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Research paper

Synthesis and biological evaluation of a new class of benzothiazines as neuroprotective agents

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ABSTRACT

Neurodegenerative diseases are disorders related to the degeneration of central neurons that gradually lead to various, severe alterations of cognitive and/or motor functions. Currently, for no such diseases does any pharmacological treatment exist able to arrest its progression. Riluzole (**1**) is a small molecule able to interfere with multiple cellular and molecular mechanisms of neurodegeneration, and is the only approved treatment of amyotrophic lateral sclerosis (ALS), the progression of which proved to significantly slow, thus increasing somewhat average survival. Here we report the synthesis of differently functionalized 4*H*-3,1-benzothiazine (**5**–**6**) and 2*H*-1,4-benzothiazine (**7**) series as superior homologues of **1**. Biological evaluation demonstrated that amidine 4*H*-3,1-benzothiazine derivatives **5b**-**d** can reduce glutamate and LDH release in the oxygen/glucose deprivation and reperfusion model (OGD/R) applied to brain slices with a higher potency than **1**. Moreover the mentioned compounds significantly reduce glutamate- and 6-hydroxydopamine (6-OHDA)-induced cytotoxicity in neuroblastoma cells. In addition, the same compounds limit ROS formation in both neuronal preparations. Finally, **5c** proved effective in inhibiting neuronal voltage-dependent Na^+ and Ca^{2+}-channels, showing a profile comparable with that of **1**.

1. Introduction

Chronic neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), as well as acute neurodegenerative diseases like brain ischemia, represent severe pathologies in the developed world. The impact of these neurodegenerative diseases is dramatically increased due to the society ageing with huge human and economic costs [1]. Currently, the pathogenesis of the mentioned disorders is not fully understood, and none of the currently available treatments can stop their progression. In this scenario, it is claimed that the administration of drugs able to modulate multiple pathways involved in the onset and progression of the pathology may slow and definitely stop the evolution of the disease itself [2]. The aetiology of diverse neurodegenerative diseases, however, is a complex process involving conjoint genetic and cellular mechanisms, which dynamically interact to cause a common final, neurodegenerative outcome [3]. In particular, several experimental data strongly suggest that excitotoxic-mediated neuronal damage plays a crucial role in the early pathogenesis of chronic as well as acute neurodegenerative diseases [4]. Overactivation of glutamate receptors impairs cellular calcium homeostasis with consequent activation of neuronal nitric oxide synthase (nNOS). Nitric oxide (NO) overproduction generates free radicals and trigger programmed cell death. Simultaneous inhibition of key signals involved in excitotoxic cell death will provide interesting and efficacious potential approaches for therapeutic intervention.

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This can be attained either by means of a combination of drugs each endowed with different mechanisms of action, or by means of multi-target-direct ligands [5,6].

Since each step of the excitotoxic cascade could represent an attractive drug target for the development of neuroprotective agents beneficial for the treatment of numerous chronic and acute brain diseases, we developed new compounds endowed with neuroprotective activity and particularly focused on those potentially useful in the treatment of ALS. Our attention was firstly attracted by riluzole (**1**, 2-amino-6-(trifluoromethoxy)benzothiazole) (Fig. 1), which has been shown to possess neuroprotective effects in animal models of PD [7], HD [8] and cerebral ischemia [9].

Interestingly, **1** is a small molecule (MW = 234.2) that *in vitro* may elicit multiple molecular actions, among which those clinically relevant are inhibition of voltage-gated sodium channels [10–13], which can lead to reduced neurotransmitter release, non-competitive inhibition of NMDA receptors [14,15], inhibition of glutamate release [16], and enhanced astrocytic uptake of extracellular glutamate [17].

Currently, **1** is the only drug that has proved able to modify the progression of ALS [18–20] and the only one approved for the treatment of this disease.

On these bases we designed and reported [21,22] the synthesis and the biological evaluation of amidine and guanidine derivatives **2** and **4**, respectively (Fig. 1), as **1**-like compounds aiming to conjugate the neuroprotective effects of **1** with the neuroprotective and anti-inflammatory activity of 1400W [23] and the NOS-inhibiting properties of aminoguanidine [24] and L-NAME [25] (Fig. 1). Thiourea derivatives **3** (Fig. 1) were synthesized since several thioureas showed potent free radical scavengers activity, preventing oxidative damage [26].

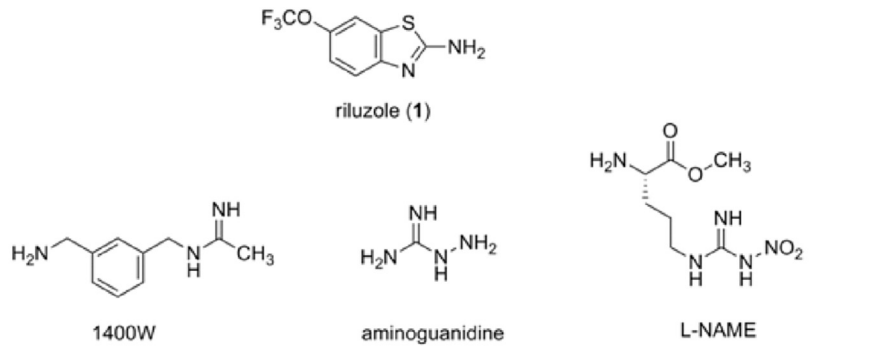
Derivatives **2**–**4** were tested using an *in vitro* protocol of ischemia/reperfusion injury and the results revealed that **2c** and **3a**-**d** meaningfully reduced neuronal injury. In particular, compounds **3a**-**d** were selected for evaluating their antioxidant properties. The results proved that the mentioned compounds were endowed with a direct ROS scavenging activity. Compounds **3b** and **3d** underwent electrophysiological studies in order to assess their potential activities on voltage-dependent Na^+ and Ca^{2+} currents in neurons from rat piriform cortex. Compound **3b** inhibited the transient Na^+ current at 50 μM, but to a much smaller extent than **1** [22].

Encouraged by our results, we decided to pursue the synthesis of two different series of benzothiazines **5**–**7** (Fig. 2), as cyclic analogues of benzothiazole.

2. Results and discussion

2.1. Chemistry

The synthesis of 2-amino-4*H*-3,1-benzothiazines **15** and **16** has been accomplished as reported in Scheme 1. The protection of the amino group of *p*-trifluoromethoxyaniline **8** by means of pivaloyl acid chloride in the presence of Et_3N gave pivaloyl amide **9** [27] that



	R	R'
2a	H	H
2b	H	Me
2c	Me	Me
2d	Et	Et
2e	*n*-Pr	*n*-Pr

	R
3a	Et
3b	*n*-Pr
3c	*i*-Pr
3d	*n*-Bu
3e	Ph
3f	4-F-C_6H_4

	R
4a	Et
4b	*n*-Pr

Fig. 1. Reference compounds.