5-Arylbenzothiadiazine Type Compounds as Positive Allosteric Modulators of AMPA/Kainate Receptors

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ⓢ *Supporting Information*

ABSTRACT: The potential therapeutic benefit of compounds able to activate AMPA receptors (AMPAr) has led to the search for new AMPAr positive modulators. On the basis of crystallographic data of the benzothiadiazines binding mode in the S1S2 GluA2 dimer interface, a set of 5-aryl-2,3-dihydrobenzothiadiazine type compounds has been synthesized and tested. Electrophysiological results suggested that 5-heteroaryl substituents on the benzothiadiazine core like 3-furanyl and 3-thiophenyl dramatically enhance the activity as positive modulators of AMPAr with respect to IDRA21 and cyclothiazide. Mouse brain microdialysis studies have suggested that 7-chloro-5-(3-furyl)-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide crosses the blood−brain barrier after intraperitoneal injection. Biological results have been rationalized by a computational docking simulation that it has currently employed to design new AMPAr-positive modulator candidates.

KEYWORDS: AMPA modulators, IDRA21, benzothiadiazines, cognitive enhancers
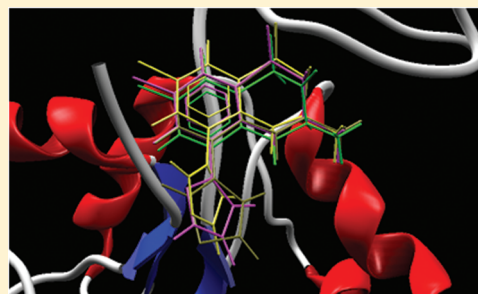
T he principal excitatory neurotransmitter in the mammalian central nervous system (CNS) is L-glutamate, and its signal transduction is mediated by ionotropic and metabotropic receptors.[1] Different studies suggest that ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAr) is involved in learning processes and in memory establishment.[2] The therapeutic potential of compounds able to activate AMPAr has led to the search for new AMPAr-positive modulators.[3,4] Among these, one of the most investigated chemical classes of compounds are benzothiadiazine derivatives such as cyclothiadiazide (CTZ) (1), (±)7-chloro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (IDRA21) (2), and (±) 8-chloro-2,3,5,6-tetrahydro-3,6-dimethyl-pyrrolo[1,2,3-de]-1,2,4-benzothiadiazine 1,1-dioxide (3), which are able to inhibit desensitization of AMPAr-potentiating ionotropic glutamatergic neurotransmission (Figure 1).[5−7]

IDRA21, the first benzothiadiazine effective in increasing learning and memory performance in behavioral tests, represents an important lead compound since it is able to cross the blood−brain barrier (BBB).[8,9] Recent crystallographic studies regarding the binding mode of benzothiadiazine derivatives on S1S2 GluA2 subunits of AMPAr suggest that these compounds bind to the dimer interface, leading to
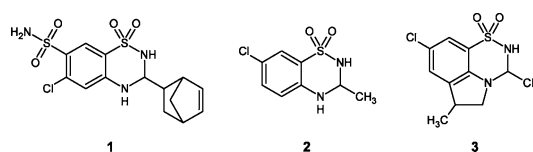

Figure 1. Cyclothiazide (**1**), IDRA21(**2**), and 8-chloro-2,3,5,6-tetrahydro-3,6-dimethyl-pyrrolo[1,2,3-*de*]-1,2,4-benzothiadiazine 1,1-dioxide (**3**).

inhibition of the receptor desensitization by stabilization of the ligand-binding domain within a dimer interface.[10,11] Notwithstanding structure similarities of IDRA21 and cyclothiazide, crystal structures of their complexes with S1S2 GluA2 domain reveal different binding modes.[10,11] In particular, IDRA21 and cyclothiazide bind to the dimer interface S1S2 GluA2 with a different orientation probably due to lower steric hindrance of C3 methyl group of IDRA21 with respect to the C3 norbornyl substituent of cyclothiazide (Figure 2).[10,11]
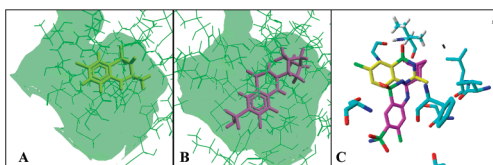
Figure 2. Comparison of binding modes of IDRA21 (A) (PDB code: 3IL1) and cyclothiazide (B) (PDB code: 1LBC) in the ligand binding site of S1S2 GluA2 dimer interface. Overlap view of IDRA21 and cyclothiazide (C).

The crystal structure of cyclothiazide in complex with S1S2 GluA2 subunits (PDB code: 1LBC) reveals that the benzene ring and C7 sulfonamidic group of cyclothiazide fit into a hydrophilic pocket defined by Lys763, Tyr424, Ser497, Phe495 and Ser729 residues. In contrast, the same hydrophilic pocket is unfilled in the crystal structure of IDRA21 in complex with S1S2 GluA2 subunits.

Detailed analysis of the latter crystal structure reveals that the pocket contains four water molecules ordered to form a network of hydrogen bonds with neighboring residues. One of these water molecules is positioned on the plane of IDRA21 aromatic ring at a distance of 3.2 Å from the C5 atom. Therefore, we hypothesized that the affinity of IDRA21 derivatives toward AMPAr could be increased by a substituent at C5 position of IDRA21 core that could accommodate the empty hydrophilic pocket and form additional hydrogen bonds with the receptor and water molecules present in this cavity.

Molecular modeling studies indicate that heteroaromatic substituents, like thiophenyl or furanyl, and aromatic substituents like anilinic or benzoyl on C5 position of IDRA21 can fit into the unfilled hydrophilic pocket, miming the binding mode of benzene ring and C7 sulfonamidic group of cyclothiazide (Figure 3).[12]
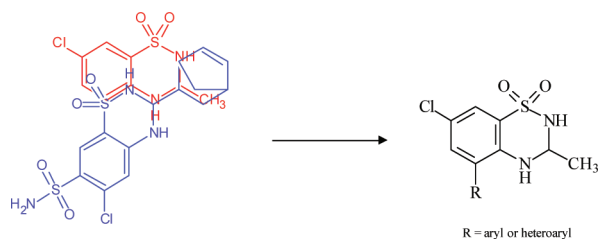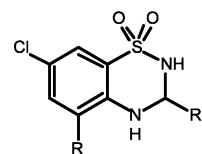

Figure 3. Overlay of IDRA21 (red) and cyclothiazide (blue) led to the design of new IDRA21 analogues.

Hence, several 5-aryl-7-chloro-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide derivatives were prepared by a synthetic route based on the Pd-catalyzed Suzuki–Miyaura coupling reaction (Table 1). The general synthetic pathway employed is shown in Scheme 1.

Halogenation of 2-amino-5-chlorobenzensulfonamide (**4a**) in acidic conditions gave **4b**, which subsequently afforded intermediate **4c** by Pd-catalyzed cross-coupling reaction with a suitable boronic acid. The 5-aryl-7-chloro-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide derivatives were obtained by ring closure with a selected aldehyde under catalytic acidic conditions. Compounds **11** and **12** were obtained by an alternative synthetic pathway: The coupling reaction with 3-aminophenylboronic acid was conducted on 7-chloro-5-iodo-3-alkyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide, since aldehyde reacted with the nitrogen atom of
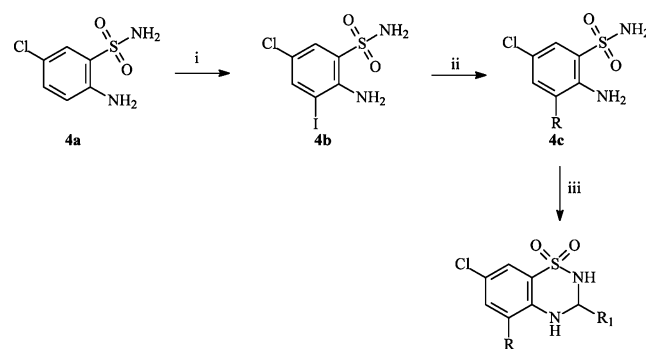
Table 1. Potentiation of Kainate (100 μM)-Evoked Current Induced by the Synthesized Compound at 10 μM

Compound	R1	R	% Potentation (%)[a]
3	-	-	+53±11
5	-CH₃	furan	+80±16
6	-CH₂CH₃	furan	<10
7	-H	furan	+20±5
8	-CH₃	thiophene	<10
9	-CH₃	furan	+358±80
10	-CH₃	thiophene	+214±41
11	-CH₃	aniline	<10
12	-H	aniline	<10
13	-CH₃	benzoic acid	<10
14	-CH₂CH₃	benzoic acid	<10
15	-H	benzoic acid	<10

[a]Each value is the mean ± SEM of 5–8 experiments.

Scheme 1. [a]

[a](i) ICl, acetic acid, DMF. (ii) Na₂CO₃, RB(OH)₂ tetrakis palladium, H₂O/dioxane. (iii) R₁CHO, 2-propanol, HCl.

the anilinic group of the aromatic substituents at C5 during benzothiadiazine ring closure (Scheme 2).

The prepared compounds described in Table 1, as well as their intermediates, were fully characterized by IR, ESI, and NMR spectroscopic analysis. Subsequently, the activities of synthesized compounds were evaluated by patch-clamp technique in primary cultures of cortical neurons as allosteric modulators of AMPA/kainate receptors.[13,14] The capability of potentiating kainate-evoked current was tested for all of the newly synthesized compounds. Kainate-evoked current was mainly mediated by AMPA receptor activation because

Scheme 2. [a]
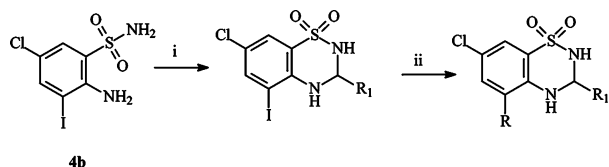
[a]R$_1$CHO, 2-propanol, HCl. (ii) Na$_2$CO$_3$, RB(OH)$_2$ tetrakis palladium, H$_2$O/dioxane.

application of GYKI 53655 (100 μM), an AMPA receptors antagonist, almost completely abolished the current (data not shown). Because **3** is one of the most active benzothiadiazine derivatives reported in literature as a AMPAr-positive allosteric modulator, it was selected as reference compound.[4] Data obtained indicate that **5**, **9**, and **10**, at 10 μM, potentiate by 80 ± 16, 358 ± 80, and 214 ± 41%, respectively, the kainate-evoked currents at 10 μM (Tab.1). The dose−response curve of the most active compound **9** reveals an EC$_{50}$ of 1.3 μM, suggesting that this benzothiadiazine derivative is one of the most potent within this class of AMPAr-positive allosteric modulators (Figure 4).
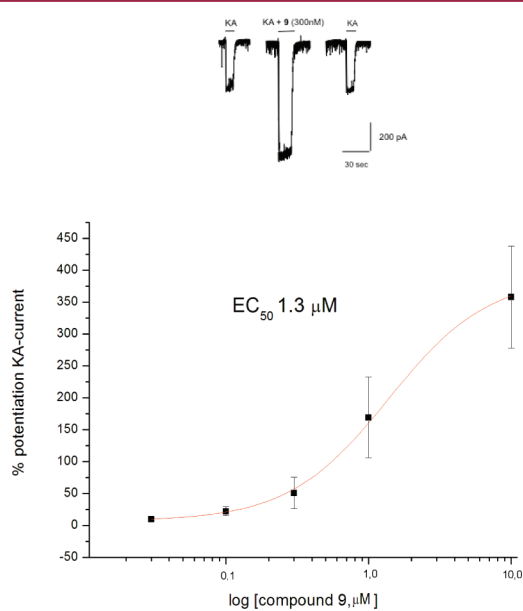

Figure 4. Modulation of KA-evoked current by compound **9**. (a) Representative whole cell recording showing KA (100 μM)-evoked current recorded from a cortical neuron in culture in control conditions (left traces), after application of **9** (300 nM) (middle trace) and after washing (right trace). (b) Dose−response curve of the effect of **9** on KA-evoked current. Each data point is the mean ± SEM of at least five cells.

The analysis of these preliminary biological results suggests a net of different molecular effects of these ligands, which affects the potentiation of KA-current activity. In particular, the activity significantly increases with a five-membered aromatic ring as the C5 substituent, whereas compounds with a six-membered aromatic ring show a very low activity. This effect is probably due to a sterical hindrance that prevent them from fitting into the hydrophilic pocket at S1S2 GluA2 dimer interface. Moreover, the maximum of activity is achieved when the heteroatom is at 3-position of the heteroaryl substituent. The activities of compounds **5**−**7** indicate that the C3 substituent

has a critical influence . The preferred substituent appears to be CH$_3$ (**5**) since H or CH$_2$CH$_3$ led to compounds (**6** and **7**) with lower activity as KA-current potentiators. Because of its activity, **9** represents a promising lead compound for the development of new potent AMPAr-positive allosteric modulators.

Molegro Virtual Docker (MVD) software has been employed to dock **5**, **9**, and **10** into the ligand binding site of S1S2 GluA2 dimer (PDB code: 3IL1) to understand their binding mode.[12] The software MVD was evaluated on cyclothiazide, triflumethiazide, diazoxide, althiazide, hydrochlorothiazide, chlorothiazide, trichlormethiazide, and IDRA21. The average root-mean-square distance (rmsd) of the best ranking pose of tested compounds as compared to their binding pose in the respective crystal structures was found to be 0.81 Å, proving that MVD is able to accurately dock this type of compounds (see the Supporting Information). Compounds **5**, **9**, and **10** compounds were built by the software Spartan'08 and energy minimized using Hartree−Fock ab initio method employing at the SCF level with the 6-31G* basis set.

The docking output clearly indicates that **5**, **9**, and **10** bind similarly to IDRA21 at the dimer interface of S1S2 GluA2 subunits with the C3 methyl substituent placed into the hydrophobic cleft defined by Val750, Leu751, and Leu259 residues, the hydrogen atom on N4 of benzothiadiazine gave an hydrogen bond with Ser754 (H-bond length 3.39 Å) residue, and the sulfone oxygen atom of benzothiadiazine gave a polar interaction with Gly731 residue (Figure 5) since it is located
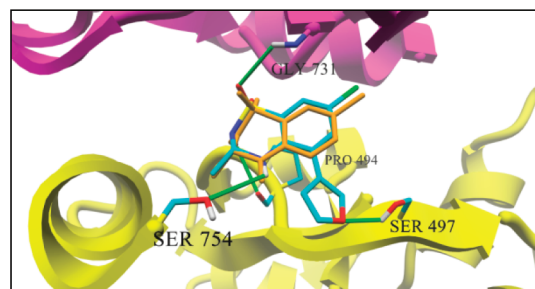

Figure 5. Binding of **9** and IDRA21 to the S1S2 GluA2 dimer interface; the secondary structures of the two S1S2 monomers are colored in yellow and magenta; green sticks illustrate plausible hydrogen bond interactions between **9** and the receptor residues, both colored in element color code; the IDRA21 molecule present in the crystal model is colored in orange.

within 2.78 Å from the backbone of this residue. These interactions are observed also in the crystal structure of IDRA21 in complex with S1S2 GluA2 subunits.

The increased activity of **5**, **9**, and **10** with respect to IDRA21 can be explained by additional interactions with the ligand binding site due to heteroaryl substituent in the C5 position of the benzothiadiazine core that can be accommodated in the unfilled hydrophilic pocket previously recognized in the crystal structure of S1S2 GluA2 domain in complex with IDRA21.

Compounds **9** and **10** have shown higher activity with respect to **5** probably because of a H-bond between the heteroatom of 3-furanyl or 3-thiophenyl substituent with the Ser497 residue of S1S2 GluA2 domain. In particular, the heteroatom of the C5 substituent of **9** and **10** is within hydrogen bond distance (2.71 Å) to the hydroxyl group of Ser497. Moreover, **9** is more active than **10** probably due to the major electronegativity of oxygen atom of 3-furanyl that allows

a stronger H-bond with respect to the sulfur atom of the 3-thiophenyl substituent.

The good activity of **9** as positive allosteric modulator of AMPAr in vitro suggests that it could exert cognition enhancing properties when administered in vivo. With the aim to evaluate if **9** can elicit AMPAr activity on the CNS, its capability to cross the BBB was investigated. Recently, it has been reported that 3,4-dihydro-2*H*-1,2,4-benzothiadiazine derivatives in vivo can hydrolyze in acidic conditions to corresponding 2-amino-benzensulfonamide.[15] Moreover, Pirrotte et al. have recently reported that 4-alkyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine derivatives undergo the action of hepatic cytochrome P450, giving the corresponding 4-alkyl-2*H*-1,2,4-benzothiadiazine derivative.[16] As a consequence, it is possible that **9** in vivo can be hydrolyzed to 5-chloro-3-(3-furyl)-2-aminobenzensulfonamide (**16**) and metabolized to give 7-chloro-5-(3-furyl)-3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**17**).

In the attempt to evaluate if **9** crosses the BBB and if it produces **16** and **17** in vivo, cerebral microdialysis experiments have been performed in mice to measure cerebral levels of **9**, **16**, and **17** after intraperitoneal administration of **9**. A liquid chromatography tandem mass spectrometry (LC/MS/MS) method has been developed to evaluate concentrations of parent ompound **9** and of their possible metabolites **16** and **17** in cerebral mouse dialysates. The identity of the compounds **9**, **16**, and **17** was confirmed by comparing their retention times and their MS/MS profiles after injection of the corresponding synthetic authentic compounds in the HPLC system. Figure 6
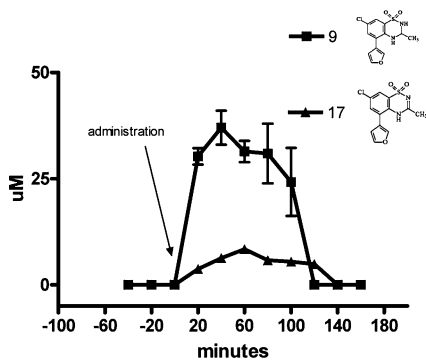

Figure 6. Time course of **9** and **17** outputs from mouse nucleus accumbens after a single administration of **9** (20 mg/kg ip). Values are expressed as μM of mean values $\pm$ SEMs of dialysates concentrations.

shows the extracellular levels of **9** and **17** in mouse nucleus accubens after intraperitoneal administration of **9** (20 mg/kg).

Dialysate concentrations of **16** were under the limit of detection of the analytical method and are not reported in the graph. The concentrations of **9** increased in the first 40 min after administration to reach the concentration of 37 μM and then gradually decreased and became undetectable after 2 h. Levels of **17** increased in the first 1 h to reach the concentration of 8 μM, then decreased slowly for the next 2 h to be undetectable at 140 min. The microdialysis results indicate that **9** reaches the CNS after intraperitoneal injection. Moreover, **9** is subject to a moderate action of cytocrome P450 to give **17**, while it does not undergo hydrolysis after intraperitoneal administration. In summary, a novel class of positive allosteric modulators of AMPAr has been designed based on crystal structures of IDRA21 and cyclothiazide in complex with the S1S2 GluA2 dimer interface. An efficient and versatile synthetic

route has been developed to prepare the designed 5-aryl-3,4-dihydrobenzothiadiazine compounds. Electrophysiological results suggested that 5-heteroaryl substituents like 3-furanyl and 3-thiophenyl dramatically enhance the activity as positive modulators of AMPAr with respect to IDRA21 and cyclo-thiazide. Biological results have been rationalized by a computational docking simulations that are employed to design new AMPAr-positive modulator candidates. Moreover, mouse cerebral microdialysis studies suggested that **9** crosses the BBB after intraperitoneal injection since it has been detected at a micromolar concentration in mouse nucleus accumbens dialysates. Low levels of the corresponding unsatured **17** derivative have been detected in mouse nucleus accumbens dialysates, suggesting a moderate action of cytocrome P450 on **9**. Efforts aimed at a further optimization, as well as in-depth biological studies, of the novel compounds are ongoing, and the results will be reported in due course.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, compound characterizations, computational methods, and pharmacology. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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