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# Synthesis and Biological Evaluation of Novel Neuroprotective Pyridazine Derivatives as Glutamate Transporter EAAT2 Activators

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**KEYWORDS:** *Glutamate Transporter EAAT2, Neuroprotective agents, Neurodegenerative disease.*

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**ABSTRACT:** Glutamate is an excitatory neurotransmitter responsible for memory formation, learning and superior cognitive function. Because an excess of glutamate in the synaptic cleft results in neuronal injury known as excitotoxicity, a small molecule pyridazine derivative 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. Having in mind that the pharmacologic activation of EAAT2 could be a valuable strategy to relieve neuropathic pain, a small series of more lipophilic bicyclic aromatic and heteroaromatic thioethers was synthesized as novel activators (**4a-f**) of glutamate transporter EAAT2. These compounds, based on the pyridazine scaffold, were analysed in comparison with reference compound **3** in different rat models of oxaliplatin-induced neuropathic pain and among them compound **4f** (3.21 mg kg<sup>-1</sup>) showed the better anti-hypersensitive profile being able to fully counteract the oxaliplatin-induced neuropathy on day 14.

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Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system,<sup>1</sup> essential for normal brain function including memory, cognition and learning. However, the extracellular concentration of glutamate must remain below excitotoxic levels (~1 μM) to avoid overstimulation of glutamate receptors, leading to neuronal **damages** or death. Inappropriate regulation of glutamatergic neurotransmission and the consequent excitotoxicity leads to a spectrum of somatic, neurological and psychiatric disorders, including neuropathic pain.<sup>1</sup>

One of the key components of normal sensory neurotransmission is the tight coupling of excitatory neurotransmitter release with rapid reuptake. Interplay between the increase in primary afferent activity and the impairment of glutamate reuptake at afferent synapses, has been proposed as a possible mechanism for the development of neuropathy.<sup>1</sup> The recovery of synaptic glutamate is mediated by a family of specific high-affinity membrane transporters, the excitatory amino acid transporters (EAATs). Five mammalian EAATs have been cloned and characterized: EAAT1 (known as GLAST in rodents), EAAT2 (known as GLT-1 in rodents), EAAT3 (known as EAAC1 in rodents), EAAT4 and EAAT5.<sup>2</sup> In the central nervous system (CNS), EAAT1 and **EAAT2** are found primarily on presynaptic processes of astrocytes closely associated with excitatory synaptic contacts and are responsible for maintaining low extracellular

glutamate concentrations. In particular, EAAT2 is responsible for up to 80–90% of all extracellular glutamate uptake activity.<sup>2</sup>

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 associated with hyperalgesia induced by chronic nerve **pain**, chemotherapy, and morphine tolerance. Furthermore, the pharmacological inhibition of the glial glutamate transporter in the dorsal **horn** hyper sensitized animals to peripheral stimulation.<sup>3</sup>

Due to the abundant distribution in the CNS, all the transporter subtypes, GLT-1/EAAT2, represent novel potential targets for preventing excessive glutamate **accumulation**.<sup>4</sup>

Although there is scarce information about the mechanisms that regulate GLT-1/EAAT2 or the other Na<sup>+</sup>-dependent glutamate transporters, findings suggest that the expression of GLT-1/EAAT2 is regulated by transcriptional and/or post-transcriptional processes.<sup>5</sup>

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

Although beta-lactams have been historically used as antimicrobials, a notable ancillary effect in the host was identified by Rothstein et al.<sup>8</sup> These studies identified

beta-lactam antibiotics as potent stimulators of GLT-1 expression and among them **1**, was identified as transcriptional activator of EAAT2 being able to increase the expression of GLT-1/EAAT2 in a concentration-dependent manner. Neuroprotective activity of **1** from glutamate toxicity was effective in *in vitro* models of ischemic injury and motor neuron degeneration. Furthermore, **1** has been shown to induce EAAT2 gene expression and to enhance glutamate transport in primary human fetal astrocytes, to delay loss of neurons, to increase muscle strength, and enhance mouse survival in an animal model of amyotrophic lateral sclerosis (ALS).<sup>9</sup> The activation of the expression of glial GLT-1 protein induced an anti-nociceptive effect in different rodent **pains** including neuropathic.<sup>10</sup> It was reported that extracellular levels of glutamate significantly increased after repeated chemotherapeutic bortezomib administration to rats. Beta-lactam antibiotics such as **1** can greatly and selectively promote glutamate transporter expression and function potentiating glutamate uptake and finally regulating extracellular glutamate homeostasis at spinal level.<sup>11</sup> It was found that in rats co-treated with **1**, bortezomib-induced hyperalgesic effect could be prevented and SNCV (*i. e.* **Sensory Nerve Conduction Velocity, an established neuropathy marker**) could be maintained at basal **values** establishing that the neuropathic state was correlated with glutamate homeostasis.<sup>12</sup>

Moreover, all these actions appear to be mediated through **an** increased transcription of the EAAT2 gene.<sup>13</sup> Antibiotic **1** promoted nuclear translocation of p65 and activation of the transcription nuclear factor-kB (NF-kB).<sup>13</sup> Activated NF-kB then binds to the NF-kB binding site of the GLT-1/EAAT2 promoter region and up-regulates the transcription of this gene, thereby decreasing glutamate concentration in the synaptic cleft and alleviating the potentially neurotoxic effects of excessive glutamate.<sup>13</sup>

However, the precise mechanism by which **1** enhances EAAT2 gene expression remained unclear. So, because EAAT2 can be upregulated by transcriptional or translational activation the continuous search for EAAT2 activators has led to the discovery of a series of small molecules.<sup>14</sup> In this way, Colton et al.<sup>15</sup> by means of a high-throughput screening (HTS) in search of compounds able to increase EAAT2 translation resulted in the development of sixteen classes of compounds from which a pyridazine-based series (**2a-e**) was selected for further investigation, including a structure-activity (SAR) study for elevating EAAT2 protein levels.<sup>16</sup> 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 also 2-(2-Cl-6-F-phenylethyl) substituent on the sulfur linker significantly increased EAAT2 protein levels.<sup>16</sup>

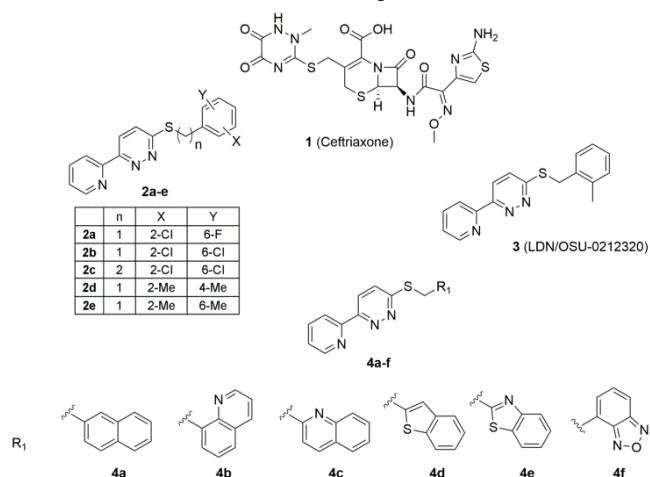
Thiopyridazine **2a** was confirmed to show a dose-dependent increase in EAAT2 protein levels after a 24 h exposure. Modification of the benzylthioether resulted in several derivatives (**2b-d**) that enhanced EAAT2 levels by > 6-fold at concentrations < 5  $\mu$ M after 24 h. In addition,

one of the derivatives (**2a**) enhanced EAAT2 levels 3.5–3.9-fold after 24 h with an EC<sub>50</sub> of 0.5  $\mu$ M. In this **series**, compound **3** (3-[(2-methylbenzyl)sulfonyl]-6-(pyridine-2-yl)pyridazine) **subsequently** reported as LDN/OSU-0212320<sup>17</sup> enhanced EAAT2 levels only 3.5–0.3-fold after 24 h and successive studies by Kong et al. demonstrated that in a murine model, compound **3** had good potency, adequate pharmacokinetic properties, no observed toxicity at the doses examined, and low side effect/toxicity potential. Additionally, compound **3** protected cultured neurons from glutamate-mediated excitotoxic injury and death via translational EAAT2 activation.<sup>17</sup>

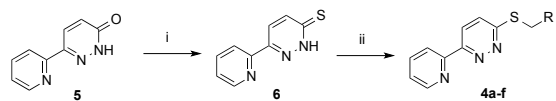
Importantly, **3** evidenced a lot of additional effects that resulted in beneficial ALS and epilepsy animal models suggesting that this novel approach has therapeutic potential for both acute and chronic neurodegenerative diseases.<sup>1</sup>

On the basis of these findings we expanded our main interest on neuroprotective agents<sup>18–20</sup> focusing our attention on the search for new EAAT2 activators based on the pyridazine scaffold of **3**. In this study we report the synthesis and the biological evaluation 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.



Briefly, as outlined in Scheme 1, compounds **4a-f** were prepared using pyridyl-pyridazinone **5** as the starting material synthesized according to a previously reported procedure.<sup>21</sup> The transformation of ketone **5** by means of Lawesson's reagent in dry **toluene** at reflux instead of P<sub>2</sub>S<sub>5</sub> in pyridine at 120 °C<sup>16</sup> 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. Reagents and Conditions:** (i) Lawesson reagent, dry **toluene**, reflux, 4 h; (ii) K<sub>2</sub>CO<sub>3</sub>, dry DMF, BrCH<sub>2</sub>R, r. t., from 1 h to 20 h.

The pain reliever profile of the novel synthesized EEAT<sub>2</sub> activators (**4a-f**) was evaluated in a rat model of oxaliplatin-induced neuropathic pain in comparison to **3**.<sup>16</sup> The anticancer drug oxaliplatin is a cytotoxic agent belonging to the diaminocyclohexane platinum family, it has become a first-line chemotherapy in metastatic colorectal cancer and a valid option as adjuvant therapy in different types of cancer. The limiting side effect is a painful neuropathy that persists between cycles correlating with characteristic alterations of the nervous system.<sup>22</sup> Oxaliplatin repeated treatment (2.4 mg kg<sup>-1</sup> i.p., daily) induced alterations of pain threshold starting from the 7<sup>th</sup> day of administration. In the present study, the response to a noxious mechanical stimulus was evaluated by the Paw pressure test<sup>23</sup> (Table 1).

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).

**Table 1.** Effect of repeated administration of compounds **3** and **4a-f** on oxaliplatin induced hyperalgesia in the rat Paw pressure test.

Treatments	Weight (g)		
	Dose mg kg <sup>-1</sup> s.c.	Day	
		7 after treatment	14 after treatment
vehicle + vehicle		62.5 ± 0.5	61.4 ± 0.7
oxaliplatin + vehicle		46.1 ± 0.5 <sup>***</sup>	44.7 ± 1.2 <sup>***</sup>
oxaliplatin + <b>3</b>	<b>3</b>	57.1 ± 0.5 <sup>^^^</sup>	57.5 ± 0.3 <sup>^^^</sup>
oxaliplatin + <b>4a</b>	<b>3.37</b>	51.9 ± 1.0 <sup>^^</sup>	51.4 ± 0.7 <sup>^^</sup>
oxaliplatin + <b>4b</b>	<b>3.37</b>	51.3 ± 1.3 <sup>^^</sup>	52.1 ± 0.5 <sup>^^</sup>
oxaliplatin + <b>4c</b>	<b>3.37</b>	48.4 ± 1.2	50.0 ± 0.5 <sup>^</sup>
oxaliplatin + <b>4d</b>	<b>3.42</b>	55.3 ± 0.9 <sup>^^</sup>	59.1 ± 1.0 <sup>^^^</sup>
oxaliplatin + <b>4e</b>	<b>3.43</b>	54.7 ± 0.9 <sup>^^</sup>	56.8 ± 1.0 <sup>^^</sup>
oxaliplatin + <b>4f</b>	<b>3.28</b>	58.6 ± 0.6 <sup>^^^</sup>	62.8 ± 1.3 <sup>^^^</sup>

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

Compound **3** (3 mg kg<sup>-1</sup> s.c.) 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** (3.21 mg kg<sup>-1</sup>) showed the better anti-hypersensitive profile. It was able to fully counteract the oxaliplatin-induced neuropathy on day 14. Compound **4d** (3.35 mg kg<sup>-1</sup>) displayed a higher anti-hypersensitivity 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 3.36 mg kg<sup>-1</sup> of **4e** (72.5%) while **4a** (3.37 mg kg<sup>-1</sup>), **4b** (3.38 mg kg<sup>-1</sup>) and **4c** (3.30 mg kg<sup>-1</sup>) 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,<sup>24</sup> applying a heat stimulation to the posterior paw with a 30 s cut-off time (Table 2). Oxaliplatin 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).

**Table 2.** Effect of repeated administration of compounds **3** and **4a-f** on oxaliplatin induced thermal hyperalgesia in the rat Plantar test.

Treatments	Withdrawal latency (s)		
	Dose mg kg <sup>-1</sup> s.c.	Day	
		7 after treatment	14 after treatment
vehicle + vehicle		16.3 ± 1.0	17.8 ± 0.2
oxaliplatin + vehicle		9.0 ± 1.0 <sup>**</sup>	10.6 ± 0.5 <sup>***</sup>
oxaliplatin + <b>3</b>	<b>3</b>	14.1 ± 0.5	18.3 ± 0.3 <sup>^^^</sup>
oxaliplatin + <b>4a</b>	<b>3.37</b>	14.2 ± 0.3	15.5 ± 0.6 <sup>^^</sup>
oxaliplatin + <b>4b</b>	<b>3.37</b>	12.5 ± 1.5	13.8 ± 1.2
oxaliplatin + <b>4c</b>	<b>3.37</b>	11.6 ± 0.8	13.7 ± 1.5
oxaliplatin + <b>4d</b>	<b>3.42</b>	12.8 ± 1.4	15.1 ± 0.8 <sup>^^</sup>
oxaliplatin + <b>4e</b>	<b>3.43</b>	14.3 ± 2.1	15.8 ± 0.7 <sup>^^</sup>
oxaliplatin + <b>4f</b>	<b>3.28</b>	15.2 ± 0.6 <sup>^^</sup>	18.7 ± 1.3 <sup>^^^</sup>

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

Von Frey<sup>25</sup> and Cold plate test<sup>22</sup> allowed evaluating the sensitivity to stimuli which normally do not provoke pain (Tables 3 and 4, respectively). The withdrawal threshold to a non-noxious mechanical stimulus was decreased in

oxaliplatin-treated animals from  $23.7 \pm 0.6$  g (vehicle + vehicle) to  $12.4 \pm 1.2$  g (oxaliplatin + vehicle) on day 7 and from  $23.1 \pm 0.4$  g (vehicle + vehicle) to  $11.6 \pm 0.5$  g (oxaliplatin + vehicle) on day 14 (Table 3). On day 14, repeated treatment with  $3 \text{ mg kg}^{-1}$  of **3** increased the withdrawal threshold by 75.9% while  $3.21 \text{ mg kg}^{-1}$  of **4f** fully prevented the pain threshold alteration, reaching a value of  $23.8 \pm 1.0$  g. Compounds **3** and **4f** were partially effective also at day 7 (withdrawal threshold increase of 51.3% and 62.8%, respectively).

**Table 3.** Effect of repeated administration of compounds **3** and **4a-f** on oxaliplatin-induced allodynia in the rat Von Frey test.

Treatments	Withdrawal latency (s)		
	Dose mg kg <sup>-1</sup> s.c.	Day	
		7 after treatment	14 after treatment
vehicle + vehicle		$23.7 \pm 0.6$	$23.1 \pm 0.4$
oxaliplatin + vehicle		$12.4 \pm 1.2^{***}$	$11.6 \pm 0.5^{***}$
oxaliplatin + <b>3</b>	<b>3</b>	$18.2 \pm 0.3^{\wedge}$	$20.6 \pm 0.6^{\wedge\wedge}$
oxaliplatin + <b>4a</b>	<b>3.37</b>	$17.6 \pm 0.7^{\wedge}$	$17.7 \pm 0.4^{\wedge\wedge}$
oxaliplatin + <b>4b</b>	<b>3.37</b>	$17.6 \pm 0.7^{\wedge}$	$19.5 \pm 0.5^{\wedge\wedge}$
oxaliplatin + <b>4c</b>	<b>3.37</b>	$13.5 \pm 1.0$	$15.4 \pm 0.7^{\wedge}$
oxaliplatin + <b>4d</b>	<b>3.42</b>	$18.8 \pm 0.5^{\wedge\wedge}$	$19.0 \pm 0.6^{\wedge\wedge}$
oxaliplatin + <b>4e</b>	<b>3.43</b>	$16.4 \pm 0.4^{\wedge}$	$19.4 \pm 0.8^{\wedge\wedge}$
oxaliplatin + <b>4f</b>	<b>3.28</b>	$19.5 \pm 0.6^{\wedge\wedge\wedge}$	$23.8 \pm 1.0^{\wedge\wedge\wedge}$

Pain: mechanical non-noxious stimulus. Oxaliplatin ( $2.4 \text{ mg kg}^{-1}$ , i.p.) was dissolved in 5% glucose solution and administered daily i.p. for two weeks. Compounds were dissolved in 1% DMSO/1% polyethylene glycol 400/0.2% Tween 80/10% hydroxypropyl- $\beta$ -cyclodextrin/saline and daily s.c. administered for two 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 noxious stimulus on day 7 and 14.  $^{***}P < 0.001$  vs vehicle + vehicle treated animals;  $^{\wedge}P < 0.05$ ,  $^{\wedge\wedge}P < 0.01$  and  $^{\wedge\wedge\wedge}P < 0.001$  vs oxaliplatin + vehicle treated rats. Each value represents the mean of 10 rats.

Treatment with compounds **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  $14.3 \pm 0.3$  s on day 7 and  $12.0 \pm 1.2$  s on day 14. Repeated administration of **3** and **4f** fully prevented the pain threshold alteration on day 14 ( $22.5 \pm 0.5$  s and  $22.0 \pm 0.8$  s, respectively). Compound **4b** reached a value of  $18.0 \pm 0.2$  s while compounds **4a**, **4d**, and **4e** partially counteracted the thermal hypersensitivity induced by oxaliplatin (Table 4).

**Table 4.** Effect of repeated administration compounds **3** and **4a-f** on oxaliplatin induced allodynia in the rat Cold plate test.

Treatments	Withdrawal latency (s)		
	Dose mg kg <sup>-1</sup> s.c.	Day	
		7 after treatment	14 after treatment
vehicle + vehicle		$19.0 \pm 0.6$	$18.3 \pm 0.9$
oxaliplatin + vehicle		$14.3 \pm 0.3^{**}$	$12.0 \pm 1.2^{***}$
oxaliplatin + <b>3</b>	<b>3</b>	$17.0 \pm 0.2^{\wedge}$	$22.5 \pm 0.5^{\wedge\wedge\wedge}$
oxaliplatin + <b>4a</b>	<b>3.37</b>	$16.0 \pm 0.6$	$16.5 \pm 0.4$
oxaliplatin + <b>4b</b>	<b>3.37</b>	$18.0 \pm 1.0^{\wedge}$	$18.0 \pm 0.2^{\wedge\wedge}$
oxaliplatin + <b>4c</b>	<b>3.37</b>	$14.5 \pm 1.2$	$15.6 \pm 0.3$
oxaliplatin + <b>4d</b>	<b>3.42</b>	$17.8 \pm 0.6^{\wedge}$	$17.5 \pm 0.4^{\wedge\wedge}$
oxaliplatin + <b>4e</b>	<b>3.43</b>	$15.8 \pm 0.4$	$16.3 \pm 1.3$
oxaliplatin + <b>4f</b>	<b>3.28</b>	$19.4 \pm 1.3^{\wedge\wedge}$	$22.0 \pm 0.8^{\wedge\wedge\wedge}$

Pain: thermal non-noxious stimulus. Oxaliplatin ( $2.4 \text{ mg kg}^{-1}$ , i.p.) was dissolved in 5% glucose solution and administered daily i.p. for two weeks. Compounds were dissolved in 1% DMSO/1% polyethylene glycol 400/0.2% Tween 80/10% hydroxypropyl- $\beta$ -cyclodextrin/saline and daily s.c. administered for two 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 day 7 and 14.  $^{**}P < 0.01$  and  $^{***}P < 0.001$  vs vehicle + vehicle treated animals;  $^{\wedge}P < 0.05$ ,  $^{\wedge\wedge}P < 0.01$  and  $^{\wedge\wedge\wedge}P < 0.001$  vs oxaliplatin + vehicle treated rats. Each value represents the mean of 10 rats.

## Conclusions

Any imbalance between the release and reuptake of glutamate may lead to disturbances in the neuronal signalling between the neurons, which may lead to neurological and psychiatric disorders, including neuropathic pain. The recovery of synaptic glutamate is mediated by a specific high-affinity membrane excitatory amino acid transporter, the EAAT2, responsible for up to 80–90% of all extracellular glutamate uptake activity. When EAAT2 function is impaired or altered in the vicinity of afferent synapses, glial pathological changes may occur, developing neuropathic pain. Considering EAAT2 a potential target, a small series of EAAT2 activators **4a-f**, was developed and tested in a rat model of oxaliplatin-induced neuropathic pain. Using **3** as the reference compound, we evaluated the response to different noxious and non-noxious mechanical and thermal stimuli after repeated treatment ( $2.4 \text{ mg kg}^{-1}$  i.p., daily). Among the novel compounds, **4f** ( $3.21 \text{ mg kg}^{-1}$ ) showed the best anti-hypersensitive 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** can be considered an interesting candidate for further preclinical studies.



## 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 both a Bruker AC 200 or a 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 either a ThermoFinnigan LCQ-Deca or an Agilent 1100 LC/MSD. 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 x 150 mm, 5  $\mu$ m) 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** (0.10 g, 0.53 mmol) with the suitable bromomethyl derivative (0.80 mmol) in dry DMF (5.0 mL) using  $K_2CO_3$  (0.15 g, 1.1 mmol) as the base. The resulting mixture was stirred at room temperature for the indicated 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 x 10 mL), dried over anhydrous  $Na_2SO_4$  and concentrated *in vacuo*. The residue 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 target 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 (m. p. 177.4-178.2  $^{\circ}C$ ).  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  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 ( $M + H^+$ ). 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.

## ASSOCIATED CONTENT

**Supporting Information.** Experimental procedures and X-ray crystallography studies. This material is available free of charge via the Internet at <http://pubs.acs.org>

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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## ABBREVIATIONS

EAATs excitatory amino acid transporters; CNS central nervous system; SNCV sensory nerve conduction velocity; GLT-1 glutamate transporter 1; GLAST glutamate-aspartate transporter.

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