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Development of Thiophenic Analogues of Benzothiadiazine Dioxides as New Powerful Potentiators of 2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic Acid (AMPA) Receptors

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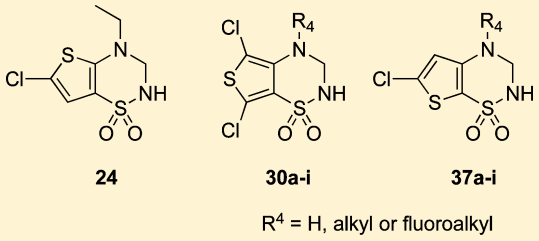
Supporting Information

ABSTRACT: On the basis of the results obtained in previous series of AMPA potentiators belonging to 3,4-dihydro-2*H*-benzo- and 3,4-dihydro-2*H*-pyrido-1,2,4-thiadiazine 1,1-dioxides, the present work focuses on the design of original isosteric 3,4-dihydro-2*H*-thieno-1,2,4-thiadiazine 1,1-dioxides. Owing to the sulfur position, three series of compounds were developed and their activity as AMPA potentiators was characterized. In each of the developed series, potent compounds were discovered. After screening the selected active compounds on a safety in vivo test, 6-chloro-4-ethyl-3,4-dihydro-2*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**24**) appeared as the most promising compound and was further evaluated. Its effects on long-term potentiation in vivo and on AMPA-mediated noradrenaline release were measured to predict its potential cognitive enhancing properties. Finally, an object recognition test performed in mice revealed that **24** was able to significantly enhance cognition, after oral administration, at doses as low as 0.3 mg/kg. This study validates the interest of the isosteric replacement of the benzene or pyridine nuclei by the thiophene nucleus in the ring-fused thiadiazine dioxides class of AMPA potentiators.



INTRODUCTION

L-Glutamic acid is the major excitatory neurotransmitter in our central nervous system, activating metabotropic (coupled to G-proteins) and ionotropic receptors (coupled to an ionic channel). Among the latter, AMPA-type receptors mediate fast excitatory postsynaptic currents at the great majority of the synapses and are involved in the expression of long-term potentiation, a phenomenon closely linked to learning and memory processes. On this basis, the AMPA receptor (AMPAR) has been proposed as an interesting target to develop cognitive enhancers. Given the fact that excessive glutamatergic activity generates toxic effects, such as excitotoxicity and seizure, the enhancement of glutamate signals through AMPAR modulators has been anticipated to produce unacceptable side effects. This hypothesis was however rejected in the beginning of the 1990s when cyclothiazide (**1**), diazoxide (**2**), IDRA-21 (**3**), and S18986 (**4**) were characterized as AMPA positive allosteric modulators[1−4] and when it was shown that this new pharmacological class enhances memory without causing severe side effects (Figure 1).[4−7] These compounds, called ampakines or "AMPApams", are now well-known to act through binding to allosteric sites located at the level of the ligand binding domain of the AMPA receptor.[8] They have thus no agonist or antagonist effects but instead stabilize the receptor in its channel-open state following the binding of the released transmitter (L-glutamate).

On the basis of these findings and considering the potential therapeutic interest of such compounds (e.g., Alzheimer's disease treatment, cognitive disorders, schizophrenia, or depression),[9−11] several distinct chemical classes of compounds were investigated, among which the 1,2,4-benzothiadiazine 1,1-dioxide-type chemical class (i.e., compounds **3**−**6**, **8**, and **10**) is one of the most studied.[12−14] Structural modifications starting from IDRA-21 (**3**) were achieved, leading to the preparation of pyridinic analogues (pyrido-1,2,4-thiadiazine 1,1-dioxides) such as **7**.[15] This first work was followed by a SAR study focused on benzenic compounds **8**, studying the impact of substitution at the 7-position.[16] The combination of SARs established from pyridinic and benzenic series led to the design of a second generation of pyrido-1,2,4-thiadiazine 1,1-dioxides such as **9**.[17] Furthermore, 4-fluoroalkylated benzo-1,2,4-thiadiazine 1,1-dioxides characterized by an enhanced metabolic stability were prepared.[18] Among the compounds designed in this study, S70340 (**10**) was found to be active in vivo in the object recognition test after oral administration in rats at the dose of 0.1 mg/kg. AMPA potentiation mediated by this compound has

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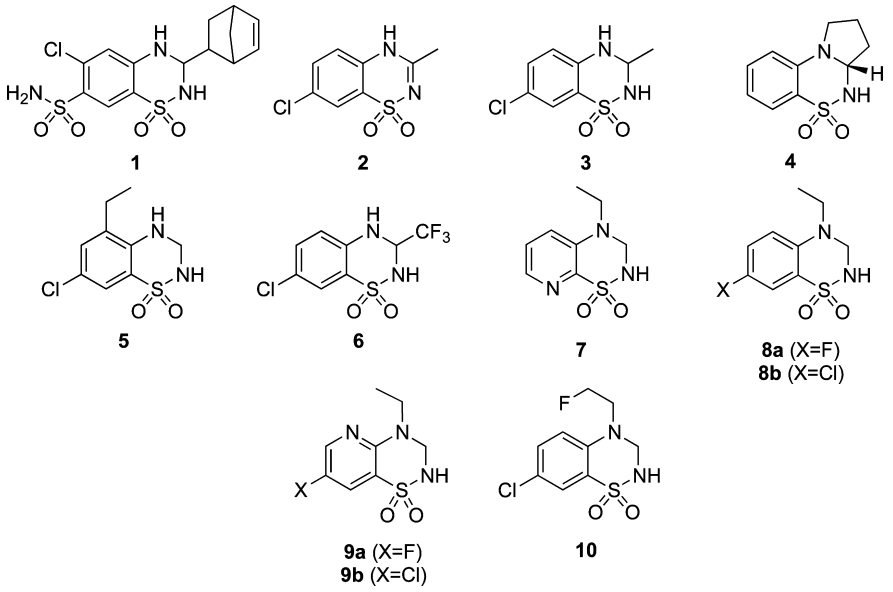

Figure 1. An overview of benzothiadiazines and related pyridinic isosteres developed as AMPApams.

been recently shown to activate genes and functional processes involved in neuroprotective and cognitive effects.[19] In particular, it was confirmed that one of the most relevant effects of **10**, like other ampakines, was the increase of the brain-derived neurotrophic factor (BDNF) expression, a major feature which determines the therapeutic potentialities of ampakines (neuroprotection, neural stem cells differentiation).

In the present paper, we disclose the results of our studies on a new series of AMPApams. With the aim of obtaining compounds with a better activity and taking into account that a small change of a ligand can easily alter receptor functionality,[20] we have focused our efforts on the aromatic part of benzo-1,2,4-thiadiazine 1,1-dioxides and prepared new compounds in which the benzene ring of known benzothiadiazines was substituted by thiophene providing thieno-1,2,4-thiadiazine 1,1-dioxide isosteres. This pharmacomodulation has been used with success in many therapeutic fields, such as the benzodiazepine class (leading to clotiazepam) or the oxicam class (leading to tenoxicam).

The benzene/thiophene switch is expected to give rise to three different thiophenic regioisomeric systems: 3,4-dihydro-2*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides ("5-thieno" series, **11**), 3,4-dihydro-2*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-dioxides ("6-thieno" series, **12**), and 3,4-dihydro-2*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides ("7-thieno" series, **13**) (Figure 2).

Keeping in mind that the best in vitro results were previously obtained with a halogen atom, i.e., a fluorine or a chlorine atom, at the 7-position of benzo-1,2,4-thiadiazine 1,1-dioxides, we focused our effort toward 5-thieno and 7-thieno compounds bearing a chlorine atom at the 6-position (the closest to the 7-position in the benzenic series). For the 6-thieno system, we envisaged a series of thieno-1,2,4-thiadiazine 1,1-dioxides bearing two chlorine atoms at the 5- and 7-positions.

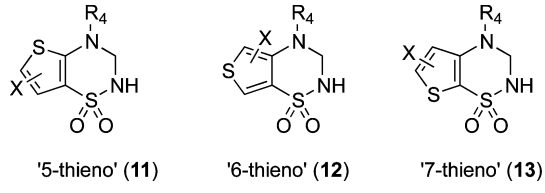

Figure 2. The three thiophenic regioisomeric systems investigated in this work.

CHEMISTRY

Considering at first that 2-amino-5-chlorothiophene-3-sulfonamide (**15**) is the key intermediate in the access to the 6-chloro-3,4-dihydro-2*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide series, we first followed the approach reported by Nielsen[21] to prepare 6-chloro-3-imino-3,4-dihydro-2*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide **14**, which had to be hydrolyzed to give the expected key product **15**. Despite numerous attempts, this step was unsuccessful and we finally based our synthesis on a strategy described by Topliss.[22] The reaction between 1-(5-chlorothiophen-2-yl)ethanone **16** and hydroxylamine provided a mixture of two oximes in an equimolar proportion (Scheme 1). Then, these two isomers underwent a Beckmann rearrangement, resulting in a mixture of the carboxamide **19** and the acetamide **20**, depending on the starting oxime. After a chromatographic separation of the isomers, a chlorosulfonation was achieved on the acetamide **20**, directly followed by a reaction with gaseous ammonia (interestingly, reaction with an aqueous solution of ammonia led to a complete loss of the material). Because the acidic hydrolysis of the amide **21** into the expected **15** was characterized by a very low yield, the amide was reduced using lithium aluminum hydride to obtain **22**. The usual ring formation with triethyl orthoformate and the subsequent reduction using sodium borohydride gave rise to the obtention of the final compound **24**. While other 5-acyl-2-chlorothiophenes could theoretically have given access to final compounds bearing a bigger alkyl group on the nitrogen atom at the 4-position, we limited our exploration in this series to **24**, considering the SAR trends observed in pyridinic and in benzenic series.[12−14]

While the strategy for the synthesis of the key intermediate 4-amino-2,5-dichlorothiophene-3-sulfonamide (**28**) could follow a previously reported scheme approach,[23] we preferred starting from the commercially available sulfonyl chloride derivative **25** (Scheme 2). Nitration was directly performed on the sulfonyl

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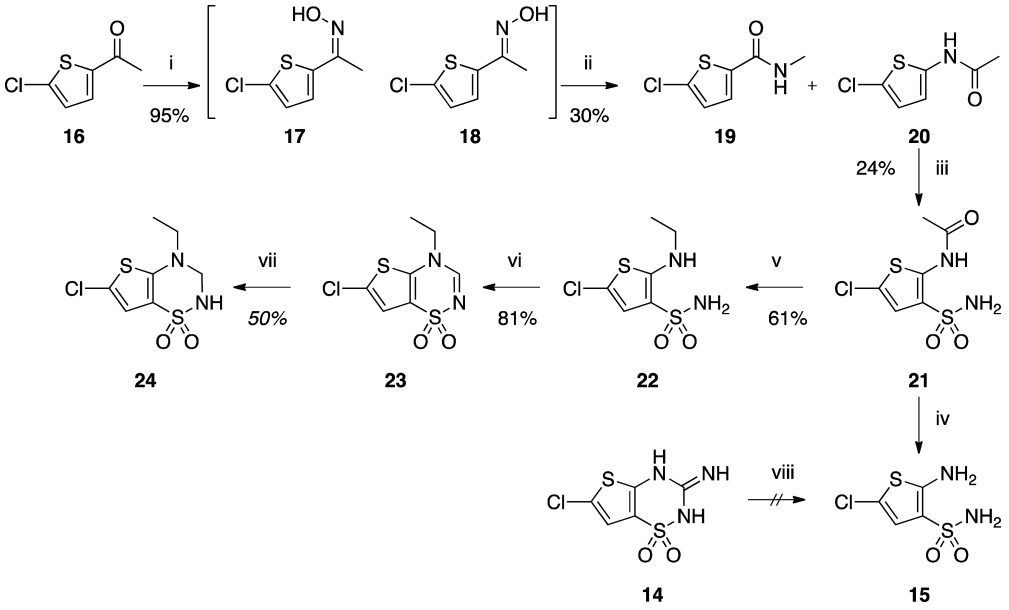
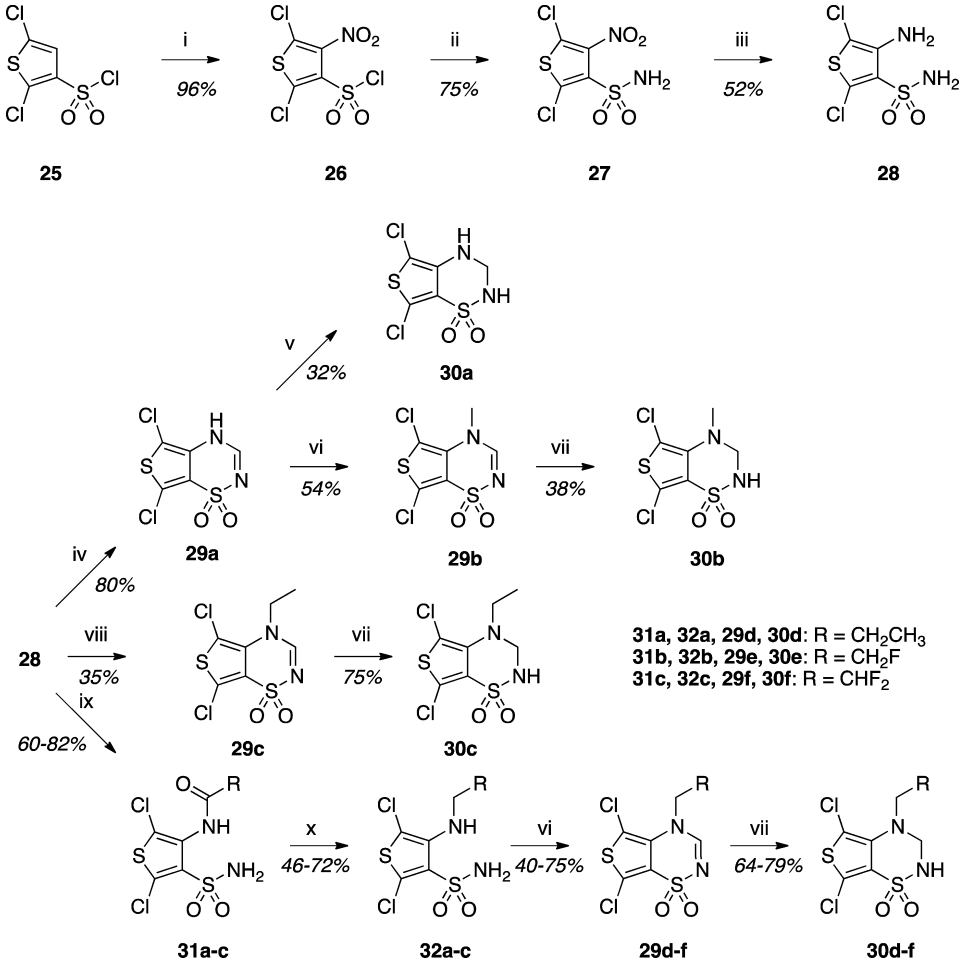
Scheme 1[a]



[a](i) NH_2OH; (ii) PCl_5; (iii) (1) $ClSO_3H$, (2) NH_3 gas; (iv) HCl; (v) $LiAlH_4$; (vi) $HC(OEt)_3$, 170 °C, 30 min; (vii) $NaBH_4$; (viii) HCl.

Scheme 2[a]



[a](i) HNO_3/H_2SO_4; (ii) NH_4OH; (iii) Fe/NH_4Cl; (iv) $HC(OEt)_3$, 150 °C, 90 min; (v) $NaBH_4$, H_2O; (vi) CH_3I, K_2CO_3, $C_2H_5NO_2$/DMF; (vii) $NaBH_4$, 2-propanol; (viii) $HC(OEt)_3$, 170 °C, 35 min; (ix) RCOCl, pyridine, dioxane; (x) $LiAlH_4$.

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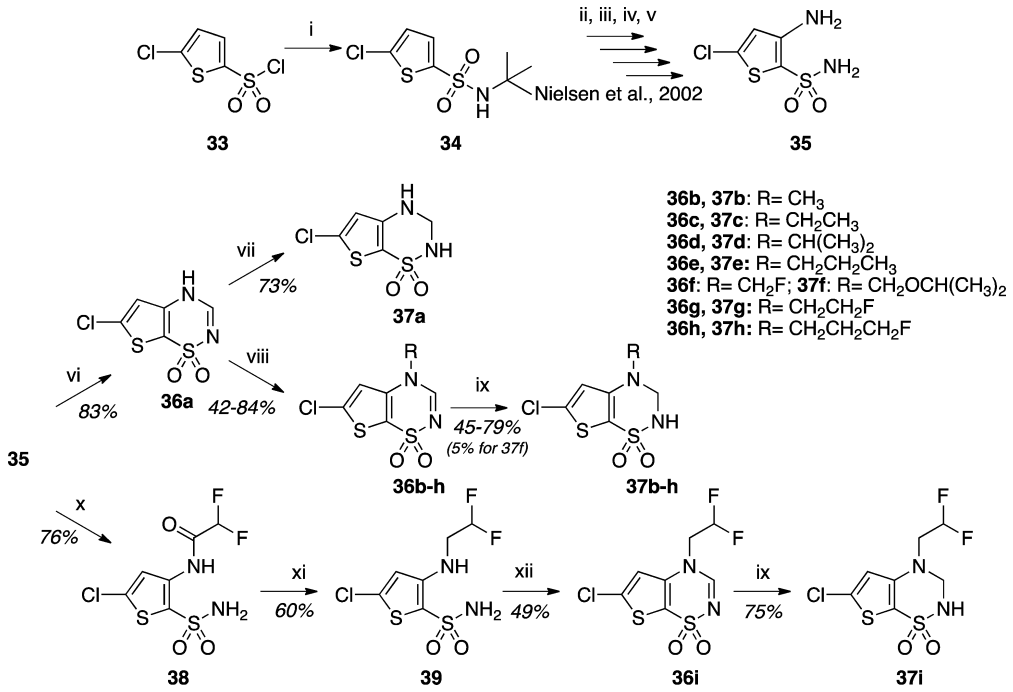
Scheme 3[a]



[a](i) $(CH_3)_3CNH_2$; (ii) *n*-BuLi; (iii) *p*-toluenesulfonyl azide; (iv) hexadecyltributylphosphonium bromide, $NaBH_4$; (v) HCl; (vi) $HC(OEt)_3$, 160 °C, 30 min; (vii) $NaBH_4$, water; (viii) R-X, K_2CO_3, CH_3CN/DMF; (ix) $NaBH_4$, 2-propanol; (x) CHF_2COCl, pyridine, dioxane; (xi) $LiAlH_4$, Et_2O; (xii) HCOOH, 80 °C, 5 h.

chloride derivative to obtain the 4-nitro-substituted compound **26**. Reaction of the latter with diluted ammonia followed by reduction of the nitro group using powdered iron in acidic medium led to **28**. From this intermediate, ring closure was performed with triethyl orthoformate, affording **29a**. While the methylation of the latter on the nitrogen atom at the 4-position (using methyl iodide and potassium carbonate in nitroethane and dimethylformamide) required 2 h at 60 °C to give **29b**, ethylation in the same conditions did not occur at all, probably due to the steric hindrance induced by the chlorine atom at the 5-position. On the basis of this hypothesis, *N*-methylation could have occurred at the 2-position, leading to the obtention of an isomer of **29b**. Selectivity of the alkylation at the 4-position was checked after the final reduction step: as expected, **30b** was found soluble in alkaline water as this compound has a weak acidic function (NH at the 2-position included in a sulfonamide).

Unexpectedly, **29c** was obtained from a side reaction taking place when prolonging the heating of **28** in triethyl orthoformate above 150 °C. Considering the reactivity of **29a** toward alkylating agents, an alternative strategy[18] was followed to insert a *n*-propyl chain or fluoroalkyl chains at the 4-position: selective acylation on the amino function of **28** with the appropriate acyl chloride at low temperature afforded the corresponding amides **31a−c**. Then, owing to the nature of the acyl chain, **31a−c** were reduced either using lithium aluminum hydride or borane in diethyl ether at low temperature, affording **32a−c**. Ring closure into the corresponding thieno-1,2,4-thiadiazine 1,1-dioxides **29d−f** was performed with triethyl orthoformate. Because of instability issues of intermediates **31b** and **32b**, the conversion of **28** into **29e** was achieved through a one-pot synthesis.

The reduction step using sodium borohydride was successfully performed with **29a** using water as the solvent, while this reaction was carried out in 2-propanol with **29b−f**.

Concerning the 6-chloro-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide series, the reaction between 5-chlorothiophene-2-sulfonyl chloride (**33**) and *tert*-butylamine afforded *N*-(*tert*-butyl)-5-chlorothiophene-2-sulfonamide (**34**) (Scheme 3). The latter was then converted into the key intermediate **35** in four steps, as previously reported.[24] Then, the ring formation using triethyl orthoformate (leading to **36a**) followed by alkylation of the 4-position (leading to **36b−h**) and finally reduction of the CN double bond of the thiadiazine ring by means of $NaBH_4$ led to expected final compounds **37b−h**. Attempts to prepare the 4-fluoromethyl derivative by this way were unsuccessful because **37f** was found to result from the substitution of the fluorine atom of intermediate **36f** by 2-propanol during the reduction step. The alternative pathway described in the 6,7-dichloro-5-thieno series was used to obtain the 4-(2,2-difluoroethyl)-substituted derivative **37i**. In this case, the ring formation was performed using formic acid instead of triethyl-orthoformate.

RESULTS AND DISCUSSION

The thieno-1,2,4-thiadiazine 1,1-dioxides were evaluated as AMPA potentiators using the voltage-clamp method and a FlipR technique developed by the Servier Institute. For each compound, among other parameters, the EC_{2x} value was determined, which corresponds to the concentration of drug responsible for a 2-fold increase of the amplitude of the current induced by AMPA (at 10 μM).

Activity data on AMPA receptors obtained by means of the voltage-clamp protocol on *Xenopus* oocytes with compounds bearing an ethyl chain at the 4-position and one chlorine atom

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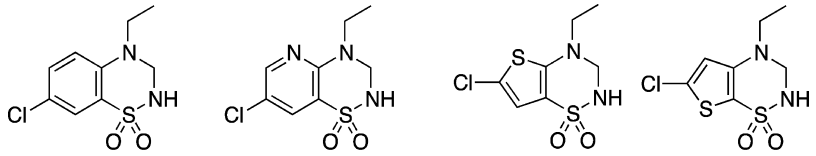
Table 1. Effects of the Different Thiophenic Compounds on the Amplitude of the Current Induced by (*S*)-AMPA (10 μM) in *Xenopus laevis* Oocytes Injected with Rat Cortex mRNA



8b **9b** **24** **37c**

compd	EC_{2X}[a] (μM)	EC_{5X}[b] (μM)	EC_{50}[c] (μM)	max effect[d]
8b	5.6 ± 0.9[e]	14 ± 3[e]	28 ± 2[e]	3682[e]
9b	20 ± 3[e]	44 ± 4[e]	nd[e]	1093 ± 302[e]
24	4.4 ± 0.6	24 ± 4	11 ± 3	2830 ± 1086
37c	3.6 ± 0.1	18.5 ± 2.3	9.2 ± 0.7	2266 ± 88

[a]Concentration of drug giving a 2-fold increase of the magnitude of the current induced by (*S*)-AMPA (10 μM; mean ± SEM; $n \geq 3$). [b]Concentration of drug giving a 5-fold increase of the magnitude of the current induced by (*S*)-AMPA (10 μM; mean ± SEM; $n \geq 3$). [c]Concentration of drug responsible for 50% of the maximal effect (mean ± SEM; $n \geq 3$). [d]Maximum effect of the drug on the AMPA-evoked current (expressed in % of the current evoked by AMPA, taken as 100%). [e]Published results.[16,17] nd: not determined.

on the aromatic ring appears to be within the same range (see Table 1). Whereas no marked difference was observed comparing **8b** and **24**, a slight improvement of in vitro potency must be pointed out after switching from the "7-chloro-benzo" to the "6-chloro-7-thieno" series (see **8b** vs **37c**). However, **37c** displayed a distinct pharmacological profile at high concentrations (from 30 to 300 μM) because it was able to act as an agonist. These trends were not observed in one of our previous studies[17] focused on pyridinic series (compare **8b** and **9b**).

The two thiophenic series developed in this work clearly displayed distinct trends.

Taking into account the in vitro results reported in Table 2, SARs in the 6-chloro-7-thieno series (compounds **36** and **37**) were in accordance with the trends observed in our previous published studies. First of all, the absence of an alkyl substituent at the 4-position was clearly unfavorable for activity on AMPA receptors (see **37a**), as already reported for pyrido-1,2,4-thiadiazine 1,1-dioxides.[15] It was also noticed that the in vitro activity improved with the size of the alkyl chain at the 4-position, with the optionally mono or difluorinated ethyl chains (**37c**, **37g**, and **37i**) being optimal for in vitro activity. Interestingly, the insertion of a third carbon at the end of the ethyl chain (*n*-propyl chain) was clearly deleterious for the activity (see **37e**), while the 4-isopropyl-substituted compound **37d** displayed an activity close to that observed with the 4-ethyl-substituted compound **37c**. Finally, a dramatic increase of the maximum effect was observed with the 4-monofluoroalkyl-substituted compounds (see **37g** and **37h**). In particular, **37g** was able to potentiate more than 50 times the AMPA current in the voltage clamp experiment (max increase: 5604%).

Unexpectedly, the most active compound in the 5,7-dichloro-6-thieno series was **30b** (with a methyl group at the 4-position). The ethyl analogue **30c** displayed a high EC_{2X} value, identical to that observed with **30a**. The poor activity of **30c** seemed surprising, considering the usual observation made in previous series and correlated in the 6-chloro-7-thieno series that an ethyl chain is preferred to hydrogen or the methyl chain at the 4-position (**37c** vs **37a**/**37b**). This atypical activity trend could be explained by the chlorine atom via steric interactions changing the binding orientation of the ethyl group into a less favorable binding interaction. The "interference" exerted by this chlorine atom could also be responsible for the surprising activity displayed by the compound devoid of an alkyl chain at the 4-position (**37a**) by replacing the hydrophobic interaction normally occupied by the small alkyl group.

In contrast to previous observations that saturated compounds were systematically found to be more active than their corresponding unsaturated precursors (focused on pyridinic, benzenic, and thiophenic series),[13,15−18] it was interesting to note that the unsaturated derivatives in the 5,7-dichloro-6-thieno series were found to be more active than their saturated counterparts (see **29c** and **29e** vs **30c** and **30e**). Considering the two thiophenic series developed here, the insertion of fluorine atoms at the alkyl chain did not significantly alter the EC_{2X} parameter (compare **29c** vs **29e**, **30c** vs **30e**, **37c** vs **37g** or **37i**, **37e** vs **37h**) while it was found to double the maximal increase in some cases (see **37c**/**37g**, **37e**/**37h**). Taken together and contrary to observations made in the benzenic series, these data suggest that insertion of one or two fluorine atoms at such position of 7-thieno compounds do not have a deleterious impact on the binding interactions.

It could be deduced from the EC_{2X} value, the EC_{5X} value, and the maximal effect obtained with **24** and **37c** (see Table 2) that the two isomers displayed a similar pharmacological profile. However, it is worth mentioning that **37c**, but not **24**, was able to act as an AMPA agonist at high drug concentrations (30−300 μM). This property precluded any further interest of **37c** as a potential cognitive enhancer.

Comparing the data obtained using the voltage clamp and the flipR methods, we may observe that the EC_{2X} values obtained by voltage clamp and FlipR approaches corroborate each other and may be thus both considered as a predictive value, allowing a preliminary screening. The FlipR method confirms the in vitro potency of **24**, **37g**, and **37i** on AMPA receptors expressed on rat primary brain cells cultures.

According to their pronounced activity as AMPA potentiators revealed by the in vitro screening, three compounds (**24**, **37g**, and **37i**) were selected for further evaluations, on rat hippocampal slices, in an ex vivo electrophysiological test already described in previous studies.[16,18] We studied the effects of **24**, **37g**, and **37i** at 10 μM on the excitatory postsynaptic field potentials (EPSfPs) evoked in the CA1 region after electrical stimulation of the Schaffer collateral. Because of the presence of 1.2 mM Mg^{2+} in the perfusion medium, EPSfPs recorded in this test are primarily mediated by AMPAR activation. Data in Table 3 clearly show that the three

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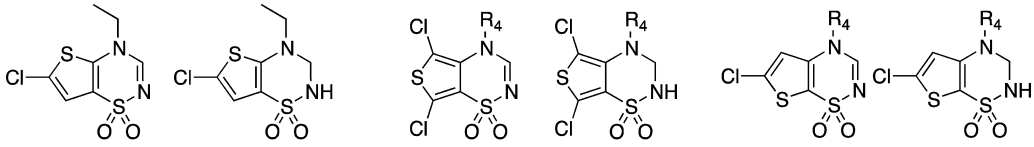
Table 2. Effects of the Different Thiophenic Compounds on the Amplitude of the Current Induced by (*S*)-AMPA (10 μM) in *Xenopus laevis* Oocytes Injected with Rat Cortex mRNA and on the Fluorescence induced by 300 μM AMPA on Primary Cultures of Neurons from Rat Embryonic Cortex



23 (NS) **24** (S) **29** (NS) **30** (S) **36** (NS) **37** (S)

compd	R_4	NS/S[a]	EC_{2X}[b] (μM)	max increase[c] (%)	FlipR[d] (μM)
23	CH_2CH_3	NS	nd	nd	>100
24	CH_2CH_3	S	4.4 ± 0.6	2830	10.4
29a	H	NS	>100	nd	nd
30a	H	S	72 ± 18	>973	>100
29b	CH_3	NS	81[e]	>413	nd
30b	CH_3	S	5.4 ± 2.8	2916	nd
29c	CH_2CH_3	NS	42 ± 15	>1003	nd
30c	CH_2CH_3	S	71 ± 14	>955	>55
29d	$CH_2CH_2CH_3$	NS	nd	nd	>100
30d	$CH_2CH_2CH_3$	S	>300[e]	>126	>100[e]
29e	CH_2CH_2F	NS	26.5[e]	>2327	20.3[e]
30e	CH_2CH_2F	S	113[e]	>485	>100[e]
29f	CH_2CHF_2	NS	nd	nd	>100[e]
30f	CH_2CHF_2	S	235[e]	>227	>100[e]
37a	H	S	216[e]	253	>100[e]
36b	CH_3	NS	166; >300		>100[e]
37b	CH_3	S	36.0 ± 4.4	>462	nd
36c	CH_2CH_3	NS	172[e]	>175	>100[e]
37c	CH_2CH_3	S	3.6 ± 0.1	2266	
36d	$CH(CH_3)_2$	NS	>100	>277	>100[e]
37d	$CH(CH_3)_2$	S	4.2 ± 0.7	217	9.55
36e	$CH_2CH_2CH_3$	NS	nd	2066	>100[e]
37e	$CH_2CH_2CH_3$	S	15[e]	2092	25.1
36f	CH_2F	NS	nd	nd	>100[e]
37f	$CH_2OCH(CH_3)_2$	S	nd	nd	nd
36g	CH_2CH_2F	NS	>300	204	>100[e]
37g	CH_2CH_2F	S	2.0 ± 0.6	5604	3.32
36h	$CH_2CH_2CH_2F$	NS	>300[e]	107	>100[e]
37h	$CH_2CH_2CH_2F$	S	12[e]	>3781	45.7 (2)
36i	CH_2CHF_2	NS	nd	nd	>100[e]
37i	CH_2CHF_2	S	1.6[e]	2558	2.9 ± 0.1

[a]NS, not saturated; S, saturated. [b]Drug concentration giving a 2-fold increase of the amplitude of the current induced by AMPA (10 μM) with the voltage clamp method (n = 3, mean ± SEM) . [c]Maximum effect of the drug on the AMPA-evoked current (expressed in % of the current evoked by AMPA, taken as 100%). [d]Drug concentration giving a 2-fold increase of the fluorescence induced by AMPA (300 M) with the flipR method (n = 3, mean ± SEM) . [e]n = 2, no SEM; nd, not determined.

compounds increased the signals at the concentration of 10 μM, suggesting that they were able to interact with postsynaptic AMPAR located on hippocampal CA1 neurons, as it was demonstrated for our previous hit compound **10**.[18] However epileptiform activity was observed after application of **37g**, probably reflecting additional effects on other targets as demonstrated for cyclothiazide[25] and CX546.[26] We can also not exclude that **37g**, **37i**, and **24** may represent different subclasses of AMPApams, with different modulations of AMPA receptors kinetics (e.g., deactivation or desensitization) or different subunits preferences (GluR1−4, flip and flop splice variants) that may also explain why **37g** as other ampakines (cyclothiazide[25] or CX546[26]) was observed to cause epileptiform discharges in hippocampal slices. This activity did not permit measurement of the half-width and constituted a first warning for a potential toxicity.

Before evaluating the in vivo efficacy of the three compounds (**24**, **37g**, and **37i**), their safety was assessed with the Irwin's test, well-known in the pharmaceutical industry to estimate the potential acute neurotoxicity of new compounds and performed to select nontoxic doses and most promising candidates for in vivo testing. After oral administration of a high dose of compound (30 mg/kg), the changes in the behavioral and physiological states of the mice were recorded. This screening first highlighted **37g**'s acute toxicity with five cases of convulsion followed by apathy after administration in five animals. Administration of **37i** triggered hypothermia in 60% of the tested animals and convulsion, leading to death in 2/5 animals. **24** was found to be atoxic, although a reversible 2 °C decrease in body temperature was observed 30 min after its oral administration (30 mg/kg). It should be noted here that the observed hypothermia could not be related to activity on

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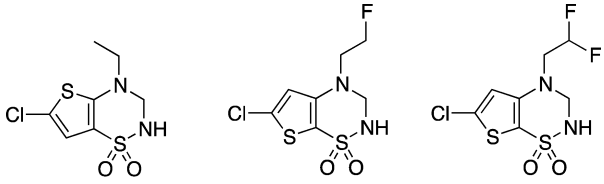
Table 3. Effects of Compounds 24, 37g, and 37i (10 μM) on the AMPA-Mediated Postsynaptic Response Recorded in CA1 Area of Rat Hippocampal Slices



24 **37g** **37i**

compd	area (%)[a]	amplitude (%)[a]	half-width (%)[a]
24	18.3 ± 5.2	3.7 ± 2.0	11.7 ± 7.2
37g	9.3 ± 5.5	33.7 ± 3.8	nd[b]
37i	22.7 ± 3.5	15.3 ± 4.3	14.3 ± 5.0

[a]Area, amplitude, and half-width of the AMPA-mediated EPSfP induced by the compounds were normalized as a percentage of that evoked before application (basal value taken as 100%), $n = 3$. [b]nd: not determined.

AMPA receptors because **36e**, inactive in vitro (FlipR data, Table 2), was able to induce a 4.6 °C hypothermia after intraperitoneal injection (30 mg/kg). This trend was concordant with hypothermic effects observed with a previously published series of AMPApams.[18] Further studies should be performed to elucidate the mechanism underlying such effect.

As **24** was atoxic compared to **37g** and **37i**, further in vivo evaluations were focused on **24**. No in vitro metabolic stability or in vivo evaluation of the blood−brain barrier crossing of the compound could unfortunately be performed in order to confirm the adequate brain levels of **24** due to difficulties in detecting the compound using LC-MS-MS methods. Therefore the first test that was performed in vivo was electrophysiological recording on the hippocampus of anesthetized Wistar rats in order to confirm the previous in vitro effects observed on EPSfPs on rats hippocampal slices and also to measure its effects on long-term potentiation. Within 1 h after intraperitoneal injection of **24** (30 mg/kg) in the animals, recordings of the EPSfPs in the dentate gyrus assessed the results obtained in the in vitro test (see Figure 3A). Then, 1 h after ip administration, LTP was induced by a tetanic stimulation and the EPSfPs continued to be measured during 3 h. As can be seen in Figure 3, **24** increased the amplitude of the measured potentials and extended the maintenance of LTP compared to vehicle rats. This activity on LTP is a key feature for a potential new cognitive enhancer. Moreover, these first in vivo results clearly highlighted the ability of **24** to cross the blood−brain barrier and to reach the CNS.

Because the role of presynaptic AMPA receptors has been established with noradrenaline release,[27] several AMPApams have been reported to potentiate noradrenaline release in rat hippocampal slices.[16,28,29] This activity could explain the cognitive enhancing properties of AMPA potentiators. Therefore, the neurochemical effects of **24** were investigated on rat hippocampal slices. When tested alone at 300 μM, **24** was found to slightly increase the [^{3}H]-NA release compared to basal DMSO control release levels (+34%). Moreover, **24** induced a 281% enhancement of the (*S*)-AMPA evoked [^{3}H]-NA release (100% representing the effects shown by (*S*)-AMPA alone). This result clearly indicated that the selected compound was able to act on presynaptic AMPA receptors.

To assess its potential interest as a new cognitive enhancer, effects of **24** were evaluated in vivo in an object recognition test with CD1 mice. This three-session test, considered as a paradigm for episodic memory in rodents, was inspired by the model developed by Ennaceur and Delacour[30] and was based on the fact that animals remembering a familiar object spend less time exploring it compared to exploring a new object. As can be seen in Figure 4, oral administration of **24** 1 h before the three sessions significantly increased the performance of mice at doses as low as 0.3 mg/kg. This activity became highly significant at 1 mg/kg. However, also in the absence of corroborative brain penetration studies, the effect of **24** both on EPSFPs recorded in vivo on anesthetized rats and on object recognition test in mice strongly suggest that **24** is absorbed in mice after oral administration and is able to reach the CNS.

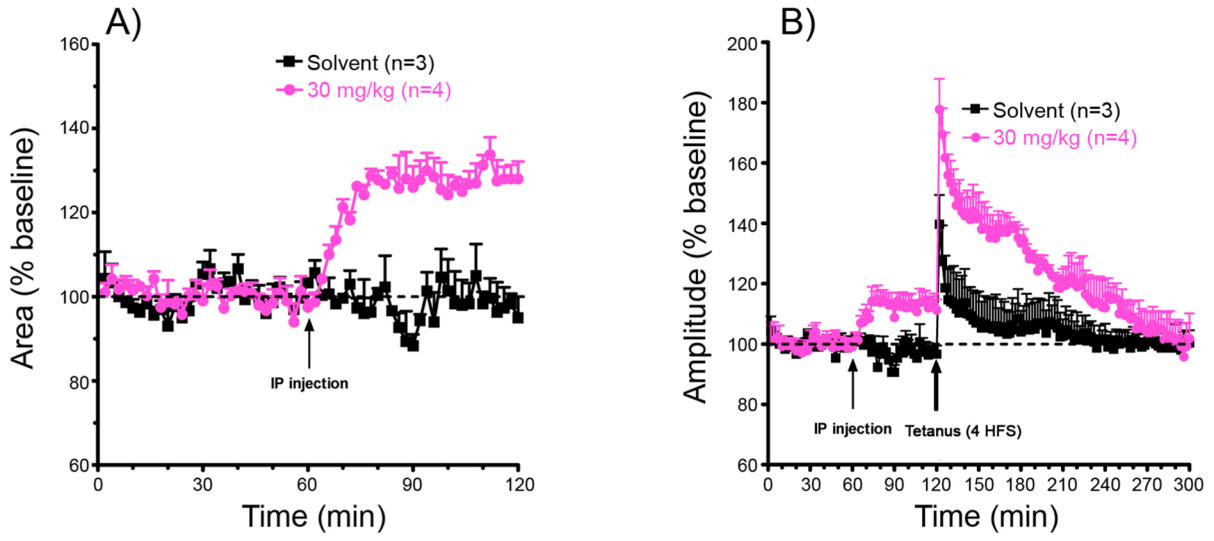


Figure 3. (A) Effects of **24** on the amplitude of the EPSfP recorded in the dentate gyrus of the hippocampus in vivo on anesthetized rats. (B) Effects of **24** on LTP induced in the dentate gyrus of the hippocampus on anesthetised Wistar rat. The amplitude of the EPSfP was averaged over four stimuli and was normalized as a percentage of the averaged amplitude of the response during the 1 h baseline period prior to ip injection (control value taken as 100%).

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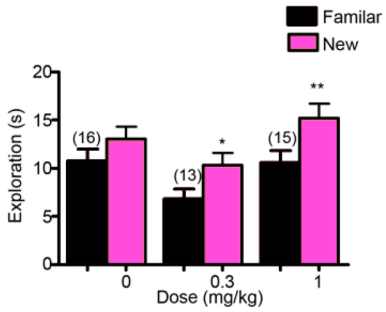

Figure 4. Effect of treatment with **24** per os on the object recognition test in CD1 mice. The discrimination index (δnew − fam) was the difference between the exploration times of the new and familiar objects on the last session with intersessions interval of 24 h. Under such conditions, control rats did no longer recognize the familiar object and spent similar times in exploring the familiar and new objects. Compound **24** significantly improved at 0.3 and 1 mg/kg per os the recognition of the familiar object as evidenced by increased exploration toward the new object (n = 13). *$p \leq 0.05$, **$p \leq 0.01$ vs control, one way ANOVA.

CONCLUSION

The present work explored new 3,4-dihydro-2*H*-thieno-1,2,4-thiadiazine 1,1-dioxides bearing at least one chlorine atom on the aromatic ring and one alkyl/fluoroalkyl chain on the nitrogen atom at the 4-position of the heterocycle. Three series of compounds were examined by varying the position of the sulfur atom in the thiophene ring.

The 6-chloro-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide series appeared to possess SAR close to that observed with their benzo-1,2,4-thiadiazine 1,1-dioxides counterparts. However, 5,7-dichloro-3,4-dihydro-2*H*-thieno-[3,4-*e*]-1,2,4-thiadiazine 1,1-dioxides clearly displayed an atypical behavior, with some saturated final compounds being found to be less active than their corresponding unsaturated counterparts.

Different attempts to prepare examples of 6-chloro-3,4-dihydro-2*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides resulted in the synthesis of **24**, bearing an ethyl chain on the nitrogen atom at the 4-position. In vitro and ex vivo tests revealed its strong biological efficiency on postsynaptic AMPA receptors and demonstrated its safety in vivo up to a dose of 30 mg/kg (no plasma/brain concentration data available). Therefore, **24** appeared to possess the most interesting pharmacological profile among all the new synthesized thiophenic compounds. Compound **24** was further explored in vivo and was found to modulate LTP and to increase AMPA-mediated noradrenaline release, two mechanisms underlying potential cognition-enhancing properties. This assumption was confirmed thanks to the object recognition test in mice, where **24** was found to improve cognition after oral administration of doses of the drug as low as 0.3 mg/kg.

Taken as a whole, 3,4-dihydro-2*H*-thieno-1,2,4-thiadiazine 1,1-dioxides constitute a new class of powerful AMPA potentiators. Further exploration of this series will be performed with the objective of discovering new candidates for the treatment of cognitive deficits.

EXPERIMENTAL SECTION

Synthesis. Melting points were determined on a Stuart smp3 capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1750 FT spectrophotometer. The ^{1}H NMR spectra were taken on a Bruker Advance 500 (500 MHz) instrument in DMSO-d_6 with TMS as an internal standard. Chemical shifts are reported in δ values (ppm) relative to internal TMS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, quint = quintuplet, m = multiplet, and b = broad are used throughout. Elemental analysis results (C, H, N, S) were realized on a Carlo-Erba EA 1108 elemental analyzer or on a FlashEA 1112 series (Thermo-Interscience) and were within (0.4% of the theoretical values (see Supporting Information). All compounds showed >95% purity, according to the elemental analyses. All reactions were routinely checked by TLC on silica gel Merck 60F254.

***N*-(5-Chloro-2-thienyl)acetamide (20).** The title compound was obtained according to the process described in the literature;[22] mp 177−178 °C (lit. 179 °C).[31] ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.06 (3H, s, CH_3), 6.43 (1H, d, 3-*H*), 6.85 (1H, d, 4-*H*), 11.36 (1H, s, N*H*).

***N*-[3-(Aminosulfonyl)-5-chloro-2-thienyl]acetamide (21).** To PCl_5 (3.5 g, 17 mmol) dissolved in chlorosulfonic acid (18 mL) was added in the cold state (+5 °C) *N*-(5-chloro-2-thienyl)-acetamide (**20**) (3.5 g, 20 mmol). The resulting mixture was heated at 70 °C for 45 min. The reaction medium was poured on ice and extracted with diethylether. Gaseous ammonia was bubbled into the dried organic phase during 15 min. After clarification of the ether suspension, the solvents were removed under reduced pressure. The solid residue was taken up in water (20 mL) and the insoluble material was collected by filtration, washed with water, and dried to yield the expected compound (1.20 g, 24%) used in the next step without further purification. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.24 (3H, s, CH_3), 7.09 (1H, s, 4-*H*), 7.60 (2H, s, SO_2NH_2), 10.19 (1H, s, N*H*).

5-Chloro-2-(ethylamino)-thiophene-3-sulfonamide (22). *N*-[3-(Aminosulfonyl)-5-chloro-2-thienyl]-acetamide (**21**) (500 mg, 1.96 mmol) was suspended in dry ether (20 mL) prior to the addition of $LiAlH_4$ (250 mg). After being stirred during 40 min at room temperature, the medium was cooled in an ice bath and water was slowly added. The mixture was then carefully adjusted to pH 4 by adding concentrated HCl prior extraction with ethyl acetate (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and filtered, and the filtrate was concentrated under reduced pressure. After treatment with charcoal in methanol, the expected compound was crystallized from a mixture of methanol/water (1:2) (290 mg, 61%); mp 134−140 °C (dec.). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.19 (3H, t, NHCH$_2$CH_3), 3.12 (2H, m, NHCH_2CH$_3$), 6.49 (1H, t, N*H*CH$_2$CH$_3$), 6.83 (1H, s, 4-*H*), 7.18 (2H, s, SO_2NH_2). Anal. ($C_6H_9ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-ethyl-4*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (23). 5-Chloro-2-(ethylamino)-thiophene-3-sulfonamide (**22**) (380 mg, 1.58 mmol) was suspended in triethyl orthoformate (20 mL). The mixture was heated at 170 °C in an open vessel for 30 min. After the mixture was cooled, the insoluble material that appeared was collected by filtration, washed with diethyl ether, and dried (0.32 g, 81%); mp 226−228 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.37 (3H, t, CH$_2$CH_3), 3.97 (2H, q, CH_2CH$_3$), 7.61 (1H, s, 7-*H*), 8.11 (1H, s, 3-*H*). Anal. ($C_7H_7ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-ethyl-3,4-dihydro-2*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (24). A solution of 6-chloro-4-ethyl-4*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**23**) (180 mg, 0.72 mmol) in 2-propanol (15 mL) was supplemented under stirring with sodium borohydride (150 mg, 3.97 mmol). After the mixture was stirred for 5 min at room temperature, the solvent was removed by distillation under reduced pressure and the residue was suspended in water (25 mL). The alkaline suspension was adjusted to pH 7 with 6 N HCl and extracted 3-fold with chloroform (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure, and the residue of the title compound was recrystallized in methanol/water, 1:2 and dried (0.09 g, 50%); mp 150−154 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.16 (3H, t, CH$_2$CH_3), 3.30 (2H, q, CH_2CH$_3$), 4.70 (2H, d, 3-H_2), 7.13 (s, 1H, 7-*H*), 7.90 (t, 1H, N*H*). Anal. ($C_7H_9ClN_2O_2S_2$) C, H, N, S.

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2,5-Dichloro-4-nitrothiophene-3-sulfonyl Chloride (26). 2,5-dichlorothiophene-3-sulfonyl chloride (**25**) (10 g, 40 mmol) was slowly added to an ice-cold mixture of nitric acid (30 mL) and sulfuric acid (30 mL). After 1 h of stirring on an ice bath, the mixture was poured on ice. The resulting solid was collected by filtration, washed with water, and dried. The title compound was used in the next step without further purification. (11.3 g, 96%).

2,5-Dichloro-4-nitrothiophene-3-sulfonamide (27). A solution of 2,5-dichloro-4-nitrothiophene-3-sulfonyl chloride (**26**) (11 g, 37 mmol) in dioxane (150 mL) was added dropwise to a 10% w/v ammonium hydroxide solution (300 mL). After 1 h, ammonia and dioxane were removed by evaporation under reduced pressure. The white precipitate that formed was collected by filtration, washed with water, and dried (7.7 g, 75%); mp 125−126 °C. IR (KBr): 3390, 3291, 1505, 1541, 1306, 1369, 1170 cm^{-1}. Anal. ($C_4H_2Cl_2N_2O_4S_2$) C, H, N, S.

4-Amino-2,5-dichlorothiophene-3-sulfonamide (28). 2,5-Dichloro-4-nitrothiophene-3-sulfonamide (**26**) (7.25 g, 26 mmol) was suspended in a 1:1 ethanol/water mixture (375 mL). The suspension was heated until the product dissolved. Then ammonium chloride (4.5 g) and powdered iron (15 g) were added. After refluxing for 10 min, the reaction was complete. The insoluble material was removed by filtration and rinsed with a small amount of hot ethanol. The filtrate was concentrated under reduced pressure. The beige precipitate that formed was collected by filtration, washed with water, and dried (3.4 g, 52%); mp 120 °C (dec.). IR (KBr): 3440, 3412, 3354, 3290, 1600, 1557, 1336, 1166 cm^{-1}. Anal. ($C_4H_4Cl_2N_2O_2S_2$) C, H, N, S.

5,7-Dichloro-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (29a). 4-Amino-2,5-dichlorothiophene-3-sulfonamide (**28**) (250 mg, 0.97 mmol) was dissolved in triethyl orthoformate (2.5 mL). The solution was brought to boiling in an open vessel. After 1.5 h, the suspension was allowed to cool, yielding a beige precipitate, which was collected by filtration, washed with diethyl ether, and dried (0.21 g, 80%); mp 200−203 °C. IR (KBr): 3147, 1597, 1318, 1175 cm^{-1}. Anal. ($C_5H_2Cl_2N_2O_2S_2$) C, H, N, S.

5,7-Dichloro-3,4-dihydro-2*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (30a). The suspension of 5,7-dichloro-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-dioxide (**29a**) (0.8 g, 3.1 mmol) in water (15 mL) was supplemented under stirring with sodium borohydride (2.0 g, 53 mmol). After 10 min stirring at room temperature, the alkaline suspension was adjusted to pH 7 with 6 N HCl and extracted 3-fold with dichloromethane (3 × 25 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure, and the residue of the title compound was recrystallized in methanol/water 1:2 (260 mg, 32%); mp 125−130 °C. IR (KBr): 3372, 3221, 1583, 1524, 1325, 1173 cm^{-1}. Anal. ($C_5H_4Cl_2N_2O_2S_2$) C, H, N, S.

5,7-Dichloro-4-methyl-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (29b). 5,7-Dichloro-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-dioxide (**29a**) (200 mg, 0.78 mmol) was dissolved in a mixture of nitroethane (2 mL) and dimethylformamide (2 mL). The solution was heated to 60 °C. Then potassium carbonate (0.2 g) was added, followed by iodomethane (0.2 mL, 2.5 mmol). After stirring for 2 h, the solvents were removed under reduced pressure. The residue was taken up in water. The insoluble material was quickly collected by filtration, washed with water, and dried (120 mg, 54%); mp 202−207 °C. IR (KBr): 2960, 1607, 1551, 1308, 1145 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz) δ 3.74 (s, 3H, NCH_3), 7.73 (s, 1H, 3-*H*). Anal. ($C_6H_4Cl_2N_2O_2S_2$) C, H, N, S.

5,7-Dichloro-4-methyl-3,4-dihydro-2*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (30b). The title compound was obtained as described for **24** starting from 5,7-dichloro-4-methyl-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-dioxide (**28b**) (500 mg, 1.8 mmol) (190 mg, 38%); mp 150−154 °C (dec.). IR (KBr): 3231, 2972, 1547, 1328, 1179 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz) δ 2.91 (3H, s, 4-CH_3), 4.40 (2H, s, 3-H_2). Anal. ($C_6H_6Cl_2N_2O_2S_2$) C, H, N, S.

5,7-Dichloro-4-ethyl-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (29c). 4-Amino-2,5-dichlorothiophene-3-sulfonamide (**28**) (4 g, 16 mmol) was suspended in triethyl orthoformate (20 mL) and heated at 170 °C for 35 min in an open vessel. The suspension was then cooled on ice. The precipitate that formed was collected by filtration, washed with *n*-hexane, and dried (1.55 g, 35%); mp 172−175 °C. IR (KBr): 1605, 1547, 1314, 1147 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz) δ 1.30 (t, 3H, NCH_2CH_3), 4.20 (q, 2H, NCH_2CH_3), 7.90 (s, 1H, 3-*H*). Anal. ($C_7H_6Cl_2N_2O_2S_2$) C, H, N, S.

5,7-Dichloro-4-ethyl-3,4-dihydro-2*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (30c). The title compound was obtained as described for **24** starting from 5,7-dichloro-4-ethyl-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-dioxide (**29c**) (0.5 g, 1.7 mmol) (380 mg, 75%); mp 144−147 °C. IR (KBr): 3313, 3281, 2963, 1582, 1548, 13224, 1172 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz) δ 1.10 (t, 3H, NCH_2CH_3), 3.20 (q, 2H, NCH_2CH_3), 5.40 (s, 2H, 3-H_2), 8.30 (s, 1H, N*H*). Anal. ($C_7H_8Cl_2N_2O_2S_2$) C, H, N, S.

2,5-Dichloro-4-(propionamido)thiophene-3-sulfonamide (31a). To 4-amino-2,5-dichlorothiophene-3-sulfonamide (**28**) (500 mg, 2.0 mmol) dissolved in dioxane (10 mL) were added in the cold state (+5 °C) pyridine (0.2 mL) and propionyl chloride (0.22 mL, 2.5 mmol). The flask was hermetically closed immediately, and contents were vigorously stirred at ambient temperature for 30 min. The solvents were then removed under reduced pressure. The solid residue was taken up in water (12 mL), and the insoluble material was collected by filtration, washed with water, and dried to yield the expected compound (500 mg, 82%). ^1H NMR (DMSO-d_6, 500 MHz) δ 1.07 (3H, t, $NHCOCH_2CH_3$), 2.31 (2H, q, $NHCOCH_2CH_3$), 7.58 (2H, s, SO_2NH_2), 9.34 (1H, s, $NHCOCH_2CH_3$).

2,5-Dichloro-4-(propylamino)thiophene-3-sulfonamide (32a). 2,5-Dichloro-4-(propionamido)-thiophene-3-sulfonamide (**31a**) (450 mg, 1.8 mmol) was suspended in dry ether (20 mL) prior to the addition of $LiAlH_4$ (250 mg). After being stirred for 60 min, additional $LiAlH_4$ (100 mg) was added. After 30 min of stirring, the mixture was cooled in an ice bath and water was slowly added. The mixture was then carefully adjusted to pH 4 by adding concentrated HCl. The mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and filtered, and the filtrate was concentrated under reduced pressure. The dry residue was then recrystallized from a mixture of acetone/water (1:10) (270 mg, 46%); mp 160 °C (dec). ^1H NMR (DMSO-d_6, 500 MHz) δ 0.92 (3H, t, $NHCH_2CH_2CH_3$), 1.60 (2H, h, $NHCH_2CH_2CH_3$), 3.47 (2H, qt, $NHCH_2CH_2CH_3$), 7.68 (2H, bs, SO_2NH_2), 8.33 (1H, s, $NHCH_2CH_2CH_3$).

5,7-Dichloro-4-propyl-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (29d). 2,5-Dichloro-4-(propylamino)-thiophene-3-sulfonamide (**32a**) (310 mg, 1.1 mmol) was suspended in triethyl orthoformate (7 mL). The mixture was heated at 120−130 °C in an open vessel for 90 min. After the mixture was cooled, the insoluble material that appeared was collected by filtration, washed with diethyl ether, and dried (240 mg, 75%); mp 179−180 °C. ^1H NMR (DMSO-d_6, 500 MHz) δ 0.90 (3H, t, 4-$CH_2CH_2CH_3$), 1.74 (2H, m, 4-$CH_2CH_2CH_3$), 4.17 (2H, t, 4-$CH_2CH_2CH_3$), 7.93 (1H, s, 3-*H*). Anal. ($C_8H_8Cl_2N_2O_2S_2$) C, H, N, S.

5,7-Dichloro-4-propyl-3,4-dihydro-2*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (30d). The title compound was obtained as described for **24** starting from 5,7-dichloro-4-propyl-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-dioxide (**29d**) (180 mg, 0,6 mmol) (130 mg, 72%); mp 135−138 °C. ^1H NMR (DMSO-d_6, 500 MHz) δ 0.90 (3H, t, 4-$CH_2CH_2CH_3$), 1.64 (2H, m, 4-$CH_2CH_2CH_3$), 3.21 (2H, t, 4-$CH_2CH_2CH_3$), 4.50 (2H, d, 3-H_2), 8.42 (1H, s, 2-*H*). Anal. ($C_8H_{10}Cl_2N_2O_2S_2$) C, H, N, S.

5,7-Dichloro-4-(2-fluoroethyl)-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (29e). To 4-amino-2,5-dichlorothiophene-3-sulfonamide (**28**) (1 g, 4.0 mmol) dissolved in dry ether (20 mL) was added in the cold state potassium carbonate (2 g). The mixture was cooled at −30 °C, and (+5 °C) pyridine (0.2 mL) and fluoroacetyl chloride (1 mL, 14 mmol) were added. After 90 min of stirring, the insoluble material was collected by filtration and washed with methanol. The combined filtrates were then concentrated under reduced pressure. This crude residue was suspended in dry ether (20 mL) and cooled at 0 °C prior to the addition of a 1.0 M borane solution in THF (10 mL, 10 mmol). After being stirred during 3 h at ambient temperature, water was slowly added. The organic layer was

separated, dried over $MgSO_4$, and filtered, and the filtrate was concentrated under reduced pressure. The dry residue was then suspended in triethyl orthoformate (8 mL) and heated at 150 °C for 60 min in an open vessel. The resulting mixture was concentrated under reduced pressure. The residue was dissolved in acetone and treated with charcoal. The resulting solution was concentrated and cooled on ice. The insoluble material was collected by filtration and dried to yield the expected compound to give the expected title compound (250 mg, 20%); mp 177−179 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 4.65 (2H, dt, NCH_2CH$_2$F), 4.79 (2H, dt, NCH$_2$CH_2F), 7.89 (1H, s, 3-*H*). Anal. ($C_7H_5Cl_2FN_2O_2S_2$) C, H, N, S.

5,7-Dichloro-4-(2-fluoroethyl)-3,4-dihydro-2*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (30e). The title compound was obtained as described for **24** starting from 5,7-dichloro-4-(2-fluoroethyl)-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-dioxide (**29e**) (150 mg, 0.5 mmol) (120 mg, 79%); mp 170−174 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.61 (2H, dt, NCH_2CH$_2$F), 4.56 (2H, s, 3-H_2), 4.71 (2H, dt, NCH$_2$CH_2F), 8.47 (1H, s, N-*H*). Anal. ($C_7H_7Cl_2FN_2O_2S_2$) C, H, N, S.

2,5-Dichloro-4-(2,2-difluoroacetamido)thiophene-3-sulfonamide (31c). To 4-amino-2,5-dichlorothiophene-3-sulfonamide (**28**) (800 mg, 3.2 mmol) dissolved in dioxane (15 mL) were added in the cold state (+10 °C) pyridine (0.4 mL) and 2,2-difluoroacetyl chloride (0.4 mL, 3.8 mmol). The flask was hermetically closed immediately, and contents were vigorously stirred at ambient temperature for 5 min. The solvents were then removed under reduced pressure. The resulting oil was taken up in water (12 mL) and extracted 3-fold with ethyl acetate (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure, and the residue of the title compound was recrystallized in methanol/water on an ice bath (630 mg, 60%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 6.42 (1H, t J = 53.09 Hz, NHCOC*H*F$_2$), 7.78 (2H, s, SO$_2$NH_2), 10.37 (1H, s, N*H*COCHF$_2$).

2,5-Dichloro-4-(2,2-difluoroethylamino)thiophene-3-sulfonamide (32c). The title compound was obtained as described for **32a** starting from 2,5-dichloro-4-(2,2-difluoroacetamido)thiophene-3-sulfonamide (**31c**) (700 mg, 2.1 mmol) (480 mg, 72%) and was used in the next step without further purification.

5,7-Dichloro-4-(2,2-difluoroethyl)-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (29f). 2,5-Dichloro-4-(2,2-difluoroethylamino)thiophene-3-sulfonamide (**32c**) (480 mg, 1.5 mmol) was suspended in triethyl orthoformate (20 mL). The mixture was heated at 180 °C in an open vessel for 7 h. After cooling, *n*-hexane was added to the mixture, and the resulting insoluble material that appeared was collected by filtration and was recrystallized in methanol (200 mg, 40%); mp 152−154 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 4.83 (2H, t, 4-CH_2CHF$_2$), 6.53 (1H, t J = 54.20 Hz, 4-CH$_2$C*H*F$_2$), 7.93 (1H, s, 3-*H*). Anal. ($C_7H_4Cl_2F_2N_2O_2S_2$) C, H, N, S.

5,7-Dichloro-4-(2,2-difluoroethyl)-3,4-dihydro-2*H*-thieno-[3,4-*e*]-1,2,4-thiadiazine 1,1-Dioxide (30f). The title compound was obtained as described for **24** starting from 5,7-dichloro-4-(2,2-difluoroethyl)-4*H*-thieno[3,4-*e*]-1,2,4-thiadiazine 1,1-dioxide (**29f**) (140 mg, 0.4 mmol) (90 mg, 64%); mp 168−170 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 4.58 (2H, s, 3-H_2), 4.83 (2H, t, 4-CH_2CHF$_2$), 6.37 (1H, t, J = 55.53 Hz, 4-CH$_2$C*H*F$_2$), 8.55 (1H, s, N*H*). Anal. ($C_7H_6Cl_2F_2N_2O_2S_2$) C, H, N, S.

6-Chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (36a). 3-Amino-5-chlorothiophene-2-sulfonamide (**35**)[24] (250 mg, 1.0 mmol) was suspended in triethyl orthoformate (2.5 mL). The resulting mixture was heated at about 160 °C for 30 min. A granular yellow precipitate formed and was collected by filtration, washed with diethyl ether, and dried (185 mg, 83%); mp 260−262 °C. IR (KBr): 3214, 3103, 3061, 1602, 1569, 1515, 1378, 1147 cm^{-1}. Anal. ($C_5H_3ClN_2O_2S_2$) C, H, N, S.

6-Chloro-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (37a). The title compound was obtained as described for **30a** starting from 6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36a**) (150 mg, 0,07 mmol) (110 mg, 73%); mp 154−156 °C. Anal. ($C_5H_5ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-methyl-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (36b). 6-Chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36a**) (250 mg, 1.1 mmol) was dissolved in a mixture of acetonitrile (5 mL) and dimethylformamide (0.5 mL). Potassium carbonate (500 mg) and methyl iodide (0.3 mL, 0.68 g, 4.8 mmol) were added, and the mixture was heated at 55 °C for 7 h. At the end of the reaction, the solvent was removed under reduced pressure and the residue was taken up in water. The insoluble material was collected by filtration, washed with water, and dried (205 mg, 77%); mp 252−254 °C. IR (KBr): 3091, 1607, 1519, 1485, 1297, 1150 cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.64 (s, 3H, NCH_3), 7.58 (s, 1H, 5-*H*), 8.05 (s, 1H, 3-*H*). Anal. ($C_6H_5ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-methyl-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (37b). The title compound was obtained as described for **24** starting from 6-chloro-4-methyl-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36a**) (200 mg, 0.85 mmol) (100 mg, 49%); mp 174−176 °C. IR (KBr): 3236, 3089, 1562, 1322, 1154 cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.00 (s, 3H, NCH_3), 4.63 (d, 2H, 3-H_2), 7.17 (s, 1H, 5-*H*), 8.12 (t, 1H, N*H*). Anal. ($C_6H_7ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-ethyl-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (36c). The title compound was obtained as described for **36b** (250 mg, 1.1 mmol) using ethyl bromide (0.4 mL, 0.58 g, 5.4 mmol) instead of methyl iodide (190 mg, 68%); mp 189−190 °C. IR (KBr): 3098, 2982, 1609, 1518, 1300, 1161 cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.32 (t, 3H, NCH$_2$CH_3), 4.08 (q, 2H, NCH_2CH$_3$), 7.68 (s, 1H, 5-*H*), 8.12 (s, 1H, 3-*H*). Anal. ($C_7H_7ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-ethyl-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (37c). The title compound was obtained as described for **24** starting from 6-chloro-4-ethyl-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36c**) (250 mg, 0.80 mmol) (90 mg, 45%); mp 119−120 °C. IR (KBr): 3235, 2980, 2931, 2872, 1558, 1324, 1154 cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.06 (t, 3H, 4-CH$_2$CH_3), 3.40 (q, 2H, 4-CH_2CH$_3$), 4.65 (d, 2H, 3-H_2), 7.14 (s, 1H, 5-*H*), 7.95 (t, 1H, N*H*). Anal. ($C_7H_9ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-isopropyl-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (36d). The title compound was obtained as described for **36b** (250 mg, 1.1 mmol) using isopropyl iodide (0.5 mL, 0.92 g, 5.4 mmol) instead of methyl iodide (200 mg, 42%); mp 149−150 °C. IR (KBr): 3112, 2989, 1605, 1515, 1306, 1170 cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.43 (d, 6H, NCH(CH_3)$_2$), 4.08 (sept, 1H, NC*H*(CH$_3$)$_2$), 7.72 (s, 1H, 5-*H*), 8.18 (s, 1H, 3-*H*). Anal. ($C_8H_9ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-isopropyl-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (37d). The title compound was obtained as described for **24** starting from 6-chloro-4-isopropyl-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36d**) (250 mg, 0.9 mmol) (130 mg, 51%); mp 100−102 °C. IR (KBr): 3267, 3241, 2970, 2954, 1545, 1332, 1156 cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.15 (d, 6H, NCH(CH_3)$_2$), 4.03 (sept, 1H, NC*H*(CH$_3$)$_2$), 4.62 (d, 2H, 3-H_2), 7.21 (s, 1H, 5-*H*), 7.79 (t, 1H, N*H*). Anal. ($C_8H_{11}ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-propyl-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (36e). To 6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36a**) (450 mg, 2.0 mmol) in acetonitrile (15 mL) were added potassium carbonate (900 mg) and *n*-propyl iodide (0.6 mL, 6.2 mmol), and the mixture was heated at 90 °C for 150 min. At the end of the reaction, the solvent was removed under reduced pressure and the residue was taken up in water. The insoluble material was collected by filtration, washed with water, and recrystallized in methanol (450 mg, 84%); mp 155−157 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 0.87 (3H, t, N-CH$_2$CH$_2$CH_3), 1.68 (2H, m, N-CH$_2$CH_2CH$_3$), 3.97 (2H, t, N-CH_2CH$_2$CH$_3$), 7.68 (1H, s, 5-*H*), 8.09 (1H, s, 3-*H*). Anal. ($C_8H_9ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-propyl-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (37e). The title compound was obtained as described for **24** starting from 6-chloro-4-propyl-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36e**) (200 mg, 0.76 mmol) (150 mg, 74%); mp 134−136 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 0.86 (3H, t, N-CH$_2$CH$_2$CH_3), 1.51 (2H, m, N-CH$_2$CH_2CH$_3$), 3,29 (2H, t, N-CH_2CH$_2$CH$_3$), 4.66 (2H, d, 3-H_2), 7.14 (1H, s, 5-*H*), 7.95 (1H, s, N-*H*). Anal. ($C_8H_{11}ClN_2O_2S_2$) C, H, N, S.

6-Chloro-4-(fluoromethyl)-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (36f). To 6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadia-

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zine 1,1-dioxide (**36a**) (500 mg, 2.2 mmol) in ice-cold acetonitrile (15 mL) were added potassium carbonate (900 mg) and bromofluoromethane (1 mL, 16 mmol). The mixture was placed in a closed vessel and heated at 70 °C for 8 h. At the end of the reaction, the solvent was removed under reduced pressure and the residue was taken up in water. The insoluble material was collected by filtration and recrystallized in methanol (380 mg, 66%); mp 198−200 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 6.10 (2H, d, N-CH_2F), 7.71 (1H, s, 5-*H*), 8.37 (1H, s, 3-*H*). Anal. ($C_6H_4ClFN_2O_2S_2$) C, H, N, S.

6-Chloro-4-(isopropoxymethyl)-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (37f). A solution of 6-chloro-4-(fluoromethyl)-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36f**) (200 mg, 0.8 mmol) in 2-propanol (8 mL) at 50 °C was supplemented under stirring with sodium borohydride (400 mg, 10.6 mmol). After the mixture was stirred for 10 min, the solvent was removed by distillation under reduced pressure and the residue was suspended in ethyl acetate (15 mL). After removal of the solid by filtration on Celite and evaporation of the solvent, the residue was purified on a column using ethyl acetate/*n*-hexane (15/5) as a mobile phase followed by a methanol/water recrystallization on an ice bath (0.01 g, 5%); mp 65−68 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.10 (6H, d, OCH(C*H*3)2), 3.70 (1H, m, OC*H*(CH3)2), 4.75 (4H, s, 3-*H*2/N-CH_2-O), 7.11 (1H, s, 5-*H*). Anal. ($C_6H_6ClFN_2O_2S_2$) C, H, N, S.

6-Chloro-4-(2-fluoroethyl)-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (36g). The title compound was obtained as described for **36b** (600 mg, 2.7 mmol) using 1-fluoro-2-iodoethane (0.6 mL, 1.3 g, 7.4 mmol) instead of methyl iodide (440 mg, 61%); mp 185−187 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 4.38 (2H, dt, N-CH_2CH$_2$F), 4.70 (2H, dt, N-CH$_2$CH_2F), 7.66 (1H, s, 5-*H*), 8.05 (1H, s, 3-*H*). Anal. ($C_7H_6ClFN_2O_2S_2$) C, H, N, S.

6-Chloro-4-(2-fluoroethyl)-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (37g). The title compound was obtained as described for **24** starting from 6-chloro-4-(2-fluoroethyl)-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36g**) (400 mg, 0.76 mmol) (290 mg, 65%); mp 145−147 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.70 (2H, dt, N-CH_2CH$_2$F), 4.55 (2H, dt, N-CH$_2$CH_2F), 4.73 (2H, s, 3-H_2), 7.14 (1H, s, 5-*H*), 8.06 (1H, s, N-*H*). Anal. ($C_7H_8ClFN_2O_2S_2$) C, H, N, S.

6-Chloro-4-(3-fluoropropyl)-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (36h). To 6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36a**) (450 mg, 2.0 mmol) in acetonitrile (20 mL) were added potassium carbonate (900 mg) and 1-fluoro-3-bromopropane (0.9 mL, 0.77 g, 5.5 mmol), and the mixture was heated at 100 °C for 9 h. At the end of the reaction, the solvent was removed under reduced pressure and the residue was taken up in water. The insoluble material was collected by filtration, washed with water, and recrystallized in methanol (420 mg, 74%); mp 181−183 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.08 (2H, m, N−CH$_2$CH_2CH$_2$F, 4.13 (2H, t, N-CH_2CH$_2$CH$_2$F), 4.55 (2H, dt, N-CH$_2$CH$_2$CH_2F), 7.64 (1H, s, 5-*H*), 8.09 (1H, s, 3-*H*). Anal. ($C_8H_8ClFN_2O_2S_2$) C, H, N, S.

6-Chloro-4-(3-fluoropropyl)-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (37h). The title compound was obtained as described for **24** starting from 6-chloro-4-(3-fluoropropyl)-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36h**) (400 mg, 1.4 mmol) (320 mg, 79%); mp 125−126 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.90 (2H, m, N-CH$_2$CH_2CH$_2$F), 3.44 (2H, t, N-CH_2CH$_2$CH$_2$F), 4.51 (2H, dt, N-CH$_2$CH$_2$CH_2F), 4.68 (2H, d, 3-H_2), 7.09 (1H, s, 5-*H*), 7.98 (1H, t, N-*H*). Anal. ($C_8H_{10}ClFN_2O_2S_2$) C, H, N, S.

5-Chloro-3-(2,2-difluoroacetamido)thiophene-2-sulfonamide (38). To 3-amino-5-chlorothiophene-2-sulfonamide (**35**) (1.00 g, 4.7 mmol) dissolved in dioxane (30 mL) was added 2,2-difluoroacetyl chloride (0.75 mL, 7.1 mmol). The flask was hermetically closed immediately, and contents were vigorously stirred at ambient temperature for 60 min. The solvents were then removed under reduced pressure. The residue was taken up in water (15 mL). The resulting solid material was collected by filtration and used in the next step without further purification (1.04 g, 76%).

5-Chloro-3-(2,2-difluoroethylamino)thiophene-2-sulfonamide (39). The title compound was obtained as described for **32a** starting from 5-chloro-3-(2,2-difluoroacetamido)thiophene-2-sulfonamide (**38**) (1.0 g, 3.4 mmol) (570 mg, 60%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.67 (2H, m, N-CH_2CHF$_2$), 6.09 (1H, tt, N-CH$_2$CHF$_2$), 7.08 (1H, s, 4-*H*), 7.45 (2H, bs, SO$_2$NH_2). Anal. ($C_6H_7ClF_2N_2O_2S_2$) C, H, N, S.

6-Chloro-4-(2,2-difluoroethyl)-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (36i). 5-Chloro-3-(2,2-difluoroethylamino)-thiophene-2-sulfonamide (**39**) (860 mg, 3.1 mmol) was heated at 80 °C in formic acid (30 mL) for 5 h. After cooling, the solvent was removed under reduced pressure. The residue was taken up in water, and the resulting insoluble material that appeared was collected by filtration (440 mg, 49%); mp 157−158 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 4.60 (2H, t, N-CH_2CHF$_2$), 6.46 (1H, t, N-CH$_2$CHF$_2$), 7.70 (1H, s, 5-*H*), 8.08 (1H, s, 3-*H*). Anal. ($C_7H_5ClF_2N_2O_2S_2$) C, H, N, S.

6-Chloro-4-(2,2-difluoroethyl)-3,4-dihydro-2*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (37i). The title compound was obtained as described for **24** starting from 6-chloro-4-(2,2-difluoroethyl)-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**36i**) (480 mg, 1.7 mmol) (360 mg, 75%); mp 131−133 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.86 (2H, m, N−CH_2CHF$_2$), 4.78 (2H, s, 3-H_2), 6.21 (1H, tt, N-CH$_2$CHF$_2$), 7.18 (1H, s, 5-*H*), 8.14 (1H, bs, N-*H*). Anal. ($C_7H_7ClF_2N_2O_2S_2$) C, H, N, S.

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

Effect on AMPA Currents in *Xenopus Laevis* Oocytes. Electrophysiological recordings were performed at room temperature on *Xenopus laevis* oocytes expressing rat cortex poly(A$^+$) mRNA using a previously described procedure.[18] S22286 (100 μM, used as positive potent reference as AMPA potentiator with rapid wash-out) was first bath-applied on the same oocyte for 45 s before, 30 s during, and 30 s after the application of 10 μM AMPA in order to select for the experiment oocytes presenting sufficient potentiation of the AMPA-evoked current. Oocytes presenting less than 130-fold increase of the AMPA-mediated current in presence of 100 μM S22286 were excluded and the experiment stopped.

Effect on Excitatory Postsynaptic Response Evoked in CA1 Area on Rat Hippocampal Slices in Vitro. This assay was performed following our previously published procedure[18]

Effect on Long-Term Potentiation (LTP) of the Postsynaptic Response Evoked in the Dentate Gyrus on Anesthetized Rats. Extracellular excitatory postsynaptic field potentials (EPSfP) were recorded in the dentate gyrus using our previously published procedure.[18]

Effect on AMPA-Mediated Release of Noradrenaline on Rat Hippocampal Slices. Male Wistar rat (200−300 g) was decapitated, the brain was rapidly removed, and both hippocampi were carefully isolated. Transversal hippocampal slices of 300 μm thickness were cut with a tissue chopper. Hippocampal slices were incubated with [^{3}H]-noradrenaline ([^{3}H]-NAD, 0.16 Ci/mL) for 30 min at 37 °C, rinsed, and placed in reaction chambers of a B18 Superfusion System (Brandel, USA). Slices were superfused with Krebs buffer, with (*S*)-AMPA or with (*S*)-AMPA in the presence of compound. The superfusate was collected in 5 min fractions. The quantity of radioactivity was estimated for each fraction with a Beckman scintillation counter and the fractional NAD release was normalized as the percentage relative to the total radioactivity from all fractions plus the tissue sample.

Effect on Object Recognition Test in Mice. The one-trial object recognition paradigm measures a form of episodic memory in the mouse and was achieved following our previously described procedure.[17,18]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Elemental analysis results for the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

§B.P. and P.d.T. equally supervised the work.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; AP/ML/DV, anterior-posterior/medial-lateral/dorsal-ventral; BDNF, brain-derived neurotrophic factor; CA1, Cornu Ammonis 1; CD1, cluster of differentiation 1; EPSfP, excitatory postsynaptic field potential; FlipR, fluorometric imaging plate reader; MΩ, megaohm; mV, millivolt; ms, millisecond; NAD, noradrenaline; nL, nanoliter

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