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Synthesis and cholinesterase inhibitory potential of 2-phenoxy-N-substituted-acetamide derivatives

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Abstract

The research studies worldwide on the identification of novel molecules having the potential to inhibit cholinesterase enzymes generated many compounds with promising results for some of them. Since there are limited number of drugs used in the treatment of Alzheimer's Disease in particular, the research studies continue. Within the scope of this study, four 2-phenoxy-N-substituted-acetamide derivatives were synthesized and their structures were identified employing spectroscopic techniques. The title molecules were further evaluated for their cholinesterase inhibitory potential in modified Ellman's method. The results displayed that the compounds have moderate activity and the simple scaffold employed might be used in future studies for more promising compounds.

Keywords

2-phenoxy-N-substituted-acetamide derivatives, Alzheimer, cholinesterase

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INTRODUCTION

Despite the fact that Alzheimer's disease (AD) was initially identified and diagnosed more than a century ago, many questions still persist, especially regarding its pathophysiological mechanisms. Dementia is a common symptom of many diseases (Gulcan and Orhan, 2021). Unfortunately, the most common type of dementia is AD-related dementia, resulting in cognitive impairment. With respect to the progressive nature of AD, dementia symptoms worsen in time as it is categorized as mild, moderate, and final stages of the disease (Pillai and Cummings, 2013).

Although the pathophysiology of the disease is too complex to fully understand, many validated and non-validated targets have been offered so far for the treatment of AD (Erdogan *et al.*, 2021). Enzymes involved in beta-amyloid cascade, kinases having function in tau-protein phosphorylation, oxidative stress mechanisms are just some of them. None of these targets studies so far ended up with promising molecules possessing disease modifying feature to stop the progression of the disease (Gulcan *et al.*, 2019). Furthermore, they have not been shown to be involved in the reconstruction of cognitive disabilities. From this perspective, cholinesterase inhibitors are still important and they are the only drugs used in the treatment of AD, beside memantine, the single representative of NMDA receptor antagonism (Pepeu, and Giovannini, 2009).

Many studies have pointed out the significance of cholinergic system in the maintenance of cognitive functions. Therefore, since 1980s, four cholinesterase inhibitors have been introduced to the clinic for the treatment of AD-related dementia. Beside the withdrawal story of tacrine, the rest three of them display diverse properties in terms of source, target, dose, pharmacokinetic and pharmacodynamic perspectives (Wilkinson *et al.*, 2004). Therefore, there has been a continuous interest on the screening of diverse structures.

In this study, the design of a previous work of our research group has been adapted on more simple molecules (Shukur *et al.*, 2021). Briefly, the 2-aryloxy-N-substituted-acetamide motif has been successfully applied in the design of multiple-target acting urolithin analogues in our previous works. This scaffold has been assessed in simple compounds, 2-phenoxy-N-substituted-acetamides. The structure of the title molecules are presented in Figure 1.

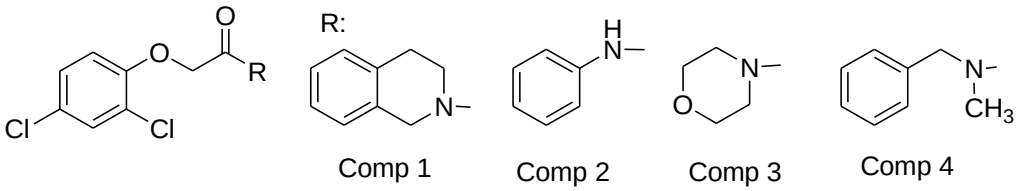

Figure 1: Title molecules.

MATERIALS AND METHODS

All reagents and organic solvents were purchased from Sigma Aldrich through the aid of local vendors. Using ethyl acetate/cyclohexane as the mobile phase (1:1, 2:1, and 1:2 ratios) thin-layer chromatography (TLC) was used to monitor the reactions on Merck aluminum-packed silica gel plates. In order to get the compounds' IR spectra, a Shimadzu FT-IR Prestige model spectrophotometer was used. 1H NMR (at 400 MHz) spectra were recorded on a Bruker-400 NMR spectrometer using tetramethylsilane as an internal standard and dimethyl sulfoxide (DMSO; d6) as a solvent; all chemical shifts were reported in parts per million (ppm, δ). The elemental analysis was performed on a Thermo Fisher Scientific Model Flash Smart CHNS elemental analyzer.

Synthesis of the ethyl 2-(2,4-dichlorophenoxy)acetate: 25mmol of 2,4-dichlorophenol was dissolved in 40mL of DMF. The solution was added 31mmol of NaH and stirred for 5 min at rt. The reaction was started through the addition of 30mmol of ethyl 2-chloroacetate into the solution prepared. At the end of 1h stirring at rt, the reaction mixture was added 50mL of distilled water. The product was extracted into the organic solution through 3 times extraction with ethyl acetate. The product was obtained via the evaporation of organic solvent at reduced pressure.

Synthesis of 2-(2,4-dichlorophenoxy)acetic acid: Ethyl 2-(2,4-dichlorophenoxy)acetate obtained in the previous step was hydrolyzed to yield out 2-(2,4-dichlorophenoxy)acetic acid. Accordingly, 20mmol of ethyl 2-(2,4-dichlorophenoxy)acetate was mixed in 5%KOH containing methanol solution. The mixture was refluxed for 3h. At the end of the reaction the organic solvent was evaporated and the residue left was added 30mL 1N HCl aqueous solution. The product was obtained through extraction with ethyl acetate (25mL x 3) and the evaporation of ethyl acetate combined fractions under reduced pressure.

Synthesis of the title molecules: In situ two steps were achieved to synthesize the final compounds. Accordingly, 20mmol of 2-(2,4-dichlorophenoxy)acetic acid was

dissolved in 25mL of dichloromethane. 3ML of thionyl chloride was added into this solution and the reaction was refluxed for 5h. At the end of the reaction time, the acyl halide (i.e., 2-(2,4-dichlorophenoxy)acetyl chloride) obtained was directly used for title molecule synthesis without further purification. Mainly, 18mmol of the appropriate amine was dissolved in 20mL of dichloromethane. Into this solution at 0ºC was added dropwise the acyl halide solution obtained in dichloromethane. The total addition was achieved in 10 min. At the end of this time, dichloromethane was evaporated. The residue was added 25mL of aqueous. The aqueous solution was further extracted with ethyl acetate (25mL) for three times. The combined organic fractions were evaporated under reduced pressure. The final compounds were obtained with column chromatography employing n-hexane/ethyl acetate (2:1) mobile phase mixture.

Comp 1 [2-(2,4-dichlorophenoxy)-1-(3,4-dihydroisoquinolin-2(1H)-yl)ethanone]: Mp: 167.3 ºC. FT-IR (major peaks) cm^{-1}: 3403, 2983, 1734, 1639. 1H-NMR (DMSO-d6) δ 7.14-6.97 (m, 6H), 6.63 (s, 1H), 4.91 (s, 2H), 4.69 (s, 2H), 3.35-2.80 (m, 4H). Anal. calc. for C17H15Cl2NO2: C, 60.73; H, 4.50; N, 4.17. Found C, 60.81; H, 4.58; N, 4.21.

Comp 2 [2-(2,4-dichlorophenoxy)-N-phenylacetamide]: Mp: 174.8 ºC. FT-IR (major peaks) cm^{-1}: 3395, 3041, 1651. 1H-NMR (DMSO-d6) δ 8.9 (bs, 1H), 7.18-6.99 (m, 7H), 6.65 (s, 1H), 4.87 (s, 2H). Anal. calc. for C14H11Cl2NO2: C, 56.78; H, 3.74; N, 4.73. Found C, 57.12; H, 3.82; N, 4.69.

Comp 3 [2-(2,4-dichlorophenoxy)-1-morpholinoethanone]: Mp: 181.0 ºC. FT-IR (major peaks) cm^{-1}: 3404, 2978, 1737, 1639. 1H-NMR (DMSO-d6) δ 7.18 (s, 1H), 7.00 (s, 1H), 6.63 (s, 1H), 4.84 (s, 2H), 3.75-3.59 (m, 4H). Anal. calc. for C12H13Cl2NO3: C, 49.68; H, 4.52; N, 4.83. Found C, 50.01; H, 4.50; N, 4.87.

Comp 4 [2-(2,4-dichlorophenoxy)-N-benzyl-N-methylacetamide]: Mp: 160.5 ºC. FT-IR (major peaks) cm^{-1}: 3412, 2928, 1737, 1637. 1H-NMR (DMSO-d6) δ 7.20-6.99 (m, 7H), 6.62 (s, 1H), 4.84 (s, 2H), 4.55 (s, 2H), 2.95 (s, 3H). Anal. calc. for C16H15Cl2NO2: C, 59.28; H, 4.66; N, 4.32. Found C, 59.65; H, 4.57; N, 4.44.

Enzyme assays

The title compounds' ability to inhibit cholinesterases was measured using a modified Ellmann's method (AChE, and BuChE) (Ellman 1958). Accordingly, each combination comprised 200 L total volume of 168 L of 50 mM Tris HCl buffer (pH 8.0), 10 L of 6.8 mM DTNB solution (0.34 mM final), 20 mM MgCl2, and 100 mM NaCl, 10 L of AChE or BuChE solution

(0.4 U/mL from Human recombinant AChE or 1.64 U/mL from Human 10 mL of either 10 mM acetylthiocholine iodide or 10 mL of 1.5 mM butyrylthiocholine iodide were added to start the reactions. Using a 96-well microplate reader, readings were taken at 412 nm following incubation for 15 minutes at 27°C (Varioskan Flash, Thermo Scientific, USA). By comparing the rates of reaction of samples relative to blank samples (DMSO and methanol) and using the formula (E S)/E100, where E is the activity of the enzyme without the test sample and S is the activity of the enzyme with the test sample, the percentage of inhibition of AChE and BuChE was calculated. Plotting the percent inhibition against the concentration of test materials allowed us to identify the concentration of test compounds and reference materials (IC50) that determined 50% inhibition of the AChE or BuChE activities. To acquire three IC50s under the experimental conditions, each concentration was evaluated in triplicate using each measurement. The mean ± standard deviation of IC50s were represented.

RESULTS AND DISCUSSION

The title compounds have been synthesized via the synthesis of 2-(2,4-dichlorophenoxy)acetyl chloride and its employment in a Schotten Bauman reaction application (Blanke, and Blanke, 1984). The general synthetic scheme is shown in Figure 2.

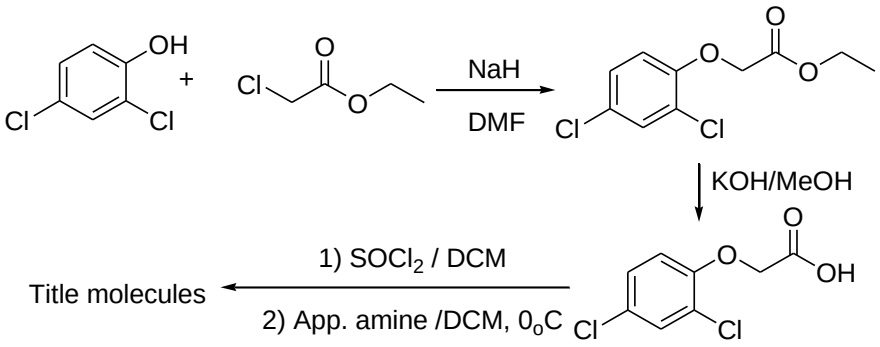

Figure 2: The synthetic scheme.

The yields of each step were in parallel with the literature findings or similar reactions. The overall (total) yields for final molecules were found higher than 40% (Gulcan *et al.*, 2003). The structures of the final molecules were identified employing IR and 1H NMR techniques. Following the synthesis work, the title molecules were assessed for their potential to inhibit cholinesterase enzymes. The results obtained are shown in Table 1.

Table 1: The potential of compounds to inhibit cholinesterase.

Compound	IC50 (AChE) (µM)	IC50 (BuChE) (µM)
Comp 1	24.5 ± 0.1	> 40
Comp 2	20.7 ± 0.8	> 40
Comp 3	22.3 ± 0.5	> 40
Comp 4	16.1 ± 0.2	29.2 ± 0.6
Donepezil	0.021 ± 0.001	8.9 ± 0.1
Rivastigmine	19.8 ± 0.3	13.4 ± 0.5

Accordingly, the title compounds were found to possess particularly acetylcholinesterase inhibitory potential. The most active compound among them was found compound 4. The N-benzyl moiety has been found an important scaffold in many cholinesterase inhibitor molecule designs. Moreover, beside compound 4, the title compounds displayed no activity against butyrylcholinesterase, implying the acetylcholinesterase selectivity of the molecules. Donepezil, one of the current cholinesterase inhibitor drugs, displayed the superior activity among the compounds tested. However, the activities of the title molecules were found comparable to the activity of rivastigmine, particularly against acetylcholinesterase enzyme.

CONCLUSION

Within this limited research work, four 2-phenoxy-N-substituted-acetamide derivatives were synthesized and their activities were assessed in cholinesterase inhibition assays. In parallel to the previous findings, the N-substituted acetamide function has been shown to be an available scaffold for the design of cholinesterase inhibitor molecules. The research warrants further work for the generation of full structure activity relationship outcomes.

DECLARATION

This manuscript has been prepared on the basis of the graduation thesis works of four EMU Faculty of pharmacy students (i.e., Kiana Harati, Seyedeh Mahta Kiaei, Tina Mahdipour Amjad, Zahra Nobavar) under the supervision of Prof. H. Ozan Gulcan. Some of the title compounds are previously synthesized for other scientific investigations and they are not original. The authors declare no conflict of interest.

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