Structural Exploration around 4-Cyclopropyl-substituted 1,2,4-Benzothiadiazine 1,1-Dioxides: Impact of the Dihalo-substitution of the Benzene Ring on α-Amino-3-hydroxy-5-methylisoxazole-4-propionic Acid (AMPA) Receptor Potentiation

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KEYWORDS

Ionotropic glutamate receptors, positive allosteric modulators, mono/dihalo-substituted 4-cycloalkyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides, cognition improvement, neuroprotection

Dedication to Prof. Bernard Pirotte on the occasion of his retirement

ABSTRACT

The present study investigates the AMPA receptor potentiation and cognitive-enhancing effects of novel halosubstituted 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides. Mono-halosubstituted 4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides were initially identified for their promising activity. In this article, a new pharmacomodulation explored the addition of a second halogen atom on the benzene nucleus and the variation of the cycloalkyl group at the 4-position. Compound **14o** (the 6,7-dichloro-4-cyclopropyl-substituted derivative) was selected based on potency and safety. It selectively potentiated AMPA receptors in human and rat models, enhanced AMPA-mediated excitatory post-synaptic responses in rat hippocampal slices, and increased brain-derived neurotrophic factor (BDNF) expression in rat cortical neurons. *In vivo*, **14o** in animal models significantly improved long-term potentiation

(LTP) and enhanced cognitive performance in memory-related behavioral tasks at low doses. Notably, **14o** also exhibited neuroprotective effects in rats, delaying hippocampal neurodegeneration following transient ischemia, without proconvulsant activity. These findings support **14o** as a promising candidate for the treatment of cognitive disorders.

INTRODUCTION

L-Glutamate (L-Glu), the major excitatory neurotransmitter in the central nervous system (CNS), is involved in many central biological functions. Glutamatergic transmission is the key to the rapid synaptic excitation and synaptic plasticity.[1,2] It takes an essential part in memory and learning mechanisms.[1,2,3] Dysfunctional glutamatergic transmission can be responsible for neurological disorders.[1,2] Among these, Alzheimer's disease is a major public health concern according to the World Health Organization (WHO). Indeed, with the increase in life expectancy, an increase in the number of cases is expected over the next few years.[4]

Glutamatergic neural transmission involves both ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). Four iGluRs subtypes are key to excitatory synaptic transmission and hold a central significance in synaptic plasticity mechanisms: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs), kainic acid receptors (KARs), *N*-methyl-D-aspartic acid receptors (NMDARs) and delta receptors.[5,6,7,8]

AMPARs are ligand-gated ion channels which are assembled from four subunits (GluA1, GluA2, GluA3 and GluA4). Each of these subunits is made up of an *N*-terminal domain, a transmembrane domain, and a C-terminal domain. The extracellular *N*-terminal domain is followed by the ligand-binding domain (LBD).[9] Homo- or heterotetrameric receptors are formed by dimerization of subunit dimers.[9,10] L-Glu is the endogenous agonist of the AMPARs. It binds to the orthosteric agonist-binding site within the LBD and causes the channel to open.[9] These receptors are permeable to Na^+ and K^+ ions.[2] The permeability to Ca^{2+} ions is modulated

according to composition in subunits forming the ion channel.[2,9] Positive allosteric modulators of AMPARs (AMPAR PAMs) connect to the interface between two LBD regions, forming the allosteric site, and enhance the ionic current in the presence of agonists.[9] Given the variety of ways to fit subunits together and the different alternative splice isoforms of each subunit, a wide variety of AMPARs with different functional properties and synaptic responses are found in the CNS.[11]

AMPARs are involved in long-term potentiation (LTP) enabling memory consolidation and learning through stimulation of synaptic plasticity. Through their involvement in the cognitive process, AMPARs are attractive targets to fight against dementia.[12,13] Indeed, the cognitive impairment observed in the early stages of Alzheimer's disease is mainly due to a loss of functional synapses in the hippocampus.[14]

In this article, the cognitive enhancement potential of the AMPAR PAMs is probed. They have been postulated to be a more promising and secure option than AMPAR agonists. In fact, since the AMPAR PAMs binding site within the receptor is different from the orthosteric-binding site of the agonist, these compounds potentiate the agonist effect of L-Glu without having own intrinsic agonist activity. This property is interesting to reduce the risk of toxic effect by excitotoxicity observed in the events of AMPARs overactivation.[15,16] The AMPAR PAMs reduce AMPARs desensitization observed while L-Glu remains bound to the receptor. They can also allow to slow down receptors deactivation phenomenon observed after elimination of L-Glu at the receptor.[17,18] In summary, in the presence of L-Glu, these enhancers act as AMPAR stabilizers and ultimately promote synaptic transmission and neuronal plasticity by increasing the likelihood of inducing LTP.[19]

Initially used for its diuretic effect, cyclothiazide (**1**, Figure 1) showed a reduction of the L-Glu receptors desensitization.[20,21] Resulting from the saturation of the C=N double bond of the thiadiazine ring of diazoxide (**2**, Figure 1), IDRA-21 (**3**, Figure 1) manifested an improvement

of cognition performance *in vivo* in animal models thanks to its ability to cross the blood-brain barrier unlike cylcothiazide.[22,23] Following these discoveries, 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (BTDs) have been the subject of numerous studies in view of developing new allosteric modulators with improved specificity and potency.[22]

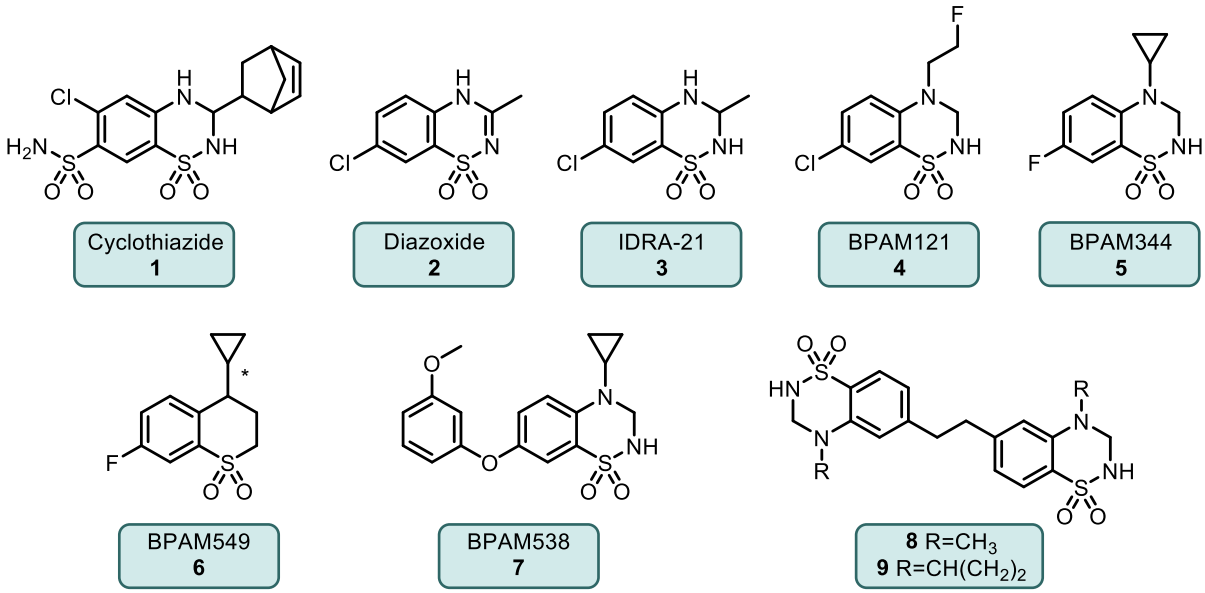


Figure 1. BTD-type modulators and isosteres acting as AMPAR PAMs: (**1**) cyclothiazide, (**2**) diazoxide, (**3**) IDRA-21, (**4**) BPAM121, (**5**) BPAM344, (**6**) BPAM549, (**7**) BPAM538, (**8**) and (**9**) dimeric BTD analogues.

These last years, many works were focused on the development of BTDs as AMPAR PAMs bearing an alkyl/cycloalkyl group at the 4-position and various substituents at the 7-position. BPAM121 (**4**, Figure 1), with a fluorine atom on the ethyl chain at the 4-position of the heterocycle, was synthesized so as to answer to the lack of metabolic stability of the corresponding compound with an ethyl chain.[24] The replacement of the 4-ethyl/4-fluoroethyl substituent by a 4-cyclopropyl group led to a 10-fold increase in *in vitro* potency. New compounds with various substituents at the 7-position were evaluated and the most potent BTD was the 7-fluoro-substituted compound BPAM344 (**5**, Figure 1).[25] The isostere concept was applied to this last compound leading to the emergence of the thiochroman 1,1-dioxide BPAM549 (**6,** Figure 1).[26] From X-ray data showing the proximity of the binding of two BTD

« monomers » at the LBD dimer interface of the AMPA receptors, BPAM538 (**7**, Figure 1) and dimers **8** and **9** (Figure 1) were developed and were found to exert an improved AMPAR potentiator activity.[27,28]

In the present paper, the 7-fluoro-4-cyclopropyl-substituted BTD **5** was taken as the starting point for the investigation of new pharmacomodulations with the aim at identifying new analogues with improved therapeutic promise. Compound **5** has already shown an improvement of the biological activity on AMPA receptors, but also on KA receptors.[29] However, the 7-chloro-substituted BTD analogue of **5** was chosen to be pre-clinically evaluated according to better benefit at the level of toxicological (Irwin's test) properties resulting from outstanding *in vitro* and *in vivo* activities.[30]

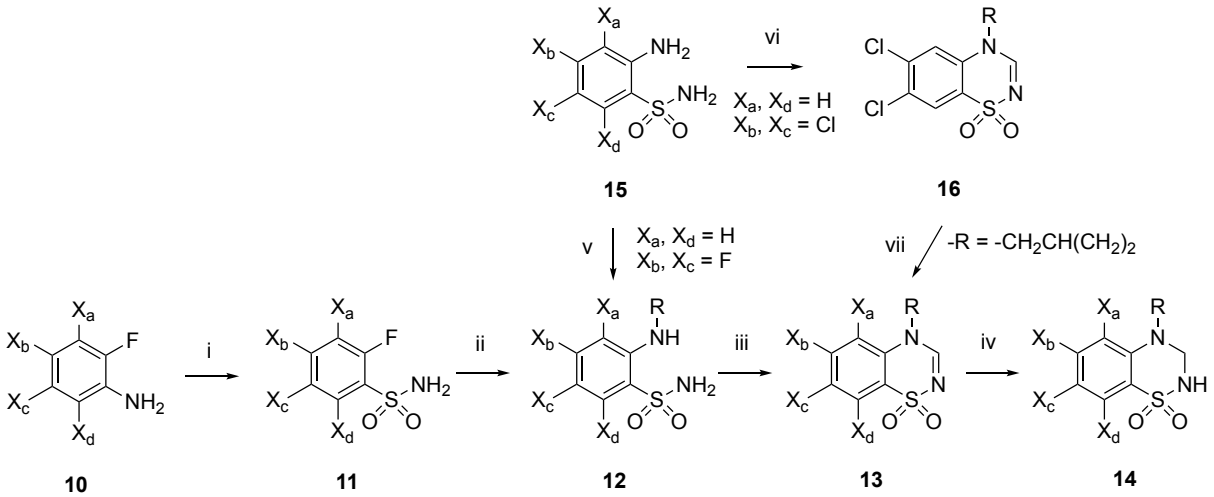
As part of our continuous effort to develop more potent drug candidates, the impact on biological activity of the nature and the position of a halogen atom on the BTD benzene ring (monosubstitution by F, Cl, Br) was first examined in this work and revealed that the best choice of halo-substituent was a fluorine or a chlorine atom. Di-substitution by chlorine or fluorine atoms linked to the benzene ring was therefore studied to probe the impact of this structural modification on biological activity and toxicity. Moreover, in previous works, the X-ray crystallographic studies of the molecular interaction of **5** at the ligand binding domain of the AMPAR GluA2 subunit (GluA2-LBD) showed that the cyclopropyl group at the 4-position possessed shape complementarity to its binding site that would explain differences in affinity compared to 4-ethyl-substituted analogues.[25] The influence of the size of the alicyclic ring at the 4-position was therefore also explored in order to study the impact of the steric hindrance in this position on AMPAR potentiator activity.

RESULTS AND DISCUSSION

Chemistry

The synthetic pathway to prepare the compounds of interest (**14**) is illustrated in Scheme 1. Most bromo-substituted and chloro/fluoro-disubstituted 4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides as well as 4-(cyclo)alkyl-substituted analogues were prepared starting from the appropriate commercially available mono/di-halo-substituted 2-fluoroaniline (**10**). These starting products were converted into the corresponding 2-fluorobenzenesulfonamides (**11**) through the formation of diazonium salts in the presence of cupric chloride and sulfur dioxide in acetic acid according to the Meerwein variation of the Sandmeyer reaction.[31] The nucleophilic substitution of the fluorine atom by cyclopropylamine or other cycloalkylamines gave access to intermediates **12**. The 4*H*-1,2,4-benzothiadiazine 1,1-dioxide intermediates **13** were obtained from ring closure of **12** by heating them in triethyl orthoformate. Reduction of the N2=C3 double bond with sodium borohydride in isopropanol led to the conversion of intermediates **13** into the corresponding saturated analogues **14.** The introduction of a cyclopropylamino group to obtain compound **12e** was performed starting from the commercially available 2-amino-4,5-difluorobenzenesulfonamide (**15a**): a hemiaminal ether intermediate was obtained by using (1-ethoxycyclopropyloxy)trimethylsilane followed by a transacetalization reaction in methanol. The cyclopropylamino group was finally formed on **12e** by reaction of the hemiaminal intermediate with boron trifluoride etherate. Intermediate **13w** was prepared by reaction of **16** with cyclopropylmethyl bromide in the presence of potassium carbonate. Intermediate **16** itself was obtained by ring closure of the commercially available 2-amino-4,5-dichlorobenzenesulfonamide **15b** in hot triethyl orthoformate.

Scheme 1. Synthetic pathway to the mono- and di-halosubstituted 4-cycloalkyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides.[a]



10a, **11a**: X_a=H, X_b=H, X_c=H, X_d=Br
10b, **11b**: X_a=H, X_b=Br, X_c=H, X_d=H
10c, **11c**: X_a=F, X_b=F, X_c=H, X_d=H
10d, **11d**: X_a=F, X_b=H, X_c=F, X_d=H
10e, **11e**: X_a=H, X_b=F, X_c=H, X_d=F
10f, **11f**: X_a=Cl, X_b=F, X_c=H, X_d=H
10g, **11g**: X_a=H, X_b=F, X_c=Cl, X_d=H
10h, **11h**: X_a=H, X_b=F, X_c=H, X_d=Cl
10i, **11i**: X_a=H, X_b=H, X_c=F, X_d=F
10j, **11j**: X_a=H, X_b=Cl, X_c=F, X_d=H
10k, **11k**: X_a=H, X_b=H, X_c=F, X_d=Cl
10l, **11l**: X_a=Cl, X_b=H, X_c=H, X_d=F
10m, **11m**: X_a=Cl, X_b=Cl, X_c=H, X_d=H
10n, **11n**: X_a=H, X_b=Cl, X_c=Cl, X_d=H

12a, **13a**, **14a**: X_a=H, X_b=H, X_c=H, X_d=Br, -R = -CH(CH_2)$_2$
12b, **13b**, **14b**: X_a=H, X_b=Br, X_c=H, X_d=H, -R = -CH(CH_2)$_2$
12c, **13c**, **14c**: X_a=F, X_b=F, X_c=H, X_d=H, -R = -CH(CH_2)$_2$
12d, **13d**, **14d**: X_a=F, X_b=H, X_c=F, X_d=H, -R = -CH(CH_2)$_2$
12e, **13e**, **14e**: X_a=H, X_b=F, X_c=F, X_d=H, -R = -CH(CH_2)$_2$
12f, **13f**, **14f**: X_a=H, X_b=F, X_c=H, X_d=F, -R = -CH(CH_2)$_2$
12g, **13g**, **14g**: X_a=Cl, X_b=F, X_c=H, X_d=H, -R = -CH(CH_2)$_2$
12h, **13h**, **14h**: X_a=H, X_b=F, X_c=Cl, X_d=H, -R = -CH(CH_2)$_2$
12i, **13i**, **14i**: X_a=H, X_b=F, X_c=H, X_d=Cl, -R = -CH(CH_2)$_2$
12j, **13j**, **14j**: X_a=H, X_b=H, X_c=F, X_d=F, -R = -CH(CH_2)$_2$
12k, **13k**, **14k**: X_a=H, X_b=Cl, X_c=F, X_d=H, -R = -CH(CH_2)$_2$
12l, **13l**, **14l**: X_a=H, X_b=H, X_c=F, X_d=Cl, -R = -CH(CH_2)$_2$
12m, **13m**, **14m**: X_a=Cl, X_b=H, X_c=H, X_d=F, -R = -CH(CH_2)$_2$
12n, **13n**, **14n**: X_a=Cl, X_b=Cl, X_c=H, X_d=H, -R = -CH(CH_2)$_2$
12o, **13o**, **14o**: X_a=H, X_b=Cl, X_c=Cl, X_d=H, -R = -CH(CH_2)$_2$
12p, **13p**, **14p**: X_a=H, X_b=Cl, X_c=Cl, X_d=H, -R = -C(CH_3)(CH_2)$_2$
12q, **13q**, **14q**: X_a=H, X_b=Cl, X_c=Cl, X_d=H, -R = -CH(CH_2)$_3$
12r, **13r**, **14r**: X_a=H, X_b=Cl, X_c=Cl, X_d=H, -R = -CH(CH_2)$_4$
12s, **13s**, **14s**: X_a=H, X_b=Cl, X_c=Cl, X_d=H, -R = -CH(CH_2)$_5$
12t, **13t**, **14t**: X_a=H, X_b=Cl, X_c=Cl, X_d=H, -R = -CH(CH_2)$_6$
12u, **13u**, **14u**: X_a=H, X_b=Cl, X_c=Cl, X_d=H, -R = -CH(CH_2)$_7$
12v, **13v**, **14v**: X_a=H, X_b=Cl, X_c=Cl, X_d=H, -R = -CH(CH_3)$_2$
13w, **14w**: X_a=H, X_b=Cl, X_c=Cl, X_d=H, -R = -CH_2CH(CH_2)$_2$

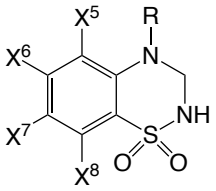
[a]Reagents and conditions: (i) $NaNO_2$, HCl/HOAc, -5 °C; SO_2, $CuCl_2$, HOAc, 0 °C, 15 min; NH_4OH, dioxane, 0 °C; (ii) cyclopropylamine or (cyclo)alkylamine, dioxane, 80-110 °C, 24-72 h; (iii) triethyl orthoformate, 130-150 °C, 1-72 h; (iv) $NaBH_4$, isopropanol, 50-55 °C, 5-10 min; (v) (1-ethoxycyclopropyloxy)trimethylsilane, HOAc, MeOH, reflux, 24 h; $NaBH_4$, BF_3.Et_2O, THF, reflux, 24 h; (vi) ethyl orthoformate, 130°C, 1 h; (vii) cyclopropylmethyl bromide, K_2CO_3, CH_3CN, reflux, 24 h.

In vitro evaluation

The efficacy of the newly synthesized compounds as AMPA potentiators was assessed using a previously established *in vitro* fluorescence assay (FDSS) conducted on primary cell cultures derived from rat embryonic cortex.[24] The EC_{2x} value was determined for each compound,

corresponding to the drug concentration required to induce a twofold increase of the amplitude of the current triggered by 300 µM AMPA (Tables 1 and 2).

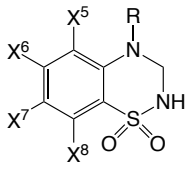
Table 1. Effects of Mono(fluoro/chloro/bromo)-substituted 4-Cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxides on the Fluorescence Induced by 300 µM AMPA on Primary Cultures of Neurons from Rat Embryonic Cortex.



Compd.	X^5	X^6	X^7	X^8	R	EC_{2x} (FDSS[a]) (µM)
17[b]	F	H	H	H	$-CH(CH_2)_2$	2.14 [1.55; 2.96] (n=5)
18[b]	H	F	H	H	$-CH(CH_2)_2$	2.11 [1.01; 4.39] (n=3)
19[b]	H	H	F	H	$-CH(CH_2)_2$	0.26 [0.15; 0.47] (n=3)
20[b]	H	H	H	F	$-CH(CH_2)_2$	0.33 [0.22; 0.50] (n=3)
21[c]	Cl	H	H	H	$-CH(CH_2)_2$	27.14 [7.29; 101.02] (n=3)
22[c]	H	Cl	H	H	$-CH(CH_2)_2$	5.19 [1.85; 14.54] (n=3)
23[c]	H	H	Cl	H	$-CH(CH_2)_2$	0.68 [0.25; 1.83] (n=4)
24[c]	H	H	H	Cl	$-CH(CH_2)_2$	1.96 [0.45; 8.45] (n=3)
14b	H	Br	H	H	$-CH(CH_2)_2$	4.79 [3.05; 7.53] (n=6)
25[d]	H	H	Br	H	$-CH(CH_2)_2$	1.17 [0.36; 3.83] (n=3)
14a	H	H	H	Br	$-CH(CH_2)_2$	5.57 [3.49; 8.88] (n=4)

[a] EC_{2x}: concentration of the modulator that results in a twofold increase in fluorescence induced by AMPA (300 µM) using the FDSS fluorescence assay conducted on primary brain cultures derived from rats (n = 3-6). The results are reported as the geometric mean accompanied by the corresponding 95% confidence intervals enclosed in brackets. [b,c,d] Published compounds and results ([b] ref.[32], [c] ref.[30], [d] ref.[25]).

Table 2. Effects of Di(fluoro/chloro)-substituted 4-Cycloalkyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxides on the Fluorescence Induced by 300 µM AMPA on Primary Cultures of Neurons from Rat Embryonic Cortex.



Compd.	X^5	X^6	X^7	X^8	-R	EC_{2x} (FDSS[a]) (µM)
14c	F	F	H	H	$-CH(CH_2)_2$	5.10 [3.00; 8.67] (n=5)
14d	F	H	F	H	$-CH(CH_2)_2$	1.14 [0.64; 2.05] (n=3)
14e	H	F	F	H	$-CH(CH_2)_2$	1.76 [0.21; 14.44] (n=4)
14f	H	F	H	F	$-CH(CH_2)_2$	0.77 [0.30; 1.94] (n=3)
14g	Cl	F	H	H	$-CH(CH_2)_2$	11.89 [3.23; 43.80] (n=3)
14h	H	F	Cl	H	$-CH(CH_2)_2$	0.19 (n=2)
14i	H	F	H	Cl	$-CH(CH_2)_2$	3.83 [3.16; 4.63] (n=3)
14j	H	H	F	F	$-CH(CH_2)_2$	0.83 [0.70; 0.99] (n=3)
14k	H	Cl	F	H	$-CH(CH_2)_2$	2.59 [0.61; 11.03] (n=4)
14l	H	H	F	Cl	$-CH(CH_2)_2$	0.53 [0.41; 0.69] (n=3)
14m	Cl	H	H	F	$-CH(CH_2)_2$	7.35 [5.96; 9.06] (n=3)
14n	Cl	Cl	H	H	$-CH(CH_2)_2$	>100 (n=3)
14o	H	Cl	Cl	H	$-CH(CH_2)_2$	0.96 [0.64; 1.44] (n=6)
14p	H	Cl	Cl	H	$-C(CH_3)(CH_2)_2$	8.94 (n=2)
14q	H	Cl	Cl	H	$-CH(CH_2)_3$	>100 (n=3)
14r	H	Cl	Cl	H	$-CH(CH_2)_4$	>100 (n=3)
14s	H	Cl	Cl	H	$-CH(CH_2)_5$	>100 (n=3)
14t	H	Cl	Cl	H	$-CH(CH_2)_6$	>100 (n=3)
14u	H	Cl	Cl	H	$-CH(CH_2)_7$	>100 (n=3)
14v	H	Cl	Cl	H	$-CH(CH_3)_2$	Inhibition
14w	H	Cl	Cl	H	$-CH_2CH(CH_2)_2$	>100 (n=3)

[a] EC_{2x}: concentration of the modulator that results in a twofold increase in fluorescence induced by AMPA (300 µM) using the FDSS fluorescence assay conducted on primary brain cultures derived from rats (n = 2-6). The results are reported as the geometric mean accompanied by the corresponding 95% confidence intervals enclosed in brackets when n>2.

According to Table 1, the enhancement of AMPA receptor activity by mono-halo-substituted 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (**14a-b**, **17-25**), bearing a cyclopropyl group at the 4-position of the heterocycle, was significantly influenced by both the position and the nature of the halogen atom on the benzene ring. The ranking order for the preferential position of the halogen atom on the heterocycle was found to be 7-position > 8-position > 6- or 5-positions. Moreover, the ranking order for the best choice of halogen atom was always found to be F > Cl > Br. Considering this observation, we decided to explore the impact of the di-substitution of the benzene ring of 1,2,4-benzothiadiazine 1,1-dioxides with fluorine and/or chlorine atoms, supporting the view that bromo-substituted compounds could provide less potent modulators.

Many examples of di(fluoro/chloro)-substituted 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides were synthesized and examined as putative AMPAR potentiators (**14c-w**, Table 2). The substitution of the cyclopropyl ring with progressively larger cycloalkyl groups was also assessed on 6,7-dichloro-substituted compounds. The results obtained indicate a preference for developing AMPAR PAMs with a cyclopropyl moiety at the 4-position of the heterocycle. The presence of one of the two halogen atoms (fluorine or chlorine) at the 5-position of the heterocycle was responsible for a decrease of the potentiator effect on AMPA receptors (see compounds **14c**, **14d**, **14g**, **14m**, **14n**) compared to the di-substitution in the other positions. On the contrary, the presence of halogen atoms at the 6,7-, 6,8- or 7,8-positions provided potent modulators expressing EC_{2x} values below or around 1 μM (see compounds **14f**, **14h**, **14j**, **14l** and **14o**). Interestingly, looking at the compounds bearing halogen atoms at the 6,7-positions (**14e**, **14h**, **14k** and **14o**), the presence of a chlorine atom at the 7-position was always found to be preferred over that of a fluorine atom. The introduction of two fluorine atoms at positions 6,8 (**14f**) significantly enhances the activity on the AMPA receptor compared to the similar compound bearing a chlorine atom at the 8-position (**14i**). The nature of the atom at the 8-

position of dihalo-substituted compounds at the 7,8-positions (**14j**, **14l**) had no noticeable impact on the activity, as both compounds remained very active. According to these compelling results, compounds **14f**, **14h**, **14j**, **14l**, and **14o** can be considered as promising AMPAR potentiators, all demonstrating a marked *in vitro* activity.

Considering its proper safety profile in rodents *in vivo*, compound **14o** (BPAM363) was selected among these promising AMPAR potentiators for further *in vitro* and *in vivo* evaluations as a candidate for development as a new drug. Indeed, Among the most active compounds revealed by the *in vitro* fluorescence assay (EC_{2x} below or around 1 µM), compound **14o** was found to express the best safety profile at very high oral doses (100 mg/kg) in mice (see discussion below; no proconvulsant effect, no marked effect on body temperature).

*Additional in vitro evaluations of compound **14o***

Compound **14o** did not display affinity for the glutamate binding site on AMPA, kainate or NMDA receptors (Ki values greater than 10 µM, n=2). In order to check its selectivity, compound **14o** was evaluated *in vitro* on about a hundred receptors, enzymes, transporters and channels (CEREP safety profile). The percentage of inhibition of control value in presence of 10 µM of compound **14o** was lower than 50%, indicating that Ki values in all these assays were greater than 10 µM, except for 5-HT2B receptors (IC_{50} = 8.5 µM, n=2), thus suggesting no affinity of compound **14o** for these studied receptors, enzymes, transporters and channels besides 5-HT2B receptors.

Compound **14o** had no effect on the holding current of *Xenopus laevis* oocytes injected with rat cortex poly(A+) mRNA, but enhanced AMPA-evoked inward current up to about 26 fold in a concentration-dependent manner, taken AMPA-evoked current alone as unity, with an EC_{2x} [CI90] of 1.7 µM [1.0; 2.9] and a concentration that induced 50% of maximal effect, EC_{50} [CI90] of 9.9 µM [5.6; 17.5] (n=5; Figure 2A). Potentiation evoked by compound **14o** is

selective for the AMPA-evoked currents as shown by the absence of effect at NMDA- or kainate-evoked currents (n = 2 and 4, respectively, Figure 2A). Similar activity was observed on oocytes injected with human hippocampal poly(A+) mRNA (n=4; Figure 2B) with equivalent EC_{50}'s value and similar maximal AMPA potentiation (human EC_{50} [CI90] of 18.0 µM [10.8; 30.2]; $p>0.05$ versus rat EC_{50}, Unpaired T-test).

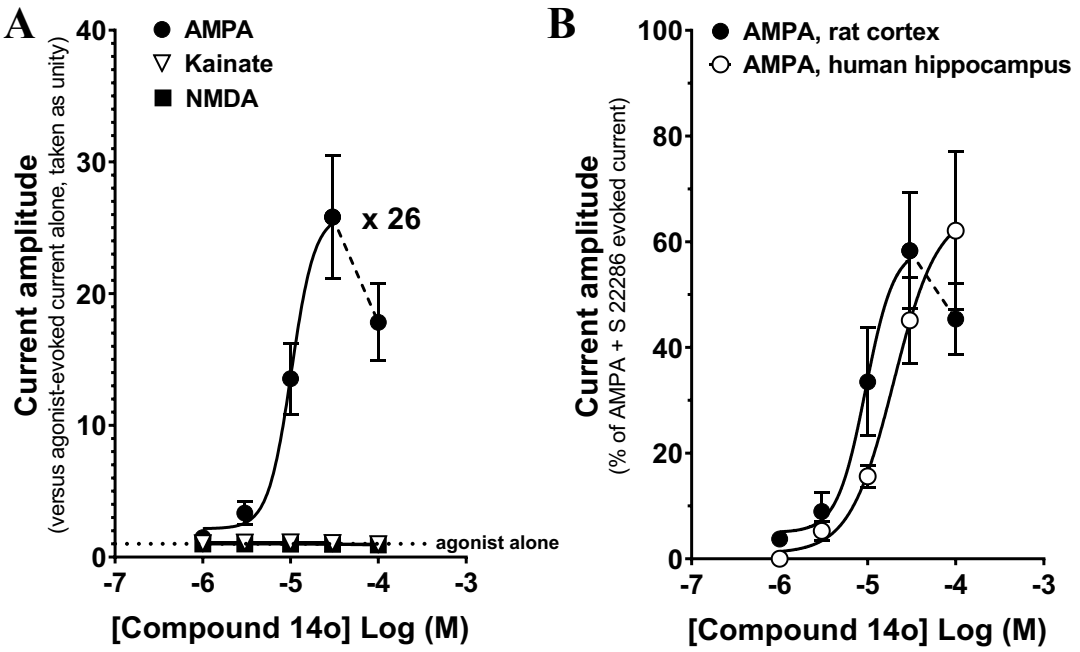


Figure 2. Effects of compound **14o** on AMPA-, kainate- or NMDA-evoked current on oocytes injected with rat cortex mRNA (n=5, 4 and 3 respectively) (**A**) and on AMPA-evoked current on oocytes injected with either rat cortex (n=5) or human hippocampus mRNA (n=4) (**B**). (**A**) The amplitude of current evoked in the presence of compound **14o** was normalized to the control current evoked by the agonist alone on the same oocytes (either 10 µM AMPA; 1 mM Kainate or 300 µM NMDA/10 µM glycine), taken as unity. (**B**) The amplitude of current evoked in the presence of compound **14o** was normalized to a control current evoked by 10 µM AMPA in the presence of 100 µM S22286, one in-house reference as AMPA receptor potentiator, on the same oocytes, as in contrast to oocytes injected with rat cortex mRNA, AMPA, applied alone, generated little or no current on oocytes injected with human hippocampal mRNA.

As reported for AMPA positive modulators[13,24], compound **14o** enhanced in a concentration dependent manner the excitatory postsynaptic response evoked in the CA1 area of the hippocampus by stimulation of the Schaeffer collaterals on Wistar rat hippocampal slices *in*

vitro. At 10 and 30 µM, compound **14o** enhanced the area of the post-synaptic response evoked in the CA1 area of the hippocampus in a concentration-dependent manner (n=5, Figure 3A). Compound **14o** had little effect on the amplitude of the response (Figure 3B) and mainly enhanced the duration of the synaptic response, as measured by the increase of the half-width (Figure 3C).

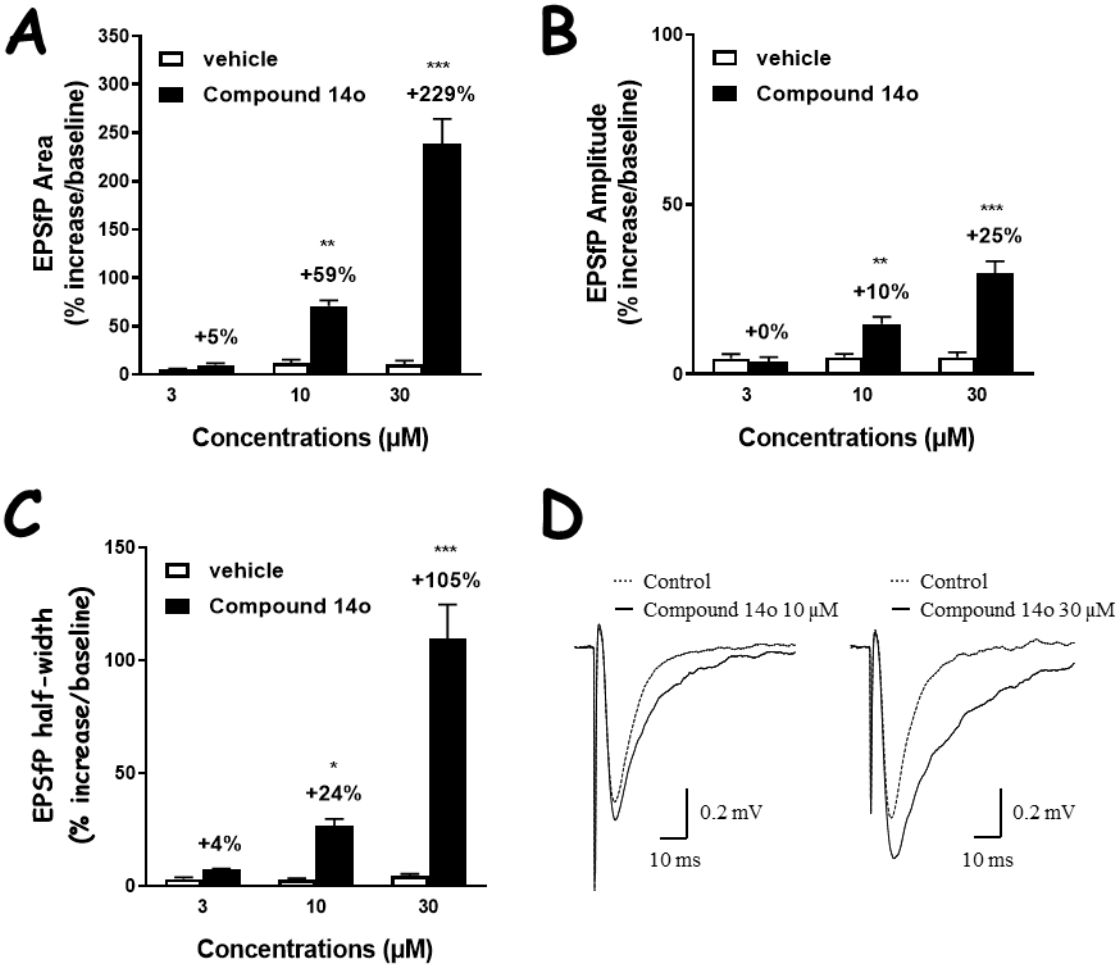


Figure 3. Effect of compound **14o** on the AMPA-mediated post-synaptic responses evoked in CA1 area on hippocampal slices (n=5). Percentage of increase induced by compound **14o** (3, 10 and 30 µM) or vehicle on the area (**A**), amplitude (**B**) and half-width (**C**) of the synaptic response. (**D**) Typical examples on post-synaptic responses evoked under control and after compound **14o** (10 and 30 µM). * p≤0.05, ** p≤0.01 and *** p≤0.001 versus vehicle control, two-way ANOVA (Treatment x Concentration).

BDNF is a member of the neurotrophin family of nerve growth factors that plays a critical role during development, retains a profound influence on structural and synaptic plasticity in the mature brain, and participates in plasticity phenomena, such as Long-Term Potentiation and learning.[33,34] As previously reported for several AMPA potentiators[35,36,37,38], compound **14o** increased in a dose-dependent manner the expression of BDNF protein when applied alone in rat primary cortical neuronal cultures (0.3-10 µM, n=3) (Figure 4). At 10 µM it increased by 10-fold the expression of BDNF protein compared with basal expression.

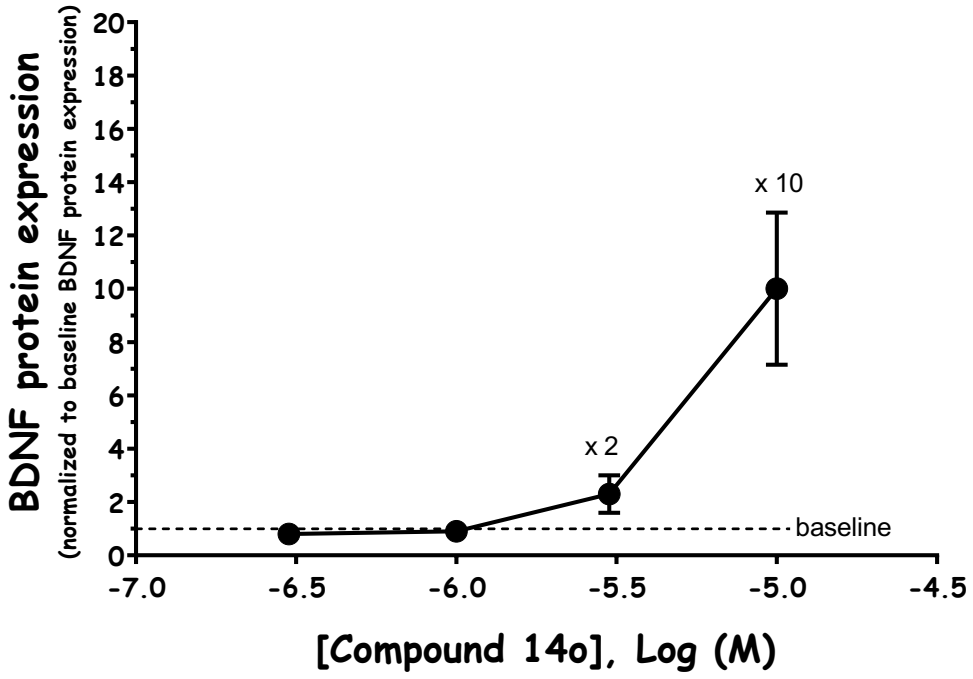


Figure 4. Effects of **14o** on BDNF protein level in rat primary cortical brain cells cultures. Cortical cells were exposed to different concentrations of **14o**. BDNF protein level induced in the presence of **14o** was normalized to the basal BDNF protein level.

*In vivo evaluation of compound **14o***

Since it is known that high doses of AMPAR PAMs could induce proconvulsant effects,[39,40] an *in vivo* safety study was conducted on NMRI mice to evaluate compound **14o** after administering a high oral dose of the drug (100 mg/kg, n=5) . The results showed that compound **14o** did not exhibit any proconvulsant effects or significant changes on body temperature

(Figure 5) even at this high dosage. The good safety profile of compound **14o** was further confirmed in the Irwin test in Wistar rats (n=5). Rats were observed for changes in autonomic function (lacrimation, salivation, piloerection), awareness (hypo or hyperreactivity), motor activity (catalepsy, hypo or hyperactivity), motor coordination/tonus (gait, tremor) and rectal temperature. Observations are made before and at regular intervals up to 3 hours after administration of the compound. When either orally (10, 30 and 100 mg/kg, n=5) or i.p. (10 and 30 mg/kg, n=5) administered, compound **14o** had no relevant effect on body temperature (Figure 5) and autonomic function. Decrease of the muscular tone was observed only at the highest dose of 100 mg/kg following p.o. administration in the strength test for 4/5 and 5/5 rats at 2- and 3-hours post-treatment, respectively.

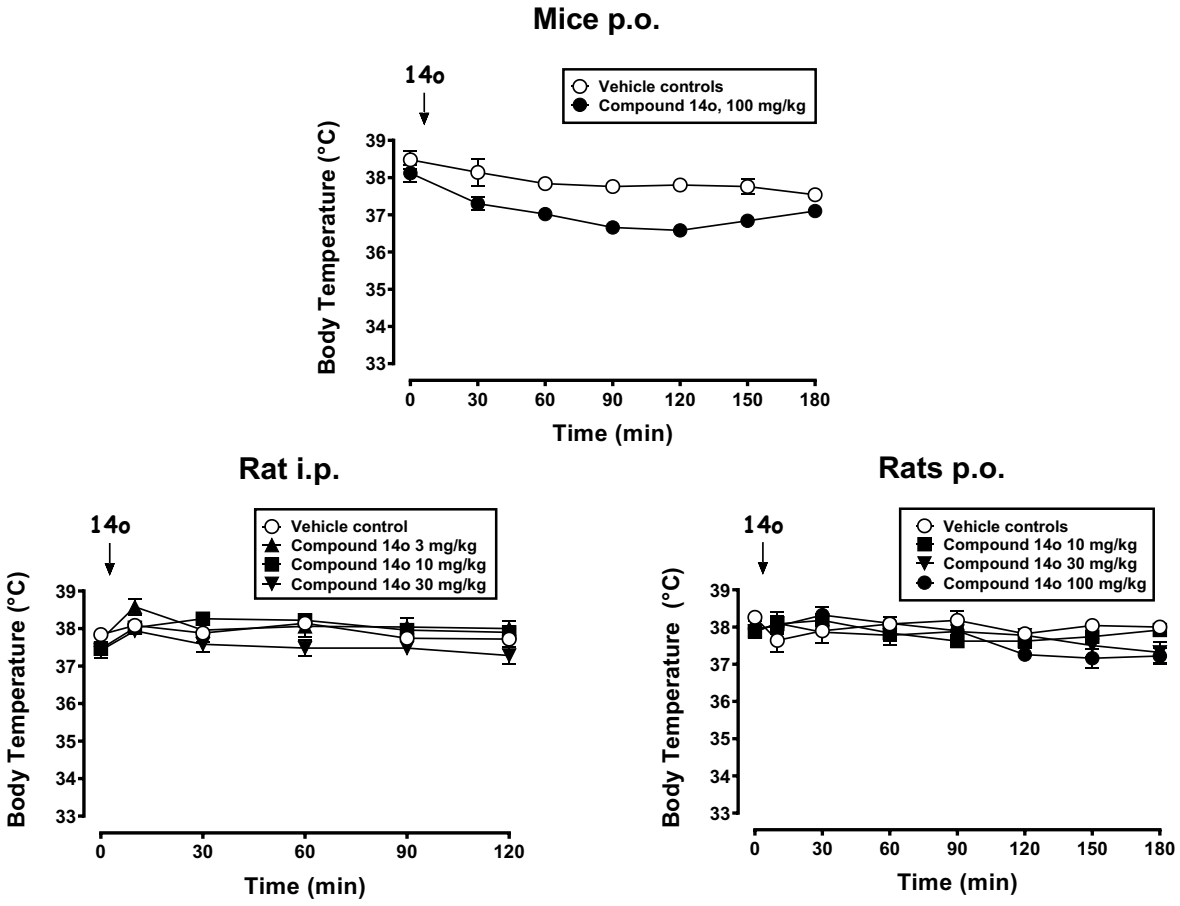


Figure 5. *In vivo* effects of **14o** on the body temperature after p.o. administration in NMRI mice and after p.o. and i.p. administration in Wistar rats; values are expressed as means +/- SEM. For each study, p>0.05 versus vehicle control group, two-way ANOVA (Treatment x Time)

with repeated measures on factor Time performed on the delta of the temperature compared to T 0min just before administration of the compound or its vehicle.

The measurement of the post-synaptic potentiation after *in vivo* tetanic stimulation in Wistar rats was conducted on compound **14o** (Figure 6). This measurement was used to assess the cognitive potential of compound **14o**. Indeed, the phenomenon of long-term potentiation (LTP) of synaptic response is involved in strengthening synaptic connections underlying learning and memory processes.[41,42,43] The LTP of **14o** was investigated *in vivo* within the dentate gyrus of the hippocampus using anesthetized rats. LTP was induced through brief high frequent stimulation or tetanus in the perforant path one hour after intraperitoneal administration of the drug. Subsequent excitatory postsynaptic field potentials (EPSPs) were recorded over a period of 3 hours. Compound **14o** significantly increased both the induction and maintenance of the potentiation of synaptic response compared to the vehicle control group (n=8, Figure 6).

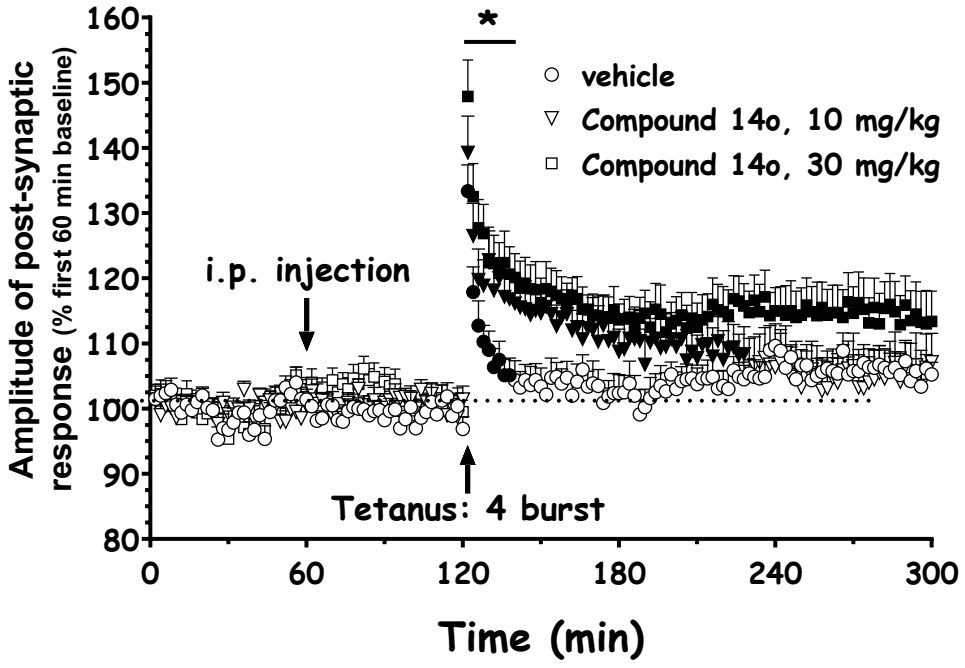


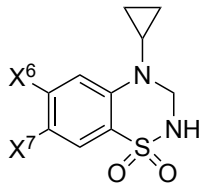
Figure 6. Effect of compound **14o** on Long-Term Potentiation (LTP) of the Postsynaptic Response Evoked in the Dentate Gyrus by a tetanic stimulation of the perforant path on anesthetized Rats (n=8 per group). In each rat, synaptic responses were averaged over 8 successive stimulations and normalized to the average amplitude of synaptic responses obtained

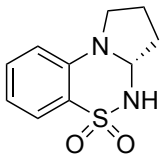
during the baseline 60 min period (control value taken as 100 %). * p≤0.05, compound **14o** at 30 mg/kg vs vehicle, Dunnett test after significant two-way ANOVA (Time x Dose). Closed symbols: p≤0.05 versus the average amplitude of EPSfP obtained during a six minutes-period preceding the tetanus, Dunnett test after significant one-way ANOVA (Time).

Potentiation was significant during more than 3h and had a magnitude of 148 ± 5 % at the highest dose of compound **14o**, whereas it lasted significantly only 18 min and reached 133 ± 4 % in the vehicle control group. The dose of 10 mg/kg i.p. has an intermediate effect. It was demonstrated that this compound, like other BTD-type AMPAR PAMs, can effectively cross the blood-brain barrier (BBB) and reach the central nervous system (CNS), where it may exert a cognitive action.[24,44]

The *in vivo* tissue distribution of **14o** was investigated by measuring the partition coefficient ([brain]/[plasma]) in mice to comprehensively assess the ability of this compound to freely cross the blood-brain barrier. Table 3 reports the brain/plasma distribution measured in mice for a selection of mono- and dihalo-substituted BTDs listed in Tables 1 and 2, as well as for the reference compound S18986 (**26**), a well-known BTD-type AMPAR modulator previously selected for clinical trials, and for which detailed pharmacokinetic studies in animal models were described.[45] The results on Table 3 reveal that, after oral administration in mice of 3 mg/kg of **14o**, it shows homogeneous distribution ([brain]/[plasma] ≈ 1) between the brain and the plasma, irrespective of the sampling time. This result confirmed that compound **14o** was able to easily cross the BBB and to be freely distributed in the CNS. Moreover, 6,7-dichloro-substitution (compound **14o**) appeared to be more favorable than 6- or 7-monohalosubstitution (compounds **18**, **14b** and **23**) to bring the compound into the CNS (Table 3), but all drugs show a brain/plasma ratio close to the unity. Compared to the well-known BTD S18986 (**26**), the brain and plasma concentrations of the latter were found to be slightly higher than **14o** after 20 minutes, but quite similar after 60 minutes.

Table 3. CNS distribution of a selection of BTDs after p.o. administration (3 mg/kg; n =3) in NMRI mice.





14b : X^6 = Br; ,X^7 = H
14o : X^6 = Cl; ,X^7 = Cl
18 : X^6 = F; ,X^7 = H
23 : X^6 = H; ,X^7 = Cl

26 (S18986)

Compound	**14b**	**18**	**23**	**14o**		**26**	
Time after p.o. administration (min)	*20*	*20*	*20*	*20*	*60*	*20*	*60*
Concentration in brain [brain] (ng/mL)	64.48	90.48	75.92[a]	191.36	83.2	267.28	75.92
Concentration in plasma [plasma] (ng/mL)	69	108	65[a]	186	79	255	59
Brain/plasma ratio [brain]/[plasma]	*0.93*	*0.84*	*1.17*	*1.03*	*1.05*	*1.05*	*1.29*

[a] Published results (ref.[30]).

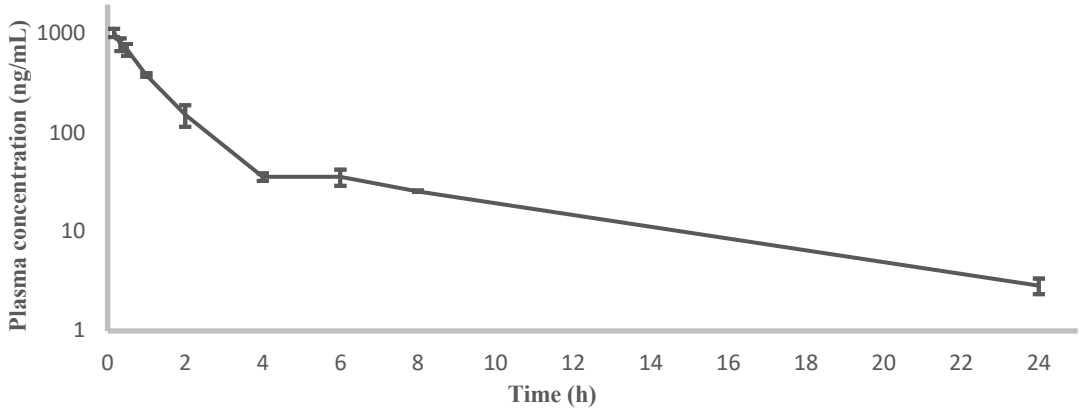
To complete the comparison between **14o** and **26**, we also examined the ability of **14o** to express a high oral bioavailability by determining the apparent permeability (P_{app}) on human Caco-2 cells.[46,47] The P_{app} value obtained with **14o** was found to be 27 x 10^{-6} cm/s, which is quite similar to that previously obtained with **26** (23.1 x 10^{-6} cm/s).[48] According to the literature, a substance is classified as a high-permeability drug if its P_{app} is higher that 10 x 10^{-6} cm/s.[47] As a result, this observation confirms the favorable oral bioavailability of compound **14o**.

In order to concretize this observation, a pharmacokinetic study was carried out with **14o** to determine the main pharmacokinetic parameters obtained in rats after i.v. (1.5 mg/kg) and *per os* (3 mg/kg) administration of the compound. Table 4 and figure 7A/B summarize the results obtained.

Table 4. Main pharmacokinetic parameters obtained with **14o** in rats after i.v. (1.5 mg/kg) and *per os* (3 mg/kg) administration.

Route of administration	IV	PO
Absorption mean values (n=3)		
C_{max} (ng/mL)	1038	189
T_{max} (h)	-	6
AUC (ng/mL.h)	1363	1887
F (%)	-	69.2
Distribution mean values (n=3)		
V_{ss} (L/kg)	3.89	-
Elimination mean values (n=3)		
$T_{1/2}$ effective (h)	2.9	-
CL_P (mL/min.kg)	18	-
CL_R (mL/min.kg)	0.00549	0.0017
$CL_{Hep\ blood}$ (mL/min.kg)	24.1	-

A: 14o IV administration 1.5 mg/kg



B: 14o PO administration 3 mg/kg

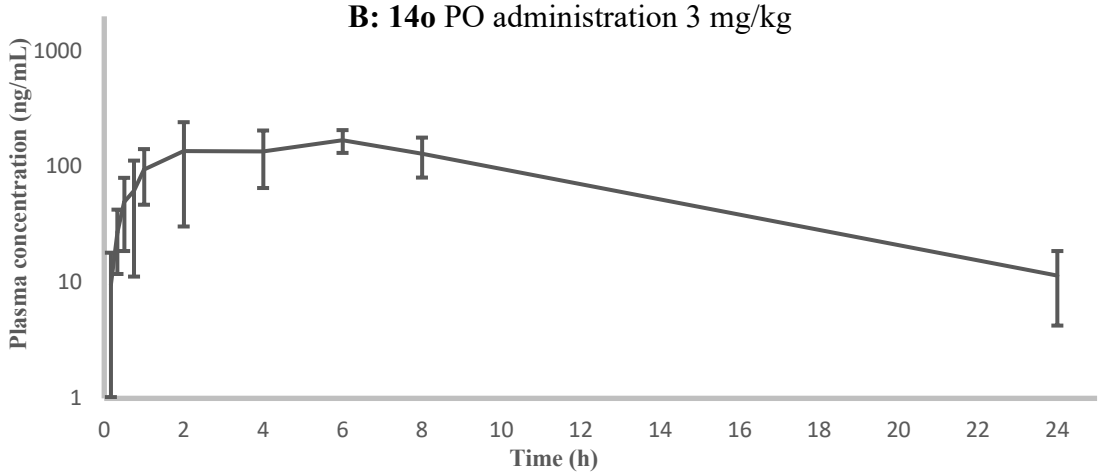


Figure 7. A: plasma concentrations versus time of compound **14o** after i.v. administration in Wistar rats (1.5 mg/kg); B: plasma concentrations versus time of compound **14o** after *per os* administration in Wistar rats (3 mg/kg).

Following the oral administration of compound **14o** in a 1% hydroxyethylcellulose solution, the absorption rate was slow, with a mean time to maximum plasma concentration (T_{max}) of 6 hours and a maximum plasma concentration (C_{max}) of 189 ng/mL. This reflects a gradual absorption profile, including a plateau in plasma concentrations observed between 1 and 6 hours after dosing. This kinetic behavior may be due to limited water solubility and/or a more distal absorption window. No formulation optimization was applied to compound **14o** in the present study, which could explain the slower absorption observed. However, this absorption profile did not affect systemic exposure: the oral bioavailability (F) observed after a 3 mg/kg dose was high (69.2%). This bioavailability is consistent with a relatively low hepatic blood clearance ($CL_{Hep\ blood}$ = 24.1 mL/min/kg). These characteristics are compatible with low to moderate hepatic extraction and support good preservation of systemic exposure after first-pass metabolism.

Compound **14o** exhibited extensive plasma protein binding, with a fraction unbound (Fu) of approximately 1.1% over a broad concentration range (100–10,000 ng/mL). This strong binding substantially limits the free drug concentration available for hepatic uptake and metabolism, which in turn explains the observed moderate hepatic clearance.

Following intravenous administration, compound **14o** displayed a large volume of distribution (Vss ≈ 3.9 L/kg) and a moderate effective half-life of about 2.9 hours. The low plasma clearance (CLP = 18 mL/min/kg) can be explained by the limited free fraction available for elimination processes. Renal clearance was negligible (CLR < 0.006 mL/min/kg), suggesting minimal urinary excretion likely due to the strong plasma protein binding. This profile is consistent with primarily non-renal elimination, likely to be of a metabolic nature, although this is limited due

to low hepatic extraction largely resulting from the strong plasma protein binding that restricts the fraction of free drug accessible to hepatic enzymes, thus limiting clearance despite intrinsic metabolic capacity.

The improvement of the cognitive performance induced by **14o** was investigated *in vivo* by evaluating the effects of this compound 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.[13,49,50] According to Figure 8, oral administration of compound **14o** 1 h before the three sessions significantly increased with a similar efficacy the cognition performance of mice at doses of 1 mg/kg and 3 mg/kg. The effect of compound **14o** in this test 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.

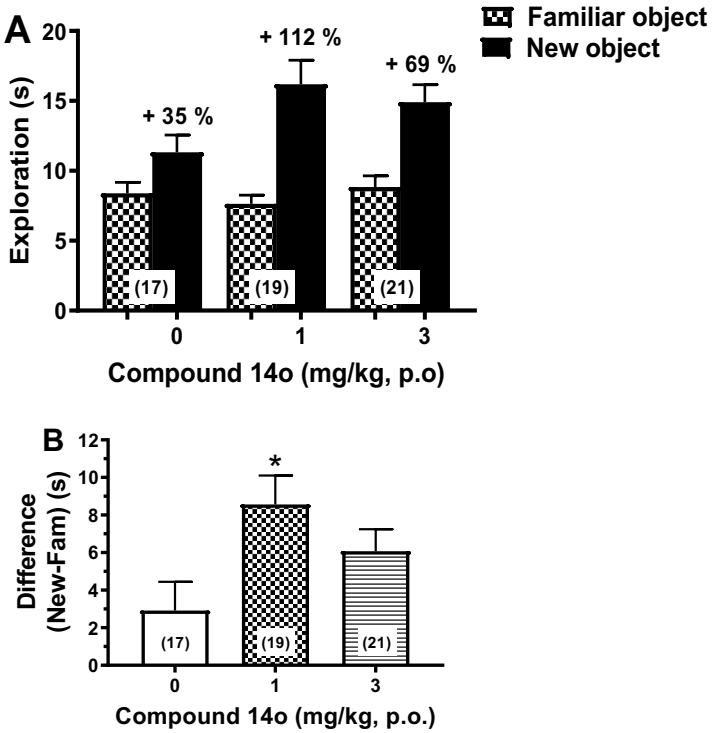


Figure 8. Effect of compound **14o** (1 and 3 mg/kg, *per os*) on the exploration time spent on either the Familiar (Fam) or the New object during the recognition phase in the object recognition task in CD1 mice with a natural forgetting setting (episodic memory). Compound **14o** was administered by oral route 1 hour before habituation, familiarization and recognition sessions, given at 24h intervals. **(A)** Results are expressed means and SEM of the durations of

exploration of both New and Familiar objects, the number in brackets refers to the number of animals. An additional indication of activity was given by the % increase of New object exploration duration relatively to Familiar object. This percentage of effect was calculated as followed for each group: (MeanNew x100 / MeanFam) - 100. During the recognition phase, compound **14o** at either 1 or 3 mg/kg *per os* increased the duration exploration of the new object of 112% and 69%, respectively whereas it was only 35% in the vehicle control group. **(B)** Results are expressed as means and SEM of the difference of New and Familiar (Fam) object durations of exploration; the number in brackets refers to the number of animals. Compound **14o** improved the discrimination between the new object and the familiar one. *p≤0.05 versus vehicle controls, Dunnett test after significant one-way ANOVA (dose).

A second *in vivo* test in C57BI/6 mice confirmed the ability of compound **14o** to improve working memory performance, specifically in a spontaneous alternation task conducted in a T-maze (Figure 9). This task assesses the natural tendency of rodents to explore new environments and their ability to remember specific information. Normally, mice tend to alternate between the two arms of the T-maze, especially when the inter-trial interval is short. However, when this interval is increased to 180 seconds, young mice show a decrease in the percentage of alternations.[50]

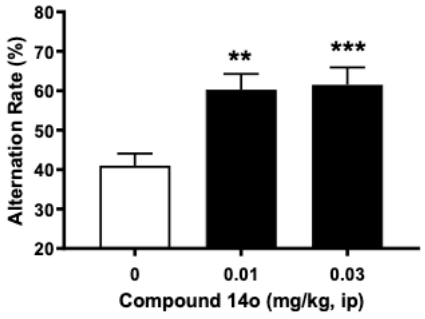


Figure 9. Effects of compound **14o** (0.01 and 0.03 mg/kg i.p.) on the spontaneous alternation in T-maze in C57BI/6 mice (spatial working memory). The results are shown as means and SEM. The level of spontaneous alternation is lowered when a long delay (180 seconds) is imposed between the successive trials of visits. In this delay-induced deficit version of the spontaneous alternation paradigm, compound **14o** increased the increased the level of alternation. ** p<0.01, *** p≤0.001 versus vehicle control group (0), Dunnett test after significant one-way ANOVA (dose).

In this study, Figure 8 shows that intraperitoneal administration of **14o** at very low doses (0.01 and 0.03 mg/kg i.p.) improved spontaneous alternation performance compared to vehicle-treated mice. This improvement in spontaneous alternation is directly related to working memory, as the ability to alternate between trials requires the retention of specific information. These results further support the potential of compound **14o** as a compound that improves cognitive performance, particularly in tasks that rely on working memory.

Lastly, it is well known that AMPAR PAMs can increase brain-derived neurotrophic factor (BDNF) protein levels in the CNS, an effect that could explain their neuroprotective effect.[51,52] Indeed, BDNF has been reported to be a survival factor for various neuronal cell types.[52] Figure 10 shows that the i.p. administration of compound **14o** (10 mg/kg) 30 minutes before a short transient ischemia was able to significantly delay hippocampal neuronal cell death observed 7 days after transient global ischemia by a four-vessels occlusion in Wistar rats (n= 11; $p \leq 0.01$, Tukey test following one way ANOVA) (Figure 10A). No significant decrease in the delta of body temperature was observed following compound **14o** administration (Figure 10B).

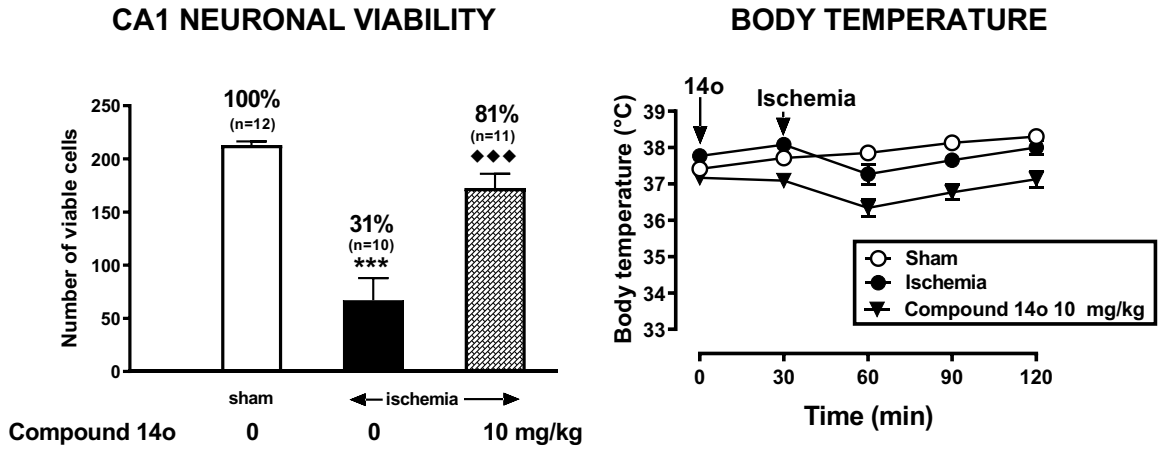


Figure 10. Effect of compound **14o** (10 mg/kg i.p.) on a rat model of delayed hippocampal neuronal cells death. **(A)** Effect of compound **14o** on CA1 hippocampal neuronal cells viability evaluated 7 days after transient global cerebral ischemia in Wistar rat. Number of viable cells are expressed as mean ± SEM, the number in brackets refer to the number of animals. Ischemia after vehicle administration reduced the number of viable cells to 31% compared to sham controls taken as 100 %. Compound **14o** had a potent neuroprotective effect as it was able to

preserve the viability of 81% of the hippocampal neurons. ***: p≤0.001, vs sham controls and ◆◆◆: p≤ 0.001 vs 0 mg/kg under ischemia, Tukey test following significant one-way ANOVA (Treatment). **(B)** Body temperature evaluated before and after transient global cerebral ischemia, after i.p. administration of either 10 mg/kg compound **14o** or its vehicle. Results are expressed as means and SEM. No significant effect was noticed on the delta of body temperature compared to T-30 min between ischemia compound **14o** and ischemia vehicle control groups 30, 60 and 90 min after ischemia (p≥0.05, two-way ANOVA (Time x Treatment)).

It should be noted that a short ischemia of 5 minutes was chosen in this study in order to induce only a partial loss of cells in CA1 area of the hippocampus. Ischemia after vehicle administration (n=10) significantly reduced the number of viable cells in the CA1 hippocampal area 7 days after occlusion compared to sham control rats (n=12): 31% viable cells remained compared to sham control taken as 100 % (p≤0.001, Tukey test following one way ANOVA) (Figure 9). The neuroprotective effect of compound **14o** in this rat model of hippocampal vulnerability was found to be significantly higher than other known AMPAR PAMs (i.e. S18986; data not shown) since compound **14o** was able to preserve the viability of more than 80% of the hippocampal neurons.

CONCLUSIONS

The synthesis of new mono- and dihalosubstituted 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides completes the structural exploration of BTDs, opening new perspectives in the search of positive allosteric modulators of AMPA receptors (AMPAR PAMs) as drug candidates expressing cognitive enhancement and neuroprotection. The underlying potential of di-halosubstitution of the benzene ring was initially motivated by the promising biological properties of mono-halogenated derivatives.

Particular emphasis was placed on compound **14o**, a BTD compound disubstituted at the 6- and 7-positions with two chlorine atoms, due to its favorable safety profile characterized by the absence of proconvulsive effects after massive oral administration (100 mg/kg). *In vivo* results

clearly demonstrated the ability of compound **14o** to exhibit a high oral bioavailability and to penetrate the central nervous system (CNS), significantly impacting cognitive function. These findings were reinforced by a series of additional *in vivo* tests in animal models, including assessments of object recognition and spontaneous alternation. Furthermore, it is important to note that compound **14o** exhibited neuroprotective properties by successfully delaying neuronal degeneration in the hippocampal region following ischemic events, thereby preserving a high percentage of intact neurons.

Taken together, these results indicate that compound **14o** could potentially play a beneficial role in the treatment of cognitive disorders, further enhancing its appeal as a promising candidate for future clinical applications.

EXPERIMENTAL SECTION

Chemistry

General Procedures.

Melting points were determined on a Büchi Tottoli capillary apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance (500 MHz) instrument using deuterated dimethyl sulfoxide (d_6-DMSO) 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, p = pentuplet, h = hexuplet, hept = heptuplet, o = octuplet, m = multiplet, dd = doublet of doublet, td = triplet of doublet, dt = doublet of triplet, tt = triplet of triplet, tq = triplet of quadruplet, ddd = doublet of doublet of doublet, ddt = doublet of doublet of triplet, dtt = doublet of 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; a higher tolerance (± 0.6%) was admitted for sulfur, considering its corresponding peak shape. 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.

2-Bromo-6-fluorobenzenesulfonamide (11a).

In a 500 ml round-bottom flask, a portion of glacial acetic acid (30 mL) was saturated for 30 minutes with a current of gaseous sulfur dioxide. To the resulting solution there was added a solution of cupric chloride (1.50 g, 11.16 mmol) in water (10 mL) (suspension A). 2-Bromo-6-fluoroaniline (**10a**: 5.28 g, 27.80 mmol) was dissolved in a mixture of glacial acetic acid (30 mL) and concentrated hydrochloric acid (15 mL). The resulting solution was cooled to -5 °C on a bath of ice and salt. A solution of sodium nitrite (2.50 g, 36.24 mmol) in water (10 mL) was then added dropwise with constant stirring. The resulting mixture was slowly added to suspension A and stirred on an ice bath for 15 minutes. The mixture was then poured into a mixture of water (200 mL) and diethylether (200 mL). The ethereal phase was separated off and washed with water (100 mL). The organic phase was concentrated to dryness by distillation under reduced pressure and the residue was re-dissolved in dioxane (25 mL). The solution was poured slowly, whilst stirring, into a mixture of concentrated ammonia (25 mL) and water (10 mL) cooled on an ice bath. After 30 minutes, the solution was evaporated to dryness by distillation under reduced pressure and the residue obtained was dissolved in methanol. The methanolic solution was treated with charcoal, filtered, and the filtrate was evaporated to dryness. The residue was recrystallized from a methanol/water mixture. mp : 185-187 °C. ^{1}H NMR (DMSO-d_6) δ 7.44 (m, 1H, 5-H), 7.51 (td, J=8.2 Hz/5.6 Hz, 1H, 4-H), 7.66 (d, J=8.0 Hz, 1H, 3-H), 7.89 (s, 2H, SO_2NH_2).

4-Bromo-2-fluorobenzenesulfonamide (11b).

The title compound was obtained as described for **11a** starting from 4-Bromo-2-fluoroaniline (**10b**). mp : 128-130 °C. ^{1}H NMR (DMSO-d_6) δ 7.61 (dd, J=8.4 Hz/1.5 Hz, 1H, 5-H), 7.73 (t, J=8.1 Hz, 1H, 6-H), 7.74 (bs, 2H, SO_2NH_2), 7.83 (dd, J=9.8 Hz/1.7 Hz, 1H, 3-H).

2,3,4-Trifluorobenzenesulfonamide (11c).

The title compound was obtained as described for **11a** starting from 2,3,4-Trifluoroaniline (**10c**). mp : 111-114 °C. ^{1}H NMR (DMSO-d_6) δ 7.51 (m, 1H, 6-H), 7.67 (m, 1H, 5-H), 7.92 (s, 2H, SO_2NH_2).

2,3,5-Trifluorobenzenesulfonamide (11d).

The title compound was obtained as described for **11a** starting from 2,3,5-trifluoroaniline (**10d**). mp : 115-117 °C. ^{1}H NMR (DMSO-d_6) δ 7.45 (m, 1H, 4-H), 7.89 (m, 1H, 6-H), 8.02 (s, 2H, SO_2NH_2).

2,4,6-Trifluorobenzenesulfonamide (11e).

The title compound was obtained as described for **11a** starting from 2,4,6-trifluoroaniline (**10e**). mp : 102-106 °C. ^{1}H NMR (DMSO-d_6) δ 7.42 (t, J=9.2 Hz, 2H, 3-H/5-H), 7.79 (bs, 2H, SO_2NH_2).

3-Chloro-2,4-difluorobenzenesulfonamide (11f).

The title compound was obtained as described for **11a** starting from 3-chloro-2,4-difluoroaniline (**10f**). mp : 128-132 °C. ^{1}H NMR (DMSO-d_6) δ 7.50 (td, J=8.8 Hz/1.4 Hz, 1H, 5-H), 7.84 (m, 1H, 6-H), 7.90 (bs, 2H, SO_2NH_2).

5-Chloro-2,4-difluorobenzenesulfonamide (11g).

The title compound was obtained as described for **11a** starting from 5-chloro-2,4-difluoroaniline (**10g**). mp : 119.5-122 °C. ^{1}H NMR (DMSO-d_6) δ 7.83 (t, J=9.6 Hz, 1H, 3-H), 7.84 (bs, 2H, SO_2NH_2), 7.94 (t, J=7.7 Hz, 1H, 6-H).

2-Chloro-4,6-difluorobenzenesulfonamide (11h).

The title compound was obtained as described for **11a** starting from 2-chloro-4,6-difluoroaniline (**10h**). mp : 116-120 °C. ^{1}H NMR (DMSO-d_6) δ 7.56 (m, 2H, 3-H/5-H), 7.99 (s, 2H, SO_2NH_2).

2,3,6-Trifluorobenzenesulfonamide (11i).

The title compound was obtained as described for **11a** starting from 2,3,6-trifluoroaniline (**10i**). mp : 148-151 °C. ^{1}H NMR (DMSO-d_6) δ 7.34 (m, 1H, 5-H), 7.78 (m, 1H, 4-H), 8.18 (s, 2H, SO_2NH_2).

4-Chloro-2,5-difluorobenzenesulfonamide (11j).

The title compound was obtained as described for **11a** starting from 4-chloro-2,5-difluoroaniline (**10j**). mp : 154-157 °C. ^{1}H NMR (DMSO-d_6) δ 7.77 (dd, J=8.4 Hz/6.2 Hz, 1H, 6-H), 7.78 (bs, 2H, SO_2NH_2), 7.95 (dd, J=9.3 Hz/5.8 Hz, 1H, 3-H).

2-Chloro-3,6-difluorobenzenesulfonamide (11k).

The title compound was obtained as described for **11a** starting from 2-chloro-3,6-difluoroaniline (**10k**). mp: 175-178 °C. ^{1}H NMR (DMSO-d_6) δ 7.52 (m, 1H, 4-H), 7.76 (m, 1H, 5-H), 8.14 (s, 2H, SO_2NH_2).

3-Chloro-2,6-difluorobenzenesulfonamide (11l).

The title compound was obtained as described for **11a** starting from 3-chloro-2,6-difluoroaniline (**10l**). mp: 144-146 °C. ^{1}H NMR (DMSO-d_6) δ 7.37 (td, J=9.8 Hz/1.9 Hz, 1H, 5-H), 7.91 (m, 1H, 4-H), 8.16 (bs, 2H, SO_2NH_2).

3,4-Dichloro-2-fluorobenzenesulfonamide (11m).

The title compound was obtained as described for **11a** starting from 3,4-dichloro-2-fluoroaniline (**10m**). mp : 162-164 °C. ^{1}H NMR (DMSO-d_6) δ 7.71 (d, J=8.7 Hz, 1H, 5-H), 7.78 (m, 1H, 6-H), 7.94 (bs, 2H, SO_2NH_2).

4,5-Dichloro-2-fluorobenzensulfonamide (11n).

The title compound was obtained as described for **11a** starting from 4,5-dichloro-2-fluoroaniline (**10n**). mp : 144-145 °C. ^{1}H NMR (DMSO-d_6) δ 7.05 (bs, 2H, SO_2NH_2), 7.55 (d, J=6.7 Hz, 1H, 6-H), 7.67 (d, J=9.3 Hz, 1H, 3-H).

2-Bromo-6-cyclopropylaminobenzenesulfonamide (12a).

A solution of 2-bromo-6-fluorobenzenesulfonamide (**11a**: 3.13 g, 12.31 mmol) in dioxane (30 mL) supplemented with cyclopropylamine (3 mL, 43.30 mmol) was heated at 100-110° C in a hermetically closed vessel for 24 hours. The solvent and the excess of amine were removed by distillation under reduced pressure and the residue was dissolved in methanol (20 mL). The methanolic solution was cooled on an ice bath, and water (60 mL) was added. The precipitate obtained (the title product) was collected by filtration, washed with water, and dried. It was used in the next step without further purification. ^{1}H NMR (DMSO-d_6) δ 0.44 (m, 2H, $CH(CH_2)_2$), 0.81 (m, 2H, $CH(CH_2)_2$), 2.44 (m, 1H, $CH(CH_2)_2$), 7.00 (d, J=7.5 Hz, 1H, 5-H),

7.18 (d, J=8.5 Hz, 1H, 3-H), 7.23 (t, J=8.0 Hz, 1H, 4-H) 7.47 (s, 1H, NH), 7.56 (bs, 2H, SO_2NH_2).

4-Bromo-2-cyclopropylaminobenzenesulfonamide (12b).

The title compound was obtained as described for **12a** starting from 4-Bromo-2-fluorobenzenesulfonamide (**11b**). ^{1}H NMR (DMSO-d_6) δ 0.53 (m, 2H, $CH(CH_2)_2$), 0.82 (m, 2H, $CH(CH_2)_2$), 2.50 (m, 1H, $CH(CH_2)_2$), 6.21 (s, 1H, NH), 6.90 (dd, J=8.4 Hz/1.8 Hz, 1H, 5-H), 7.25 (d, J=1.8 Hz, 1H, 3-H), 7.42 (bs, 2H, SO_2NH_2), 7.52 (d, J=8.4 Hz, 1H, 6-H).

2-Cyclopropylamino-3,4-difluorobenzenesulfonamide (12c).

The title compound was obtained as described for **12a** starting from 2,3,4-trifluorobenzenesulfonamide (**11c**). ^{1}H NMR (DMSO-d_6) δ 0.59 (m, 2H, $CH(CH_2)_2$), 0.71 (m, 2H, $CH(CH_2)_2$), 2.94 (m, 1H, $CH(CH_2)_2$), 5.90 (s, 1H, NH), 6.81 (td, J=9.3 Hz/6.9 Hz, 1H, 5-H), 7.49 (td, J=6.4 Hz/3.0 Hz, 1H, 6-H), 7.57 (s, 2H, SO_2NH_2).

2-Cyclopropylamino-3,5-difluorobenzenesulfonamide (12d).

The title compound was obtained as described for **12a** with a heating time of 40 hours, starting from 2,3,5-trifluorobenzenesulfonamide (**11d**). ^{1}H NMR (DMSO-d_6) δ 0.52 (m, 2H, $CH(CH_2)_2$), 0.65 (m, 2H, $CH(CH_2)_2$), 2.86 (m, 1H, $CH(CH_2)_2$), 5.47 (s, 1H, NH), 7.30 (d, J=7.0 Hz, 1H, 6-H), 7.47 (m, 1H, 4-H), 7.72 (s, 2H, SO_2NH_2).

2-Cyclopropylamino-4,5-difluorobenzenesulfonamide (12e).

To a solution of 2-amino-4,5-difluorobenzenesulfonamide (**15a**: 1.00 g, 4.80 mmol) in methanol (20 mL) were added (1-ethoxycyclopropyloxy)trimethylsilane (4 mL) and glacial acetic acid (4 mL). The reaction mixture was refluxed for 24 hours. The mixture was then evaporated to dryness under reduced pressure and taken up in water (30 mL). The precipitate

obtained was collected by filtration, washed with water, and dried. The product was then dissolved in THF (50 mL). Sodium borohydride (2.00 g) and boron trifluoride etherate (2 mL) were added, and the mixture was heated at reflux for 24 hours. The solvent was removed by evaporation under reduced pressure and the residue was taken up in water (30 mL), adjusted to slightly acid pH by adding 6N HCl and then extracted with chloroform (3x30 mL). The organic phases were collected, dried over anhydrous $MgSO_4$ and filtered, and the filtrate was evaporated under reduced pressure. The residue was taken up in ethyl acetate (5 mL). Hexane (50 mL) was added to the solution and the precipitate which appears was collected by filtration, washed with hexane and dried. ^{1}H NMR (DMSO-*d*$_6$) δ 0.52 (m, 2H, CH(CH$_2$)$_2$), 0.81 (m, 2H, CH(CH$_2$)$_2$), 2.46 (m, 1H, CH(CH$_2$)$_2$), 6.14 (s, 1H, NH), 7.07 (dd, J=13.6 Hz/6.8 Hz, 1H, 3-H), 7.50 (bs, 2H, SO_2NH_2), 7.58 (dd, J=10.7 Hz/8.9 Hz, 1H, 6-H).

2-Cyclopropylamino-4,6-difluorobenzenesulfonamide (12f).

The title compound was obtained as described for **12a** starting from 2,4,6-trifluorobenzenesulfonamide (**11e**). ^{1}H NMR (DMSO-*d*$_6$) δ 0.47 (m, 2H, CH(C*H*$_2$)$_2$), 0.82 (m, 2H, CH(C*H*$_2$)$_2$), 2.46 (m, 1H, C*H*(CH$_2$)$_2$, 6.58 (m, 1H, 5-*H*), 6.70 (d, J=12.2 Hz, 1H, 3-*H*)), 7.27 (s, 1H, N*H*), 7.73 (s, 2H, SO_2NH_2).

3-Chloro-2-cyclopropylamino-4-fluorobenzenesulfonamide (12g).

The title compound was obtained as described for **12a** starting from 3-chloro-2,4-difluorobenzenesulfonamide (**11f**). ^{1}H NMR (DMSO-*d*$_6$) δ 0.56 (m, 2H, CH(CH$_2$)$_2$), 0.69 (m, 2H, CH(CH$_2$)$_2$), 3.12 (m, 1H, CH(CH$_2$)$_2$), 5.91 (s, 1H, NH), 6.95 (t, J=8.7 Hz, 1H, 5-H), 7.69 (s, 2H, SO_2NH_2), 7.69 (dd, J=8.9 Hz/6.3 Hz, 1H, 6-H).

5-Chloro-2-cyclopropylamino-4-fluorobenzenesulfonamide (12h).

The title compound was obtained as described for **12a** starting from 5-chloro-2,4-difluorobenzenesulfonamide (**11g**). ^{1}H NMR (DMSO-d_6) δ 0.53 (m, 2H, CH$(CH_2)_2$), 0.82 (m, 2H, CH$(CH_2)_2$), 2.50 (m, 1H, CH$(CH_2)_2$), 6.32 (s, 1H, NH), 7.06 (d, J=12.4 Hz, 1H, 3-H), 7.53 (bs, 2H, SO_2NH_2), 8.23 (d, J=8.2 Hz, 1H, 6-H).

2-Chloro-6-cyclopropylamino-4-fluorobenzenesulfonamide (12i).

The title compound was obtained as described for **12a** starting from 2-chloro-4,6-difluorobenzenesulfonamide (**11h**). ^{1}H NMR (DMSO-d_6) δ 0.46 (m, 2H, CH$(CH_2)_2$), 0.83 (d, J=6.2 Hz, 2H, CH$(CH_2)_2$), 2.46 (m, 1H, CH$(CH_2)_2$), 6.75 (d, J=8.0 Hz, 1H, 5-H), 6.88 (d, J=12.0 Hz, 1H, 3-H), 7.64 (s, 1H, NH), 7.69 (bs, 2H, SO_2NH_2).

2-Cyclopropylamino-5,6-difluorobenzenesulfonamide (12j).

The title compound was obtained as described for **12a** starting from 2,3,6-trifluorobenzenesulfonamide (**11i**). ^{1}H NMR (DMSO-d_6) δ 0.47 (m, 2H, CH$(CH_2)_2$), 0.68 (m, 2H, CH$(CH_2)_2$), 2.88 (m, 1H, CH$(CH_2)_2$), 6.62 (td, J=10.1 Hz/3.3 Hz, 1H, 5-H), 6.78 (bs, 1H, NH), 7.33 (ddd, J=13.6 Hz/9.0 Hz/5.1 Hz, 1H, 4-H), 7.83 (bs, 2H, SO_2NH_2).

4-Chloro-2-cyclopropylamino-5-fluorobenzenesulfonamide (12k).

The title compound was obtained as described for **12a** starting from 4-chloro-2,5-difluorobenzenesulfonamide (**11j**). ^{1}H NMR (DMSO-d_6) δ 0.52 (m, 2H, CH$(CH_2)_2$), 0.81 (m, 2H, CH$(CH_2)_2$), 2.50 (m, 1H, CH$(CH_2)_2$), 6.09 (s, 1H, NH), 7.21 (d, J=6.2 Hz, 1H, 3-H), 7.54 (d, J=9.2 Hz, 1H, 6-H), 7.56 (bs, 2H, SO_2NH_2).

2-Chloro-6-cyclopropylamino-3-fluorobenzenesulfonamide (12l).

The title compound was obtained as described for **12a** starting from 2-chloro-3,6-difluorobenzenesulfonamide (**11k**). ^{1}H NMR (DMSO-d_6) δ 0.44 (m, 2H, CH(CH_2)$_2$), 0.80 (m, 2H, CH(CH_2)$_2$), 2.43 (m, 1H, CH(CH_2)$_2$), 7.16 (dd, J=9.5 Hz/4.7 Hz, 1H, 5-H), 7.25 (bs, 1H, NH), 7.49 (m, 1H, 4-H), 7.78 (bs, 2H, SO_2NH_2).

3-Chloro-2-cyclopropylamino-6-fluorobenzenesulfonamide (12m).

The title compound was obtained as described for **12a** starting from 3-chloro-2,6-difluorobenzenesulfonamide (**11l**). ^{1}H NMR (DMSO-d_6) δ 0.47 (m, 2H, CH(CH_2)$_2$), 0.67 (m, 2H, CH(CH_2)$_2$), 3.07 (m, 1H, CH(CH_2)$_2$), 6.73 (d, J=3.5 Hz, 1H, NH), 6.81 (dd, J=10.2 Hz/9.0 Hz, 1H, 4-H), 7.56 (dd, J=8.9 Hz/5.7 Hz, 1H, 3-H), 7.85 (bs, 2H, SO_2NH_2).

3,4-Dichloro-2-cyclopropylaminobenzenesulfonamide (12n).

The title compound was obtained as described for **12a** starting from 3,4-dichloro-2-fluorobenzenesulfonamide (**11m**). ^{1}H NMR (DMSO-d_6) δ 0.53 (m, 2H, CH(CH_2)$_2$), 0.67 (m, 2H, CH(CH_2)$_2$), 3.11 (m, 1H, CH(CH_2)$_2$), 5.80 (s, 1H, NH), 7.22 (d, J=8.6 Hz, 1H, 5-H), 7.64 (d, J=8.6 Hz, 1H, 6-H), 7.72 (s, 2H, SO_2NH_2).

4,5-Dichloro-2-cyclopropylaminobenzenesulfonamide (12o).

The title compound was obtained as described for **12a** starting from 4,5-dichloro-2-fluorobenzenesulfonamide (**11n**). ^{1}H NMR (DMSO-d_6) δ 0.54 (m, 2H, CH(CH_2)$_2$), 0.83 (m, 2H, CH(CH_2)$_2$), 2.50 (m, 1H, CH(CH_2)$_2$), 6.25 (s, 1H, NH), 7.28 (s, 1H, 3-H), 7.58 (bs, 2H, SO_2NH_2), 7.71 (s, 1H, 6-H).

4,5-Dichloro-2-(1-methyl)cyclopropylaminobenzenesulfonamide (12p).

The title compound was obtained as described for **12a** starting from 4,5-dichloro-2-fluorobenzensulfonamide (**11n**), with addition of (1-methyl)cyclopropylamine. mp: 130-132 °C. ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 4H, $CCH_3(CH_2)_2$), 1.33 (s, 3H, $CCH_3(CH_2)_2$), 6.37 (s, 1H, NH), 7.19 (s, 1H, 3-H), 7.72 (s, 1H, 6-H), 7.90 (bs, 2H, SO_2NH_2).

4,5-Dichloro-2-cyclobutylaminobenzenesulfonamide (12q).

The title compound was obtained as described for **12a** starting from 4,5-dichloro-2-fluorobenzensulfonamide (**11n**), with addition of cyclobutylamine. ^{1}H NMR (DMSO-d_6) δ 1.75 (m, 2H, $CH(CH_2)_3$), 1.87 (p, J=8.7 Hz, 2H, $CH(CH_2)_3$), 2.40 (m, 2H, $CH(CH_2)_3$), 4.01 (h, J=7.4 Hz, 1H, $CH(CH_2)_3$), 6.12 (d, J=6.1 Hz, 1H, NH), 6.85 (s, 1H, 3-H), 7.64 (bs, 2H, SO_2NH_2), 7.71 (s, 1H, 6-H).

4,5-Dichloro-2-cyclopentylaminobenzenesulfonamide (12r).

The title compound was obtained as described for **12a** starting from 4,5-dichloro-2-fluorobenzensulfonamide (**11n**), with addition of cyclopentylamine. ^{1}H NMR (DMSO-d_6) δ 1.45 (m, 2H, $CH(CH_2)_4$), 1.60 (m, 2H, $CH(CH_2)_4$), 1.67 (m, 2H, $CH(CH_2)_4$), 1.99 (m, 2H, $CH(CH_2)_4$), 3.90 (h, J=6.5 Hz, 1H, $CH(CH_2)_4$), 5.99 (d, J=6.7 Hz, 1H, NH), 7.01 (s, 1H, 3-H), 7.63 (bs, 2H, SO_2NH_2), 7.71 (s, 1H, 6-H).

4,5-Dichloro-2-cyclohexylaminobenzenesulfonamide (12s).

The title compound was obtained as described for **12a** starting from 4,5-dichloro-2-fluorobenzensulfonamide (**11n**), with addition of cyclohexylamine which was heated at 80 °C in a hermetically closed vessel for 72 hours. ^{1}H NMR (DMSO-d_6) δ 1.26 (m, 3H, $CH(CH_2)_5$), 1.40 (m, 2H, $CH(CH_2)_5$), 1.57 (m, 1H, $CH(CH_2)_5$), 1.67 (m, 2H, $CH(CH_2)_5$), 1.89 (m, 2H,

$CH(CH_2)_5$), 3.49 (m, 1H, $CH(CH_2)_5$), 5.97 (d, J=7.6 Hz, 1H, NH), 7.05 (s, 1H, 3-H), 7.60 (bs, 2H, SO_2NH_2), 7.71 (s, 1H, 6-H).

4,5-Dichloro-2-cycloheptylaminobenzenesulfonamide (12t).

The title compound was obtained as described for **12a** starting from 4,5-dichloro-2-fluorobenzensulfonamide (**11n**), with addition of cycloheptylamine. ^{1}H NMR (DMSO-d_6) δ 1.47-1.64 (m, 10H, $CH(CH_2)_6$), 1.88 (m, 2H, $CH(CH_2)_6$), 3.65 (m, 1H, $CH(CH_2)_6$), 6.00 (d, J=7.7 Hz, 1H, NH), 6.94 (s, 1H, 3-H), 7.63 (bs, 2H, SO_2NH_2), 7.72 (s, 1H, 6-H).

4,5-Dichloro-2-cyclooctylaminobenzenesulfonamide (12u).

The title compound was obtained as described for **12a** starting from 4,5-dichloro-2-fluorobenzensulfonamide (**11n**), with addition of cyclooctylamine which was heated at 80 °C in a hermetically closed vessel for 72 hours. ^{1}H NMR (DMSO-d_6) δ 1.50-1.67 (m, 10H, $CH(CH_2)_7$), 1.81 (m, 2H, $CH(CH_2)_7$), 3.65 (m, 1H, $CH(CH_2)_7$), 6.00 (d, J=7.7 Hz, 1H, NH), 6.93 (s, 1H, 3-H), 7.61 (bs, 2H, SO_2NH_2), 7.72 (s, 1H, 6-H).

4,5-Dichloro-2-isopropylaminobenzenesulfonamide (12v).

To a solution of 4,5-dichloro-2-fluorobenzensulfonamide (**11n**: 3.00 g, 10.59 mmol) in dioxane (20 ml) were added isopropylamine (3 ml). The mixture was heated at 100 °C in a sealed vessel for 72 hours. The solvent was then removed by evaporation under reduced pressure. The oil obtained was used in the following step without further purification. ^{1}H NMR (DMSO-d_6) δ 1.13 (d, J=6.3 Hz, 2H, $CH(CH_3)_2$), 3.55 (o, 1H, $CH(CH_3)_2$), 5.99 (d, J=7.5 Hz, 1H, NH), 6.67 (s, 1H, 3-H), 7.25 (s, 1H, 6-H), 7.37 (bs, 2H, SO_2NH_2).

6,7-Dichloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (16).

A mixture of 2-amino-4,5-dichlorobenzenesulfonamide (**15b**: 3.00 g, 14,52 mmol) and ethyl orthoformate (40 mL) was heated at boiling for 1 hour in an open vessel. The volume of the mixture was reduced by half under reduced pressure. The precipitate obtained was collected by filtration, washed with ether and dried. mp: 237-240 °C. ^{1}H NMR (DMSO-*d*$_6$) δ 7.57 (s, 1H, 5-H), 8.06 (s, 1H, 3-H), 8.15 (s, 1H, 8-H), 12.47 (bs, 1H, NH).

8-Bromo-4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13a).

In a round-bottom flask, a mixture of 2-Bromo-6-cyclopropylaminobenzenesulfonamide (**12a**) (2.59 g, 8.90 mmol) and ethyl othoformate (25 mL) was heated in the open state at 150° C for 1,5 hours. The suspension obtained was cooled on an ice bath, and the insoluble material was collected by filtration, washed with diethylether and dried. The solid was redissolved in a hot mixture of acetone and methanol and the hot solution was treated with charcoal, filtered, and the filtrate was concentrated to dryness. The residue was recrystallised from methanol. mp : 244-255 °C. ^{1}H NMR (DMSO-*d*$_6$) δ 1.04 (m, 2H, CH(CH$_2$)$_2$), 1.16 (d, J=6.0 Hz, 2H, CH(CH$_2$)$_2$), 3.36 (tt, J=7.3 Hz/3.8 Hz, 1H, CH(CH$_2$)$_2$), 7.69 (t, J=8.2 Hz, 1H, 6-H), 7.79 (d, J=7.9 Hz, 1H, 5-H), 7.90 (d, J=8.6 Hz, 1H, 7-H), 8.12 (s, 1H, 3-H).

6-Bromo-4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13b).

The title compound was obtained as described for **13a** with a heating time of 24 hours at 130 °C, starting from 4-Bromo-2-cyclopropylaminobenzenesulfonamide (**12b**). mp : 268-271 °C. ^{1}H NMR (DMSO-*d*$_6$) δ 1.03 (m, 2H, CH(CH$_2$)$_2$), 1.17 (d, J=6.4 Hz, 2H, CH(CH$_2$)$_2$), 3.40 (tt, J=7.1 Hz/3.7 Hz, 1H, CH(CH$_2$)$_2$), 7.76 (dd, J=8.4 Hz/1.6 Hz, 1H, 7-H), 7.84 (d, J=8.4 Hz, 1H, 8-H), 7.98 (d, J=1.5 Hz, 1H, 5-H), 8.16 (s, 1H, 3-H).

4-Cyclopropyl-5,6-difluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13c).

The title compound was obtained as described for **13a** with a heating time of 6 hours, starting from 2-cyclopropylamino-3,4-difluorobenzenesulfonamide (**12c**). mp : 182-184 °C. ^{1}H NMR (DMSO-d_6) δ 1.07 (d, J=5.5 Hz, 4H, $CH(CH_2)_2$), 3.77 (m, 1H, $CH(CH_2)_2$), 7.65 (td, J=9.3 Hz/6.7 Hz, 1H, 7-H), 7.78 (m, 1H, 8-H), 8.22 (s, 1H, 3-H).

4-Cyclopropyl-5,7-difluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13d).

The title compound was obtained as described for **13a** with a heating time of 3 hours at 130 °C, starting from 2-cyclopropylamino-3,5-difluorobenzenesulfonamide (**12d**). mp : 160-163 °C. ^{1}H NMR (DMSO-d_6) δ 1.03 (m, 4H, $CH(CH_2)_2$), 3.76 (m, 1H, $CH(CH_2)_2$), 7.69 (ddd, J=6.9 Hz/2.6 Hz/1.5 Hz, 1H, 8-H), 7.88 (m, 1H, 6-H), 8.20 (s, 1H, 3-H).

4-Cyclopropyl-6,7-difluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13e).

The title compound was obtained as described for **13a** with a heating time of 1 hour, starting from 2-cyclopropylamino-4,5-difluorobenzenesulfonamide (**12e**). mp : 140-142 °C. ^{1}H NMR (DMSO-d_6) δ 1.03 (m, 2H, $CH(CH_2)_2$), 1.18 (m, 2H, $CH(CH_2)_2$), 3.35 (m, 1H, $CH(CH_2)_2$), 7.95 (dd, J=12.2 Hz/6.7 Hz, 1H, 5-H), 8.15 (m, 2H, 3-H/8-H).

4-Cyclopropyl-6,8-difluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13f).

The title compound was obtained as described for **13a** with a heating time of 24 hours, starting from 2-cyclopropylamino-4,6-difluorobenzenesulfonamide (**12f**). mp : 167-168 °C. ^{1}H NMR (DMSO-d_6) δ 1.06 (m, 2H, $CH(CH_2)_2$), 1.17 (q, J=6.9 Hz, 2H, $CH(CH_2)_2$), 3.32 (m, 1H, $CH(CH_2)_2$), 7.57 (m, 2H, 5-H/7-H), 8.16 (s, 1H, 3-H).

5-Chloro-4-cyclopropyl-6-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13g).

The title compound was obtained as described for **13a** with a heating time of 2 hours at 130 °C., starting from 3-chloro-2-cyclopropylamino-4-fluorobenzenesulfonamide (**12g**). mp : 147-148 °C. ^{1}H NMR (DMSO-d_6) δ 0.86 (m, 2H, $CH(CH_2)_2$), 1.14 (q, J=6.5 Hz, 2H, $CH(CH_2)_2$), 4.04 (dt, J=6.8 Hz/3.4 Hz, 1H, $CH(CH_2)_2$), 7.63 (t, J=9.7 Hz, 1H, 7-H), 7.94 (t, J=8.8 Hz/5.7 Hz, 1H, 8-H), 8.38 (s, 1H, 3-H).

7-Chloro-4-cyclopropyl-6-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13h).

The title compound was obtained as described for **13a** with a heating time of 5 hours at 130 °C., starting from 5-chloro-2-cyclopropylamino-4-fluorobenzenesulfonamide (**12h**). mp : 217-219 °C. ^{1}H NMR (DMSO-d_6) δ 1.04 (m, 2H, $CH(CH_2)_2$), 1.18 (m, 2H, $CH(CH_2)_2$), 3.36 (m, 1H, $CH(CH_2)_2$), 7.89 (d, J=11.1 Hz, 1H, 5-H), 8.18 (s, 1H, 3-H), 8.21 (d, J=7.6 Hz, 1H, 8-H).

8-Chloro-4-cyclopropyl-6-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13i).

The title compound was obtained as described for **13a** with a heating time of 3 hours at 130 °C., starting from 2-chloro-6-cyclopropylamino-4-fluorobenzenesulfonamide (**12i**). mp : 252-255 °C. ^{1}H NMR (DMSO-d_6) δ 1.06 (m, 2H, $CH(CH_2)_2$), 1.17 (d, J=5.5 Hz, 2H, $CH(CH_2)_2$), 3.33 (m, 1H, $CH(CH_2)_2$), 7.71 (m, 2H, 5-H/7-H), 8.14 (s, 1H, 3-H).

4-Cyclopropyl-7,8-difluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13j).

The title compound was obtained as described for **13a** with a heating time of 2 hours at 130 °C., starting from 2-cyclopropylamino-5,6-difluorobenzenesulfonamide (**12j**). mp : 205-306 °C. ^{1}H NMR (DMSO-d_6) δ 1.04 (m, 4H, $CH(CH_2)_2$), 3.73 (m, 1H, $CH(CH_2)_2$), 7.46 (td, J=9.1 Hz/2.9 Hz, 1H, 5-H), 7.77 (m, 1H, 6-H), 8.16 (s, 1H, 3-H).

6-Chloro-4-cyclopropyl-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13k).

The title compound was obtained as described for **13a** with a heating time of 2 hours, starting from 4-chloro-2-cyclopropylamino-5-fluorobenzenesulfonamide (**12k**). mp : 205-206 °C. ^{1}H NMR (DMSO-d_6) δ 1.04 (m, 2H, CH(CH_2)$_2$), 1.17 (m, 2H, CH(CH_2)$_2$), 3.39 (tt, J=7.1 Hz/3.8 Hz, 1H, CH(CH_2)$_2$), 8.05 (d, J=6.1 Hz, 1H, 5-H), 8.08 (d, J=7.9 Hz, 1H, 8-H) , 8.16 (s, 1H, 3-H).

8-Chloro-4-cyclopropyl-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13l).

The title compound was obtained as described for **13a** with a heating time of 24 hours at 130 °C., starting from 2-chloro-6-cyclopropylamino-3-fluorobenzenesulfonamide (**12l**). mp : 260-263 °C. ^{1}H NMR (DMSO-d_6) δ 1.05 (m, 2H, CH(CH_2)$_2$), 1.15 (m, 2H, CH(CH_2)$_2$), 3.88 (t, J=7.1 Hz/4.0 Hz; 1H, CH(CH_2)$_2$), 7.90 (m, 1H, 5-H), 7.94 (dd, J=9.5 Hz/4.5 Hz, 1H, 6-H), 8.13 (s, 1H, 3-H).

5-Chloro-4-cyclopropyl-8-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13m).

The title compound was obtained as described for **13a** with a heating time of 6 hours, starting from 3-chloro-2-cyclopropylamino-6-fluorobenzenesulfonamide (**12m**). mp : 201-204 °C. ^{1}H NMR (DMSO-d_6) δ 0.90 (m, 2H, CH(CH_2)$_2$), 1.11 (m, 2H, CH(CH_2)$_2$), 3.98 (tt, J=7.1 Hz/3.6 Hz, 1H, CH(CH_2)$_2$), 7.46 (t, J=9.0 Hz, 1H, 7-H), 7.95 (dd, J=9.1 Hz/5.7 Hz, 1H, 6-H), 8.31 (s, 1H, 3-H).

5,6-Dichloro-4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13n).

The title compound was obtained as described for **13a** with a heating time of 2 hours at 130 °C., starting from 3,4-dichloro-2-cyclopropylaminobenzenesulfonamide (**12n**). mp : 183-185 °C. ^{1}H NMR (DMSO-d_6) δ 0.78 (m, 2H, CH(CH_2)$_2$), 1.12 (d, J=6.7 Hz, 2H, CH(CH_2)$_2$), 4.05 (tt, J=6.9 Hz/3.5 Hz, 1H, CH(CH_2)$_2$), 7.86 (m, 2H, 7-H/8-H), 8.42 (s, 1H, 3-H).

6,7-Dichloro-4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13o).

The title compound was obtained as described for **13a** with a heating time of 1 hour, starting from 4,5-dichloro-2-cyclopropylaminobenzenesulfonamide (**12o**). mp : 260-262 °C. ^{1}H NMR (DMSO-d_6) δ 1.05 (m, 2H, $CH(CH_2)_2$), 1.18 (q, J=7.1 Hz, 2H, $CH(CH_2)_2$), 3.39 (tt, J=7.1 Hz/3.8 Hz, 1H, $CH(CH_2)_2$), 8.05 (s, 1H, 5-H), 8.18 (s, 1H, 3-H), 8.20 (s, 1H, 8-H).

6,7-Dichloro-4-(1-methyl)cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13p).

The title compound was obtained as described for **13a** with a heating time of 24 hours, starting from 4,5-Dichloro-2-(1-methyl)cyclopropylaminobenzenesulfonamide (**12p**). mp : 230-232 °C. ^{1}H NMR (DMSO-d_6) δ 1.05 (m, 1H, $CH(CH_2)_2$), 1.11 (m, 1H, $CH(CH_2)_2$), 1.26 (m, 1H, $CH(CH_2)_2$), 1.35 (m, 1H, $CH(CH_2)_2$), 1.54 (s, 3H, CH_3), 7.92 (s, 1H, 5-H), 8.22 (s, 1H, 3-H), 8.33 (s, 1H, 8-H).

6,7-Dichloro-4-cyclobutyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13q).

The title compound was obtained as described for **13a** with a heating time of 24 hours, starting from 4,5-dichloro-2-cyclobutylaminobenzenesulfonamide (**12q**). mp : 238-240 °C. ^{1}H NMR (DMSO-d_6) δ 1.80 (m, 2H, $CH(CH_2)_3$), 2.36 (m, 2H, $CH(CH_2)_3$), 2.50 (m, 2H, $CH(CH_2)_3$), 4.79 (m, 1H, $CH(CH_2)_3$), 7.17 (s, 1H, 5-H), 8.09 (s, 1H, 3-H), 8.19 (bs, 1H, 8-H).

6,7-Dichloro-4-cyclopentyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13r).

In a sealed autoclave, a mixture of 4,5-dichloro-2-cyclopentylaminobenzenesulfonamide (**12r**) (2.50 g, 8.08 mmol) and ethyl orthoformate (25 ml) was heated at 140 °C for 72 hours. The mixture was cooled on an ice bath, and the insoluble material was collected by filtration, washed with diethylether and dried. The solid was re-dissolved in a hot mixture of acetone and methanol

and the hot solution was treated with charcoal, filtered, and the filtrate was concentrated to dryness. The residue was recrystallised from methanol. mp : 189-191 °C. ^{1}H NMR (DMSO-d_6) δ 1.68 (m, 2H, CH(CH$_2$)$_4$), 1.82 (m, 4H, CH(CH$_2$)$_4$), 2.19 (m, 2H, CH(CH$_2$)$_4$), 4.85 (p, J=7.5 Hz, 1H, CH(CH$_2$)$_4$), 8.13 (s, 1H, 5-H), 8.17 (s, 1H, 3-H), 8.20 (s, 1H, 8-H).

6,7-Dichloro-4-cyclohexyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13s).

The title compound was obtained as described for **13s** starting from 4,5-dichloro-2-cyclohexylaminobenzenesulfonamide (**12s**). mp : 219-222 °C. ^{1}H NMR (DMSO-d_6) δ 1.20 (m, 1H, CH(CH$_2$)$_5$), 1.56 (m, 2H, CH(CH$_2$)$_5$), 1.67 (m, 1H, CH(CH$_2$)$_5$), 1.78 (m, 4H, CH(CH$_2$)$_5$), 1.96 (m, 2H, CH(CH$_2$)$_5$), 4.47 (m, 1H, CH(CH$_2$)$_5$), 8.13 (s, 1H, 5-H), 8.17 (s, 1H, 3-H), 8.30 (s, 1H, 8-H).

6,7-Dichloro-4-cycloheptyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13t).

The title compound was obtained as described for **13s** starting from 4,5-dichloro-2-cycloheptylaminobenzenesulfonamide (**12t**). mp : 222-224 °C. ^{1}H NMR (DMSO-d_6) δ 1.54-1.75 (m, 8H, CH(CH$_2$)$_6$), 1.97 (m, 4H, CH(CH$_2$)$_6$), 4.56 (tt, J=9.4 Hz/4.4 Hz, 1H, CH(CH$_2$)$_6$), 8.09 (s, 1H, 5-H), 8.16 (s, 1H, 3-H), 8.28 (s, 1H, 8-H).

6,7-Dichloro-4-cyclooctyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13u).

The title compound was obtained as described for **13s** starting from 4,5-dichloro-2-cyclooctylaminobenzenesulfonamide (**12u**). mp : 229-231 °C. ^{1}H NMR (DMSO-d_6) δ 1.53 (m, 3H, CH(CH$_2$)$_7$), 1.66 (m, 5H, CH(CH$_2$)$_7$), 1.76 (m, 2H, CH(CH$_2$)$_7$), 1.92 (m, 2H, CH(CH$_2$)$_7$), 2.08 (m, 2H, CH(CH$_2$)$_7$), 4.57 (t, J=9.9 Hz, 1H, CH(CH$_2$)$_7$), 8.00 (s, 1H, 5-H), 8.16 (s, 1H, 3-H), 8.30 (s, 1H, 8-H).

6,7-Dichloro-4-isopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13v).

A mixture of 4,5-dichloro-2-isopropylaminobenzenesulfonamide (**12v**) and ethyl othoformate (20 mL) was heated in a sealed vessel at 150° C for 48 hours. The reaction mixture was then evaporated under reduced pressure and the residue was recrystallised from methanol. mp : 221-223 °C. ^{1}H NMR (DMSO-d_6) δ 1.46 (d, J=6.6 Hz, 6H, $CH(CH_3)_2$), 4.85 (hept, J=6.6 Hz, 1H, $CH(CH_3)_2$), 8.10 (s, 1H, 5-H), 8.18 (s, 1H, 3-H), 8.30 (s, 1H, 8-H).

6,7-Dichloro-4-cyclopropylmethyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (13w).

To a solution of 6,7-dichloro-4*H*-1,2,4-benzothiadiazine 1,1 dioxide (**16**: 1.00 g, 3,98 mmol) in acetonitrile (40 ml) were added potassium carbonate (2.15 g, 15.56 mmol) and cyclopropylmethyl bromide (0.6 mL, 6.19 mmol), and the mixture was heated at reflux for 24 hours. The reaction mixture was evaporated under reduced pressure and the residue was taken up in water (40 mL). The insoluble material was collected by filtration, washed with water and dried, and was recrystallised from methanol. mp: 192-195 °C. ^{1}H NMR (DMSO-d_6) δ 0.43 (m, 2H, $CH_2CH(CH_2)_2$), 0.56 (m, 2H, $CH_2CH(CH_2)_2$), 1.27 (dtt, J=12.5 Hz/7.6 Hz/3.9 Hz, 1H, $CH_2CH(CH_2)_2$), 4.02 (d, J=7.2 Hz, 2H, $CH_2CH(CH_2)_2$), 8.09 (s, 1H, 5-H), 8.17 (s, 1H, 3-H), 8.20 (s, 1H, 8-H).

8-Bromo-4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14a).

Finely ground $NaBH_4$ (1.00 g, 26.43 mmol) was added to a solution of 8-Bromo-4-cyclopropyl-4H-1,2,4-benzothiadiazine 1,1-dioxide (**13a**) (1.97 g, 6.54 mmol) in isopropanol (50 mL) and then heated at 50-55° C for 5-10 minutes. The solvent was removed by evaporation under reduced pressure. The residue was taken up in water (50 mL) and brought to acid pH by adding 6N HCl. The title product was extracted with dichloromethane (3x30 mL). The organic phase was dried over $MgSO_4$ and filtered. The filtrate was evaporated to dryness and the residue

obtained was recrystallized from methanol/water 1/1 (60 mL). mp : 189-191 °C. ^{1}H NMR (DMSO-*d*$_6$) δ 0.67 (m, 2H, CH(CH$_2$)$_2$), 0.92 (m, 2H, CH(CH$_2$)$_2$), 2.49 (tt, J=6.7 Hz/3.7 Hz, 1H, CH(CH$_2$)$_2$), 4.62 (s, 2H, NCH$_2$NH), 7.09 (dd, J=5.2 Hz/3.6 Hz, 1H, 5-H), 7.30 (m, 2H, 6-H/7-H), 8.04 (bs, 1H, NH). ^{13}C NMR (DMSO-*d*$_6$) δ 8.8 (CH(CH$_2$)$_2$), 30.4 (CH(CH$_2$)$_2$), 60.3 (C-3), 114.4 (C-5), 118.8 (C-8), 122.8 (C-7), 123.2 (C-8a), 133.0 (C-6), 146.8 (C-4a). Anal. ($C_{10}H_{11}BrN_2O_2S$) theoretical: C, 39.62; H, 3.66; N, 9.24; S, 10.57. Found: C, 39.70; H, 3.71; N, 9.50; S, 10.34.

6-Bromo-4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14b).

The title compound was obtained as described for **14a** starting from 6-Bromo-4-cyclopropyl-4H-1,2,4-benzothiadiazine 1,1-dioxide (**13b**). mp : 180-181 °C. ^{1}H NMR (DMSO-*d*$_6$) δ 0.67 (m, 2H, CH(CH$_2$)$_2$), 0.93 (m, 2H, CH(CH$_2$)$_2$), 2.54 (tt, J=6.7 Hz/3.7 Hz, 1H, CH(CH$_2$)$_2$), 4.69 (s, 2H, NCH$_2$NH), 7.02 (dd, J=8.3 Hz/1.8 Hz, 1H, 7-H), 7.38 (d, J=1.8 Hz, 1H, 5-H), 8.34 (d, J=8.3 Hz, 1H, 8-H), 7.98 (bs, 1H, NH). ^{13}C NMR (DMSO-*d*$_6$) δ 8.4 (CH(CH$_2$)$_2$), 29.7 (CH(CH$_2$)$_2$), 61.0 (C-3), 116.6 (C-5), 119.8 (C-7), 122.3 (C-8a), 126.2 (C-8), 126.4 (C-6), 145.3 (C-4a). Anal. ($C_{10}H_{11}BrN_2O_2S$) theoretical: C, 39.62; H, 3.66; N, 9.24; S, 10.57. Found: C, 39.81; H, 3.68; N, 9.44; S, 10.25.

4-Cyclopropyl-5,6-difluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14c).

The title compound was obtained as described for **14a** starting from 4-cyclopropyl-5,6-difluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13c**). mp : 160-162 °C. ^{1}H NMR (DMSO-*d*$_6$) δ 0.79 (m, 2H, CH(CH$_2$)$_2$), 0.84 (m, 2H, CH(CH$_2$)$_2$), 3.02 (m, 1H, CH(CH$_2$)$_2$), 4.73 (s, 2H, NCH$_2$NH), 6.98 (td, J=9.4 Hz/6.7 Hz, 1H, 7-H), 7.47 (ddd, J=8.7 Hz/5.8 Hz/2.0 Hz, 1H, 8-H), 8.14 (bs, 1H, NH). ^{13}C NMR (DMSO-*d*$_6$) δ 9.1 (CH(CH$_2$)$_2$), 35.5 (CH(CH$_2$)$_2$), 62.1 (C-3), 107.3 (C-7), 120.8 (C-8), 123.4 (C-8a), 135.3 (C-4a), 138.8-140.8 (C-5), 152.0-154.0 (C-6). Anal.

($C_{10}H_{10}F_2N_2O_2S$) theoretical: C, 46.15; H, 3.87; N, 10.76; S, 12.32. Found: C, 46.36; H, 4.05; N, 11.06; S, 12.18.

4-Cyclopropyl-5,7-difluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14d).

The title compound was obtained as described for **14a** starting from 4-cyclopropyl-5,7-difluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13d**). mp : 149-151 °C. ^{1}H NMR (DMSO-d_6) δ 0.73 (m, 2H, $CH(CH_2)_2$), 0.82 (m, 2H, $CH(CH_2)_2$), 2.88 (tq, J=6.7 Hz/3.6 Hz, 1H, $CH(CH_2)_2$), 4.69 (s, 2H, NCH_2NH), 7.41 (ddd, J=7.4 Hz/2.8 Hz/1.6 Hz, 1H, 8-H), 7.53 (ddd, J=12.2 Hz/8.8 Hz/2.9 Hz, 1H, 6-H), 8.31 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 8.7 ($CH(CH_2)_2$), 35.2 ($CH(CH_2)_2$), 62.3 (C-3), 106.6 (C-6), 109.2 (C-8), 128.9 (C-8a), 130.6 (C-4a), 151.7-153.7 (C-7), 153.6-155.5 (C-5). Anal. ($C_{10}H_{10}F_2N_2O_2S$) theoretical: C, 46.15; H, 3.87; N, 10.76; S, 12.32. Found: C, 46.37; H, 4.14; N, 10.80; S, 12.81.

4-Cyclopropyl-6,7-difluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14e).

The title compound was obtained as described for **14a** starting from 4-cyclopropyl-6,7-difluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13e**). mp : 146-148 °C. ^{1}H NMR (DMSO-d_6) δ 0.66 (m, 2H, $CH(CH_2)_2$), 0.93 (m, 2H, $CH(CH_2)_2$), 2.54 (m, 1H, $CH(CH_2)_2$), 4.68 (s, 2H, NCH_2NH), 7.22 (m, 1H, 5-H), 7.73 (m, 1H, 8-H), 8.06 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 8.4 ($CH(CH_2)_2$), 30.0 ($CH(CH_2)_2$), 61.1 (C-3), 103.4 (C-5), 113.5 (C-8), 118.6 (C-8a), 140.6-142.5 (C-7), 142.4 (C-4a), 151.1-153.1 (C-6). Anal. ($C_{10}H_{10}F_2N_2O_2S$) theoretical: C, 46.15; H, 3.87; N, 10.76; S, 12.32. Found: C, 46.06; H, 3.83; N, 11.06; S, 11.86.

4-Cyclopropyl-6,8-difluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14f).

The title compound was obtained as described for **14a** starting from 4-cyclopropyl-6,8-difluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13f**). mp : 161-164 °C. ^{1}H NMR (DMSO-d_6) δ 0.68 (m,

2H, $CH(CH_2)_2$), 0.93 (m, 2H, $CH(CH_2)_2$), 2.54 (ddt, J=8.9 Hz/5.9 Hz/2.9 Hz, 1H, $CH(CH_2)_2$), 4.67 (s, 2H, NCH_2NH), 6.75 (ddd, J=10.5 Hz/9.4 Hz/2.4 Hz, 1H, 7-H), 7.02 (m, 1H, 5-H), 8.19 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 8.6 ($CH(CH_2)_2$), 30.2 ($CH(CH_2)_2$), 60.8 (C-3), 93.4 (C-7), 97.2 (C-5), 109.3 (C-8a), 147.4 (C-4a), 158.9-160.9 (C-8), 163.3-165.3 (C-6). Anal. ($C_{10}H_{10}F_2N_2O_2S$) theoretical: C, 46.15; H, 3.87; N, 10.76; S, 12.32. Found: C, 46.38; H, 3.86; N, 11.01; S, 11.91.

5-Chloro-4-cyclopropyl-6-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14g).

The title compound was obtained as described for **14a** starting from 5-chloro-4-cyclopropyl-6-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13g**). mp : 141-143 °C. ^1H NMR (DMSO-d_6) δ 0.80 (m, 4H, $CH(CH_2)_2$), 3.18 (m, 1H, $CH(CH_2)_2$), 4.77 (s, 2H, NCH_2NH), 7.06 (t, J=8.7 Hz, 1H, 7-H), 7.66 (dd, J=8.8 Hz/6.1 Hz, 1H, 8-H), 8.16 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 10.0 ($CH(CH_2)_2$), 36.5 ($CH(CH_2)_2$), 62.4 (C-3), 108.2 (C-7), 111.2 (C-5), 124.3 (C-8), 125.8 (C-8a), 143.5 (C-4a), 159.5-161.4 (C-6). Anal. ($C_{10}H_{10}ClFN_2O_2S$) theoretical: C, 43.40; H, 3.64; N, 10.12; S, 11.59. Found: C, 43.64; H, 3.73; N, 10.30; S, 11.33.

7-Chloro-4-cyclopropyl-6-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14h).

The title compound was obtained as described for **14a** starting from 7-chloro-4-cyclopropyl-6-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13h**). mp : 155-158 °C. ^1H NMR (DMSO-d_6) δ 0.68 (m, 2H, $CH(CH_2)_2$), 0.94 (m, 2H, $CH(CH_2)_2$), 2.56 (tt, J=6.7 Hz/3.8 Hz, 1H, $CH(CH_2)_2$), 4.71 (s, 2H, NCH_2NH), 7.19 (d, J=12.5 Hz, 1H, 5-H), 7.76 (d, J=8.0 Hz, 1H, 8-H), 8.07 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 8.4 ($CH(CH_2)_2$), 30.0 ($CH(CH_2)_2$), 61.0 (C-3), 102.7 (C-5), 107.7 (C-7), 120.3 (C-8a), 126.3 (C-8), 145.2 (C-4a), 158.4-160.4 (C-6). Anal.

($C_{10}H_{10}ClFN_2O_2S$) theoretical: C, 43.40; H, 3.64; N, 10.12; S, 11.59. Found: C, 43.26; H, 3.85; N, 9.87; S, 11.50.

8-Chloro-4-cyclopropyl-6-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14i).

The title compound was obtained as described for **14a** starting from 8-chloro-4-cyclopropyl-6-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13i**). mp : 190-192 °C. ^{1}H NMR (DMSO-d_6) δ 0.69 (m, 2H, $CH(CH_2)_2$), 0.94 (m, 2H, $CH(CH_2)_2$), 2.54 (ddd, J=10.4 Hz/6.7 Hz/3.8 Hz, 1H, $CH(CH_2)_2$), 4.65 (s, 2H, NCH_2NH), 6.92 (dd, J=8.6 Hz/2.5 Hz, 1H, 7-H), 7.02 (dd, J=12.1 Hz/2.5 Hz, 1H, 5-H), 8.16 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 8.8 ($CH(CH_2)_2$), 30.5 ($CH(CH_2)_2$), 60.4 (C-3), 100.3 (C-5), 107.1 (C-7), 118.7 (C-8a), 132.7 (C-8), 148.3 (C-4a), 162.2-164.2 (C-6). Anal. ($C_{10}H_{10}ClFN_2O_2S$) theoretical: C, 43.40; H, 3.64; N, 10.12; S, 11.59. Found: C, 43.51; H, 3.52; N, 10.19; S, 11.23.

4-Cyclopropyl-7,8-difluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14j).

The title compound was obtained as described for **14a** starting from 4-cyclopropyl-7,8-difluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13j**). mp : 199-201 °C. ^{1}H NMR (DMSO-d_6) δ 0.75 (m, 2H, $CH(CH_2)_2$), 0.85 (m, 2H, $CH(CH_2)_2$), 2.99 (tq, J=7.3 Hz/3.8 Hz, 1H, $CH(CH_2)_2$), 4.71 (s, 2H, NCH_2NH), 6.82 (td, J=9.1 Hz/3.0 Hz, 1H, 5-H), 7.40 (ddd, J=13.0 Hz/9.1 Hz/5.3 Hz, 1H, 6-H), 8.33 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 9.0 ($CH(CH_2)_2$), 35.0 ($CH(CH_2)_2$), 61.9 (C-3), 105.8 (C-5), 116.5 (C-8a), 120.2 (C-6), 134.7 (C-4a), 147.1-149.1 (C-7), 153.7-155.7 (C-8). Anal. ($C_{10}H_{10}F_2N_2O_2S$) theoretical: C, 46.15; H, 3.87; N, 10.76; S, 12.32. Found: C, 46.31; H, 3.82; N, 11.18; S, 12.04.

6-Chloro-4-cyclopropyl-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14k).

The title compound was obtained as described for **14a** starting from 6-chloro-4-cyclopropyl-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13k**). mp : 170-171 °C. ^{1}H NMR (DMSO-*d*$_6$) δ 0.66 (m, 2H, CH(CH$_2$)$_2$), 0.93 (m, 2H, CH(CH$_2$)$_2$), 2.55 (m, 1H, CH(CH$_2$)$_2$), 4.68 (s, 2H, NCH$_2$NH), 7.36 (d, J=6.2 Hz, 1H, 5-H), 7.68 (d, J=8.3 Hz, 1H, 8-H), 8.11 (bs, 1H, NH).^{13}C NMR (DMSO-*d*$_6$) δ 8.4 (CH(CH$_2$)$_2$), 29.9 (CH(CH$_2$)$_2$), 61.1 (C-3), 112.3 (C-8), 115.9 (C-5), 122.0 (C-8a), 124.6 (C-6), 141.8 (C-4a), 148.1-150.0 (C-7). Anal. (C$_{10}$H$_{10}$ClFN$_2$O$_2$S) theoretical: C, 43.40; H, 3.64; N, 10.12; S, 11.59. Found: C, 43.02; H, 3.54; N, 10.40; S, 11.15.

8-Chloro-4-cyclopropyl-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14l).

The title compound was obtained as described for **14a** starting from 8-chloro-4-cyclopropyl-7-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13l**). mp : 198-200 °C. ^{1}H NMR (DMSO-*d*$_6$) δ 0.67 (m, 2H, CH(CH$_2$)$_2$), 0.92 (m, 2H, CH(CH$_2$)$_2$), 2.50 (m, 1H, CH(CH$_2$)$_2$), 4.62 (s, 2H, NCH$_2$NH), 7.30 (dd, J=9.5 Hz/4.3 Hz, 1H, 5-H), 7.54 (m, 1H, 6-H), 8.21 (bs, 1H, NH). ^{13}C NMR (DMSO-*d*$_6$) δ 8.8 (CH(CH$_2$)$_2$), 30.2 (CH(CH$_2$)$_2$), 60.6 (C-3), 115.2 (C-5), 117.1 (C-8), 120.3 (C-6), 122.0 (C-8a), 143.1 (C-4a), 149.1-151.0 (C-7). Anal. (C$_{10}$H$_{10}$ClFN$_2$O$_2$S) theoretical: C, 43.40; H, 3.64; N, 10.12; S, 11.59. Found: C, 43.48; H, 3.63; N, 10.28; S, 11.48.

5-Chloro-4-cyclopropyl-8-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14m).

The title compound was obtained as described for **14a** starting from 5-chloro-4-cyclopropyl-8-fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13m**). mp : 171-174 °C. ^{1}H NMR (DMSO-*d*$_6$) δ 0.76 (m, 2H, CH(CH$_2$)$_2$), 0.88 (m, 2H, CH(CH$_2$)$_2$), 3.15 (tt, J=7.2 Hz/4.0 Hz, 1H, CH(CH$_2$)$_2$),

4.76 (s, 2H, NCH_2NH), 6.95 (t, J=9.0 Hz, 1H, 7-H), 7.62 (dd, J=9.0 Hz/6.0 Hz, 1H, 6-H), 8.35 (bs, 1H, NH). ^{13}C NMR (DMSO-*d*$_6$) δ 9.7 ($CH(CH_2)_2$), 36.9 ($CH(CH_2)_2$), 62.0 (C-3), 108.9 (C-7), 118.6 (C-8a), 119.8 (C-5), 135.2 (C-6), 143.0 (C-4a), 156.2-158.2 (C-8). Anal. ($C_{10}H_{10}ClFN_2O_2S$) theoretical: C, 43.40; H, 3.64; N, 10.12; S, 11.59. Found: C, 43.26; H, 3.74; N, 10.09; S, 11.89.

5,6-Dichloro-4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14n).

The title compound was obtained as described for **14a** starting from *5,6*-dichloro-4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13n**). mp : 132-133 °C. ^1H NMR (DMSO-*d*$_6$) δ 0.77 (m, 4H, $CH(CH_2)_2$), 3.15 (tt, J=6.7 Hz/4.4 Hz, 1H, $CH(CH_2)_2$), 4.78 (s, 2H, NCH_2NH), 7.31 (d, J=8.5 Hz, 1H, 7-H), 7.60 (d, J=8.5 Hz, 1H, 8-H), 8.22 (bs, 1H, NH). ^{13}C NMR (DMSO-*d*$_6$) δ 9.7 ($CH(CH_2)_2$), 36.7 ($CH(CH_2)_2$), 62.4 (C-3), 122.0 (C-7), 123.0 (C-5), 123.2 (C-8), 128.7 (C-8a), 137.1 (C-6), 143.1 (C-4a). Anal. ($C_{10}H_{10}Cl_2N_2O_2S$) theoretical: C, 40.97; H, 3.44; N, 9.56; S, 10.94. Found: C, 41.16; H, 3.46; N, 9.90; S, 10.81.

6,7-Dichloro-4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14o).

The title compound was obtained as described for **14a** starting from 6,7-dichloro-4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13o**). mp : 174-176 °C. ^1H NMR (DMSO-*d*$_6$) δ 0.68 (m, 2H, $CH(CH_2)_2$), 0.94 (m, 2H, $H(CH_2)_2$), 2.58 (tt, J=6.7 Hz/3.7 Hz, 1H, $CH(CH_2)_2$), 4.71 (s, 2H, NCH_2NH), 7.41 (s, 1H, 5-H), 7.76 (s, 1H, 8-H), 8.14 (bs, 1H, NH). ^{13}C NMR (DMSO-*d*$_6$) δ 8.4 ($CH(CH_2)_2$), 29.8 ($CH(CH_2)_2$), 61.0 (C-3), 116.0 (C-5), 118.8 (C-7), 22.9 (C-8a), 125.6 (C-8), 135.5 (C-6), 143.9 (C-4a). Anal. ($C_{10}H_{10}Cl_2N_2O_2S$) theoretical: C, 40.97; H, 3.44; N, 9.56; S, 10.94. Found: C, 41.21; H, 3.44; N, 9.79; S, 11.08.

6,7-Dichloro-4-(1-methyl)cyclopropyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14p).

The title compound was obtained as described for **14a** starting from 6,7-dichloro-4-(1-methyl)cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13p**). mp : 162-163 °C. ^{1}H NMR (DMSO-d_6) δ 0.82 (m, 2H, C(CH_2)$_2$), 0.99 (m, 2H, C(CH_2)$_2$), 1.29 (s, 3H, CH_3), 4.72 (s, 2H, NCH_2NH), 7.31 (s, 1H, 5-H), 7.76 (s, 1H, 8-H), 8.13 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 13.8 (C(CH_2)$_2$), 17.0 (C(CH_2)$_2$), 36.0 (CH_3), 59.9 (C-3), 115.7 (C-5), 118.2 (C-7), 122.8 (C-8a), 125.9 (C-8), 135.6 (C-6), 142.1 (C-4a). Anal. ($C_{11}H_{12}Cl_2N_2O_2S$) theoretical: C, 43.01; H, 3.94; N, 9.12; S, 10.44. Found: C, 42.84; H, 3.94; N, 9.21; S, 9.72.

6,7-Dichloro-4-cyclobutyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14q).

The title compound was obtained as described for **14a** starting from 6,7-dichloro-4-cyclobutyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13q**). mp : 161-163 °C. ^{1}H NMR (DMSO-d_6) δ 1.65 (m, 2H, CH(CH_2)$_3$), 2.11-2.27 (m, 4H, CH(CH_2)$_3$), 4.25 (m, 1H, CH(CH_2)$_3$), 4.75 (s, 2H, NCH_2NH), 7.17 (s, 1H, 5-H), 7.74 (s, 1H, 8-H), 8.11 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 14.2 (C-3'), 27.9 (C-2'/C-4'), 51.6 (C-1'), 56.3 (C-1'), 115.6 (C-5), 117.9 (C-7), 122.7 (C-8a), 125.7 (C-8), 135.8 (C-6), 142.4 (C-4a). Anal. ($C_{11}H_{12}Cl_2N_2O_2S$) theoretical: C, 43.01; H, 3.94; N, 9.12; S, 10.44. Found: C, 43.14; H, 3.98; N, 9.26; S, 10.34.

6,7-Dichloro-4-cyclopentyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14r).

The title compound was obtained as described for **14a** starting from 6,7-dichloro-4-cyclopentyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13r**). mp : 221-224 °C. ^{1}H NMR (DMSO-d_6) δ 1.59 (m, 4H, CH(CH_2)$_4$), 1.68 (m, 2H, CH(CH_2)$_4$), 1.89 (m, 2H, CH(CH_2)$_4$), 4.33 (p, J=7.6 Hz, 1H, CH(CH_2)$_4$), 4.67 (s, 2H, NCH_2NH), 7.38 (s, 1H, 5-H), 7.71 (s, 1H, 8-H), 8.09 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 23.6 (C-3'/C-4'), 28.3 (C-2'/C-5'), 55.5 (C-3), 57.7 (C-1'), 115.4 (C-5), 117.1 (C-7), 122.5 (C-8a), 125.7 (C-8), 136.1 (C-6), 143.3 (C-4a). Anal. ($C_{12}H_{14}Cl_2N_2O_2S$) theoretical: C, 44.87; H, 4.39; N, 8.72; S, 9.98. Found: C, 45.02; H, 4.45; N, 8.88; S, 9.90.

6,7-Dichloro-4-cyclohexyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14s).

The title compound was obtained as described for **14a** starting from 6,7-dichloro-4-cyclohexyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13s**). mp : 207-209 °C. ^{1}H NMR (DMSO-d_6) δ 1.13 (m, 1H, $CH(CH_2)_5$), 1.53 (m, 5H, $CH(CH_2)_5$), 1.73 (m, 4H, $CH(CH_2)_5$), 3.80 (m, 1H, $CH(CH_2)_5$), 4.70 (s, 2H, NCH_2NH), 7.36 (s, 1H, 5-H), 7.73 (s, 1H, 8-H), 8.08 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 25.4 ($CH(CH_2)_5$), 25.6 ($CH(CH_2)_5$), 29.8 ($CH(CH_2)_5$), 55.5 (C-3), 56.4 ($CH(CH_2)_5$), 115.6 (C-5), 117.8 (C-7), 122.8 (C-8a), 126.4 (C-8), 136.8 (C-6), 143.2 (C-4a). Anal. ($C_{13}H_{16}Cl_2N_2O_2S$) theoretical: C, 45.57; H, 4.81; N, 8.36; S, 9.56. Found: C, 46.56; H, 4.83; N, 8.53; S, 9.07.

6,7-Dichloro-4-cycloheptyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14t).

The title compound was obtained as described for **14a** starting from 6,7-dichloro-4-cycloheptyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13t**). mp : 191-192 °C. ^{1}H NMR (DMSO-d_6) δ 1.51 (m, 4H, $CH(CH_2)_6$), 1.67 (m, 6H, $CH(CH_2)_6$), 1.79 (m, 2H, $CH(CH_2)_6$), 3.91 (m, 1H, $CH(CH_2)_6$), 4.67 (s, 2H, NCH_2NH), 7.33 (s, 1H, 5-H), 7.71 (s, 1H, 8-H), 8.06 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 24.4 ($CH(CH_2)_7$), 26.8 ($CH(CH_2)_7$), 31.4 ($CH(CH_2)_6$), 55.5 (C-3), 57.8 ($CH(CH_2)_6$), 115.0 (C-5), 117.0 (C-7), 122.5 (C-8a), 125.9 (C-8), 136.1 (C-6), 142.5 (C-4a). Anal. ($C_{14}H_{18}Cl_2N_2O_2S$) theoretical: C, 48.14; H, 5.19; N, 8.02; S, 9.18. Found: C, 48.25; H, 5.22; N, 8.24; S, 8.71.

6,7-Dichloro-4-cyclooctyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14u).

The title compound was obtained as described for **14a** starting from 6,7-dichloro-4-cyclooctyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13u**). mp : 154-155 °C. ^{1}H NMR (DMSO-d_6) δ 1.48-1.84 (m, 14H, $CH(CH_2)_7$), 3.90 (m, 1H, $CH(CH_2)_7$), 4.68 (s, 2H, NCH_2NH), 7.25 (s, 1H, 5-H), 7.72 (s, 1H, 8-H), 8.08 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 24.3 ($CH(CH_2)_7$), 25.1

($CH(CH_2)_7$), 26.1 ($CH(CH_2)_7$), 30.5 ($CH(CH_2)_7$), 56.0 (C-3), 57.2 ($CH(CH_2)_7$), 114.9 (C-5), 117.1 (C-7), 122.6 (C-8a), 126.0 (C-8), 136.0 (C-6), 142.4 (C-4a). Anal. ($C_{15}H_{20}Cl_2N_2O_2S$) theoretical: C, 49.59; H, 5.55; N, 7.71; S, 8.82. Found: C, 49.63; H, 5.56; N, 7.98; S, 8.99.

6,7-Dichloro-4-isopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14v).

The title compound was obtained as described for **14a** starting from 6,7-dichloro-4-isopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13v**). mp : 184-185 °C. ^{1}H NMR (DMSO-d_6) δ 1.19 (d, J=6.6 Hz, 6H, $CH(CH_3)_2$), 4.23 (hept, J=6.6 Hz, 1H, $CH(CH_3)_2$), 4.68 (s, 2H, NCH_2NH), 7.33 (s, 1H, 5-H), 7.72 (s, 1H, 8-H), 8.05 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 19.2 ($CH(CH_3)_2$), 47.5 ($CH(CH_2)_2$), 54.3 (C-3), 115.0 (C-5), 117.1 (C-7), 122.6 (C-8a), 125.9 (C-8), 136.1 (C-6), 142.7 (C-4a). Anal. ($C_{10}H_{10}Cl_2N_2O_2S$) theoretical: C, 40.69; H, 4.10; N, 9.49; S, 10.86. Found: C, 40.78; H, 4.10; N, 9.87; S, 10.80.

6,7-Dichloro-4-cyclopropylmethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (14w).

To a solution of 6,7-dichloro-4-cyclopropylmethyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13w**: 0.70 g, 2.29 mmol) in isopropanol (50 mL) was added sodium borohydride (0.17 g, 4.49 mmol), and the mixture was heated at 50 °C for 5 minutes. The reaction mixture was evaporated under reduced pressure and the residue was taken up in water (30 mL). The pH of the medium was adjusted to 5-6 by addition of 6N hydrochloric acid and the suspension was extracted three times with chloroform (3x30 mL). The organic phases were collected, dried over anhydrous $MgSO_4$ ad filtered. The filtrate was evaporated under reduced pressure and the residue was taken up in methanol (5 mL). Water (50 mL) was added to the methanolic solution and the precipitate which appears was collected by filtration, washed with water and dried. mp: 171-173 °C. ^{1}H NMR (DMSO-d_6) δ 0.29 (m, 2H, $CH(CH_2)_2$), 0.48 (m, 2H, $CH(CH_2)_2$), 1.03 (m,

1H, $CH(CH_2)_2$), 3.33 (m, 2H, NCH_2CH), 4.79 (s, 2H, NCH_2NH), 7.27 (s, 1H, 5-H), 7.72 (s, 1H, 8-H), 8.28 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 3.3 ($CH(CH_2)_2$), 8.5 ($CH(CH_2)_2$), 52.6 (NCH_2CH), 61.0 (C-3), 115.4 (C-5), 117.4 (C-7), 122.2 (C-8a), 125.6 (C-8), 136.0 (C-6), 142.8 (C-4a). Anal. ($C_{11}H_{12}Cl_2N_2O_2S$) theoretical: C, 43.01; H, 3.94; N, 9.12; S, 10.44. Found: C, 42.90; H, 3.90; N, 9.11; S, 10.04.

Biological evaluation

Animals were housed in collective cages with continuous access to tap water and food on a 12 h light-dark cycle, in a temperature-controlled (21 ± 1°C) and ventilated room. On completion of studies, subjects were euthanized by either CO_2 inhalation or intraperitoneal (i.p.) lethal pentobarbital injection. All animal experimentations were performed in accordance with local ethical committees following the principles of laboratory animal care with the European Communities Council Directive 2010/63/UE. All efforts were made to minimize animal suffering and to reduce the number of animals used. This study including different animal models was conducted following institutional review and in accordance with institutional and national guidelines at Servier.

Impact on AMPA-induced membrane depolarization (FDSS) in rat primary brain cultures.

This study involved examining AMPA-triggered membrane depolarization conducted on primary cell cultures derived from rat embryonic cortex. Membrane potential measurements were conducted with the new compounds using fluorescent dyes and an imaging technology-based plate reader, following our previously published procedure.[24]

Effect of compound 14o on AMPA, NMDA and Kainate currents evoked in *Xenopus laevis* oocytes

Xenopus Laevis oocytes were injected with rat cortex or human hippocampus poly(A+) mRNA. Currents evoked by either AMPA, NMDA or kainate in absence or presence of different concentrations of compound **14o** applied on the same oocytes were recorded in a Plexiglas chamber at room temperature using 2-electrode voltage-clamp recordings, as previously described.[13,24,50]

Effect of compound 14o on AMPA-mediated excitatory postsynaptic responses evoked in the CA1 area on rat hippocampal slices

Wistar rat hippocampal slices were maintained in a rapid-flow rectangular recording chamber, continuously superfused with Artificial Cerebro-Spinal Fluid solution. AMPA-mediated excitatory postsynaptic responses were elicited in the CA1 region of the hippocampus by stimulating the Schaeffer collaterals every 30 sec with a stimulus intensity that evoked approximately 50% of the maximum evoked response. DMSO and one concentration of compound **14o** (3, 10 or 30 μM) were bath-applied during 15 min on the same slice. For more details see our previous references.[13,24]

Effect on BDNF protein expression in rat primary brain cortical cells cultures

Dissociated rat primary cerebral cortical cells were prepared from embryo (E16-E17) and were added to poly-D-Lysine coated 24-well culture plates in MEM containing 10 % FCS/5 % HS/5 % NS/0.6 % D-glucose/25 % HBSS for 11-12 days at 37°C/ 5% CO_2 (5 10^5 cells/well). Culture medium was replaced two times a week. On the day of the experiment (11-12 days later), the culture medium was replaced with MEM/Glucose and primary cortical cells were exposed for 24 h to different concentrations of compound **14o** (0.3-10 μM). Each plate included a well with

untreated cells in order to measure basal BDNF protein expression. After removal of the supernatant, 150 µl/well lyse buffer were added to the cells. Samples were removed and stored at -80 °C until BDNF assay. Expression of intracellular BDNF protein was measured by ELISA performed with human BDNF Immunoassay kit (R&D System), according to manufacturer's instructions. Internal standards for the BDNF ELISA Kit were used to perform a standard curve (62.5 to 4000 pg/ml) to allow BDNF protein quantification. Titer 96-well plates coated with monoclonal antibody specific for BDNF were incubated with standards of BDNF added in triplicate and with samples. Optical density of each well was evaluated within 30 min using a microplate reader set to 450 nm (Sunrise, Tecan). BDNF protein expression in each sample was determined in pg/ml using the standard curve. The expression of BDNF protein in presence of compound **14o** was normalized to unity versus the averaged basal expression obtained on the same culture.

Influence on body temperature in NMRI mice and Wistar rats.

The impact of the compound **14o** on the body temperature was measured in NMRI mice after oral administration at a dose of 100 mg/kg, and in Wistar rats following both oral and intraperitoneal administration at doses of 10 mg/kg, 30 mg/kg, and 100 mg/kg, and 10 mg/kg and 30 mg/kg respectively. The methodologies followed those described in the referenced literature.[53,54,55]

Influence on long-term potentiation (LTP) of the postsynaptic response induced in the dentate gyrus of anesthetized rats.

Excitatory postsynaptic field potentials (EPSFP) and its LTP evoked by brief high-frequent stimulation were measured with compound **14o** (10 and 30 mg/kg i.p.) and its vehicle in the

dentate gyrus of anesthetized rats after stimulating the perforant path following our previously described protocol.[13, 24]

Impact of 14o on episodic memory using the object recognition test in CD1 mice.

The one-trial object recognition paradigm appraising a type of episodic memory in mice was conducted using a previously described methodology.[24,56] The impact of compound **14o** on CD1 mice following oral administration was evaluated at doses of 1 and 3 mg/kg and compared to vehicle controls.

Effects of 14o on spatial working memory using the spontaneous alternation test in C57BI/6 mice.

The evaluation of spontaneous alternation behavior in mice placed in a T-maze was carried out following a previously documented protocol.[50] Compound **14o** was administered to C57BI/6 mice through intraperitoneal injections at varying doses (0.01, and 0.03mg/kg) and compared to vehicle controls.

Determination of cerebral and plasma concentration of selected compounds after oral administration in NMRI mice.

Twenty and sixty minutes after oral administration of 3 mg/kg of the tested drug, NMRI mice were sacrificed by decapitation (n = 3). Brain and blood samples were rapidly removed and frozen. Cerebral and plasmatic concentrations of the compound were determined by using LC−MS/MS detection.[30]

Determination of the apparent permeability of 14o on human Caco-2 cells.

The Caco-2 apparent permeability value (P_{app}) of **14o** was determined according to a previously described protocol.[47]

Pharmacokinetic studies of compound 14o in Wistar rats.

Adult male Wistar rats were used for the pharmacokinetic studies of compound **14o**. The animals were divided into two groups for intravenous (IV, n = 3) and oral (PO, n = 3) administration. Compound **14o** was administered as a single dose without prior fasting. For the IV group, the compound was formulated in a mixture of PEG 300, ethanol 95% and 0.9% NaCl (40:10:50, v/v/v) and infused over 10 minutes at a volume of 3 mL/kg, corresponding to a dose of 1.5 mg/kg. The oral formulation consisted of a 1% aqueous hydroxyethylcellulose solution (w/v), administered by gavage at a volume of 4 mL/kg (3 mg/kg). Blood samples were collected via femoral vein puncture into lithium heparinized tubes for plasma isolation. In the IV group, samples were collected at 10, 20 and 30 minutes and at 1, 2, 4, 6, 8 and 24 hours after the start of the infusion. In the PO group, sampling was performed at 10, 20 and 30 minutes and at 1, 2, 4, 6, 8 and 24 hours after administration. Urine was collected over a 0–24 hour interval in both groups. Plasma and urine concentrations of compound **14o** were quantified using a validated ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method. Plasma samples (25 µL) were thawed, centrifuged (5 min, 4 °C, 3000 rpm), and mixed with internal standard (100 µL, 2.5 ng/mL) and 0.1 N NaOH (25 µL). After vortexing (1 min) and centrifugation (5 min, 10 °C, 1000 rpm), solid-phase extraction was performed (Waters Oasis HLB). The eluate was evaporated under nitrogen at 37 °C and reconstituted in the mobile phase (10 mM ammonium formate and 0.1% formic acid in acetonitrile, 50:50 v/v) prior to injection. Urine samples (20 µL) were thawed, vortexed and centrifuged (5 min, 4 °C, 3000 rpm). An internal standard (180 µL, 5 ng/mL), prepared in the mobile phase, was then added, followed by direct injection of 4 µL. Chromatographic analysis was performed using an

Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm), maintained at 40 °C. Separation was achieved under gradient conditions using a mobile phase consisting of 10 mM ammonium formate with 0.1% formic acid (aqueous phase) and acetonitrile with 0.1% formic acid (organic phase). The flow rate was set at 0.4 mL/min with a total run time of 10 minutes. Detection was carried out by tandem mass spectrometry using negative electrospray ionization (ESI−). Quantification was based on a validated calibration curve. Lower limits of quantification were 0.25 ng/mL in plasma and 2 ng/mL in urine.

Determination of the neuroprotective effects of compound 14o on hippocampal neurons in Wistar rats after ischemia.

Male adult Wistar anaesthetized rats (pentobarbital 60 mg/kg IP) were placed in a stereotaxic device and the vertebral arteries were definitively occluded by electrocauterization. Body temperature was maintained at 37 °C with a heating pad connected to a temperature control unit. Loose atraumatic silastic ligatures were placed around the common carotid arteries without interrupting the blood flow. On the following day, a transient global cerebral ischemia was induced in conscious animal by tightening the ligatures during 5 min. Circulation was then restored by releasing the ligatures. The rats were kept in a warm place throughout the duration and 90-120 min after the ischemia. Compound **14o** (10 mg/kg, i.p.) or vehicle were administered 30 min before the induction of the transient global cerebral ischemia. Body temperature was measured via a rectal probe before compound **14o** or vehicle injection (T-30 min), before occlusion (T0 min) and then every 30 min for 1.5 hours after occlusion (T30 min-T60 min-T90 min), as a long-lasting decrease of body temperature is neuroprotective. The effect of Treatment on the delta of temperature compared to T-30min was analysed from T30min to T90min with a two-way (Treatment x Time) analysis of variance with repeated measures on time. If Treatment effect was significant, complementary analyses were done with

Dunnett test versus Ischemia group. The significance threshold was set to 5 %. Seven days after ischemia the animals were sacrificed, brains were removed. Seven μm thick sections were cut on a cryostat, collected on slides, postfixed by immersion in 4 % paraformaldehyde and stained with hematoxilyn and eosin to identify dead cells with cytoplasmic eosinophilia and nuclear pyknosis. Under microscope, a neuronal viable cells count was performed in the CA1 hippocampal area in the 2 hemispheres at 3 levels (5.7, 5.55 and 5.4 mm anterior to interaural line[57]). The effect of treatment on the total number of viable cells was assessed with a one-way (Treatment) analysis of variance, followed in case of significance of the treatment effect by a Tukey test for pairwise comparisons. All the groups (even the sham one) were included in the analysis. The significance threshold was set to 5 %.

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ABBREVIATIONS

AMPARs, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor; AMPAR PAMs, Positive allosteric modulators of AMPARs; BDNF: brain derived neurotrophic factor; BBB, blood-brain barrier; BTDs, 1,2,4-benzothiadiazine 1,1 dioxides; CNS, central nervous system; d_6-DMSO, deuterated dimethyl sulfoxide; FDSS, *in vitro* fluorescence assay; GluA2-LBD, ligand binding domain of the AMPAR GluA2 subunit; iGluRs, ionotropic glutamate receptors; LBD, ligand-binding domain; L-Glu, L-glutamate; LTP, long-term potentiation; mGluRs, metabotropic glutamate receptors; NA, noradrenaline; TMS, tetramethylsilane; WHO, World Health Organization.

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