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Brief Article

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Synthesis and Biological Evaluation of Novel Neuroprotective Pyridazine Derivatives as Excitatory Amino Acid Transporter 2 (EAAT2) Activators

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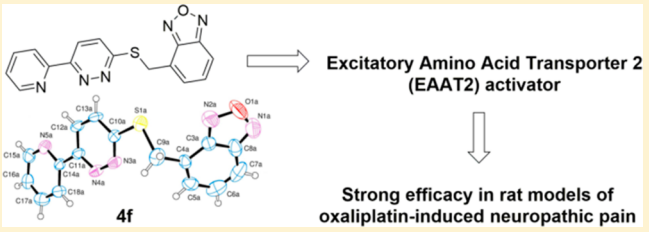
Supporting Information

ABSTRACT: LDN-212320 (**3**) was found to be a potent EAAT2 activator at a translational level, restoring the normal clearance of glutamate and providing neuronal protection. Since the pharmacologic activation of EAAT2 represents a valuable strategy to relieve neuropathic pain, we synthesized novel activators (**4a**–**f**) of EAAT2. Among them **4f**, analyzed in comparison with **3** by different paradigms in a rat model of oxaliplatin-induced neuropathic pain, showed the better antihypersensitive profile being able to fully counteract the oxaliplatin-induced neuropathy.



INTRODUCTION

In mammals, the glutamate is the prevalent excitatory neurotransmitter in the central nervous system (CNS).[1] It is necessary for regular brain activity including memory, learning, and cognition. In order to avoid overstimulation of glutamate receptors that can lead to neuronal damages or death, the concentration of glutamate in the extracellular environment must be lower than ∼1 μM, which represents the excitotoxic level. Inappropriate regulation of glutamatergic neurotransmission and the consequent excitotoxicity lead to an array of somatic, psychiatric, and neurological diseases, including neuropathic pain.[1]

The tight coupling of excitatory neurotransmitter release with rapid reuptake represents a pivotal element of the regular sensory neurotransmission.

Interplay between the increase in primary afferent activity and the impairment of glutamate reuptake at afferent synapses was suggested as a possible mechanism of neuropathy.[1] The excitatory amino acid transporters (EAATs), a family of specific high-affinity membrane transporters, mediate the recovery of synaptic glutamate. Five EAATs (EAAT1–5) from mammals have been discovered. EAAT1–3 correspond to rodents GLAST, GLT-1, and EAAC1, respectively.[2] In the CNS, EAAT1 and EAAT2 are found principally on presynaptic processes of astrocytes tightly linked to excitatory synaptic interactions being able to keep low extracellular glutamate concentrations. In particular, EAAT2 is accountable for up to 80–90% of the total extracellular glutamate uptake activity.[2]

The neuronal pathological changes observed during the neuropathic pain suggested an impaired or altered function of EAAT2 in the proximity of afferent synapses. The down-regulation of EAAT2 in the dorsal horn of the spinal cord was related to hyperalgesia induced by chronic nerve pain, chemotherapy, and morphine tolerance. Furthermore, the inhibition of the glial glutamate transporter in the dorsal horn, elicited by drugs, hypersensitized animals to peripheral stimulation.[3]

Due to the abundant distribution in the CNS, GLT-1/EAAT2 represents novel potential targets for preventing excessive glutamate accumulation.[4]

Although there is scarce information regarding the exact mechanism that modulates GLT-1/EAAT2 or the other Na^+-dependent glutamate transporters, findings suggest that the transcriptional and/or post-transcriptional events control the expression of GLT-1/EAAT2.[5]

The upregulation of EAAT2 induced by ceftriaxone (**1**)[6] or gene transfer[7] prevented the development of pathological pain evoked by nerve injury.

Albeit β-lactams have been traditionally employed as antimicrobial agents, a remarkable additional effect in the

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host was described by Rothstein et al.[8] The results indicated β-lactam antibiotics as effective enhancer of GLT-1 expression, and among them **1** was identified as transcriptional activator of EAAT2, being able to increase GLT-1/EAAT2 expression in a concentration-dependent manner. Neuroprotective activity of **1** from glutamate toxicity was effective in in vitro models of motor neuron deterioration and ischemic injury. Furthermore, **1** was found to alter EAAT2 gene expression, to enhance glutamate transport in primary human fetal astrocytes, to delay loss of neuronal cells, to increase muscle strength, and to enhance mouse survival in an in vivo model of amyotrophic lateral sclerosis (ALS).[9] The activation of the expression of glial GLT-1 led to an antinociceptive effect in diverse rodent pains comprising neuropathic pain.[10] It was reported that extracellular levels of glutamate were considerably boosted after repeated chemotherapeutic bortezomib administration to rats. β-Lactam antibiotics (i.e., **1**) can significantly and selectively stimulate glutamate transporter expression and function, enhancing glutamate uptake and modulating extracellular glutamate homeostasis at the spinal level.[11] It was found that in rats co-treated with **1**, bortezomib-induced hyperalgesic effect could be precluded and SNCV (i.e., sensory nerve conduction velocity, a well-known neuropathy marker) might be kept at basal value showing that the neuropathy was associated with glutamate homeostasis.[12]

Even if the possibility of a direct stimulation of EAAT2 activity has been suggested,[13] these actions appear mediated by means of an augmented transcription of the EAAT2 gene.[14] Antibiotic **1** stimulated nuclear translocation of p65 and initiation of the transcription nuclear factor κB (NF-κB).[14] Activated NF-κB then binds to the NF-κB binding site of the GLT-1/EAAT2 promoter region and upregulates the transcription of this gene. So a decrease of glutamate concentration in the synaptic space relieves the neurotoxic effects of excessive glutamate.[14]

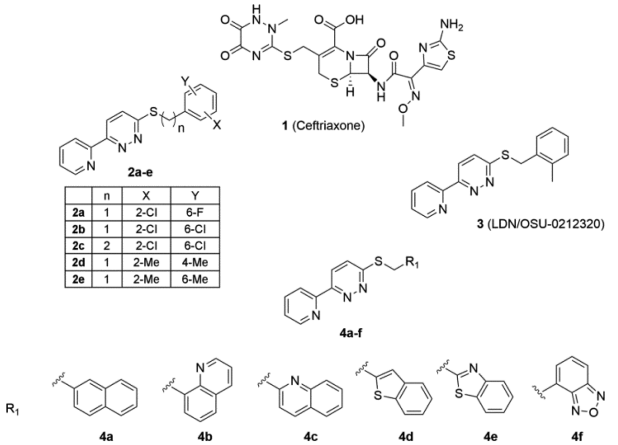
Unfortunately, the exact mechanism by which **1** increases EAAT2 gene expression is still not clarified. Because EAAT2 can be upregulated by transcriptional or translational activation, the continuous efforts to develop EAAT2 activators have spurred the discovery of novel chemical entities.[15] In this way, Colton et al.[16] by means of a high-throughput screening (HTS) in search of compounds able to increase EAAT2 translation succeeded in the development of 16 classes of structurally unrelated molecules from which a pyridazine-based series (**2a–e**) was further investigated by means of structure–activity relationship (SAR) analysis for elevating EAAT2 protein levels.[17] It was demonstrated that the 2-pyridyl, the pyridazine, and the benzyl thioether moieties were required for activity and that compounds containing a methyl substituted benzyl group, 2,6-dichlorobenzyl ring, and 2-(2-Cl-6-F-phenylethyl) substituent on the sulfur linker significantly increased EAAT2 protein levels.[17]

Thiopyridazine **2a** caused a dose-dependent increase in EAAT2 protein levels after a 24 h exposure. Modification of the benzyl thioether resulted in several derivatives (**2b–d**) that increased EAAT2 levels by >6-fold at <5 μM after 24 h. Furthermore, **2a** increased EAAT2 levels 3.5- to 3.9-fold after 24 h with an EC_{50} of 0.5 μM. In this series, **3** (3-[(2-methylbenzyl)sulfenyl]-6-(pyridine-2-yl)pyridazine) subsequently reported as LDN/OSU-0212320[18] enhanced EAAT2 levels only 3.5- to 0.3-fold after 24 h, and successive studies by Lin et al. demonstrated that in an in vivo study, **3** showed strong potency along with satisfactory pharmacokinetic parameters without relevant toxic profile at the tested doses. Moreover, **3** was able to protect cultured neuronal cells from glutamate-mediated excitotoxic injury and death via translational EAAT2 activation.[18] Notably, **3** evidenced a lot of additional effects that resulted in beneficial epilepsy and ALS animal models proposing that this new approach is potentially useful for treating both chronic and acute neurodegenerative disorders.[1]

RESULTS AND DISCUSSION

On the basis of these findings, we expanded our main interest in neuroprotective agents,[19−21] focusing our attention on the search for new EAAT2 activators based on the pyridazine scaffold of **3**. Presently, we describe the chemical synthesis and the analysis of biological profile of hitherto unknown compounds **4a–f** (Chart 1) in which the benzyl moiety of **3** was replaced by more lipophilic aryl or heteroaryl substituents.

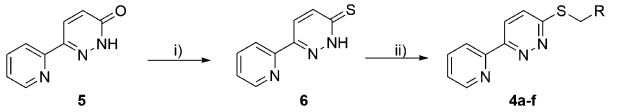
Chart 1. Reference and Title Compounds



	n	X	Y
2a	1	2-Cl	6-F
2b	1	2-Cl	6-Cl
2c	2	2-Cl	6-Cl
2d	1	2-Me	4-Me
2e	1	2-Me	6-Me

Briefly, as outlined in Scheme 1, **4a–f** were prepared using pyridyl-pyridazinone **5** as the starting material synthesized according to a previously reported procedure.[22] The transformation of ketone **5** by means of Lawesson's reagent in dry DMF at reflux instead of P_2S_5 in pyridine at 120 °C[17] afforded pyridazinthione **6** that was in turn alkylated by treatment with the appropriate aryl- or heteroarylmethyl bromide to give the expected thioethers **4a–f**.

Scheme 1[a]



[a]Reagents and conditions: (i) Lawesson reagent, dry toluene, reflux, 4 h; (ii) K_2CO_3, dry DMF, $BrCH_2R$, rt, from 1 to 20 h.

All six compounds were screened for pan assay interference compounds (PAINS),[23] and none of them were found to contain structural features recognized as "frequent hitters" in high throughput screens.

The pain relieving effects of the novel synthesized EAAT2 activators (**4a–f**) were preclinically evaluated in a model of neuropathic pain induced by oxaliplatin in comparison to **3**.[17] The anticancer drug oxaliplatin is a cytotoxic agent belonging to the diaminocyclohexane platinum family. The compound is

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currently used as first-line chemotherapy in metastatic colorectal cancer, and it represents a relevant option as adjuvant therapy for neoplastic diseases of different tissues. A painful neuropathy that persists between cycles is the limiting side effect, tightly correlated with specific alterations of the nervous system.[24] The repeated administration of oxaliplatin (2.4 mg kg^{-1} ip, daily) induced decrease of pain threshold starting from day 7 of treatment. In the present study, the assessment of a response to a noxious mechanical stimulus was attained by means of the paw pressure test[25] (Table 1).

Table 1. Effect of Repeated Administration of Compounds 3 and 4a−f on Oxaliplatin Induced Hyperalgesia in the Rat Paw Pressure Test[a]

		weight (g)	
		day	
treatment	dose, mg kg^{-1} sc	7 after treatment	14 after treatment
vehicle + vehicle		62.5 ± 0.5	61.4 ± 0.7
oxaliplatin + vehicle		46.1 ± 0.5***	44.7 ± 1.2***
oxaliplatin + **3**	3	57.1 ± 0.5^^^	57.5 ± 0.3^^^
oxaliplatin + **4a**	3.37	51.9 ± 1.0^^	51.4 ± 0.7^^
oxaliplatin + **4b**	3.37	51.3 ± 1.3^^	52.1 ± 0.5^^
oxaliplatin + **4c**	3.37	48.4 ± 1.2	50.0 ± 0.5^
oxaliplatin + **4d**	3.42	55.3 ± 0.9^^	59.1 ± 1.0 ^^^
oxaliplatin + **4e**	3.43	54.7 ± 0.9^^	56.8 ± 1.0^^
oxaliplatin + **4f**	3.28	58.6 ± 0.6^^^	62.8 ± 1.3^^^

[a]Pain: mechanical noxious stimulus. Oxaliplatin (2.4 mg kg^{-1}, ip) was administered daily ip for 2 weeks dissolved in glucose solution (5%). Compounds were dissolved in 1% DMSO/1% polyethylene glycol 400/0.2% Tween 80/10% hydroxypropyl-β-cyclodextrin/saline and administered daily sc for 2 weeks, starting from the first day of oxaliplatin administration. Control animals received vehicles. Paw pressure test was used to measure the sensitivity to a mechanical noxious stimulus on days 7 and 14. ***P < 0.001 vs vehicle + vehicle treated animals; ^P < 0.05, ^^P < 0.01, and ^^^P < 0.001 vs oxaliplatin + vehicle treated rats. Each value represents the mean of 10 rats.

In oxaliplatin + vehicle treated rats, the weight tolerated on the posterior paw progressively decreased to 46.1 ± 0.5 g on day 7 and to 44.7 ± 1.2 g on day 14 in comparison to the control value of about 62 g (Table 1).

Compound **3** (3 mg kg^{-1} sc) was administered daily starting from the first day of oxaliplatin treatment. Compounds **4a−f** were subcutaneously administered at doses equimolar to **3**, following the same experimental protocol. Compound **3** limited the mechanical hypersensitivity induced by oxaliplatin treatment and increased the weight tolerated on posterior paw by 67.1% on day 7 and 76.4% on day 14. Among the new compounds, **4f** (3.28 mg kg^{-1}) showed the better antihypersensitive profile. It was able to fully counteract the oxaliplatin-induced neuropathy on day 14. Compound **4d** (3.42 mg kg^{-1}) displayed a higher antihypersensitivity effect in comparison to **3**, increasing the pain threshold by 86.2% on day 14. A lower efficacy was recorded on the same day with 3.43 mg kg^{-1} of **4e** (72.5%), while **4a** (3.37 mg kg^{-1}), **4b** (3.37 mg kg^{-1}), and **4c** (3.37 mg kg^{-1}) counteracted the oxaliplatin-induced mechanical hypersensitivity by 40.1%, 44.3%, and 31.7%, respectively (Table 1).

The pain threshold to thermal noxious stimulus was evaluated by plantar test,[26] applying a heat stimulation to the posterior paw with a 30 s cutoff time (Table 2). Oxaliplatin

Table 2. Effect of Repeated Administration of Compounds 3 and 4a−f on Oxaliplatin Induced Thermal Hyperalgesia in the Rat Plantar Test[a]

		withdrawal latency (s)	
		day	
treatment	dose, mg kg^{-1} sc	7 after treatment	14 after treatment
vehicle + vehicle		16.3 ± 1.0	17.8 ± 0.2
oxaliplatin + vehicle		9.0 ± 1.0**	10.6 ± 0.5***
oxaliplatin + **3**	3	14.1 ± 0.5	18.3 ± 0.3^^^
oxaliplatin + **4a**	3.37	14.2 ± 0.3	15.5 ± 0.6^^
oxaliplatin + **4b**	3.37	12.5 ± 1.5	13.8 ± 1.2
oxaliplatin + **4c**	3.37	11.6 ± 0.8	13.7 ± 1.5
oxaliplatin + **4d**	3.42	12.8 ± 1.4	15.1 ± 0.8^^
oxaliplatin + **4e**	3.43	14.3 ± 2.1	15.8 ± 0.7^^
oxaliplatin + **4f**	3.28	15.2 ± 0.6^^	18.7 ± 1.3^^^

[a]Pain: thermal noxious stimulus. Oxaliplatin (2.4 mg kg^{-1}, ip) was dissolved in 5% glucose solution and administered daily ip for 2 weeks. Compounds were dissolved in 1% DMSO/1% polyethylene glycol 400/0.2% Tween 80/10% hydroxypropyl-β-cyclodextrin/saline and administered daily sc for 2 weeks, starting from the first day of oxaliplatin administration. Control animals received vehicles. Plantar test was used to measure the sensitivity to a thermal noxious stimulus on days 7 and 14. **P < 0.01 and ***P < 0.001 vs vehicle + vehicle treated animals; ^^P < 0.01 and ^^^P < 0.001 vs oxaliplatin + vehicle treated rats. Each value represents the mean of 10 rats.

reduced the withdrawal latency from about 17 s (vehicle + vehicle) to 9.0 ± 1.0 s on day 7 and 10.6 ± 0.5 s on day 14. Compounds **3** and **4f** fully counteracted thermal hypersensitivity induced by oxaliplatin on day 14. Compounds **4a**, **4d**, and **4e** were partially active while **4b** and **4c** were not effective (Table 2).

Von Frey[27] and cold plate test[24] allowed evaluating the sensitivity to stimuli which usually do not provoke pain (Tables 3 and 4, respectively). The withdrawal threshold to a non-noxious mechanical stimulus diminished in oxaliplatin-treated animals from 23.7 ± 0.6 g (vehicle + vehicle) to 12.4 ± 1.2 g (oxaliplatin + vehicle) on day 7 and from 23.1 ± 0.4 g (vehicle + vehicle) to 11.6 ± 0.5 g (oxaliplatin + vehicle) on day 14 (Table 3). On day 14, repeated treatment with 3 mg kg^{-1} of **3** increased the withdrawal threshold by 75.9% while 3.28 mg kg^{-1} of **4f** fully prevented the pain threshold alteration, reaching a value of 23.8 ± 1.0 g. Compounds **3** and **4f** were partially effective also at day 7 (withdrawal threshold increase of 51.3% and 62.8%, respectively).

Treatment with **4b**, **4d**, and **4e** partially counteracted the neuropathic state on day 14, with an increase of the pain threshold of 68.7%, 64.3%, and 67.8%, respectively. Compounds **4a** and **4c** showed lower efficacy (53% and 33%, respectively) (Table 3). The sensitivity to a cold non-noxious stimulus is shown in Table 4 (cold plate test). The licking latency decreased from about 19 s (vehicle + vehicle) to 14.3 ± 0.3 s on day 7 and 12.0 ± 1.2 s on day 14. Repeated administration of **3** and **4f** fully prevented the pain threshold alteration on day 14 (22.5 ± 0.5 s and 22.0 ± 0.8 s, respectively). Compound **4b** reached a value of 18.0 ± 0.2 s while **4a**, **4d**, and **4e** partially counteracted the thermal hypersensitivity induced by oxaliplatin (Table 4).

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Table 3. Effect of Repeated Administration of Compounds 3 and 4a−f on Oxaliplatin-Induced Allodynia in the Rat Von Frey Test[a]

		withdrawal latency (s)	
		day	
treatment	dose, mg kg^{-1} sc	7 after treatment	14 after treatment
vehicle + vehicle		23.7 ± 0.6	23.1 ± 0.4
oxaliplatin + vehicle		12.4 ± 1.2***	11.6 ± 0.5***
oxaliplatin + **3**	3	18.2 ± 0.3^	20.6 ± 0.6^^^
oxaliplatin + **4a**	3.37	17.6 ± 0.7^	17.7 ± 0.4^^
oxaliplatin + **4b**	3.37	17.6 ± 0.7^	19.5 ± 0.5^^^
oxaliplatin + **4c**	3.37	13.5 ± 1.0	15.4 ± 0.7^
oxaliplatin + **4d**	3.42	18.8 ± 0.5^^	19.0 ± 0.6^^^
oxaliplatin + **4e**	3.43	16.4 ± 0.4^	19.4 ± 0.8^^
oxaliplatin + **4f**	3.28	19.5 ± 0.6^^^	23.8 ± 1.0^^^

[a]Pain: mechanical non-noxious stimulus. Oxaliplatin (2.4 mg kg^{-1}, ip) was administered daily ip for 2 weeks dissolved in glucose solution (5%). Compounds were dissolved in 1% DMSO/1% polyethylene glycol 400/0.2% Tween 80/10% hydroxypropyl-β-cyclodextrin/saline and administered daily sc for 2 weeks, starting from the first day of oxaliplatin administration. Control animals were treated with vehicles. Von Frey test was used to measure the sensitivity to a mechanical non-noxious stimulus on days 7 and 14. ***P < 0.001 vs vehicle + vehicle treated animals; ^P < 0.05, ^^P < 0.01, and ^^^P < 0.001 vs oxaliplatin + vehicle treated rats. Each value represents the mean of 10 rats.

Table 4. Effect of Repeated Administration of Compounds 3 and 4a−f on Oxaliplatin Induced Allodynia in the Rat Cold Plate Test[a]

		withdrawal latency (s)	
		day	
treatment	dose, mg kg^{-1} sc	7 after treatment	14 after treatment
vehicle + vehicle		19.0 ± 0.6	18.3 ± 0.9
oxaliplatin + vehicle		14.3 ± 0.3**	12.0 ± 1.2***
oxaliplatin + **3**	3	17.0 ± 0.2^	22.5 ± 0.5^^^
oxaliplatin + **4a**	3.37	16.0 ± 0.6	16.5 ± 0.4
oxaliplatin + **4b**	3.37	18.0 ± 1.0^	18.0 ± 0.2^^
oxaliplatin + **4c**	3.37	14.5 ± 1.2	15.6 ± 0.3
oxaliplatin + **4d**	3.42	17.8 ± 0.6^	17.5 ± 0.4^^
oxaliplatin + **4e**	3.43	15.8 ± 0.4	16.3 ± 1.3
oxaliplatin + **4f**	3.28	19.4 ± 1.3^^	22.0 ± 0.8^^^

[a]Pain: thermal non-noxious stimulus. Oxaliplatin (2.4 mg kg^{-1}, ip) was administered daily ip for 2 weeks dissolved in glucose solution (5%). Compounds were dissolved in 1% DMSO/1% polyethylene glycol 400/0.2% Tween 80/10% hydroxypropyl-β-cyclodextrin/saline and administered daily sc for 2 weeks, starting from the first day of oxaliplatin administration. Control animals were treated with vehicles. Cold plate test was used to measure the sensitivity to a thermal non-noxious stimulus on days 7 and 14. **P < 0.01 and ***P < 0.001 vs vehicle + vehicle treated animals; ^P < 0.05, ^^ P < 0.01 and ^^^P < 0.001 vs oxaliplatin + vehicle treated rats. Each value represents the mean of 10 rats.

CONCLUSIONS

The imbalance concerning the release and reuptake of glutamate may cause disturbances in the neuronal signaling, leading to neurological and psychiatric disorders, including neuropathic pain. The high-affinity membrane excitatory amino acid transporter EAAT2 mediates the recovery of glutamate from the synaptic space. When EAAT2 function is impaired or altered in the proximity of afferent synapses, neuronal and glial pathological changes occur, evoking painful hypersensitivity. Considering EAAT2 a potential target, a small series of EAAT2 activators **4a−f** was developed and tested in animal model of neuropathic pain induced by oxaliplatin. Using **3** as the reference compound, we assessed the response to different noxious and non-noxious mechanical and thermal stimuli after repeated administration of the anticancer drug (2.4 mg kg^{-1} ip, daily). Among the novel compounds, **4f** (3.28 mg kg^{-1}) showed the best antihypersensitive profile being able to fully counteract the oxaliplatin-induced neuropathy on day 14. These data suggested that the introduction of an appropriate and more lipophilic heteroarylmethyl thioether in the pyridyl-pyridazine scaffold of **3** results in an increase in the pain threshold.

In this way, **4f** is a candidate to deep preclinical studies aimed to characterize its potentiality against pain. The pharmacodynamic profile of the molecule deserves investigations about the possible modulation of EAAT2. Both the enhancement of glutamate transporter function and expression should be considered.

EXPERIMENTAL SECTION

General Methods. All chemicals used were of reagent grade. Yields refer to purified products and are not optimized. Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Merck silica gel 60 (230−400 mesh) was used for column chromatography. Merck TLC plates, silica gel 60 F254 were used for TLC. NMR spectra were recorded by means of Bruker AC 200 and Bruker DRX 400 AVANCE spectrometers in the indicated solvents (TMS as internal standard); the values of the chemical shifts are expressed in ppm and the coupling constants (J) in Hz. Mass spectra were recorded on a ThermoFinnigan LCQ-Deca or an Agilent 1100 LC/MSD instrument. High resolution mass spectra (HMRS) were recorded on LTQ Orbitrap (ThermoFisher). The purity of compounds **4a−f** was assessed by RP-HPLC and was found to be higher than 95%. An Agilent 1100 series system equipped with a Zorbax Eclipse XDB-C8 (4.6 mm × 150 mm, 5 μm) column was used in the HPLC analysis with acetonitrile−methanol−water (50:30:20) as the mobile phase at a flow rate of 1.0 mL/min. UV detection was achieved at 254 nm.

General Procedure for the Synthesis of Pyridyl-pyridazine Thioethers (4a−f). Compounds **4a−f** were synthesized by reaction of **6** (0.53 mmol) with the suitable bromomethyl derivative (0.80 mmol) in dry DMF (5.0 mL) using K_2CO_3 as the base. The resulting mixture was stirred at room temperature for the suitable time (from 90 min to 20 h). At the end of reaction the inorganic material was filtered off and washed with EtOAc. The resulting organic filtrate was washed with water (3 × 10 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude was purified by flash chromatography on silica gel, eluting with petroleum ether/EtOAc (from 8:2 to 6:4 v/v) to give the expected compounds as solids.

4-((6-(Pyridin-2-yl)pyridazin-3-ylthio)methyl)benzo[*c*][1,2,5]-oxadiazole (4f). Compound **4f** was obtained after 3 h of reaction time (yield 99%) as a yellow solid (mp 177.4−178.2 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.04 (s, 2H), 7.29−7.37 (m, 2H),7.43 (d, J = 8.8, 1H), 7.60 (d, J = 7.2, 1H), 7.70 (d, J = 8.8, 1H), 7.85 (t, J = 7.8, 1H), 8.34 (d, J = 8.8, 1H), 8.57 (d, J = 8.0, 1H), 8.67 (d, J = 4.8, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): 29.8, 115.3, 121.1, 124.1, 124.6, 126.7, 126.9, 130.5, 131.6, 137.1, 149.0, 149.4, 153.2, 156.1, 160.8. MS (ESI): m/z 321.8 (M + H$^+$). An analytical sample was obtained by recrystallization from ethyl acetate. HRMS (ESI): m/z calculated for [$C_{16}H_{11}N_5OS$ + H$^+$] requires 322.0757, found 322.0760.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b00383.

Experimental procedures and X-ray crystallography studies (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

In memory of our beloved colleague, Prof. Jacopo Magistretti, Neurophysiologist (University of Pavia, Italy), who recently passed away.

■ ABBREVIATIONS USED

EAAT, excitatory amino acid transporter; CNS, central nervous system; SNCV, sensory nerve conduction velocity; GLT-1, glutamate transporter 1; GLAST, glutamate−aspartate transporter

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