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NEW INSIGHTS IN THE DEVELOPMENT OF POSITIVE ALLOSTERIC MODULATORS OF A-AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLEPROPIONIC ACID (AMPA) RECEPTORS BELONGING TO 3,4-DIHYDRO-2*H*-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDES: INTRODUCTION OF (MONO/DIFLUORO)METHYL GROUPS AT THE 2-POSITION OF THE THIADIAZINE RING

Eric Goffin [a], Pierre Fraikin [a], Dayana Abboud [b], Pascal de Tullio [a], Caroline Beaufour [c], Iuliana Botez [c], Julien Hanson [a,b], Laurence Danober [c], Pierre Francotte [a], Bernard Pirotte [a]

[a] *Center for Interdisciplinary Research on Medicines (CIRM) - Laboratory of Medicinal Chemistry, University of Liège, Avenue Hippocrate 15 (B36), B-4000, Liège, Belgium*
[b] *Laboratory of Molecular Pharmacology, GIGA-Molecular Biology of Diseases, University of Liège, Avenue Hippocrate 1/11 (B34), B-4000, Liège, Belgium*
[c] *Institut de Recherches Servier, 125 Chemin de Ronde, F-78290, Croissy-sur-Seine, France*

Abstract

Positive allosteric modulators of the AMPA receptors (AMPAR PAMs) have been proposed as new drugs for the management of various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, attention deficit hyperactivity disorder, depression, and schizophrenia. The present study explored new AMPAR PAMs belonging to 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (BTDs) characterized by the presence of a short alkyl substituent at the 2-position of the heterocycle and by the presence or absence of a methyl group at the 3-position. The introduction of a monofluoromethyl or a difluoromethyl side chain at the 2-position instead of the methyl group was examined. 7-Chloro-4-cyclopropyl-2-fluoromethyl-3,4-dihydro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**15e**) emerged as the most promising compound associating high *in vitro* potency on AMPA receptors, a favorable safety profile *in vivo* and a marked efficacy as a cognitive enhancer after oral administration in mice. Stability studies in aqueous medium suggested that **15e** could be considered, at least in part, as a precursor of the corresponding 2-hydroxymethyl-substituted analogue and the known AMPAR modulator 7-chloro-4-cyclopropyl-3,4-dihydro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**3**) devoid of an alkyl group at the 2-position.

Keywords : Ionotropic glutamate receptors, 1,2,4-Benzothiadiazine 1,1-dioxides, Positive allosteric modulator, Cognitive enhancer, N-Mono/difluoromethyl-substituted compounds

1. Introduction

L-Glutamate is well known as the major excitatory neurotransmitter in the brain, interacting with ionotropic (ligand-gated ion channel, iGluRs) and metabotropic (G-protein coupled, mGluRs) receptors. iGluRs have been divided into four subclasses: the *N*-methyl-D-aspartic acid (NMDA), the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), the kainic acid (KA) and the delta receptors [1,2]. These receptors are essential in neurophysiology, e.g. synaptic plasticity, learning and memory [3]. A decrease of AMPA receptor signaling has been shown to play a role in neurological disorders such as Alzheimer's disease, Parkinson's disease, attention deficit hyperactivity disorder, depression, and schizophrenia [4]. Therefore, positive allosteric modulators of the AMPA receptors (AMPAR PAMs) have been proposed as new drugs for the management of such neurodegenerative diseases [5–7]. Moreover, AMPAR PAMs were also found to be neuroprotectants through stimulation of brain-derived neurotrophic factor (BDNF) release [8].

AMPARs are tetrameric combinations of four subunits (GluA1-4) that arrange themselves in various stoichiometries, most of which being symmetric dimer-of-dimers of GluA2 and either GluA1, GluA3 or GluA4 [9,10]. Each subunit comprises an extracellular N-terminal domain (NTD), a ligand-binding domain (LBD) incorporating the L-glutamate binding site as well as the AMPAR PAM allosteric binding sites, and a transmembrane domain (TMD) located in the cell membrane and prolonged in the cytosol as the C-terminal domain (CTD) [11,12].

Distinct classes of AMPAR PAMs have been described in recent years, among which benzamides, 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (BTDs) and *N*-biaryl(cyclo)alkyl-2-propanesulfonamides [5, 7]. Depending on their molecular structure, these modulators interact with distinct allosteric binding sites at the level of the LBD of the receptor [11–15].

In previous works, we developed extensive research on original AMPAR PAMs belonging to the BTD-type family of modulators, and more specifically, to compounds structurally related to the well-known orally active AMPAR potentiator IDRA 21 (**1**, Fig. 1) [16–28]. These works led to the discovery of potent and orally active compounds such as **2** and **3** (Fig. 1) that showed improved *in vitro* properties and *in vivo* efficacy [18,21,22]. The 2,3,4-trimethyl-substituted analogue of IDRA 21, compound **4** (Fig. 1), was surprisingly found to be markedly potent *in vitro* as an AMPA receptor potentiator [24]. More recently, we reported 7-phenoxy-substituted BTDs such as **5** (Fig. 1) [26] and dimers such as **6** (Fig. 1) [27] amongst which were identified the most potent AMPAR PAMs to date, potentiating the effect of glutamate on AMPARs in the nanomolar range [26,27]. Finally, thiochroman 1,1-dioxides like compound **7** designed to be isosteric analogues of potent BTD modulators such as **8** were found to express an interesting potentiator activity on the AMPA receptors [28].

The present study will explore new BTDs structurally related to compounds **3** and **4** bearing a short alkyl substituent at the 2-position of the heterocycle and characterized by the presence or absence of a methyl group at the 3-position. The introduction of the unusual monofluoromethyl or difluoromethyl side chain at the 2-position instead of the methyl group will be examined. The substituent at the 4-position will be chosen among a methyl, an ethyl or a cyclopropyl chain expected to be the best choice of short (cyclo)alkyl chains in accordance with previously established structure-activity relationships

[16–27]. For the same reasons, the halogen atom introduced at the 7-position will be chosen between a fluorine or a chlorine atom.

2. Results and discussion

2.1. CHEMISTRY

The synthetic pathway used to prepare the new 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides reported here is illustrated in Scheme 1.

Starting from the appropriate 5-halo-substituted 2-fluorobenzenesulfonamides **9**, the corresponding 2-(cyclo)alkylamino-substituted benzenesulfonamides **10** were obtained after nucleophilic substitution, with the appropriate (cyclo)alkylamine, of the fluorine atom at the *ortho*-position of the electron-withdrawing sulfonamide group. The latter intermediates were engaged in two distinct pathways. The first one led to the desired 2,3,4-trialkyl-substituted 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **12** in two steps: ring closure reaction with acetaldehyde provided intermediates **11**; alkylation of the latter with the appropriate (mono/difluoro)alkyl halide at the 2-position of the thiadiazine ring provided the target compounds **12**. The second pathway led to the 4*H*-1,2,4-benzothiadiazine 1,1-dioxide intermediates **13** by ring closure after reaction with triethyl orthoformate. Saturation of the double bond at the 2,3-positions of intermediates **13** was achieved in 2-propanol using sodium borohydride to give the expected products **14**. The alkylation at the 2-position with the appropriate (mono/difluoro)alkyl halide allowed to obtain the target compounds **15**.

It is interesting to note that compounds bearing a monofluoromethyl or a difluoromethyl moiety on a heteroatom (O, N, S) are not usual in medicinal chemistry. Nevertheless, several examples of S-monofluoromethyl- and O-monofluoromethyl-substituted compounds have reached the clinical use. The corticosteroid fluticasone propionate **16** (Fig. 2) with a SCH_2F group is an example of such compound currently used in medicine [29]. The general anesthetic sevoflurane **17** (Fig. 2) with a good clinical safety profile is an example of drug bearing the unusual OCH_2F group [29]. Looking at compounds tightly related to the original structures described in this work, it must be noted that *N*-fluoromethyl- and *N*-difluoromethylbenzenesulfonamides are little described in the literature. To our knowledge, only one published work reported the synthesis of a series of *N*-difluoromethylbenzenesulfonamides i.e. compound **18** (Fig. 2) [30]. On the other hand, *N*-(fluoromethyl)saccharin **19** (Fig. 2) is a rare example of *N*-fluoromethylbenzenesulfonamide studied as a serine protease inhibitor [31,32]. This observation could justify the attraction for such compounds in the drug discovery process always guided by a need for novelty and structural originality. Several review articles have described the chemical access to O-, S- and N-monofluoromethyl-substituted compounds [29,33–35]. However, little is known on the chemical and metabolic stability of such compounds [36]. Monofluoromethylated amines bearing an α-H ($R–NHCH_2F$) are reported to be thermally unstable, undergoing dehydrofluorination reactions [33]. In the case of the N-monofluorinated secondary sulfonamides described in this work (**12i**, **15b**, **15e**), absence of a hydrogen atom in the α-position could justify a better chemical stability.

As a potential drug candidate, the stability of compound **15e** in different solvents was examined. The drug was found to be chemically stable in most organic solvents even after several days in solution (i.e. in DMSO-d_6 after one week at room temperature followed by NMR). However, when **15e** was solubilized in DMSO-d_6/D_2O 1:1 at room temperature, the evolution of the NMR spectra indicated that **15e** was slowly converted (stability half-life = 14.0 h) into the hypothetical 2-hydroxymethyl-substituted analogue **20**, and finally into the corresponding unsubstituted analogue **3** (Fig. 3). After 24 h, the proportion of **15e**, **20** and **3** in solution was estimated to be quite similar (30–35%). In the same conditions, **15b** was found to be transformed more rapidly (stability half-life = 9.2 h).

An interesting observation can be retrieved from the NMR data of the non-fluoro-substituted (**15a**, **15d**), the monofluoro-substituted (**15b**, **15e**) and the difluoro-substituted (**15c**, **15f**) target compounds to appreciate the deshielding capacity of the electronegative fluorine atoms. Table 1 reports, for the selected compounds **15a-f**, the chemical shifts of the protons located on the intracyclic C^3 carbon atom (in blue) and on the exocyclic carbon atom linked at the 2-position of the thiadiazine ring (in red). Although the methylene protons located at the 3-position of the heterocycle were only slightly influenced by the presence of one or two fluorine atoms in the vicinity, the electron density of the protons linked to the carbon atoms bearing the fluorine atoms was strongly affected (0 F: 2.6 ppm; 1 F: 5.6 ppm; 2 F: 7.2 ppm). The increased deshielding was in line with the increase of the number of the fluorine atoms, and the signals appeared as singlets (no coupling with F), doublets (coupling with one F) and triplets (coupling with 2 F), respectively. The strong deshielding of the lonely proton of the N-difluoromethylsulfonamide group was already observed by Petko et al. [30].

Considering that **15e** could be a precursor of the well-known potent AMPAR modulator **3** with a different safety profile, we decided to compare the two compounds in a panel of pharmacological *in vitro* and *in vivo* tests to appreciate the advantage of the presence of an unusual N-fluoromethyl-substituted sulfonamide group in terms of activity, bioavailability, and toxicity.

2.2. IN VITRO EVALUATION

The new 1,2,4-benzothiadiazine 1,1-dioxides (BTDs) were evaluated as AMPAR potentiators using a previously described *in vitro* fluorescence assay (FlipR or FDSS) on rat cortical primary cell cultures [18]. For each compound, the EC2x value was determined, which corresponds to the concentration of drug responsible for a 2-fold increase of the amplitude of the current induced by AMPA at 300 µM (Table 2). Moreover, the potentiating effect of the new compounds on the calcium influx induced by 1 mM glutamate on HEK293 cells stably expressing the GluA2 (Q) flop subunit, which is an isoform forming channels permissive for calcium ions [37], was also measured (Table 2).

Lastly, AMPAR PAMs have been reported to potentiate noradrenaline (NA) release in rat hippocampal slices, an effect linked to their interaction with presynaptic AMPA receptors [38,39]. This activity could be responsible for the cognition-enhancing properties of AMPAR potentiators. Potentiation of noradrenaline ([^{3}H]NA) release was measured on rat hippocampal slices with the new compounds at 300 µM in the presence of (*S*)-AMPA (10 µM), 100% representing the effects shown by (S)-AMPA alone (Table 2).

Table 2 indicates that the potentiation effect on AMPA receptors of 7-fluoro-substituted compounds **12a-c** bearing a methyl group at the 2- and 3-positions of the heterocycle was impacted by the nature of the substituent at the 4-position. The presence of a cyclopropyl group instead of a short non bulky methyl or ethyl radical at this position was responsible for a strong decrease of potentiator activity on AMPA receptors. This impact was already observed with the corresponding previously reported 7-chloro-substituted 2,3-dimethyl BTDs **12d** and **12h** [24]. In accordance with known structure-activity relationships established with pyridothiadiazine dioxides [40], replacement of the methyl side chain at the 2-position by an ethyl side chain (**12e** and **12g**) decreased the AMPAR potentiator activity. Indeed, previous works indicated that the increase of the size of the alkyl chain introduced at the 2-position of the heterocycle induced a progressive decrease of potency on AMPA receptors. Surprisingly, the replacement of the 2-methyl chain with a 2-fluoromethyl one (**12i**) considerably increased the AMPAR potentiator activity on rat cortical primary cell cultures and on HEK293 cells [see Table 2; **12i**: EC2x = 2.5 µM/pEC_{50} = 5.67 (EC_{50} = 2.1 µM) *vs* **12h**: EC2x = 65 µM/pEC_{50} < 4 (EC_{50} > 100 µM)] as well as the ability to increase glutamate-mediated current amplitude on GluA2 subunit and the noradrenaline release (see Table 2; **12i**: NA release = 329% *vs* **12h**: NA release <100%) (Table 2).

In the series of 4-cyclopropyl-substituted BTDs devoid of a methyl group at the 3-position (compounds **15a-f**), it was also observed that the replacement of the methyl group at the 2-position by a fluoromethyl group (compare **15a** *vs* **15b** and **15d** *vs* **15e**) was responsible for an increase of biological activity as AMPAR PAMs. Moreover, deletion of the 3-methyl substituent was logically found to improve the potentiator activity of the 7-fluorosubstituted BTDs (see **12c** *vs* **15a**), as previously observed with the 7-chloro-substituted analogues (**12h** *vs* **15d**) [18]. Introduction of a second fluorine atom on the 2-methyl group, leading to 2-difluoromethyl-substituted BTDs, provided active compounds (EC2x values and NA release) albeit less potent on AMPA receptors than the corresponding 2-fluoromethyl-substituted BTDs (see **15b** *vs* **15c** and **15e** *vs* **15f**). Among the new compounds developed in this study, the 2-fluoromethyl-substituted BTDs **15b** and **15e** were found to be the most potent compounds expressing biological activities on AMPA receptors close to that of their corresponding unsubstituted analogues **8** and **3** [see Table 2: **15b**: EC2x = 0.6 µM/pEC_{50} = 6.55 (EC_{50} = 0.28 µM) *vs* **8**: EC2x = 0.2 µM (FlipR) – 0.3 µM (FDSS)/pEC50 = 6.56 (EC50 = 0.28 µM); **15e**: EC2x = 2.1 µM (FlipR) – 1.5 µM (FDSS)/pEC50 = 5.66 (EC50 = 2.2 µM) *vs* **3**: EC2x = 2.2 µM (FlipR) – 0.7 µM (FDSS)/pEC_{50} = 6.10 (EC_{50} = 0.79 µM)].

We also observed in the calcium influx assay that the most potent compounds **12i**, **15b** and **15e** displayed similar maximal efficacies (E_{max}) compared to the reference compound **3** (**3**: E_{max} = 100%; **15e**: Emax = 90 ± 8%).

Concerning the ability of the compounds to increase noradrenaline release from rat hippocampal slices induced by AMPA (300 µM), **15e** was compared to compound **3** at various concentrations. The concentration-response curve indicated that the two compounds were quite equipotent (see Fig. 4).

Taken together, these new *in vitro* results complete structure-activity relationships in the BTD class of AMPA receptor positive allosteric modulators. These are summarized in Fig. 5.

2.3. IN VIVO EVALUATION

The *in vitro* biological data highlighted the potential interest of the two 2-fluoromethyl-substituted BTDs **15b** and **15e** as candidates for a development as new drugs. To select the best candidate for further investigations, we compared the new 2-fluoromethyl-substituted BTDs **15b** and **15e** with their previously reported analogues **8** and **3** devoid of an alkyl substituent at the 2-position and we examined their safety profile *in vivo* in NMRI mice after a massive *per os* drug administration (30 mg/kg and 100 mg/kg). It is well known that a major concern with AMPAR PAMs is their possible pro-convulsant effect at high doses resulting from an excessive stimulation of the glutamatergic neurotransmission in the CNS [41,42]. The absence of pro-convulsant effect in mice after *per os* administration of 30 mg/kg was observed with the 7-chloro-substituted BTDs **3** and **15e**, such an observation constituting a first positive safety criterion. But only **15e** was found to be non-pro-convulsant after a massive *per os* dose of 100 mg/kg, supporting the view that **15e** could present a better safety profile. Under the latter conditions, however, hypothermia was observed with **15e** (see Fig. 6), a phenomenon probably linked to a CNS effect of the drug. Hypothermia induced in mice by medicines is usual with neuroactive drugs like benzodiazepines, antipsychotics, and general anesthetics [43]. This side effect observed in mice does generally not justify stopping the development of a new drug candidate at an early stage of development but need to be mitigated by further investigations in rodents and in other large animal species.

Considering the potential benefit of **15e** over the previously studied **3**, we decided to compare the two drugs in various *in vivo* experiments.

We first compared the effects of compound **3** and compound **15e** on the potentiation of the post-synaptic response induced *in vivo* by a tetanic stimulation in Wistar rats (Fig. 7). The Long-Term Potentiation (LTP) of synaptic response is considered to be one of the synaptic plastic mechanisms that underlies learning and memory processes [44,45]. The effect of **3** and **15e** on the LTP was examined *in vivo* in the dentate gyrus of the hippocampus in anesthetized rats following an electrical stimulation of the perforant path. One hour after the intraperitoneal administration of the drugs, the LTP was induced by a tetanic stimulation (brief high frequent stimulation), and the subsequent excitatory postsynaptic field potentials (EPSfPs) were then recorded during 3 h. The two compounds were found to increase the duration of the LTP at a dose of 10 mg/kg (Fig. 7). It was noted however that the effect of 10 mg/kg i. p. Of **3** was close to 30 mg/kg i. p. Of **15e**. Although the doses of compounds examined in this test were quite important, the efficacy observed with the two drugs was comparable to that obtained with previously described BTDs [18]. Anyway, this effect demonstrated that the two compounds were able to cross the blood-brain barrier and to reach the CNS supporting the view that they may exert a cognition-enhancing effect.

To demonstrate the improvement of the cognitive performance induced by these drugs in an *in vivo* animal model, effects of **3** and **15e** were evaluated *in vivo* in an object recognition test in CD1 mice. The three-session test performed, considered as a paradigm for episodic memory in rodents, was based on the fact that animals remembering a familiar object seen in the previous session spend less time exploring it compared to exploring a new object [46]. According to Fig. 8, oral administration of **3** and **15e** 1 h before the three sessions significantly increased with a similar efficacy the cognition

performance of mice at doses as low as 1 mg/kg (the result of **3** was previously reported [22]). The effect of **15e** on the object recognition test in mice confirmed its interest as a cognitive-enhancing drug and strongly suggests that the compound is absorbed in mice after oral administration and reach the CNS.

A second *in vivo* test in mice confirmed the ability of the new drug **15e** as well as compound **3** to improve also working memory performance. In the alternation test in a T-maze [47], in which a long inter-trial delay (180 s) induced an impairment of the percentage of alternation in young mice, Fig. 9 showed that intraperitoneal acute administration of both drugs at low doses (0.3 mg/kg i. p. For **3** and 0.1 mg/kg for **15e**) improved the performance of spontaneous alternation compared to vehicle-treated mice. Spontaneous alternation relies on working memory since the ability to alternate from trial to trial requires the retention of specific information.

3. Conclusions

We have synthesized new AMPAR PAMs belonging to 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (BTDs) characterized by the presence of a short alkyl substituent at the 2-position of the heterocycle and by the presence or absence of a methyl group at the 3-position. The introduction of a monofluoromethyl or a difluoromethyl side chain at the 2-position instead of the methyl group was examined.

The introduction of a monofluoromethyl chain at the 2-position of the thiadiazine ring improved the AMPAR potentiation effect *in vitro* compared to the impact of the introduction of a 2-methyl or a 2-difluoromethyl chain, instigating further biological investigations on the 2-fluoromethyl-substituted BTDs.

7-Chloro-4-cyclopropyl-2-fluoromethyl-3,4-dihydro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**15e**) emerged as the most promising 2-fluoromethyl-substituted BTD associating high *in vitro* potency on AMPA receptors, and a favorable safety profile *in vivo* compared to its analogue **3** devoid of the fluoromethyl chain at the 2-position of the thiadiazine ring. The two compounds were also found to express a similar efficacy as cognitive enhancers after oral administration in mice at very low doses (0.3 mg/kg and 1 mg/kg), as they can counteract both episodic and working memory deficits.

Lastly, stability studies suggested that **15e** was less stable in water than in organic solvents. A slow conversion of the 2-fluoromethyl-substituted BTD compound into the corresponding unsubstituted analogue **3** in aqueous solution was observed. As a result, **15e** could be considered, at least in part, as a precursor of the known AMPAR potentiator 7-chloro-4-cyclopropyl-3,4-dihydro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **3**, with a different bioavailability and safety profile. However, further *in vitro* and *in vivo* studies are needed to decipher the hypothesis of the formation of an active metabolite from *N*-fluoromethyl-substituted BTDs.

4. Experimental section

4.1. CHEMISTRY

4.1.1. GENERAL PROCEDURES

Melting points were determined on a Büchi Tottoli capillary apparatus and are uncorrected. The ^{1}H and ^{13C} NMR spectra were recorded on a Bruker Avance (500 MHz for ^{1}H; 125 MHz for ^{13}C) instrument using deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent with tetramethylsilane (TMS) as an internal standard; chemical shifts are reported in δ values (ppm) relative to that of internal TMS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, dd = doublet of doublet, qd = quadruplet of doublet, dt = doublet of triplet, tt = triplet of triplet, and bs = broad singlet are used throughout. Elemental analyses (C, H, N, S) were realized on a Thermo Scientific Flash EA 1112 elemental analyzer and were within ±0.4% of the theoretical values for carbon, hydrogen, and nitrogen. This analytical method certified a purity of ≥95% for each tested compound. All reactions were routinely checked by TLC on silica gel Merck 60 F254.

The synthesis of compounds **10c**, **11d-f** and **14a-b** was previously described [21,24].

4.1.2. 5-FLUORO-2-(METHYLAMINO)BENZENESULFONAMIDE (10A)

The solution of 2,5-difluorobenzenesulfonamide **9a** (1.0 g, 5.18 mmol) and methylamine (2 mL of a 40% water solution, 15.76 mmol) in dioxan (10 mL) was heated in a sealed vessel at 100–110 °C for 24 h. The solvents and excess of amine were then removed by distillation under reduced pressure and the residue was solubilized in methanol (7 mL). The methanolic solution was cooled on an ice bath and water (20 mL) was added under stirring. The resulting precipitate was collected by filtration, washed with water, and dried (yields: 90–95%). The pure solid of **10a** was used in the next step without further purification. White solid; ^{1}H NMR (DMSO-d_6) δ 2.81 (d, J = 4.9 Hz, 3H, CH_3), 5.72 (q, J = 5.0 Hz, 1H, NH), 6.74 (dd, J = 9.1 Hz/4.4 Hz, 1H, 3-H), 7.29 (td, J = 8.6 Hz/3.2 Hz, 1H, 4-H), 7.38 (dd, J = 8.9 Hz/3.1 Hz, 1H, 6-H), 7.43 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ 30.1 (CH$_3$), 112.5 (d, J = 8 Hz, C-3), 114.1 (d, J = 25 Hz, C-6), 120.3 (d, J = 22 Hz, C-4), 124.9 (d, J = 6 Hz, C-1), 142.6 (C-2), 151.1–153.0 (d, J = 233 Hz, C-5).

4.1.3. 5-FLUORO-2-(ETHYLAMINO)BENZENESULFONAMIDE (10B)

The title product was obtained as described for **10a** starting from **9a** (1 g, 5.18 mmol) and ethylamine (1 mL of a 70% water solution, 15.52 mmol) (yields: 90–95%). White solid; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7.1 Hz, 3H, CH$_2$CH_3), 3.17 (qd, J = 7.1 Hz/5.1 Hz, 2H, CH_2CH$_3$), 5.63 (t,J = 5.3 Hz, 1H, NH), 6.78 (dd, J = 9.2 Hz/4.4 Hz, 1H, 3-H), 7.27 (td, J = 8.6 Hz/3.1 Hz, 1H, 4-H), 7.38 (dd, J = 9.0 Hz/3.2 Hz, 1H, 6-H), 7.46 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ 14.1 (CH$_2$CH$_3$), 37.5 (CH$_2$CH$_3$), 113.0 (d, J = 7 Hz, C-3), 114.1 (d, J = 25 Hz, C-6), 120.3 (d, J = 22 Hz, C-4), 124.9 (d, J = 6 Hz, C-1), 141.7 (C-2), 151.1–153.0 (d, J = 233 Hz, C-5).

4.1.4. R/S-3,4-DIMETHYL-7-FLUORO-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (11A)

The solution of **10a** (0.44 g, 2.15 mmol) in acetonitrile (10 mL) was supplemented with acetaldehyde (0.5 mL; 8.94 mmol) and a catalytic amount of camphorsulfonic acid (5 mg). After stirring in a closed vessel for 1–2 h at room temperature, the solvent was removed by distillation under reduced pressure. The resulting white solid was solubilized in a small volume of methanol. The addition to this stirred solution of an equal volume of distilled water led to the precipitation of the title compound, which was collected by filtration, washed with water and dried. Due to its instability in aqueous medium, the title compound was immediately used in the next step without further purification. ^{1}H NMR (DMSO-d_6) δ 1.48 (d, *J* = 6.4 Hz, 3H, CHC*H*$_3$), 2.90 (s, 3H, NC*H*$_3$), 4.79 (dq, *J* = 9.1 Hz/6.4 Hz, 1H, C*H*CH$_3$), 6.96 (dd, *J* = 9.3 Hz/4.3 Hz, 1H, 5-*H*), 7.34 (dd, *J* = 9.3 Hz/8.4 Hz/3.2 Hz, 1H, 6-*H*), 7.39 (dd, *J* = 7.8 Hz/3.1 Hz, 1H, 8-*H*), 8.15 (d, *J* = 9.1 Hz, 1H, N*H*). ^{13}C NMR (DMSO-d_6) δ 18.3 (CH*C*H$_3$), 34.8 (N*C*H$_3$), 67.9 (*C*HCH$_3$), 109.9 (d, *J* = 24 Hz, C-8), 116.9 (d, *J* = 7 Hz, C-5), 120.7 (d, *J* = 22 Hz, C-6), 123.2 (d, *J* = 6 Hz, C-8a), 141.2 (C-4a), 152.3–154.1 (d, *J* = 238 Hz, C-7).

4.1.5. R/S-4-ETHYL-7-FLUORO-3-METHYL-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (11B)

The title compound was obtained as described for **11a** starting from **10b**. Due to its instability in aqueous medium, the title compound was immediately used in the next step without further purification. ^{1}H NMR (DMSO-d_6) δ 1.09 (t, *J* = 7.0 Hz, 3H, CH$_2$C*H*$_3$), 1.51 (d, *J* = 6.5 Hz, 3H, CHC*H*$_3$), 3.31–3.55 (m, 2H, C*H*$_2$CH$_3$), 4.84 (dq, *J* = 9.5 Hz/6.4 Hz, 1H, C*H*CH$_3$), 6.99 (dd, *J* = 9.4 Hz/4.2 Hz, 1H, 5-*H*), 7.31 (dd, *J* = 9.4 Hz/8.2 Hz/3.2 Hz, 1H, 6-*H*), 7.37 (dd, *J* = 7.7 Hz/3.2 Hz, 1H, 8-*H*), 8.06 (d, *J* = 9.4 Hz, 1H, N*H*). ^{13}C NMR (DMSO-d_6) δ 12.3 (CH$_2$*C*H$_3$), 19.3 (CH*C*H$_3$), 41.2 (*C*H$_2$CH$_3$), 66.5 (*C*HCH$_3$), 110.1 (d, *J* = 24 Hz, C-8), 116.4 (d, *J* = 7 Hz, C-5), 120.8 (d, *J* = 23 Hz, C-6), 122.9 (d, *J* = 6 Hz, C-8a), 140.1 (C-4a), 151.9–153.8 (d, *J* = 238 Hz, C-7).

4.1.6. R/S-4-CYCLOPROPYL-7-FLUORO-3-METHYL-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (11C)

The title compound was obtained as described for **11a** starting from **10c** obtained as previously described [21]. Due to its instability in aqueous medium, the title compound was immediately used in the next step without further purification. ^{1}H NMR (DMSO-d_6) δ 0.45 (m, 1H, CH (C*H*$_2$)$_2$), 0.78 (m, 1H, CH(C*H*$_2$)$_2$), 0.88 (m, 1H, CH(C*H*$_2$)$_2$), 1.01 (m, 1H, CH(C*H*$_2$)$_2$), 1.52 (d, *J* = 6.6 Hz, 3H, CHC*H*$_3$), 2.44 (tt, *J* = 6.8 Hz/3.8 Hz, 1H, C*H*(CH$_2$)$_2$), 4.80 (q, *J* = 6.7 Hz, 1H, C*H*CH$_3$), 7.25 (dd, *J* = 9.1 Hz/4.4 Hz, 1H, 5-*H*), 7.34–7.39 (m, 2H, 6-*H*/8-*H*), 8.07 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6) δ 7.6 (CH(*C*H$_2$)$_2$), 10.3 (CH(*C*H$_2$)$_2$), 16.8 (CH*C*H$_3$), 28.1 (*C*H(CH$_2$)$_2$), 67.6 (*C*HCH$_3$), 110.0 (d, *J* = 25 Hz, C-8), 117.4 (d, *J* = 7 Hz, C-5), 120.4 (d, *J* = 22 Hz, C-6), 123.2 (d, *J* = 6 Hz, C-8a), 140.1 (C-4a), 152.6–154.5 (d, *J* = 238 Hz, C-7). Anal. ($C_{11}H_{13}FN_2O_2S$) theoretical: C, 51.55; H, 5.11; N, 10.93; S, 12.51. Found: C, 51.63; H, 4.89; N, 11.04; S, 12.32.

4.1.7. SYNTHETIC PATHWAY TO R/S-4-ALKYL-7-FLUORO-2-METHYL-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDES 12A-C

The solution of the appropriate *R/S*-4-alkyl-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **11** (0.3 g, 1.2–1.3 mmol) in acetonitrile (20 mL) was supplemented with potassium carbonate

(1 g, 7.2 mmol) and methyl iodide (0.5 mL, 8 mmol). The resulting suspension was heated at 70− 80 °C under stirring for 1–3 h. The solvent and excess reagent were removed by distillation under reduced pressure and the residue was dissolved in a small volume of methanol. The addition to this stirred solution of an equal volume of distilled water led to the precipitation of the title compound, which was collected by filtration, washed with water, and dried (yields: 80–90%).

According to this general synthetic pathway, the compounds listed below were obtained.

4.1.7.1. ***R/S-7-fluoro-2,3,4-trimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (12a).***

White solid; m. p, 71–72 °C. ^{1}H NMR (DMSO-d_6) δ 1.54 (d, J = 6.6 Hz, 3H, CHCH_3), 2.63 (s, 3H, NCH_3), 2.95 (s, 3H, SO_2NCH_3), 5.11 (q, J = 6.6 Hz, 1H, CHCH_3), 6.99 (dd, J = 9.3 Hz/4.2 Hz, 1H, 5-*H*), 7.38 (td, J = 8.8 Hz/3.1 Hz, 1H, 6-*H*), 7.43 (dd, J = 7.8 Hz/3.1 Hz, 1H, 8-*H*). ^{13}C NMR (DMSO-d_6) δ 17.7 (CHCH$_3$), 32.5 (NCH$_3$), 34.7 (SO_2NCH$_3$), 72.6 (CHCH$_3$), 111.4 (d, J = 24 Hz, C-8), 116.4 (d, J = 7 Hz, C-5), 119.9 (d, J = 6 Hz, C-8a), 121.4 (d, J = 23 Hz, C-6), 140.2 (C-4a), 152.6–154.5 (d, J = 238 Hz, C-7). Anal. ($C_{10}H_{13}FN_2O_2S$) theoretical: C, 49.17; H, 5.36; N, 11.47; S, 13.12. Found: C, 49.09; H, 5.24; N, 11.53; S, 13.17.

4.1.7.2. ***R/S-4-ethyl-7-fluoro-2,3-dimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (12b).***

White solid; m. p, 63–65 °C. ^{1}H NMR (DMSO-d_6) δ 1.10 (t, J = 7 Hz, 3H, CH_2CH_3), 1.57 (d, J = 6.6 Hz, 3H, CHCH_3), 2.59 (s, 3H, NCH_3), 3.37 (m, 1H, CH*a*CH$_3$), 3.55 (m, 1H, CH*b*CH$_3$), 5.16 (q, J = 6.7 Hz, 1H, CHCH$_3$), 7.02 (dd, J = 9.4 Hz/4.2 Hz, 1H, 5-*H*), 7.36 (dd, J = 9.5 Hz/8.2 Hz/3.2 Hz, 1H, 6-*H*), 7.41 (dd, J = 7.8 Hz/3.1 Hz, 1H, 8-*H*). ^{13}C NMR (DMSO-d_6) δ 12.2 (CH_2CH$_3$), 18.1 (CHCH$_3$), 32.1 (NCH$_3$), 40.7 (CH$_2$CH$_3$), 71.2 (CHCH$_3$), 111.5 (d, J = 24 Hz, C-8), 116.0 (d, J = 7 Hz, C-5), 119.3 (d, J = 6 Hz, C-8a), 121.4 (d, J = 23 Hz, C-6), 138.7 (C-4a), 152.1–154.0 (d, J = 238 Hz, C-7). Anal. ($C_{11}H_{15}FN_2O_2S$) theoretical: C, 51.15; H, 5.85; N, 10.84; S, 12.41. Found: C, 50.79; H, 5.54; N, 11.02; S, 12.15.

4.1.7.3. ***R/S-4-cyclopropyl-7-fluoro-2,3-dimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (12c).***

White solid; m. p, 149–151 °C. ^{1}H NMR (DMSO-d_6) δ 0.57 (m, 1H, CH(CH_2)$_2$), 0.73 (m, 1H, CH(CH_2)$_2$), 0.89 (m, 1H, CH(CH_2)$_2$), 1.03 (m, 1H, CH(CH_2)$_2$), 1.56 (d, J = 6.8 Hz, 3H, CHCH_3), 2.46 (dq, J = 6.8 Hz/3.4 Hz, 1H, CH(CH$_2$)$_2$), 2.68 (s, 3H, NCH_3), 4.96 (q, J = 6.9 Hz, 1H, CHCH$_3$), 7.33 (dd, J = 9.3 Hz/4.4 Hz, 1H, 5-*H*), 7.38–7.45 (m, 2H, 6-*H*/8-*H*). ^{13}C NMR (DMSO-d_6) δ 7.5 (CH(CH$_2$)$_2$), 10.0 (CH(CH$_2$)$_2$), 17.1 (CHCH$_3$), 28.2 (CH(CH$_2$)$_2$), 34.9 (NCH$_3$), 73.8 (CHCH$_3$), 111.1 (d, J = 24 Hz, C-8), 117.3 (d, J = 7 Hz, C-5), 120.9 (d, J = 22 Hz, C-6), 121.0 (d, J = 6 Hz, C-8a), 139.2 (C-4a), 153.1–155.0 (d, J = 239 Hz, C-7). Anal. ($C_{12}H_{15}FN_2O_2S$) theoretical: C, 53.32; H, 5.59; N, 10.36; S, 11.86. Found: C, 53.21; H, 5.33; N, 10.47; S, 11.64.

4.1.8. R/S-7-CHLORO-2-ETHYL-3,4-DIMETHYL-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (12E)

The title compound was obtained as described for **12a-c** starting from *R/S*-7-chloro-4-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **11d** [24] and ethyl iodide instead of methyl iodide (yields: 70%). White solid; m. p, 100–102 °C. ^{1}H NMR (DMSO-d_6) δ 1.14 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.55 (d, J = 6.7 Hz, 3H, CHCH_3), 2.95 (m, 4H, CH*a*CH$_3$/NCH_3), 3.25 (m, 1H, CH*b*CH$_3$), 5.10 (q, J = 6.8 Hz, 1H, CHCH$_3$), 6.91 (d, J = 9.1 Hz, 1H, 5-*H*), 7.47 (dd, J = 9.1 Hz/2.6 Hz, 1H, 6-*H*), 7.54 (d, J = 2.6 Hz, 1H, 8-*H*). ^{13}C NMR (DMSO-d_6) δ 14.8 (CH_2CH_3), 18.2 (CHCH$_3$), 34.8 (NCH$_3$), 42.9 (CH$_2$CH$_3$), 72.6 (CHCH$_3$), 115.9 (C-

8), 119.9 (C-7), 121.3 (C-8a), 124.0 (C-8), 133.3 (C-6), 141.5 (C-4a). Anal. ($C_{11}H_{15}ClN_2O_2S$) theoretical: C, 48.08; H, 5.50; N, 10.20; S, 11.67. Found: C, 48.29; H, 5.90; N, 10.19; S, 11.09.

4.1.9. R/S-7-CHLORO-2,4-DIETHYL-3-METHYL-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (12 G)

The title compound was obtained as described for **12e** starting from *R/S*-7-chloro-4-ethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **11e** [24] (yields: 70%). White solid; m. p, 72–74 °C. ^{1}H NMR (DMSO-d_6) δ 1.10 (t, J = 7.0 Hz, 3H, $SO_2NCH_2CH_3$), 1.16 (t, J = 7.1 Hz, 3H, NCH_2CH_3), 1.58 (d, J = 6.7 Hz, 3H, $CHCH_3$), 2.83 (m, 1H, $SO_2NCHaCH_3$), 3.21 (m, 1H, $SO_2NCHbCH_3$), 3.38 (m, 1H, $NCHaCH_3$), 3.54 (m, 1H, $NCHbCH_3$), 5.14 (q, J = 6.7 Hz, 1H, $CHCH_3$), 6.96 (d, J = 9.3 Hz, 1H, 5-*H*), 7.45 (dd, J = 9.2 Hz/2.7 Hz, 1H, 6-*H*), 7.53 (d, J = 2.6 Hz, 1H, 8-*H*).

^{13}C NMR (DMSO-d_6) δ 12.1 ($SO_2NCH_2CH_3$), 15.0 (NCH_2CH_3), 19.3 ($CHCH_3$), 41.3 ($SO_2NCH_2CH_3$), 42.5 (NCH_2CH_3), 71.4 ($CHCH_3$), 115.8 (C-8), 119.6 (C-7), 120.9 (C-8a), 124.4 (C-8), 133.5 (C-6), 140.4 (C-4a). Anal. ($C_{12}H_{17}ClN_2O_2S$) theoretical: C, 49.91; H, 5.93; N, 9.70; S, 11.10. Found: C, 49.99; H, 5.83; N, 9.75; S, 10.56.

4.1.10. 7-CHLORO-4-CYCLOPROPYL-2-(FLUOROMETHYL)-3-METHYL-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (12I)

The solution of *R/S*-7-chloro-4-cyclopropyl-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide 11f [24] (0.35 g, 1.28 mmol) in acetonitrile was supplemented with potassium carbonate (0.7 g, 5.07 mmol) and bromofluoromethane (0.5 mL, 7.97 mmol). The reaction mixture was stirred in a sealed vessel at room temperature for 24 h. The solvent and excess of reagent were removed by distillation under reduced pressure and the residue was treated with water (10 mL). The resulting suspension of the title compound was collected by filtration, washed with water and dried (yields: 70–80%). White solid; m. p, 126–127 °C. ^{1}H NMR (DMSO-d_6) δ 0.49 (m, 1H, $CH(CH_2)_2$), 0.91 (m, 2H, $CH(CH_2)_2$), 1.04 (m, 1H, $CH(CH_2)_2$), 1.61 (d, J = 6.8 Hz, 3H, $CHCH_3$), 2.52 (m, 1H, $CH(CH_2)_2$), 5.42 (q, J = 6.8 Hz, 1H, $CHCH_3$), 5.50–5.71 (m, 2H, CH_2F), 7.32 (d, J = 9.2 Hz, 1H, 5-H), 7.54 (dd, J = 9.2 Hz/2.6 Hz, 1H, 6-H), 7.64 (d, J = 2.5 Hz, 1H, 8-H). ^{13}C NMR (DMSO-d_6) δ 7.3 ($CH(CH_2)_2$), 10.1 ($CH(CH_2)_2$), 17.9 (CH_3), 29.0 ($CH(CH_2)_2$), 73.1 ($CHCH_3$), 88.7–90.3 (d, J = 205 Hz, CH_2F), 117.6 (C-5), 121.3 (C-7), 122.7 (C-8a), 123.2 (C-8), 133.4 (C-6), 141.2 (C-4a). Anal. ($C_{12}H_{14}ClFN_2O_2S$) theoretical: C, 47.29; H, 4.63; N, 9.19; S, 10.52. Found: C, 46.94; H, 4.46; N, 9.21; S, 10.54.

4.1.11. 4-CYCLOPROPYL-7-FLUORO-2-METHYL-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (15A)

The solution of 4-cyclopropyl-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **14a** [21] (0.7 g, 2.89 mmol) in acetonitrile (25 mL) was supplemented with potassium carbonate (0.7 g, 5.07 mmol) and iodomethane (1 mL, 16.06 mmol). The reaction mixture was stirred in a closed vessel at 70 °C for 3 h. The solvent and excess of reagent were then removed by distillation under reduced pressure and the residue was treated with water (20 mL). The insoluble material of the title compound was collected by filtration, washed with water and recrystallized in methanol (yields: 65–70%). White solid; m. p, 130–131 °C. ^{1}H NMR (DMSO-d_6) δ 0.68 (m, 2H, $CH(CH_2)_2$), 0.91 (m, 2H, $CH(CH_2)_2$), 2.57 (tt, J = 6.8 Hz/3.7

Hz, 1H, C*H*(CH_2)$_2$), 2.63 (s, 3H, C*H*$_3$), 4.87 (s, 2H, NC*H*$_2$N), 7.35 (dd, *J* = 9.1 Hz/4.5 Hz, 1H, 5-*H*), 7.43 (m, 2H, 6-*H*/8-*H*). ^{13}C NMR (DMSO-d_6) δ 8.2 (CH(*C*H_2)$_2$), 29.7 (*C*H(CH_2)$_2$), 33.9 (*C*H_3), 66.8 (C-3), 111.2 (d, *J* = 24 Hz, C-8), 116.4 (d, *J* = 7 Hz, C-5), 119.6 (d, *J* = 6 Hz, C-8a), 121.0 (d, *J* = 23 Hz, C-6), 140.2 (C-4a), 153.0–154.9 (d, *J* = 239 Hz, C-7). Anal. ($C_{11}H_{13}FN_2O_2S$) theoretical: C, 51.55; H, 5.11; N, 10.93; S, 11.51. Found: C, 51.08; H, 5.04; N, 10.95; S, 12.36.

4.1.12. 4-CYCLOPROPYL-7-FLUORO-2-(FLUOROMETHYL)-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (15B)

The title compound was obtained as described for **12i** starting from 4-cyclopropyl-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **14a** [21] and recrystallized in methanol (yields: 65–70%). White solid: m. p, 125–127 °C. ^{1}H NMR (DMSO-d_6) δ 0.72 (m, 2H, CH (C*H*$_2$)$_2$), 0.92 (m, 2H, CH(C*H*$_2$)$_2$), 2.57 (tt, *J* = 6.8 Hz/3.7 Hz, 1H, C*H* (CH_2)$_2$), 5.15 (s, 2H, NC*H*$_2$N), 5.58 (d, *J* = 54.1 Hz, 2H, C*H*$_2$F), 7.35 (dd, *J* = 9.4 Hz/4.5 Hz, 1H, 5-*H*), 7.42 (dd, *J* = 9.0 Hz/8.5 Hz/3.0 Hz, 1H, 6-*H*), 7.51 (dd, *J* = 7.9 Hz/3.0 Hz, 1H, 8-*H*). ^{13}C NMR (DMSO-d_6) δ 8.5 (CH(*C*H_2)$_2$), 30.2 (*C*H(CH_2)$_2$), 65.0 (C-3), 88.2–89.9 (d, *J* = 204 Hz, *C*H_2F), 110.3 (d, *J* = 25 Hz, C-8), 117.0 (d, *J* = 7 Hz, C-5), 121.2 (d, *J* = 23 Hz, C-6), 122.2 (d, *J* = 6 Hz, C-8a), 140.9 (C-4a), 153.0–154.9 (d, *J* = 239 Hz, C-7). Anal. ($C_{11}H_{12}F_2N_2O_2S$) theoretical: C, 48.17; H, 4.41; N, 10.21; S, 11.69. Found: C, 47.86; H, 4.14; N, 10.20; S, 11.28.

4.1.13. 4-CYCLOPROPYL-2-(DIFLUOROMETHYL)-7-FLUORO-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (15C)

The solution of **14a** [21] (0.5 g, 2.06 mmol) in DMF (2 mL) was stirred on a water bath maintained at 15 °C and chlorodifluoromethane was bubbled for 4 min. Finely crushed potassium hydroxide (0.6 g, 10.69 mmol) was added under stirring and chlorodifluoromethane was bubbled for 5 min. After a few minutes, distilled water (20 mL) was added under stirring and the insoluble material of the title compound was collected by filtration, washed with water and recrystallized in acetone-water (yields: 30%). White solid; m. p, 93–94 °C. ^{1}H NMR (DMSO-d_6) δ 0.70 (m, 2H, CH(C*H*$_2$)$_2$), 0.95 (m, 2H, CH(C*H*$_2$)$_2$), 2.59 (tt, *J* = 6.8 Hz/3.7 Hz, 1H, C*H*(CH_2)$_2$), 5.15 (s, 2H, NC*H*$_2$N), 7.15 (t, *J* = 59.3 Hz, 1H, C*H*F$_2$), 7.40 (dd, *J* = 9.4 Hz/4.5 Hz, 1H, 5-*H*), 7.48 (dd, *J* = 9.4 Hz/8.3 Hz/3.0 Hz, 1H, 6-*H*), 7.60 (dd, *J* = 7.9 Hz/3.0 Hz, 1H, 8-*H*). ^{13}C NMR (DMSO-d_6) δ 8.3 (CH(*C*H_2)$_2$), 30.3 (*C*H(CH_2)$_2$), 60.0 (C-3), 110.5 (d, *J* = 25 Hz, C-8), 117.3 (d, *J* = 7 Hz, C-5), 121.3 (d, *J* = 7 Hz, C-8a), 121.9 (d, *J* = 23 Hz, C-6), 140.4 (C-4a), 153.2–155.1 (d, *J* = 239 Hz, C-7). Anal. ($C_{11}H_{11}F_3N_2O_2S$) theoretical: C, 45.20; H, 3.79; N, 9.58; S, 10.97. Found: C, 44.89; H, 3.65; N, 9.75; S, 10.75.

4.1.14. 7-CHLORO-4-CYCLOPROPYL-2-(FLUOROMETHYL)-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (15E)

The title compound was obtained as described for **12i** starting from 7-chloro-4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **14b** [21] (yields: 85–90%). White solid: m. p, 139–140 °C. ^{1}H NMR (DMSO-d_6) δ 0.73 (m, 2H, CH(C*H*$_2$)$_2$), 0.93 (m, 2H, CH(C*H*$_2$)$_2$), 2.60 (tt, *J* = 6.7 Hz/3.7 Hz, 1H, C*H*(CH_2)$_2$), 5.18 (s, 2H, NC*H*$_2$N), 5.58n(d, *J* = 54.1 Hz, 2H, C*H*$_2$F), 7.33 (d, *J* = 9.2 Hz, 1H, 5-*H*), 7.54 (dd, *J* = 9.2 Hz/2.6 Hz, 1H, 6-*H*), 7.63 (d, *J* = 2.5 Hz, 1H, 8-*H*). ^{13}C NMR (DMSO-d_6) δ 8.4 (CH(*C*H_2)$_2$), 29.9 (*C*H(CH_2)$_2$), 64.8 (C-3), 88.2–89.9 (CH_2F), 116.9 (C-5), 121.1 (C-7), 123.0 (C-8a), 123.1 (C-8), 133.3 (C-6),

142.7 (C-4a). Anal. ($C_{11}H_{12}ClFN_2O_2S$) theoretical: C, 45.44; H, 4.16; N, 9.64; S, 11.03. Found: C, 45.37; H, 4.19; N, 10.02; S, 11.54.

4.1.15. 7-CHLORO-4-CYCLOPROPYL-2-(DIFLUOROMETHYL)-3,4-DIHYDRO-2H-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE (15F)

The title compound was obtained as described for **15c** starting from 7-chloro-4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **14b** [21] (yields: 25%). White solid: m. p, 110–112 °C. ^{1}H NMR (DMSO-d_6) δ 0.71 (m, 2H, CH(CH_2)$_2$), 0.93 (m, 2H, CH(CH_2)$_2$), 2.62 (tt, J = 6.8 Hz/3.6 Hz, 1H, CH(CH$_2$)$_2$), 5.18 (s, 2H, NCH_2N), 7.15 (t, J = 59.2 Hz, 1H, CHF$_2$), 7.38 (d, J = 9.2 Hz, 1H, 5-*H*), 7.60 (dd, J = 9.1 Hz/2.5 Hz, 1H, 6-*H*), 7.72 (d, J = 2.5 Hz, 1H, 8-*H*). ^{13}C NMR (DMSO-d_6) δ 8.2 (CH(*C*H$_2$)$_2$), 30.1 (*C*H(CH$_2$)$_2$), 59.6 (C-3), 117.1 (C-5), 121.5 (C-7), 122.0 (C-8a), 123.3 (C-8), 133.9 (C-6), 142.2 (C-4a). Anal. ($C_{11}H_{11}ClF_2N_2O_2S$) theoretical: C, 42.79; H, 3.59; N, 9.07; S, 10.38. Found: C, 42.69; H, 3.56; N, 9.19; S, 10.17.

4.2. STABILITY STUDY IN DMSO-D_6/D_2O

Compound **15e** (± 1 mg) and maleic acid (± 1 mg; internal reference) introduced in an NMR tube were solubilized in DMSO-d_6 (0.5 mL). Then D_2O (0.5 mL) was added, and the solution was homogenized just before starting the NMR recordings at room temperature (Bruker 500 MHz). The integrations of the peaks of maleic acid (singlet at 6.33 ppm) and the N^2–CH_2–N^4 methylene protons of **15e** (singlet at 5.19 ppm) were recorded at different time periods (T = 0, T = 5 h, T = 24 h, T = 48 h). The ratio of the peak surface of maleic acid with the peak surface of the N^2–CH_2–N^4 methylene protons of **15e** at T = 0 corresponded to 100% of the starting compound **15e**. The same ratio measured at different time periods allowed the calculation of the residual % of **15e** in solution. The presence of new species in solution was made visible by the appearance of a new singlet at 5.00 ppm (probably the N^2–CH_2–N^4 methylene protons of **20**) and later another singlet at 4.74 ppm (N^2–CH_2–N^4 methylene protons of **3** as certified by the NMR recording of an authentic sample of **3** in DMSO-d_6/D_2O 1:1). The same protocol was followed with compound **15b**.

4.3. BIOLOGICAL EVALUATION

4.3.1. EFFECT ON AMPA-EVOKED MEMBRANE DEPOLARIZATION (IN VITRO FLUORESCENCE ASSAY)

This assay investigating AMPA-evoked membrane depolarization, measured by fluorescent membrane potential dyes and an imaging-based plate reader on rat primary brain cultures, was achieved following our previously published procedure [18].

4.3.2. FLUORESCENCE-BASED CALCIUM ASSAY ON GLUA2(Q) CELLS

HEK293 cells stably expressing AMPA tetrameric channel form A2 (Q) flop isoform (GluA2o(Q)) were prepared and used in the fluorescence-based Calcium assay as previously described [26,27].

4.3.3. EFFECT ON AMPA-MEDIATED RELEASE OF NORADRENALINE ON RAT HIPPOCAMPAL SLICES

Potentiation of noradrenaline release on rat hippocampal slices was measured according to our previously reported procedure [20].

4.3.4. EFFECT IN VIVO OF ON THE BODY TEMPERATURE IN NMRI MICE

The effect of compounds **3** and **15e** on the body temperature in male NMRI mice was measured after *per os* administration (30 and 100 mg/ kg) according to previously described procedures [48–50].

4.3.5. EFFECT ON LONG-TERM POTENTIATION (LTP) OF THE POSTSYNAPTIC RESPONSE EVOKED IN THE DENTATE GYRUS ON ANESTHETIZED RATS

Extracellular excitatory postsynaptic field potentials (EPSfP) were recorded in the dentate gyrus using our previously published procedure [18].

4.3.6. EFFECT ON OBJECT RECOGNITION TEST IN MICE

The one-trial object recognition paradigm measures a form of episodic memory in the mouse and was achieved following our previously described procedure [17,18]. The effects of compounds **3** and **15e** was measured in CD1 mice after *per os* administration (1 and 3 mg/kg).

4.3.7. EFFECTS ON THE SPATIAL WORKING MEMORY (SPONTANEOUS ALTERNATION TEST) IN C57BI/6 MICE

The spontaneous alteration in a T-maze in mice was performed as previously described [47]. The tested compounds **3** and **15e** were administered in C57BI/6 mice by intraperitoneal injection (0.3, 1 and 3 mg/kg).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pirotte Bernard reports financial support was provided by Laboratoires Servier. Pirotte Bernard reports a relationship with Laboratoires Servier that includes: funding grants.

Data availability

Data will be made available on request.

Acknowledgements

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Appendix A. Supplementary data

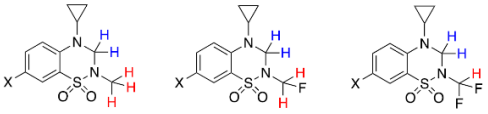
Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2023.115221.

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	AMPA receptor
AMPAR PAM	AMPAR positive allosteric modulator
BDNF	brain-derived neurotrophic factor
BTD	3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide
CTD	C-terminal domain
EPSfP	excitatory postsynaptic field potential
HEK	human embryonic kidney
FlipR	fluorescent imaging plate reader
iGluR	ionotropic glutamate receptor
KA	kainic acid
LBD	ligand-binding domain
LTP	long-term potentiation
mGluR	metabotropic glutamate receptor
NA	noradrenaline
NMDA	*N*-methyl-$_D$-aspartic acid
NTD	N-terminal domain
TMD	transmembrane domain
TMS	tetramethylsilane

Tables

Table 1. *Chemical shifts of the protons located on the intracyclic C^3 carbon atom (in blue) and on the exocyclic carbon atom linked at the 2-position of the thiadiazine ring (in red).*

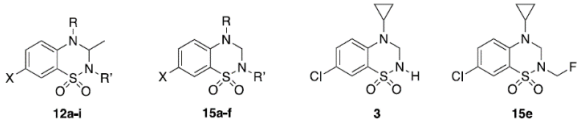


15a,15d **15b,15e** **15c,15f**

compound	X	N^2–CH_2–N^4 protons	N^2–CH–Y protons
15a	F	4.87 (s)	2.63 (s)
15d	Cl	4.90 (s)[a]	2.62 (s)[a]
15b	F	5.15 (s)	5.58 (d)
15e	Cl	5.18 (s)	5.58 (d)
15c	F	5.15 (s)	7.15 (t)
15f	Cl	5.18 (s)	7.15 (t)

a NMR data from ref. 24.

Table 2. *Effects of 2,4-dialkyl-, and 2,3,4-trialkyl-substituted 7-halo-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxides on the fluorescence induced by 300 µM AMPA on primary cultures of neurons from rat embryonic cortex (FlipR/FDSS), on the calcium influx induced by 1 mM glutamate on HEK293 cells stably expressing the GluA2 (Q) subunit, and on AMPA-mediated presynaptic noradrenaline (NA) release on rat hippocampal slices.*



12a-i **15a-f** **3** **15e**

	X	R	R'	EC2x (FlipR[a]/FDSS[b]) (µM)	Ca^{2+} influx[c] pEC_{50} (M)	NA rel.[d] (% increase)
12a	F	CH_3	CH_3	4.1 [2.8; 5.9] (2)[a]	4.82 ± 0.17	167
12b	F	CH_2CH_3	CH_3	1.4 [0.09–22.5] (3)[b]	5.06 ± 0.23	135
12c	F	$CH(CH_2)_2$	CH_3	70.9 [53.5; 93.9] (2)[a]	<4.00	<100
4 (12d)[e]	Cl	CH_3	CH_3	4.3 [3.3–5.6] (2)[a,f]	4.09 ± 0.79	112
12e	Cl	CH_3	CH_2CH_3	11.2 [1.3; 95.2] (3)[b]	<4.00	<100
12f[e]	Cl	CH_2CH_3	CH_3	6.2 [3.0; 12.6] (3)[a]	4.50 ± 0.27	103
12g	Cl	CH_2CH_3	CH_2CH_3	12.2 [1.6; 93.8] (3)[b]	<4.00	<100
12h[e]	Cl	$CH(CH_2)_2$	CH_3	65 (1)[a]	<4.00	<100
12i	Cl	$CH(CH_2)_2$	CH_2F	2.5 [2.1; 3.0] (2)[a]	5.67 ± 0.12	329
15a	F	$CH(CH_2)_2$	CH_3	9.3 [7.0; 12.3] (2)[a]	4.24 ± 0.47	<100
15b	F	$CH(CH_2)_2$	CH_2F	0.6 [0.5; 0.8] (2)[a]	6.55 ± 0.89	427
15c	F	$CH(CH_2)_2$	CHF_2	6.9 [1.1; 43.1] (2)[b]	4.78 ± 0.10	345
15d[e]	Cl	$CH(CH_2)_2$	CH_3	13.4 [7.7; 23.3] (3)[a]	<4.00	–
15e	Cl	$CH(CH_2)_2$	CH_2F	2.1 [0.6; 7.3] (2)[a,f]	5.66 ± 0.07	394
15f	Cl	$CH(CH_2)_2$	CHF_2	6.0 [6.0; 6.0] (2)[b]	<4.00	340
8 (14a)[e]	F	$CH(CH_2)_2$	H	0.2 [0.04; 1.3] (2)[a,f]	6.56 ± 0.30	230
3 (14b)[e]	Cl	$CH(CH_2)_2$	H	2.2 [1.3; 3.8] (2)[a,f]	6.10 ± 0.11	311

[a] EC2x: concentration of modulator giving a 2-fold increase of the fluorescence induced by AMPA (300 µM) with the FlipR method on rat primary brain cultures (n = 2–3); results are expressed as the geometric mean with its 95% confidence intervals in brackets.

[b] EC2x values obtained by the FDSS fluorescence assay, a variant of the FlipR fluorescence assay using another plate reader.

[c] pEC50: negative logarithm of AMPAR potentiator concentration responsible for 50% of the maximal effect (mean ± SEM (n ≥ 3)).

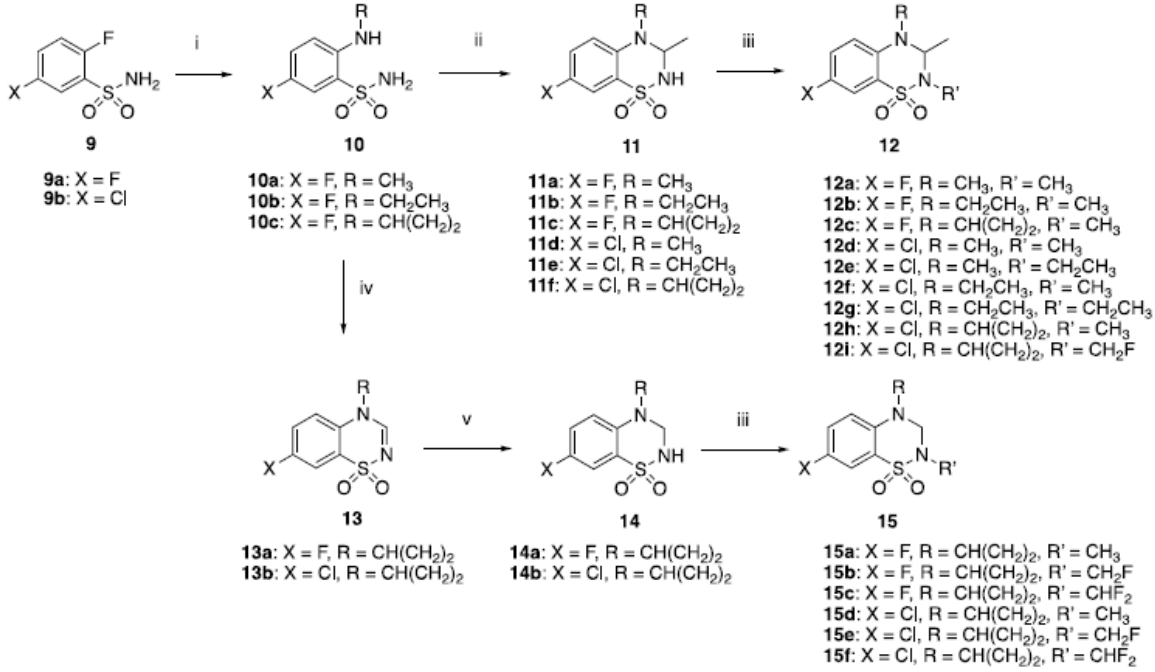
[d] NA rel: potentiation of noradrenaline release on rat hippocampal slices; 100% corresponds to AMPA alone.

[e] Published compounds [21,24].

[f] These compounds tested with the FDSS fluorescence assay gave the following results; 4: 0.8 µM [0.6; 1.1] (5), 15e: 1.5 µM [1.1; 2.0] (5), 8: 0.3 µM [0.2; 0.5] (3), 3: 0.7 µM [0.3; 1.8] (4).

Scheme

Scheme 1. *Synthetic pathways to the target compounds* ***12*** *and* ***15***. ***Reagents****: (i) R–NH_2, dioxane, 100–110 °C, 24 h; (ii) CH_3CHO, H^+, CH_3CN, rt, 1–2 h; (iii) R' = CH_3, CH_2F, CH_2CH_3: R' -X, K_2CO_3, CH_3CN, 70–80 °C, 1–3 h; R' = CHF_2: R' -X, KOH, DMF, rt; (iv) $HC(OEt)_3$, 130–150 °C, 24–48 h; (v) $NaBH_4$, isopropanol, 50–55 °C, 5–10 min.*



Figures

Fig. 1. *Examples of 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxides (**1**–**6**, **8**) and one example of thiochroman 1,1-dioxide (**7**) reported as potent AMPAR PAMs.*

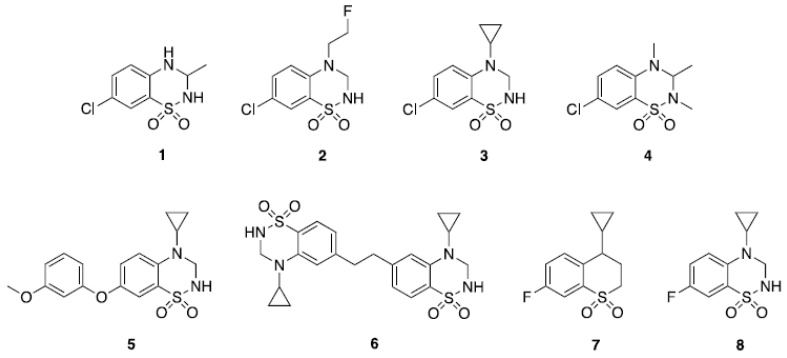


Fig. 2. *Examples of compounds bearing a monofluoromethyl or a difluoromethyl moiety on a heteroatom (O, N, S): **16**: the clinically used corticoid fluticasone propionate; **17**: the clinically used general anesthetic sevoflurane; **18**: example of N-difluoromethylbenzenesulfonamide; **19**: example of N-fluoromethylbenzenesulfonamide.*

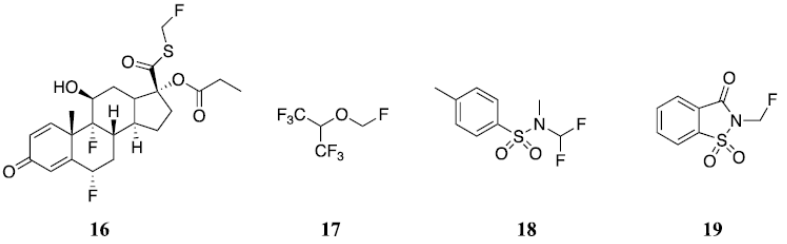


Fig. 3. *Hypothetic slow conversion of **15e** in aqueous medium into the corresponding 2-hydroxymethyl-substituted analogue **20**, and into the unsubstituted analogue **3**.*

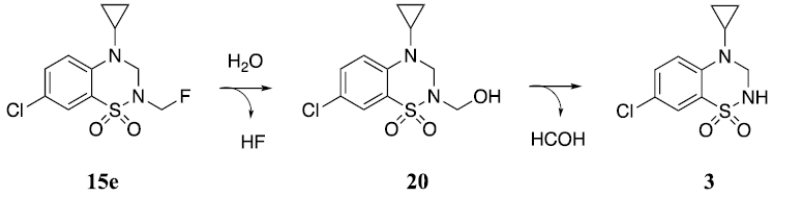


Fig. 4. *Effects of compound **3** and compound **15e** on the noradrenaline release from rat hippocampal slices induced by AMPA (300 µM). Results are expressed as % release compared to AMPA alone corresponding to 100%. EC2x corresponds to the concentration of drug responsible for a 2-fold increase of NA release (or 200%).*

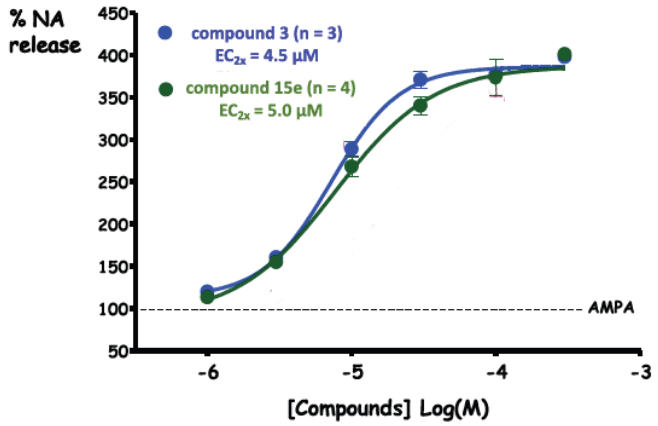


Fig. 5. *Update of structure-activity relationships established for the 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (BTD) class of AMPA receptor positive allosteric modulators according to the newly reported in vitro results.*

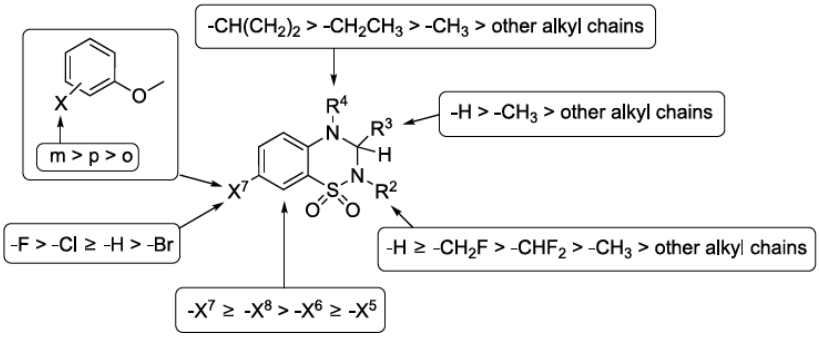


Fig. 6. *Effect of compound **3** and compound **15e** after per os administration (30 mg/kg and 100 mg/kg) on the body temperature In NMRI mice. Data are expressed as means ± sem (n = 5/group) (**p ≤ 0.01 and ***p ≤ 0.001 versus vehicle controls [Dunnett test after a significant two-way (time × treatment) ANOVA]).*

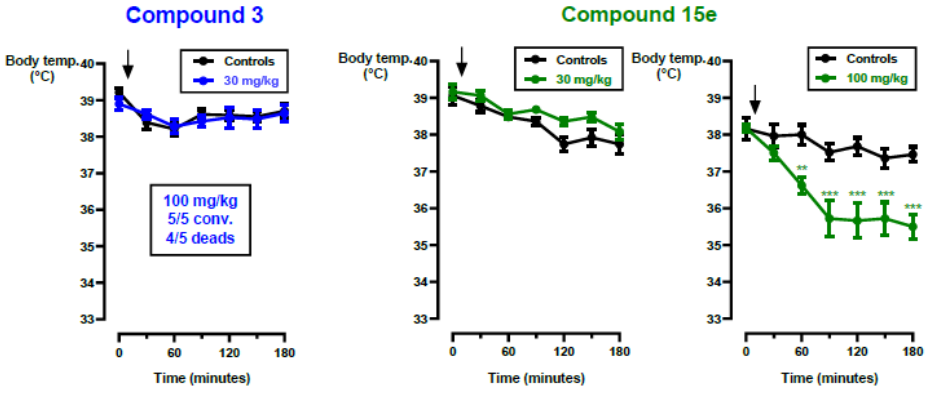


Fig. 7. *Post-synaptic response induced in vivo in the anesthetized rat. Effect of compound **3** (3 and 10 mg/ kg i. p.) and compound **15e** (10 and 30 mg/kg i. p.) on the potentiation of the synaptic response evoked in the dentate gyrus of the hippocampus by a high-frequency stimulation (HFS) delivered in the perforant path. In each rat, synaptic responses were averaged over eight successive stimulations (every 2 min) and normalized to the average amplitude of synaptic responses obtained during the first 60 min baseline period before the injection of the compounds (control value taken as 100%). Results are expressed as mean ± SEM. *p ≤ 0.05 versus vehicle [Dunnett test after a significant two-way (time × treatment) ANOVA].*

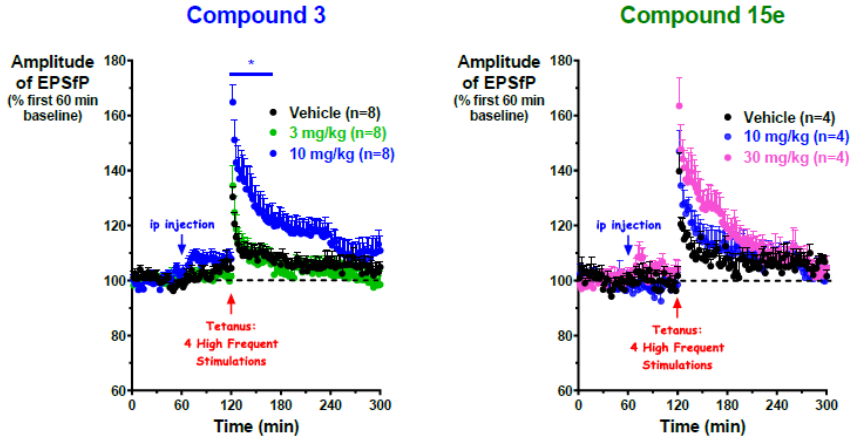


Fig. 8. *Effect of compound **3** and compound **15e** (1 and 3 mg/kg, per os) on the exploration time spent on either the Familiar or the New object during the recognition phase in the object recognition task in CD1 mice. During the recognition phase, compound **3** and compound **15e** at either 1 or 3 mg/kg per os increased the duration exploration of the new object versus the familiar one, whereas there is no difference in the vehicle-treated group (on the left). The number in brackets refers to the number of animals. For compound **3**, the % of effect was +40%, +82% and +79% for vehicle, 1 and 3 mg/kg, respectively. For compound **15e**, the % of effect was –3%, +64% and +78% for vehicle, 1 and 3 mg/kg, respectively. When considering difference of exploration between New and Familiar objects (New-Fam) (on the right), the two compounds at both 1 and 3 mg/kg increased this parameter. For compound **3**, ANOVA was just above statistical significance (p = 0.055); but the difference (New-Fam) was significant at the dose of 1 and 3 mg/ kg (p = 0.08 and p = 0.05 versus vehicle controls, Dunnett). For compound **15e**, ANOVA reached statistical significance (p ≤ 0.01), with a statistically significant difference (New-Fam) at both 1 and 3 mg/ kg (*p ≤ 0.05 and **p ≤ 0.01 respectively, versus vehicle controls, Dunnett test).*

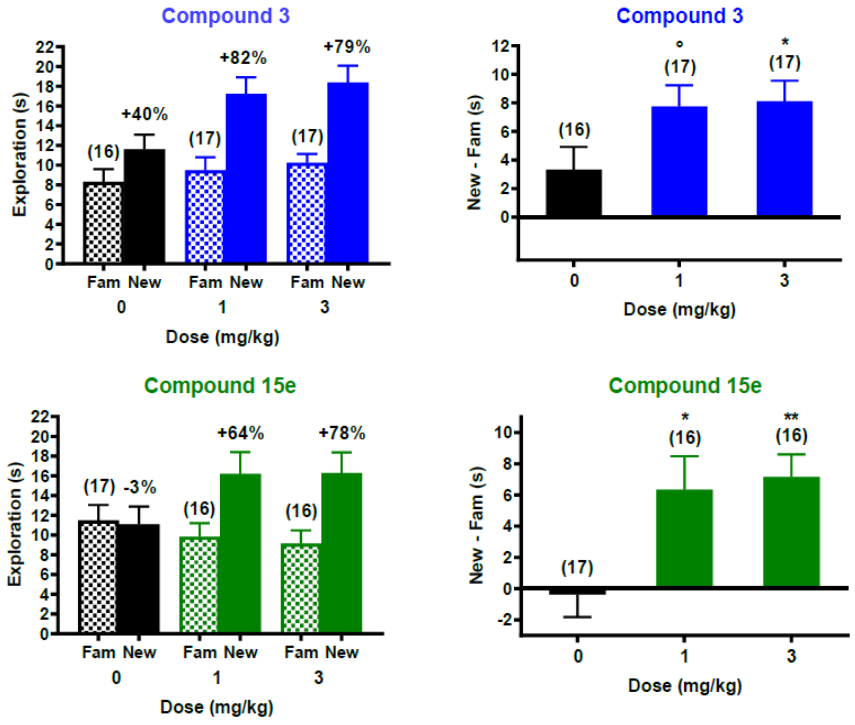
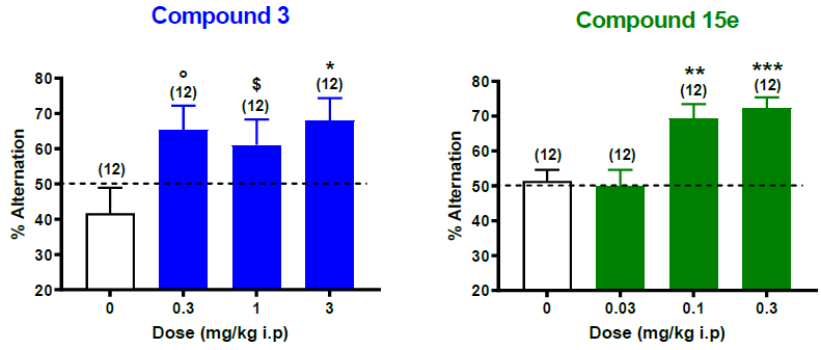


Fig. 9. *Effects of compound* ***3*** *(0.3, 1 and 3 mg/kg i. p.) and compound* ***15e*** *(0.03, 0.1 and 0.3 mg/kg i. p.) on the spontaneous alternation in T-maze in C57Bl/6 mice (spatial working memory). Histograms illustrate the mean ± SEM of the percentage of spontaneous alternation of mice [° p = 0.053; $ p = 0.133; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001 vs control 0 mg/kg group, Dunnett test after a significant one-way ANOVA].*



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