

Neuroprotective activity studies of some phenylacetamide derivatives bearing $1H$ -pyrazole or $1H$ -1,2,4-triazole

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Abstract: Neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases are one of the most studied diseases in health sciences. Although each of them has their own mechanisms and outcomes, they share molecular mechanisms like oxidative stress which plays the key role in neurodegeneration. Therefore, alleviating the oxidative stress and improving the antioxidant defense capacity of the brain tissue helps to reduce the progression of neurotoxicity in neurodegenerative diseases including the Alzheimer's disease. In this regard, we have studied the neuroprotective activity of the compounds that were determined as the most active derivatives against AChE enzyme in our previous study. The results revealed that Compounds **3** and **4** restore the cell viability especially at lower doses, hence, these two molecules may be a promising lead for further studies with their neuroprotective potential and AChE inhibitory activity.

Keywords: phenylacetamide; pyrazole; 1,2,4-triazole; neuroprotectivity; neurodegenerative diseases ©2021 ACG Publication. All right reserved.

1. Introduction

Brain, compared to other tissues in the body, consumes the most oxygen and glucose, has insufficient antioxidant defense capacity, and contains high levels of iron (Fe^{2+}), ascorbate and unsaturated fatty acids,¹⁻³ therefore it is the most vulnerable tissue to oxidative stress. Oxidative stress that plays the key role in neurodegeneration, progressive loss of specific neuronal cells in CNS leading to functional loss as ataxia or sensory dysfunction as dementia,^{4,5} is originated from the imbalance between mitochondrial reactive oxygen species (ROS) production and antioxidant defense capacity.⁵⁻⁹ Oxidative stress is involved in the pathogenesis of progressive neurodegenerative disorders and increased ROS has been reported⁴ in Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD).¹⁰

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AD is the major cause of dementia and it is defined by the memory loss and deterioration in cognition, function and behavior. The neuropathological features of the AD brain are the loss of cholinergic neurotransmission, formation of extracellular amyloid plaques and intracellular neurofibrillary tangles. Several treatment strategies including the cholinergic hypothesis were suggested to fight the symptoms of AD. Cholinergic hypothesis claims that the decreased concentration of pre-synaptic neurotransmitter acetylcholine (ACh) is one of the causes of the cognitive disorder. Therefore, inhibition of the AChE enzyme is one of the treatment methods that catalyzes the degradation of ACh. In this manner, tacrine, donepezil, rivastigmine, galantamine and memantine were established as AChE inhibitors used for symptomatic treatment.¹¹⁻¹³

As mentioned before, increase of the mitochondrial ROS also causes neurodegenerative disorders including AD, acting as a major contributor to neuronal loss.⁶ Besides literature survey reveals that antioxidants help to treat ROS-mediated damage and delay the progression of AD.^{11,14} Furthermore, there are quite a lot of studies supporting the cause of AD as oxidative stress but arguing whether it is the primary or a secondary cause.⁹ Therefore, neuroprotection became essential to prevent or treat the neurodegenerative conditions in AD.¹⁵⁻¹⁷

In our previous study, we have designed and synthesized a group of acetamide derivatives bearing pyrazole and 1,2,4-triazole as five membered heterocyclic rings and studied for their cholinesterase activity.¹⁸ The results revealed that all the compounds have selective moderate to good AChE inhibitory activity. In this study, we have chosen the most active five compounds among them and tested their neuroprotective potential on SH-SY5Y cell line after inducing neurotoxicity by 6-hydroxydopamine (6-OHDA) (Figure 1).

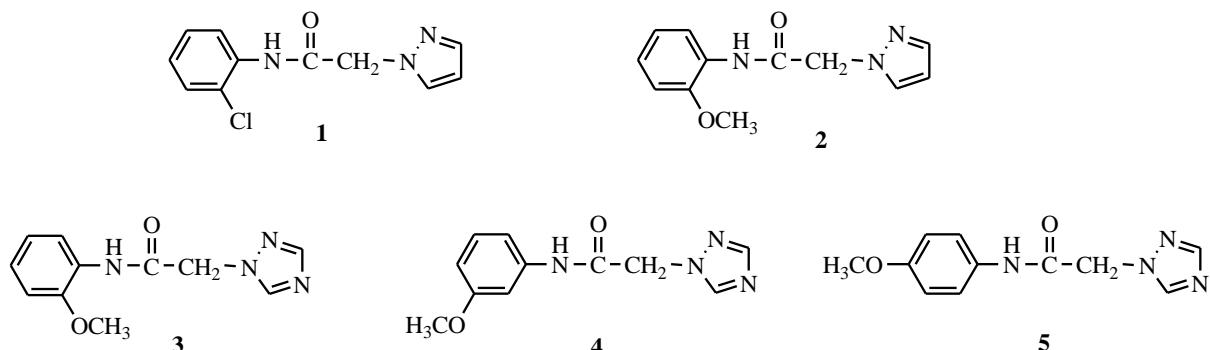


Figure 1. Molecular formula of the title compounds

2. Experimental

2.1. Biological Materials and Apparatus

SH-SY5Y cells were obtained from American Type Culture Collection (ATCC) and cultured in DMEM (Dulbecco's modified Eagle's medium; Biological Ind., Israel) supplemented with 10% FBS (fetal bovine serum, Panbiotech, Germany) and 2 mM *L*-glutamine (Biological Ind., Israel) at 37°C under humidified 5% CO₂.

Cells were seeded at a density of 2x10⁴ cells in 96 well plates. In the neuroprotective study, the cells were pre-treated with increasing concentration of synthesized compounds for 12 h and followed by the treatment of 6-OHDA (50 µm; Sigma Aldrich, UK) for an additional 12 h. Cells were treated with DMSO (Dimethyl sulfoxide) as a solvent control.

6-OHDA stock solution was prepared in sterile water. The synthesized compounds were prepared in DMSO (as 1000x stock). The final concentrations of DMSO were below 0.1%.

Neuroprotective activity studies of some phenylacetamide derivatives

2.2. Chemistry

2.2.1. General procedure for the synthesis of the compounds

As mentioned in reference ¹⁸, *o*-chloro, *o*-methoxy, *m*-methoxy and *p*-methoxy aniline (1 eq.) was dissolved in glacial acetic acid. Chloroacetyl chloride (1 eq.) was added dropwise in ice bath. After stirring in ice bath for 1h and at room temperature for an additional hour the reaction mixture was poured into saturated sodium acetate solution. The precipitate was filtered and washed with water. After intermediate product dried, the obtained product was crystallized from ethanol: water mixture.

In the second step, acetamide intermediates (1 eq.) and pyrazole or 1,2,4-triazole (5 eq.) were refluxed in toluene. After the confirming the end of the reaction by TLC, the solvent was evaporated. The residue was dissolved in chloroform and extracted with water. The organic phases were combined, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The product was crystallized from water. Spectral data of the compounds were reported in our previous study.¹⁸

2.3. Biological Assay

2.3.1. Cell Viability Assay

To evaluate the effects of these compounds on the neurotoxicity mediated by 6-OHDA, we performed cell viability assay via WST-1 reagent (Roche, Switzerland) according to the supplier's instructions. After cells were incubated for desired concentration of compounds/6-OHDA for indicated times, the mixture of WST-1 and medium (1:9) was replaced with old media. The absorbance was measured with a microplate reader at 440 nm (Varioscan, Thermo Fisher Scientific, US) and 690 nm as a reference wavelength. Cell viability was showed as a percentage cell viability compared to cells treated with DMSO as a solvent control. The WST-1 experiment was performed by triplicate samples. Student's *t* test was used to determine the significance of the differences (***p* ≤ 0.001, ****p* ≤ 0.005).¹⁹

3. Results and Discussion

3.1. Biological Assay

3.1.1. The Neuroprotective Effects of the Synthesized Compounds Against 6-OHDA Mediated Neurotoxicity

In this study, we have tested the neuroprotectivity potential of the most active five compounds against AChE that were synthesized in our previous study¹⁸ (Table 1).

Table 1. AChE inhibitory activity results of the title compounds

Compounds	AChE $\text{IC}_{50} \pm \text{SEM}$ (μM) ^a
1	8.97 ± 0.25
2	8.32 ± 0.19
3	7.41 ± 0.19
4	6.68 ± 0.21
5	8.14 ± 0.39
Galanthamine	0.43 ± 0.03

^a Data are means \pm standard error of the mean of triplicate independent experiments.

In order to investigate the potential effects of the compounds on neurotoxic mediated by 6-OHDA, cells were pre-treated with indicated concentrations of compounds for 12 h then treated with 6-OHDA for 12 h. Treatment of 50 μM 6-OHDA on SH-SY5Y cells decreased the cell viability by $57.60 \pm 1.08\%$ (Figure 2). The viability assays showed that compounds **1,2** and **5** did not reverse the toxic effect of 6-OHDA, moreover decreased the cell viability synergistically with 6-OHDA in each

concentration. However, compound **3** and **4** attenuated the toxic effect of 6-OHDA significantly especially at concentrations between 1 and 5 μ M. Compound **3** increased the viability to 105.46 ± 1.48 (1 μ M), 104.28 ± 1.57 (2.5 μ M) and 102.67 (10 μ M), while compound **4** treatment ameliorated the cell viability to 102.15 ± 0.14 (1 μ M), 102.85 ± 0.85 (5 μ M) and 104.18 ± 0.34 (10 μ M). The highest concentrations of these compounds did not restore the cell viability as much as lower doses (Figure 2).

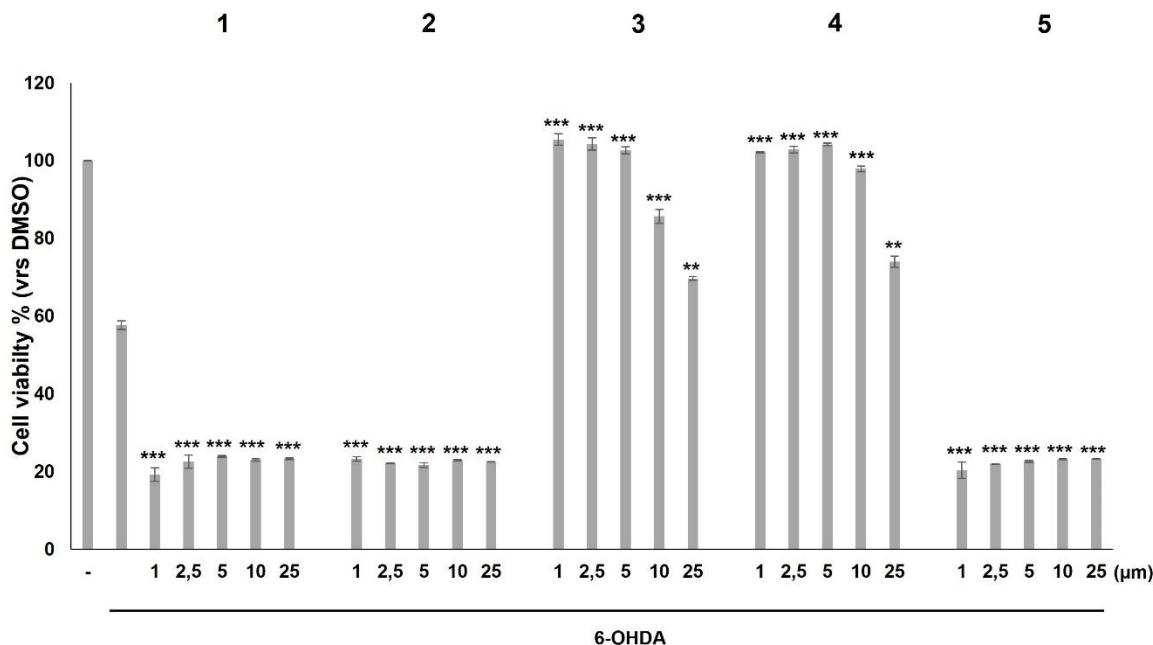


Figure 2. Compounds **3** and **4** reverse the toxic effects of 6-OHDA. SH-SY5Y cells were pre-treated with increasing concentrations of compounds for 12 h then treated with 6-OHDA for 12 h. Cell viability was done by WST-1 reagent. The absorbance of cells treated with DMSO used as a solvent control was considered as 100% and cell viability of the applied concentrations was calculated. The WST-1 assay was performed by triplicate samples. ANOVA analysis with Dunnett's post hoc test was used to determine the significance of the differences compared to the control cells (* $p \leq 0.05$, ** $p \leq 0.001$, *** $p \leq 0.005$).

The results of the neuroprotectivity study are remarkable because not only compound **3** and compound **4** have good neuroprotective activity but also have the most AChEI activity.

4. Conclusion

In this study, we have tested the neuroprotective potential of the five compounds that were active against AChE. The results demonstrated that compounds **3** and **4** which have AChEI activity below 8 μ M restore the cell viability especially at lower doses. Therefore, we can speculate that compound **3** and **4** both have AChE inhibitory activity and has neuroprotective potential, and these two molecules may be a promising lead for further studies.

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References

- [1] Dringen, R. Metabolism and functions of glutathione in brain. *Prog. Neurobiol.* **2000**, *62*(6), 649-671.
- [2] Floyd, R.A.; Carney, J.M. Free radical damage to protein and DNA: Mechanisms involved and relevant observations on brain undergoing oxidative stress. *Ann. Neurol.* **1992**, *32*(1), 22-27.
- [3] Gerlach, M.; Ben-Shachar, D.; Riederer, P.; Youdim, M.B. Altered brain metabolism of iron as a cause of neurodegenerative diseases. *J. Neurochem.* **1994**, *63*(3), 793-807.
- [4] Patten, D.A.; Germain, M.; Kelly, M.A.; Slack, R.S. Reactive oxygen species: Stuck in the middle of neurodegeneration. *J. Alzheimers Dis.* **2010**, *20*(1), 357-367.
- [5] Lopes, F.M.; Londero, G.F.; Marengo de Medeiros, L.; Lisbôa da Motta, L.; Behr, G.A.; Aguiar de Oliveira, V.; Ibrahim, M.; Moreira, J.C.F.; Porciuncula, L.O.; Teixeira da Rocha, J.B.; Klamt, F. Evaluation of the neurotoxic/neuroprotective role of organoselenides using differentiated human neuroblastoma SH-SY5Y cell line challenged with 6-hydroxydopamine. *Neurotox. Res.* **2012**, *22*(2), 138-149.
- [6] Tanay, D.; Bhaskar, D.; Gopal, A.; Dattatray, S.S.; Alok, J.; Akshay, S.; Bichismita, S. Design, synthesis, and *in-vitro* evaluation of fluorinated triazoles as multi-target directed ligands for Alzheimer's disease. *Bioorg. Med. Chem. Lett.* **2021**, *42*(1), 127999.
- [7] Umeno, A.; Biju, V.; Yoshida, Y. *In vivo* ROS production and use of oxidative stress-derived biomarkers to detect the onset of diseases such as Alzheimer's disease, Parkinson's disease, and diabetes. *Free Rad. Res.* **2017**, *51*(4), 413-427.
- [8] Cacciato, I.; Fornasari, E.; Baldassarre, L.; Cornacchia, C.; Fulle, S.; Di Filippo, E.S.; Piertangelo, T.; Pinnen, F. A potent (R)-alpha-bis-lipoil derivative containing 8-hydroxyquinoline scaffold: Synthesis and biological evaluation of its neuroprotective capabilities in SH-SY5Y human neuroblastoma cells. *Pharmaceutics.* **2013**, *6*(1), 54-69.
- [9] Cacciato, I.; Baldassarre, L.; Fornasari, E.; Cornacchia, C.; Di Stefano, A.; Sozio, P.; Cerasa, L.S.; Fontana, A.; Fulle, S.; Di Filippo, E.S.; La Rovere, R.M.; Pinnen, F. (R)- α -lipoil-glycyl-L-prolyl-L-glutamyl dimethyl ester codrug as a multifunctional agent with potential neuroprotective activities. *ChemMedChem.* **2012**, *7*(11), 2021-2029.
- [10] Koufaki, M.; Kiziridi, C.; Nikoloudaki, F.; Alexis, M.N. Design and synthesis of 1,2-dithiolane derivatives and evaluation of their neuroprotective activity. *Bioorg. Med. Chem. Lett.* **2007**, *17*(15), 4223-4227.
- [11] Bhatt, S.; Puli, L.; Patil, C.R. Role of reactive oxygen species in the progression of Alzheimer's disease. *Drug Discov. Today.* **2021**, *26*(3), 794-803.
- [12] Reitz, C.; Brayne, C.; Mayeux, R.; Epidemiology of Alzheimer's disease. *Nat. Rev. Neurol.* **2011**, *7*(3), 137-152.
- [13] Parlar, S.; Bayraktar, G.; Tarikogullari, A.H.; Alptuzun, V.; Erciyas, E. Synthesis, biological evaluation, and molecular docking study of hydrazone-containing pyridinium salts as cholinesterase inhibitors. *Chem. Pharm. Bull.* **2016**, *64*(9), 793-807.
- [14] Dumont, M.; Beal, M.F. Neuroprotective strategies involving ROS in Alzheimer's disease. *Free Radical Bio. Med.* **2011**, *51*(5), 1014-1026.
- [15] González-Muñoz G.C.; Arce, M.P.; López, B.; Pérez, C.; Romero, A.; del Barrio, L.; Martín-de-Saavedra, M.D.; Egea, J.; León, R.; Villarroya, M.; López, M.G.; García, A.G.; Conde, S.; Rodríguez-Franco, M.I. *N*-acylaminophenothiazines: Neuroprotective agents displaying multifunctional activities for a potential treatment of Alzheimer's disease. *Eur. J. Med. Chem.* **2011**, *46*(6), 2224-2235.
- [16] Robertson, G.S.; Crocker, S.J.; Nicholson, D.W.; Sculz, J.B. Neuroprotection by the inhibition of apoptosis. *Brain Pathol.* **2000**, *10*(2), 283-292.
- [17] Vajda, F.J.E. Neuroprotection and neurodegenerative diseases. *J. Clin. Neurosci.* **2002**, *9*(1), 4-8.
- [18] Tarikogullari, A.H.; Çizmecioğlu, M.M.; Saylam, M.; Parlar, S.; Alptüzün, V.; Soyer Z. Synthesis and cholinesterase inhibitory activity of some phenylacetamide derivatives bearing 1*H*-pyrazole or 1*H*-1,2,4-triazole. *J. Res. Pharm.* **2016**, *20*(1), 21-27.
- [19] Khan, N.; Yilmaz, S.; Aksoy, S.; Uzel, A.; Tosun, Ç.; Ballar Kirmizibayrak, P.; Bedir, E. Polyethers isolated from the marine actinobacterium *streptomyces cacaoi* inhibit autophagy and induce apoptosis in cancer cells. *Chem-Biol. Interact.* **2019**, *307*(1), 167-178.