17% (n = 5) and 23 ± 2% (n = 5), respectively (Fig. 3).

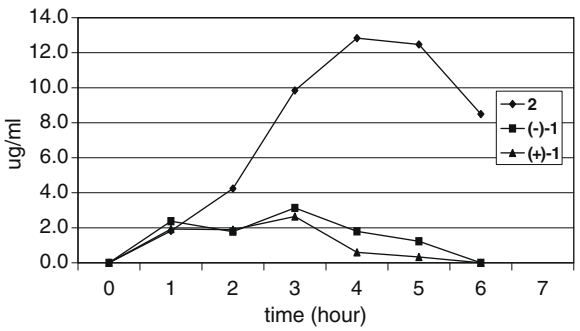


Figure 2. Time course of **2**, (−)**1** and (+)**1** outputs from rat hippocampus after a single administration of (±)**1** (100 mg/kg po). Values were expressed as μg/ml of mean value. SEM were always lower then 14% and omitted from the graph.

These results suggest that IDRA 21 could elicit its pharmacological activity in vivo via its hydrolysis product **2** or it could undergoes in vivo an "exchange" mechanism as part of its action and activity, as in vivo there exist a number of carbonyl moieties which may be used for transport or activity.

Using **2** as lead compound a series of 2-aminobenzensulfonamides (**3–16**) (Table 1) with different substituent at C^4, C^5, N^1 and N^2 has been prepared and studied for their activity as allosteric modulators of KA-activated currents in primary cultures of cerebellar granule neurons, highlighting the molecule's determinants that confer the modulatory activity at AMPA receptors.

Compounds **3–5** were obtained from 5-chloro-2-nitrobenzenesulfonyl chloride by reaction with the appropriate amine and subsequently reduction as outlined in Scheme 2.

The synthesis of compounds **6–9** were conducted starting from 2,5-dichlorosulfonyl chloride with alkylamine and by heating the product in a sealed vessel with the appropriate amine (Scheme 3).

Compounds **10–16** were obtained by methods previously described.[25–27]

As summarized in Table 2 and Figure 3, among the 2-aminobenzensulfonamide derivatives, tested at 200 μM, **6–9** and **12** acted as positive modulators of KA-evoked currents.

The most active derivatives were **6**, **7** and **8** that potentiate of KA currents by 69 ± 14% (n = 8), 91 ± 13% (n = 8) and 80 ± 13% (n = 8), respectively.[28]

The analysis of these preliminary biological results suggests a net of different molecular effects of these ligands, which affects observed potentiation of KA-current activity. First of all, significantly increased activity of N-alkylated derivatives **6–8**, which are 3–4 times more potent comparing to compound **2**, most likely is a result of inductive effect on the sulfonamide moiety produced by the secondary amine in *ortho* position. High activity of compound **1**, which also contains secondary amino group additionally supports this hypothesis. The trend of activities for compounds **6–8** shows that the N-alkylating moiety longer than methyl additionally increase KA-current potentiation activity, however, the compound **9** with isopropyl substituent shows lower activity comparable to that of compound **2**. This suggests the second effect,

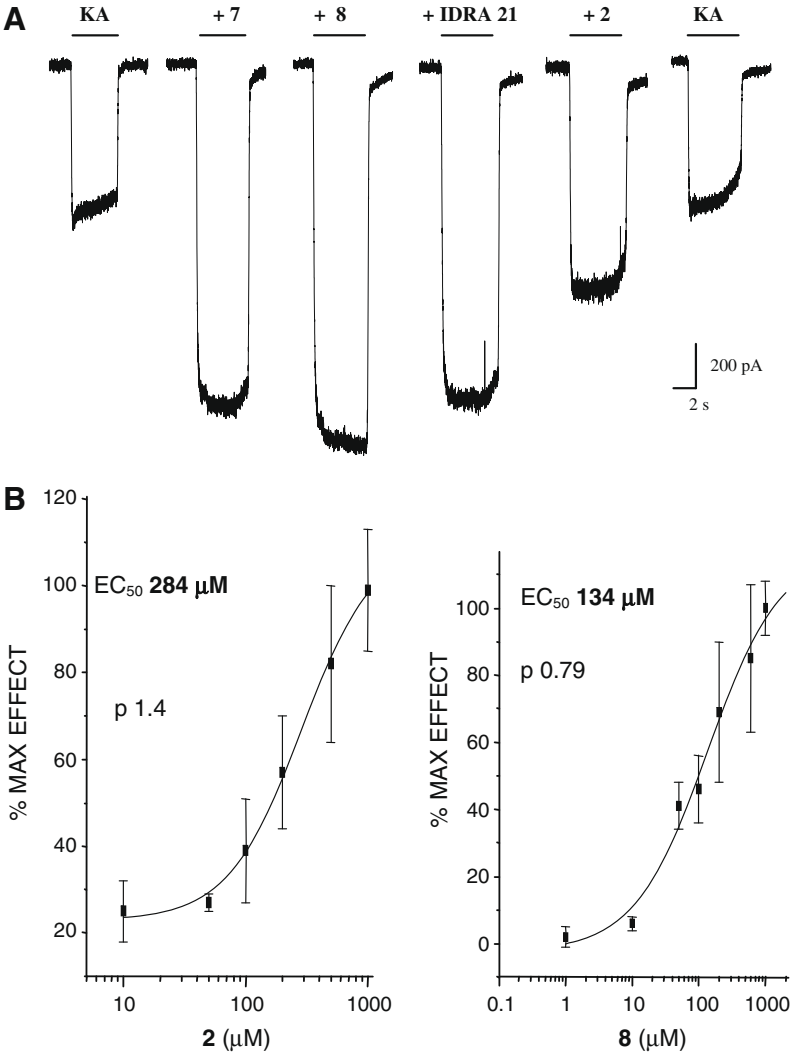
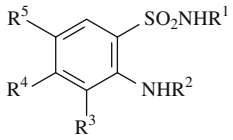


Figure 3. (A) KA-evoked currents from a cerebellar granule neuron in presence of 2-aminobenzensulfonamide derivatives and IDRA21 (all drugs are at 200 μM). Bar indicates the duration of application of KA 100 μM; holding potential −60 mV. (B) Dose–response curves of **2** and **8** expressed as percentage of the maximal effect. The curve was fitted with a sigmoidal function using Origin 4.1. Each data point is the mean ±SE of at least five cells.

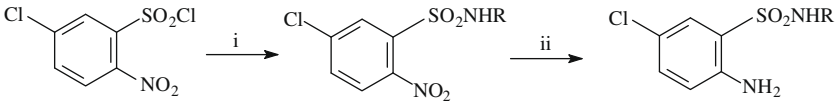
Table 1
2-Aminobenzenesulfonamides studied



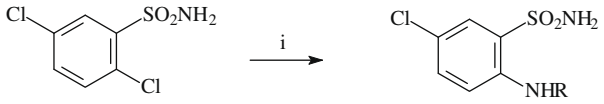
Compound	R^1	R^2	R^3	R^4	R^5
2	–H	–H	–H	–H	–Cl
3	$–CH_3$	–H	–H	-H	–Cl
4	$–CH_2CH_3$	–H	–H	–H	–Cl
5	$–CH_2CH_2CH_3$	–H	–H	–H	–Cl
6	–H	$–CH_3$	–H	–H	–Cl
7	–H	$–CH_2CH_3$	–H	–H	–Cl
8	–H	$–CH_2CH_2CH_3$	–H	–H	–Cl
9	–H	$–CHCH_3CH_3$	–H	–H	–Cl
10	–H	–H	–H	–H	–H
11	–H	–H	–H	–Cl	–H
12	–H	–H	–H	–Cl	–Cl
13	–H	–H	–H	$–OCH_3$	–H
14	–H	–H	–H	–H	$–NO_2$
15	–H	–H	–H	–Cl	$–SO_2NH_2$
16	–H	–H	–CH3	–H	–H

the negative steric influence of the N-alkylation by the branched substituent. It may either prevent the rotation of moieties of the ligand molecule and/or the receptor's active site has some steric restraints that are felt by the larger volume of the isopropyl group and not by the *n*-alkyl groups.

Molecular modeling studies of ligands confirm this interpretation. Compounds **1**, **6–9** has higher dipole moments calculated from the atom's partial charges (6.75 D, 5.43 D, 5.59 D, 5.63 D, 5.66 D, respectively) than derivatives **2–5** (5.03 D, 4.89 D, 4.98 D, 4.88 D, respectively). Similarly, a significant difference was observed comparing point charge distributed on nitrogen atom of the *ortho* amino group among these molecules. The partial charge of nitrogen atom in derivatives **1, 6–9** ranged from −0.33 to −0.32 whereas in derivatives **2–5** was calculated as −0.36. Results of this simulation indicate inductive effect of *N-alkyl* moiety on the sulfonamide group which promotes activity. In case of compound **9**, this positive effect is balanced by the negative steric effect of branched substituent. The presence of the chlorine atom in position 5 seems to be another essential requisite for the action of 2-aminobenzenesulfonamide type compounds as AMPA allosteric positive modulators. The absence of chlorine atom (compound



Scheme 2. Reagents and conditions: (i) methylamine, ethylamine or propylamine for **3**, **4**, and **5**, respectively; (ii) Fe, HCl.



Scheme 3. Reagents and conditions: (i) methylamine, ethylamine, propylamine or isopropylamine for **6**, **7**, **8**, and **9**, respectively, Δ.

Table 2
Variation of KA-evoked currents induced by IDRA21 (**1**) and by 2-aminosulfonamide derivatives **2**–**16**

Compound	Variation of KA current, %	Compound	Variation of KA current, %
1	80 ± 17	**9**	32 ± 5
2	23 ± 2	**10**	−5 ± 5
3	5 ± 11	**11**	8 ± 11
4	12 ± 11	**12**	35 ± 11
5	4 ± 5	**13**	−5 ± 2
6	69 ± 14	**14**	−8 ± 5
7	91 ± 13	**15**	−6 ± 3
8	80 ± 13	**16**	7 ± 3

10) or its substitution at C^5 with a nitro or sulfamoyl group (compounds **14** and **15**) resulted in a loss of activity. A chlorine or methoxy group in position 4 (compounds **11** and **13**) leads to compounds without positive modulator activity on AMPA receptor. Chlorine atom at the *meta* position should not produce significant inductive effect on the sulfamoyl group (compound **11** is less active than **2** and compound **12** is as active as **2**). On the other hand, the chlorine groups can form hydrogen bond, have some hydrophobic characteristics and are bulky, thus, one should assume that this substituent at C^5 helps to position the compounds within the active site of the molecule. The lack of activity of compounds **13**–**16** supports this assumption.

The results of this study suggest that in vivo IDRA21 (**1**) is rapidly hydrolyzed to 2-amino-5-chlobenzensulfonamide (**2**), which display in vitro biological activity similar to that of IDRA21. Taking 2-amino-5-chlobenzensulfonamide (**2**) as the lead compound, a novel class of AMPA positive allosteric modulators has been prepared, in which compounds **7** and **8** had the highest biological activity. The analysis of the relationship between the structures of the synthesized compounds and their biological activity has indicated structural features that may be manipulated to increase biological activity. This possibility is currently being investigated and will be expanded and confirmed. The objective will be the development of new lead drug candidates for the treatment of cognitive deficits.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.12.094.

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