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Design, stereoselective synthesis, configurational stability and biological activity of 7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide

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ABSTRACT

Chiral 5-arylbenzothiadiazine derivatives have recently attracted particular attention because they exhibit an interesting pharmacological activity as AMPA receptor (AMPAr) positive modulators. However, investigations on their configurational stability suggest a rapid enantiomerization in physiological conditions. In order to enhance configurational stability, preserving AMPAr activity, we have designed the novel compound (*R,S*)-7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide bearing a pyrrolo moiety coupled with the 5-(furan-3-yl) substituent on benzothiadiazine core. A stereoselective synthesis was projected to obtain single enantiomer of the latter compound. Absolute configuration was assigned by X-ray crystal structure. Patch clamp experiments evaluating the activity of single enantiomers as AMPAr positive allosteric modulator showed that *R* stereoisomer is the active component. Molecular modeling studies were performed to explain biological results. An on-column stopped-flow bidimensional recycling HPLC procedure was applied to obtain on a large scale the active enantiomer with enantiomeric enrichment starting from the racemic mixture of the compound.

1. Introduction

L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). Two distinct families of L-Glutamate receptor, widely distributed in the CNS, have been presented: the ionotropic (ligand-gated ion channel, iGluRs) and metabotropic (G-protein coupled) receptors.[1–4] On the basis of their sensitivity to selective agonists, iGluRs have been further classified into three subclasses: kainic acid (KA), *N*-methyl-D-aspartic acid (NMDA) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors.[5] A dysfunction of glutamatergic neurotransmission has been implicated in a number of neurological and psychiatric diseases.[6] Many studies have been carried out to develop compounds able to enhance glutamatergic function without causing excitotoxicity. In this context, there is a rapid growing interest in positive allosteric modulators of AMPA receptors as potentiators of glutamatergic function.[1–6] Compared to direct AMPA receptor agonists, allosteric modulators are anticipated to possess finer tuning to increase glutamatergic function since they have no effects in the absence of the natural ligand in the synapse. These agents, binding to an allosteric site, enhance receptor function by decreasing desensitization and/or deactivation. Several chemical classes of positive modulators of AMPA receptors have been described in the last years as reported in many review articles.[5,7–13] Among the different classes of positive allosteric modulators of AMPA receptors, benzothiadiazines represent one of the most investigated since they proved to be beneficial in the management of neurological disorder such as schizophrenia, depression, Alzheimer's disease, Parkinson's disease, attention-deficit/hyperactivity disorder (ADHD) and respiratory depression.[14–21] Among benzothiadiazine derivatives, IDRA21, a compound resulting from saturation of the nitrogen—carbon double

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bond of the thiadiazide ring of the antihypertensive diazoxide, was demonstrated as a promising clinical candidate.

IDRA21 has attracted particular clinical interest despite its modest in vitro activity since it is effective in increasing learning and memory performances in behavioral tests, representing an important lead compound able to cross the blood–brain barrier (BBB).[22,23] Recent crystallographic studies regarding the binding mode of benzothiadiazine derivatives on S1S2 GluA2 subunits of AMPAr suggested that these compounds bind to the dimer interface, leading to inhibition of the receptor desensitization by stabilization of the ligand-binding domain within a dimer interface.[24,25] In particular detailed analysis of the crystal structure of IDRA21 in complex with S1S2 GluA2 subunits reveal the presence of an unfilled hydrophilic pocket that could accommodate an aryl or heteroaryl substituent at C5 atom of IDRA21 core.[26] Molecular modeling studies have suggested that heteroaromatic substituents, like thiophenyl or furanyl, and aromatic substituents, on C5 position of IDRA21 can fit into the unfilled hydrophilic pocket increasing the affinity of IDRA21derivatives towards AMPA receptor. Therefore a series of 5-arylbenzothiadiazine derivatives were recently designed and synthesized by our research group.[26] Among them, 7-chloro-5-(3-furanyl)-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**1**, Fig. 1) has attracted particular attention because it exhibited an interesting pharmacological activity as AMPAr positive allosteric modulator.[26] Since we have recently reported a rapid enantiomerization in aqueous solution and a rapid hydrolysis in acid medium of IDRA21 and related compounds, a primary goal of the present work is to develop a compound with high configurational and chemical stability under conditions similar to those that they will meet in vivo.[27–35] One of the most relevant IDRA21 derivatives, 2,3,3a,4-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,2,4]benzothiadiazine 5,5-dioxide (**2**, Fig. 1) reached clinical trials due to its ability to act as cognitive enhancing agent in normal young and aged rodents.[36–38]

Further studies have demonstrated that all activity resided in the *S* enantiomer of **2**; moreover, it showed high chemical and configurational stability.[33] Starting from this interesting chemical and pharmacological profile, we have designed a new compound bearing the pyrrolo moiety of compound **2** coupled with 5-aryl substituent on benzothiadiazine core following the approach of combining in a single molecule two different pharmacophores. 7-Chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (**3**) was designed and synthesized in order to enhance configurational and chemical stability preserving the relevant biological activity (Fig. 1).

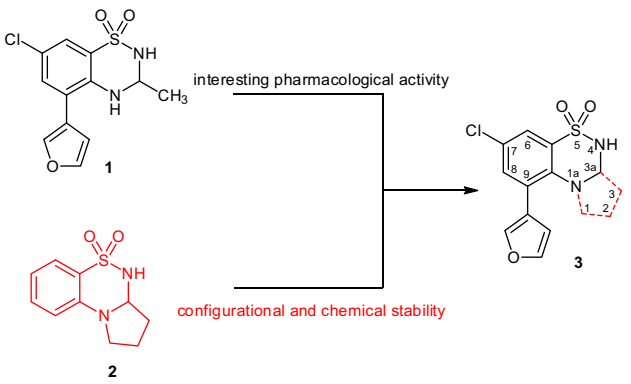


Figure 1. Design of 7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (**3**).

2. Results and discussion

2.1. Design and synthesis of (±)-7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (3)

Recently (*S*)-**2** (S18986) has attracted particular attention since in vivo experiments demonstrated its cognition enhancing properties when it is administered orally at low dose.[36–38] Subsequent stereo and chemical evaluation studies have suggested that (*S*)-**2** (S18986) is stable to enantiomerization and hydrolysis.[33] Thus in order to enhance stability towards enantiomerization and hydrolysis, preserving AMPAr affinity, 7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (**3**) was designed and synthesized combining several chemical features of compounds **1** and **2**. Hence, the designed **3** was prepared following the synthetic route reported in Scheme 1. The intermediate (±)(*R*,*S*)-7-chloro-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (**7**) was prepared as previously described by Cameroni et al. with minor modifications.[39] Subsequently, **7** was halogenated in acidic conditions to give **8**, that was coupled with 3-furanyl boronic acid by Suzuki Pd-catalyzed reaction giving the desired compound **3** (Scheme 1). The latter was fully characterized by IR, GC–MS and NMR spectroscopy analysis.

2.2. Configurational and chemical stability of (±)-7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (3)

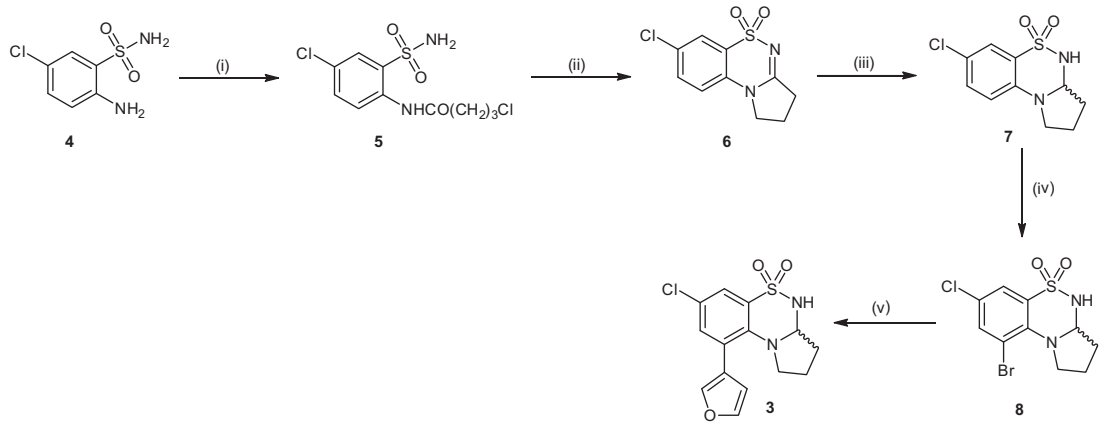
Recent studies conducted by dynamic chromatography on compound (±)-**1** underlined its configurational and chemical lability.[35] Indeed during the chiral separation on a Chiralcel OD-R column at temperatures between 25 and 45 °C employing aqueous mobile phases (water/acetonitrile) at different pH a pronounced plateau was observed between the peaks corresponding to the two enantiomers, indicating a rapid enantiomerization of (±)-**1**.[35] Differently the same experiments applied to (±)-**3** evidenced its stereo stability during chromatographic separation in aqueous mobile phases. No enantiomerization and hydrolysis occurred when (±)-**3** was chromatographed with similar conditions (see Fig. 2).

In order to evaluate stereo and chemical stability of (±)-**3** in absence of chiral stationary phase a recently developed method was employed.[32]

Stopped-flow bidimensional recycling HPLC (sf-BD-rHPLC) method was applied employing immobilized artificial membrane (IAM) stationary phase as reactor column. No enantiomerization and/or hydrolysis product peaks appeared in the sf-BD-rHPLC chromatograms performed in IAM column at both pH 2.2 and 7.4 after 90 min at 37.5 °C. A moderate racemization occurs only at pH 1 at 45 °C for 90 min suggesting a great increase of the configurational stability of (±)-**3** with respect to compound (±)-**1**. Moreover the hydrolysis product of (±)-**3** was not observed suggesting a complete chemical stability of the latter compound. A similar trend was previously observed for compound **2** confirming the stabilizing effect of the pyrrolo moiety.[33]

2.3. Stereoselective synthesis of (*S*)-7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide and (*R*)-7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide

Since previous studies suggested that chiral benzothiadiazines display a stereoselective pharmacological action, it was important to evaluate the activity of single stereoisomers of (±)-**3** as AMPAr modulators.[40,41]



Scheme 1. Synthesis of **3**. Reagents and conditions: (i) γ-chlorobutyryl chloride (2 equiv), *N*,*N*-dimethylacetamide, 0 °C; (ii) NaOH (2%), 100 °C, 15 min; (iii) $LiAlH_4$ (2 equiv), diethylether, −10 °C; (iv) *N*-bromosuccinimide (6 equiv), acetic acid, acetonitrile, room temperature; (v) Na_2CO_3 (3 equiv), 3-furanyl boronic acid (1.2 equiv), tetrakis palladium (0.5 equiv), H_2O/dioxane, 110 °C.

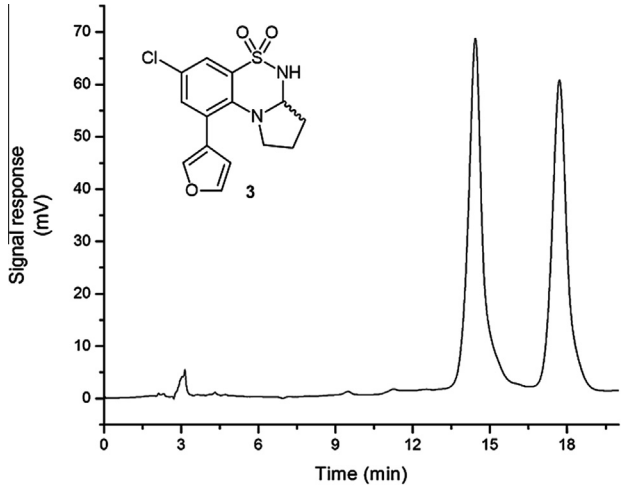


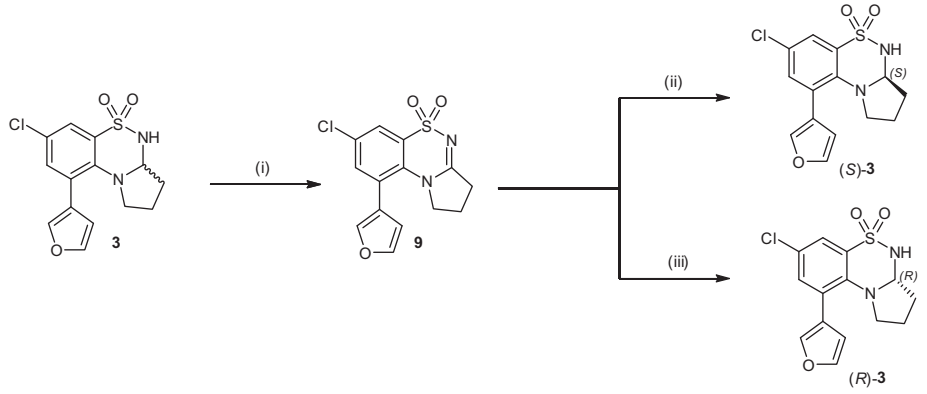
Figure 2. On column racemization of (±)-**3**. Condition: column Chiralcel OD-RH; mobile phase water/acetonitrile (50:50 v/v), *T* = 45 °C.

Moreover, the configurational stability observed for (±)-**3** prompted us to develop an asymmetric synthesis. The synthetic pathway employed is shown in Scheme 2. Once obtained racemic **3**, it was oxidized in presence of potassium permanganate in basic conditions to give **9**, which was subjected to asymmetric reduction.

The stereoselective reduction of **9** to obtain (*S*)-**3** was performed in presence of aminocarbinol-(+)-(2*S*,3*R*)-4-dimethylamino-3-methyl-1,2-diphenyl-2-butanol (Chirald®) as chiral selector and $LiAlH_4$ as reductive agent (Scheme 2).

A similar protocol was employed by Desos et al. to obtain S18986 with high optical purity.[41] The enantiomeric excess achieved in this reaction was about 70% as calculated by chiral HPLC. Since enantiomeric excess obtained by asymmetric synthesis was insufficient to conduct biological experiments, a semipreparative chromatographic method was developed in order to purify the enantiomer of **3**. The enriched enantiomeric mixture was chromatographed on semipreparative Chiralcel OD column with hexane/2-propanol 95:5 (v/v) as mobile phase. The collected fractions containing the single enantiomer show high enantiomeric excess values (ee > 99%). Enantiomeric excess of (+)-**3** was calculated employing an OD-R column with water/acetonitrile 50:50 (v/v) as mobile phase. Subsequently, optical rotation values for the enantiomer obtained was calculated and the specific rotation in chloroform was $[\alpha]_D$ +32.4° (26 mg/mL; chloroform, 24 °C). In order to assign the absolute configuration, single crystal X-ray diffraction analysis were performed on the isolated dextrorotatory enantiomer (Fig. 3). The data analysis indicated that the enantiomer obtained employing Chirald® as chiral auxiliary had *S* configuration. Thus the reaction proceeds with the same stereochemical outcome observed for S18986.[41]

In order to obtain (*R*)-**3** the procedure described by Cohen et al. and Deeter et al., was applied to **9**.[42–44]



Scheme 2. Reagents and conditions: (i) $KMnO_4$, KOH 2%; (ii) Chirald (3 equiv), $LiAlH_4$ (1.5 equiv), diethylether, 0 °C; (iii) (2*R*,3*S*)-(−)-4-dimethylamino-1,2-diphenyl-3-methyl-2-butanol (3 equiv), $LiAlH_4$ (1.5 equiv), toluene 0 °C.

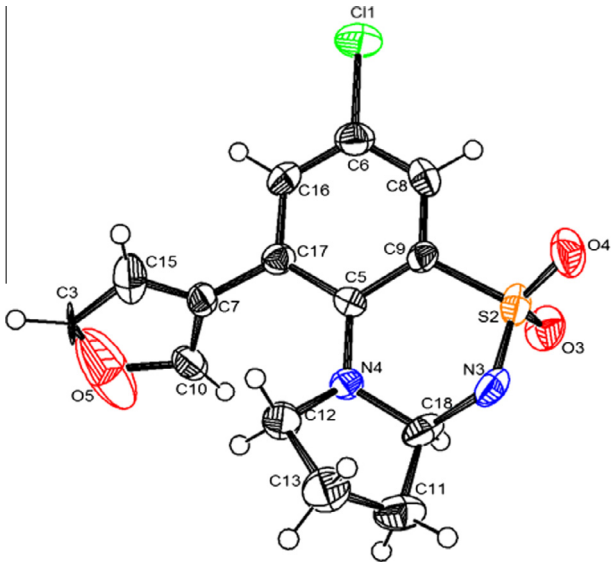


Figure 3. Molecular structure of (+)-(*S*)-**3** at 293 K. Anisotropic displacements are depicted at the 50% probability level.

They demonstrated that by choosing Chirald® or its enantiomer (2*R*,3*S*)-(−)-4-dimethylamino-1,2-diphenyl-3-methyl-2-butanol in complex with $LiAlH_4$ for the reduction of acetylenic ketones it was possible to obtain selectively alcohols with opposite configuration with good enantioselectivity.[43] This strategy applied to compound **9** furnished the desired (*R*)-**3** with good enantiomeric excess (73%); further purification on semipreparative Chiralcel OD column gave enantiopure (*R*)-**3** (ee > 99%) with $[\alpha]_D$ −33.8° (34 mg/mL; chloroform, 24 °C).

2.4. Biological activity

The activity of compound **3** and its enantiomers as allosteric modulators of AMPA/kainate receptors was evaluated by patch-clamp technique in primary cultures of cerebellar neurons. Kainate (KA)-evoked current was mainly mediated by AMPAr activation because application of GYKI 53655 (100 μM), an AMPA receptors antagonist, almost completely abolished the current (data not shown). Since IDRA 21 is one of the most relevant benzothiadiazine derivatives reported in literature as AMPAr-positive allosteric modulator, it was selected as reference compound. Data obtained indicate that compound **3** and IDRA21 potentiate by 30 ± 9% ($n = 4$) and 8 ± 11% ($n = 4$) KA-evoked currents at 10 μM (Fig. 4). In order to highlight possible stereospecific interactions of **3** with AMPA/kainite receptors, (+)-(*R*)-**3** and (−)-(*S*)-**3** were also tested.

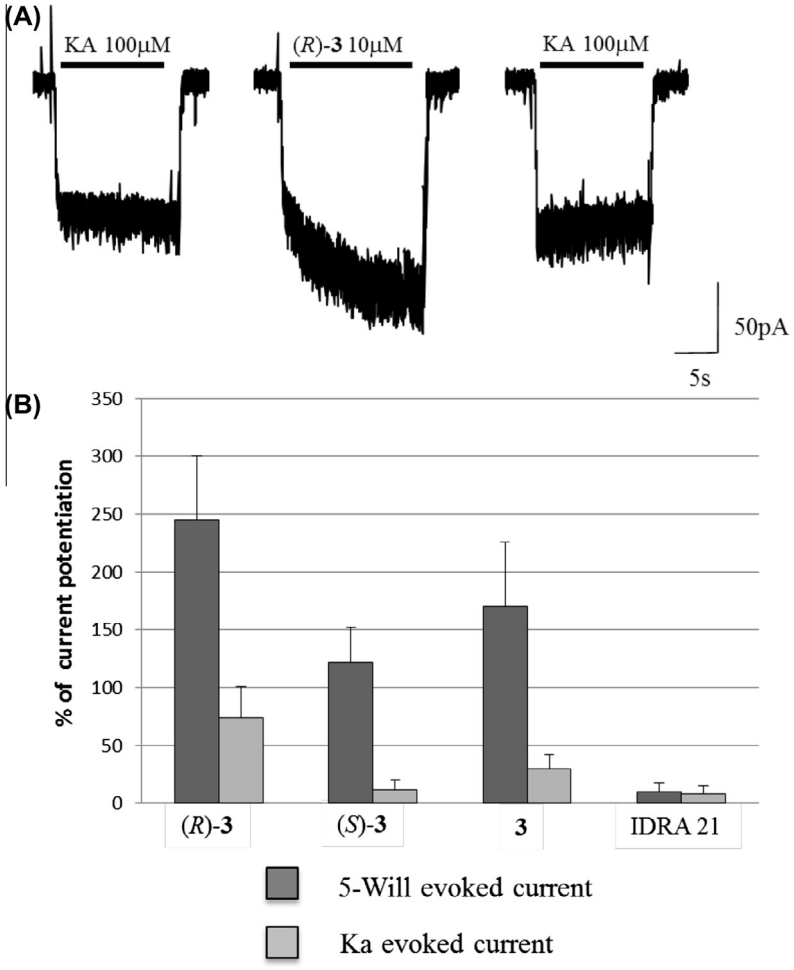


Figure 4. (A) Representative whole cell recording showing KA (100 μM)-evoked current recorded from a cerebellar neuron in culture in control conditions (left traces), after application of (*R*)-**3** (10 μM) (middle trace) and after washing (right trace). (B) Histogram showing the potentiation of (*R*)-**3**, (*S*)-**3**, **3** and IDRA 21 of 5-Will-evoked current and of KA-evoked current. Each data point is the mean ± SEM of at least five cells. Significative differences among (*R*)-**3** and (*S*)-**3** enantiomers were obtained (*T*-test, $p < 0.05$).

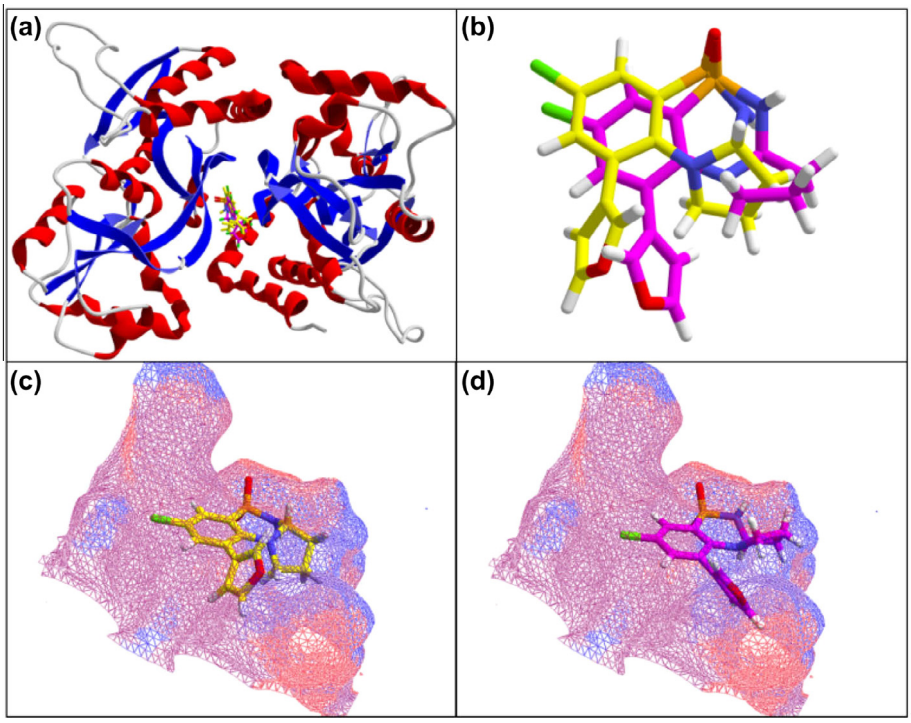


Figure 5. (a) (*S*)-**3** (magenta) and (*R*)-**3** (yellow) in GluA2 dimer interface. (b) Binding mode of (*S*)-**3** (magenta) and (*R*)-**3** (yellow). (c) Position of the pyrrole ring of (*R*)-**3** (yellow) in the binding pocket. (d) Position of the pyrrole ring of (*S*)-**3** (magenta) in the binding pocket. The hydrophobic regions are coloured in blue, the hydrophilic regions in red.

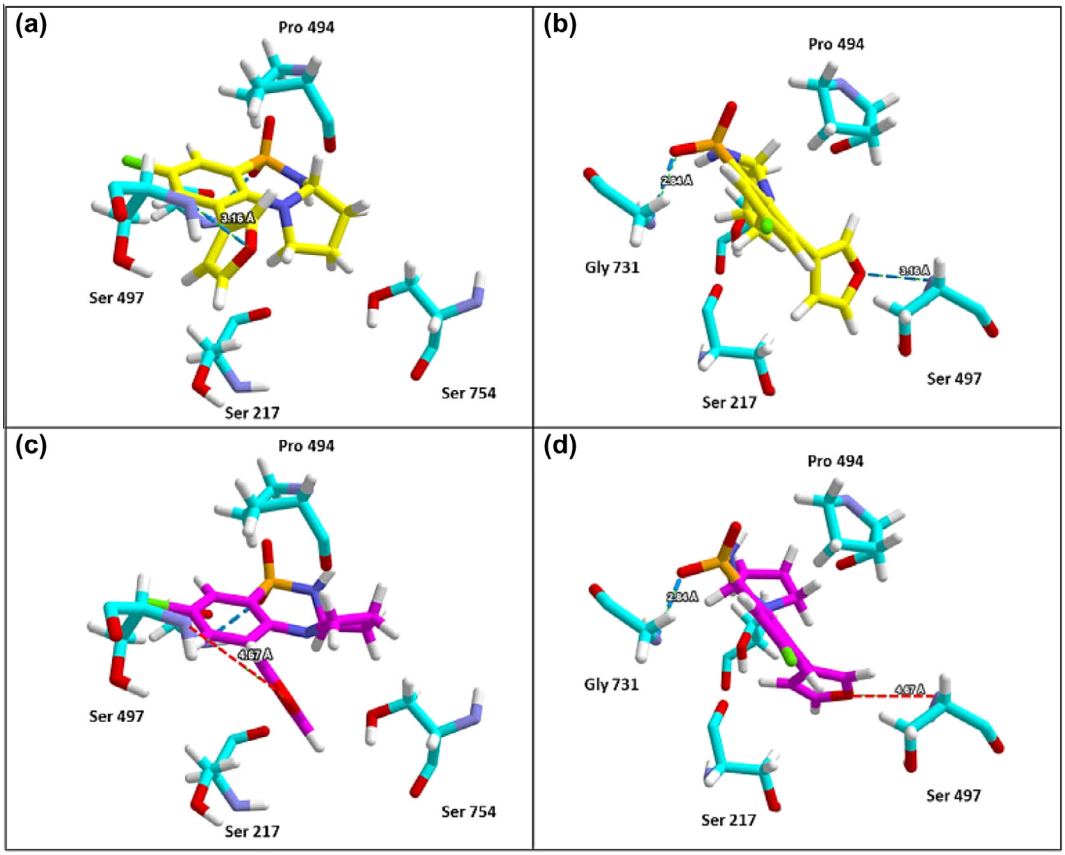


Figure 6. Principal polar interactions of (*R*)-**3** (yellow, panel a and b) and (*S*)-**3** (magenta, panel c and d). Blue dashes indicate H-bond interactions. Red dashes indicate absence of H-bond interactions.

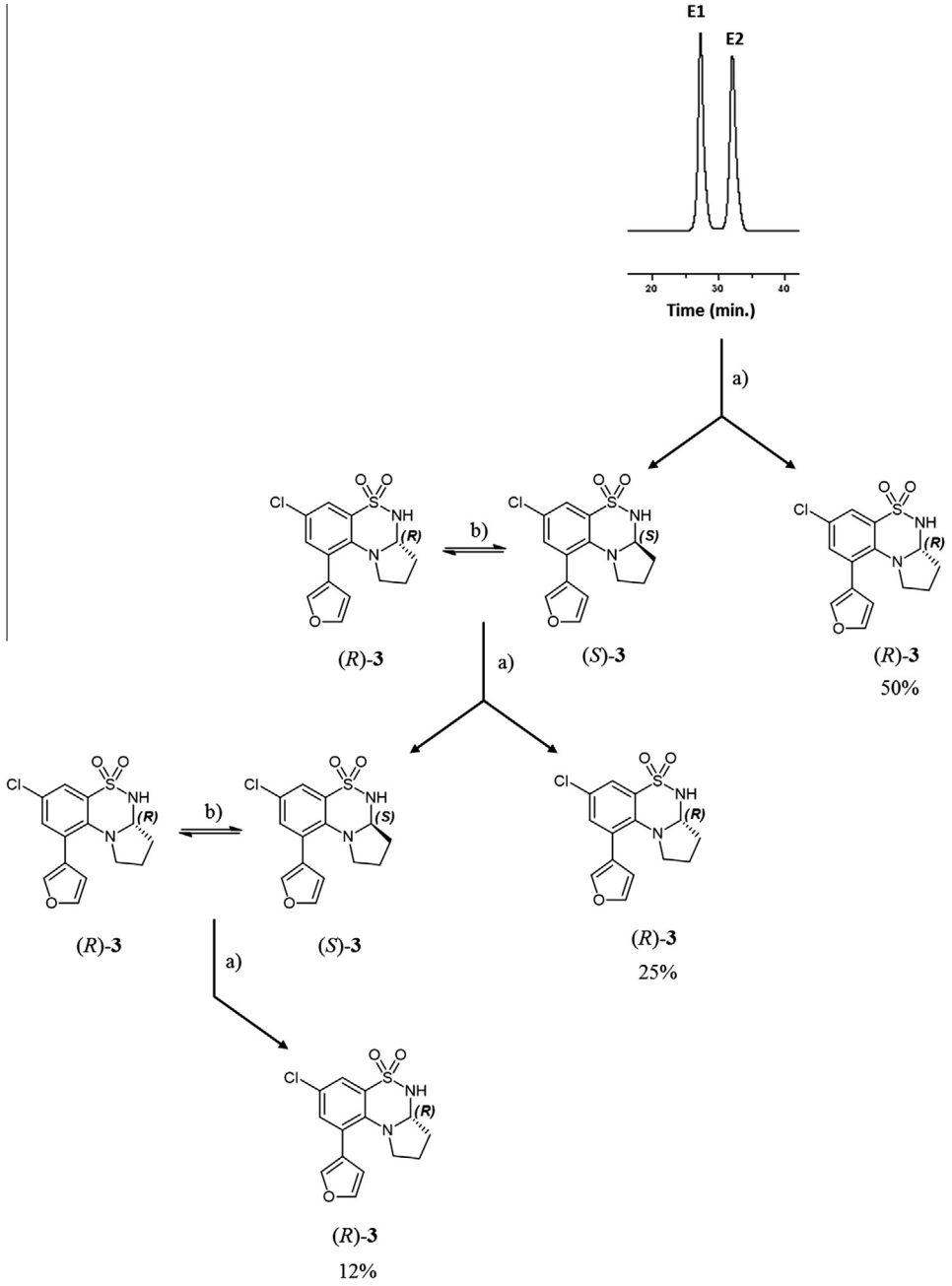


Figure 7. 'Deracemization' of (±)-**3** to obtain (−)-(*R*)-**3**. Conditions: a) Chiralcel OD-R, hexane:2-propanol 95:5 (v/v), 0 °C; b) IAM, IPA (1%TFA), 45 °C.

Data obtained indicate a marked difference in activity: (+)-(*R*)-**3** enhances the current by 74 ± 27% while (−)-(*S*)-**3** by 12 ± 7%. These results indicate that (+)-(*R*)-**3** is more active than (−)-(*S*)-**3** while Desos et al. found, for a structurally similar compound (S18986), that the active component was the *S* enantiomer.[41] Probably these two allosteric modulators have different orientations in the ligand binding domain that promote different interactions with the receptor. Subsequently compound **3** and its enantiomers were tested as modulators of the current evoked by (−)-(*S*)-5-Fluorowillardiine (5-Will), a potent and selective AMPAr agonist in order to evaluate and confirm their AMPAr selectivity.[45]

The racemate potentiate 5-Will evoked current of 170 ± 56% (mean ± SE) ($n = 7$) while (−)-(*R*)-**3** and (+)-(*S*)-**3** enhance the current of 245 ± 55% ($n = 5$) and 122 ± 30% ($n = 7$), respectively. IDRA 21 potentiation of the current was 10 ± 21% ($n = 4$) at 10 μM. The stereoselective activity previously observed on KA-evoked currents was confirmed since (−)-(*R*)-**3** was more active than (+)-(*S*)-**3**. The extent of potentiation of the KA-evoked currents for **3** and its enantiomers was lower than that obtained on 5-Will-evoked current, suggesting a selectivity towards GluA1 and GluA2 AMPAr subunits.

2.5. Docking studies

Several and various binding modes have been observed for benzothiadiazine type compounds active as AMPA positive allosteric modulators.[25,24] Thus in order to understand the possible stereospecific interactions of (−)-(*R*)-**3** and (+)-(*S*)-**3** docking studies were performed. Taking advantage of crystal structures of the AMPA GluA2 ligand binding domain co-crystallized with several benzothiadiazines, Molegro Virtual Docker (MVD) software was applied to dock (−)-(*R*)-**3** and (+)-(*S*)-**3** within the binding pocket

of the GluA2 dimer interface. The software MVD was previously evaluated on several crystal structure of benzothiadiazine.[26,34] The average root mean square distance (RMSD) of the best ranked pose of tested compounds compared to their binding pose in their respective crystal structures was found to be less than 1.0 Å proving that MVD is able to accurately dock this class of compounds. The crystal structure obtained for (+)-(*S*)-**3** was employed as input file to build the ligands with Spartan Wavefunction 08. Among the tested crystal structures, the GluA2 dimer in complex with Cyclothiazide and IDRA21 (PDB codes: 1LBC and 3IL1) were selected for docking studies of both enantiomers **3**.[25] The docking protocol template was built using the following chemical properties of Cyclothiazide and IDRA21: ring atoms, hydrogen-bond acceptors, hydrogen-bond donors and steric interactions. Minimization was performed after docking in order to increase the precision of the analysis.

The docking output clearly demonstrated that (−)-(*R*)-**3** and (+)-(*S*)-**3** adopt a binding mode close to that of IDRA21 (Fig. 5). This result is particularly significant since it was obtained for both the crystal structures employed.

The primary polar interaction partners for both the enantiomers of **3** are Pro494 and Gly731 (Fig. 6). The carbonyl oxygen atom of Pro494 forms a polar interaction with the N4 atom of (−)-(*R*)-**3** and (+)-(*S*)-**3**, whereas the amidic backbone of Gly731 makes an hydrogen bond with the oxygen of the sulphone moiety since it's located for both the stereoisomer within 3 Å.

However, in contrast to what observed in IDRA21 crystal structure, the N1a hydrogen atom in compound **3** has been substituted with a pyrrole group; therefore, the hydrogen bond to Ser754 is not possible. The lack of this interaction could explain the moderate decrease of activity showed for both the enantiomer of **3** with respect to **1**. The main difference observed for (−)-(*R*)-**3** and (+)-(*S*)-**3** is the position of the pyrrole. The *S* configuration of the C3a atom imposes a spatial orientation on the five membered ring such that favored steric interactions with hydrophobic cleft defined by Val750, Leu751, and Leu259 residues (Fig. 5). As described by Ptak et al. the substituent at C3 and thus the hydrophobic interactions played an important role in the orientation and in the position of the benzothiadiazine rings. In compound (+)-(*S*)-**3** the pyrrole moiety is buried deep inside in the lipophilic cavity previously described preventing the formation of the hydrogen bond between the heteroatom of 3-furanyl fragment and the amidic backbone of Ser497 (Figs. 5 and 6). This interaction, as recently reported, is crucial in conferring high activity as AMPA positive allosteric modulator.[26,44] On the other hand, (−)-(*R*)-**3** poorly interact with the hydrophobic region of the ligand binding domain since the pyrrole is located diametrically opposite to Val750, Leu751, and Leu259 residues (Fig. 5). Therefore the furan moiety could be accommodated within the hydrophilic pocket lined by Lys763, Tyr424, Ser729, Phe495 and Ser497 residues interacting via hydrogen bond with the latter amino acid.

2.6. Synthesis of (−)-(*R*)-3 and (+)-(*S*)-3 by on-line racemization

Notwithstanding the asymmetric synthesis previously described proved to be successful, the modest enantiomeric excess obtained with the reaction, the high costs of the chiral auxiliaries employed and the moderate yields obtained led us to develop a new strategy to gain access to enantiopure (−)-(*R*)-**3**.

Our goal was to achieve (−)-(*R*)-**3** on a preparative scale in order to conduct full and detailed pharmacological in vivo experiments.

The good enantioselectivity achieved during the chromatography of (±)-**3** on a semipreparative Chiralcel OD column and the moderate racemization observed at pH 1 suggested the possible application of a recently developed enantiomeric enrichment process.[46]

The stopped-flow bidimensional recycling HPLC method consists in a 3-step experimental protocol. In step 1, the racemate was injected and quantitatively separated on chiral column. Subsequently, one of the two enantiomers was trapped into a reactor achiral column, which was filled with appropriate racemization solvent. Afterwards, the mobile phase flow was reinforced into the achiral column and the two enantiomers were separated in the chiral column. The cycle can be repeated by trapping one of the two eluted enantiomers in the achiral column (Fig. 7).

(±)-**3** was injected on a semipreparative Chiralcel OD with hexane/2-propanol 95:5 (v/v) as mobile phase. The first eluted stereoisomer (−)-(*R*)-**3** of the racemic mixture injected was initially collected. The second eluted enantiomer, the undesired (+)-(*S*)-**3**, was trapped in the achiral column, that was subsequently filled with 2-propanol added of 1% (v/v) of trifluoroacetic acid as catalyst. The trapped enantiomer was left to racemize for 60 min at 45 °C. Afterwards the original mobile phase (hexane/2-propanol 95:5 (v/v)) was introduced in the achiral column forcing the original enantiomer (+)-(*S*)-**3** and its interconversion product (−)-(*R*)-**3** to the semipreparative Chiralcel OD where they was enantioseparated. Thus by harvesting again the selected stereoisomer it is possible to collect additional 25% of (−)-(*R*)-**3**. The racemization/separation cycle could be repeated several times in order to increase the yield of (−)-(*R*)-**3**. 200 mg of racemic compound by consecutive injections afforded approximately 170 mg of the desired enantiomer.

This method will assist us to gain access to relevant data on the pharmacokinetic profile of (−)-(*R*)-**3**.

3. Conclusions

Recently, we designed and synthesized compound **1** that has attracted particular attention because it exhibited an interesting pharmacological activity as AMPA receptor positive modulator. Preliminary configurational stability studies suggested a rapid enantiomerization in condition similar to those it will meet in vivo.

In order to enhance stability towards enantiomerization and hydrolysis, preserving AMPAr affinity, 7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (±)-**3** was synthesized. Studies on (±)-**3** confirmed a great increase in the configuration stability and a complete suppression of the hydrolysis in physiological conditions. A stereoselective synthesis was developed to obtain the single enantiomers of (±)-**3**. The absolute configuration of the enantiomers of **3** was assigned thanks to X-ray diffraction spectroscopy. Biological studies suggested a stereospecific interaction of (−)-(*R*)-**3** with AMPA receptor. Molecular modeling experiments performed on AMPA GluA2 ligand binding domain identified the probable binding mode of (−)-(*R*)-**3** and the source of the stereospecific recognition with the receptor. The 'on line racemization' technique was applied to synthesize the eutomer (−)-(*R*)-**3** on preparative scale. In vivo pharmacokinetic experiments in rats are in development in order to confirm the stereo-pharmacological activity of **3**.

4. Experimental

4.1. Instrumentation

The chromatographic apparatus was a Shimadzu LC-10AD Pump (Shimadzu Italia, Milan), a Merck Hitachi L-6200A Pump (Merck KGaA, Darmstadt, Germany), a Rheodyne 7725 manual injector equipped with a 20 µl sample loop (Jasco Europe, Italy, Milan). A Merck Hitachi L-7400UV (Merck KGaA, Darmstadt, Germany) was used as detector. Chromatograms were recorded with a Jasco J-700 program (Jasco Europe, Italy, Milan). Two Rheodyne 7000 valves were used to switch the mobile phase flow

(Jasco Europe, Italy, Milan). Column temperature regulation was obtained with a Jasco CO-2067 column oven (Jasco Europe, Italy, Milan).

The columns used were Chiralcel OD-R [cellulose tris (3,5-dimethylphenylcarbamate); 250 × 4.6 mm I.D.; 10 μm], Chiralcel OD [cellulose tris (3,5-dimethylphenylcarbamate); 250 × 10 mm I.D.; 10 μm] purchased from Chiral Technologies Europe, Illkirch, France.

Optical rotation (α) was measured with the P-2000 Digital Polarimeter (cell-length 100 mm, volume 1 ml) from Jasco Europe, Italy, Milan.

Melting points were determined with an Electrothermal Apparatus and they are uncorrected.

IR spectra were recorded on a PerkinElmer Model 1600 FT-IR spectrometer. ^{1}H NMR spectra were recorded with a Bruker DPX 400 spectrometer with $CDCl_3$ as solvent and tetramethylsilane (TMS) as external standard. Chemical shifts (δ) are in part per million and coupling constant (J) in hertz. Multiplicities are abbreviated as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. The electrospray ionization (HR-ESI-MS) experiments were carried out in a hybrid QqTOF mass spectrometer (PE SCIEX-QSTAR) equipped with an ion spray ionization source. MS (+) spectra were acquired by direct infusion (5 mL/min) of a solution containing the appropriate sample (10 pmol/mL), dissolved in a solution 0.1% acetic acid, methanol/water 50:50 at the optimum ion voltage of 4800 V.

All pH measurements were made using Orion Research EA940 pH-meter.

HPLC-grade acetonitrile, *n*-hexane and 2-propanol were obtained from Sigma–Aldrich (Milan, Italy).

4.2. Synthesis

4.2.1. 7-Chloro-5-(2-furanyl)-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (1)

Compound **1** was obtained as described by Battisti et al.[26] Yield 45% (three steps).

Mp: 195–197 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.62 (d, J = 6.1 Hz, 3H), 4.51 (d, J = 13.0 Hz, 1H), 5.10–5.13 (m, 1H), 5.87 (s, 1H, broad), 6.56 (dd, J = 1.8 Hz, 3.3 Hz, 1H), 6.65 (d, J = 3.3 Hz, 1H), 7.50 (d, J = 2.4 Hz, 1H), 7.55 (d, J = 1.3 Hz, 1H), 7.59 (d, J = 2.4 Hz, 1H).

GC–MS (70 eV): m/z 298 (100) [M+], 283 (67), 255 (17), 192 (52), 164 (35), 128 (68).

4.2.2. 7-Chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (3)

4.2.2.1. (±)(*R*,*S*)-7-Chloro-2,3,3a,4-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,2,4]benzothiadiazine 5,5-dioxide (7). The compound was synthesized as previously described by Cameroni et al. starting from 2-amino-5-chlorobenzensulfonamide.[39] Yield 70% (three steps).

Mp: 226–228 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.88–1.99 (m, 1H), 2.00–2.12 (m, 2H), 2.20–2.24 (m, 1H), 2.51–2.57 (m, 1H), 3.33–3.40 (m, 1H), 3.52–3.55 (m, 1H), 5.15–5.18 (m, 1H), 6.53 (d, J = 8.4 Hz, 1H,), 7.29 (dd, J = 3.1 Hz, 8.4 Hz, 1H), 7.61 (d, J = 3.1 Hz, 1H).

GC–MS (70 eV): m/z 258 (90) [M$^+$], 193 (100), 165 (89), 138 (40), 75 (50).

4.2.2.2. 9-Bromo-7-chloro-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (8). To a stirring solution of **7** (1 mmol) in ACN (4 ml), a solution of *N*-bromosuccinimide (1.5 mmol) in AcOH (1.2 ml) was added dropwise. The reaction was stirred at room temperature until no starting material was detected by TLC. Subsequently water was added and the resulting precipitate was recovered by filtration. The filtrate provides pure compound as a white solid. Yield 75%.

Mp: 214–216 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.01–2.15 (m, 3H), 2.38–2.45 (m, 1H), 3.17–3.22 (m, 1H), 4.24–4.31 (m, 1H), 4.86 (d, J = 12.4 Hz, 1H), 5.13–5.18 (m, 1H), 7.55 (d, J = 2.3 Hz, 1H), 7.61 (d, J = 2.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.8, 31.1, 52.2, 71.8, 114.8, 123.1, 126.4, 129.1, 137.6, 141.5.

GC–MS 70 eV: m/z 338 (100) [M+2], 273 (60), 257 (30), 246 (58), 193 (56), 166 (58), 109 (35).

HRMS-ESI: calcd for $C_{10}H_{11}BrClN_2O_2S$: 336.9407 found: 336.9412.

4.2.2.3. 7-Chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (3). To a stirring solution of 9-bromo-7-chloro-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (**8**) (1 mmol) and 3-furanylboronic acid (1.2 mmol) in water/dioxane (1:1 v/v), tetrakis(triphenylphosphine)palladium (5% mol) and Na_2CO_3 (3 mmol) were added. The reaction mixture was heated at 100 °C for 3 h and then cooled down to room temperature. The mixture was neutralized with HCl 1 M and extracted with ethyl acetate. Combined organic layers were dried over anhydrous sodium sulfate and concentrate under vacuum. Column chromatography (petroleum ether/ethyl acetate 7:3 (v/v)) provides the pure compound as a yellow solid. Yield 75%.

Mp: 218–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.88–1.99 (m, 3H), 2.33–2.43 (m, 1H), 2.76–2.82 (m, 1H), 3.28–3.34 (m, 1H), 4.86 (d, J = 12.4 Hz, 1H), 5.21–5.27 (m, 1H), 6.61 (d, J = 2.6 Hz, 1H,), 7.32 (d, J = 2.6 Hz, 1H,), 7.47–7.49 (m, 2H), 7.63 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.4, 32.0, 51.8, 71.8, 110.9, 122.5, 123.6, 125.9, 127.4, 127.9, 134.6, 140.6, 141.2, 143.4.

GC–MS 70 eV: m/z 324 (100) [M$^+$], 295 (25), 259 (23), 205 (30), 113 (20).

HRMS-ESI: calcd for $C_{14}H_{14}ClN_2O_3S$: 325.0408; found: 325.0415.

4.2.3. Asymmetric synthesis

4.2.3.1. 7-Chloro-9-(3-furanyl)-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (9). The compound was synthesized as described by Cameroni et al. starting from 7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (**3**).[39] Yield 50%.

Mp: 222–224 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.05 (dd, J = 7.8 Hz, 7.9 Hz, 2H,), 2.92 (t, J = 7.9 Hz, 2H), 3.78 (t, J = 7.8 Hz, 2H), 6.49 (d, J = 2.3 Hz, 1H), 7.41 (d, J = 2.3 Hz, 1H), 7.51–7.53 (m, 2H), 7.60 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.9, 33.5, 54.2, 113.3, 123.6, 124.4, 127.8, 130.4, 132.6, 134.1, 136.9, 140.3, 141.1, 164.5.

GC–MS 70 eV: m/z 322 (84) [M$^+$], 293 (100), 259 (14), 229 (51), 190 (31).

HRMS-ESI: calcd for $C_{14}H_{11}ClN_2O_3S$: 322.0179; found: 322.0178.

4.2.3.2. (*S*)-(+)-7-Chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (*S*)-(3). The compound was synthesized following the protocol reported by Cannazza et al.[33,41] Yield 85%.

Mp: 218–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.88–1.99 (m, 3H), 2.33–2.43 (m, 1H), 2.76–2.82 (m, 1H), 3.28–3.34 (m, 1H), 4.86 (d, J = 12.4 Hz, 1H), 5.21–5.27 (m, 1H), 6.61 (d, J = 2.6 Hz, 1H), 7.32 (d, J = 2.6 Hz, 1H), 7.47–7.49 (m, 2H), 7.63 (s, 1H).

$[\alpha]_D$ +32.4° (15 mg/mL; chloroform, 24 °C).

4.2.3.3. (*R*)-(−)-7-Chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (*R*)-(3). A dry toluene solution, containing the chiral amino alcohol (2*R*,3*S*)-(−)-4-dimethylamino-1,2-diphenyl-3-methyl-2-butanol (3.0 mmol) was added at 0 °C to a magnetically stirred solution of LiA1H$_4$ (1.5 mmol)

in dry toluene. The reaction mixture was vigorously stirred at 0 °C during 15 min. Compound **9** (1.0 mmol) was added in several portions and the mixture was stirred for 2 h at 0 °C. The reaction was hydrolysed by adding dropwise 1 N HCl (vigorous evolution of gas). Then the reaction mixture was neutralized with1 N NaOH and extracted with ethyl acetate. The organic extracts were dried over Na_2SO_4 and evaporated under vacuum to give reduced product as a white solid (ee: 76%). The compound was further purified by chiral HPLC to increase enantiomeric excess. Yield 69%.

Mp: 217–219 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.88–1.99 (m, 3H), 2.33–2.43 (m, 1H), 2.76–2.82 (m, 1H), 3.28–3.34 (m, 1H), 4.86 (d, J = 12.4 Hz, 1H), 5.21–5.27 (m, 1H), 6.61 (d, J = 2.6 Hz, 1H), 7.32 (d, J = 2.6 Hz, 1H), 7.47–7.49 (m, 2H), 7.63 (s, 1H).

$[\alpha]_D$ −33.8° (15 mg/mL; chloroform, 24 °C).

4.3. X-ray crystallography

X-ray diffraction experiments were carried out at room temperature (293°K) by a Bruker-Nonius KappaCCD single crystal diffractometer, equipped α with a charge-coupled device (CCD detector), using monochromatized MoK (λ radiation = 0.71073 Å). The automatic data collection strategy was defined by the COLLECT software, cell determination and refinement by DIRAX and data reduction by EVAL.[47,48] Absorption effects were corrected by SADABS, exploiting a semi-empirical approach. Crystal structure was solved by SIR2011 and refined by SHELXL-97.[49–51]

4.4. Chromatography

The separation factor (α) was calculated as k_2/k_1 and retention factors (k_1 and k_2) as $k_1 = (t_1 - t_0)/t_0$ where t_1 and t_2 refer to the retention times of the first and second eluted enantiomers. The resolution factor (R_s) was calculated by the formula $R_s = 2(t_2 - t_1)/(w_1 + w_2)$ where w_1 and w_2 are the peak widths at base for the first and second eluted enantiomers. The dead time of the columns (t_0) was determined by injection of 1,3,5-tri-*tert*-butylbenzene.

Pure (+)(*S*) and (−)(*R*) enantiomers of 7-chloro-9-(furan-3-yl)-2,3,3a,4-tetrahydro-1*H*-benzo[*e*]pyrrolo[2,1-*c*][1,2,4]thiadiazine 5,5-dioxide (**3**) were obtained by semipreparative HPLC on Chiralcel OD semipreparative column with fraction collection of the respective peaks. The mobile phase consisted of *n*-hexane and 2-propanol 95:5 (v/v). The compound was dissolved in *n*-hexane. The injection volume was 500 µl. The detector was set at 254 nm. The collected fractions corresponding to the enantiomers were analyzed by injection on the same column and in the same chromatographic conditions.

Separation of enantiomers of compound **3** was carried out isocratically at 25–45 °C on Chiralcel OD-RH column using water/acetonitrile (50:50, v/v) as mobile phase. The compound was dissolved in ethanol and subsequently diluted 1:10 (v/v) with mobile phase at final concentration of 100 µg/ml. The injection volume was 50 µl. The detector was set at 254 nm.

The sf-BD-rHPLC method was previously described.[24] The racemic mixture of 3 was injected on Chiralcel OD. The mobile phase consisted of *n*-hexane and 2-propanol 95:5 (v/v). The injection volume was 500 µl. The detector was set at 254 nm. The racemization of the trapped enantiomer was conducted employing an IAM column as reactor column and isopropanol added of 1% (v/v) of trifluoroacetic acid as racemization solvent. The reactor column was left 1 h at 45 °C.

4.5. Electrophysiology

Primary cultures of cerebellar granule neurons were prepared from 7 days old Sprague–Dawley rats as reported in the literature.[52] Briefly, cells from the cerebellum were dispersed with trypsin (0.24 mg/mL; Sigma Aldrich, Milan, Italy) and plated at a density of 0.8×10^6 cells/mL on 35 mm Falcon dishes coated with poly-L-lysine (10 µg/mL, Sigma Aldrich). Cells were plated in basal Eagle's Medium (BME; Celbio, Milan, Italy), supplemented with 10% fetal bovine serum (Celbio), 2 mM glutamine, 25 mM KCl and 100 µg/mL gentamycin (Sigma Aldrich) and maintained at 37 °C in 5% CO_2. After 24 h in vitro, the medium was replaced with 1:1 mixture of BME and Neurobasal medium (Celbio, Milan) containing 2% B27 supplement, 1% antibiotic, and 0.25% glutamine (Invitrogen). At 5 days in vitro (DIV5), cytosine arabinofuranoside (Ara-C) was added at a final concentration of 1 µM.

Recordings were performed at room temperature, under voltage-clamp in the whole-cell configuration of the patch-clamp technique on cells. Electrodes pulled from borosilicate glass (Heidelberg, FRG) on a vertical puller (PB-7, Narishige) and had a resistance of 5–6 M Ω. Currents were amplified with an Axopatch 1D amplifier (Axon Instruments, Foster). The recording chamber was continuously perfused at 5 mL/min with an artificial extracellular solution composed of (mM): 145 NaCl, 5 KCl, 1 $CaCl_2$, 5 Hepes, 5 Glucose, 20 Sucrose, pH 7.4 with NaOH. Electrode intracellular solution contained (mM): 140 KCl, 3 $MgCl_2$, 5 EGTA, 5 Hepes, 2 ATP-Na, pH 7.3 with KOH. Drugs were applied directly by gravity through a Y-tube perfusion system.

4.6. Docking studies

The GluA2-S1S2J crystal structures in complex with Cyclothiazide and IDRA21 were retrieved from the Protein Data Bank (PDB codes: 1LBC and 3IL1) and imported into MVD.[25] All water molecules and co-factors were deleted. (*S*)-**3** and (*R*)-**3** were built in Spartan employing the crystal structure of (*S*)-**3** as input file. Then they were exported as mol2 files and docked in GluA2 by using MVD. We used the template docking available in MVD and evaluated MolDock score, Rerank score, and protein–ligand interaction score from MolDock and MolDock [GRID] options. Cyclothiazide and IDRA21 were selected as reference compounds for the template. It was used the default settings, including a grid resolution of 0.30 Å, the MolDock optimizer as a search algorithm, and the number of runs was set to 10. A population size of 50, maximum iteration of 2000, scaling factor of 0.50, crossover rate of 0.90. The maximum number of poses to generate was increased to 10 from a default value of 5.

References and notes

1. Krogsgaard-Larsen, P.; Ebert, B.; Lund, T. M.; Brauner-Osborne, H.; Slk, F. A.; Johansen, T. N.; Brehm, L.; Madsen, U. *Eur. J. Med. Chem.* **1996**, *31*, 515.
2. *Excitatory Amino Acid Receptors, Design of Agonists and Antagonists*; Krogsgaard-Larsen, P., Hansen, J. J., Eds.; Ellis Horwood: Chichester, 1992.
3. *The NMDA Receptor*; Collingridge, G. L., Watkins, J. C., Eds.; Oxford University Press: Oxford, 1994.
4. Kew, J. N. C.; Kemp, J. A. *Psychopharmacology* **2005**, *179*, 4.
5. Francotte, P.; de Tullio, P.; Fraikin, P. *Recent Pat. CNS Drug Discov.* **2006**, *1*, 239.
6. Mayer, M. L.; Armstrong, N. *Annu. Rev. Physiol.* **2004**, *66*, 161.
7. O'Neill, M. J.; Bleakman, D.; Zimmerman, D. M.; Nisenbaum, E. S. *Curr. Drug Targets CNS Neurol. Disord.* **2004**, *3*, 181.
8. O'Neill, M. J.; Dix, S. *IDrugs* **2007**, *10*, 185.
9. Zarate, C. A.; Manji, H. K. *Exp. Neurol.* **2008**, *211*, 7.
10. Hashimoto, K. *Brain Res. Rev.* **2009**, *61*, 105.
11. Pirotte, B.; Francotte, P.; Goffin, E. *Curr. Med. Chem.* **2010**, *17*, 3575.
12. Ward, S. E.; Harries, M. *Curr. Med. Chem.* **2010**, *17*, 3503.
13. Pirotte, B.; Francotte, P.; Goffin, E.; de Tullio, P. *Expert Opin. Ther. Patents* **2013**, *23*, 615.
14. Grove, S. J. A.; Jamieson, C.; Maclean, J. C. F.; Morrow, J. A.; Rankovic, Z. *J. Med. Chem.* **2010**, *53*, 7271.
15. Ward, S. E.; Bax, B. D.; Harries, W. *Br. J. Pharmacol.* **2010**, *160*, 181.
16. Philips, D.; Sonnenberg, J.; Arai, A. C.; Vaswani, R.; Krutzik, P. O.; Kleisli, T.; Kessler, M.; Granger, R.; Lynch, G.; Chamberline, A. R. *Bioorg. Med. Chem.* **2002**, *10*, 1229.
17. Braghiroli, D.; Puia, G.; Cannazza, G.; Tait, A.; Parenti, C.; Losi, G.; Baraldi, M. *J. Med. Chem.* **2002**, *45*, 2355.
18. Pirotte, B.; Podona, T.; Diouf, O.; de Tullio, P.; Lebrun, P.; Dupont, L.; Somers, F.; Delarge, J.; Morain, P.; Lestage, P.; Lepagnol, J.; Spedding, M. *J. Med. Chem.* **1998**, *41*, 2946.

19. Graindorge, E.; Francotte, P.; Boverie, S.; de Tullio, P.; Pirotte, B. *Curr. Med. Chem.* **2004**, *4*, 95.
20. Hashimoto, K.; Okamura, N.; Shimizu, E.; Iyo, M. *Curr. Med. Chem.* **2004**, *4*, 147.
21. Francotte, P.; de Tullio, P.; Goffin, E.; Dintilhac, G.; Graindgorge, E.; Fraikin, P.; Lestage, P.; Danober, L.; Thomas, J. Y.; Caignard, D. H.; Pirotte, B. *J. Med. Chem.* **2007**, *50*, 3153.
22. Arai, A.; Guidotti, A.; Costa, E.; Lynch, G. *NeuroReport* **1996**, *7*, 2211.
23. Thompson, D. M.; Guidotti, A.; DiBella, M.; Costa, E. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7667.
24. Sun, Y.; Olson, R.; Horning, M.; Armstrong, N.; Mayer, M.; Gouaux, E. *Nature* **2002**, *417*, 245.
25. Ptak, C. P.; Ahmed, A. H.; Oswald, R. E. *Biochemistry* **2009**, *48*, 8594.
26. Battisti, U. M.; Jozwiak, K.; Cannazza, G.; Puia, G.; Stocca, G.; Braghiroli, D.; Parenti, C.; Brasili, L.; Carrozzo, M. M.; Citti, C. *Med. Chem. Lett.* **2012**, *3*, 25.
27. Cannazza, G.; Braghiroli, D.; Tait, A.; Baraldi, M.; Parenti, C.; Lindner, W. *Chirality* **2001**, *13*, 94.
28. Cannazza, G.; Carrozzo, M. M.; Braghiroli, D.; Parenti, C. *J. Chromatogr., A* **2008**, *1212*, 41.
29. Carrozzo, M. M.; Cannazza, G.; Battisti, U.; Braghiroli, D.; Parenti, C. *Chirality* **2010**, *22*, 389.
30. Cannazza, G.; Carrozzo, M. M.; Battisti, U.; Braghiroli, D.; Parenti, C.; Troisi, A.; Troisi, L. *Chirality* **2010**, *22*, 789.
31. Carrozzo, M. M.; Cannazza, G.; Battisti, U. M.; Braghiroli, D.; Troisi, L.; Parenti, C. *J. Chromatogr., A* **2010**, *1217*, 8136.
32. Cannazza, G.; Battisti, U. M.; Carrozzo, M. M.; Brasili, L.; Braghiroli, D.; Parenti, C. *Chirality* **2011**, *23*, 851.
33. Cannazza, G.; Carrozzo, M. M.; Braghiroli, D.; Parenti, C. *J. Chromatogr., B* **2008**, *875*, 42.
34. Battisti, U. M.; Carrozzo, M. M.; Cannazza, G.; Puia, G.; Troisi, L.; Braghiroli, D.; Parenti, C.; Jozwiak, K. *Bioorg. Med. Chem.* **2011**, *19*, 7111.
35. Cannazza, G.; Battisti, U. M.; Carrozzo, M. M.; Cazzato, A. S.; Braghiroli, D.; Parenti, C.; Troisi, L. *J. Chromatogr., A* **2014**, in press, http://dx.doi.org/doi:10.1016/j.chroma.2014.05.073.
36. Rosi, S.; Giovannini, M. G.; Lestage, P. J.; Munoz, C.; Corte, L. D.; Pepeu, G. *Neurosci. Lett.* **2004**, *361*, 120.
37. Vandesquille, M.; Krazem, A.; Louis, C.; Pierre, L.; Beracochea, D. *Psychopharmacology* **2011**, *215*, 709.
38. Lebrun, C.; Pilliere, E.; Lestage, P. *Eur. J. Pharm.* **2000**, *401*, 205.
39. Cameroni, R.; Bernabei, M. T.; Forni, F.; Baggi, G. *Il Farmaco Ed. Sci.* **1976**, *31*, 508.
40. Harpsoe, K.; Varming, T.; Gouliiaev, A. H.; Peters, D.; Liljefors, T. *J. Mol. Graphics Modell.* **2007**, *26*, 213.
41. Desos, P.; Serkiz, B.; Morain, P.; Lepagnol, J.; Cordi, A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 3003.
42. Yamaguchi, S.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 1870.
43. Cohen, N.; Lopresti, R. J.; Neukom, C.; Saucy, G. *J. Org. Chem.* **1980**, *45*, 582.
44. Deeter, J.; Frazier, J.; Staten, G.; Staszak, M.; Weigel, L. *Tetrahedron Lett.* **1990**, *131*, 7101.
45. Jane, D. E.; Hoo, K.; Kamboj, R.; Deverill, M.; Bleakman, D.; Mandelzys, A. *J. Med. Chem.* **1997**, *40*, 3645.
46. Cannazza, G.; Carrozzo, M. M.; Battisti, U.; Braghiroli, D.; Parenti, C. *J. Chromatogr., A* **2009**, *1216*, 5655.
47. Nonius *COLLECT and EVAL*; Nonius BV: Delft, The Netherlands, 2002.
48. Duisenberg, A. J. *J. Appl. Crystallogr.* **1992**, *25*, 92.
49. Sheldrick, G. M. *SADABS*; University of Göttingen: Germany, 2002.
50. Burla, M. C.; Caliandro, R.; Camalli, M.; Carrozzini, B.; Cascarano, G. L.; Giacovazzo, C.; Mallamo, M.; Mazzone, A.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **2012**, *45*, 357.
51. Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112.
52. Murase, K.; Ryu, P. D.; Randic, M. *Neurosci. Lett.* **1989**, *103*, 56.