Macedonian pharmaceutical bulletin, 68 (Suppl 1) 395 - 396 (2022)
Online ISSN 1857 - 8969
DOI:10.33320/maced.pharm.bull.2022.68.03.190
Short communication

Targeting the oxidative stress in neurodegenerative disorders with multifunctional benzimidazole and indole hybrids

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Introduction

Neurodegenerative disorders comprise a broad group of neurological diseases with diverse symptomatic manifestations, of which Alzheimer's and Parkinson's diseases are with the highest prevalence. Despite years of extensive research and considerable advances made towards a better understanding, nowadays the underlying cause still remains unknown. What has been revealed is highly complex pathogenesis with multiple contributing genetic and environmental factors. Commonly the following are observed: protein misfolding and plaque aggregation, mitochondrial dysfunction, transition metal ions dysregulation, oxidative stress, neuroinflammation, leading to neuronal death (Angelova, 2021). The true apex (if one) may yet be unknown, but there is compelling evidence of the early events. Post-mortem studies of patients' brains show increased peroxidation of biomolecules, suggesting that oxidative damage precedes the senile plaques formation (Van Bulck et al., 2019). However, the currently approved therapies remain single-target and provide only a symptomatic treatment with adverse side effects unable to arrest the disease's progression. A more recent alternative and promising approach beyond the 'one-molecule, one-target' paradigm is the Multi-Target-Directed Ligand (MTDL) strategy. In order to make a real impact and be efficient in interrupting, or even have a chance of curing the degenerative process, the MTDLs must be aimed as high as possible at the pathological cascade's hierarchy. The current review aims to provide a brief summary of our achievements so far and any future prospects regarding the drug-design of benzimidazole and indole based multi-target compounds for the treatment of neurodegenerative disorders. Our research is mainly inspired by the hypothesis that the inhibition of oxidative stress with a simultaneous rendering of neuroprotection might delay the degeneration progression. The structural design represents a carefully planned molecular hybridization of pharmacophore subunits (arylhydrazone, propargyl, catechol, hydroxy and methoxy moieties, etc.) with proven activity targeted upstream the neurotoxic cascade, with special emphasis on antioxidant activity, intended to eliminate the major oxidative stress sources through three different mechanisms: i) direct free radical scavenging; ii) inhibiting monoamine oxidase-B (MAO-B); iii) balancing the metal dyshomeostasis - processes coinciding with three different major causes of protein aggregation and overall toxicity. Broad series of benzimidazole and indole arylhydrazone hybrids have been synthesized (Anastassova et al., 2021; Anastassova et al., 2022) and evaluated through a variety of neurobiological, antioxidant and theoretical methods. The following is an overview of the neurotoxicological and neuroprotective effects of the *N,N'*-disubstituted benzimidazole-2-thione and the *N*-alkylated benzimidazole derivatives.

Neurobiological properties evaluation

Establishing a safety profile is an essential part of the potential drug characterization. The neurotoxicolocial potential of the compounds was assessed by measuring the cell viability of the neuroblastoma SH-SY5Y cell line and isolated rat brain synaptosomes using melatonin and rasagiline as reference compounds. Series I constitutes a

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small library of 19 disubstituted benzimidazole arylhydrazone containing a variety of methoxy and hydroxy combinations. Six of them bearing residues of syringaldehyde, salicylaldehyde, 2,3-dihydroxy-, 2,4-dihydroxy-, 2-hydroxy-6-methoxy-, 2-hydroxy-4-methoxy-, 2-hidoxy-2-methoxy and 4-hydroxy-2-methoxybenzaldehyde had IC_{50} values > 200 μM and were considered of low toxic potential and thus most perspective for further studies. In the monosubstituted Series II, the isomers containing one hydroxy and one methoxy group had the highest IC_{50} values of 256.12 and 311.43 μM. Similarly, the compounds did not show any toxic effect on the functional-metabolic status of rat brain synaptosomes, neither on the synaptosomal viability, nor the levels of the reduced glutathione (GSH). The neuroprotective effects were evaluated on a model of H_2O_2-induced oxidative stress in SH-SY5Y cells generating ROS and leading to cellular lipids, proteins and DNA damage. The compounds bearing two OH groups (2,3- and 2,4diOH, 50 μM) demonstrated remarkable activity, more pronounced than melatonin and rasagiline, restoring the cell viability up to 79% and 80% whereas for the reference compounds it was barely 52% and 39%. On the same model, among Series II the strongest effects were exhibited by the 2-hydroxy-4-methoxy derivative (10 μM) - also surpassing the ones by the references by restoring the cell viability to 71%, further confirmed by a microscope observation on the cell morphology. The studies on a model of 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in synaptosomes showed that all of the tested compounds from Series I had protective properties, yet the 2,3diOH arylhydrazone (10 μM) outstood significantly inhibiting the decrease in synaptosomal viability by 71% and GSH level to 62% compared to 6-OHDA treatment. In series II the same compound (2OH-4OMe, 10 μM) displayed again activity that exceeded the reference compounds, preserving the synaptosomal viability up to 75%.

All of the tested arylhydrazones showed a capability to inhibit MAO-B on the fluorimetric AmplexRed method, as the most potent among Series I was the catecholic compound with 2,3diOH residues, whereas in Series II was the 2OH-4OMe derivative whose activities were statistically identical to the clinically used rasagiline and selegiline. The docking studies confirmed that the hydrazone group is significant for the effective interaction ligand orientation. The potent activity of the 2OH-4-OMe was explained by its structure being appropriate for entering deep in the narrow and mostly lipophilic active site pocket of the enzyme, interacting favorably with the key amino acid residues Tyr326 and Cys172. In comparison, the better MAO-B inhibition of the catecholic disubstituted compound is explained by their longer chains enabling them to reach closer proximity of FAD and fill the whole active site with one of the phenolic rings in close proximity to flavine moiety.

Radical-scavenging properties evaluation

The radical-scavenging activity has been studied by a variety of methods. Using iron-induced oxidative degradation of lecithin both Series showed lower absorbance values compared to melatonin, *i.e.* lower extent of molecular damage. As the most potent protectors of Series I effective up to 40%, the ones bearing fragments of syringaldehyde, 2,3-dihydroxy and 3,4-dihydoxy moieties were denoted. In a similar fashion in Series II the catecholic compounds were distinguished as the 3,4diOH's effect was up to 48%. Additionally, studies were carried out using chemiluminescent and spectrophotometric assays. In chemiluminescent model systems with hypochlorite and superoxide radicals hybrids from Series I with residues of vanillin and syringaldehyde had significantly higher scavenging activity compared to melatonin. At the non-enzymatic superoxide generated in the KO2 system, only the 2,3diOH decreased significantly the SPh-SI compared to controls. In the xanthine/xanthine oxidase model system again the dihydroxyl compounds 2,3- and 2,4diOH) decreased significantly the SPh-SI indicating a capability to prevent the NBT reduction. At the maximal tested concentration compound 10 decreased the SPh-SI to 64%.

Acknowledgements

This work was supported by The National Science Fund of Bulgaria, Young scientist project КП-06-ПМ59/2.

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