

Design and Evaluation of Selective Positive Allosteric Modulators Targeting Kainate Receptors

Thomas Colson,^{*,||} Yasmin Bay,^{||} Alexandra Manos-Turvey, Giacomo Baudino, Bastiaan Poldermans, Eric Goffin, Marine Piette, Cindy Lesenfants, Marie Leclercq, Jette S. Kastrup, Bernard Pirotte, Anders S. Kristensen, and Pierre Francotte*



Cite This: *ACS Omega* 2026, 11, 55299–55315



Read Online

ACCESS |



Metrics & More

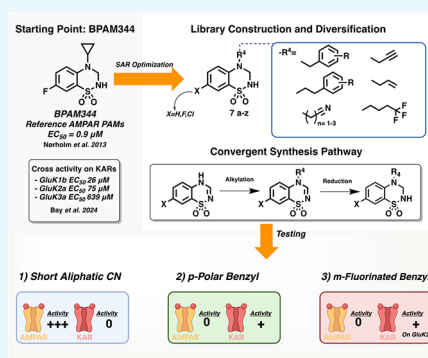


Article Recommendations



Supporting Information

ABSTRACT: Kainate-type ionotropic glutamate receptors (KARs) are emerging therapeutic targets for the modulation of excitatory neurotransmission in epilepsy, psychiatric disorders, and neurodegenerative diseases, yet selective positive allosteric modulators (PAMs) remain scarce. Starting from the benzothiadiazine AMPA receptor (AMPA) PAM BPAM344 (1), we pursued a structure-based scaffold repurposing strategy to identify KAR-directed analogues. A focused library of benzothiadiazine 1,1-dioxides (7a–7z) was designed through systematic diversification at the N4 position and modulation of the C7 substituent (H, F, and Cl). Compounds were synthesized via a convergent route involving benzothiadiazine core assembly, N4 alkylation with aliphatic (including nitrile-terminated), trifluorinated, benzyl, or phenethyl substituents, followed by reduction to the corresponding 3,4-dihydro derivatives. All compounds were fully characterized and evaluated for modulatory activity at GluA2 and GluK1–3 receptors using Ca^{2+} fluorescence assays in HEK cells. Clear structure–activity relationships (SAR) were established. Short nitrile-terminated aliphatic substituents (7a–7g) retained GluA2 potentiation but lacked KAR activity, indicating that flexible polar chains favor AMPAR selectivity. In contrast, the N4-benzyl series revealed a more complex structure–activity relationship. The para-nitro benzyl analogue 7y was inactive across the receptor subtypes tested, whereas the 3-fluoro-4-nitrobenzyl derivative 7q showed preferential potentiation of GluK1 and GluK3, with minimal GluA2 modulation. Notably, the meta-fluorinated benzyl analogue 7u preferentially potentiated GluK3 while remaining inactive at GluK1, GluK2, and GluA2, whereas the other fluorinated derivatives were devoid of detectable activity across the receptor subtypes tested. These findings reveal a distinct subtype-dependent SAR and support the existence of a spatially constrained polar microenvironment within the GluK1/3 allosteric sites, which appears to be optimally engaged by rigid para-polar benzyl substituents. In line with our docking observations, acidic substituents may further exploit this polar region while the core scaffold remains favorably accommodated in the surrounding hydrophobic pocket. On the basis of the pharmacological testing, meta-fluorination appears to further refine subtype selectivity toward GluK3 and may represent a promising starting point for further exploratory work. Collectively, these results establish 1,2,4-benzothiadiazine 1,1-dioxides as a tractable chemotype for the development of subtype-selective KAR PAMs and provide a medicinal chemistry framework for second-generation optimization, while the docking-derived contacts should be regarded as plausible structural hypotheses rather than experimentally confirmed interactions, given that they are based on SwissDock-predicted poses rather than a crystallographic ligand–receptor complex.



INTRODUCTION

Most of the excitatory signals in the central nervous system (CNS) are mediated by the amino acid *L*-glutamate through the activation of ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs).¹ iGluRs are classified into three main categories according to their interaction with specific agonists. The first class includes receptors that respond to *N*-methyl-*D*-aspartic acid (NMDA), known as NMDA receptors (NMDARs). The second class consists of receptors sensitive to 2-amino-3-(5-methyl-3-hydroxy-1,2-oxazol-4-yl)propanoic acid (AMPA), known as AMPA receptors (AMPARs). The third class includes receptors that respond to kainic acid (2-carboxy-3-carbox-

ymethyl-4-isopropenylpyrrolidine), known as kainate receptors (KARs). Additionally, there is a lesser-known type of iGluRs, the δ (GluD) receptors.^{1,2,3}

iGluRs are composed of different subunits: for the NMDARs, GluN1, GluN2A-D, and GluN3A/B; for the AMPARs, GluA1–4; and for the KARs, GluK1–5.^{2,4,5,6}

Received: April 1, 2026

Revised: August 19, 2026

Accepted: August 26, 2026

Published: September 10, 2026



AMPA receptors (AMPA receptors) are tetrameric assemblies of GluA1–4 subunits in which the amino-terminal domain (ATD) and ligand-binding domain (LBD) form an intertwined dimer-of-dimers arrangement with domain-swapped subunit pairs, whereas the transmembrane domain (TMD) forms a 4-fold symmetric tetrameric ion channel.^{7,8,9} KAR assemblies can be composed of the five different subunits, GluK1–5.^{10,11} The GluK1, GluK2, and GluK3 subunits can form heterotetrameric as well as homotetrameric receptors. The GluK4 and GluK5 subunits necessarily form heterotetramers by association with GluK1–3 subunits.^{3,12,13} In contrast to NMDARs, which are obligatory heterotetramers,⁴ AMPARs and KARs can thus exist both as heterotetramers and homotetramers.⁴

AMPA receptors and KARs comprise a large extracellular region containing two distinct domains: the amino-terminal domain (ATD) and the ligand-binding domain (LBD), which harbors the glutamate-binding site.⁴ There is also a transmembrane domain (TMD), consisting of three hydrophobic membrane-spanning helices (M1, M3, and M4) and an intramembrane reentrant loop (M2) that forms part of the ion channel pore.^{5,14} Finally, an intracellular carboxyl-terminal domain (CTD) is present, with motifs for binding and phosphorylation of transmembrane regulatory proteins.¹⁵

Alternative splicing in AMPARs^{3,16,17} gives rise to two variants, flip (which is dominant during early development) and flop (the most abundant variant in adults¹⁸).

AMPA receptors are believed to regulate the rapid excitation required to remove the magnesium block of nearby NMDARs, thereby allowing information transmission.¹⁹ The physiology of KARs is less understood than that of AMPARs. KARs seem to show similar properties to AMPARs, allowing ion flow immediately after glutamate binding, but they are generally less permeable to calcium ions than GluA2-lacking AMPARs and more permeable than GluA2-containing AMPARs.¹⁹ AMPARs are primarily located on the postsynaptic membrane, while research indicates that KARs can be found on the presynaptic^{20,21} as well as the postsynaptic side of the neuronal synapse.^{20,22,23} Postsynaptic KARs, like AMPARs, can depolarize the membrane sufficiently to relieve the Mg²⁺ block of NMDARs, although KAR-mediated excitatory postsynaptic currents (EPSCs) exhibit slower kinetics than those mediated by AMPARs.¹⁹

In addition to their importance for rapid excitatory communication in the brain, iGluRs also play a role in synaptic plasticity, which is critical for learning and memory.^{5,20} Also, iGluRs have vital functions in neural development and neurodegeneration, and their abnormal activity is often associated with a wide range of neurological disorders, such as epilepsy, ischemic stroke, and ADHD, and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease,²⁴ Huntington's disease, and amyotrophic lateral sclerosis.^{5,19,25} Dysregulation may also be associated with psychiatric disorders such as schizophrenia, obsessive-compulsive disorder, major depressive disorder, and bipolar disorder.²⁶ Because of these multiple implications, iGluRs have attracted considerable interest in drug development.^{5,25} Several studies indicate that KAR subtypes, defined by their subunit composition, differ in function and may be differentially implicated in disease. In particular, heterotetrameric receptors composed of low-affinity subunits (GluK1–3) together with high-affinity subunits (GluK4 or GluK5) have been associated with neurological disorders such as epilepsy, autism, schizophrenia, and depression.^{27,28,29,30} These observations

highlight KARs as potential therapeutic targets and have motivated efforts to develop pharmacological tools to modulate their function.

Initial studies in the field of iGluR modulators focused on the synthesis of positive allosteric modulators of AMPARs (AMPA PAMs), and some of them were also tested on KARs. Among such modulators, BPAM344 (**1**) showed marked activity as a potentiator of KARs.⁶ This project aimed to produce a set of new positive allosteric modulators of KARs (KAR PAMs) based on the structure of BPAM344 (**1**). Our main scaffold (**A**) illustrated in Figure 1 was inspired by

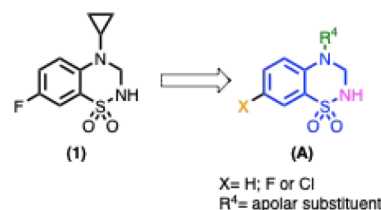


Figure 1. Structure of BPAM344 (**1**) and the primary scaffold of the new KAR PAMs (**A**).

Frydenvang et al.,¹² who suggested targeting a region in the receptors where differences are observed between AMPARs (GluA2 Asp781) and KARs (GluK1 His792) to achieve selectivity by extending PAMs that bind to the conventional site with substituents that reach this region. The new compounds contain a hydrogen atom or a small halogen atom, such as fluorine or chlorine, at the 7-position of the benzothiadiazine ring. The presence of a H-bond donor group at the 2-position appears to be important¹² and therefore has been retained.

The 4-position of the benzothiadiazine ring allows the introduction of a broad range of substituents.^{31,32} Here, we address the effect of introducing apolar substituents (R⁴) on the modulatory efficacy of the compounds on AMPARs (GluA2) and KARs (GluK1–3) using Ca²⁺ fluorescence-based assays. Initially, short aliphatic chains with steric and hydrophobic properties comparable to the cyclopropyl group of BPAM344 were explored (Figure 2), including alkene and

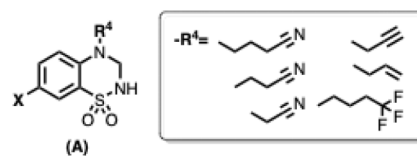


Figure 2. Structures of benzothiadiazine derivatives with aliphatic and polar side chains.

alkyne analogues designed to mimic its compact apolar volume. A second series, including nitrile-terminated aliphatic chains, was conceived as “polar probing” substituents to explore the potential formation of additional interactions within the GluK1–3 binding pocket. Similarly, the 4,4,4-trifluorobutyl moiety was introduced with the same exploratory rationale, aiming to evaluate whether polarizable or partially electron-withdrawing substituents could favor new protein–ligand contacts.

Indeed, it has been investigated that the cyclopropyl has the ability to interact with neighboring π -electron systems and π -electron centers.³³ The goal of this second pool was to

generate molecules capable of establishing π interactions with their surroundings, similar to those observed with the cyclopropyl chain.

To complete this approach, substituted benzyl and phenethyl groups were also fixed at the 4-position of the benzothiadiazine ring (Figure 3). Tolerated apolar R^4 substituents may be further explored by the introduction of charged groups.

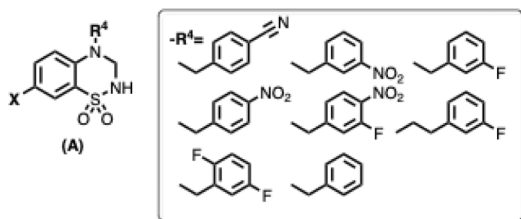


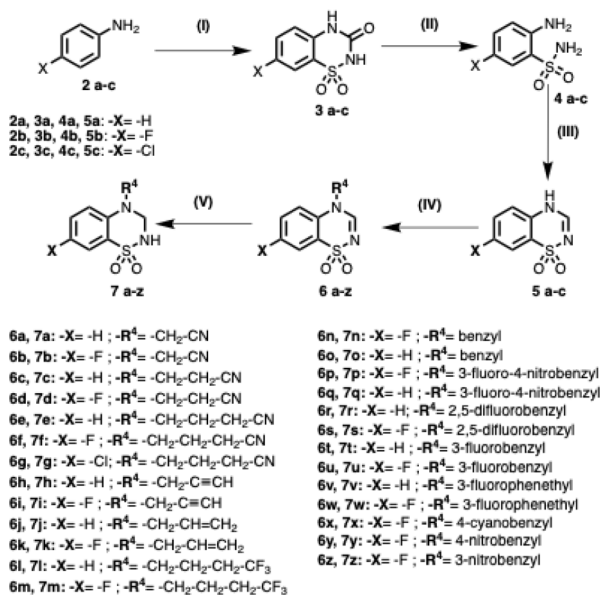
Figure 3. Structure of benzothiadiazine derivatives with aromatic benzyl and phenethyl substituents.

RESULTS AND DISCUSSION

Synthesis

The target compounds **7a–z** were synthesized starting from aniline (**2a**; $X = H$), 4-fluoroaniline (**2b**; $X = F$), or 4-chloroaniline (**2c**; $X = Cl$) (Scheme 1). Initially, the reaction of

Scheme 1. (I) **a**: Chlorosulfonyl Isocyanate (1.2 equiv), CH_2Cl_2 , **b**: $AlCl_3$ (1.3 equiv); (II) H_2SO_4 , H_2O (50% v/v), reflux, 3 h; (III) Triethyl Orthoformate; (IV) Alkylating Agent (2.2 equiv), K_2CO_3 (4 equiv), ACN; (V) $NaBH_4$ (9–18 equiv), Isopropanol



anilines with chlorosulfonyl isocyanate in the presence of aluminum chloride ($AlCl_3$) led to the formation of the ring-closed “3-oxo” intermediates **3a–c**. Subsequent hydrolysis under strongly acidic conditions of these intermediates yielded the corresponding aminobenzenesulfonamides **4a–c**. Next, the latter underwent a ring closure reaction with triethyl orthoformate to generate the 1,2,4-benzothiadiazine 1,1-dioxides **5a–c**. These key intermediates were then selectively

alkylated at the 4-position using the appropriate aliphatic/benzylic/phenethyl alkylating agent and potassium carbonate (K_2CO_3), afforded an aliphatic/benzylic/phenethyl chain at the 4-position of the 1,2,4-benzothiadiazine 1,1-dioxides **6a–z**. Finally, reduction of the $C=N$ bond of this ring by means of sodium borohydride ($NaBH_4$) provided the target structures **7a–z**.

Biological Testing of KAR Modulators

The potentiating activity of the compounds was evaluated in HEK293 cells expressing recombinant homomeric GluA2 AMPA receptors or KARs GluK1–3 using a Ca^{2+} -sensitive fluorescence assay (Figure 4). Cells were stimulated with saturating concentrations of agonist in the absence or presence of test compounds, and agonist-evoked responses were quantified as peak fluorescence increases. Potentiation was normalized to the response produced by the reference modulator BPAM344 (100%). Compounds were tested at 100 μM at GluA2, GluK1, and GluK2 receptors and at 300 μM at GluK3 receptors. Compounds in series 1 (Figure 4B), containing linear aliphatic R^4 substituents, generally retained potentiating activity at GluA2 comparable to BPAM344. Within this series, several analogues also potentiated GluK3 responses, whereas activity at GluK1 and GluK2 was largely absent. In contrast, compounds in series 2 (Figure 4C), bearing aryl-based R^4 substituents, showed no potentiation at GluA2 overall, but a subset showed activity at GluK1 and/or GluK3. Based on the primary screening, four compounds (**7l**, **7m**, **7q**, and **7u**) that displayed significant GluK1, GluK2, or GluK3 potentiation were further evaluated in concentration–response experiments to assess their potency and receptor subtype selectivity (Figure 4D). Increasing concentrations of compounds **7l**, **7m**, **7q**, and **7u** produced concentration-dependent potentiation of agonist-evoked responses, with clear subtype-dependent differences in efficacy and apparent potency. As indicated by the screening, compounds **7l** and **7m** showed preferential potentiation at GluK3 and GluA2, and minimal activity at GluK1 and GluK2. Notably, compound **7q** exhibited pronounced potentiation at GluK1 and GluK3 receptors at the maximum tested concentration of 300 μM , as well as significant potentiation at GluK2, but no potentiation of the GluA2 AMPA receptor, indicating a degree of KAR selectivity. Compound **7u** displayed concentration-dependent potentiation primarily at GluK3 receptors, with comparatively weaker effects at GluA2 and no detectable activity at GluK1 or GluK2. For all compounds, full saturation of the concentration–response curves could not be achieved, as concentrations above 300 μM were not attainable due to limited solubility in the assay buffer. Consequently, maximal responses should be interpreted as apparent estimates, and EC_{50} values could not be reliably determined from these data.

Docking Exploration

To gain preliminary insight into the binding mode of compound **7q** within the allosteric pocket, molecular docking was performed using the GluK1 crystal structure 5MFQ.⁶ This template was used after the removal of one of the two cocrystallized BPAM344 molecules present in the pocket. The top-ranked SwissDock pose was then selected for interaction analysis using PLIP to identify the types of contacts that could account for the marked interest in this compound within the acidic KAR PAM series.

Prior to selecting the pose retained for Protein–Ligand Interaction Profiler (PLIP) analysis, we first verified that

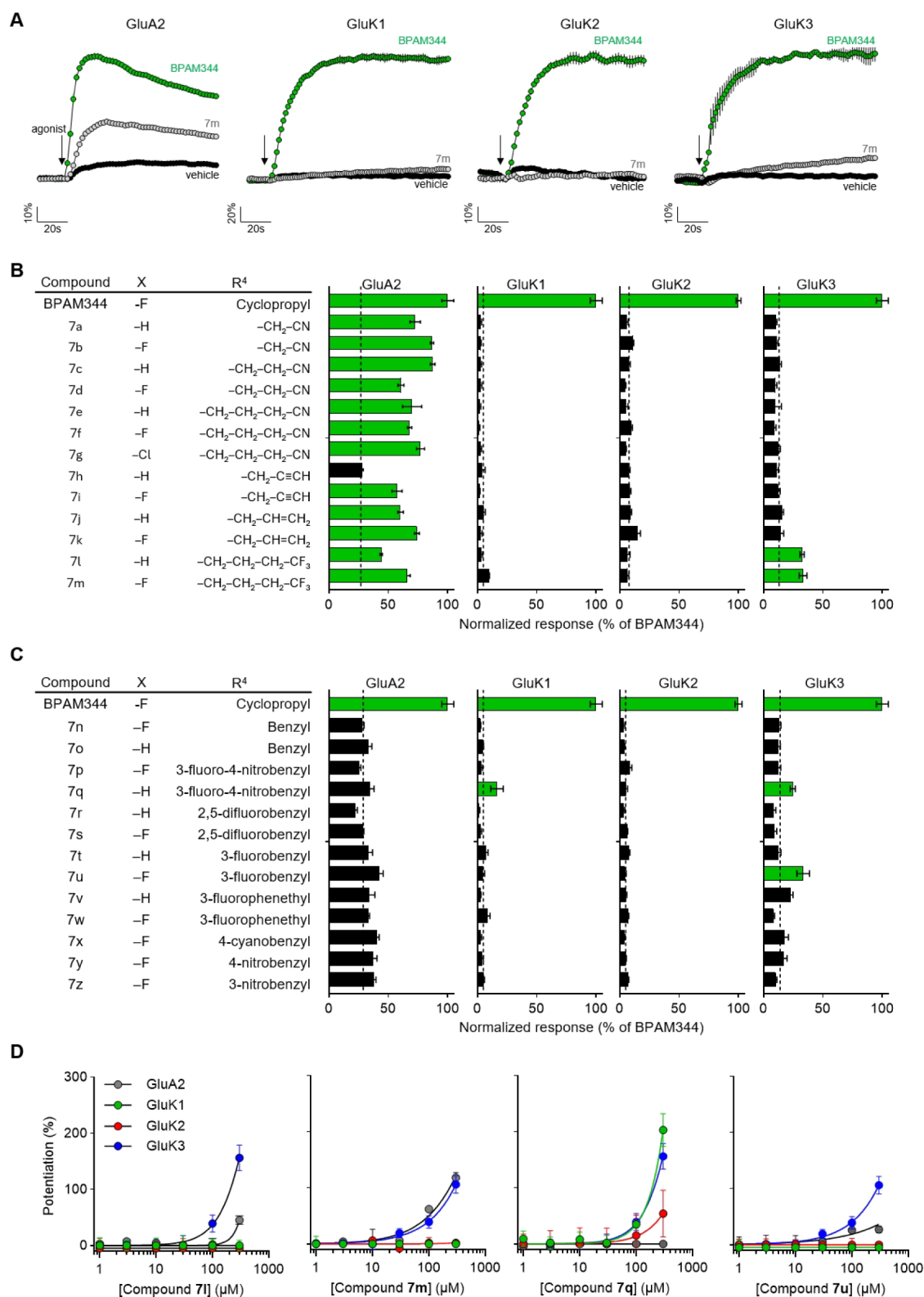


Figure 4. Screening and concentration–response characterization of modulators at recombinant AMPA and kainate receptors using a Ca^{2+} -sensitive fluorescence assay. (A) Representative fluorescence traces from HEK293 cells expressing homomeric GluA2, GluK1, GluK2, or GluK3 receptors following application of agonist (arrow) in the absence (vehicle, black) or presence of the reference modulator BPAM344 (green) or compound 7m. Data points represent mean fluorescence from three replicate wells. Error bars indicate SEM (shown when larger than symbol size). Vertical and horizontal scale bars denote percent fluorescence change relative to baseline and time, respectively. (B–C) Graphical summaries of the screening of series 1 (panel B; linear aliphatic R⁴ substituents) and series 2 (panel C; aryl-based R⁴ substituents) compounds for potentiation of agonist-evoked responses at GluA2 and GluK1–3 receptors. The responses to agonist application in the presence of test compounds are expressed as peak fluorescence normalized to the response in the presence of BPAM344 (100%). Bars represent mean $\pm$ SEM from at least $n = 6$ independent determinations. The black dashed line indicates the response to the agonist alone. Green bars indicate compounds that produced a statistically

Figure 4. continued

significant increase in response compared with agonist alone (one-way ANOVA with appropriate post hoc test, $p < 0.05$), whereas responses that are not significantly different from agonist-alone are shown in black. Compounds were tested at 100 μM at GluA2, GluK1, and GluK2 receptors and at 300 μM at GluK3 receptors. (D) Concentration–response relationships for selected compounds (**7l**, **7m**, **7q**, and **7u**) at GluA2 and GluK1–3 receptors. Data points represent mean values from independent experiments, and error bars indicate SEM (shown when larger than symbol size). Full saturation of the curves could not be achieved due to limited compound solubility, restricting the maximal test concentration to 300 μM .

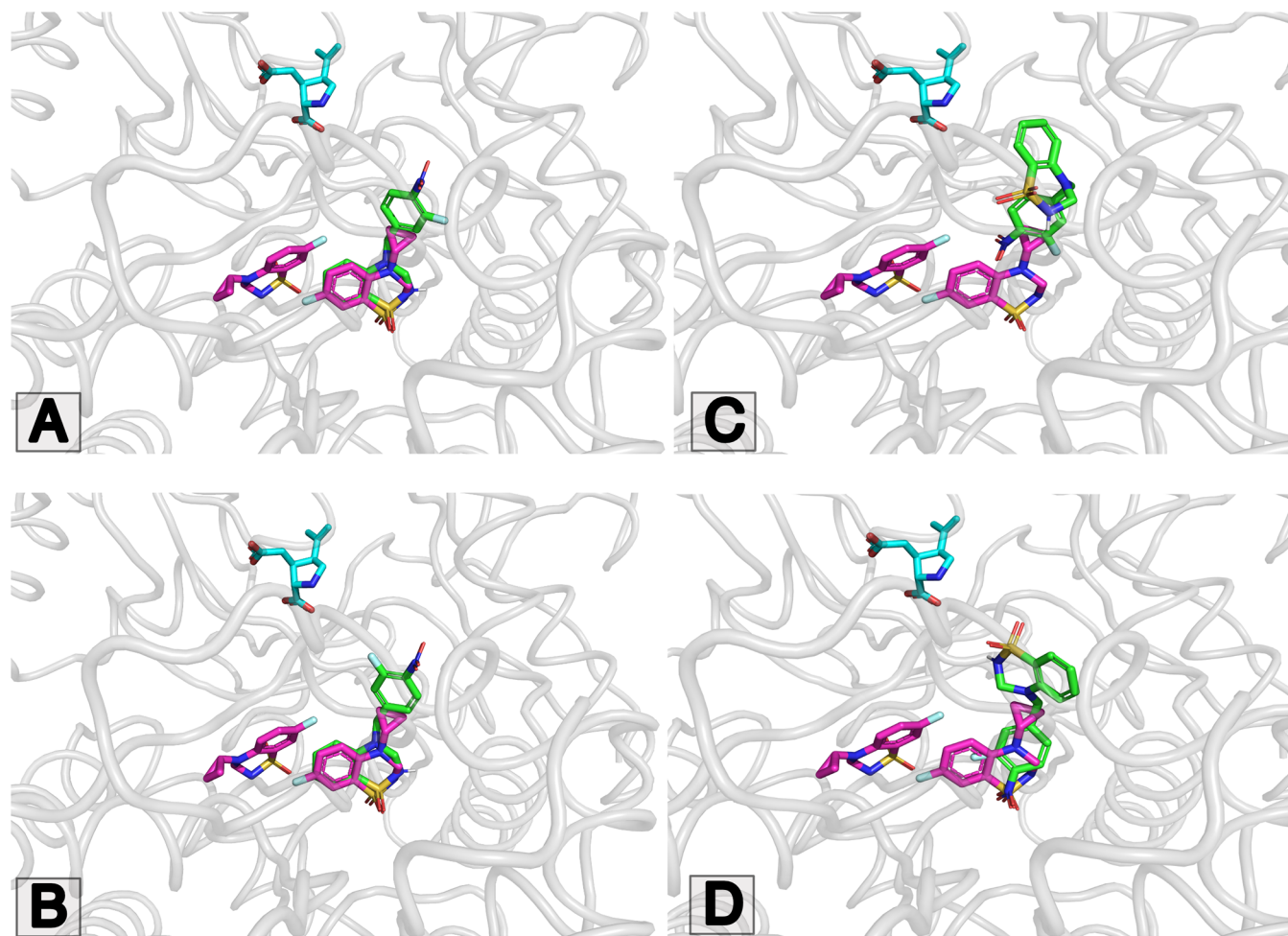


Figure 5. Superposition of docked **7q** pose 1 (A), pose 2 (B), pose 3 (C), and pose 4 (D) with BPAM344 (**1**) in the GluK1 allosteric pocket. Compound **7q** is shown as green sticks, BPAM344 as rose-colored sticks, and a molecule of kainic acid in the orthosteric site in turquoise. Heteroatoms are displayed using conventional color coding (for example, red for oxygen and cyan for other distinct colors for additional substituents), facilitating visualization of polar groups and potential interaction sites in the cavity.

docked conformations of **7q** could reproduce the binding mode of the cocrystallized PAM BPAM344 in 5MFQ. In particular, several of the highest-ranked poses showed a close overlap of the benzothiadiazine core of **7q** with the corresponding bicyclic scaffold of BPAM344, while maintaining a comparable orientation of the 4-substituent toward the central region of the allosteric pocket (illustrated for poses 1–4 in Figure 5A–D).

Among these four poses, only poses 1 and 2 were retained on the basis of their binding modes. The two selected poses were visually inspected to verify that no steric clashes were present (Figure 6).

Among these alternative solutions, pose 1 combined this favorable superposition with a slightly lower predicted binding free energy than that of pose 2, as reflected by the SwissDock affinity scores (Table 1). On this basis, pose 1 was selected as

the most plausible binding mode and was used for subsequent interaction analysis and design considerations.

Based on this selection, pose 1 was then submitted to PLIP for detailed interaction analysis. The predicted binding mode suggests that **7q** is accommodated within the allosteric cavity through a combination of hydrophobic and polar interactions. According to the PLIP analysis, the ligand displays hydrophobic contacts with Pro 532A, Thr 535B, Gln 786B, and Leu 791B, with distances ranging from 3.17 to 3.77 Å, consistent with a favorable insertion of the scaffold into the pocket (Table 2 and Table 3).

Hydrogen Bonds

In addition to these hydrophobic contacts, one direct hydrogen bond is predicted between the ligand and His792B, with the protein acting as a donor toward the ligand atom O3. Notably, this interaction pattern points to a polar microenvironment in

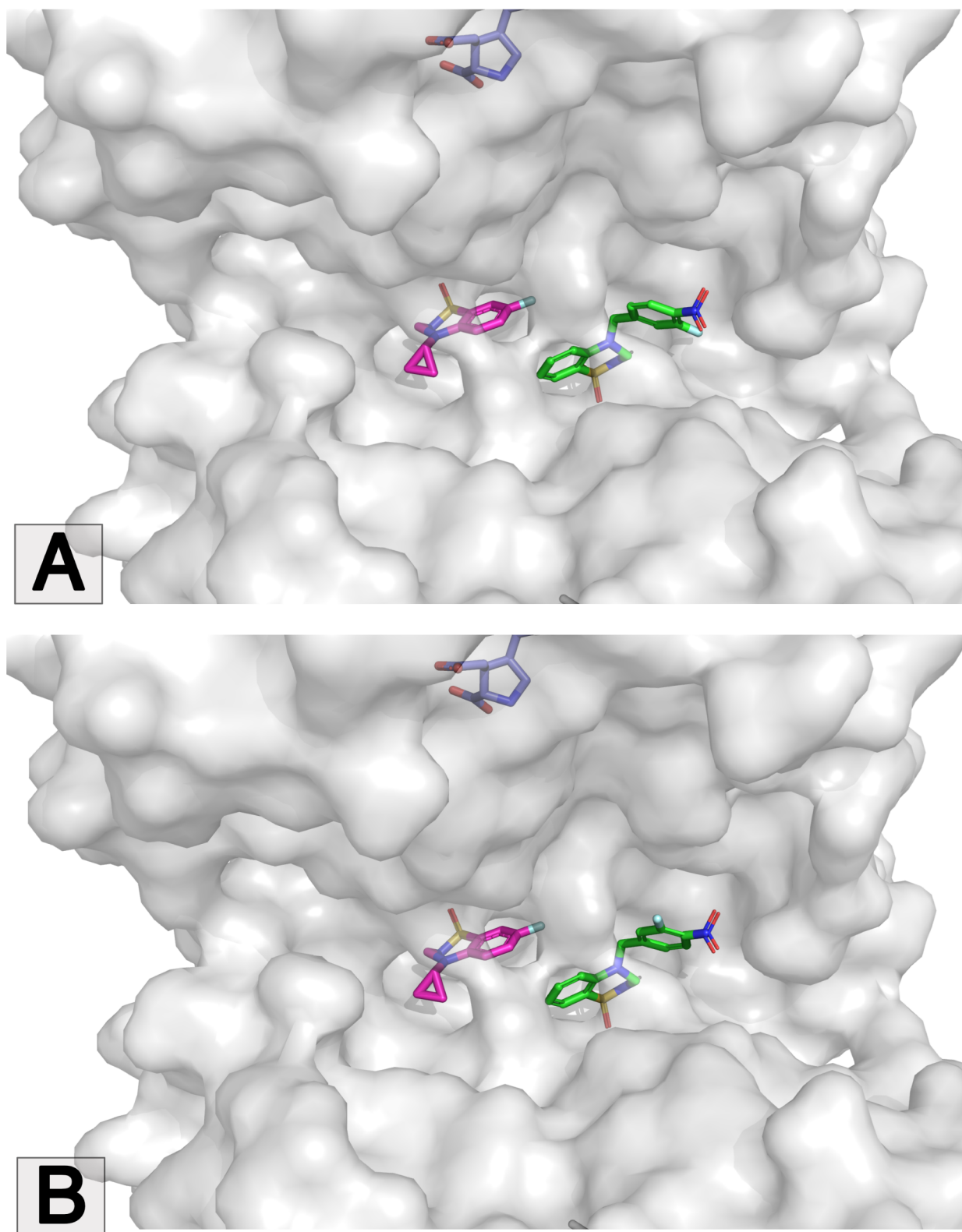


Figure 6. Structural validation of the selected docking poses. Pose 1 (A) fits within the binding site without steric clashes with surrounding residues, and pose 2 (B) showed the same behavior. Compound 7q is shown as green sticks and BPAM344 (1) as rose-colored sticks.

the vicinity of the 4-substituted region of the benzothiadiazine core, in particular near a histidine side chain, while the rest of the scaffold remains anchored in a largely hydrophobic environment. This observation was a key element of our design rationale and motivated the development of a second

series of 4-substituted acidic derivatives designed to probe this polar subpocket more effectively. In particular, these analogues were designed to favor the formation of a strong salt bridge with His792B, with the aim of enhancing the selectivity or potency of the newly synthesized KAR PAMs (Table 4).

Table 1. Calculated Affinity of the Four Poses Predicted by SwissDock

Model	Calculated affinity (kcal/mol)
1	−9.587
2	−9.570
3	−6.842
4	−6.725

Table 2. Hydrophobic Interactions between the Ligand and Surrounding Residues in the Binding Site, Including Residue Number, Amino Acid Type, Interatomic Interaction Distance, and Interacting Ligand and Protein Atoms

Index	Residue	AA	Distance (Å)	Ligand Atom	Protein Atom
1	532A	PRO	3.47	4791	831
2	535B	THR	3.17	4788	3237
3	786B	GLN	3.49	4780	4286
4	791B	LEU	3.77	4780	4327

Water Bridges

The PLIP output also reveals several water-mediated interactions involving mainly Ser761A and Gln786B, further supporting the presence of a structured polar network around the acidic region of the ligand. Taken together, these data suggest that the favorable behavior of **7q** may not arise from a single dominant contact but rather from a cooperative interaction network. Within this network, hydrophobic anchoring of the benzothiadiazine scaffold is complemented by localized polar recognition and solvent-mediated stabilization of the N4-acidic substituent (Figure 7).

DISCUSSION

The newly synthesized KPAM library was evaluated for its ability to potentiate AMPA and kainate receptor subtypes, using BPAM344 as the reference ligand. BPAM344 is known for its modulatory activity on KARs (GluK1b $EC_{50} = 26 \mu\text{M}$; GluK2a $EC_{50} = 75 \mu\text{M}$; GluK3a $EC_{50} = 639 \mu\text{M}$)³⁴ but differs markedly in its activity at AMPARs ($EC_{50} = 0.9 \mu\text{M}$).³²

At first, the subset comprising **7a–g**, characterized by a short aliphatic linker terminated with a nitrile group, yielded an unexpected pharmacological outcome. Although originally designed as polar chemical probes to explore new interactions within the GluK1–3 binding site, these compounds, tested at $100 \mu\text{M}$ at GluK1 and GluK2 and at $300 \mu\text{M}$ at GluK3, showed minimal activity on KARs while potentiating the GluA2 AMPA receptor, albeit less than that of BPAM344. These findings indicate that the nitrile-terminated chain favors AMPA receptor engagement and may represent a structural feature biasing selectivity toward AMPARs rather than KARs.

Attempts to mimic the electronic contribution of the cyclopropyl (**7h–k**, **7n**, and **7o**) substituent in BPAM344 resulted in inactive compounds across all tested kainate receptor subtypes. Taken together, these data do not allow us to attribute the effect of the cyclopropyl motif solely to electronic factors. Instead, the cyclopropyl group likely contributes to ligand–receptor fit through a combination of steric and conformational constraints and shape and electrostatic complementarity that are not fully reproduced by the corresponding π -extended analogues. In line with this view, the alkyne derivatives **7h** and **7i**, the allyl analogues **7j** and **7k**, and the benzyl derivatives **7o** and **7n** all show little or no activity at KARs, whereas the CF_3 -substituted compounds **7l** and **7m** display activity at GluK3, and **7m** shows weak activity on GluK1 but they also gain GluA2 activity, indicating a different balance between potency and subtype selectivity. The aryl-substituted series (Figure 4C) reveals an interesting SAR governing KAR selectivity within the benzothiadiazine scaffold. The most pronounced activity was observed for derivatives bearing a 3-fluoro-4-nitrobenzyl substituent, exemplified by **7q**, which display clear potentiation at GluK1 and GluK3 while remaining essentially inactive at GluA2. Importantly, however, the presence of a polar, electron-withdrawing substituent in the para position is not sufficient on its own to confer activity. Indeed, compounds **7y** and **7z**, which also bear a nitro substituent, are inactive across all receptor subtypes. This observation demonstrates that KAR engagement requires a precise combination of electronic, positional, and scaffold-dependent parameters rather than a single polar functionality.

Additional complexity emerges from the behavior of **7u**, which bears a meta-fluoro substituent on the aromatic ring and selectively potentiates GluK3. Notably, its direct analogue **7t**, which lacks the C7-fluorine substituent, is devoid of activity. This discontinuous SAR suggests a delicate interplay between (i) the electronic modulation imparted by meta-fluorination and (ii) the substitution at the C7 position of the benzothiadiazine core, highlighting cooperative effects that drive a finely tuned three-dimensional recognition process rather than additive substituent contributions.

Collectively, these findings strongly support the existence of a spatially restricted polar microenvironment within the GluK1 and GluK3 allosteric sites. Productive engagement appears to require (i) a benzyl-type vector capable of projecting a polar group to a defined depth, (ii) precise angular orientation of the substituent (para vs meta effects), and (iii) an appropriate electronic environment modulated by both ring substitution and core decoration. The inactivity of several closely related analogues underscores the narrow tolerance of the pocket.

Importantly, the *p*-polar benzyl derivatives remain inactive at GluA2, further supporting the view that structural differences between KAR and AMPAR allosteric sites are sufficient to enable selective modulation. This divergence provides a

Table 3. Hydrogen Bonds Formed between the Ligand and the Protein, Specifying Residue, Amino Acid, H–A and D–A Distances, Donor Angle, Protein Donor/Side-Chain Involvement, and Donor/Acceptor Atoms

Index	Residue	AA	Distance H–A (Å)	Distance D–A (Å)	Donor Angle (°)	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	792B	HIS	3.15	3.99	144.32	✓	✓	4336 [Nar]	4792]

Table 4. Water-Mediated Bridges between the Ligand and Protein Residues, Indicating Residue and Amino Acid, Geometrical Parameters (A–W and D–W Distances, Donor Angle, Water Angle), Protein Donor Character, and the Corresponding Donor, Acceptor, and Water Atoms

Index	Residue	AA	Dist. A–W (Å)	Dist. D–W (Å)	Donor Angle (°)	Water Angle (°)	Protein donor?	Donor Atom	Acceptor Atom	Water Atom
1	761A	SER	3.13	3.86	104.39	121.40	✓	1701 [Nam]	4775 [Ntr]	4555
2	761A	SER	3.55	2.82	154.37	96.87	✓	1706 [O3]	4775 [Ntr]	2228
3	761A	SER	3.88	2.82	154.37	91.56	✓	1706 [O3]	4793 [O3]	2228
4	786B	GLN	3.80	2.85	143.87	109.01	✓	4288 [Nam]	4777 [N3]	4623
5	786B	GLN	2.70	2.56	108.49	127.29	✗	4793 [O3]	4289 [O2]	4555

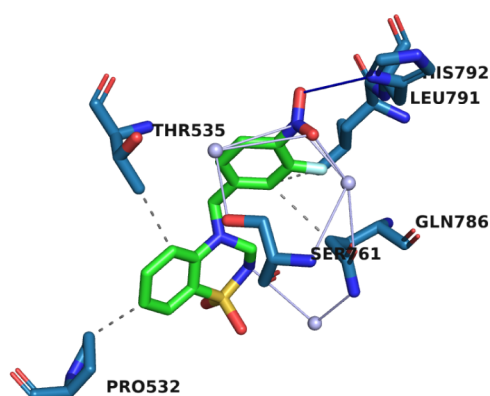


Figure 7. Predicted interaction pattern of compound **7q** in the GluK1 allosteric pocket obtained from the top-ranked SwissDock pose and analyzed with PLIP. Hydrophobic contacts are observed with Pro532A, Thr535B, Gln786B, and Leu791B, together with one direct hydrogen bond to His792B and several water-mediated interactions involving Ser761A and Gln786B.

mechanistically grounded opportunity to drive subtype selectivity within an AMPAR-biased chemotype.

Altogether, the aryl series defines a nontrivial, cooperatively controlled selectivity motif rather than a simple substituent effect. An N4-benzyl appendage bearing a properly oriented polar functionality emerges as a necessary but not sufficient condition for KAR potentiation, with subtype selectivity further modulated by meta-fluorination and adapted C7 substitution. These insights establish a structurally coherent framework for second-generation optimization aimed at enhancing potency while preserving KAR selectivity.

Finally, it should be emphasized that the docking analysis provides only a qualitative hypothesis of the binding mode of **7q** at the GluK1 allosteric site. Because the calculations were performed on a single, crystallographically derived receptor conformation, they do not account for the conformational changes that are expected to accompany positive allosteric modulator binding at the dimer interface. As a consequence,

the docked poses and the corresponding interaction patterns should be regarded as plausible models that are consistent with the SAR rather than as experimentally validated structures. A more realistic description of the allosteric recognition process would require molecular dynamics simulations.

EXPERIMENTAL SECTION

General Procedures

Melting points were determined on a Buchi M-565 capillary apparatus and were uncorrected. Elemental analyses (C, H, N, S) were performed on a Thermo Scientific Flash EA 1112 elemental analyzer and were within $\pm 0.4\%$ of the theoretical values for carbon, hydrogen, and nitrogen; a higher tolerance ($\pm 0.6\%$) was accepted for sulfur, considering its corresponding peak shape. This analytical method confirmed a purity of $\geq 95\%$ for all final compounds submitted for biological evaluation. The ^1H and ^{13}C NMR spectra were recorded on Bruker Avance instruments operating at ^1H frequencies of 400, 500, and 700 MHz and ^{13}C frequencies of 125 and 126 MHz using deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$) as the solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts were reported in δ values (ppm) relative to the internal TMS standard. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and bs = broad singlet were used throughout. All reactions were routinely checked by TLC on silica gel 60 F_{254} (Merck).

Biological Evaluation

To assess positive allosteric modulation of AMPA and kainate receptors, compound activity was evaluated using a Ca^{2+} -sensitive fluorescence-based assay adapted from previous studies.³⁴ GripTite HEK293 cells transiently expressing homomeric recombinant rat GluA2, GluK1, GluK2, or GluK3 receptors (as described by Bay et al.³⁴) were seeded in poly-D-lysine-coated 96-well plates and loaded with the Ca^{2+} -sensitive dye Fluo-8 AM (AAT Bioquest). Following dye loading and washing, fluorescence signals were recorded in a FlexStation I plate reader (Molecular Devices) at excitation/emission wavelengths of 485/538 nm. Agonist-evoked Ca^{2+} responses were measured in the absence and presence of test modulators added in an assay buffer containing 100 mM choline chloride, 10 mM NaCl, 5 mM KCl, 1 mM MgCl_2 , 20 mM CaCl_2 , and 10 mM HEPES (pH 7.4). Compounds were dissolved in DMSO and diluted into buffer immediately before use (final DMSO concentration $\leq 1\%$ v/v). Peak

fluorescence changes following application of agonist (typically 100 μ M L-glutamate or kainate) were quantified as relative fluorescence units (RFU) and normalized to the reference modulator BPAM344. Data were obtained from multiple independent experiments with three or four technical replicates per plate and analyzed using SoftMax Pro and GraphPad Prism (GraphPad Inc.) to calculate mean $\pm$ SEM values and statistical significance.

Molecular Docking

Molecular docking studies were carried out using SwissDock with the AutoDock Vina engine, employing the GluK1 crystal structure (PDB ID: 5MFQ) as the receptor template. Prior to docking, one of the two BPAM344 molecules cocrystallized in the allosteric pocket was removed, and the resulting protein structure (5MFQ_1lig.pdb) was used for the calculations. The docking search space was defined around the allosteric site, with a box centered at (−4, 40, 3) and dimensions of 13 $\times$ 14 $\times$ 13, and the sampling exhaustivity was set to 28. Compound 7q was docked into this binding pocket, and the generated poses were ranked according to their predicted binding free energies.

Among the solutions obtained, four representative poses displayed calculated affinities of −9.587, −9.570, −6.842, and −6.725 kcal/mol, respectively. The best-scoring pose (−9.587 kcal/mol), which also showed no steric clashes and a favorable overlap of the benzothiadiazine scaffold with the cocrystallized BPAM344 orientation, was selected for subsequent analysis. Protein–ligand contacts for the selected pose were characterized using the online version of PLIP (Protein–Ligand Interaction Profiler), which identified hydrophobic contacts with Pro532A, Thr535B, Gln786B, and Leu791B, together with one direct hydrogen bond to His792B and several water-mediated interactions involving Ser761A and Gln786B. Three-dimensional visualization and figure generation were performed using the commercial executable build of PyMOL on an Apple system (Apple M-series GPU, OpenGL 2.1/Metal backend).

Methods

7-Fluoro-2H-benzo[e][1,2,4]thiadiazin-3(4H)-one 1,1-dioxide (3b). To a 100-mL flask containing 20 mL of dichloromethane, 2.1 mL of chlorosulfonyl isocyanate (24.36 mmol; 1.2 equiv) was added. The mixture was then sealed and immersed in an ice bath until a temperature of −5 °C was reached. In addition, 3.79 mL of 4-fluoroaniline (20.30 mmol; 1 equiv) was dissolved in 8 mL of dichloromethane. This solution was added dropwise to the chlorosulfonyl isocyanate solution over 5 min with stirring and was left stirring for 15 min after the end of the addition. 3.55 g of AlCl₃ (26.39 mmol; 1.3 equiv) was then added to the reaction medium, and the mixture was brought to reflux for 1 h. The hot solution was poured onto 200 g of ice. Once the ice had melted, the precipitate was filtered under vacuum and washed with 2 $\times$ 25 mL of water. The solid was then dissolved in 100 mL of a 10% w/v NaHCO₃ solution. After addition of 1 g of charcoal, the medium was boiled for 60 min. The charcoal was removed by filtration under vacuum. The filtrate was placed in an ice bath and acidified by adding 6 N HCl (pH = 1). The white precipitate obtained was filtered through a sintered funnel (1.7 g, 39%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 11.33 (s, 1H), 7.68 (dd, *J* = 7.6, 2.9 Hz, 1H), 7.55 (td, *J* = 8.7, 2.9 Hz, 1H), 7.29 (dd, *J* = 9.0, 4.3 Hz, 1H).

2H-Benzo[e][1,2,4]thiadiazin-3(4H)-one 1,1-dioxide (3a). The title compound was obtained as described for 3b, starting from aniline (2a) (3 g, 32.2 mmol) (1.24 g, 20%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 11.18 (s, 1H), 7.77 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.64 (ddd, *J* = 8.5, 7.4, 1.5 Hz, 1H), 7.28 (td, *J* = 7.6, 1.0 Hz, 1H), 7.24 (dd, *J* = 8.3, 1.0 Hz, 1H).

7-Chloro-2H-benzo[e][1,2,4]thiadiazin-3(4H)-one 1,1-dioxide (3c). The title compound was obtained as described for 3b, starting from 4-chloroaniline (2c) (5.10 g, 39.9 mmol) (4.2 g, 45%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 11.40 (s, 3H), 7.83 (d, *J* = 2.4 Hz, 3H), 7.70 (dd, *J* = 8.8, 2.4 Hz, 3H), 7.46 – 7.36 (m, 1H), 7.25 (dd, *J* = 8.8, 6.0 Hz, 4H).

2-Amino-5-fluorobenzenesulfonamide (4b). To 40 mL of a 50% v/v sulfuric acid solution (18 N), 3b (2.0 g, 9.25 mmol, 1 equiv) was added. The reaction mixture was heated to reflux for 3 h and then cooled in an ice bath. Neutralization was performed by the dropwise addition of a 50% w/v sodium hydroxide solution until the pH reached 7. The solvent was evaporated under reduced pressure, and the resulting solid was suspended in 100 mL of ethanol. The suspension was filtered under vacuum, and the filtrate was evaporated under reduced pressure, yielding a white solid (1.4 g, 78%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 7.36 (s, 2H), 7.29 (dd, *J* = 9.1, 3.1 Hz, 1H), 7.16 (td, *J* = 9.1, 8.2, 3.1 Hz, 1H), 6.81 (dd, *J* = 9.0, 4.7 Hz, 1H), 5.73 (s, 2H).

2-Amino-5-chlorobenzenesulfonamide (4c). The title compound was obtained as described for 4b, starting from 7-chloro-2H-benzo[e][1,2,4]thiadiazin-3(4H)-one 1,1-dioxide (3c) (2.0 g, 9.7 mmol) (1.4 g, 71%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 7.49 (d, *J* = 2.6 Hz, 1H), 7.20 (dd, *J* = 8.7, 2.6 Hz, 1H), 6.78 (t, *J* = 7.4 Hz, 3H), 5.98 (s, 2H).

4H-Benzo[e][1,2,4]thiadiazine 1,1-dioxide (5a). To a suitable reaction flask, 4a (5 g, 27.4 mmol) and triethyl orthoformate (TEOF, 10 mL) were added successively. The reaction mixture was heated at 130 °C for 3 h in an open vessel. The resulting precipitate was collected by filtration, washed with diethyl ether (2 $\times$ 10 mL), and dried in an oven (4.6 g, 92%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 12.30 (s, 1H), 7.98 (s, 1H), 7.81 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.68 (ddd, *J* = 8.6, 7.3, 1.5 Hz, 1H), 7.46 (td, *J* = 7.7, 1.1 Hz, 1H), 7.32 (dd, *J* = 8.3, 1.0 Hz, 1H).

7-Fluoro-4H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (5b). The title compound was obtained as described for 5a, starting from 2-amino-5-fluorobenzenesulfonamide (4b) (1.0 g, 5.3 mmol, 1 equiv) with triethyl orthoformate (TEOF, 10 mL) added successively. The reaction mixture was heated at 130 °C for 3 h in an open vessel. The resulting precipitate was collected by filtration, washed with diethyl ether (2 $\times$ 10 mL), and dried in an oven (0.74 g, 70%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 12.42 (s, 1H), 8.01 (s, 1H), 7.72 (dd, *J* = 7.6, 2.9 Hz, 1H), 7.61 (td, *J* = 8.8, 2.9 Hz, 1H), 7.42 (dd, *J* = 9.1, 4.5 Hz, 1H).

7-Chloro-4H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (5c). The title compound was obtained as described for 5a, starting from 2-amino-5-chlorobenzenesulfonamide (4c) (2 g, 9.7 mmol) (1.2 g, 60%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 12.46 (s, 1H), 8.02 (s, 1H), 7.88 (d, *J* = 2.3 Hz, 1H), 7.75 (dd, *J* = 8.9, 2.4 Hz, 1H), 7.37 (d, *J* = 8.9 Hz, 1H).

4-(Cyanomethyl)-4H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (6a). In a 20 mL sealed vial, 5a (0.40 g, 2.00 mmol, 1 equiv), potassium carbonate (K₂CO₃) (1.10 g, 8.00 mmol, 4 equiv), and bromoacetonitrile (0.580 g, 4.40 mmol, 2.2 equiv, 0.336 mL) were added sequentially. The reaction mixture was dissolved in acetonitrile (13 mL) and heated at reflux until completion, as monitored by TLC.

After completion, the reaction mixture was filtered to remove the insoluble material. The filtrate was concentrated to dryness under reduced pressure. The crude solid obtained was dissolved in a minimal volume of methanol and cooled in an ice bath. Diethyl ether (30 mL) was slowly added to induce precipitation. The resulting solid was collected by vacuum filtration, washed with cold diethyl ether (2 $\times$ 10 mL), and dried to afford the desired product 6a (0.420 g, 1.73 mmol, 90%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.39 (s, 1H), 7.63 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.55 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 4.76 (s, 2H), 4.68 (s, 2H); ¹³C NMR (DMSO-*d*₆, 125 MHz) δ 143.94, 135.92, 127.05, 127.01, 121.26, 118.77, 116.74, 63.71, 39.15.

7-Fluoro-4-(cyanomethyl)-4H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (6b). The title compound was obtained as described for 6a, starting from 5b (0.40 g, 2.00 mmol, 1 equiv), potassium carbonate (K₂CO₃) (1.21 g, 8.80 mmol, 4.0 equiv), and bromoacetonitrile (0.580 g, 4.84 mmol, 2.2 eq., 0.336 mL) at a temperature of 50 °C and a reaction time of 24 h to obtain 6b (0.48 g, 1.76 mmol, 80%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.15 (s, 1H),

7.92 (dd, $J = 7.3$, 2.9 Hz, 1H), 7.88–7.80 (m, 1H), 7.73 (dd, $J = 9.3$, 4.1 Hz, 1H), 5.39 (s, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 160.59, 158.61, 150.78, 130.63, 124.05, 121.66, 119.13, 115.10, 111.04.

4-(2-Cyanoethyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6c). In a 20 mL sealed vial, **5a** (0.400 g, 2.20 mmol, 1.0 equiv), cesium carbonate (Cs_2CO_3) (2.86 g, 8.80 mmol, 4 equiv), and 3-bromopropionitrile (0.72 mL, 4.84 mmol, 2.2 equiv) were added sequentially. The reaction mixture was dissolved in acetonitrile (13 mL) and heated at 60 °C for 16 h. At the end of the reaction, the insoluble material was removed by filtration and washed with 2×10 mL MeOH. The filtrate was concentrated to dryness under reduced pressure. The resulting crude solid was dispersed in 50 mL of water. The insoluble material formed was collected by vacuum filtration to afford the desired product **6c** (0.320 g, 1.17 mmol, 53%); ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.18 (s, 1H), 7.92 (dd, $J = 7.9$, 1.5 Hz, 1H), 7.78 (ddd, $J = 8.8$, 7.2, 1.6 Hz, 1H), 7.67 (d, $J = 8.6$ Hz, 1H), 7.57 (t, $J = 7.6$ Hz, 1H), 4.45 (t, $J = 6.8$ Hz, 2H), 3.08 (t, $J = 6.8$ Hz, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 151.01, 134.13, 133.40, 127.06, 124.50, 122.86, 117.87, 116.35, 45.11, 40.01, 39.85, 39.68, 39.51, 39.34, 39.17, 38.99, 16.80.

4-(2-Cyanoethyl)-7-fluoro-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6d). The title compound was obtained as described for **6c**, starting from **5b** (0.40 g, 2.00 mmol, 1 equiv), cesium carbonate (Cs_2CO_3 , 2.61 g, 8.00 mmol, 4.0 equiv), and 3-bromopropionitrile (0.72 mL, 4.40 mmol, 2.2 equiv), with a reaction time of 17 h to afford the desired product **6d** (0.31 g, 1.11 mmol, 56%); ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.18 (s, 1H), 7.83 (dd, $J = 7.4$, 2.9 Hz, 1H), 7.79 (dd, $J = 9.4$, 4.2 Hz, 1H), 7.70 (ddd, $J = 9.3$, 8.1, 2.9 Hz, 1H), 4.45 (t, $J = 6.8$ Hz, 2H), 3.07 (t, $J = 6.8$ Hz, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 160.32, 158.34, 150.86, 130.95, 123.87, 121.13, 119.64, 117.84, 110.58, 45.33, 16.82.

4-(3-Cyanopropyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6e). The title compound was obtained as described for **6a**, starting from **5a** (0.40 g, 2.20 mmol, 1 equiv), potassium carbonate (K_2CO_3) (1.21 g, 8.80 mmol, 4.0 equiv), and 4-bromobutyronitrile (0.71 g, 4.84 mmol, 2.2 equiv) at a temperature of 65 °C and a reaction time of 24 h to afford the desired product **6e** (0.35 g, 1.30 mmol, 59%); ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.11 (s, 1H), 7.90 (dd, $J = 7.9$, 1.6 Hz, 1H), 7.79 (ddd, $J = 8.8$, 7.3, 1.6 Hz, 1H), 7.63–7.51 (m, 2H), 4.19–4.11 (m, 2H), 2.64 (t, $J = 7.3$ Hz, 2H), 2.04 (p, $J = 7.5$ Hz, 2H).

4-(3-Cyanopropyl)-7-fluoro-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6f). The title compound was obtained as described for **6a**, starting from **5b** (0.40 g, 2.00 mmol, 1 equiv), potassium carbonate (K_2CO_3 , 1.10 g, 8.00 mmol, 4.0 equiv), and 4-bromobutyronitrile (0.710 g, 4.84 mmol, 2.2 equiv) at a temperature of 65 °C and a reaction time of 48 h to afford the desired product **6f** (0.21 g, 0.80 mmol, 40%); ^1H NMR (400 MHz, DMSO) δ 8.11 (s, 1H), 7.90 (dd, $J = 7.9$, 1.6 Hz, 1H), 7.79 (ddd, $J = 8.8$, 7.3, 1.6 Hz, 1H), 7.63–7.51 (m, 2H), 4.19–4.11 (m, 2H), 2.64 (t, $J = 7.3$ Hz, 2H), 2.04 (p, $J = 7.5$ Hz, 2H).

7-Chloro-4-(3-cyanopropyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6g). In a 50 mL round-bottom flask, **5c** (1.00 g, 4.62 mmol, 1 equiv) was suspended in acetonitrile (28 mL), and potassium carbonate (3.83 g, 27.7 mmol, 6.0 equiv) was added. 4-Bromobutyronitrile (1.4 mL, 14.0 mmol, 3.0 equiv) was then added dropwise. The reaction mixture was heated at 80 °C overnight.

At the end of the reaction, the mixture was filtered, and the solid residue was rinsed with acetonitrile. The combined filtrates were concentrated under reduced pressure to yield a yellow oil that partially crystallized under vacuum. The crude solid was washed successively with hexane and diethyl ether, followed by a minimal amount of ethyl acetate to remove a yellow impurity. After drying under vacuum, a white solid **6g** was obtained (433 mg, 1.53 mmol, 33%); ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.13 (s, 1H), 7.95 (d, $J = 2.5$ Hz, 1H), 7.85 (dd, $J = 9.1$, 2.5 Hz, 1H), 7.67 (d, $J = 9.2$ Hz, 1H), 4.14 (t, $J = 7.6$ Hz, 2H), 2.63 (t, $J = 7.3$ Hz, 2H), 2.02 (quintet, $J = 7.5$ Hz, 2H).

4-(Prop-2-yn-1-yl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6h). The title compound was obtained as described for **6a**, starting

from **5a** (0.40 g, 2.20 mmol, 1 equiv), potassium carbonate (1.21 g, 8.80 mmol, 4 equiv), and propargyl bromide (0.660 g, 5.06 mmol, 2.2 equiv) at a temperature of 60 °C and a reaction time of 1.5 h to afford the desired product **6h** (0.20 g, 0.91 mmol, 41%); ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.19 (s, 1H), 7.93 (dd, $J = 8.3$, 1.5 Hz, 1H), 7.84 (ddd, $J = 9.6$, 7.0, 1.6 Hz, 1H), 7.63–7.55 (m, 2H), 5.02 (d, $J = 2.5$ Hz, 2H), 3.61 (t, $J = 2.4$ Hz, 1H).

7-Fluoro-4-(prop-2-yn-1-yl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6i). The title compound was obtained as described for **6a**, starting from **5b** (0.40 g, 2.20 mmol, 1 equiv), potassium carbonate (1.10 g, 8.00 mmol, 4 equiv), and propargyl bromide (0.523 g, 4.40 mmol, 2.2 equiv) at a temperature of 60 °C and a reaction time of 3 h to afford the desired product **6i** (0.32 g, 1.38 mmol, 69%); ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.19 (s, 1H), 7.85 (dd, $J = 7.4$, 2.9 Hz, 1H), 7.78 (ddd, $J = 9.4$, 8.2, 2.9 Hz, 1H), 7.69 (dd, $J = 9.4$, 4.3 Hz, 1H), 5.04 (d, $J = 2.5$ Hz, 2H), 3.63 (t, $J = 2.4$ Hz, 1H).

4-Allyl-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6j). The title compound was obtained as described for **6a**, starting from **5a** (0.40 g, 2.20 mmol, 1 equiv), potassium carbonate (1.21 g, 8.80 mmol, 4 equiv), and allyl bromide (0.58 g, 4.84 mmol, 2.2 equiv) at a temperature of 60 °C and a reaction time of 3 h to afford the desired product **6j** (0.43 g, 1.93 mmol, 88%); ^1H NMR (DMSO- d_6 , 700 MHz) δ 8.16 (s, 1H), 7.91 (dd, $J = 7.9$, 1.5 Hz, 1H), 7.76 (ddd, $J = 8.8$, 7.3, 1.6 Hz, 1H), 7.57–7.52 (m, 1H), 7.49 (d, $J = 8.6$ Hz, 1H), 6.02 (ddt, $J = 17.3$, 10.3, 5.0 Hz, 1H), 5.28 (dd, $J = 8.3$, 1.5 Hz, 1H), 5.22 (dt, $J = 17.3$, 1.5 Hz, 1H), 4.79 (dt, $J = 5.0$, 1.7 Hz, 2H).

4-Allyl-7-fluoro-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6k). The title compound was obtained as described for **6a**, starting from **5b** (0.40 g, 2.20 mmol, 1 equiv), potassium carbonate (1.10 g, 8.00 mmol, 4 equiv), and allyl bromide (0.53 g, 4.40 mmol, 2.2 equiv) at a temperature of 60 °C and a reaction time of 3 h to afford the desired product **6k** (0.34 g, 1.41 mmol, 71%); ^1H NMR (DMSO- d_6 , 700 MHz) δ 8.16 (s, 1H), 7.81 (dd, $J = 7.4$, 3.0 Hz, 1H), 7.69 (ddd, $J = 9.3$, 8.3, 3.0 Hz, 1H), 7.59 (dd, $J = 9.4$, 4.2 Hz, 1H), 6.01 (ddt, $J = 17.3$, 10.3, 5.0 Hz, 1H), 5.28 (dq, $J = 10.4$, 1.4 Hz, 1H), 5.21 (dq, $J = 17.2$, 1.6 Hz, 1H), 4.80 (dt, $J = 5.1$, 1.7 Hz, 2H).

4-(4,4,4-Trifluorobutyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6l). The title compound was obtained as described for **6a**, starting from **5a** (0.40 g, 2.20 mmol, 1 equiv), potassium carbonate (1.21 g, 8.80 mmol, 4 equiv), and 1-bromo-4,4,4-trifluorobutane (0.92 g, 4.84 mmol, 2.2 equiv) at a temperature of 75 °C and a reaction time of 24 h to afford the desired product **6l** (0.22 g, 0.75 mmol, 34%); ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.13 (s, 1H), 7.91 (dd, $J = 7.9$, 1.5 Hz, 1H), 7.80 (ddd, $J = 8.7$, 7.3, 1.6 Hz, 1H), 7.64 (d, 1H), 7.56 (t, $J = 7.0$ Hz, 1H), 4.17 (t, $J = 15.3$ Hz, 1H), 2.47–2.33 (m, 2H), 1.98–1.88 (m, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 151.11, 134.49, 133.39, 128.28, 126.88, 126.08, 124.43, 122.88, 116.24, 48.51, 29.38, 20.54.

7-Fluoro-4-(4,4,4-trifluorobutyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6m). The title compound was obtained as described for **6a**, starting from **5b** (0.40 g, 2.20 mmol, 1 equiv), potassium carbonate (1.10 g, 8.00 mmol, 4 equiv), and 1-bromo-4,4,4-trifluorobutane (0.84 g, 4.40 mmol, 2.2 equiv) at a temperature of 75 °C and a reaction time of 72 h to afford the desired product **6m** (0.46 g, 1.48 mmol, 74%); ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.13 (s, 1H), 7.82 (dd, $J = 7.4$, 2.8 Hz, 1H), 7.75 (td, $J = 9.1$, 3.6 Hz, 1H), 7.72–7.63 (m, 1H), 4.17 (t, $J = 7.7$ Hz, 2H), 2.46–2.28 (m, 2H), 2.00–1.87 (m, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 160.19, 158.21, 150.95, 131.32, 128.27, 126.08, 123.91, 121.37, 119.47, 110.52, 48.76, 44.01, 29.37, 20.53.

4-Benzyl-7-fluoro-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6n). In a 20 mL sealed vial, **5b** (0.50 g, 2.50 mmol, 1 equiv), potassium carbonate (1.38 g, 10.0 mmol, 4.0 equiv), and benzyl bromide (0.652 mL, 5.50 mmol, 2.2 equiv) were added sequentially. The reaction mixture was dissolved in acetonitrile (13 mL) and heated under reflux for 4 h. At the end of the reaction, the insoluble material was removed by filtration. The filtrate was concentrated to dryness under reduced pressure. The resulting crude solid was redissolved in a minimal volume of methanol and cooled in an ice bath. Diethyl ether (30 mL) was slowly added. The precipitate that

formed was collected by vacuum filtration, washed with cold diethyl ether (2×10 mL), and dried to afford the desired product **6n** as a white solid (0.42 g, 1.45 mmol, 58%); ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.38 (s, 1H), 7.81 (dd, $J = 7.4, 3.0$ Hz, 1H), 7.59 (ddd, $J = 9.4, 8.2, 3.0$ Hz, 1H), 7.49 (dd, $J = 9.4, 4.2$ Hz, 1H), 7.39 (dd, $J = 8.6, 6.4$ Hz, 2H), 7.31 (td, $J = 7.5, 7.0, 1.7$ Hz, 3H), 5.42 (s, 2H).

4-Benzyl-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6o). In a 20 mL sealed vial, **5a** (0.30 g, 1.65 mmol, 1 equiv), potassium carbonate (0.912 g, 6.60 mmol, 4.0 equiv), and benzyl bromide (0.43 mL, 3.63 mmol, 2.2 equiv) were added sequentially. The reaction mixture was dissolved in acetonitrile (13 mL) and heated under reflux for 2 h. At the end of the reaction, the insoluble material was removed by filtration. The filtrate was concentrated to dryness under reduced pressure. The resulting crude solid was dissolved in a minimal volume of methanol and cooled in an ice bath. Diethyl ether (30 mL) was slowly added. The precipitate formed was collected by vacuum filtration, washed with cold diethyl ether (2×10 mL), and dried to afford the desired product **6o** as a white solid (0.10 g, 0.331 mmol, 20%); ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.39 (s, 1H), 7.90 (dd, $J = 7.8, 1.5$ Hz, 1H), 7.67 (ddd, $J = 8.8, 7.3, 1.6$ Hz, 1H), 7.50 (t, $J = 7.6$ Hz, 1H), 7.39 (t, $J = 7.9$ Hz, 3H), 7.32 (d, $J = 6.4$ Hz, 2H), 7.30 (d, $J = 5.1$ Hz, 1H), 5.41 (s, 2H).

7-Fluoro-4-(3-fluoro-4-nitrobenzyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6p). In a 20 mL sealed vial, **5b** (0.40 g, 2.00 mmol, 1 equiv), potassium carbonate (1.21 g, 8.80 mmol, 4.0 equiv), and 3-fluoro-4-nitrobenzyl bromide (1.13 g, 4.84 mmol, 2.42 equiv) were added sequentially. The reaction mixture was dissolved in acetonitrile (13 mL) and heated at 60°C for 6 h. At the end of the reaction, the insoluble material was removed by filtration. The filtrate was concentrated to dryness under reduced pressure. The resulting crude solid was dissolved in a minimal volume of methanol and cooled in an ice bath. Diethyl ether (30 mL) was slowly added. The precipitate that formed was collected by vacuum filtration, washed with cold diethyl ether (2×10 mL), and dried to afford the desired product **6p** as a yellow solid (0.47 g, 1.33 mmol, 67%); ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.35 (s, 1H), 8.18 (t, $J = 8.2$ Hz, 1H), 7.84 (dd, $J = 7.3, 2.9$ Hz, 1H), 7.65–7.56 (m, 2H), 7.41 (dd, $J = 9.4, 4.1$ Hz, 1H), 7.30 (dd, $J = 8.6, 1.8$ Hz, 1H), 5.53 (s, 2H).

4-(3-Fluoro-4-nitrobenzyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6q). In a 20 mL sealed vial, **5a** (0.40 g, 2.20 mmol, 1 equiv), potassium carbonate (1.21 g, 8.80 mmol, 4.0 equiv), and 3-fluoro-4-nitrobenzyl bromide (1.13 g, 4.84 mmol, 2.2 equiv) were added sequentially. The reaction mixture was dissolved in acetonitrile (13 mL) and heated at 60°C for 6 h. At the end of the reaction, the insoluble material was removed by filtration. The filtrate was concentrated to dryness under reduced pressure. The resulting crude solid was dissolved in a minimal volume of methanol and cooled in an ice bath. Diethyl ether (30 mL) was slowly added. The precipitate formed was collected by vacuum filtration, washed with cold diethyl ether (2×10 mL), and dried to afford the desired product **6q** as a yellow solid (0.45 g, 1.38 mmol, 63%); ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.36 (s, 1H), 8.18 (t, $J = 8.2$ Hz, 1H), 7.92 (dd, $J = 7.9, 1.6$ Hz, 1H), 7.72–7.58 (m, 2H), 7.52 (td, $J = 7.7, 0.9$ Hz, 1H), 7.35–7.27 (m, 2H), 5.52 (s, 2H).

4-(2,5-Difluorobenzyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6r). In a 25 mL vial, compound **5a** (0.40 g, 2.20 mmol, 1 equiv), potassium carbonate (1.21 g, 8.74 mmol, 4 equiv), and 2,5-difluorobenzyl bromide (0.61 mL, 4.84 mmol, 2.2 equiv) were added successively. The reaction medium was supplemented with 13 mL of acetonitrile and heated at 60°C for 3 h with stirring. At the end of the reaction, the insoluble material was removed by filtration through filter paper, and the filtrate was evaporated to dryness under reduced pressure. The resulting solid was collected and dissolved in a minimal amount of methanol, then cooled in an ice bath. Thirty milliliters of diethyl ether were added to induce precipitation. The precipitate was collected by vacuum filtration, washed with 2×10 mL of diethyl ether, and dried (0.46 g, 67%); mp $205.4\text{--}207.0^\circ\text{C}$; ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.33 (d, $J = 1.3$ Hz, 1H), 7.91 (dd, $J = 7.9, 1.5$ Hz, 1H), 7.72 (td, $J = 8.8, 7.3, 1.6$ Hz, 1H), 7.56–7.50 (m,

1H), 7.43 (d, $J = 8.6$ Hz, 1H), 7.35 (td, $J = 9.4, 4.5$ Hz, 1H), 7.31–7.18 (m, 2H), 5.45 (s, 2H).

4-(2,5-Difluorobenzyl)-7-fluoro-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6s). The title compound was obtained as described for **6r**, starting from **5b** (0.4 g, 1.23 mmol, 1 equiv), potassium carbonate (1.1 g, 7.96 mmol, 6.5 equiv), and 2,5-difluorobenzyl bromide (0.49 mL, 2.7 mmol, 2.2 equiv) (0.33 g, 83%); mp $178.2\text{--}184.1^\circ\text{C}$; ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.33 (d, $J = 1.5$ Hz, 1H), 7.82 (dd, $J = 7.4, 3.0$ Hz, 1H), 7.64 (ddd, $J = 9.4, 8.2, 3.0$ Hz, 1H), 7.53 (dd, $J = 9.4, 4.2$ Hz, 1H), 7.40–7.31 (m, 1H), 7.25 (F, $J = 8.7, 4.2$ Hz, 2H), 5.46 (s, 2H).

4-(3-Fluorobenzyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6t). In a 10–20 mL vial, compound **5a** (0.4 g, 1 equiv), potassium carbonate (1.21 g, 4 equiv), and 3-fluorobenzyl bromide (0.61 mL, 4.60 mmol, 2.2 equiv) were successively added. The reaction medium was then supplemented with 13 mL of acetonitrile, and the mixture was heated at 60°C for 3 h with stirring.

After completion, the insoluble material was removed by filtration through filter paper, and the filtrate was evaporated to dryness under reduced pressure. The resulting solid was collected and dissolved in a minimal amount of methanol, then cooled in an ice bath. Thirty milliliters of diethyl ether were added to the cold solution to induce precipitation. The resulting solid was collected by vacuum filtration, washed with 2×10 mL of diethyl ether, and dried (0.455 g, 67%); mp $205.4\text{--}207.0^\circ\text{C}$; ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.36 (d, $J = 3.0$ Hz, 1H), 7.91 (dd, $J = 7.9, 1.6$ Hz, 1H), 7.68 (td, $J = 8.8, 7.3, 1.6$ Hz, 1H), 7.51 (t, $J = 7.6$ Hz, 1H), 7.43 (d, $J = 7.2$ Hz, 1H), 7.39 (d, $J = 8.7$ Hz, 1H), 7.23–7.09 (m, 2H), 5.41 (s, 2H).

7-Fluoro-4-(3-fluorobenzyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6u). In a 10 mL sealed vial, **5b** (0.4 g, 1.23 mmol, 1 equiv), potassium carbonate (1.1 g, 7.96 mmol, 6.5 equiv), and 3-fluorobenzyl bromide (0.53 mL, 2.7 mmol, 2.2 equiv) were added sequentially. The reaction mixture was dissolved in acetonitrile (13 mL) and heated at 60°C for 3 h. After completion of the reaction, the insoluble material was removed by filtration. The resulting filtrate was evaporated to dryness under reduced pressure. The crude solid was recovered, dissolved in a minimal volume of methanol, and cooled in an ice bath. Then, diethyl ether (30 mL) was slowly added. The resulting precipitate was collected by vacuum filtration and washed with cold diethyl ether (2×10 mL) and dried (0.30 g, 75%); mp $188.6\text{--}191.6^\circ\text{C}$; ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.37 (s, 1H), 7.83 (dd, $J = 7.4, 2.9$ Hz, 1H), 7.61 (ddd, $J = 9.4, 8.3, 3.0$ Hz, 1H), 7.52–7.40 (m, 2H), 7.21 (dt, $J = 10.0, 2.2$ Hz, 1H), 7.16 (td, $J = 8.6, 2.7$ Hz, 1H), 7.11 (dd, $J = 7.6, 1.5$ Hz, 1H), 5.43 (s, 2H).

4-(3-Fluorophenethyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6v). In a 10–20 mL vial, compound **5a** (0.40 g, 2.20 mmol, 1 equiv), potassium carbonate (1.21 g, 8.74 mmol, 4 equiv), and 3-fluorophenethyl bromide (0.61 mL, 0.952 g, 4.60 mmol, 2.1 equiv) were added successively. The reaction medium was supplemented with 13 mL of acetonitrile and heated at 75°C for 36 h with stirring. After 24 h, an additional portion of 3-fluorophenethyl bromide (0.61 mL, 0.952 g, 4.60 mmol, 2.1 equiv) was added to the reaction medium. At the end of the reaction, the insoluble material was removed by filtration through filter paper, and the filtrate was evaporated to dryness under reduced pressure. The resulting solid was collected and dissolved in a minimal amount of methanol, then cooled in an ice bath. Thirty milliliters of diethyl ether were added to the cold solution to induce precipitation. The precipitate was collected by vacuum filtration, washed with 2×10 mL of diethyl ether, and dried in an oven (0.34 g, 50%); mp $160.2\text{--}163.8^\circ\text{C}$; ^1H NMR (DMSO- d_6 , 400 MHz) δ 7.96–7.91 (m, 1H), 7.90 (d, $J = 8.3$ Hz, 1H), 7.85–7.77 (m, 1H), 7.72 (d, $J = 8.3$ Hz, 1H), 7.57 (t, $J = 7.5$ Hz, 1H), 7.34 (q, $J = 7.4$ Hz, 1H), 7.17 (d, $J = 10.2$ Hz, 1H), 7.07 (dd, $J = 8.4, 3.7$ Hz, 2H), 4.35 (t, $J = 7.5$ Hz, 2H), 3.05 (t, $J = 7.4$ Hz, 2H).

7-Fluoro-4-(3-fluorophenethyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6w). In a sealed 10 mL vial, **5b** (0.4 g, 1.23 mmol, 1 equiv), potassium carbonate (1.1 g, 7.96 mmol, 6.5 equiv), and 3-fluorophenethyl bromide (0.61 mL, 2.2 equiv) were added sequentially. The reaction mixture was dissolved in acetonitrile (13

mL) and heated at 75 °C for 36 h. After 24 h of reaction, a second portion of 3-fluorophenethyl bromide (0.61 mL, 2.2 equiv) was added to the reaction mixture. At the end of the reaction, the insoluble material was removed by filtration. The filtrate was concentrated to dryness under reduced pressure. The resulting crude solid was recovered, redissolved in a minimal volume of methanol, and cooled in an ice bath. Diethyl ether (30 mL) was then slowly added. The precipitate formed was collected by vacuum filtration, washed with cold diethyl ether (2 × 10 mL), and dried (0.35 g, 79%): mp 202.1–203.9 °C; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.92 (s, 1H), 7.87–7.76 (m, 2H), 7.70 (td, *J* = 9.2, 8.8, 3.0 Hz, 1H), 7.34 (td, *J* = 8.0, 6.2 Hz, 1H), 7.16 (dt, *J* = 10.1, 2.2 Hz, 1H), 7.07 (dd, *J* = 10.4, 7.0 Hz, 2H), 4.37 (t, *J* = 7.4 Hz, 2H), 3.04 (t, *J* = 7.3 Hz, 2H).

4-(4-Cyanobenzyl)-7-fluoro-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6x). Potassium carbonate (1.04 g, 7.49 mmol, 3 equiv), **5b** (0.500 g, 2.50 mmol, 1 equiv), and 4-cyanobenzyl bromide (0.734 g, 3.75 mmol, 1.5 equiv) were placed in a 25 mL microwave vial sealed with a Suba-Seal and then subjected to vacuum. Anhydrous acetonitrile (MeCN, 12 mL) was added directly to the mixture. The vial was flushed with nitrogen, the Suba-Seal was removed, and the vial was sealed. The reaction mixture was heated at 70 °C for 22 h. The mixture was filtered, and the residue was rinsed with MeCN (×3). The combined filtrate was concentrated under reduced pressure to yield a colorless viscous oil. This oil was dissolved in minimal methanol (MeOH), resulting in the formation of a white precipitate. The solid was collected by vacuum filtration, rinsed with minimal cold MeOH, and dried to afford the final product **6x** (0.51 g, 65%); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.37 (s, 1H), 7.89 (bs, 1H), 7.86–7.76 (m, 2H), 7.64–7.55 (m, 3H), 7.46 (dd, *J* = 9.4, 4.2 Hz, 1H), 5.46 (s, 2H); ¹³C NMR (DMSO-*d*₆, 126 MHz): δ 159.4, 151.8, 136.8, 131.9, 131.4, 131.3, 130.6, 130.2, 124.3, 121.3, 120.0, 118.5, 111.9, 110.8, 52.4.

7-Fluoro-4-(4-nitrobenzyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6y). Potassium carbonate (K₂CO₃, 0.518 g, 3.75 mmol, 1.5 equiv), **5b** (0.500 g, 2.50 mmol, 1 equiv), and 4-nitrobenzyl bromide (0.809 g, 3.75 mmol, 1.5 equiv) were placed in a 25 mL microwave vial sealed with a Suba-Seal and placed under vacuum. Anhydrous acetonitrile (MeCN, 12 mL) was added directly to the mixture, followed by flushing with nitrogen, removal of the Suba-Seal, and sealing of the vial. The reaction was then heated at 70 °C for 1 h. The reaction mixture was cooled and then rinsed with MeCN and filtered. The filtrate was concentrated under reduced pressure to give a yellow solid. The filter cake was washed with water to dissolve the excess K₂CO₃, leaving no further solid. The yellow crude product was recrystallized from methanol (MeOH) and rinsed with diethyl ether. A final wash with MeOH yielded the desired compound **6y** as an off-white crystalline solid (0.75 g, 84%); ¹H NMR (DMSO-*d*₆, 500 MHz): δ 8.39 (s, 1H), 8.25 (d, *J* = 9.0 Hz, 2H), 7.85 (dd, *J* = 7.5, 3.0 Hz, 1H), 7.61–7.56 (m, 1H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.41 (dd, *J* = 9.5, 4.0 Hz, 1H), 5.57 (s, 2H); ¹³C NMR (DMSO-*d*₆, 125 MHz): δ 159.4, 151.8, 147.2, 142.8, 131.4, 127.8, 124.3, 124.1, 121.3, 120, 111.9, 52.6.

7-Fluoro-4-(3-nitrobenzyl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (6z). In a 25 mL microwave vial, potassium carbonate (K₂CO₃, 0.518 g, 3.75 mmol, 1.5 equiv), **5b** (1.00 g, 5.00 mmol, 1 equiv), and 3-nitrobenzyl bromide (0.809 g, 3.75 mmol, 1.5 equiv) were added. The vial was sealed with a Suba-Seal, evacuated under vacuum, and anhydrous acetonitrile (12 mL) was added. The vial was flushed with nitrogen, the Suba-Seal was removed, and the vial was sealed tightly. The reaction mixture was stirred and heated at approximately 50 °C for 1 h. TLC revealed the formation of two product spots and the complete consumption of the starting material. The reaction was allowed to cool and the resulting solid was collected by filtration. The filter cake was washed successively with water (to remove residual K₂CO₃), 1 M HCl, and diethyl ether, leaving the title compound as a white solid (1.04 g, 3.10 mmol, 62%); ¹H NMR (DMSO-*d*₆, 500 MHz): δ 8.41 (s, 1H), 8.28 (bs, 1H), 8.18 (dt, *J* = 7.8, 1.9 Hz, 1H), 7.84 (dd, *J* = 7.3, 2.9 Hz, 1H), 7.75–7.65 (m, 2H),

7.59 (td, *J* = 8.6, 3.0 Hz, 1H), 7.51 (dd, *J* = 9.4, 4.2 Hz, 1H), 5.55 (s, 2H).

4-(Cyanomethyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7a). In a 100 mL round-bottom flask containing 30 mL of water, the starting material **6a** (0.40 g, 1.81 mmol, 1 equiv) was dispersed. Sodium borohydride (0.62 g, 16.31 mmol, 9 equiv) was then added portionwise. The reaction mixture was stirred until complete conversion was observed by TLC monitoring. The pH of the medium was adjusted to approximately 3 using 6 N HCl, and the flask was cooled in an ice bath. The suspension was evaporated to dryness under reduced pressure. The resulting solid residue was suspended in acidified water and collected by vacuum filtration, washed with water, and dried to afford the desired product **7a** (0.103 g, 0.46 mmol, 25%); mp 134.8–135.5 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.39 (s, 1H), 7.63 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.55 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 4.76 (s, 2H), 4.68 (s, 2H); ¹³C NMR (DMSO-*d*₆, 125 MHz) δ 143.94, 135.92, 127.05, 127.01, 121.26, 118.77, 116.74, 63.71, 39.15.

7-Fluoro-4-(cyanomethyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7b). The title compound was obtained as described for **7a** starting from **6b** (0.48 g, 2.01 mmol, 1 equiv) and sodium borohydride (0.68 g, 18.09 mmol, 9 equiv) to afford the desired product **7b** (0.30 g, 1.24 mmol, 62%); mp 144.7–147.4 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.48 (s, 1H), 7.54 (dd, *J* = 7.7, 3.1 Hz, 1H), 7.49 (ddd, *J* = 9.2, 8.3, 3.1 Hz, 1H), 7.14 (dd, *J* = 9.3, 4.2 Hz, 1H), 4.73 (s, 2H), 4.68 (s, 2H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 155.48, 153.56, 138.38, 124.90, 121.08, 116.70, 116.23, 110.89, 61.23, 37.04.

4-(2-Cyanoethyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7c). The title compound was obtained as described for **7a**, starting from **6c** (0.32 g, 1.36 mmol, 1 equiv) and sodium borohydride (0.47 g, 12.24 mmol, 9 equiv) to afford the desired product **7c** (0.23 g, 0.97 mmol, 71%); mp 138.4–140.1 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 7.98 (s, 1H), 7.55 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.41 (ddd, *J* = 8.7, 7.2, 1.7 Hz, 1H), 7.04 (d, *J* = 8.6 Hz, 1H), 6.83 (t, *J* = 7.5 Hz, 1H), 4.79 (s, 2H), 3.74 (t, *J* = 6.9 Hz, 2H), 2.78 (t, *J* = 6.9 Hz, 2H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 142.15, 133.21, 124.37, 123.09, 119.21, 116.86, 113.98, 60.65, 44.76, 15.38.

4-(2-Cyanoethyl)-7-fluoro-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7d). The title compound was obtained as described for **7a** starting from **6d** (0.30 g, 1.18 mmol, 1 equiv) and sodium borohydride (0.40 g, 10.62 mmol, 9 equiv) to afford the desired product **7d** (0.16 g, 0.62 mmol, 53%); mp 161.7–164.4 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.11 (s, 1H), 7.42 (dd, *J* = 7.8, 3.1 Hz, 1H), 7.34 (ddd, *J* = 9.3, 8.3, 3.1 Hz, 1H), 7.12 (dd, *J* = 9.4, 4.2 Hz, 1H), 4.77 (s, 2H), 3.74 (t, *J* = 7.0 Hz, 2H), 2.78 (t, *J* = 6.9 Hz, 2H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 154.39, 152.50, 139.25, 123.26, 120.67, 119.21, 116.45, 110.32, 60.64, 45.23, 15.50.

4-(3-Cyanopropyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7e). The title compound was obtained as described for **7a**, starting from **6e** (0.20 g, 0.80 mmol, 1 equiv) and sodium borohydride (0.27 g, 7.20 mmol, 9 equiv) to afford the desired product **7e** (0.13 g, 0.52 mmol, 65%); mp 116.7–122.8 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 7.94 (t, *J* = 8.0 Hz, 1H), 7.54 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.40 (ddd, *J* = 8.7, 7.0, 1.7 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 1H), 6.78 (t, *J* = 7.5 Hz, 1H), 4.70 (d, *J* = 8.0 Hz, 2H), 3.41 (t, *J* = 7.4 Hz, 2H), 2.59 (t, *J* = 7.4 Hz, 2H), 1.85 (p, *J* = 7.4 Hz, 2H), 1.47 (s, 1H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 142.65, 133.21, 124.41, 122.61, 120.35, 116.15, 113.41, 60.81, 47.35, 22.60, 13.50.

4-(3-Cyanopropyl)-7-fluoro-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7f). The title compound was obtained as described for **7a**, starting from **6f** (0.2 g, 0.75 mmol, 1 equiv) and sodium borohydride (0.25 g, 6.73 mmol, 9 equiv) to afford the orange desired product **7f** (0.14 g, 0.51 mmol, 69%); mp 152.1–154.4 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 7.41 (dd, *J* = 7.8, 3.1 Hz, 1H), 7.33 (ddd, *J* = 9.3, 8.2, 3.1 Hz, 1H), 6.98 (dd, *J* = 9.4, 4.2 Hz, 1H), 4.69 (s, 2H), 3.40 (t, *J* = 7.4 Hz, 2H), 2.58 (t, *J* = 7.4 Hz, 2H), 1.84

(h, $J = 7.4, 6.8$ Hz, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 153.96, 152.07, 139.77, 120.90, 120.34, 115.78, 110.35, 60.83, 47.83, 22.65, 13.49.

7-Chloro-4-(3-cyanopropyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]-thiadiazine 1,1-dioxide (7g). Sodium borohydride (0.05 g, 1.32 mmol, 3.8 equiv) was added to a stirred suspension of **6g** (0.10 g, 0.35 mmol) in isopropanol (4 mL), and the mixture was heated at 50 °C for 5 min in an unsealed 25 mL microwave tube. The reaction mixture was then transferred to a round-bottom flask and the solvent removed in vacuo before the addition of water and slow neutralization of the mixture by dropwise addition of 1 N HCl. At pH 6–7, ethyl acetate ($\times 3$) was used to extract the aqueous layer. The combined organic layers were dried (MgSO_4), filtered, and the solvent was removed in vacuo to give the desired product **7g** (97 mg, 0.34 mmol, 97%); mp 186.6–187.8 °C; ^1H NMR (DMSO- d_6 , 500 MHz): δ 8.11 (t, $J = 8.1$ Hz, 1H), 7.54 (d, $J = 2.6$ Hz, 1H), 7.44 (dd, $J = 9.2, 2.6$ Hz, 1H), 6.98 (d, $J = 9.2$ Hz, 1H), 4.72 (d, $J = 8.1$ Hz, 2H), 3.41 (t, $J = 7.3$ Hz, 2H), 2.58 (t, $J = 7.4$ Hz, 1H), 1.83 (quintet, $J = 7.4$ Hz, 2H); ^{13}C NMR (DMSO- d_6 , 125 MHz): δ 141.7, 133.2, 123.5, 123.4, 120.4, 119.8, 115.8, 60.9, 22.7, 13.6.

4-(Prop-2-yn-1-yl)-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7h). The title compound was obtained as described for **7a**, starting from **6h** (0.2 g, 0.91 mmol, 1 equiv) and sodium borohydride (0.309 g, 8.17 mmol, 9 equiv), to afford the desired product **7h** (0.11 g, 0.495 mmol, 55%); mp 117.1–118.5 °C; ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.25 (t, $J = 8.1$ Hz, 1H), 7.56 (dd, $J = 7.8, 1.6$ Hz, 1H), 7.46 (ddd, $J = 8.7, 7.2, 1.7$ Hz, 1H), 7.01 (dd, $J = 8.7, 0.9$ Hz, 1H), 6.88 (td, $J = 7.6, 1.0$ Hz, 1H), 4.69 (d, $J = 8.1$ Hz, 2H), 4.26 (d, $J = 2.4$ Hz, 2H), 3.24 (t, $J = 2.3$ Hz, 1H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 142.41, 133.06, 124.20, 124.00, 117.61, 114.71, 78.88, 75.01, 60.85, 37.42.

7-Fluoro-4-(prop-2-yn-1-yl)-4H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7i). The title compound was obtained as described for **7a**, starting from **6i** (0.2 g, 0.91 mmol, 1 equiv) and sodium borohydride (0.309 g, 8.17 mmol, 9 equiv) to afford the desired product **7i** (0.22 g, 0.916 mmol, 68%); mp 78.8–85.7 °C; ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.39 (s, 1H), 7.48–7.34 (m, 2H), 7.08 (dd, $J = 9.3, 4.2$ Hz, 1H), 4.67 (s, 2H), 4.26 (d, $J = 2.4$ Hz, 2H), 3.28–3.23 (m, 1H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 154.82, 152.92, 139.43, 124.18, 120.73, 117.06, 110.55, 78.74, 75.21, 60.87, 37.72.

4-Allyl-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7j). The title compound was obtained as described for **7a**, starting from **6j** (0.20 g, 0.90 mmol, 1 equiv) and sodium borohydride (0.31 g, 8.10 mmol, 9 equiv) to afford the desired product **7j** (0.17 g, 0.758 mmol, 84%); mp 99.7–102.8 °C; ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.04 (s, 1H), 7.52 (dd, $J = 7.8, 1.7$ Hz, 1H), 7.36 (ddd, $J = 8.7, 7.2, 1.7$ Hz, 1H), 6.82–6.78 (m, 1H), 6.78–6.73 (m, 1H), 5.84 (ddt, $J = 17.2, 10.0, 4.8$ Hz, 1H), 5.25–5.13 (m, 2H), 4.70 (s, 2H), 4.02 (dt, $J = 4.9, 1.8$ Hz, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 142.81, 133.03, 132.67, 124.03, 122.42, 116.29, 115.99, 113.95, 60.88, 50.72.

4-Allyl-7-fluoro-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7k). The title compound was obtained as described for **7a**, starting from **6k** (0.20 g, 0.83 mmol, 1 equiv) and sodium borohydride (0.28 g, 7.50 mmol, 9 equiv) to afford the desired product **7k** (0.14 g, 0.578 mmol, 69%); mp 78.8–85.7 °C; ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.16 (d, $J = 7.9$ Hz, 1H), 7.39 (dd, $J = 7.8, 3.1$ Hz, 1H), 7.30 (ddd, $J = 9.3, 8.3, 3.1$ Hz, 1H), 6.84 (dd, $J = 9.3, 4.2$ Hz, 1H), 5.83 (ddt, $J = 17.2, 10.0, 4.8$ Hz, 1H), 5.24–5.14 (m, 2H), 4.69 (s, 2H), 4.01 (dt, $J = 4.8, 1.8$ Hz, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 153.88, 152.00, 139.85, 132.71, 120.56, 116.43, 116.14, 110.02, 60.90, 51.12.

4-(4,4,4-Trifluorobutyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7l). The title compound was obtained as described for **7a**, starting from **6l** (0.22 g, 0.75 mmol, 1 equiv) and sodium borohydride (0.26 g, 6.78 mmol, 9 equiv) to afford the desired product **7l** (0.20 g, 0.68 mmol, 90%); mp 138.0–139.5 °C; ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.97 (s, 1H), 7.53 (dd, $J = 7.8, 1.7$ Hz, 1H), 7.40 (ddd, $J = 8.8, 7.2, 1.7$ Hz, 1H), 6.93 (d, $J = 8.5$ Hz, 1H), 6.77 (t, $J = 7.4$ Hz, 1H), 4.71 (s, 2H), 3.42 (t, $J = 7.4$ Hz, 2H),

2.44–2.30 (m, 2H), 1.79–1.69 (m, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 142.63, 133.21, 128.67, 126.47, 124.39, 122.51, 115.96, 113.30, 60.88, 47.07, 30.11, 19.37.

7-Fluoro-4-(4,4,4-trifluorobutyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7m). The title compound was obtained as described for **7a**, starting from **6m** (0.20 g, 0.64 mmol, 1 equiv) and sodium borohydride (0.22 g, 7.59 mmol, 9 equiv) to afford the desired product **7m** (0.14 g, 0.45 mmol, 70%); mp 127.7–130.7 °C; ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.08 (d, $J = 7.4$ Hz, 1H), 7.41 (dd, $J = 7.8, 3.1$ Hz, 1H), 7.35–7.27 (m, 1H), 7.00 (dd, $J = 9.4, 4.2$ Hz, 1H), 4.70 (d, $J = 6.7$ Hz, 2H), 3.42 (t, $J = 7.4$ Hz, 2H), 2.43–2.27 (m, 2H), 1.73 (p, $J = 7.7$ Hz, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 153.85, 151.96, 139.69, 128.65, 126.45, 122.37, 120.98, 115.63, 110.55, 60.78, 47.49, 29.64, 19.88.

4-Benzyl-7-fluoro-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7n) (CAS 2568187-95-9). In a 100 mL round-bottom flask, **6n** (0.300 g, 1.08 mmol, 1 equiv) was dispersed in water (30 mL). Sodium borohydride (0.232 g, 6.14 mmol, 9 equiv) was added in one portion. The reaction mixture was stirred at room temperature for 24 h until complete conversion was observed by TLC. The pH of the reaction medium was adjusted to 3 by dropwise addition of 6 N hydrochloric acid, and the flask was cooled in an ice bath. The aqueous solution was evaporated to dryness under reduced pressure. The resulting residue was scraped from the flask walls and recovered using acidified water. The precipitate was collected by vacuum filtration, washed with cold water (2×10 mL), and dried to yield the desired product **7n** as a pale solid (0.100 g, 0.359 mmol, 33%); mp 190.2–192.6 °C; ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.33 (t, $J = 8.1$ Hz, 1H), 7.43 (dd, $J = 7.8, 3.1$ Hz, 1H), 7.43–7.31 (m, 4H), 7.33–7.23 (m, 2H), 7.27–7.20 (m, 1H), 6.71 (dd, $J = 9.4, 4.2$ Hz, 1H), 4.86 (d, $J = 8.1$ Hz, 2H), 4.63 (s, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 153.96, 152.07, 139.89, 137.32, 128.55, 126.92, 126.32, 122.28, 120.66, 115.99, 110.38, 61.64, 52.42.

4-Benzyl-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7o) (CAS 1428268-29-4). In a 100 mL round-bottom flask, **6o** (0.190 g, 0.682 mmol, 1 equiv) was dispersed in water (30 mL). Sodium borohydride (0.232 g, 6.14 mmol, 9 equiv) was added in one portion. The reaction mixture was stirred at room temperature for 24 h until complete conversion was observed by TLC. The pH of the reaction medium was then adjusted to 3 by dropwise addition of 6 N hydrochloric acid, and the flask was cooled in an ice bath. The aqueous solution was evaporated to dryness under reduced pressure. The resulting residue was scraped from the flask walls and recovered using acidified water. The precipitate was collected by vacuum filtration, washed with cold water (2×10 mL), and dried to yield the desired product **7o** as a pale solid (30 mg, 0.108 mmol, 16%); mp 161.6–163.6 °C; ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.19 (s, 1H), 7.55 (dd, $J = 7.8, 1.6$ Hz, 1H), 7.39–7.23 (m, 6H), 6.76 (dd, 1H), 6.69 (d, $J = 8.5$ Hz, 1H), 4.88 (s, 2H), 4.63 (s, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 142.33, 136.85, 132.53, 127.97, 126.39, 125.83, 123.56, 121.93, 115.60, 113.30, 61.14, 51.53.

7-Fluoro-4-(3-fluoro-4-nitrobenzyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (7p). In a 100 mL round-bottom flask, **6p** (0.100 g, 0.298 mmol, 1 equiv) was dispersed in water (30 mL). Sodium borohydride (0.193 g, 2.68 mmol, 9 equiv) was added in one portion. The reaction mixture was stirred at room temperature for 10 h until complete conversion was observed (monitored by TLC). The pH of the mixture was then adjusted to 3 by the dropwise addition of 6 N hydrochloric acid, and the flask was cooled in an ice bath. The aqueous layer was evaporated to dryness under reduced pressure. The walls of the flask were scraped, and the resulting solid was recovered with acidified water. The precipitate was collected by vacuum filtration, washed with cold water (2×10 mL), and dried to afford the desired product **7p** as a yellow solid (0.100 g, 0.298 mmol, 100%); mp 179.3–181.3 °C; ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.40 (s, 1H), 8.16 (t, $J = 8.2$ Hz, 1H), 7.60 (dd, $J = 12.4, 1.8$ Hz, 1H), 7.44 (ddd, $J = 22.3, 8.2, 2.5$ Hz, 2H), 7.33–7.20 (m, 1H), 6.59 (dd, $J = 9.4, 4.2$ Hz, 1H), 4.89 (s, 2H), 4.72 (s, 2H); ^{13}C NMR (DMSO- d_6 , 126 MHz) δ 155.89, 154.29, 153.81, 152.40, 148.24, 139.47, 135.59, 126.50, 123.02, 122.99, 120.90, 115.94, 110.66, 61.70, 51.71.

4-(3-Fluoro-4-nitrobenzyl)-3,4-dihydro-2H-benzo[e][1,2,4]-thiadiazine 1,1-dioxide (7q). In a 100 mL round-bottom flask containing 30 mL of water, **6q** (0.45 g, 1.34 mmol, 1 equiv) was dispersed. Sodium borohydride (0.46 g, 12.03 mmol, 9 equiv) was then added. The reaction mixture was stirred until complete conversion was observed. The pH of the medium was adjusted to 3 by the addition of 6 N hydrochloric acid, and the flask was cooled in a refrigerator or ice bath. The solution was then evaporated to dryness under reduced pressure. The walls of the flask were scraped, and the resulting solid was recovered with acidified water. The yellow precipitate was collected by vacuum filtration and dried (0.11 g, 0.328 mmol, 25%); mp 152.5–154.3 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.28 (s, 1H), 8.16 (t, *J* = 8.1 Hz, 1H), 7.62–7.57 (m, 2H), 7.43 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.31 (ddd, *J* = 8.8, 7.2, 1.7 Hz, 1H), 6.84–6.76 (m, 1H), 6.58–6.53 (m, 1H), 4.91 (s, 2H), 4.73 (s, 2H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 155.89, 153.81, 148.30, 142.44, 135.51, 133.25, 126.49, 124.33, 122.95, 122.85, 116.76, 116.06, 113.67, 61.64, 51.36.

4-(2,5-Difluorobenzyl)-3,4-dihydro-2H-benzo[e][1,2,4]-thiadiazine 1,1-dioxide (7r). In a 100 mL round-bottom flask containing 30 mL of isopropanol, **6r** (0.20 g, 0.649 mmol, 1 equiv) was dispersed. Sodium borohydride (0.44 g, 11.67 mmol, 18 equiv) was then added. The reaction mixture was stirred until complete conversion was observed. The pH of the medium was adjusted to 3 by the addition of 6 N hydrochloric acid, and the flask was cooled in a refrigerator or an ice bath. The solution was then evaporated to dryness under reduced pressure. The walls of the flask were scraped, and the resulting solid was recovered with acidified water. The precipitate was collected by vacuum filtration and dried (0.17 g, 85%); mp 189.8–191.5 °C; ¹H NMR (DMSO-*d*₆, 500 MHz): δ 8.24 (s, 1H), 7.58 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.31 (qd, *J* = 9.3, 8.8, 3.0 Hz, 2H), 7.24–7.14 (m, 2H), 6.80 (t, *J* = 7.5 Hz, 1H), 6.61 (d, *J* = 8.6 Hz, 1H), 4.86 (s, 2H), 4.63 (s, 2H), 2.58–2.53 (m, 1H), 2.48–2.44 (m, 1H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 159.38, 157.47, 156.89, 154.98, 142.61, 133.29, 126.46, 124.25, 122.85, 117.02, 116.59, 115.32, 114.51, 113.47, 61.43, 46.09.

4-(2,5-Difluorobenzyl)-7-fluoro-3,4-dihydro-2H-benzo[e]-[1,2,4]thiadiazine 1,1-dioxide (7s). In a 100 mL round-bottom flask containing 30 mL of isopropanol, **6s** (0.2 g, 0.615 mmol, 1 equiv) was dispersed. Then, sodium borohydride (0.42 g, 11.07 mmol, 18 equiv) was added. The reaction mixture was stirred for 30 min. The pH of the mixture was adjusted to 3 by the addition of 6 N hydrochloric acid, and the flask was cooled in a refrigerator or an ice bath. The mixture was then evaporated to dryness under reduced pressure. The walls of the flask were scraped, and the resulting solid was recovered using acidified water. The precipitate was collected by vacuum filtration and dried (0.14 g, 70%); mp 149.9–152.6 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.35 (s, 1H), 7.46 (dd, *J* = 7.8, 3.1 Hz, 1H), 7.35–7.13 (m, 4H), 6.66 (dd, *J* = 9.4, 4.1 Hz, 1H), 4.84 (s, 2H), 4.63 (s, 2H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 159.37, 157.46, 156.89, 154.97, 154.21, 152.31, 139.64, 126.57, 122.83, 120.96, 116.98, 115.67, 115.17, 114.70, 110.56, 61.40, 46.49.

4-(3-Fluorobenzyl)-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (7t). In a 100 mL round-bottom flask containing 30 mL of isopropanol, **6t** (0.200 g, 0.689 mmol, 1 equiv) was dispersed. Sodium borohydride (0.47 g, 12.40 mmol, 18 equiv) was then added. The reaction mixture was stirred until complete conversion was observed. The pH of the medium was adjusted to 3 by the addition of 6 N hydrochloric acid, and the flask was cooled in a refrigerator or ice bath. The solution was then evaporated to dryness under reduced pressure. The walls of the flask were scraped, and the resulting solid was recovered with acidified water. The precipitate was collected by vacuum filtration and dried (0.18 g, 89%); mp 145.6–147.1 °C; ¹H NMR (DMSO-*d*₆, 500 MHz): δ 8.23 (s, 1H), 7.55 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.39 (td, *J* = 8.1, 6.1 Hz, 1H), 7.30 (ddd, *J* = 8.8, 7.2, 1.7 Hz, 1H), 7.23–7.16 (m, 2H), 7.08 (td, *J* = 9.4, 8.9, 2.6 Hz, 1H), 6.77 (t, *J* = 7.5 Hz, 1H), 6.63 (d, *J* = 8.6 Hz, 1H), 4.87 (s, 2H), 4.63 (s, 2H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 163.43, 161.49, 142.73, 140.78,

133.12, 130.54, 124.16, 122.60, 122.38, 116.33, 113.82, 113.05, 61.73, 51.56.

7-Fluoro-4-(3-fluorobenzyl)-3,4-dihydro-2H-benzo[e][1,2,4]-thiadiazine 1,1-dioxide (7u). In a 100 mL round-bottom flask containing 30 mL of isopropanol, compound **6u** (0.2 g, 0.565 mmol, 1 equiv) was dispersed. Then, sodium borohydride (0.44 g, 11.03 mmol, 18 equiv) was added. The reaction mixture was stirred for 30 min until the reaction was complete. The pH of the medium was adjusted to 3 with 6 N hydrochloric acid, and the flask was cooled in a refrigerator or an ice bath. The solution was then evaporated to dryness under reduced pressure. The walls of the flask were scraped, and the resulting solid was recovered using acidified water. The precipitate was collected by vacuum filtration and dried (0.17 g, 89%); mp 137.1–138.4 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.35 (s, 1H), 7.43 (dd, 1H), 7.39 (dd, 1H), 7.24 (td, *J* = 8.8, 3.1 Hz, 1H), 7.19 (t, *J* = 10.0 Hz, 2H), 7.09 (td, *J* = 8.7, 2.6 Hz, 1H), 6.67 (dd, *J* = 9.4, 4.2 Hz, 1H), 4.86 (s, 2H), 4.63 (s, 2H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 163.43, 161.49, 154.07, 152.17, 140.72, 139.76, 130.51, 122.70, 122.40, 120.83, 115.84, 113.72, 113.09, 110.45, 61.73, 51.92.

4-(3-Fluorophenethyl)-3,4-dihydro-2H-benzo[e][1,2,4]-thiadiazine 1,1-dioxide (7v). In a 100 mL round-bottom flask containing 30 mL of isopropanol, **6v** (0.20 g, 0.657 mmol, 1 equiv) was dispersed. Sodium borohydride (0.45 g, 11.82 mmol, 18 equiv) was then added. The reaction mixture was stirred until complete conversion was observed. The pH of the medium was adjusted to 3 by the addition of 6 N hydrochloric acid, and the flask was cooled in a refrigerator or ice bath. The solution was then evaporated to dryness under reduced pressure. The walls of the flask were scraped, and the resulting solid was recovered with acidified water. The precipitate was collected by vacuum filtration and dried (0.12 g, 60%); mp 137.1–138.6 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 7.95 (s, 1H), 7.52 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.42–7.27 (m, 2H), 7.22 (dt, *J* = 10.4, 2.1 Hz, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 7.05 (td, *J* = 8.7, 2.7 Hz, 1H), 6.95 (d, *J* = 8.6 Hz, 1H), 6.76 (t, *J* = 7.5 Hz, 1H), 4.69 (s, 2H), 3.59 (t, *J* = 7.8 Hz, 2H), 2.85 (t, *J* = 7.8 Hz, 2H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 162.10, 160.18, 141.50, 140.68, 132.19, 129.10, 124.05, 123.24, 121.56, 114.90, 114.70, 112.61, 111.96, 59.76, 49.11, 31.05.

7-Fluoro-4-(3-fluorophenethyl)-3,4-dihydro-2H-benzo[e]-[1,2,4]thiadiazine 1,1-dioxide (7w). In a 100 mL round-bottom flask containing 30 mL of isopropanol, compound **6w** (0.2 g, 1 equiv) was suspended. Then, sodium borohydride (0.42 g, 18 equiv) was added in one portion. The reaction mixture was stirred at room temperature until completion. The pH of the medium was adjusted to 3 using 6 N hydrochloric acid, and the flask was placed in a refrigerator or an ice bath. The solution was then evaporated to dryness under reduced pressure. The flask walls were carefully scraped, and the resulting solid was recovered using acidified water. The precipitate was collected by vacuum filtration and dried. (0.16 g, 80%); mp 137.8–139.5 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.08 (s, 1H), 7.39 (dd, *J* = 7.8, 3.1 Hz, 1H), 7.34 (td, *J* = 8.0, 6.3 Hz, 1H), 7.30 (ddd, *J* = 9.4, 8.4, 3.2 Hz, 1H), 7.22 (dt, *J* = 10.4, 2.1 Hz, 1H), 7.16 (d, *J* = 8.8 Hz, 1H), 7.05 (td, *J* = 8.4, 2.4 Hz, 1H), 6.99 (dd, *J* = 9.4, 4.2 Hz, 1H), 4.67 (s, 2H), 3.64–3.54 (m, 2H), 2.88–2.81 (m, 2H); ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 163.11, 161.18, 153.80, 151.92, 141.62, 139.61, 130.16, 125.05, 120.84, 115.72, 112.90, 110.18, 60.79, 50.50, 32.10.

4-(4-Cyanobenzyl)-7-fluoro-3,4-dihydro-2H-benzo[e][1,2,4]-thiadiazine 1,1-dioxide (7x). Sodium borohydride (0.046 g, 1.21 mmol, 3.8 equiv) was added to a stirred suspension of compound **6x** (0.100 g, 0.317 mmol, 1 equiv) in isopropanol (4.0 mL), and the reaction mixture was heated at 50 °C for 25 min in an open flask. Upon completion, the solvent was removed under reduced pressure, yielding a white solid. This residue was dissolved in water and neutralized by the dropwise addition of 1 N hydrochloric acid, affording a white suspension. The solid was collected by vacuum filtration, washed with water (3 ×), and dried to yield the title compound as a white solid (0.099 g, 0.302 mmol, 95%); mp 190.0–191.8 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.37 (s, 1H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 2H), 7.45 (dd, *J* = 8.3, 3.0 Hz, 1H), 7.23 (ddd, *J* = 9.0, 9.0, 3.5 Hz, 1H), 6.61 (dd, *J* = 9.5, 4.0 Hz,

1H), 4.87 (bs, 2H), 4.71 (s, 2H); ¹³C NMR (DMSO-*d*₆, 125 MHz) δ 153.3, 143.8, 139.7, 132.6, 127.5, 122.7, 121.0, 118.8, 115.9, 110.6, 109.9, 61.8, 52.3.

7-Fluoro-4-(4-nitrobenzyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]-thiadiazine 1,1-dioxide (7y). Sodium borohydride (0.13 g, 3.40 mmol, 3.8 equiv) was added to a stirred suspension of **6y** (0.30 g, 0.895 mmol, 1 equiv) in isopropanol (12 mL), and the reaction mixture was heated at 50 °C for 15 min in an open flask. Shortly after heating, the mixture turned bright yellow. The reaction mixture was then slowly neutralized by dropwise addition of 1 N hydrochloric acid until the pH reached 6–7. A yellow solid precipitated and was collected by vacuum filtration and washed with water. The resulting product, **7y**, was obtained as a yellow solid (0.29 g, 91%); mp 183.0–184.9 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.39 (s, 1H), 8.21 (d, *J* = 8.5 Hz, 2H), 7.60 (d, *J* = 9.0 Hz, 2H), 7.46 (dd, *J* = 7.8, 3.1 Hz, 1H), 7.24 (ddd, *J* = 9.3, 8.4, 3.2 Hz, 1H), 6.63 (dd, *J* = 9.4, 4.2 Hz, 1H), 4.90 (s, 2H), 4.77 (s, 2H); ¹³C NMR (DMSO-*d*₆, 125 MHz): δ 153.4, 146.8, 146.0, 139.7, 127.7, 123.8, 122.8, 120.9, 116.0, 110.6, 61.8, 52.2.

7-Fluoro-4-(3-nitrobenzyl)-3,4-dihydro-2H-benzo[*e*][1,2,4]-thiadiazine 1,1-dioxide (7z). To a stirred suspension of **6z** (0.500 g, 1.49 mmol, 1 equiv) in a mixture of ethanol and DMF (3:1, 7.5 mL) in a 25 mL microwave vial, sodium borohydride (0.141 g, 3.73 mmol, 2.5 equiv) was added. The sealed vial was heated at approximately 60 °C for 10 min. The suspension almost completely dissolved, and the solution turned yellow. The reaction mixture was then diluted with water (2× the initial volume) and transferred into a round-bottom flask. A solution of acetic acid (1 mL in 20 mL of H₂O) was added to quench excess borohydride. The resulting yellow precipitate was collected by vacuum filtration, washed with water, and dried (0.46 g, 1.36 mmol, 91%); mp 196.5–198.3 °C; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.42 (s, 1H), 8.21 (app bs, 1H), 8.13 (dd, *J* = 7.7, 2.2 Hz, 1H), 7.79 (bd, *J* = 7.8, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.46 (dd, *J* = 7.8, 3.1 Hz, 1H), 7.24 (ddd, *J* = 9.3, 8.3, 3.2 Hz, 1H), 6.71 (dd, *J* = 9.4, 4.2 Hz, 1H), 4.88 (s, 2H), 4.75 (s, 2H); ¹³C NMR (DMSO-*d*₆, 125 MHz) δ 153.4, 148.2, 140.3, 139.9, 133.2, 130.2, 122.9, 122.2, 121.5, 121.0, 116.1, 110.6, 61.8, 52.0.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.6c03618>.

NMR spectra of all synthesized compounds (ZIP)

■ AUTHOR INFORMATION

Corresponding Authors

Pierre Francotte — University of Liège, Faculty of Medicine, Department of Pharmacy, Laboratory of Medicinal Chemistry (CIRM), Liège B-4000, Belgium; orcid.org/0000-0001-9252-7798; Email: Pierre.Francotte@uliege.be

Thomas Colson — University of Liège, Faculty of Medicine, Department of Pharmacy, Laboratory of Medicinal Chemistry (CIRM), Liège B-4000, Belgium; orcid.org/0000-0003-4828-1289; Email: t.colson@uliege.be

Authors

Yasmin Bay — University of Copenhagen, Department of Drug Design and Pharmacology, Molecular and Cellular Pharmacology, Copenhagen 2100, Denmark

Alexandra Manos-Turvey — University of Liège, Faculty of Medicine, Department of Pharmacy, Laboratory of Medicinal Chemistry (CIRM), Liège B-4000, Belgium

Giacomo Baudino — University of Liège, Faculty of Medicine, Department of Pharmacy, Laboratory of Medicinal Chemistry (CIRM), Liège B-4000, Belgium

Bastiaan Poldermans — University of Copenhagen, Department of Drug Design and Pharmacology, Molecular and Cellular Pharmacology, Copenhagen 2100, Denmark

Eric Goffin — University of Liège, Faculty of Medicine, Department of Pharmacy, Laboratory of Medicinal Chemistry (CIRM), Liège B-4000, Belgium

Marine Piette — University of Liège, Faculty of Medicine, Department of Pharmacy, Laboratory of Medicinal Chemistry (CIRM), Liège B-4000, Belgium

Cindy Lesenfants — University of Liège, Faculty of Medicine, Department of Pharmacy, Laboratory of Medicinal Chemistry (CIRM), Liège B-4000, Belgium

Marie Leclercq — University of Liège, Faculty of Medicine, Department of Pharmacy, Laboratory of Medicinal Chemistry (CIRM), Liège B-4000, Belgium

Jette S. Kastrup — University of Copenhagen, Department of Drug Design and Pharmacology, Structural Biology, Copenhagen 2100, Denmark; orcid.org/0000-0003-2654-1510

Bernard Pirotte — University of Liège, Faculty of Medicine, Department of Pharmacy, Laboratory of Medicinal Chemistry (CIRM), Liège B-4000, Belgium

Anders S. Kristensen — University of Copenhagen, Department of Drug Design and Pharmacology, Molecular and Cellular Pharmacology, Copenhagen 2100, Denmark; orcid.org/0000-0001-8955-6790

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/acsomega.6c03618>

Author Contributions

[†]T.C. and Y.B. equally contributed to the work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

[‡]A.S.K. and P.F. equally supervised the work.

■ ACKNOWLEDGMENTS

This study was supported in part by a grant from the Leon Fredericq Foundation and the National Fund for Scientific Research (F.N.R.S., Belgium), of which T. Colson and C. Lesenfants are Research Fellows. Y. Bay and J. S. Kastrup acknowledge financial support from the Independent Research Fund Denmark — Medical Sciences (0134-00162B). The technical assistance of S. Counerotte is gratefully acknowledged.

■ ABBREVIATIONS

¹ H/ ¹³ C NMR	Proton/carbon-13 NMR
ADHD	Attention-deficit/hyperactivity disorder
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPA PAM(s)	AMPA receptor positive allosteric modulator(s)
AMPA(s)	AMPA receptor(s)
CNS	Central nervous system
CTD	C-terminal domain
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide

DMSO- <i>d</i> ₆	Deuterated dimethyl sulfoxide
EC ₅₀	Half-maximal effective concentration
EPSC(s)	Excitatory postsynaptic current(s)
EtOAc	Ethyl acetate
GluA1–4	AMPA receptor subunits 1–4
GluD	Delta (δ) glutamate receptor
GluK1–5	Kainate receptor subunits 1–5
HEK cells	Human embryonic kidney cells
iGluR(s)	Ionotropic glutamate receptor(s)
KAR PAM(s)	Kainate receptor positive allosteric modulator(s)
KAR(s)	Kainate receptor(s)
LBD	Ligand-binding domain
MeCN (ACN)	Acetonitrile
MeOH	Methanol
mGluR(s)	Metabotropic glutamate receptor(s)
mp	Melting point
NMDAR(s)	NMDA receptor(s)
NMDA	N-methyl-D-aspartic acid
NMR	Nuclear magnetic resonance
ATD	amino-terminal domain
PAM(s)	Positive allosteric modulator(s)
SAR	Structure–activity relationship
s, d, t, q, m, bs, dd, dt	singlet, doublet, triplet, quadruplet, multiplet, broad singlet, doublet of doublets, doublet of triplets
TEOF	Triethyl orthoformate
TLC	Thin-layer chromatography
TMD	Transmembrane domain
TMS	Tetramethylsilane

REFERENCES

- (1) Francotte, P.; de Tullio, P.; Fraikin, P.; Counerotte, S.; Goffin, E.; Pirotte, B. In Search of Novel AMPA Potentiators. *Recent Patents CNS Drug Discovery* **2006**, *1* (3), 239–246.
- (2) Pirotte, B.; Francotte, P.; Goffin, E.; de Tullio, P. AMPA Receptor Positive Allosteric Modulators: A Patent Review. *Expert Opin. Ther. Pat.* **2013**, *23* (5), 615–628.
- (3) Hansen, K. B.; Wollmuth, L. P.; Bowie, D.; Furukawa, H.; Menniti, F. S.; Sobolevsky, A. I.; Swanson, G. T.; Swanger, S. A.; Greger, I. H.; Nakagawa, T.; McBain, C. J.; Jayaraman, V.; Low, C.-M.; Dell'acqua, M. L.; Diamond, J. S.; Camp, C. R.; Perszyk, R. E.; Yuan, H.; Traynelis, S. F. Structure Function, and Pharmacology of Glutamate Receptor Ion Channels. *Pharmacol. Rev.* **2021**, *73* (4), 1469–1658.
- (4) Sobolevsky, A. I.; Rosconi, M. P.; Gouaux, E. X-Ray Structure, Symmetry and Mechanism of an AMPA-Subtype Glutamate Receptor. *Nature* **2009**, *462* (7274), 745–756.
- (5) Traynelis, S. F.; Wollmuth, L. P.; McBain, C. J.; Menniti, F. S.; Vance, K. M.; Ogden, K. K.; Hansen, K. B.; Yuan, H.; Myers, S. J.; Dingledine, R. Glutamate Receptor Ion Channels: Structure, Regulation, and Function. *Pharmacol. Rev.* **2010**, *62* (3), 405–496.
- (6) Larsen, A. P.; Fièvre, S.; Frydenvang, K.; Francotte, P.; Pirotte, B.; Kastrup, J. S.; Mulle, C. Identification and Structure-Function Study of Positive Allosteric Modulators of Kainate Receptors. *Mol. Pharmacol.* **2017**, *91* (6), 576–585.
- (7) Greger, I. H.; Watson, J. F.; Cull-Candy, S. G. Structural and Functional Architecture of AMPA-Type Glutamate Receptors and Their Auxiliary Proteins. *Neuron* **2017**, *94* (4), 713–730.
- (8) Zhao, Y.; Chen, S.; Swensen, A. C.; Qian, W.-J.; Gouaux, E. Architecture and Subunit Arrangement of Native AMPA Receptors Elucidated by Cryo-EM. *Science* **2019**, *364* (6438), 355–362.
- (9) Kamalova, A.; Nakagawa, T. AMPA Receptor Structure and Auxiliary Subunits. *J. Physiol.* **2021**, *599* (2), 453–469.
- (10) Lerma, J.; Marques, J. M. Kainate Receptors in Health and Disease. *Neuron* **2013**, *80* (2), 292–311.
- (11) Pinheiro, P.; Mulle, C. Kainate Receptors. *Cell Tissue Res.* **2006**, *326* (2), 457–482.
- (12) Frydenvang, K.; Pickering, D. S.; Kastrup, J. S. Structural Basis for Positive Allosteric Modulation of AMPA and Kainate Receptors. *J. Phys.* **2022**, *600* (2), 181–200.
- (13) Kristensen, O.; Kristensen, L. B.; Møllerud, S.; Frydenvang, K.; Pickering, D. S.; Kastrup, J. S. The Structure of a High-Affinity Kainate Receptor: GluK4 Ligand-Binding Domain Crystallized with Kainate. *Structure* **2016**, *24* (9), 1582–1589.
- (14) Gouaux, E. Structure and Function of AMPA Receptors. *J. Physiol.* **2004**, *554* (2), 249–253.
- (15) Collingridge, G. L.; Isaac, J. T. R.; Wang, Y. T. Receptor Trafficking and Synaptic Plasticity. *Nat. Rev. Neurosci.* **2004**, *5* (12), 952–962.
- (16) Sommer, B.; Keinänen, K.; Verdoorn, T. A.; Wisden, W.; Burnashev, N.; Herb, A.; Kohler, M.; Takagi, T.; Sakmann, B.; Seeburg, P. H. Flip and Flop: A Cell-Specific Functional Switch in Glutamate-Operated Channels of the CNS. *Science* **1990**, *249* (4976), 1580–1585.
- (17) Pei, W.; Huang, Z.; Wang, C.; Han, Y.; Park, J. S.; Niu, L. Flip and Flop: A Molecular Determinant for AMPA Receptor Channel Opening. *Biochemistry* **2009**, *48* (17), 3767–3777.
- (18) Crovato, T. E.; Egebjerg, J. ASF/SF2 and SC35 Regulate the Glutamate Receptor Subunit 2 Alternative Flip/Flop Splicing. *FEBS Lett.* **2005**, *579* (19), 4138–4144.
- (19) Lau, A.; Tymianski, M. Glutamate Receptors, Neurotoxicity and Neurodegeneration. *Pflüg. Arch. - Eur. J. Physiol.* **2010**, *460* (2), 525–542.
- (20) Madden, D. R. The Structure and Function of Glutamate Receptor Ion Channels. *Nat. Rev. Neurosci.* **2002**, *3* (2), 91–101.
- (21) Chittajallu, R.; Vignes, M.; Dev, K. K.; Barnes, J. M.; Collingridge, G. L.; Henley, J. M. Regulation of Glutamate Release by Presynaptic Kainate Receptors in the Hippocampus. *Nature* **1996**, *379* (6560), 78–81.
- (22) Castillo, P. E.; Malenka, R. C.; Nicoll, R. A. Kainate Receptors Mediate a Slow Postsynaptic Current in Hippocampal CA3 Neurons. *Nature* **1997**, *388* (6638), 182–186.
- (23) Vignes, M.; Collingridge, G. L. The Synaptic Activation of Kainate Receptors. *Nature* **1997**, *388* (6638), 179–182.
- (24) Gautam, D.; Naik, U. P.; Naik, M. U.; Yadav, S. K.; Chaurasia, R. N.; Dash, D. Glutamate Receptor Dysregulation and Platelet Glutamate Dynamics in Alzheimer's and Parkinson's Diseases: Insights into Current Medications. *Biomolecules* **2023**, *13* (11), 1609.
- (25) Fu, H.; Chen, Z.; Josephson, L.; Li, Z.; Liang, S. H. Positron Emission Tomography (PET) Ligand Development for Ionotropic Glutamate Receptors: Challenges and Opportunities for Radiotracer Targeting N-Methyl-D-Aspartate (NMDA), α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid (AMPA), and Kainate Receptors: Miniperspective. *J. Med. Chem.* **2019**, *62* (2), 403–419.
- (26) Zarate, C. A.; Manji, H. K. The Role of AMPA Receptor Modulation in the Treatment of Neuropsychiatric Diseases. *Exp. Neurol.* **2008**, *211* (1), 7–10.
- (27) Das, A.; Wallace, G. C.; Holmes, C.; McDowell, M. L.; Smith, J. A.; Marshall, J. D.; Bonilha, L.; Edwards, J. C.; Glazier, S. S.; Ray, S. K.; Banik, N. L. Hippocampal Tissue of Patients with Refractory Temporal Lobe Epilepsy Is Associated with Astrocyte Activation, Inflammation, and Altered Expression of Channels and Receptors. *Neurosci.* **2012**, *220*, 237–246.
- (28) Aller, M. I.; Pecoraro, V.; Paternain, A. V.; Canals, S.; Lerma, J. Increased Dosage of High-Affinity Kainate Receptor Gene *Grik4* Alters Synaptic Transmission and Reproduces Autism Spectrum Disorders Features. *J. Neurosci.* **2015**, *35* (40), 13619–13628.
- (29) Greenwood, T. A.; Lazzaroni, L. C.; Calkins, M. E.; Freedman, R.; Green, M. F.; Gur, R. E.; Gur, R. C.; Light, G. A.; Nuechterlein, K. H.; Olincy, A.; Radant, A. D.; Seidman, L. J.; Siever, L. J.; Silverman, J. M.; Stone, W. S.; Sugar, C. A.; Swerdlow, N. R.; Tsuang, D. W.; Tsuang, M. T.; Turetsky, B. I.; Braff, D. L. Genetic Assessment of Additional Endophenotypes from the Consortium on the Genetics of Schizophrenia Family Study. *Schizophr. Res.* **2016**, *170* (1), 30–40.

- (30) Catches, J. S.; Xu, J.; Contractor, A. Genetic Ablation of the GluK4 Kainate Receptor Subunit Causes Anxiolytic and Antidepressant-like Behavior in Mice. *Behav Brain Res.* **2012**, *228* (2), 406–414.
- (31) Francotte, P.; De Tullio, P.; Goffin, E.; Dintilhac, G.; Graindorge, E.; Fraikin, P.; Lestage, P.; Danober, L.; Thomas, J.-Y.; Caignard, D.-H.; Pirotte, B. Design, Synthesis, and Pharmacology of Novel 7-Substituted 3,4-Dihydro-2 H -1,2,4-Benzothiadiazine 1,1-Dioxides as Positive Allosteric Modulators of AMPA Receptors. *J. Med. Chem.* **2007**, *50* (13), 3153–3157.
- (32) Nørholm, A.-B.; Francotte, P.; Olsen, L.; Krintel, C.; Frydenvang, K.; Goffin, E.; Challal, S.; Danober, L.; Botez-Pop, I.; Lestage, P.; Pirotte, B.; Kastrup, J. S. Pharmacological and Structural Characterization, and Thermodynamic Aspects of GluA2-Positive Allosteric Modulators with a 3,4-Dihydro-2 H -1,2,4-Benzothiadiazine 1,1-Dioxide Scaffold. *J. Med. Chem.* **2013**, *56* (21), 8736–8745.
- (33) Gagnon, A.; Duplessis, M.; Fader, L. Arylcyclopropanes: Properties, Synthesis and Use in Medicinal Chemistry. *Org. Prep. Proced. Int.* **2010**, *42* (1), 1–69.
- (34) Bay, Y.; Venskutonytė, R.; Frantsen, S. M.; Thorsen, T. S.; Musgaard, M.; Frydenvang, K.; Francotte, P.; Pirotte, B.; Biggin, P. C.; Kristensen, A. S.; Boesen, T.; Pickering, D. S.; Gajhede, M.; Kastrup, J. S. Small-molecule Positive Allosteric Modulation of Homomeric Kainate Receptors GluK1–3: Development of Screening Assays and Insight into GluK3 Structure. *FEBS J.* **2024**, *291* (7), 1506–1529.