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Design and synthesis of benzo[*d*] thiazol-2-yl-methyl-4-(substituted)-piperazine-1-carbothioamide as novel neuronal nitric oxide inhibitors and evaluation of their neuroprotecting effect in 6-OHDA-induced unilateral lesioned rat model of Parkinson's disease

Saurabh Agrawal , Rita Kumari , Tuithung Sophronea , Namrata Kumari, Pratibha Mehta Luthra *

Neuropharmaceutical Chemistry Laboratory, Dr BR Ambedkar Centre for Biomedical Research, University of Delhi, Delhi 110007, India

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ABSTRACT

Neuronal nitric oxide synthase (nNOS) is an enzyme constitutively expressed in the mammalian brain and skeletal muscles. The excessive activation of nNOS in the neurons results in oxidative and nitrosative stress associated with neuronal loss in various neurological disorders. Several nNOS inhibitors have been reported to limit the excessive activation of nNOS. In the present work, we have designed and carried the synthesis of benzo [*d*]thiazol-2-yl-methyl-4-(substituted)-piperazine-1-carbothioamide as novel neuronal nitric oxide inhibitors (**5–28**, twenty-four compounds). Stably transfected HEK 293 cells expressing NOS isoforms treated with the compounds (**5–28**) showed that the eight compounds exhibited > 95% cell survival in the MTT assay. nNOS inhibition assay of the eight compounds illustrated that the compound **18** was most selective for nNOS (nNOS−66.73 ± 1.51; eNOS−28.70 ± 1.39; iNOS −13.26 ± 1.01) in HEK 293 cells expressing NOS isoforms. 6-OHDA-induced unilaterally lesioned rats treated with the compound **18** showed the improvement in motor and non-motor functions. Furthermore, the compound **18** showed the increased levels of dopamine and decreased levels of glutamate and nitrite ions in the isolated rat brain. In the docking analysis, the compound **18** showed the significant binding affinity with the nNOS binding site (the ΔG value − - 9.0 kcal/mol). Overall results demonstrated that the N-(benzo[*d*]thiazol-2-ylmethyl)-4-(4-nitrophenyl) piperazine-1-carbothioamide (the compound **18**) possessed significant nNOS inhibiting activity and neuroprotecting potential in 6-OHDA-induced unilaterally lesioned rat model of PD and more work will be required to establish the role of the compound **18** in the therapy of PD and other neurodegenerative disorders.

1. Introduction

Nitric Oxide ($NO^{\cdot}$), a highly soluble and diffusible free radical generated endogenously, is induced by nNOS activation in a calcium/calmodulin (Ca^{2+}/CaM)-dependent manner by the conversion of L-arginine into L-citrulline in brain [1–3]. Neuronal damage activates nNOS resulting in increased levels of NO in the brain leading to NO-mediated neurotoxicity and impairing of the cellular energy production via interaction with the iron-sulfur centers in the mitochondrial electron transport chain [4,5]. Excessively produced NO reacted with the superoxide radicals to give peroxynitrite triggering the nitrergic signaling which irreversibly reacted with the tyrosine (Tyr) residues to

Abbreviations: RNS, Reactive Nitrogen Species; NOS, Nitric Oxide Synthase; NO, Nitric Oxide; TLC, Thin Layer Chromatography; HPLC, High Performance Liquid Chromatography; NMR, Nuclear Magnetic Resonance; MTT, 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide; LPS, Lipopolysachharide; DMSO, Dimethyl sulfoxide; HEK 293 cells, Human Embryonic Kidney cells; DAF-FM DA, 4-Amino-5-methylamino-2',7'-difluorofluorescein diacetate; HBSS, Hank's balanced salt solution; CaM, Calmodulin; PD, Parkinson's Disease; FAD, Flavin Adenine Dinucleotide; FMN, Flavin Monocleotide; NADPH, Nicotinamide adenine dinucleotide phosphate; PPH_3, Polyphosphoric acid; IR, Infrared; PE, Petroleum Ether; PBS, Phosphate Buffer Saline; SDS, Sodium Dodecyl Sulphate; TGS, Tris glycine SDS; UV, Ultra-Violet; Δ, Heat.

* Corresponding Author.
E-mail address: pmluthra@acbr.du.ac.in (P.M. Luthra).

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